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QUALITY CHANGES IN HEAT TREATED SWEET ORANGE FRUIT DURING STORAGE AT LOW TEMPERATURE

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ABSTRACT

The quality changes in heat treated sweet orange fruit during storage at low temperature were evaluated by subjecting sweet orange fruit to heat treatment for 0-20 minutes and storage at low temperature (5±1 °C) for 0-75 days. Low temperature storage for more than 30 days significantly increased the weight loss, surface pitting, disease incidence, TSS, TSS/Acid ratio. In contrast, acidity, reducing sugars and ascorbic acid declined during low temperature storage of sweet orange fruits, while non-reducing sugars were not affected significantly. Heat treatment, for 5-10 minutes at 50 °C, retarded the storage-associated changes in sweet orange fruit but longer heat treatments durations (15-20 minutes) declined the physical and chemical quality attributes. Ascorbic acid, however, continued to decrease even with modest heat treatment. The interaction of heat treatment and low temperature storage duration indicated no significant effect on weight loss, TSS, acidity, reducing sugars and ascorbic acid content but surface pitting, disease incidence and TSS/acid ratio increased significantly with 75 days storage at low temperature. While the maximum weight loss (8.83%) in control fruits after 75 days storage was at par with the heat treatment for 5-20 minutes. Disease incidence, after 75 days storage, was the highest (15.33%) with heat treatment for 20 followed by heat treatment for 15 minutes (10%) and control (8%) but was the least (5.33%) with heat treatment for 5 minutes which was at par with 10 minutes heat treatment.

Key words: Ascorbic acid, Heat treatment, Low temperature Storage, Sweet orange, TSS/ Acid ratio, Ascorbic Acid

INTRODUCTION

In Pakistan, citrus fruits are grown over an area of 194.5 thousand hectares with a total production of 1982.25 thousand tons (MINFAL, 2012). Citrus fruits are important for both domestic consumption and as a source of foreign exchange earnings. Recently, Pakistan exported 214764.6 tons of citrus (Kinnow and others) fruit to different countries (MINFAL, 2012). The bulk of the sweet orange is harvested during the months of December–January and its supply as well as quality decline after the peak production season is over. Weight loss and decline in sugars and ascorbic acid are common in stored oranges (Rab *et al*., 2010). While low temperature storage slow down the deterioration of temperate fruits (Lee and Kader, 2000), tropical and subtropical fruit are damaged by low temperature due to chilling sensitivity (Rab and Saltveit, 1996). The citrus fruits are also injured by extended exposure to temperatures below 5±1 °C (Rodov *et al*., 1995), though citrus cultivars may differ in susceptibility and severity to chilling injury. For example, Fortune mandarin are found to has higher susceptibility to chilling injury as compared to Valencia orange (Lafuente *et al*., 2003). Chilling injury symptoms in citrus fruits include increased weight loss, surface pitting, enhanced disease susceptibility (Rodov *et al*., 1995) and increased electrolyte leakage of skin tissue (Woolf, 1997). Attempts have been made to reduce chilling sensitivity by different postharvest techniques such as heat shock (Rab and Saltveit, 1996), anaerobic shock treatments (Pesis *et al*., 1994), chemicals treatment (Saltveit *et al*., 2004) and hot air, vapor and water treatments (Woolf, 1997), storage conditions (Rab *et al*., 2010), packaging and waxing (Petracek *et al*., 1998). High temperature treatment is commonly recommended in citrus fruit for eliminating insects' infestation (Gould and McGouri, 2000), disease incidence (Mansour *et al*., 2006), inducing chilling tolerance (Rab and Saltveit, 1996; Ritenour *et al*., 2004) and maintaining quality during storage (Erkan *et al*., 2005). The effect of heat treatments is believed to be due to induction of protective heat shock proteins (HSP) (Ding *et al*., 2002). Generally, the fruits are heated to achieve the core temperature of 43 to 46.7 °C, with exposure times varying from 35 to 90 minutes (Gould and McGuire, 2000). Significant decrease in chilling injury has been reported when Fortune mandarin were heat treated with a temperature of 47.5 °C for 2 or 5 minutes and at 50 °C for 2 minutes before storage for 8 weeks at 2 °C (Ghasemnezhad *et al*., 2008). The effectiveness of heat treatment, however, may vary from species to specie and different fruit require different temperatures and treatment duration for desired benefits (Barki-Golan and Phillips, 1991). The present study was, therefore, was initiated to evaluate the effects of heat treatment duration on the physico-chemical quality attributes of sweet orange fruits stored at low temperature (5±1°C).

MATERIALS AND METHODS

Experimental Materials: The fruit of sweet orange *cv*. Blood red was procured from an orchard in district Mardan. The fruit were carefully transported to University of Agriculture, Peshawar. Fruits, free from mechanical or pathological damage were selected and exposed to heat treatments in the postharvest laboratory of University of Agriculture, Peshawar.

Heat treatment of fruit: The heat treatment was applied by immersion of sweet orange fruit for 0 (Control), 5, 10, 15 or 20 minutes in water heated to 50 °C. After the heat treatment, the surface moisture was removed with a gentle breeze from a fan. The fruits were left for 3 hours at room temperature and then packed in cardboard packages having 2 holes on each side to remove excessive moisture and heat. The fruit were then stored at 5±1 °C for different duration and data was recorded on physico-chemical quality attributes of fruits at 15 days interval for 75 days.

Quality attributes studied: Weight loss was approximated by selecting 5 fruits in each treatment in each replication and recording their initial and final weight at 15 days interval. The weight loss data for five fruits was then averaged to represent weight loss in corresponding treatments and replications and presented as percent weight loss.

$$Percent\,weight\,loss = \frac{Initial\,Fruit\,Weight - Final\,Fruit\,Weight}{Initial\,Fruit\,Weight} x100$$

Juice content: Percent juice content was estimated by extracting juice from five randomly selected fruit from each treatment with the help of juice extracting machine, weighed and the percentage was computed.

Surface pitting and disease incidence: Surface pitting was assessed by visual observation of the fruits and counting the number of fruits showing the symptoms of surface pitting. Disease incidence was estimated by observing the fruits and any fruit showing any disease symptom was regarded as diseased fruit.

TSS: The total soluble solids (TSS) content of the fruit was determined at each storage interval with a hand refractometer (Kernco, Instruments Co. Texas). A drop of orange juice was uniformly spread over the prism for each reading. The prism of refractometer was washed with double distilled water after each reading. Acidity, TSS/Acid ratio, reducing and non-reducing sugars and ascorbic acid content were determined according to AOAC (1990).

Reducing and non reducing sugars (%): Reducing and non-reducing sugars were determined by Lane and Eyenon (1923) method as described in AOAC (1990) by extracting the juice from 10 fruit in each replication. The juice, 25 grams of filtered (whattman-4) was transferred to 250 ml volumetric flask and diluted with 100 ml water. The solution was neutralized with 1 N NaOH and 2 ml of lead acetate solution was added. The solution was shaken and then allowed to stand for 10 minutes. The necessary amount of potassium oxalate was added to remove the excess lead and the volume made with water.
Sugars were calculated as:

$$\text{Re}\,ducing\ Sugars\ (\%) = \frac{Factor\ x\ Dilution\ x\,100}{Titer\ x\,Wt\ or\ Vol\ of\ Sampel}\ x100$$

$$Non\ \text{Re}\,ducing\ \ Sugars\ (\%) = (\%\ Total\ \ Sugars\ - \%\ \text{Re}\,ducing\ \ sugars\ )\ x\ 0.95$$

Ascorbic acid: Ascorbic acid was determined by diluting one ml of orange juice in 0.4% oxalic acid solution and volume was made up to 10 ml. This 10 ml diluted sample was titrated against the standard dye solution until light pink color appeared, which persisted for 15 seconds.

Statistical procedures: The experiment was designed in two factors CRD and the data on different parameters were subjected to analysis of variance (ANOVA) technique to observe the differences between the different treatment as well as their interactions Steel *et al*. (1997). In cases where the differences were significant, the means were further assessed for differences through least significant difference (LSD) test. Statistical computer software, MSTATC (Michigan State University, USA), was applied for computing both the ANOVA and LSD.

RESULTS AND DISCUSSION

Weight loss: The weight loss was significantly affected by storage duration but heat treatment duration had no significant effect on the weight loss in citrus fruits. Weight loss continued to increase with prolonged storage duration so that each 15 days interval caused significant increase in cumulative weight loss. The weight loss increased to the maximum of (8.57%) with 75 days storage (Table 1). Heat treatment duration has no significant effect on percent weight loss during low temperature storage of citrus fruits. The interaction of heat treatment and storage duration was also not significant (Table 1). The fruit continue to lose water after harvest resulting in increased weight loss during storage. The increasing weight loss with prolonged storage duration is due to moisture loss from the fruit (Ali *et al*., 2011), yet the incremental increase during storage at low temperature indicates greater sensitivity of sweet orange fruits to water loss despite low temperature. According to Cohen *et al*. (1994), removing the fruits from cold storage (2°C) and holding at 20°C increased the rate of water loss in citrus fruits due to cracks around the stomata of chilling injured fruits. Thus, the increase in weight loss during low temperature storage can be used as an indicator of chilling injury (CI) in sweet orange fruits. Erkan *et al*. (2000) reported that heat treatments increase weight loss in Valencia oranges (*Citrus sinensis*). However, no significant effect of heat treatments on weight loss during storage at chilling temperature was observed in the present study.

Surface pitting (%): Differences in surface pitting were non-significant between control and low temperature storage for 30 days but extending the storage beyond 30 days increased the surface pitting significantly to the maximum of 16.27% after 75 days storage (Table 1). The heat treatment duration also significantly affected the surface pitting. Heat treatment for 5 minutes resulted in the minimum mean surface pitting (3.44%) that was non-significant with heat treatment for 10 (3.56%) and 15 minutes (5.33%) respectively. By contrast, surface pitting in control (6.78%) and heat treatment for 20 minutes (5.78%) was statistically at par with one another. The interaction of heat treatment and storage duration revealed the maximum surface pitting (20.67%) in control fruits stored for 75 days was followed by 18% with the same storage duration and heat treatment for 20 minutes. The least surface pitting (3.44%) was observed with heat treatment for 5 minutes which was at par with 3.56% with heat treatment for 10 minutes (Fig. 1). Surface pitting is a symptom of chilling injury in citrus fruits, which is expressed as pitting, staining and necrotic areas in the rind tissue that may increase in number and size over time (Sanchez-Ballesta *et al*., 2003; Ritenour *et al*., 2004). Storage at 5±1°C for 30 days had no significant effect on surface pitting, which increased significantly with further increase in storage duration, indicating relatively modest susceptibility of sweet orange fruit to chilling injury (Wang, 1990). Surface pitting was the highest in control that decreased significantly with heat treatments for 5-15 minutes. However, heat treatment for 20 minutes was statistically at par with control (Table 2), indicating that prolong heat treatments may also cause surface pitting (Ghasemnezhad *et al*., 2008). It is likely that both chilling and excessive heat treatments may cause surface pitting (Reitenor *et al*., 2004).

Disease incidence (%): Disease incidence did not increase significantly with storage duration for 30 days but further extension in storage duration resulted in significant increase to the maximum of 8.87 % after 75 days storage at low temperature. The heat treatment durations also significantly affected the disease incidence on sweet orange fruit. Disease incidence in control fruits 2.67%, decreased significantly to 1.44 and 1.06% with heat treatment for 5 and 10 minutes respectively. But heat treatment for 15 or 20 minutes caused significant increase (4.11 and 5.44% respectively) in disease incidence. Similarly, the interaction of storage and heat treatment duration revealed the maximum disease incidence (15.33%) was after 75 days storage at low temperature with heat treatment for 20, followed by 10 and 8% with 15 minutes heat treatment and control respectively. The same storage duration (75 days), however had the least (5.33%) disease incidence when fruit were heat treatment for 5 minutes, which was at par with 5.67% disease incidence observed with heat treatment for 10 minutes and storage for 75 days (Fig. 2). Since chilling exposure increase disease susceptibility, the non-significant changes in disease incidence for 30 days storage indicates that the incidence and severity of diseases increases when storage is increased beyond the critical length required to induce chilling injury (D'hallewin and Schirra, 2000). While the decay is more rapid at higher than lower temperatures (Kader, 2002), but the disease symptoms on citrus fruits were observed at temperature as low as 5°C. The heat treatments have been shown to decrease disease incidence (Ritenour *et al*., 2004), yet its success depend on the moisture content and metabolic activity of the spores, exposure temperature and duration (Barki-Golan and Phillips, 1991). The decrease in disease incidence with modest heat treatments can be attributed to inactivation of spores, thus, control postharvest decay (Ritenour *et al*., 2004) or enhanced synthesis of scoparone that have anti-fungal properties (Kim *et al*., 1991). However, prolonged heat treatment (15-20 minutes) may injure the rind tissue resulting in increased cuticular cracks (Joyce *et al*., 2003), thus allowing the pathogens to initiate the infection process.

Total soluble solids: The minimum TSS (10.28%) at day 0 remained almost constant with storage duration up to 30 days (10.51%) but thereafter increased significantly to 10.90% with 45 days storage and to the maximum of 11.39% after 75 days storage, though the difference between 60 and 75 days was non-significant (Table 2). The heat treatment also significantly affected the TSS of the sweet orange, but its influence was duration-dependent. The mean TSS content of control fruits (11.11%) decreased to 10.49 and 10.5% with heat treatment for 5 and 10 minutes accordingly increased to 10.82 and 10.90% with heat treatment for 15 and 20 minutes respectively (Table 4). The TSS content recorded in control fruit was statistically at far with heat treatment for 15 and 20 minutes. The TSS contents of citrus fruits, generally, increase during storage (Lee and Kader, 2000) due to the break down of complex carbohydrates

(Brummell *et al*., 2004). While, the heat treatment for 5 and 10 minutes had significantly lower TSS, but 15 and 20 minutes heat treatment resulted in significantly higher TSS content of the fruits that was at par with control. The heat treatments slow down the ripening (Lurie, 1998) hence retain TSS at low levels with modest heat treatments (5-10 minutes) as compared to control or longer heat treatments (15 and 20 minutes). It indicate that while modest heat treatment may slow down the increase in TSS during storage, longer heat treatment duration (15 and 20 minutes) may promote the ripening and thus increase the total soluble solids during storage of sweet orange fruit (Jacobi *et al*., 2001).

Acidity (%): The acidity of the fruit was non-significant between day 0 and 30 days of storage but then decreased significantly with increase in storage duration (Table 2). The maximum acidity recorded on day 0 (1.65%) decreased non-significantly to 1.58% with 30 days storage and significantly with increasing storage duration beyond 30 days. The least mean acidity (1.27%) was recorded with 75 days storage at low temperature. The acidity was not significantly affected by heat treatment for 5 or 10 minutes and it was at par with control fruit (1.55%). However, it decreased significantly to 1.49 and 1.46% with increasing heat treatment duration to 15 or 20 minutes. The non-significant decrease in acidity of the citrus fruit between day 0 and 30 days but significant decline with increase in storage duration (confirm the previous reports that total acidity of citrus fruits decrease during storage (Rab *et al*., 2010) even at low temperature. Similarly, the changes in acidity were also non-significant with heat treatment for 5 or 10 minutes but longer heat treatment durations (15-20 minutes) resulted in significant decrease in percent acidity. While, it has been reported that heat treatments had no consistent effects on titratable acidity in Valencia oranges (Ekran *et al.*, 2000), the acidity in this study declined significant with prolonged heat treatment duration (Lurie, 1998).

TSS/Acid ratio: The mean TSS/acid ratio increased non-significantly from the minimum of 6.25 on day 0 to 6.67 after 30 days storage but then significantly increased during the storage duration and finally to the maximum of 9.00 after 75 days storage at low temperature (Table 2). The heat treatment for 5 and 10 minutes decreased the TSS/acid ratio to 6.89 and 7.09, but it increased to 7.59 with heat treatment for 20 minutes as compared to control fruits (7.24). The TSS/acid is a function of TSS and acidity of the fruit. Since, the TSS increased while acidity decreased during storage, thus, the TSS/Acid ratio also increased with increasing storage duration (Lee and Kader, 2000). The heat treatment effect was also significant but duration dependent. Whereas, modest heat treatment (5-10 minutes) was effective in retaining lower TSS/acid ratio, prolonged heat treatment increased it. The inhibition of increase in TSS/acid with modest heat treatment may be due to the inhibition of ripening (Lauri, 1998). However, extended heat treatments may damage the tissue and thus, enhance ripening during storage (Torres *et al*., 2009).

Reducing sugar (%): The maximum reducing sugar (5.12%) on day 0 of storage continued to decrease gradually but non-significantly with increasing storage duration to the minimum of (4.94%) with 75 days storage (Table 2). The heat treatments resulted in significant decrease in reducing sugars. The reducing sugar content of control fruit (5.43%) was significantly higher than heat treatment durations 5-20 minutes with all the heat treatment durations at par with each other. The interaction of storage duration and heat treatments was also non-significant. The non reducing sugar was not significantly affected by both storage and heat treatment duration or its interaction (data not shown). The non-significant increase during 75 days storage is indicative of the fact that low temperature storage decreased the metabolic activities and hence delayed the storage associated changes in reducing sugars (Sajid *et al*., 2013). Chilling injury is also reported to causes various metabolic alterations in the fruits (Maldonado *et al*., 2004), such as the inhibition of electron transport chain (Atkin and Tjoelker, 2003) and, hence, respiration (Kurets *et al*., 2003). The heat treatment resulted in significant decrease in reducing sugars, indicating that heat treatment may inhibit the chilling induced inhibition of respiration (Atkin and Tjoelker, 2003; Kurets *et al*., 2003).

Ascorbic acid (mg/100mg): The fresh fruits (day 0 of storage) had the maximum ascorbic acid (43.70 mg/100g), which decreased significantly with increasing storage duration. The Ascorbic acid decreased to 35.03 and 30.60 mg/100g with 45 and 60 days storage and finally to the lowest of 27.77 mg/100g with 75 days storage (Table 2). The heat treatment also resulted in significant decline in ascorbic acid. The maximum ascorbic acid (37.27 mg/100g) in control was at par with (37.07 mg/100g) in heat treatment for 5 minutes but significantly higher than heat treatment for 10 minutes. The ascorbic acid declined further to the minimum of 34.28 mg/100g with heat treatment for 20 minutes (Table 4). The interaction of storage duration and heat treatment duration was non significant. The Ascorbic acid is an important component of sweet orange fruit quality (Ladania *et al*., 2003). The ascorbic acid decreased significantly with increase in storage duration and increasing heat treatment duration (Kaul and Saini, 2000). The decrease in ascorbic acid with increase in storage or heat treatment durations indicates that ascorbic acid is particularly sensitive to chilling as well as high temperature (Kaul and Siani, 2000).

It can be concluded that sweet orange fruits can safely be stored for 30 days at low temperature (5±1°C) but increasing storage duration may cause chilling injury which appears as enhanced moisture loss, surface pitting, diseases incidence, TSS, TSS/Acid ratio and decreased acidity and Ascorbic acid content. Heat treatments for 5-10 minutes at 50°C may decrease the chilling related changes but longer durations (15 -20 minutes) may enhance the rate of deterioration. The loss of ascorbic acid is enhanced with both increase in low temperature storage and heat treatment duration.

Table 1. Effect of low temperature storage and heat treatment durations on weight loss, surface pitting, disease incidence and TSS contents of sweet orange fruit

Storage Duration (Days at 05°C)	**Weight Loss (%)**	**Surface Pitting**	**Disease Incidence**	**TSS (%)**
0	0 f	0.00 d	0.00d	10.28 c
15	0.93 e	0.00 d	0.13d	10.41 c
30	1.80 d	1.20 d	1.20d	10.51 c
45	3.41 c	3.60 c	2.53c	10.90 b
60	6.39 b	8.93 b	4.93b	11.09 a
75	8.57 a	16.27a	8.87a	11.39 a
LSD α 0.05	**0.229**	**1.311**	**1.73**	0.329
H T Durations (Minutes at 50 ºC)				
0	3.57	6.78 a	2.67c	11.11 a
5	3.47	3.44 b	1.44d	10.49 b
10	3.43	3.56 b	1.06d	10.51 b
15	3.57	5.33 b	4.11b	10.82 ab
20	3.54	5.78 ab	5.44a	10.90 a
LSD at α 0.05	ns	1.197	1.81	0.239
		SD x HT Interactions		
Significance	ns	* Fig 1	* Fig 2	Ns

Table 2. Influence of heat treatment and low temperature storage duration on acidity, TSS/Acid ratio, reducing sugars and ascorbic acid content of sweet orange fruits.

Storage Duration (Days at 05°C)	**Acidity (%)**	**TSS/Acid Ratio**	**Reducing Sugars (%)**	**Ascorbic Acid (mg/100g)**
0	1.65 a	6.25 d	5.12	43.70 a
15	1.63 a	6.40 d	5.06	41.55 b
30	1.58 ab	6.67 d	5.01	37.53 c
45	1.52 b	7.21 c	5.07	35.03 d
60	1.40 dc	7.93 b	5.01	30.64 e
75	1.27 d	9.00 a	4.94	27.77 f
LSD α 0.05	0.096	0.485	NS	0.981
H T Durations (Minutes at 50ºC)				
0	1.55 a	7.24 b	5.43 a	37.27a
5	1.53a	6.89 c	4.85 b	37.07 a
10	1.50ab	7.09 bc	4.80 b	36.34 b
15	1.49b	7.40 ab	5.04 b	35.22 c
20	1.46b	7.59 a	5.07 b	34.28 d
LSDs	0.042	0.255	0.301	0.981
SD x HT Interactions				
Significance	ns	ns	* Fig 3	ns

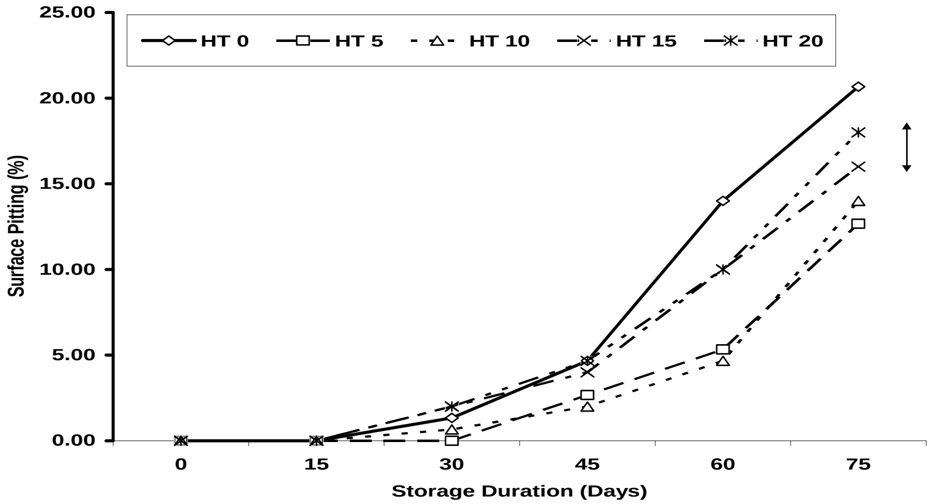

Fig. 1. The influence of heat treatment and low temperature storage duration on surface pitting of sweet orange fruit. The vertical arrow represents LSD (2.93) at P > 0.05.

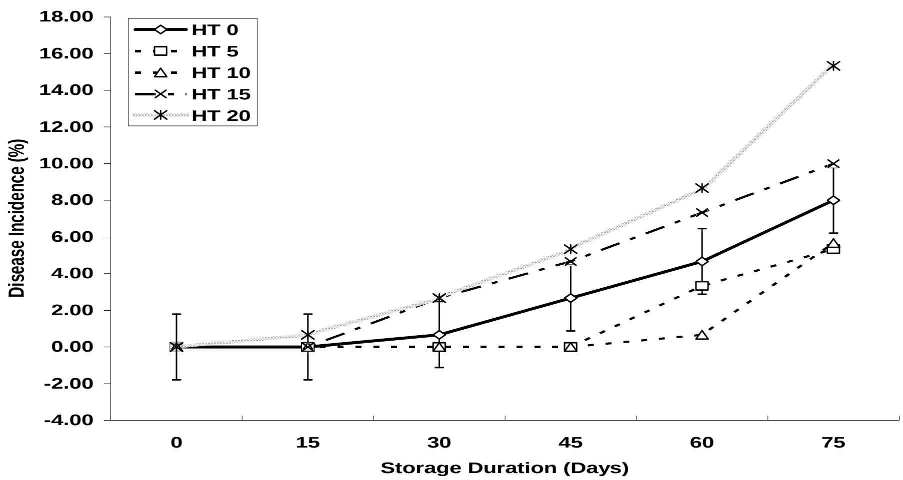

Fig. 2 The influence of heat treatments and low temperature storage duration on disease incidence on sweet orange fruit. The vertical bars on control data represents LSD (1.79) at P > 0.05.

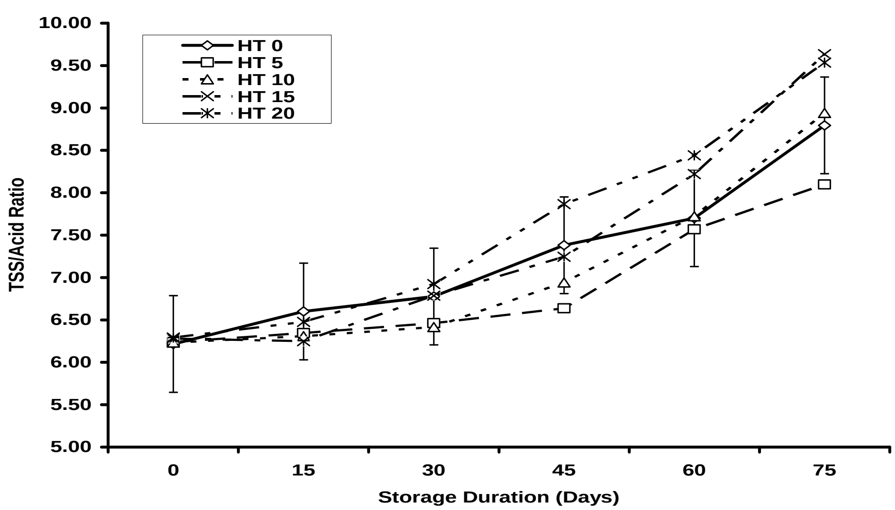

Fig. 3 The influence of heat treatments and low temperature storage durations on TSS/Acid ratio of sweet orange fruit. The vertical bars on control data represents LSD (0.67) at P > 0.05.

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