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β -Cryptoxanthin and Zeaxanthin Pigments Accumulation to Induce Orange Color on Citrus Fruits

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Abstract. Degreening, a transformation process of green color on citrus peel to be orange color on tropical low-land citrus fruits often fails. Orange color of the citrus peel comes from the mixture carotenoid pigments, such as zeaxanthine and mainly β -cryptoxanthin and β -citaurin. The accumulation of β -citaurin occurs when the fruits are exposed to low temperature, and otherwise, it will fail to occur. Precooling treatment on lowland tropical citrus fruits is expected to stimulate the accumulation of β -citaurin. The results showed the most favorable color obtained from precooling and 24-hour ethylene exposure duration. This treatment could decrease total chlorophyll and β -carotene content as well as proven to increase 3 times the accumulation of β -cryptoxanthin in accelerating the appearance of bright orange color on citrus peel. Degreening gave no significant effect to internal quality of *Citrus reticulata*.

Keywords: *Citrus reticulata*; degreening; β -cryptoxanthin; zeaxanthin; β -carotene; Citrus color Index

1. Introduction

The increasing public awareness of the nutritional value effects on the increasing demand for citrus fruits. This increase led to importing large amounts of fresh citrus, which made Indonesia as the second largest importer in ASEAN after Malaysia. Increasing consumer demand for imported citrus fruits was due to consumer interest in the fruit appearance and the taste of orange citrus instead of non-uniform citrus (yellowish green). [15] and [7] reported that 85% of respondents in Bogor chose to buy imported citrus, while 15% of respondents preferred to buy local ones. This decision was determined by the attractive appearance of imported citrus fruits compared to local fruits. Another existing problems are



very low availability of orange *Citrus reculata* and low yield which could not meet the demand. Therefore, this research is needed to improve color quality to make it more consumer-attractive/orange.

Efforts to increase the quantity and quality of citrus fruits are needed to meet the market demand. One of the efforts to meet the consumer favorable taste is the formation of orange color on citrus fruits. Therefore, degreening treatment with ethylene should be done after harvesting. Degreening is the destruction process of green color on citrus peel with orange color. Degreening so far can not stimulate the formation of orange color because the fruits only managed to turn into yellow. Yellow-colored fruits are considered unripe even almost rotten so that it is less desired by consumers. Yellow-colored fruit peel formed by accumulation of cryptoxanthin [5, 2, 19], orange-colored fruit peel comes from the mixture of β -cryptoxanthin with β -citraurin on carotenoids. Pigments contributed in the formation of β -citraurin is β -cryptoxanthin and zeaxanthin [17, 3, 13].

The research regarding the transformation of pigments on citrus peel in Indonesia has never been done before. According to the literature mentioned above, the failure of degreening in tropical regions to form orange color due to the temperature which β -citraurin will be activated, which is at 18 – 24 °C. Citrus tree planted in different elevation will be exposed to different temperature level during growth. Precooling treatment right after harvest is expected to substitute low temperature not found during its growth time. It is expected to induce the β -citraurin and β -cryptoxanthin synthesis, because once harvested, fruit metabolisms persist.

Basically, the role of ethylene hormones to stimulate the rapid degreening process in color change is enormous [18]. Ethylene is a substance commonly used in degreening citrus fruits [13, 19, 9, 10]. [12, 11] reported that ethylene has been shown to play a role in reducing chlorophyll content in fruit peel, causing discoloration of citrus peel from green to orange

Therefore, a degreening technology should be developed to induce orange color formation, as well as the identification of β -cryptoxanthin and zeaxanthin on carotenoids as the agent of orange color formation. Here, we reported the effect of precooling and precise ethylene exposure duration in the formation of orange color in citrus peels, and the changes of β -cryptoxanthin, zeaxanthin, and β -carotene pigments content in citrus peel before and after degreening.

2. Research Methodology

2.1 The degreening treatment and fruit quality observation were done at PKHT IPB and Center for Biological Research-LIPI Cibinong. The research took place from January 2016 to July 2017.

The materials used were citrus fruits and ethylene gas, *Citrus reticulata* “tejakula”. The tools used consists of refractometer, degreening containers, cool storage, syringe, colorreader, HPLC (High Performance Liquid Chromatography), spectrophotometry, and chromatography.

Randomized Factorial Block Design was used as the research design. The treatment consists of 2 factors, precooling treatment and exposure duration. There were 2 levels of precooling factor that is precooling and without precooling. There were 3 levels of exposure duration factor, without exposure, 24 hour-exposure, and 48-hour exposure. As a result, there were 6 combinations repeated 3 times and 18 units of experiment.

Citrus reticulata “tejakula” was obtained from smallholdings in Tuban, East Java. The estates located in lowland region at 40 m AMSL. The fruits were distributed to Laboratory of PKHT IPB Bogor. Fruits were placed into degreening containers then tightly sealed and kept airtight. Ethylene gas at 100 ppm concentration was injected into the degreening containers using 5 ml-syringe. The containers then put in cold storage at 15 °C. The fans were turned on during the degreening treatment, for control, 24 hours, and 48 hours. The fruits then were inspected and the color changes were observed.

2.2 Hue Angle identification

Changes in the color quality of citrus peel was measured with Minolta Color Reader type 310. This tool color notation system is hunter notation system (color system L, a, and b). Hunter color notation system is characterized by three parameters of color, the brightness symbolized by L, the

chromatic colors symbolized by ‘a’ notation and color intensity with the notation ‘b’ (1). The measurement results were expressed in the hue angle. The results of the measurements of a and b values are converted into chromatic units of hue angle ($^{\circ}\text{hue}$). The $^{\circ}\text{hue}$ angle describes the pure color which indicates the dominant color in a mixture of several colors. According to [8], $^{\circ}\text{hue}$ can be obtained using the following equation:

$$^{\circ}\text{hue} = \tan^{-1} \left(\frac{b}{a} \right)$$

2.3 Material Preparation

Citrus peels are separated from the edible portion, then immediately cooled with nitrogen liquid in the dewar tube until next step.

Carotenoid Extraction

The identification of carotenoids was performed by the method described by Kato *et al* (2004). The pigment was extracted from the sample with hexane solution: acetone: ethanol (2:1:1, v/v) containing 0.1% (w/v) 2,6 di-tert butyl 4 methylphenol and 10% (w/v) magnesium carbonate. After the organic solvent evaporates entirely, the carotenoid-containing extract, which is esterified into fatty acid, is disacrated with 20% (w/v) methanolic KOH. Water-soluble extracts are removed by saturated NaCl. The pigments were partitioned in the diethyl ether phase and evaporated.

2.4 Analysis of β -cryptoxanthin, zeaxanthin and β -carotene

The residue is redissolved in methyl tert butyl ether solution: methanol (4:6, v/v). An aliquot (30 μL) is separated by an HPLC reversed phase (Shimadzu, Ascentis) equipped with type C-18, at a 1 mL min^{-1} flow rate. The standards of β -cryptoxanthin, zeaxanthin and β -carotene were prepared based on the method described with fresh weight micrograms per gram (preparing for calibration of the standard). Quantification of β -cryptoxanthin, zeaxanthin and β -carotene was performed in 3 replications. The data were obtained from average HPLC values and calculated using the calibration equation. With standard pigments (Figure 1).

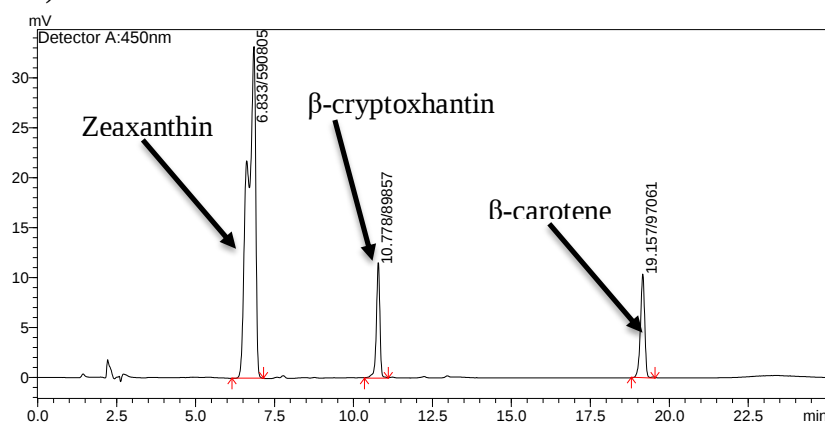


Figure 1. HPLC chromatogram for mixture of authentic sample of Zeaxanthin β -cryptoxanthin, and β -carotene.

3. Results and Discussions

There were two different experiments conducted in this research: to observe the effect of precooling and ethylene exposure duration on the change of citrus *reticulata* color. The effect of precooling and exposure duration to *Citrus reticulata* of Tuban origin showed the best color change was on the duration of 24 hours exposure with precooling and degreening treatment. Citrus peel color with precooling treatment on 24-hour ethylene exposure resulted in orange peel color, whereas without precooling showed a yellowish orange color (Figure 2).

Citrus reticulata “tejakula” with precooling and degreening treatment on 24-hour ethylene exposure duration showed uniform orange color, whereas the control fruits (without precooling and degreening) showed the fruits color stayed green (Figure 3).

Total soluble solids and total acid

The measurement for measuring citrus sweetness level is expressed in the content of total soluble solids. Based on the precooling treatment and ethylene exposure duration at the final observation, it was shown that the treatments gave no significant effects to total soluble solids, total acid and citrus firmness (the data is not shown).

Hue Value

The decrease in Hue value indicates that there is a change of color from green to yellow to orange. Orange color on citrus peel was shown with Hue ≤ 71.5 . Hue ($^{\circ}$ Hue) is the conversion result of a and b values and becomes an important parameter in the citrus measurement method (6).

Citrus reticulata “tejakula” treated with precooling treatment with degreening on 24-hour exposure duration on day 9 after treatment showed rapid decrease in hue value compared with other treatments, with a value of 68.07. Hue values with treatment without precooling on 24-hour exposure duration on day 12 showed a decrease in hue value compared to control and 48-hour exposure duration, with a value of 68.11 (Figure 4).

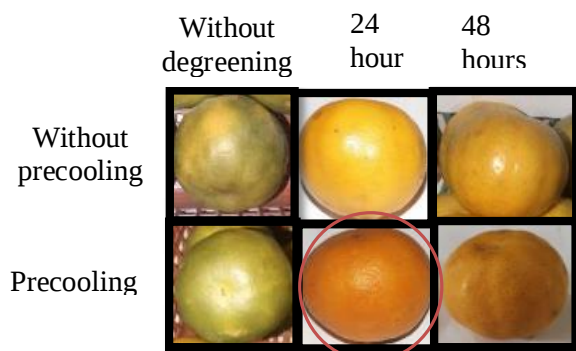


Figure 2. The change of *Citrus reticulata* “tejakula” peel color with degreening treatment on several ethylene exposure durations (9 days after treatment)

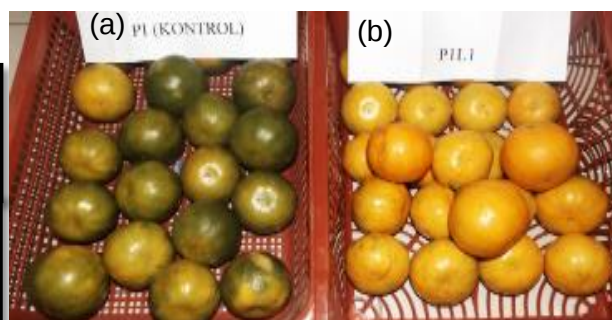


Figure 3. The color of *Citrus reticulata* “tejakula” peel, precooling without degreening (a), precooling with degreening (b) 24-hour ethylene exposure duration (9 days after treatment)

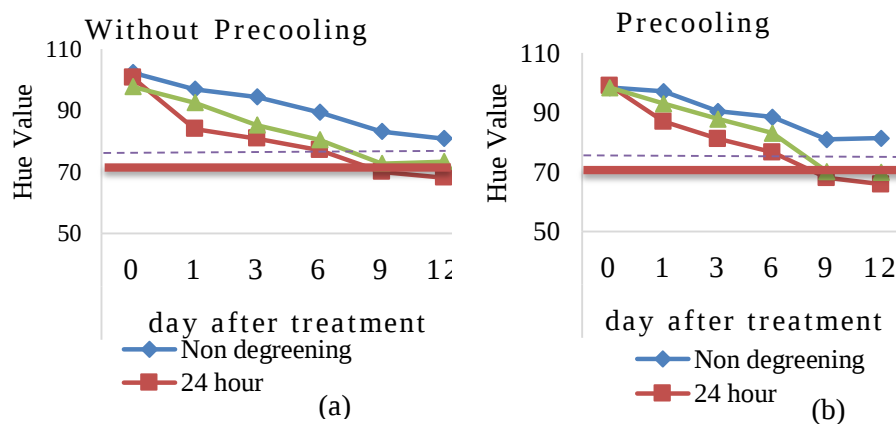


Figure 4. The trend of Hue Value changes of *Citrus reticulata* “tejakula” peel color on degreening without precooling treatment (a) and with precooling (b) at several exposure duration

Identification of β -Carotene, β -Cryptoxanthin and Zeaxanthin

Carotenoid biosynthesis contributes to the increase of citrus color from green to orange, the amount of β -cryptoxanthin and zeaxanthin content increase while chlorophyll and β -carotene content decrease during storage and these changes are accelerated by precooling and degreening applications with 4-hour exposure duration in *Citrus reticulata* “tejakula”. The HPLC chromatograms for β -carotene, β -cryptoxanthin and zeaxanthin pigments are shown below (Figure 5 and 6).

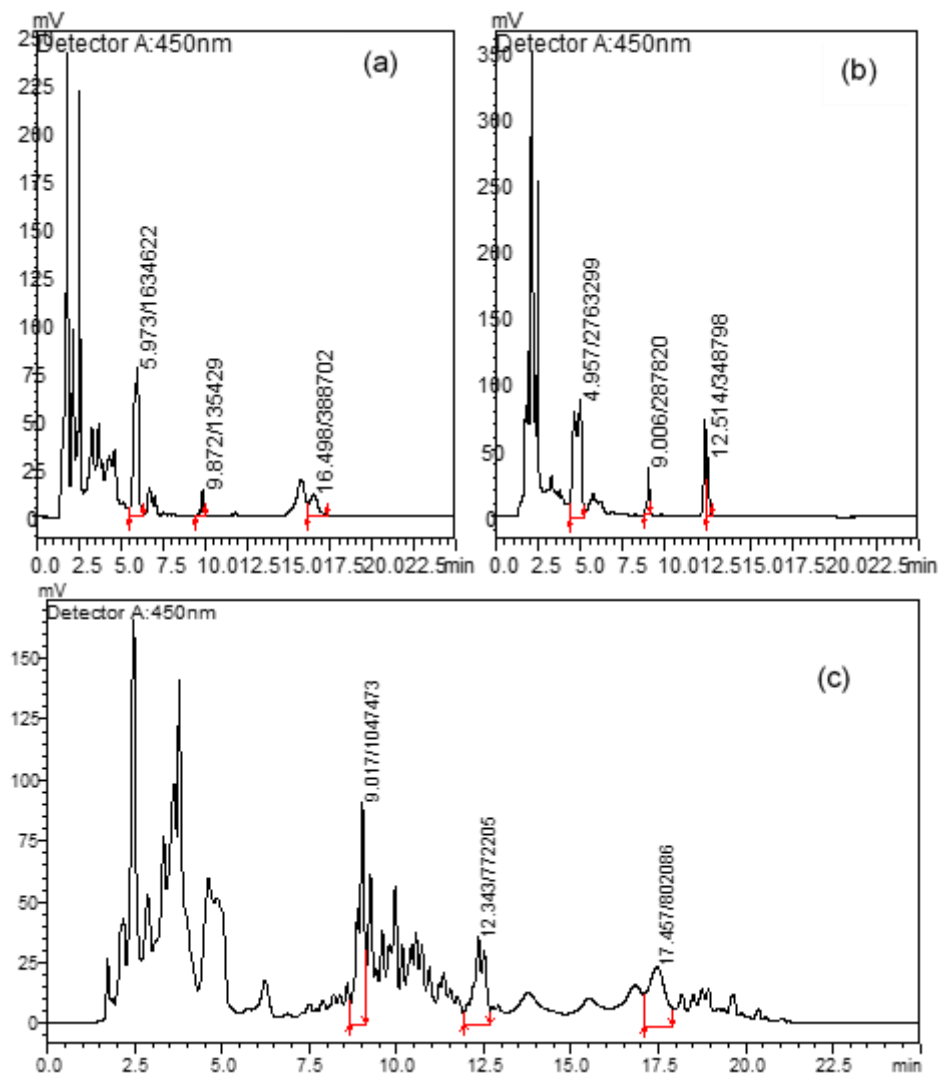


Figure 5. HPLC chromatograms for identification of zeaxanthin, β -cryptoxanthin, and β -carotene pigment of reticulata (keprok tejakula) without precooling at harvest time (before treatment) (a), without degreening (9 days after treatment), and with degreening without precooling (9 days after treatment).

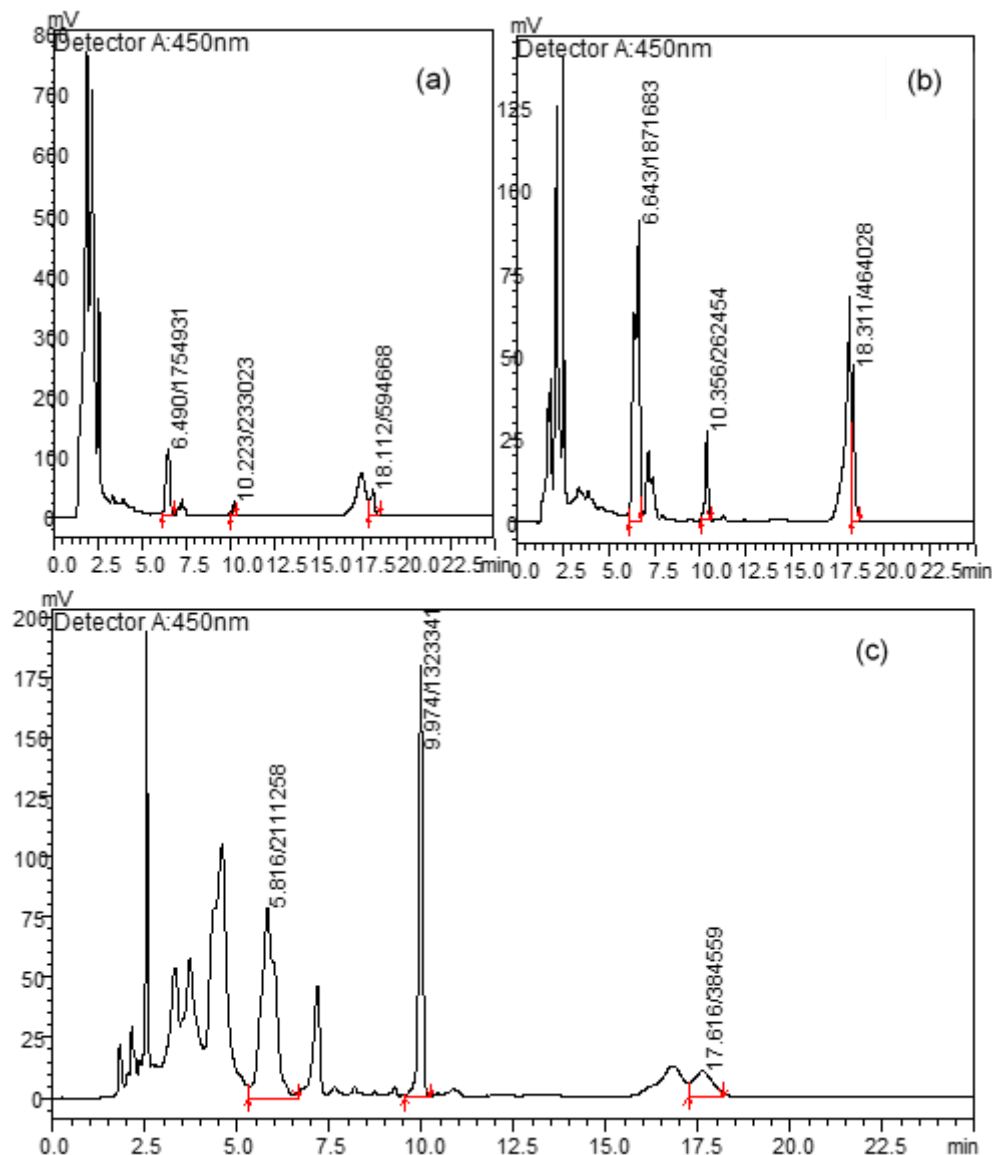


Figure 6. HPLC chromatograms for identification of *zeaxanthin*, *β -cryptoxanthin*, and *β -carotene* pigment of *reticulata* (keprokajakula) with precooling at harvest time (before treatment) (a), without degreening (9 days after treatment), dan precooling with degreening (9 days after treatment).

The value of β -cryptoxanthin content in citrus peel with precooling without degreening treatment before treatment was $1.93 \text{ ug g FW}^{-1}$, while treatment of precooling and degreening could significantly increase the β -cryptoxanthin content to $37.16 \text{ ug g FW}^{-1}$ on day-9 after treatment. The content of β -cryptoxanthin in citrus without precooling also increased but only $6.35 \text{ ug g FW}^{-1}$ (Figure 7). [4, 13] also observed similar results that the orange peel color changed from green to orange due to the nonphotosintetic characteristic of carotenoid synthesis, which is β -citaurin which causes reddish orange color on Mandarin citrus peel. The accumulation of these compounds is determined by the availability of photosynthetic carotenoid precursor such as zeaxanthin, β -cryptoxanthin, nonphotosintetic carotenoids (β -citaurin) which occur only at low temperatures.

The results showed that the formation of orange color on citrus peel required precooling treatment prior to degreening treatment. Without precooling, degreening of *Citrus reticulata* “tejakula” would be slower. Precooling was needed because β -citaurin could be induced only at low temperature. The content of β -carotene on citrus peel after precooling and degreening treatment sowed rapid decrease

compare to the content of β -carotene on citrus peel without precooling. Before degreening treatment, the content of β -carotene was 5.32 $\mu\text{g g FW}^{-1}$, while 9 days after precooling treatment and 24-hour ethylene exposure duration caused the content to decrease to 44.82 $\mu\text{g g FW}^{-1}$.

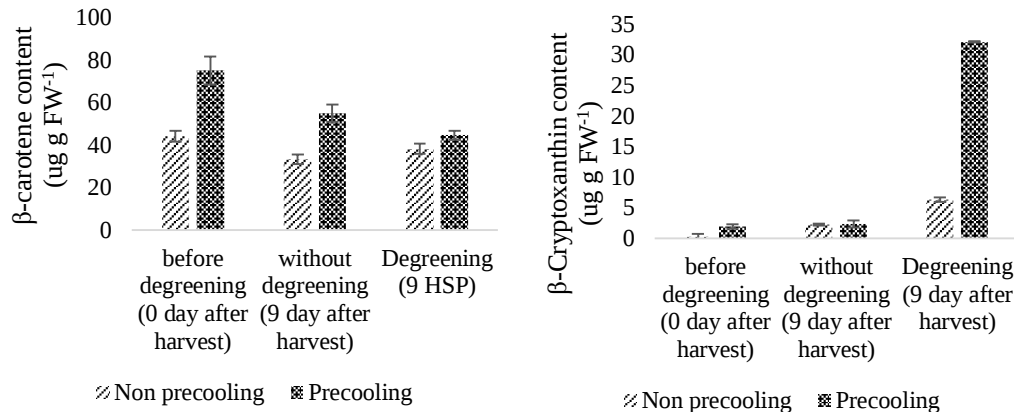


Figure 7. The change of β -carotene and β -Cryptoxanthin accumulation in citrus peel with degreening and 24-hour exposure duration

The value of zeaxanthin content on precooling and degreening treatment with 24-hour exposure duration gave result 37.65 $\mu\text{g g FW}^{-1}$ on day-9 after treatment. The value of zeaxanthin treatment content without degreening showed a lower value, with a value of 12.22 $\mu\text{g g FW}^{-1}$. While the zeaxanthin value at initial condition before degreening without precooling (26.96 $\mu\text{g g FW}^{-1}$), with precooling (27.88 $\mu\text{g g FW}^{-1}$) can be seen in Figure 8.

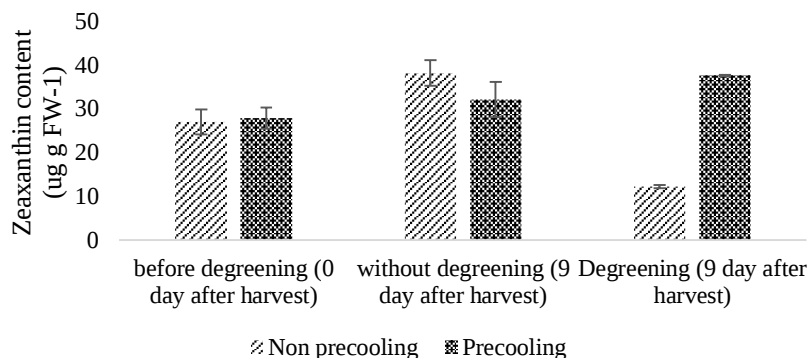


Figure 8. The change of Zeaxanthin accumulation on citrus peel with degreening 24-hour exposure duration

4. Conclusions

The most favorable result was obtained from precooling and 24-hour ethylene exposure duration. This treatment could decrease the β -carotene content and was proven to increase 3 times the content of β -cryptoxanthin pigment to accelerate the formation of citrus peel to turn orange. Degreening treatment gave no significant effect to internal quality of citrus peel.

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