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Fruit Sorting Based on Maturity Reduces Internal Disorders in Vapor Heat-Treated 'B74' Mango

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Abstract: Postharvest internal disorders (IDs) in mango fruit present a significant challenge to the industry, with their underlying causes still unclear. This study investigated the relationship between fruit maturity and the susceptibility of vapor heat-treated (VHT) 'B74' mangoes to IDs in three experiments. In the first experiment, fruit were categorized into three maturity groups based on dry matter content (DMC): <15%, 15–17%, and >17%, using a handheld near-infrared device. Half of the fruit in each group underwent VHT, while the remainder were untreated controls. Flesh cavity with white patches (FCWP) was the only disorder observed exclusively in VHT fruit. The incidence and severity of FCWP was significantly higher ($p < 0.05$) in fruit with <15% DMC, with 12.4% incidence and a severity score of 0.2 on a 0–3 scale (0: healthy and 3: severely affected), compared to more mature fruit. In the second experiment, the fruits were harvested at early and late maturity stages, with average DMC values of 14.5% and 17.4%, respectively. The fruit was subjected to no VHT, VHT, and VHT following a 12 h pre-conditioning period at 37 ± 1 °C. Consistent with the first experiment, FCWP was observed only in VHT fruit, with early-harvested fruit displaying a significantly higher ($p < 0.05$) FCWP incidence (26.9%) and severity (0.3) compared to late-harvested fruit (8.3% incidence and 0.1 severity). Pre-conditioning significantly reduced FCWP, particularly in early-harvested fruit. In the third experiment, fruit maturity sorted based on density was assessed, followed by VHT and simulated sea freight under controlled (CA) and ambient atmospheres. Fruit density did not effectively differentiate maturity considering DMC as a maturity indicator. Storage conditions significantly reduced ($p < 0.05$) flesh browning incidence from 71.1% under ambient conditions to 33.3% under CA. This study highlights fruit maturity as a key factor in the susceptibility of 'B74' mangoes to postharvest IDs following VHT. Therefore, sorting fruit based on DMC at harvest or at the packing facility prior to VHT serves as a valuable decision support for reducing IDs in VHT fruit. Further research will explore advanced technologies to enable rapid and efficient fruit sorting based on DMC.

Keywords: dry matter content; internal disorders; mango; vapor heat treatment

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1. Introduction

Tephritid fruit flies pose a serious quarantine risk [1], and freedom from infesting insects must be guaranteed for international trade with protocol countries. Postharvest

heat disinfestation by vapor heat (VHT) is a viable, non-chemical, and internationally acceptable control method for fruit flies in mango fruit [2,3]. VHT involves heating fruit with saturated hot vapor to a core temperature of 47 °C for 15 min [3]. However, VHT can result in internal disorders (IDs) characterized by cavities with white starchy tough lesions in the inner mesocarp [2,4–9]. VHT may damage fruit due to induced internal O_2 deficiency that results in fermentation [4,10].

IDs in mango fruit are influenced by harvest maturity along with genetic, environmental, and management factors [11–13]. Relatively less mature mango fruit were prone to developing flesh cavity with white patches (FCWP) following hot water treatment (HWT) or VHT [7–9,14]. Immature 'Carabao' mango fruit were more susceptible to internal cavity and starchy lesions following VHT (fruit core temperature 46 °C for 10 min) than mature fruit [4]. Less mature 'B74' mango fruits were also more susceptible to FCWP after VHT (47 °C fruit core for 15 min) than the more mature fruit [7–9]. Similarly, immature 'Kensington Pride' were more susceptible to FCWP following HWT (47 °C fruit core for 15 min) than mature fruit [15].

In the above-mentioned studies, the maturity assessment was subjective, making it difficult to identify fruit sensitive to IDs. Ideally, objective indicators of maturity stages sensitive to IDs are required. Dry matter content (DMC; represented as a percentage of dry weight to fresh weight) is one such objective indicator of maturity [16]. It is used for maturity prediction in various fruit crops, including mango [17].

A 24 h delay under typical ambient temperature before treatment [14] or a temperature conditioning treatment at 32–42 °C for 8–12 h before VHT/HWT helped reduce heat-induced disorders in mangoes [2,3,14,18–20], especially in less mature fruit [7,14]. Temperature conditioning involves pre-treating the product at an elevated temperature above the prescribed storage temperature for a specific duration [3].

Flesh browning (FB) is another important ID in 'B74' mango fruit [7,9]. It is thought to be associated with extended storage periods and/or significant delays between harvest and commencement of cold storage [21]. The exact causes and mechanisms of FB in mango remain unresolved. However, FB in temperate fruits, such as apples and pears, has been studied extensively [22–25]. It is influenced by combinations of specific pre- and postharvest factors [22]. FB has been linked to fruit maturity (viz., higher in more mature fruit) and storage conditions (i.e., longer storage and low O_2 with elevated temperature) [22,24,26]. Nonetheless, studies on the role of maturity and storage conditions in mango fruit are limited.

Likewise, limited information exists on the influences of maturity on the susceptibility of 'B74' mango fruit to IDs. It was hypothesized that the sensitivity of 'B74' mango fruit to IDs is largely a function of fruit maturity. Sensitivity to FCWP after VHT seemingly decreased with more advanced fruit maturity. Conversely, fruit sensitivity to FB apparently increased with a higher maturity irrespective of VHT. Several studies have explored the relationship between fruit maturity and IDs in mango fruit [7–9,15,19,27], offering a prospective selection criterion to mitigate IDs.

2. Materials and Methods

This study investigated the relationship between fruit maturity and the susceptibility of 'B74' mangoes to IDs in three serial experiments.

2.1. Experiment 1: Harvest Maturity (DMC) Effects on IDs of 'B74' Mango Fruit

2.1.1. Fruit Source

Fruit was sourced from a commercial mango farm near Childers, Queensland, Australia (−25.14° S, 152.37° E). The 7-year-old 'B74' mango trees were on 'Kensington Pride' rootstock. Tree spacing was 7 m between rows and 4 m within rows.

2.1.2. Fruit Harvest

Fruit was harvested with short peduncles using hand-held secateurs and picking poles. Fruit was placed in commercial fibreboard mango trays lined with polypropylene

inserts. They were held in a vehicle overnight at ambient temperature (ca. 25 °C) and then transported ~365 km for ~5 h in an air-conditioned vehicle to the postharvest laboratory at Gatton Campus within 20 h of harvest. After overnight storage at ~16 °C, fruit were de-stemmed and de-sapped in 2% (*w*/*v*) Mango-Plus® aqueous solution (580 g/kg alkaline salts and 500 ppm available chlorine; Avanti Chemicals, Ormeau, QLD, Australia) for 90 s and then dipped in (0.05%) aqueous Sportak® (450 g a.i./L prochloraz; FCM Australasia Pty. Ltd., Murarrie, QLD, Australia) fungicide for 30 s. Fruit were then air-dried and held at ~16 °C overnight.

2.1.3. Treatments

Fruit was classified into three maturity grades based on DMC: less mature: <15% DMC, optimum maturity: 15–17% DMC, and more mature: >17% DMC (Table 1). Half of the fruit from each maturity class underwent VHT and the remainder were untreated control fruit (Table 1). The next day fruit were transported by air-conditioned vehicle (~72 km in ~1 h) to a commercial VHT facility at Rocklea, Brisbane (−27.53° S, 152.99° E) for VHT. The control fruit were maintained at ~20 °C and ~90% RH at the laboratory. Individual fruit maturity in terms of DMC was assessed by portable near-infrared (NIR) (F-750 Produce Quality Meter, CID Bio-Science, Inc., Washington, DC, USA) device [28].

Table 1. Experiment 1 treatments to assess fruit maturity as the dry matter content (DMC) effect on incidence and severity of flesh cavity with white patches (FCWP) and flesh browning (FB) for 'B74' mango fruit in the 2022 season. DMC was assessed by a portable near-infrared F-750 Produce Quality Meter.

Maturity (DMC %)	VHT
<15	+
15–17	+
>17	+
<15	-
15–17	-
>17	-

2.1.4. Disinfestation Treatment (Vapor Heat Treatment, VHT)

VHT was performed in a commercial VHT facility of Perfection Fresh Pty Ltd. Rocklea, Brisbane (−27.53° S, 152.99° E). The fruit was tipped into bins and placed in the commercial VHT chamber. A pulp temperature probe was inserted into the center of a random fruit in the middle of each bin. VHT was applied to fruit in the bin as per the commercial protocol that the fruit core temperature is maintained at 47 °C for 15 min under >90% relative humidity (RH) [29]. The temperature of the fruit was gradually increased from ~18 °C to 47 °C and RH was recorded to be ~88% to 93% during the ~4 h treatment. Thereafter, fruit were maintained at 47 °C and RH for 15 min to complete the disinfestation process. Fruit was removed from the treatment chamber immediately after treatment.

2.1.5. Internal Disorder Assessment

After VHT, all fruit were transported in an air-conditioned vehicle to the postharvest laboratory at The University of Queensland, Gatton (~72 km; ~1 h) and stored at ~20 °C and ~90% RH until reaching the eating soft stage as per hand firmness ratings of 0–4 [21]. The time taken for fruit to reach firmness stage 4 (eating soft) was recorded as its shelf-life. At the eating soft stage/end of shelf-life, individual fruit were sliced longitudinally on both sides of the seed and visually assessed for incidence and severity of the IDs' flesh cavity with white patches (FCWP) and flesh browning (FB). FCWP and FB severity for individual fruit were rated on a scale 0–3, whereby 0: healthy, 1: slight, 2: moderate, and 3: severe [7].

2.1.6. Starch Iodine Staining

Three fruits from each maturity class were randomly sampled after VHT. Fruit was longitudinally sliced on both sides of the seed. Lugol's solution (1 g potassium iodide plus 0.25 g iodine in 100 mL water; Plant Essentials, Deeragun, QLD, Australia) was applied to the fruit halves by paint brush and left for 30 s. Starch reacts as a dark stain after Lugol's solution application [30]. During ID assessment, fruits with FCWP were stained with Lugol's solution to test whether white patches in VHT-induced FCWP represented starchy lesions.

2.1.7. Experiment Design and Data Analysis

The experiment consisted of two factors: three maturity class and +/− VHT (Table 1). Each treatment was replicated three times, with each replicate consisting of a tray of 20 fruit. An analysis of variance between treatments was performed to compare treatments, with means separated by Tukey's HSD at $p < 0.05$. For the +/− VHT factor, means were separated by a *t*-test at $p < 0.05$. Data were analyzed using JMP Pro 16.0.0 (JMP Statistical Discovery LLC, 920 SAS Campus Drive, Cary, NC, USA).

2.2. Experiment 2: Effects of Maturity Stage on IDs of 'B74' Mango Fruit

2.2.1. Fruit Source and Treatments

Fruit was sourced and handled as described for experiment 1. Two factors were tested in this experiment: fruit maturity stage and conditioning treatment prior to VHT. Fruit was harvested at two maturity stages, i.e., early (1-week before commercial harvest) and late (1-week after commercial harvest), over a 2-week period (Table 2). Fruit was then placed at ~16 °C, except those subjected to conditioning treatment, which were stored at 37 ± 1 °C for 12 h. The treatments imposed are shown in Table 2. The fruits were then transported for VHT, as described in experiment 1. Fruit maturity, disinfestation treatment, firmness, shelf-life, starch staining, and ID assessment were as described above.

Table 2. Experiment 2 treatments to assess maturity stages and the temperature conditioning effects on the incidence and severity of flesh cavity with white patches (FCWP) and flesh browning (FB) on 'B74' mango fruit in the 2022 season. Fruit was harvested at two maturity stages, i.e., early (1-week before commercial harvest) and late (1-week after commercial harvest), over a 2-week period.

Harvest	Conditioning at 37 ± 1 °C for 12 h	VHT
Early	−	−
Early	−	+
Early	+	+
Late	−	−
Late	−	+
Late	+	+

2.2.2. Starch Iodine Staining

Before taking fruit to the VHT, three fruit from each conditioned and non-conditioned treatment were randomly selected and treated with Lugol's solution (Plant Essentials, Deeragun, QLD, Australia), as in Section 2.1.6. This was conducted to observe the effect of conditioning on starch hydrolysis prior to VHT.

2.2.3. Experiment Design and Data Analysis

The experiment consisted of six treatments (Table 2), with each treatment replicated three times, and each replicate consisting of a tray with 20 fruits. An analysis of variance was performed to compare treatments, with mean differences separated by Tukey's HSD at $p < 0.05$. For maturity effect comparison, means were separated by a *t*-test at $p < 0.05$. Data were analyzed using JMP Pro 16.0.0 (JMP Statistical Discovery LLC, 920 SAS Campus Drive, Cary, NC, USA).

2.3. Experiment 3: Effect of Fruit Maturity (Sorted Based on Density) on IDs of 'B74' Mango Fruit with Controlled Atmosphere Storage

2.3.1. Fruit Source and Treatments

Fruit was sourced as described above. Fruit were sorted into two maturity classes based on density [31]. Fruit was harvested as in experiment 10 and placed in 25 L of aqueous salt solution (2% *w*/*v*) (Saxa iodized table salt; distributed by Salpak Pty Ltd., Seven Hills, NSW, Australia). Floaters were considered less mature and sinkers were considered more mature [31]. Fruit was held overnight and transported the next day in an air-conditioned car to the Maroochy Research Facility (MRF; ~213 km; ~3 h). Fruits were de-stemmed, de-sapped, and fungicide-treated as in experiment 10. Fruits were then air-dried and held at ~16 °C overnight. On the next day, they were transported by air-conditioned vehicle over ~116 km in ~2 h to a commercial VHT facility at Rocklea, Brisbane (−27.53° S, 152.99° E). VHT was performed as in experiment 10, after holding fruit for 21 h at ambient conditions. After VHT, fruit were transported to the MRF in an air-conditioned car and placed at ~16 °C overnight. After an initial fruit DMC assessment as in experiment 1, fruit from each maturity class were stored at two different conditions, i.e., controlled atmosphere (CA: Temperature 11 °C; gas composition 3% O_2 and 5% CO_2), and the control (Temperature: 11 °C; ambient atmosphere), for 21 days (Table 3). After this period, the fruits were placed in a shelf-life room at ~20 °C and ~90% RH till the eating soft stage. ID assessment was conducted as described in experiment 1.

Table 3. Experiment 3 treatments to assess maturity (sorted based on density) effect on flesh cavity with white patches (FCWP) and flesh browning (FB) incidence and severity on 'B74' mango fruit in the 2022 season.

Maturity	VHT	Storage for 21 Days at 11 °C
Floating	+	CA (3% O_2 and 5% CO_2)
Floating	+	Control (ambient atmosphere)
Sinking	+	CA (3% O_2 and 5% CO_2)
Sinking	+	Control (ambient atmosphere)

2.3.2. Experiment Design and Data Analysis

The experiment was designed with two maturity levels and two storage conditions (Table 3). Each treatment was replicated three times, with each replicate consisting of a tray containing 15 fruits. An analysis of variance was conducted to compare treatments, and mean differences were separated using a *t*-test at a significance level of $p < 0.05$. Data were analyzed using JMP Pro 16.0.0 (JMP Statistical Discovery LLC, 920 SAS Campus Drive, Cary, NC, USA).

3. Results

3.1. Experiment 1: Harvest Maturity (DMC) Effects on IDs of 'B74' Mango Fruit

3.1.1. Incidence and Severity of Internal Disorders

The maturity effects on FCWP incidence and severity were significant ($p < 0.05$). FCWP was observed only in VHT fruit. VHT fruit with <15% DMC had a significantly ($p < 0.05$) higher FCWP incidence and severity (Table 4). However, FCWP incidence and severity in VHT fruit with >15% DMC were similar ($p > 0.05$) to the untreated controls (Table 1). FB was not observed in this experiment.

3.1.2. Starch Iodine Staining

The starch iodine staining of fruit at different maturity after VHT showed that, in mature fruit, starch hydrolysis commenced in the inner mesocarp near the seed, where FCWP is typically observed (Figure 1). In less mature fruit, starch hydrolysis did not progress appreciably. Iodine staining confirmed that the white lesions within the mesocarp were starch (Figure 2). Thus, the hydrolysis of starch was disrupted by VHT.

Table 4. Fruit maturity (dry matter content—DMC), vapor heat treatment (VHT), and interaction effects for incidence and severity (means ± SE) of flesh cavity with white patches (FCWP) in 'B74' mango fruit in the 2022 season. Analysis of variance between treatments was tested and significant means were separated by Tukey's HSD at $p < 0.05$. In the case of +/− VHT, means were separated by a *t*-test at $p < 0.05$.

Maturity (DMC)		FCWP Incidence (%)	FCWP Severity (0–3)
<15 (n = 60)		12.4 ± 5.7 [a]	0.2 ± 0.1 [a]
15–17 (n = 60)		3.9 ± 2.6 [b]	0.1 ± 0.1 [b]
>17 (n = 60)		2.4 ± 2.4 [b]	0.1 ± 0.1 [b]
Treatment			
Non-VHT (n = 90)		0.0 ± 0.0 [b]	0.0 ± 0.0 [b]
VHT (n = 90)		12.4 ± 3.7 [a]	0.2 ± 0.1 [a]
Treatment × Maturity (DMC)			
VHT	<15 (n = 30)	24.6 ± 2.4 [a]	0.4 ± 0.1 [a]
	15–17 (n = 30)	7.7 ± 4.4 [b]	0.1 ± 0.0 [ab]
	>17 (n = 30)	4.8 ± 4.8 [b]	0.1 ± 0.1 [b]
Non-VHT	<15 (n = 30)	0.0 ±0.0 [b]	0.0 ± 0.0 [b]
	15–17 (n = 30)	0.0 ±0.0 [b]	0.0 ± 0.0 [b]
	>17 (n = 30)	0.0 ±0.0 [b]	0.0 ± 0.0 [b]

Mean values followed by the same letter(s) are not significantly ($p < 0.05$) different.

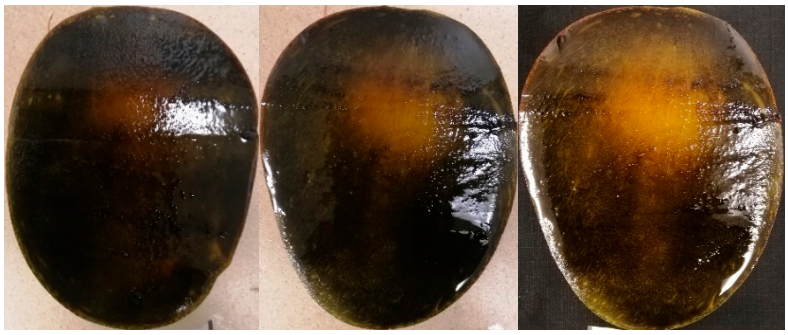

Figure 1. Starch iodine staining for starch after VHT in less mature (starch hydrolysis not initiated; **left**), optimum maturity (starch hydrolysis initiated in the middle of the flesh; **middle**), and more mature (advanced stage of starch hydrolysis as indicated by yellow flesh; **right**) fruit. Starch stains black.

3.2. Experiment 2: Effects of Maturity Stages on IDs of 'B74' Mango Fruit

3.2.1. Fruit Maturity

The average DMC values were 14.5% (range of 12.2–17.4%) and 17.4% (range of 14.6–21.2%) for early- and late-harvested fruit, respectively.

3.2.2. Incidence and Severity of Internal Disorders

The maturity stage significantly ($p < 0.05$) affected the incidence and severity of FCWP (Table 5). Late-harvested fruit showed a 70% reduction in FCWP versus early-harvested fruit. As in the previous experiment, the untreated fruit had no FCWP. Fruit conditioned at 37 ± 1 °C for 12 h prior to VHT had significantly reduced FCWP disorder (Table 5), with seven- and four-fold reductions in the early and late harvest, respectively. FB was observed only in a few late-harvested fruits (Table 5).

Figure 2. Starch iodine staining affirming disruption of starch hydrolysis in fruit expressing flesh cavity with white patches. White lesions in flesh after VHT (**left**) are starchy as confirmed by black starch-iodine staining (**right**).

Table 5. Fruit maturity, vapor heat treatment (VHT) with and without temperature preconditioning at 37 ± 1 °C, and interactive effect on incidence and severity (mean ± SE) of flesh cavity with white patches (FCWP) and flesh browning (FB) on 'B74' mango fruit in the 2022 season. Analysis of variance across treatments was tested and means were separated by Tukey's HSD at $p < 0.05$. In the case of maturity, means were separated by a *t*-test at $p < 0.05$.

Harvest/Maturity		FCWP Incidence (%)	FCWP Severity (0–3)	FB Incidence (%)	FB Severity (0–3)
Early (14.5% DMC)		26.9 ± 11.2 ^{a}	0.3 ± 0.1 ^{a}	0.0 ± 0.0	0.0 ± 0.0
Late (17.4% DMC)		8.3 ± 3.4 ^{b}	0.1 ± 0.1 ^{b}	2.9 ± 1.5	0.1 ± 0.1
Treatments					
Non-VHT		0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	1.7 ± 1.7	0.0 ± 0.0
VHT		45.0 ± 11.9 ^{a}	0.5 ± 0.2 ^{a}	1.7 ± 1.7	0.0 ± 0.0
Conditioning + VHT		7.8 ± 3.0 ^{b}	0.1 ± 0.0 ^{b}	0.8 ± 0.8	0.0 ± 0.0
Harvest × Treatments					
Early harvest	Non-VHT	0.0 ± 0.0 ^{c}	0.0 ± 0.0 ^{b}	0.0 ± 0.0	0.0 ± 0.0
	VHT	70.0 ± 7.6 ^{a}	0.8 ± 0.1 ^{a}	0.0 ± 0.0	0.0 ± 0.0
	Conditioning + VHT	10.6 ± 5.2 ^{bc}	0.1 ± 0.1 ^{b}	0.0 ± 0.0	0.0 ± 0.0
Late harvest	Non-VHT	0.0 ± 0.0 ^{c}	0.0 ± 0.0 ^{b}	3.3 ± 3.3	0.0 ± 0.0
	VHT	20.0 ± 5.0 ^{b}	0.2 ± 0.0 ^{b}	3.3 ± 3.3	0.0 ± 0.0
	Conditioning + VHT	5.0 ± 2.9 ^{c}	0.03 ± 0.0 ^{b}	1.7 ± 1.7	0.0 ± 0.0

Mean values followed by the same letter(s) are not significantly ($p < 0.05$) different.

3.2.3. Starch Iodine Staining

The initiation of starch hydrolysis was confirmed by the starch iodine staining of fruit exposed to the conditioning treatment. Non-conditioned fruit had more black stains in the whole flesh, signifying that starch hydrolysis had not begun. In contrast, conditioned fruit had no black stain in the center (~15% of fruit flesh), signifying starch hydrolysis underway (Figure 3).

Figure 3. Starch iodine staining for starch in mango fruit flesh with (starch hydrolysis initiated represented by yellow flesh inside red circle; (**right**)) and without (starch hydrolysis not initiated; (**left**)) temperature conditioning of 37 ± 1 °C prior to vapor heat treatment (VHT).

3.3. Experiment 3: Effect of Fruit Maturity (Sorted Based on Density) on IDs of 'B74' Mango Fruit

3.3.1. Fruit Weight and Maturity

The average fruit weight of floaters (496.9 g) and sinkers (514.0 g) did not differ significantly ($p > 0.05$). Similarly, the average DMC of floaters and sinkers were 15.3% and 15.9%, respectively, and they were not different ($p > 0.05$).

3.3.2. Incidence and Severity of Disorders

The effect of fruit maturity classes sorted based on density on the incidence and severity of FCWP and FB were not significant ($p < 0.05$) (Table 6). Storage conditions had significant effects ($p < 0.05$) on the incidence and severity of FB (Table 7). CA significantly ($p < 0.05$) reduced FB incidence and severity in ambient storage. There was no interaction ($p < 0.05$) between maturity and storage conditions on the incidence and severity of FB (Table 7).

Table 6. Fruit maturity (sorted based on density) effect on flesh cavity with white patches (FCWP) incidence and severity (mean ± SE) on 'B74' mango fruit in the 2022 season. Analysis of variance between treatments was tested and means were separated by a *t*-test at $p < 0.05$.

Maturity	FCWP Incidence (%)	FCWP Severity (0–3)
Floating ($n = 90$)	33.33 ± 3.4	0.3 ± 0.0
Sinking ($n = 90$)	36.67 ± 6.6	0.34 ± 0.1
Significance	NS	NS

NS: non-significant.

Table 7. Fruit maturity (sorted based on density) and storage conditions effect on FB incidence and severity (mean ± SE) on 'B74' mango fruit in the 2022 season. Analysis of variance between treatments was tested and means were separated by a *t*-test at $p < 0.05$.

Maturity	FB Incidence (%)	FB Severity (0–3)
Floating ($n = 90$)	57.8 ± 11.4	0.6 ± 0.2
Sinking ($n = 90$)	46.7 ± 8.4	0.5 ± 0.1
	NS	NS
Storage conditions		
Control ($n = 90$)	71.1 ± 5.1 [a]	0.8 ± 0.1 [a]
CA ($n = 90$)	33.3 ± 6.7 [b]	0.3 ± 0.1 [b]
Maturity × storage conditions	NS	NS

Mean values followed by the same letter are not significantly ($p < 0.05$) different. NS: non-significant.

4. Discussion

4.1. Flesh Cavity with White Patches

The overall results indicated that less mature fruit (<15% DMC) were more susceptible to FCWP after VHT (Tables 4 and 5). Likewise, Jacobi, MacRae [3] reported that less mature 'Kensington Pride' mango fruits were prone to starchy layers and spots in the mesocarp and that tolerance to HWT-induced disorders increased as maturity increased. Similarly, Esguerra, Brena [4], Jacobi, MacRae [15], and Brecht, Sargent [14] reported that less mature mango fruit were prone to cavity formation after VHT/HWT.

White starchy layers or spots in the flesh of VHT fruit may be attributable to the disruption of starch hydrolysis due to heat. Enzymes that convert starch into sugar may be heat-sensitive [2]. Disrupted starch degradation could thereby lead to white starchy regions in the flesh. This hypothesis was supported by starch iodine staining. White regions reflected high starch levels by staining with Lugol's solution (Figure 2, right). Fuchs, Pesis [32] found a significant increase in alpha-amylase activity in mango fruit from the 6th day of harvest, peaking on the 8th day postharvest. Peak alpha-amylase activity occurred earlier in mature than in less mature mango fruit. The acceleration of ripening processes in more mature fruit may produce sugar, particularly sucrose, sufficient to reduce injury from heat treatment [15]. Reduced starch content in more mature fruit than less mature fruit was evident by lesser starch iodine staining after VHT (Figure 1). Support for a protective role of sugar in different stresses is considered in Section 4.3.

Generally, injuries caused by VHT occur when the duration and/or temperature of heat treatment surpass recommended guidelines [14]. Towards avoiding VHT-induced injuries, it is important to monitor and manage the treatment process followed by prompt cooling. To prevent excessive water loss, which may be exacerbated by heat treatment, it is recommended to maintain a relative humidity of 90–95% and/or utilize plastic film liners or bags to protect the fruit [14].

The mechanism of cavity formation in heat-treated mango is elusive. Heat evidently disrupts cellular integrity and may reduce cell-to-cell adhesion. As surrounding tissue 'ripens', starch conversion into sugar may osmotically draw water away from the afflicted regions [2]. Cells damaged by heat in specific regions of the flesh would thereby experience dehydration and desiccated mesocarp tissue may collapse to create cavities in the flesh [2].

4.2. Flesh Browning

No relationship of fruit maturity to FB was discerned in this study. However, storage conditions influenced FB in mango fruit (Table 7). For example, the fruits in experiments 1, 2, and 3 were sourced from the same farm and block and handled in same way before allocation to treatments. A very high FB incidence was observed only in experiment 3 (Table 7). The differences between the experiments were the storage conditions after VHT. In experiments 1 and 2, the fruits were held at 20–22 °C and >95% RH immediately after VHT for ripening (Section 2.1.2 and 2.2.1). In contrast, in experiment 3, after VHT, the fruits were held at 16 °C overnight, followed by storage at 11 °C with and without CA for 21 days (Section 2.3.1). The fruit were then removed from storage conditions and held at 20–22 °C and >95% RH until eating ripe stage. Given that fruits stored for 21 days in CA and ambient conditions were the only ones that had FB, this suggests that FB is associated with a long storage time (Table 7). CA was able to significantly reduce the FB incidence. Other studies also suggest that FB is a function of storage time and temperature [14,21,33–35]. Longer storage durations can increase polyphenol oxidase activity in mango fruit, suggesting an intensification of oxidative processes [34]. Costa, Figueiredo Neto [36] reported that the FB index in 'Palmer' mangoes increased from 41.2 after 7 days to 79.3 after 28 days of storage at 16 °C.

FB in mangoes may be attributed to over-ripening [37]. Ripening involves alterations in the physiochemical properties of the cell wall, resulting in compositional and structural changes [38]. However, when fruit over-ripen, cell senescence may manifest as tissue browning. More mature fruits were more susceptible to FB disorder, possibly because diffusivity

to gases reduced as fruit mature and ripen [39]. Changes in the microstructure of fruit tissue during ripening include decreasing pore size, increasing pore fragmentation, and increasing pore specific surface area, with likely concomitant effects on respiratory metabolism [40]. The reduction in the ability of O_2 to diffuse into the fruit would result in localized O_2 starvation and build-up of CO_2 in the inner mesocarp towards fermentation [39].

4.3. Temperature Conditioning

Fruit conditioned at 37 ± 1 °C for 12 h prior to VHT had a significantly ($p < 0.05$) reduced FCWP disorder (Table 5). Unconditioned VHT fruit had a higher FCWP incidence compared to conditioned fruit. Jacobi, MacRae [15] and Klein and Lurie [20] also reported that conditioning before heat treatment protects fruit from stress injury and physiological disorders. Compared to maintaining fruit at ambient temperatures, HWT-treated mango fruit stored at 13 °C between harvest and treatment became unmarketable [41].

Elevated temperature conditioning affects fruit physiology. For instance, the acceleration of ripening is associated with starch degradation and the concomitant accumulation of sucrose. Starch iodine staining confirmed that conditioning treatment led to starch hydrolysis initiation in fruit. Non-conditioned fruits showed black stains throughout the mesocarp, while conditioned fruits had an unstained inner mesocarp (Figure 3). Researchers have also reported that partially ripe mango fruit is relatively more tolerant to physiological disorders, including chilling injury [42], under skin browning [43,44], and irradiation damage [27,45]. Collectively, these studies show that an elevated sugar content is important in enhanced fruit tolerance to stress. Sucrose protects cells from osmotic stress by stabilizing cellular structures and maintaining turgor pressure. It prevents damage to cell membranes under stress conditions, ensuring membrane integrity through its protective effect.

Overall, time delay or conditioning prior to heat treatment should be more beneficial for less mature fruit [14]. Nevertheless, pre-conditioning prior to VHT may not eliminate the FCWP disorder. In the present study, conditioned fruits had seven-fold reductions in FCWP incidence when harvested early compared to four-fold reductions in late-harvested fruit (Table 5). The relatively greater susceptibility in less mature fruit may be because more mature fruit have expressed enzymes for starch degradation, e.g., alpha amylase. Conversely, the activation of these enzymes in relatively immature fruit might not have progressed much before heat treatment, and hence, a subsequent VHT could inhibit their activity and contribute to the starchy layers observed.

However, for an effective commercial phytosanitary treatment, it is essential that treatments meet quarantine restrictions. In this regard, enhancing the thermotolerance of fruit through temperature conditioning may also enhance the thermotolerance of targeted insect pests [46]. Beckett and Evans [46] noted that conditioning led to an increased time required for conditioned *B. tryoni* larvae and eggs to reach mortality levels equivalent to those of non-conditioned larvae. Moreover, the efficacy of conditioning toward providing protection to fruit depends on several factors, including the season, fruit ripeness, pre-treatments, and temperatures [47].

5. Conclusions

FCWP and FB are the major IDs observed in this study. FCWP was induced by VHT, a mandatory disinfestation treatment for fruit export. VHT had differential effects on fruits of varying maturity. Less mature fruits (<15% DMC) were relatively more susceptible to FCWP after VHT. Temperature conditioning prior to VHT ameliorated heat injury more in less mature fruits than in more mature fruits but did not eliminate it. Overall, the physiological stage (i.e., age of fruit) at time of treatment affects fruit sensitivity to VHT injury. FB was observed in mango fruit at the end of shelf-life and a relationship with fruit maturity was not established in this study. FB in mango fruit was a function of postharvest time and temperature. Maintaining optimum temperature and/or targeting short supply chains may help to assure a reduced incidence of FB.

Sorting fruit based on DMC at harvest or at packing facility prior to VHT could serve as a valuable decision-making tool for reducing IDs in VHT fruit. Further research should explore the use of advanced technologies that enable effective and efficient fruit sorting based on DMC, e.g., in-line NIR. The more comprehensive characterization and quantification of positive and negative effectors and their interplay would inform better practice for supply of best consumer quality 'B74' fruit in export markets.

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