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Effect of pre - and post-harvest factors on 'Benny' Valencia fruit rind phenolics on mitigation of chilling and non-chilling disorders during cold storage

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ABSTRACT

Purpose: This study investigated the effect of harvest time, postharvest dehydration + waxing and storage temperature on rind-free and conjugated phenolics and their ability to alleviate chilling injury and pitting of 'Benny' Valencia oranges during cold storage. **Research method:** Fruit were harvested at early, mid- and late season, and thereafter, divided into control, dehydrated, waxed + dehydrated portions. After treatment, fruit were stored at -0.6 and 4.5°C for 28 days, thereafter, 7 days at ambient temperature (25°C). **Findings:** In general, peel pitting index (PPI) was significantly higher for late season fruit, while, CI was higher for early season fruit, especially at -0.6°C storage. Furthermore, dehydration stress without waxing resulted in significantly higher PPI and CI at -0.6°C when compared with 4.5°C storage. With respect to both free and soluble conjugated phenolics, the control fruit showed higher levels of rind phenolics, especially at late harvest across all the storage temperatures. Therefore, untreated fruit appeared to tolerate cold stress by up-regulating endogenous systems of total rind phenolics. Postharvest dehydration repressed endogenous phenolics synthesis. In conclusion, susceptibility to pitting disorder increases with harvest time, dehydration stress, while fruit harvested early were highly susceptible to CI. **Research limitations:** The main limitation of this study is the lack of specific phenolics. **Originality/Value**: The study found that dehydration plus waxing has a significant effect on chilling and non-chilling citrus 'Benny' Valencia fruit. Furthermore, these treatments induced an increase in rind total phenolics to mitigate rind physiological disorders during extended cold storage.

INTRODUCTION

In South Africa, citrus is the third largest horticultural industry after deciduous fruit and vegetable industries (Dodd et al., 2010; CGA, 2015). Globally, the industry is the second highest exporter of citrus fruit after Spain, exporting approximately 1,7 million tons annually (CGA, 2015). Exported citrus fruit from South Africa to USA, Korea, Thailand and China must be cold sterilised at -0.6°C for 22 days in order to comply with the phytosanitary requirements (Dodd et al., 2010; Hordijk, 2013). In general, cold sterilisation has been proved by Hordijk (2013) to be the best practice that kills insect larvae of major pest in citrus, including false codling moth (*Cryptophlebia leucotreta*) and Mediterranean fruit fly (*Ceratitis capitata*), However, it has previously been found that cold sterilisation may result in rind physiological disorder such as; chilling injury (CI) and rind pitting disorder (RPD) which manifest during shipment; and therefore, affecting fruit quality (Dou, 2004; Dou, 2005; Magwaza, 2008).

According to Magwaza et al. (2013), symptoms of non-chilling (pitting) and chilling rind disorders are non-distinguishable. Chilling injury manifest in the citrus rind initially as small sunken lesion that later enlarge and become dark-brown sport, thus reducing fruit marketability (Dou, 2004; Dou, 2005). While non-chilling rind disorders manifest as the collapse of the sub-epidermal rind cells with no discoloration taking place at early stages, at later stages, the clusters of oil gland are affected which release intercellular content that change colourless lesions to brown colour (Alquezar et al., 2010). Previous studies have attempted to study and understand CI and RPD mostly in citrus fruit types such as lemon fruit (Mathaba, 2012), mandarin fruit (Medeira et al., 1999; Joubert, 2016), navel oranges (Alférez, & Zacarías, 2000; Agustí et al., 2001) and 'Marsh' grapefruit (Alférez & Burns, 2004; Olarewaju et al., 2020). Recently, 'Benny' Valencia were found to be highly susceptible to peel pitting in South Africa (Ehlers, 2016; Cronje et al., 2017; Mothapo et al., 2022), of which the cause is unknown. However, as part of solution, various factors have been previously related with the occurrence of rind physiological disorders on other cultivars with no clear solutions.

Previously researchers investigated various factors that relate to development of rind physiological disorders on other citrus cultivars. In Spain and Florida, researchers investigated post-harvest RPD on grapefruit (*Citrus paradisi* Macfadyen) and found that the fluctuation in relative humidity at harvest and during storage caused rind physiological disorder (Alférez & Burns, 2004; Alférez et al., 2010; Alquezar et al., 2010) and similar findings were observed in 'Brigitta' norther highbush blueberry (*Vaccinium corymbosum*) (Moggia et al., 2023). According to Alférez et al. (2003), exposure to low relative humidity followed by rehydration at high relative humidity induces difference in cellular water potential between the rind tissues (flavedo and albedo), thereby, leading to albedo cells collapse. In contrast, Alférez and Zacharías (2014) reported that changes in rind morphological and their potential relation to the alteration in water status as induced by fluctuations of relative humidity during post-harvest handling is dependent on fruit maturity at harvest. The effect of maturity has yielded various conclusions in different citrus cultivars (Gonzalez-Aguilar et al., 2000).

Several researchers studied the effect of fruit maturity on physiological disorder in citrus and in accordance to their results early and late harvest times usually leads to more post-harvest RPD (Dou, 2005; Khumalo, 2006; Magwaza et al., 2013) and 'Brigatta' blueberries harvested early, peak, and late (Moggia et al., 2023). Furthermore, fruit maturity has been found to also influence phytochemical substances (alkaloids, cyanogenic glycosides, flavonoids, terpenoids and phenolic compounds) with antioxidant capacity. In citrus, phenolics are the most effective phytochemical substances and bioactive compounds with antioxidant properties, playing a crucial role to maintain fruit quality (Mditshwa, 2012). Under stress conditions, phenolics act as scavenging compounds against various reactive oxygen species (ROS), especially during cold storage (Sala, 1998). Oxidative stress occurs when production of ROS exceeds the capacity of the cell to scavenge and resulting in the occurrence of physiological disorders (Huang et al., 2007; Sala, 1998).

In pomegranate fruit, Fawole and Opara (2013) found that as maturation continues, several antioxidants (scavengers) are synthesized and such compounds determines the fruit quality attributes under cold storage. In the study of Wang and Lin (2000), higher total phenolics concentrations and total antioxidant activity were reported on late harvested blackberry, raspberry, and strawberry fruit. Conversely, there is sufficient evidence suggesting that waxing play a significant role in regulation of total phenolic metabolism (Meighani et al., 2015). However, the information is unclear. Thus, the aim of this study was to investigate the combined effect of harvest time, postharvest dehydration plus waxing and storage temperature on rind phenolics in order to alleviate manifestation of chilling injury and pitting of ‘Benny Valencia sweet oranges after cold sterilization.

MATERIALS AND METHODS

Experimental sites and procedures

Fruit were randomly harvested at early (3 weeks before commercial harvest - June 2021), mid- (commercial harvest on the - July 2021) and late (three weeks after commercial harvest - August 2021) from Mahela Boerdery commercial farm at Letsitele in Limpopo province, South Africa (23° 88′ 36″ S, 30° 82′ 34″ E). After harvest, fruit were transported to the Agricultural Research Council - Institute for Tropical and Subtropical Crops (ARC-ITSC) postharvest laboratory in Nelspruit (25° 45′ 18″ S, 30° 96′ 97″ E) and drenched with water containing Sporekill® (Dimethyldidecyl ammonium chloride, Hygrotech Pty LTD, South Africa) (120 mg L^{-1}), thereafter air dried. After air-drying, fruit were packed inside 18 crates (0.14 m^{-2}), each containing 25 fruit. At each harvest, ten fruit sample were collected before storage and used for immediate external and internal quality, while the additional ten fruit per each treatment were stored for analysis after storage.

Post-harvest treatments

The experiment comprised of three treatment factors: harvest time (early, mid- and late), three post-harvest treatments [Wax only (A), wax plus dehydration (B) and dehydration only (C)] and two storage temperatures (-0.6±0.1 and 4.5±0.1°C). From the 18 crates, one crate fruit (n = 25) were randomly allocated to each of the three treatments, which were replicated three times. Fruit allocated for treatment A (n = 25) served as control and were waxed using Citrishine® (Citrishine Pty Ltd, Johannesburg, South Africa), thereafter transferred directly to cold storage temperature (0.6±0.1 and 4.5±0.1°C) with 90±1% relative humidity (RH). Fruits for treatment B (n = 25) were also waxed before storage. However, prior to storage, 3 days’ dehydration stress was applied to fruit allocated for treatment B by dehydrating fruit at constant condition of 25°C with 45±1% RH for 3 days, in order to create an environment conducive to induce high vapour pressure deficit (0.7-1.1 kPa) (Alférez et al., 2003). Fruit allocated for treatment C (n =25) received similar dehydration stress as treatment B; however, did not receive wax application. Thereafter, fruit were exposed to 3 days of dehydration stress (Treatments B and C) thereafter transferred to different cold storage temperature (-0.6±0.1 and 4.5±0.1°C), for up to 28 days. After 28 days of cold storage, fruit were kept at room temperature for 7 days to allow citrus rind pitting and chilling injury to manifest. Storage temperature and relative humidity were monitored by means of temperature/relative humidity logger (Tinytag View 2 TV-4500 Gemini Data Loggers (UK) Ltd. After 7 days' shelf-life, fruit were evaluated for rind physiological disorder (peel pitting and chilling injury), electrolyte leakage, weight loss, firmness loss, rind colour and internal fruit quality.

Estimation of Peel pitting/ chilling injury rind physiological disorders

Fruit were evaluated visually before cold storage and after 7 days' shelf-life. In each fruit, number and cluster of sunken glands were counted. Visual appearance (peel pitting) was assessed based on a 4-point hedonic scale: [0 (no post-harvest pitting: 0%), 1 (low post-harvest pitting: 25%), 2 (low to moderate post-harvest pitting: 50%), 3 (moderate to high pitting: 75%) and 4 (severe post-harvest pitting: 100%)]. Peel pitting incidence was calculated using the formula previously reported by Lafuente and Sala (2002) as follow (1):

$$\text{Peel Pitting Index (PPI)} = \sum(\text{Peel damage scale (0–4)} \times \text{number of fruit within each class}) / \text{Total number of fruit} \qquad (1)$$

Weight loss

Fruit weight was measured before cold storage (W_0) and after 7 days' shelf-life (W_1) using weighing scale (Scaltec, SBA, Heilingenstadt, Germany). Weight loss was calculated using the following formula (2):

$$\text{Weight loss (\%)} = \left[\frac{W0-W1}{W0}\right] \times 100\% \qquad (2)$$

Electrolyte leakage

Membrane leakage was determined using fruit disks following the method of Bajji et al., (2002) and calculated using the following formula (3):

$$\text{Total electrolyte leakage (\%)} = (EC_1/EC_2) \times 100 \qquad (3)$$

Firmness loss

Fruit firmness loss was measured before cold storage (F_1) and after 7 days of shelf-life (F_2) using Sinclair desktop machine (Model: 51DFTB, International LTD, Jarrold, Bowthorpa, Norwich, NR5, 9.D, England) and calculated using the following formula (4):

$$\text{Firmness loss (\%)} = \left[\frac{F1-F2}{F1}\right] \times 100\% \qquad (4)$$

Determination of total phenolic concentration

Total phenolic concentration (TPC) was measured using the Folin–Ciocalteu (Folin–C) method as described by Abeysinghe et al. (2007) with some modification. Pulverized lemon peel (0.5 g) was accurately weighed in a test tube. For both free and conjugated phenolics, samples were extracted with 5 ml of 50% DMSO: 50% of 1.2 M HCl in 80% methanol/water, vortexed for 1 minute and centrifuged at 10 000 rpm for 10 min to remove the solid fraction. The resultant supernatant was used for determination of free phenolics. Therefore, for extraction of conjugated phenolics, samples were heated at 90°C for 3 hours, with vortexing every 30 minutes and after allowed to cool down to room temperature, following which they were diluted to 10 ml with methanol and centrifuged at 10 000 rpm for 10 minutes to remove the solid fraction. Thereafter, Folin-Ciocalteu reagent (0.5 ml) was added to the solution and allowed to react for 3 minutes. The reaction was neutralized with 1 ml of sodium carbonate (2%) solution. The mixture was then vortexed, and absorbance read at 760 nm using a UV–visible spectrophotometer (Thermo Scientific Technologies, Madison, Wisconsin). Gallic acid was used as standard (0.02−0.10 mg mL^{-1}) and data were expressed as milligram gallic acid equivalent per 100 mL DW (mg GAE 100 mL^{-1} DW).

Statistical analysis

Analysis of variance was performed using using GenStat 16th edition (VSN international, UK). Means were separated using Least Significant Difference Test (LSD) at probability level of ($P < 0.05$). Relationship between weight loss vs peel pitting index and chilling injury index vs electrolyte leakage was performed using Pearson correlation method.

RESULTS AND DISCUSSION

Peel pitting

In this study, peel pitting symptoms appeared as cluster of sunken areas (‘pits’) on the flavedo that eventually affected oil glands and were higher in fruit stored at -0.6°C when compared with fruit stored at 4.5°C, irrespective of harvest time (Fig. 1). This could be as result of rind ultrastructural changes and breakdown of the external cellular strata due to chilling temperature (Vercher et al., 1994). Moreover, peel pitting index (PPI) increased with maturity and was significantly higher at late harvest, especially for unwaxed fruit exposed to dehydration stress after removal from storage at -0.6 and 4.5°C. These results were similar to those previously reported by Alférez and Zacarías (2014), whereby, peel pitting incidence were significantly higher for late season and dehydrated ‘Navelin’ navel fruit stored at 20°C for 28 days. Meanwhile, waxed fruit either with dehydration or non-dehydration treatment showed less susceptibility to the incidence of peel pitting when compared with unwaxed fruit, irrespective of harvest time and storage temperature. Generally, fruit exposed to dehydration stress at low RH experience higher moisture loss, reducing flavedo and later albedo cells water potential (Agustí et al., 2001; Alférez et al., 2003; Alférez et al., 2005). In the findings of Alquezar et al., (2010), higher rind pitting was observed on ‘Nave’ orange fruit stored at 45% RH when compared with 95% RH at 20°C for 5 weeks. According to Alférez et al. (2003), Alférez et al. (2004), and Ehlers (2016), when fruit is transferred to the water-saturated atmosphere (95% RH) where vapour pressure deficit (VPD) was low, water moves from the surroundings to the epidermal cells, resulting into rehydration of cells. Flavedo and outer albedo cells rehydrate fast when compared with subtending albedo cells, creating variation in water potential between the cells (Alférez et al., 2003; Alférez & Burns, 2004), which in turn depends on fruit maturity (Storey & Treeby, 1994). This variation creates a suction force between cells; and subsequently, cause collapse of internal flavedo and external albedo cell layers, resulting into peel pitting development (Agustí et al., 2001; Alquezer et al., 2010). Alférez and Zacarías (2014), reported that late harvested ‘Navelate’ navel fruit albedo cells were shapeless and compacted after ehydration, thereby failed to recover after it was transferred to higher RH. This could be the reason for the occurrence of peel pitting on unwaxed late harvested fruit exposed to dehydration stress prior storage. So far, findings have shown contradictory wax effects on pitting, whereby, non-waxed citrus fruit also showed higher pitting due to prior dehydration (Lado et al., 2019; Strano et al., 2022).

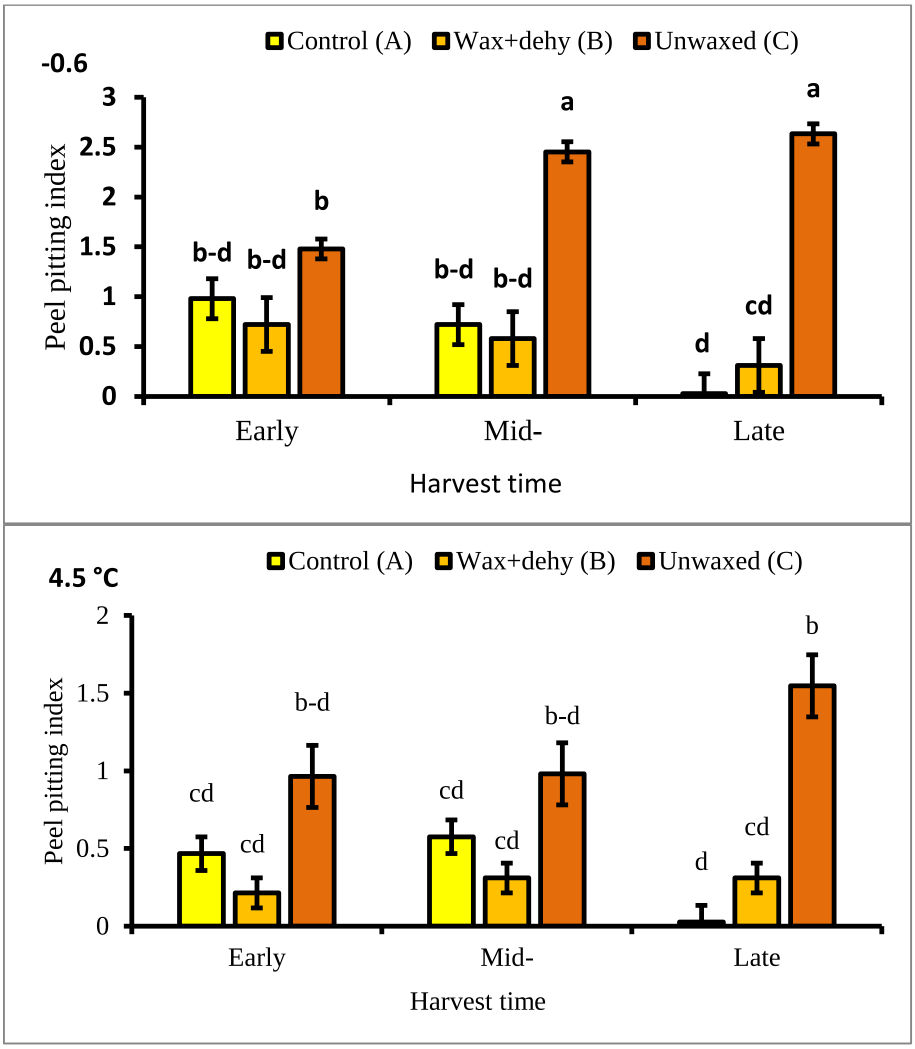

Fig. 1. Effect of harvest time and post-harvest treatment in peel pitting index (PPI) (0-4) of 'Benny' Valencia fruit cold stored at -0.6 and 4.5°C. Means with same letter are not significantly different at $P < 0.05$ (±SE, n = 25).

Chilling injury

Several studies have shown that chilling susceptibility of citrus fruit could be affected by harvest time (Lafuente et al., 1997), waxing and dehydration of fruits and extended cold storage (Mothapo et al., 2018). In the present study, the combination of harvest time, post-harvest treatment (waxing plus dehydration) and cold storage temperature had a significant effect ($P < 0.05$) on chilling injury (CI) of 'Benny' Valencia fruit (Fig. 2). CI symptoms manifested as skin browning lesion after withdrawal from cold storage, particularly on fruit stored at -0.6°C. However, fruit stored at 4.5°C did show no chilling symptoms, irrespective of wax plus dehydration, although, slight symptoms manifested in unwaxed dehydrated fruit at early harvest. This could be an indication that cold storage temperature caused an increase in reactive oxygen species (ROS) activities while reducing antioxidant activity (Sala, 1998; Lado et al., 2019; Liang et al., 2020). Unwaxed dehydrated fruit harvested early and stored at -0.6°C showed higher chilling injury index (CII) when compared with mid- and late harvested fruit of the same treatments. Furthermore, wax treatment effectively reduced chilling injury on fruit stored at -0.6°C, as waxed fruit had lower chilling injury symptoms when compared to untreated fruit at all harvest times. These results agreed with Wild (1993), who observed lower chilling symptoms on waxed ‘Washington’ navel oranges when compared with unwaxed fruit stored at 1°C for 8 weeks with additional 1 week at 20°C. In pineapple (*Anana comosus* (L.), wax application was shown to improve the chilling injury in two cultivars (Star-Fresh and 2952 and Sta-Fresh 7055) for 21 days cold storage at 7°C and 90% relative humidity (RH) (Hu et al., 2011). The effectiveness of wax in reducing the fruit susceptibility to chilling injury may be as a result of anti-transpiration effect caused by wax (Wang, 1993; Dou, 2004; Germano et al., 2019). Therefore, this study confirmed the result previously reported by Hu et al. (2011), that wax treatment could be a potentially method to alleviate chilling injury.

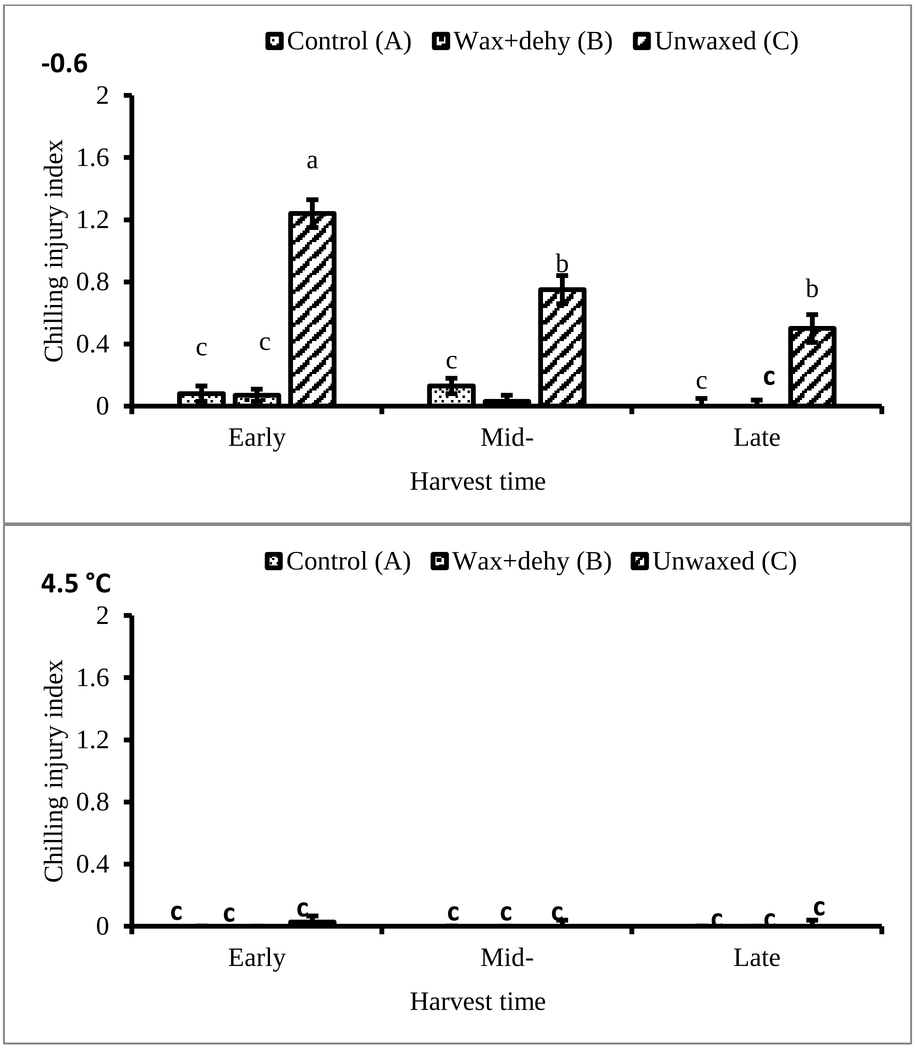

Fig. 2. Effect of harvest time and post-harvest treatment on chilling injury index (CII) (0-4) of ‘Benny’ Valencia fruit cold stored at -0.6 and 4.5°C. Means with same letter are not significantly different at $P < 0.05$ (±SE, n = 25).

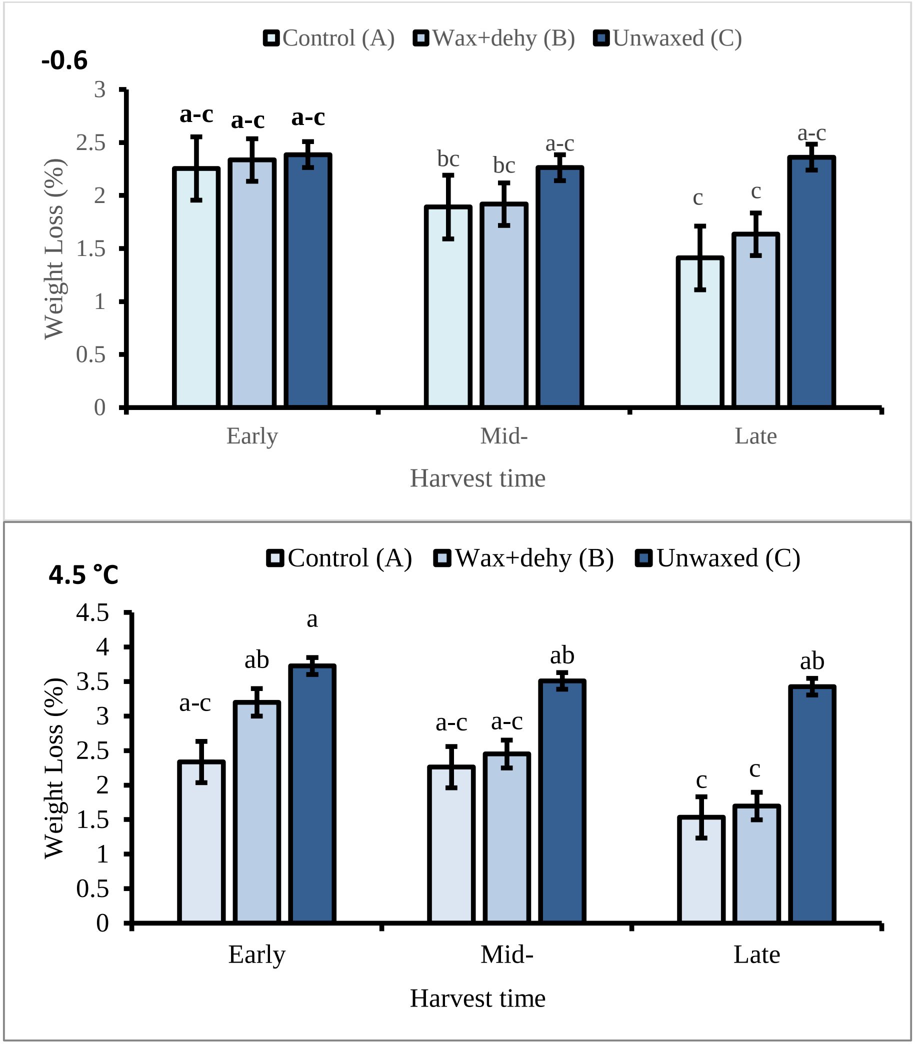

Fig. 3. Effect of harvest time and post-harvest treatment in weight loss of 'Benny' Valencia fruit stored at -0.6 and 4.5°C. Means with same letter are not significantly different at $P < 0.05$ (±SE, n = 25).

Weight loss

There was significant interaction effect ($P<0.05$) between harvest time, post-harvest treatment and cold storage temperature on fruit weight loss. The weight loss in fruit stored at 4.5°C was higher when compared with those stored at -0.6°C across all post-harvest treatment and harvest time (Fig. 3). These results agreed with those reported by Undurraga et al. (2014), who observed lower weight loss in 'Eureka' lemon fruit stored at lower temperature (3°C) compared to fruit stored at higher temperature (7 and 11°C). This could be due to high respiration under high evaporative demand (Yaman & Bayoindurh, 2002). Furthermore, higher weight loss was observed in early harvested fruit in comparison to mid- and late harvested fruit stored at both temperatures for all post-harvest treatment. Mid- and late unwaxed harvested fruit exposed to dehydration stress and stored at -0.6°C showed higher percentage of weight loss, while there was no variation between control and waxed plus dehydrated fruit. These findings were agreeing with those reported by Pailly et al. (2004), whereby, higher weight loss rate for early harvested 'Star Ruby' grapefruit stored at 6°C when compared with late harvested fruit. Moreover, passion fruit (*Passiflora edulis*) weight loss was significantly reduced in response to bees and carnauba wax when compared with waxed fruit (Sang & Hai, 2020), and shellac and decco wax reduced 'Ngowe' mango fruit weight loss during storage at 12°C during 28 days' storage (Maina et al., 2019). It is presumed by some that higher weight loss on early harvested fruit relates to poor development of wax layering on the fruit (Moon et al., 2003; Sala, 1998). Generally, the rate of weight loss in wax treated fruit progressively decreased with the delay of harvest time; and consequently, increased the postharvest behavioural differences between waxed fruit and unwaxed fruit. In general, wax act as a semi permeable barrier against oxygen, carbon dioxide and moisture loss on the fruit, thus, reducing the rate of weight loss (Baldwin et al., 1999; Sang & Hai, 2020).

Firmness loss

There was a significant interaction effect ($P<0.05$) between harvest time, post-harvest treatment and cold storage temperature on fruit firmness loss. The results revealed that storage at -0.6°C reduced fruit firmness loss when compared with 4.5°C across all post-harvest treatment and harvest time (Fig. 4). Moreover, late harvested fruit showed higher firmness loss, while lower firmness loss was observed in early harvested fruit stored at -0.6 and 4.5°C for unwaxed and waxed dehydrated fruit. It has been found that an increase in firmness loss at late harvest occur due to high enzymatic activity by polygalacturonase (PG) breaking down pectin substances in the soluble fraction (Seymour et al., 1996). The breakdown of pectin substances in the middle lamellae and cell wall occur as maturity continues and has been reported to cause loss of cell wall integrity and firmness (Tzoutzoukou & Bouranis, 1997). Interestingly, the present study, showed waxed fruit had lower firmness loss when compared with unwaxed dehydrated fruit stored at both storage temperatures. Our results agreed with previous findings, whereby, wax significantly reduced ‘Ngowe’ mango fruit (Hu et al., 2011) and ‘Sta-Fresh 2952 and ‘Sta-Fresh 7055’ pineapple fruit during cold storage (Maina et al., 2019). In these fruit, firmness loss was retained by waxing treatment, which retarded enzymatic pectin degradation (Zhou et al., 2011).

Electrolyte leakage (EL)

In this study, there was a significant interaction effect ($P< 0.05$) between harvest time, post-harvest treatment and cold storage temperature on fruit electrolyte leakage. Fruit stored at -0.6°C had higher electrolyte leakage when compared with those stored at 4.5°C at all harvest times, regardless of post-harvest treatment (Fig. 5). Generally, low storage temperature is a major factor that increases fruit membrane permeability and the subsequent rate of ion leakage (Raison & Orr, 1990; Saltveit, 2000). Cold storage temperature induces lipid phase transition from a more flexible liquid crystalline structure to a solid gel (Hordijk, 2013). According to Raison and Orr (1990), when gel phase coexists, the lipids do not pack well, causing cracks and result into high solutes leakage. In accordance to these findings, the present study found that storing fruit at -0.6°C resulted in higher electrolyte leakage after removal from storage.

A decreasing trend of electrolyte leakage was observed as harvest time progressed after removal from both cold storage temperatures. Late harvested fruit showed the lowest electrolyte leakage when compared with early and mid-harvested fruit stored at -0.6 and 4.5°C for all post-harvest treatments. Fruit exposed to dehydration stress without wax showed higher electrolyte leakage when compared with control and waxed plus dehydrated fruit, irrespective of harvest time (Fig. 5). These results were similar to those previously reported by Negi and Roy (2000), whereby, electrolyte leakage was higher for dehydrated ‘Nantes’ carrots stored at 7.5°C than non-dehydrated fruit due to higher water loss. In addition to our result, we found that waxing treatment repressed electrolyte leakage across all harvest time and storage temperature. These results were supported to those found by Zhou et al. (2011), whereby, electrolyte leakage during storage could effectively be inhibited by wax treatment due to higher peroxidase (POD) activities induced by wax on ‘Huanghua’ pears fruit stored at

4°C. In addition, relative leakage was correlated with membrane damage (malondialdehyde (MDA) content) and found to be significant lower in waxed pineapple fruit during cold storage at 7°C plus 90% relative humidity (RH) for 21 days (Hu et al., 2011). Peroxidase activity has the ability to scavenge peroxides (superoxide and peroxides) that contribute in damaging cell membrane and increase membrane permeability (Yuan et al., 2010).

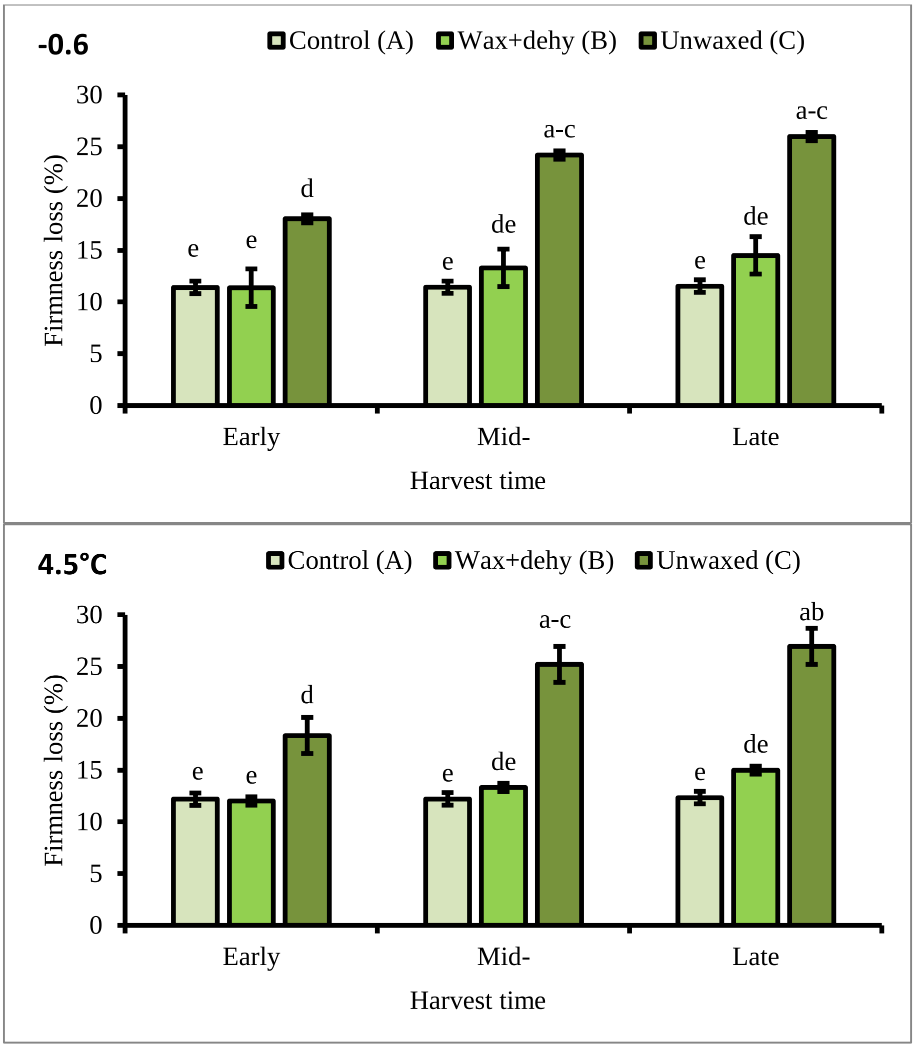

Fig. 4. Effect of harvest time and post-harvest treatment in firmness loss of 'Benny' Valencia fruit stored at -0.6 and 4.5°C. Means with same letter are not significantly different at $P < 0.05$ (±SE, n = 25).

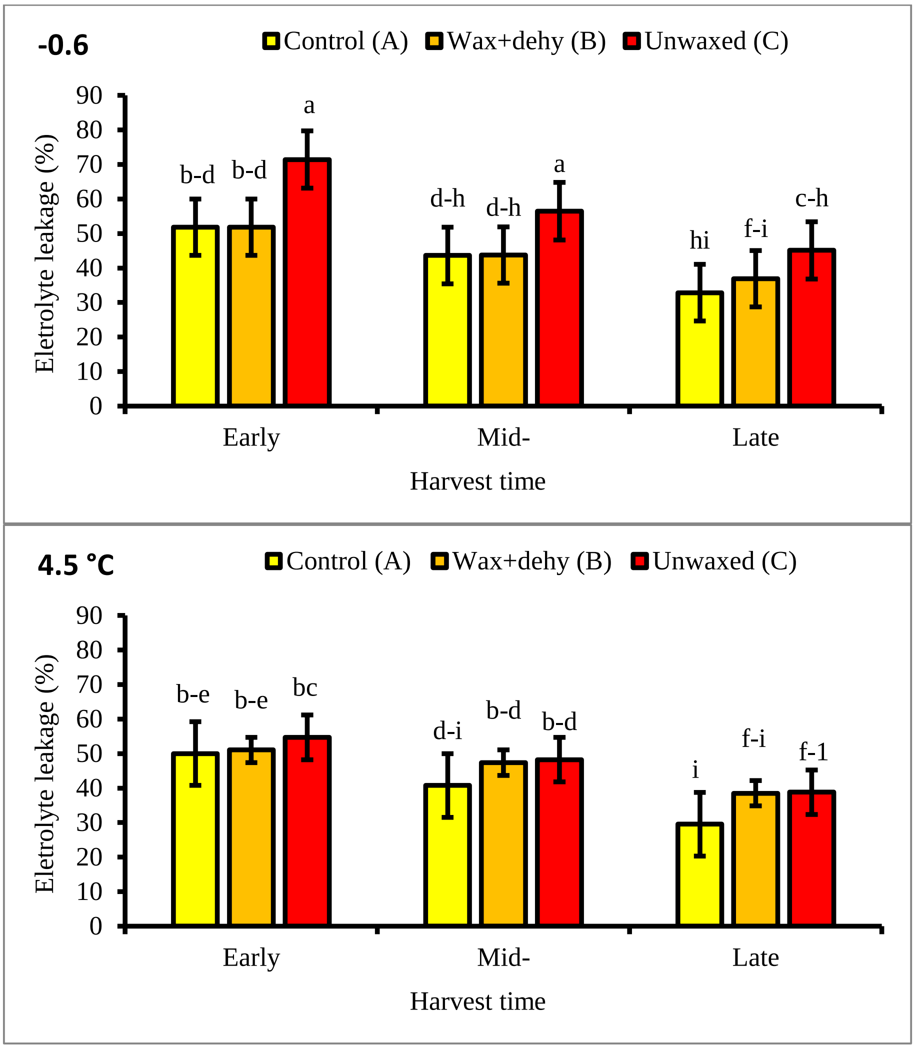

Fig. 5. Effect of harvest time and post-harvest treatment in electrolyte leakage (%) of 'Benny' Valencia fruit stored at -0.6 and 4.5°C. Means with same letter are not significantly different at $P < 0.05$ (±SE, n = 5).

Total phenolic concentration

Free phenolic

The interaction of harvest time, wax plus dehydration and storage temperature had no significant effect on total phenolic concentration of 'Benny' Valencia fruit ($P>0.05$) (Table 1). However, the free phenolic concentration increased at late harvested fruit, both stored at -0.6 and 4.5°C, especially in control fruit. These results agreed with Davis and Albrigo (1994), who indicated that over matured fruits have high percentage of amino acids, vitamin C, minerals and small quantities of lipids, proteins, and secondary metabolites. Fruit stored at 4.5°C showed lower free phenolic content when compare with fruit stored at -0.6°C, across all harvest time and post-harvest treatment. This result was similar to those previously reported by Chaudhary et al. (2016) who found high total phenolic in 'Rio Red' grapefruit stored at 5°C compared with fruit stored at 11°C due to high CI observed on fruit stored at 5°C. Across all harvest time and storage temperature, unwaxed fruit exposed to 3 days dehydration stress, showed lower total phenolic concentration when compared with control and waxed dehydrated fruit. Similar results were reported in the study of Li et al. (2017), whereby, higher levels of total phenolics were found in 'Sweet Charlie' strawberry fruit waxed with polysaccharide and stored at 3°C when compared with unwaxed fruit, thereby significantly inhibited fruit decay and respiration after 12 days of storage. In ‘Muscat’ grapes, dehydration slightly increased total phenolics and flavonoids, thereby increasing oxidative stress tolerance (Corona et al., 2020). In addition, Petriccione et al. (2015) suggested that waxing could be used to prolong postharvest fruit life through reducing moisture, respiration, gas exchange, and oxidative reaction rates by regulating antioxidant activity.

Soluble conjugated phenolic

There was no significant interaction effect (P>0.05) between harvest time, post-harvest treatment and cold storage temperature on soluble conjugated phenolic (Table 1). Conjugated phenolic content was higher in fruit harvested late compared with early and mid- harvested fruit (Table 1). Storage temperature had no effect on the level of conjugated phenolics. For all harvest time and storage temperatures, fruit that received wax application showed higher conjugated phenolics. The study of Tavarini et al. (2008) associated moisture loss with total antioxidant activity. In generally, the study emphasized that moisture loss led to antioxidant degradation. Therefore, high soluble conjugated phenolic observed on this study could have resulted due to rind moisture content.

Correlation

Weight Loss vs. Peel pitting

Additionally, this study further suggested that weight loss after removal from storage temperature can serve as a non-destructive predictor of peel pitting, since it was significantly (P < 0.05) correlated with peel pitting incidence during mid- (r^2 = 0.98) and late (r^2 = 0.70) harvest (Fig. 2). These findings were supported by previous work by Cronjé et al. (2017), whereby, weight loss and peel pitting of ‘Navelate Navel’ and ‘Tangor Ortanique’ mandarin fruit correlated during storage for 3 weeks at 30 or 90% relative humidity.

Table 1. Effect of wax plus dehydration on conjugated and free phenolics of ‘Benny’ Valencia fruit harvested at three different times and stored at -0.6 and 4.5°C.

Storage Temperature	Harvest time	Treatment	Conjugated (mg GAE 100 ml^{-1} DW)	Free
-0.6°C	Early	Control (A)	409^{abc}	392.5^{abc}
		Waxed-dehydrated (B)	431^{abc}	332.7^{abc}
		Unwaxed-dehydrated (C)	309.7^{abc}	309.7^{abc}
	Mid-	Control (A)	443.1^{abc}	423^{abc}
		Waxed-dehydrated (B)	395.7^{abc}	376.4^{abc}
		Unwaxed-dehydration (C)	263.8^{c}	215.5^{c}
	Late	Control (A)	508.6^{abc}	471.6^{a}
		Waxed-dehydrated (B)	477.7^{abc}	355.7^{abc}
		Unwaxed-dehydration (C)	447.6^{abc}	394.8^{abc}
4.5°C	Early	Control (A)	390.2^{abc}	413.2^{bc}
		Waxed-dehydrated (B)	348.8^{abc}	254.6^{bc}
		Unwaxed-dehydrated (C)	337.3^{abc}	351.1^{abc}
	Mid-	Control (A)	362.6^{abc}	334.8^{abc}
		Waxed-dehydrated (B)	390.2^{abc}	420.1^{ab}
		Unwaxed-dehydration (C)	275.3^{c}	289.3^{abc}
	Late	Control (A)	456.5^{abc}	437.8^{ab}
		Waxed-dehydrated (B)	446.5^{abc}	420.1^{ab}
		Unwaxed-dehydration (C)	397.1^{abc}	344.7^{abc}
	P value		0.350	0.550

Chilling Injury vs. Electrolyte Leakage

The positive correlation between electrolyte leakage and chilling injury was observed on fruit stored at -0.6°C and only strong and significant for early and mid-harvested fruit (Table 2). Electrolyte leakage on fruit stored at 4.5°C was observed; however, fruit did not show any chilling symptoms. In this case, it was noticeable that electrolyte leakage could not be used as predictor for chilling injury in citrus as previously reported by Cohen et al. (1994).

Table 2. Correlation between fruit peel pitting index (PPI) vs weight loss (%) and chilling injury index (CII) vs electrolyte leakage (EL).

Harvest time	Variables	-0.6°C		4.5°C	
		Pearson (r)	t distribution	Pearson (r)	t distribution
Early harvest	PPI vs WL	0.39	0.4	0.68	1.1
	CII vs EL	0.99	7.1	N/A	N/A
Mid-harvest	PPI vs WL	0.98	4.9	0.71	1.3
	CII vs EL	0.98	4.9	N/A	N/A
Late harvest	PPI vs WL	0.95	3.2	0.99	7.1
	CII vs EL	0.88	1.7	N/A	N/A

For 3 degree of freedom and one tailed test, t critical value = 2.93

CONCLUSION

This study demonstrated that peel pitting and chilling injury occurrence together with the overall quality of ‘Benny’ Valencia fruit are influenced by harvest time, dehydration stress, waxing and cold storage temperature. Exposing fruit to dehydration stress at low relative humidity (RH) (45%) as previously documented to other cultivars is indeed a key factor leading to the occurrence of rind physiological disorders during storage. Therefore, maintaining of a rind constant water status prior to and during postharvest handling by waxing and/or other factors may have a crucial role in retaining peel quality and reduce the occurrence of rind disorders in ‘Benny’ Valencia fruit. However, for the successful reduction of these disorders during commercial shipment of ‘Benny’ Valencia fruit, the focus should not be on a single factor but rather a strategy that encompasses multiple factors known to decrease the impact of rind disorders. With respect to both free and soluble conjugated phenolics, the control fruit showed higher rind phenolics, especially at late harvest, across all the storage temperatures. Therefore, late harvested, waxed (control) fruit seem to be naturally responding to cold stress by up-regulating endogenous systems of total rind phenolics. Contrarily, postharvest treatments (dehydration) suppressed endogenous phenolics synthesis, therefore, poor defence against chilling and non-chilling disorders of ‘Benny’ Valencia fruit.

Conflict of interest

The authors declare that there is no conflict of interest.

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