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Effect of nitric oxide on postharvest quality of lime fruit (*Citrus aurantifolia* Swingle)

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Abstract

The effect of different concentrations of nitric oxide (NO) on postharvest life of lime (*Citrus aurantifolia* Swingle) was investigated. Lime was immersed with nitric acid at concentration 0, 5, 10 and 20 $\mu\text{g/l}$ for 1 minute then kept at 4 C°. Changes in weight loss, total soluble solids (TSS), fruit firmness, ascorbic acid (vitamin C), and Chlorophyllase activity were evaluated periodically with 7 day intervals during storage. The results showed that lime immersed with 5 $\mu\text{g/l}$ SNP was significantly delayed the decrease of weight loss and firmness, also maintained the highest ascorbic acid and delayed the increase of total soluble solids contents during storage. Moreover, lime fruits treated with 20 $\mu\text{g/l}$ SNP increased more slowly Chlorophyllase activity than the other treatments. This application of NO may be a useful method for extending shelf-life and maintaining quality of lime.

Keywords : *Nitric oxide, weight loss, storage life, lime*

1. Introduction

Lime (*Citrus aurantifolia* Swingle) is an important horticulture crop in Thailand. Thousands of tons of this fruit are consumed yearly, indicating its importance in Thai economy. The production of lime is from July to September in Thailand (Pranmornkith et al., 2005) and it is mainly used for domestic consumption. It has been used both for enhancing the taste of various Thai foods and is popular for use in Thai traditional medicine. In 2552, Thailand domestic consumption of lime products

accounted for 99.48 percent of total output. Exported to foreign countries. 0.52 percent of total output. Lime has a short period life, causing a great demand during the time of scarcity. The price of lime depends on its quality characteristics. Usually, lime fruits were harvested when the rind is still green, with highly aromatic compounds. Postharvest quality of lime fruit deteriorates quickly after harvest. The most visible deterioration factor is the loss of peel greenness that usually occurs with chlorophyll (Chl) degradation (Kaewsuksaeng et al., 2011; Srilaong et al.,

2011). For the maintenance of postharvest quality, it is necessary to retain the green color in the peel of lime fruit as long as possible. The maintenance of green color in the peel of lime fruits during storage and shelf-life is required for maintaining their value prices (Pranmornkith et al., 2005).

The key issue during lime storage after harvested is short dehydration, which resulted withered. The peel will be changing from green to yellow and brown within 2 weeks at room temperature. When lime fruits are in a state of dehydration, the ethylene biosynthesis will be increasing rapidly from respiration rate. Ethylene plays a role to catalyst of enzymes involved in ripening and aging such as chlorophyllase, which is broken down chlorophyll and pectin methylesterase resulting to soften and discoloration of lime. The chlorophyll degradation stimulated by ethylene, the green substance becomes a colorless. It is yellow carotene carotenoids substances which have been shown when the green of chlorophyll degrading. In addition, the soluble solids content (total soluble solids: TSS) of lime will increase, while vitamin C will decrease after the harvest.

The shelf life of lime fruits, it depends on the physiological and biochemical processes, as well as the environment after harvest. Nitric oxide (NO) is a highly reactive free radical gas, a low concentration of NO gas has been shown to extend the postharvest life of various intact fresh fruits and vegetables (Zhu et al., 2007; Zhu and Zhou, 2007). In addition, nitric oxide inhibits the synthesis of ethylene, reduce respiratory rate and 1- aminocyclopropane-1-carboxylic acid (ACC) activity. The aim of this work was to investigate the effects of nitric oxide on postharvest quality of lime fruits.

2. Material and Methods

2.1 Sample preparation

The lime fruits were obtained from a market (Talaad Thai) located at Khlong Luang, Pathum Thani, Thailand. After transportation to the laboratory, lime fruits were selected for uniformity in maturity, size, shape, peel color and lack of defects.

2.2 Nitric oxide (NO) treatments and experimental design

Sodium nitroprusside (SNP), the donor of NO, was purchased from Merck Co (Darmstadt, Germany). Lime fruits were immersed with 5, 10 and 20 $\mu\text{g/l}$ SNP aqueous solutions for 1 minute at room temperature then allowed to dry. After treated, the fruits were kept in polyethylene (PE) bags with holes 4 holes (diameter of the hole is equal to 1 cm) and stored at 4°C. Control lime fruits were immersed with distilled water for 1 minute. The experiment was completely randomized with 4 treatments, 18 lime fruits of each treatment. Each treatment was repeated thrice. Determinations were carried out after 7 to 35 days after the beginning of storage. The visual color was determined daily during the storage period. The fresh weight of each lime fruit was monitored every 7 days and data were expressed as percent of weight loss.

Determination of fruit firmness. The firmness of fruit was evaluated by using a texture analyzer with 1 cm diameter conical head thrust deep into lime fruit 0.5 cm to determine firmness, then means were expressed as kilograms per square centimeter.

Determination of total soluble solids (TSS). This is a measure of the total soluble solids in the juice. These soluble solids are

primarily sugars; sucrose, fructose, and glucose. Lime juice was squeezed very carefully to avoid any contamination and screened through a fine cloth. The fine suspended pulp was removed by centrifuging the juice. The total soluble solids (TSS) were determined with a hand refractometer reported as “degrees Brix”. For vitamin C was determined titrimetrically using 2,6-dichlorophenolindophenol (AOAC, 2000).

Determination of enzyme activity. For the enzyme assays, lime fruits 5 g was grounded with 50 ml of cold acetone concentrations of 80% for five minutes and then filtered on a Buchner funnel with paper filter Whatman No.1. The residue was washed with cold diethyl ether until to white residue then dried at room temperature become to acetone powder. Chlorophyllase enzyme was extracted from acetone powder followed Yamauchi (1991). The resulting supernatants were used for the determination of enzymatic activity, it was spectrophotometrically detected by chlorophyll A formation at 663 nm per unit per mg protein.

The statistical analyses were performed using SAS 9.1.3 and the means were compared by Duncan’s multiple range test.

3. Results and Discussion

The quality of limes differed according to the treatment applied. Weight loss of lime increased throughout the period of storage and dramatically increases the range of 14 and 21 days after storage (Figure 1), a statistically significant difference. Weight loss in the control treatment (distilled water) was greater than the other treatment (Figure 1) which was clearly observed after 21 days of storage.

During 28 days after storage, the concentration of SNP at 5 μ g/l showed minimal weight loss as 3.80%, following the concentration of SNP at 10 and 20 μ g/l as 4.40% and 4.50%, respectively. While, the maximum weight loss obtained from the control treatment of 5.10% (Figure 1). For 35 days after storage, weight loss of the control and SNP treated fruit increased. Lime fruit immersed with SNP concentration of 5 μ g/l showed minimal weight loss as 4.80% when compared with the control as 6.67%, which no significantly different statistically with lime fruit treated with SNP concentration of 10 μ g/l as 6.70% and 20 μ g/l as 6.20%, respectively (Figure 1). The results indicated that SNP had an effect by decreasing weight loss of lime fruits by reducing water loss in limes. This result similar to Nipa (2541) demonstrated that coating lemon surface reduces the rate of dehydration. The coating makes the surface to be protected and covered the openings nature or the baptist cells (lenticel) as a result, less water loss (Danai, 2540). In addition, The NO had an affects to control the ripening of Strawberries by reducing the production of ethylene, reduced respiratory rate and the activity of the enzyme (ACC) (Shuhua el at., 2007).

The firmness of lime, there is increasing trend over the period of storage in all treatments (Figure 2). Before storage, an average of the firmness was 0.60 to 0.80 kilograms per square centimeter. After 35 days of fruit storage, the firmness of lime decrease was 0.5 to 0.6 kilograms per square centimeter. All SNP immersed fruit did not show any significant difference in fruit firmness as compared to the control (Figure 2). SNO immersed treatments at 5 μ g/l resulted in a minimal rate of firmness as 0.6 kilograms per square. The reduction

in firmness may be ascribed to reduced activities of fruit softening enzymes induced with the suppression of ethylene production in NO-treated fruit. Similarly, Khan and Singh (2008) reported that increased activities of fruit softening enzymes such as pectin esterase, endoglucanase, endo - and exo-polygalacturonase were associated with increased climacteric ethylene production during plum fruit ripening. NO fumigation of plum and banana slices with 1 and 5mMNO, respectively, has been retarded fruit softening during storage and ripening delays in fruit ripening by 3 and 4 days at $21\pm 1^\circ\text{C}$ (Yong-Sheng et al., 2008; Cheng et al., 2009).

Total Soluble Solids (TSS) was determined by hand refractometer and expressed as Brix percentage. In the initial phase and final phase of storage, SNP immersed treatments at 10 $\mu\text{g/l}$ resulted in minimal of TSS as 7.5% (Figure 3). SNP immersed fruit of concentration 5 $\mu\text{g/l}$, 10 and control did not show any significant difference in TSS as 7.8, 8.0 and 8.2, respectively after storage 35 days (Figure 3). This results according to Wills et al. (2006) demonstrated that nitric oxide can inhibit the loss of tissue and decreased levels of Total soluble solids including, vitamin C inhibits its activity polyphenoloxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL). The amount of phenolic compounds indicates the amount of lime substances which necessary for respiration. They were used as energy and the various functions. Therefore, the total soluble solids decrease in breathing process (Saichol, 2528; Ting et al., 1980). In citrus fruits sugars and acids constitute approximately 85% of the organic food reserves (Wardowski et al.,

1979). The sugars and acids, together with small amounts of dissolved vitamins, proteins, pigments and minerals, are commonly referred as "soluble solids". The term Brix is used interchangeably with "total soluble solids". In Lime, however, sugars constitute only 25% of the soluble solids the remaining being citric and malic acid. Differences in sugars concentration and proportions are also greatly influenced by rootstock, geographical location, weather and cultural practices. The concentration of TSS varies considerably during fruit development.

The highly total ascorbic acid (Vitamin C) found in control treatment at 43.15 mg, following the lime treated with SNP concentration of 10, 20 and 5 $\mu\text{g/l}$ at 42.78, 30.20 and 28.81 mg, respectively at 7 days after storage (Figure 4). After 35 days of storage, lime immersed with SNP concentration of 10, 20 and 5 $\mu\text{g/l}$ showed high total ascorbic acid as 81.41, 79.56 and 78.11 mg, respectively, while, the low total ascorbic acid as 71.39 mg obtained from control (Figure 4). Similarly to Zhang et al. (2007) found that NO treatment maintained high levels of ascorbic acid and decreased the activity of antioxidant enzymes in longan. This result showed NO immersion seems to be influencing the organic acid metabolism in lime fruit during storage period. Usually, the fruits coated will have less water loss because allows gas exchange by natural opening, so it is chemical little from the breathing process (Zaharah et al., 2011). The content of total ascorbic acid increasing, It may have been caused by oxidation with many enzymes, such as ascorbic oxidase, polyphenol oxidase and peroxidase, also was catalyzed by heavy metals such as copper. Moreover, the wound cause damage to fruit, resulting in a loss of

water, the texture and chemical reaction changes, accelerate the oxidation of ascorbic acid causing the loss of vitamin C rapidly. This may affect on the quality of the produce (Danai, 2540).

SNP treatment delayed the increase of Chlorophyllase activity in limes as shown in Figure 5. Chlorophyllase activity in lime treated with NO increased during storage, but less than the control. The lime fruit treated with SNP concentrate at 20 ug/l had a lower activity of Chlorophyllase enzymes than other treatments and control fruit from beginning to end storage. The Chlorophyllase activity increased in the control treatment throughout the storage time and remained high at the end of storage. Lime fruits have changed color from green to yellow during storage (Figure 6). Changing the color of lime is mainly due to the use of ethylene to stimulate the decomposition of chlorophyll (Barmore, 1975). The results, according to Dominguez et al. (1993), found that the decomposition of chlorophyll is influenced by the production of ethylene through the intermediary of chlorophyllase enzyme system to synthetic of enzyme chlorophyllase.

Trebitsh (1993) found that the biosynthetic enzymes chlorophyllase. The ethylene to stimulate faster in the process of translation. Jacob-Wilk et al. (1999) reported that mRNA of chlorophyllase enzyme increase after treated with ethylene for three hours.

4. Results

The effect of difference concentration of NO as a safe compound, derived from sodium nitroprusside (SNP) on postharvest quality of lime fruit found that the lime fruits treated with 5 ug/l SNP have lowest weight loss and firmness at the end of storage. The highly total soluble solid (TSS) and ascorbic acid was detected from lime fruits immersed with SNP concentration at 5 ug/l. Lime fruits treated with 20 ug/l SNP increased more slowly Chlorophyllase activity than the other treatments. The findings obtained in the present study show that NO treatment effectively on postharvest quality and could be a useful method to prolong the postharvest quality of Thai lime fruit during storage.

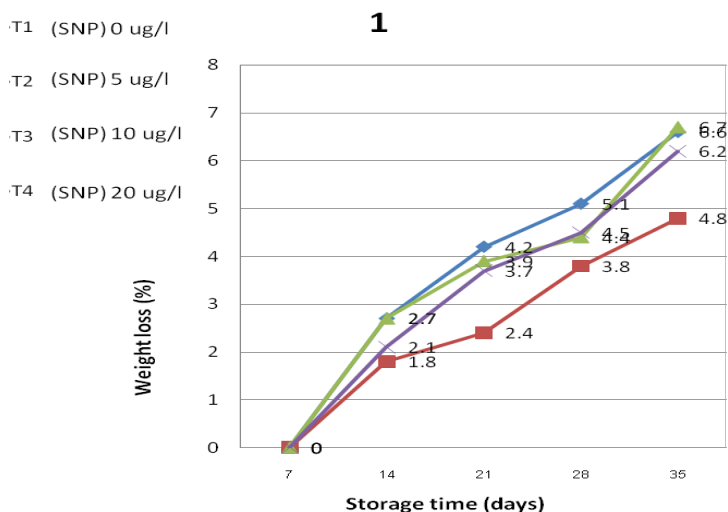


Figure 1. Weight loss of lime fruits treated with different concentration of SNP for 1 minute. T1= control , T2= 5ug/l SNP, T3= 10ug/l SNP and T4= 20ug/l SNP

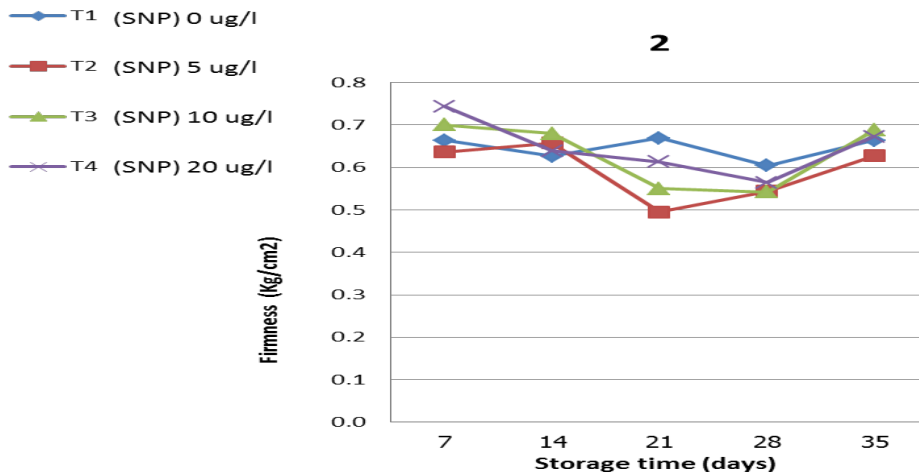


Figure 2. Firmness of lime fruits treated with different concentration of SNP for 1 minute. T1= control , T2= 5ug/l SNP, T3= 10ug/l SNP and T4= 20ug/l SNP

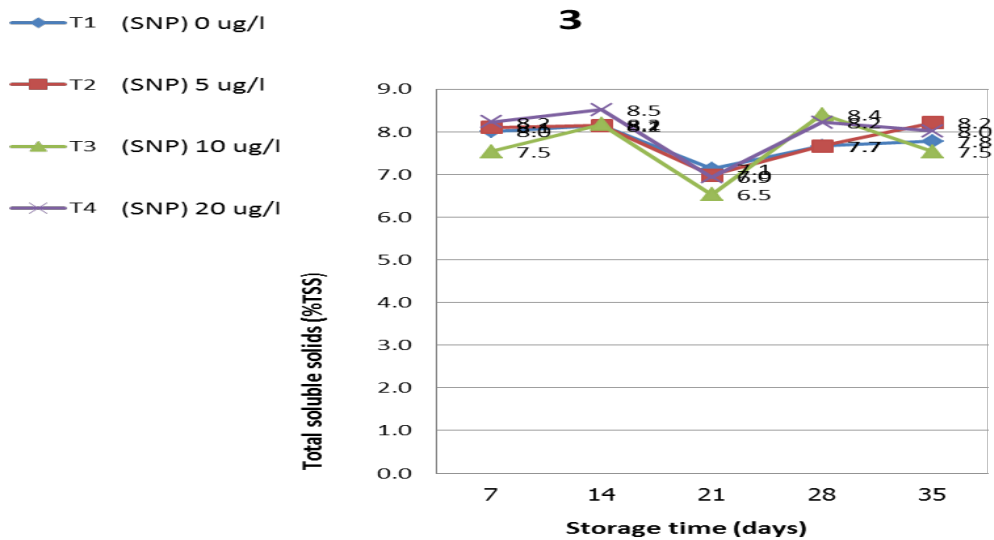


Figure 3. Total soluble solids of lime fruits treated with different concentration of SNP for 1 minute. T1= control , T2= 5ug/l SNP, T3= 10ug/l SNP and T4= 20ug/l SNP

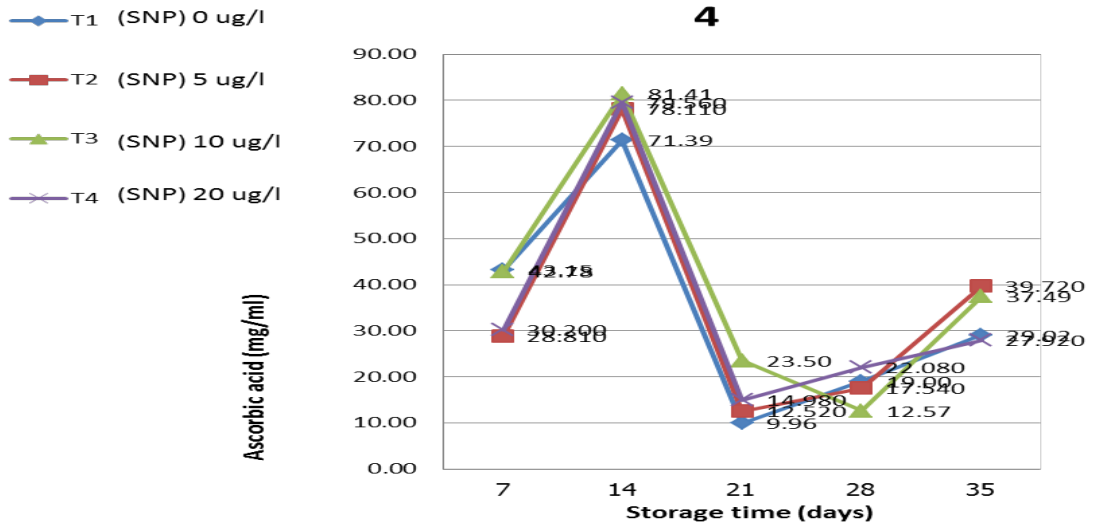


Figure 4. Ascorbic acid of lime fruits treated with different concentration of SNP for 1 minute. T1= control , T2= 5ug/l SNP, T3= 10ug/l SNP and T4= 20ug/l SNP

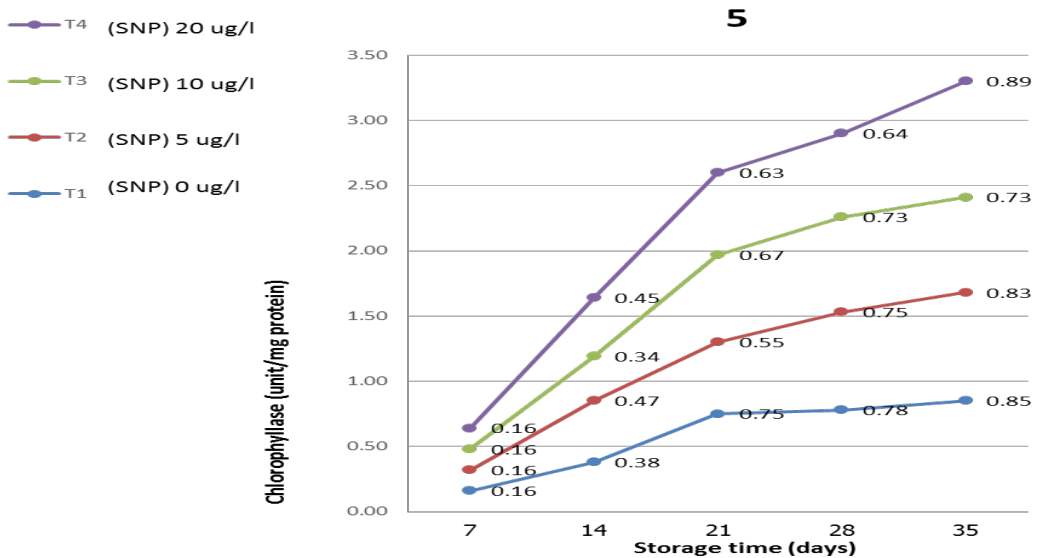


Figure 5. Chlorophyllase activity of lime fruits treated with different concentration of SNP for 1 minute. T1= control , T2= 5ug/l SNP, T3= 10ug/l SNP and T4= 20ug/l SNP



Figure 6. Lime fruits immersed with different concentration of SNP for 1 minute kept at 4°C during. A, B = Lime fruits treated with concentration of 0 (Control) and 5 ug/l SNP storage 7 days. C, D = Lime fruits treated with concentration of 10 ug/l and 20 ug/l SNP storage 14 days. E, F= Lime fruits treated with concentration of 0,5 ,10 and 20 ug/l SNP storage 21 days. H, I= Lime fruits treated with concentration of 0 and 5 ug/l SNP storage 28 days. J, K= Lime fruits treated with concentration of 10 and 20 ug/l SNP storage 35 days.

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