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Improvement berry color skin profile by exogenous cyanocobalamin treatment of 'Crimson seedless' grapevines

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ABSTRACT

The experiment was conducted to study the effect of cyanocobalamin (B_{12}) treatments (0, 3, 6, and 9 mM B_{12}) on *Vitis vinifera* L. 'Crimson seedless' which conducted during two seasons 2014 and 2015. The study aims to regenerate berry color during growth and preserve it during shelf-life at room temperature for four days. The results showed that B_{12} treatments were significantly effective in reducing weight loss. Berry shatter, rachis browning index, while it preserved another quality parameter high such as berry firmness, separation force, total phenol content (TPC), total sugar content (TSC), total anthocyanin content (TAC), B-Carotene, ascorbic acid (AA) and color hue angle during shelf-life for four days. The previous results were significantly observed with B_{12} at 9 mM compared to control and other B_{12} concentrations. However, total solid content (SSC%), titratable acidity (TA%), and SSC/TA ratio were significantly affected by B_{12} at 9 mM up to end the shelf-life period. In contrast, the lowest values of total chlorophyll (Chl_{ab}) content during shelf-life compared with other B_{12} concentrations. Therefore, cyanocobalamin (B_{12}) is an effective vitamin for improving or generating berry color at harvest time and maintaining cluster quality of 'Crimson seedless' grapes during shelf-life (marketing).

1. Introduction

'Crimson Seedless' is a late maturing red seedless grape cultivar with firm berries. It ripens in mid-September and can be stored on vines until mid-November. Also, it possible the storage grapes until early winter under Egyptian climate [1]. The color of red grape berries is an important factor for the market acceptance of crimson table grapes but it remains poorly colored, especially when vines grown in regions or seasons with high or fluctuation temperature [2]. Sometimes, 'Crimson seedless' grapes develop red color on some berries while others remain green and unripe or some cluster had different berry color stages [3]. Anthocyanin is reflected the berry skin color in crimson seedless grape. It accumulates in berries at the beginning of the véraison stage of berry development. Continually, accumulation during berry development related to abscisic acid (ABA) metabolism by which berry skin anthocyanin content increased then color appeared [4]. Generally, there are five anthocyanin substituents that found in crimson skin grapes such as *malvidin-3-glucosides, delphinidin-3-glucosides, peonidin-3-glucosides, cyanidin-3-glucosides, and petunidin-3-glucosides* [5]. The *cyanidin-3-glucoside* and *peonidin-3-glucoside* are major two substituents that were responsible for color appearing. Then they acylated derivatives of these anthocyanins higher content in fresh grape skins [6]. Many studies focused on the application of plant growth regulation (PGRs), abscisic acid (ABA), auxins, cytokinins, ethylene and gibberellic acid (GA3). These hormones all have different functions and peak at different stages during vine and berry development as they are responsible for the regulation of growth and ripening can further inhibit coloring [7].

Cobalamin, also called vitamin B_{12}, is a water-soluble vitamin [8]. Also, it found in the plant cell organs such as cytosol, plastids, and mitochondria [9]. Higher plants neither synthesize nor require vitamin B_{12} because they contain cobalamin-independent methionine synthase (Met) [10]. Methionine synthase catalyzes the final reaction of the Met biosynthetic pathway in two steps, the first step, is catalyzed by the enzyme cystathionineγ-synthase (*CgS*) to form cystathionine from the substrates cysteine and *O*-phosphohomoserine. It is important to note that *O*-phospho-homoserine is also the immediate precursor of threonine so that methionine synthesis and threonine synthesis compete for a common substrate. The reaction catalyzed by *CgS* is followed by the conversion of cystathionine to homocysteine by the enzyme cystathionine β-lyase. In the last step, a methyl group is transferred to plants from N5-methyl-tetrahydrofolic acid to homocysteine by a vitamin-B12-independent methionine synthase to yield Met [11]. In plants, Met serves as a precursor for a variety of metabolic processes, including protein synthesis, as the prime methyl donor for a large number of biological methylations, polyamine synthesis, and ethylene synthesis. Since methionine synthase is also required for

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both the regeneration and the de novo biosynthesis of Met. It is the convergence point for two major biochemical domains in cellular metabolism. The Met biosynthetic pathway and the one-carbon cycle as to photosynthesis capacity [11]. Recently, the application of cyanocobalamin to many fruit in order to improve fruit quality during shelf life and to reduce fruit losses such as during cold storage of mango to alleviate chilling injury incorporation with ascorbic acid [12] also extend shelf life of kaki fruit [13,14], 'Thompson seedless' grapevines [15], and increasing storability and marketing of guava [16] and productivity and quality attributes of 'Williams' banana cultivar [17].

To enlarge the knowledge of cyanocobalamin (Vit.B_{12}) to evaluate the effect of B_{12} at different concentrations to improve berry skin color on 'Crimson' grapes during handling or marketing. So, the study aims to exploit the physiological roles of cyanocobalamin (B_{12}) in a plant cell for enhancing and improving coloration of berry skin on 'Crimson during shelf-life.

2. Materials and methods

2.1. Plant materials and experimental setup

Cluster samples were harvested on November 2014 and 2015 from trees 10 years old growing in the sandy soil of a commercial orchard. It was located in Sadat city, Egypt. Crimson seedless vines 36 were treated by three doses of cyanocobalamin at 0, 3, 6 and 9 mM. Vitamin B_{12} or cobalamin in Cyanocobalamin form (1255.3 molecular weight) is water-soluble vitamin and was purchased from El-Gomhoreya Com. suppliers, EGY, purity 98%. Each treatment contained 9 vines which distributed on three replicates. The doses were applied three times (at the véraison stage, after véraison 14 days and before harvest 14 days at sunset due to the sensitivity of vitamin to sunlight. Upon arrival in pomology department lab, the 200 clusters were picked at the soluble solid content (SSC 17% in orchid). Samples were divided into two main batches. The first batch was composed of 120 clusters fruits. 30 clusters per treatment were distributed in three replicates, for non-distractive measurements such as rachis browning index and color hue angle. Distractive measurements were measured on four treatments, each treatment composed 20 fruit clusters in three replicates. Two fruit clusters of each replicate were picked every day for measure chemical analysis up to 4th day of shelf life at ambient conditions at 25 ± 1C and air humidity average during shelf-life period 57 ± 2) (imitating marketing).

2.2. Physical quality analysis

Berry firmness was recorded using fruit texture Effegi-penetrometer supplemented with a plunger 2 mm diameter penetrator and separation force was measured using a hook instead of a plunger. Firmness and separation force of berries were expressed an *N*. The Berry shattering percentage and rachis browning index was recorded as described by [15]. Water loss percentage was recorded [15]. The color was recorded according to [18], and thereafter, all images were analyzed by using software ImageJ Ver. 1.43u USA to get RGB signals to calculate hue angle of clusters as reported to [19].

2.3. Chemical quality analysis

Quality elements were determined, berries were randomly removed from several cluster samples and were divided into three replicates to measure soluble solid content (SSC%) using Carlzeiss hand refractometer, acidity as tartaric acid (TA) was determined by titration with 0.1 N NaOH and ascorbic acid content (vitamin C) was measured by titrimetric method using 2,6-dichlorophenol indophenol and 6% oxalic acid as substrate according to [20], SSC/TA ratio was calculated as defined maturity index. Total phenol (TP) in the treated fruits were measured spectrophotometrically using the Folin–Ciocalteu reagent with gallic acid as standard [21]. The phenols were measured at the wavelength 750 nm. The results were reported as mg of gallic acid equivalents (GAE) 100 g^{-1} FW. β-carotene and chlorophyll a and b was spectrophotometrically determined by modified methods [22]. The extraction method was modified by using *N*,*N*-dimethylformamide (DMF) instead of acetone. Samples were stored at 4 °C for 16 h to allow the DMF to extract the pigments from the sample. Finally, samples were centrifuged for 5 min at 16,000 rpm, and then samples were determined for wavelengths 452 nm for β-Carotene. It was expressed in mg 100 g^{-1} FW. Chlorophyll a and b were measured at wavelength 663.8 and 646.8 nm and it presented in mg 100 g^{-1} FW. Anthocyanin was determined spectrophotometry at wavelength 535 nm and it presented in mg 100 g^{-1} FW [23]. Total sugars were measured by using phenol 18% and sulphuric acid 96% and the absorbance was recorded with spectrophotometer at 490 nm as it described by [24].

2.4. Statistical analysis

Data for evaluating of physical and chemical analysis were analyzed using two ways incomplete block randomize (ANOVA). The means were compared using the least significant differences (L.S.D.) at $p \leq 0.05$ level of probability. The statistical software package GenStat ver. 11 (Lawes Agricultural Trust, Rothamsted Experimental station, UK) was used.

3. Results and discussion

3.1. Physical quality attributes

Table 1 shows a significant at $P \leq 0.05$ when B_{12} concentrations were considered. Considering the different B_{12} concentrations, it is clear that the B_{12} treatment (9 mM) is more effective to improve physical quality attributes compared to the other B_{12} treatments. The water loss is approximately stable during shelf life when vine treated with high B_{12} dose during shelf life time. It was recorded at 2nd day 2.89% and reached to 10.76% at the 4th day of shelf life compared to control vines (5.79% up to 22.48%). At harvest time, there was no evidence for BS before the 2nd day and for RBI 3rd day of shelf life time. In this case, B_{12} at 9 mM delays BS up to a 2nd day (5.66%) and increase gradually up to a 4th day (30.13%) compared to other treatments. While RBI was detected at 4th day (2.20 = more than slight browning incidence), while control treatment has severed brown (4.06). However, the hue angle value is a quite dark red (h° 16.96), when vines treated with high B_{12} concentration compared to other treatments at harvest time. Continuously, the h° decrease more rapidly up to end shelf life period.

Taken as a whole these results show that the physical quality attributes can be affected differ. It seems plausible that B_{12} control water loss during shelf life. Basically, berries have a somewhat, thick epidermis which covered with waxes layer on berries surface, acting as a protective layer against dehydration [15]. It could be that the highest B_{12} treatment at 9 mM increases waxes during berries development. Moreover, the responses of berries to B_{12} treatments could be related to that B_{12} keeps enhancing ascorbic acid, β-carotene and α-Tocopherol synthesis in berries tissues [25]. Since the last vitamins are considered as antioxidants which play a role to scavenge active oxygen species during shelf-life [8,26]. The important roles of these vitamins are to maintain the right functions of the cell membrane of cells/tissue of berries. How-

Table 1
Effect of preharvest cyanocobalamin (B_{12}) application on cluster weight losses, berry shattering %, rachis browning index, Hue angle (ho), Berry firmness (N) and separating force (N) of 'Crimson Seedless' grapes during four-day shelf-life at 2014 and 2015 seasons.

Treatments	Shelf-life time (days)							
	D1	D2	D3	D4	D1	D2	D3	D4
Water loss %				Berry shattering %				
Treatments				Treatments				
0	0.00	5.79^{a}	11.95^{a}	22.48 ^{a}	4.48^{a}	20.21^{a}	44.06^{a}	52.30^{a}
3	0.00	4.17^{b}	9.12^{a}	18.08^{ab}	1.63^{b}	12.09^{b}	33.89^{b}	48.17^{a}
6	0.00	3.56^{bc}	9.79^{a}	13.14^{bc}	1.10^{b}	9.32^{c}	27.14^{c}	36.07^{b}
9	0.00	2.89^{c}	7.22^{a}	10.76^{c}	1.00^{b}	5.66^{d}	23.11^{d}	30.13^{b}
LSD		**0.717**	**4.885**	**6.576**	**0.81**	**1.64**	**3.70**	**7.89**
Rachis browning index					Hue angle (h^{o})			
Treatments					Treatments			
0	1.00	1.17^{a}	2.61^{a}	4.06^{a}	108.87^{a}	105.60^{a}	95.93^{a}	85.64^{a}
3	1.00	1.07^{b}	1.67^{b}	3.04^{b}	68.13^{b}	65.08^{b}	62.14^{b}	59.13^{b}
6	1.00	1.00^{c}	1.28^{c}	2.95^{b}	47.88^{c}	46.00^{c}	43.48^{c}	40.26^{c}
9	1.00	1.00^{c}	1.00^{d}	2.20^{c}	16.96^{d}	15.55^{d}	13.33^{d}	10.67^{d}
LSD		**0.06**	**0.23**	**0.16**	LSD	**2.19**	**4.56**	**3.82**	**8.20**
Berry firmness (N)					Berry separation forces (N)			
Treatments					Treatments			
0	7.69^{def}	7.27^{fg}	6.06^{h}	4.92^{i}	6.67^{d}	5.89^{e}	4.42^{h}	4.25^{i}
3	8.48^{bc}	7.95^{cd}	6.97^{g}	5.92^{h}	7.00^{c}	6.64^{d}	5.17^{g}	4.49^{h}
6	8.62^{b}	8.27^{bc}	7.34^{efg}	6.38^{h}	7.45^{b}	7.08^{c}	5.61^{f}	5.04^{g}
9	13.08^{a}	8.74^{b}	7.91^{cde}	7.08^{g}	7.68^{a}	7.43^{b}	5.96^{e}	5.56^{f}
LSD 5%	**0.61**				LSD 5%	**0.35**		

Means in a column are significantly different at ($P < 0.05$) according to the LSD. Each value represents mean of 3 replicates during two seasons of 2014 and 2015.
The superscript letters, differ ($P < 0.05$) according to the LSD of two way using two ways incomplete block randomize according to Duncan's test.
Cyanocobalamin treatments at 0 mM, 3 mM, 6 mM and 9 mM were sprayed at three times.
Rachis browning was used to determine stem condition (1 = cap stem healthy; 2 = cap stem slightly brown; 3 = cap stem and secondary stem moderately brown; and 4 = cap stem, severe brown, and 5 = primary stem fully brown).

ever, results may be suggested that the B_{12} at 9 mM maintained insoluble pectin content in berry brush and pedicel [27], and reduce the formation of an abscission layer at the distal end of pedicel of berry [28].

The visual appearance of the stem and pedicel of berry was no detected with vine treated by high B_{12} before the 4th day of shelf life. It could be suggested that the B_{12} protected plasma membranes from peroxidation lipid process by reacting directly with reactive oxygen and nitrogen species during shelf life [8]. So, the plant cell reserved by keeping water in cluster tissues resulted in less water loss and berry shattering percentages.

Berries color Hue angle ($h°$) value is easily perceived by human eye. The color of the berries of vines treated with B_{12} at 9 mM, was visually more red ($h°$ = 16.96) than other treatments at harvest time and decreased gradually up to end the shelf-life period. It could be suggested that hue correlated with anthocyanin content. It may be that B_{12} activated cDNA and protein soluble in plant cell during berry development [27], then, by which increasing accumulation of peonidin and the acylated derivatives of anthocyanins content in fresh grape skins [6].

Table 1 shows the berry firmness (N) and separation force (N) plotted as a function of shelf life time (days) at different B_{12} concentrations. The interaction at $P \leq 0.001$ was significant between shelf life time and B_{12} treatments. The results show that the berry firmness and separation force are initially firmer and higher separation force at harvest time. Vines treated by B_{12} at 9 mM, is firmer (13.08 N) and high separation force (7.43N) compared to other B_{12} treatments. Berry firmness and separation force are the most important physical quality attributes determining cluster acceptability to the consumers. Firmness and separation force depends on many properties of the plant tissue such as water content, the nature of cell wall and turgor are clearly important sources of firmness. Based on these, an illustration could be suggested for the higher berry firmness at harvest time that B_{12} (9 mM) could be increased cell wall polysaccharide during berry development (Lo'ay, 2011). Moreover, it could be pointed out for higher separation force that it related to insoluble pectin content in brush and pedicel of berries [29]. Furthermore, B_{12} could be reacted as an antioxidant in this respect by maintaining insoluble pectin content during shelf-life [27].

3.2. Chemical quality attributes

Table 2 depicts the variation of soluble solid content (SSC), titratable acidity (TA%) and SSC/TA ratio and total sugars (TSC), ascorbic acid (AA) and total phenol content (TP) as a function of shelf life time (days) at four preharvest B_{12} applications. In fact, the previous parameters show a significant interaction $P \leq 0.001$ when the shelf time and B_{12} concentrations were considered. The main significant effect is observed at harvest time with vines treated with high B_{12} concentration (9 Mm) compared to other treatments. Initially, SSC, SSC/TA ratio, TSC, AA and TP content were observed to be higher than other B_{12} treatments, while TA% decreased at harvest time. It is most likely that the increases in chemical parameters content might be affected by high concentration of B12. It could be that B_{12} enhance activation of carbohydrate enzymes synthesis and keeping them in an active status in a plant cell in equilibrium [30]. Therefore, the activation of Calvin cycle, the pentose phosphate pathway and glycolysis might be activated by applying high B_{12} concentration [8], resulting in increasing in total sugars [14].

3.3. Skin berry pigments: β-carotene (β-Car), total Chlorophyll$_{ab}$ (Chl$_{ab}$) and anthocyanin (TAC)

Table 3 shows the changes of berry skin pigments content as a function of shelf life time (days). A significant interaction $P \leq 0.001$between shelf life time and B_{12} concentrations. Berry skin pigments decreased with increasing shelf life time up to end of the experiment. Pre-harvest B_{12} treatment at 9 mM presented more β-Car (2.28 mg 100 g^{-1} FW) and TAC (36.11 mg 100 g^{-1} FW), on the contrary, the total Chl_{ab} content 0.66 mg 100 g^{-1} FW at harvest

Table 2
Effect of preharvest cyanocobalamin (B_{12}) application on SSC%, AT%, SSC/AT ratio, total sugar content (TSC), ascorbic acid (AA mg 100 g^{-1} FW) and Total phenol content (TP mg 100 g^{-1} FW) of 'Crimson Seedless' grapes during four-day shelf-life at 2014 and 2015 seasons.

Treatments	Shelf-life time (days)							
	D1	D2	D3	D4	D1	D2	D3	D4
SSC%					TA%			
Treatments					Treatments			
0	17.00^{e}	18.00^{bcd}	17.33^{de}	17.66^{cde}	0	0.77^{a}	0.75^{ab}	0.74^{b}	0.67^{cd}
3	17.67^{cde}	18.33^{abc}	17.00^{e}	18.00 ^{bcd}	3	0.67^{c}	0.66^{cd}	0.64^{d}	0.61^{e}
6	18.00^{bcd}	18.67^{ab}	17.00^{e}	18.33^{abc}	6	0.61^{e}	0.59^{ef}	0.57^{fg}	0.55^{gh}
9	19.00^{a}	18.66^{ab}	17.67^{cde}	18.33^{abc}	9	0.54^{h}	0.54^{hi}	0.43^{hi}	0.52^{i}
LSD 5%	**0.89**				LSD 5%	**0.04**			
SSC/TA ratio					Total sugars (TSC%)				
Treatments					Treatments				
0	22.08^{i}	24.00^{hi}	23.42^{gh}	26.36^{f}	0	10.83^{f}	10.90^{f}	10.11^{g}	9.92^{g}
3	26.37^{gh}	27.77^{g}	26.56^{ef}	29.51^{de}	3	12.18^{cd}	12.30^{cd}	11.74^{de}	11.07^{ef}
6	29.51^{f}	31.64^{cd}	29.82^{b}	33.33^{bc}	6	12.86^{bc}	12.85^{bc}	12.23^{cd}	11.75^{de}
9	35.19^{cd}	34.56^{b}	41.09^{a}	35.25^{a}	9	15.33^{a}	15.12^{a}	13.46^{b}	12.00^{cd}
LSD 5%	**1.90**				LSD 5%	**0.71**			
Ascorbic acid content (AS mg 100 g^{-1} FW)					Total phenol content (TPC mg 100 g^{-1} FW)				
Treatments					Treatments				
0	1.96^{h}	1.66^{i}	1.29^{j}	1.10^{k}	0	0.26^{ab}	0.23^{cd}	0.23^{d}	0.154^{e}
3	2.11^{h}	2.00^{h}	1.58^{i}	1.35^{j}	3	0.27^{ab}	0.26^{abc}	0.25^{bcd}	0.23^{d}
6	2.85^{e}	2.47^{f}	2.26^{g}	2.07^{h}	6	0.27^{ab}	0.26^{ab}	0.26^{abc}	0.25^{bcd}
9	3.96^{a}	3.73^{b}	3.41^{c}	3.00^{d}	9	0.28^{a}	0.27^{ab}	0.26^{ab}	0.26^{abc}
LSD	**0.18**				LSD	**0.03**			

Means in a column are significantly different at ($P < 0.05$) according to LSD. Each value represents mean of 3 replicates during two seasons of 2014 and 2015. The superscript letters, differ ($P < 0.05$) according to the LSD of two way using two ways incomplete block randomize according to Duncan's test.

Table 3
Effect of preharvest cyanocobalamin (B_{12}) application on β-Carotene (β-Car), Total chlorophyll $_{a\ and\ b}$ (Chl_{ab}), and Total anthocyanin content (TAC) of 'Crimson Seedless' grapes during four-day shelf-life at 2014 and 2015 seasons.

Treatments	Shelf-life time (days)			
	D1	D2	D3	D4
β-Carotene content (CAR mg 100 g^{-1} FW)				
Treatments				
0	1.30^{h}	1.96^{d}	1.59^{g}	1.12^{i}
3	1.99^{d}	2.15^{c}	1.85^{e}	1.34^{h}
6	2.11^{c}	2.30^{b}	1.99^{d}	1.54^{g}
9	2.28^{b}	2.53^{a}	2.11^{c}	1.69^{f}
LSD	**0.14**			
Chlorophyll (Chl_{ab} mg 100 g^{-1} FW)				
Treatments				
0	1.12^{a}	0.94^{b}	0.80^{cd}	0.66^{f}
3	0.94^{b}	0.84^{c}	0.77^{de}	0.64^{fg}
6	0.85^{c}	0.78^{de}	0.73^{e}	0.60^{g}
9	0.64^{fg}	0.63^{fg}	0.54^{h}	0.48^{i}
LSD	**0.05**			
Total anthocyanin content (TAC mg 100 g^{-1} FW)				
Treatments				
0	16.43^{gh}	17.80^{fg}	14.69^{h}	10.43^{i}
3	19.95^{e}	20.92^{e}	16.58^{gh}	12.14^{i}
6	25.84^{c}	30.66^{b}	19.55^{ef}	15.76^{gh}
9	36.11^{a}	37.70^{a}	23.66^{d}	15.50^{h}
LSD	**2.98**			

Means in a column are significantly different at ($P < 0.05$) according to LSD. Each value represents mean of 3 replicates during two seasons of 2014 and 2015. The superscript letters, differ ($P < 0.05$) according to the LSD of two way using two ways incomplete block randomize according to Duncan's test.

time was observed. The changes in β-Car and TAC during shelf life are noticed owing to the continued synthesis of β-Car and TAC which occurred for a limited period at all B_{12} treatments at 2nd day of shelf life. Therefore, in β-Car and TAC which were then followed by a decrease in up to end shelf life time.

Total chlorophyll responds differently to B_{12} treatments. The reductions of Chl_{ab} is evenly distributed across all components, however, with B_{12} at 9 mM treatment, the decreasing of Chl_{ab} suggests a breakdown of Chl_b containing chlorophyll binding protein such as the LHC (light harvest center) of photosystem I or II [31]. While β-Car behave quite differently which increases up to 2nd day and then decrease up to end experiment time. It could be due to the effect of high concentration of B_{12} (9 mM) activates many metabolic processes are enhanced which are diverted towered the lipid-soluble vitamin A [32]. Also, the increases TAC at 2nd day of shelf life can be related to activating major precursors of anthocyanin are responsible for increasing TAC such as cyanidin, peonidin and the acylated derivatives in berry skin and pulp [3,6]. Continuously, the degradation of chlorophyll might be due to senesce of berry and deterioration during shelf life period, thereafter, TAC was more appearance [2].

4. Rachis browning index

Rachis browning index: rachis quality of bunches has been investigating extensively among producers and exporters because of its high impact on the cluster freshness that determines consumers. Table 1 shows a significant at $P < 0.05$ when the B_{12} concentrations were considered as a factor. It is clear that the treatments at 9 mM are more effective to reduce cluster rachis browning compared to other treatments. At harvest time, there was no evidence for RBI up to 3rd days of shelf life time. In this case, RBI was detected only at 4th day. It was minimized RBI round slight browning incidence (2.20) compared to other control fruit (4.06 severity symptoms) and B_{12} treatments (3 mM, 3.04 and 6 mM, 2.95). It is clear that B_{12} treatment at 9 mM has a good potential beneficial against rachis browning of detached grape clusters. It could be due to activating enzymatic carbohydrate synthesis during berry development [30]. Therefore, the activation processes such as Calvin cycle, the pentose phosphate, and Glycolysis pathway might constitute the increase sugar content in berry development. So, total sugars increased (Glucose and fructose) which it could be a precursor toward to increase AA synthesis in berry juice [8]. The increases of TP might be explained that the increases of AA berry content by which maintaining the amount of TP as antioxidant properties [33] also, cyanocobalamin may modulate the active oxygen species [26].

5. Conclusion

It might be concluded that the application of cyanocobalamin (B_{12}), especially preharvest treatment at 9 mM had a positive impact in improving anthocyanin pigment content of berries and maintaining cluster quality during shelf life. Moreover, it minimizes the cluster/riche browning incidence by preserving phenolic compounds from oxidation during four days of shelf life. it is due to increase ascorbic acid synthesis by increasing metabolic carbohydrates during berry development. Hence, the preharvest application of B_{12} at 9 mM can be applied as an effective method for improving anthocyanin pigment and postharvest quality attributes of 'Crimson seedless' at harvest time and during marketing.

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