The Effect of Different Concentrations and Exposure Durations of Ethylene Gas on Peel Colour Change of Citrus (Citrus nobilis) in the De-greening Process

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Abstract. De-greening using ethylene gas is one of the methods to change the peel colour of an in-attractive fruit appearance. Therefore, this research aims to investigate the effect of ethylene concentration and exposure duration on the peel colour changes of Citrus. Ethylene concentrations of 0, 50, 100, and 150 ppm and exposure durations of 24, 48, and 72 h were researched in factorial design 4 x 3 with three replications. Green ripe Citruses were cleaned and selected as the samples which were treated with ethylene gas in a closed box, then removed and stored in the open room until the peel changed uniformly to orange. The colours were measured using a colourimeter (3nh, SC-10) every 12 h for 4 days. It was found that as the storage time passed, the redness value turned from negative to positive, while yellowness increased consistently. Statistical analysis indicated that ethylene concentrations and exposure durations had a significant effect on redness, yellowness, chroma, and hue angle. The largest value of the observed parameter was found at 150 ppm concentration and 72 h exposure duration. This de-greening also altered the Colour Index value from -5.51 to 6.77.

Keywords: de-greening, ethylene, concentration, exposure duration, and colour.

1. Introduction

Colour is one of the most important qualities of fresh fruits and vegetables. Furthermore, the visual quality encompasses a product’s appearance [1] such as in Citrus fruits that have in-attractive colour. Hence, some efforts are needed to increase their appearance to attract consumers’ demand. De-greening is a process of chlorophyll degradation or destruction and carotenoids formation in Citrus peels. This is carried out chemically for taste improvement and reduction in juice acidity [2]. It is a necessary method especially for agricultural products which have in-attractive peel colour despite being ripe. Musdalifah et al. [3] reported that consumers do not mind spending more money on Citrus that have orange peels compared to green ones. The de-greening process is usually carried out by exposing the products to ethylene gas (C₂H₄) at a certain concentration and duration. This accelerates external colour development and renders the fruit more attractive [4]. However, de-greening has not yet been widely applied to fruits in Indonesia. Citrus nobilis is one of the famous local Citrus in the country, but the
appearance is not attractive, as the peel appears green while ripened. De-greening has the potential to be applied in this case to increase consumers’ demand. Porat [4], stated that de-greening treatments application is especially important for early varieties to extend the marketing season, and for fruit grown in warm tropical climates where natural colour development is late and weak. The improvement of visual appearance by this process leads to scarification of other fruit quality attributes, such as shorter shelf life, increased susceptibility to decay, enhanced weight loss, and acceleration of rind and calyx senescence. Therefore, further investigation on ethylene application during de-greening is needed to determine the best method, especially related to concentration levels and exposure durations. This research aims to investigate the effect of ethylene concentrations and exposure durations on the peel colour change of Citrus during ripening.

2. Research Methods

2.1. Materials

The main materials used were green ripened tangerine Citrus (Citrus nobilis) bought from the local market at Yogyakarta, Indonesia, which were transported to the laboratory, cleaned and then carefully selected. The ones used as samples were homogeneous in shape, size, colour as well as free from defects and diseases. Based on CIELAB colour space, the initial colour attributes of these samples were lightness (L*) 39.46, redness (a*) -4.20, yellowness (b*) 19.33, Citrus Colour Index (CCI) -5.51, hue angle (h°) 102.26°, and chroma (C*) 19.78 which were all determined from five replications. Visually, these samples were still green despite being ripened. Closed thick plastic containers (25x17.5x8cm) capable of holding 8 fruits, also equipped with a small hole and rubber tip on the top cover were prepared for applying ethylene in the de-greening process.

2.2. Experimental procedures

Selected Citrus samples were loaded into the prepared plastic containers and the desired ethylene concentrations were injected through the cover tip using a syringe and then kept for certain exposure hours. The applied concentrations were 0 ppm (control), 50 ppm, 100 ppm, and 150 ppm, while the exposure durations were 24 h, 48 h, and 72 h. After the exposure, the samples were removed and then stored in other opened plastic containers at room temperature. The peel colours were measured daily for 4 days of observation using a handheld digital colourimeter (3nh, Sucolor, SC-10) and three replications were used for each treatment combination.

2.3. Expression of fruit colour

The Citrus colour was determined based on CIELAB colour space with the parameters of L*, a*, and b*. For every sample’s peel, the colour was taken at 3 different positions on the equator and the average values were used in the data analysis. From these, some other colour parameters’ values including CCI [5], hue angle, and chroma were then calculated [6].

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\begin{align*}
\text{CCI} & = \frac{1000 \cdot \text{a*}}{\text{L*}} \cdot \text{b*} \quad \text{…………………………… (1)} \\
\text{h°} & = 180 + \tan^{-1} \left( \frac{\text{b*}}{\text{a*}} \right) \quad \text{if a*} < 0 \text{ and b*} > 0 \quad \text{……………… (2)} \\
\text{h°} & = \tan^{-1} \left( \frac{\text{b*}}{\text{a*}} \right) \quad \text{if a*} > 0 \text{ and b*} \geq 0 \quad \text{……………. (3)} \\
\text{C*} & = \left( \text{a*}^2 + \text{b*}^2 \right)^{1/2} \quad \text{……………………………… (4)} 
\end{align*}
\]

2.4. Statistical analysis

The values of L*, a*, b*, CCI, h°, and C* were subjected to three-way repeated measure analysis of variance (ANOVA) using IBM-SPSS ver. 26 software. The first factor was the storage time, the second was ethylene concentrations and the third was exposure duration. Subsequently, the means among each treatment were compared using the Duncan’s Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Effect of de-greening on L*, a*, and b*

The values of L* increased with the increase of storage times in all ethylene concentrations and exposure durations. However, the increment rates were different, and 24 h exposure duration had the largest average increment. Fattahi et al. [7] reported that generally, skin colour values (L*, a*, b*) except in hue angle for all Citrus varieties investigated, had progressive enhancement during ripening. The results
showed that the storage time, ethylene concentration, and exposure duration significantly affected the value of L*. But, interaction only occurred between storage time and exposure duration (p<0.05). In the other words, ethylene concentration did not influence storage time’s effect on L* values. Comparison of means indicated that L* values for 100 and 150 ppm ethylene concentrations were not different, while the others differ. Meanwhile, the mean values of L* differed for the three exposure durations tested (p<0.05). The largest L* value was found for 48 h exposure duration and 100 ppm ethylene concentration. The mean values of a* consistently increased with the storage time for all treatment combinations, where the increment rates were nearly the same. Messina et al. [8] reported a continuous increase in a* values of tomato kept at 19 ± 0.5°C and 85% RH until 14 days observation. Statistical analysis indicated that storage time, ethylene concentration, exposure time, and all interaction combinations were significantly different (p<0.05). The values of a* changed from negative on the first day to positive in the second and afterwards, except for 24 h exposure duration. Means comparison analysis revealed the different values of a* in all ethylene concentrations and exposure durations and the maximum was for 100 ppm and 72 h. The mean values of b* indicated the same phenomenon as a*, where they increased along with the storage time for all treatment combinations, but the differences were only in the magnitude and increment rates. These gave nearly the same results as the a* values, except there was no interaction between concentration and exposure time. Means comparison analysis of b* also indicated the maximum was for 100 ppm and 72 h. Considering the results, it seemed the values of a* and b* were more influenced by the treatments applied than L*. It was proven that as the storage time passed, a* and b* increased consistently with larger values than L*. This might be caused by green chlorophyll degradation in the Citrus peel as the de-greening process proceeded.

Table 1. Mean values of peel colour attributes according to ethylene concentration and exposure time

| Concentration (ppm) | L*     | a*     | b*     | CCI    | h°     | C°     |
|---------------------|--------|--------|--------|--------|--------|--------|
| Initial             | 39.46±1.06 | -4.20±0.40 | 19.33±0.37 | -5.51±0.54 | 102.26±1.07 | 19.78±0.40 |
| 0                   | 45.1±1.62 | 0.27±1.20 | 27.8±2.51  | -0.54±0.97   | 90.98±2.04 | 27.98±2.49 |
| 50                  | 49.4±3.14 | 4.69±1.22 | 31.9±2.62  | 0.45±1.19    | 82.72±2.58 | 32.16±2.60 |
| 100                 | 54.5±3.58 | 7.25±0.99 | 47.9±2.46  | 3.54±0.48    | 80.88±1.57 | 49.20±3.51 |
| 150                 | 54.2±3.40 | 8.49±1.33 | 43.9±4.04  | 4.19±0.86    | 80.54±1.43 | 45.29±3.96 |
| Exposure time (h)   | L*     | a*     | b*     | CCI    | h°     | C°     |
| 24                  | 45.69±3.06 | 0.68±1.33 | 31.76±3.12 | 1.03±1.22 | 90.43±2.84 | 32.33±3.11 |
| 48                  | 54.46±2.21 | 6.68±1.32 | 39.29±2.90 | 2.36±0.67 | 80.66±1.29 | 40.12±2.87 |
| 72                  | 52.26±3.53 | 8.17±0.91 | 42.54±3.45 | 2.34±0.73 | 80.24±1.60 | 43.53±3.45 |

*) Means in the same column with the same letter are not significantly different from one another at p=0.05

Table 1 shows the mean values of L*, a*, and b* where the values of L* for 100 and 150 ppm were not different, but the others differed. Also, it was found that the largest a* and b* values were for 150 ppm and 72 h. This possibly means that as ethylene concentration and exposure duration increased, the faster the green Citrus samples changed toward ripe colour. Figure 1 illustrates examples of the colour appearance.
| Days | Exposure Time Duration (h) |
|------|---------------------------|
|      | 24 | 48 | 72 |
| 1    | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| 2    | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |
| 3    | ![Image](image7.png) | ![Image](image8.png) | ![Image](image9.png) |
| 4    | ![Image](image10.png) | ![Image](image11.png) | ![Image](image12.png) |

**Fig. 1** Photographs of Citrus samples’ appearance from day one to four (top to bottom) and exposure durations of 24, 48, and 72 h (left to right) for 100 ppm ethylene concentration.

### 3.2. Effect of de-greening on Citrus Colour Index (CCI)

CCI values increased consistently as the storage time passed (Figure 2). Messina et al. [8] also reported a continuous increase in CCI values of tomato kept at 19 ± 0.5°C and 85% RH until 14 days observation. Repeated measure statistical analysis indicated that the storage time and its interaction with exposure duration and concentration as well as ethylene concentration, exposure time and their interaction significantly affected CCI values. These results were the same as for a* value, meaning that CCI was easily affected by the treatment applied. CCI is often used to measure the ripeness level of fruits based on peel colour appearance, such that as the values increase the fruit is closer to the ripe condition. Sdiri et al. [9] classified the ripeness stage based on CCI values, where ‘Clemenules’ mandarins were divided into CCI-1 from −16 to −10 (deep green), CCI-2 from −9 to −3 (yellowish-green), CCI-3 from −2 to 3 (greenish-yellow), and CCI-4 > 4 (yellowish-orange). Furthermore, ‘Navelina’ oranges were divided into CCI-1 from −12 to −5 (green), CCI-2 from −4 to 0 (greenish-yellow), and CCI-3 from 0 to 4 (orange).

Table 1 shows that the largest mean of CCI value was 4.19 for 150 ppm ethylene concentration and 48 or 72 h exposure period (p>0.05 when those two were not different), meaning this treatment was the best in altering Citrus peel colour toward the ripening stage. This treatment’s CCI values ranged from 4.19 to 6.77, meaning yellowish orange and the maximum ripeness grade of the classification above. However, from visual observation, Citrus with 72 h ethylene exposure experienced faster deterioration, hence 48 h duration was defined as a better treatment.
3.3. Effect of de-greening on hue angle (h°)

The applied de-greening treatment reduced the h° values in all ethylene concentrations and exposure durations (Figure 3). Cox et al. [10] also reported the same phenomenon where h° values of avocado decreased along with storage period during ripening at 15, 20, and 25°C room temperatures. Machado et al. [11] also stated the decrease of h° values when de-greening orange with combined treatment of ethylene gas and storage temperature. Repeated measure analysis indicated that the storage time, ethylene concentration, exposure duration, and all interactions, except the one between storage time and ethylene concentration significantly affected the h° values (p<0.05). DMRT analysis indicated that h° values of the ethylene concentration’s means differed from one another, except for 100 and 150 ppm. However, 48 and 72 h of exposure durations had no difference, but they significantly differed from 24 h (p<0.05). The h° value decreased from 102.26° at the initial condition to around 75-85° on the fourth day of the ripening process. Based on the colour chart, this h° value is included in the yellow region, in other words, the treated Citrus samples were totally ripened.

3.4. Effect of de-greening on Chroma (C*)

Chroma is used to determine a hue’s degree of difference in comparison to grey colour with the same lightness. The higher the values the higher the colour intensity of samples perceived by humans [1]. Figure 4 shows the change in the samples’ C* values which consistently increased with all the treatments’ storage times. This meant that as the storage time passed, the peel samples’ colour became thicker. Radzevičius et al. [12] stated that in the study of tomato ripening, C* increased as the colour changed from green to light red and finally declined at the red stage. But, chroma of the hybrid ‘Vaisa’ increased at all ripening stages. It was statistically indicated that the storage time, ethylene concentration, exposure duration, and all interactions, except the one between storage time and ethylene concentration significantly affected the C* values (p<0.05). This result was the same as h° values analysis, meaning the trend of C* values’ change was similar to that of h° values. However, DMRT analysis indicated that C* values differed from one another considering ethylene concentration and exposure duration, where the largest C* was found for 100 ppm and 72 h. The sample’s initial C* value was 19.78, which increased by twofold to more than 40 during the de-greening process. Based on the colour chart, this C* value is included in the yellow region, in other words the treated Citrus samples were totally ripened.
4. Conclusions

Generally, storage times, ethylene concentrations, and exposure durations significantly affected Citrus nobilis peel colour attributes. The colour values consisting of L*, a*, b*, CCI, and C* increased with the storage time, while h° values decreased. Based on h° and C* values, the Citrus samples reached the yellow and yellowish-orange region respectively or the maximum ripeness grade on the fourth day. However, from visual observation, Citrus with 72 h ethylene exposure experienced faster deterioration. Therefore, the 48 h duration was defined as a better treatment.

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