http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (3): 428-433, 2006
ISSN 1028-8880

The Effects of Light, Storage Temperature, pH and Variety on Stability of Anthocyanin Pigments in Four *Malus* Varieties

M.A. Bakhshayeshi, M. Khayami, R. Heidari and R. Jamei
Department of Biology, Faculty of Science, Urmia University, Iran

Abstract: The anthocyanin pigments was extracted from the four *Malus* varieties such as *M. domestica* cv. red starking, *M. domestica* cv. red delicious, *M. domestica* cv. jonathan and *M. domestica* cv. abbasi mashhad using the soaking in Ethanol (1% acidified).The extracted anthocyanin pigments then were exposed to number of environmental factors which could destabilize the anthocyanin molecules. These environmental factors were included three different pH (1, 2.5 and 4), four various temperatures (5, 20, 30 and 40°C) and presence or absence of light. The results showed that increasing in pH, temperature or exposure to light is able to destruction the anthocyanin pigments. Another factor affecting the tolerance of anthocyanin towards the environmental condition is the role of different varieties. Among the various *Malus* varieties anthocyanin pigment in *M. domestica* cv. red starking, showed the greatest resistance to destruction by environmental conditions, other varieties followed: *M. domestica* cv. red delicious *M. domestica* cv. jonathan and *M. domestica* cv. abbasi mashhad consecutively.

Key words: *Malus*, anthocyanin, stability, pH, light and temperature

INTRODUCTION

The anthocyanins comprises the largest group of water soluble pigments in the plant kingdom and are especially characteristic of the angiosperms or flowering plants, which themselves provide our major source of food crops in food plants, anthocyanins are widespread occurring in at least 27 families, 73 genera and a multitude of species and can be classified chemically as both flavonoid (related to flavone/isoflavone, $C_{15}H_{10}O_2$) and phenolic related to phenol, C_6H_5OH) (Malien-Aubert *et al*., 2001). Anthocyanins are synthesized in the cytoplasm of plant cells and actively transported across the tonoplast into the vacuole, where they accumulate (Leng *et al*., 2000). They are responsible for the red, pink, mauve, purple and blue colours found in the flowers fruits and leaves of Angiosperms, as well as some Gymnosperms and some Bryophytes (Yamazaki and Saito, 2002). There has been a great increase in utilization of natural plant pigments in the food industries, replacing the implementation of artificial coloring agents, in order to provide a healthier food for consumers. However, in comparison with the natural coloring agents, the artificial coloring agents show greater resistance and stability when exposed to oxidation changes in temperature, pH and other factors (Navidra *et al*., 2002). Although anthocyanins are less stable in various environmental condition, they include varieties of colors such as orange, red, maroon and blue which make them an attractive alternative as coloring agents in food industries (Mazza and Minitiati, 1993). The intensity and stability of the anthocyanin pigments is dependent on various factors including structure and concentration of the pigments, pH, temperature, light intensity, quality and presence of other pigments together, metal ions, enzymes, oxygen, ascorbic acid, sugar and sugar metabolites, sulfur oxide etc. (Lee, 2002). Anthocyanins have four different structures, which are in equilibrium and include flavylium cation, quinoidalbase, carbinolpseudobase and chalcone. The relative amounts of these structures in equilibrium are varied and depend on the pH and anthocyanin structure (Spayd *et al*., 2002). Some anthocyanins are more stable than other depends on their molecular structure. The example of this is the Malvidin glycosides, the major anthocyanin in grape, which due to dimethyloxylation of t he molecules are more stable than other anthocyanins (Bridle and Timberlake, 1997). Moreover hydroxylation of organic acids results in more stable molecules in most cases (Cooper-Driver, 2001). Anthocyanins have applications in chemotaxonomical and ecological studies. They have bright attractive colours. Another factor in increasing the stability of the anthocyanin is the co-pigmentationv (Cordenunsi *et al*., 2003). The skin of *Malus* of red fruit is also among the plants, which contains large amount of anthocyanin. Transformation of these pigments to other forms by enzymes, oxidation, light, temperature, etc., during storage

Corresponding Author: M. Khayami, Department of Biology, Faculty of Science, Urmia University, Iran
Fax: +984412776707

cause color change from red to brown in *Malus* fruits, which has a negative impact on appearance of the product.

There are also more stable color pigments exist in the fruits and vegetables which their phyto-chemical structure and anthocyanin properties need to be investigated with regard to importance of anthocyanin in different industries in the current research, effects of some environmental factors were evaluated on the stability of anthocyanin in four *Malus* varieties in uromieh such as: *M. domestica* cv. jonathan *M. domestica* cv. red starking, *M. domestica* cv. red delicious and *M. domestica* cv. abbasi mashhad.

MATERIALS AND METHODS

Sample preparation: Samples of *Malus* varieties such as M. domestica cv. red starking, *M. domestica* cv. red delicious, *M. domestica* cv. jonathan and *M. domestica* cv. abbasi mashhad gathered in urmia city area of Ghasemlo in provence of West Azarbayjan in Iran. Then fruits were washed with distilled water and dried and kept frozen at -8°C till use.

Methods: We used the methods of Chiriboga and Francis (1970) using ethanol, acidified with 0.1% hydrochloric acid 1% for extraction of anthocyanin. Samples were taken out of the freezer, left at room temperature for 30 min to defrost. Three hundred grams skin of each *Malus* variety were put in a mixer, solvent added and a mixed for 10 min. The products then filtered in vacuum using Bochner funnel and wattman filter (grade 1), the remain of the mixture on the filter paper was washed again with the solvent and filter again to get a clear liquid. The filtered product then placed in a balloon container in a vacuum evaporator at 35°C to separate the ethanol-acid solvent. The balloon container was separated from the vacuum evaporator and distilled water was added to dissolve the powder which was formed at the bottom of the balloon container. The product then transferred to a 500 mL container and brought the volume to 500 mL using distilled water. The product then centrifuged at 8000 rpm, the supernatant was separated and kept for further analysis. The anthocyanin contents of the extracts was measured by Spectrophotometer (UV/Visible). The following two buffers were used: a) 0.13 M HCl-0.05 M KCl, pH = 1.0 and (b) 0.05 M HCl -0.5M CH_3COONa, pH = 5. The mixture of buffer and samples were equilibrated in darkness for 1 h and their absorbance was measured at 520 nm by spectrophotometer.

RESULTS AND DISCUSSION

The role of *Malus* varieties on the stability f anthocyanin: There are great inter-genus and inter-species variations between the level of phenolic compounds in fruits. Depends on the *Malus* varieties the stability of anthocyanin varies. Stability of anthocyanin extracted from four different *Malus* varieties in a fixed temperature of 25°C and pH = 2 measured. The results show that *M. domestica* cv. red starking contains the most stable form of anthocyanin and *M. domestica* cv. abbasi mashhad has the least stable form of anthocyanin. The level of anthocyanin destruction after 90 days in *Malus* varieties was 51.92, 64.67, 68.77 and 94.49% for *M. domestica* cv. red starking, *M. domestica* cv. red delicious, *M. domestica* cv. jonathan and *M. domestica* cv. abbasi mashhad consecutively (Fig. 1 and Table 1).

The effect of pH on the stability of anthocyanin: Another factor which affects the stability of anthocyanin is the pH (1, 2.5 and 4). All the experiments were performed in a fixed temperature of 25°C in 90 days period and total of 5 separate measurements for each variety. Present results show that increasing pH cause greater destruction of anthocyanin in samples (Fig. 2 and Table 2). The degree of anthocyanin destruction between pH = 2.5 and 4 is considerably greater than that of pH = 1 to pH = 2.5. Flavylium salts are stable only in highly acidic conditions. These salts loose the proton in higher pH and transform into quinoidal base, which is an unstable pigment and immediately bound to water and form colourless compound called chromenol. Delpech (2000) have reported that in warm agricultural areas, high pH of the grapes at the time of harvest could cause problem for the juice making industry. Higher pH in grapes can cause fading the colour and decrease in stability of the products. Little (1997) studied the combination of the colour pigments in strawberry, jam and packaged strawberries at the 37.7°C during time. Also recorded the data for pH of 2, 3 and <1 and showed that destruction of anthocyanin pigments increases with increase in pH.

The effect of temperature on the destruction of anthocyanin: Temperature is also another factor, which has a role in destabilising the anthocyanin molecular structure; with increase in temperature we see a greater degree of destruction in anthocyanin. In a fixed pH of 2, the effect of four different temperatures of 5, 20, 30 and 40°C on level of anthocyanin extracted from four different

Table 1: Comparison effect of varieties on the percentage of destruction of anthocyanin in four *Malus* varieties

	Days			
Malus varieties	0	21	42	63
Malus domestica cv. red starking	0.00	22.19	37.20	51.92
Malus domestica cv. red delicious	0.00	19.24	38.96	64.67
Malus domestica cv. jonathan	0.00	21.78	36.69	68.77
Malus domestica cv. abbasi mashhad	0.00	49.36	91.79	94.49

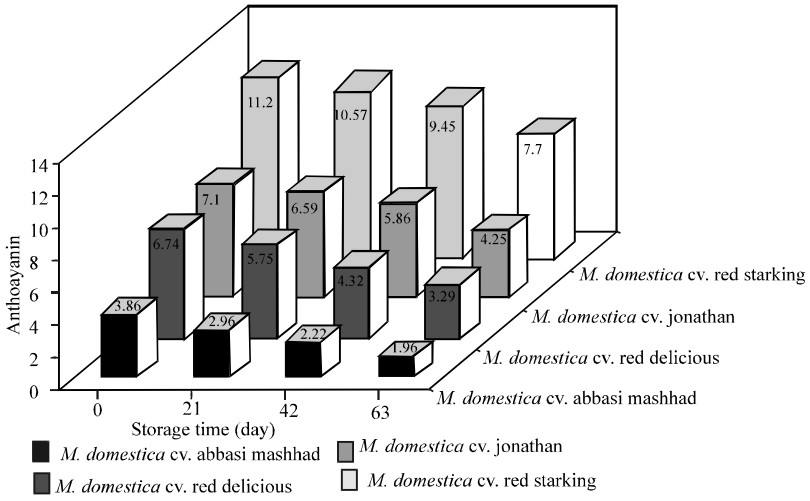

Fig. 1: Comparison of Anthocyanin in four *Malus* varieties

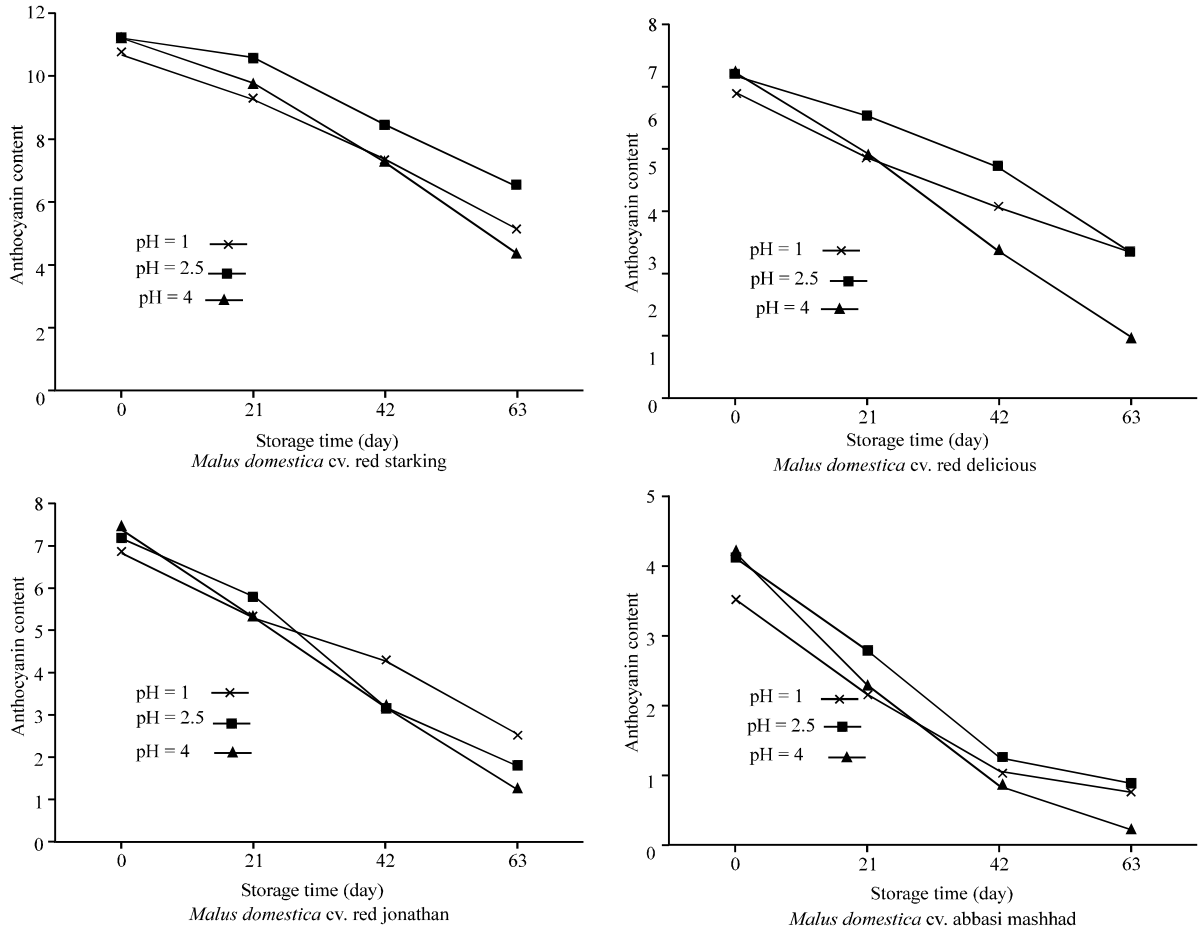

Fig. 2: pH effects on the stability of Anthocyanin in four *Malus* varieties

Table 2: The percentage of anthocyanin destruction in pH of 1, 2.5 and 4 in varieties of *Malus*

	Days								
	pH = 1			pH = 2.5			pH = 4		
Malus varieties	21	42	63	21	42	63	21	42	63
Malus domestica cv. jonathan	22.56	37.70	63.61	19.95	55.82	75.62	28.40	57.74	83.85
Malus domestica cv. red delicious	21.19	37.20	53.05	13.01	28.32	54.77	24.82	54.38	81.64
Malus domestica cv. red starking	13.25	30.92	51.88	5.82	24.53	41.81	13.10	35.83	61.23
Malus domestica cv. abbasi mashhad	38.42	69.77	78.53	32.28	69.66	78.52	46.70	79.19	94.27

Table 3: The percentage of anthocyanin destruction in temperatures of 5, 20, 30 and 40°C in varieties of *Malus*

	Days											
	5°C			20°C			30°C			40°C		
Malus varieties	21	42	63	21	42	63	21	42	63	21	42	63
Malus domestica cv. jonathan	09.58	13.8	25.07	7.19	17.46	40.14	8.17	26.90	47.04	20.56	55.07	87.38
Malus domestica cv. red delicious	10.39	14.09	30.71	14.69	35.9	51.19	19.14	31.45	52.52	30.03	53.25	73.67
Malus domestica cv. red starking	4.19	16.58	25.13	5.79	15.78	31.37	4.10	17.83	33.51	13.10	45.28	63.46
Malus domestica cv. abbasi mashhad1	16.58	24.35	46.89	23.32	42.29	69.69	27.72	65.80	83.16	41.97	57.25	91.11

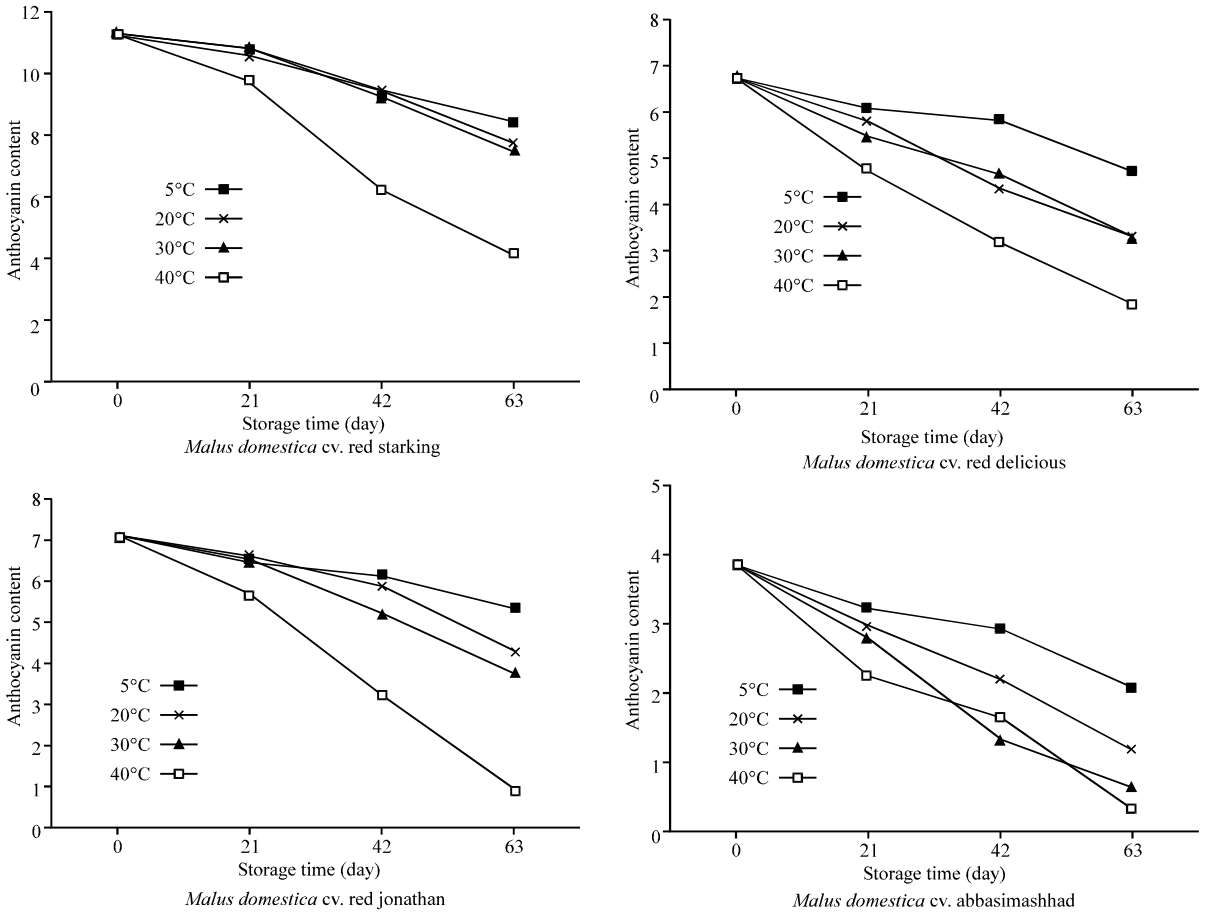

Fig. 3: The effects of temperature on the stability of Anthocyanin in four *Malus* varieties

Malus varieties during 90 days period were measured in 5 separate instances (Fig. 3). The results show that percentage of destruction of anthocyanin in 5, 20, 30 and 40°C which are presented in the Table 3. We suggest that the speedy destruction of anthocyanin in higher temperatures could be due to hydrolisation of 3-Glycoside

Table 4: Effect of presence or absence of light the percentage of destruction of anthocyanin in four *Malus* varieties

	Days							
	Absence of light				Presence of light			
Malus varieties	0	21	42	63	0	21	42	63
Malus domestica cv. jonathan	0.00	8.12	27.45	49.16	0.00	3.08	19.33	41.60
Malus domestica cv. red delicious	0.00	9.62	25.36	40.38	0.00	10.79	23.62	30.76
Malus domestica cv. red starking	0.00	7.82	18.35	28.33	0.00	2.79	10.70	21.58
Malus domestica cv. abbasi mashhad	0.00	25.77	70.21	82.13	0.00	18.71	49.17	73.29

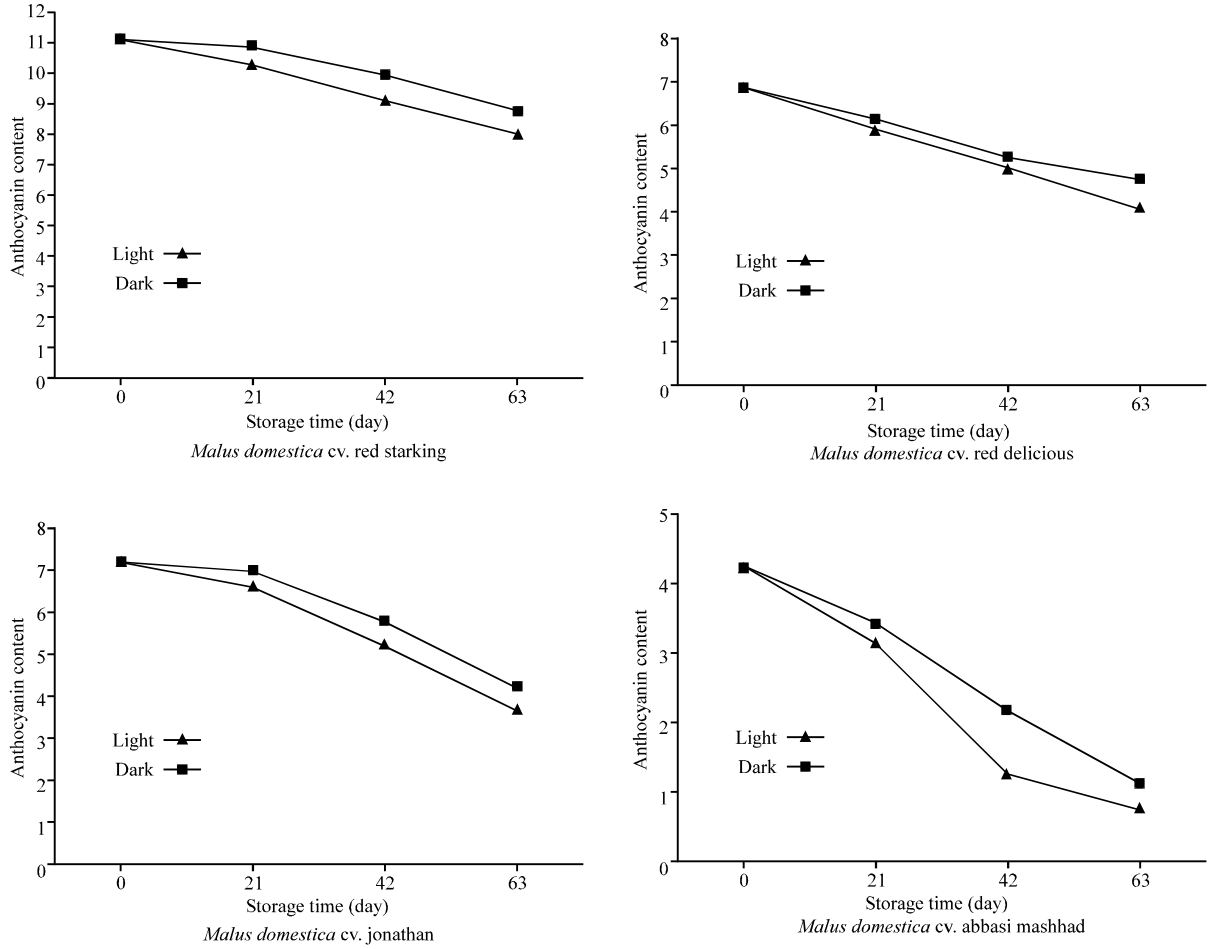

Fig. 4: The effects of Light on the stability of Anthocyanin in four *Malus* varieties

structure, which has a protective effect in unstable anthocyanin. The other suggestion is that the hydrolisation of the pyrilium ring resulted in production of chalcone, which are responsible for brown colour developed in food containing anthocyanin (Giusti and Wrolstad, 2000). Palamidis and Markakis (1975) has studied the effect of temperature on the stability of anthocyanin in soft drinks and have shown that increase in the storage temperature greatly accelerate the destruction of pigments in soft drinks. Spayd *et al.* (2002) found that the increase in temperature accelerates the destruction of anthocyanins.

The effect of presence or absence of light on the stability of anthocyanin: Light is another factor, which affects the stability of anthocyanin. pH in all samples was 2 and temperature was kept at 25°C (Fig. 4). The period of experiments was 90 days and data was recorded in 5 separate instances. In the presence or absence of light (400 Lux) the percentage of destruction of anthocyanin is shown in Table 4 for four *Malus* varieties. Similar results for grapes anthocyanin had been reported by Palamidis and Markakis (1975). They exposed the pigments of the light for 135 days at 20°C and observed 50% of pigments destruction, at the same time in dark the level of

destruction was 30%. Timberlake (1989) suggested that light increase the flavylium cation construction, but in the absence of light the amount of chalcone in the extract containing anthocyanin was higher than its flavylium cation.

REFERENCES

Bridle, P. and C.F. Timberlake, 1997. Anthocyanin as natural food colors-selected aspects. Food Chem., 58: 103-109.

Chiriboga, C. and F.J. Francis, 1970. An anthocyanin recovery system from cranberry pomace. J. Am. Soc. Hortic. Sci., 9: 223-223.

Cooper-Driver, G.A., 2001. Contribution of Jeffrey Harbone and Co-works to the study of anthocyanins. Phytochemistry, 56: 229-236.

Cordenunsi, B.R., J.R.O. Nascimento and F.M. Lajolo, 2003. Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage. Food Chem., 83: 167-173.

Delpech, R., 2000. Anthocyanins, extraction chemistry: Methodology plant cell and tissue. J. Biol. Edn., 34: 206-210.

Giusti, M.M. and R.E. Wrolstad, 2000. Characterization and measurement of anrhocyanins by UV-visible spectroscopy. Food. Anal. Chem., Home page.

Lee, H.S., 2002. Characterization of major anthocyanins and the color of red-fleshed budd blood orange (*Citrus sinensis)*. J. Agric. Food Chem., 50: 1243-1246.

Leng, P., H. Itamura, H. Yamamura and X.M. Deng, 2000. Anthocyanins accumulation in apple and peach shoots during cold acclimation. Sci. Hortic., 83: 43-50.

Little, A.C., 1997. Calorimetry of anthocyanin pigmented products: Changes in pigment composition with time. J. Food. Sci., 42: 1570-1574.

Malien-Aubert, C., O. Dangles and M.J. Amiot, 2001. Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra and intermolecular copigmentation. Agric. Food Chem., 49: 170-176.

Mazza, G. and E. Minitiati, 1993. Introduction. CRC Press, Boca Raton, Florida, pp: 1-28.

Navidra, P.S., R.S.A. Chandra and M.G. Nair, 2002. Characterization, quantification and bioactivities of anthocyanins in cornus species. J. Agric. Food Chem., 50: 2519-2523.

Palamidis, N. and T. Markakis, 1975. Structure of anthocyanin. Food Sci., 40: 104-104.

Spayd, S.E., J.M. Tarara, D.L. Mee and J.C. Feguson, 2002. Mee and J.C. Feguson, 2002. Saparation of sunlight and temperature effects on the composition of *Vitis vinifera*. J. Food Sci., 53: 3-3.

Timberlake, C., 1989. Plant pigments for coloring food. J.B.N.F. Nutr., 14: 113-117.

Yamazaki, M. and K. Saito, 2002. Biochemistry and molecular biology of the late stage of biosynthesis of anthocyanin. Lessons from *Perilla frutescens* as a model plant. J. New Phytol., 155: 9-23.