

Full Length Research Paper

Effect of kinetin on quality and harvest date of loquat fruit

Heqiang Lou¹, Ping Chen², Hong Zheng¹, Cuicui Xu² and Hongfei Lu^{1*}

¹College of Chemistry and Life Science, Zhejiang Normal University, 321004 Jinhua, China.

²Chuyang Honors College, Zhejiang Normal University, 321004 Jinhua, China.

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Loquat fruit is a sweet-acid fruit and rich in natural antioxidants. However, the harvest season of loquat fruit is rather short and it has a short shelf life after harvesting. Therefore, an effective method is needed for postponing fruit harvest date in order to avoid peak sales. In our study, we investigated the effect of kinetin (Kn) treatment on fruit color, size, weight and levels of chlorophyll (Chl), total phenolics (TP), ascorbic acid (AA) and antioxidant activity in 'Jiajiao' loquat fruit during development. Our results showed that the Kn-treated fruit exhibited significantly greener color and significantly higher levels of Chl, TP and AA than control fruit. Meanwhile, the treatment also increased fruit size and weight and maintained significantly higher antioxidant activity as measured by the scavenging capacity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) compared to the control. Therefore, Kn treatment provide a new fresh-keeping technology in loquat fruit on the tree and it also can postpone harvest date and increase fruit quality and yield of loquat fruit.

Key words: Loquat, Jiajiao, kinetin, fruit quality, antioxidant activity, harvest date.

INTRODUCTION

Loquat (*Eriobotrya japonica* Lindl.) fruit is a sweet-acid fruit when harvested at the right point of ripeness and it is widely cultivated in the subtropical regions of southern China, Japan, northern India, Israel, and the Mediterranean. However, the harvest season of loquat in China and Japan is rather short, lasting only from mid-May to mid-June in open field cultivation. In addition, loquat fruit has a short shelf life after harvesting and it is susceptible to decay, mechanical damage and nutritional losses during postharvest life. On the other hand, as the loquat fruit ripens and senesces, titratable acidity declines, accompanied by a loss of flavor and taste, browning and hardening of the fruit flesh, decreased juiciness and increased ion leakage from the skin tissue (Lin et al., 1999; Zheng and Xi, 1999; Zheng et al., 2000). Therefore, an effective method for maintaining or improving loquat fruit quality and keeping fruit fresh on the tree in order to prolong sale dates of loquat fruit is needed.

Evidence suggests that kinetin (Kn) belongs to a group of plant hormones called cytokinin and its role is connected with the growth and development of plants. It is also implicated in the vascular development and synthesis of secondary metabolites like indol alkaloids and anthocyanins. It influences chloroplast differentiation and chlorophyll (Chl) biosynthesis by stimulation of 5-aminolevulinic acid synthesis (Duszka et al., 2009). When exogenously applied, Kn has been shown to result in increased plant height (Letham, 1969; Richards and Wilkinson, 1984), pod length and pod area (Powell and Howell, 1985; Mukherjee and Kumar, 2007), leaf area (Abdullah et al., 1986; Goswami and Srivastava, 1987), branching (Mulgrew and Williams, 1985; Tanimoto and Harada, 1986), seed mass (Carlson et al., 1987) and yield (Ray et al., 1983). Moreover, Yamauchi et al. (2004) reported that Kn repressed Chl degradation by repressed C4 isoperoxidase (Rf 0.40). Sayed (1999) also found that Kn-treated plants had higher Chl. Therefore, Kn could be a useful technique to maintain or improve loquat fruit quality, keep fruit fresh on the tree and increase fruit yield. However, little information is known about the effect of Kn on loquat fruit on the tree.

*Corresponding author. E-mail: luhongfei63@yahoo.com.cn.
Tel: +86-0579-8228-2284.

Therefore, we studied the effects of Kn application on 'Jiajiao' loquat fruit changes in fruit color, fruit size, fruit weight, Chl content, ascorbic acid (AA), total phenolics (TP) and antioxidative ability during growth and ripening in this work. The results will help to postpone harvest date and keep fruit fresh on the tree in order to prolong sale dates of loquat fruit, and the success of this research will provide the fruit industries with a reference for increasing economic benefits of various fruit.

MATERIALS AND METHODS

Samples and procedures

Nine trees of loquat (*Eriobotrya japonica* Lindl. cv. Jiajiao), similar in vigor, age and size, were selected on the experimental farm (Jinhua, Zhejiang, P.R. China). We conducted the experiments on 14 March, 2010, 11 April, 2010, 25 April, 2010, 9 May, 2010 and 23 May, 2010 during the development of Loquat. Kn, at the concentrations of 5, 15, 30 ppm, were applied from 11 April, 2010. A sample of 90 intact and healthy fruits from different directions of each tree was harvested randomly and delivered to the laboratory for analysis within 3 h at 8°C by refrigeration in each experiment. These samples were located away from the edge of the orchard in order to avoid a border effect. Their longitudinal diameter, transverse diameter, volume, fresh weight and dry weight were measured in each experiment. Longitudinal diameter and transverse diameter of fruit were measured with a vernier caliper; the fruit volume was determined by water displacement technique and the fruit weight was measured by digital balance.

Determination of chlorophyll (Chl) content

Chl a and Chl b content were determined according to the method of Lichtenthaler (1987) with modifications. The peel of loquat was sliced into small pieces, then 10 ml 80% acetone and 0.5 mg the peel were added to each tube and the tubes were placed in dark (4°C, 12 h). The absorbance was recorded at wavelengths of 646.8 and 663.2 nm by a UV-Vis spectrophotometer. Chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equations (results were expressed as mg/100 g of fresh weight):

$$\text{Chl a} = (12.25A_{663.2} - 2.79A_{646.8})$$

$$\text{Chl b} = (21.21A_{646.8} - 2.79A_{663.2})$$

$$\text{Chl a+b} = \text{Chl a} + \text{Chl b}$$

Determination of ascorbic acid (AA)

Each 10 g fruit flesh were ground with 2% oxalic acid. The slurry was filtered and the final volume of filtrate was brought up to 100 ml with 2% oxalic acid. AA content was determined using 2,6-dichlorophenol indophenols by visual titration (AOAC, 2000). Results of AA content were expressed as milligram ascorbic acid mg/100 g of fresh weight. Measurements were done in triplicate.

Determination of total phenolics (TP)

Before TP determination, the samples were dried in the electric thermal dryer at 105°C for 12 h until the weight did not change any more. Then TP content was analyzed using the Folin-Ciocalteu colorimetric method (Martin et al., 2009) with modifications. The

extract (1 ml) of loquat fruit flesh in ethanol was diluted ten times. The diluted extract (0.5 ml), water (6 ml) and Folin-Ciocalteu reagent (0.5 ml of 0.5 M) were added to tubes and the solutions were mixed and incubated at room temperature for 10 min followed by addition of 0.75 ml 20% (w/v) Na₂CO₃. The final volume of solutions was brought up to 10 ml with distilled water. After mixing, tubes were incubated at 40°C for 40 min, and then cooled to room temperature. All samples were analyzed at 755 nm by UV-Vis spectrophotometry. Gallic acid was used as a standard, and results were expressed as milligram gallic acid equivalent (GAE)/100 g of dry weight (DW).

Determination of antioxidative ability

Antioxidative ability was analyzed by the method of free radical scavenging capacity (DPPH) which was carried out according to the method of Lu et al. (2010) with modifications. The extracts (0.1 ml) of loquat fruit flesh in ethanol were reacted with 10 ml of 0.03 g/l DPPH (2,2-diphenyl-1-picrylhydrazyl) ethanol solution at room temperature. The extract (0.1 ml) with 10 ml distilled water was used as control. The absorbance was measured at 517 nm after 30 min of reaction in the dark. The antioxidative ability was expressed as DPPH radical-scavenging activity (%) = 100*(1 - As/Ac), where Ac is the absorbance of 10 ml DPPH with 0.1 ml distilled water, As is the absorbance of 10 ml DPPH added to 0.1 ml sample.

Statistical analysis

Each assay was done at least three times from the same extract, and statistical analyses were carried out using the Statistical Analysis Systems (SAS, version 9.0) software package and Excel statistical tools (Microsoft software). Significant differences were determined using Duncan's new multiple range test at $p = 0.05$. Pearson's correlation coefficient (r) was used to calculate correlations among data obtained.

RESULTS

Effects of Kn on fruit size and weight of loquat during fruit development

In controls and all the treatments, fruit growth was characterized by a sigmoid curve (Figure 1). Fruit longitudinal diameter, transverse diameter, volume, fresh weight and dry weight at harvest increased with increasing concentration up to 30 ppm (Figure 1). An increment of about 3 g in final fresh weight per fruit was obtained with fruits treated at 30 ppm Kn which is an increment of approximately 21.3% (Figure 2A). The final fruit volume treated at 30 ppm Kn was about 1.5 times as large as the control (Figure 1E).

Effects of Kn on antioxidant compounds, antioxidant activity and color of loquat fruit during fruit development

Change during fruit development in AA content is shown in Figure 3A. AA content increased slowly from 14 March to 25 April, reaching its peak value rapidly on 9 May, the

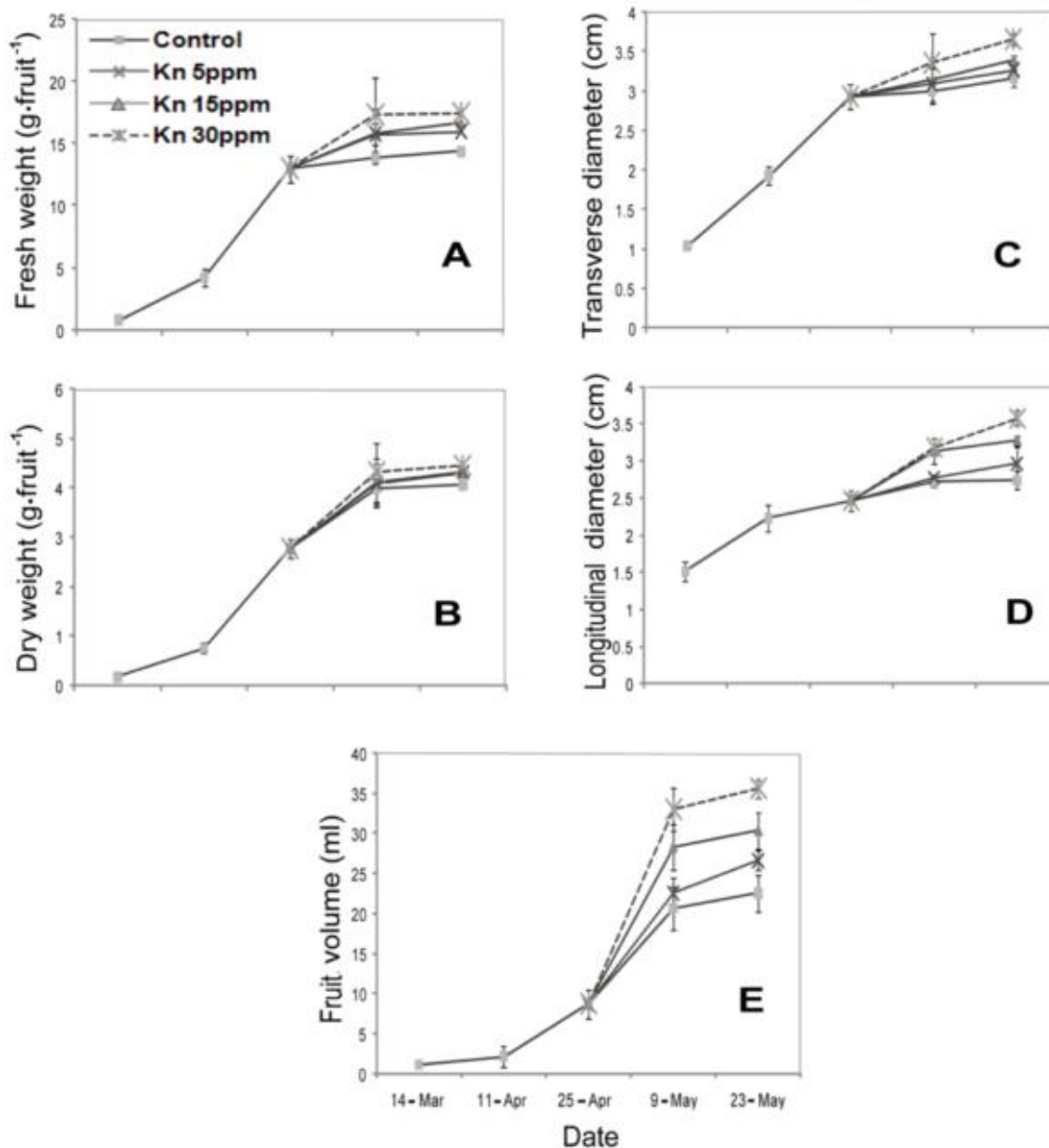


Figure 1. Effects of Kn on fresh weight (A), dry weight (B), transverse diameter (C), longitudinal diameter (D) and volume (E) of loquat fruit during development.

AA content increased from 1.12 mg/100 g fresh weight on 25 April to 10.34 mg/100 g fresh weight on 9 May. The AA contents of Kn-treated fruit were significantly higher than control fruit except the 15 and 30 ppm Kn-treated fruit on 9 May, even the AA content in 30 ppm Kn-treated fruit was lower than in control fruit on 9 May. It is clear from Figure 3A that the 5 ppm Kn was the most effective concentration in increasing the ascorbic acid content. We also found that ascorbic acid content in Kn-treated fruit decreased from 9 May to 23 May except for an increase in 30 ppm Kn-treated fruit (Figure 3A).

TP content decreased from 14 March to 25 April, then

increased with fruit development and this increase was enhanced by 5 ppm Kn application and delayed by 15 and 30 ppm Kn treatment on 9 May. At the later stages of fruit development, the TP content decreased again. The peak value appeared on 14 March. The TP content of 5 and 15 ppm Kn-treated fruit were significantly higher than control fruit on 23 May. The strongest effect was obtained by 5 ppm Kn, and there is no difference between 30 ppm Kn-treated fruit and control fruit on 23 May. We also found that TP content in all Kn-treated fruit increased from 9 May to 23 May (Figure 3B).

Figure 3C shows that DPPH radical scavenging activity

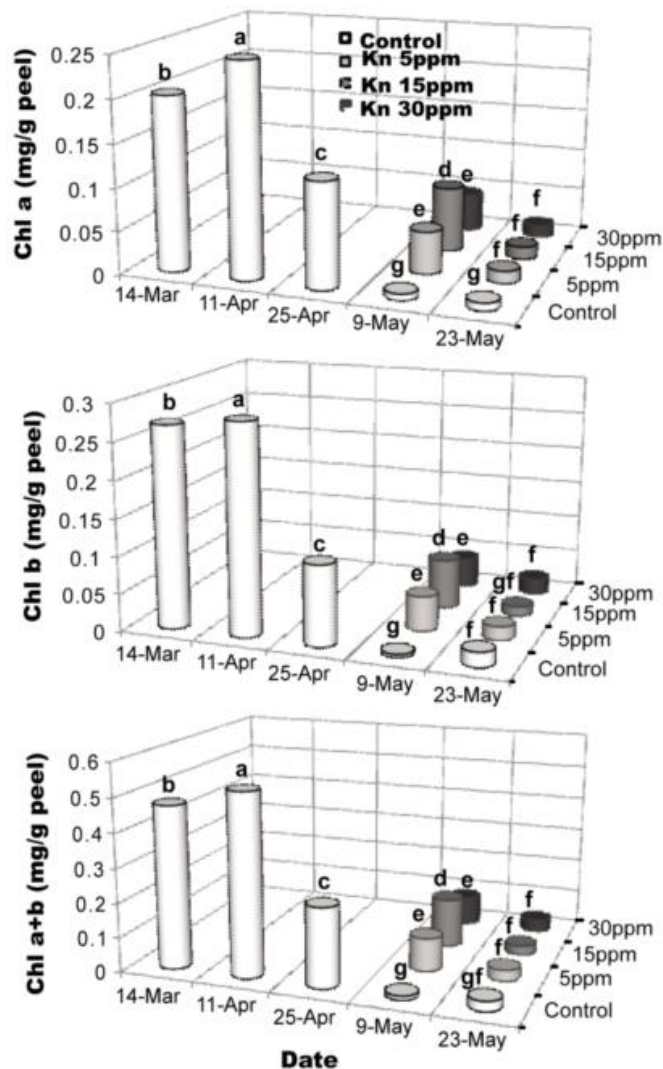


Figure 2. Effects of Kn on chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Chl a+b) content of loquat fruit during fruit development. Data were subjected to one-way ANOVA and different letters mean that values are statistically different $p < 0.05$.

of loquat fruit declined gradually from 69.5 to 16.6% with fruit development. DPPH radical scavenging activities of all Kn-treated fruit were significantly higher than control fruit on 9 May and 23 May. The strongest effect was obtained by 15 ppm Kn on 9 May and 5 ppm on 23 May, and 30 ppm had a lesser effect than the lower concentration on both 9 May and 23 May.

Chl a and Chl b contents in loquat fruit increased from 14 March to 11 April and then decreased as fruit developed. It is noteworthy that Chl a and Chl b contents decreased rapidly to a low level on 9 May and staying more or less unchanged through the rest of the sampling dates (Figure 2). The Chl a and Chl b contents of 5, 15 and 30 ppm Kn-treated fruit were significantly higher than the control fruit on 9 May. The strongest effect was

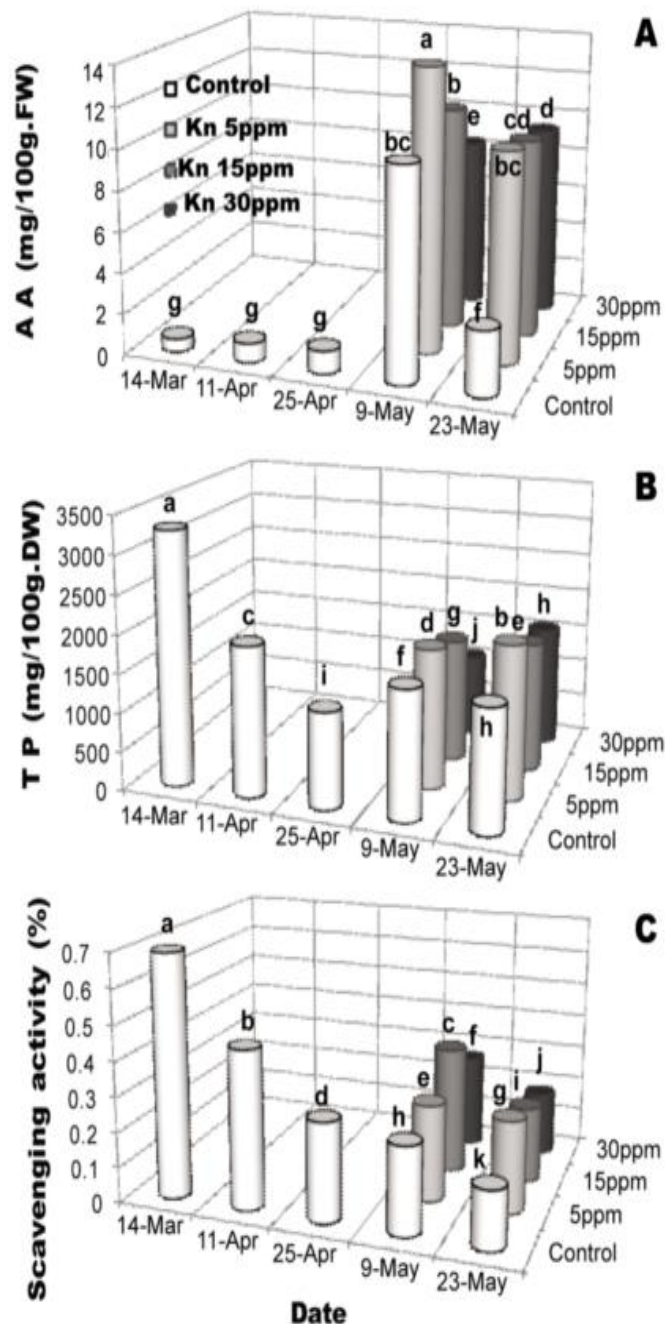


Figure 3. Effects of Kn on ascorbic acid (AA), total phenolics (TP) and scavenging activity of loquat fruit during fruit development. Data were subjected to one-way ANOVA and different letters mean that values are statistically different $p < 0.05$.

obtained by 15 ppm Kn, and 30 ppm had a lesser effect than the lower concentration on 9 May (Figure 2). After two weeks, the effects of Kn, at the concentrations of 5, 15, 30 ppm, on the increase of Chl content became similar and slight (Figures 2 and 4). We also found that fruits treated with Kn were greener than control fruits (Figure 4).

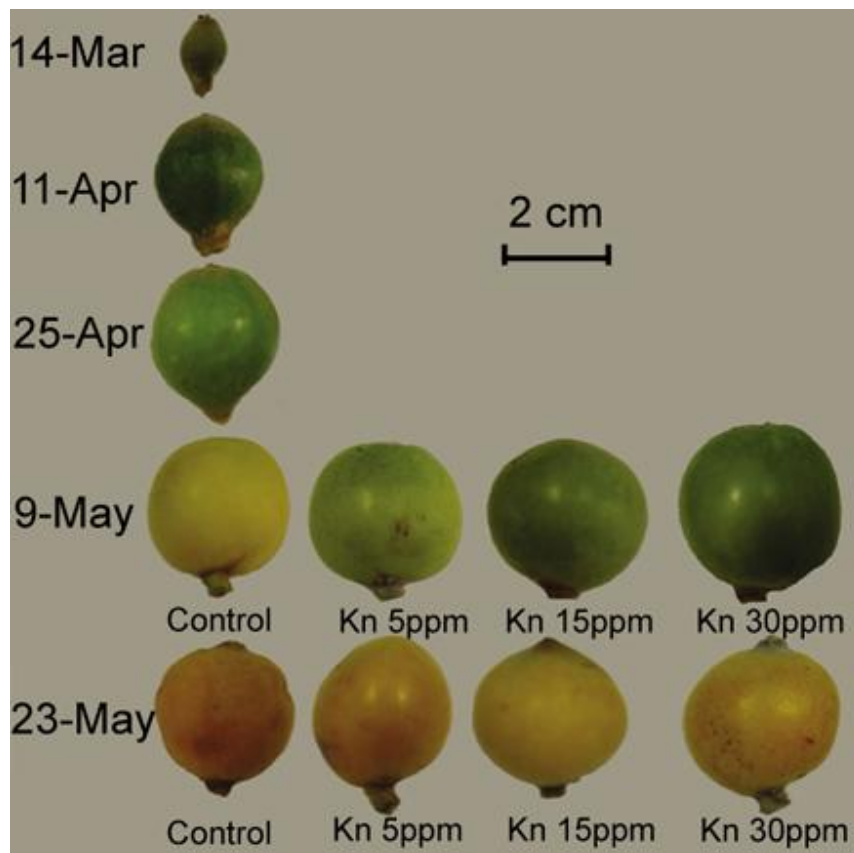


Figure 4. Effects of Kn, at the concentrations of 5, 15, 30 ppm, on the size and color of loquat fruit.

Relationships among Chl, antioxidant activity, TP and AA

The Pearson correlation coefficients among Chl, antioxidant activity, TP content and AA content of loquat fruit were calculated in Table 1. Correlations among Chl a, Chl b and Chl (a+b) were positive and ranged between 0.983 and 0.996 ($P < 0.0001$). The correlations between antioxidant activity and Chl were positive as well, especially between Chl b and antioxidant activity ($r = 0.873$, $P < 0.0001$). In addition, antioxidant activity was positively correlated with TP ($r = 0.914$, $P < 0.0001$). Significant correlations were also found between TP and Chl, especially between Chl b and TP ($r = 0.664$, $P < 0.01$). However, AA showed a negative correlation with Chl ranging between -0.725 and -0.709, in addition, antioxidant activity and TP were not significantly correlated with AA.

DISCUSSION

Our results fully coincide with Cuevas et al. (2003) who described fruit growth pattern of loquat cultivars 'Algerie' as a single sigmoid model. In our experiments, AA in

loquat fruit increased slowly at the early stages of fruit development, and reached a maximum rapidly and then declined. This is in agreement with the results obtained for tomato fruit (Yahia et al., 2001). In young loquat fruit, TP concentration was high (Figure 3). TP content decreased from 14 March to 25 April. Similar phenomenon has been found in apple (CoSeteng and Lee, 1987; Burda et al., 1990), peach (Lee and Jaworski, 1988) and grapes (Romeyer et al., 1983). Interestingly, the TP content rose again at the later stages of loquat fruit development and leveled off in the last two weeks (Figure 3). This is in agreement with the report that TP in loquat fruit cv. Tanaka decreased steadily during growth between 4 and 2 weeks, and rose again at about 2 weeks before harvest and leveled off in the last week before harvest (Ding et al., 2001). We found that scavenging activity of loquat fruit declined gradually with fruit development during the experiment. Kulkarni and Aradhya (2005) also found that the pomegranate arils showed a rapid decrease in antioxidant activity from 20 to 60 days of fruit development.

Our results show that Chl contents of 5, 15 and 30 ppm Kn-treated fruit were higher than the controls at the later stages of fruit development (Figure 2). The effectiveness of Kn in minimizing Chl loss was also found by Mukherjee and Kumar (Mulgrew and Williams, 1985). Our study as

Table 1. Pearson correlation coefficients among chlorophyll (Chl), antioxidant activity, total phenolics (TP) content and ascorbic acid (AA) content of loquat fruit^a.

Correlation coefficient	Chl a (mg/g-peel)	Chl b (mg/g-peel)	Chl (a+b) (mg/g-peel)	Antioxidant activity	TP (mg/100 g DW)
Chl b (mg/g-peel)	0.983****				
Chl (a+b) (mg/g-peel)	0.995****	0.996****			
Antioxidant activity	0.797***	0.873****	0.842****		
TP (mg/100 g DW)	0.526*	0.664**	0.604*	0.914****	
AA (mg/100 g FW)	-0.725**	-0.709**	-0.719**	-0.49	-0.308

^a * p < 0.05, ** p < 0.01, *** p < 0.001 and **** p < 0.0001.

well as the report on wheat cv. Uqab-2000 (Rafiq et al., 2006) show that Kn effectively increased the AA content. Kn at inhibiting concentrations decreased the synthesis of soluble phenolic compounds in tissue culture of *Cassia fistula* L. (Shah et al., 1976) and increased it in the long-passaged tissue culture of *Camellia sinensis* (Zagoskina and Zaprometov, 1983). Our study show that the increase of TP was enhanced by 5 ppm Kn application and delayed by 15 and 30 ppm Kn treatment on 9 May (Figure 3B). It is clear from Figure 3B that TP content in all Kn-treated fruit increased from 9 May to 23 May (Figure 3B). Several authors (Zagoskina and Zaprometov, 1983; Angelova et al., 2001) also found a negative correlation between growth induced by different concentrations of Kn and the accumulation of TP. Our data show that scavenging activity of all Kn-treated fruit decreased from 9 May to 23 May, and scavenging activity of all Kn-treated fruit were significantly higher than control fruit on 9 May and 23 May. We found that 5 ppm kinetin could slow down the decline in scavenging activity effectively (Figure 3C).

In this study, we found that Chl concentration was significantly correlated with antioxidant activity and TP (Table 1). Hunter and Fletcher (2002) reported that Chl could contribute to the total antioxidant activity in peas. Therefore, Chl may be as an indicator of the change in the internal quality of loquat fruit. In addition, antioxidant activity was positively and significantly correlated with TP with an r of 0.914 (P < 0.0001), as shown in Table 1. The high correlation between antioxidant activities and TP is not surprising as such correlations were measured in many other fruit species (Gil et al., 2002; Özgen et al., 2007; Celik et al., 2008; Özgen et al., 2008a; Özgen et al., 2008b; Özgen et al., 2009; Polat et al., 2010). In addition, we found AA was not significantly correlated with TP and antioxidant activity. This is in agreement with the earlier report that there was no correlation between AA and antioxidant activity as determined by DPPH or FRAP assay in nectarines, peaches and plums (Gil et al., 2002). However, high correlation between antioxidant activity using any method and AA was found in only few fruits that contain high AA such as orange (Gardner et al., 2000) and guava (Thaipong et al., 2006).

In all species studied so far, synthetic auxin had the

potential for increasing fruit size without inducing thinning (Stern et al., 2007). We also found application of Kn increased loquat fruit size and no thinning effect was derived from Kn application to whole trees in this study. In addition, Kn treatment also significantly increased loquat weight. Therefore the yield of loquat fruit increased by Kn treatment. The synthetic auxin 2,4-DP on fruit development of loquat was studied and results indicated that 2,4-DP could increase fruit size further, fruit color break and maturation were encouraged and harvest time was earlier than in untreated trees (Agusti et al., 2003). However, our study showed that Kn could improve loquat fruit quality as the fruits treated with Kn retain their green color longer than control fruits; this is an important effect of Kn since a greener color of the fruit is considered a positive quality characteristic of the fruit. This result is in accordance with the known effect of cytokinins on promoting the greening of plant tissue (Binns, 1994). Since the harvest time is mainly determined by fruit size and color, loquat fruits can be harvested later.

Conclusion

The findings in this study suggest that Kn provide us an effective method for improving both the quality and yield of loquat fruit. Moreover, the harvest date of loquat fruit was prolonged by Kn treatment. Therefore, Kn can assist loquat growers to avoid peak sales and improve economic benefits.

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