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Integrated Control of Postharvest Decay on Blood Orange Fruits by Curing, Hot Water and Sodium Carbonate Applications

[1]Dorria M. Ahmed, Omaima M. Hafez and [2]Mohei EL-Din Solliman

[1]Pomology Department, Agriculture, and Biological Division,
National Research Center, Dokki, Giza, Cairo, Egypt.
[2]Plant Biotechnology Department, Genetic Engineering, and Biotechnology Division,
National Research Center, Dokki, Giza, Cairo, Egypt.

Abstract: The effectiveness of biological control of postharvest decay on the quality of strain A and B of blood orange fruits (*Citrus sinensis*) was studied under cold storage and shelf –life periods. At first, the RAPD and electrophoresis analysis to both unknown strains were reported. It can be concluded that we have two different strains A and B of blood orange fruits used for this investigation. Using treatments of 3 % sodium carbonate (SC) in hot water at 20 and 45°C under curing conditions at 30 and 35°C for 72 hours against physiological and pathological decay incidence were studied. Blood orange fruits were stored after the previous applications at 5°C and 90 % relative humidity (RH) for 42 days and an additional week at 20°C as a simulated marketing period (shelf-life). Fruit quality characteristics (weight loss and decay percentages, soluble solids (SSC), total acidity (TA), vitamin C and anthocyanine contents were evaluated. Treated and control fruits withstand free from pathogenic rots or microbial fruit deterioration up to 42 days of cold storage at 5°C. Sodium carbonate applications at 20°C were more effective in reducing weight loss and physiological decay percent than SC at 45°C. Curing temperatures exposure at 30°C showed higher response of controlling orange fruit deterioration compared with application at 35°C. Untreated fruits had intermediate values between cured and non-cured blood orange fruits. Fruit quality properties as soluble solids content and total acidity appeared a response varied between insignificant or slight effect values due to SC and curing treatments. During cold storage period, ascorbic acid content was decreased significantly in all treated and control fruits due SC, curing application, and storage duration. The anthocyanine concentrations showed the opposite trend, developing higher content in fruits of strain A than that of strain B. In general, the influence of all treatments examined under storage periods tested was more effective in fruits of strain B than those of strain A.

Keywords: Blood orange, strains , sodium carbonate, curing, decay ,export fruit, quality, marketable life, RAPD.

INTRODUCTION

Injuries sustained by citrus fruit during harvest allow the entry of wound pathogens, including green and blue molds. These pathogens occur in almost regions of the world where citrus is grown, and cause serious postharvest losses annually[1,2,3]. Biological control is increasingly becoming an effective alternative to the use of chemicals in plant disease control[4]. Decay pathogens enter fruit through wounds sustained during harvesting and handling. This implies that the pathogen is already in the fruit before treatment is applied. For any antagonist to be beneficial to growers, it has to be able to stop further development of a pathogen that is already in the fruit[5,2,6].

Carbonic acid salts such as sodium carbonate (SC) can be useful tool to manage postharvest decay because they are inexpensive, readily available, and can be used with a minimal risk of injury to the fruit. It is common food additive allowed with no restrictions for many applications by European and North American regulations[7,8]. Sodium carbonate ($NaCO_3$) which have the antimicrobial activity and listed as approved ingredient on products labeled “organic” as proposed by the United States Department of agriculture[7]. Applying sodium carbonate for 150s at 45°C at 3 or 4 % reduced decay more than 90 %. Sodium carbonate was superior to hot water. Temperatures of sodium carbonate solutions influenced effectiveness more than concentration or immersion period[7]. The benefits of hot water treatment for control decay were reported to inhibit spore germination and subsequently growth of *P. digitatum*[5,2].

Corresponding Author: Dorria M. Ahmed, Pomology Department, Agriculture, and Biological Division, National Research Center, Dokki, Giza, Cairo, Egypt.

Curing involves holding the produce at relatively high temperatures (15° to 40°) and high humidity[9]. An alternative approach is to enhance host defense mechanisms at wound site, by holding fruit at high temperatures and humidity (curing) conductive to wound healing and detrimental pathogen development[10,7]. The exact mode of action of curing in reducing decay of citrus fruit is not clear, although heat treatments induce accumulation of antifungal compounds lignin, lignin-like materials, and phenolic compounds, and cause thermal inhibition of the pathogen[7,11]. Maintenance of high humidity level was essential to the development of the defense mechanism. Below 75% RH, the cells surrounding the injury were damaged by desiccation and did not synthesize lignin[9].

Regarding RAPD (random amplified polymorphic DNA) are DNA fragments amplified by the Polymerase Chain Reaction (PCR) using short synthetic primers of random sequence (generally 10 bp). These oligonucleotides serve as both forward and reverse primer and usually are able to amplify fragments from 3-10 genomic sites simultaneously. Amplified fragments (within the 0.5-5 kb range) are separated by gel-electrophoresis and polymorphisms are detected as the presence or absence of bands of particular size.

Welsh and McClell and Williams *et al.,*[12,13] demonstrated that the utility of single short oligonucleotide primers of arbitrary sequence for the amplification of DNA segments distributed randomly through the genome. Welsh and McClell[12] showed that the pattern of amplified bands could be used for fingerprinting[13] and the differences in the pattern of bands amplified from genetically distinct individuals behaved as Mendelian genetic markers.

RAPD is one of its applications, this technique is based on the finding that single DNA primers of arbitrary nucleotide sequence can amplify genomic DNA fragment in PCR. This happens whenever the primers find regions of sufficient homology at a favorable distance and in converging orientation on the two DNA strands[13]. The result is the production of DNA fragments that can be analyzed in gel electrophoresis. RAPDs do not depend on the identification of RFLP probes or on DNA sequence information. Furthermore, it is require no-radioactive, use nanogram of DNA and require merely PCR and agarose gel.

Blood oranges, so called because of a pigment that gives the red flesh color reminiscent of blood, red color is light or dark depending upon variety. It is juicy, sweet and is slightly less acidic with higher economical and nutritional values than regular table oranges and commonly cultivated in the Mediterranean area, especially in Italy and Spain. Recently, a blood orange has been popular for numerous years in Europe and now it has gained in popularity in the US and can be found fresh or in juice[14]. In Egypt, export strategies are planned and great interest to satisfy the needs of international markets demands in fresh products, as dictated by the markets demand requirements and the fruit needs to be in high quality.

The objectives of this study were:

- (i) utilizing the biological integrated materials as effective alternatives to synthetic fungicides and chemicals to control the postharvest decay of blood orange fruits.
- (ii) Determine the response of sodium carbonate immersion and curing temperatures exposure for maintaining orange fruit quality throughout cold storage duration as simulation conditions of organic citrus exportation.

MATERIALS AND METHODS

Fruit: Two unknown strains (A and B) of blood orange fruits (*Citrus sinensis*) were obtained from a private orchard (Dina), Giza Governorate. Fruits were picked from trees grown in sand–loam soil, similar in growth and received common horticulture practices. Mature blood orange fruits, undamaged, free from apparent pathogen infection and were uniformed in shape; weight and color were harvested at the late of January of 2005 and 2006 in the full color stage and transported to the laboratory of Agriculture Development Systems (ADS) project in the Faculty of Agriculture, Cairo University. The initial quality of the two strains after harvest was determined as shown in (Table 1).

Curing and Sodium Carbonate Treatments: The selected fruits from each strain, washed, air dried and placed into plastic baskets, and immersed for 2 min in 20 liter tanks containing sodium carbonate solutions (pH11.3 to11.5; Sigma-Aldrich). The treatments equipment consisted stainless steel tanks, each one individually fitted with an electrical heater, a temperature sensor, and a mechanical agitation system. Sodium carbonate concentration at 3% (w/v) in two hot water temperatures (20 and 45°C), and immersion period for 2 min were tested. The temperature of the solutions did not change more than 0.5°C during treatment. After treatment, the fruit were rinsed with 10 ml of deionized water per fruit. Each treatment was applied to four replicates of 5 fruit per each and the experiment was repeated twice. Treated fruit with sodium carbonate and hot water were placed into plastic cavity trays and cured by two temperatures (30 and 35°C) and 90 % relative humidity for 72 h.

After that, all treated and untreated fruits stored at 5°C + 1 and 90 % RH up to 42 days. The initial fruit quality characteristics of both blood orange strains after treatments were measured (0 storage day).

After 21 days intervals, fruit samples (20 fruits in each treatment) were removed from cold storage and fruit quality measurements were assessed. Shelf-life as a simulation to marketability was examined for decay percent at 20°C up to 7 days.

Quality Assessments:

Weight Loss (WL): Fruits were periodically weighed and the loss in mass weight was recorded for each replicate. Data were calculated as percentage from the initial weight.

Decay Percentage: evaluated by type, by skin appearance, shriveling, chilling injury, and pathogenic rots. In every inspection, decayed fruits were discarded and the number of fruits per replicate was used to express decay percentage. Storage was stopped when decay assessment reached 25 % in stored fruits. After each sample, shelf –life as marketability indicator was determined at 20°C for one week.

Soluble Solids Content (SSC): measured using a T/C hand refractometer Instrument (Model 10430 Brix – readings 0 - 30 ranges Bausch & Lomb Co. Calif., USA) according to[15].

Total Acidity (TA): (expressed as citric acid) and determined by titrating 5 – ml juice with 0.1N sodium hydroxide using phenolphthalein as an indicator[15].

Ascorbic Acid Content (VC): measured using 2-6 dichlorophenol indophenols blue dye, method described by[15].

Total Anthocyanin: One gram from the mixture of fruits skin was grounded with 95% ethyl alcohol and HCl. The mixture then filtered through centered glass funnel (G3) and the extract was transferred to 25 m/volumetric flask and completed to volume with the acidified alcohol. The increase in optical density at 550 mm represents the concentration of total anthocyanin[16].

Randomly Amplified Polymorphic DNA (RAPD) Analysis: DNA isolation was performed using the Cetyl Trimethyl Ammonium Bromide (CTAB) method of[17].

The polymerase chain reaction (PCR) mixture (25 ul) consisted of 0.8 units of Taq DNA polymerase, 25 pmol dNTPs, and 25 pmol of random primer, and 50 mg of genomic DNA. The reaction mixture was placed on a DNA thermal cycler. The PCR program included an initial denaturation step at 94°C for 2 mins followed by 45 cycles with 94°C for 1 min for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 seconds and final extension at 72 °C for 10 minutes were carried out. The amplified DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. Four 10-mer primers (Operon technologies Inc., Alameda, California) randomly selected were used in RAPD analysis. A 100 bp DNA ladder (Promga) was used as a Marker with molecular size of 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp. The amplified pattern was visualized on a UV transilluminator and photographed.

Electrophoresis: The amplification products were analyzed by electrophoresis according to [18] in 2% agarose in TAE buffer (for each liter of 50X TAE Stock solution: 242 g Tris Base, 57.1 mL Glacial Acetic Acid and 100 mL 0.5 MEDTA), stained with 0.2 mg/ml ethidium bromide. Nucleic acids bands were photographed and detected under short wave UV light.

Experimental Design and Statistical Analysis: The design for this experiment was a Completely Randomized Design (CRD) with three replications. Data were analyzed with the Analysis of Variance (ANOVA) procedure of MSTAT-C program. When significant differences were detected, means of treatments were compared by LSD range test at the 5 % level of probability in the two investigated seasons[19].

RESULTS AND DISCUSSIONS

Strain A and B: It is commercially important to identify the two strains of blood orange fruits utilized in this study. Both strains (A and B) received the same sodium carbonate treatments and equally exposed to curing temperatures and cold storage periods. This study provided us with an acceptable quality of the two strains and their efficiency against postharvest decay of orange fruits during cold storage periods.

Fruit quality as physical and chemical characteristics of blood orange fruits of both strains (A and B) at harvest was shown in Table 1. It is clear from such results that fruits of strain B have larger size, shape, weight and peel thickness than that of strain A. On the opposite hand, fruits of strain A have higher chemical properties (SSC, TA, VC and anthocyanin content) than that of strain B fruits. Also, the blood orange fruits of strain A have higher respiration rate than that of strain A.

Table1: Fruit quality characteristics of blood orange strains A and B at harvest.

	Fruit quality characteristics at harvest										
	Physical properties							Chemical properties			
Blood orange strains	Fruit weight gm	Fruit size cm^3	Fruit shape/cm Height	Width	Peel thickness cm	Fruit juice %	Respiration rate ($ml^{-1}/kg^{-1}/h$)	SSC %	Total acidity %	Vitamin C (mg /100g)	Anthoc-yanin (mg/100g)
Strain A	128.8	137	6.7	6.48	0.54	49.23	11.65	10.6	1.1	48.33	0.009
Strain B	172.5	195.33	7.58	6.94	0.63	48.17	8.62	10.4	1.07	45.55	0.006
LSD at 0.05	18.930	18.580	0.278	0.203	0.358	NS	0.405	NS	0.020	1.704	0.006

Data are means of four replicates of 5 fruits each (average of two seasons)

Table 2: Primers used and their annealing temperatures. Annealing Tm 36 °C / Sec

Primer Sequence 5'- 3', K1: TGGCGACCTG, K2: GAGGCGTCGC, K3: CCCTACCGAC, K4: TCGTTCCGC, K5: CACCTTTCCC, K6: GAGGGAGAG and K7: GTTCCGCTCC.

Table 3: Weight loss percentage of strains A and B of blood orange fruits as affected by 3% sodium carbonate treatments for 3 min. and exposed to curing temperatures at 30 and 35°C for 72h after treatments and during storage at 5°C for 42 days

		Weight loss %					
		Strain A			Strain B		
		Storage at 5°C for 42 days					
Sodium Carbonate Treatments	Curing temperatures	0	21	42	0	21	42
Untreated		0.00 d	5.23 c	10.21 c	0.00 d	4.61ba	7.93 c
NA_2CO_3 at 20°C	Curing at 30°C	2.38 c	3.74 e	6.96 e	2.15 c	3.82 d	5.79 e
NA_2CO_3 at 45°C		2.40 c	3.76 e	6.03 f	2.24 c	4.12 cd	5.83 e
NA_2CO_3 at 20°C	Curing at 35°C	3.67 b	4.40 d	7.00 e	3.48 b	4.38 bc	6.14 de
NA_2CO_3 at 45°C		3.47 b	4.50 d	7.52 d	3.45 b	4.68 b	6.33 d
NA_2CO_3 at 20°C	Without curing	5.06 a	7.00 b	11.90 b	3.74 ab	6.40 a	11.00 b
NA_2CO_3 at 45°C		5.27 a	7.50 a	12.70 a	3.98 a	6.90 a	11.90 a
LSD at a 0.05		0.39	0.46	0.35	0.31	0.52	0.39

Data are means of three replicates of 5 fruits each (average of two seasons)

The products of RAPD reaction using short arbitrary primers are expected to exhibit very specific banding pattern to individual strains[20,21]. Since no polymorphism could be detected at intraspecific levels by RAPD arbitrary primers tested, the primer from k1 to k7 showed the complete homology between strain A, strain B and also all the seven primer which precisely differentiates the strain A, strain B and control, so that this primers can be used as a reliable and reproducible method to designate the strain A, strain B and control. The interspecific polymorphism, which was also reproducible, can be attributed to the stringent PCR conditions adopted. Thus, the ability of the two primers can be exploited to identify the strain A, strain B and control as shown in (Figure1). Our results suggest that random primers based RAPD profiles can be useful in the strain A, strain B and control differentiation.

Thus there may be reason to view with caution systematic conclusions based on RAPD analysis alone. On the other hand, the possibility of carrying out compatibility analysis with unlimited numbers of primers, each detecting variation at several regions in the genome, provides an advantage over other techniques. Even if some primers amplify identical regions of the genome or if the technique itself is noisy, it should be possible to build up quickly a consensus from patterns of inter-population variation.

Fruit Quality Characteristics:

Weight Loss: The average weight loss percentage of blood orange fruits was significantly increased gradually with extending storage period of cold storage at 5°C. However, fruits of both strain (A and B) showed similar significant loss, but with lower values in fruits of strain B than that in fruits of strain A during the two successive seasons of investigation as shown in Table 3.

Generally, fruits of both strains treated with sodium carbonate at 20°C for 3 min had lower significant effect on weight loss percent than that of fruits dipped

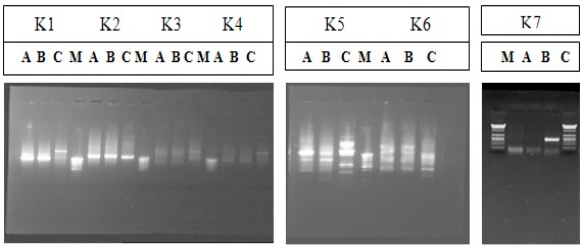

Fig. 1: RAPD patterns in 3 samples of blood oranges strains A and B and control (Baldi cultivar) were obtained with the seven primers. Lanes 1, 2 and 3 are strain A, strain B and control respectively and (M) is DNA marker, Fig (1-A) with K1, K2, K3 and K4 primers. Fig (1-B) with K5 and K6 primers. Fig 1-(C) with K7 primer. Lanes (A) are the samples of strain A, lanes (B) are the samples of strain B and lanes (C) are the sample of control plant. The genetic similarity between the strain A, strain B and control ranged from 0 to 100% using our random primer from K1 to K7. The banding profiles obtained suggested that both K2 and K4 are genetically distinguish between strain A, strain B and control and different with the control plant.

in SC at 45°C for the same period. Meanwhile, curing fruits at 30°C was more effective than that of 35°C in reducing weight loss percent in the two strains of orange, but with less percent in strain B. Moreover, after sodium carbonate treatments and curing temperature exposure, fruits lost weight percent ranged between 2.38 - 5.27 and 2.15 - 3.98 % in strain A and B, respectively as an average of two seasons of our study. In addition, holding fruits in cold storage at 5°C for 42 days caused further increase in weight loss percent with directly proportional to the storage period. At the end of cold storage period, the highest loss percent (12.70%) was obtained in orange fruits of strain A after Na_2CO_3 treatment at 45°C without curing, followed by 11.90% in fruits of strain B received the same application. On the other side, the most pronounced loss (5.79%) was recorded in orange fruits of strain B cured at 30°C after sodium carbonate treatment at 20°C. Untreated fruits (control) showed intermediate values of weight loss percent between cured and non-cured fruits.

In this concern,[7,22] reported that Valencia orange fruits cured at 40 to 55°C and stored at 5°C, showed a significant reduction on weight loss percent. On the other hand, the results obtained by[6,23], revealed higher weight loss values in cured fruits of mandarin than non-cured ones. Moreover, Palou *et al.,*[1] found that temperatures of sodium carbonate solutions influenced effectiveness more than concentration or immersion period. Furthermore, Henroid, *et al.,*[14] reported that Navel orange fruits stored for 55 days at 5°C and 21 day at 21°C lost 3% and 13%, respectively of their initial weight.

Decay Percent: Blood orange fruits previously treated with sodium carbonate and curing temperatures before storage at 5°C and also control fruits withstand free from pathogenic rots or microbial fruit deterioration. It can be cleared that decay injuries noticed in the peel of fruits influenced by postharvest treatments were identify as physiological disorders symptoms. Generally, blood orange fruits of strain A appeared higher decay percent than that of strain B in the two successive seasons of current work as shown in (Table 4). Moreover, orange fruits treated after harvest with sodium carbonate at 45°C, showed higher physiological decay percent than those treated with SC at 20°C at removal from cold storage for 42 as well as plus one week at 20°C.

Orange fruits of strain B treated previously with 3% Na_2CO_3 at 20°C and cured either at 30 or 35°C, maintain in a good quality or even free from peel injuries discoloration after 42 days at 5°C plus 7 days at 20°C as shelf-life. Meanwhile, orange fruits received 3% sodium carbonate treatment at 45°C, had the least peel damage (2.30%) at transfer from cold storage for 42 days. This percent was increased by 3.72 % after one week at 20°C. Control fruits of strain B recorded 13.75% at the end of cold storage and increased to 16.65 % after 7 days at 20°C.

It is evident that, fruits cured at 30°C after previously treated with SC caused significant showed lower decay incidence compared with that cured at 35°C in both strains. Furthermore, non-cured fruits only received 3 % SC at 45°C recoded the highest damage percent (11.16 and 13.89 %) of all SC treatments and curing temperatures applications after 42 days of cold storage at 5°C as well as shelf-life period, respectively.

These results are in harmony with those reported by[9,10] and[2,7]. They found that control green and blue molds of oranges and mandarins were reduced by curing treatments at 30-36°C and 90-98 % RH during postharvest storage at different temperatures. Moreover,[4,1,22] added that biological control is increasingly becoming an effective alternative to the use of chemicals in citrus disease control. Furthermore, the investigators[24,2,6], all agree Sodium carbonate and hot water applied for 150s at 45°C for 3 or 4 % reduced decay more than 90% on mandarin, lemons and orange fruits, respectively. It was concluded that pre-storage hot water dip and curing at high temperatures might be beneficial in preventing chilling injury and decay of 'Valencia' oranges for 6 month of storage at 4°C[6].

Table 4: Physiological decay percent of strains A and B of blood orange fruits as affected by 3% sodium carbonate treatments and curing temperatures at 30 and 35°C for 72h after transfer from cold storage at 5°C for 42 days and plus 7 days at 20°C as shelf-life.

		Physiological decay %			
		Strain A		Strain B	
		Storage at 5°C for 42 days			
Sodium Carbonate Treatments	Curing temperatures	At transfer	Plus 7 d. at 20°C	At transfer	Plus 7 d. at 20°C
Untreated		15.67 a	18.03 a	13.75 a	16.65 a
Na_2CO_3 at 20°C	Curing at 30°C	5.28 g	6.25 g	0.00 e	0.00 f
Na_2CO_3 at 45°C		6.58 e	7.75 e	0.00 e	1.64 e
Na_2CO_3 at 20°C	Curing at 35°C	5.59 f	7.15 f	0.00 e	0.00 f
Na_2CO_3 at 45°C		8.53 c	10.83 c	2.29 d	3.72 d
Na_2CO_3 at 20°C	Without curing	7.78 d	10.00 d	4.93 c	6.25 c
Na_2CO_3 at 45°C		11.16 b	13.89 b	6.67 b	9.53 b
LSD at a 0.05		0.157	0.136	0.111	0.157

Data are means of three replicates of 5 fruits each (average of two seasons)

Table 5: Fruit quality characteristics of strain A of blood orange fruits as affected by 3% sodium carbonate treatments for 3 min. and exposed to curing temperatures at 30 and 35°C for 72h after treatments and during storage at 5°C for 42 days.

		Strain A											
		Fruit quality characteristics											
		Soluble solids %			Total acidity %			Vitamin C (mg/100g)			Anthocyanin (mg/100g)		
Sodium Carbonate Treatments	Curing temper-atures	Storage in days at 5°C for 42 days											
		0	21	42	0	21	42	0	21	42	0	21	42
Untreated		10.40	11.50	11.70	1.20	1.11	1.00	48.50	46.83	44.42	0.019	0.048	0.066
Na_2CO_3 at 20°C	Curing at 30°C	10.50	11.60	11.80	1.13	1.13	1.12	46.90	42.60	41.90	0.007	0.036	0.061
Na_2CO_3 at 45°C		10.40	11.10	11.30	1.20	1.10	1.00	46.83	42.80	39.40	0.009	0.032	0.048
Na_2CO_3 at 20°C	Curing at 35°C	10.30	10.90	11.50	1.13	1.04	0.97	47.60	44.60	41.50	0.021	0.048	0.051
Na_2CO_3 at 45°C		10.00	10.60	11.30	1.10	1.06	1.00	48.90	43.21	39.60	0.007	0.023	0.045
Na_2CO_3 at 20°C	Without curing	10.60	10.70	11.60	1.22	1.16	0.99	46.20	44.60	43.80	0.014	0.017	0.016
Na_2CO_3 at 45°C		10.30	10.50	11.50	1.12	1.13	0.97	45.60	43.00	42.20	0.017	0.026	0.017
LSD at a 0.05		N.S	0.72	N.S	N.S	N.S	0.08	N.S	0.85	0.84	0.002	0.004	0.005

Data are means of three replicates of 5 fruits each (average of two seasons)

Table 6: Fruit quality characteristics of strain B of blood orange fruits as affected by 3% sodium carbonate treatments for 3 min. and exposed to curing temperatures at 30 and 35°C for 72h after treatments and during storage at 5°C for 42 days.

		Strain B											
		Fruit quality characteristics											
		Soluble solids %			Total acidity %			Vitamin C (mg/100g)			Anthocyanin (mg/100g)		
Sodium Carbonate Treatments	Curing temperatures	Storage in days at 5°C for 42 days											
		0	21	42	0	21	42	0	21	42	0	21	42
Untreated		10.40	10.70	11.20	1.22	1.10	1.06	46.14	43.21	40.50	0.012	0.027	0.058
Na_2CO_3 at 20°C	Curing at 30°C	10.50	10.70	11.20	1.13	1.03	0.95	42.90	42.50	40.60	0.002	0.029	0.050
Na_2CO_3 at 45°C		10.40	10.40	10.90	1.20	1.19	1.00	44.13	42.80	41.00	0.003	0.015	0.053
Na_2CO_3 at 20°C	Curing at 35°C	10.40	10.80	11.30	1.04	0.93	0.86	41.90	41.11	40.32	0.002	0.031	0.046
Na_2CO_3 at 45°C		10.50	10.70	11.00	1.10	1.10	0.99	42.30	41.14	40.10	0.002	0.026	0.039
Na_2CO_3 at 20°C	Without curing	10.40	10.80	11.30	1.22	0.96	0.87	44.60	41.50	39.00	0.012	0.017	0.022
Na_2CO_3 at 45°C		10.50	10.70	11.00	1.03	0.96	0.93	42.40	40.50	38.20	0.010	0.014	0.024
LSD at a 0.05		N.S	0.34	N.S	N.S	0.15	0.10	N.S	0.51	1.47	0.002	0.015	N.S

Data are means of three replicates of 5 fruits each (average of two seasons)

Soluble Solids Content: The response of postharvest treatments of sodium carbonate (SC) and curing exposures on soluble solids content (SSC) of blood orange fruits appeared a slight insignificant increase during cold storage period up to 42 days at the two successive seasons of such study as shown in Tables 5 and 6. It can be stated that fruits of the two strains (A and B) showed the same trend of soluble solids content, but with higher SSC values in fruits of strain A than those noticed in fruits of strain B due to SC and curing applications before cold storage duration for 42 days at 5°C.

Moreover, at the end of cold storage, fruits of strain A previously dipped in 3% SC at 45°C had equal values and the least SSC value (11.30%) either cured at 30 or 35°C. On the other hand, the highest soluble solids content (11.80%) was recorded in fruits received 3% SC at 20°C and cured at 30°C for 72 h. It is followed by 11.70% SSC recorded in untreated fruits of strain A. In addition, orange fruits treated with sodium carbonate at 45°C without curing had higher soluble solids content (11.60%) compared with insignificant percent (11.50%) due to the application of SC at 20°C. Moreover, it seemed to have negative and insignificant correlation between curing exposure at 30 or 35°C and soluble solids content of blood orange fruits throughout cold storage.

The present results are inconformity with that reported by[2,8] revealed that soluble solids content of cured 'Clementine' mandarin fruits was higher than non-cured ones. Meanwhile,[23] found non significant effect to curing application in compared to control fruits. In addition[25] reported that no adverse affects on quality attributes as soluble solids content to hot water treatments of blood orange fruits.

Total Acidity: According to Tables 5 and 6, it is clear that blood orange fruits of strain A and B had gradual and slight significant decrease in total acid content due to sodium carbonate and curing temperatures and appeared more decline as storage period progress. It can be stated that, sodium carbonate treatment at 45°C showed higher acid values than that fruits received SC applications at 20°C in any tested storage period and both strains examined. It had the minimum acid values (1.13 and 1.04 %) and (1.13 and 1,13 %) of strain A and B fruits before curing at 30 and 35°C, respectively. Such results were significantly equal to that obtained in untreated fruits neither in fruits of strain A nor fruits of strain B.

At the end of storage period, curing temperature at 30°C caused less and minimum acid content (0.86 and 0.99 %) in fruits of strain B compared with those of strain A which recorded total acid values (0.97 and 1.00 %) after Na_2CO_3 treatments at 20 and 45°C, respectively before curing exposure. In addition, fruits treated with SC and cold stored without curing showed intermediate acid content between their values and untreated fruits, but with more effective in SC at 45°C than that received SC at 20°C. Also, fruits of strain B without curing were more effective in reducing total acid content than those of strain A throughout cold storage duration at the first and second seasons of such investigation.

The results of sodium carbonate (SC) and curing treatments confirmed the previous findings of[26] which noticed that after cold storage at 5°C, treated citrus fruits showed significant reduction in acid content. Moreover, they added that heat treatments had no consistent and adverse effects on titratable acidity and ph values [26]. Overall, hot water and curing applications were affective to induce tolerance of treatments for Clementine mandarins without impairing any other quality attributes[6,8]. At the end of shelf-life, significant lower acidity was observed in cured treated Clementine fruits than non-cured fruits[11].

Ascorbic Acid Content: Data presented in Tables 5 and 6 demonstrated that ascorbic acid content (vitamin C) of blood orange fruits revealed significant and gradual decrease due to postharvest treatments of sodium carbonate and curing temperatures under cold storage periods at 5°C including untreated fruits. In general, fruits of all treatments of SC at 20°C were more effective in keeping ascorbic acid content than those of SC at 45°C application either cured at 30°C or at 35°C of the two strains examined.

After 42 days of cold storage, it can be stated that orange fruits of strain A treated with sodium carbonate and hot water treatments contain the least significant and approximately equal ascorbic acid values (39.40 and 39.6 mg/100FW) after curing at 30°C, respectively. Moreover, untreated fruits (control) had the highest vitamin C content (44.42 mg/100g) followed by (43.80 and 42.20 mg/100g) showed in non-cured fruits which previously received Na_2CO_3 at 20°C, respectively. The same trend was observed in blood orange fruits of strain B during the two successive seasons of current study. However, the least vitamin C values (38.20 and 39.0 mg/100FW) were obtained in non-cured fruits treated previously with sodium carbonate at 45 and 20°C, respectively.

Similarly, ascorbic acid, and overall quality of Clementine mandarin fruits were lower in cured fruit than control and hot water dipped fruits[14]. Moreover, [25] [[23] noticed that ascorbic acid levels decreased in all stored fruits along shelf-life, with slightly lower values in cured treated mandarin fruits than in the control ones. On the other side, negative effects were found in relation to vitamin C retention of curing treated

mandarin fruits by[11]. In addition,[6,3] confirmed the previous results when they found that heat treatments had no consistent effects on ascorbic acid of Valencia oranges and Satsuma mandarin to maintain postharvest quality during storage and marketing.

Total Anthocyanin Content: From data presented in Tables 5 and 6, it can stated that total anthocyanin concentrations in the pulp of blood orange fruits of both strains showed significant and continuous increase in all treatments of sodium carbonate and curing temperatures parallel to the progress of cold storage periods at 5°C. Among the two strains studied (A and B) it is obvious that fruits of strain A kept higher anthocyanin content than those of strain B up to 42 of cold storage during the two successive seasons of investigation.

However, fruits treated with sodium carbonate at 20°C contain further and significant anthocyanin concentrations than those fruits received SC at 45°C either cured after SC application at 30 or 35°C. Control fruits kept at cold storage period for 42 days without Na_2CO_3 or curing applications had the highest content of anthocyanin values (0.66 and 0.058 mg/100g) of fruits of strain A and B, respectively. Meanwhile, the least anthocyanin content (0.019 mg/100g) was obtained in non-cured fruits of strain B treated with SC at 20°C, followed by those received the same treatments of strain A which showed anthocyanin values (0.022 mg/100g FW) at the end of cold storage period

These results are in harmony with those reported by[27,2] found that sodium carbonate application decreased color and anthocyanin content of orange fruits. Meanwhile,[3] added that hot water dipping applied to Satsuma mandarin was an effective pretreatment to maintain postharvest quality during storage and marketing life. On the contrary,[6] reported that heat treatments had no consistent effects on peel color. Moreover,[8] found that mandarin fruit quality was lower in cured fruits than control and hot water dipped ones. In addition, the intermittent curing treatment seems to be a feasible treatment to control blue mold development during shelf-life of Clementine mandarin fruits, without impairing quality parameters such as color[23].

Concluions: From the current study, it can be concluded that using electrophoresis and RAPD techniques proved that we have two different strains A and B of blood orange fruits. Sodium carbonate as organic and inexpensive materials used successfully to manage and control postharvest decay and maintaining quality of blood orange fruits for long term storage duration. Moreover, the combinations of SC, hot water and curing treatments prevented pathological rots and reduced physiological peel injuries of ruts up to 42 days at 5°C. Finally, it can be concluded that the influence of all treatments examined under storage periods tested was more effective in blood orange fruits of strain B than those of strain A.

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REFERENCES

1. Korsten, L., E.S. De Jager, I. Paul, J. Obagwu and A. El-Ghaouth,. 2000. Alternative control of citrus postharvest diseases. Proc. Int. Soc. Citriculture, in press.
2. Palou, L., J.L. Smilanick, J. Usall and I. Vinas., 2001. Control of postharvest blue and green molds of oranges by hot water, sodium carbonate and sodium bicarbonate. Plant Dis,. 85: 371-376.
3. Hong, S.I., H.H. Lee and D. kim., 2007. Effect of hot water treatment on the storage stability of Satsuma mandarin as a postharvest decay control. Postharvest Biology and Technology, 2: 271-279.
4. El-Ghaouth, A., J.L. Smilanick, and C.L. Wilson., 2000. Enhancement of the performance of *Candida satioana* by the addition of glycolchitosan for the control of postharvest decay of apple and citrus fruit. Postharvest Biology and Technology, 19: 103-110.
5. Porat, R., A. Daus, A. Weiss, B. Cohen, L. Fallik and S. Droby, 2000. Reduction of postharvest decay in organic citrus fruit by a short hot water brushing treatment. Postharvest Biology and Technology, 18: 151-157.
6. Erkan, M., M. Pekmezci and C.Y. Wang, 2005a. Hot water and curing treatments reduce chilling injury and maintain postharvest quality of 'Valencia' oranges. International Journal and Food Science and Technology, 1: 91-96.
7. Plaza, P., J. Usall, R. Torresw, N. Lamarca, A. Asensio and I. Vinas, 2003. Control of green and blue mould by curing on oranges during ambient and cold storage. Postharvest Biology and Technology, 28: 195-198.
8. Erkan, M., M. Pekmezci, I. Karasahin and H. Uslu, 2005b. Reducing chilling injury and decay in stored 'Clementine' mandarins with hot water and

curing treatments. European Journal of horticultural Science, 4: 183-188.
9. Ben-Yehoshua, S., E. Barak and B. Shapiro, 1987. Postharvest curing at high temperatures reduces decay of individually sealed lemons, pomelos, and other citrus fruit. J. Amer. Soc. Hort. Sci., 112(4): 658-663.
10. Stange, R.R. and J.W. Eckert, 1994. Influence of postharvest handling and surfactants on control of green mold of lemons by curing. Phytopathology, 84: 612-616.
11. Perez, A.G., P. Luaces, J. Oliva, J.J. Rios and C. Sanz, 2005a. Changes in vitamin C and flavor components of mandarin juice due to curing of fruits. Food Chem., 1: 19-24.
12. Welsh J, and M. McClell, 1990. Fingerprinting genome-using PCR with arbitrary primers. Nucleic Acid Res., 18: 7213–7218.
13. Williams G, A Kubelik, K Livak, J. Rafalski and S. Tingey, 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res., 18: 6532-6535.
14. Henriod, R.E., 2006. postharvest characteristics of novel oranges following high humidity and low temperature storage and transport. Postharvest Biology and Technology, 1: 57-64.
15. A.O.A.C., 1990. Official methods of analysis.Association of Official Analytical Chemists., Washington, DC. U.S.A.
16. Hsia, G.L., B.S. Luh and C.O. Chichester, 1965. Anthocyanin in free stone peaches. J. Food Sci., 30: 5-12.
17. Doyle, J.J. and. L. Doyle, 1990. Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.
18. Maniatis T., E. Fritsch and L. Sambrook, 1989. Molecular cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
19. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th edition lowa State Univ. press Ames. Low USA.
20. Lee, G.P., C.H. Lee and CS. Kim, 2004. Molecular markers derived from RAPD, SCAR, and the conserved 18 SrDNA sequences for classification and identification in *Pyrus pyrifolia* and *P. communis*. Theor Appl Genet, 108: 1487-1491.
21. Ordon, F., A. Schiemann and W. Friedt, 1997. Assessment of the genetic relatedness of barley accessions (*Hordeum vulgare s.I.*) resistant to soil borne mosaic-inducing viruses (BaMMV, BaYMV, BaYMV-2) using RAPDs. Theor. Appl. Genet., 94: 325-330.
22. Nunes, C., J. Usall, T. Manso, R. Terres, M. Olmo and J.M. Garcia, 2007. Effect of high temperature treatments on growth of penicillium spp. and their development on 'Valencia' oranges. Food Sci. Tech., 1: 63-68.
23. Perez, A.G., P. Luaces, M. Olmo, C. Sanz and J.M. Garcia, 2005. Effect of intermittent curing on mandarin quality. J. Food Sci., 1: 64-68.
24. Smilanick, J.L., D.A. Margosan, F. Milkota, j. Usall and I.F. Michale, 1999. Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficiency. Plant Dis., 83: 139-145.
25. Saidani, M. and B. Marzouk, 2003. Biochemical characterization of blood orange, sweet orange, lemon, bergamot and bitter orange. Phtochemistry, 62: 1283-1289.
26. Obagwu, J. and L. korsten, 2003. Integrated control of citrus green and blue molds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water. Postharvest Biology and Technology, 28: 187-194.
27. Emanuele, M., A. Maccarrone and P. Rapisarda, 1985. Stabilization of anthocyanins of blood orange fruit juice. J. Food Sc., 50: 901-904.