Effects of Storage and Preservation of Postharvest Gonggan Mandarin (*Citrus reticulata*) Fruit Immersion in Salicylic Acid Combined with Calcium Chloride

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Abstract [**Objectives**] To investigate the effects and mechanism of fruit immersion treatment with salicylic acid (SA), calcium chloride ($CaCl_2$) solutions at different concentrations or their combinations on post-harvest preservation of Gonggan Mandarin (*Citrus reticulata*) fruit. [**Methods**] 13 experiments (2 storage temperatures, 3 control treatments, 3 concentrations of SA solution, 4 concentrations of $CaCl_2$ solution and 3 combinations of SA + $CaCl_2$ solution for fruit immersion treatment) were conducted for 90 d. Gonggan fruit weight loss rate, rotting rate, disease index, disease type, soluble solids and organic acids content, and the differences in the activity of six enzymes (APX, PPO, CHI, CAT, SOD, PAL) related to fruit disease resistance were detected regularly. [**Results**] With the increase of storage days, the weight loss rate, rotting rate and disease index increased, the content of soluble solids and organic acids decreased, the activity of APX, PPO, CHI and SOD increased, and the activity of CAT and PAL decreased. However, the increase or decrease of fruit loss at low temperature was the smallest, and the increase or decrease of fruit at natural room temperature was the largest. Gonggan fruit cleaning before storage is an effective preservation and fresh-keeping technology, but its preservation and fresh-keeping effect is far less than that of fruit immersion by SA, $CaCl_2$ and SA + $CaCl_2$ compound solution. The effect of fruit immersion by SA solution was greater than that of fruit immersion by $CaCl_2$ solution; the best concentration of SA alone was 6.0 mmol/L, which was not significantly different from 3.0 mmol/L, but the effect of 9.0 mmol/L decreased; the best concentration of $CaCl_2$ alone was 6.0 mmol/L, which was significantly better than 3.0 and 9.0 mmol/L; the optimal concentration combination of SA + $CaCl_2$ compound solution for fruit immersion treatment was 6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$. [**Conclusions**] The fruit immersion by SA, $CaCl_2$ and their compound solutions induced the increase of activity of six enzymes related to disease resistance in Gonggan fruit, and PPO and CHI were judged to be two key enzymes based on response intensity.

Key words Gonggan Mandarin (*Citrus reticulata*), Blue mold, Green mold, Preservation and fresh-keeping, Induced resistance

1 Introduction

Salicylic acid (SA) is a fat-soluble organic acid with the properties of aromatic acid and phenol. It is an important antiseptic material and raw material for fine chemical industry and pharmaceutical industry. SA participates in many physiological and biochemical processes of plants, and can regulate metabolic processes such as growth and development, ripening and senescence, disease resistance, *etc.* It is the most important signal substance in enzyme-activated chain reaction related to plant defense responses such as resisting viruses, fungi and bacteria[1-2]. Calcium chloride ($CaCl_2$) is a typical ionic halide and an important source of bioavailable calcium (Ca^{2+}). As a food ingredient, $CaCl_2$ can act as a multivalent chelating agent and curing agent. Ca^{2+} is an important second messenger in cells. Organelles are important storage sites of Ca^{2+} in cells. When plants are stimulated by external environment, the concentration of Ca^{2+} in cytoplasm increases specifically, and cells transmit information downstream through calcium signals, thus generating response. Many organelles not only have specific calcium signals, but also participate in the formation of calcium signals in cytoplasm[3-4].

The treatment with proper concentration of SA can induce disease resistance, inhibit ethylene synthesis, delay ripening and senescence of fruits, and reduce the storage period and rotting rate of fruits during post-harvest storage and transportation[5-6]. Spraying Ca on leaves before harvest or soaking fruits with Ca after harvest have obvious effects on the storage and preservation of various fruits, and can significantly improve the good fruit rate during storage[7-8]. The combination of SA and $CaCl_2$ can further maintain fruit firmness, prolong shelf life, and slow down the decline of fruit quality[9-10]. However, the concentration of different kinds of fruits varies greatly. Gonggan Mandarin (*Citrus reticulata*) is a famous special citrus in Zhaoqing, Guangdong Province, which has won the honorary title of "One of Top Ten Excellent Fruits in Lingnan", but there are few studies on its storage and preservation at present[11-12]. The purpose of this paper is to study the effect and mechanism of fruit immersion by SA combined with chlorine $CaCl_2$ on preservation and fresh-keeping, and to provide a reference for their scientific application.

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2 Materials and methods

2.1 Experimental materials When Gonggan is in season (December 18th, 2015), 9-year-old Gonggan trees with good growth were selected in the orchard (23°24′ N, 111°26′ E) of Mawei Town, Deqing County, Guangdong Province. The fruit (280 d after full bloom) in the middle of the crown was collected with regular fruit shape, uniform size and yellow surface, and was put into a box and brought back to the laboratory. It was temporarily stored indoors to release field heat and reduce water content appropriately. At 72 h (3 d) after harvest, the fruits without obvious damage were selected for experimental treatment. The chemical reagents SA, $CaCl_2$, concentrated H_2SO_4, $C_2HCl_3O_2$, H_3PO_4, $FeCl_3$, NaCl, phenolphthalein and NaOH (chemically pure) used in the experiment were produced by Shanghai Shenbo Chemical Co., Ltd.; ethanol, glucose, citric acid, ascorbic acid, o-phenanthroline, 3, 5-dinitrosalicylic acid, 2, 6-dichlorophenol indophenol (analytically pure) were produced by Shanghai Sinopharm Chemical Reagent Co., Ltd.

2.2 Fruit design and treatment methods 13 treatments were designed, including three controls, three concentrations of SA solution, four concentrations of $CaCl_2$ solution and three kinds of SA + $CaCl_2$ compound solutions. The controls are: (i) CK1: Storage at (6.5 ± 0.5) ℃ without fruit immersion; (ii) CK2: Storage at natural room temperature [(13.5 ± 3.5) ℃] without fruit immersion; (iii) CK3: Storage at natural room temperature after fruit immersion in pure water (45 s, dried). SA, $CaCl_2$ and their combinations were used for fruit immersion (45 S), and the fruit was dried and stored at room temperature. There were 10 treatments: (i) 1.5 mmol/L SA; (ii) 3.0 mmol/L SA; (iii) 6.0 mmol/L SA; (iv) 9.0 mmol/L SA; (v) 3.0 mmol/L $CaCl_2$; (vi) 6.0 mmol/L $CaCl_2$; (vii) 9.0 mmol/L $CaCl_2$; (viii) 3.0 mmol/L SA + 6.0 mmol/L $CaCl_2$; (ix) 6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$; (x) 6.0 mmol/L SA + 6.0 mmol/L $CaCl_2$.

After treatment, the fruits were packed into modified atmosphere bags (100 cm × 60 cm) made of polyethylene plastic film (3 mm in diameter, 18 holes in each bag) and stored for 90 d. The temperature of the refrigerating chamber was automatically controlled by the equipment. In the natural temperature storage room, the floor was cleaned by wet mop once every morning, and the window was ventilated from 21:00 to 23:00 in the evening. The relative humidity of all storage rooms was ⩾ 85%. The weight loss rate, rotting rate, disease index, disease type, soluble solid content and organic acid content of fruits were tested and analyzed every 30 from 0 d (storage); on the 30th d, the activity of six enzymes related to fruit disease resistance was tested and analyzed, including ascorbate peroxidase (APX), chitinase (CHI), polyphenol oxidase (PPO), catalase (CAT), superoxide dismutase (SOD) and L-phenylalalanin ammonia-lyase (PAL).

2.3 Analytical test methods Fruit incidence = number of diseased fruits/number of fruits tested in corresponding treatments (15). The fruit disease index was calculated according to formula (1). The disease index was divided into 8 levels based on the degree of fruit rotting after inoculation—the percentage of rotting area to fruit surface area (%): 0 for Level 0, 0 – 1% for Level 1, 1% – 5% for Level 2, 5% – 10% for Level 3, 10% – 20% for Level 4, 20% – 50% for Level 5, 50% – 80% for Level 6, over 80% for Level 7.

Disease index = [Σ(Number of rotten fruits at all levels × Representative value at all levels)/(Total number of fruits at corresponding treatments × representative value at the highest level (7))] × 100 (1)

At 0, 30, 60 and 90 d after storage, the weight loss rate, soluble sugar and organic acid content of fruit in each treatment were tested and analyzed. Measurement of fruit weight loss rate: On the day of storage, each bag of fruits were numbered and marked, and the weight (fresh weight) was measured regularly after storage. It was expressed by the percentage of the weight reduction of each marked fruit to its weight on the day of storage. It was calculated according to formula (2):

The fruit weight loss rate (%) = Weight at storage – Weight after storage × 100 (2)

Measurement of fruit rotting rate: Fruit with disease spots ⩾ 0.50 cm in diameter on the surface was regarded as rotten fruit. After storage, the number of rotten fruits in each bag was counted regularly according to the interval of days, and the rotting rate was the percentage of the number of rotten fruits in the bag to the total number of fruits stored in the bag. It was calculated according to the formula (3):

Rotting rate (%) = Number of rotten fruits ÷ Total number of fruits stored × 100 (3)

Measurement of soluble total sugar, organic acid and vitamin C in fruits: Juice vesicle was taken as sample for determination according to NY/T 2742-2015 *Determination Method of Soluble Sugar in Fruits and Products* (3, 5-Dinitrosalicylic Acid Colorimetry), GB/T 12293-1990 *Determination Method of Titratable Acidity of Fruits and Vegetable Products* (NaOH Titration Method), and GB 5009.86-2016 *Determination Method of Ascorbic Acid in Foods* (2, 6-Dichlorophenol Indophenol Method).

APX activity (U/(g · min) related to disease resistance of Gonggan fruit was determined according to the method described by Wang Haibo *et al.* and Sun Dezhi *et al.*, $1U = 0.01\Delta OD_{290}$. CHI activity (U/g FW · h) was determined according to the method described by Zhang Shaoying *et al.* [13], and 1U refers to the decomposition of colloidal chitin per gram of fresh plant tissue to produce 1.0 μg N-acetylglucosamine per hour.

PPO activity (U/(g · min) was determined according to the method of Zauberman *et al.* [14], $1U = 0.01\Delta OD_{420}$; SOD and CAT activity was determined according to the method of Li Ling *et al.* [15], and 1 U means 50% and $0.01\Delta OD_{240}$ of NBT photochemical reduction inhibition per 1 g of fresh weight, respectively. PAL activity (U/(g · h) was determined based on the method of Ye Maobing *et al.* [16].

2.4 Statistical analysis of data According to the test results,

the average and standard deviation were calculated and plotted by Microsoft Office Excel 2013, and the significant difference between different treatments was analyzed by Duncan's new multiple range method.

3 Results and analysis

3.1 Effects on weight loss rate of Gonggan fruit The fruit weight loss rate of Gonggan increased significantly with the increase of storage days, but the weight loss rate of fruit stored at low temperature (CK1) was the lowest, and the weight loss rates at 30, 60 and 90 d were only 23.6%, 31.4% and 41.8% of those stored at natural room temperature (CK2), respectively, while the weight loss rates in all other treatments were significantly higher than those stored at low temperature (Table 1). At the same time, even at natural room temperature, the weight loss rate in Gonggan fruit cleaning treatment (CK3) significantly decreased by 52.9%, 21.3% and 18.2% compared with that in CK2 at 30, 60 and 90 d, respectively, but the weight loss rate in 10 treatments after immersion with SA, $CaCl_2$ or their compound solutions was further significantly lower than that in CK3. It can be seen from Table 1 that the effects of fruit immersion by SA solution were greater than that of fruit immersion by $CaCl_2$ solution. Among the four SA solution concentrations, the weight loss rate in fruit immersion treatment at 6.0 mmol/L was the lowest, which was significantly different from that at 1.5 mmol/L, equivalent to that at 3.0 mmol/L with no significant difference, but the weight loss rate in fruit immersion treatment at 9.0 mmol/L significantly increased; among the three $CaCl_2$ solution concentrations for fruit immersion, the weight loss rate at 6.0 mmol/L was the lowest, which was equivalent to that at 3.0 mmol/L with no significant difference, but the weight loss rate in fruit immersion treatment at 9.0 mmol/L significantly increased; among the three combination treatments, the weight loss rate in the treatment of 6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$ was the lowest, while the weight loss rate in the treatment of 6.0 mmol/L SA + 6.0 mmol/L $CaCl_2$ was the highest, which indicated that the optimum concentration in the combination treatment was lower than that in the single treatment.

3.2 Effects on rotting rate of Gonggan fruit Compared with the change characteristics of fruit weight loss rate, the rotting rate of Gonggan fruit increased more sharply with the increase of storage days. However, the rotting rate of fruits stored at low temperature (CK1) was the lowest, and the rotting rate of fruits stored at 30, 60 and 90 d was only 19.6%, 20.9% and 20.5% of that stored at room temperature (CK2), respectively, while the rotting rate of fruits in all other treatments was significantly higher than that stored at low temperature, which further indicated that low temperature storage was the most basic condition for post-harvest storage of Gonggan. At the same time, even at the natural room temperature, the rotting rate of the fruits treated with cleaning (CK3) before storage could be significantly reduced. The rotting rate was 52.5%, 30.4% and 23.3% lower than that in CK2 at 30, 60 and 90 d, respectively. However, the rotting rate of the fruits stored after being treated with immersion by SA, $CaCl_2$ or their compound solutions was further reduced compared with that in CK3. Among the 4 concentrations of SA solution for fruit immersion, 6.0 mmol/L led to the lowest rotting rate, which was not significantly different from 3.0 mmol/L, and 9.0 mmol/L could increase rotting rate by 53.9%; among the three $CaCl_2$ solution concentrations for fruit immersion, the rotting rate was the lowest in 6.0 mmol/L treatment, which was significantly lower than that in 3.0 and 9.0 mmol/L treatments; among the three combination treatments, the rotting rate was the lowest in 6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$ treatment, and the rotting rate of fruit decreased by 73.7%, 77.3% and 61.9%, respectively at 30, 60 and 90 d compared with that in CK3 (Table 2).

Table 1 Effects of salicylic acid and calcium chloride on fruit weight loss rate of Gonggan

Treatment	Fruit weight loss rate//%		
	30 d	60 d	90 d
CK1	0.32 ± 0.03 h	0.75 ± 0.08 j	1.32 ± 0.02 i
CK2	1.36 ± 0.12 a	2.39 ± 0.11 a	3.16 ± 0.07 a
CK3	0.64 ± 0.02 b	1.88 ± 0.06 b	2.75 ± 0.05 b
1.5 mmol/L SA	0.45 ± 0.02 ef	1.43 ± 0.05 ef	2.25 ± 0.02 d
3.0 mmol/L SA	0.39 ± 0.04 fgh	1.29 ± 0.01 g	1.98 ± 0.05 f
6.0 mmol/L SA	0.36 ± 0.02 gh	1.15 ± 0.03 h	1.85 ± 0.03 g
9.0 mmol/L SA	0.48 ± 0.02 de	1.58 ± 0.03 d	2.21 ± 0.04 d
3.0 mmol/L $CaCl_2$	0.42 ± 0.04 efg	1.51 ± 0.02 de	2.02 ± 0.02 f
6.0 mmol/L $CaCl_2$	0.44 ± 0.03 efg	1.42 ± 0.03 ef	1.88 ± 0.02 g
9.0 mmol/L $CaCl_2$	0.68 ± 0.02 b	1.68 ± 0.07 c	2.39 ± 0.03 c
3.0 mmol/L SA + 6.0 mmol/L $CaCl_2$	0.48 ± 0.03 de	1.55 ± 0.03 d	2.03 ± 0.04 f
6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$	0.44 ± 0.03 efg	1.03 ± 0.06 i	1.68 ± 0.08 h
6.0 mmol/L SA + 6.0 mmol/L $CaCl_2$	0.58 ± 0.05 c	1.38 ± 0.06 fg	2.12 ± 0.07 e

Note: CK1: Direct storage at (6.5 ± 0.5) ℃ (low temperature) after fruit selection; CK2: Direct storage at (12.5 ± 3.5) ℃ (natural room temperature) after fruit selection; CK3: Fruit immersion in pure water for 45 s, drying and storing at natural room temperature; the other treatments were that the fruit was soaked in SA, $CaCl_2$ or compound solution for 45 s and then stored at room temperature. Different lowercase letters followed by the values of the same column indicate significant differences ($P < 0.05$). The same below.

Table 2 Effects of salicylic acid and calcium chloride on rotting rate of Gonggan fruit

Treatment	Fruit rotting rate//%		
	30 d	60 d	90 d
CK1	5.5 ± 3.0 cd	16.0 ± 5.2 e	25.6 ± 8.9 f
CK2	28.0 ± 0 a	76.6 ± 2.4 a	100.0 ± 0 a
CK3	13.3 ± 2.2 b	53.3 ± 9.4 b	79.5 ± 4.7 cd
1.5 mmol/L SA	10.5 ± 4.7 bc	26.7 ± 4.7 d	76.7 ± 9.4 cd
3.0 mmol/L SA	6.7 ± 2.2 cd	26.0 ± 4.7 d	73.3 ± 9.4 d
6.0 mmol/L SA	3.3 ± 0 d	13.5 ± 4.5 b	43.3 ± 9.4 e
9.0 mmol/L SA	7.5 ± 0 cd	28.2 ± 5.4 cd	80.8 ± 6.5 bcd
3.0 mmol/L $CaCl_2$	13.0 ± 4.4 b	53.3 ± 4.7 b	87.0 ± 1.9 bc
6.0 mmol/L $CaCl_2$	6.5 ± 0 cd	15.0 ± 8.2 e	36.3 ± 5.2 ef
9.0 mmol/L $CaCl_2$	10.0 ± 4.5 bc	50.0 ± 8.2 b	90.0 ± 0 ab
3.0 mmol/L SA + 6.0 mmol/L $CaCl_2$	8.9 ± 3.5 bc	37.8 ± 3.2 c	55.0 ± 8.2 de
6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$	3.5 ± 0.5 d	12.1 ± 5.0 e	30.3 ± 4.7 f
6.0 mmol/L SA + 6.0 mmol/L $CaCl_2$	12.5 ± 2.0 b	34.8 ± 6.8 cd	70.0 ± 8.2 d

3.3 Effects on disease index of Gonggan fruit Compared with the change characteristics of fruit rotting rate, the disease index of Gonggan fruit increased more and more sensitively with the increase of storage days, but it was the smallest in CK1, the largest in CK2, and the disease index in CK3 was smaller than that in CK2; the disease index was only 49.1%, 41.7% and 36.7% of that in CK2 at 30, 60, 90 d of storage, and the disease index decreased by 22.7%, 34.6% and 11.9% in CK3 compared with that in CK2 at the same time (Table 3). It can be seen from Table 3 that the disease index of the fruits stored after being treated with immersion by SA, $CaCl_2$ or their compound solutions was further reduced compared with that in CK3. Among the 4 concentrations of SA solution for fruit immersion, 6.0 mmol/L treatment led to the lowest rotting rate, the longer the storage time, the greater the difference, and after 90 d of storage, the rotting rate was 37.8%, 32.0% and 41.4% lower than that in 1.5, 3.0 and 9.0 mmol/L SA treatment, respectively; among the three concentrations of $CaCl_2$ solution for fruit immersion, the disease index in 6.0 mmol/L treatment was the smallest, which was 36.2% and 29.4% lower than that in 3.0, 9.0 mmol/L SA treatment at 90 d of storage; among the three combination treatments, the disease index in 6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$ treatment was the smallest, and it was 81.4%, 61.4% and 60.6% lower than that in CK3 treatment at 30, 60 and 90 d of storage, equivalent to that in 6.0 mmol/L SA treatment, 61.2%, 23.5% and 60.1% lower than that in 6.0 mmol/L $CaCl_2$ treatment at the same time.

3.4 Effects on disease type of Gonggan fruit It can be seen from Table 4 that green mold showed the highest fruit disease incidence of Gonggan stored at natural room temperature (CK2) for 30, 60 and 90 d, and the disease incidence for green mold was obviously lower than that for green mold, especially before 30 d of storage; after storage for 60 and 90 d, the diseased fruits subject to blue mold and compound infections increased greatly, and the incidence of the three diseases judged from external signs was equivalent; low temperature storage had different inhibitory effects on three kinds of diseases, especially on green mold infection in the first 30 d of storage, and then the difference was reduced, but the inhibitory effect on compound infection was enhanced; the effect of cleaning fruit (CK3) before storage on inhibiting the disease type was not very clear, but the main inhibited type before storage for 60 d was green mold. The fruit immersion with SA, $CaCl_2$ or their compound solutions had no significant specificity on the incidence of diseases during storage of Gonggan, but green mold and compound infections were mainly controlled at the early stage, and 6.0 mmol/L SA, 6.0 mmol/L $CaCl_2$ and the compound solution 6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$ also showed similar characteristics in the middle and late storage (Table 4).

Table 3 Effects of salicylic acid and calcium chloride on fruit disease index of Gonggan

Treatment	Fruit disease index//%		
	30 d	60 d	90 d
CK1	10.6 ± 1.7 bcd	25.3 ± 2.5 cde	36.0 ± 5.6 ef
CK2	21.6 ± 2.4 a	60.6 ± 4.1 a	98.0 ± 0.8 a
CK3	16.7 ± 0.5 ab	39.6 ± 2.6 b	86.3 ± 3.7 a
1.5 mmol/L SA	11.7 ± 3.1 bcd	25.6 ± 4.8 cde	54.7 ± 3.8 cd
3.0 mmol/L SA	6.3 ± 8.9 de	21.0 ± 4.9 def	50.0 ± 7.2 d
6.0 mmol/L SA	3.3 ± 4.7 e	19.7 ± 1.5 ef	34.0 ± 4.9 f
9.0 mmol/L SA	7.5 ± 2.1 cde	38.9 ± 4.6 b	58.0 ± 5.4 cd
3.0 mmol/L $CaCl_2$	14.0 ± 2.2 bc	31.3 ± 3.8 c	85.2 ± 11.8 b
6.0 mmol/L $CaCl_2$	8.0 ± 0.8 cde	20.0 ± 2.9 ef	54.4 ± 6.9 cd
9.0 mmol/L $CaCl_2$	11.5 ± 3.7 bcd	28.3 ± 2.8 c	77.0 ± 5.1 b
3.0 mmol/L SA + 6.0 mmol/L $CaCl_2$	12.3 ± 2.6 bcd	26.0 ± 6.2 cde	62.3 ± 3.9 c
6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$	3.1 ± 0.6 e	15.3 ± 2.8 f	34.0 ± 7.6 f
6.0 mmol/L SA + 6.0 mmol/L $CaCl_2$	10.0 ± 2.2 cd	28.0 ± 3.6 cd	46.0 ± 10.7 de

Table 4 Effects of salicylic acid and calcium chloride on disease type composition of Gonggan fruit %

Treatment	30 d			60 d			90 d		
	Fruit infected with blue mold	Fruit infected with green mold	Fruit with compound infection	Fruit infected with blue mold	Fruit infected with green mold	Fruit with compound infection	Fruit infected with blue mold	Fruit infected with green mold	Fruit with compound infection
CK1	4.5 ± 0.9 bc	1.0 ± 0.5 c	1.0 ± 0.5 c	6.8 ± 0.8 ef	8.0 ± 1.0 ef	1.2 ± 0.1 d	5.2 ± 0.5 d	12.6 ± 1.2 d	10.8 ± 1.1 f
CK2	3.3 ± 0.5 c	12.7 ± 5.2 a	4.0 ± 1.5 a	22.3 ± 6.5 a	33.3 ± 6.0 a	21.0 ± 4.9 a	33.3 ± 5.8 a	36.7 ± 6.2 a	30.0 ± 4.5 bc
CK3	7.7 ± 2.1 a	4.5 ± 1.5 b	1.1 ± 0.8 c	16.5 ± 4.8 bc	20.1 ± 5.2 b	16.7 ± 3.9 b	18.1 ± 3.2 c	26.6 ± 4.0 bc	32.0 ± 4.5 b
1.5 mmol/L SA	6.2 ± 1.5 abc	3.4 ± 1.0 bc	0.9 ± 0.5 c	8.7 ± 0.6 def	13.3 ± 2.1 de	4.7 ± 1.0 d	19.5 ± 2.0 bc	25.8 ± 1.9 bc	31.4 ± 1.9 b
3.0 mmol/L SA	4.3 ± 1.2 bc	2.4 ± 0.5	0 c	6.3 ± 0.5 ef	16.7 ± 3.2 cd	3.0 ± 0.5 d	10.9 ± 0.9 d	23.6 ± 2.3 c	38.8 ± 3.2 a
6.0 mmol/L SA	3.3 ± 0.5 c	0 c	0 c	3.5 ± 0.5 f	8.0 ± 1.2 ef	2.0 ± 0.5 d	9.4 ± 0.8 de	14.6 ± 1.1 d	19.3 ± 2.0 e
9.0 mmol/L SA	5.5 ± 0.5 abc	2.0 ± 0.5 bc	0 c	8.6 ± 1.9 def	15.5 ± 1.9 cd	4.1 ± 2.0 d	10.2 ± 1.0 d	40.1 ± 2.6 a	30.5 ± 2.2 bc
3.0 mmol/L $CaCl_2$	6.7 ± 2.2 ab	5.0 ± 3.0 b	1.0 ± 0 c	20.0 ± 5.0 ab	23.3 ± 4.8 b	10.0 ± 4.2 c	20.0 ± 2.5 bc	41.2 ± 5.5	25.8 ± 4.1 cd
6.0 mmol/L $CaCl_2$	4.5 ± 0 bc	2.0 ± 0.5 bc	0 c	6.8 ± 0.6 ef	4.9 ± 0.5 f	3.3 ± 0.5 d	7.9 ± 0.9 de	16.2 ± 1.8 d	12.2 ± 1.0 f
9.0 mmol/L $CaCl_2$	6.7 ± 3.2 ab	2.2 ± 1.8 bc	1.1 ± 0.6	13.3 ± 2.9 cd	20.0 ± 3.5 bc	16.7 ± 2.8 b	23.5 ± 2.2 b	36.5 ± 2.7 a	30.0 ± 2.2 bc
3.0 mmol/L SA + 6.0 mmol/L $CaCl_2$	5.5 ± 2.0 abc	4.8 ± 3.0 b	3.0 ± 1.0 b	10.0 ± 1.5 de	16.1 ± 1.9 cd	11.7 ± 2.0 c	18.5 ± 3.5 c	40.3 ± 6.2 a	21.2 ± 3.8 de
6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$	3.5 ± 0.5 c	0 c	0 c	5.6 ± 0.6 ef	6.2 ± 0.5 f	0.3 ± 0.1 d	10.7 ± 1.2 d	13.1 ± 0.9 d	4.5 ± 0.5 g
6.0 mmol/L SA + 6.0 mmol/L $CaCl_2$	8.5 ± 2.2 a	4.0 ± 2.0 bc	0 c	8.3 ± 1.2 def	15.3 ± 1.2 cd	11.2 ± 2.8 c	16.5 ± 1.1 c	30.3 ± 2.2 b	23.2 ± 2.8 de

3.5 Effects on fruit quality of Gonggan With the increase of storage days, the content of soluble solids and organic acids in Gonggan fruit decreased continuously, especially for the content of organic acids. The decrease of soluble solids content was not as large as that of organic acids, but low temperature storage significantly inhibited this process, and washing fruits before storage had a certain inhibition effect on it. Fruit immersion with appropriate concentrations of SA, $CaCl_2$ or their compound solution could significantly inhibit this

even when stored at natural room temperature, and the post-effect was more significant (Table 5). It can be seen from Table 5 that after 30, 60 and 90 d of storage, the soluble solids content of fruits in CK2 decreased by 12.9%, 30.1% and 35.7%, respectively, and the organic acid content of fruits decreased by 26.6%, 37.5% and 61.1%, respectively compared with CK1; compared with CK2, the soluble solid content in CK3 increased by 3.3%, 10.5% and 11.1% respectively, and the organic acid content increased by 9.1%, 8.0% and 14.3%, respectively; compared with CK3, the soluble solid and the organic acid content of the fruits treated with 6.0 mmol/L SA and 6.0 mmol/L $CaCl_2$ showing the best fruit immersion performance with single solution, increased by 2.4%, 1.6%, 12.6%, 3.2%, 15.0% and 11.3%, and 16.7%, 19.4%, 18.5%, 11.1%, 43.8% and 25.0%, respectively; in the 6.0 mmol/L SA +3.0 mmol/L $CaCl_2$ treatment showing the best fruit immersion performance with compound solution, the soluble solids increased by 2.4%, 17.9% and 27.5%, respectively, and the organic acid content increased by 19.4%, 25.9% and 62.5%, respectively.

Table 5 Effects of salicylic acid and calcium chloride on the content of soluble solids and organic acids in Gonggan fruit

Treatment	Soluble solid content//%				Organic acid content//%			
	0 d	30 d	60 d	90 d	0 d	30 d	60 d	90 d
CK1	13.4±0.3 a	13.9±0.6 a	12.3±0.8 a	11.2±0.9 a	0.46±0.02 a	0.45±0.02 a	0.40±0.02 a	0.36±0.02 a
CK2	13.4±0.3 a	12.1±1.2 bc	8.6±0.9 f	7.2±1.8 e	0.46±0.02 a	0.33±0.02 d	0.25±0.02 c	0.14±0.02 d
CK3	13.4±0.3 a	12.5±0.9 bc	9.5±0.8 cd	8.0±1.4 de	0.46±0.02 a	0.36±0.03 cd	0.27±0.03 bc	0.16±0.03 cd
1.5 mmol/LSA	13.4±0.5 a	12.6±0.5 bc	9.2±0.6 de	8.8±0.5 cd	0.46±0.02 a	0.38±0.03 bc	0.28±0.04 bc	0.17±0.04 cd
3.0 mmol/LSA	13.4±0.3 a	12.8±0.3 b	10.6±0.9 bc	9.1±0.6 bc	0.46±0.02 a	0.40±0.01 ab	0.30±0.02 bc	0.19±0.02 bc
6.0 mmol/LSA	13.4±0.3 a	12.8±0.5 b	10.7±0.7 bc	9.2±0.5 bc	0.46±0.02 a	0.42±0.02 ab	0.32±0.01 bc	0.23±0.01 bc
9.0 mmol/LSA	13.4±0.3 a	12.8±0.6 b	10.6±0.5 bc	9.1±0.5 bc	0.46±0.02 a	0.41±0.03 ab	0.30±0.04 bc	0.21±0.04 bc
3.0 mmol/L $CaCl_2$	13.4±0.3 a	12.0±0.3 bc	9.0±0.5 ef	8.6±0.6 cd	0.46±0.02 a	0.39±0.04 bc	0.28±0.02 bc	0.17±0.02 cd
6.0 mmol/L $CaCl_2$	13.4±0.3 a	12.7±0.3 b	9.8±0.5 cd	8.9±0.7 cd	0.46±0.02 a	0.43±0.03 ab	0.30±0.04 bc	0.20±0.04 bc
9.0 mmol/L $CaCl_2$	13.4±0.3 a	11.5±0.3 c	9.3±0.6 de	8.7±0.6 cd	0.46±0.02 a	0.40±0.03	0.31±0.05 bc	0.21±0.05 bc
3.0 mmol/LSA +6.0 mmol/L $CaCl_2$	13.4±0.3 a	12.2±0.5 bc	10.2±0.7 bc	9.6±0.6 bc	0.46±0.02 a	0.43±0.03 ab	0.33±0.06 ab	0.23±0.06 bc
6.0 mmol/LSA +3.0 mmol/L $CaCl_2$	13.4±0.3 a	12.8±0.5 bc	11.2±0.5 ab	10.2±0.6 ab	0.46±0.02 a	0.43±0.03 ab	0.34±0.07 ab	0.26±0.07 b
6.0 mmol/LSA +6.0 mmol/L $CaCl_2$	13.4±0.3 a	12.6±0.5 bc	10.5±0.5 bc	9.8±0.6 bc	0.46±0.02 a	0.40±0.03 ab	0.31±0.08	0.20±0.08 bc

3.6 Effects on the activity of enzymes related to disease resistance of Gonggan fruit In all 13 treatments, the activity of APX, CHI, PPO and SOD related to disease resistance in the peel of Gonggan fruit after 30 d of storage was significantly higher than that after 0 d of storage. The activity of CAT and PAL was significantly lower than that at 0d of storage, but the extent of increase or decrease varied in different treatments. Compared with CK1, the activity of APX, CHI, PPO and SOD in CK2 decreased by 21.6%, 62.9%, 15.5% and 53.6%, respectively, while the activity of CAT and PAL increased by 81.5% and 85.0%, respectively; the activity of APX, CHI, PPO and SOD in CK3 was 63.8%, 197.1%, 53.1% and 208.3% higher than that in CK2, while the activity of CAT and PAL in CK3 was 39.3% and 41.6% lower than that in CK2; in the 6.0 mmol/L SA treatment showing the best performance of fruit immersion with single solution, the activity of APX, CHI, PPO and SOD increased by 76.3%, 62.2%, 392.0% and 87.8%, respectively compared with CK3, while the activity of CAT and PAL increased by 45.9% and 71.3%, respectively compared with CK3; in the 6.0 mmol/L SA +3.0 mmol/L $CaCl_2$ treatment showing the best performance of fruit immersion with compound solution, the activity of APX, CHI, PPO and SOD increased by 96.6%, 125.7%, 520.0% and 147.7%, respectively compared with CK3, while the activity of CAT and PAL increased by 139.6% and 136.7%, respectively compared with CK3.

Table 6 Effects of salicylic acid and calcium chloride on the activity of enzymes related to disease resistance of Gonggan fruit

Treatment	Activity of enzymes related to disease resistance					
	APX U/(g·min)	CHI U/(g·h)	PPO U/(g·min)	SOD U/(g·min)	CAT U/(g·min)	PAL U/(g·h)
CK1	8.39±2.85 de	10.18±1.42 i	0.58±0.11 f	2.07±0.16 ef	74.46±6.26 e	35.44±4.34 e
CK2	6.58±1.48 e	3.78±0.39 j	0.49±0.02 f	0.96±0.19 f	135.12±7.34 c	65.56±3.34 b
CK3	10.78±5.37 cde	11.23±0.89 hi	0.75±0.21 f	2.96±0.09 de	82.03±8.18 e	38.27±5.75 de
1.5 mmol/LSA	15.02±4.38 bcd	13.93±0.75 efg	1.51±0.42 def	3.24±0.56 de	92.66±7.33 f	45.84±4.29 d
3.0 mmol/LSA	16.02±3.05 abc	15.69±1.83 de	2.08±0.63 de	3.93±0.75 cd	108.05±6.34 e	55.29±6.28 c
6.0 mmol/LSA	19.02±2.28 ab	18.22±0.98 c	3.69±0.96 ab	5.56±1.05 ab	119.66±9.35 d	65.56±6.29 b
9.0 mmol/LSA	16.02±4.96	12.35±1.25 gh	2.05±0.75 de	3.05±0.86 de	98.66±3.86 ef	60.29±7.22 bc
3.0 mmol/L $CaCl_2$	13.39±3.29 bcd	14.87±0.55 def	2.63±0.73 bcd	2.87±0.17 de	93.58±9.14 f	35.32±0.19 de
6.0 mmol/L $CaCl_2$	16.03±4.29 abc	16.65±0.65 cd	2.63±0.63 bcd	3.68±0.45 d	119.36±6.89 d	43.62±0.04 de
9.0 mmol/L $CaCl_2$	10.36±2.58 cde	13.36±0.33 fg	1.18±0.41 ef	3.03±0.68 de	105.52±0.14 e	37.67±0.35 de
3.0 mmol/LSA +6.0 mmol/L $CaCl_2$	20.46±5.30 ab	20.93±0.74 b	3.40±0.92 bc	5.43±0.93 ab	165.47±0.12 b	68.85±7.25 b
6.0 mmol/LSA +3.0 mmol/L $CaCl_2$	21.19±3.73 a	25.35±1.16 a	4.65±0.81 a	6.59±0.75 a	196.54±0.26 a	90.60±6.10 a
6.0 mmol/LSA +6.0 mmol/L $CaCl_2$	17.45±2.88 abc	16.25±1.03 d	238.00±0.97 cde	4.86±0.89 bc	135.18±0.21 c	60.57±6.86 bc

At 0 d of storage, the activity of APX, CHI, PPO, SOD, CAT and PAL in Gonggan fruit (peel) was (4.77±1.07) U/(g·min), (2.56±0.42) U/(g·min), (0.37±0.08) U/(g·min), (0.68±0.32) U/(g·min), (152.12±6.26)

U/(g · min), (67.47 ±0.39) U/(g · min), respectively.

4 Discussion

With the increase of storage days, the weight loss rate, rotting rate and disease index of Gonggan fruit increased, the content of soluble solids and organic acids decreased, the activity of APX, PPO, CHI and SOD increased, and the activity of CAT and PAL decreased. However, the increase or decrease at low temperature was the smallest, and the increase or decrease at natural room temperature was the largest. Cleaning Gonggan fruit before storage was an effective preservation and fresh-keeping technology, but its preservation and fresh-keeping effect was far less than that of fruit immersion with SA, $CaCl_2$ and SA + $CaCl_2$ compound solution. The effect of fruit immersion by SA solution was greater than that of fruit immersion by $CaCl_2$ solution; the best concentration of SA alone was 6.0 mmol/L, which was not significantly different from 3.0 mmol/L, but the effect of 9.0 mmol/L decreased; the best concentration of $CaCl_2$ alone was 6.0 mmol/L, which was significantly better than 3.0 and 9.0 mmol/L; the optimal concentration combination of SA + $CaCl_2$ compound solution for fruit immersion treatment was 6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$. The results showed that SA, $CaCl_2$ and their mixed solutions induced the increase of activity of six enzymes related to disease resistance of Gonggan fruit. These results provide an important basis for scientific and efficient storage and preservation of Gonggan after harvest, and have outstanding value.

The role of SA in fruit preservation has been recognized by researchers at home and abroad. SA is a key signal molecule involved in regulating many physiological functions in plants, such as seed germination, seedling growth and various biotic and abiotic stress responses. In the past few years, it has been found that SA plays an important role in signal regulation of plant disease resistance, such as inducing the expression of plant disease resistance gene, activating allergic reaction and systemic acquired resistance[17-18]. It has been reported for a long time that exogenous SA plays a role in plant disease resistance. At present, it is generally believed that SA plays an important role in plant disease resistance. Studies on exogenous SA in tobacco, cucumber, potato, kidney bean, cowpea, rice and Arabidopsis thaliana showed that exogenous SA induced plant disease resistance; induced inoculation of cucumber and Arabidopsis thaliana showed that salicylic acid level in plants was closely related to the production of plant disease resistance. In this paper, the study of Gonggan, a special product in Xijiang River Basin of Guangdong Province, was confirmed again. However, from the induction of the activity response of six enzymes related to disease resistance, all of them were effective, but which one was the most important enzyme can not be confirmed and need to be further studied.

According to the response intensity of enzyme activity during storage, PPO and CHI may be two key enzymes to Gonggan fruit. PPO can oxidize phenolic substances such as tannin, catechol, pyrogallol and hydroquinone into corresponding quinones, which can inhibit and kill pathogenic microorganisms and have certain antibacterial ability. Therefore, when plants are infected by exogenous pathogenic bacteria, PPO activity will be improved to varying degrees to resist further invasion of pathogenic microorganisms. CHI is a secondary hydrolase related to defense in plants, and is a component of broad-spectrum defense mechanism of plants. It can catalyze the hydrolysis of chitin, an important component of fungal cell wall, thus inhibiting the growth and proliferation of fungi and improving the antifungal ability of plants[19-20]. CHI mainly hydrolyzes chitin polymer β-1, 4 bonds to produce N-acetylglucosamine oligomer, which can be exogenous or endogenous. When plants are infected by pathogenic fungi, bacteria and viruses, mechanical trauma or ethylene treatment, chitinase expression activity will be significantly enhanced.

There have been many reports on the role of Ca in delaying post-harvest senescence of fruits and vegetables. Early studies have shown that Ca^{2+} can form water-insoluble calcium pectinate with pectinic acid, and form covalent bond bridges between cells to maintain tissue texture and inhibit softening; pre-harvest or post-harvest Ca treatment can significantly increase the content of calcium in pulp tissue, inhibit VC loss, improve fruit quality, and increase the rate of good stored fruit and commercial fruit. There are many literature reports on prolonging the storage period of fruits. The concentration of post-harvest treatment is mostly 1.5% -2.0% ($CaCl_2$), the best concentration in this paper is 6.0 mmol/L ($CaCl_2$), and is converted into a percentage concentration of 0.684% ($CaCl_2$), which is significantly lower than that reported in previous literature. However, there is no report on post-harvest Ca treatment in citrus fruits so far, so whether this treatment is universal needs further verification. In any case, the effect of Ca treatment on preservation and fresh-keeping in post-harvest storage of Gonggan is positive, especially the effective prevention and control of green mold infection in the early stage provides an important foundation for good control effect in the whole storage period.

5 Conclusions

Low temperature environment is the basic condition for post-harvest storage and preservation of Gonggan. Cleaning Gonggan fruit before storage is an effective preservation and fresh-keeping technology. SA and $CaCl_2$ alone or in combination for fruit immersion could significantly induce the disease resistance of Gonggan fruit during storage, thus improving the preservation and fresh-keeping effect. The induction effect of SA on disease resistance of Gonggan was higher than that of $CaCl_2$, the optimum concentration in both treatments (alone) was 6.0 mmol/L, and the optimum concentration combination for fruit immersion (combination) was 6.0 mmol/L SA + 3.0 mmol/L $CaCl_2$. The results showed that the fruit immersion by SA, $CaCl_2$ and their compound solutions induced the increase of activity of six enzymes related to disease resistance in Gonggan fruit, and PPO and CHI were judged to be two key enzymes based on response intensity.

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(From page 31)

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