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Impact of vapor heat treatment on immature stages of Peach Fruit Fly, *Bactrocera Zonata* (*Diptera*: *Tephritidae*) on quality alteration of Keitt mango variety (*Mangifera indica* L.).

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Abstract
Keitt Mango variety (*Mangifera indica* L.) was artificially infested with immature stages (eggs and three larval instars) of peach fruit fly, *Bactrocera zonata*. Infested fruits were exposed to vapor heat treatment until temperature of fruit core reached 46.2C°. Then treatment continued for 30 minutes at this temperature. Vapor heat treatment induced 100% mortality for all immature stages of *B. zonata*. Quality changes of treated fruits were observed over four weeks of storage after application. The observed quality parameters included weight loss, skin color, flesh color, total soluble solids (TSS), titratable acidity (TA), and skin/flesh firmness. The results showed that vapor heat treatment did not induce any adverse the effect mango quality.

Keywords: Keitt mango, peach fruit fly, vapor heat treatment, mango quality

Introduction
Mango (*Mangifera indica* L.) is considered one of the most economically important tropical fruits in the world in terms of both worldwide production and cultivated area (Bally and Dillon, 2018) [4]. In Egypt, mango is a major fruit crop with high potential for export. Egypt's mango exports increased from 31.36 thousand tons in 2010 reaching 51.55 thousand tons in 2019 (FAO, 2023) [9].
The peach fruit fly, *Bactrocera zonata* (Saunders), poses an obstacle for the export of fruit from countries where it is endemic, such as Egypt, India, Pakistan, and Vietnam, into countries where it does not occur but could become established, such as the United States (Mohamed and El-Wakkad 2009) [25].
Heat treatment is one of the numerous effective quarantine treatments for *Bactrocera* flies (Heather and Hallman 2008) [10]. Vapor heat treatment (VHT) is a promising alternative which provided high efficacy against several species of fruit flies without damaging the mango fruit host (Merino *et al*. 1986, Sunagawa *et al.* 1987) [24, 31]. Such as treatment requires vaporizing equipment to provide efficacious quarantine security against *Bactrocera* infestation risk in fruits (Seo *et al*. 1974, Hansen and Armstrong 1990, Armstrong *et al*. 1995, Chan *et al.* 1996) [1, 6, 12, 29].
The International Standard for Phytosanitary Measures (ISPM) no. 28 suggests VH treatment as a viable method for eliminating fruit flies (IPPC, 2007) [15]. This treatment involves exposing fruits to water vapor at temperatures around 40-50 C°, aiming to kill either eggs or immutre stages of insects. Additionally, it can serve as a quarantine treatment before shipping fruits to their intended countries (Le *et al*., 2010; APHIS, 2011) [3, 20].
The present research aimed to assess the impact of VH treatment on immature stages of PFF, as well as in mango fruit quality.

Material and Methods
Insects Rearing
The peach fruit fly, *Bactrocera zonata* used in this research were obtained from laboratory of Horticultural Insects Research Department (PPRI). Adult flies were kept in cages (35×35×35cm) under laboratory conditions at 25±0.5C°,65±5% RH and a photoperiod 12:12 (L:D). Adult flies were fed on a diet composed of sugar, enzymatic yeast hydrolysate at rate of 3:1, respectively in addition moist sponge for water supply. Eggs deposited by sexually mature females (10-14days old) in artificial receptacle within an hour were collected.

Artificial Infestation of Mango Fruits
Export-graded mangoes (Keitt variety) were obtained from Al-Bakrawi station for exporting vegetables and fruits, Sadat City, Menoufia Governorate, Egypt. The fruits were cleaned, washed thoroughly with tap water, and allowed to air dry.
In order to determine the effect of VHT on different immature stages of *B. zonata*, fruits should have only one stage before exposure to treatment. The achieve this goal 40 fruits each mounted a black filter paper (1×1cm) loaded with 50 eggs and inserted into fruits. These fruits will contain 3rd larval instar after 7days from infestation. After 2 day 40 fruits were prepared as described before which will contain 2nd larval instar at the day of treatment. Two days later the same procedure was repeated to obtain 1st larval instar at the day of treatment. One day of treatment (at the 7 day from first fruits infection) anther 40fruits were loaded with fresh eggs. Twenty fruits contained different immature stags (5 fruits from each stage) were kept under room condition and considered untreated control. Infested fruits were kept laboratory condition at 25±1°C and 65±5% RH to allow development all immature stages of *B. zonata.*

Vapor Heat chamber
Dimensions of treatment chamber were (9m x 5.5m x 4.5 m). The Vapor Heat chamber was provided with five heat sensors connected with Testo device outside the chamber to record temperatures, one free to measurement room

temperature and four ones for measuring temperature inside the pulp of fruits, where they were placed at different heights and locations. Heat temperature was adjusted at 46.2C° by an electronic panel fixed outside the chamber. Plastic boxes loaded with infected fruits were distributed randomly in 8 sites inside of VHT chamber representing different places and heights. Each site contained 20 fruits representing the four immature stages of *B. zonata* (5fruits loaded with eggs, five fruits contain 1st, 5 fruits contain 2nd and 5 fruits contain 3rd larval instars).

Then hot water vapor is gradually pumped in until the temperature of the fruit core reaches the required degree of temperature (46.2°C) in all sensors. After the treatment, mango fruits were immediately cooled by a fan for 20 minutes to restore them to normal conditions then transferred for examination.

Mango quality analysis

The quality of both treated and an untreated fruit was evaluated throughout a four-week storage period. Measurements were conducted weekly. For each week of storage, ten mango fruits from both treated and untreated groups were selected as samples to analyze changes in weight loss, skin color, flesh color, total soluble solids (TSS), pH, titratable acidity (TA), and skin/flesh firmness.

Weight loss was calculated using the following formula:

$$\text{Weight loss, \%} = \frac{\text{Initial fruit weight} - \text{Final fruit weight}}{\text{Initial fruit weight}} \times 100 \quad \ldots\ldots (1)$$

The color of the fruits' skin and flesh, represented by L*, a*, and b* values, was measured using a colorimeter.

Three points on each fruit were measured for color, and the average values were used (Saad *et al*., 2016, Saad *et al*., 2022) [27, 28].

To determine the total soluble solids (TSS) as °Brix, the juice obtained from each mango fruit was measured using a digital refractometer. Total titratable acidity (TA) was determined following the AOAC official method 942.15. TA was expressed as a percentage of citric acid per 100 g of mango juice by titrating a 10 g sample of juice extract with 0.1 N NaOH until reaching a final pH of 8.1.

The firmness of the fruits' skin and flesh was measured using a 6.35 mm plunger diameter of penetrometer (ST 308 model) in kg/cm^2. The results were then converted to N/mm^2.

Statistical analysis

The collected data from both the treated and untreated groups underwent analysis of variance (ANOVA). Additionally. Moreover, using SPSS software to assess the alteration in fruit quality properties during storage was evaluated by comparing the least significant difference (LSD) test at a significance level of 5%.

Results and Discussions

Results obtained from Table (1) showed that vapor heat treatment at 46.2°C for 30 minutes as post-harvest treatment was effective as it caused 100% kill to all immature (eggs, 1st larval instar, 2nd larval instar and 3rd larval instar) *B. zonata*.

Table 1: Total number of immature stages of the *B. zonata* for control compared with vapor heat treatment

Stage	Control treatment			Vapor heat treatment	
	No. eggs treated	No. live larvae recovered	Mortality %	No. live larvae recovered	Mortality %
Eggs	250	230	8	0	100
1st larval	250	200	20	0	100
2nd larval	250	180	28	0	100
3rd larval	250	153	38.8	0	100

Lestari *et. al* (2017) [21] showed that vapor heat treatment at 47C for 40 minutes was effective to eradicate of eggs and larvae of *B. dorsalis*.

Yu-Lin Hsu *et.al* (2018) [33] found that when the papaya fruit core temperature increased at a heating rate of 0.0925C°/min from room temperature to 47.2C° in 3 h, *Bactrocera dorsalis* and *Bactrocera cucurbitae* showed 100% mortality.

Effect of VH treatment on Mango quality during storage

Weight loss

There was a minimal disparity in weight loss between the treated mangoes and those that did not. Specifically, after 4 weeks of storage, the treated fruits exhibited a weight loss of 7.18%, while the untreated fruits experienced a weight loss of 7.12%. The mangoes that underwent VH treatment exhibited a higher weight loss (3.09%) during the initial week compared to the untreated mangoes (2.71%) due to their accelerated ripening process (Table1). However, there was no significant difference in weight loss between the treated and untreated mangoes after seven days from storage, indicating a stable rate of weight loss (Figure 1). This outcome aligns with previous studies conducted by (Hasbullah *et al*. (2001) [13] regarding the mango, where they found that VH treatment did not significantly influence weight loss during storage. The effects of VH treatment on weight loss can vary, as it may reduce weight loss by altering the structure of the outer wax layer and filling cracks in the fruit's surface (Lufu *et al.*, 2020) [22]. Conversely, VH treatment can also lead to increased weight loss, particularly when applied for longer durations, resulting in wider cuticle cracks (D'hallewin & Schirra, 2000) [7].

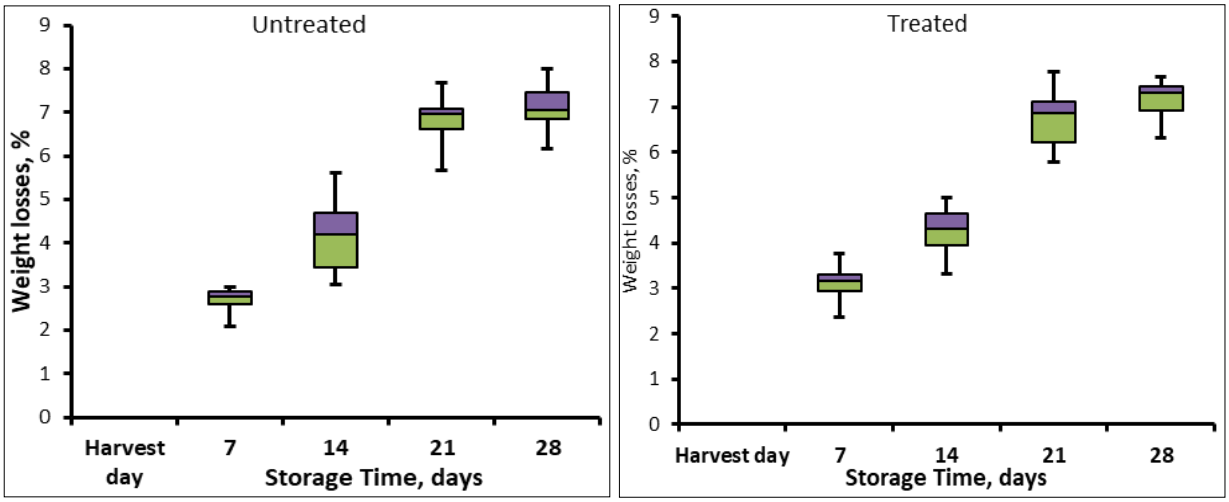

Fig 1: Weight losses of untreated/ treated mango fruits during four-week of storage

Table 1: Weight losses, %

Weight losses, %		Storage time, days				
		Harvest day	7	14	21	28
Untreated	Mean	0	2.71	4.15	6.76	7.12
	Max	0	2.99	5.61	7.69	8
	Min	0	2.10	3.05	5.68	6.16
	SD	0	0.25	0.79	0.54	0.49
	CV %	0	9.25	18.99	7.10	6.85
Treated	Mean	0	3.09	4.24	6.81	7.18
	Max	0	3.76	4.99	7.78	7.66
	Min	0	2.36	3.31	5.78	6.33
	SD	0	0.33	0.50	0.59	0.39
	CV %	0	10.61	11.72	8.68	5.36

Fruit skin color

A color meter was used to measure the skin color of the fruit, providing values for L* (lightness), a* (red/green color), and b* (yellow/blue color).

The findings from (Table 2) indicated that VH treatment did not have a significant influence on the fruit skin color until 14 days into the storage period. However, after two weeks of storage, significant changes were observed in the values of L*, a*, b*, and a*/b* (Figure 2). Throughout the storage duration, there was a noticeable improvement in color, with a shift from green to yellow (Figure 3). Similar results were reported by Hasbullah *et al*. (2001) [13], who found that the duration of VH treatment did not significantly affect the color change of Irwin mangoes during storage. The change in mango skin color from green to yellow was attributed to the degradation of chlorophyll. According to Karanjalker *et al*. (2018) [17], the yellowing color resulted from the breakdown of chlorophyll during the fruit's maturation, which revealed the presence of carotene pigment. This pigment remained stable in the fruit's skin but was concealed by chlorophyll.

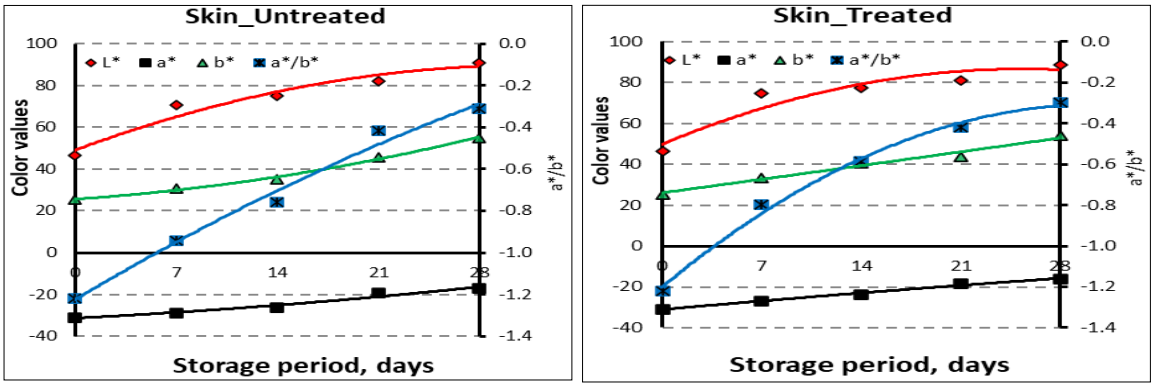

Fig 2: Skin color change of mango during four-week of storage (Untreated/Treated fruits)

Harvest day After 7 days After 14 days After 21 days After 28 days

Untreated Keitt mango during storage

Harvest day After 7 days After 14 days After 21 days After 28 days

Ttreated Keitt mango during storage

Fig 3: Skin/ flesh color alteration observed during four-week of storage for untreated and treated mango fruits

Table 2: Skin color values

Mean of skin color values		Storage time, days				
		Harvest day	7	14	21	28
Untreated	L*	46.57	70.71	75.06	81.97	91.03
	a*	-31.01	-28.83	-26.46	-19.08	-17.15
	b*	25.44	30.65	34.92	45.81	55.04
	a*/b*	-1.22	-0.94	-0.76	-0.42	-0.31
Treated	L*	46.57	74.67	77.27	80.97	88.86
	a*	-31.01	-26.75	-23.72	-18.37	-16.09
	b*	25.44	33.56	40.47	43.74	53.91
	a*/b*	-1.22	-0.80	-0.59	-0.42	-0.30

Fruit flesh color

Based on data in Table 3 and Figure 4, results illustrated the change in flesh color of mango during four-week of storage comparing between untreated and treated samples. Both untreated and treated fruits were statistically similar without significant differences on L*, a*, b*, and a*/b* values.

Table 3: Flesh color values

Mean of flesh color values		Storage time, days				
		Harvest day	7	14	21	28
Untreated	L*	86.26	80.77	77.07	73.24	75.31
	a*	-7.79	-2.49	2.74	4.20	7.54
	b*	64.25	69.58	75.69	79.71	84.19
	a*/b*	-0.12	-0.04	0.04	0.05	0.09
Treated	L*	86.26	78.69	76.57	75.82	74.17
	a*	-7.79	-1.94	0.12	3.16	6.48
	b*	64.25	70.92	71.46	80.51	83.84
	a*/b*	-0.12	-0.03	0.00	0.04	0.08

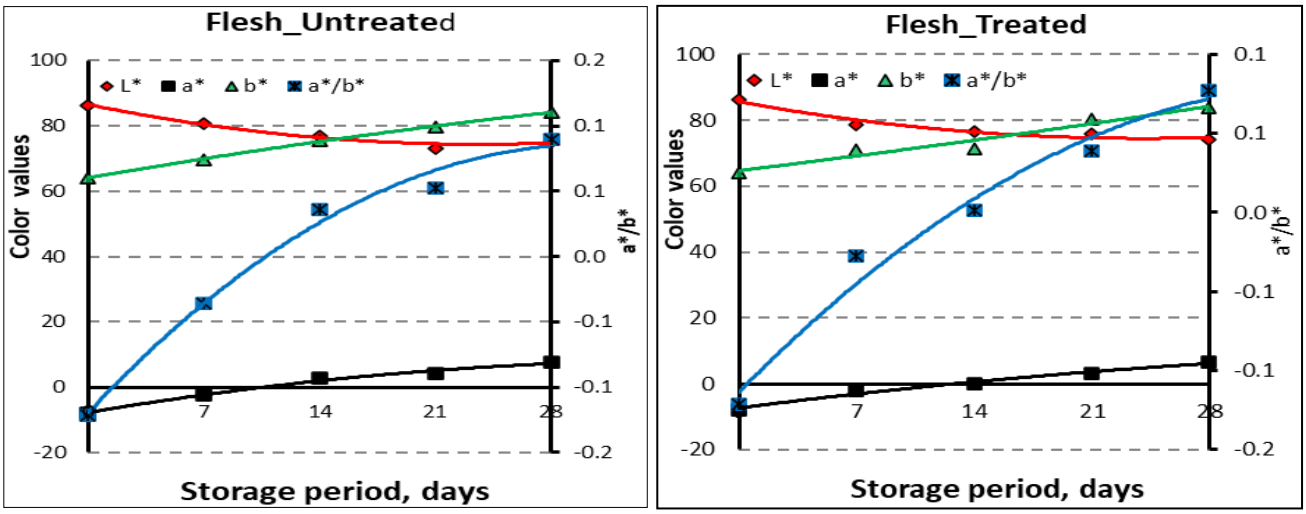

Fig 4: Flesh color change of mango during four-week of storage (Untreated/Treated fruits)

Skin / flesh firmness

Fruit firmness is a widely used parameter for assessing fruit quality. The findings indicated that the VH treatment did not have a significant impact on the reduction of fruit firmness in both the skin and flesh (Figure 5). As the storage period increased, the firmness of the mango fruit decreased (Table 4). Throughout the storage period of mangoes, no notable differences in firmness were observed between the treated and untreated fruits. This observation implies that the presence of obstacles and the enhanced activity of pectin hydrolysis due to the VH treatment might have contributed to this outcome. The importance of these factors in determining fruit hardness after heat treatment was emphasized (Shalom *et al*., 1996) [30]. Conversely, Jacobi and Giles (1997) [16] reported that Kensington mangoes treated for 15 minutes were softer than untreated ones. On the other hand, Le *et al*. (2010) [20] documented that both VH treatment and hot water treatment applied to mangoes could maintain firmness for one week during storage, but it declined after three weeks. Omoba and Onyekwere (2016) [26] state that fruit softening is a biochemical process involving the enzymatic hydrolysis of pectin and starch by cell wall hydrolases. In this context, the effective VH treatment employed in this study may have inhibited the activity of these enzymes, thereby preserving the firmness of the treated fruits. Furthermore, the similarity in firmness between the treated fruits and the untreated fruits can be attributed to the antifungal properties of the VH treatment, which likely reduced infection, respiration, and other ripening processes in the treated fruits.

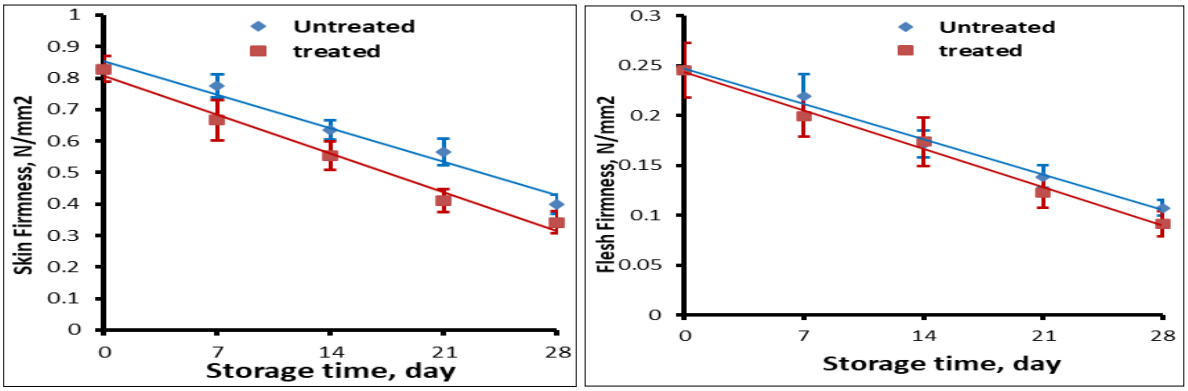

Fig 5: Firmness change of mango (Skin /Flesh) during four-week of storage

Table 4: Skin/ flesh firmness of mango during storage period

			Storage time, days				
			Harvest day	7	14	21	28
Skin firmness, N/mm^2	Untreated	Mean	0.829	0.774	0.634	0.566	0.399
		Max	0.952	0.821	0.696	0.664	0.475
		Min	0.752	0.681	0.579	0.505	0.352
		SD	0.041	0.037	0.031	0.043	0.031
		CV %	5.014	4.753	4.911	7.537	7.651
	Treated	Mean	0.829	0.666	0.553	0.411	0.341
		Max	0.952	0.769	0.65	0.503	0.389
		Min	0.752	0.516	0.479	0.357	0.257
		SD	0.041	0.063	0.046	0.037	0.035
		CV %	5.014	9.506	8.265	9.062	10.156
Flesh firmness, N/mm^2	Untreated	Mean	0.245	0.219	0.171	0.139	0.108
		Max	0.294	0.263	0.198	0.166	0.124
		Min	0.205	0.177	0.151	0.121	0.09
		SD	0.028	0.022	0.013	0.012	0.008
		CV %	11.225	10.043	7.828	8.41	7.386
	Treated	Mean	0.245	0.199	0.174	0.123	0.092
		Max	0.294	0.238	0.212	0.154	0.112
		Min	0.205	0.167	0.127	0.096	0.071
		SD	0.028	0.020	0.024	0.016	0.013
		CV %	11.225	10.25	13.946	12.698	13.745

Total soluble solids (TSS) of Mango fruit

The findings from the study showed that the application of VH treatment had no significant impact on the level of fruit sugar, as indicated in Table 5 and Figure 6. During the storage period, a minor elevation in total soluble solids (TSS) was noted, which could be attributed to the conversion of starch into simpler sugars through hydrolysis (Yaman and Bayoindirli, 2002) [32]. This aligns with the findings of Le *et al*. (2010) [20], who reported that VH treatment did not induce changes in the TSS of mangoes stored for three weeks. Similarly, Kim *et al.* (2009) [18] observed that subjecting mangoes to heat treatment at 46.1°C for various durations (70, 90, and 110 minutes) did not have a significant effect on the TSS. Also, Ekran *et al.* (2005) [8] documented that heat treatment did not consistently affect the TSS. However, it is worth noting that the non-significant increase in TSS observed in the treated fruits during storage coincided with the findings of Alikhan *et al*. (2007) [2]. In contrast, Helmiyesi *et al.* (2008) [11] demonstrated that an increase in TSS during the post-harvest period was attributed to the breakdown of polysaccharides into sugars (sucrose, glucose, fructose), while a decrease in TSS was associated with a depletion of polysaccharide reserves.

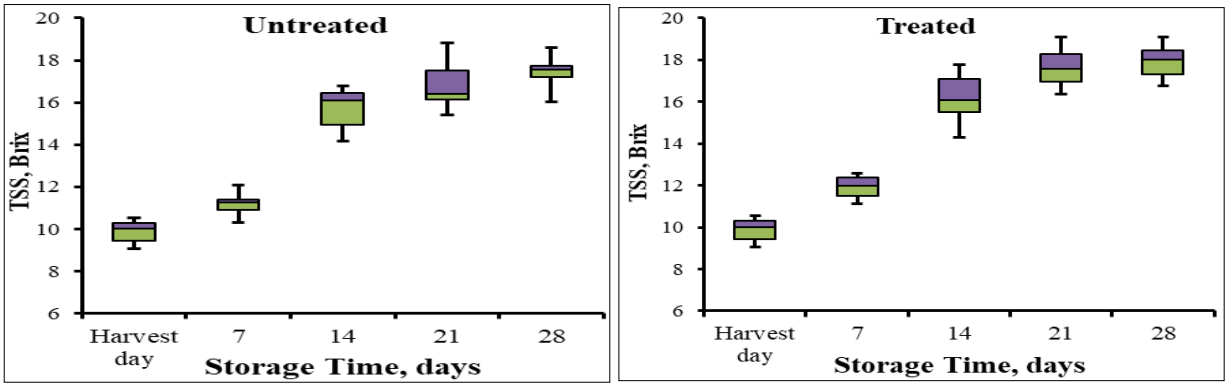

Fig 6: TSS change of mango fruits during four-week of storage

Table 5: TSS change of mango during storage period

TSS, Brix		Storage time, days				
		Harvest day	7	14	21	28
Untreated	Mean	9.90	11.20	15.68	16.72	17.42
	Max	10.5	12.1	16.8	18.8	18.6
	Min	9.1	10.3	14.2	15.4	16
	SD	0.47	0.43	0.89	0.95	0.66
	CV %	4.75	3.80	5.67	5.65	3.77
Treated	Mean	9.90	11.90	16.20	17.61	17.91
	Max	10.5	12.6	17.8	19.1	19.1
	Min	9.1	11.1	14.3	16.4	16.8
	SD	0.47	0.50	0.99	0.72	0.71
	CV %	4.75	4.21	6.09	4.07	3.96

Titratable acidity (TA) of Mango fruit

During the storage period, there was no significant impact on TA observed between untreated and treated mango fruits, as indicated by Table 6 and Figure 7. As the storage duration increased, the acidity of the mango fruits decreased. Mango fruits harvested on the first day exhibited the highest acidity (0.62%), while the lowest acidity (0.30%) was found in untreated mango fruits stored for 28 days (Figure 7).

The reduction in TA could be attributed to the significant depletion of organic acids during extended storage, as noted by Kelany *et al.* (2010) [19]. McCollum *et al.* (1993) [23] also observed similar findings regarding TA in heated "Keitt" mango fruits at 38°C for two days, reporting lower acidity. Additionally, Hoa *et al.* (2010) [14] reported that subjecting fruits to hot air treatment at 47ºC for 120-180 minutes resulted in decreased ascorbic acid content compared to untreated fruits.

Table 6: TA change of mango during storage period

TA, %		Storage time, days				
		Harvest day	7	14	21	28
Untreated	Mean	0.62	0.53	0.48	0.35	0.30
	Max	0.66	0.57	0.52	0.39	0.32
	Min	0.57	0.49	0.44	0.32	0.28
	SD	0.03	0.02	0.03	0.02	0.01
	CV %	4.75	3.80	5.67	5.65	3.77
Treated	Mean	0.62	0.50	0.40	0.38	0.32
	Max	0.66	0.53	0.44	0.41	0.34
	Min	0.57	0.46	0.35	0.35	0.30
	SD	0.03	0.02	0.02	0.02	0.01
	CV %	4.75	4.21	6.09	4.14	3.96

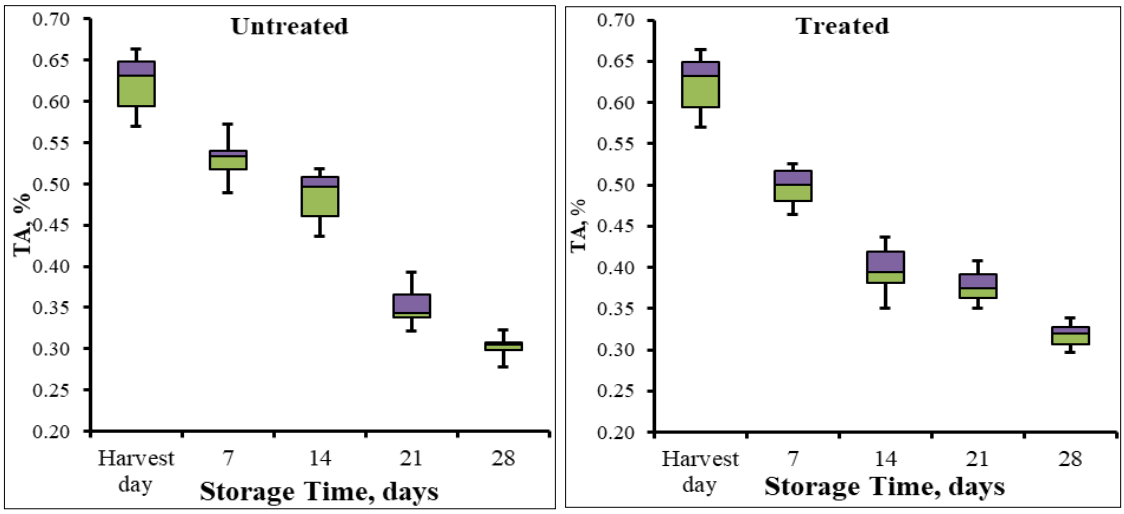

Fig 7: TA change of mango fruits during four-week of storage

Conclusion

The implementation of the quarantine VH treatment (46.2°C for 30 minutes) demonstrated a beneficial impact in eliminating the mortality of PFF eggs and larvae in Keitt mangoes. Both untreated and treated mango fruits exhibited no significant adverse alterations in quality throughout a four-week storage period. Therefore, the VH treatment at 46.2°C for 30 minutes can be successfully employed as a postharvest quarantine treatment for Keitt mango fruits intended for export to other countries using a similar VH treatment protocol.

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