การใช้ไคโตซานและฟิล์มพอลิโพรพิลีนดัดแปรเพื่อยืดเวลาการเก็บรักษามะนาว *Citrus aurantifolia* Swingle หลังการเก็บเกี่ยว

นางสาวพรจันทร์ จงศรี

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USE OF CHITOSAN AND MODIFIED POLYPROPYLENE FILM TO PROLONG POSTHARVEST STORAGE OF LIME *Citrus aurantifolia* Swingle

Miss Pornchan Jongsri

A Thesis Submitted in Partial Fulfillment of the Requirements

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ไคโตซานเป็นไบโอพอลิเมอร์ที่ไม่มีความเป็นพิษต่อสิ่งแวดล้อม ไคโตซานสามารถช่วยยืดอายุการเก็บรักษาและชะลอการเสื่อมของผักและผลไม้ได้ ในงานวิจัยนี้มุ่งหาความเข้มข้นของไคโตซานที่เหมาะสมในการยืดอายุการเก็บรักษาผลมะนาว (*Citrus aurantifolia* Swingle) ภายหลังการเก็บเกี่ยว ร่วมกับการใช้ฟิล์มพอลิโพรพิลีนดัดแปรในการเก็บรักษา พบว่า ผลมะนาวที่จุ่มในสารละลายไคโตซาน (80%DD) ความเข้มข้น 10 ppm ช่วยลดความเสียหายจากโรคได้เมื่อเก็บที่อุณหภูมิ 25 องศาเซลเซียส และผลมะนาวที่จุ่มในสารละลายไคโตซานความเข้มข้น 5 ppm สามารถชะลอการสูญเสียน้ำหนักสดและการเปลี่ยนแปลงสีผิวได้เมื่อเก็บที่อุณหภูมิ 10 องศาเซลเซียส การจุ่มผลมะนาวในสารแขวนลอยของรา *Penicillium* sp. ก่อนจุ่มในสารละลายไคโตซานความเข้มข้น 10 ppm แล้วใส่ในถุงพอลิโพรพิลีนดัดแปร organic clay polypropylene (Org/PP) และ polypropylene porous clay heterostructure (PPPCH) เปรียบเทียบกับถุงพอลิโพรพิลีนทั่วไป (PP) เก็บที่อุณหภูมิ 25 และ 10 องศาเซลเซียส เป็นเวลา 4 และ 6 สัปดาห์ตามลำดับ พบว่า การเปลี่ยนแปลงสีผิวของผลมะนาวไม่มีความแตกต่างกันที่อุณหภูมิ 25 และ 10 องศาเซลเซียส นอกจากนี้ผลมะนาวที่ใส่ในถุง PP มีเปอร์เซ็นต์การสูญเสียน้ำหนักสดน้อยกว่าถุงพอลิโพรพิลีนดัดแปรทั้ง 2 อุณหภูมิ ในขณะที่มะนาวที่ใส่ในถุง PP มีการเกิดโรคมากกว่ามะนาวที่ใส่ในถุงพอลิโพรพิลีนดัดแปรที่อุณหภูมิ 25 องศาเซลเซียส สำหรับอัตราการหายใจและค่าปริมาณของแข็งที่ละลายน้ำไม่มีความแตกต่างกัน การทำงานของเอนไซม์ catalase และ ascorbate peroxidase ปริมาณฟีนอลิกทั้งหมด และวิตามินซี ในผลมะนาว มีความแตกต่างกันในบางชุดการทดลองระหว่างการเก็บรักษา

ภาควิชา พฤกษศาสตร์ ลายมือชื่อนิสิต.....

สาขาวิชา พฤกษศาสตร์ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

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PORNCHAN JONGSRI: USE OF CHITOSAN AND MODIFIED POLYPROPYLENE FILM TO PROLONG POSTHARVEST STORAGE OF LIME *Citrus aurantifolia* Swingle. ADVISOR: TEERADA WANGSOMBOONDEE, Ph.D., CO-ADVISOR: ASST.PROF. KANOGWAN SERAYPHEAP, Ph.D., 109 pp.

Chitosan is a non-poisonous biopolymer which is safe for the environment. Chitosan is known to have the potential to prolong the storage life and control the decay of fruit and vegetable. In this study, we aimed to investigate an appropriate concentration of chitosan to prolong postharvest storage of lime (*Citrus aurantifolia* Swingle). Limes dipped in 10 ppm chitosan (80%DD) could reduce fungal decay at 25°C and 5 ppm chitosan treatment resulted in a delay in weight loss and change in peel color at 10°C. Limes inoculated with *Penicillium* sp. and treated with 10 ppm chitosan at 25°C and 10°C and packed in modified polypropylene films; organic clay polypropylene (Org/PP) and polypropylene porous clay heterostructure (PPPCH) were compared with commercial polypropylene (PP) bag stored at 25°C and 10°C for 4 and 6 weeks, respectively. Peel color change of limes did not show any significantly difference among treatments during storage at 25°C and 10°C. The control PP resulted in lower percentage of weight loss than modified polypropylene films in all treatments in both temperatures. While lime disease incidence increased in PP more than modified polypropylene films at 25°C. No significant difference in modified PP or normal PP treatments in term of respiration rate, and total soluble solids were observed. Catalase and ascorbate peroxidase activities, total phenolic content and ascorbic acid content of citrus fruits were different in some treatments during storage.

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CHAPTER I
INTRODUCTION

Lime (*Citrus aurantifolia* Swingle) is one of an economic plant in Thailand with high demand throughout the year. However, during summer time (March and April), lime production is sharply decreased due to the hot and dry season resulting in ten-fold increase in price about 5-10 baht/fruit (Department of Internal Trade of Samutsakorn, 2009). Thus, prolonging shelf life of lime during the dry season and for exporting can lead to higher income. Lime fruit injuries during harvest can be sustained by the entry of wound pathogens, including *Penicillium* spp., which are the causal agents of blue mold and green mold. Moreover, peel color change of lime is one of the important problems. Previous studies have tried to resolve this problem but no effective results were reported to decrease physiological change of limes.

Peel coating is one way to prolong shelf-life of fruit. Lime has natural oil coating on the peel which can prevent weight loss of the fruit. Chitosan (poly-β-(1,4)-D-glucosamine), a deacetylated form of chitin, is a natural antimicrobial compound which can be used as peel coating. Chitosan can be obtained from crustacean shells (crabs, shrimp and crayfishes) either by chemical or microbiological processes and on the other hand it can be produced by some fungi (Devlieghere et al., 2004). Chitosan coating of postharvest products is safe (Hirano et al., 1990) and shows antifungal activity against several fungi (El-Ghaouth et al., 1992b; Liu et al., 2007). Significant reduction of storage rots has been recorded in apples, kiwi fruit, pears (Du et al., 1997; Bautista-Banos et al., 2004) and other fruits treated with chitosan. Previous studies indicated that chitosan coating had the potential to prolong storage life and control decay of many fruits, such as strawberry, peach and table grape (El-Ghaoth et al. 1991; Du et al., 1997; Romanazzi et al., 2002). Zhang and Quantick (1997) and Jiang and Li (2001) reported that application of 2 g chitosan/100 g solution was the most effective treatment in delaying browning in litchi and longan fruits stored at low temperature. However, there are few reports on induction of resistance by chitosan in lime fruit during storage, and little

information is available on whether the positive effect of chitosan on postharvest diseases by pathogens.

In 2007, Chien et al. showed the effects of coating with low molecular weight (LMWC) and high molecular weight chitosan (HMWC) on the decay of "Murcott" tangor (sweet orange hybrid). The experiments showed that increasing LMWC concentration promoted the retention of firmness and water content. The LMWC effectively inhibited the growth of *P. digitatum*, *P. italicum*, *Botrytis lecanidion* and *B. cinerea*; its effectiveness increased with its concentration. Mangosteen sprayed with chitosan resulted in delayed peel color change and off-flavor for 30 days (Postharvest Technology Innovation Center Chiang Mai University, 2003). Moreover, the use of low concentration of chitosan could prolong shelf-life of asparagus (Patai Charoonnart, 2007).

Use of packaging in lime was reported to reduce dehydration, gas exchange, respiration rate and ethylene synthesis (Kunsongkeat, 1988). Sweet corn (*Zea mays* L.cv. Jubilee) sealed with Polyolefin films (K-400T and AM films) showed a significant reduction in weight loss and reduced the decay incidence compared with polyvinyl chloride (PVC) film (Aharoni et al., 1995). In 2008, Srithammaraj et al. showed the effect of polypropylene film mixed with clay and modified porous could absorb ethylene from fruits and vegetables. Thus, this film is very attractive for an application in postharvest packaging.

Therefore, we investigated an appropriate concentration of chitosan in combination with modified polypropylene film packaging to prolong postharvest storage of lime (*Citrus aurantifolia* Swingle). Catalase and ascorbate peroxidase which are antioxidant enzymes were also determined for their roles in prolonging shelf-life of limes.

Objectives

To determine the effects of chitosan and modified polypropylene film packaging on postharvest qualities of limes

CHAPTER II

LITERTURE REVIEWS

Lime *(Citrus aurantifolia* Swingle) is a non-climateric fruit which has high economical value in many countries. India is the number one in lemon and lime producing country which the amount is about 16% the production followed by Mexico (~14.5%), Argentina (~10%), Brazil (~8%), and Spain (~7%) (Table 1).

Table 1. Top ten lemon and lime production in 2007.

Country	Production (Tonnes)	Production (%)
India	2060000[F]	~16.0
Mexico	1880000[F]	~14.5
Argentina	1260000[F]	~10.0
Brazil	1060000[F]	~8.0
Spain	880000[F]	~7.0
People's Republic of China	745100[F]	~6.0
United States	722000[P]	~5.5
Turkey	706652[P]	~5.0
Iran	615000[F]	~4.5
Italy	546584[P]	~4.0
World	13032388[A]	

F = FAO estimate

P = Official figure

A = Aggregate (may include official, semi-official or estimates)

Source: Food And Agricultural Organization of United Nations: Economic And Social Department: The Statistical Division, 2007

Lime is used for extraction of juice, concentrates, beverages and other by-products, such as citric acid and pectin etc. In the Middle East, whole lime fruit, which is dried to a charred color, is used in rice-based biryani/pulav-type preparations, as well as curry preparations. The powdered fruit is also sprinkled over roasted meat (Yadav et al., 2004). Lime volatile extract also showed insecticidal properties (Ezeonu et al., 2001). In Thailand, lime is also considered as a medical plant and use as one of the ingredients to add sour taste to many different dishes.

The green color of lime peel is caused by chlorophyll presence in mature green fruit. These pigments are located in the flavedo tissue of the peel and juice vesicles (pulp). The maintenance of green color in peel of lime throughout the postharvest supply chain is required if fruit are to attract premium prices (Pranmornkith et al., 2005). The chlorophyll degradative process occurring after harvest and is relatively rapid at ambient temperature during marketing. Yellowing is a consequence of alterations in the physiological and biochemical processes occurring in the flavedo tissue of the lime peel (Tin et al., 2006), thus leading to changing of peel color. Moreover, lime fruit injuries during harvest sustained by the entry of wound pathogens, including *Penicillium* spp., which are the causal agents of blue mold and green mold.

1. Postharvest management of citrus fruits

In order to prolong shelf-life of citrus produces, there are several approaches to retain the postharvest quality of citrus fruits.

1.1 Waxing

Wax and coating can reduce water loss in citrus fruits. Coating can prolong shelf life of fruits that control permeability of CO_2, O_2, and water vapor, which reduce metabolic rate and water loss. Permeability of citrus coatings should be high to O_2 and low to water vapor to reduce transpiration as much as possible and not overly restrict respiration. The respiration is the important problem in physiological change that results in off-flavor. Effective protective coating reduces weight loss by 30-40 percent (Johnson, 1991). In a study of 'Nagpur' mandarins, respiration rate is reduced to about

30 mg/kg/h in aqueous wax emulsion coated fruit compared with non-coated fruit (42 mg/kg/h).

Solvent waxing was broadly used in Australia during the 1950s and 1960s (Long and Leggo, 1959). The first time of wax emulsions were used to extend the shelf life of citrus fruits in India was started in 1960s ; the method was manual dipping of fruit placed in the buckets into drums containing a wax emulsion. Eaks and Ludi (1960) reported that temperature, cleaning, and the method of waxing also affect the physiological change of citrus fruits. The method to apply waxes are brushed, sprayed, fogged, or foamed onto produce.

The use of polyethylene wax mixed with Imazalil on Florida-grown Valencia oranges could lower weight loss over 22 weeks of storage at 3-4ºC and also maintained low anaerobic respiratory of product with good internal quality (Peeples et al., 2000). Wax coatings also reduced chilling injury in grapefruit and mandarins (Pantastico et al., 1968). When coated 'Nagpur' mandarin fruit stored at 3.5ºC, chilling injury was found less than in non-coated fruit (Ladaniya et al., 2005). Effects of carbohydrate and lipid emulsions and carboxy-methylcellulose emulsions used on citrus fruits were reported. Carbohydrate and lipid emulsions gave the better weight-loss control. The shelf-life of orange fruit ranged from 4-8 weeks for fruit coated with sucrose ester (2 percent) and carbohydrate and lipid emulsion compared with control ranged from 3-5 weeks (Yicheng and Tingfu, 1990). Sucrose esters favored the development of yeast populations (support survival of the yeast *Candida oleophila*, a bioagent that controls postharvest decay).

1.2 Plant growth regulators and chemical treatments

Plant growth regulators (PGRs) control physiological processes at extremely low concentrations. Most of these compounds occur naturally and hence their use in postharvest citrus treatments is expected to receive consumer acceptance. Auxins, gibberellins (GA), cytokinins, abscissic acid (ABA), and ethylene are five important types of PGRs that occur naturally in fruits. The first three types of compounds are used to extend the vitality of fruit tissues while the last two are known to promote aging and

senescence processes. Gibberellic acid (GA_3) and 2,4-dichloro phenoxy acetic acid (2,4-D) are the most widely and commercially used PGRs in citrus. Both of these chemicals have pre and postharvest applications. As a preharvest application, 2,4-D delays and reduces abscission of mature fruit and increases fruit size. As a postharvest application in lemons, 2,4-D delays button abscission by maintaining its vitality and thus reduces *Alternaria* rot (Coggins, 1991).

GA_3 is primarily used as preharvest spray on navels and Minneola tangelos to delay peel senescence and fruit maturity in lemons while postharvest application in lemons is aimed to delay coloration and reduce sour rot (Coggins, 1991). GA_3 is observed to be highly persistent in orange peel (half life = 80 days). Ethylene causes a slight enhancement of GA_3 metabolism in orange fruit (Shechter et al., 1989). Application of GA_3 and low temperature storage are very important factors required to retain green color in citrus fruit (Porat et al., 2000) and sometimes, cytokinins and GA_3 can increase regreening (Lewis, 1982). In California, GA_3 application is recommended for lemons since it delays ripening and improves storage quality.

Postharvest application of GA_3 (50 ppm) could reduce the incidence of *Geotrichum* decay, probably by delaying senescence and thus retaining resistance of fruit to the decay pathogen (Coggins et al., 1992). The quality of GA_3 dip–treated (100 ppm) mature green fruits of the Mahaley orange was better than that of the controls throughout the storage period at 4ºC or 7ºC (Al-Doori et al., 1990). GA_3 (250 ppm) treatment of *C. latifolia* fruits prevented degreening at low temperatures (8°C or 10ºC) more than at ambient temperature (Mizobutsi et al., 2000).

In addition, treatments with ethylene inhibitors can be applied in citrus fruit to delay postharvest senescence. However, limes dipped in $KMnO_4$ then stored at 10°C couldn't delay peel color change while limes dipped in GA_3 could delay peel color change. Use of solutions of chlorine 200 mg/l and bennomyl solution (1,000 mg/l) to

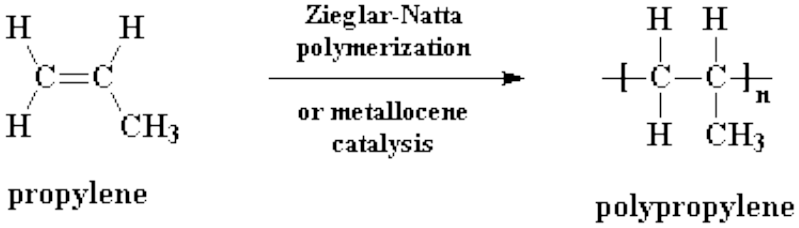
wash limes before storage for a period of 3 months showed that the bennomil could control the disease appearance. Moreover, Kunprom et al. (2002) reported that fungus found on citrus fruit after harvest may not associate with microorganisms that caused disease during storage

1-methylcyclopropene (1-MCP) at 250, 500, 750 and 1000 nl/l were used in West Indian limes (*Citrus aurantifolia*, Swingle cv. 'Paan') and fruits were examined under ambient conditions (24-31°C and 73–81% RH). 1-MCP (250 or 500 nl/l) effectively retarded yellowing of limes for 21 days at ambient storage. Ascorbic acid content was also reduced in fruits treated with 1000 nl/l of 1-MCP but not in fruits treated with 250, 500 or 750 nl/l. Chlorophyllase and chlorophyll degrading peroxidase activities in flavedo tissue of lime peel were delayed in 1-MCP treated fruit at concentrations of 250 and 500 nl /l of 1-MCP (Tin et al., 2006).

1.3 Film packaging

1.3.1 Polypropylene (PP)

Molecular formula of PP is $(C_3H_6)_n$. Polypropylene have density of 0.855 g/cm^3 and have melting point at 130–171°C. PP is resistant to high temperature (100°C) and most chemicals. PP can be a very good insulator even at high temperatures (Clive and Teresca, 1998). Structurally, it's a vinyl polymer, and is similar to polyethylene, only that on every other carbon atom in the backbone chain has a methyl group attached to it. Polypropylene can be made from the monomer propylene by Ziegler-Natta polymerization and by metallocene catalysis polymerization (Fig. 1)



Polymerization

Figure 1. Polymerization of propylene

1.3.2 Modified polypropylene film

Polypropylene can be modified by adding some materials to get a specific property of a film. For example; Organic clay polypropylene (Org/PP) is polypropylene mixed with clay. Polypropylene porous clay heterostructure (PPPCH) have been prepared by the surfactant-directed assembly of mesostructured silica within the two-dimensional galleries of clays. The PPPCH is an interesting material to use as entrapping system such as ethylene scavenger, owing to its high surface area with uniform and specific pore size. In the present work, the PPPCH was synthesized within the galleries of Na-bentonite clay by the polymerization of tetraethoxysilane (TEOS) in the presence of surfactant micelles. According to pore characterization, PPPCHs have surface areas of 421-551 m^2/g, an average pore diameter in the supermicropore to small mesopore range of 4.79-5.02 nm and a pore volume of 0.57-0.66 cc/g (Srithammaraj et al., 2008).

1.3.3 Effect of plastic film on postharvest storage of fruits and vegetables

The use of modified atmosphere packaging (MAP) is one of the techniques to maintain qualities of fruits in postharvest (Beaudry, 1999). This technique was to seal amount of fruits in plastic bags which have permeability to gas exchange to control O_2 and CO_2 concentrations by decrease in O_2 and

increase in CO_2 levels inside the bags (Kader et al., 1989). Modified atmosphere (MA) can directly or indirectly reduce postharvest diseases (Barkai-Golan, 1990). Polyvinylchloride (PVC) wraps that are in commercial use do not maintain the desired atmosphere within the package to control decay development. Effects of PVC and the two polyolefin films on fresh sweet corn (*Zea mays* L. cv. Jubilee) placed on either cardboard or polystyrene trays and stored at 1°C for 12 days and then transferred for an additional two shelf-life days at 20°C were studied. The result showed that PVC packaging could keep the freshness of sweet corn by maintaining high humidity within the package which reduces moisture loss of the kernels and husks. Increased levels of CO_2 and decreased O_2 atmospheres were generated through the use of the new films compared with PVC in which it reduced decay, and maintained quality during 12 days storage at 1°C and two days at 20°C (Aharoni et al., 1995). However, the main limiting factor affecting the shelf-life of fresh sweet corn cobs is the rapid development of pathogens on the cut ends, kernels and flag leaves trimmed according to export criteria (Temkin-Gorodeiski and Barkai-Golan, 1980).

Application of plastic films on different citrus species (lemons, oranges and grapefruits) has given better response than waxing in preserving overall quality, shrinkage, softening, deformation and flavor loss (D'Aquino et al., 2000). In addition, consumers prefer to buy wrapped fruits because of their better appearance and the hygienic function of the films.

In 2007, Elsabee et al. studied antifungal and antibacterial properties of PP films which were irradiated with corona discharge or modified by chitosan and chitosan/pectin extracted from different sources. The result showed that the native PP had no biocidal activity; the corona treated ones showed slight activity however the films coated with chitosan and its derivatives showed much higher antifungal, as well as, antibacterial activity. Using these films for tomato packaging showed no apparent rotting infection for 13 days. This test indicates

the potential of this treatment of PP films for the production of antimicrobial packaging.

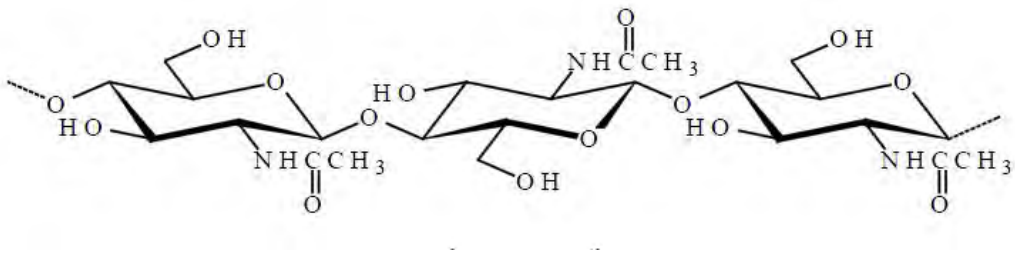
2. Chitosan

2.1 Chitosan

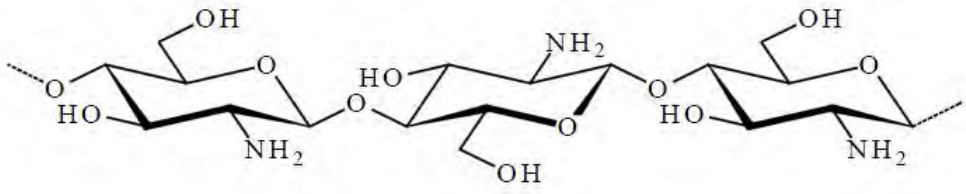
Chitosan (poly-β-(1,4)-D-glucosamine) is a derivative of chitin (poly-N-acetylglycosamine) which is a modified polysaccharide that contains nitrogen (Horton et al., 1993). It is synthesized from units of *N*-acetylglucosamine (GlcNAc). These units form covalent β-1,4 linkages (similar to the linkages between glucose units forming cellulose). The raw materials of chitosan are the main component of the cell walls of fungi, the exoskeletons of arthropods such as crustaceans (e.g., crabs, lobsters and shrimps) and insects, the radulas of mollusks, and the beaks of cephalopods, including squid and octopuses (Chankrajang, 2000). Chitosan can be obtained by partial de-N-acetylation of chitin through the process of deacetylation. Acetamide group of chitin is converted into amino group ($-NH_2$) so chitosan can be more soluble than chitin at the same pH (Li et al., 1997). Chitosan is not expensive, non-toxic and natural compound, and holds great potential for food applications with its unique physiological and biological properties (Shahidi et al., 1999).

Chitosan has three types of reactive functional groups: an amino group, as well as primary and secondary hydroxyl groups at the C-2, C-3, and C-6 positions (Fig. 2) (Furusaki et al., 1996), which makes chitosan useful for many applications (Galed et al., 2001). Chemical modifications of these groups increase the numerous applications of chitosan in different fields, including bioconversion for the production of value added food products (Shahidi and Synowiecki, 1991), preservation of foods from microorganisms deterioration (Chen et al., 1998; Galed et al., 2001), formation of

biodegradable films (Wong et al., 1992), and the clarification and de-acidification of fruit juices (Chen et al., 1996).



(A)



(B)

Figure 2. (A) Structure of chitin, (B) Structure of chitosan (Furusaki et al., 1996)

2.2 Applications of chitosan

Chitosan's biofunctionalities are highly related to its molecular weight and degree of deacetylation. The antibacterial functions of chitosan and its derivatives represent their primary utility in biological applications. Regardless of the source of chitosan, its antimicrobial efficacy is influenced by a number of factors, which include degree of polymerization, microorganism species and the degree of deacetylation at which antimicrobial activity increases (Tsai et al., 2002). Accordingly, the antimicrobial activity of chitosan will depend on several factors such as the kind of chitosan, used, the pH of the medium, the temperature, the presence of several food components, etc. (Devlieghere et al., 2004).

The applications of chitosan and its derivatives are widely used in agriculture, medicine, environment, food, etc. (Devlieghere et al., 2004). Coating fruit and vegetables with chitosan helps to long-term storage of food (El-Ghaouth et al., 1992a), because a chitosan film provides a type of active package, as preservatives are released from the film deposited on the surface of the food and these inhibit fungal growth.

2.2.1 Applications of chitosan in food and medicine

Apart from its antimicrobial effect, chitosan is also used in food (Root and Johnson, 1978; Soto-Peralta et al.,1989; Boguslawski et al., 1990), antioxidant in sausages (Xie et al., 2001) and enzymatic browning inhibitor in apple and pear juices (Saper, 1992). Chitosan's properties allow it to rapidly clot blood, and has recently gained approval in the United States and Europe for use in bandages and other hemostatic agents. Chitosan hemostatic products reduce blood loss in comparison to gauze dressings and increase patient survival (Pusateri et al., 2003). Chitosan's properties also allow it to be used in trans-dermal drug delivery. The most important property of chitosan with regards to drug delivery is its positive charge under acidic conditions. This positive charge comes from protonation of its free amino groups. Lack of a positive charge means chitosan is insoluble in neutral and basic environments. However, in acidic environments, protonation of the amino groups leads to an increase in solubility. The implications of this are very important to biomedical applications. This is a molecule that will maintain its structure in a neutral environment but will solubilize and degrade in an acidic environment. This means that chitosan can be used to transport a drug to an acidic environment, where the chitosan packaging will then degrade, releasing the drug to the desired environment. One example of this drug delivery has been seen in the transport of insulin (Sunil et al., 2004).

Chitosan is frequently sold as tablet form at health stores as a "fat binder". It is supposed to have the capability to interact with lipids (fat) from the digestive system and limit their absorption in the body. In an experimental model of the stomach and duodenum tract, chitosan has shown to interact with oil, which inhibited duodenal absorption and enhanced lipid excretion (Rodríguez et al., 2005). However, the mechanism of interaction between chitosan and fat is not very well understood and has not been really proved clinically.

2.2.2 Applications of chitosan in agriculture

Chitosan was first characterized as an elicitor in plants. It has been shown to activate plant defense genes through the octadecanoid pathway (Doares et al., 1995), to increase the activity of a wounding responsive protein in tomatoes (Stratmann and Ryan, 1997) and has acted as an elicitor in slash pine (*Pinus elliotti*) (Mason and Davis, 1997) and oats (Tada et al., 2001). Chitosan activates several defense processes in the host tissue (El-Ghaouth et al., 1992c), and acts as a water-binding agent and inhibits various enzymes (Young et al., 1982). Agricultural applications of chitosan can reduce environmental stress due to drought and soil deficiencies, strengthen seed vitality, improve stand quality, increase yields, and reduce fruit decay of vegetables, fruits and citrus crops (Linden et al., 2007). Chitosan was also involved in stomatal response. The stomatal opening provides access to inner leaf tissue for many plant pathogens. Therefore, the narrowing stomatal aperture may be advantageous in plant defense. The stomatal aperture of tomato and *Commelina communis* was reduced when the epidermis was treated with chitosan (Lee et al., 1999). The result showed that foliar application of chitosan could decrease transpiration in pepper plants, resulting in a reduction in water use by 26–43%, while their biomass production and yield still remained unchanged (Bittelli et al., 2001), suggesting that chitosan could be an effective antitranspirant to conserve water use in agriculture.

In tissue culture studies, concentration and molecular weight of chitosan applied influenced the meristematic bud growth in the shoots of orchid plants (*Dendobrium phalaenopsis*) (Nge et al., 2006). Application of foliar applications with chitosan resulted in higher vegetative growth of the leaves and weight of the inflorescences and improvement in the postharvest quality of curcuma (*Curcuma alismatifolia* x *Curcuma cordata*) cv. 'Laddawan' (Tamala et al., 2007). For other cultivated plants, chitosan seed coating promoted the emergence as well as increased the vigor of wheat (*Triticum aestivum*) (Reddy et al., 1999) and the vegetative growth in pearl millet (*Pennisetum glaucum*) (Sharathchandra et al., 2004).

Chitosan's effects on plant growth have also been shown in *Eustoma grandiflorum* (Raf.) Shinn (Ohta et al., 1999). Chitosan application to the soil mix at sowing time remarkably enhanced plant growth and the treated plants flowered 15 days earlier than the controls. Moreover, a greater number and weight of flowers was produced by chitosan-treated plants. Chitosan application in soil mixture also promoted seedling growth of *Torenia fournieri* Linden ex E. Fourn., *Exacumaffine* Balf., *Begonia hiemalis* Fotsch, *Sinningia speciosa* (Lodd.*), Lobelia erinus* L. and *Mimulus hybridus* hort.ex A. Siebert et Voss (Ohta et al., 2004).

The biocontrol of chitosan elicits natural innate defense responses within plant to resist insects, pathogens, and soil-borne diseases when applied to foliage or the soil (Linden et al., 2005). Chitosan increases photosynthesis, promotes and enhances plant growth and stimulates nutrient uptake. When used as seed treatment or seed coating on cotton, corn, seed potatoes, soybeans, sugar beets, tomatoes, wheat and many other seeds, it elicits an innate immunity response in developing roots which destroys parasitic cyst nematodes without harming beneficial nematodes and other organisms (Stoner et al., 2006). According to the defense gene induction activity, chitosan has been proven to induce disease resistance in several plants, with pathogen and

plant cultivar specificity (Bell et al., 1998; Elkemo et al., 2003). The role of chitosan in plant protection may also result from its antifungal activity. Fifty parts per million chitosan almost completely inhibited *Botrytis cinerea* conidia germination in vitro, and it was shown to be able to control gray mould, caused by *B. cinerea* in cucumber plants (Ben-Shalom et al., 2003).

2.2.3 Applications of chitosan in postharvest

Chitosan coating is known to have the potential to prolong shelf-life and control the decay of strawberries, tomatoes, peaches, pears, kiwifruit, litchi, apples and longan fruits (El-Ghaouth et al., 1991; El-Ghaouth et al.,1992b; Du et al., 1997; Zhang and Quantick, 1997; Ippolito et al., 2000; Jiang and Li, 2001). Postharvest treatments of chitosan in fruits and vegetables were used as a semi-permeable film that delays fruit ripening and prolongs its shelf-life (Bautista-Banos et al., 2006). Moreover, its property antifungal activity so can limit fungal decay (El-Ghaouth et al., 1997; Bautista- Banos et al., 2006), or by its capability for induction of the host resistance to pathogens (Wilson et al., 1994; Fajardo et al., 1998).

Significantly reduction of storage rots has been recorded in apples, kiwifruit, pears (Du et al., 1997; Bautista- Banos et al., 2004) and other fruits treated with chitosan. Chitosan treatments induced an increase in phenolic compounds, flavonoid and lignin contents (Zhang and Quantick, 1997; Vander et al., 1998; Liu et al., 2007; Meng et al., 2008; Sun et al., 2008). After treated with chitosan, defense responses have been induced in several fruits, including the elicitation of phenylalanine ammonia-lyase (PAL) activity in grape berries (Romanazzi et al., 2002), and chitinase and β-1,3-glucanase in oranges, strawberries and raspberries (Zhang and Quantick, 1997), thereby promoting protection from further fungal infection (Liu et al., 2007). Among potential additives, chitosan (β-1,4-glucosamine polymer) could be a useful additive to antagonistic microorganisms. Chitosan and its derivatives such as glycolchitosan and carboxymethylchitosan are known to form a semipermeable film and are inhibitory to a number of pathogenic fungi, and also induce host-

defense responses (Allan and Hadwiger, 1979; El-Ghaouth et al., 1994). Combining chitosan with antagonists will make it possible to exploit the antifungal and eliciting property of chitosan-chloride and the biological activity of the antagonist. The effect of molecular weight on the physical properties of chitosan membranes has been reported. Low molecular weight chitosans (LMWC) have permeability higher than that of high molecular weight chitosans (HMWC) (Chen and Hwa, 1996). Recently, LMWC with an average MW in the range 5000–20,000 Da, were shown to exhibit superior biological activities than chitosan (Muzzarelli and Muzzarelli, 2002). LMWC have also been shown to modulate plant resistance to disease (Vasyukova et al., 2001).

In 2000, El-Ghaouth et al. studied biocontrol activity of the combination of *Candida saitoana* with chitosan compounds in apple and citrus fruits. They found that combination with *C. saitoana and* 0.2% glycolchitosan effected in reduction of green mold incidence on light green and yellow lemons and was more effective in controlling postharvest decay than *C. saitoana* or 0.2% glycolchitosan alone. Moreover, concentration of glycolchitosan did not affect in controlling postharvest decay when combination with *C. saitoana*. In apple fruit, the combination of *C. saitoana* and 0.2% glycolchitosan when inoculated with *Botrytis cinerea* or *Penicillium expansum* showed no visible symptoms of infection until 10 days of storage at 24°C, while in control fruit lesions were visible in 4th day of storage .

Postharvest diseases caused by *Penicillium digitatum* (green mould) and *P. italicum* (blue mould) are the most important negative factors affecting handling and marketing of citrus fruits. In 2007, Chien et al. investigated the effects of coating with low molecular weight chitosan (LMWC, Mw=15 kDa) and high molecular weight chitosan (HMWC, Mw=357 kDa) on the decay of 'Murcott' tangor (Honey Tangerine; Citrus spp.; Mandarin x *Citrus sinensis*, sweet orange hybrid) and the maintenance of its quality and stored at 15ºC for 56 days. Treated Murcott tangor with fungicide thiabendazole (TBZ) was used as control.

They found that weight loss with 0.1% LMWC treatment was slower than that of the TBZ-treated control. LMWC coating beneficially influenced firmness, total soluble solid content, titratable acidity, ascorbic acid content and water content of citrus fruits. Increasing LMWC concentration resulted in an increase in firmness and water content. Moreover, the two chitosans were more effective in inhibiting the rate of growth of *P. digitatum, P. italicum, B. lecanidion* and *B. cinerea* than TBZ-treated control. Effectiveness of LMWC increased with its concentration.

CHAPTER III

MATERIALS AND METHODS

Experiment 1. Effects of chitosan treatments on postharvest quality of limes during storage.

1.1 Plant materials

Limes (*Citrus aurantifolia* Swingle) were harvested from lime orchard in Phetchaburi province. Mature limes were selected for uniformity in shape, color, size, and any blemished or diseased fruits were discarded.

1.2 Fungal culture

Penicillium sp. Was isolated from diseased lime fruits and pure culture was maintained on potato dextrose agar (PDA). Conidial suspension of the fungus was prepared by flooding the 7-day-old culture dishes incubated at room temperature. The conidial suspension was counted using a hemocytometer and adjusted to 1×10^{6} conidia ml^{-1} with sterile distilled water.

1.3 Chitosan treatments

The experiment was designed as a complete randomized design (CRD) with four treatments and four replicates per treatment at each storage temperature (25ºC and 10ºC). Limes were divided and dipped in different concentrations of chitosan as followed,

Treatment 1 dipping lime fruit in distilled water (control)

Treatment 2 dipping lime fruit in 5 ppm chitosan

Treatment 3 dipping lime fruit in 10 ppm chitosan

Treatment 4 dipping lime fruit in 15 ppm chitosan

Limes were dipped in all treatments for 5 min. Then fruits were air dried at 25ºC.

After that fruits were packed in polypropylene bag and stored at 25ºC and 10ºC with 90–95% RH for 4 weeks and 6 weeks, respectively. Fruits were sampling every week for determination in physiological changes.

1.4 Measurement of some physiological changes of limes

1.4.1 Peel color change

Peel colors were detected by Minolta Chroma Meter (10-CR series, Minolta Co. Ltd., Japan) at three equidistant locations on each fruit along the equator of the fruit and expressed as L*, a* and b* values. Hue values were calculated from a* and b* values using the following formula:

$$\text{Hue} = \text{arc tangent } (a^*/b^*)$$

1.4.2 Percentage of weight loss (AOAC, 1984)

Weight loss was calculated as percentage loss of initial weight. The fruits were weighed regularly to determine weight loss using the following formula:

$$\text{Percentage of weight loss} = \frac{\text{weight before storage-weight after storage}}{\text{weight before storage}} \times 100$$

1.4.3 Disease incidence

Disease incidence was measured by counting diseased limes in each week and calculated to percentage as follow:

$$\text{Percentage of disease incidence} = \frac{\text{No. of diseased fruits X 100}}{\text{No. of total fruits}}$$

1.5 Statistical analysis

All Statistical analyses were performed using SPSS 11.0 software. Statistical comparisons were made by one-way analysis of variance (ANOVA). Differences were regarded as significant when the p-values were less than 0.05. Mean separations were performed by employing Duncan's Multiple Range Test comparison procedure.

Experiment 2. Effects of chitosan and modified polypropylene film packaging on postharvest quality of limes during storage at 25°C for 4 weeks.

The best concentrations of chitosan from each temperature were selected to use in the second and third experiments. Limes were dipped in conidial suspension of *Penicillium* sp. (1×10^{6} conidia ml^{-1}) for 5 min then stored at room temperature for 24 h. Then, limes were dipped in chitosan for 5 min. After air dried, limes were packed in three different packaging; polypropylene (PP), organic clay polypropylene (Org/PP) and polypropylene porous clay heterostructure (PPPCH) films.

2.1 Treatments

The experiment was designed as a CRD with nine treatments and four replicates per treatment. Limes were dipped in different solutions and packed in three different types of films as followed:

Treatment 1 dipping lime fruit in distilled water and packed in PP

Treatment 2 dipping lime fruit in distilled water and packed in Org/PP

Treatment 3 dipping lime fruit in distilled water and packed in PPPCH

Treatment 4 dipping lime fruit in *Penicillium* sp. and packed in PP

Treatment 5 dipping lime fruit in *Penicillium* sp. and packed in Org/PP

Treatment 6 dipping lime fruit in *Penicillium* sp. and packed in PPPCH

Treatment 7 dipping lime fruit in *Penicillium* sp.+chitosan and packed in PP

Treatment 8 dipping lime fruit in *Penicillium* sp.+chitosan and packed in Org/PP

Treatment 9 dipping lime fruit in *Penicillium* sp.+chitosan and packed in PPPCH

After air dried, limes were stored at 25ºC with 90-95% RH for 4 weeks. Fruits were sampling every week for physicochemical analysis.

2.2 Measurement of some physicochemical changes of limes.

2.2.1 Peel color change

Peel colors were detected by Minolta Chroma Meter (CR10- series,

Minolta Co. Ltd., Japan) at three equidistant locations on each fruit along the equator of the fruit and expressed as L*, a* and b* values. Hue values were calculated from a* and b* values using the following formula as the first experiment.

2.2.2 Percentage of weight loss (AOAC, 1984)

Limes were weighed and calculated the same as the first experiment.

2.2.3 Disease incidence

Disease incidence was calculated using the same formula as the first experiment.

2.2.4 Respiration rate

Five fruits from each treatment were weighed and placed in 0.7 L jars for three hours at 25°C. Ten mL sample of the internal atmosphere was withdrawn by inserting the needle of a syringe from the stilar end. Gas was kept in saturated saline in 50 ml glass bottle until analysis. CO_2 was detected by Gas Chromatography (GC-8A, Japan) at Kasetsart University.

2.2.5 Ascorbic acid (Shin, 2007)

The 2,6-dichloroindophenol titrimetric method (Shin, 2007) was used to determine the vitamin C content of peel extract. Peel (0.1 g) was cut and 10 mL of metaphosphoric acid-acetic acid solution was added. After appropriate dilutions with metaphosphoric acid-acetic acid solution and filtration as determined by the extract colour intensity, five mL of the diluted solution was titrated against standard indophenol solution. Results are expressed in µg ascorbic acid/g fresh weight (showed in appendix A).

2.2.6 Total soluble solids

Lime juice was used to measure total soluble solids (TSS) with a hand refractometer (N-1E, Japan) and TSS was expressed as ºBrix.

2.2.7 Total phenolic content (Ramful et. al., 2010)

Folin–Ciocalteu assay adapted from Ramful et.al., (2010) was used for determination of total phenolics presented in the lime extracts. Total phenolics were calculated with respect to gallic acid standard curve (concentration range: 0-12 µg/mL). Results are expressed in µg of gallic acid/g fresh weight (showed in appendix A).

2.2.8 Antioxidant enzymes (Nittaya Umrat, 2005)

Catalase assay and ascorbate peroxidase assay were measured following by Nittaya Umrat (2005) to determine enzyme activities. Peel extract (0.1 g) was homogenized in 1 mL of ice-cold extraction buffer. Homogenates were centrifuged at 14,000 g for 15 min at 4ºC and the resulting supernatants were used for assay (showed in appendix A).

2.2.9 Protein assay (Nittaya Umrat, 2005)

The method was adapted from Nittaya Umrat (2005). Bovine serum albumin (BSA) was as the standard protein (showed in appendix A).

2.3 Statistical analysis

Statistical comparisons were made by one-way analysis of variance (ANOVA). Differences were regarded as significant when the p-values were less than 0.05. Mean separations were performed by employing Duncan's Multiple Range Test comparison procedure.

Experiment 3. Effects of chitosan and modified polypropylene film packaging on postharvest quality of limes during storage at 10°C for 6 weeks

The most appropriate concentration of chitosan from the first experiment was used to pretreat lime fruits. The experiment was designed as same as the second experiment but storage temperature was changed from 25°C to 10°C and stored for 6 weeks.

CHAPTER IV

RESULTS

1. Effects of chitosan treatments on postharvest quality of limes after storage

1.1 Peel color change

The chitosan-untreated (control) and chitosan-treated limes at 5, 10 and 15 ppm of concentrations stored at 25°C and 10°C for 4 weeks and 6 weeks, respectively, were measured for peel color change reported by hue value and L value. L value increased in all treatments during storage until the final week of storage and didn't show any significant difference among treatments at 25°C and 10°C (Fig. 3-4). Hue values were also decreased in all treatments and were not significant difference (Fig. 5-6).

1.2 Percentage of weight loss

Percentage of weight loss was high in limes treated with 5 ppm chitosan stored at 25°C compared with all other treatments while 15 ppm chitosan was the best treatment to reduce percentage of weight loss in limes. At 10°C storage, concentration of chitosan at 5 ppm significantly resulted in the lowest percentage of weight loss of limes (Fig. 7-8).

1.3 Disease incidence

Limes stored at 10°C from all treatments didn't show any disease incidence until the final week of storage whereas disease incidence of limes stored at 25°C occurred since the second week of storage in all treatments. In the final week, 5 ppm chitosan treated limes resulted in the highest fruits disease incidence and 10 ppm chitosan treated limes resulted in the lowest fruits disease incidence (Fig. 9).

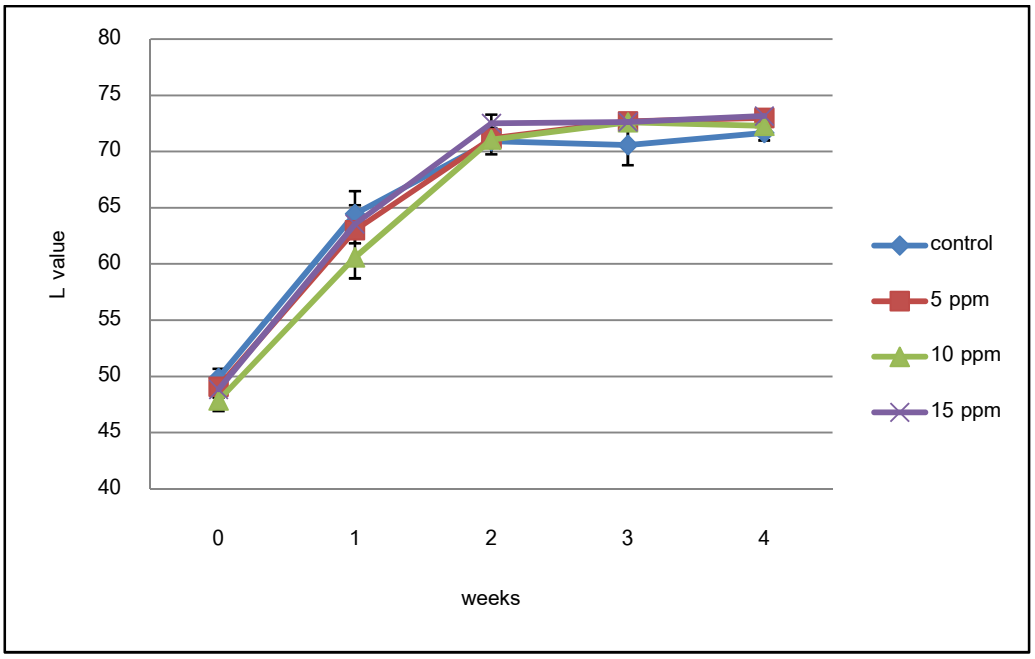


Figure 3. Effect of chitosan on L value of limes during storage at 25°C for 4 weeks.

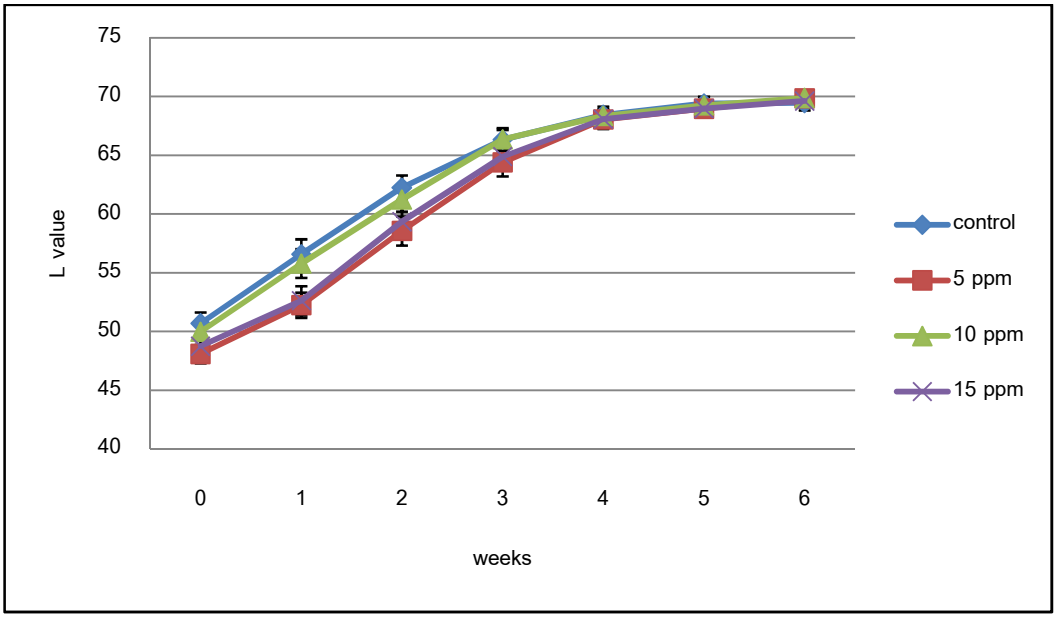


Figure 4. Effect of chitosan on L value of limes during storage at 10°C for 6 weeks.

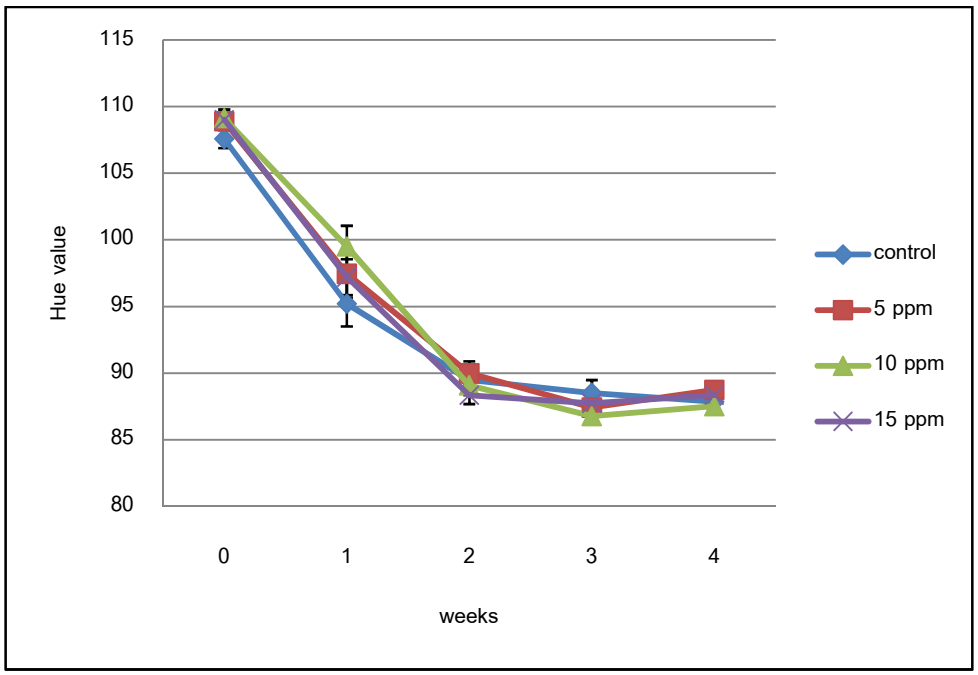
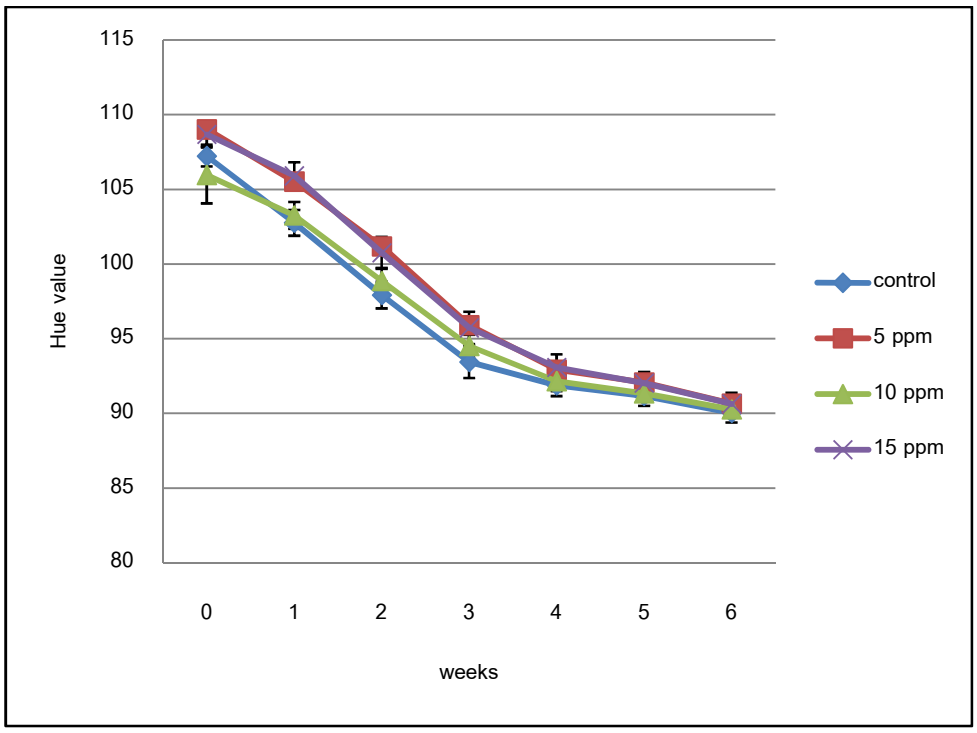


Figure 5. Effect of chitosan on hue value of limes during storage at 25°C for 4 weeks.



Figuer 6. Effect of chitosan on hue value of limes during storage at 10°C for 6 weeks.

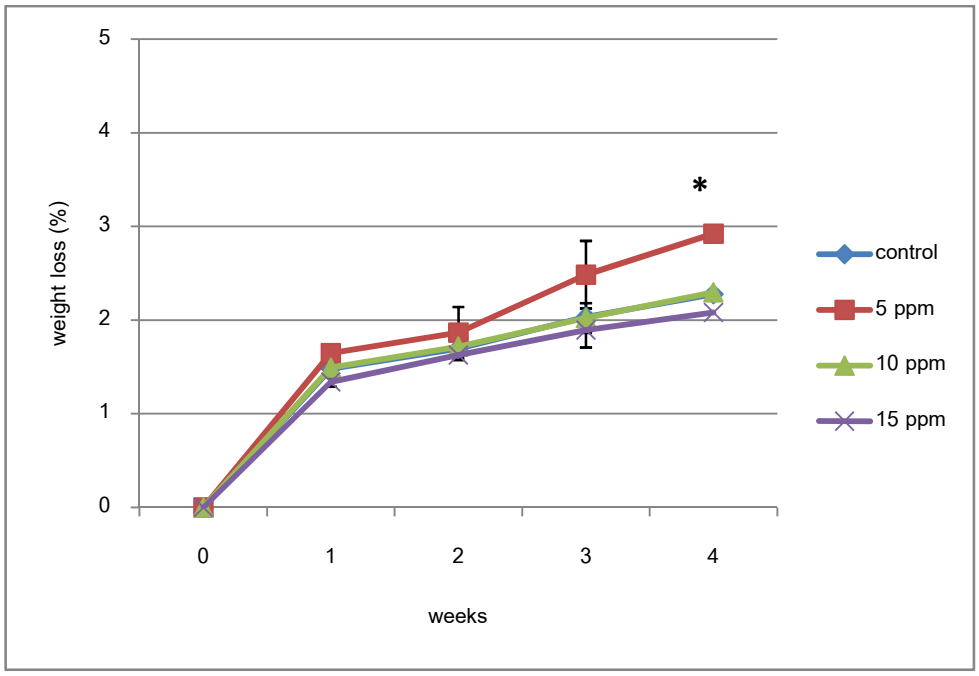


Figure 7. Effect of chitosan treatments on percentage of weight loss of limes during storage at 25°C for 4 weeks.

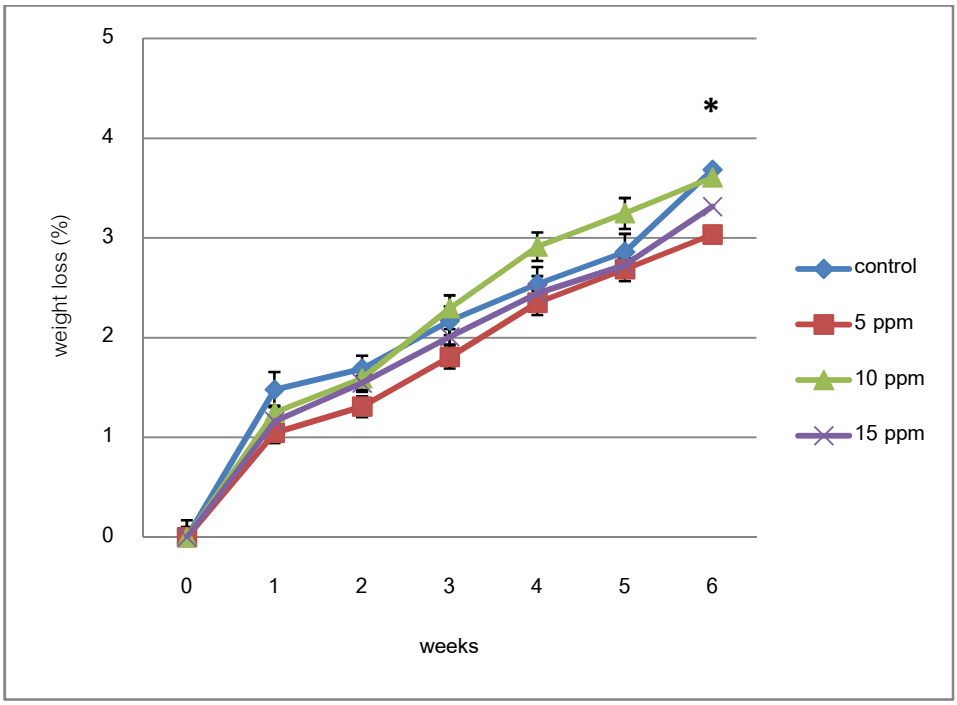


Figure 8. Effect of chitosan treatments on percentage of weight loss of limes during storage at 10°C for 6 weeks.

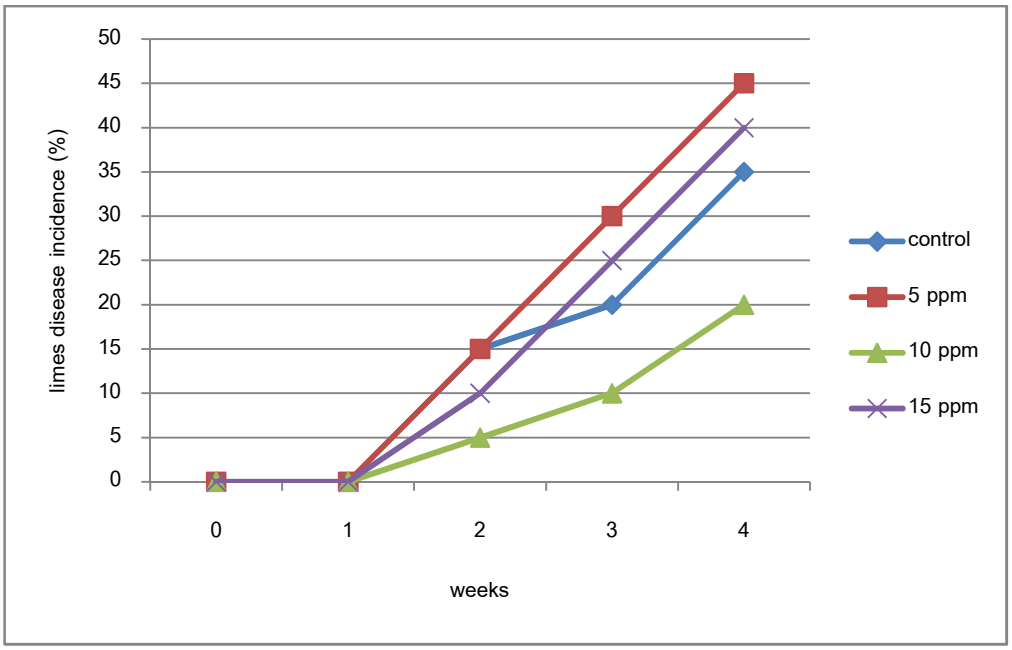


Figure 9. Percentage of lime disease after treated with chitosan during storage at 25ºC for 4 weeks.

2. films packaging on postharvest quality of limes during storage at 25°C for 4 weeks

2.1 Peel color change

Chitosan treated limes packed in modified polypropylene films showed an increasing trend of L value in all treatments since the first week through the final week of storage (Fig.10). In contrast, hue value decreased in all treatments during storage. There were no significant difference in terms of hue or L values of treated fruits in all treatments (Fig.11).

2.2 Percentage of weight loss

The control PP gave the best result by having the lowest percentage of weight loss. Fruits inoculated with the fungus and placed in modified PP were resulted in higher percentage of weight loss than the control PP. Treated fruits with chitosan after fungal inoculation did not maintain fresh weight of limes (Fig.12-15).

2.3 Disease incidence

Lime disease appeared in the second week of storage. Fruits inoculated with the fungus then treated with chitosan and packed in the control PP had the highest percentage of disease incidence and no disease incidence was found in non-treated limes packed in PPPCH (Fig. 16). When treatments were grouped by sets of packaging and treated methods, the highest disease incidence (20-40%) was showed in treatments packed in the control PP. Disease incidence was low in non-treated and treated limes with the fungus and chitosan then both packed in PPPCH (0 and 8%, respectively) and in fruits inoculated with the fungus and packed in Org/PP (4%) (Fig. 17, 18 and 19).

2.4 Respiration rate

The respiration rate of lime fruit steadily increased during storage. Limes inoculated with fungus and treated with chitosan then packed in PPPCH and PP had the highest CO_2 production rate in the first and second week, respectively. While non-

inoculated and inoculated limes with fungus and packed in Org/PP showed the lowest respiration rate in the first and second week (Fig. 20). Only limes inoculated with fungus and treated with chitosan and packed in Org/PP had CO_2 production rate lower than others in the first week when compared within group of packaging (Fig.21-23).

2.5 Ascorbic acid

The amount of ascorbic acid content of treated limes varied during storage. However, ascorbic acid content had a tendency to increase during the first and second week of storage then decreased afterward. In the final week, non-treated lime packed in Org/PP had the highest ascorbic acid content which was significant difference from other treatments (Fig. 24). It did not show any difference in set of packaging (Fig. 25-27).

2.6 Total Soluble Solids

The total soluble solid content was not significant difference in all treatments until the final week of storage. Only limes inoculated with fungus and treated with chitosan then packed in PPPCH significantly had different TSS from the control PP (Fig. 28).

2.7 Total phenolic content

Total phenolic content did not show any significant difference between treatments until the third week of storage. Limes inoculated with fungus and packed in Org/PP and limes treated with chitosan and packed in PPPCH had higher total phenolic content than other treatments (Fig. 29-32).

2.8 Antioxidant enzymes

2.8.1 Catalase activity

CAT activity gradually decreased in all treatments during storage, and fruits that were inoculated with fungus and packed in Org/PP showed slightly higher activity than the other treatments. The significant difference was shown on the last week of storage (Fig. 33). CAT activity had a tendency to decrease in all

treatments but did not show any difference in the control PP or modified PP which similar to limes inoculated with fungus or treated with chitosan then packed in different PP (Fig.34-36).

2.8.2 Ascorbate peroxidase activity

APX activity in all fruits decreased during storage (Fig. 37). APX showed different activity in the second week of storage. Lime packed in modified PP had higher APX activity than control PP (Fig. 38). Limes inoculated with fungus and pack in PPPCH had different APX activity from the others in the second and third week of storage (Fig. 39). The result did not show any difference in limes treated with chitosan and packed in different PP (Fig. 40).

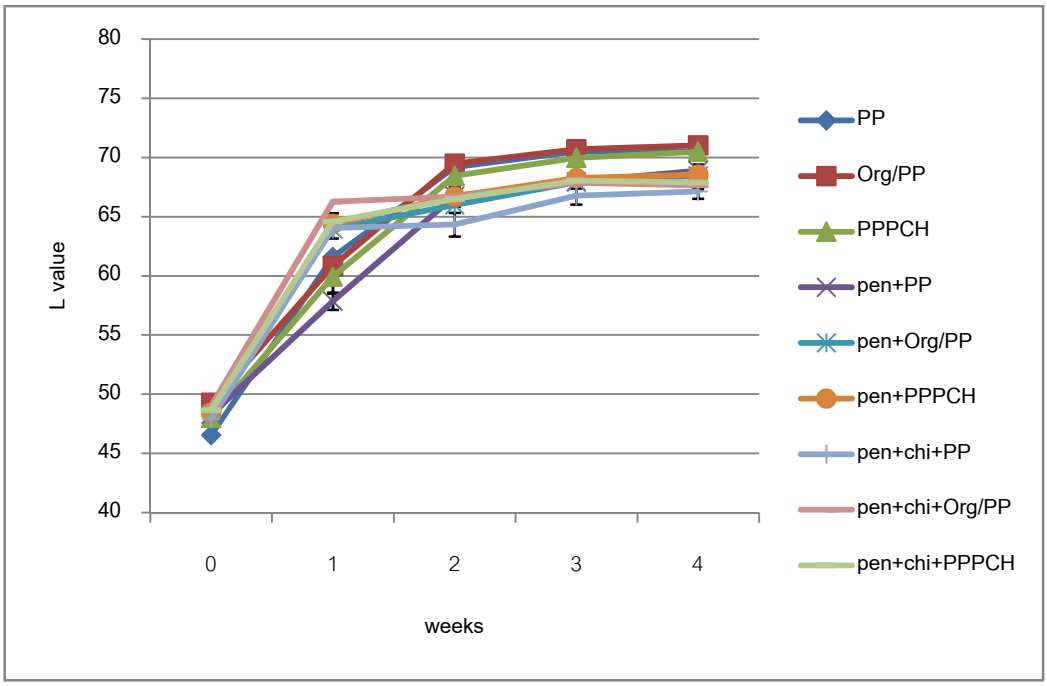


Figure 10. L value of limes in different treatments during storage at 25ºC for 4 weeks.

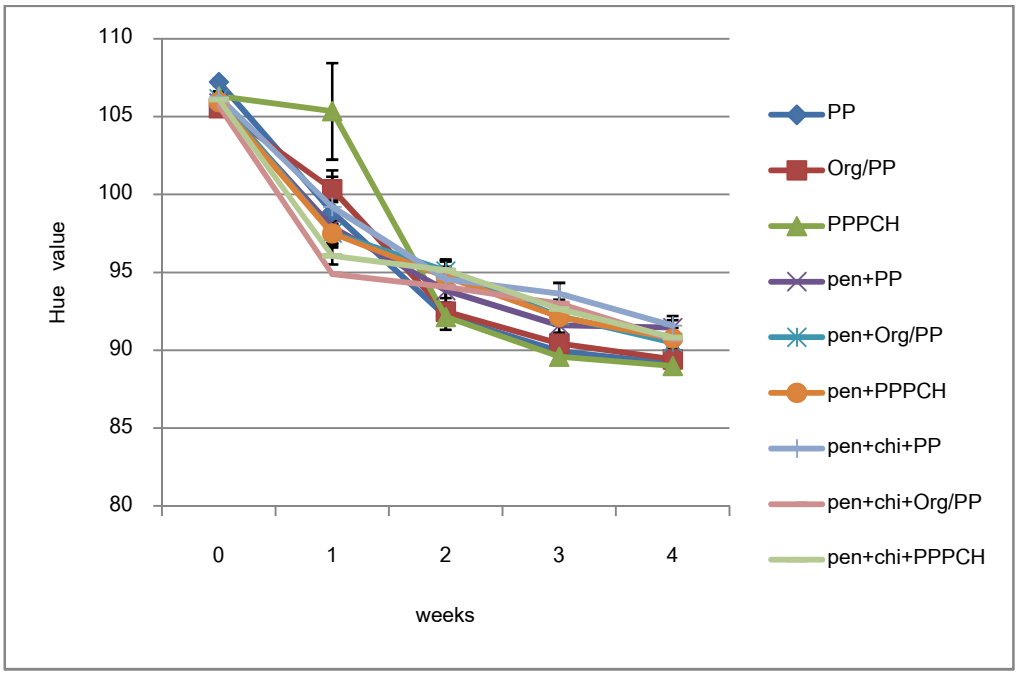


Figure 11. Hue value of limes in different treatments during storage at 25ºC for 4 weeks.

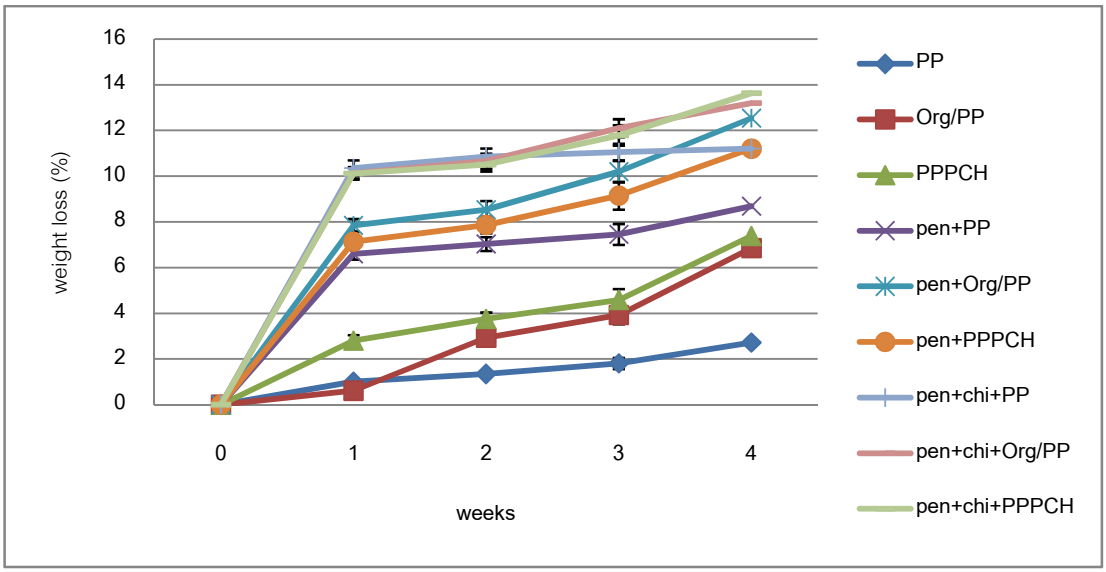


Figure 12. Percentage of weight loss of limes in different treatments during storage at 25ºC for 4 weeks.

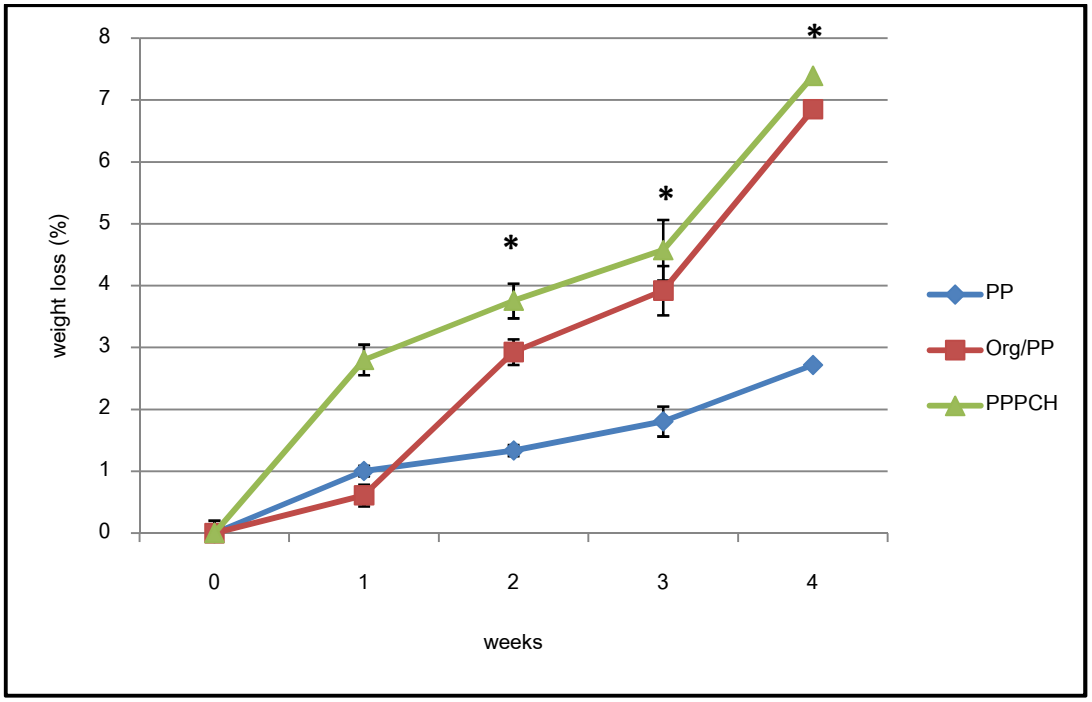


Figure 13. Percentage of weight loss of limes in control treatments packed in modified polypropylene films during storage at 25ºC for 4 weeks.

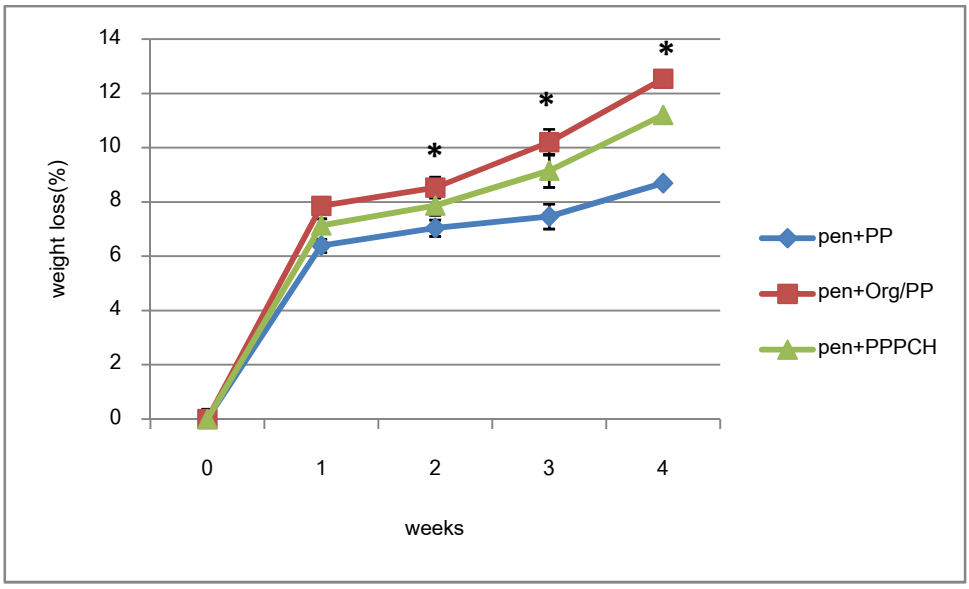


Figure 14. Percentage of weight loss of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25°C for 4 weeks.

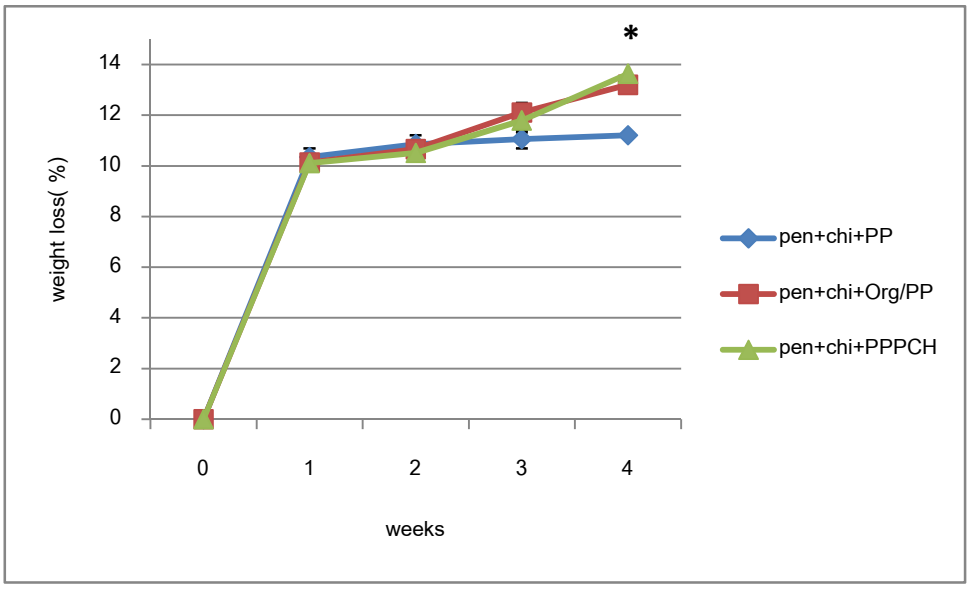


Figure 15. Percentage of weight loss of limes after treated with chitosan and packed in modified polypropylene films during storage at 25°C for 4 weeks.

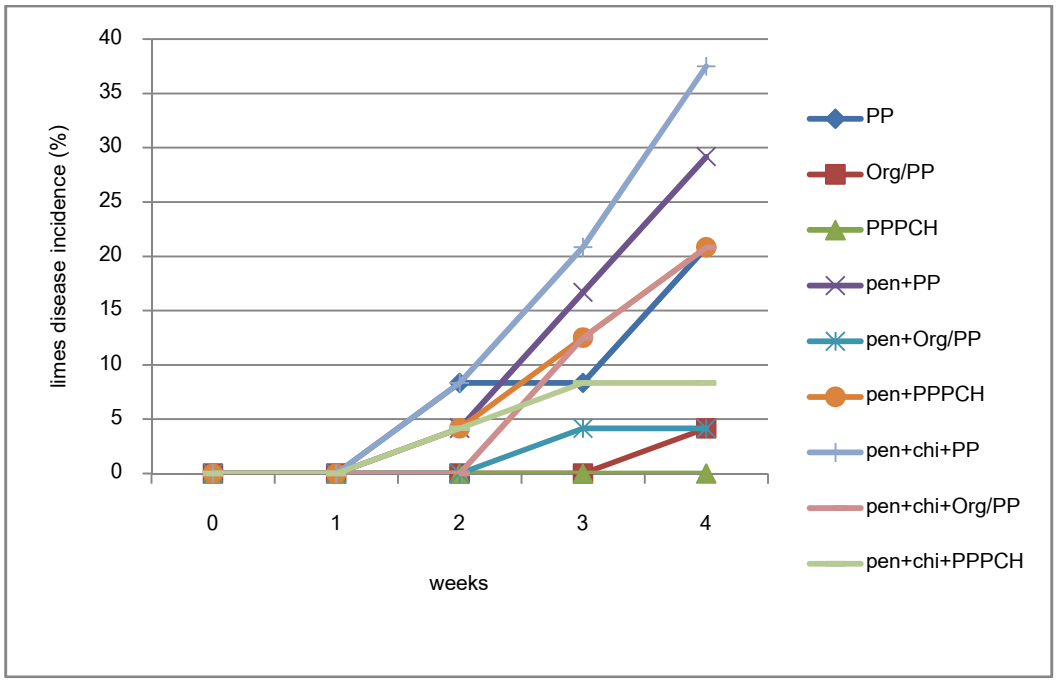


Figure 16. Percentage of lime disease in different treatments during storage at 25ºC for 4 weeks.

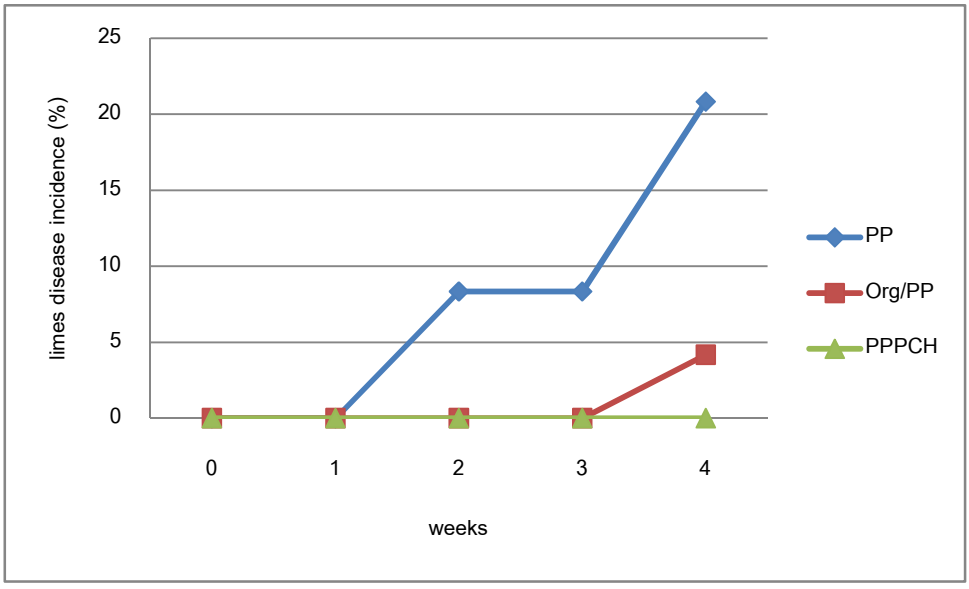


Figure 17. Percentage of lime disease in control treatments packed in modified polypropylene films during storage at 25ºC for 4 weeks.

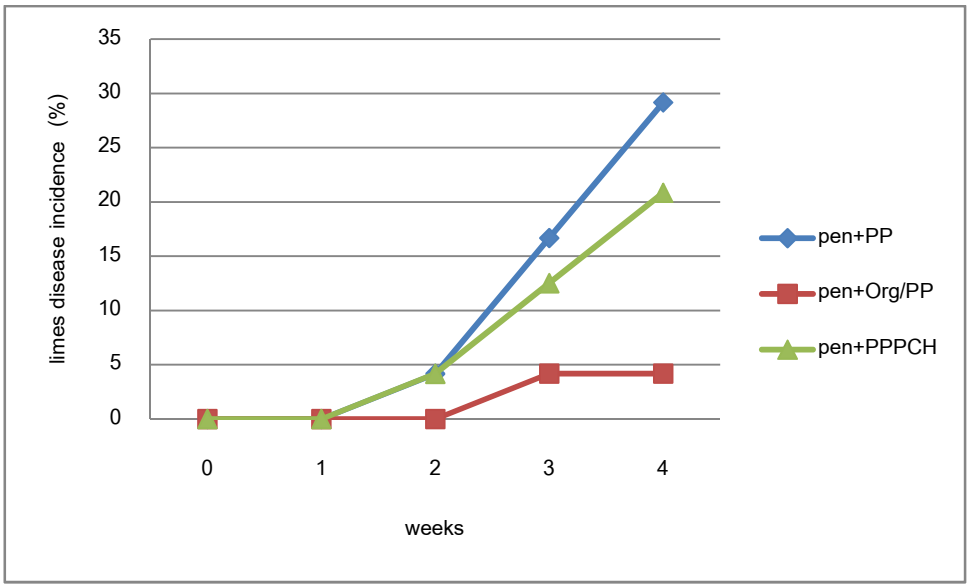


Figure 18. Percentage of lime disease after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

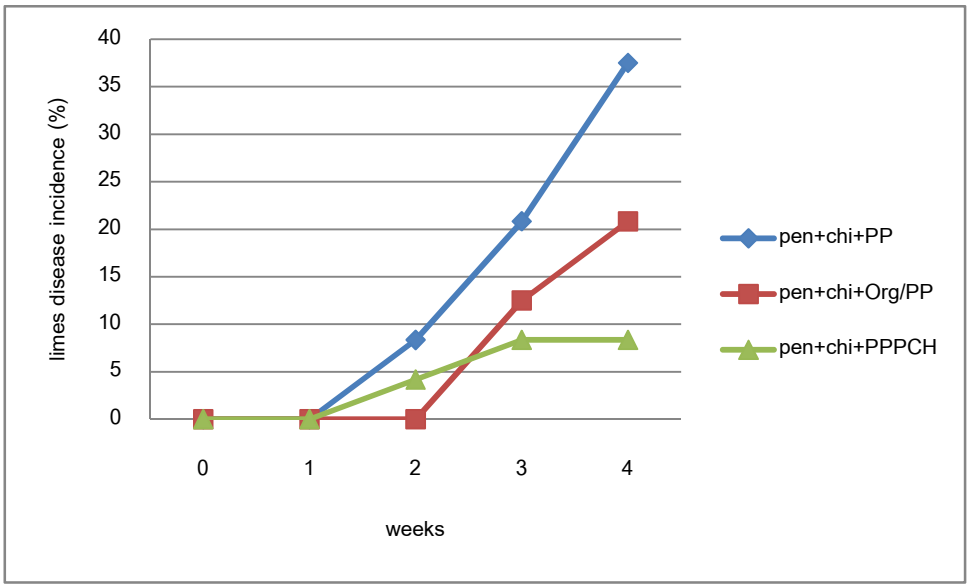


Figure 19. Percentage of lime disease after treated with chitosan and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

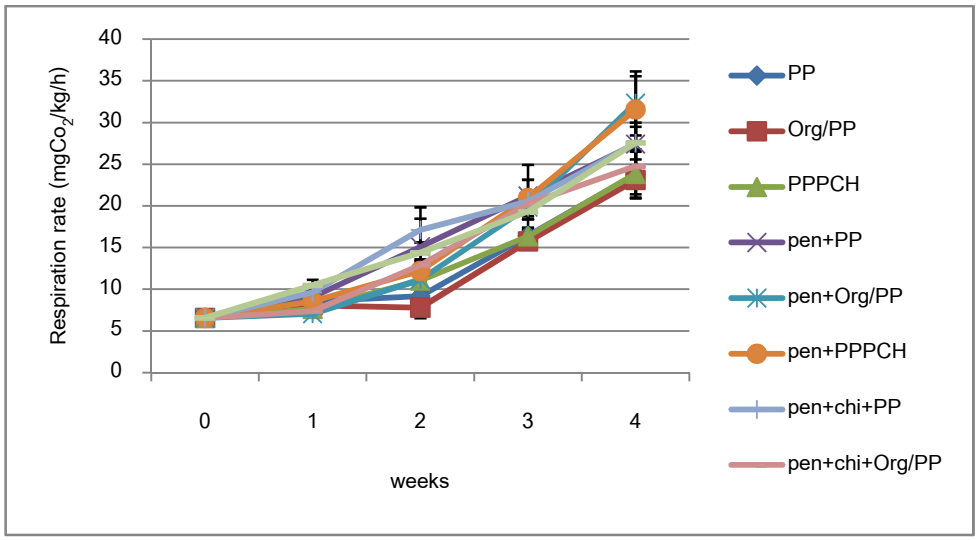


Figure 20. Respiration rate ($mgCo_2$/kg/h) of limes in different treatments during storage at 25°C for 4 weeks.

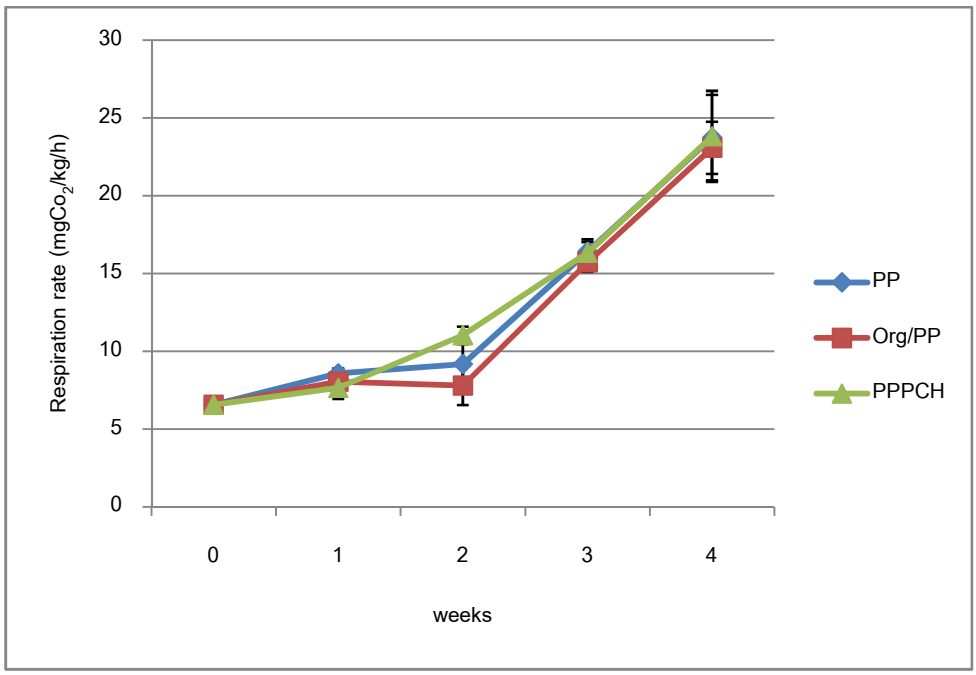


Figure 21. Respiration rate ($mgCo_2$/kg/h) of limes in control treatments packed in modified polypropylene films during storage at 25°C for 4 weeks.

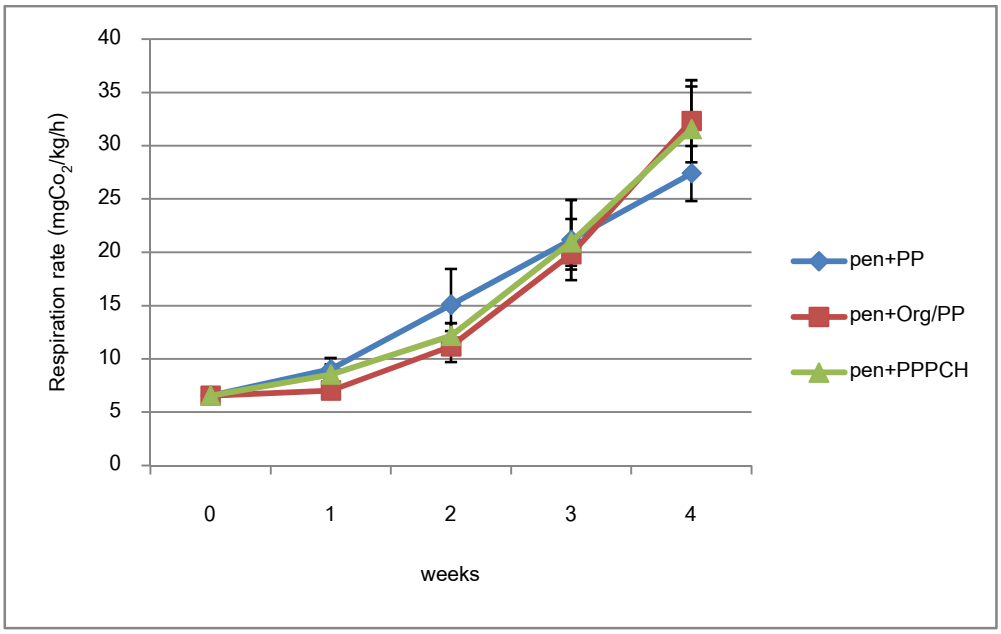


Figure 22. Respiration rate ($mgCo_2$/kg/h) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25°C for 4 weeks.

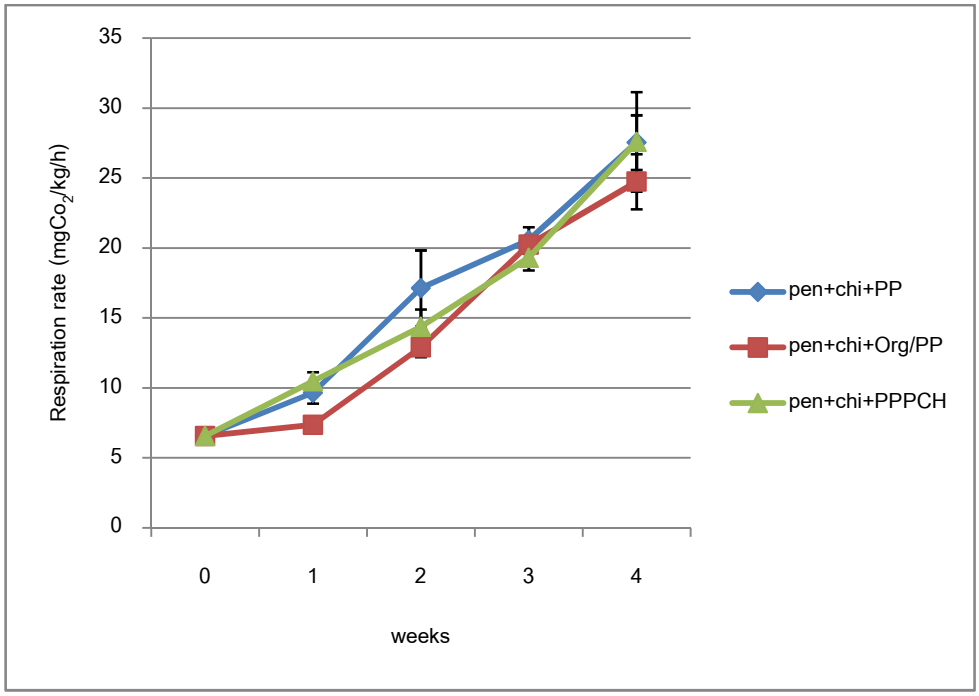


Figure 23. Respiration rate ($mgCo_2$/kg/h) of limes after treated with chitosan and packed in modified polypropylene films during storage at 25°C for 4 weeks.

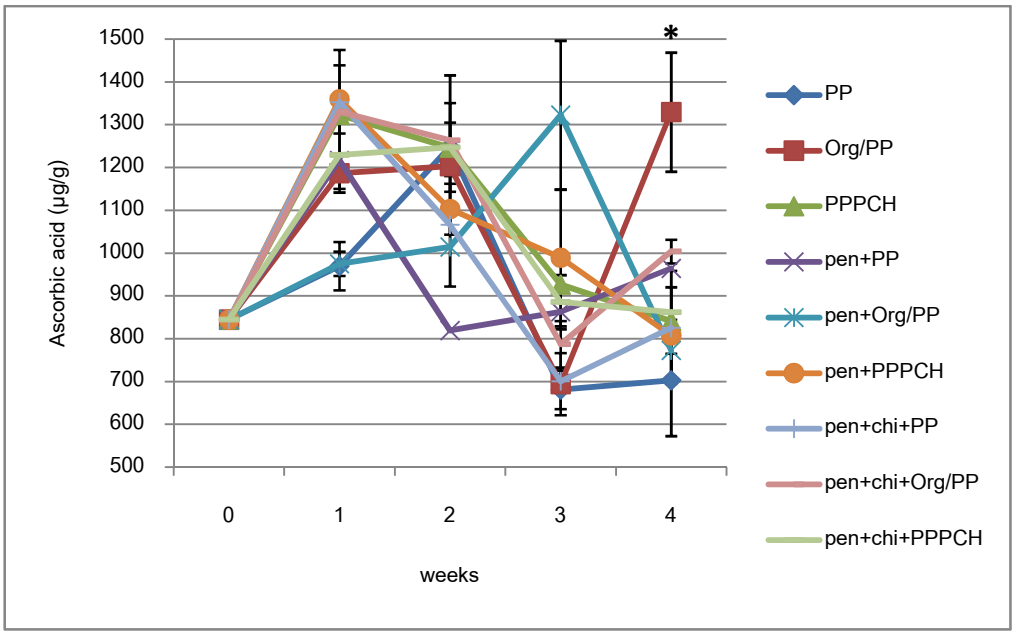


Figure 24. Ascorbic acid concentration (µg/g FW) of limes in different treatments during storage at 25°C for 4 weeks.

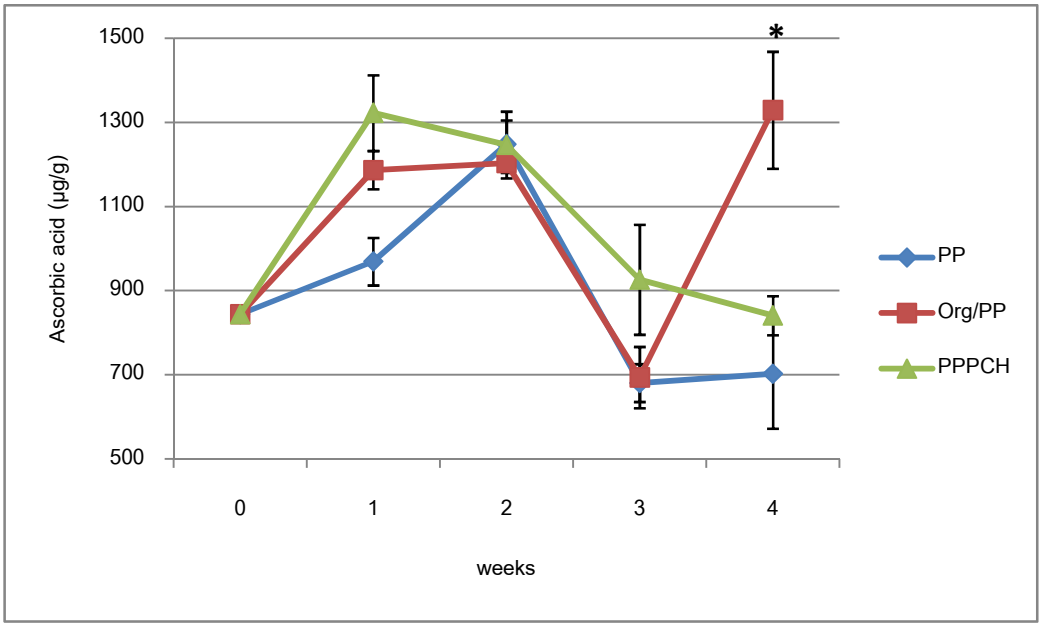


Figure 25. Ascorbic acid concentration (µg/g FW) of limes in control treatments packed in modified polypropylene films during storage at 25°C for 4 weeks

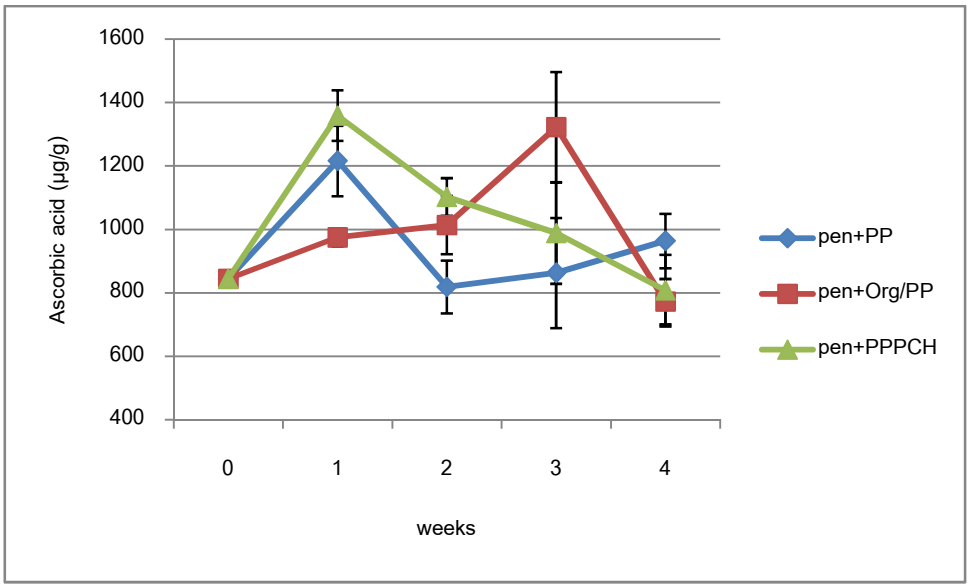


Figure 26. Ascorbic acid concentration (µg/g FW) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25°C for 4 weeks.

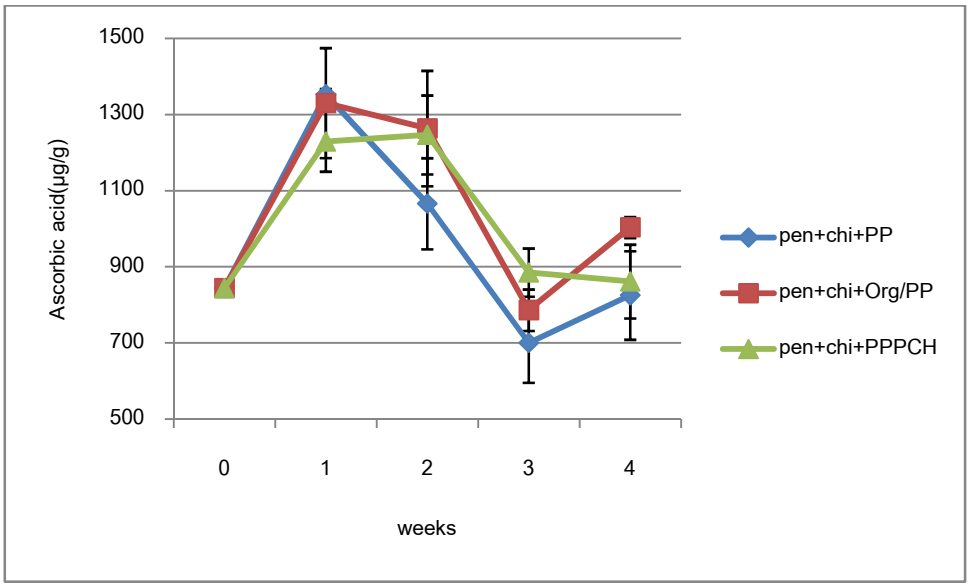


Figure 27. Ascorbic acid concentration (µg/g FW) of limes after treated with chitosan and packed in modified polypropylene films during storage at 25°C for 4 weeks.

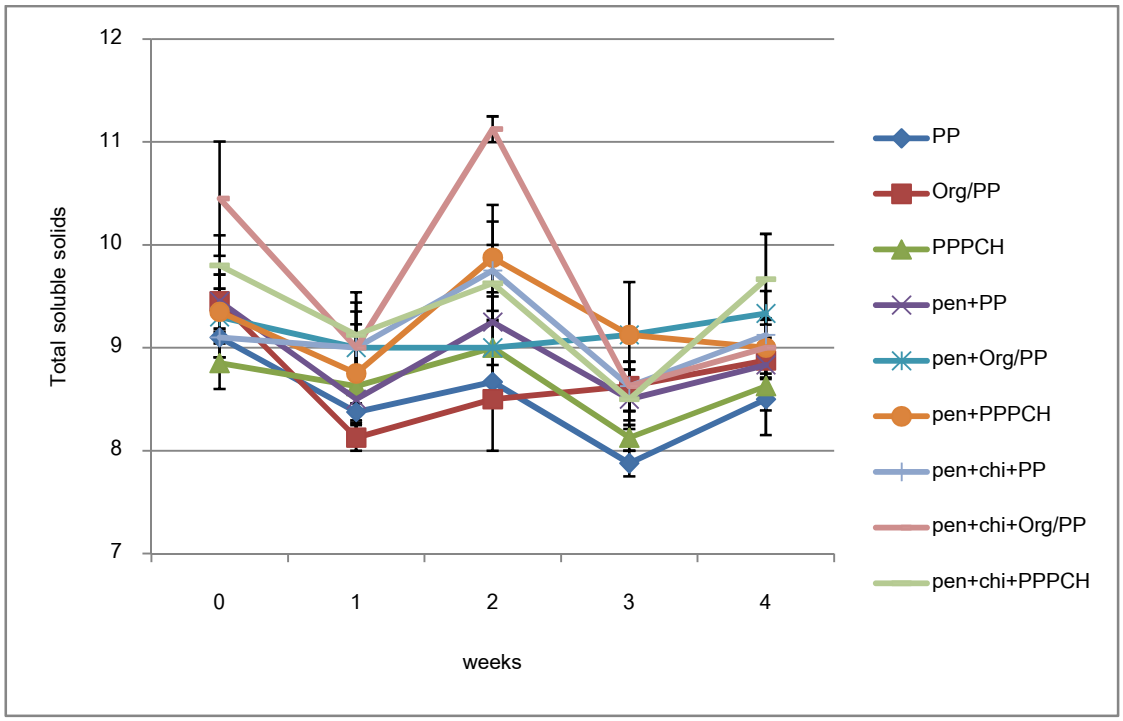


Figure 28.Total soluble solids (TSS) of limes in different treatments during storage at 25ºC for 4 weeks.

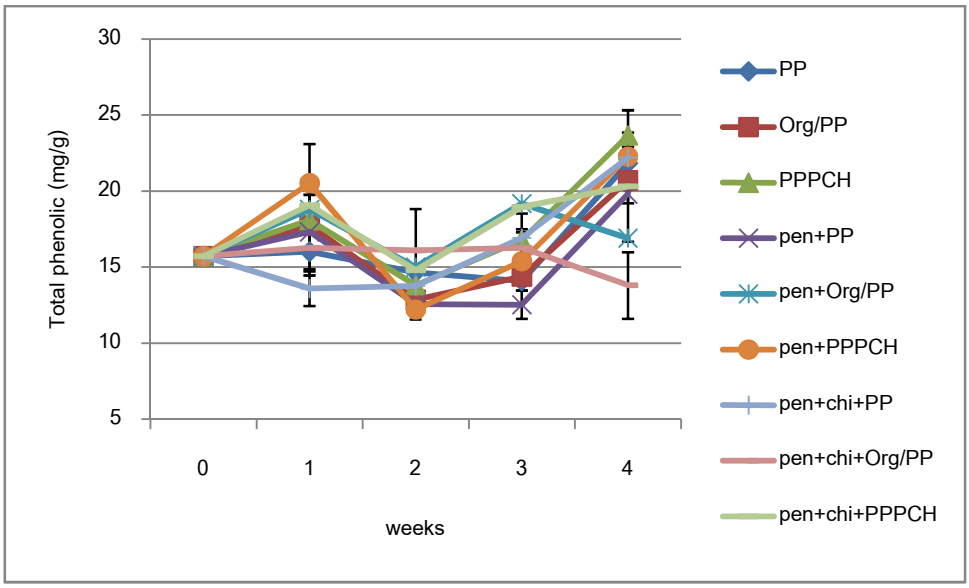


Figure 29.Total phenolic concentration (mg/g FW) of limes in different treatments during storage at 25°C for 4 weeks.

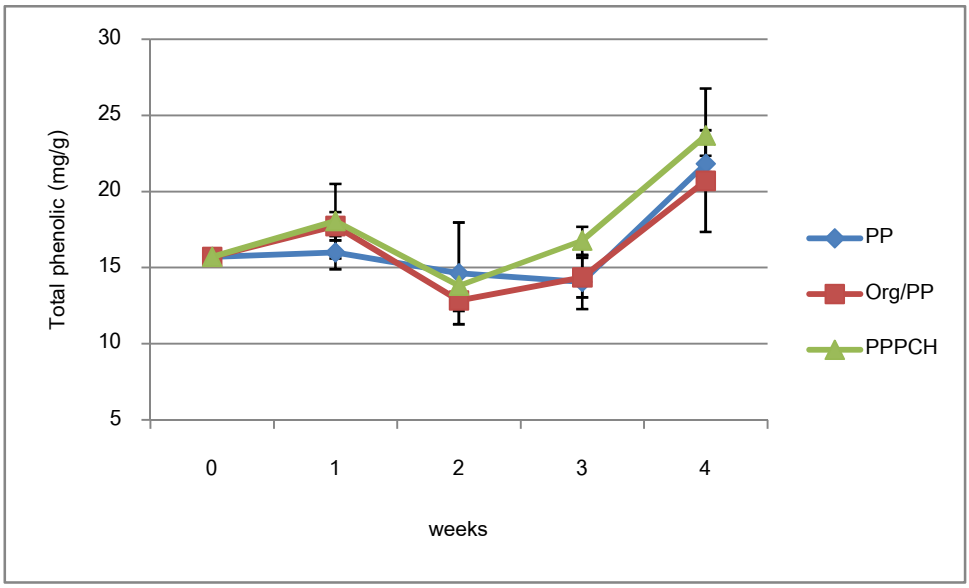


Figure 30. Total phenolic concentration (mg/g FW) of limes in control treatments packed in modified polypropylene films during storage at 25°C for 4 weeks.

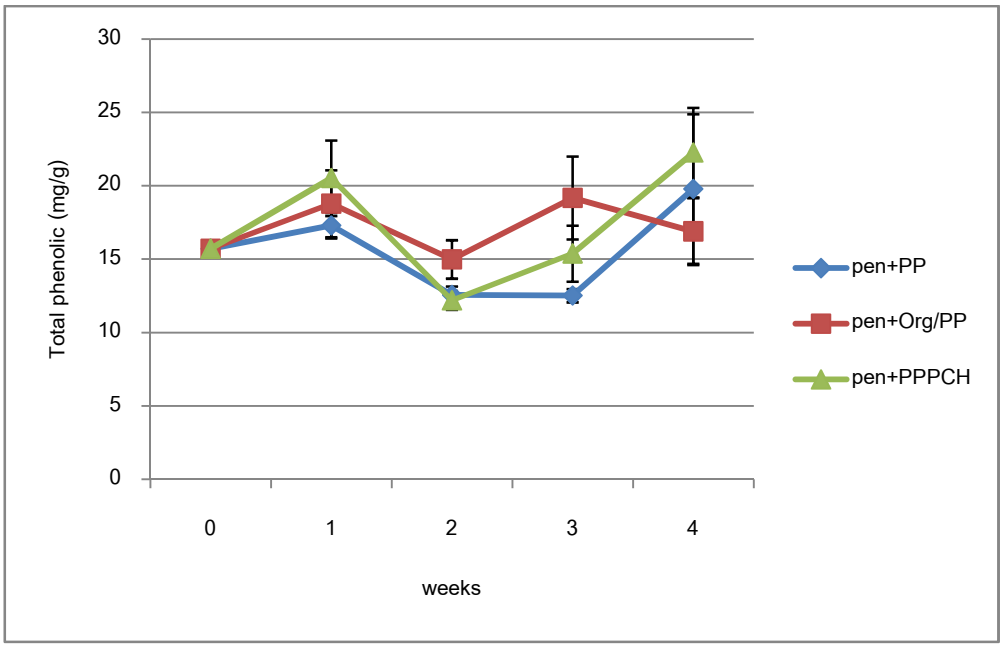


Figure 31. Total phenolic concentration (mg/g FW) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25°C for 4 weeks.

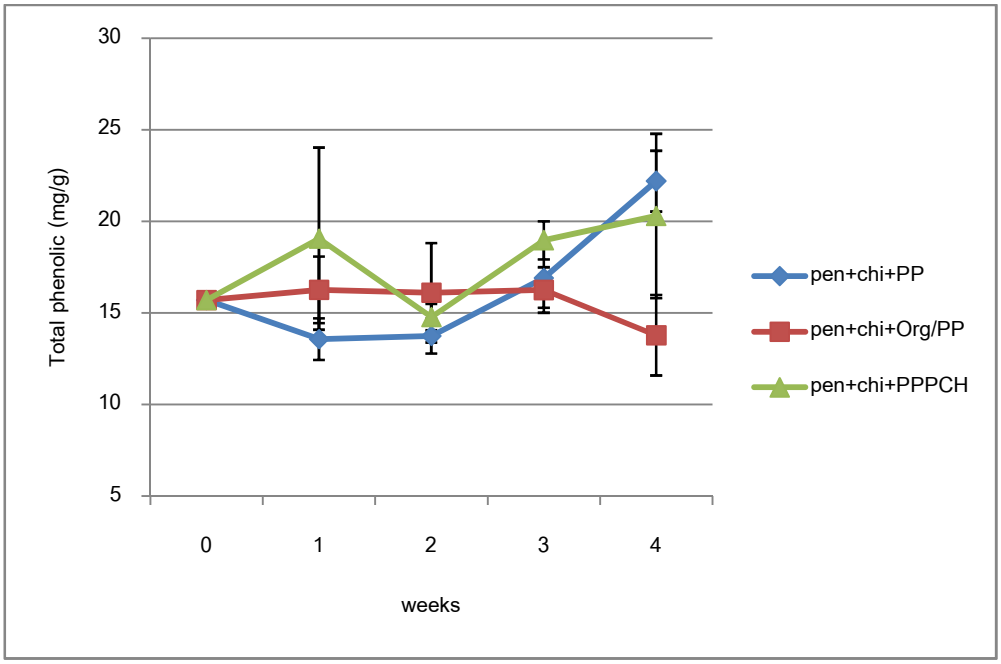


Figure 32. Total phenolic concentration (mg/g FW) of limes after treated with chitosan and packed in modified polypropylene films during storage at 25°C for 4 weeks.

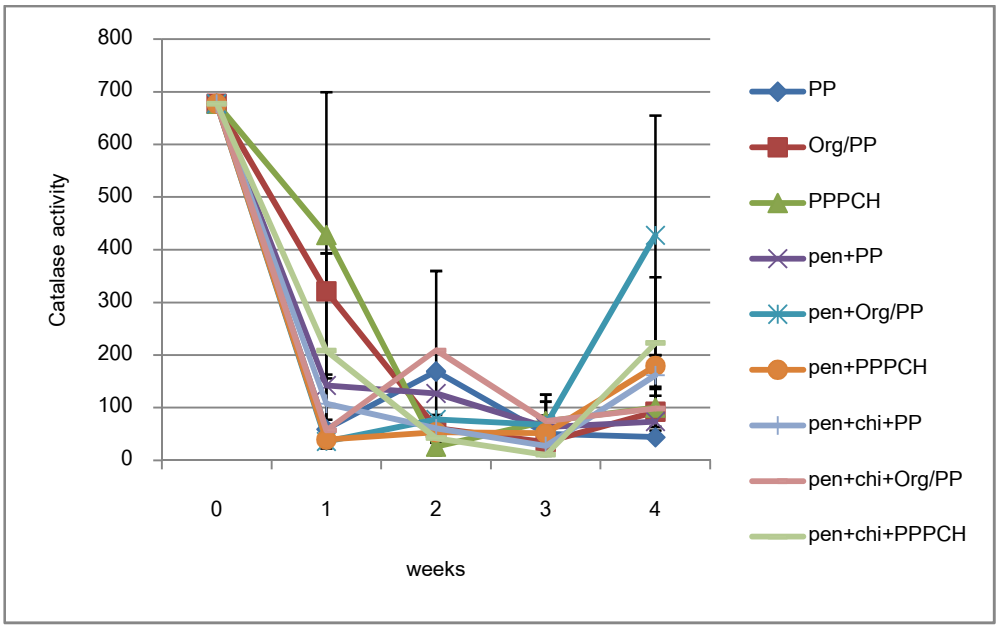


Figure 33. CAT activity of limes in different treatments during storage at 25ºC for 4 weeks.

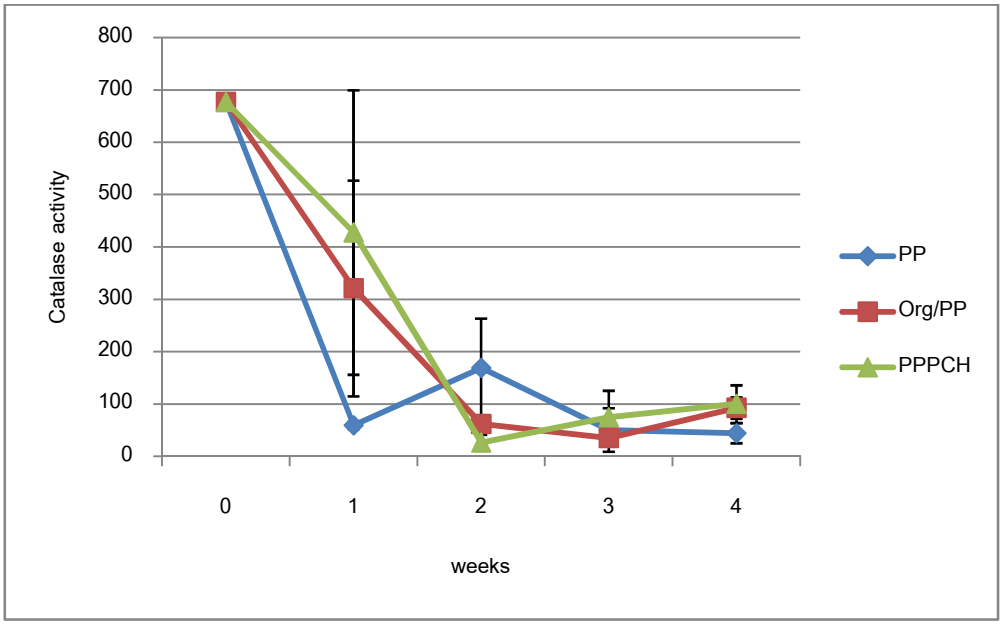


Figure 34. CAT activity of limes in control treatments packed in modified polypropylene films during storage at 25ºC for 4 weeks.

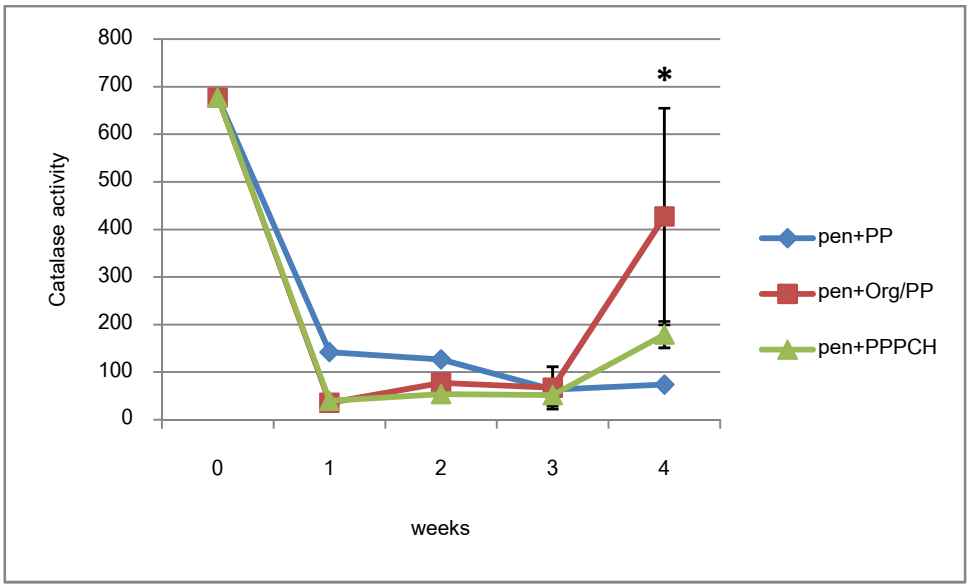


Figure 35. CAT activity of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

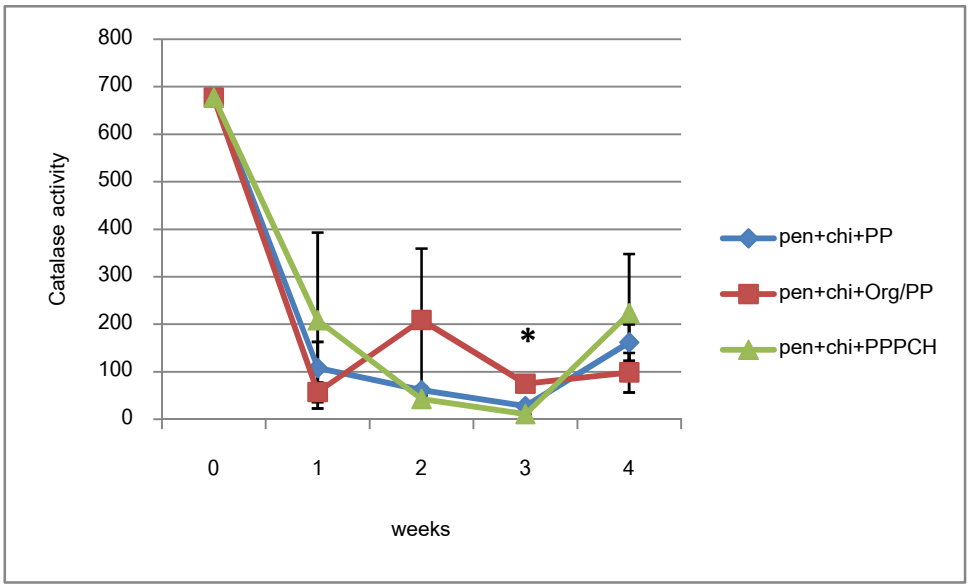


Figure 36. CAT activity of limes after treated with chitosan and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

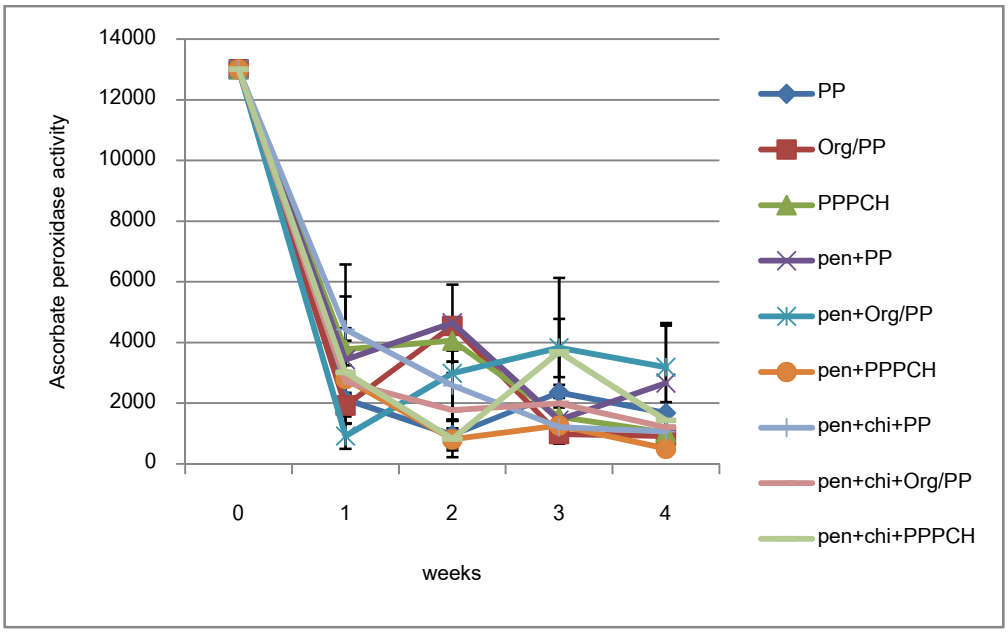


Figure 37. APX activity of limes in different treatments during storage at 25°C for 4 weeks.

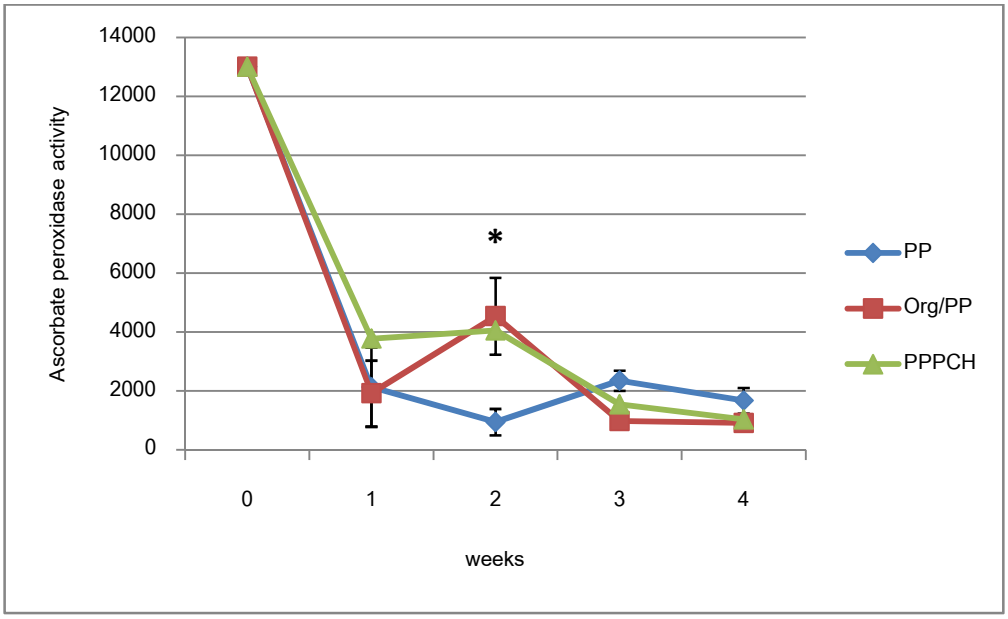


Figure 38. APX activity of limes in control treatments packed in modified polypropylene films during storage at 25°C for 4 weeks.

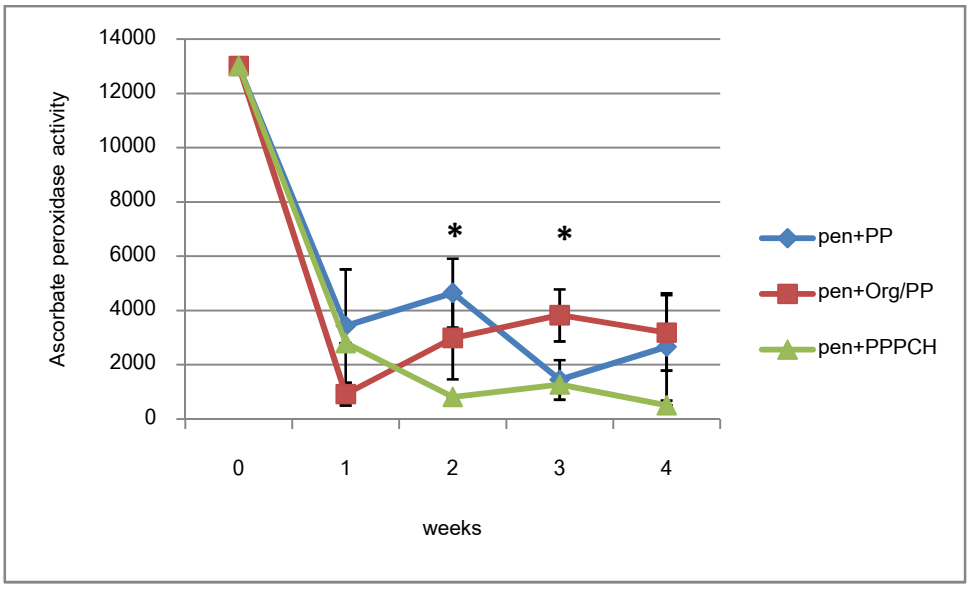


Figure 39. APX activity of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

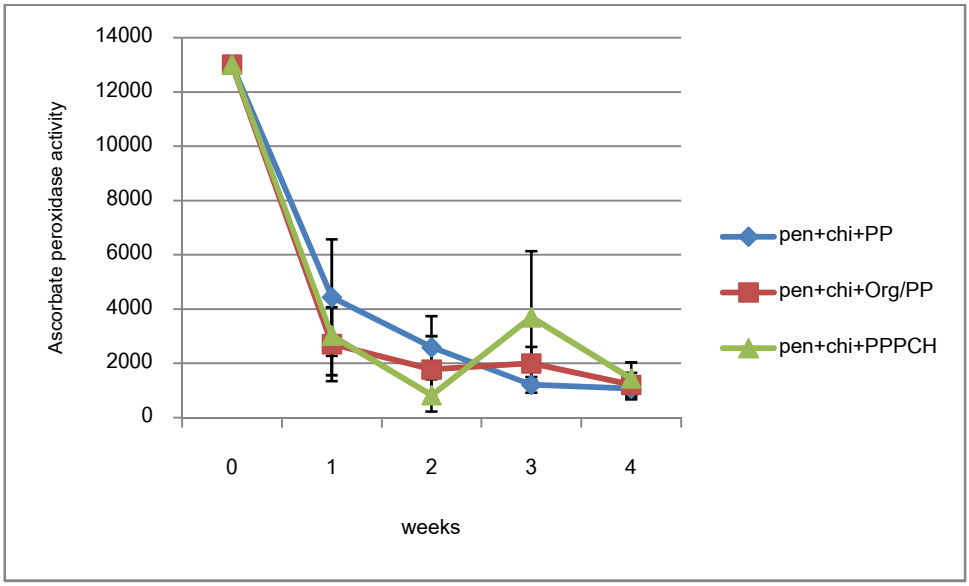


Figure 40. APX activity of limes after treated with chitosan and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

3. **Effect of chitosan and modified polypropylene film packaging on postharvest quality of limes during storage at 10°C for 6 weeks**

3.1 Peel color change

L value of limes increased in all treatments in which the difference was non-significant during storage times. Hue value measured in all treatments showed a tendency to decrease. The results showed no significant difference in term of peel color change in all treatments (Fig.41-42).

3.2 Percentage of weight loss

Percentage of weight loss was higher in fruits packed in modified PP than control PP. With increased in storage interval, weight loss increased significantly (Fig. 43). Limes packed in normal PP had the lowest percentage of weight loss compared with modified PP (Fig. 44). Fruits inoculated with only fungus or chitosan and placed in different packages resulted in higher percentage of weight loss in modified PP than in control PP (Fig. 45-46).

3.3 Disease incidence

There was no disease incidence of lime throughout storage time at 10ºC.

3.4 Respiration rate

Respiration rate of limes decreased during storage in the first and second week then increased afterward in all treatments (Fig. 47). Limes packed in Org/PP had the highest CO_2 respiration rate in the third week of storage when compared within set of packaging and lime inoculated with fungus and packed in Org/PP had the highest CO_2 respiration rate in the fourth week of storage. However, it did not show any difference in chitosan treatments (Fig. 48-50).

3.5 Ascorbic acid

Figure 51 showed amounts of ascorbic acid of limes presented in nine treatments. The trend of ascorbic acid content increased during storage and showed

difference in the second and the third weeks of storage. No significant difference in ascorbic acid content was observed in modified PP or normal PP treatments (Fig. 52). Limes inoculated with fungus and packed in Org/PP resulted in higher ascorbic acid content than other treatments in the second week (Fig. 53). Limes inoculated with fungus and treated with chitosan then packed in PP showed more ascorbic acid content than the others in the fourth week (Fig. 54).

3.6 Total Soluble Solids

Difference in total soluble solids was shown in the third and fourth week of storage. Limes inoculated with fungus and packed in Org/PP had the highest TSS from the others in the third week of storage. Limes inoculated with fungus and packed in PP had the lowest TSS and showed significant difference from other treatments in the fourth week of storage (Fig. 55).

3.7 Total phenolic content

Total phenolic content decreased during storage in all treatments (Fig. 56). Lime packed in PP had higher total phenolic content than Org/PP or PPPCH, and significant difference could be found in the third week of storage (Fig. 57). Inoculation with fungus of limes in different PP did not show any significant difference between treatments (Fig. 58). Limes inoculated with fungus and treated with chitosan then packed in PPPCH resulted in the highest total phenolic content in the first week (Fig. 59).

3.8 Antioxidant enzymes

3.8.1 Catalase activity

During storage, CAT activity decreased in all treatments. In addition, some treatments increased during storage (Fig. 60), while there was no significant difference between PP and modified PP packagings (Fig. 61). Limes inoculated with fungus and packed in PP were significantly decreased in CAT activity in the fourth week (Fig. 62). Furthermore, CAT activity in chitosan treated

fruits and packed in PP was significant difference from modified PP in the second week (Fig. 63).

3.8.2 Ascorbate peroxidase activity

APX activity gradually decreased during storage, while a significant increase was detected in lime inoculated with fungus and treated with chitosan then packed PPPCH in the third week (Fig. 64). APX activity in PPPCH was higher than other treatments in the fifth week (Fig. 65). The significant difference of APX activity was found in the fourth and fifth weeks in limes inoculated with fungus and packed in PP and PPPCH (Fig. 66). It did not show any significant difference in lime treated with chitosan (Fig. 67).

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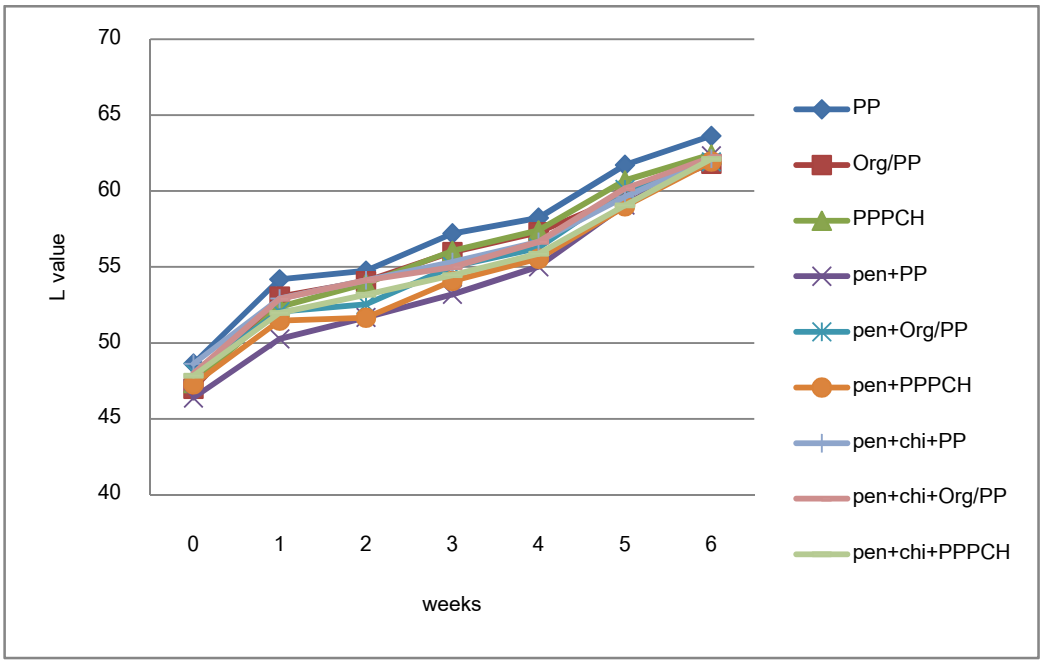

Figure 41. L value of limes in different treatments during storage at 10ºC for 6 weeks.

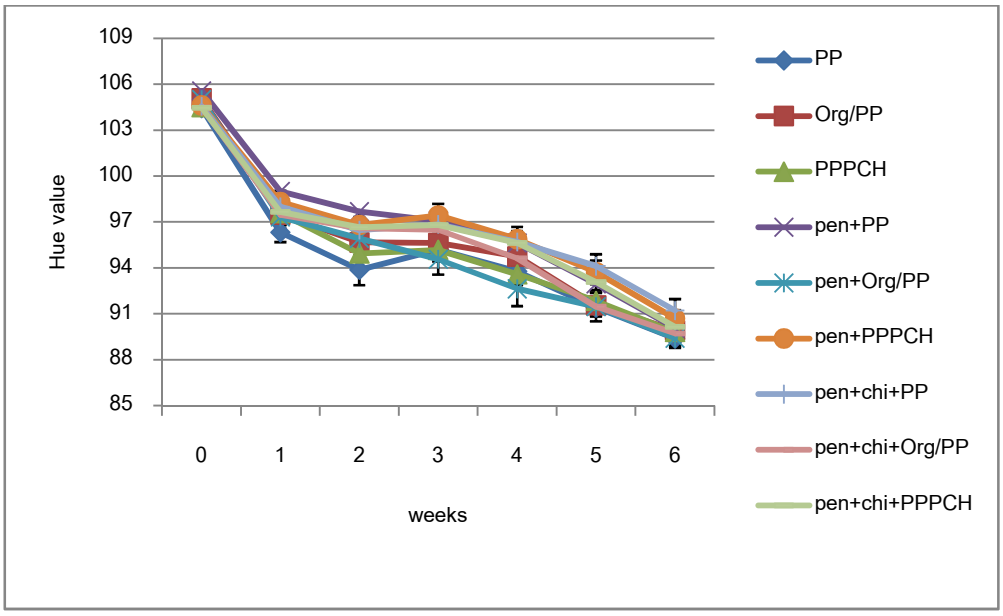

Figure 42. Hue value of limes in different treatments during storage at 10ºC for 6 weeks.

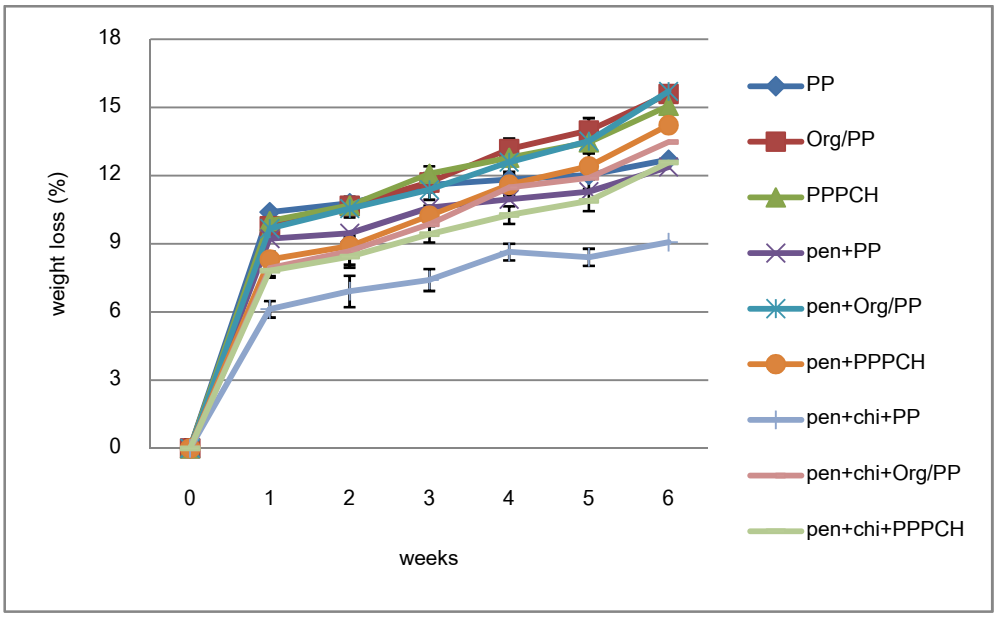

Figure 43. Percentage of weight loss of limes in different treatments during storage at 10°C for 6 weeks.

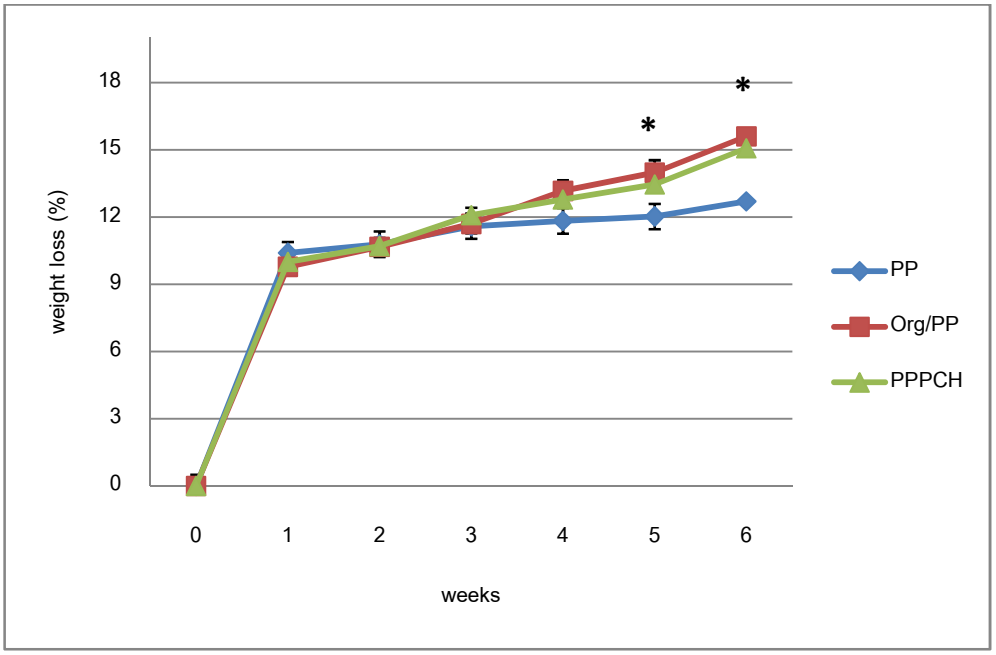

Figure 44. Percentage of weight loss of limes in control treatments packed in modified polypropylene films during storage at 10°C for 6 weeks.

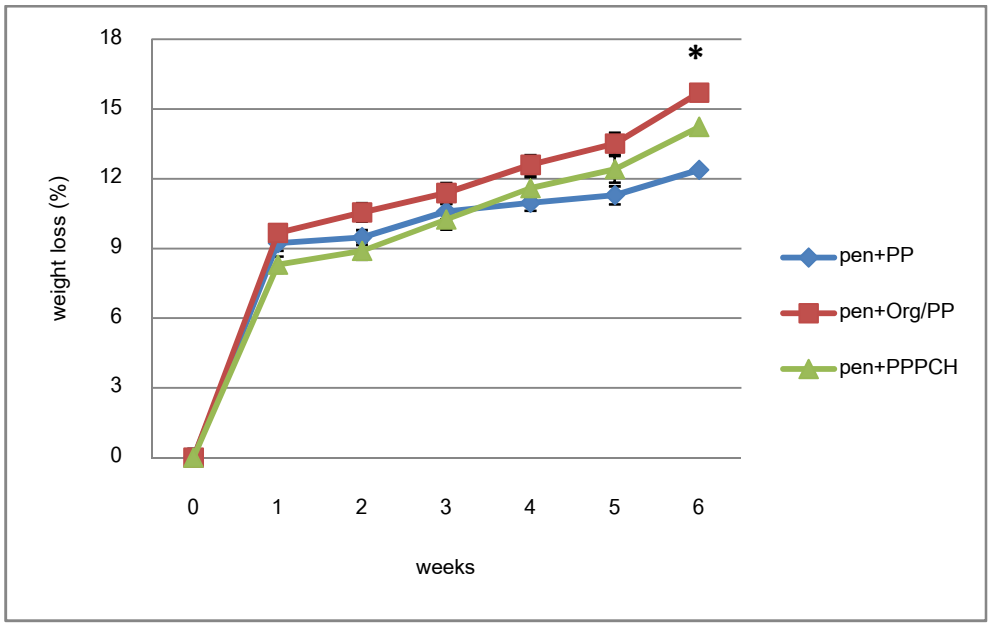

Figure 45. Percentage of weight loss of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 10°C for 6 weeks.

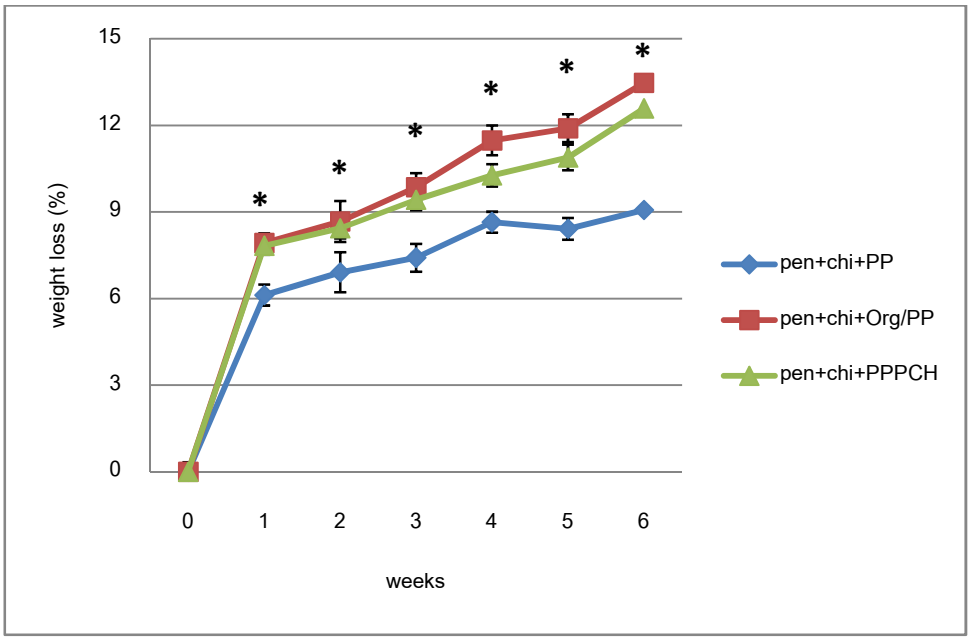

Figure 46. Percentage of weight loss of limes after treated with chitosan and packed in modified polypropylene films during storage at 10°C for 6 weeks.

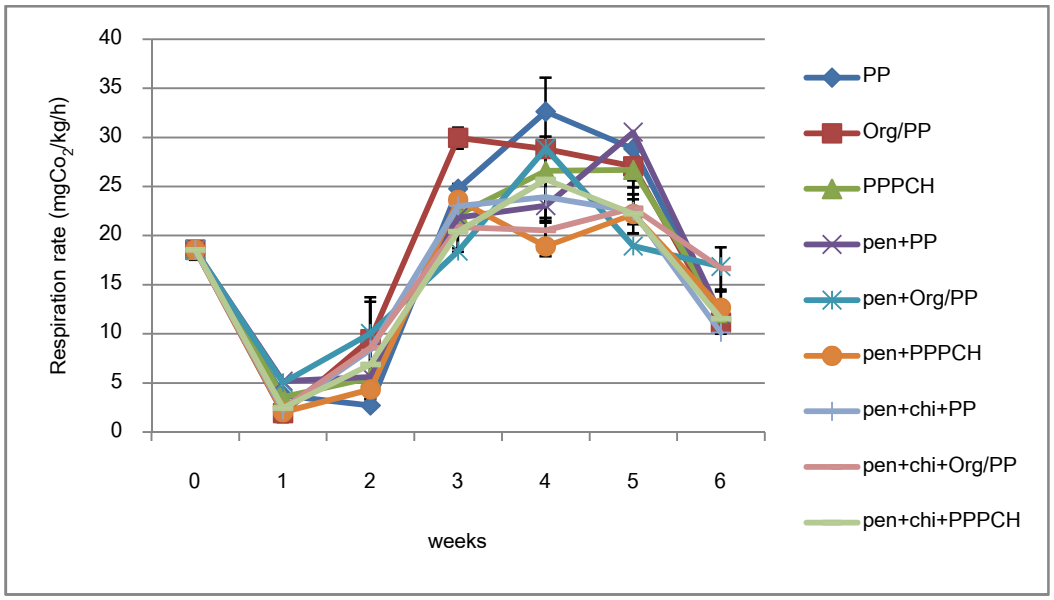

Figure 47. Respiration rate ($mgCo_2$/kg/h) of limes in different treatments during storage at 10ºC for 6 weeks.

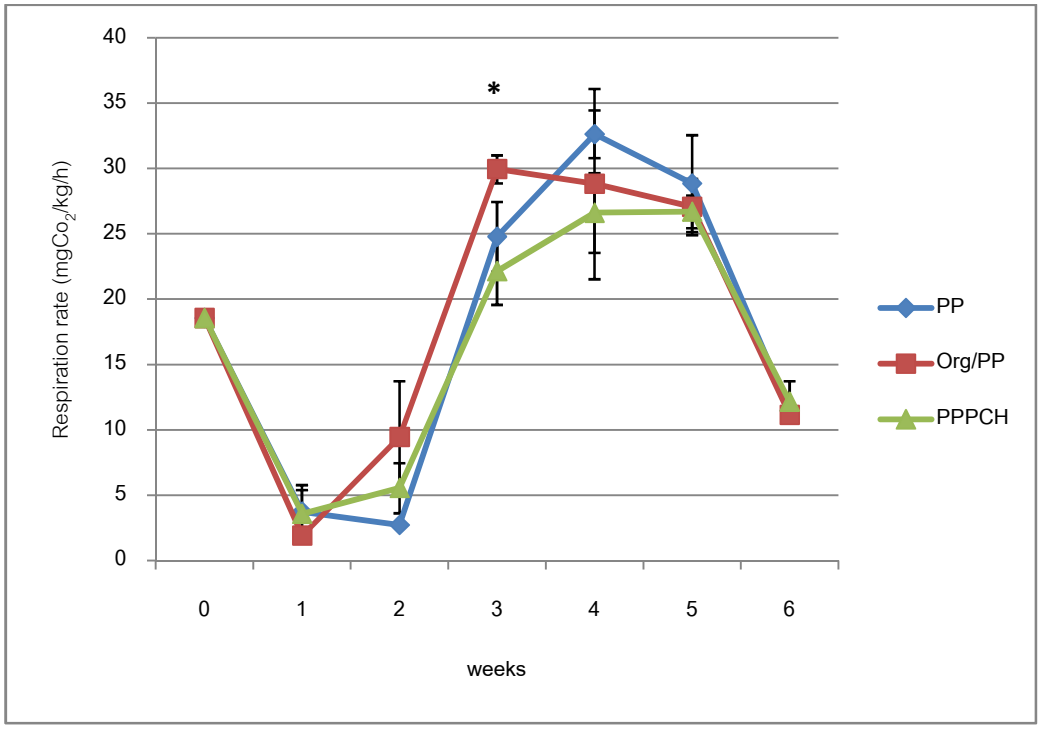

Figure 48. Respiration rate ($mgCo_2$/kg/h) of limes in control treatments packed in modified polypropylene films during storage at 10ºC for 6 weeks.

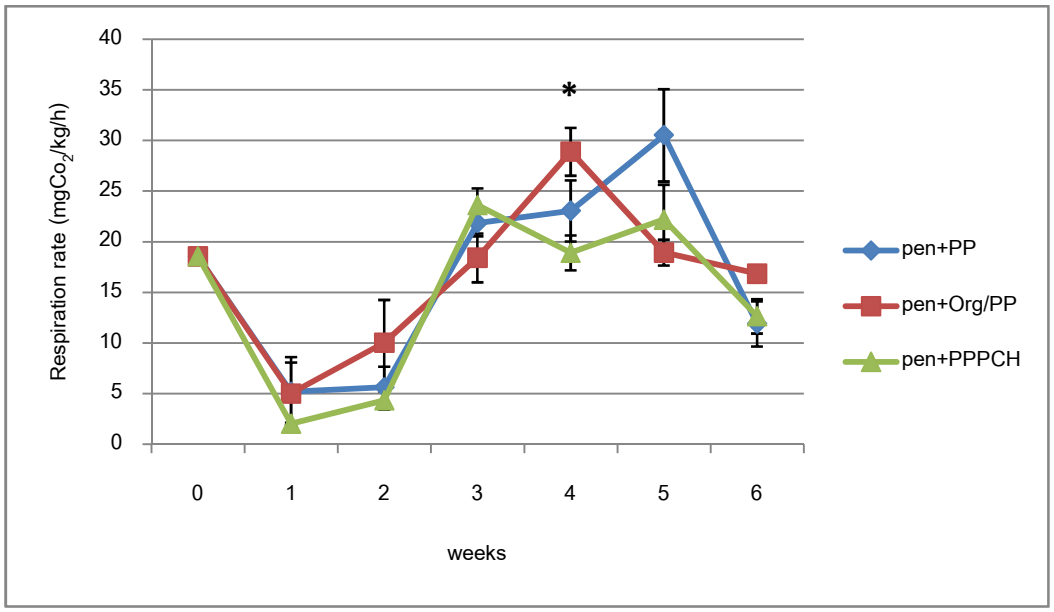

Figure 49. Respiration rate (mg.Co_2/kg./h) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 10°C for 6 weeks.

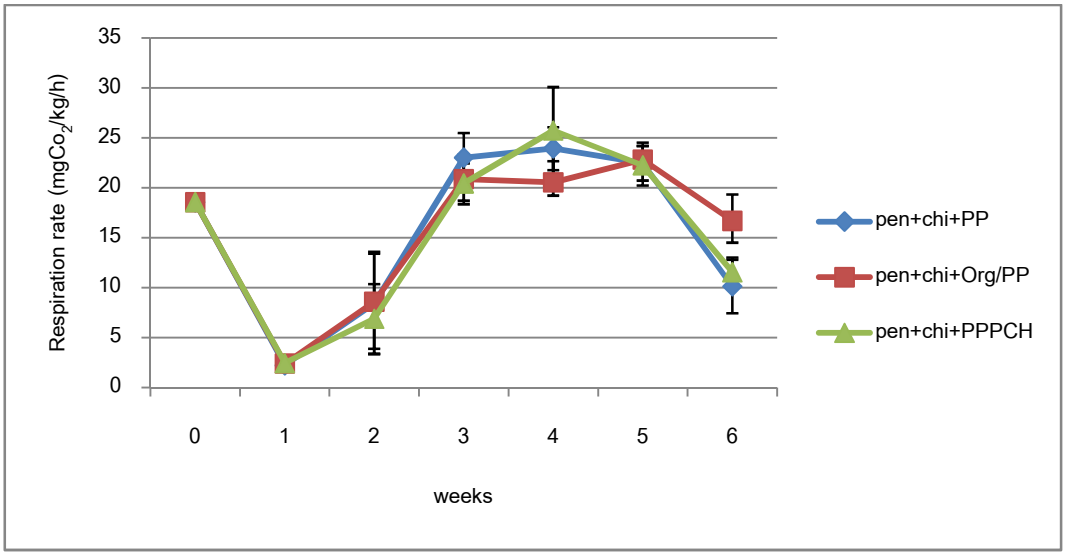

Figure 50. Respiration rate (mgCo_2/kg/h) of limes after treated with chitosan and packed in modified polypropylene films during storage at 10°C for 6 weeks.

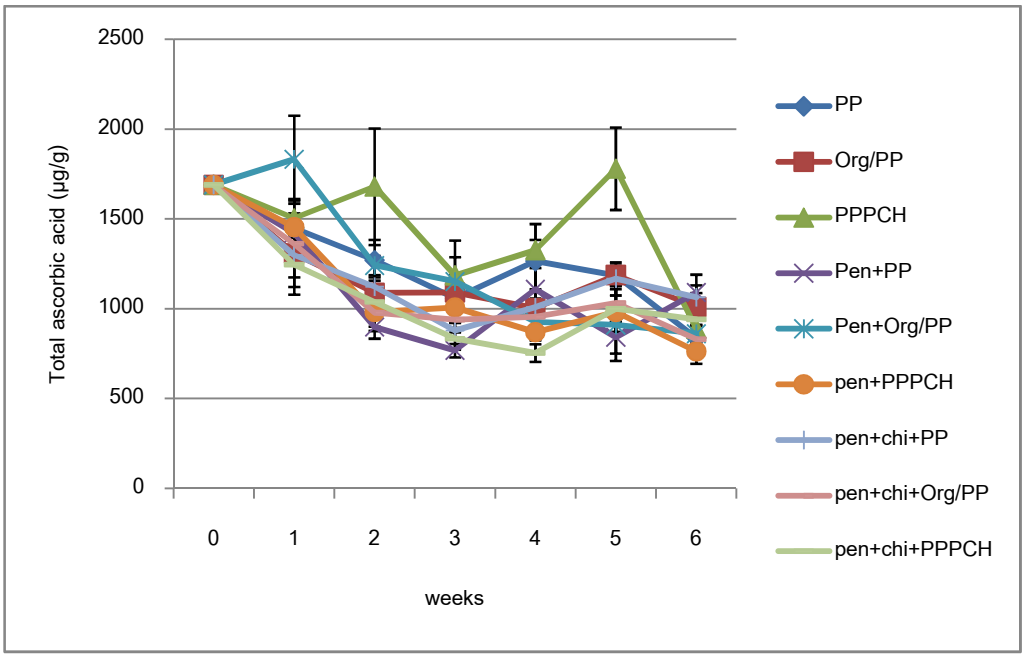

Figure 51. Ascorbic acid concentration (µg/g FW) of limes in different treatments during storage at 10°C for 6 weeks.

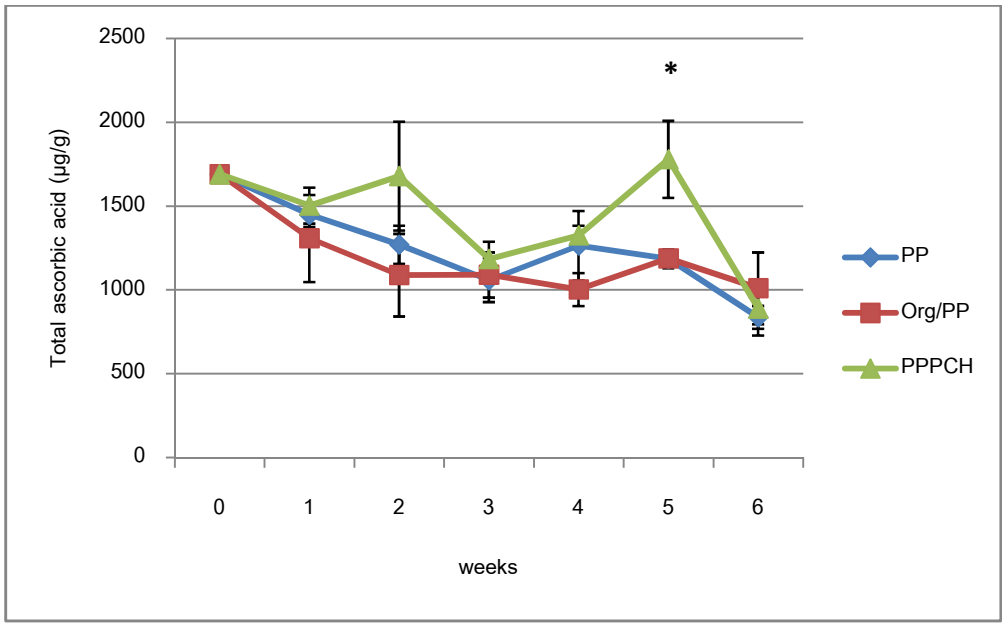

Figure 52. Ascorbic acid concentration (µg/g FW) of limes in control treatments packed in modified polypropylene films during storage at 10°C for 6 weeks.

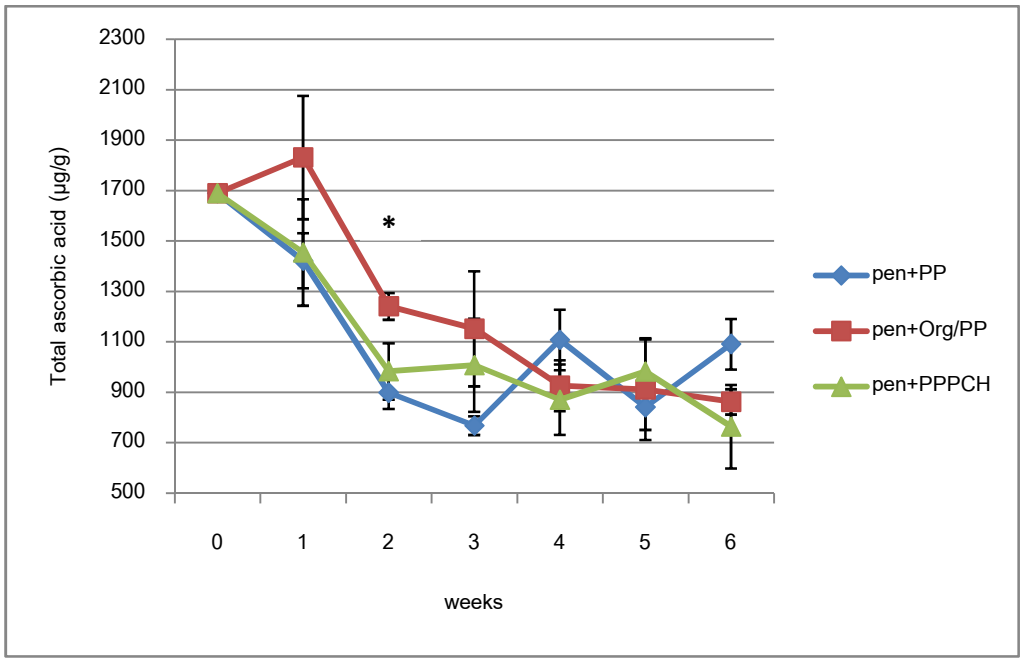

Figure 53. Ascorbic acid concentration (µg/g FW) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 10°C for 6 weeks.

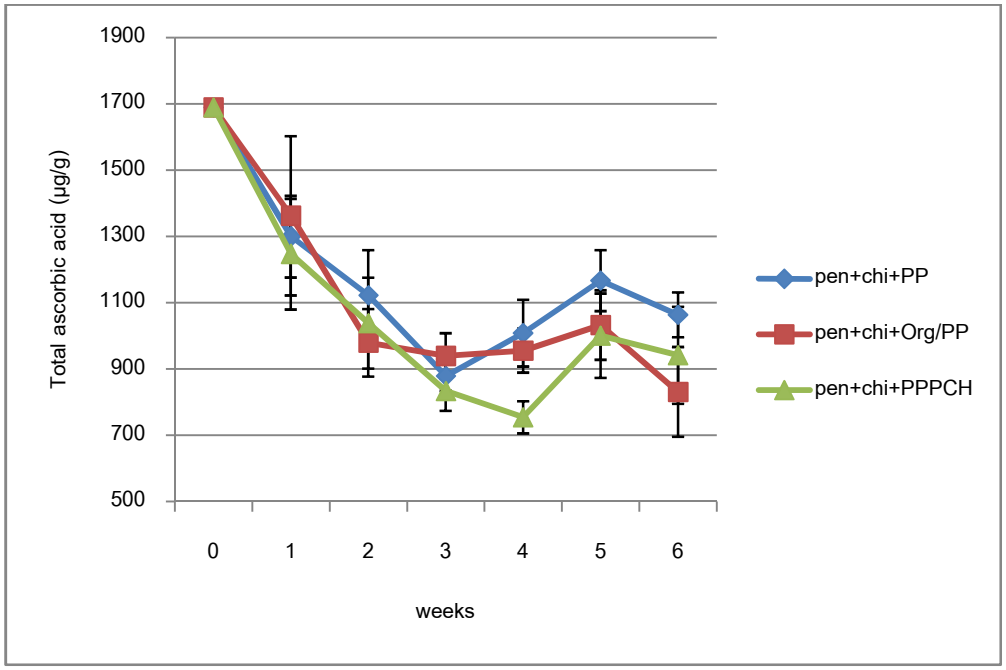

Figure 54. Ascorbic acid concentration (µg/g FW) of limes after treated with chitosan and packed in modified polypropylene films during storage at 10°C for 6 weeks.

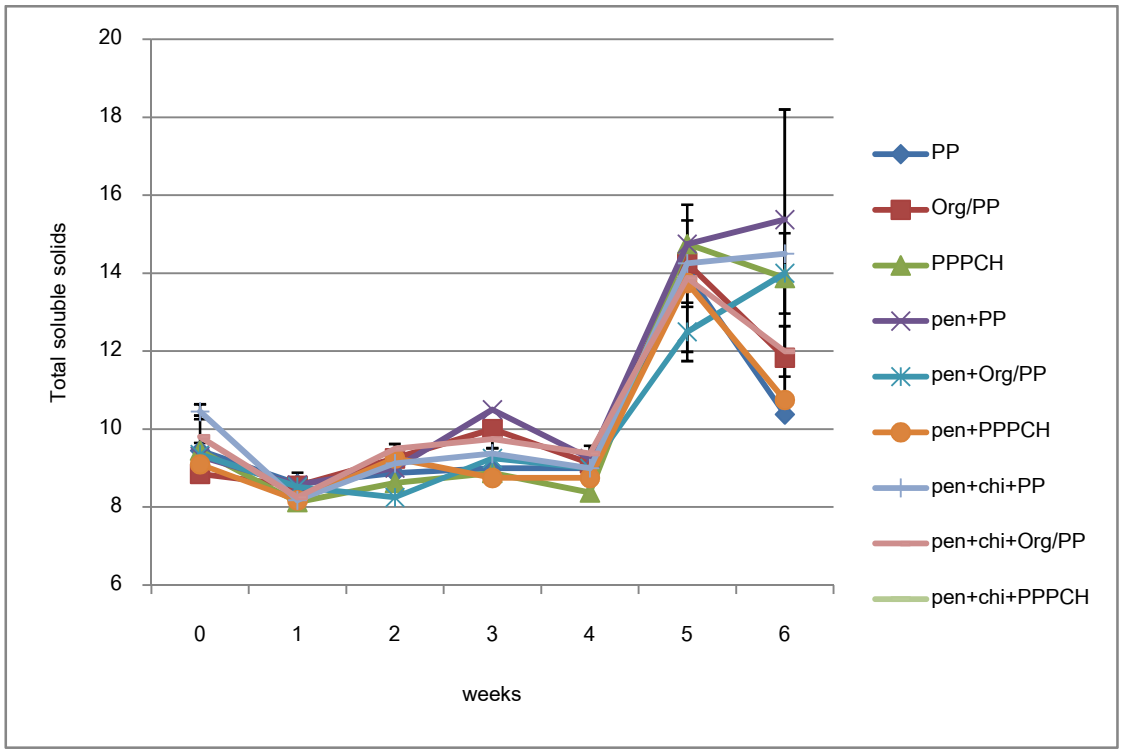

Figure 55. Total soluble solids (TSS) of limes in different treatments during storage at 10°C for 6 weeks.

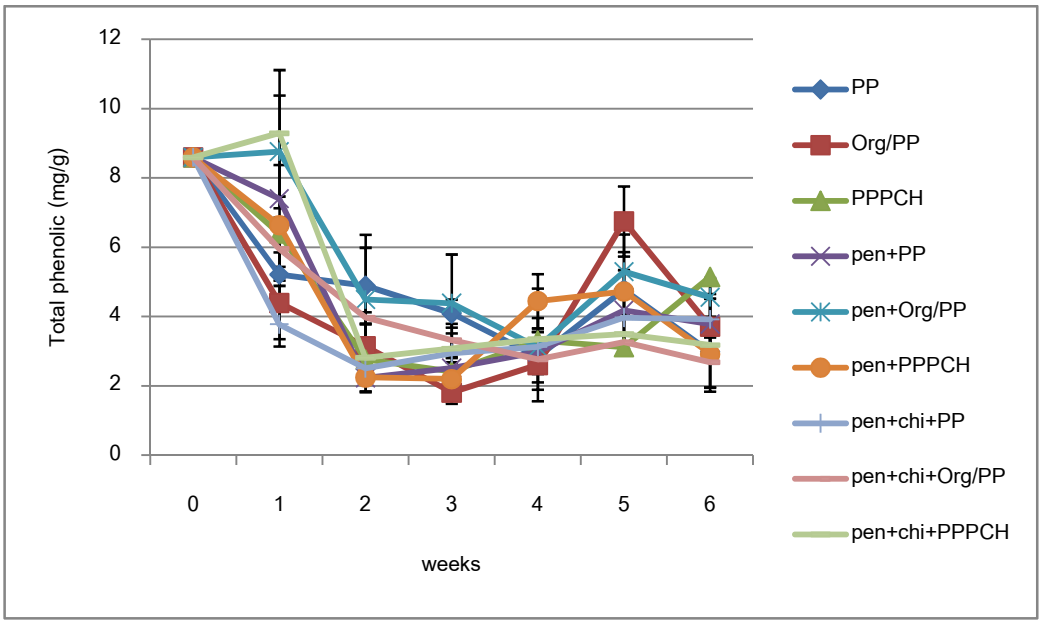

Figure 56.Total phenolic concentration (mg/g FW) of limes packed in modified polypropylene films during storage at 10°C for 6 weeks.

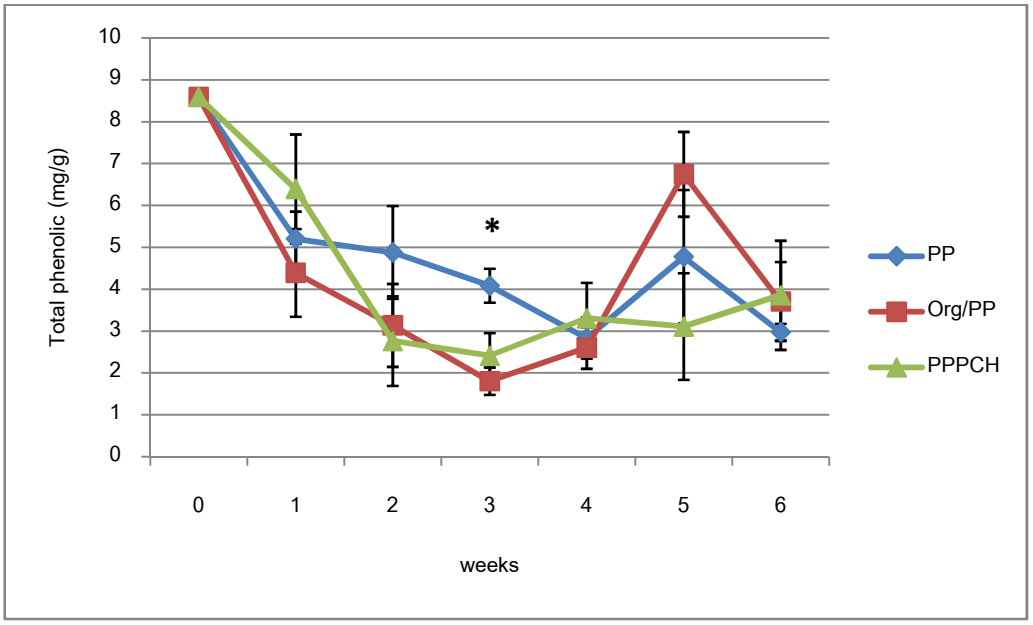

Figure 57. Total phenolic concentration (mg/g FW) of limes in control treatments packed in modified polypropylene films during storage at 10°C for 6 weeks.

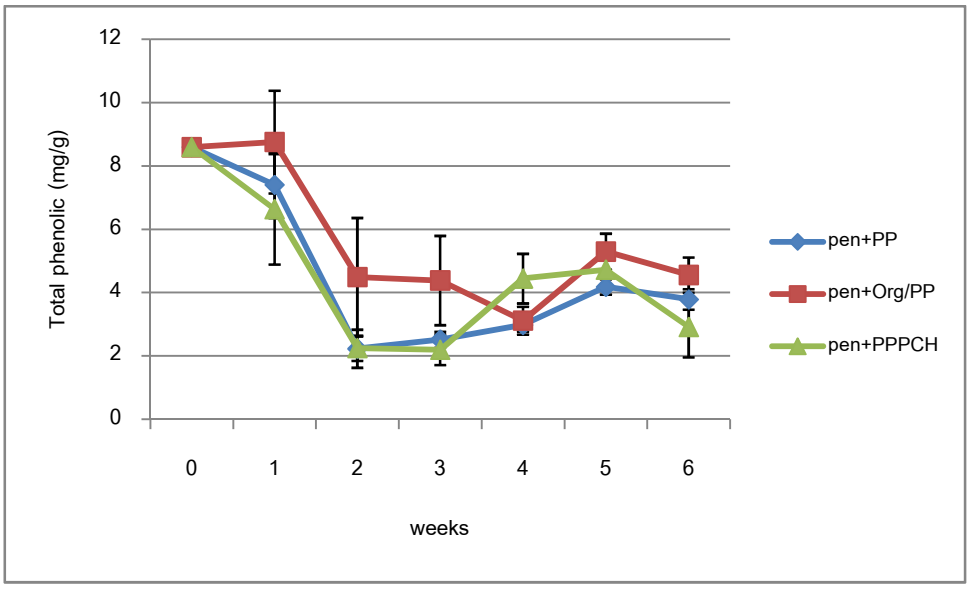

Figure 58. Total phenolic concentration (mg/g FW) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 10°C for 6 weeks.

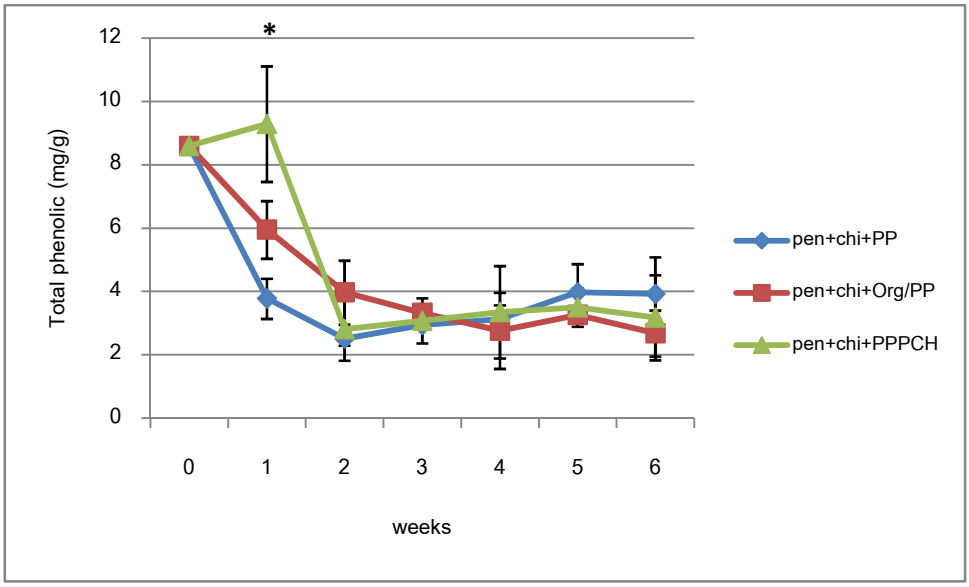

Figure 59. Total phenolic concentration (mg/g FW) of limes after treated with chitosan and packed in modified polypropylene films during storage at 10°C for 6 weeks.

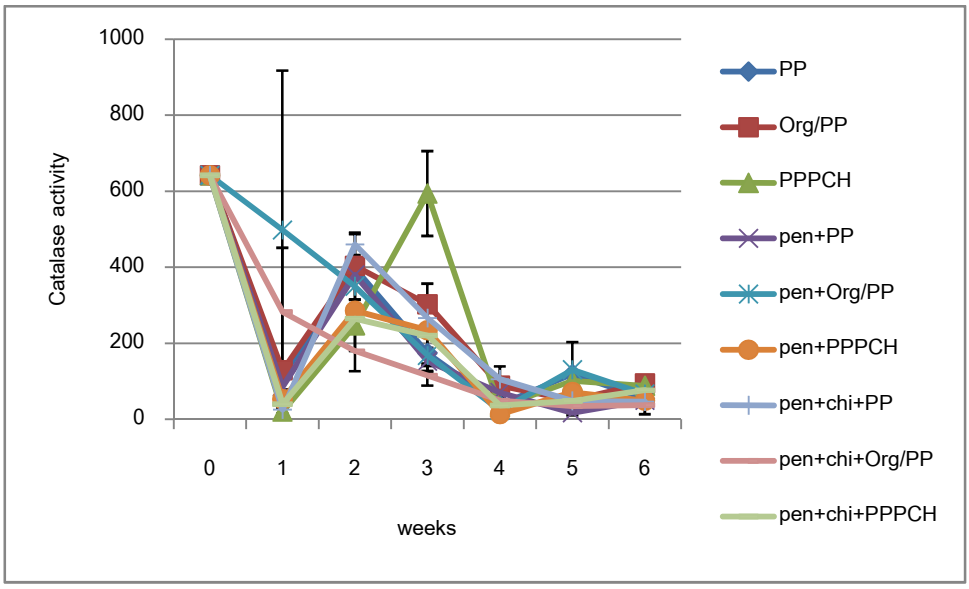

Figure 60. CAT activity of limes in different treatments during storage at 10°C for 6 weeks.

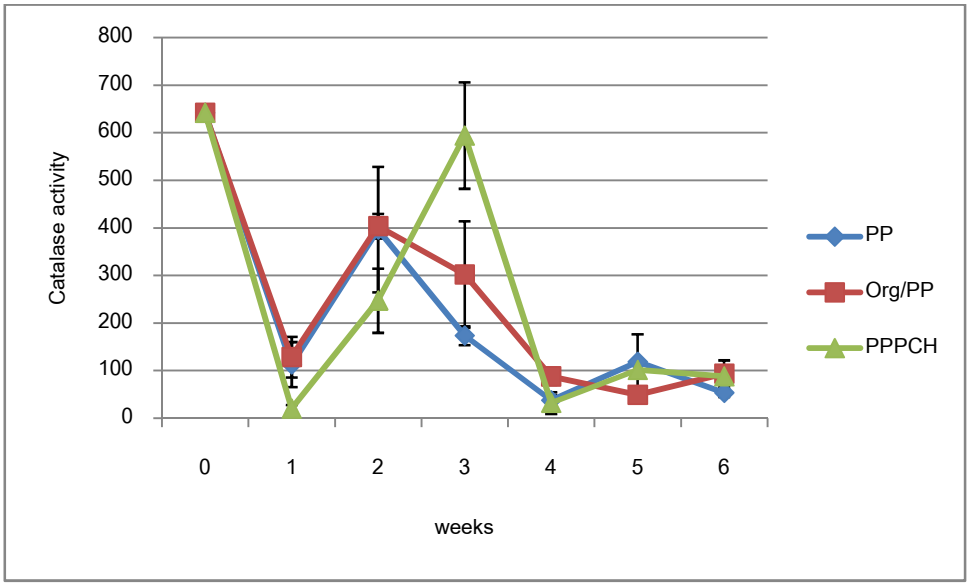

Figure 61. CAT activity of limes in control treatments packed in modified polypropylene films during storage at 10°C for 6 weeks.

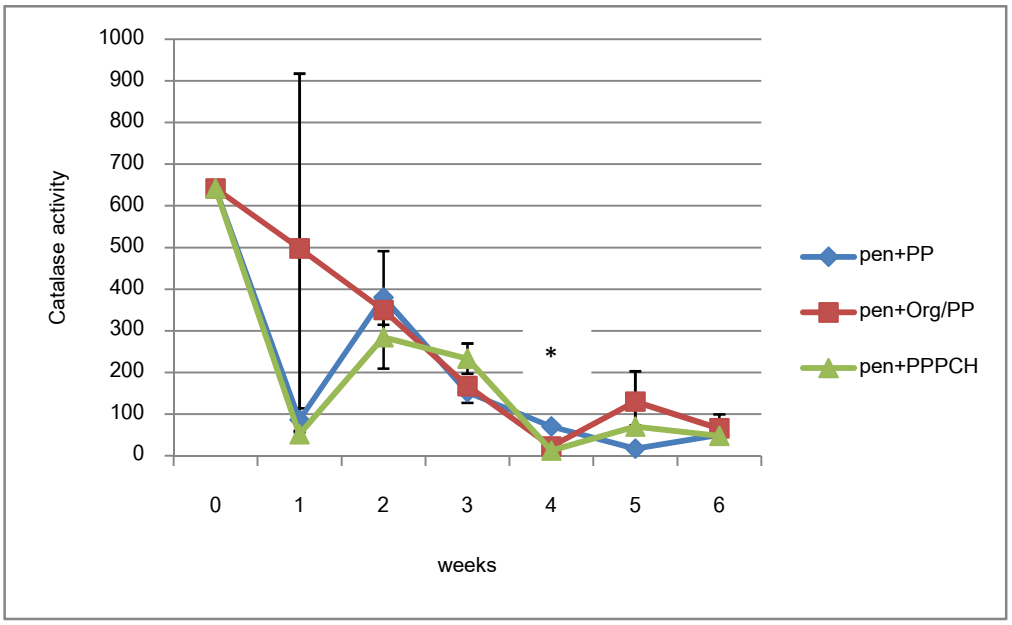

Figure 62. CAT activity of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

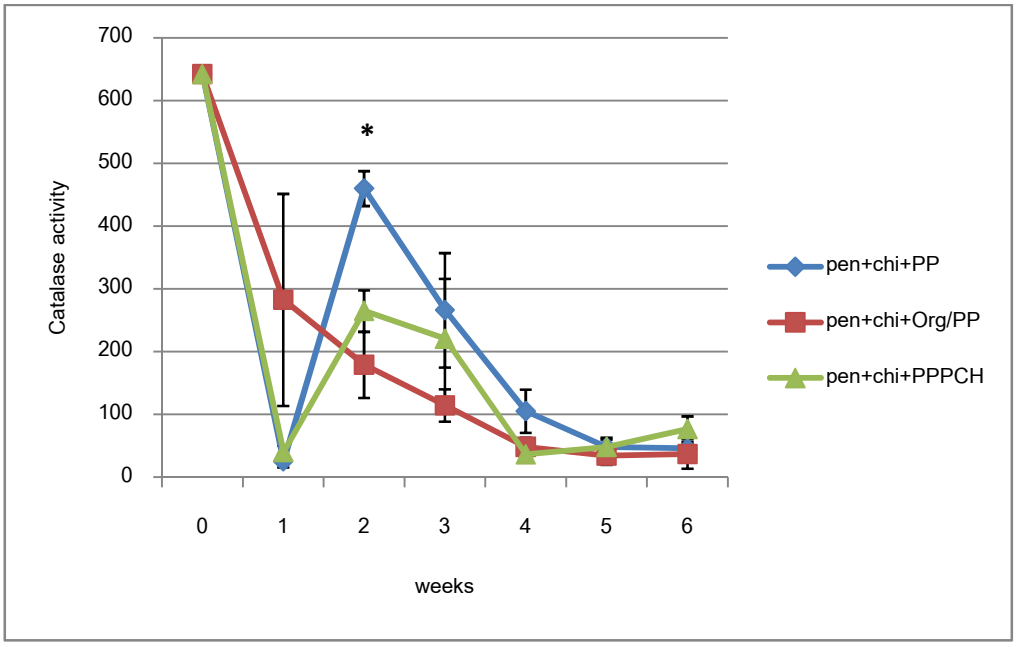

Figure 63. CAT activity of limes after treated with chitosan and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

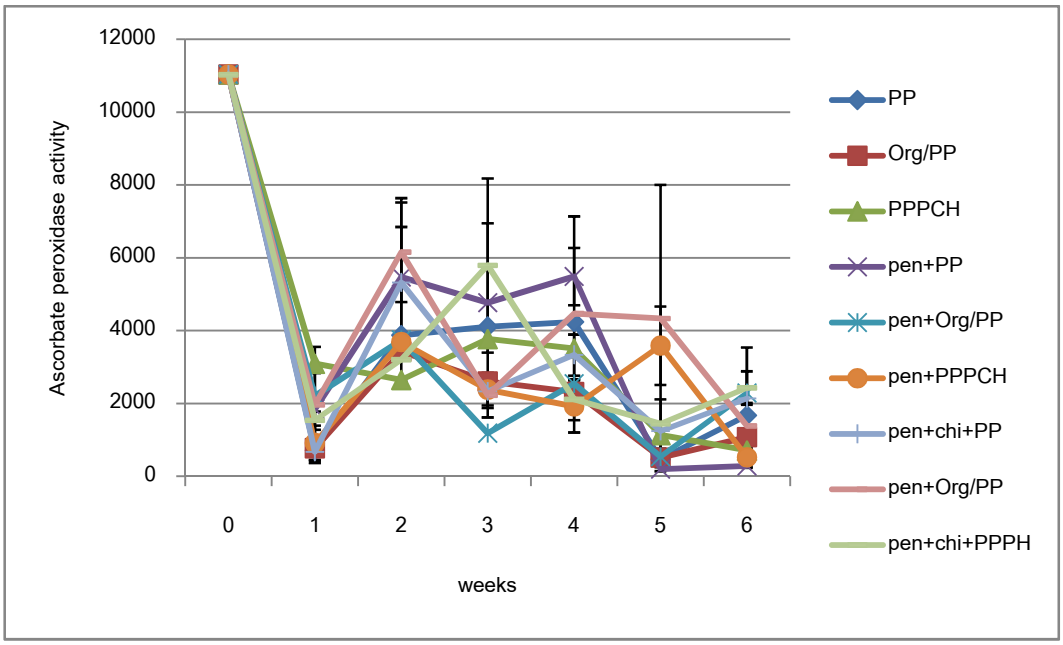

Figure 64. APX activity of limes in different treatments during storage at 10ºC for 6 weeks.

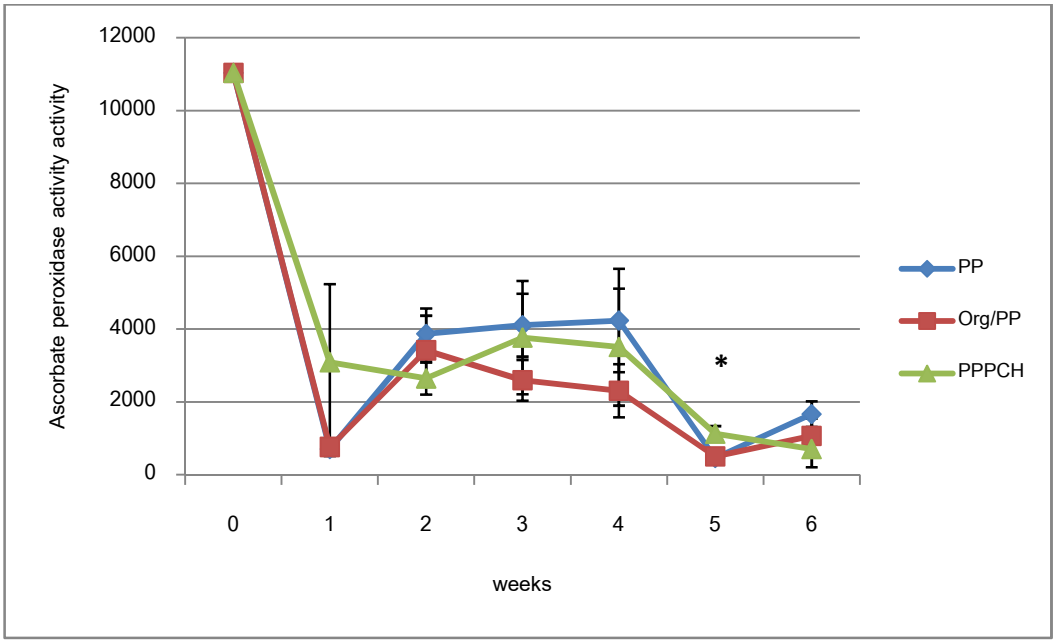

Figure 65. APX activity of limes in control treatments packed in modified polypropylene films during storage at 10ºC for 6 weeks.

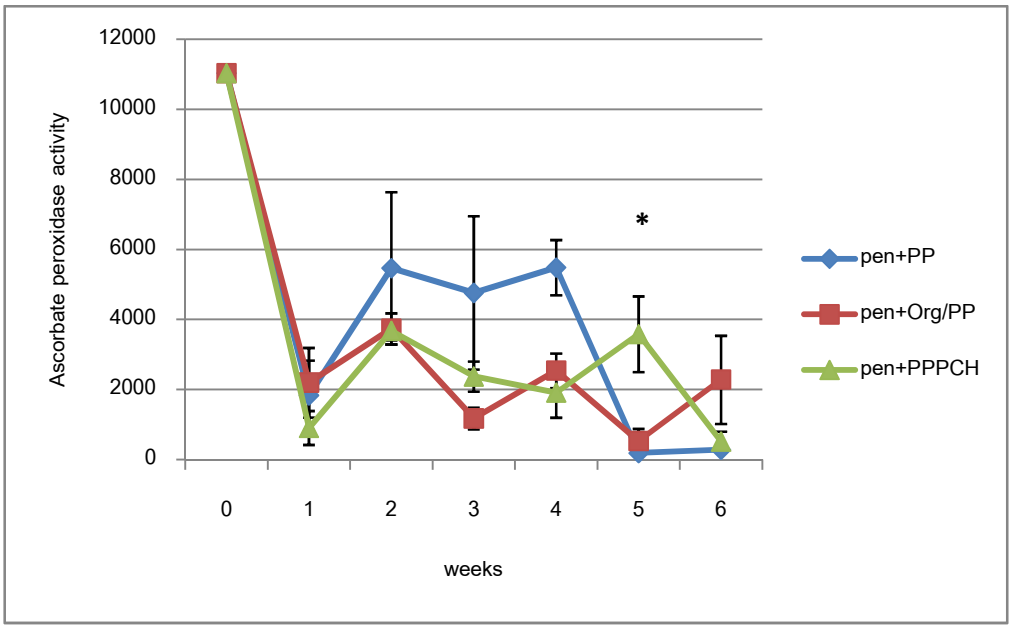

Figure 66. APX activity of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

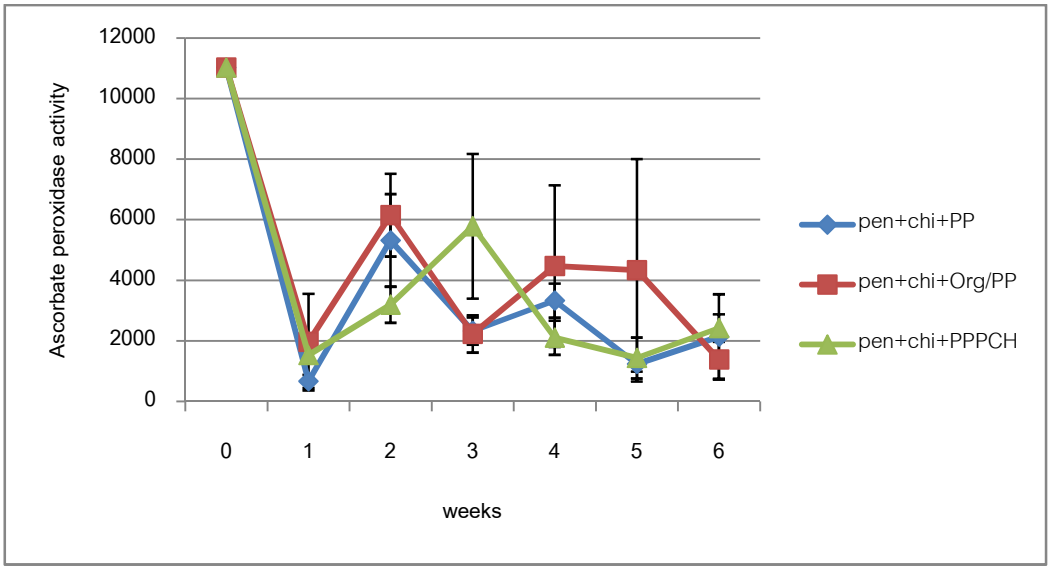

Figure 67. APX activity of limes after treated with chitosan and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

CHAPTER V

DISCUSSION

Peel color change

Peel color changes of lime were showed as L and hue value. L value increased in all treatments and hue value of peel rapidly declined in all treatments. Peel color changed from green to yellow when fruits and vegetables were stored for a long time (Jingtae, 1995) and yellowing of peel is a consequence of alterations in the physiological and biochemical processes occurring in the flavedo tissue of the lime peel (Tin et al., 2006). Both L value and hue value did not show any difference among all treatments which treated with chitosan solution during storage at 25°C and 10°C. Similarly, lime packed in modified PP and control PP did not show any difference in term of peel color change of limes in all treatments. Thus, chitosan and polypropylene packagings did not have any effect on peel color changes of lime in this experiment. Since lime is non-climacteric fruit, its peel color doesn't change from the beginning of storage which is difference from climacteric fruit (Martinez et al., 2002).

Percentage of weight loss

The weight loss of 5 ppm chitosan treated fruits stored at 25°C was higher than all other treatments while 5 ppm chitosan treatment at 10°C resulted in the lowest percentage of weight loss. This shows that low concentration of chitosan were appropriate for low temperature storage of limes. This study agrees with the previous report in which asparagus treated with 5 ppm chitosan then stored at 4°C for 15 days could slowly decrease weight loss compared with higher concentration of chitosan and control (water) (Patai Charoonnart, 2007) and citrus fruits treated with 0.1% concentration of low molecular weight chitosan (LMWC) had weight loss less than the fungicide thiabendazole (TBZ) treatement (Chien et al., 2007). Correspondingly, fresh cut strawberries (*Fragaria ananassa* Duchesne) cv. 329 dipped in 1.0% chitosan solution resulted in delay percentage of weight loss compared with 1.0% carboxymethyl

cellulose (CMC) when stored at 2°C for 10 days (Inkha et al., 2005). Chitosan was also involved in stomatal response. The stomatal aperture of tomato and *Commelina communis* was reduced when the epidermis was treated with chitosan (Lee et al., 1999). Moreover, chitosan could decrease transpiration in pepper plants, resulting in decrease water loss (Bittelli et al., 2001), suggesting that chitosan could be an effective antitranspirant to conserve water in plant. In addition, previous studies revealed that the chitosan coating functioned as a self control atmosphere and selectively permeated C_2H_4, CO_2 and O_2 inside and out of the fruit, thus reducing fruit respiration metabolism (El-Ghaouth et al., 1991) so could protect water loss in fruits.

The second and third experiments showed higher percentage of weight loss in fruits packed in modified PP than control PP in both temperatures. From the observation in packaging characteristic, we noticed that modified PP are thicker than normal PP thus, temperature within modified PP might be higher than normal PP. So limes packed in modified PP had higher transpiration rate than normal PP which might lead to weight loss.

Limes disease incidence

Limes showed disease incidence in the second week of storage at 25ºC. Chitosan treatment at 10 ppm was the best concentration to decrease disease incidence of limes. Treatment with chitosan on disease of fruit is in agreement with the results reported by Pilar et al. (2006) on strawberries dipped with either 1.5% chitosan or 1.5% chitosan and 1% CaGlu (calcium gluconate) mixture in which the treatment did not show any sign of fungal decay after a storage period of 4 days at 20°C compared with control. High concentration of chitosan could inhibit growth and formation of spores of *Rhizopus stolonifer* and *Botrytis cinerea* (El-Ghaouth et al., 1992b). In 2007, Chien et al. also reported the effect of chitosan in citrus fruits treated with 0.1% concentration of low molecular weight chitosan (LMWC) which reduced fungal rot compared with control treatment (water) during storage at 15ºC for 40 days. In addition, chitosan was more effective in inhibiting the rate of growth of *Penicillium digitatum, P. italicum, Botrytis lecanidion* and *B. cinerea* than thiabendazole (TBZ), the fungicide used as control and

effectiveness of LMWC increased with its concentration. Furthermore, El-Ghaouth et al. (2000) studied biocontrol activity of the combination of *Candida saitoana* with chitosan compounds in apple and citrus fruit. They found that combination of *C. saitoana* and 0.2% glycolchitosan had an effect on reducing green mold incidence in light green and yellow lemons. In addition, chitosan treatment could induce defense responses in several fruits including the elicitation of phenylalanine ammonia-lyase (PAL) activity in grape berries (Romanazzi et al., 2002), and chitinase and β-1,3-glucanase in oranges, strawberries and raspberries (Zhang and Quantick, 1997), thereby promoting protection from further fungal infection (Liu et al., 2007). Chitosan and its derivatives showed property a semipermeable film and are inhibitory to a number of pathogenic fungi, and also induce host-defense responses (Allan and Hadwiger, 1979; El-Ghaouth et al., 1994). Previous studies reported that reactive oxygen species (ROS) were the events correlated with plant resistance to pathogens (Baker and Orlandi, 1995) and developed of disease resistance in fruit (Torres et al., 2003). Our research was supported by El-Ghaouth et al. (1994), because of its film property, chitosan may act as a barrier to in and out flux of nutrients so may reduce the availability of nutrient to a level that was not enough for growth of the pathogen.

Postharvest diseases caused by *Penicillium digitatum* (green mould) and *P. italicum* (blue mould) are the most important negative factors affecting handling and marketing of citrus fruits (Porat et al., 2000). Our result showed that lime treated with chitosan and packed in modified PP could lower percentage of disease incidence than other treatments at 25°C, indicating that chitosan and modified PP might reduce pathogen growth. Muksing et al. (2008) reported that Org/PP had higher ethylene absorbtion property than normal PP and could control permeability of O_2 within packaging thus modified PP could delay senescence of limes and control growth of the fungi. Limes stored at 10°C did not show disease incidence during storage, because low temperature is not suitable for the fungal growth.

Respiration rate

The trend of respiration rate increased during storage in all treatments but it did not show any significant difference among treatments in both temperatures. The initial respiration rate was different between two temperatures because of harvest time. In the second experiment, limes were harvested in winter and the third experiment, limes were harvested in summer. Therefore, limes in the second experiment had the initial respiration rate lower than the third experiment. Taken together, chitosan and polypropylene packaging did not effect on respiration rate of lime in this experiment.

Phenolic compound

Phenolic compound decreased during storage in all treatments but it did not show any significant difference among all treatments in both temperatures. Thus chitosan and modified PP did not affect on phenolic compound of lime in this experiment. However, previous report showed that chitosan could induce formation of phenolic compounds, which controlled development and growth of *Aspergillus flavus* and production of aflatoxin BI (Fajardo et al. 1995). Also, chitosan had an effect on limitation of ability to colonization of *Pythium aphanidermatum*. Moreover preharvest chitosan spray treatment could enhance phenolic compounds content at the end of the storage period in table grape fruit (Meng et al., 2008).

Total soluble solid (TSS)

Total soluble solid (TSS) increased during storage but it did not show any significant difference among treatments at 25°C and 10°C storage. TSS level in limes can be correlated to higher respiration rate during storage which accelerates a process of changing carbohydrate to sugars. Breakdown of complex carbohydrates due to irradiation can also add to the soluble sugars, thus increasing TSS content of the juice in lime (Ladaniya et al., 2005). However, in this experiment, no difference of TSS in all treatments was detected which may due to non-climacteric characteristic of lime fruit. So, respiration rate increased slowly during storage and lime did not have high amount

of carbohydrates in juice thus TSS did not show difference among all treatments in both temperatures.

Ascorbic acid (vitamin C)

Limes packed in Org/PP had higher ascorbic acid than other packaging at 25°C. Limes inoculated with fungus and treated or non-treated with chitosan then packed in PPPCH had higher ascorbic acid than other packagings at 10°C. Thus, modified PP could delay a decrease of ascorbic acid more than normal PP. Modified PP could control permeability of O_2 within packaging and had higher ethylene absorbtion property than normal PP so modified PP could prolong shelf life of limes more than PP that improved ascorbic concentration (Muksing et al., 2008).

Catalase and ascorbate peroxidase activites

Lime inoculated with fungus and treated or non-treated with chitosan then packed in Org/PP had catalase and ascorbate peroxidase activities higher than other packaging at 25°C. While lime packed in PPPCH had higher catalase and ascorbate peroxidase activities than other packaging at 10°C. Corresponding with Zeng et al. (2010) report in that the activity of peroxidase (POD) in the navel orange fruit was significantly enhanced by chitosan coating, which could protect the tissues from injury of excessive high levels of ROS induced by chitosan in the fruit. Therefore, modified PP could prolong shelf life of limes which was a result of high antioxidant activities leading to a protection of limes from senescence.

CHAPTER VI

CONCLUSIONS

In the first experiment, 10 ppm chitosan solution was the best concentration to reduce fungal decay at 25°C and could delay weight loss of limes. Fruit treated with 5 ppm chitosan stored at 10°C resulted in delay weight loss and change in peel color, therefore chitosan can be used for prolonging the storage life and preventing loss of the products after harvest.

In the second experiment, Org/PP was the best packaging to increase ascorbic acid content, CAT and APX activities during storage at 25°C. Modified PP resulted in a decrease of lime disease incidence when compared with control PP.

In the third experiment, PPPCH was the best packaging to increase total phenolic content, ascorbic acid content, CAT and APX activities during storage at 10°C. Control PP could affect the delay of weight loss of limes in both temperatures. No disease incidence was found during storage in all treatments at 10°C.

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APPENDICES

APPENDIX A

1. **Ascorbic acid (Shin, 2007)**

One gram of fruit tissue was added to 10mL of a mixture of 6% metaphosphoric acid in 2 mol/ L acetic acid. The mixture was centrifuged at 17,600×*g* for 15 min at 4 ˚C the supernatant was filtered through #1 Whatman filter paper. A 1mL aliquot of the supernatant was mixed with 0.05mLof 0.2% 2,6-dichlorophenolindolphenol (DCIP) and the solution was incubated in dark room for 1 h at room temperature. After that, 1mL of 2% thiourea in 5% metaphosphoric acid and 0.5mL of 2% DNPH in 4.5 mol/ L sulfuric acid were added to the solution, and then incubated at 60 ˚C for 2 h. The reaction was stopped by placing the tubes in an ice bath and slowly adding 2.45mL of ice cold 90% sulfuric acid. Total AA was measured by absorbance at 540 nm using a standard curve. The concentrations were expressed as ascorbic acid on a fresh weight.

2. **Total phenolic content (Ramful et. al., 2010)**

The Folin–Ciocalteu assay, adapted from Ramful et. al., (2010), was used for the determination of total phenolics present in the citrus fruit extracts. One part ten gram of fruit tissue was added to 1.5 ml of 80% methanol. Then tube contents were vortexed at 9000 rpm for 20 min at 4˚C . To 100 µL of plant extract, 3.65 mL of distilled water was added followed by 0.25mL of Folin–Ciocalteu reagent (Merck). A blank was prepared using 100 µL of 80% methanol instead of plant extract. After 3min, 1mL of 20% sodium carbonate was added. Tube contents were vortexed before being incubated for 40 min in a waterbath set at 40˚C. The absorbance of the blue coloration formed was read at 685nm against the blank standard. Total phenolics were calculated with respect to gallic acid standard curve (concentration range: 0–12µg/mL). Results are expressed in µg of gallic acid/g fresh weight of plant material.

3. **Antioxidant activity (Nittaya Umrat, 2005)**

Peel lime (0.1g) was homogenized in 1 ml of ice-cold extraction buffer and 1% (w/v) polyvinyl polypyrrolidone (PVPP), 1 mg/ml dithiothreitol (DTT),100 mM phenylmethylsulfonyl fluoride (PMSF) with 50 mM sodium phosphate (pH 7.0) was used as extraction buffer. The homogenate was centrifuged at 13,000g for 15 min at 4 ˚C and the resulting supernatants were used directly for assay.

a. Catalase activity (CAT)

CAT activity measured by the decline in absorbance at 240 nm caused by the decomposition of H_2O_2 with slight modifications. The reaction mixture consisted of 1.78 ml sodium phosphate buffer (50 mM, pH 7.0), 0.2 ml H_2O_2 (100 mM) and 40 µl extract enzyme. The specific activity was expressed as U/mg protein, CAT activity was calculated as follows:

$$\text{U/mg protein} = \frac{(\Delta \text{ A240/min})(1000)}{(43.6)(\mu\text{l plant extract})(\text{mg protein/ } \mu\text{l plant extract})}$$

b. Ascorbate peroxidase activity (APX)

APX activity was assayed by determining the oxidised ascorbate by the method of Nakano and Asada (1989). The reaction mixture consisted of 1.58 ml sodium phosphate buffer (50 mM, pH 7.0), 0.2 ml H_2O_2 (100 mM), 20 µl EDTA (500mM, pH 8.0), 0.2 ml ascorbate (2mM) and 20 µl extract enzyme. The reaction rate was monitored by the decrease in absorbance at 290 nm. The rate constant was calculated as follows:

$$\text{U/mg protein} = \frac{(\Delta \text{ A290/min})(1000)}{(2.8)\ )(\mu\text{l plant extract})(\text{mg protein/ } \mu\text{l plant extract}}$$

3.3 Total protein assay (Nittaya Umrat, 2005)

The method was used to determine the protein content of the samples (Nittaya Umrat, 2005). Bradford dye reagent (BioRad) 50 µl was added to test

tubes containing 50 µl enzyme extract samples and distilled water 100 µl and the tubes were incubated at room temperature for 5 min. The samples were then thoroughly mixed and read at a wavelength of 595 nm in a spectrophotometer. Protein content was using bovine serum albumin (BSA) as the standard protein.

4. Respiration rate

Percentage of CO_2 were measure by Gas Chromatography (GC-8A).

Calculate : Air 100 mL have CO_2 A mL

Air (in jar) 700 mL have CO_2 $\frac{Ax700}{100}$ mL

Lime B kg. have CO_2 $\frac{Ax700}{100}$ mL

Lime 1 kg. have CO_2 $\frac{Ax700}{100B}$ mL

Formular : PV=nRT (Boyle 's law ; at 25°C)

CO_2 24453 mL weight 44000 mg.

CO_2 $\frac{Ax700}{100B}$ mL weight $\frac{(44000)(Ax700)}{(24453)(100B)}$ mg.

APPENDIX B

Table B1. Respiration rate ($mgCo_2/kg/h$) of limes in different treatments during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
PP	6.56±0.00^{a}	8.57±0.31^{abc}	9.18±1.42^{ab}	16.42±0.61^{a}	23.75±2.75^{a}
Org/PP	6.56±0.00^{a}	8.04±0.83^{abc}	7.80±1.25^{a}	15.73±0.60^{a}	23.09±1.68^{a}
PPPCH	6.56±0.00^{a}	7.64±0.69^{ab}	11.03±0.57^{abc}	16.33±0.88^{a}	23.83±2.93^{a}
Pen+PP	6.56±0.00^{a}	9.04±0.47^{abc}	15.10±3.37^{cd}	21.18±3.76^{a}	27.41±2.58^{a}
Pen+Org/PP	6.56±0.00^{a}	7.05±0.30^{a}	11.18±1.46^{abc}	19.83±1.44^{a}	32.31±3.85^{a}
Pen+PPPCH	6.56±0.00^{a}	8.54±1.57^{abc}	12.19±1.19^{abcd}	20.96±2.19^{a}	31.56±4.01^{a}
Pen+chi+PP	6.56±0.00^{a}	9.65±0.75^{bc}	17.12±2.72^{d}	20.55±0.95^{a}	27.54±1.95^{a}
Pen+chi+Org/PP	6.56±0.00^{a}	7.36±0.28^{ab}	12.90±0.69^{abcd}	20.24±0.51^{a}	24.74±1.96^{a}
Pen+chi+PPPCH	6.56±0.00^{a}	10.47±0.67^{c}	14.37±1.25^{bcd}	19.30±0.89^{a}	27.59±3.56^{a}

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at $P \leq 0.05$

Table B2. Respiration rate of lime ($mgCo_2$/kg/h) of limes packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
PP	6.56±0.00[a]	8.57±0.31[a]	9.18±1.42[a]	16.42±0.61[a]	23.75±2.75[a]
Org/PP	6.56±0.00[a]	8.04±0.83[a]	7.80±1.25[a]	15.73±0.60[a]	23.09±1.68[a]
PPPCH	6.56±0.00[a]	7.64±0.69[a]	11.03±0.57[a]	16.33±0.88[a]	23.83±2.93[a]

Table B3. Respiration rate of lime ($mgCo_2$/kg/h) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25ºC for 4 weeks

.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
Pen+PP	6.56±0.00[a]	9.04±0.47[a]	15.10±3.37[a]	21.18±3.76[a]	27.41±2.58[a]
Pen+Org/PP	6.56±0.00[a]	7.05±0.30[a]	11.18±1.46[a]	19.83±1.44[a]	32.31±3.85[a]
Pen+PPPCH	6.56±0.00[a]	8.54±1.57[a]	12.19±1.19[a]	20.96±2.19[a]	31.56±4.01[a]

Table B4. Respiration rate of lime ($mgCo_2$/kg/h) of limes after treated with chitosan and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
Pen+chi+PP	6.56±0.00[a]	9.65±0.75[b]	17.12±2.72[a]	20.55±0.95[a]	27.54±1.95[a]
Pen+chi+Org/PP	6.56±0.00[a]	7.36±0.28[a]	12.90±0.69[a]	20.24±0.51[a]	24.74±1.96[a]
Pen+chi+PPPCH	6.56±0.00[a]	10.47±0.67[b]	14.37±1.25[a]	19.30±0.89[a]	27.59±3.56[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at $P \leq 0.05$

Table B5. Ascorbic acid concentration (μg/g FW) of limes in different treatments during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
PP	844.38±0.00[a]	969.52±56.55[a]	1247.87±57.32[b]	680.68±44.96[a]	702.64±130.29[a]
Org/PP	844.38±0.00[a]	1186.80±45.15[ab]	1203.18±22.72[b]	693.95±72.76[a]	1329.50±139.09[b]
PPPCH	844.38±0.00[a]	1322.62±89.97[b]	1246.67±79.48[b]	926.37±130.67[a]	840.79±46.43[a]
Pen+PP	844.38±0.00[a]	1216.45±111.13[ab]	819.04±83.28[a]	862.88±173.29[a]	964.11±85.77[a]
Pen+Org/PP	844.38±0.00[a]	975.09±28.18[a]	1014.23±91.91[ab]	1322.45±174.03[b]	772.50±71.63[a]
Pen+PPPCH	844.38±0.00[a]	1359.44±79.64[b]	1102.80±59.45[ab]	988.99±159.92[ab]	807.41±112.94[a]
Pen+chi+PP	844.38±0.00[a]	1352.67±15.46[b]	1066.29±119.72[ab]	700.40±104.29[a]	825.42±116.38[a]
Pen+chi+Org/PP	844.38±0.00[a]	1330.60±144.52[b]	1264.03±151.49[b]	786.70±54.36[a]	1003.90±27.69[a]
Pen+chi+PPPCH	844.38±0.00[a]	1229.33±78.84[ab]	1247.06±103.62[b]	885.44±63.20[a]	861.93±97.00[a]

The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B6. Ascorbic acid concentration (µg/g FW) of limes packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
PP	844.38±0.00[a]	969.52±56.55[a]	1247.87±57.32[a]	680.68±44.96[a]	702.64±130.29[a]
Org/PP	844.38±0.00[a]	1186.80±45.15[ab]	1203.18±22.72[a]	693.95±72.76[a]	1329.50±139.09[b]
PPPCH	844.38±0.00[a]	1322.62±89.97[b]	1246.67±79.48[a]	926.37±130.67[a]	840.79±46.43[a]

Table B7. Ascorbic acid concentration (µg/g FW) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
Pen+PP	844.38±0.00[a]	1216.45±111.13[ab]	819.04±83.28[a]	862.88±173.29[a]	964.11±85.77[a]
Pen+Org/PP	844.38±0.00[a]	975.09±28.18[a]	1014.23±91.91[a]	1322.45±174.03[a]	772.50±71.63[a]
Pen+PPPCH	844.38±0.00[a]	1359.44±79.64[b]	1102.80±59.45[a]	988.99±159.92[a]	807.41±112.94[a]

Table B8. Ascorbic acid concentration (µg/g FW) of limes after treated with chitosan and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
Pen+chi+PP	844.38±0.00[a]	1352.67±15.46[a]	1066.29±119.72[a]	700.40±104.29[a]	825.42±116.38[a]
Pen+chi+Org/PP	844.38±0.00[a]	1330.60±144.52[a]	1264.03±151.49[a]	786.70±54.36[a]	1003.90±27.69[a]
Pen+chi+PPPCH	844.38±0.00[a]	1229.33±78.84[a]	1247.06±103.62[a]	885.44±63.20[a]	861.93±97.00[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B9. Total soluble solids (TSS) of limes in different treatments during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
PP	9.10±0.06^{a}	8.38±0.20^{a}	8.67±0.17^{ab}	7.88±0.12^{a}	8.50±0.00^{a}
Org/PP	9.45±0.26^{a}	8.13±0.13^{a}	8.50±0.50^{a}	8.63±0.24^{ab}	8.88±0.13^{ab}
PPPCH	8.85±0.25^{a}	8.63±0.43^{a}	9.00±0.54^{ab}	8.13±0.13^{a}	8.63±0.47^{ab}
Pen+PP	9.45±0.13^{a}	8.50±0.20^{a}	9.25±0.25^{ab}	8.50±0.29^{ab}	8.83±0.44^{ab}
Pen+Org/PP	9.30±0.30^{a}	9.00±0.00^{a}	9.00±0.00^{ab}	9.13±0.24^{b}	9.33±0.17^{ab}
Pen+PPPCH	9.35±0.44^{a}	8.75±0.48^{a}	9.88±0.52^{b}	9.13±0.52^{b}	9.00±0.29^{ab}
Pen+chi+PP	9.10±0.19^{a}	9.00±0.54^{a}	9.75±0.48^{ab}	8.63±0.24^{ab}	9.13±0.43^{ab}
Pen+chi+Org/PP	10.45±0.56^{ab}	9.00±0.35^{a}	11.13±0.13^{c}	8.63±0.24^{ab}	9.00±0.00^{ab}
Pen+chi+PPPCH	9.80±0.29^{ab}	9.13±0.31^{a}	9.63±0.38^{ab}	8.50±0.20^{ab}	9.67±0.44^{b}

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B10. Total phenolic concentration (mg/g FW) of limes in different treatments during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
PP	15.71±0.00[a]	16.01±1.09[a]	14.64±3.35[a]	14.07±1.78[ab]	21.83±0.54[a]
Org/PP	15.71±0.00[a]	17.74±0.93[a]	12.84±0.67[a]	14.36±1.30[ab]	20.70±3.34[a]
PPPCH	15.71±0.00[a]	18.07±2.45[a]	15.80±0.73[a]	16.77±0.93[ab]	23.67±3.12[a]
Pen+PP	15.71±0.00[a]	17.31±0.88[a]	11.78±0.59[a]	12.52±0.45[a]	19.80±5.09[a]
Pen+Org/PP	15.71±0.00[a]	18.79±2.28[a]	13.61±1.31[a]	19.18±2.83[b]	16.90±2.29[a]
Pen+PPPCH	15.71±0.00[a]	20.53±2.57[a]	12.20±0.64[a]	15.39±1.91[ab]	22.27±3.06[a]
Pen+chi+PP	15.71±0.00[a]	13.58±1.14[a]	13.75±0.95[a]	16.91±1.62[ab]	22.20±1.66[a]
Pen+chi+Org/PP	15.71±0.00[a]	18.94±2.96[a]	16.11±2.71[a]	16.26±1.24[ab]	13.79±2.20[a]
Pen+chi+PPPCH	15.71±0.00[a]	16.50±4.36[a]	14.78±0.72[a]	18.97±1.03[b]	20.30±4.49[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B11. Total phenolic concentration (mg/g FW) of limes packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
PP	15.71±0.00[a]	16.01±1.09[a]	14.64±3.35[a]	14.07±1.78[a]	21.83±0.54[a]
Org/PP	15.71±0.00[a]	17.74±0.93[a]	12.84±0.67[a]	14.36±1.30[a]	20.70±3.34[a]
PPPCH	15.71±0.00[a]	18.07±2.45[a]	15.80±0.73[a]	16.77±0.93[a]	23.67±3.12[a]

Table B12. Total phenolic concentration (mg/g FW) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
Pen+PP	15.71±0.00[a]	17.31±0.88[a]	11.78±0.59[a]	12.52±0.45[a]	19.80±5.09[a]
Pen+Org/PP	15.71±0.00[a]	18.79±2.28[a]	13.61±1.31[a]	19.18±2.83[a]	16.90±2.29[a]
Pen+PPPCH	15.71±0.00[a]	20.53±2.57[a]	12.20±0.64[a]	15.39±1.91[a]	22.27±3.06[a]

Table B13. Total phenolic concentration (mg/g FW) of limes after treated with chitosan and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
Pen+chi+PP	15.71±0.00[a]	13.58±1.14[a]	13.75±0.95[a]	16.91±1.62[a]	22.20±1.66[a]
Pen+chi+Org/PP	15.71±0.00[a]	18.94±2.96[a]	16.11±2.71[a]	16.26±1.24[a]	13.79±2.20[a]
Pen+chi+PPPCH	15.71±0.00[a]	16.50±4.36[a]	14.78±0.72[a]	18.97±1.03[a]	20.30±4.49[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B14. CAT activity of limes in different treatments during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
PP	677.10±0.00[a]	59.14±6.58[a]	168.89±94.23[a]	50.37±41.47[a]	44.11±19.10[a]
Org/PP	677.10±0.00[a]	320.77±206.17[a]	61.14±15.79[a]	34.50±11.40[a]	92.26±20.43[a]
PPPCH	677.10±0.00[a]	427.69±271.79[a]	25.94±15.52[a]	74.15±51.16[a]	99.84±35.91[a]
Pen+PP	677.10±0.00[a]	142.13±53.07[a]	126.78±65.53[a]	63.23±32.29[a]	73.67±8.12[a]
Pen+Org/PP	677.10±0.00[a]	35.93±8.17[a]	77.80±8.93[a]	67.32±44.55[a]	427.37±227.74[b]
Pen+PPPCH	677.10±0.00[a]	39.54±7.98[a]	53.35±11.90[a]	51.44±22.36[a]	179.21±27.86[ab]
Pen+chi+PP	677.10±0.00[a]	107.73±55.51[a]	60.94±8.00[a]	27.56±6.22[a]	161.73±38.46[ab]
Pen+chi+Org/PP	677.10±0.00[a]	56.95±20.75[a]	208.77±151.08[a]	74.48±10.81[a]	98.37±41.73[a]
Pen+chi+PPPCH	677.10±0.00[a]	208.06±185.26[a]	42.27±8.17[a]	10.54±1.64[a]	222.81±125.46[ab]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B15. CAT activity of limes packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
PP	677.10±0.00[a]	59.14±6.58[a]	168.89±94.23[a]	50.37±41.47[a]	44.11±19.10[a]
Org/PP	677.10±0.00[a]	320.77±206.17[a]	61.14±15.79[a]	34.50±11.40[a]	92.26±20.43[a]
PPPCH	677.10±0.00[a]	427.69±271.79[a]	25.94±15.52[a]	74.15±51.17[a]	99.84±35.91[a]

Table B16. CAT activity of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
Pen+PP	677.10±0.00[a]	142.13±53.06[a]	126.77±65.52[a]	63.23±32.29[a]	73.67±8.12[a]
Pen+Org/PP	677.10±0.00[a]	35.94±8.17[a]	77.80±8.93[a]	67.32±44.55[a]	427.37±227.74[a]
Pen+PPPCH	677.10±0.00[a]	39.54±7.98[a]	53.36±11.90[a]	51.44±22.36[a]	179.21±27.86[a]

Table B17. CAT activity of limes after treated with chitosan and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
Pen+chi+PP	677.10±0.00[a]	107.73±55.51[a]	60.94±8.00[a]	27.56±6.22[a]	161.73±38.46[a]
Pen+chi+Org/PP	677.10±0.00[a]	56.95±20.75[a]	208.77±151.08[a]	74.48±10.81[b]	98.37±41.73[a]
Pen+chi+PPPCH	677.10±0.00[a]	208.06±185.26[a]	42.27±8.17[a]	10.54±1.64[a]	222.81±125.46[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B18. APX activity of limes in different treatments during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
PP	13006.84±0.00[a]	2127.86±616.74[a]	945.11±519.04[a]	2351.34±1052.30[a]	1672.52±543.10[a]
Org/PP	13006.84±0.00[a]	1919.24±1114.94[a]	4540.22±1307.18[b]	977.24±248.00[a]	913.23±196.11[a]
PPPCH	13006.84±0.00[a]	3777.84±1346.81[a]	4059.13±447.85[ab]	1545.89±343.56[a]	1032.47±434.19[a]
Pen+PP	13006.84±0.00[a]	3431.20±2087.91[a]	4645.10±1270.43[b]	1444.25±730.98[a]	2659.69±1981.33[a]
Pen+Org/PP	13006.84±0.00[a]	913.62±410.87[a]	2981.18±1516.79[ab]	3822.36±960.99[a]	3178.05±1387.91[a]
Pen+PPPCH	13006.84±0.00[a]	2795.43±455.88[a]	811.43±363.11[a]	1265.45±599.24[a]	500.47±45.35[a]
Pen+chi+PP	13006.84±0.00[a]	4429.91±2148.29[a]	2585.69±1154.24[ab]	1210.02±288.24[a]	1063.41±389.60[a]
Pen+chi+Org/PP	13006.84±0.00[a]	2705.37±1361.78[a]	1773.00±1230.71[ab]	1995.02±614.10[a]	1205.51±450.50[a]
Pen+chi+PPPCH	13006.84±0.00[a]	3017.49±1453.99[a]	820.24±594.10[a]	3693.40±2443.06[a]	1430.91±608.07[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B19. APX activity of limes packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
PP	13006.84±0.00[a]	2127.86±616.74[a]	945.11±519.04[a]	2351.34±1052.30[a]	1672.52±543.10[a]
Org/PP	13006.84±0.00[a]	1919.24±1114.94[a]	4540.22±1307.18[b]	977.24±248.00[a]	913.23±196.11[a]
PPPCH	13006.84±0.00[a]	3777.84±1346.81[a]	4059.13±447.85[b]	1545.89±343.56[a]	1032.47±434.19[a]

Table B20. APX activity of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
Pen+PP	13006.84±0.00[a]	3431.20±2087.91[a]	4645.10±1270.43[b]	1444.25±730.98[ab]	2659.69±1981.33[a]
Pen+Org/PP	13006.84±0.00[a]	913.62±410.87[a]	2981.18±1516.79[ab]	3822.36±960.99[b]	3178.05±1387.91[a]
Pen+PPPCH	13006.84±0.00[a]	2795.43±455.88[a]	811.43±363.11[a]	1265.45±599.24[a]	500.47±45.35[a]

Table B21. APX activity of limes after treated with chitosan and packed in modified polypropylene films during storage at 25ºC for 4 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
Pen+chi+PP	13006.84±0.00[a]	4429.91±2148.29[a]	2585.69±1154.24[a]	1210.02±288.24[a]	1063.41±389.60[a]
Pen+chi+Org/PP	13006.84±0.00[a]	2705.37±1361.78[a]	1773.00±1230.71[a]	1995.02±614.10[a]	1205.51±450.50[a]
Pen+chi+PPPCH	13006.84±0.00[a]	3017.49±1453.99[a]	820.24±594.10[a]	3693.40±2443.06[a]	1430.91±608.07[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B22. Respiration rate ($mgCo_2/kg/h$) of limes in different treatments during storage at 10°C for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PP	18.55±0.00[a]	3.73±2.06[a]	2.71±0.12[a]	24.79±2.68[ab]	32.64±1.82[b]	28.86±3.71[b]	11.46±0.47[ab]
Org/PP	18.55±0.00[a]	1.91±0.27[a]	9.45±4.29[a]	29.95±1.07[b]	28.83±7.28[ab]	27.07±2.14[ab]	11.15±0.41[a]
PPPCH	18.55±0.00[a]	3.57±1.84[a]	5.55±1.92[a]	22.15±2.56[a]	26.62±3.05[ab]	26.70±1.25[ab]	12.15±1.59[ab]
Pen+PP	18.55±0.00[a]	5.18±3.43[a]	5.62±2.05[a]	21.85±1.29[a]	23.06±3.01[ab]	30.53±4.56[b]	11.90±2.23[ab]
Pen+Org/PP	18.55±0.00[a]	5.01±3.07[a]	10.02±4.24[a]	18.42±2.41[a]	28.90±2.37[ab]	18.93±1.28[a]	16.85±0.59[b]
Pen+PPPCH	18.55±0.00[a]	2.02±0.10[a]	4.34±0.90[a]	23.62±1.67[ab]	18.91±1.72[a]	22.19±3.46[ab]	12.64±1.70[ab]
Pen+chi+PP	18.55±0.00[a]	2.22±0.23[a]	8.39±5.03[a]	23.03±2.49[a]	23.94±2.15[ab]	22.47±1.73[ab]	10.13±2.68[a]
Pen+chi+Org/PP	18.55±0.00[a]	2.41±0.17[a]	8.59±4.70[a]	20.85±2.12[a]	20.54±1.30[a]	22.81±0.92[ab]	16.68±2.15[b]
Pen+chi+PPPCH	18.55±0.00[a]	2.45±0.14[a]	6.89±3.49[a]	20.43±2.05[a]	25.73±4.38[ab]	22.24±1.99[ab]	11.54±1.50[ab]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at $P \leq 0.05$

Table B23. Respiration rate ($mgCo_2/kg/h$) of limes packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PP	18.55±0.00[a]	3.73±2.06[a]	2.71±0.12[a]	24.79±2.68[ab]	32.64±1.82[a]	28.86±3.71[a]	11.46±0.47[a]
Org/PP	18.55±0.00[a]	1.91±0.27[a]	9.45±4.29[a]	29.95±1.07[b]	28.83±7.28[a]	27.07±2.14[a]	11.15±0.41[a]
PPPCH	18.55±0.00[a]	3.57±1.84[a]	5.55±1.92[a]	22.15±2.56[a]	26.62±3.05[a]	26.70±1.25[a]	12.15±1.59[a]

Table B24. Respiration rate ($mgCo_2/kg/h$) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Pen+PP	18.55±0.00[a]	5.18±3.43[a]	5.62±2.05[a]	21.85±1.29[a]	23.06±3.01[ab]	30.53±4.56[b]	11.90±2.23[a]
Pen+Org/PP	18.55±0.00[a]	5.01±3.07[a]	10.02±4.24[a]	18.42±2.41[a]	28.90±2.37[b]	18.93±1.28[a]	16.85±0.59[a]
Pen+PPPCH	18.55±0.00[a]	2.02±0.10[a]	4.34±0.90[a]	23.62±1.67[ab]	18.91±1.72[a]	22.19±3.46[ab]	12.64±1.70[a]

Table B25. Respiration rate ($mgCo_2/kg/h$) of limes after treated with chitosan and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Pen+chi+PP	18.55±0.00[a]	2.22±0.23[a]	8.39±5.03[a]	23.03±2.49[a]	23.94±2.15[a]	22.47±1.73[a]	10.13±2.68[a]
Pen+chi+Org/PP	18.55±0.00[a]	2.41±0.17[a]	8.59±4.70[a]	20.85±2.12[a]	20.54±1.30[a]	22.81±0.92[a]	16.68±2.15[a]
Pen+chi+PPPCH	18.55±0.00[a]	2.45±0.14[a]	6.89±3.49[a]	20.43±2.05[a]	25.73±4.38[a]	22.24±1.99[a]	11.54±1.50[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at $P \leq 0.05$

Table B26. Ascorbic acid concentration (mg/g FW) of limes in different treatments during storage at 10°C for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PP	1.69±0.00^{a}	1.45±0.07^{a}	1.27±0.11^{ab}	1.06±0.13^{a}	1.27±0.21^{bc}	1.19±0.05^{a}	0.84±0.07^{a}
Org/PP	1.69±0.00^{a}	1.31±0.26^{a}	1.09.±0.25^{a}	1.09±0.13^{a}	1.00±0.10^{abc}	1.19±0.04^{a}	1.01±0.21^{a}
PPPCH	1.69±0.00^{a}	1.50±0.11^{a}	1.68±0.324^{b}	1.19±0.10^{a}	1.33±0.06^{c}	1.78±0.23^{b}	0.89.±0.16^{a}
Pen+PP	1.69±0.00^{a}	1.42±0.11^{a}	0.90±0.06^{a}	0.77±0.04^{a}	1.11±0.12^{abc}	0.84±0.09^{a}	1.09±0.10^{a}
Pen+Org/PP	1.69±0.00^{a}	1.83±0.24^{a}	1.24±0.05^{ab}	1.15±0.23^{a}	0.93±0.10^{ab}	0.91±0.20^{a}	0.86±0.05^{a}
Pen+PPPCH	1.69±0.00^{a}	1.46±0.21^{a}	0.98±0.11^{a}	1.01±0.18^{a}	0.87±0.14^{a}	0.98±0.13^{a}	0.76±0.17^{a}
Pen+chi+PP	1.69±0.00^{a}	1.30±0.12^{a}	1.12±0.13^{a}	0.88.±0.04^{a}	1.01±0.10^{abc}	1.12±0.09^{a}	1.06±0.07^{a}
Pen+chi+Org/PP	1.69±0.00^{a}	1.36±0.24^{a}	0.98±0.10^{a}	0.94±0.07^{a}	0.96±0.07^{abc}	1.03±0.10^{a}	0.83±0.14^{a}
Pen+chi+PPPCH	1.69±0.00^{a}	1.25±0.17^{a}	1.04±0.14^{a}	0.833±0.06^{a}	0.75±0.05^{a}	1.00±0.13^{a}	0.94±0.15^{a}

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at $P \leq 0.05$

Table B27. Ascorbic acid concentration (mg/g FW) of limes packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PP	1.69±0.00[a]	1.45±0.07[a]	1.27±0.11[a]	1.06±0.13[a]	1.27±0.21[a]	1.19±0.05[a]	0.84±0.07[a]
Org/PP	1.69±0.00[a]	1.31±0.26[a]	1.09.±0.25[a]	1.09±0.13[a]	1.00±0.10[a]	1.19±0.04[a]	1.01±0.21[a]
PPPCH	1.69±0.00[a]	1.50±0.11[a]	1.68±0.324[a]	1.19±0.10[a]	1.33±0.06[a]	1.78±0.23[b]	0.89.±0.16[a]

Table B28. Ascorbic acid concentration (µg/g FW) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Pen+PP	1.69±0.00[a]	1.42±0.11[a]	0.90±0.06[a]	0.77±0.04[a]	1.11±0.12[a]	0.84±0.09[a]	1.09±0.10[a]
Pen+Org/PP	1.69±0.00[a]	1.83±0.24[a]	1.24±0.05[b]	1.15±0.23[a]	0.93±0.10[a]	0.91±0.20[a]	0.86±0.05[a]
Pen+PPPCH	1.69±0.00[a]	1.46±0.21[a]	0.98±0.11[a]	1.01±0.18[a]	0.87±0.14[a]	0.98±0.13[a]	0.76±0.17[a]

Table B29. Ascorbic acid concentration (mg/g FW) of limes after treated with chitosan and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Pen+chi+PP	1.69±0.00[a]	1.30±0.12[a]	1.12±0.13[a]	0.88±0.04[a]	1.01±0.10[b]	1.12±0.09[a]	1.06±0.07[a]
Pen+chi+Org/PP	1.69±0.00[a]	1.36±0.24[a]	0.98±0.10[a]	0.94±0.07[a]	0.96±0.07[ab]	1.03±0.10[a]	0.83±0.14[a]
Pen+chi+PPPCH	1.69±0.00[a]	1.25±0.17[a]	1.04±0.14[a]	0.833±0.06[a]	0.75±0.05[a]	1.00±0.13[a]	0.94±0.15[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B30. Total soluble solids (TSS) of limes in different treatments during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PP	9.10±0.06^{a}	8.25±0.14^{a}	9.13±0.92^{a}	9.13±0.31^{ab}	9.25±0.25^{b}	14.88±1.28^{a}	12.13±0.66^{a}
Org/PP	9.45±0.26^{a}	8.60±0.29^{a}	8.88±0.43^{a}	9.00±0.41^{ab}	9.00±0.35^{ab}	14.00±1.08^{a}	10.38±0.38^{a}
PPPCH	8.85±0.25^{a}	8.55±0.17^{a}	9.25±0.52^{a}	10.00±0.46^{bc}	9.13±0.31^{ab}	14.25±0.97^{a}	11.83±0.60^{a}
Pen+PP	9.45±0.13^{a}	8.13±0.13^{a}	8.63±0.13^{a}	8.88±0.31^{ab}	8.38±0.24^{a}	14.75±1.53^{a}	13.88±0.31^{a}
Pen+Org/PP	9.30±0.30^{a}	8.58±0.36^{a}	9.00±0.00^{a}	10.50±0.65^{c}	9.25±0.14^{b}	14.75±0.75^{a}	15.38±1.03^{a}
Pen+PPPCH	9.35±0.44^{a}	8.53±0.20^{a}	8.25±0.25^{a}	9.25±0.25^{ab}	9.00±0.20^{ab}	12.50±1.04^{a}	14.00±0.58^{a}
Pen+chi+PP	9.10±0.19^{a}	8.18±0.12^{a}	9.25±0.25^{a}	8.75±0.14^{a}	8.75±0.14^{ab}	13.75±1.11^{a}	10.75±3.71^{a}
Pen+chi+Org/PP	10.45±0.56^{b}	8.18±0.12^{a}	9.13±0.13^{a}	9.38±0.24^{ab}	9.00±0.20^{ab}	14.25±1.89^{a}	14.50±0.65^{a}
Pen+chi+PPPCH	9.80±0.29^{ab}	8.25±0.25^{a}	9.50±0.20^{a}	9.75±0.14^{abc}	9.38±0.31^{b}	13.88±1.69^{a}	12.00±1.08^{a}

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B31. Total phenolic concentration (mg/g FW) of limes packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PP	8.59±0.00^{a}	5.21±0.65^{ab}	4.88±1.11^{a}	4.08±0.41^{bc}	2.83±0.49^{a}	4.78±1.59^{ab}	2.98±0.20^{a}
Org/PP	8.59±0.00^{a}	4.39±1.05^{a}	3.14±0.99^{a}	1.80±0.33^{a}	2.60±0.50^{a}	6.75±1.01^{b}	3.71±0.94^{a}
PPPCH	8.59±0.00^{a}	6.40±1.30^{ab}	2.76±1.07^{a}	2.41±0.54^{abc}	3.31±0.85^{a}	3.11±1.27^{a}	3.86±1.30^{a}
Pen+PP	8.59±0.00^{a}	7.40±1.04^{ab}	2.23±0.60^{a}	2.51±0.26^{abc}	2.98±0.22^{a}	4.18±0.24^{ab}	3.78±0.32^{a}
Pen+Org/PP	8.59±0.00^{a}	8.76±1.63^{b}	4.49±1.87^{a}	4.38±1.41^{c}	3.11±0.44^{a}	5.29±0.57^{ab}	4.55±0.56^{a}
Pen+PPPCH	8.59±0.00^{a}	6.63±1.74^{ab}	2.24±0.40^{a}	2.19±0.49^{ab}	4.44±0.78^{a}	4.72±0.62^{ab}	2.91±0.96^{a}
Pen+chi+PP	8.59±0.00^{a}	3.77±0.64^{a}	2.50±0.21^{a}	2.94±0.58^{abc}	3.12±0.45^{a}	3.97±0.90^{ab}	3.92±1.16^{a}
Pen+chi+Org/PP	8.59±0.00^{a}	5.95±0.91^{ab}	3.96±1.01^{a}	3.31±0.48^{abc}	2.76±1.20^{a}	3.25±0.22^{a}	2.67±0.73^{a}
Pen+chi+PPPCH	8.59±0.00^{a}	9.29±1.83^{b}	2.81±0.99^{a}	3.07±0.27^{abc}	3.35±1.46^{a}	3.49±0.60^{a}	3.17±1.34^{a}

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B32. Total phenolic concentration (mg/g FW) of limes packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PP	8.59±0.00[a]	5.21±0.65[a]	4.88±1.11[a]	4.08±0.41[b]	2.83±0.49[a]	4.78±1.59[a]	2.98±0.20[a]
Org/PP	8.59±0.00[a]	4.39±1.05[a]	3.14±0.99[a]	1.80±0.33[a]	2.60±0.50[a]	6.75±1.01[a]	3.71±0.94[a]
PPPCH	8.59±0.00[a]	6.40±1.30[a]	2.76±1.07[a]	2.41±0.54[a]	3.31±0.85[a]	3.11±1.27[a]	3.86±1.30[a]

Table B33. Total phenolic concentration (mg/g FW) of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Pen+PP	8.59±0.00[a]	7.40±1.04[a]	2.23±0.60[a]	2.51±0.26[a]	2.98±0.22[a]	4.18±0.24[a]	3.78±0.32[a]
Pen+Org/PP	8.59±0.00[a]	8.76±1.63[a]	4.49±1.87[a]	4.38±1.41[a]	3.11±0.44[a]	5.29±0.57[a]	4.55±0.56[a]
Pen+PPPCH	8.59±0.00[a]	6.63±1.74[a]	2.24±0.40[a]	2.19±0.49[a]	4.44±0.78[a]	4.72±0.62[a]	2.91±0.96[a]

Table B34. Total phenolic concentration (mg/g FW) of limes after treated with chitosan and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Pen+chi+PP	8.59±0.00[a]	3.77±0.64[a]	2.50±0.21[a]	2.94±0.58[a]	3.12±0.45[a]	3.97±0.90[a]	3.92±1.16[a]
Pen+chi+Org/PP	8.59±0.00[a]	5.95±0.91[ab]	3.96±1.01[a]	3.31±0.48[a]	2.76±1.20[a]	3.25±0.22[a]	2.67±0.73[a]
Pen+chi+PPPCH	8.59±0.00[a]	9.29±1.83[b]	2.81±0.99[a]	3.07±0.27[a]	3.35±1.46[a]	3.49±0.60[a]	3.17±1.34[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B35. CAT activity of limes in different treatments during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PP	642.18±0.00^{a}	112.90±47.25^{ab}	396.74±131.87^{ab}	173.63±19.70^{a}	37.63±11.22^{ab}	118.22±58.66^{ab}	53.43±8.68^{a}
Org/PP	642.18±0.00^{a}	128.59±42.75^{ab}	403.88±25.96^{ab}	302.26±111.98^{a}	88.08±17.62^{bc}	49.43±13.86^{ab}	93.96±27.90^{a}
PPPCH	642.18±0.00^{a}	20.15±7.98^{a}	247.36±67.51^{ab}	594.33±111.75^{b}	32.13±22.74^{ab}	101.34±13.84^{ab}	87.84±11.70^{a}
Pen+PP	642.18±0.00^{a}	85.83±28.76^{a}	380.32±111.37^{ab}	152.33±24.74^{a}	70.19±4.95^{abc}	16.87±6.65^{a}	50.07±9.53^{a}
Pen+Org/PP	642.18±0.00^{a}	498.15±419.80^{b}	350.04±35.17^{ab}	167.55±20.69^{a}	22.60±5.54^{a}	129.89±73.32^{b}	65.90±34.17^{a}
Pen+PPPCH	642.18±0.00^{a}	52.08±8.55^{a}	285.03±75.27^{ab}	233.73±36.39^{a}	12.86±2.85^{a}	70.59±3.13^{ab}	48.31±12.29^{a}
Pen+chi+PP	642.18±0.00^{a}	24.89±9.34^{a}	459.96±27.76^{b}	266.00±91.19^{a}	105.05±34.33^{c}	47.87±15.08^{ab}	45.62±6.54^{a}
Pen+chi+Org/PP	642.18±0.00^{a}	282.47±169.07^{ab}	178.93±52.55^{a}	114.22±25.74^{a}	48.23±14.56^{abc}	34.27±14.75^{ab}	36.85±23.58^{a}
Pen+chi+PPPCH	642.18±0.00^{a}	39.75±10.56^{a}	264.85±32.91^{ab}	220.21±95.98^{a}	36.05±10.59^{ab}	47.99±11.99^{ab}	76.28±20.62^{a}

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B36. CAT activity of limes packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PP	642.18±0.00[a]	112.90±47.25[a]	396.74±131.87[a]	173.63±19.70[a]	37.63±11.22[a]	118.22±58.66[a]	53.43±8.68[a]
Org/PP	642.18±0.00[a]	128.59±42.75[a]	403.88±25.96[a]	302.26±111.98[a]	88.08±17.62[a]	49.43±13.86[a]	93.96±27.90[a]
PPPCH	642.18±0.00[a]	20.15±7.98[a]	247.36±247.36[a]	594.33±111.75[b]	32.13±22.74[a]	101.34±13.84[a]	87.84±11.70[a]

Table B37. CAT activity of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Pen+PP	642.18±0.00[a]	85.83±28.76[a]	380.32±111.37[a]	152.33±24.74[a]	70.19±4.95[b]	16.87±6.65[a]	50.07±9.53[a]
Pen+Org/PP	642.18±0.00[a]	498.15±419.80[a]	350.04±35.17[a]	167.55±20.69[a]	22.60±5.54[a]	129.89±73.32[a]	65.90±34.17[a]
Pen+PPPCH	642.18±0.00[a]	52.08±8.55[a]	285.03±75.27[a]	233.73±36.39[a]	12.86±2.85[a]	70.59±3.13[a]	48.31±12.29[a]

Table B38. CAT activity of limes after treated with chitosan and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Pen+chi+PP	642.18±0.00[a]	24.89±9.34[a]	459.96±27.76[b]	266.00±91.19[a]	105.05±34.33[a]	47.87±15.08[a]	45.62±6.54[a]
Pen+chi+Org/PP	642.18±0.00[a]	282.47±169.07[a]	178.93±52.55[a]	114.22±25.74[a]	48.23±14.56[a]	34.27±14.75[a]	36.85±23.58[a]
Pen+chi+PPPCH	642.18±0.00[a]	39.75±10.56[a]	264.85±32.91[a]	220.21±95.98[a]	36.05±10.59[a]	47.99±11.99[a]	76.28±20.62[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B39. APX activity of limes in different treatments during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PP	11030.75±0.00[a]	711.94±201.15[a]	3869.61±696.96[a]	4105.92±862.25[ab]	4236.36±1420.79[a]	464.51±33.40[a]	1666.96±351.3[a]
Org/PP	11030.75±0.00[a]	761.15±132.38[a]	3415.15±953.49[a]	2595.18±559.68[ab]	2305.96±727.84[a]	503.43±166.96[a]	1063.00±480.8[a]
PPPCH	11030.75±0.00[a]	3085.39±2146.9[a]	2642.31±442.91[a]	3764.57±1554.13[ab]	3505.62±1607.64[a]	1125.09±211.09[a]	704.44±499.15[a]
Pen+PP	11030.75±0.00[a]	1838.37±993.31[a]	5468.00±2172.22[a]	4763.59±2188.49[ab]	5486.13±788.19[a]	192.35±53.30[a]	284.65±33.47[a]
Pen+Org/PP	11030.75±0.00[a]	2201.13±993.97[a]	3736.06±445.11[a]	1177.73±308.24[a]	2539.98±493.74[a]	533.54±350.20[a]	2283.87±1258.16[a]
Pen+PPPCH	11030.75±0.00[a]	908.83±484.23[a]	3678.98±267.18[a]	2376.71±430.49[ab]	1916.61±715.47[a]	3584.85±1079.50[a]	518.02±285.82[a]
Pen+chi+PP	11030.75±0.00[a]	662.57±214.12[a]	5318.06±1531.75[a]	2324.89±448.61[ab]	3329.87±563.97[a]	1230.37±246.29[a]	2130.07±1407.8[a]
Pen+chi+Org/PP	11030.75±0.00[a]	1960.85±1595.99[a]	6154.45±1367.10[a]	2227.06±612.80[ab]	4467.63±2670.67[a]	4331.60±3674.47[a]	1379.28±631.89[a]
Pen+chi+PPPCH	11030.75±0.00[a]	1528.06±249.30[a]	3195.57±596.01[a]	5788.14±2391.51[b]	2104.23±564.41[a]	1435.34±677.17[a]	2424.51±457.07[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

Table B40. APX activity of limes packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PP	11030.75±0.00[a]	711.94±201.15[a]	3869.61±696.96[a]	4105.92±862.25[a]	4236.36±1420.79[a]	464.51±33.40[a]	1666.96±351.3[a]
Org/PP	11030.75±0.00[a]	761.15±132.38[a]	3415.15±953.49[a]	2595.18±559.68[a]	2305.96±727.84[a]	503.43±166.96[a]	1063.00±480.8[a]
PPPCH	11030.75±0.00[a]	3085.39±2146.9[a]	2642.31±442.91[a]	3764.57±1554.13[a]	3505.62±1607.64[a]	1125.09±211.09[b]	704.44±499.15[a]

Table B41. APX activity of limes after treated with *Pencillium* sp. and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Pen+PP	11030.75±0.00[a]	1838.37±993.31[a]	5468.00±2172.22[a]	4763.59±2188.49[a]	5486.13±788.19[b]	192.35±53.30[a]	284.65±33.47[a]
Pen+Org/PP	11030.75±0.00[a]	2201.13±993.97[a]	3736.06±445.11[a]	1177.73±308.24[a]	2539.98±493.74[a]	533.54±350.20[a]	2283.87±1258.16[a]
Pen+PPPCH	11030.75±0.00[a]	908.83±484.23[a]	3678.98±267.18[a]	2376.71±430.49[a]	1916.61±715.47[a]	3584.85±1079.50[b]	518.02±285.82[a]

Table B42. APX activity of limes after treated with chitosan and packed in modified polypropylene films during storage at 10ºC for 6 weeks.

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Pen+chi+PP	11030.75±0.00[a]	662.57±214.12[a]	5318.06±1531.75[a]	2324.89±448.61[a]	3329.87±563.97[a]	1230.37±246.29[a]	2130.07±1407.8[a]
Pen+chi+Org/PP	11030.75±0.00[a]	1960.85±1595.99[a]	6154.45±1367.10[a]	2227.06±612.80[a]	4467.63±2670.67[a]	4331.60±3674.47[a]	1379.28±631.89[a]
Pen+chi+PPPCH	11030.75±0.00[a]	1528.06±249.30[a]	3195.57±596.01[a]	5788.14±2391.51[a]	2104.23±564.41[a]	1435.34±677.17[a]	2424.51±457.07[a]

*The alphabet showed significant difference between treatments (in column) when compared by DMRT at P≤0.05

BIOGRAPHY

Miss Pornchan Jongsri was born on February 27, 1987 in Prachuabkirikhan province. She finished the secondary school from Princess Chulabhorn's Collage Phetchaburi in 2004. Then she got scholarship from Research Professional Development Project under the Science Achievement Scholarship of Thailand (SAST) to study in Bachelor' s Degree, majored in Biology, Faculty of Science, at Silpakorn University from 2005-2008 and . After that she continued on Master Degree in the Department of Botany, Faculty of Science, at Chulalongkorn University in 2009.