International Journal of Agricultural Technology 2014 Vol. 10(5):1273-1288
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Citrus Fruit Decay and Its Control

El-Sayed, M. E.[1*] and Hagag, L. F. [2]

[1]Plant Pathology Department, National Research Centre, Cairo, Egypt, [2]Pomology Department, National Research Centre, Cairo, Egypt.

El-Sayed, M. E. and Hagag, L. F. (2014). Citrus fruit decay and its control. International Journal of Agricultural Technology 10(5):1273-1288.

Abstract Four fungal species belonging three genera were isolated and identified from collected citrus rotted fruit samples which collected from two localities i.e. Beheira and Qalyoubia Governorates in Egypt. These are *Alternaria citri, Botryodiblodia theobromae, Penicillium digitatum and Penicillium italicum.* Hot water treatments at 45, 50, and 55^{o}C for 5 minutes were found to reduce significantly the percentage of spore viability % with all tested fungi compared with untreated spores (control). The most effect was recorded with 55 ^{o}C followed by 50 ^{o}C, while 45 ^{o}C was less effective. All hot water treatments were found to be protected the inoculated fruits and increased significantly the shelf life of Navel orange fruits for 30 days comparing with non-treated control. Hot water treatments were found to be decreased significantly the fruit rot percent (disease incidence %) as well as disease severity (%) in inoculated fruits. No significant was recorded in between hot water treatments. All the three tested temperatures i.e. 45, 50, and 55 ^{o}C were found to be reduced significantly all changes in the physical and chemical composition of navel orange citrus fruits. Hot water at 55 ^{o}C was the best than others.

Keyword: Citrus Fruit Quality, Fungi, Hot water

Introduction

Citrus is an important fruit crop grown commercially in more than 135 countries in different agro-climatic conditions for its diversified use and increasing demand world over with about 102.64 million tones total world production and probably stands first largest among the fruit crop. The fruits are rich in vitamin C (ascorbic acid), various fruit acids (especially citric acid), and fruit sugar (Milind, 2008). Post-harvest diseases account to about 50% losses in fruits stored in poor storage conditions especially under high humidity. The most important fungi causing post-harvest diseases include: *Penicillium* spp, *Aspergillus* spp, *Alternaria* spp, *Botrytis cinerea*, *Monilinia lax* and *Rhizopus stolonifer* (Agrios, 2005). Green and blue moulds, due to the pathogenic action of *Penicillium digitatum* and *Penicillium italicum* respectively are the main

[*] **Corresponding author:** El-Sayed, M. E.**; E-mail:** embaby.elsayed@yahoo.com

cause of orange losses during postharvest. Under Mediterranean climate conditions, both together are responsible for 80% of total postharvest citrus fruit decay (Nunes *et al.,* 2010 and Munoz *et al.*, 2011).

Alternaria rot of citrus is a serious problem in citrus production worldwide. Alternaria rot occurs primarily as a stem-end rot on fruit held in cold storage. The disease can significantly reduce yield, and annual fruit losses have been estimated at 0.5 box per tree. In terms of fruit quality, this disease can be a serious problem for the fresh fruit market as well as for the processing industry (Barry Pryor *et al.*, 2003). Alternaria is found on citrus fruits as SER (stem-end rot) and ICR (internal core rot) or black rot. *Alternaria citri* Ellis & Pierce or *A. alternata* Fr. (Keissler) cause these rots. Alternaria can be recovered from sound fruits (stylar and stem-end)– hence it is also called core rot (Milind, 2008 and Shideh and Naser, 2012). Also, the commonly known Diplodia stem-end rot is caused by *Botryodiplodia theobromae* (*Physalospora rhodina* Berk & Curt.) Cooke (Milind, 2008).

Hot-Water Treatment: Hot water treatment (45 ℃) for 150 s, in addition to controlling rot, to a great level improves the fruit cortex (Larrigaudiere *et al*., 2002). Fruits were passed through hot dips for a few minutes at 49 oC to kill mold spores on citrus fruit. Practical systems have used either vapor heat or hot water. Fruits are dipped in water at 50-55oC for 15 min before storage for control of fungus. (Irtwange, 2006). Short-term heating, where the fruit or vegetable is dipped in hot water at temperatures above 40 ℃ (generally 44-55 ℃) for a short time (from a few minutes to 1 h), has been the main heat treatment studied over the years. Fruits and vegetables commonly tolerate such temperatures for 5-10 min, and that even shorter exposure to these temperatures is sufficient to control many of the post-harvest pathogens. Pre–storage hot water dips of fruit at temperatures above 40 ℃ have been shown to be effective in controlling storage decay, not only by reducing the pathogen but also by enhancing the resistance of fruit tissue, influencing host metabolism and ripening.

Aim of this study: Interestingly, this work centered on the fungal pathogens associated with citrus fruits affecting fruit production and fruit quality (causing citrus fruit decay). Also, the aim of this study was to assess the effect of hot water treatments on some of the quality characteristics and control of core rot, blue and green mold decay orange.

Materials and methods

Source of pathogens

Alternaria citri, Botrydiplodia theobromae, Penicillium digitatum and Penicillium italicum were isolated from rotten citrus fruits and then were grown on potato dextrose agar (PDA) at 22±2^{o}C. for 10 days Embaby *et al.* (2013).

Effects of heat treatments on germination (viability) in vitro

Sterile glass tubes containing 1.8 ml distilled water were placed in water baths at 45, 50, and 55 ℃, and allowed to equilibrate for 5 min. afterwards; 0.2 ml of a concentrated spore suspension was added to the tubes, to achieve a final concentration of 2 x 105 spore's ml_1. After 5min., tubes were removed from the water baths and placed immediately on ice. Aliquots (50 ml) of the spore suspensions were transferred to Potato Dextrose Agar (PDA) kept in Petri dishes padded with treated spore suspension, and incubated for 48 or 72 h at 25 ℃ in darkness. The numbers of colony-forming were counted after 3 days of incubation at 25 ℃. and the number of viable spores was calculated for each treatment (Porat *et al.*, 2000 and Plaza *et al.*, 2004). Treatments included: hot water (45, 50 and 55 ℃) for 5 min, and control treatment (Fatemi and Borji, 2011).

Effects of hot water in vivo

"Washington" Navel orange fruits were wounded with a dissecting needle (1–2 mm deep) at three sites around the stem end. The wounded sites were inoculated with 20 ml of spore suspension of tested fungi each (10^5 spores ml1), and the fruits were kept for incubation in plastic trays, at 24 ℃ under humid conditions. After 24 h, the fruit were rinsed in hot water at 45, 50 and 55 ℃ for 5min., and kept for incubation under the same conditions. The percentage of infected wounds was determined 7, 15, 21 and 30 days after inoculation. Twenty fruits were used for each treatment (Porat *et al*., 2000). Treatments included: hot water (45, 50 and 55 ℃) for 5 min and control (untreatment) (Fatemi and Borji, 2011). Following drying, (lapse of 12 h), each treatment was separately packed in plastic bags and along with two control. At the end of the experiment, appraised parameters including fruit rot percentage, percentage of total soluble solid (TSS); vitamin C percentage and total acidity (TA), pH and decreased weight loss of fruits were evaluated. Fruit rot percentage was determined through separating polluted treatments from healthy fruits and counting them. To determine fruit weight loss decrease, healthy fruits remained of each replication, were counted and weighed with a scale of 1/100 precision. Then, the secondary weight that remained of healthy fruits was deducted from their primary weight and obtained figures were added up and averaged. pH was determined with hand-digital pH-meter and to measure percent percentage of total soluble solid (TSS), the hand refractometer was used and results were expressed in Breaks degree. To determine total acidity (TA) according to (A.O.A.C 2005) methods, amount of 10 ml fruit extract was titrated with 0.3 N sodium hydroxide (NaOH) in the presence of Phenolphthalein and expressed as a percent of citric acid and vitamin C level was assessed with titration according to (AOAC, 2005) methods. The disease incidence was calculated as percentage of infected navel orange fruit in relation to the total number of fruits in each replicate according to the following formula:

Infection (%) = Number of rotted fruits /Total number of tested fruits x 100

Severity of infection was determined according to Townsend and Heuberger (1943) and Embaby *et al*. (2013).

Decay of some citrus fruit quality caused by fungi (fruit Quality attributes)

Physical Attributes (Physical Parameters): These attributes of quality are measured by applying principles of physics and measuring the response of fruit Embaby *et al.* (2013).

Fruit Weight: Fruit weight is a basic parameter of quality and has to be measured precisely on digital balance/weighing machine to calculate loss in weight of fruit (Milind, 2008 and Rab *et al.,* 2012). The percentage of weight loss was calculated using the following formula:

$$\text{Percent weight loss} = \frac{\text{Weight of fresh fruit} - \text{Weight after interval}}{\text{Weight of fresh fruit}} \times 100$$

Juice Percentage: Juice percentage of citrus fruit is generally expressed on the basis of weight. Citrus fruits are valued for their juice content. Weight of juice is taken. Percentage of juice is calculated as the weight of juice divided by the weight of fruit multiplied by 100. Juice is squeezed and measured in milliliters or cubic centimeters and percentage is calculated as the volume of juice divided by the volume of the fruit multiplied by 100 (Milind, 2008).

Chemical Attributes (Chemical Parameters): These attributes of quality are measured by applying certain principles of chemistry and based on response of fruit internal parts/composition to chemical reactions. These quality attributes for citrus include total titratable acidity, ascorbic acid and pH (Milind, 2008).

Total (Titratable) Acidity: Phenolphthalein is generally used as a visual endpoint indicator; it gives a pink-colored endpoint. Total acidity can be determined by using 0.1 NaOH (or 0.3125 N NaOH) for a large number of samples Embaby *et al.* (2013).

Total Soluble Solids: Total soluble solids (TSS) of orange juice constitute mainly sugars (80–85 percent). Juice, a specific gravity of 1.04 is used, assuming the average soluble solids to be 10_Brix (Milind, 2008).

TSS-to-Acid Ratio: The relative sweetness or sourness of citrus fruit is determined by its ratio of sugars to acids. It is calculated by dividing TSS (Brix) with titratable acidity. The soluble solids concentration (SSC) with a hand refractometer and the titratable acidity with an automatic titrator that measured the volume of 0.1 N NaOH required by 10 ml of juice to reach pH 8.0 were also measured. Data were expressed as °Brix and percentage of citric acid, respectively. Maturity index (MI) was evaluated by the ratio: °Brix / % Citric acid for each extracted juice (Porat *et al.,* 2000, Milind, 2008 and Nunes *et al.*, 2010).

Ascorbic Acid: Ascorbic acid can be estimated using 2.6-dichlorophenol indophenol dye that is reduced by ascorbic acid. Ascorbic acid is calculated as well as Vitamin C content was measured as mentioned in AOAC (2005).
All obtained data was analyzed using SPSS software and means were compared using Duncan Fatemi and Borji (20011).

Results and discussions

Three fungal genera belonging four species were isolated and identified from collected citrus rotted fruit samples which collected from two localities i.e. Beheira and Qalyoubia Governorates in Egypt. These are *Alternaria citri, Botryodiblodia theobromae, Penicillium digitatum and Penicillium italicum* Fig (1a& b).

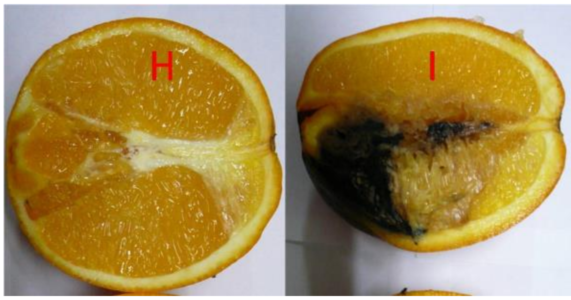

Fig. 1.a. Internal symptom of black or core rot

Fig. 1.b. External symptoms of blue and green mould comparing with healthy one. H= Healthy I= Infected

In vitro test

Effects of hot water treatments on spore Viability of fungal pathogens *in vitro* presented that, hot water treatments at 45, 50, and 55^{o}C for 5 minutes were found to significantly reduce the percentage of total fungal count (Viability %) with all tested fungi compared with untreated (control) (Table 1.a&b). The most temperature effect was recorded with 55^{o}C than other treatments, followed by 50 ^{o}C, while 45 ^{o}C was less effective. The average mean of spore count (Viability %) was decreased from 61.33% with un-treated control to 25.33% when treated spore suspension of each fungus with hot water at 45 ^{o}C for 5 min. then decreased more with 50 and 55 ^{o}C. to 17.67 and 8.67%, respectively. On the other hand, *A. citri* was higher fungal decreasing level and affected which record 18.75% followed by *P. italicum* 26.25%, while *P. digitatum* was less decreasing level and affected 39.75 %. Hot water treatments showed a significant difference in between deferent temperature as will as between all the tested fungi.

On the other hand, hot water treatment were found to reduce significantly the percentage of *A. citri* spore Viability % from 70 spores (equal 100 Viability %) to 20 (equal 28.57Viability %), 10 (equal 14.29 Viability %) and 5(equal 7.14 Viability %) when treated *A. citri* spore suspension with 45, 50 and 55 ^{o}C for 5min, respectively. Significantly decreased the percentage of *P. digitatum* Viability % from 79 spores (equal 100 Viability %) to 40 (equal 50.63 Viability %), 30 (equal 37.97 Viability %) and 10 (equal 12.66 Viability %) when treated *P. digitatum* spore suspension with the same treatments at the same period time while; hot water treatment were found to reduce significantly the percentage of *P. italicum* spore Viability% from 35 spores (equal 100 Viability %) to 16 (45.71Viability %), 13 (equal 37.14 %) and 11 (equal 31.42 Viability %) when treated with the same treatments at the same period time respectively. The same results were recorded by (Porat *et al.*, 2000; Auret, 2001; Fallik, 2004; Lemessa *et al.*, 2004; Irtwange, 2006 and Zamani *et al.*, 2009).

Lemessa *et al.* (2004) and Irtwange, (2006) reported that, Fruits were passed through hot dips for a few minutes at 49 ^{o}C to kill mold spores on citrus fruit. Also, Auret (2001); Zamani *et al.* (2009) reported that, pre–storage hot water dips of fruit at temperatures above 40 ℃ have been shown to be effective in controlling storage decay, not only by reducing the pathogen but also by enhancing the resistance of fruit tissue, influencing host metabolism and ripening. Postharvest dips are applied for a few minutes at high temperatures, because fungal spores and latent infections of the pathogen are either on the surface or in the first few cell layers under the peel of the fruit. (Porat *et al*, 2000; Fallik, 2004 and Irtwange, 2006) found that, pre-storage dipping of ' Fortune ' mandarins in water at 50, 52 or 54 ^{o}C for 3 min reduced decay and simulated shelf-life at 20 ^{o}C without causing adverse effect to the rind surface. Physiological behavior and the internal quality attributes between the untreated and those treated at 50-54 ^{o}C were minimal. Also, Irtwange (2006) found that, fruits are dipped in water at 50-55 ^{o}C for 15 min before storage for control of fungus.

Table 1. Effect of hot water treatment on spore Viability of fungal pathogens *in vitro*

Temperatures [^{o}C]	*A. citri*		*P. digitatum*		*P. italicum*		Mean
	T.C	%V	T.C	%V	T.C	%V	
45	20 cd	28.57	40 b	50.63	16 de	45.71	25.33 B
50	10de	14.29	30 bc	37.97	13 de	37.14	17.67 C
55	5 e	7.14	10 de	12.66	11 b	31.42	8.67 D
Control	70 a	100.00	79 a	100.00	35 b	100.00	61.33 A
Mean	18.75 C		39.75 A		26.25 B		

T.c = Total count, %V = Percentage of spore Viability, L.S.D Temperatures = 5.034, L.S.D Fungi = 5.813, L.S.D T$_*$ F = 10.07

In vivo test

Effect of hot water on disease incidence (infection %): Effect of various treatments on fruit rots percentage (disease incidence %) in both inoculated and un-inoculated cases showed a significant difference. The highest fruit rot percent (disease incidence %) was that of the inoculated control fruits (100% after 30 days) and the least rot level was relevant to fruits treated with hot water. Also, in inoculated fruits, 55 ℃ hot water treatment performed better compared with other hot water treatments, while 45 ℃ hot water treatment was less effective than others (Table 2).

On the other hand, all hot water treatments were found to be protected the inoculated fruits and increased significantly the shelf life of Navel orange fruits for 30 days comparing with non-treated control. Data in this table show that, hot water treatments were found to be decreased significantly the fruit rot percent (disease incidence %) from 100% average72.22, 54.63 and 22.22 % in inoculation fruits treated with hot water at 45, 50 and 55 ℃ for 5 minutes respectively. No significant were recorded in between hot water treatments and different fungal tested which gave 55.56, 59.03 and 59.72 % of disease incidence (rot infection) with either molds i.e. *A. citri, P. digitatum and P. italicum*, respectively. Similar results were obtained by (Porat *et al.*, 2000; Auret, 2001; Fallik, 2004; Lemessa *et al.*, 2004, Irtwange, 2006 and Zamani, *et al.*, 2009).

Nafussi *et al*. (2001) found that, hot water dip for 2 min at 52–53 ℃ inhibited the development of decay in lemons inoculated with *P. digitatum*. Green mould incidence caused by *P. digitatum* was reduced by hot water (HW) from 97.9 and 98% on untreated Eureka lemons (*Citrus limon*) and Valencia oranges (*Citrus sinensis*) to 14.5 and 9.4%, respectively. While, short-term heating, where the fruit or vegetable is dipped in hot water at temperatures above 40 ℃ (generally 44-55 ℃) for a short time (from a few minutes to 1 h). Fruits and vegetables commonly tolerate such temperatures for 5-10 min, and that even shorter exposure to these temperatures is sufficient to control many of the post-harvest pathogens. Also, Auret (2001) and Zamani *et al*. (2009) found that, Pre–storage hot water dips of fruit at temperatures above 40 ℃ have been shown to be effective in controlling storage decay, not only by reducing the pathogen but also by enhancing the resistance of fruit tissue, influencing host metabolism and ripening.

Table 2. Reducing disease incidence (%) of "Washington" Navel orange fruits caused by tested moulds using hot water *in vivo*

Fungi	Temp. [℃]	Disease incidence (%) 7	15	21	30	Mean
A. citri	45	44.44 cdef	55.56 bcde	77.78 abc	88.89 ab	55.56 A
	50	33.33 defg	44.44 cdef	55.56 bcde	66.67 abcd	
	55	00.00 g	11.11 fg	33.33 defg	44.44 cdef	
P. digitatum	45	44.44 cdef	66.67 abcd	88.89 ab	100.00 a	59.03 A
	50	22.22 efg	44.44 cdef	66.67 abcd	77.78 abc	
	55	11.11 fg	22.22 efg	22.22 efg	22.22 efg	
P. italicum	45	44.44 cdef	66.67 abcd	88.89 ab	100.00 a	59.72 A
	50	22.22 efg	55.56 bcde	77.78 abc	88.89 ab	
	55	00.00 g	11.11 fg	22.22 efg	44.44 cdef	
Control		66.67 abcd	77.78 abc	88.89 ab	100.00 a	
Mean		35.19 D	50.93 C	67.59 B	78.70 A	
Mean	45	72.22 A				
	50	54.63 B				
	55	22.22 C				

L.S.D Fungi = 7.250, L.S.D Temperature degree = 8.372, L.S.D Days = 8.372

Effect of hot water on disease severity (%): Effect of various treatments on fruit rot disease severity (%) in both inoculated and un-inoculated cases showed a significant difference. In inoculated fruits, the highest fruit rot severity (%) was that of the un-treated control fruits (71.11 % after 30 days) and the least rot level was relevant to fruits treated with hot water. Also, in inoculation fruits, 55 ℃ hot water treatment performed better compared with other hot water treatments, while 45 ℃ hot water treatment was less effective than others (Table 3). On the other hand, all hot water treatments were found to be protected the inoculation fruits and increased significantly the shelf life of Navel orange fruits for 30 days comparing with non-treated control. Data in this table show that, hot water treatments were found to be decreased significantly the fruit rot severity (%) average 32.22, 23.52 and 7.78 % in inoculated fruits treated at 45, 50 and 55 ℃ for 5 minutes respectively. No significant were recorded in between and *A. citri* and *P. digitatum* treated by hot water treatments which gave 24.17 and 25.28 exhibited *P. italicum* which record 29.86 % of disease incidence (rot infection) respectively. These results were agreement with (Porat *et al.*, 2000; Auret, 2001; Fallik, 2004; Lemessa *et al.*, 2004; Irtwange, 2006 and Zamani *et al.*, 2009).

Physical treatment with hot water can eradicate quiescent infection of Diplodia. Hot water (53 ℃ for 5 min) controlled green mold and SER by *Phomopsis* and *Diplodia natalensis* in oranges. The least success was reported with grapefruit, especially if degreened. Hot-water dips (52 _ 1 ℃ for 5 min) in Hamlin sweet oranges. Hot-water treatment (40 ℃ water for a 5-min dip) has been reported to increase levels of phytoalexin (scoparone) in the peel, thus controlling P. digitatum.

Table 3. Reducing disease severity (%) of "Washington" Navel orange fruits caused by tested moulds using hot water

Fungi	Temp.	Disease incidence (%				Mean
	[℃]	7	15	21	30	
	45	15.55 def	20.00 cdef	24.45 cde	53.34 ab	
A. citri	50	13.33 def	15.55 def	22.22 cdef	26.67 cd	24.17 B
	55	00.00 f	2.22 ef	11.11 def	20.00 cdef	
	45	15.55 def	20.00 def	24.45 cde	53.34 ab	
P. digitatum	50	8.87 def	15.55 def	20.00 cdef	40.00 bc	25.28 B
	55	2.22 ef	8.87 def	11.11 def	15.56 def	
	45	15.55 def	22.22 ef	53.34 ab	68.89 a	
P. italicum	50	8.87 def	20.00 cdef	37.78 bc	53.34 ab	29.86 A
	55	00.00 f	4.44 def	8.87 def	15.55 def	
Control		20.00 cdef	24.45 cde	53.34 ab	71.11 a	
Mean		11.67 D	16.85 C	31.11 B	46.11 A	
	45	32.22 A				
Mean	50	23.52 B				
	55	7.78 C				

L.S.D Fungi = 4.454, L.S.D Temperature degree = 5.143, L.S.D Days = 5.143, L.S.D F*T*D = 17.82

Effect of hot water on some physical characteristics of "Washington" Navel orange fruits

Effect on weight loss %: Effect of treatments on fruit weight loss % in treated and non- treated cases, showed a significant difference (Table 4). The highest weight loss was recorded with non- treated fruits (control 2.96%). The least weight loss was recorded with fruit samples treated for 5 min with hot water 55 °C. (0.42 %) followed by 50 °C. (1.44%). Also data show that, significantly decreasing weight loss % in Navel orange fruits with increasing the temperature of water used from 45, 50 and 55 °C which recorded 2.28, 1.44 and 0.42%, respectively. As a whole, no significant difference was seen between the three tested fungi *A. citri* 1.74%, *P. digitatum* 1.70 % *and P. italicum*1.88 %. The same results were reported by (Porat *et al.,* 2000a; Auret, 2001; Fallik, 2004, Lemessa, *et al.,* 2004; Irtwange, 2006 and Zamani *et al.*, 2009).

Larrigaudiere *et al*. (2002) found that, hot water treatment (45 °C) for 150 s, in addition to controlling rot to a great level improves the fruit cortex. Also, immersing fruits in hot water for 3 min in a temperature of 52 °C significantly decreased green mold and fruit loss and also preserved fruit firmness and ascorbic acid content. Auret (2001); Zamani *et al*. (2009) reported that, pre–storage hot water dips of fruit at temperatures above 40 °C have been shown to be effective in controlling storage decay, not only by reducing the pathogen but also by enhancing the resistance of fruit tissue, influencing host metabolism and ripening.

Table 4. Effect of hot water on weight loss (%)

Temperature degree [°C]	*A. Citri*	*P. digitatum*	*P. italicum*	Mean
45	2.39 b	2.05 b	2.39 b	2.28 A
50	1.53 c	1.23 c	1.56 c	1.44 B
55	0.07 e	0.57 d	0.63 d	0.42 C
Control	2.96 a	2.96 a	2.96 a	
Mean	1.74 A	1.70 A	1.88 A	

L.S.D Fungi = 0.1912, L.S.D Temperature degree = 0.2208, L.S.D F*T = 0.3824

Effect of hot water treatments on Juice percentage (%): Data were recorded in Table (5).The highest fruit Juice percent (av. 82.03 %) was that fruit treated with 55 °C. hot water for 5min followed by 77.63 and 70.51% when treated fruits with hot water at 50 and 45 °C. for 5min respectively. The least level of Juice percent was relevant to non- treated fruits (control 59.82%). As a whole, no significant difference was seen between *A. Citri* 75.29% and *P. digitatum* 72.40% also, between *P. digitatum*72.40% and *P. italicum* 69.80%.

The same results were obtained by (Porat *et al.*, 2000; Auret, 2001; Fallik, 2004; Lemessa *et al.*, 2004; Irtwange, 2006 and Zamani *et al.*, 2009).

Tow min dip in water at 53 ℃ or 56 ℃ markedly reduced decay caused by green and blue molds in kumquats (Nagami cultivar). Higher temperatures (59 ℃ or 61 ℃) increased decay. Drenching the fruits for 30 sec with water at 53 ℃ or 56 ℃ gave similar results to dipping for 2 min and is recommended as the preferred commercial treatment because of the enhanced quality of uniform, continuous heat application. Hot-water treatment (50–55 ℃ for 2–3 min) also results in the redistribution of the epicuticular wax layer and a reduction of cuticular micro lesions (cracks), thus improving physical barriers to pathogen penetration. Also, (Porat *et al*., 2000; Fallik, 2004; Irtwange, 2006) found that, pre-storage dipping of ' Fortune ' mandarins in water at 50, 52 or 54oC for 3 min reduced decay both during cold storage at 6oC and simulated shelf-life at 20oC without causing adverse effect to the rind surface. Physiological behavior and the internal quality attributes between the untreated and those treated at 50-54oC were minimal. Those dipped at 58oC however, developed off-flavor, which was probably due to the increased ethanol level. (Smilanick *et al.,* 2008) reported that, pre-storage application of hot water treatment for a short time (about a few minutes), is only effective on pathogenic agents found on external layers of fruit skin. The results showed that floating of fruits in hot water 55 ℃ for 2 to 3 min caused the control of green mould and improved the post harvest quality in tangerine. Also Inkha *et al*. (2009) reported that, Immersion of fruits in 55 ℃ hot water for 2 and 3 min and 50 ℃ for 3 min had the best effect in controlling and delaying activity of the tangerine green mold activity.

Table 5. Effect of hot water treatments on Juice percentage (%)

Temperature degree [℃]	*A. Citri*	*P. digitatum*	*P. italicum*	Mean
45	74.64 bc	67.57 d	69.32 cd	70.51 C
50	82.87 a	77.72 ab	73.29 bcd	77.63 B
55	83.82 a	84.5 a	77.77 ab	82.03 A
Control	59.82 e	59.82 e	59.82 e	
Mean	75.29 A	72.40 AB	69.80 B	

L.S.D Fungi = 3.228, L.S.D Temperature degree = 3.727

Effect of hot water treatments on Fiber (g) of citrus: Effects of hot water treatments on Fiber (g) were recorded in Table (6). Data show that all hot water treatments were found to be increased significantly fruit fiber content (g) comparing with non-treated control. Also, significant difference was seen between different temperatures. The highest fruit fiber content(g) (av. 98.73 %) was that fruit treated with 55 ℃. hot water for 5min followed by 90.54 and 84.14 % when treated fruits with hot water at 50 and 45 ℃. for 5min respectively. The least level of Juice percent was relevant to non- treated fruits (control 61.03 %). As a whole, no significant difference was seen between *A. Citri* 80.69 % and *P. digitatum* 83.43 %. Similar results were recorded by (Porat *et al.,* 2000; Auret, 2001; Fallik, 2004; Lemessa *et al.,* 2004; Irtwange, 2006 and Zamani *et al.,* 2009).

Table 6. Effects of hot water treatments on Fiber (gm)

Temperature degree[℃]	*A. Citri*	*P. digitatum*	*P. italicum*	Mean
45	77.85 e	82.57 de	92.00 bc	84.14 C
50	87.77 cd	89.62 bc	94.24 abc	90.54 B
55	96.1 ab	100.51 a	99.58 a	98.73 A
Control	61.03 f	61.03 f	61.03 f	
Mean	80.69 B	83.43 B	86.71 A	

L.S.D Fungi = 3.252, L.S.D Temperature degree = 3.755, L.S.D F*T = 6.504

VI-Effect of hot water treatments on some chemical characteristics: Effects of hot water treatments on TSS (%) character of "Washington" Navel orange fruits were tabulated in Table (7). Data show that all hot water treatments were found to be increased significantly TSS (%) of fruit content comparing with non-treated control. Also, significant difference was seen between different temperatures. The highest TSS (%) of fruit content (av. 12.56 %) was that fruit treated with 55 ℃. hot water for 5min followed by 12.00 and 11.22 % when treated fruits with hot water at 50 and 45 ℃. for 5min respectively. The least level of TSS percent was relevant to non- treated fruits (control 9.00 %). As a whole, no significant difference was seen between *A. Citri* 11.25 % and *P. italicum* 11.58 %. The same results were obtained by (Porat *et al.,* 2000; Auret, 2001; Fallik, 2004; Lemessa *et al.,* 2004; Irtwange, 2006 and Zamani *et al.,* 2009).

Table 7. Effects of hot water treatments on TSS (%)

Temperature degree[℃]	*A. Citri*	*P. digitatum*	*P. italicum*	Mean
45 ^{o}C	11.67 cd	10.33 e	11.67 cd	11.22 C
50 ^{o}C	12.00 bcd	11.33 d	12.67 ab	12.00 B
55 ^{o}C	12.33 abc	12.33 abc	13.00 a	12.56 A
Control	9.00 f	9.00 f	9.00 f	
Mean	11.25 A	10.75 B	11.58 A	

L.S.D Fungi = 0.3429, L.S.D Temperature degree = 0.3959, L.S.D F*T = 0.6857

Effects of hot water treatments on TA (%): Data show that all hot water treatments were found to be changing significantly TA (%) of fruits comparing with non-treated control Table (8). Also, significant difference was seen between different temperatures. The av. percentage of TA was 0.15, 0.35 and

0.52% that fruit treated with hot water at 55, 50 and 45℃. for 5min respectively comparing with non- treated fruits (control 1.07 %). As a whole, no significant difference was seen between *A. Citri* 0.46 % and *P. digitatum* 0.49 %. The same results were obtained by (Porat *et al.*, 2000; Auret, 2001; Fallik, 2004; Lemessa *et al.*, 2004; Irtwange, 2006 and Zamani *et al.*, 2009).

Table 8. Effects of hot water treatments on TA (%)

Temperature degree[℃]	*A. Citri*	*P. digitatum*	*P. italicum*	Mean
45 ^{o}C	0.40 c	0.43 c	0.73 b	0.52 A
50 ^{o}C	0.28 d	0.3 d	0.47 c	0.35 B
55 ^{o}C	0.11 e	0.17 e	0.17 e	0.15 C
Control	1.07 a	1.07 a	1.07 a	
Mean	0.46 B	0.49 B	0.61 A	

L.S.D Fungi = 0.04637, L.S.D Temperature degree = 0.05355, L.S.D F*T = 0.09275

Effects of hot water treatments on TSS/TA ratio (%) character of "Washington" Navel orange fruits were tabulated in Table (9). Data show that all hot water treatments were found to be increased significantly TSS/TA ratio (%) of fruit content comparing with non-treated control. Also, no significant difference was seen between both different temperatures 45 & 50℃. while significant difference was seen between these temperatures and 55℃. The highest TSS/TA ratio (%) of fruit content (av. 106.0 %) was that fruit treated with 55℃. hot water for 5min followed by 36.29 and 23.11 % when treated fruits with hot water at 50 and 45℃. for 5min respectively. The least level of TSS/TA (%) was relevant to non- treated fruits (control 8.44 %). As a whole, no significant difference was seen between all tested fungi *A. citri* gave 52.89 %, *P. digitatum* 36.57 and *P. italicum* 40.90 %. These results were agreement by (Porat *et al.*, 2000; Auret, 2001; Fallik, 2004; Lemessa *et al.*, 2004; Irtwange, 2006 and Zamani *et al.*, 2009).

Table 9. Effects of hot water treatments on TSS/TA (%)

Temperature degree[℃]	*A. Citri*	*P. digitatum*	*P. italicum*	Mean
45 ^{o}C	29.06 bc	24.33 bc	15.95 bc	23.11 B
50 ^{o}C	43.27 bc	38.06 bc	27.52 bc	36.29 B
55 ^{o}C	130.77 a	75.44 ab	112.03 a	106.0 A
Control	8.44 c	8.44 c	8.44 c	
Mean	52.89 A	36.57 A	40.90 A	

L.S.D Fungi = 27.68, L.S.D Temperature degree = 31.97, L.S.D F*T = 55.37

Effects of hot water treatments on Vitamin C (mg/100 ml juice) of "Washington" Navel orange fruits were recorded in Table (10). Data show that all hot water treatments were found to be increased significantly Vitamin C (mg/100 ml juice) of fruit content comparing with non-treated control. Also, significant difference was seen between different temperatures. The highest Vitamin C (mg/100 ml juice) of fruit content (av. 67.22 %) was that fruit treated with 55 ℃. hot water for 5min followed by 60.44 and 57.44 % when treated fruits with hot water at 50 and 45 ℃. for 5min respectively. The least level of TSS percent was relevant to non- treated fruits (control 55.33%). As a whole, no significant difference was seen between *A. citri* 62.00 % and *P. italicum* 71.17 *%*. While significant difference was seen between these molds and *P. digitatum* which record57.17%. Similar results were recorded by (Porat *et al.*, 2000; Auret, 2001; Fallik, 2004; Lemessa *et al.*, 2004; Irtwange, 2006 and Zamani *et al.*, 2009).

Auret (2001); Zamani *et al*. (2009) reported that, pre–storage hot water dips of fruit at temperatures above 40 ℃ have been shown to be effective in controlling storage decay, not only by reducing the pathogen but also by enhancing the resistance of fruit tissue, influencing host metabolism and ripening.

Table 10. Effects of hot water treatments on L-ascorbic acid Vitamin C (mg/100 ml juice)

Temperature degree[℃]	*A. Citri*	*P. digitatum*	*P. italicum*	Mean
45 ^{o}C	58.00 cde	82.57 e	60.33 cd	57.44 C
50 ^{o}C	61.67 bc	89.62 cde	62.67 bc	60.44 B
55 ^{o}C	73.00 a	100.51 bc	66.33 b	67.22 A
Control	55.33 de	55.33 de	55.33 de	
Mean	62.00 A	57.17 B	71.17 A	

L.S.D Fungi = 2.548, L.S.D Temperature degree = 2.942, L.S.D F*T = 5.096

Conclusion

Hot water treatment increased the protective effect of fruits and increased significantly the shelf life of Navel orange fruits. Pre–storage hot water dips of fruit at temperatures above 40 ℃ have been shown to be effective in controlling storage decay, not only by reducing the pathogen but also by enhancing the resistance of fruit tissue, influencing host metabolism and ripening.

References

AOAC. (2005). Association of official analytical chemists. Official Methods of Analysis of AOAC International 17th edition. Nature Toxins. AOAC International, Arlington, Virginia, USA.

Agrios, G. N. (2005). Plant pathology. New York: Academic Press.

Auret, E. E. (2001). Control strategies for citrus postharvest diseases. (Master's thesis). University of Pretoria, South Africa.

Barry, P., Mike, M. and Patricia, F. (2003). This is a part of 2003 citrus research report, the university of arizona college of agriculture and life sciences. Retrieved from http://cals.arizona. edu/pubs/crops/az1331.

Embaby, E. M., Hazaa, M., Hagag, L. F., Ibrahim, T. E. and El-Azem, F. S. A. (2013). Decay of Some Citrus Fruit Quality Caused by Fungi. Journal of Applied Sciences Research 9:5920-5929.

Fallik, E. (2004). Hot water treatments for control of fungal decay on fresh produce. In Sapers, G. M., Gorny, J. R. and Yousef, A. E. (Eds.), Microbiology of Fruits and Vegetables. Boca Raton, Florida: CRC Press, Inc.

Fatemi, S. and Borji, H. (2011). The effect of physical treatments on control of *Penicillium digitatum* decay orange cv. Valencia during storage period. African Journal of Agricultural Research 6:5757-5760.

Inkha, S., Boonyakita, D. and Srichuwong, S. (2009). Effect of treatment on green infection in tangerine fruit cv. SaiNumPung. Cmu Journal Natural Science. Retrieved from http://cmuj.chiangai.ac.th/cmujvol8_1/09%20Journal%202009%20 pp. 8-1.

Irtwange, S. V. (2006). Hot water treatment: a non-chemical alternative in keeping quality during postharvest handling of citrus fruits. Agricultural engineering international: the cigr ejournal. Invited Overview. pp. 5.

Larrigaudiere, C., Pons, J., Torres, R. and Usall, J. (2002). Storage performance of clementines treated with hotwater, sodium carbonate and sodium bicarbonate dips. Journal of Horticulture 77:314-319.

Lemessa, F., Shunli, W., Agravante, J., Amuoh, C. and Kiyimba, F. (2004). Effect of hot water treatment on the quality of citrus fruit. Unpublished Group Project Report. International Research and Development Course in Postharvest Biology and Technology, the Volcani Center, Israel. Postharvest Biology and Technology 18:235-244.

Milind, S. L. (2008). Citrus fruit biology, technology and evaluation. First ed book, Copyright Elsevier Inc. All Rights Reserved Academic Press is an Imprint of Elsevier.

Munoz, A. M., Garcia, B. L., Candelas, A. V. and Marcos, J. F. (2011). Comparative analysis of the sensitivity to distinct antimicrobials among *Penicillium* spp. causing fruit postharvest decay. Phytopathol Mediter 50:392−407.

Nafussi, B., Ben-Yehoshua, V., Rodov, J., Peretz, and B. Dhallewin, G. (2001). Mode of action of hot-water dip in and reducing decay of lemon fruit. Journal of Agricultural and Food Chemistry 49:107-113.

Nunes, C., Duarte, A., Manso, T., Garc ía, J., Cayuela, J., Yousfi, K., Mart ínez, M., Weiland, C. and Salazar, M. (2010). Relationship between post-harvest diseases resistance and mineral composition of citrus fruit. Acta Horticulturae 868 pp.

Plaza, P., Usall, J., Torres, R., Abadias, M., Smilanik, J. and Vi ñas (2004). The use of sodium carbonate to improve curing treatments against green and blue moulds on citrus fruits. Pest Management Science 60:815–821.

Porat, R., Daus, A., Weiss, B., Cohen, L., Fallik, E. and Droby, S. (2000). Reduction of post-harvest decay in organic citrus fruit by a short hot water brushing treatment. Post-harvest Biology and Technology 18:151–157.

Rab, A., Sajid, M., Khan, N., Nawab, K., Arif, M. and Khattak, M. (2012). Influence of storage temperature on fungal prevalence and quality of citrus fruit (CV. Blood Red). Pakistan Journal of Botany 44:831-836.

Shideh, M. and Naser, S. (2012),. Phylogenetic analysis of *alternaria* species associated with citrus black rot in Iran. Plant Pathology and Microbiology 3:1-7.

Smilanick, J. L., Mansour, M. F., Mlikota, F. L., Gabler, F. F. and Sorenson, D. B. (2008). Control of citrus post-harvest green mould and sour rot by potassium sorbate combined with heat and fungicides. Post-harvest Biology and Technology 47:226-238.

Townsend, G. K. and Hunburger, T. W. (1943). Methods for estimating losses caused by diseases in fungicide experiments. Plant Disease Report 27:340-343.

Zamani, M., Sharifi-Tehrani, A., Ahmadzadeh1, M., Hosseininaveh, V. and Mostofy, Y. (2009). Control of *penicillium digitatum* on orange fruit combining *pantoea agglomerans* with hot sodium bicarbonate dipping. Plant Pathology 91:437-442.

(Received 15 July 2014; accepted 31 August 2014)