Final Report

Project Title Effect of silver nanoparticles-longkong peel extract coating on quality of Longkong

By Dr. Intira Lichanporn

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Researcher	Institute
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This project granted by the Thailand Research Fund

Abstract

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Project Title : **Effect of silver nanoparticles-longkong peel extract coating on quality of Longkong**

Investigator : **Dr. Intira Lichanporn**

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Project Period : **2 year**

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Bio-inspired silver nanoparticles were synthesized with the aid of a novel, non-toxic, eco-friendly biological material namely, longkong peel extract crushed, acetone precipitated, air-dried peel powder was used for reducing silver nitrate. Silver nanoparticles were formed when the reaction conditions were altered with respect to pH, LPE content and concentration of silver nitrate mixtures turned yellow at pH 2.0 and when increased to pH 5.0 a dark brown color was observed. The effect of concentration of silver nitrate on nanoparticle synthesis was evaluated. The results indicate that silver nitrate concentration at 0.125 and 0.5 mM had yellow color and darker shades of brown when the silver nitrate concentration ranged between 1.0, 1.5 and 2.0 mM. Effects of concentration of LPE powder on nanoparticle synthesis were also evaluated. The addition of 0.5 1.0 2.0 4.0 or 10.0 mg of LPE had no significant effect on the absorbance but maximums absorbance was at 600 nm. The color of LPEs was obtained. The effects of the concentration of LPE coating on the preservation of raw longkong were measured. Alginate coating was used as a component of SLN-LPE. Longkong was coated with 0, 0.5, 1.0, 2.0, 4.0 and 10.0 mg SLN-LPE and then stored at 13°C and 90-95% relative humidity for 9 days. Every 3 days, longkong samples were analyzed for changes in browning, weight loss and chemical quality. It was established that alginate coating as a SLN-LPE maintained the quality of longkong. The best results were obtained with SLN-LPE concentrations of 1.0 and 2.0 mg, longkong showed the lowest range of browning, weight loss and activities of POD and PPO compared to the control fruit. The total phenolic content of longkong coating with 1.0 and 2.0 mg SLN-LPE was higher than that of the control. However, Longkong coating with

SLN-LPE of all treatment maintained titratable acidity and total soluble solid during storage. These results indicated that SLN-LPE stored at 13°C was a promising approach in inhibiting browning and maintaining the quality of longkong.

Key words: longkong peel extract, silver nanoparticles, coating

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บทคัดย่อ

การสังเคราะห์ซิลเวอร์นาโนพาทิเคิลจากชีวภาพ ซึ่งเป็นสารที่ไม่เป็นพิษ เป็นวัสดุที่เกี่ยวข้องกับสารชีวภาพได้แก่ สารสกัดจากเปลือกลองกองโดยถูกนำมาบดและตกตะกอนด้วยอะซีโตน นำไปทำให้แห้งจะได้ผงเปลือกลองกองซึ่งใช้เป็นตัวรีดิวซ์เงินไนเตรท ซิลเวอร์นาโนพาทิเคิลถูกฟอร์มตัวขึ้นเมื่อมีการเปลี่ยนแปลงเงื่อนไขปฏิกิริยาเกี่ยวกับค่าความเป็นกรดด่าง ปริมาณสารสกัดเปลือกลองกอง และความเข้มข้นของของส่วนผสมซิลเวอร์ไนเตรท สีของปฏิกิริยาส่วนผสมนี้เปลี่ยนเป็นสีเหลืองเมื่อ มีค่ากรดด่างเท่ากับ 2.0 และเมื่อเพิ่มค่ากรดด่างเป็น 5.0 สีจะเป็นสีน้ำตาลเข้ม ผลของความเข้มข้นของซิลเวอร์ไนเตรทต่อการสังเคราะห์นาโนพาทิเคิลชี้ให้เห็นว่าความเข้มข้นของซิลเวอร์ไนเตรทที่ 0.125 และ 0.5 มิลลิโมลาร์ มีสีเหลืองและสีน้ำตาลเข้มเมื่อความเข้มข้นของซิลเวอร์ไนเตรทอยู่ในช่วงระหว่าง 1.0 1.5 และ 2.0 มิลลิโมลาร์ นอกจากนี้ยังได้ประเมินผลกระทบของความเข้มข้นของผงเปลือกลองกองต่อการสังเคราะห์นาโนพาทิเคิล พบว่าการเพิ่ม 0.5 1.0 2.0 4.0 หรือ 10.0 มิลลิกรัม ของผงเปลือกลองกองไม่มีผลต่อค่าการดูดกลืนคลื่นแสง แต่ที่ 600 นาโนเมตร มีค่าการดูดกลืนคลื่นแสงสูงที่สุด โดยวัดจากสีของสารสกัดเปลือกลองกอง ผลของความเข้มข้นของการเคลือบผิวจากสารสกัดจากเปลือกลองกองต่อการป้องกันคุณภาพของผลลองกอง โดยใช้สารเคลือบผิวแอลจิเนตเป็นส่วนประกอบของซิลเวอร์นาโนพาทิเคิลที่มีสารสกัดจากเปลือกลองกอง ลองกองถูกเคลือบด้วย 0 0.5 1.0 2.0 4.0 และ 10.0 มิลลิกรัม ของซิลเวอร์นาโนพาทิเคิลที่มีสารสกัดจากเปลือกลองกองและต่อมาเก็บรักษาที่อุณหภูมิ 13°C และความชื้นสัมพัทธ์ที่ 90-95% เป็นเวลา 9 วัน ตรวจสอบผลลองกองทุกๆ 3 วัน เพื่อวิเคราะห์การเปลี่ยนแปลงสีน้ำตาล การสูญเสียน้ำหนัก และคุณภาพทางเคมี พบว่าสารเคลือบผิวแอลจิเนตที่มีซิลเวอร์นาโนพาทิเคิลที่มีสารสกัดจากเปลือกลองกองรักษาคุณภาพของลองกองได้ โดยผลลองกองที่เคลือบด้วยซิลเวอร์นาโนพาทิเคิลที่มีสารสกัดจากเปลือกลองกอง ความเข้มข้น 1.0 และ 2.0 มิลลิกรัมแสดงการเกิดสีน้ำตาล การสูญเสียน้ำหนักและกิจกรรมของเอนไซม์เปอร์ออกซิเดสและโพลีฟีนอลออกซิเดส ต่ำสุดเมื่อเปรียบเทียบกับชุดควบคุม ปริมาณสารประกอบฟีนอลิกของลองกองเคลือบผิวด้วยแอลจิเนตที่มีซิลเวอร์นาโนพาทิเคิลที่มีสารสกัดจากเปลือกลองกองที่ความเข้มข้น 1.0 และ 2.0 มิลลิกรัม สูงกว่าชุดควบคุม อย่างไรก็ตามลองกองเคลือบด้วยซิลเวอร์นาโนพาทิเคิลที่มีสารสกัดจากเปลือกลองกองในทุกสิ่งทดลองมีปริมาณกรดที่ไตเตรทได้ และปริมาณของแข็งที่ละลายน้ำได้ค่อนข้างคงที่ระหว่างการเก็บรักษา ผลการทดลองนี้แสดงให้เห็นว่าซิลเวอร์นาโนพาทิเคิลที่มีสารสกัดจากเปลือกลองกองที่เก็บไว้ที่ 13°C เป็นวิธีที่สามารถยับยั้งการเกิดสีน้ำตาล และการรักษาคุณภาพของลองกองได้

คำสำคัญ : สารสกัดเปลือกลองกอง ซิลเวอร์นาโนพาทิเคิล สารเคลือบผิว

Executive summary

Longkong (*Aglaia dookkoo* Griff.) is a subtropical fruit, which can be grown in various parts of Thailand. It is one of the most popular fruits due to its sweet and slightly sour taste. It is also an economically important fruit that is distributed to consumers all over the country. The major problems of longkong are rapid pericarp browning and desiccation a few days after harvesting. Because of its susceptibility to browning and desiccation, it is difficult to keep longkong in good appearance at room temperature without proper postharvest conditions. Browning is an undesirable physiological disorder that occurs in many kinds of fruits. The underlying mechanism of this process may be either an enzymatic or non-enzymatic reaction. However, browning in fresh fruits and vegetables is generally caused by enzymatic reaction. Consumable coatings are well known for their ability to protect perishable food products from deterioration by slowing dehydration, suppressing respiration, improving the quality of the texture, helping retain volatile flavor compounds and reducing microbial growth. Specially formulated consumable coatings provide increased protection against contamination of microorganism while having a similar effect as modified atmosphere storage in modifying internal gas composition. Among noble-metal nanomaterials, silver nanoparticles have received positive attention because their desirable physicochemical properties. It is well known that silver in various forms has strong toxicity to a vast range of microorganisms. Notably, silver nanoparticles have proven to be promising antimicrobial material. longkong peels that are naturally rich in polymers such as phenolic, lignin and antioxidant could be used in the synthesis of silver nanoparticles. Even though the coating has been studied extensively to increase the shelf life of many fresh fruits, there is currently no information available regarding the application of silver nanoparticles-LPE extracts coating for longkong fruits. These results indicated that silver nitrate longkong peel extract (SLN-LPE) stored at 13°C was a promising approach in inhibiting browning and maintaining the quality of longkong.

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CHAPTER 1

INTRODUCTION

1.1 Research background

Longkong (*Aglaia dookkoo* Griff.) is a subtropical fruit, which can be grown in various parts of Thailand. It is one of the most popular fruits due to its sweet and slightly sour taste. It is also an economically important fruit that is distributed to consumers all over the country. The major problems of longkong are rapid pericarp browning and desiccation a few days after harvesting. Because of its susceptibility to browning and desiccation, it is difficult to keep longkong in good appearance at room temperature without proper postharvest conditions. Browning is an undesirable physiological disorder that occurs in many kinds of fruits. The underlying mechanism of this process may be either an enzymatic or non-enzymatic reaction. However, browning in fresh fruits and vegetables is generally caused by enzymatic reaction.

Postharvest browning of fruit is primarily attributed to the oxidation of phenolic compounds by polyphenol oxidase (PPO) (Walker, 1995) and/or peroxidase (POD) (Jiang, 1999; Jiang and Li, 2001). The activity of phenylalanine ammonia lyase (PAL) converts phenylalanine to free phenolic substrates for PPO (Camm and Towers, 1977), which enable enzymatic browning by catalyzing the oxidation of mono- and di-phenols to *o*-quinones. These quinones polymerize to produce brown pigments (McEvily et al., 1992). Lin et al. (1988), Chen and Wang (1989), and Underhill and Critchley (1995) reported increased in POD activity during litchi pericarp browning. POD catalyzes the oxidation of several kinds of phenols when exposed to oxygen, which results in enzymatic browning of postharvest fruits. In addition, fruit tissue browning can result from cellular breakdown, causing to the mixing of browning-related enzymes and their

substrates, which results in enzymatic oxidation when in the presence of oxygen (Ju and Zhu, 1988).

Consumable coatings are well known for their ability to protect perishable food products from deterioration by slowing dehydration, suppressing respiration, improving the quality of the texture, helping retain volatile flavor compounds and reducing microbial growth (Debeaufort et al, 1998). Specially formulated consumable coatings provide increased protection against contamination of microorganism while having a similar effect as modified atmosphere storage in modifying internal gas composition (Park, 1999). Among noble-metal nanomaterials, silver nanoparticles have received positive attention because their desirable physicochemical properties. It is well known that silver in various forms has strong toxicity to a vast range of microorganisms (Liau et al, 1997). Notably, silver nanoparticles have proven to be promising antimicrobial material (Sondi and Salopek-Sondi, 2004). Longkong peels that are naturally rich in polymers such as phenolic, lignin and antioxidants could be used in the synthesis of silver nanoparticles. Even though the coating has been studied extensively to increase the shelf life of many fresh fruits, this research provides the only information regarding the application of silver nanoparticles-LPE extracts coating for longkong fruits. The objective of this research was to investigate the effect of pH and silver concentration nitrate and LPE powder content on nanoparticle synthesis and to evaluate the effect of a silver nanoparticles-LPE coating on the weight loss, total phenolic content, polyphenol oxidase, peroxidase, color, total soluble solid and titratable acidity of longkong fruits stored at 13 ºC.

1.2 Objectives

The main objectives of this research were:

1.2.1 To investigate the effects of pH on nanoparticle synthesis.

1.2.2 To investigate the effects of the concentration of silver nitrate on nanoparticle synthesis.

1.2.3 To investigate the effects of the quantity of LPE powder on nanoparticle synthesis.

1.2.4 To study the effect of the concentration of LPE coating on the preservation of raw longkong.

1.3 Scopes

1.3.1 To investigate effects of pH on nanoparticle synthesis by adjusting the pH of the reaction mixtures (10mg LPE, 1.0mM silver nitrate) to 2.0, 3.0, 4.0 and 5.0. Characterization of silver nanoparticles synthesized after 3min of incubation was carried out with the help of a UV–Vis spectrophotometer (Jasco V-530).

1.3.2 To investigate the effects of concentration of silver nitrate on nanoparticle synthesis by varying the concentration of silver nitrate (0.125, 0.5, 1.0, 1.50 and 2.0 mM). Characterization of silver nanoparticles synthesized after 3min of incubation was carried out with the help of a UV–Vis spectrophotometer (Jasco V-530).

1.3.3 To investigate the effects of the concentration of LPE powder (0.5, 1.0, 2.0, 4.0 or 10.0 mg) while keeping the silver nitrate concentration at a level of 1.0mM. for nanoparticle synthesis. Characterization of silver nanoparticles synthesized after 3min of incubation was carried out with the help of a UV–Vis spectrophotometer (Jasco V-530).

1.3.4 To study the effects of LPE concentration coating (0.5, 1.0, 2.0, 4.0 or 10.0 mg of silvernanopaticles-LPE) on pericarp browning of longkong fruit. Color evaluation and browning assessment were performed throughout the experimental period. Total phenolic content, extraction and assay of PPO and POD activities, weight loss, titratable acidity and soluble solid contents of longkong were determined during the browning of longkong at 13 ºC.

1.4 Expected benefits

This research provided better understanding of the influence of silvernano-longkong peel extract coating on browning in longkong. This will be practically applied to commercial use. After these studies, the results will be used as a guideline for farmers and exporters to improve the quality and increase the market value of longkong.

CHAPTER 2

LITERATURE REVIEW

2.1 Longkong

Longkong (*Aglaia dookkoo* Griff.), belonging to the Meliaceae family, is one of the well-known fruits in Thailand. This fruit originated in the southern part of Thailand and has become economically important due to being distributed and cultivated widely in southern and eastern regions of the country. Also, the fruit is cultivated in many countries such as Australia, Sri Lanka, Vietnam, Myanmar, India and Puerto Rico (Paull, 2004). Longkong fruits contain a wide variety of nutrients, including proteins, carbohydrates and are low fat with a high percentage of vitamins and minerals (Sabah, 2004). The fruit pulp is juicy with an aromatic smell and a sweet but slightly sour taste.

Longkong is a non-climacteric subtropical fruit with high marketable value. However, fruits rapidly deteriorate after harvest due to pericarp browning resulting in the reduction of the price.

2.2 Enzymatic browning

Enzymatic browning is one of the most devastating reactions for many exotic fruits and vegetables, in particular tropical and subtropical varieties. It is estimated that over 50 percent loss occurs in fruits as a result of enzymatic browning (Whitaker and Lee, 1995). Lettuce, potatoes, other green leafy vegetables, other starchy staples such as sweet potato and yam and varieties of the tropical and subtropical fruits and vegetables, for instance, breadfruit, apples, avocados, bananas, grapes, peaches or mushrooms are all susceptible to browning and therefore, lead to economic losses for the agriculturalists. The control of browning from harvester to consumer is therefore very

critical to minimize losses and maintain economic value to the agriculturalist and food producers. Moreover, browning can also adversely affect flavor and nutritional values.

For enzymatic browning occurrence, the appropriate enzymes, substrates and an oxygen supply must be presented. Access to copper, which acts as a catalyst is, also a prerequisite (Macheix et al,. 1990).

2.3 Browning mechanisms in plants

Phenolic compounds are a diverse range of secondary metabolites derived from the shikimate pathway and phenylpropanoid metabolism, which consist of three early steps in the conversion of L-phenylalanine to various hydroxycinnamic acids. The enzymes catalyze the individual steps in this sequence, which are respectively phenylalanine ammonia lyase, cinnamate-4-hydroxylase and 4-coumarate CoA ligase (Haslam, 1998). Postharvest browning in fruits and vegetables has been mainly attributed by the oxidation of phenolic compounds such as polyphenol oxidase (PPO) (Walker, 1995) and/or peroxidase (POD) (Jiang, 1999; Jiang and Li, 2001). These quinones are polymerized to produce brown pigments (McEvily et al., 1992) (Fig 1).

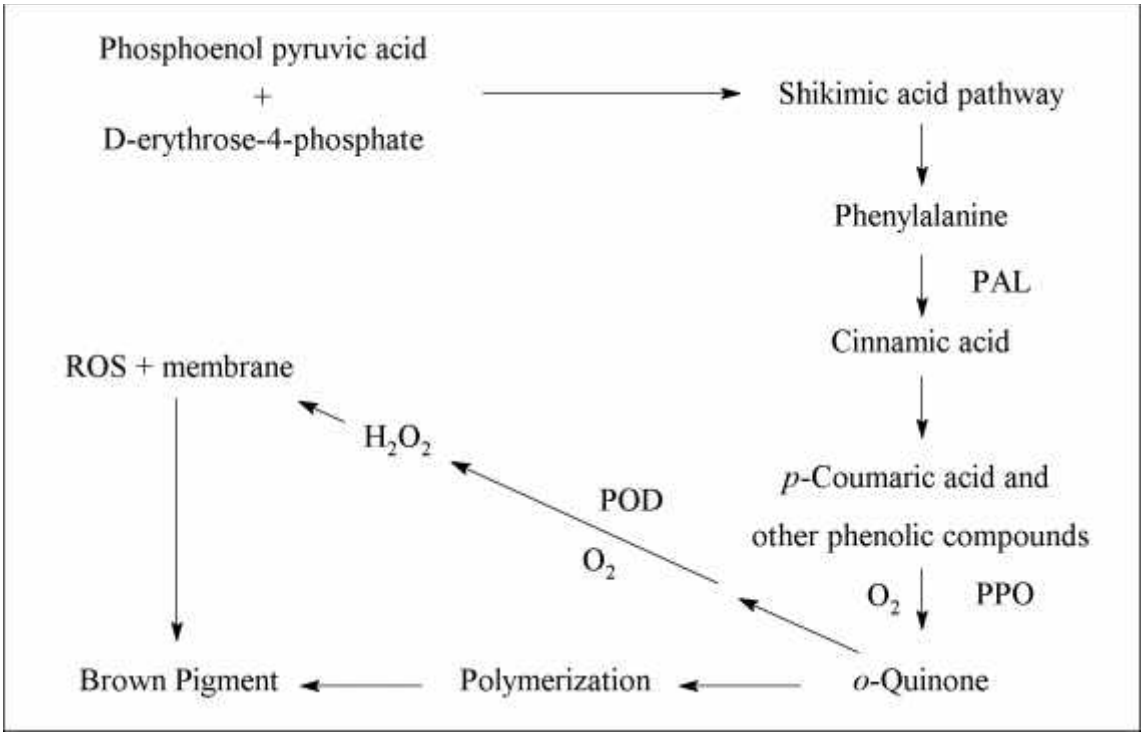

Fig. 2.1 Enzymatic browning pathway (McEvily et al., 1992).

2.4 Phenolic compounds

Phenolic compounds are plant-based materials, phytochemicals and widely distributed in the plant kingdom. Plant tissues may contain up to several grams per kilogram of phenolic compounds. There may be 4,000 of these plant compounds, and only a few, such as Vitamin C and E, are publicly discussed to any significant degree. External stimuli such as microbial infections, ultraviolet radiation, and chemical stressors induce their synthesis

Plants produce a great variety of organic compounds which are not directly involved in primary metabolic processes of growth and development. The roles of these natural products or secondary metabolites in plants have only recently come to be appreciated in an analytical context. Natural products appear to function primarily in the defense against predators and pathogens and in providing reproductive advantage as

attractants of pollinators and seed dispersers. They may also act to create competitive advantage as poisons of rival species.

Most natural products can be classified into three major groups; terpenoids, alkaloids, and phenolic compounds (mostly phenylpropanoids). Terpenoids are composed of five carbon units synthesized by the acetate/mevalonate pathway. Many plant terpenoids are toxins, feeding deterrents to herbivores and attractants of various sorts. Alkaloids, nitrogen-containing compounds, are principally synthesized from amino acids. These compounds protect plants from a variety of herbivorous animals, and many possess pharmacologically important activity. Phenolic compounds, which are primarily synthesized from products of the shikimic acid pathway, play several important roles in plants. Tannins, lignans, flavonoids, and some simple phenolic compounds serve as defenses against herbivores and pathogens. Flavonoids are found to comprise mainly quercetin and kaempferol, and the major dietary contributor was black tea, followed by onions and apples. Multivariate regression analysis revealed a significant relationship between flavonoid intake and the risk of mortality from coronary heart disease after adjustment for age and other risk factors (Harbborne, 2008).

The phenolic compounds are found in all parts of the plants but their nature and concentration varies greatly among the various tissues contributing to their color and flavor, mainly in the astringent and bitter attributes (Macheix et al., 1990). The phenolic compounds are the diverse range of secondary metabolites derived from the shikimate pathway and phenylpropanoid metabolism, which consists of three early steps in the conversion of L-phenylalanine to various hydroxycinnamic acids. The enzymes catalyze the individual steps in this sequence are respectively phenylalanine ammonia lyase, cinnamate-4-hydroxylase and 4-coumarate: CoA ligase (Haslam, 1998).

2.5 Polyphenol oxidase (PPO)

Polyphenol oxidase (1,2-benzenediol: oxygen oxidoreductase; EC 1.10.3.1), is a Cu-containing enzyme which is also known as tyroxinase, diphenol oxidase, *o*-diphenolase, catechol oxidase, catecholase and phenolase. PPO is present in some bacteria and fungi. PPOs are also found in almost all higher plants, including tea, potato, lettuce, mango and apple. PPO catalyzes the oxidation of *o*-phenolic substrates to *o*-quinones, which are subsequently polymerized to dark-coloured pigments (Billaud et al., 2004).

2.6 Peroxidase (POD)

POD (EC1.11.1.7) is iron-porphyrin organic catalysts and members of the group of enzymes described as oxidoreductases. They decompose hydrogen peroxide in the presence of a hydrogen donor. They are widespread in nature and those occurring in fruits and vegetables are iron containing. POD has been implicated in lignification, ethylene biosynthesis, praline hydroxylation, IAA degradation and other functions (Pressey, 1990). Changes in POD may be brought about by wounding, physiological stress and infections. POD can catalyze oxidation of many kind of phenols in the presence of oxygen and result in enzymatic browning of harvested fruit, such as pear (Richard and Gauillard, 1997), pineapple (Selvarajah et al., 1998) and peach (Sutte, 1989). In addition, POD are involved in several metabolic plant processes such as the catabolism of auxins, the formation of bridges between components of the cell wall and oxidation of the cinnamyl alcohols before their polymerization during lignin and suberin formation (Quiroga et al., 2000).

2.7 Methods for preventing enzymatic browning (Marshall et al., 2000)

Enzymatic browning does not occur in intact plant cells since phenolic compounds in cell vacuoles are separated from the polyphenol oxidase present in the cytoplasm. Once tissue is damaged by slicing, cutting or pulping, the formation of brown pigments occurs. Therefore, both the organoleptic and biochemical characteristics of fruits and vegetables are altered by pigment formation. The rate of enzymatic browning in fruit and vegetables is governed by the active polyphenol oxidase content and phenolic content, pH, temperature and oxygen availability within tissue (Marshall et al., 2000). Polyphenol oxidase catalyses the oxidation reaction to change from phenol to *o*-quinone which is a highly reactive compound. Thus, *o*-quinone forms the spontaneous polymerization to produce high-molecular-weight compounds or brown pigments (melanins). These melanins may in turn react with amino acids and proteins leading to the enhancement of the brown color product. Many studies have focused on either inhibiting or preventing polyphenol oxidase activity in foods. Various techniques and mechanisms have been developed over the years for the control of these undesirable enzyme activities (Marshall et al., 2000).

2.8 The techniques controlling enzymatic browning

2.8.1. The elimination of oxygen from the cut surface of fruits or vegetables greatly retards the browning reaction. However, browning occurs rapidly upon exposure to oxygen. Exclusion of oxygen is possible by immersion in water, syrup, brine, and a vacuum treatment.

2.8.2. The copper prosthetic group of polyphenol oxidases must be presented for the enzymatic browning reaction to occur. Therefore, chelating agents are effective in removing copper.

2.8.3. Inactivation of the polyphenol oxidases by heat treatments such as steam blanching is effectively applied for the control of browning in fruits and vegetables to be canned or frozen. Heat treatments are not, however, practically applicable in the storage of fresh produce.

2.8.4. Polyphenol oxidase catalyses the oxidation of phenolic substrates such as caffeic acid, protocatechuic acid, chlorogenic acid and tyrosine. Chemical modification of these substrates can prevent oxidation.

2.8.5. Certain chemical compounds react with the products of polyphenol oxidase activity and inhibit the formation of the colored compounds produced in the secondary, non-enzymatic reaction steps, which lead to the formation of melanin (Marshall et al., 2000).

Many techniques are applied to prevent enzymatic browning. Relatively new techniques, such as the use of killer enzymes, naturally occurring enzyme inhibitors and ionizing radiation, have been explored and exploited as alternatives to the heat treatment and the health risks associated with certain chemical treatments. Processing technologies applied in the control of enzymatic browning in fruits and vegetables are now reviewed (Marshall et al., 2000).

2.9 Silver nanoparticles coating

Silver nanoparticles are nanoparticles of silver between 1 nm and 100 nm in size. Although frequently labeled as silver, some are composed of a large percentage of

silver oxide due to their high ratio of surface-to-bulk silver atoms. Silver nanoparticles are important materials that have been extensively studied. They can be synthesized by several physical, chemical and biological methods (Nair and Laurencin, 2007; Zhang et al, 2008; Sharma et al, 2009). These nanoparticles exhibit electrical, optical as well as biological properties and are applied in catalysis, biosensing, imaging, drug delivery, nanodevice fabrication and in medicine (Nair and Laurencin, 2007; Lee and El-Sayed, 2006; Jain et al, 2008). Over the past several years, there has been increasing interest in silver nanoparticles because of their antimicrobial properties that they display (Choi et al, 2008). They are even being projected as future generation antimicrobial agents (Rai et al, 2009). The bio-inspired synthesis of silver nanoparticles is significant for current methods. Several biological systems including bacteria, fungi and algae have been used in this regard (Pugazhentuiran et al, 2009; Fayaz et al, 2009; Xie et al, 2007). The use of soluble biopolymers as soft templates for directing crystal growth and controlling self-assembly of inorganic nanoparticles has also been proven as an important technique (Yu and Chen, 2006). With reference to silver nanoparticles for example, a procedure using diaminopyridinylated heparin and hyaluronan has been described (Kemp et al, 2009).

The main components of our everyday foods (proteins, polysaccharides and lipids) can fulfil requirements for edible films and coatings' formation. An edible film or coating is defined as any type of material used for enrobing (coating or wrapping) food to extend shelf life of the product that may be eaten together with food with or without further removal. Edible films provide replacement and/or fortification of natural layers to prevent moisture losses and they should allow passage or restrict gas exchange of oxygen, carbon dioxide, aroma volatiles. A film or coating can also provide surface sterility and prevent loss of other important components. Furthermore, during storage, it

must not ferment, coagulate, separate, develop off-flavours, or otherwise spoil (Baldwin et al, 2012).

Coatings can also be used as carriers of functional ingredients such as antimicrobial or antioxidant compounds in order to reduce, inhibit or stop the growth of microorganisms or oxidation/rancidity, respectively. Several antimicrobial compounds (essential oils and natural plant extracts), anti-browning agents, antioxidants (alpha-tocopherol, ferulic acid, etc.) or firming agents have been added to coatings to improve the microbial stability, appearance and texture of the coated product (Martin-Belloso and Rojas-Graeu, 2009; No et al, 2007; Ponce, et al, 2008). Sometimes, the coating can be antimicrobial as has been reported for chitosan coatings (Coma et al., 2002; No et al., 2007). Edible coatings may be applied to processed foods for the prevention of moisture loss, transfer of water vapour between components of different water activity in a heterogeneous food system, formation of ice in frozen foods, exposition to oxygen or diffusion of carbon dioxide (Baldwin, 2007; Cutter and Summer, 2002; Ustunol, 2009). Concerning frozen foods, coatings can also be used to coat meat and seafood (Coma, 2008; Ustunol, 2009) as well as minimally processed or fresh-cut fruits and vegetables (Rojas-Graeu, et al, 2009). Finally, coatings can be used for flavour or nutrient incorporation (Reineccius, 2009). Bio-inspired silver nanoparticles were synthesized with the aid of a novel, non-toxic, eco-friendly biological material namely, banana peel extract (BPE). Boiled, crushed, acetone precipitated, air-dried peel powder was used for reducing silver nitrate. Silver nanoparticles were formed when the reaction conditions were altered with respect to pH, BPE content, concentration of silver nitrate and incubation temperature. The colorless reaction mixtures turned brown and displayed UV–visible spectra characteristic of silver nanoparticles. Scanning electron microscope (SEM) observations revealed the predominance of silver nanosized crystallites after

short incubation periods. When the reaction mixtures were incubated for 15 days, some micro-aggregates were also observed. Energy dispersive spectrometer (EDS) studies and X-ray diffraction analysis confirmed the presence of silver nanoparticles. Fourier transform infra-red spectroscopy (FTIR) indicated the role of different functional groups (carboxyl, amine and hydroxyl) in the synthetic process. These silver nanoparticles displayed antimicrobial activity against fungal as well as bacterial cultures. (Bankar et al, 2010). In a previous research, the effect of maturation on the changes in weight loss, chemical quality, texture and color of guavas were studied. It was established that Candeuba® wax as a solid lipid nanoparticles (SLN) maintained the quality of guavas, and that the SLN concentration in the film formation depends on the characteristics of the fruits. The best results were obtained with SLN concentrations of 60 and 65 g/L since at these concentrations, guavas showed the lowest range of weight loss and preserved the best quality compared to the fruits processed at concentrations above 70 g/L. In addition high contents of SLN cause physiological damage and also delay the maturation which can be observed by the greenness color without changes. (Zambrano-Zaragoza et al, 2013)

CHAPTER 3

MATERIALS AND METHODS

3.1 Longkong peels extract (LPE) powder preparation

longkong peels (*A. dookkoo* Griff.) (120 fruit) were obtained, washed and boiled in distilled water for 30 min at 90ºC. Such peels (100 g) were crushed in 100 ml of distilled water and the extract formed was filtered through a cheese cloth. This filtrate was treated with equal volumes of chilled acetone and the resulting precipitate was centrifuged at 1000 rpm for 5 min. This was air-dried into a powder and used for further experiments.

3.2 Experimental designs

3.2.1 Experiment I Effect of pH on nanoparticle synthesis.

For all experiments, the source of silver was silver nitrate ($AgNO_3$) in distilled water. Typical reaction mixtures contained 10 mg of LPE powder in 2 ml of silver nitrate solution (1 mM). Other reaction conditions included incubation at 80ºC in a water bath for 3 min. All experiments were carried out in triplicates. The reaction mixtures were monitored at different time intervals and the nanoparticles that were formed were characterized further. The effect of pH on nanoparticle synthesis was determined by adjusting the pH of the reaction mixtures (10 mg LPE, 1.0mM silver nitrate) as follows:

(1) pH 2.0

(2) pH 3.0

(3) pH 4.0

(4) pH 5.0

The silver nanoparticles synthesized after 3min of incubation were characterized with the help of a UV–Vis spectrophotometer (Jasco V-530) at 400-800 nm.

3.2.2 Experiment II Effect of concentration of silver nitrate on nanoparticle synthesis pH on nanoparticle synthesis.

For all experiments, the source of silver was silver nitrate ($AgNO_3$) in distilled water. Typical reaction mixtures contained 10 mg of LPE powder in 2 ml of silver nitrate solution (1 mM) unless otherwise stated. Other reaction conditions included incubation at 80ºC in a water bath for 3 min. All experiments were carried out in triplicates. The reaction mixtures were monitored at different time intervals and the nanoparticles that were formed were characterized further. The effect of the silver salt was determined by varying the concentration of silver nitrate (0.125, 0.5, 1.0, 1.50 or 2.0 mM), reaction mixtures containing 10 mg LPE at pH 3.0 as follows:

(1) silver nitrate 0.125mM)

(2) silver nitrate 0.5 mM)

(3) silver nitrate 1.0 mM)

(4) silver nitrate 1.50 mM)

(5) silver nitrate 2.0 mM)

The silver nanoparticles synthesized after 3min of incubation were characterized with the help of a UV–Vis spectrophotometer (Jasco V-530) at 400-800 nm.

3.2.3 Experiment III Effect of concentration of LPE powder on nanoparticle synthesis.

For all experiments, the source of silver was silver nitrate ($AgNO_3$) in distilled water. Typical reaction mixtures contained 10 mg of LPE powder in 2 ml of silver

nitrate solution (1 mM) unless otherwise stated. Other reaction conditions included incubation at 80ºC in a water bath for 3 min. All experiments were carried out in triplicates. The reaction mixtures were monitored at different time intervals and the nanoparticles that were formed were characterized further. The LPE powder content was varied while keeping the silver nitrate concentration at a level of 1.0 mM (1.0 mM $AgNO_3$ at pH 3.0) as follows:

(1) LPE power 0.5 mg

(2) LPE power 1.0 mg

(3) LPE power 2.0 mg

(4) LPE power 4.0 mg

(5) LPE power 10.0 mg

The silver nanoparticles synthesized after 3min of incubation were characterized with the help of a UV–Vis spectrophotometer (Jasco V-530) at 400-800 nm.

3.2.4 Experiment IV effect of LPE concentration coating on the preservation of raw longkong.

Coating solutions were prepared by dissolving alginate in distilled water and heating at 70 ºC while stirring until the solution became clear. Coating solutions also contained glycerol (1.5% w/w). Silvernanoparticles longkong peel extract were then incorporated into coating solutions at the following concentrations: 0% (control), 0.5, 1.0, 2.0, 4.0 or 10.0 mg silvernanoparticles-LPE, respectively. These solutions were homogenized for 3 min at 12,500 rpm using an Ultra Turrax T25 (IKA® WERKE, Germany) homogenizer with a S25NG25G device, and degassed under vacuum. *N*-acetylcysteine (1% w/v) was added to a calcium chloride bath (2% w/v) to crosslink the carbohydrate polymers. These formulations were chosen in accordance with previous

work (Rojas-Grau et al., 2006, 2007). The longkong was coated by immersing for 1 min. They were dried at room temperature for 1 h and then immediately transferred to refrigeration storage at 13 °C. Data was recorded every 3 days for one month. Each treatment contained 120 fruits/3 replicates.

The sampled fruit was analyzed as (1)-(7).

(1) Determination of peel color changes

Peel color changes in the middle part of longkong was determined by measuring the L value with a Minolta colorimeter (Model RC-300, Minolta Co. Ltd., Osaka, Japan) and expressed as a lightness value (L value).

(2) Determination of fruit browning

Pericarp browning was estimated by measuring the extent of total browned area on each fruit surface with the following scores: 1= no browning, 2= <20% of peel surface, 3= 20-40% of peel surface, 4= 40-60% of peel surface and 5= >60% of peel surface.

(3) Total phenolic content

Quantification of the total phenolic content was carried out using the method proposed by Singleton et al. (1999). The extraction was separately prepared from the top (around calyx), middle and bottom part of longkong peel. Two grams of each peel section were homogenized with 20 mL of 80% ethanol for 1 min. The extract was filtered and centrifuged at 10,000 × g for 15 min. One mL of the supernatant was mixed with 1 mL of Folin Ciocalteu reagent (SigmaAldrich, Buchs, Switzerland) and 10 mL of 7% sodium carbonate. This was increased to 25 mL with distilled water and left to

settle for 1 h. The absorbance was then read at 760 nm by a spectrophotometer **(**UV-1601**;** Shimadsu Co., Kyoto,. Japan**)**. A standard curve of gallic acid was used for quantification of total phenolics.

(4) Extraction and assays of PPO, and POD activities

Pericarp tissues (2 g) from 20 fruit were homogenized in 20 mL of 0.05 M phosphate buffer (pH 7) and 0.2 g of polyvinylpyrrolidone (insoluble) at 4°C. After filtration of the homogenate through a cheese cloth, the filtrate was centrifuged for 20 min at 19,000 × *g* and 4°C. The supernatant was then collected for PPO and POD activity assays as the crude enzyme extract. PPO activity was assayed by measuring the oxidation of 4-methylcatechol as the substrate, according to the method of Jiang (2000). Change in absorbance at 410 nm was measured by a spectrophotometer **(**UV-1601**;** Shimadsu Co., Kyoto,. Japan**)**. One unit of PPO activity was defined as a change of 0.001 in absorbance per minute.

POD activity was assayed by the method of Zhang et al. (2005), using guaiacol as a substrate, in a reaction mixture (3 mL) containing 25 μl of enzyme extract, 2.78 mL of 0.05 M phosphate buffer (pH 7.0), 0.1 ml of 20 mM H_2O_2 and 0.1 mL of 20 mM guaiacol. The increase in the absorbance at 470 nm due to the guaiacol oxidation was recorded for 2 min. One unit of enzyme activity was defined as the amount that caused a change of 0.01 in the absorbance per minute.

(5) Weight loss

Longkong from each treatment was weighed individually before and during storage period, and the percentage of weight loss was calculated using the equation below.

$$\text{Total weight loss (\%)} = \frac{\text{initial weight of fruit} - \text{final weight of fruit}}{\text{initial weight of fruit}} \times 100$$

(6) Total titratable acidity

Total titratable acidity (TA) was measured by titration 1 mL of extracted juice diluted with 9 mL of distilled water with 0.1 M NaOH to an endpoint of pH 8.1, using an automatic titrator (AUT-501, DKK-TOA Corporation, Tokyo, Japan). Total titratable acidity was expressed as percentage of citric acid. All measurements were carried out according to AOAC procedures (Horwitz, 2000 and Nunes et al., 2009).

(7) Soluble solid content

Total soluble solid (TSS) from fruit juice was measured by a digital refractometer (PAL-1, Atago, Tokyo, Japan). The units of TSS were expressed as a percentage.

3.3 Statistical analysis

All experiments were performed in a completely randomized design (CRD) with three replications for analytical parameter of color evaluation, browning assessment, weight loss, titratable acidity and soluble solid contents. For the determinations of total phenol content, activities of PPO, and POD, 3 replicates of 20 fruits were used. The treatment means were separated using the least significant difference method. Differences at P=0.05 were considered to be significant. Data was presented as means $\pm$ standard errors (S.E.M).

CHAPTER 4

RESULTS AND DISCUSION

4.1 Effect of pH on nanoparticle synthesis.

The reaction mixtures turned yellow-brown after 3min of incubation. The color and the intensity of the peaks were pH dependent. At pH 2.0, there was neither a change in color nor a characteristic peak (Fig. 4.1). A yellow color was observed at pH 4.0 and at pH 5.0, a dark brown color and an intense peak were observed. A change in color was also associated with well-defined peaks characterized by a maxima centered around 500nm. Such peaks are known to be due to the plasmon resonance displayed by silver nanoparticles (Mulvaney, 1996). Control silver nitrate solutions (without LPE) neither developed the brown colors nor did they display the characteristic peaks. These results indicated that abiotic reduction of silver nitrate did not occur under the reaction conditions that were used. A variety of biomolecules are postulated to be involved in biological nanoparticle synthesis (Pimprikar et al, 2009). Under extremely acidic conditions (pH 2.0) such biomolecules are likely to be inactivated. The differences in the colors obtained over the range of pH could be due to a variation in the dissociation constants (pKa) of functional groups on the biomass that are involved in the reduction process. A variation in the biological material and metal salt concentration is known to influence nanoparticle synthesis (Pimprikar et al, 2009).

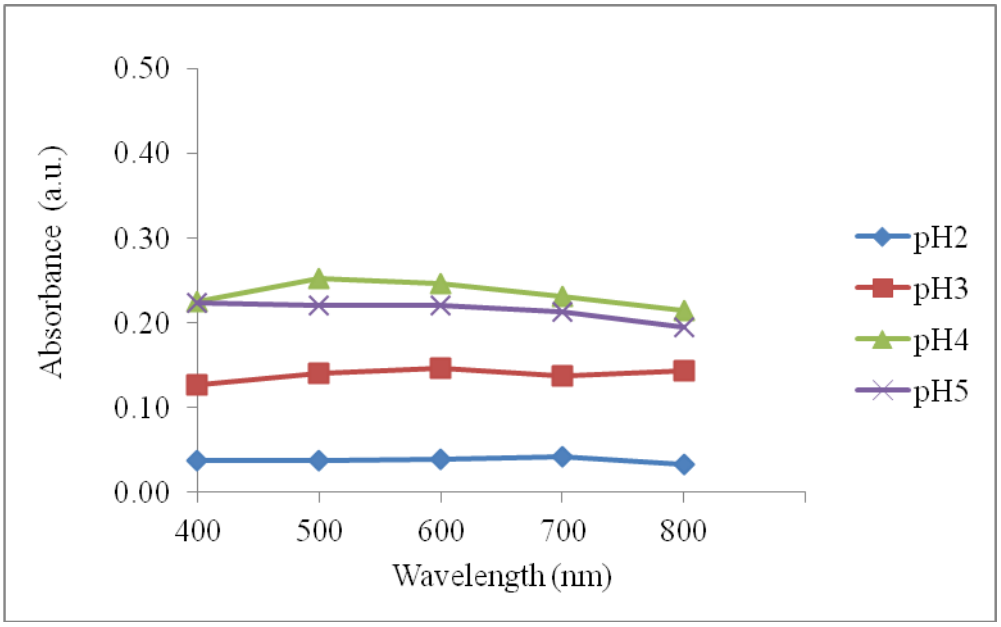

Fig 4.1 UV-visible absorption spectra of reaction mixtures containing 10 mg of LPE powder and 1.0 mM $AgNO_3$ at different pH values.

4.2 Effect of concentration of silver nitrate on nanoparticle synthesis pH on nanoparticle synthesis.

Fig. 4.2 shows the effect of varying silver nitrate concentrations on nanoparticle synthesis when the reaction was carried out at pH 3.0. With 0.125, 0.5 1.0 1.5 and 2.0mM concentrations of silver nitrate, yellow, golden brown and brown colors were observed, respectively. When the silver nitrate concentration ranged between 1.0 1.5 and 2.0mM, darker shades of brown were observed. The UV–visible spectra of reaction mixtures containing varying concentrations of silver nitrate are also shown in Fig. 4.2. The surface plasmon peak for silver nanoparticles became distinct with an increasing concentration of the silver salt.

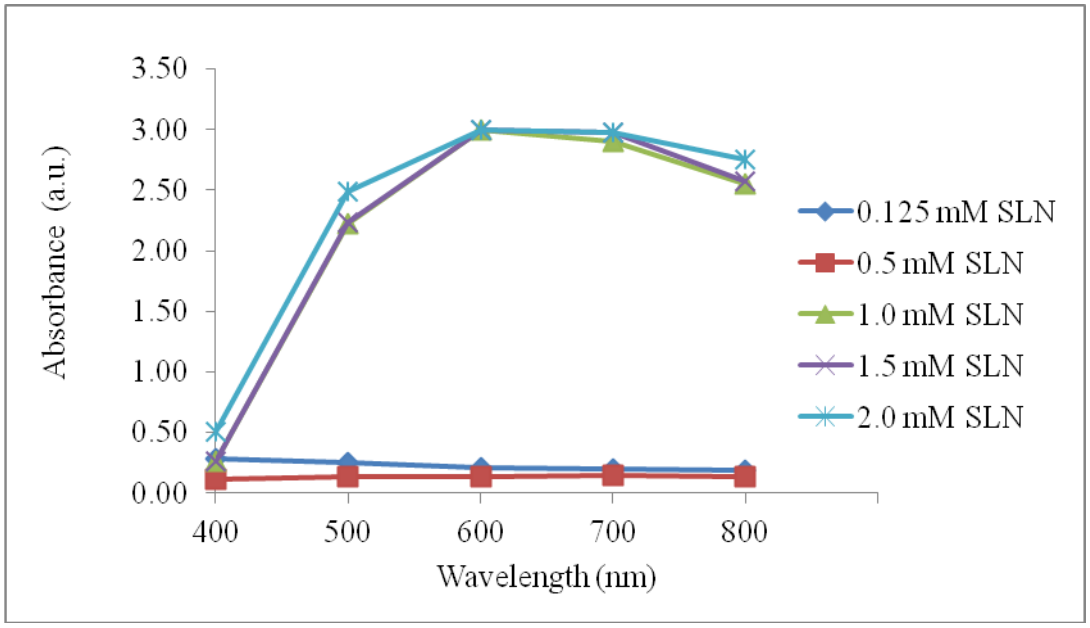

Fig 4.2 UV-visible absorption spectra of reaction mixtures containing 10.0 mg LPE powder with varying concentrations of 0.125, 0.5, 1.0, 1.5 and 2.0 mM $AgNO_3$ at pH 3.0.

4.3 Experiment III Effect of concentration of LPE powder on nanoparticle synthesis.

There was variation in the colors that developed when the content of the LPE was decreased. As stated earlier, the reaction mixtures containing 10mgml^{-1} of LPE developed a dark brown color. With 0.5 1.0 2.0 4.0 or 10.0mg of LPE, The shade of brown darkened respectively (Fig. 4.3).

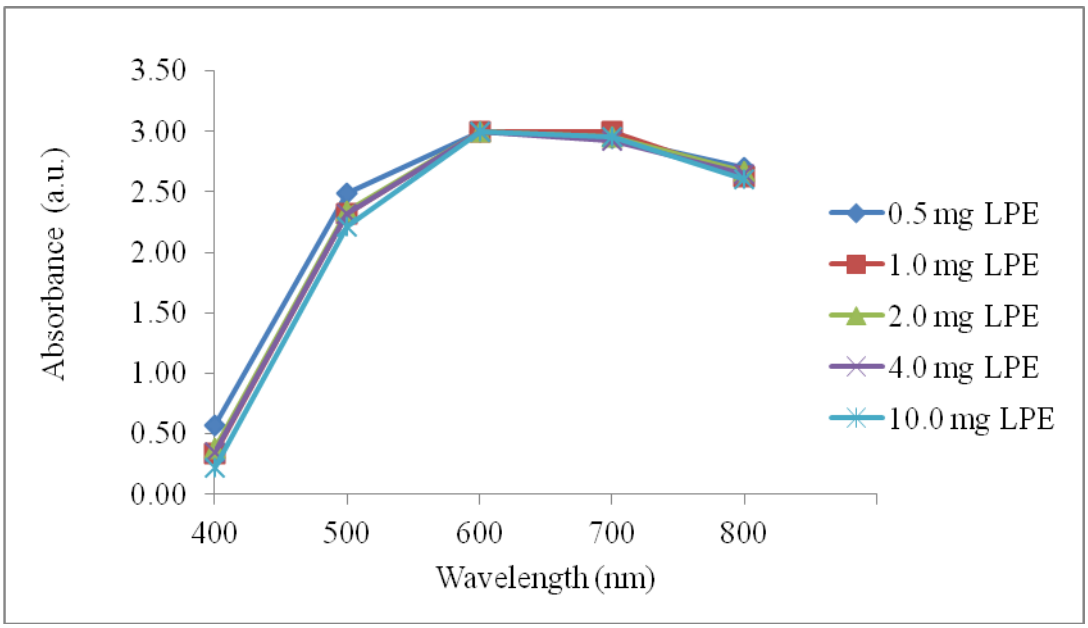

Fig 4.3 UV-visible absorption spectra of reaction mixtures containing 1.0 mM $AgNO_3$ with varying concentrations of LPE powder 0.5, 1.0, 2.0, 4.0 and 10.0 mg at pH 3.0.

4.4 Experiment IV effect of LPE concentration coating on the preservation of raw longkong.

Browning of fresh fruits and vegetables reduces quality and is often the factor limiting shelf-life and marketability of fresh – cut product. The longkong coated with SLN-LPE increased browning during storage at 13ºC for 9 days (Fig. 4.4) (Appendix A.4). Longkong coated with 10.0 mg SLN-LPE increased the browning score and after 9 days of storage the index was the same as the control at 4.0. Browning of the longkong coated with SLN-LPE at 0.5 and 1.0 mg delayed the browning of longkong and after 9 days of storage, the browning score was 3, significantly lower than the control.

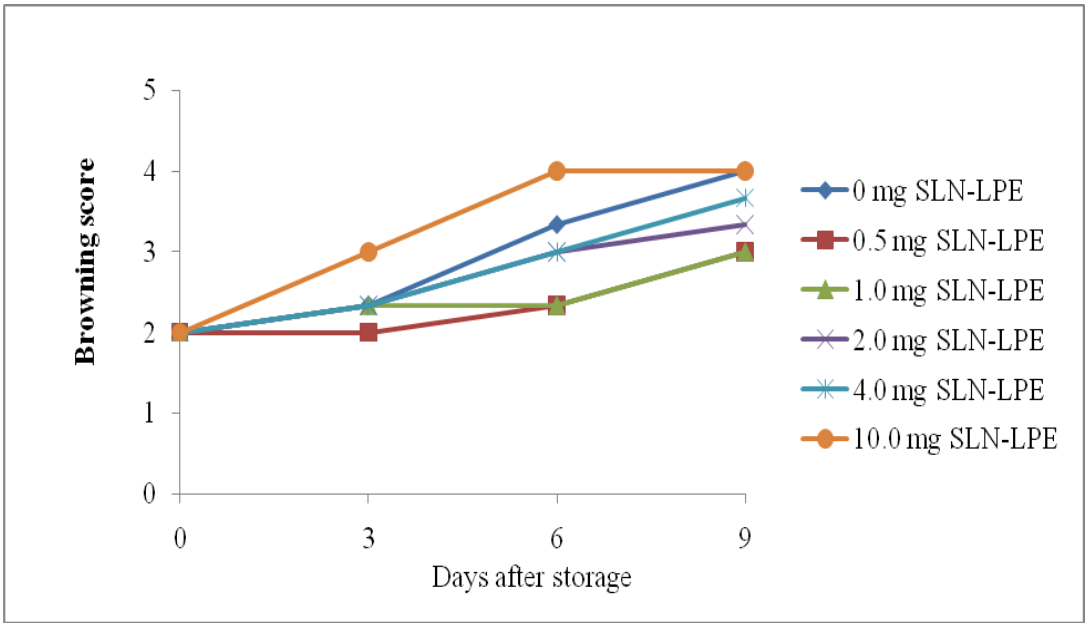

Fig 4.4 Change in browning score of longkong coated with SLN-LPE of reaction mixtures containing 1.0 mM $AgNO_3$ with varying concentrations of LPE powder 0.5, 1.0, 2.0, 4.0 and 10.0 mg at pH 3.0 and then stored at 13ºC and 90-95% RH.

L value of longkong peel from all treatments decreased gradually during 9 days of storage (Fig. 4.5) (Appendix A.5). L value of 10.0 mg SLN-LPE fruit was lower than 4.0, 2.0, 0.5, 0, 1.0 and 2.0 mg SLN-LPE respectively after 9 days of storage. The fruit treated with 2.0 mg SLN-LPE showed the highest L value at 57.93 but no significant difference had been observed. Apparently, SLN-LPE has potential applications due to their distribution in the coating on longkong surface. The most effective LPE concentrations were 2.0 and 1.0 mg where the fruits showed a slower change of color, browning and physiochemical parameters; also, they dis not show any physiological damage at the moment of transferring to room temperature for five days, with successful maturation development attributed to the homogeneous LPE distribution in the coating formed on the longkong skin allowing fruit respiration.

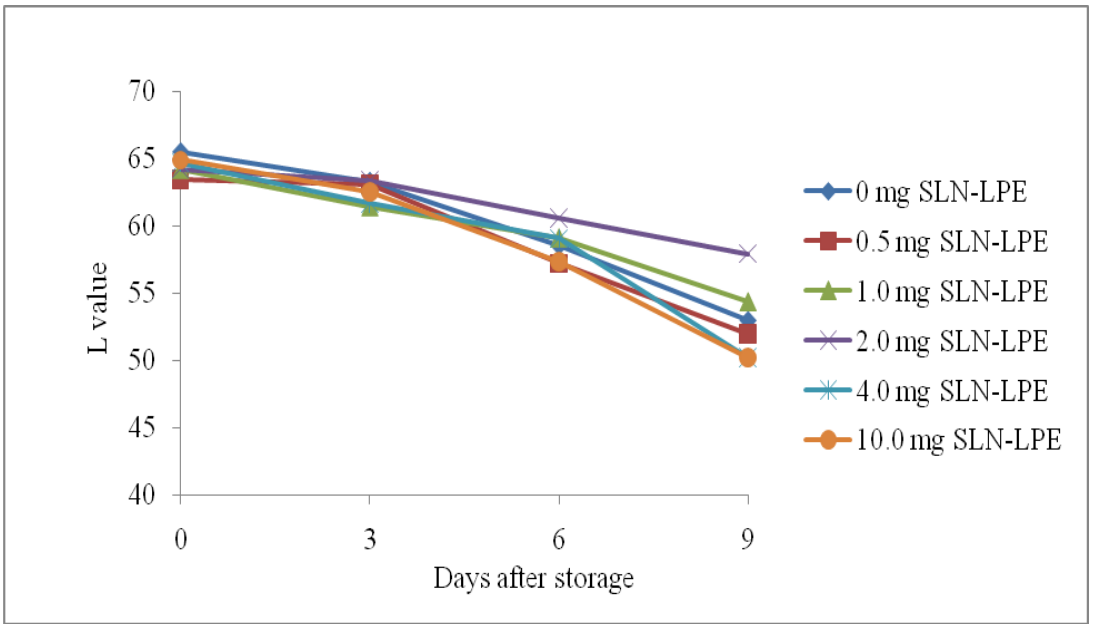

Fig 4.5 Change in L value of longkong coated with SLN-LPE of reaction mixtures containing 1.0 mM $AgNO_3$ with varying concentrations of LPE powder 0.5, 1.0, 2.0, 4.0 and 10.0 mg at pH 3.0 and then stored at 13ºC and 90-95% RH.

Total phenol content from all treatments showed a pattern of decreasing over the storage period (Fig. 4.6) (Appendix A.6). Total phenolic content of longkong coated with 0.5 and 2.0 mg SLN-LPE was significantly lower than that of 0 mg SLN-LPE at the onset of the experiment. Postharvest browning of fruit and vegetables is also related to the synthesis of phenolic compounds which were oxidized into quinines polymerized into brown polymeric (Martinez and Whitaker, 1995). Being a key enzyme in phenolic biosynthesis, PAL has been reported to play an important role in the biosynthesis of phenols in fruit and vegetables (Ke and Saltveit, 1989), and can be induced by various stress conditions (Dixon and Paira, 1995). As show in Fig 4.6, initial total phenolic content of longkong in 0.5 and 2.0 mg SLN-LPE was considerably lower than that in control; after 9 days of storage, 4.0, 10.0 and 2.0 had the lowest total phenolic content at 75.00, 89.26 and 95.35 mg /g FW.

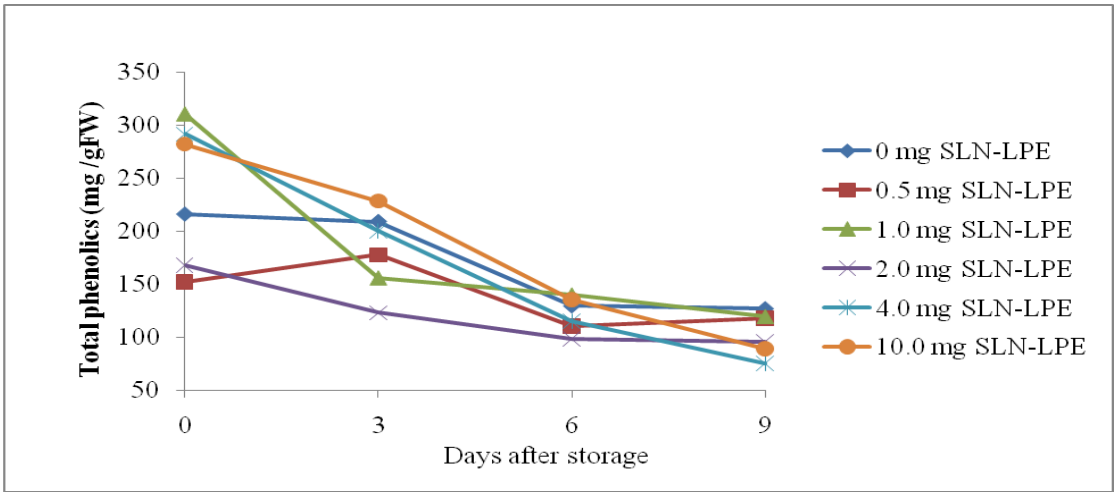

Fig 4.6 Change in total phenolics of longkong coated with SLN-LPE of reaction mixtures containing 1.0 mM $AgNO_3$ with varying concentrations of LPE powder 0.5, 1.0, 2.0, 4.0 and 10.0 mg at pH 3.0 and then stored at 13ºC and 90-95% RH.

Generally enzymatic browning in fruit and vegetable is assumed to be a total consequence of reaction between polyphenol substrates and oxygen with PPO and POD. The primary enzymes responsible for browning reaction is PPO, which is able to catalyze the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to their corresponding o-quinones (Richard and Gauillard, 1997). POD is another oxidoreductase enzyme involved in enzymatic browning since diphenols may function as reducing substrates in its reaction (Chisari et al, 2007). POD activity increased rapidly in 0.5 mg SLN-LPE treatments in the first 3 days and then was followed by a rapidly decrease. POD activity of 10.0 mg SLN-LPE decreased progressively in the first six days of storage and increased between days 6 and 9. However, the POD of all concentrations of SLN-LPE were significantly lower than that of control samples after 9days of storage, the concentrations of 1.0 and 2.0 were the lowest at 5.11 and 2.05 unit/mg protein respectively (Fig 4.7) (Appendix A.7).

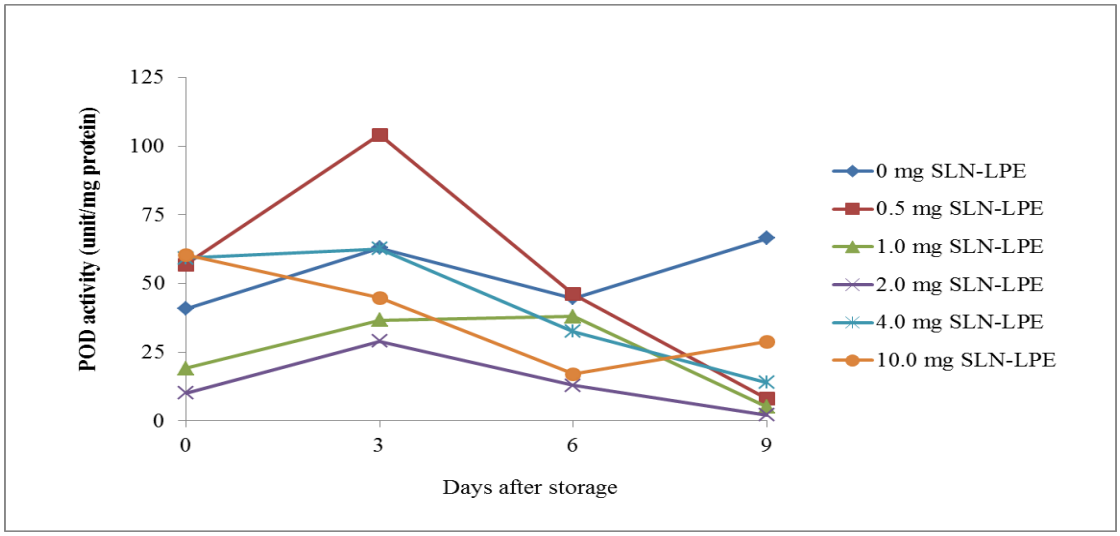

Fig 4.7 Change in POD activity of longkong coated with SLN-LPE of reaction mixtures containing 1.0 mM $AgNO_3$ with varying concentrations of LPE powder 0.5, 1.0, 2.0, 4.0 and 10.0 mg at pH 3.0 and then stored at 13ºC and 90-95% RH.

PPO activity increased quickly in all treatments in the first 3 days and then, with the exception of 1.0 mg SLN-LPE decreased until 9 days of storage. PPO activity of 1.0 and 2.0 mg SLN-LPE was lower than that of control samples until the end of storage (Fig. 4.8) (Appendix A.8). PPO is a key enzyme for enzymatic browning in many fruits. The latent form of PPO is often activated during ripening, senescence or stress condition when the membrane is damaged, which results in an increase of PPO activity (Mayer, 1987). PPO activity in longkong fruits increased in the first 3 days of storage. However, at the end of storage, the PPO activity of 0.5, 1.0 and 2.0 mg SLN-LPE was lower than the control at 1.80, 4.42 and 2.37 unit/mg protein respectively.

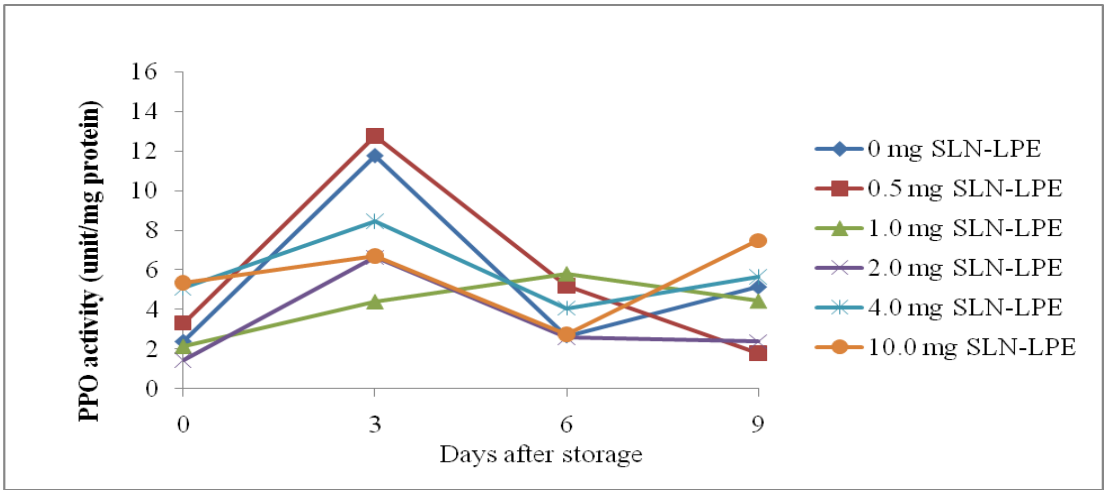

Fig 4.8 Change in PPO activity of longkong coated with SLN-LPE of reaction mixtures containing 1.0 mM $AgNO_3$ with varying concentrations of LPE powder 0.5, 1.0, 2.0, 4.0 and 10.0 mg at pH 3.0 and then stored at 13ºC and 90-95% RH.

The weight loss is indicative of a fruit dehydration process due to transpiration and it involves water transfer from the cell to the surrounding atmosphere, thus representing a way to evaluate coating efficiency in the preservation of quality (Pérez-Gago et al., 2010). In this work alginate was used as a film former, acting as a gas exchange barrier that did not modify the water transport, so, the aim of introducing LPE into the coating is to create a lipophilic environment capable of acting as a barrier against water, as happened in the samples with 0.5, 1.0, 2.0,4.0 and 10.0 mg SLN-LPE. The result showed that the weight loss of all SLN-LPE fruits had the lower weight loss than longkong treated with 0mg SLN-LPE during storage time (Fig 4.9) (Appendix A.9).

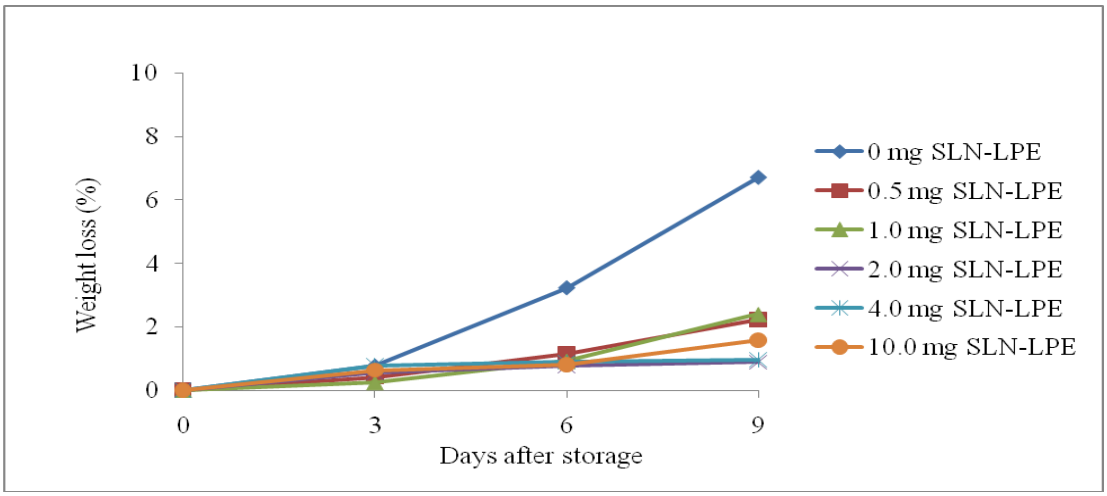

Fig 4.9 Change in weight loss of longkong coated with SLN-LPE of reaction mixtures containing 1.0 mM $AgNO_3$ with varying concentrations of LPE powder 0.5, 1.0, 2.0, 4.0 and 10.0 mg at pH 3.0 and then stored at 13ºC and 90-95% RH.

Total titratable acidity of all SLN-LPE fruit had an overall increase within 6 days and then decreased slowly until day 9 (Fig. 4.10) (Appendix A.10). While, 0.5 and 2.0 mg SLN-LPE had lower titratable acidity than that of other SLN-LPE on day 3 of storage. The decrease in acidity after day 6 of storage might be due to a rapid utilization of acids by respiration, as a result of maturity, demonstrating that the said decrease is dependent on many factors, including SLN-LPE aggregation in the coating's surface (Singh and Pal, 2008; Mercado-silva et al., 1998; Jain et al., 2003).

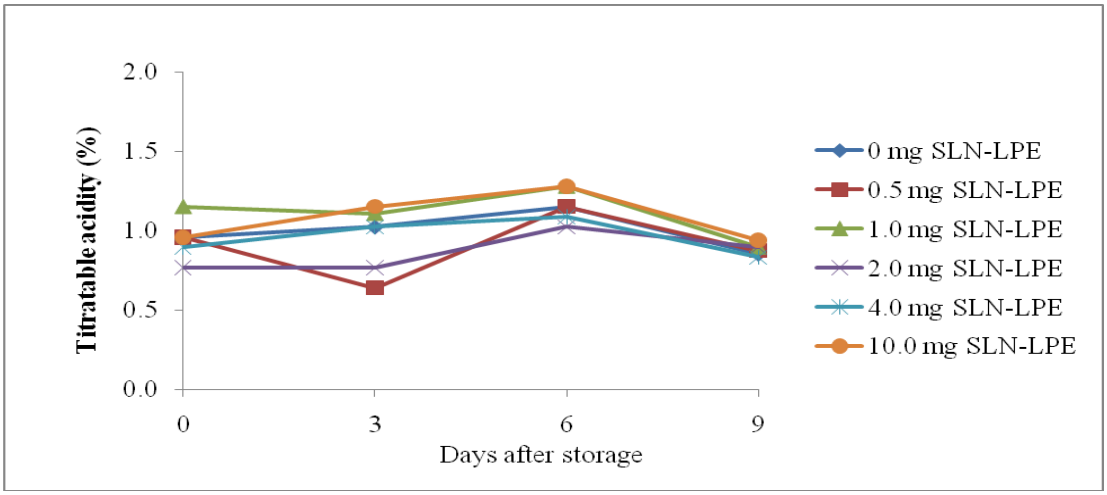

Fig 4.10 Change in titratable acidity of longkong coated with SLN-LPE of reaction mixtures containing 1.0 mM $AgNO_3$ with varying concentrations of LPE powder 0.5, 1.0, 2.0, 4.0 and 10.0 mg at pH 3.0 and then stored at 13ºC and 90-95% RH.

Total soluble solids content of chemical treated fruits changed slightly during 9 days until the end of storage (Fig 4.11) (Appendix A.11). The application of coating alginate was the most effective way to stop the conversionof the acids present in the fruit into sugar (Bassetto et al, 2005). Thus, these coatings create a new atmosphere on the surface of longkong.

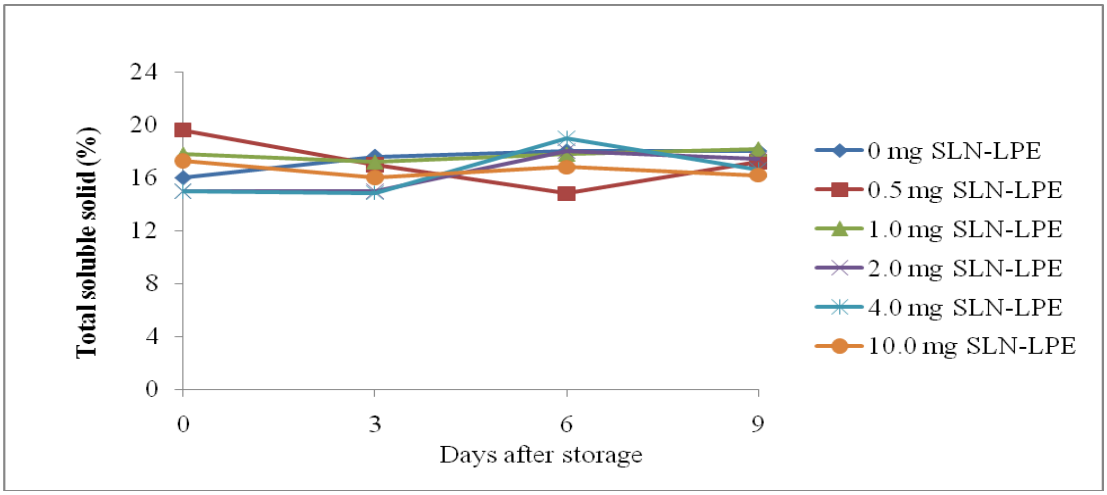

Fig 4.11 Change in total soluble solid of longkong coated with SLN-LPE of reaction mixtures containing 1.0 mM $AgNO_3$ with varying concentrations of LPE powder 0.5, 1.0, 2.0, 4.0 and 10.0 mg at pH 3.0 and then stored at 13ºC and 90-95% RH.

CHAPTER 5

CONCLUSIONS

1. The study investigated the effect of pH on nanoparticle synthesis. The color and the intensity of the peaks were pH dependent. At pH 2.0, there was clear yellow color and at pH 5.0 a dark brown color was observed. However, all pHs were maintained with no significant difference in absorbance of nanoparticle synthesis.
2. Effect of concentration of silver nitrate on nanoparticle synthesis when the reaction was carried out at pH 3.0 with 0.125 and 0.5 mM concentrations of silver nitrate, yellow was observed. When the silver nitrate concentration ranged between 1.0 and 2.0 mM, darker shades of brown were observed.
3. Effect of concentration of LPE powder on nanoparticle synthesis was observed. With 0.5, 1.0, 2.0, 4.0 or 10.0mg of LPE, lighter shades of brown were obtained and the peaks were proportionately less intense.
4. Longkong coated with 1.0 and 2.0 mg SLN-LPE delayed the browning, weight loss and activities of POD and PPO during storage. While longkong of 1.0 and 2.0 mg SLE-LPE had higher total phenolic content than that the control. Longkong with SLN-LPE at 0, 0.5, 1.0, 2.0, 4.0 and 10.0 mg maintained titratable acidity and total soluble solid during storage.

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APPENDIX A

Table A.1. UV-visible absorption spectra of reaction mixtures containing 10 mg of LPE powder and 1.0 mM $AgNO_3$ at different pH values.

pH values	Absorbance (a.u.)				
	400	500	600	700	800
pH 2	0.03	0.03	0.03	0.04	0.03
pH 3	0.12	014	0.14	0.13	0.14
pH 4	0.22	0.25	0.24	0.23	0.21
pH 5	0.22	0.22	0.22	0.21	0.19
F-test	NS	NS	NS	NS	NS
C.V. (%)	92.69	87.27	82.23	79.44	84.40

[1_/] Within the day of each treatment, means followed by the different letters are significant differences. * $p \leq 0.05$, ** $p \leq 0.01$, NS = not significant differences, - = non-detected. The absence letters indicate no statistically significant differences.

Table A.2. The effect of the silver salt will be determined by varying the concentration of silver nitrate (0.125, 0.5, 1.0, 1.50 or 2.0 mM), reaction mixtures containing 10 mg LPE at pH 3.

Silver nitrate	Absorbance (a.u.)				
	400	500	600	700	800
0.125 mM $AgNO_3$	0.28^{ab}	0.25^{c}	0.21^{b}	0.19^{b}	0.18^{b}
0.5 mM $AgNO_3$	0.11b	0.13^{c}	0.13^{b}	0.14^{b}	0.13^{b}
1.0 mM $AgNO_3$	0.26^{ab}	2.21^{b}	3.00^{a}	2.90^{a}	2.55^{a}
1.5 mM $AgNO_3$	0.25^{ab}	2.22^{b}	3.00^{a}	2.97^{a}	2.57^{a}
2.0 mM $AgNO_3$	0.50^{a}	2.49^{a}	3.00^{a}	2.97^{a}	2.74^{a}
F-test	*	**	**	**	**
C.V. (%)	44.33	9.08	2.71	3.79	7.80

[1_/] Within the day of each treatment, means followed by the different letters are significant differences. * $p \leq 0.05$, ** $p \leq 0.01$, NS = not significant differences, - = non-detected. The absence letters indicate no statistically significant differences.

Table A.3. The LPE powder content will be varied while keeping the silver nitrate concentration at a level of 1.0 mM (1.0 mM $AgNO_3$ at pH 3.0)

LPE powder	Absorbance (a.u.)				
	400	500	600	700	800
0.5 mg LPE	0.56	2.48	3.00	2.94	2.69
1.0 mg LPE	0.33	2.32	3.00	3.00	2.62
2.0 mg LPE	0.37	2.34	3.00	2.95	2.66
4.0 mg LPE	0.34	2.31	3.00	2.92	2.64
10.0 mg LPE	0.21	2.21	3.00	2.94	2.60
F-test	NS	NS	NS	NS	NS
C.V. (%)	71.25	10.50	0	2.01	7.37

1_/ Within the day of each treatment, means followed by the different letters are significant differences. * $p \leq 0.05$, ** $p \leq 0.01$, NS = not significant differences, - = non-detected. The absence letters indicate no statistically significant differences.

Table A.4. Change in browning score of longkong treated with 0, 0.5, 1.0, 2.0, 4.0 and 10.0 mg SLN-LPE and then stored at 13°C and 90-95% RH.

Treatments	Browning score			
	Days after storage[1_/]			
	0	3	6	9
0 mg SLN-LPE (Control)	2.00	2.33	3.33^{ab}	4.00^{a}
0.5 mg SLN-LPE	2.00	2.00	2.33^{c}	3.00^{c}
1.0 mg SLN-LPE	2.00	2.33	2.33^{c}	3.00^{c}
2.0 mg SLN-LPE	2.00	2.33	3.00^{bc}	3.33^{bc}
4.0 mg SLN-LPE	2.00	2.33	3.00^{bc}	3.66^{ab}
10.0 mg SLN-LPE	2.00	3.00	4.00^{a}	4.00^{ab}
F-test	-	NS	**	**
C.V. (%)	0	19.73	13.60	9.52

[1_/] Within the day of each treatment, means followed by the different letters are significant differences. * $p \leq 0.05$, ** $p \leq 0.01$, NS = not significant differences, - = non-detected. The absence letters indicate no statistically significant differences.

Table A.5. Change in L* of longkong treated with 0, 0.5, 1.0, 2.0, 4.0 and 10.0 mg SLN-LPE and then stored at 13°C and 90-95% RH.

Treatments	L* value			
	Days after storage[1_/]			
	0	3	6	9
0 mg SLN-LPE (Control)	65.50	63.3.	58.53	52.96
0.5 mg SLN-LPE	63.43	63.10	57.20	51.95
1.0 mg SLN-LPE	64.16	61.40	59.10	54.36
2.0 mg SLN-LPE	64.23	63.40	60.56	57.93
4.0 mg SLN-LPE	64.70	61.70	59.06	50.23
10.0 mg SLN-LPE	64.90	62.53	57.33	50.20
F-test	NS	NS	NS	NS
C.V. (%)	3.78	3.76	3.42	6.03

[1_/] Within the day of each treatment, means followed by the different letters are significant differences. * $p \leq 0.05$, ** $p \leq 0.01$, NS = not significant differences, - = non-detected. The absence letters indicate no statistically significant differences.

Table A.6. Change in total phenolics of longkong treated with 0, 0.5, 1.0, 2.0, 4.0 and 10.0 mg SLN-LPE and then stored at 13°C and 90-95% RH.

Treatments	Total phenolics (mg/gFW)			
	Days after storage[1_/]			
	0	3	6	9
0 mg SLN-LPE (Control)	215.86^{d}	208.97^{ab}	129.48^{b}	126.92^{a}
0.5 mg SLN-LPE	152.08^{f}	177.72^{c}	110.41^{c}	117.63^{a}
1.0 mg SLN-LPE	310.41^{a}	155.93^{d}	139.90^{a}	119.55^{a}
2.0 mg SLN-LPE	167.79^{e}	123.08^{e}	98.56^{d}	95.35^{b}
4.0 mg SLN-LPE	291.50^{b}	200.48^{b}	115.22^{c}	75.00^{c}
10.0 mg SLN-LPE	282.05^{c}	228.36^{a}	135.25^{ab}	89.26^{bc}
F-test	**	**	**	**
C.V. (%)	1.59	6.67	4.49	7.89

[1_/] Within the day of each treatment, means followed by the different letters are significant differences. * $p \leq 0.05$, ** $p \leq 0.01$, NS = not significant differences, - = non-detected. The absence letters indicate no statistically significant differences.

Table A.7. Change in POD activity of longkong treated with 0, 0.5, 1.0, 2.0, 4.0 and 10.0 mg SLN-LPE and then stored at 13°C and 90-95% RH.

Treatments	POD activity (unit /mg protein)			
	Days after storage[1_/]			
	0	3	6	9
0 mg SLN-LPE (Control)	40.74^{b}	62.95^{b}	44.59^{a}	66.41^{a}
0.5 mg SLN-LPE	56.79^{ab}	104.07^{a}	46.01^{a}	7.94^{cd}
1.0 mg SLN-LPE	19.08^{c}	36.69^{c}	38.00^{a}	5.11^{cd}
2.0 mg SLN-LPE	10.01^{c}	29.02^{c}	12.87^{c}	2.05^{d}
4.0 mg SLN-LPE	59.26^{ab}	62.62^{b}	32.46^{ab}	13.86^{c}
10.0 mg SLN-LPE	60.44^{a}	44.67^{c}	16.94^{bc}	28.81^{b}
F-test	**	**	**	**
C.V. (%)	24.35	14.92	27.46	23.17

[1_/] Within the day of each treatment, means followed by the different letters are significant differences. * $p \leq 0.05$, ** $p \leq 0.01$, NS = not significant differences, - = non-detected. The absence letters indicate no statistically significant differences.

Table A.8. Change in PPO activity of longkong treated with 0, 0.5, 1.0, 2.0, 4.0 and 10.0 mg SLN-LPE and then stored at 13°C and 90-95% RH.

Treatments	PPO activity (unit/mg protein)			
	Days after storage[1_/]			
	0	3	6	9
0 mg SLN-LPE (Control)	2.36^{bc}	11.77^{a}	2.66^{b}	5.12^{ab}
0.5 mg SLN-LPE	3.31^{b}	12.77^{a}	5.19^{a}	1.80^{c}
1.0 mg SLN-LPE	2.15^{bc}	4.30^{b}	5.79^{a}	4.42^{bc}
2.0 mg SLN-LPE	1.43^{c}	6.62^{b}	2.60^{b}	2.37^{c}
4.0 mg SLN-LPE	5.07^{a}	8.45^{ab}	4.06^{ab}	5.65^{ab}
10.0 mg SLN-LPE	5.33^{a}	6.70^{b}	2.73^{b}	7.48^{a}
F-test	**	**	*	**
C.V. (%)	20.56	28.29	33.78	32.00

[1_/] Within the day of each treatment, means followed by the different letters are significant differences. * $p \leq 0.05$, ** $p \leq 0.01$, NS = not significant differences, - = non-detected. The absence letters indicate no statistically significant differences.

Table A.9. Change in weight loss of longkong treated with 0, 0.5, 1.0, 2.0, 4.0 and 10.0 mg SLN-LPE and then stored at 13°C and 90-95% RH.

Treatments	Weight loss (%)			
	Days after storage[1_/]			
	0	3	6	9
0 mg SLN-LPE (Control)	0	0.76[b]	3.22[a]	6.69[a]
0.5 mg SLN-LPE	0	0.42[e]	1.14[b]	2.23[c]
1.0 mg SLN-LPE	0	0.24[f]	0.92[c]	2.40[b]
2.0 mg SLN-LPE	0	0.56[d]	0.77[f]	0.89[f]
4.0 mg SLN-LPE	0	0.78[a]	0.89[d]	0.97[e]
10.0 mg SLN-LPE	0	0.63[c]	0.80[e]	1.59[d]
F-test	-	**	**	**
C.V. (%)	-	0.18	0.04	0.01

[1_/] Within the day of each treatment, means followed by the different letters are significant differences. * $p \leq 0.05$, ** $p \leq 0.01$, NS = not significant differences, - = non-detected. The absence letters indicate no statistically significant differences.

Table A.10. Change in titratable acidity of longkong treated with 0, 0.5, 1.0, 2.0, 4.0 and 10.0 mg SLN-LPE and then stored at 13°C and 90-95% RH.

Treatments	Titratable acidity (%)			
	Days after storage[1_/]			
	0	3	6	9
0 mg SLN-LPE (Control)	0.96	1.02^{b}	1.15^{ab}	0.85^{b}
0.5 mg SLN-LPE	0.96	0.64^{d}	1.15^{ab}	0.87^{ab}
1.0 mg SLN-LPE	1.15	1.10^{ab}	1.28^{a}	0.89^{ab}
2.0 mg SLN-LPE	0.76	0.76^{c}	1.02^{b}	0.89^{ab}
4.0 mg SLN-LPE	0.89	1.02^{b}	1.08^{b}	0.83^{b}
10.0 mg SLN-LPE	0.96	1.15^{a}	1.28^{a}	0.93^{a}
F-test	-	**	**	*
C.V. (%)	0	5.70	5.94	4.19

[1_/] Within the day of each treatment, means followed by the different letters are significant differences. * $p \leq 0.05$, ** $p \leq 0.01$, NS = not significant differences, - = non-detected. The absence letters indicate no statistically significant differences.

Table A.11. Change in total soluble solid of longkong treated with 0, 0.5, 1.0, 2.0, 4.0 and 10.0 mg SLN-LPE and then stored at 13°C and 90-95% RH.

Treatments	Total soluble solid (%)			
	Days after storage[1/]			
	0	3	6	9
0 mg SLN-LPE (Control)	16.00^{d}	17.6^{a}	18.00^{b}	18.00^{b}
0.5 mg SLN-LPE	19.60^{a}	17.00^{c}	14.80^{e}	17.20^{d}
1.0 mg SLN-LPE	17.80^{b}	17.20^{b}	17.80^{c}	18.20^{a}
2.0 mg SLN-LPE	15.00^{e}	15.00^{e}	18.00^{b}	17.40^{c}
4.0 mg SLN-LPE	15.00^{e}	14.86^{f}	19.00^{a}	16.60^{e}
10.0 mg SLN-LPE	17.26^{c}	16.00^{d}	16.80^{d}	16.20^{f}
F-test	**	**	**	**
C.V. (%)	0.28	0.28	0	0

[1/] Within the day of each treatment, means followed by the different letters are significant differences. * $p \leq 0.05$, ** $p \leq 0.01$, NS = not significant differences, - = non-detected. The absence letters indicate no statistically significant differences.

Output (Acknowledge the Thailand Research Fund)

1. International Journal Publication

   Title : Effect of silver nanoparticles-longkong peel extract coating on quality of Longkong (รอการตีพิมพ์ ในวารสาร Postharvest Biology and Technology)

2. Application

   นำข้อมูลที่ได้จากงานวิจัยนี้ไปใช้ต่อยอดงานวิจัยเรื่อง Effect of $CaCO_3$-nanoparticles-longkong peel extracts coating on quality and browning of longkong after harvesting โดยได้รับทุนจากสำนักงานคณะกรรมการวิจัยแห่งชาติ (วช) ในปีงบประมาณ 2560