Asian Journal of Plant Sciences

ISSN 1682-3974

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2022.56.65

Research Article
Orange Colour Formation and Pigment Variation on Tropical Tangerine Peel by Precooling and Degreening

[1,2]Darda Efendi, [3]Inanpi Hidayati Sumiasih, [4]Rahmat Budiarto, [1,2]Roedhy Poerwanto, [5]Andria Agusta and [6]Sri Yuliani

[1]Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Jl. Meranti, IPB Darmaga Campus, Bogor 16680, Indonesia
[2]Center for Tropical Horticulture Studies, IPB University, Jl. Raya Pajajaran, Bogor 16144, West Java, Indonesia
[3]Study Program of Agrotechnology, Faculty of Bioindustry, Trilogi University, South Jakarta 12760, Jakarta, Indonesia
[4]Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Sumedang 45363, Indonesia
[5]Research Center for Chemistry, Indonesian Institute of Science, South Tangerang 15314, West Java, Indonesia
[6]Indonesian Center for Agricultural Postharvest Research and Development, Bogor 16124, West Java, Indonesia

Abstract
Background and Objective: Tropical tangerine (*Citrus nobilis* Lour) can not produce orange peel colour naturally due to the absence of low temperature during the pre-harvest condition. This study aimed to evaluate the effect of precooling and ethylene application on degreening and variation in pigment metabolites (chlorophyll, β-carotene, β-cryptoxanthin and zeaxanthin) of the tropical tangerine peel. **Materials and Methods:** Approximately 960 fruits of low land tropical tangerine were subjected to precooling and various ethylene concentrations (0, 100, 200 and 300 ppm) for degreening. **Results:** The result showed that both precooling and degreening induced peel colour alteration of tangerine fruit, from green to yellow and yellowish-orange. In general, chromaticity analysis resulted in the increase of the Citrus colour index and the decrease of °hue after 10 days of storage. The best treatment for the best colour (yellowish orange) achieved was the combination of precooling and degreening with100 ppm ethylene. The colour transformation was also proved by the quantitative change of chlorophyll and carotenoid (β-carotene, β-cryptoxanthin and zeaxanthin) that revealed by reverse-phase high-performance liquid chromatography. **Conclusion:** Both chlorophyll and β-carotene were significantly reduced in fruit treated by best treatment, while the β-cryptoxanthin showed a significant and multi-fold increased, ascertained by the formation of strong yellowish-orange peel colour within this treatment. The effect of a single factor either only precooling or degreening was less powerful than the best combination treatment.

Key words: Chromaticity, citrus colour index, ethylene, °hue, high-performance liquid chromatography

Citation: Efendi, D., I.H. Sumiasih, R. Budiarto, R. Poerwanto, A. Agusta and S. Yuliani, 2022. Orange colour formation and pigment variation on tropical tangerine peel by precooling and degreening. Asian J. Plant Sci., 20: 56-65.

Corresponding Author: Darda Efendi, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Jl. Meranti, IPB Darmaga Campus, Bogor 16680, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Citrus is one of the economically important and highly demanded fruit commodities worldwide. The high demand for citrus fruit is attributed to its well-known image as delicious functional fruit. Citrus fruit is rich in nutritional compounds, such as vitamin C, vitamin A, carotenoids, folate, dietary fibre, flavonoid and limonoid[1]. Moreover, citrus peel is potentially utilized as a source of nutritional dietary supplement since this part is a rich source of dietary fibre and phenolic compound[2].

The external fruit peel colour is one of the maturity indexes for citrus[3]. The Citrus peel colour is the final result that is regulated by the changes of pigments profile[4]. Under a natural condition of the lowland tropics, the tangerine cannot form orange peel colour and remain green even when ripe[5], due to the inactivation of biosynthesis of certain carotenoids pigment in association with the absence of low temperature during fruit growth and development[6]. A high temperature during citrus fruit maturation in tropical orchards produced a high chlorophyll followed by a low carotenoid in their peel[7]. Postharvest treatment is required to transform the peel colour from green to orange to gain more attractiveness to the consumer since the colour is a crucial attribute that affected consumer perception and acceptance[8].

The loss of green colour domination in fruit peel is achieved as the impact of the chlorophyll degradation process. The chlorophyll disruption can be occurred either naturally as the effect of endogenous ethylene or artificially by exogenous ethylene exposure[9]. Citrus is a non-climacteric fruit with lower ethylene produced during the post-harvest period[10]. Thus, the use of exogenous ethylene in form of degreening treatment is a promising technique to have an improvement of citrus fruit external colour[11,12]. Degreening is reported to not affect the internal quality such as the total soluble solids, vitamin C and total flavonoid of citrus[13]. In contrast, the application of ethylene for degreening is highly associated with the alteration of carotenoid profile[14-17].

Another postharvest treatment that is potentially related to fruit colouration is precooling. Postharvest precooling treatment is needed to replace the effect of low temperature that is not obtained during fruit growth and development in the lowland tropics[18]. Precooling is the cooling process of fruits immediately after harvest, to immediately remove field heat[19]. The application of precooling may vary in terms of technical perspectives, however, the basic principle is still the same, by using air and or water as cooling medium[20]. Precooling has been adopted in citrus fruit commodities[21,22]. In addition, degreening has been also applied in numerous *Citrus* spp, including *C. reticulata*/mandarin[18,23], *C. limon*/lemon[24], *C. sinensis*/orange[15], *C. changshanensis*/huyou[14], etc. A limited report is found regarding degreening and precooling on tropical *C. nobilis*/tangerine. Thus, this study aimed to evaluate the effect of precooling and ethylene concentration for degreening on the colour change, chromaticity and pigment metabolites (chlorophyll, β-carotene, β-cryptoxanthin and zeaxanthin) of the tropical tangerine peel.

MATERIALS AND METHODS

Study site: This study was conducted from February, 2016 to May, 2017 in the post-harvest laboratory of the Center for Tropical Horticulture Studies (PKHT), IPB University and phytochemical laboratory of Research Center for Biology, Indonesian Institute of Science. Tangerines fruits were harvested from a local orchard in tropical low land of Semboro subdistrict (70 m above sea levels), Jember district, East Java province, Indonesia.

Procedure: This study was arranged in a factorial completely randomized design, i.e., precooling as 1st factor (with and without precooling) and ethylene concentrations as 2nd factor (0, 100, 200 and 300 ppm). Eight combination treatments replicated three times, resulted in 24 experimental units and there were 40 fruits used for every experimental unit. Selected fruits were characterized by ready-to-harvest (6-7 months after anthesis) and pest/disease-free with no malformation in shape. For no precooling treatment, the fruit was immediately packed in the fruit box for delivery to the laboratory. Fruit treated by precooling was immersed in cold water (5-7°C) for 30 sec before the pack in the fruit box. All fruit was transported to the post-harvest laboratory in Bogor, however, only precooled fruits were stored at low temperatures (9-11°C) during a two-day trip. At the post-harvest laboratory, the fruit was transferred into a degreening plastic box (2.8 kg fruit/box) and tightly sealed. Ethylene gas was injected into the degreening box by using a 5 mL syringe and then the box was stored at the best degreening temperature for tropical tangerine 20°C for 24 hrs[18]. Tangerines fruits were then transferred to room temperature for further observation.

Measured variables

External peel colour: The external citrus peel colour was observed visually and measured quantitatively using an L*a*b colour system by Minolta Colour Reader type 310 and then calculated into the Citrus Colour Index (CCI) and °hue at 0, 1, 4, 7, 10 and 13 Days After Treatment (DAT). The CCI and °hue were calculated following a previous study by Muthmainnah *et al.*[25] and Manera *et al.*[24], respectively.

Pigment analysis: The total chlorophyll content of the tangerine peel was measured by modifying Sims and Gamon[26] method. Approximately 0.5 g of crushed tangerine peel was added with 2 mL acetris (80% acetone: 20% Tris buffer, pH solution 7.8) and well mixed by vortex for about 10 min. The 1 mL supernatant added with 3 mL acetris was prepared in the cuvette for absorbance measurement by using a spectrophotometer at three wavelengths, i.e., 537 (A_{537}), 647 (A_{647}) and 663 nm (A_{663}). In addition to chlorophyll, the present work also measured the carotenoid pigment (β-cryptoxanthin, β-carotene and zeaxanthin). The sample preparation and analysis were conducted by following Sumiasih *et al.*[23] by using a reverse-phase Shimadzu HPLC instrument and then the result was expressed in mg per g fresh weight (mg g FW^{-1}). Pigment analysis was conducted at 0 and 10 DAT.

Data analysis: Analysis of variance (ANOVA) was performed on data obtained from pigment analysis by using Statistical Analysis Software (SAS) version 9.4. If there was a significant difference between treatments, the results were tested by Duncan's Multiple Range Test (DMRT) at a 5%.

RESULTS AND DISCUSSION

Tangerine peel colour varied in response to precooling and degreening treatment. The absence of degreening treatment produced the yellowish-green colour on tangerine peel at 10 DAT, irrespective of precooling treatment. Degreening with the absence of precooling seemed to induce pale yellow colour. A combination of precooling and Degreening has successfully turned the peel into yellowish-orange. In terms of visual colour observation, the most attractive yellowish-orange colour was found in the combination of precooling and 100 ppm ethylene in Fig. 1.

Citrus fruit colour was also measured by quantitative chromaticity variables, such as CCI and °hue. The CCI was a popular colourimetric variable measured by colourimeter[27,28], colour chart[29] and image processing in a mobile device[30,31]. This variable was widely used in the citrus industry to evaluate the fruit maturity level[32] and also the success of degreening treatment[25]. Additionally, °hue was also a colourimetric variable frequently used to evaluate citrus juice[33] and fruit quality[34] and also classify citrus fruit maturity[35,36]. In general, the deeper orange peel colour induced by ethylene treatment was associated with a lower °hue and a higher CCI[36]. The CCI at 6 and °hue at 74 implied the yellowish-orange colour of citrus peel, while the CCI at 8 and °hue at 70 were associated with the orange colour of tropical tangerine peel.

The result showed that the lowest CCI (1.66) was noted in control fruit (no precooling and no degreening) at 10 DAT associated with the yellowish-green of external peel in Fig. 2a. In contrast, the highest CCI was found in the combination of precooling and 100 ppm ethylene treatment that reached 6.20, which appeared as a yellowish-orange peel in Fig. 2b. All of the no precooled fruit displayed CCI values varied from 1.66-3.04, irrespective of ethylene dosage (Fig. 2a). In terms of °hue, all treatments similarly showed the decreasing pattern of °hue during the storage period associated with the peel

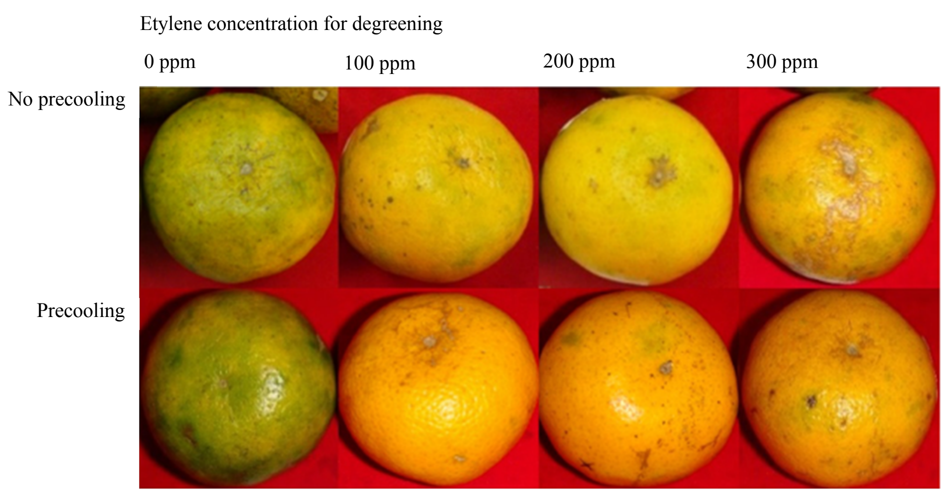

Fig. 1: Fruit peel colouration of tangerine treated by precooling and degreening at 10 DAT

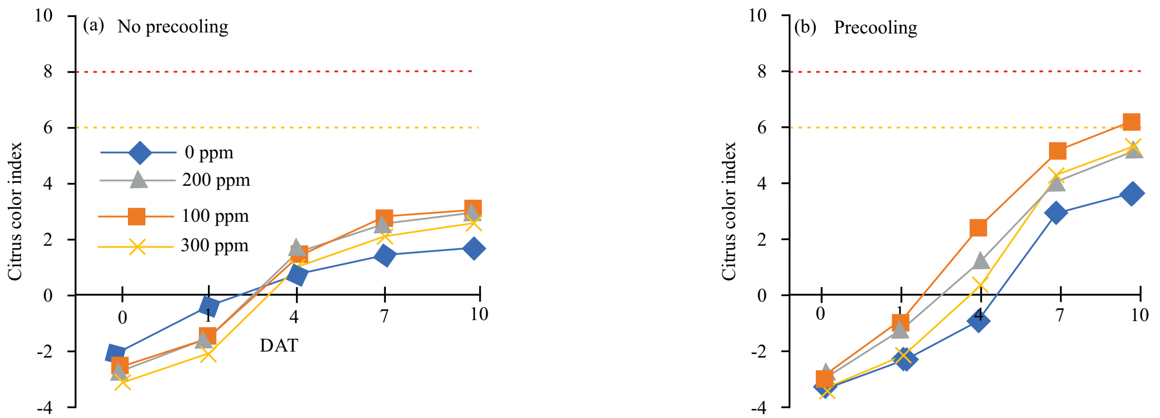

Fig. 2: Citrus colour index of tangerine treated by (a) Precooling and (b) Degreening at various observation times

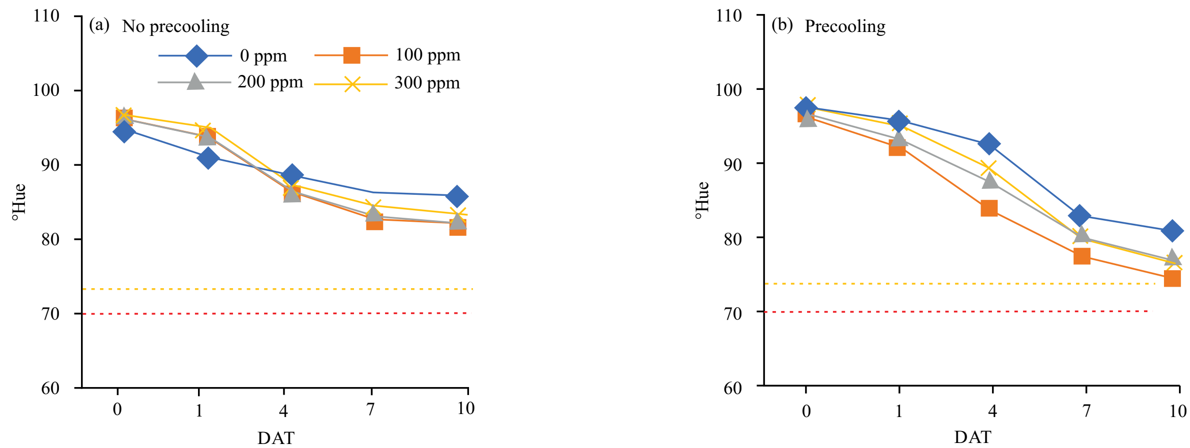

Fig. 3: °Hue of tangerine treated by (a) Precooling and (b) Degreening at various observation times

colour alteration from green to yellow. At 10 DAT, the highest °hue for about 83.85 measured in the control fruits in Fig. 3a, while the lowest °hue for about 74.37 was found in the combination of precooling and 100 ppm ethylene in Fig. 3b. Interestingly, the increase of ethylene dosage used for degreening up to 200 and 300 ppm produced a lower CCI and a higher °hue as compared to 100 ppm, implied that 100 ppm was the best ethylene dosage for tangerine degreening.

The effect of precooling and degreening on tangerine fruit might differ from the mandarin. Both mandarin and tangerine have come from the same species-level, followed by a high similarity at the seedling stage[37], however, the tangerine is more dominant in terms of cultivation area rather than mandarin, i.e., 85 and 10%, respectively[38]. The mandarin was seemed easier to turn to orange colour rather than tangerine. The precooling was not a prerequisite in mandarin, while tangerine showed the opposite[39]. The success of degreening in both citrus varieties was also associated with the altitude of the growing location. Most of the tangerine was produced in lowland, while the mandarin in medium to high land. The degreening on high land cultivated fruit was easier to succeed (form orange colour) rather than low land fruit. An earlier study by Sumiasih *et al.*[18] proved that the orange colour intensity in highland cultivated mandarin citrus var. Selayar was greater than lowland mandarin var. Tejakula. The external colour variation in citrus peel was the final result of the changes in the quantity and the ratio of the pigment metabolites. This argument was supported by the earlier studies that concluded plant metabolites were highly influenced by fruit maturation stages, soil-plant nutrient status, climatic and culture practices such as foliar feeding[40-42].

Chlorophyll is a well-known photosynthetic pigment that is frequently measured in leaves due to its association with the photosynthetic rate[43]. and nitrogen fertilization[44].

Additionally, it is also responsible for the formation of green colour in numerous unripe citrus fruit[45], e.g., tangerine[18], mandarin[46], limau[47], lime[48] and kaffir lime[49]. The total chlorophyll content in tangerine fruit peel was significantly affected not only by postharvest treatment but also by observational time. At the initial time of observation (0 DAT equal to 3 days after harvest), the total chlorophyll was generally higher than the last observation time (10 DAT). The highest total chlorophyll content (100 mg g FW^{-1}) was found in the control fruit at 0 DAT, then continuously declined into 69 mg g FW^{-1} in the control fruit at 10 DAT. The presence of a single factor was also significantly reduced chlorophyll content, into 70 and 38 mg g FW^{-1} in precooling and degreening, respectively. The lowest total chlorophyll content (8 mg g FW^{-1}), which implied the least green colour presence, was measured in the combination between precooling and degreening at 10 DAT in Fig. 4.

Chlorophyll degradation was happened naturally during the fruit ripening process[50-52] or induced artificially by using exogenous ethylene application as called degreening[53-55]. The chlorophyll degradation was summed up, started from chlorophyll b to chlorophyll a to pheophorbide to red chlorophyll catabolite to primary fluorescent catabolite[56,57]. Several genes were reported governing chlorophyll degradation in citrus species, namely Citrus Chlase1[58], SGR[59], CitPPH[50] and CitAP2/ERF[60]. The chlorophyll degradation process has occurred in the sub-epidermal layer, therefore there was no effect on fruit internal quality[39,55,61].

External peel colouration during the maturation process was not only about chlorophyll degradation but also biosynthesis and alteration of certain carotenoid content that might vary at inter- and intra-species level[62,63]. Therefore, the present study also measured quantitatively carotenoid pigments such as β-carotene, β-cryptoxanthin and zeaxanthin. β-carotene is frequently reported to stimulate orange-red colour and is mostly extracted from carrot[64]. Both β-cryptoxanthin and zeaxanthin were two main components to generate β-citraurin[45,65,66] as the main reason for the red-orange citrus peel colour[14].

The result of β-carotene analysis in the present study is significantly affected by precooling and degreening. The level of β-carotene in tangerine fruit peel declined with the running of the storage period in Fig. 5. β-carotene content of control (no degreening, no precooling) fruit peel was 708 mg g FW^{-1} at 0 DAT and then decreased up to 50% to be 352 mg g FW^{-1} after 10 days stored in room temperature. The application of a single factor either pre-cooling or degreening was also caused the reduction of β-carotene up to 61 and 51%, to be 273 and 350 mg g FW^{-1}, respectively. As compared to control at 0 DAT, the highest reduction of β-carotene for about 96% to be 28 mg g FW^{-1} was measured in the combination of precooling and 100 ppm ethylene used for degreening. The reduction of β-carotene as the fruit getting mature and treated by precooling and degreening might relate to the conversion of β-carotene into β-cryptoxanthin. An earlier study[14] reported the high expression of carotenogenic gen called β-ring hydroxylase (BCH), which encoded an enzyme to catalyze the conversion of β-carotene to β-cryptoxanthin as the effect of ethylene application and storage temperature.

The level of zeaxanthin was increased as the effect of 10 days storage at room temperature, from 31-32 mg g FW^{-1} at 0 DAT to 39-53 mg g FW^{-1} at 10 DAT in Fig. 6. The increasing pattern was not significantly different among treatments, i.e., 53 mg g FW^{-1} in fruit treated by a single factor of precooling, 39 mg g FW^{-1} in fruit by only degreening and 40 mg g FW^{-1} treated by a combination of precooling and degreening. A previous study[14] also reported the increasing pattern of zeaxanthin content in huyou (*Citrus changshanensis*) peel in response to different ethylene treatments.

Postharvest treatments in form of precooling and degreening significantly increased the content of β-cryptoxanthin in tangerine fruit peel after 10 days stored at room temperature. The highest β-cryptoxanthin for about 7.9 mg g FW^{-1} was noticed at 10 DAT in fruit treated by the combination of precooling and degreening (100 ppm ethylene), this result was almost 8 and 5 times higher than control at 0 and 10 DAT, respectively in Fig. 7. The presence of a single factor either only degreening or precooling produced a relatively similar content of β-cryptoxanthin for about 2.5 mg g FW^{-1}. The higher β-cryptoxanthin might contributed to the induction of 'more orange intensity' in citrus fruit peel. A previous study[14] showed the difference between β-cryptoxanthin in ordinary and red peel huyou (*Citrus changshanensis*) varieties, where the red peel (orange colour) huyou consisted of more β-cryptoxanthin for about 0.99 mg g FW^{-1} rather than the ordinary (yellow colour) huyou for only 0.25 mg g FW^{-1}. In addition, the β-cryptoxanthin accumulation in citrus fruit was associated with a low expression of carotenoid catabolic genes, namely CitNCED2 and CitNCED3 and a high expression of carotenoid biosynthetic genes, namely CitPSY, CitPDS, CitZDS, CitLCYb2, CitHYb and CitZEP[66].

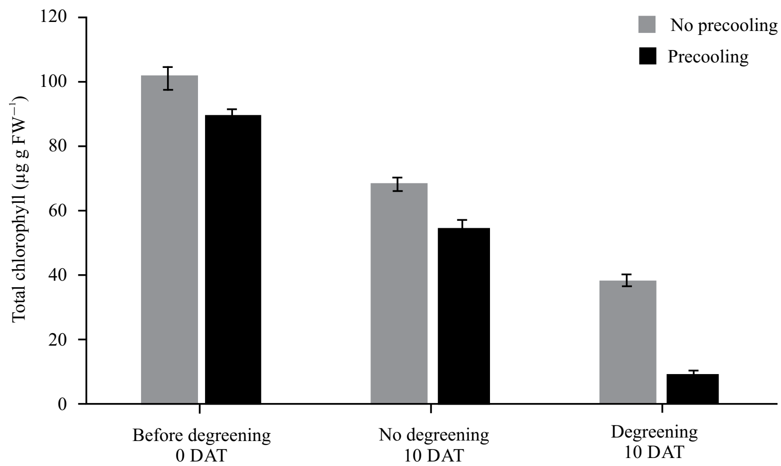

Fig. 4: Content of total chlorophyll in tangerine fruit peel treated by precooling and the best treatment of degreening using 100 ppm of ethylene at 0 and 10 DAT

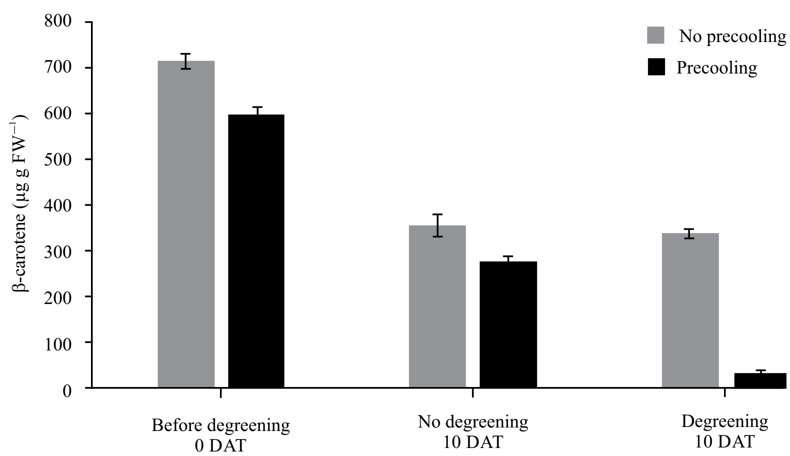

Fig. 5: Content of β-carotene in tangerine fruit peel treated by precooling and the best treatment of degreening using 100 ppm of ethylene at 0 and 10 DAT

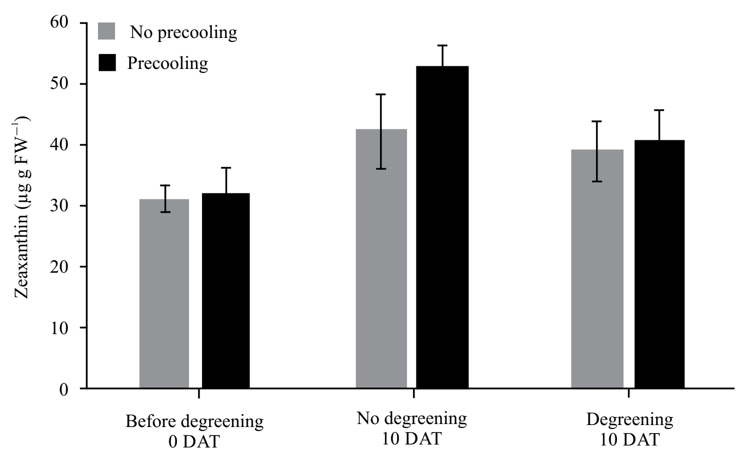

Fig. 6: Content of zeaxanthin in tangerine fruit peel treated by precooling and the best treatment of degreening using 100 ppm of ethylene at 0 and 10 DAT

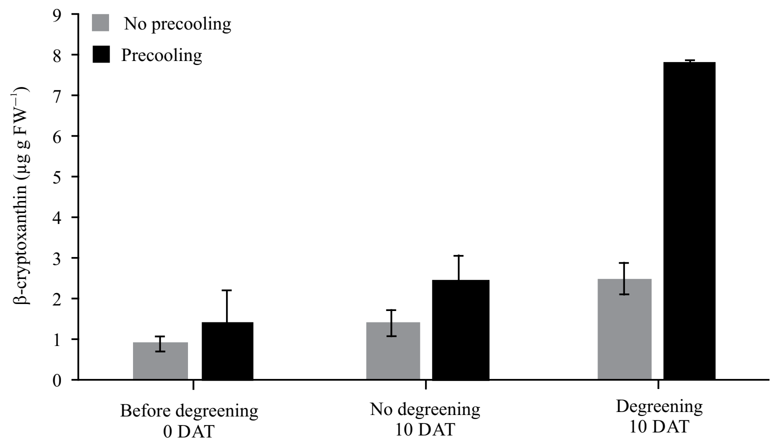

Fig. 7: Content of β-cryptoxanthin in tangerine fruit peel treated by precooling and the best treatment of degreening using 100 ppm of ethylene at 0 and 10 DAT

CONCLUSION

In conclusion, both precooling and degreening induced external colour alteration of tangerine peel fruit, indicated by the increase CCI and the decrease °hue. The best colour (yellowish-orange) was achieved by the combination of precooling and degreening by using 100 ppm of ethylene, thus determined as the best treatment for tropical tangerine. The incidence of fruit colouration was also proved by the change of chlorophyll and carotenoid (β-carotene, β-cryptoxanthin and zeaxanthin) levels. Both chlorophyll and β-carotene were significantly reduced in fruit treated by best treatment, while the β-cryptoxanthin showed the opposite result as certained by the formation of yellowish-orange peel colour within this treatment. The effect of a single factor either only precooling or degreening was less powerful than the best combination treatment.

SIGNIFICANCE STATEMENTS

This study discovered that precooling and degreening can be beneficial for the induction of orange colour formation on tropical tangerine fruit. This study will help the researchers to uncover the critical areas of pigment metabolites variation on tropical tangerine that many researchers were not able to explore. Thus a new theory on the reduction of chlorophyll and β-carotene and the increase of β-cryptoxanthin during the orange colour formation on tropical tangerine peel may be arrived at.

REFERENCES

1. Turner, T. and B.J. Burri, 2013. Potential nutritional benefits of current citrus consumption. Agriculture, 3: 170-187.
2. Rafiq, S., R. Kaul, S.A. Sofi, N. Bashir, F. Nazir and G.A. Nayik, 2018. Citrus peel as a source of functional ingredient: A review. J. Saudi Soc. Agric. Sci., 17: 351-358.
3. Itakura, K., Y. Saito, T. Suzuki, N. Kondo and F. Hosoi, 2019. Estimation of citrus maturity with florescence spectroscopy using deep learning. Horticulture, Vol. 5. 10.3390/horticulturae5010002.
4. Alós, E., M. Cercós, M.J. Rodrigo, L. Zacarías and M. Talón, 2006. Regulation of color break in citrus fruits. changes in pigment profiling and gene expression induced by gibberellins and nitrate, two ripening retardants. J. Agric. Food Chem., 54: 4888-4895.
5. Martasari, C., D. Agisimanto, Karsinah and Reflinur, 2013. Characterization of Indonesian tangerine cultivar by morphological and ISSR markers. Acta Hortic., 975: 103-110.
6. Iglesias, D.J., M. Cercos, J.M. Colmenero-Flores, M.A. Naranjo and G. Rios *et al*., 2007. Physiology of citrus fruiting. Braz. J. Plant Physiol., 19: 333-362.
7. Hasimi, N.R., R. Poerwanto and D.K. Suketi, 2016. Degreening siam tangerine fruit (*Citrus nobilis*) in ethylene concentrations and exposure durations. J. Hortic. Indonesia, 7: 111-120.
8. Morales, J., A. Tárrega, A. Salvador, P. Navarro and C. Besada, 2020. Impact of ethylene degreening treatment on sensory properties and consumer response to citrus fruits. Food Res. Int., Vol. 127. 10.1016/j.foodres.2019.108641.
9. Peng, G., X.L. Xie, Q. Jiang, S. Song and C.J. Xu, 2013. Chlorophyll a/b binding protein plays a key role in natural and ethylene-induced degreening of ponkan (*Citrus reticulata* Blanco). Sci. Hortic., 160: 37-43.
10. McAtee, P., S. Karim, R. Schaffer and K. David, 2013. A dynamic interplay between phytohormones is required for fruit development, maturation and ripening. Front. Plant Sci., Vol. 4. 10.3389/fpls.2013.00079.

11. Sdiri, S., P. Navarro, A. Monterde, J. Benabda and A. Salvador, 2012. New degreening treatments to improve the quality of citrus fruit combining different periods with and without ethylene exposure. Postharvest Biol. Technol., 63: 25-32.
12. Zhang, P. and Z. Zhou, 2019. Postharvest ethephon degreening improves fruit color, flavor quality and increases antioxidant capacity in 'eureka' lemon (*Citrus limon* (L.) burm. F.). Sci. Hortic., 248: 70-80.
13. Mayuoni, L., Z. Tietel, B.S. Patil and R. Porat, 2011. Does ethylene degreening affect internal quality of citrus fruit? Postharvest Biol. Technol., 62: 50-58.
14. Luan, Y., S. Wang, R. Wang and C. Xu, 2020. Accumulation of red apocarotenoid β-citraurin in peel of a spontaneous mutant of huyou (*Citrus changshanensis*) and the effects of storage temperature and ethylene application. Food Chem., Vol. 309. 10.1016/j.foodchem.2019.125705.
15. Carmona, L., L. Zacarías and M.J. Rodrigo, 2012. Stimulation of coloration and carotenoid biosynthesis during postharvest storage of 'Navelina' orange fruit at 12°C. Postharvest Biol. Technol., 74: 108-117.
16. Matsumoto, H., Y. Ikoma, M. Kato, N. Nakajima and Y. Hasegawa, 2009. Effect of postharvest temperature and ethylene on carotenoid accumulation in the flavedo and juice sacs of satsuma mandarin (*Citrus unshiu* Marc.) fruit. J. Agric. Food Chem., 57: 4724-4732.
17. Rodrigo, M.J. and L. Zacarias, 2007. Effect of postharvest ethylene treatment on carotenoid accumulation and the expression of carotenoid biosynthetic genes in the flavedo of orange (*Citrus sinensis* L. Osbeck) fruit. Postharvest Biol. Technol., 43: 14-22.
18. Sumiasih, I.H., T.S. Arzam, R. Poerwanto, D. Efendi, A. Agusta and S. Yuliani, 2018. Study on the accumulation of β-cryptoxanthin pigment to induce orange color on citrus fruits in the tropic. J. Hortic. Indonesia, 9: 73-83.
19. Duan, Y., G.B. Wang, O.A. Fawole, P. Verboven and X.R. Zhang *et al*., 2020. Postharvest precooling of fruit and vegetables: A review. Trends Food Sci. Technol., 100: 278-291.
20. Wang, L. and D.W. Sun, 2003. Recent developments in numerical modelling of heating and cooling processes in the food industry-a review. Trends Food Sci. Technol., 14: 408-423.
21. Elansari, A.M., D.L. Fenton and C.W. Callahan, 2019. Precooling. In: Postharvest Technology of Perishable Horticultural Commodities, Yahia, E.M. (Ed.)., Elsevier Inc., pp: 161-207.
22. Defraeye, T., R. Lambrecht, A.A. Tsige, M.A. Delele and U.L. Opara *et al*., 2013. Forced-convective cooling of citrus fruit: Package design. J. Food Eng., 118: 8-18.
23. Sumiasih, I.H., R. Poerwanto, D. Efendi, A. Agusta and S. Yuliani, 2017. The analysis of β-*cryptoxanthin* and *Zeaxanthin* using HPLC in the accumulation of orange color on lowland citrus. Int. J. Appl. Biol., 1: 37-45.
24. Manera, J., J.M. Brotons, A. Conesa and I. Porras, 2012. Relationship between air temperature and degreening of lemon (*Citrus lemon* L. Burm. F) peel color during maturation. Aust. J. Crop Sci., 6: 1051-1058.
25. Muthmainnah, H., R. Poerwanto and D. Efendi, 2014. Skin color changes on three varieties of tangerines with degreening and storage temperature treatments. J. Hortikultura Indonesia, 5: 10-20.
26. Sims, D.A. and J.A. Gamon, 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens. Environ., 81: 337-354.
27. Cao, J., C. Wang, S. Xu, Y. Chen, Y. Wang, X. Li and C. Sun, 2019. The effects of transportation temperature on the decay rate and quality of postharvest ponkan (*Citrus reticulata* Blanco) fruit in different storage periods. Sci. Hortic., 247: 42-48.
28. Bello, F., D.E. Vázquez, N. Almirón, M. Cocco and G.E. Meier, 2015. Sensorial analysis as an instrument for color measurement in 'satsuma' and 'nova' mandarin fruit. Acta Hortic., 1065: 1491-1496.
29. Gupta, A.K., M. Medhi, S. Chakraborty, M. Yumnam and P. Mishra, 2020. Development of rapid and non-destructive technique for the determination of maturity indices of Pomelo fruit (*Citrus grandis*). J. Food Meas. Charact., 15: 1463-1474.
30. Cubero, S., F. Albert, J.M. Prats-Moltalbán, D.G. Fernández-Pacheco, J. Blasco and N. Aleixos, 2018. Application for the estimation of the standard citrus colour index (CCI) using image processing in mobile devices. Biosyst. Eng., 167: 63-74.
31. Vidal, A., P. Talens, J.M. Prats-Montalbán, S. Cubero, F. Albert and J. Blasco, 2013. In-line estimation of the standard colour index of citrus fruits using a computer vision system developed for a mobile platform. Food Bioprocess Technol., 6: 3412-3419.
32. Singh, G., P.S. Aulakh and H.S. Rattanpal, 2017. Determination of maturity and genetic diversity in mandarin (*Citrus reticulata* Blanco) genotypes based on citrus colour index. Int. J. Curr. Microbiol. Appl. Sci., 6: 577-586.
33. Lee, H.S., 2000. Objective measurement of red grapefruit juice color. J. Agric. Food Chem., 48: 1507-1511.
34. Cai, N., C. Chen, C. Wan and J. Chen, 2020. Effects of pre-harvest gibberellic acid spray on endogenous hormones and fruit quality of kumquat (*Citrus japonica*) fruits. New Zealand J. Crop Hortic. Sci., 49: 211-224.

35. Iqbal, S.M., A. Gopal, P.E. Sankaranarayanan and A.B. Nair, 2015. Classification of selected citrus fruits based on color using machine vision system. Int. J. Food Prop., 19: 272-288.
36. Zhou, J.Y., C.D. Sun, L.L. Zhang, X. Dai, C.J. Xu and K.S. Chen, 2010. Preferential accumulation of orange-colored carotenoids in ponkan (*Citrus reticulata*) fruit peel following postharvest application of ethylene or ethephon. Sci. Hortiic., 126: 229-235.
37. Budiarto, R., R. Poerwanto, E. Santosa and D. Efendi, 2021. Morphological evaluation and determination keys of 21 citrus genotypes at seedling stage. Biodiversitas J. Bio. Diversity, 22: 1570-1579.
38. Purbiati, T. and A. Supriyanto, 2014. 'Borneo prima', mandarin cultivar for low and wet area of Kalimantan, Indonesia. Acta Hortic., 1024: 69-75.
39. Arzam, T.S., I. Hidayati, R. Poerwanto and Y.A. Purwanto, 2015. Precooling and etilen concentration at degreening to generate orange color of tangerine peels. J. Hortic., 25: 257-265.
40. Efendi, D., R. Budiarto, R. Poerwanto, E. Santosa and A. Agusta, 2021. Relationship among agroclimatic variables, soil and leaves nutrient status with the yield and main composition of kaffir lime (*Citrus hystrix* DC) leaves essential oil. Metabolites, Vol. 11. 10.3390/metabo11050260.
41. Mubarok, S., F. Yulianto, R. Budiarto, B.P.N. Rahmat and S.A. Khoerunnisa, 2021. Metabolite correlation with antioxidant activity in different fruit maturation stages of *Physalis peruviana*. Biodiversitas J. Bio. Diversity, 22: 2743-2749.
42. El-Monem, A.A.A., M.M.S. Abdallah, B.A. Bakry, H.M.S. El-Bassiouny and M.S. Sadak, 2020. Physiological role of iron chelators and/or arginine for improving yield and active constituents of roselle sepals. Asian J. Plant Sci., 19: 77-90.
43. Budiarto, R., R. Poerwanto, E. Santosa, D. Efendi and A. Agusta, 2019. Agronomical and physiological characters of kaffir lime (*Citrus hystrix* DC) seedling under artificial shading and pruning. Emir. J. Food Agric., 31: 222-230.
44. Poshtmasari, H.K., H. Pirdashti, M. Nasiri and M.A. Bahmanyar, 2007. Chlorophyll content and biological yield of modern and old rice cultivars in different urea fertilizer rates and applications. Asian J. Plant Sci., 6: 177-180.
45. Rodrigo, M.J., B. Alquézar, E. Alós, V. Medina and L. Carmona *et al*., 2013. A novel carotenoid cleavage activity involved in the biosynthesis of citrus fruit-specific apocarotenoid pigments. J. Exp. Bot., 64: 4461-4478.
46. Budiarto, R., R. Poerwanto, E. Santosa and D. Efendi, 2018. Shoot manipulations improve flushing and flowering of mandarin citrus in Indonesia. J. Appl. Hortic., 20: 112-118.
47. Budiarto, R., R. Poerwanto, E. Santosa and D. Efendi, 2017. The potentials of limau (*Citrus amblycarpa* Hassk. Ochse) as a functional food and ornamental mini tree based on metabolomic and morphological approaches. J. Trop. Crop Sci., 4: 49-57.
48. Srilaong, V., S. Aiamla-or, A. Soontornwat, M. Shigyo and N. Yamauchi, 2011. UV-B irradiation retards chlorophyll degradation in lime (*Citrus latifolia* Tan.) fruit. Posth. Biol. Technol., 59: 110-112.
49. Budiarto, R., R. Poerwanto, E. Santosa, D. Efendi and A. Agusta, 2019. Production, post-harvest and marketing of kaffir lime (*Citrus hystrix* DC) in tulungagung, Indonesia. J. Trop. Crop Sci., 6: 138-143.
50. Yin, X.R., X.L. Xie, X.J. Xia, J.Q. Yu, I.B. Ferguson, J.J. Giovannoni and K.S. Chen, 2016. Involvement of an ethylene response factor in chlorophyll degradation during citrus fruit degreening. Plant J., 86: 403-412.
51. Barry, C.S., 2009. The stay-green revolution: Recent progress in deciphering the mechanisms of chlorophyll degradation in higher plants. Plant Sci., 176: 325-333.
52. Yang, X., Z. Zhang, D. Joyce, X. Huang, L. Xu and X. Pang, 2009. Characterization of chlorophyll degradation in banana and plantain during ripening at high temperature. Food Chem., 114: 383-390.
53. Li, S.J., X.L. Xie, S.C. Liu, K.S. Chen and X.R. Yin, 2019. Auto- and mutual-regulation between two citerfs contribute to ethylene-induced citrus fruit degreening. Food Chem., Vol. 299 10.1016/j.foodchem.2019.125163.
54. Win, T.O., V. Srilaong, K.L. Kyu, K. Poomputsa and S. Kanlayanarat, 2006. Biochemical and physiological changes during chlorophyll degradation in lime (*Citrus aurantifolia* Swingle cv. 'paan'). J. Hortic. Sci. Biotechnol., 81: 471-477.
55. Belyaeva, O.B. and F.F. Litvin, 2009. Pathways of formation of pigment forms at the terminal photobiochemical stage of chlorophyll biosynthesis. Biochem. (Moscow), 74: 1535-1544.
56. Hörtensteiner, S. and B. Kräutler, 2011. Chlorophyll breakdown in higher plants. Biochim. Biophys. Acta (BBA) Bioenerg., 1807: 977-988.
57. Takamiya, K.I., T. Tsuchiya and H. Ohta, 2000. Degradation pathway(s) of chlorophyll: What has gene cloning revealed? Trends Plant Sci., 5: 426-431.
58. Delmas, F., S. Sankaranarayanan, S. Deb, E. Widdup and C. Bournonville *et al*., 2013. ABI3 controls embryo degreening through Mendel's I locus. Proc. Nat. Acad. Sci., 110: E3888-E3894.
59. Xie, X.L., S.L. Shen, X.R. Yin, Q. Xu and C. De Sun *et al*., 2014. Isolation, classification and transcription profiles of the AP2/ERF transcription factor superfamily in citrus. Mol. Biol. Rep., 41: 4261-4271.

60. Azoulay-Shemer, T., S. Harpaz-Saad, Y. Eyal and E.E. Goldschmidt, 2011. Pathway of chlorophyll breakdown in citrus fruit peel as compared with Arabidopsis leaves and other plant systems. Acta Hortic., 892: 335-342.
61. Alquezar, B., M.J. Rodrigo and L. Zacarías, 2008. Regulation of carotenoid biosynthesis during fruit maturation in the red-fleshed orange mutant Cara Cara. Phytochemistry, 69: 1997-2007.
62. Kato, M., 2011. Mechanism of carotenoid accumulation in citrus fruit. Chem. Biol., 49: 843-851.
63. Bogacz-Radomska, L. and J. Harasym, 2018. β-carotene-properties and production methods. Food Qual. Saf., 2: 69-74.
64. Zheng, X., K. Zhu, Q. Sun, W. Zhang and X. Wang *et al*., 2019. Natural variation in CCD4 promoter underpins species-specific evolution of red coloration in citrus peel. Mol. Plant, 12: 1294-1307.
65. Ma, G., L. Zhang, A. Matsuta, K. Matsutani and K. Yamawaki *et al*., 2013. Enzymatic formation of β-citraurin from β-cryptoxanthin and Zeaxanthin by carotenoid cleavage dioxygenase4 in the flavedo of citrus fruit. Plant Physiol., 163: 682-695.
66. Ma, G., L. Zhang, W. Yungyuen, Y. Sato and T. Furuya *et al*., 2018. Accumulation of carotenoids in a novel citrus cultivar 'Seinannohikari' during the fruit maturation. Plant Physiol. Biochem., 129: 349-356.