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DELAYING GUAVA RIPENING BY EXOGENOUS SALICYLIC ACID

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ABSTRACT

The experiment was conducted to study the effect of exogenous salicylic acid (SA) treatment (water, 100, 300 and 500 µM) on fruit repining of guava cv 'Baladi' during 12 days shelf-life at room temperature. Fruit were harvested at three different maturity stages: green mature stage (G), green yellow (GY) and yellow stage (Y). Thereafter, fruits were immersed in SA solutions for 20 min. Fruit were immersed at high level 500 µM had less IL%, degradation in total phenol, fruit browning and maintained the amount of vitamin C. Moreover, it had higher fruit firmness and color (h^{O}), compared with other concentrations of SA and water-control.

INTRODUCTION

Ripening processes are important in fruits as they involve chemical and physical changes. These changes are in color, flavor, and texture, and thereby making them most acceptable for edible purpose. Many physiological, biochemical and structure changes occur during fruit repining which induce starch degradation or other polysaccharides to produce sugars. The pigment synthesis and volatile compounds and the solubilization of cell wall (Jain *et al*., 2003).

Climacteric fruits such guava ripens rapidly and is highly perishable, a shelf-life period ranges from 2-3 days at room temperatures. So, it makes transportation and storage difficult (Bassetto *et al*., 2005). Moreover, during shelf-life, fruit ripening is characterized by green color loss, rot development, fruit softening, wilting and loss of brightness (Jacomino *et al*., 2001). Retailing of guava fruit in Egypt is usually carried out without refrigeration and therefore, the preservation of fruit at room temperature is highly desirable. Increased shelf-life period could help long-distance transportation and improve its commercialization.

Salicylic acid (SA) is a simple phenolic compound (Shafiee *et al*., 2010), also it plays a good role in post-harvest decay and disease resistance (Aghdam *et al*., 2009), increase the plant defense against oxidative stress (Xu and Tian, 2008), delaying fruit ripening (Srivastava and Dwivedi, 2000). Hence, SA treatment is used to control fruit ripening during shelf-life by which SA decreases successfully ethylene production in different fruits such as Kiwi (Aghdam *et al*., 2009), and both apple and pear (Asghari and Aghdam, 2010). The inhibition of ethylene production in fruit may be through inhibition of ethylene by decreasing both of amino cyclopropane-1-carboxylic acid

synthase (ACS) and oxidase (ACO) production and activity during early stage of fruit ripening (Zhang *et al*., 2003). Since, ethylene plays a key role in fruit ripening and senescence. Also, it triggers the induction of cell wall hydrolyzing engines leading to increases in respiration rate, fruit softening and senescence (Asghari and Aghdam, 2010).

Therefore, the aim of this study is to utilize the metabolic roles of salicylic acid to delay the ripening of guava to extend the shelf-life and to provide a better understanding of the role of SA in the control of fruit ripening.

MATERIALS AND METHODS

Fruit materials

Guava (*Psidium guajava* L.), also know Baladi fruits were harvested from the production region of Demyatta province. The determination of fruit maturity was according to fruit color change from dark to light green. Fruits maturation was classified into three maturity stages: light green (LG) stage, green yellow (GY) and yellow ripe fruit (Y) were collected for physicochemical and physiological analyses in the laboratory as shown in photograph 1. (Mondal *et al*., 2009).

Yellow (Y) **Yellow Green (YG)** **Green (G)**

Fruits (720) of uniform size clear of diseases and defect free were selected for the experiment. Fruit were divided in tow patches only each contents 360 fruits. Each patch was divided into three maturity stage G, GY and Y; only each contains 120 fruits and extra 30 fruit for browning index measurement for only each treatment. All patches (contains: G, GY and Y) were immersed in solution of SA at 100, 300 and 500 µM for 20 min and the control fruit were immersed in water in the same period. Thereafter, all patches stored at room temperature (26±2^{o}C and 71±2 RH %). The fruit samples were collected every 3-days intervals until up to end the experiment.

Color measurement

The computer vision system was used to measure fruit color (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 product location. These lamps were chosen to set the color temperature to D65 (6500 K), a common light source used in food color 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 color 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 color values (RGB) of each color tile image are converted to pixel has RGB values between 0 and 255. Thereafter, all images were analyzed by using software Image*J* Ver. 1.43u USA to get RGB signals to calculate the fruit skin according to (Khojastehnazhand *et al*., 2010).

Measurements

Fruit firmness at both fruit sides was measured 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 (Zisheng, 2006). Ion leakage was measure according to methods was described (Lo'ay, 2005). Total phenolic compounds were measured by the Folin–Ciocalteu method (Singleton and Rossi, 1965). A visual assessment of external browning-index ratting. The visual assessment of browning was chosen to score brown spots using the method of and vitamin C (Lo'ay, 2009).

Statistical analysis

Data for evaluation of parameters in time were analyzed using analysis of variance (ANOVA) when shelf-life time, fruit color maturation and treatment factors are considered. The means were compared using the least significant differences (LSD) at p≤0.05 level of probability. The statistical software package GenStat Ver. 11(Lawes Agriculture Trust, Rothamsted Experimental station, UK) was used.

RESULTS AND DISCUSSION

Fruit firmness *N*

Table 1, shows the fruit firmness plotted as a function of shelf-life time at different fruit color maturities. It is apparent from table that fruit firmness presents a significant interaction (P<0.038) when the shelf-life factors are considered.

Fruit firmness increase according to increasing SA concentrations. It has more quite firm (G: 24.19; GY: 20.59 and Y: 20.27 *N*) compared with fruit was immersed in water (G: 24.19; GY: 20.59 and Y: 20.27 *N*) at end of shelf-life period. However, fruit color maturities present differently fruit firmness at harvest time and at end of shelf-life time. It was the highest value with G (107.87 *N*) fruit compared with GY (83.36 *N*) and Y (58.84 *N*). Thereafter, fruit firmness decreased gradually with increasing SA concentration compared with fruit immersed in water.

Table 1:Effect of salicyic acid pre-storing appplication on fruits firmness (*N*) were harvested at three different color stages (G, GY and Y), that immeriesed in different concentrations of SA (100, 300 and 500 µM) and water for 20 min and stored at room temperature (26±2°C and 71±2 RH %) for 12 days.

Treatments	Maturity stages	Shelf-life time (days)				
		0	3	6	9	12
Water	Green	107.87	43.48	23.21	9.48	8.83
	G-yellow	83.36	39.88	25.17	8.50	3.92
	Yellow	58.84	27.46	22.88	2.29	2.92
100 µM SA	Green	107.87	58.19	34.32	20.92	15.04
	G-yellow	83.36	39.55	29.09	20.59	14.71
	Yellow	58.84	27.98	19.61	17.65	11.77
300 µM SA	Green	107.87	65.7	39.88	17.33	15.69
	G-yellow	83.36	48.71	30.73	21.57	11.44
	Yellow	58.84	28.11	23.54	4.58	12.42
500 µM SA	Green	107.87	77.15	49.69	29.75	24.19
	G-yellow	83.36	66.03	49.69	30.07	20.59
	Yellow	58.84	49.03	40.86	25.82	20.27
LSD at 5%	6.244					

The differences between in fruit color maturities response to SA treatments. The increasing fruit firmness with higher SA concentration may be related with the effect of SA on cell wall degradation enzymes such as cellulose, polygalacturonase and xylanase and also pectin degradation. So treating guava fruit with higher concentration of SA at 500 µM, could be decreasing of cell wall degradation enzymes activities (Srivastava and Dwivedi, 2000).

Ion leakage percentage (IL %)

Table 2, depicts a significant interaction ($P<0.001$) of shelf-life factors time, fruit color maturities and pre-storing immersing treatments of water and SA. It is clear from table that the IL% increases with shelf-life duration with all treatments. However, more deeply, IL% has less with higher SA concentration 500 µM with all fruit color maturities compared with other treatments at end shelf-life time. Also, G fruit maturity was immersed in SA at 500 µM presented less IL% at day twelfth (39.15%) compared with GY (63.66%) and Y (66.15%) and it became more leakage with fruit were immersed in water (G: 84.77; GY: 95.80 and Y: 99.67%).

The most severe ion leakages during shelf-life of fruits were harvested at three different color maturities that fruit were immersed in water. Whereas, it less with increasing SA concentration. It might be explained that SA prevents the dysfunction of cell membrane by minimizing the effect of active oxygen species (Asghari and Aghdam, 2010). So, proteins and lipids of cell membranes matrix are protected against oxidative reaction (Lo'ay, 2010), and increase the ascrobate peroxidase (APX) activity. Also, it prevents vitamin C destruction in fruits (Wang *et al*., 2006)

Table 2: Ion leakage% of fruits were harvested at three different maturity stages (G, GY and Y) that immeriesed in different concentrations of SA (100, 300 and 500 µM) and water for 20 min and stored at room temperature (26±2°C and 71±2 RH %) for 12 days.

Treatments	Maturity stages	Shelf-life time (days)				
		0	3	6	9	12
Water	Green	35.02	58.14	71.93	84.15	84.77
	G-yellow	35.12	69.88	76.54	88.84	95.80
	Yellow	37.46	87.72	95.27	90.66	99.67
100 µM SA	Green	30.91	44.70	53.99	67.35	66.02
	G-yellow	30.80	54.12	63.35	70.05	86.04
	Yellow	31.64	73.37	87.41	72.39	96.24
300 µM SA	Green	27.02	38.04	48.10	52.73	52.26
	G-yellow	26.84	39.85	46.70	60.04	76.55
	Yellow	27.12	45.58	52.81	65.25	87.59
500 µM SA	Green	21.37	34.78	41.70	43.46	39.15
	G-yellow	21.37	33.45	43.35	48.24	63.66
	Yellow	21.37	43.62	47.58	51.54	69.15
LSD at 5%		9.139				

Vitamin C (mg 100 g^{-1} FW)

Table 3, shows the changes in vitamin C content in fruits were harvested at three different color maturities stages. Vitamin C shows a significant interaction (P<0.022) when shelf-life duration, fruit color maturities and SA concentrations. Generally, Vitamin C decreased with all treatment during shelf-life period up to 12 days.

Table 3: Vitamin C content (mg 100g^{-1} FW) of fruits were harvested at three different maturity stages (G, GY and Y) that immeriesed in different concentrations of SA (100, 300 and 500 µM) and water for 20 min and stored at room temperature (26±2°C and 71±2 RH %) for 12 days.

Treatments	Maturity Stages	Shelf-life time (days)				
		0	3	6	9	12
Water	Green	90.59	80.64	75.85	67.74	59.94
	G-yellow	84.85	72.72	68.29	59.64	50.18
	Yellow	70.86	64.18	51.84	44.64	36.37
100 µM SA	Green	90.59	86.96	80.64	76.21	72.84
	G-yellow	84.85	77.33	71.98	69.96	64.06
	Yellow	70.86	67.59	61.09	59.47	52.57
300 µM SA	Green	90.59	89.93	83.77	79.92	76.83
	G-yellow	84.85	78.37	77.33	72.59	68.46
	Yellow	70.86	69.25	66.71	61.38	57.36
500 µM SA	Green	90.59	91.08	88.35	82.20	80.82
	G-yellow	84.85	80.86	77.33	77.50	70.66
	Yellow	70.86	70.31	69.25	64.94	61.22
LSD at 5%		1.687				

It was decreased rapidly with fruits which immersed in water. Then, it decreased gradually with increasing SA concentrations. However, it clear that G fruit contains the highest of vitamin C content (90.59 mg $100g^{-1}$ FW) compared with GY (84.85) and Y (70.86 mg 100 g^{-1}FW) at harvest time. Also, it clears that the higher concentration of SA prevents degradation of vitamin C content in all fruit color maturities at 500 µM until end of shelf-life period. It was in G: 80.82; GY: 70.66 and Y: 61.22 mg 100 g^{-1}FW.

The various vitamin C contents between different color maturities and SA concentrations during 12 days of shelf-life might be related with the mechanism of SA in fruit tissue. It may preventing destruction of vitamin C content of fruit were immersed in 500 µM than other treatments (Shafiee *et al*., 2010).

Fruit browning-index (BI)

The concerned results of browning index in fruits stored at room temperature that were illustrated in table 4, a significant interaction at $P \leq 0.05$ level between shelf-life duration, fruit color maturities and SA concentrations. It was cleared that a positive variation between treatments, fruit color maturities and shelf-life period. BI incidence was higher with fruit which immersed in water compared with other treatments at end of shelf-life time. Whereas, less browning incidence was presented with fruit that immersed in SA at 500 µM. it was round in moderate index (BI = 3.00), compared with other treatments. Generally, fruit color mature presents different browning incidence. It was round in moderate (BI of G = 3.00) of SA treatments up to strong browning incidence (BI of G = 4.05) of fruit was immersed in water.

Table 4: Fruit browning-index of fruits were harvested at three different maturity stages (G, GY and Y) that immeriesed in different concentrations of SA (100, 300 and 500 µM) and water for 20 min and stored at room temperature (26±2°C and 71±2 RH %) for 12 days.

Treatments	Maturity Stages	Shelf-life time (days)				
		0	3	6	9	12
Water	Green	1.00	1.00	3.14	3.65	4.05
	G-yellow	1.00	1.20	4.30	4.69	5.00
	Yellow	1.00	4.41	4.85	4.80	5.00
100 µM SA	Green	1.00	1.00	2.92	3.19	4.00
	G-yellow	1.00	1.00	2.50	3.96	4.34
	Yellow	1.00	3.49	4.16	4.36	4.91
300 µM SA	Green	1.00	1.00	1.65	2.93	3.10
	G-yellow	1.00	1.00	1.14	3.44	4.03
	Yellow	1.00	2.78	3.82	3.86	4.29
500 µM SA	Green	1.00	1.00	1.04	2.70	3.00
	G-yellow	1.00	1.00	1.01	2.89	3.04
	Yellow	1.00	2.56	3.00	3.22	3.55
LSD at 5%		…	0.244	0.281	0.229	0.191

It might be explained that SA decreased browning incidence in guava fruit by decreasing ethylene production and respiration which prevents fruit

senescence during shelf-life (Hernandez-Munoz *et al*., 2006). Also, the role of SA to prevent destruction of vitamin C in fruit tissue may be decreased browning incidence during shelf-life (Lo'ay, 2009).

Total phenol (mg GAE 100 g-1 FW)

The affected results of total phenols contain changes in fruits stored at room temperature that were illustrated in table 5, a significant interaction at $P \leq 0.05$ level between shelf-life duration, fruit color maturities and SA concentrations. It is clear that the total phenol has more decreases in fruit which were immersed in water with all fruit color maturities. The decreases were in G: 113.67; GY: 111.33 and 86.00) at end of shelf-life time.

Table 5: Total phenol (mg GAE 100 g^{-1} FW) of fruits were harvested at three different color maturities (G, GY and Y) that immeriesed in different concentrations of SA (100, 300 and 500 µM) and water for 20 min and stored at room temperature (26±2°C and 71±2 RH %) for 12 days.

Treatments	Maturity Stages	Shelf-life time (days)				
		0	3	6	9	12
Water	Green	302.67	258.33	211.33	140.00	113.67
	G-yellow	258.00	242.67	213.67	177.00	111.33
	Yellow	214.67	167.67	144.33	117.00	86.00
100 µM SA	Green	302.67	270.67	258.33	250.00	223.67
	G-yellow	258.00	250.33	235.00	223.67	206.33
	Yellow	214.67	195.33	197.33	188.33	180.67
300 µM SA	Green	302.67	278.33	268.33	262.67	246.67
	G-yellow	258.00	252.33	246.67	242.67	232.67
	Yellow	214.67	205.00	203.67	194.67	188.00
500 µM SA	Green	302.67	280.33	275.00	271.33	269.00
	G-yellow	258.00	256.33	251.33	249.00	239.00
	Yellow	214.67	214.00	212.67	204.00	190.00
LSD at 5%		13.381				

Whereas, fruits were immersed in SA at 500 µM present less degradation in total phenol at day 12 of shelf-life. Also, it clear that total phenol of fruits was differed according to fruit maturities. It was higher in G compared with GY and Y fruit maturities at harvest time up to end shelf-life period.

It is clear that the differences between fruit were immersed in various SA concentrations as to total phenol contents during shelf-life. It may be suggested that SA at higher concentration (500 µM) had increased inhibition on PPO activity, but it at lower (100 µM) concentration decreased the PPO activity in vitro. Thus, the inhibition of the PPO activity by SA could be indirect. POD is also related to surface browning of fruits. SA treatment effectively prevented browning and maintained eating quality with higher concentrations of SA (Peng and Jiang, 2006)

Fruit color h^O

Immersed application of SA affected on fruit h^O during shelf-life. It was significant higher with fruit immersed in SA at 500 µM than fruit were

immersed in water (control) as shown in table 6. The higher h^O of fruit were immersed at 500 µM of SA which decreased gradually during shelf-life at 12th days (G: 52.16; GY: 46.44 and Y: 30.67 h^O) than control fruit (G: 31.96; GY: 10.97 and Y: 9.47 h^O).

From the previous result, the higher h^O of fruit it is related to less browning incidence in fruit surfaces (Vicente *et al*., 2003)

Table 6: Fruit color (h^o) of fruits were harvested at three different color maturities (G, GY and Y) that immeriesed in different concentrations of SA (100, 300 and 500 µM) and water for 20 min and stored at room temperature (26±2^{o}C and 71±2 RH %) for 12 days.

Treatments	Maturity Stages	Shelf-life time (days)				
		0	3	6	9	12
Water	Green	79.20	57.77	50.11	41.55	31.96
	G-yellow	62.97	53.64	43.97	28.97	10.97
	Yellow	45.44	39.70	31.92	24.41	9.47
100 µM SA	Green	79.20	65.89	55.37	46.48	41.22
	G-yellow	66.31	58.91	49.97	35.83	21.97
	Yellow	45.44	41.41	37.85	27.72	24.48
300 µM SA	Green	79.20	68.43	60.11	52.40	48.00
	G-yellow	62.97	60.18	51.97	41.31	31.31
	Yellow	45.44	43.29	40.59	33.59	26.20
500 µM SA	Green	79.20	70.13	61.85	57.74	52.16
	G-yellow	62.97	64.42	57.92	50.66	46.44
	Yellow	45.44	44.88	43.59	38.12	30.67
LSD at 5%		6.244				

Conclusion

In general, SA experiment showed the effectiveness of all postharvest treatments on the guava fruit quality during shelf-life. Application of SA at 500 µM treatment improved the physical and chemical characteristics such as fruit firmness through inhibition of ethylene biosynthesis at to ACS and ACO. Chemically, SA treatment maintained vitamin C amount during shelf-life with high level of SA. This reaction decreased the cell wall degradation by decreasing the cell wall hydrolases enzymes during repining fruits and less IL%. So, no breakdown of fruit tissue occurred, therefore, browning incidence becomes less (moderate level; browning index) during the shelf-life. On the other hand, total phenol decreased slightly according to inhibit PPO by SA during ripening that reflects to increase fruit color quality (h^O). Completely, SA treatment can be easily and safe usage to delaying/shifting ripening processes of guava with improving fruit quality during shelf-life.

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تأخير نضج ثمار الجوافة بأستخدام حمض السالسيلك

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أجريت هذه الدراسة على معاملات ما بعد الحصاد خلال موسمى ٢٠٠٧ – ٢٠٠٨ على ثمار الجوافة المعروفة بالصنف البلدى المنزرعة بمزرعة خاصة بمحافظة دمياط بهدف دراسة تأخير نضج ثمار الجوافة خلال عملية التسويق من خلال التقليل من العمليات الفسيولوجية بأنسجة الثمار خلال التسويق او بمعنى أخر خلال عملية النضج. تمت هذه الدراسة بأستخدام حمض السالسيلك نظرا لما هو معروف بتأثيراته فى التحكم بنضج الثمار خلال عملية التسويق. تم تحديد اعمار الثمار من خلال درجة الون حيث طبق مقياس اللون لتحديد ثلاث درجات نمو للثمار مرحلة الخضراء و الاخضر مصفر و الاصفر. تم نقع الثمار فى تركيزات مختلفة من حمض السالسيك ١٠٠ – ٣٠٠ – ٥٠٠ ميكروكول بالاضافة الى النقع فى الماء لمدة ٢٠ دقيقة على درجة حرارة الغرفة، بعد عملية النقع تترك الثمار فى جو الغرفة. اظهرت النتائج المتحصل عليها انة يوجد اختلافات بيولوجية بين الاعمار و قت الحصاد فى جميع القياسات التى اجريت الى ان الواضح لتأثير حمض السالسيك عند التركيز العالى ٥٠٠ ميكرومول انه حافظ على صلابة الثمار دون انهيار سريع و كذلك محتوى الثمار من فيتامين سى كمية الفينولات الكلية و تحسن درجة اللون للثمار كما قللت المعامله ذاتها التبقع البنى للأسطح الثمار و النفاذية الخلايا خلال التخزين على حرارة الغرفة(٢٦±٢ درجة مئوية و رطوبة نسبية ٧١%) لمدة ١٢ يوم. اذا يمكن القول ان حمض السالسيك اخر نضج الثمار خلال هذه الفترة مما يتيح للمستهلك استهلاك الثمار فى فترة اقل من ١٢ يوم و بالتالى سوق يقلل الفاقد فى الثمار خلال مراحل عملية التسويق.

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