J. Plant Prod., Mansoura Univ., Vol. 1 (12): 1653 - 1663 , 2010

CYANOCOBALAMIN CONTROL FRUIT RIPENING OF PERSIMMON FRUITS

Lo'ay, A.A.
Pomology Dept., Faculty Of Agriculture Mansoura University.
E-mail: Loayarafat2011@gmail.com www.mans.edu.eg

ABSTRACT

Cyanocobalamin (B_{12}) may generate many different physiological processes in plant tissue such as: protein metabolism, soluble lipids, carbohydrates and ethylene. Changes in tannins and total sugars in astringent persimmons cultivar 'Costata' were investigated during shelf-life postharvest period. Soluble tannins exhibited significant decreases up to the experiment end. Total sugars increased 4 fold with fruit immersed in B_{12} 0.444 µM compared with other treatments. Fruit firmness decreases with increasing B_{12} concentration during the entire period monitored. Moreover, it also significantly retarded the increase in β-carotene content and ion leakage % while decreased the fruit firmness and increased h° up to day 10 then decreased and be constant during shelf-life. Generally, B_{12} treatments can greatly extend the postharvest life and increased fruit quality of 'Costata' persimmon fruit during shelf-life.

INTRODUCTION

Persimmon (*Diospyros kaki* L.) is one of the most important fruit was recently cultivated in Dakahlia province. It relatively has high content of dietary fibers, total and major phenolics, vitamins, and trace elements, which makes fruit preferable for healthy diet (Gorinstein*, et al.*, 2001). The most part of persimmon cultivars in Egypt is the astringent 'Costata' persimmon. It considered one of the important astringent and/early cultivar which is very easy to soften after harvesting and handling at ambient temperature (Zisheng, 2006). Although, Egypt has a great potential to produce high fruit quality and export to other countries. Its marketability is still limited to local market. This is due to the delicate nature of fruit, poor post-harvest technique for handling and transporting and storage facilities (Özdemir*, et al.*, 2009), and the common technique for controlling fruit ripening processes under ambient temperature. The rate of postharvest deterioration is affected by temperature (Arnal and del Río, 2004). Even it used to prevent many post-harvest aspects such as, controlling insect pests, fungal rots, and increasing chilling injury tolerance. Therefore, heat application is being extensively studied as commercial method to control fruit ripening (Zisheng, 2006). However, The persimmon is also considered to be a good source of ascorbic acid (Testoni, 2002) and tannins (Gu*, et al.*, 2008), and these are related to various physiological functions including a protective role against oxidative stress (Suzuki*, et al.*, 2005).

Cobalamin, also called vitamin B_{12}, is a water-soluble vitamin (Asensi-Fabado and Munne´-Bosch, 2010). Also it found in the cytosol, plastids and mitochondria (Roje, 2007). Higher plants neither synthesize nor require vitamin B_{12} because they contain cobalamin-independent

methioninesynthase (*Met*) (Smith*, et al.*, 2007). Methioninesynthase catalyses the final reaction of the *Met* biosynthetic pathway in two steps, the first step, is catalysed by the enzyme cystathionineγ-synthase (*CgS*) to form cystathionine from the substrates cysteine and *O*-phosphohomoserine. It is important to note that *O*-phosphohomoserine is also the immediate precursor of threonine so that methionine synthesis and threonine synthesis compete for a common substrate. The reaction catalysed 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 in plants from N5-methyl-tetrahydrofolic acid to homocysteine by a vitamin-B_{12}-independent methionine synthase to yield *Met* (Zeh*, et al.*, 2002). 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 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 (Zeh, *et al*., 2002)

No study has been reported on immersing 'Costata' kaki fruits in B_{12} solution. Therefore, the objective of this study is to exploit the physiological roles of B_{12} for prolonging the shelf-life of Costata and improving fruit quality.

MATERIALS AND METHODS

Fruit harvesting and shelf-life condition

Fruit were harvested on November 2007 and 2008 from trees more than 10 years old growing in clay soil of a commercial orchard. It was located in Dakahlia province. Costata cultivar fruits were harvested at maturity stage according to colour development (Badawy, 2008), when colour was become 50% yellow and 50% orange. Fruits were collected and washed with cold water to reduce the field temperature and microbial load on fruit surface. 375 uniform fruits in colour and size were divided into two batches. The first batch, 150 fruits were distributed at 5 treatments (3 replicates), only each 30 fruits for measure colour and ripening. The second batch 255 fruit extra, also divided at 5 treatments (3 replicates) for fruit quality destructive measurements. Two fruit samples from each replicate were picked every 5 days intervals for destructive fruit chemical measurements.

Cyanocobalamin B_{12} application

All fruit treated by immersing in B_{12} solution for 6-hours at room temperature 25±2 whereas, the control fruits (75 fruits) were immersed in water for the same period. 300 fruits were immersed in B_{12} solution, at different concentrations 0.075µM, 0.147 µM, 0.296 µM and 0.444 µM at room temperature.

Measurements

Total tannins were determined by titrating method as presented by (Badawy, 2008). Total tannin content present as percentage.

Fruit firmness was measured at both fruit sides using Effegi-pentrometer supplemented with a plunger 8 mm prop. Firmness was measured on the opposite side along the equatorial region of the fruit and expressed in Newton (N) (Zisheng, 2006)

Fruit colour, the computer vision system is used (Kang*, et al.*, 2008). Two fluorescence lamps (TL-D Delux, 18W/965, Philips) were attached at the top corner of the light box, in parallel, at an angle of 45° to the produce location. These lamps were chosen to set the colour temperature to D65 (6500 K), a common light source used in food colour measurement. Both lamps were covered with diffuse battens, and the internal size of the light box was 700mm (width) ×700mm (length) ×500mm (height). This light box was designed to illuminate an A3 size area. In order to illuminate this large surface as evenly as possible, the inside walls and ceiling were painted white, while the floor (and background for the photos) was painted black to prevent reflection. A colour digital camera (JVC) captured images through a hole on the top surface. The camera settings for this experiment were: manual mode ISO200; shutter speed 1/100; aperture 5.0; no zoom; no flash; resolution 2816×2112; format JPEG. Three colour values (RGB) of each colour tile image are converted to pixel has RGB values between 0 and 255. Thereafter, all images were analyzed by using software ImageJ Ver. 1.43u USA to get RGB signals to calculate the fruit skin (Khojastehnazhand*, et al.*, 2010).

β-carotene was spectrophotometrically determined by modified methods (Lo'ay, 2005). The extraction method was modified by using *N,N*-dimethylformamide (DMF) instead of acetone. Samples were stored at 4°C for 16 hours to allow the DMF to leach the pigments from the sample. Finally, samples were centrifuged for 5 min at 16000 rpm, and then samples were determined for wavelengths 452 nm for β-carotene. β-carotene was expressed in mg 100 g^{-1} FW.

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 (Shalan, 2009). Ion leakage was measure according to methods was described (Lo'ay, 2005).

Statistical analysis

Data for evaluation of parameters in time were analyzed using analysis of variance (ANOVA). The means were compared using the least significant differences (L.S.D.) at ≤0.05 level of probability. The statistical software package GenStat Ver. 11(Lawes Agriculture Trust, Rothamsted Experimental station, UK) was used.

RESULTS AND DISCUSSION

Tannin concentration %

Total tannin concentration as function of shelf-life time for all B_{12} applications is shown in figure 1. The soluble tannin concentration declined from the level of 2.0% to 1.5% at day 10 of storage time. Thereafter, the decreases of tannin content decreased more clearly from day 10 up to end of

self-life period. The significant differences in tannin concentration were observed between all B_{12} treatments. Significantly higher decreases with B_{12} application at 0.444 µM compared with other treatments and control. It is clear that the degradation of tannin by B_{12} treatments during 25 days of shelf-life especially, the high concentration of B_{12} at 0.444 µM. it suggested that B_{12} generates B_{12} –independent methionine synthase in fruit tissue by catalyzes the methylation of homocysteine to methionine with 5-methyltetrahydrofolate as a methyl group donor, then protein, lipids and nucleic acid which are related to create enzymes in fruit during shelf-life are activated (Ravanel*, et al.*, 2004).

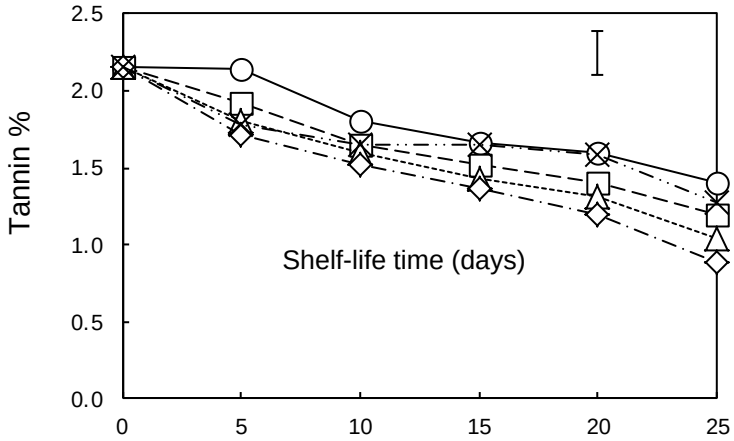


Figure 1: Tannin percentage of Costata cultivar harvested at maturity stage at shelf-life. Symbols represent the B_{12} application at different concentrations (-○- B_{12} at 0.075µM, --□-- B_{12} at 0.147 µM, -.-△- B_{12} at 0.296 µM, ---◇--- B_{12} at 0.444 µM, and --×-- control). Tannin percentage expressed as main (n=3).the vertical bar indicated to L.S.D. at p=0.05 for 25 days of shelf-life.

These activations, provoked ethylene synthesis by recycling of sulphur atom of methionine, then it activates 1-aminocyclopropane-1-carboxylate synthase to produce ethylene (Ravanel*, et al.*, 1998). So, generating fruit ripening via B_{12} application during their shelf-life by increasing ethylene synthesis, since, the kaki fruit is classified climacteric fruit (Nakano*, et al.*, 2003).

Fruit firmness (N)

Figure 2. Illustrates the fruit firmness (*N*), while fruits were immersed in four different solution of B_{12} for 25 days of shelf-life. Firmness data showed erratic changes during the whole period monitored; this led, however, to an overall decreasing tendency of the flesh consistency (Bubba*, et al.*, 2009). The significant decrease of fruit firmness becomes clear after day 5 of shelf-life. The changes were independent according to B_{12} solution concentrations. It was higher with immersing fruit in 0.444 µM; 7.47 N, and it

is less with low concentrations. Basically, fruit firmness depends on many properties of the fruit tissue: water content, the nature of the cell wall and turgor are clearly important sources related to firmness. Also the breakdown of cell wall polysaccharides during shelf-life resulted in increasing activity of some enzymes; polygalacturonases, cellulase and β-galactosidase (Lo'ay, 2005). Based on these, an explanation could be suggested for rapid decrease in firmness of fruits when they were immersed in B_{12} solution. However, fruit firmness declined much rapidly with control fruits than fruits were immersed in B_{12} solution. It could be illustrated that the hydrolysis process of cell wall polysaccharides enhanced more rapidly as affected by immersing in B_{12} solutions.

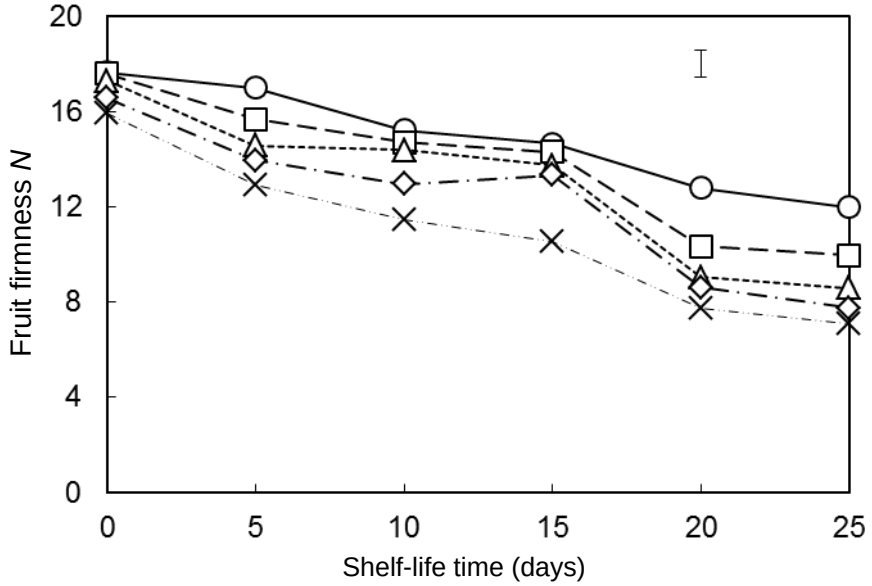


Figure 2: Firmness of Costata cultivar harvested at maturity stage at shelf-life. Symbols represent the B_{12} application at different concentrations (-○- B_{12} at 0.075μM, --□-- B_{12} at 0.147 μM, -.-△- B_{12} at 0.296 μM, ---◇--- B_{12} at 0.444 μM, and --×-- control). Firmness *N* expressed as main (n=3).the vertical bar indicated to L.S.D. at p=0.05 for 25 days of shelf-life.

β-Carotene content (β-Car)

Figure 3. debates the changes of β-Car content during shelf-life of kaki fruit during 25 days, since, β-Car shows significant interaction (P<0.001). β-Car was initially increased after 5 days of storage and continues in the same pattern till 10 days. The response of fruits to different concentrations of B_{12} showed a significant interaction. It was higher content with fruit that immersed in 0.444μM (40.80 mg 100 g^{-1} FW at day 15 of shelf-life), and it was shifted according to B_{12} concentrations. It was 39.10 mg 100 g^{-1} FW at day 15 with B_{12} 0.296 μM, at day 20 with B_{12} 0.145 μM (35.10 mg 100 g^{-1}

FW), and becomes almost stable with low B_{12} concentration 0.074 µM (round 30.10 mg 100 g^{-1} FW). However, the highest β-Car content was observed with control fruits.

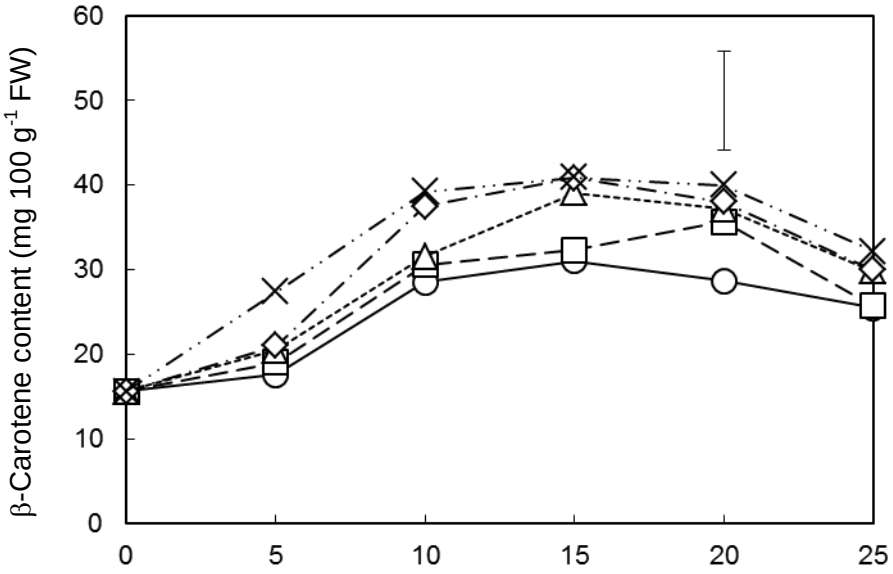


Figure 3: β-Carotene content of Costata cultivar fruits harvested at maturity stage at shelf-life. Symbols represent the B_{12} application at different concentrations (-○- B_{12} at 0.075µM, --□-- B_{12} at 0.147 µM, -.-△- B_{12} at 0.296 µM, ---◇--- B_{12} at 0.444 µM, and --×-- control).β-Carotene expressed as main (n=3).the vertical bar indicated to L.S.D. at p=0.05 for 25 days of shelf-life.

The different fruit responses to B_{12} applications during shelf life with regards to the nature of cultivar, even the β-Car is classified and acted as antioxidant with chlorophyll by quenching singlet oxygen once formed in plant. The increases of β-Car by increasing the B_{12} concentrations might suggest that the B_{12} increases the activation of both carbohydrate pathways of Calvin and pentose phosphate and glycolysis (Asensi-Fabado and Munne´-Bosch, 2010). Afterword, many metabolic processes are enhanced which are diverted toward the lipid-soluble vitamin such as β-Car.

Skin colour Hue (h^o)

Figure 4 presents the Hue angle of persimmon fruit skin colour increased after day 5 of shelf-life ripening round 40, thereafter, it declined up to day 15 of shelf-life period and became almost stable until end of period (round 30). It is clear that hue angle differs depending on B_{12} applications but the last 15 days of shelf-life is almost there difference among treatments. The highest hue at day 10 it could be related to increase β-carotene synthesis by

B_{12} application (Zisheng, 2006).This indicated that yellow colour development was enhanced by B_{12} treatments as shown in photograph 1.

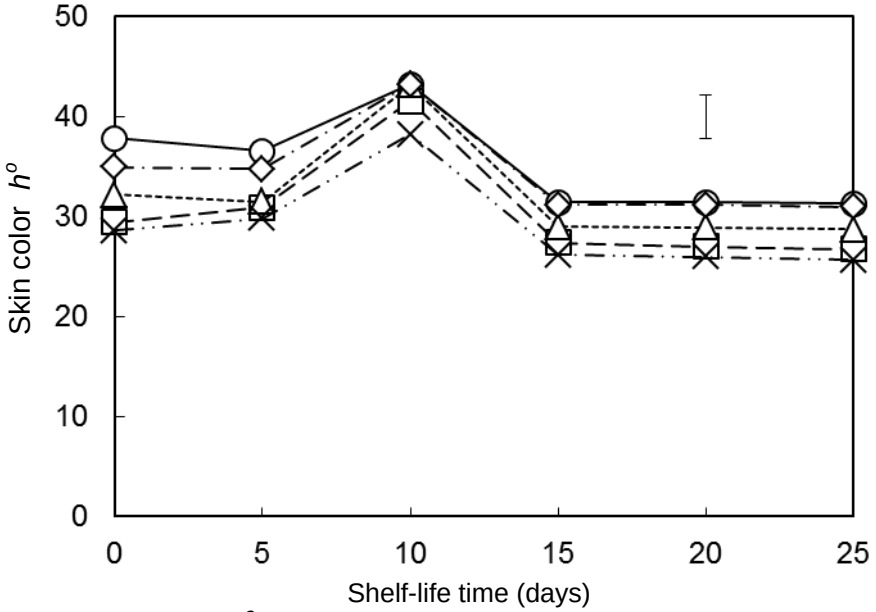
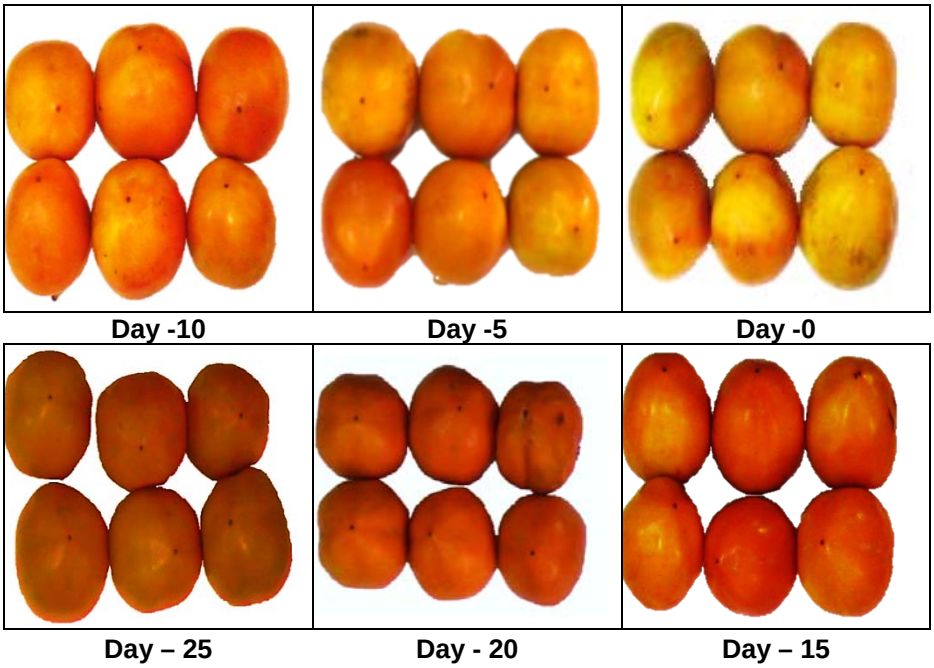


Figure 4:Colour h^o of Costata cultivar harvested at maturity stage at shelf-life. Symbols represent the B_{12} application at different concentrations (-○- B_{12} at 0.075µM, --□-- B_{12} at 0.147 µM, -.-△- B_{12} at 0.296 µM, ---◇--- B_{12} at 0.444 µM, and --×-- control Colour H^o expressed as main (n=3).the vertical bar indicated to L.S.D. at p=0.05 for 25 days of shelf-life

Ion leakage %

Figure 5 shows the ion leakage percentage plotted as function of shelf-life time (days) when kaki fruits were immersed at four different B_{12} solution applications. It apparent from the figure that fruits immersed in B_{12} solution 0.074 µM has less ion leakage than other treatment. It is very term that ion leakage increases with increasing B_{12} application and shelf-life time. These responses of fruit to B_{12} application during shelf-life could related to that B_{12} keeps enhancing ascorbic acid, β-carotene and α-Tocopherol in fruit tissues (Rébeillé*, et al.*, 2007). Since, the last vitamins are considered as antioxidants which play a role to scavenge active oxygen species during shelf-life (Asensi-Fabado and Munne´-Bosch, 2010). The important roles of these vitamins are to maintain the right functions of cell membrane of cells/tissue (of fruits), so less ion leakage was observed. However, increases the B_{12} (0.444 µM), could enhance fruit ripening so more leakage can be noticed.



Photograph 1. Presents the shin colour development during shelf-life of persimmon kaki fruits was immersed in 0.444 µM for 24 hour. Hue angle shows significant interaction (P<00.1), when days of shelf-life and B_{12} treatment are considered.

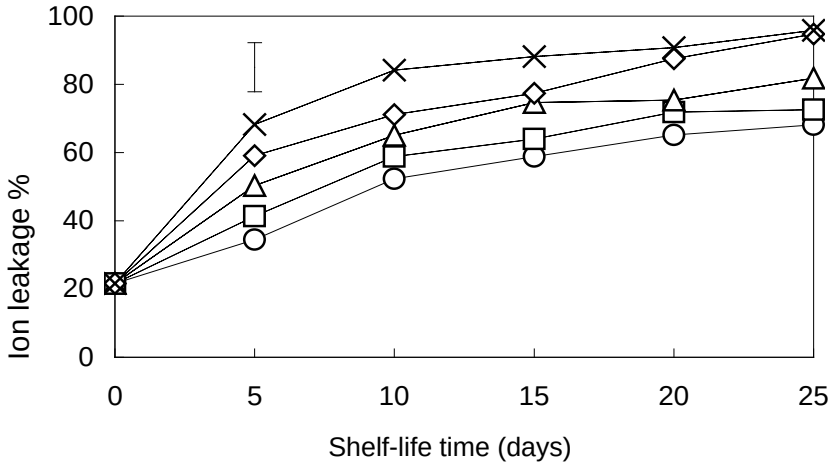


Figure 5: Ion leakage % of Costata cultivar harvested at maturity stage at shelf-life. Symbols represent the B_{12} application at different concentrations (-○- B_{12} at 0.075µM, --□-- B_{12} at 0.147 µM, -.-△- B_{12} at 0.296 µM, ---◇--- B_{12} at 0.444 µM, and --×-- control). Ion leakage percentage expressed as main (n=3).the vertical bar indicated to L.S.D. at p=0.05 for 25 days of shelf-life.

Total sugars

Total sugar concentrations determined in kaki fruits were increased independence to the B_{12} applications. It is clear from the figure that total sugars increased with immersing fruit in B_{12} solution 0.444 µM, and it has less content with the lowest B_{12} concentration 0.074 µM (other treatment. However, it is very term that the control fruit presents less content of sugars at end of the shelf-life time. It might be suggest that B_{12} treatment activation of both carbohydrate pathways of Calvin and pentose phosphate and glycolysis (Asensi-Fabado and Munne´-Bosch, 2010). Afterword, many metabolic processes are enhanced which are diverted toward glucose, fructose and sucrose increases during shelf-life period (Bubba, *et al*., 2009).

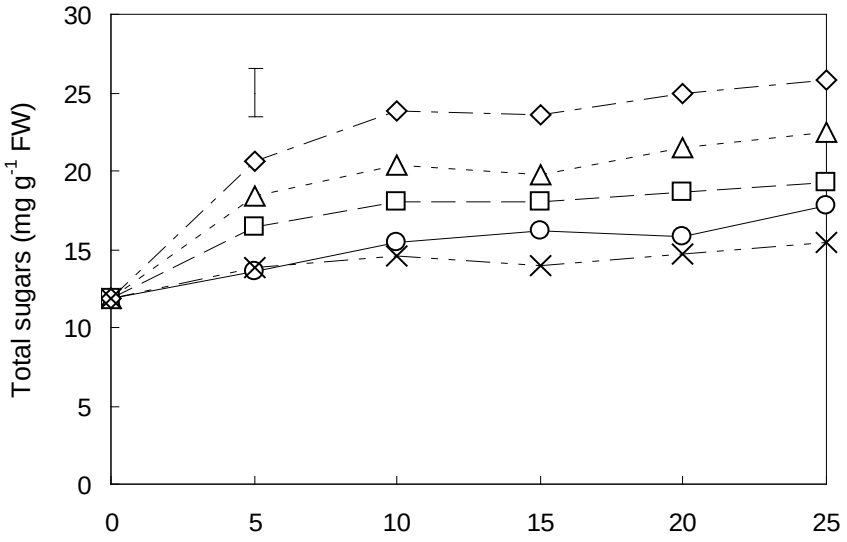


Figure 6: Total sugars of Costata cultivar harvested at maturity stage at shelf-life. Symbols represent the B_{12} application at different concentrations (-○- B_{12} at 0.075µM, --□-- B_{12} at 0.147 µM, -.-△- B_{12} at 0.296 µM, ---◇--- B_{12} at 0.444 µM, and --×-- control). Ion leakage percentage expressed as main (n=3).the vertical bar indicated to L.S.D. at p=0.05 for 25 days of shelf-life.

In conclusion, the kaki fruit presents increase in total sugars and fruit pigment (β-Carotene) and decreases in fruit firmness and total tannin after 25 days of shelf-life when fruits were immersed in different B_{12} solutions. B_{12} application was necessary for increase skin colour development and acceptable firmness loss during shelf-life. These characteristics would classify that B_{12} application as a new method to ripening kaki fruit chemically. Based to , the physiological roles of B_{12} in plant/fruit tissue were enhanced such as protein, soluble lipid and cDNA and acting as antioxidant (Asensi-Fabado and Munne´-Bosch, 2010) via addition it to the fruits.

REFERENCES

Arnal, L. and del Río, M.A., (2004). Quality of persimmon fruit cv. Rojo brillante during storage at different temperatures. Spanish Journal of Agricultural Research, 2: 243-247

Asensi-Fabado, M.A. and Munne´-Bosch, S., (2010). Vitamins in plants: occurrence, biosynthesis and antioxidant function. Trends in Plant Science, 15: 582-592

Badawy, A.A., (2008). Studies on ripening of kaki fruits. MS Thesis, Agriculture Faculty of Mansoura University, Egypt,

Bubba, M.D., Giordani, E., Pippucci, L., Cincinelli, A., Checchini, L. and Galvan, P., (2009). Changes in tannins, ascorbic acid and sugar content in astringent persimmons during on-tree growth and ripening and in response to different postharvest treatments. Journal of Food Composition and Analysis, 22: 668-677

Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zemser, M., Weisz, M., Trakhtenberg, S. and Màrtín-Belloso, O., (2001). Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples. Journal Agricultural and Food Chemistry, 49: 952-957

Gu, H., Li, C., Xu, Y., Hu, W., Chen, M. and Wan, Q., (2008). Structural features and antioxidant activity of tannin from persimmon pulp. Food Research International, 41: 208–217

Kang, S.P., East, A.R. and Trujillo, F.J., (2008). Colour vision system evaluation of bicolour fruit: A case study with 'B74' mango. Postharvest Biology and Technology, 49: 77-85

Khojastehnazhand, M., Omid, M. and Tabatabaeefar, A., (2010). Development of a lemon sorting system based on color and size. African Journal of Plant Science, 4: 122-127

Lo'ay, A.A., (2005). Chilling injury in mangoes. Ph.D. Thesis. Wageningen University., ISBN:90-8504-309-3: 1-224., 1-224

Nakano, R., Ogura, E., Kubo, Y. and Inaba, A., (2003). Ethylene biosynthesis in detached young persimmon fruit is initiated in calyx and modulated by water loss from the fruit. Plant Physiology, 131: 276-286

Özdemir, A.E., Çandır, E.E., Toplu, C., Kaplankıran, M., Yıldız, E. and Inan, C., (2009). The effects of hot water treatments on chilling injury and cold storage of fuyu persimmons. African Journal of Agricultural Research 4 1058-1063

Ravanel, S., Block, M.A., Rippert, P., Jabrin, S., Curien, G., Rébeillé, F. and Douce, R., (2004). Methionine Metabolism in Plants. The Jouranal of Biological Chemistry, 279: 22548-22557

Ravanel, S., Gakière, B., Job, D. and Douce, R., (1998). The specific features of methionine biosynthesis and metabolism in plants. The Proceedings of the National Academy of Sciences, 95: 7805-7812

Rébeillé, F., Ravanel, S., Marquet, A., Mendel, R.R., Smith, A.G. and Warren, M.J., (2007). Roles of vitamins B5, B8, B9, B12 and molybdenum cofactor at cellular and organismal levels. Natural Product Reports, 24: 949-962

Roje, S., (2007). Vitamin B biosynthesis in plants. Phytochemistry 68: 1904–1921

Shalan, A.M., (2009). Studies on bud dormancy, yeild and fruit quality of Le-Conte pears. Ph.D. Thesis, Agriculture Faculty of Mansoura University, Egypt,

Smith, A.G., Croft, M.T., Moulin, M. and Webb, M.E., (2007). Plants need their vitamins too. Current Opinion in Plant Biology, 10: 266–275

Suzuki, T., Someya, S., Hu, F. and Tanokura, M., (2005). Comparative study of catechin compositions in five Japanese persimmons (*Diospyros kaki*). Food Chemistry, 93: 149-152

Testoni, A., (2002). Post-harvest and processing of persimmon fruit. In: Bellini, E., Giordani, E. (Eds.), First Mediterranean Symposium on Persimmon. Options Mediterraneennes, Series A, 51:

Zeh, M., Leggewie, G., Hoefgen, R. and Hesse, H., (2002). Cloning and characterization of a cDNA encoding a cobalamin-independent methionine synthase from potato (*Solanum tuberosum* L.). Plant Molecular Biology, 48: 255–265

Zisheng, L., (2006). Extending shelf-life of persimmon (*Diospyros kaki* L.) fruit by hot water. Europ Food Research Technology, 222: 149-154

التحكم فى نضج ثمار الكاكى بأستخدام السيانوكوبلامين خلال فترة التسويق

لؤى عبد اللطيف

قسم الفاكهة – كلية الزراعة – جامعة المنصورة

أجريت هذه الدراسة على ثمار الكاكى صنف كوستاتا بعد الحصاد خلال موسمى 2007-2008 المنزرعة بمزرعة خاصة بمحافظة الدقهلية بهدف التحكم او تنظيم نضج ثمار الكاكى للحفاظ على جودة الثمار خلال فترة التسويق باستخدام مادة السينانوكوبلامين (فيتامين ب12) حيث تم نقع الثمار فيها لمدة 6 ساعات بتركيزات مختلفة (0.074 - 0.147 – 0.296 – 0.444 ميكرومول) ثم خزنت الثمار على درجات الحرارة الغرفة العادية (3±22، رطوبة 75%) لمدة 25 يوم.

اوضحت النتائج المتحصل عليها من هذه الدراسة ان مستوى التنينات انخفض انخفاضا معنويا فى الثمار المنقوعة فى التركيز العالى من مادة السينانوكوبلامين و كذلك لون الثمار و السكريات الكلية و الكاروتين كأهم عناصر جودة ثمار الكاكى، كما انخفضت صلابة الثمار بخلاف المعاملات الاخرى و الكنترول، كما توصى الدراسة بأن نقع الثمار الكاكى لمدة 6 ساعات فى محلول مادة السينانوكوبلامين يحسن من الصفات الكميائية و الفزيائية لثمار الكاكى كطريقة جديدة للأنضاج بعد الحصاد مع الحفاظ على صلابة الثمار و بالتالى تحملها لعمليات التداول المتكررة.

قام بتحكيم البحث

أ.د/ السيد البدوى طه البلز — كلية الزراعة – جامعة المنصورة

أ.د/ على محمد كمل الخريبى — كلية الزراعة – جامعة قناة السويس