

*Full Length Research Paper*

# Effects of bagging on sugar metabolism and the activity of sugar metabolism related enzymes during fruit development of Qingzhong loquat

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To investigate the effects of bagging on sugar metabolism and the activity of sugar metabolism related enzymes in Qingzhong loquat fruit development, the contents of sucrose, glucose and soluble solids as well as the activities of sugar metabolism related enzymes were evaluated. The content of sucrose, glucose and soluble solids increased, while the content of fructose, sorbitol and titratable acidity decreased in ripe fruit in response to bagging. In addition, the activities of acid invertase (AI) and neutral invertase (NI) in the bagged fruit were lower than that in the non-bagged fruit, and the activities of sucrose synthase (SS) and sucrose-phosphate synthase (SPS) in the bagged fruit were higher than that in the non-bagged fruit. The activities of SDH (sorbitol dehydrogenase) and SOX (sorbitol oxidase) in the bagged fruit were lower than those in the non-bagged fruit, but there was no significant difference, whereas the activities of SS and SPS in the bagged fruit were significantly higher than that in the non-bagged fruit, suggesting that bagging mainly increased the products of photosynthesis by enhancing the activities of SS and SPS.

**Key words:** Loquat, bagging, sugar metabolism.

## INTRODUCTION

Bagging is an effective method of improving fruit quality in fruit production and has been widely used to improve fruit appearance, decrease pesticide residues and increase commercial value. Moreover, bagged fruit is preferred by the consumers. Yang et al. (2009) proved that bagging could modify the microenvironment during fruit development, decreasing the rate of fruit drop and reducing the content of organic acid in longan fruit. Kim et al. (2008) reported that the appearance of bagged peaches improved, while the content of soluble solids, anthocyanin and chlorophyll increased in white coloured

bags. Lin et al. (2008) demonstrated that bagged pear fruits were more attractive with less russet and visible dots than non-bagged fruit, while the residues of chlorpyrifos carbendazim and cyhalothrin were also greatly reduced. Consumers also showed preference for bagged fruit.

Loquat (*Eriobotrya japonica* Lindl.) originated in China, is distributed in Zhejiang, Fujian and Jiangsu Province (Qiu and Zhang, 1996). 'Qingzhong' loquat is a local cultivar from Suzhou, Jiangsu Province. The fruit has good flavour and storage ability. Xu et al. (2008, 2010) and Feng et al. (2009) studied the effects of bagging on 'Baiyu' loquat and 'Ninghaibai' loquat fruit quality and antioxidative capacity. However, the effects of bagging on sugar metabolism during fruit development were not explored. The purpose of this study was to determine the effects of bagging on sugar metabolism and the activities of related enzymes during loquat fruit development, and establish a basis for the improvement of this cultivation technique.

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**Abbreviations:** AI, Acid invertase; NI, neutral invertase; SS, sucrose synthase; SPS, sucrose-phosphate synthase; SDH, sorbitol dehydrogenase; SOX, sorbitol oxidase.

## MATERIALS AND METHODS

### Materials

Thirty-year-old 'Qingzhong' loquat trees grown at Suzhou, Jiangsu Province were used in this project. The fruits were bagged (white, single-layer sulphuric acid paper) about 40 days after initial fruit set, with bagging 80 fruits per plant. Ten fruits were sampled every 15 days until harvest. Sampling was repeated three times. Flesh was separated from the fresh fruit for each treatment and frozen at -70°C for further study.

### Sugar content

Dried fruit (0.1 to 0.2 g) was powdered in a chilled mortar with 5 ml of distilled water. The homogenate was centrifuged at 5000  $\times$ g for 10 min. The supernatant was poured into a Sephadex G-25 column and re-extracted once. The supernatants were combined and the volume was increased to 25 ml. The contents of sucrose, glucose and fructose were determined by the anthrone colorimetric method. Sampling was repeated three times.

Frozen fruit was powdered in a mortar chilled with liquid nitrogen. Two grams of fruit were added to 7 ml 70% ethanol and mixed into a homogenate for about 20 min in 35°C water. Then, the homogenate was centrifuged at 10,000  $\times$ g for 5 min. The supernatant was poured into a Sephadex G-25 and re-extracted once. The supernatants were combined and the volume increased to 25 ml. A 2 ml sample of supernatant was evaporated to dryness and dissolved with 1 ml of distilled water. The content of sorbitol was quantified by HPLC (high performance liquid chromatography, Agilent 1100). A 15  $\mu$ l aliquot was passed through a CA column (10  $\mu$ m diameter). Flowing water was used for the mobile phase. The temperature of the column was 85°C. Sampling was repeated three times.

### Enzyme activity

Extraction and determination of acid invertase (AI), neutral invertase (NI), sucrose synthase (SS) and sucrose-phosphate synthase (SPS) were determined by the method described by Nielsen et al. (1991). Frozen fruit was powdered in a mortar chilled with liquid nitrogen. Fruit (1 g) was added to 5 ml of pre-cooled extraction buffer (0.2 M Tris-HCl buffer, pH = 8.8) containing 25 mM MgCl<sub>2</sub> and 15 mM NaN<sub>3</sub>, and made into a homogenate in an ice-bath. Then, the homogenate was centrifuged at 10,000  $\times$ g for 15 min. The supernatant was poured into a Sephadex G-10 and re-extracted once. The supernatants were combined and the volume was increased to 10 ml, which was used as the enzyme extract. Sampling was repeated three times.

The activity of sorbitol dehydrogenase (SDH) was determined by the following method: Enzyme solution (150  $\mu$ l) and 1.05 ml of 25 mM NAD<sup>+</sup> were combined, to which 1.05 ml of 150 mM D-sorbitol was added. The absorbance was determined at 340 nm. In the enzyme-free reaction system, 150  $\mu$ l of 0.2 M Tris-HCl (pH = 8.8) was used as the control instead of enzyme solution. The treatment was repeated three times. The units (OD  $\cdot$  mL<sup>-1</sup>  $\cdot$  min<sup>-1</sup>) of SDH activity was the change of absorbance of 1 ml reaction solution per minute. The activity of SOX (sorbitol oxidase) was determined by the method of Yamaki (1980) and Yamaki and Ishikawa (1986).

### Statistical analysis

Statistical analysis was carried out using analysis of variance and the significance was determined using SPSS 16.0 (Statistical package for the social science) with LSD (least significant difference) values at  $P < 0.05$ .

## RESULTS

### Effects of bagging on the content of sugar

The glucose content in ripe fruit increased in response to bagging ( $P < 0.05$ ), while the levels of sorbitol and titratable acid decreased (Table 1). Soluble solid to acidity ratio increased in bagged fruit. The contents of sucrose, fructose and glucose in the bagged and non-bagged fruit increased with fruit development (Figure 1 A, B and C). The content of fructose in the bagged fruit was slightly lower than that in the non-bagged fruit before 70 days after initial fruit set and decreased by 2.01 mg·g<sup>-1</sup> in ripe fruit when compared with control. The amount of sucrose and glucose in the bagged fruit were slightly lower than those in the non-bagged fruit before 85 days after initial fruit set, after which the amounts were higher for bagged than non-bagged fruit. The contents of sucrose and glucose were higher in bagged than in non-bagged fruits by 1.30 mg·g<sup>-1</sup> and 2.75 mg·g<sup>-1</sup> in ripe fruit, respectively.

The accumulation of sorbitol in the bagged fruit was very similar to that in non-bagged fruit during fruit development, decreasing at first and then increased during development. The content of sorbitol in the bagged fruit was lower than that found in the non-bagged fruit (Figure 1D).

### Effects of bagging on the activities of sucrose metabolism related enzymes

Invertase belongs to an enzyme class in which the regulation of sucrose conversion into fructose and glucose in the plant is mediated by NI and AI (Ni et al., 2009). The activities of NI and AI in the bagged fruit were quite similar during fruit development (Figure 2A and B). The activities of NI and AI in the non-bagged fruit initially increased faster and then more slowly until the fruit ripened. The activity of AI was slightly higher in the bagged fruit than in the non-bagged fruit when fruit ripening approached.

SS is an enzyme that plays a key role in sucrose decomposition in the course of sucrose metabolism and SPS is a key enzyme that regulates sucrose synthesis in the plant (Zheng et al., 2006). The activity of SS in the bagged fruit increased during fruit development and was higher than that in the non-bagged fruit (Figure 2C). The change in SS and SPS activities in the bagged and non-bagged fruit was almost the same before 85 days after the initial fruit set. After this date, the activities of SS and SPS in the bagged fruit increased more rapidly (Figure 2D).

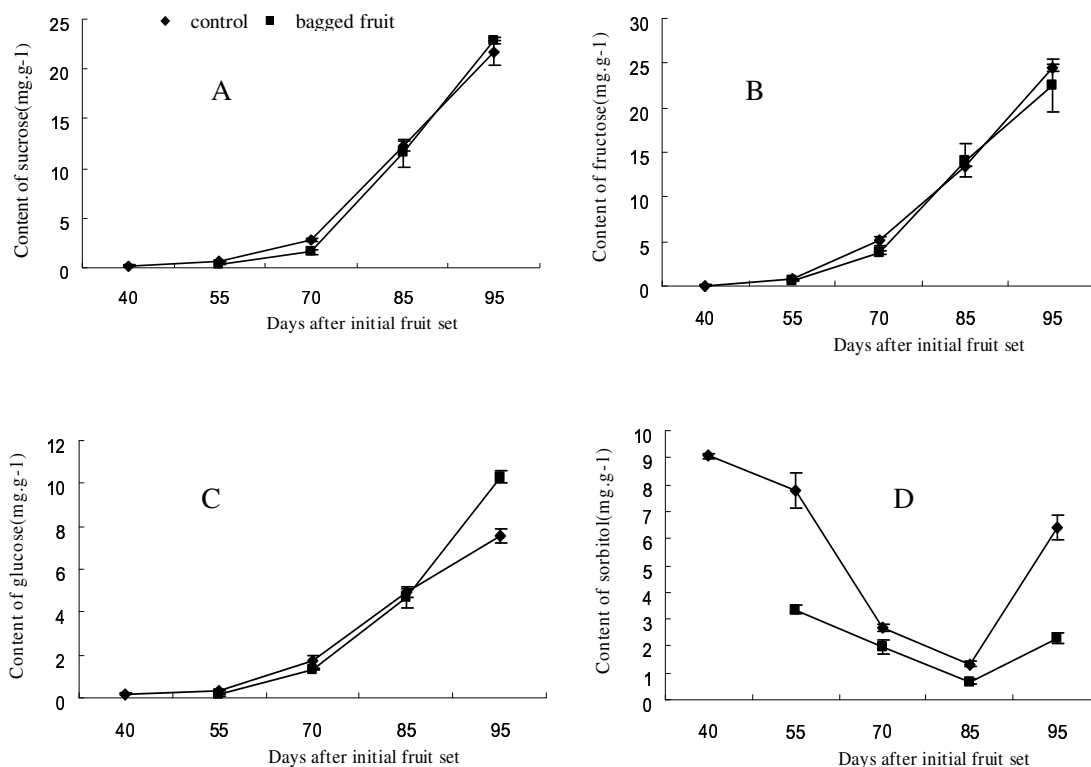
### Effects of bagging on the activities of sorbitol metabolism related enzymes

SDH is an enzyme that catalyses the conversion of sorbitol

**Table 1.** Effects of bagging on the contents of sugar, sucrose, fructose, glucose, sorbitol, soluble solids and titratable acidity in fruit of Qingzhong loquat.

Treatment	Content of sugar (mg·g <sup>-1</sup> )	Content of sucrose (mg·g <sup>-1</sup> )	Content of fructose (mg·g <sup>-1</sup> )	Content of glucose (mg·g <sup>-1</sup> )	Content of sorbitol (mg·g <sup>-1</sup> )	Soluble solid (%)	Titratable acidity (%)	Solid/acid ratio
Control	60.04 <sup>b</sup>	21.62 <sup>a</sup>	24.46 <sup>a</sup>	7.55 <sup>b</sup>	6.41 <sup>a</sup>	14.35 <sup>a</sup>	0.45 <sup>a</sup>	31.89
Bagged	57.95 <sup>a</sup>	22.92 <sup>a</sup>	22.45 <sup>a</sup>	10.3 <sup>a</sup>	2.28 <sup>b</sup>	15.13 <sup>a</sup>	0.35 <sup>b</sup>	43.23

The different letters indicate significant difference at 0.05 level; sugar = sucrose + fructose + glucose + sorbitol. Solid/acid ratio represents the ratio of soluble solid to titratable acidity.

**Figure 1.** Effects of bagging on the content of (A) sucrose, (B) fructose, (C) glucose and (D) sorbitol in fruit of Qingzhong loquat.

to fructose and SOX is an enzyme that catalyses the conversion of sorbitol to glucose (Liang et al., 2004). The activities of SDH and SOX in the bagged and non-bagged fruit were quite similar during fruit development and decreased before 85 days after initial fruit set before increasing slowly until the fruit ripened (Figure 3A and B).

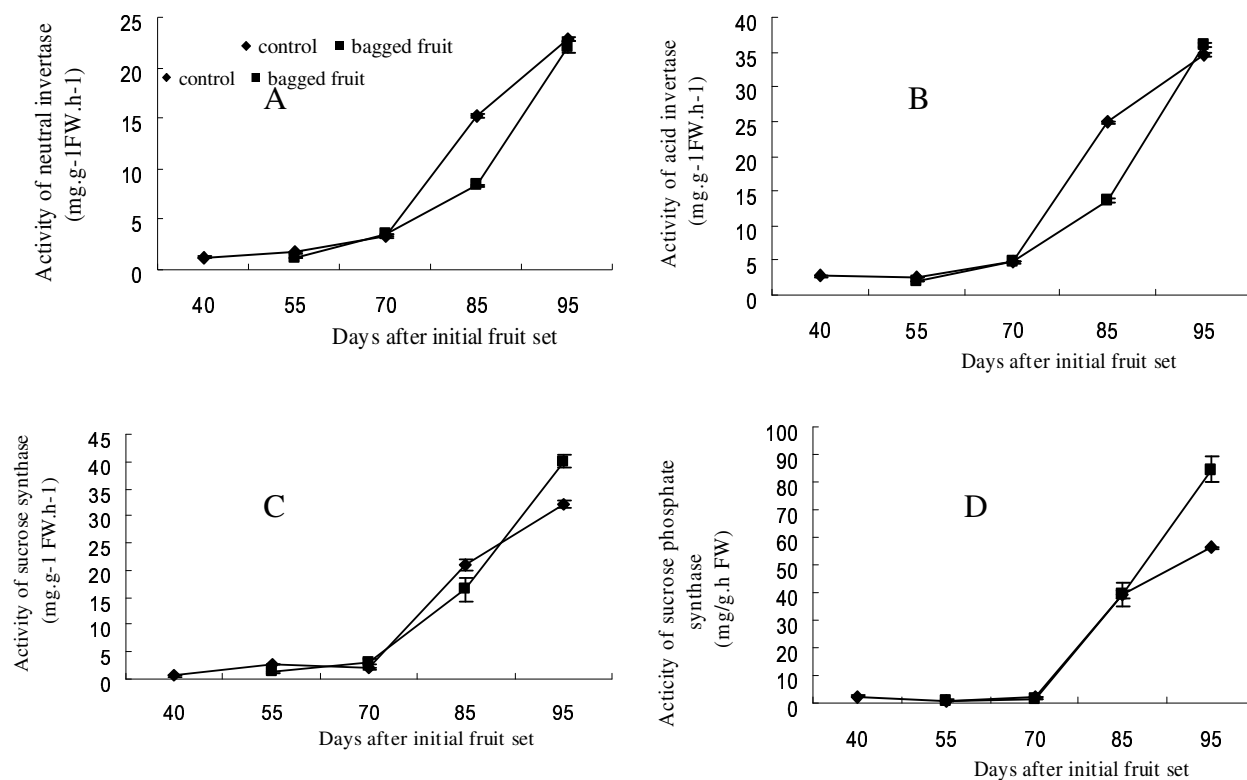
#### The correlation between sugar accumulation and activities of sucrose and sorbitol-metabolizing enzymes during fruit development

Sucrose content displayed a very significant positive correlation with the activities of SS and SPS during bagged fruit development, while glucose content also

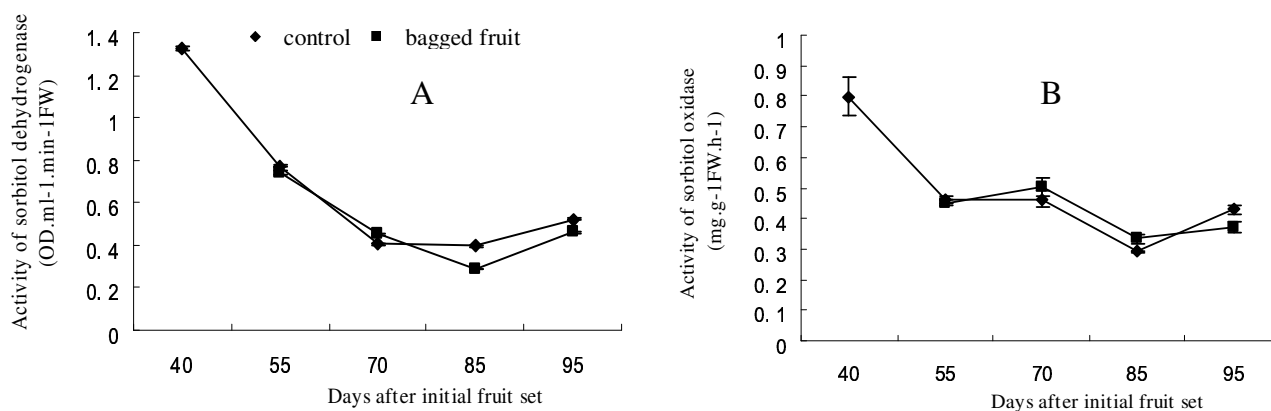
positively correlated very significantly with the activity of sucrose-metabolizing enzymes. Sorbitol content also exhibited a significant positive correlation with the activity of SDH. The content of sucrose, fructose, glucose and the activity of sucrose-metabolizing enzymes during non-bagged fruit development showed a very significant positive correlation, suggesting that bagging affects the accumulation of fructose and the activity of sucrose-metabolizing enzymes (Table 2).

#### DISCUSSION

The results demonstrated that average fruit weight in the bagged fruit was lower than that in non-bagged fruit. The



**Figure 2.** Effects of bagging on the activities of (A) neutral invertase, (B) acid invertase, (C) sucrose synthase and (D) sucrose-phosphate synthase in fruit of Qingzhong loquat.



**Figure 3.** Effects of bagging on the activities of (A) sorbitol dehydrogenase and (B) sorbitol oxidase in fruit of Qingzhong loquat.

trend in sucrose, fructose, glucose and sorbitol accumulation were quite similar but the rates of accumulation were not synchronous in the bagged and non-bagged fruit during fruit development. The accumulation of sucrose and glucose in the bagged fruit increased, while the accumulation of fructose and sorbitol decreased. The content of soluble solids in the bagged fruit increased and the content of titratable acidity decreased when compared

with the non-bagged fruit. These results coincide with what Xu et al. (2008, 2010) report on the 'Baiyu' loquat treated with a white single-layer paper bag.

This study found that the content of sorbitol in 'Qingzhong' loquat mature leaf was  $24.52 \text{ mg}\cdot\text{g}^{-1}$ , 3.83 times that of the mature fruit (data was not shown in the results), which revealed that the content of sorbitol in the mature fruit was much lower than that in the mature leaf.

**Table 2.** The correlation between sugar accumulation and activities of sucrose-metabolizing enzyme and sorbitol-metabolizing enzyme during fruit development.

Treatment	Sugar content	NI	AI	SS	SPS	SDH	SOX
Control	Sucrose	0.996**	0.990**	0.991**	0.986**	—	—
	Fructose	0.988**	0.978**	0.977**	0.970**	—	—
	Glucose	0.994**	0.989**	0.983**	0.981**	—	—
	Sorbitol	—	—	—	—	0.836	0.76
Bagging	Sucrose	0.981*	0.984*	0.994**	0.999**	—	—
	Fructose	0.957*	0.958*	0.972*	0.984*	—	—
	Glucose	0.993**	0.993**	0.997**	0.994**	—	—
	Sorbitol	—	—	—	—	0.968*	0.558

\*\*Correlation is significant at the 0.01 level (2-tailed); \*correlation is significant at the 0.05 level (2-tailed); — correlation is negative. AI, acid invertase; NI, neutral invertase; SS, sucrose synthase; SPS, sucrose-phosphate synthase; SDH, sorbitol dehydrogenase; SOX, sorbitol oxidase.

However, the accumulation of sorbitol in bagged fruit was lower than that of the non-bagged fruit. Ruan et al. (1997) reported that the difference in sugar content in fruit was independent of export from a source leaf but the transport capacity of sugar was different in different kinds of fruit trees, which suggested that the content of sorbitol entering the fruit did not depend on the output capacity of the leaf but on the gathering ability of the fruit. A possible reason why the content of sorbitol in the bagged fruit was lower than that in the non-bagged fruit is that bagging might affect the ability to transport sorbitol from the leaves to the fruits. Bagging influenced the activities of sucrose and sorbitol metabolism related enzymes. The activities of NI and AI were lower in bagged fruit than those in the non-bagged fruit and were significantly different before 85 days after initial fruit set. The activity of NI was similar in the bagged and non-bagged fruit, whereas the activity of AI was higher in the bagged than in the non-bagged fruit until the fruit ripened. The activities of SS and SPS were higher in the bagged than in the non-bagged fruit, which suggested that bagging mainly increased the activities of sucrose metabolism related enzymes.

Sugar introduced into the fruit from the leaf depends on the sink strength of the fruit to a large extent. An important physiological marker that determines sink strength is the activities of sucrose metabolism related enzymes (Vizzotto et al., 1996). As photosynthesis in fruit decreased after bagging (Xu et al., 2010), the activities of sugar related enzymes may be required to increase the sink strength of the fruit to obtain more products of photosynthesis. This study suggests that bagging mainly offsets the loss induced by lack of sunlight by increasing the activities of SS and SPS.

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