

Effect of Fruit Size and Coating Material on Quality of Tangerine Fruit cv. *Sai Nam Phueng*

Danai Boonyakiat^{1*}, Pimjai Seehanam² and Nithiya Rattanapanone³

¹Postharvest Technology Research Institute, Chiang Mai University, Chiang Mai 50200, Thailand.

²Department of Plant Sciences and Natural Resources, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

³Department of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand.

*Corresponding author. E-mail: agxxo005@chiangmai.ac.th

ABSTRACT

Small (No. 4; 92-98 g) and large (No. 6; 134-142 g) tangerine fruit coated with either Zivdar or Fomesa as well as a non-coated control were stored at room temperature (24±3°C) and 59±6% relative humidity for 10 days. The results showed that large fruit had lower weight loss, less off-flavor and better visual appearance than small fruit. Fruit size also had an effect on hue angle of peel color, pH, total soluble solids (TSS) and titratable acidity (TA) but had no effect on internal O₂, internal CO₂, ethanol content in juice, pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activity, L and chroma of peel color, TSS/TA ratio and vitamin C content. Tangerine fruit coated with Fomesa had the lowest weight loss. Fruit coated with Zivdar had higher O₂ and CO₂ exchange and lower internal ethanol in fruit juice than fruit coated with Fomesa. The coating affected the flavor and visual appearance quality of tangerine fruit, but had no effect on enzyme activities, peel color and chemical composition.*

Keywords: Tangerine fruit, Fruit size, Coating material, Visual appearance, Internal gas

INTRODUCTION

Tangerine or mandarin (*Citrus reticulata* Blanco) is the most common citrus fruit grown in Thailand. Harvested tangerines are typically brought to a packinghouse soon after harvest to begin the steps of preparing the fruit for market – cleaning, coating, grading and packing. In addition, fruit destined for export may need to be treated with the natural ripening agent ethylene in order to improve the external peel color, best done before cleaning and grading (Ministry of Fisheries, Crops and Livestock; New Guyana Marketing Corporation; and National Agricultural Research Institute, 2004).

During the process of fruit handling in the packinghouse, most of the natural wax is removed during washing. It is imperative that these natural protectants are replaced by different coating materials. Various types of citrus wax formulations are available. Waxing reduces moisture loss and shriveling of the fruit and extends the shelf life. Waxing also imparts an attractive shine to the peel (Hagenmaier and Shaw, 1991). Coating treatments modify the internal atmosphere of fruit and have significant potential to extend the shelf-life of citrus fruit (Mannheim and Soffer, 1996). Wax coatings have been shown to extend postharvest quality of fruit and vegetable crops by limiting gas exchange and reducing water loss, skin discoloration, fruit deterioration and should not cause partial anaerobic conditions (Hagenmaier and Baker, 1993; Baldwin et al., 1999)

Generally, chemical composition of citrus fruit quality is affected by location (McDonald and Hillebrand, 1980), cultivar and rootstock (Wutscher and Shull, 1972; Cameron and Soost, 1977), mineral nutrition (Koo et al., 1974), climate (Levy et al., 1974), maturity (Issarakraisila, 1984), the position of the fruit on the tree (Sites and Reitz, 1950) and fruit size (McDonald and Hillebrand, 1980; Ketsa, 1988). However, there are no reports on the effect of fruit size and coating on the quality and chemical composition of tangerine fruit.

The objective of this study was to examine the effect of fruit size and commercial coating material on the postharvest quality of tangerine fruit cv. *Sai Nam Phueng* after being coated by commercial method and stored at room temperature for 10 days.

MATERIALS AND METHODS

Fruit

Tangerine fruit cv. *Sai Nam Phueng* were harvested at commercial maturity from a commercial orchard in Fang District, Chiang Mai Province, Thailand, in February 2008. Fruit were sized immediately after harvesting using a dimension sizer. Fruit were selected for a weight range of about 92-98 g (No. 4) and 134-142 g (No. 6) as well as uniform maturity, shape, color and lack of defects. Tangerines were washed with water and rotated on a soft brush. Fruit surfaces were dried by warm air (45°C) before coating by Zivdar (Safepack Products Ltd., Israel) or Fomesa (Fomesa Fruitech, S.L., Spain), then dried again by warm air (40°C). Non-coated fruit were used as a control. Tangerine fruit were packed into cartons and transported by truck (~3 hours) to the Postharvest Horticultural Laboratory, Department of Plant Science and Natural Resources, Faculty of Agriculture, Chiang Mai University. The study consisted of a factorial design with two fruit sizes and two types of coating.

Table 1. Coating materials, main components and their sources.

Name of commercial coating	Main components	Source of products
Fomesa	10% oxidized polyethylene wax 8% glycerol ester of wood rosin and 2% ammonium hydroxide	Fomesa Fruitech, S.L., Spain
Zivdar	18% w/v waxes, shellac, polyethylene wax and imazalil	Safepack Products Ltd., Israel

Note: Components of commercial coatings were declared on the product labels.

Weight loss

Weight loss during storage was determined from twenty fruit set aside for each treatment. The percentage of weight loss was calculated from the difference between the initial and final weight.

Internal O₂ and CO₂

Ten replicates were used for each treatment. The internal gas was withdrawn by a syringe (previously flushed with helium gas to remove O₂) with the needle inserted through the blossom end into the internal space of fruit submerged in water. The internal O₂ and CO₂ concentrations were measured with a gas chromatograph (Model GC-8A, Shimadzu, Japan) equipped with a thermal conductivity detector, fitted with a CTR-1 column (2 m × 6 mm i.d.) (Alltech, Deerfield, IL., USA), consisting of an outer column (Parapak Type N; 80-100 Mesh, Shimadzu, Tokyo, Japan). The column temperature was 65°C and the thermal conductivity detector temperature was 110°C. Helium was used as the carrier gas at a flow rate of 150 mL/min. Peak areas obtained from standard gas mixtures were determined before and after analysis of samples. Oxygen concentration was calculated from the O₂-Ar peak area after correction for 0.9% Ar in the atmosphere (Hagenmaier, 2001).

Ethanol content

The juice samples for ethanol were pooled from ten tangerine fruit per treatment. The juice was extracted using a juice maker. Ethanol in the juice was determined using an ethanol assay kit (Diagnostic Chemical Limited, Charlottetown, Canada) as described by Bonnicksen and Theorell (1951).

Extraction and determination of PDC and ADH activities

Enzyme extraction. For each replicate, 5 g of tissue was obtained from five tangerines (3 segments per fruit) and homogenized in 10 ml of 100 mM 2-(N-morpholino) ethane-sulfonic acid (MES) buffer (Fluka, Lyon, France) (pH 6.5) containing 2 mM dithithreitol (Fluka, Lyon, France) and 1% (w/v) polyvinylpyrrolidone (Fluka, Lyon, France). The homogenate was centrifuged at 12,000 × g for 20 min at 4°C (Centrifuge, Universal 32 R, CE, Wisconsin, USA). The supernatant was decanted and set on ice as crude enzyme extract (Ke et al., 1994).

Enzyme assays and protein determination.

PDC activity: PDC activity was assayed through coupling with ADH reaction by mixing 0.45 ml of 100 mM MES buffer (pH 6.5), 0.1 ml of 5 mM thiamine pyrophosphate (Sigma-Aldrich, Missouri, USA), 0.1 ml of 50 mM MgCl₂ (Merck, Darmstadt, Germany), 0.05 ml of 1.6 mM NADH (Fluka, Lyon, France), 0.1 ml of commercial ADH solution (containing 13.5 enzyme units) (Sigma-Aldrich, Missouri, USA), 0.1 ml of 50 mM pyruvate (Fluka, Lyon, France), and 0.1 ml of enzyme extract. PDC oxidation was measured by recording the decrease in absorbance at 340 nm over time using a spectrophotometer (Thermo Spectronic, Model Genesys 10UV-Scanning, CE, Wisconsin, USA). Enzyme activities were expressed as unit mg⁻¹ protein (Ke et al., 1994).

ADH activity: ADH activity was measured by mixing 0.8 ml of 100 mM MES buffer (pH 6.5), 0.05 ml of 1.6 mM NADH (Fluka, Lyon, France), 0.1 ml of crude enzyme extract, and 0.05 ml of 80 mM acetaldehyde (Riedel-de Haen, Hanover, Germany). ADH, NADH oxidation was measured by recording the decrease in absorbance at 340 nm over time (5 min) using a spectrophotometer (Thermo Spectronic, Model Genesys 10UV-Scanning, CE, Wisconsin, USA). Enzyme activities were expressed as unit mg⁻¹ protein (Ke et al., 1994).

Protein determination: Soluble protein content was detected by calculating specific enzyme activity using the method described by Bradford (1976). The samples were measured at 595 nm using a spectrophotometer (Thermo Spectronic, Model Genesys 10UV-Scanning, CE, Wisconsin, USA) and protein concentrations were determined for each sample with a bovine serum albumin (BSA) (Sigma-Aldrich, Missouri, USA) standard curve.

Sensory evaluation

Fruit visual appearance. Twenty fruit per treatment were evaluated for visual quality, wilting and shriveling. The same fruit was evaluated for overall appearance. A subjective scale ranging from 1 to 5 was used, where: 5 = excellent, 4 = good, 3 = fair, 2 = poor and 1 = unusable.

Estimation of flavor. Ten untrained panelists (7 females, 3 males, ages 25- 31 years) evaluated the flavor of tangerine fruit by tasting, using a score of 1 to 4, where: 4 = excellent, 3 = slightly off-flavor, 2 = moderately off-flavor and 1 = extremely off-flavor.

Fruit appearance was rated “unacceptable” when the flavor and visual appearance scores were below 3.

Measurement of peel color

Peel color of tangerine fruit was measured with a Chroma meter (Model CR-300, Minolta, Tokyo, Japan). Ten fruit were used for each treatment. Each fruit was marked (middle of fruit) by a pen on the peel (2 positions) before measuring peel color. The lightness coefficient, L*, ranged from black (0) to white (100). A more appropriate measure of color can be obtained by chroma (C*) and hue angle (H°), an index somewhat analogous to color saturation or intensity (McGuire, 1992).

Chemical composition analysis

Three fruit from three replications per treatment were squeezed with a hand-press juicer. The juice was measured for total soluble solids (TSS) content with a digital refractometer (Model PR-101, Atago, Tokyo, Japan). The values were expressed in percentage of total soluble solids. Titratable acidity (TA) was determined by diluting 10 ml of fruit juice to 100 ml with distilled water and titrated with 0.1N NaOH (Univar, New South Wales, Australia) to a pH end point of 8.2 using a pH meter (Model CG842, Schott, Hofheim, Germany). Each treatment was replicated three times. Titratable acidity was expressed as percent citric acid per 100 ml fruit juice. The ratios of TSS to TA were calculated as the average of the ratios. The pH of the juice was measured by a pH-Meter (Model CG 842/14 pH, Schott, Hofheim, Germany) previously calibrated with buffer solutions of pH 4.0 and 7.0. The pH measurement was carried out on three replicates per treatment and the value was registered once it had stabilized.

Ascorbic acid content was determined by 2,6-dichlorophenol-indophenol titration method by standardizing 0.04% 2,6-dichlorophenol-indophenol dye solution against 0.1% ascorbic acid solution. Three 1.0 ml aliquots of ascorbic acid standard solution were transferred to each of three 50 ml Erlenmeyers flask then titrated rapidly with 2,6-dichlorophenol indophenol dye solution until a light but distinct rose pink (≥ 15 seconds). Ascorbic acid content was estimated by diluting 10 ml of juice with 90 ml of 0.4% oxalic acid (Univar, New South Wales, Australia). This was mixed thoroughly by shaking to ensure a uniform test portion, and filtered through filter paper Whatman[®] No.1 (Whatman International Ltd., Maidstone, England). Then the three test solution aliquots from each treatment were titrated until light but distinct rose pink (≥ 15 seconds). The results were expressed in milligrams of ascorbic acid per 100 ml fruit juice (Ranganna, 1986).

Statistical analysis

The experiment was a 2 \times 3 factorial in completely randomized block design, with factor A being the fruit sizes (No. 4 and No. 6) and factor B the coating treatments (Zivdar, Fomesa and non-coated). Each treatment included three replications. All data were subjected to analysis of variance and mean separation was accomplished by the least significant difference (LSD) test (significant at $p \leq 0.05$).

RESULTS AND DISCUSSION

Weight loss

Tangerine fruit No. 6 had lower weight loss than fruit No. 4 ($5.86 \pm 1.35\%$ and $6.84 \pm 1.53\%$, respectively) during 10 days of storage (Table 2). Some factors that affect transpiration in fruit are the surface area/volume or surface area/mass ratio (Ben-Yehoshua, 1987; Díaz-Pérez, 1998). Small fruit with a greater surface area/volume ratio than large fruit had a larger proportional weight loss than large fruit, over the same shelf life (Burton, 1985). The reduction of water loss rate with increases in fruit size was probably due, at least partly, to the decreases in the

surface area/fresh weight ratio, as suggested by the relationship of water loss rate with the surface area/fresh weight ratio (Díaz-Pérez et al., 2007). Ben-Yehoshua (1987) mentioned that fruit size affected water loss and the transpiration rate was greater in smaller fruit such as oranges compared to large fruit such as grapefruit. Pailly et al. (2004) stated that small *Star Ruby* grapefruit (90 to 94 mm) had a significantly higher relative weight loss than that of larger fruit (107 and 119 mm) during storage at a constant temperature (6 or 10±0.2°C) and a constant relative humidity (85%). Over the 8-day shelf-life at 31.2°C and 67.4% relative humidity of the study, small tangerines cv. *Khieo Wan* lost proportionately more weight than the large ones. The average daily weight loss as a proportion of the original weight over the 8-day period was 1.79, 1.75, 1.58, 1.29 and 1.17% per day for grades 3, 2, 1, 0 and 00, respectively (Ketsa, 1990).

Table 2. Effects of fruit size and coating material on weight loss, internal gases, ethanol content, PDC activity and ADH activity of tangerine fruit stored at room temperature (24±3°C) and 59±6% relative humidity for 10 days.

Treatments	Weight loss (%)	Internal O ₂ (%)	Internal CO ₂ (%)	Ethanol content (mg/l)	PDC activity (unit/min/mg protein)	ADH activity (unit/min/mg protein)
Factor 1: Fruit size						
No. 4	6.84±1.53a	7.74±4.37	11.44±5.61	1,093.25±452.50	1.14±0.12	3.20±0.49
No. 6	5.86±1.35b	7.95±5.08	11.66±3.66	1,215.58±484.46	2.38±0.81	2.50±1.27
Factor 2: Coating material						
Zivdar	6.16±1.06b	7.22±1.48b	10.84±1.39b	1,148.56±127.51b	0.92±0.09	2.03±0.73
Fomesa	5.50±1.00c	2.89±1.52c	15.62±4.60a	1,791.78±318.40a	2.39±1.03	2.49±0.60
Non-coated	7.41±1.72a	13.41±1.77a	7.79±3.68b	522.90±83.20c	1.96±.73	4.02±1.80
Factor 1	*	ns	ns	ns	ns	ns
Factor 2	*	*	*	*	ns	ns
Factor 1×2	ns	ns	ns	ns	ns	ns

Note: Means with different letters in the same column differ significantly ($P < 0.05$). * = significance, ns = non significance.

The minimum weight loss occurred in tangerine fruit coated with Fomesa (5.5±1.0%), followed by the fruit coated with Zivdar (6.2±1.1%) as compared with non-coated fruit (7.4±1.7%) (Table 2). The weight loss of tangerine fruit significantly increased during storage for 13 days (data not shown). Weight loss is mainly caused by evaporation of water from the fruit. The non-coated tangerines exhibited a sharp increase in percentage of weight loss at room temperature while it was less when fruits were coated with Fomesa and Zivdar and stored under the same conditions. Continuous increase in percentage of weight loss during storage contributes to fruit quality deterioration. Impairment of fruit appearance as a result of loss in weight starts after the second week of storage, turning the fruit unattractive owing to formation of wrinkles on the skin (Raghav and Gupta, 2000). This condition makes the rind leathery and unacceptable for the market (Aquino et al., 2001a, b). Coating, as an additional barrier to the peel, inhibited

water loss. Satsuma mandarin coated with Britex 505, PacRite-StorRite 101 (contained polyethylene and shellac), Primafresh 30 (contained carnauba wax and shellac), Decco Lustr 202 (contained natural and synthetic waxes and fatty acids) and Natural Zivdar (contained a carnauba wax emulsion) had lower weight loss than non-coated fruit during storage at 15°C for 28 days (Mannheim and Soffer, 1996).

Internal gases

Fruit size did not affect internal O₂ and CO₂ concentration of tangerine fruit (Table 2). The internal gases were markedly different for different coatings. The Fomesa coating resulted in very low internal O₂ and high internal CO₂. By contrast, Zivdar coating resulted in higher internal O₂ and lower internal CO₂. Non-coated fruit had the highest internal O₂ and lowest internal CO₂ concentration (Table 2). The explanation for these observations is that the coatings serve as a barrier for gases so that the concentrations of O₂ that can pass into the tangerines reduce and the CO₂ that is the product of respiration accumulates inside the fruit (Ben-Yehoshua, 1969). In addition, when coatings are applied to fruit, they form an additional barrier through which gases must pass. Because coatings differ in gas permeance and ability to block openings in the peel, they have different effects on gas exchange (Hagenmaier and Baker, 1993). Mannheim and Soffer (1996) used Natural Zivdar (containing a carnauba wax emulsion), Primafresh 30 (containing carnauba wax and shellac) and PacRite-Sun-Shine (containing shellac) to coat *Valencia* oranges and reported that treated oranges had lower O₂ and higher CO₂ concentrations than the control fruit.

Ethanol content

There was no significant difference in ethanol content of tangerine fruit size No. 4 (1,093.25±452.50 mg/L) and No. 6 (1,215.58±484.46 mg/L) (Table 2). Ethanol content in tangerine juice was lower in non-coated fruit (522.90±83.20 mg/L) than in fruit coated with Zivdar (1,148.56±127.51 mg/L) and Fomesa (1,791.78±318.40 mg/L), after 10 days of storage at room temperature. Fruit coated with Fomesa tended to reach higher concentrations of ethanol in juice than Zivdar-coated fruit during storage (data not shown). The volatile compounds ethanol and acetaldehyde underwent the greatest change during storage. Ethanol levels in juice for coated and uncoated mandarins are significantly different due to creation of a modified atmosphere, as can be seen by the lower ethanol accumulation during storage in uncoated fruit than in coated fruit (Baldwin et al., 1995a, b). *Mor* mandarin coated with commercial Tag and Modified Tag coatings and kept at 5°C for 5 weeks, followed by holding at 20°C for 5 days, had higher ethanol content in juice than non-coated fruit (Porat et al., 2005).

PDC and ADH enzyme activities

PDC activity. The PDC activity of tangerine fruit No. 4 and No. 6 were not significantly different (Table 2). The enzyme activity of both large and small tangerine fruit was quite similar during storage (data not shown). After storage

for 10 days, the results showed that tangerine fruit were not significantly different among Zivdar, Fomesa and non-coated fruit on activity of PDC enzyme (Table 2). PDC activities in tangerine fruit coated with Zivdar and Fomesa increased 2 and 5 times greater than non-coated control by days 5 and 7, respectively. PDC activity of non-coated fruit increased by day 4 of storage, and became quite variable until day 11 of storage. Both fruit sizes coated with Fomesa had higher PDC activity than other treatments. Tangerine fruit No. 4 and No. 6 coated with Zivdar had the highest PDC activity on days 5 and 8 of storage, respectively, then gradually became lower and relatively constant. Non-coated fruit No. 4 had the highest PDC activity after storage for 8 days. However, enzyme activity of non-coated fruit No. 6 was relatively constant throughout the storage period (Figure 1).

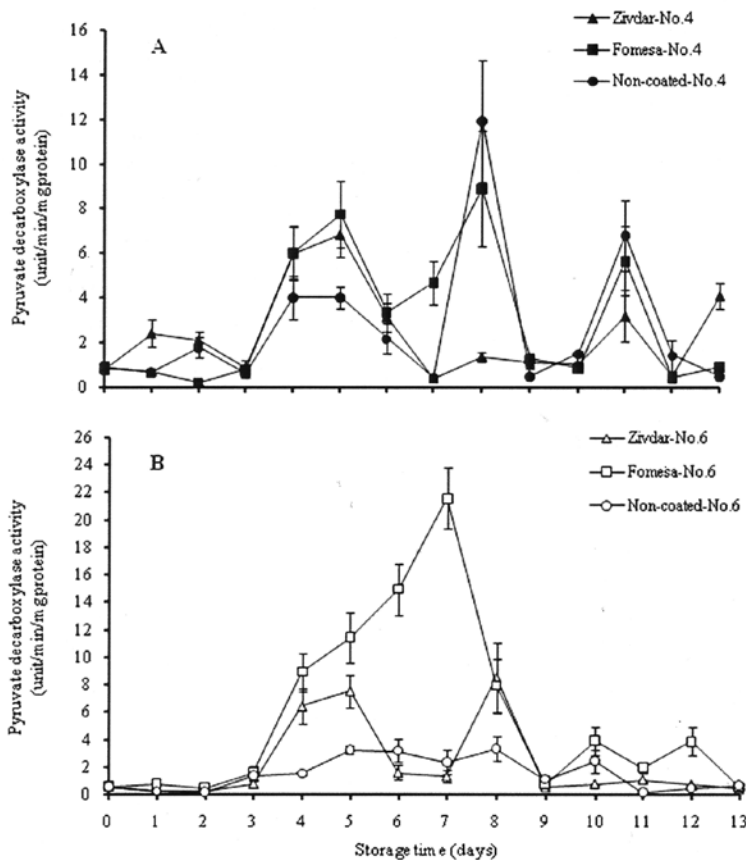


Figure 1. Change in pyruvate decarboxylase (PDC) activity of tangerine fruit cv. *Sai Nam Phueng* (A) No.4 and (B) No.6 coated with coating materials and stored at ambient temperature ($24\pm 3^{\circ}\text{C}$) and $59\pm 6\%$ RH for 13 days.

ADH activity. ADH activity of tangerine fruit No. 4 was the same as that of the fruit size No. 6 (2.5 ± 1.3 units/min/mg protein) (Table 2). ADH activity of tangerine fruit coated with Zivdar, Fomesa and non-coated fruit were not significantly different on day 10 of storage (Table 2). The results indicated that coated and non-coated control fruit stored for 1 to 8 days had high ADH activity and then dropped after 9 days. Fomesa treatment caused a large increase in ADH activity on day 8 of storage. Tangerine fruit No. 4 and No. 6 coated with Zivdar had the highest enzyme activity on day 5 of storage (Figure 2).

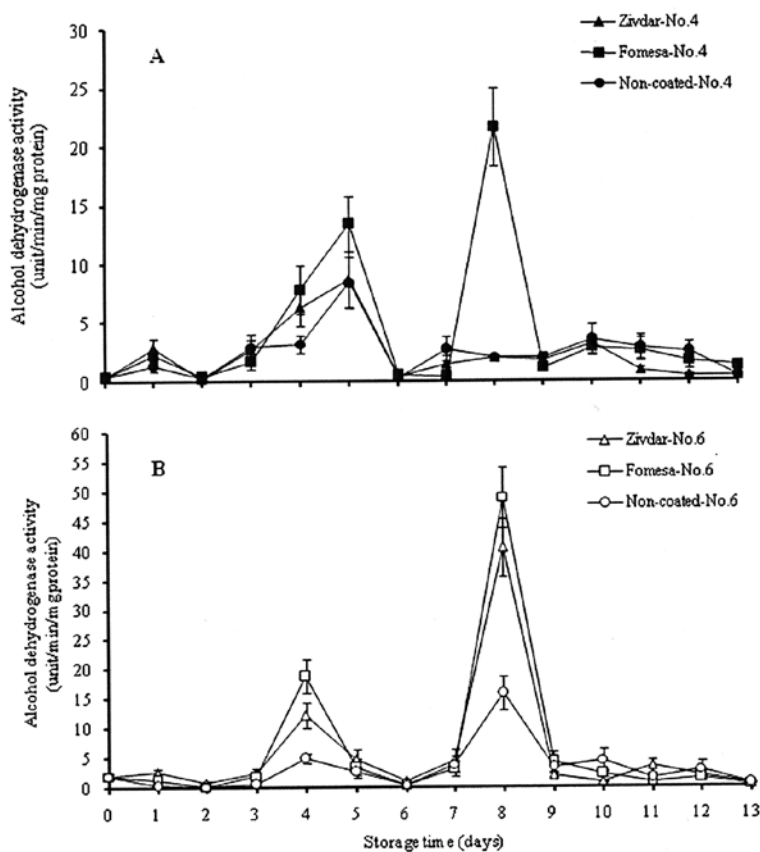


Figure 2. Change in alcohol dehydrogenase (ADH) activity of tangerine fruit cv. *Sai Nam Phueng* (A) No. 4 and (B) No. 6 coated with coating materials and stored at ambient temperature ($24 \pm 3^\circ\text{C}$) and $59 \pm 6\%$ RH for 13 days.

Plant responses to very low O_2 and/or very high CO_2 concentrations include induction of fermentation pathways, accumulation of succinate and/or alanine, and decrease in intracellular pH and ATP levels. One pathway of fermentative metabolism results in accumulation of acetaldehyde and ethanol catalyzed by the enzymes PDC and ADH, respectively (Ke et al., 1994). In some plant tissues,

lactate accumulation results from fermentation and this is catalyzed by the enzyme lactate dehydrogenase (LDH). The major function of fermentative metabolism is to use NADH and pyruvate when electron transport and oxidative phosphorylation are inhibited so that glycolysis can proceed. This will allow for the production of some ATP through substrate phosphorylation, which permits the plant tissues to survive temporarily. Kanellis et al. (1991) found that ADH isozymes could be induced by exposure of avocado fruit to 2.5%, 3.5% or 5.5% O₂. Increased activities of PDC and ADH were observed when sweet potato, *Bartlett* pear, lettuce and strawberry were kept in low O₂ or high CO₂ concentrations (Chang et al., 1983; Nanos et al., 1992; Ke et al., 1993). Kennedy et al. (1992) reviewed studies of anaerobic metabolism in plants under O₂ stress. The induction of PDC, ADH and/or LDH was regarded as one of the reasons for accumulation of anaerobic products. Imahori et al. (2003) also reported that low O₂ conditions increased PDC, ADH and LDH activities in tomato fruit during storage at 20°C for 7 days.

Sensory evaluation

Visual appearance. The visual appearance score of fruit No. 6 was higher than fruit No. 4, with statistical significance (Table 3). The visual appearance of tangerine fruit continuously decreased during storage, but the tangerine fruit coated with both commercial coatings had higher visual appearance scores than the non-coated control. Non-coated control fruit shriveled faster than coating treatments (data not shown). The visual appearance scores of fruit coated with Zivdar and Fomesa on day 10 of storage (4.0 ± 0.6 and 3.7 ± 0.5 , respectively) were not statistically different (Table 3). Coating treatments effectively retarded shriveling of tangerine fruit during storage. Coating treatments imparted an attractive natural-looking sheen to the fruit. Mandarin fruit coated with Britex, Decco, PacRite-Sunshine, Natural Zivdar, Zivdar PE, PacRite-StorRite and Primafresh was shiny and very attractive (Mannheim and Soffer, 1996).

Table 3. Effects of fruit size and coating material on flavor score, visual appearance and peel color of tangerine fruit stored at room temperature (24±3°C) and 59±6% relative humidity for 10 days.

Treatments	Visual appearance (score)	Flavor (score)	Peel color		
			L*	chroma	hue angle
Factor 1: Fruit size					
No. 4	3.33±0.69b	3.44±0.70b	64.53±2.93	58.55±6.62	73.23±5.94b
No. 6	3.67±0.69a	3.67±0.49a	63.95±2.77	57.82±6.24	76.08±6.78a
Factor 2: Coating material					
Zivdar	4.00±0.60a	3.83±0.39a	64.72±2.38	59.21±6.36	73.04±6.01
Fomesa	3.67±0.49a	2.83±0.39b	63.75±3.10	56.27±6.60	76.22±7.34
Non-coated	2.83±0.39b	4.00±0.00a	64.28±2.75	59.18±5.94	74.37±5.83
Factor 1	*	*	ns	ns	*
Factor 2	*	*	ns	ns	ns
Factor 1×2	*	*	ns	ns	ns

Note: Means with different letters in the same column differ significantly (P<0.05) * = significance, ns = non significance. Evaluation of flavor by tasting, using a scale of 1 to 4, where 4 = excellent, 3 = slightly off-flavor, 2 = moderately off-flavor and 1 = extremely off-flavor. Fruit taste was rated “unacceptable” when the taste score was below three. Evaluation of visual appearance (wilting and shriveling), using a scale of 1 to 5, where 5 = excellent, 4 = good, 3 = fair, 2 = poor and 1 = unusable. Fruit appearance was rated “unacceptable” when the score was below three.

Flavor. Fruit No. 6 had a higher flavor score (3.7±0.5 score) than No. 4 (3.4±0.7 score) (Table 3). The flavor score significantly decreased with storage period in both fruit sizes No. 4 and No. 6 (data not shown). There was no significant difference between Zivdar-coated and non-coated fruit in flavor scores (4.0±0.0 and 3.8±0.4 scores, respectively). The fruit coated with Fomesa had the lowest flavor score (Table 3). Fruit size No. 4 and No. 6 coated with Fomesa had abnormal smell and taste on day 5 and day 8 of storage, respectively. At the end of storage, non-coated tangerine retained the highest flavor score while Zivdar-coated fruit had a significantly higher flavor score than Fomesa-coated fruit (data not shown).

The flavor quality of tangerines is sensitive to the type of coatings applied to the fruit (Ahmad and Khan, 1987; Mannheim and Soffer, 1996). In general, citrus fruit tends to develop off-flavor when stored at about 20°C after application of coating with low O₂ permeability that over-restricts the exchange of O₂ and CO₂ between the atmosphere and the fruit. The internal O₂ concentration becomes too low to support anaerobic respiration, with the result that ethanol, acetaldehyde and other flavor components are produced (Hagenmaier and Baker, 1994; Hagenmaier, 2000; Hagenmaier, 2002). Tangerines are often coated in the packinghouse with high-gloss coatings that have low gas permeability (Amarante and Banks, 2001).

Waxing or application of non-wax based coatings that occurs during commercial packing can alter the internal atmosphere in citrus fruit, leading to the production of anaerobic metabolites such as ethanol and acetaldehyde (Davis and Hoffman, 1973; Hagenmaier and Baker, 1994). Accumulation of these metabolites has been linked to poor flavor in waxed citrus (Ahmad and Khan, 1987; Cohen

et al., 1990; Hagenmaier, 2002) and in citrus exposed to long-term controlled atmosphere storage (Ke and Kader, 1990). Ethanol is naturally present in unwaxed fruit and is thought to be an enhancer of flavor if present in low to moderate amounts (Nisperos-Carriedo et al., 1990), although high amounts appear to cause off-flavor (Cohen et al., 1990; Ke and Kader, 1990). Mandarin oranges are especially prone to the accumulation of ethanol and off-flavors following waxing (Hagenmaier, 2002). In a comparison of different citrus types, Shi et al. (2005) found mandarins to be much more sensitive to anaerobic stress than grapefruit and speculated that this may be a major reason for the relatively poor storability of mandarins.

A limited number of studies have also documented that alcohol and acetaldehyde are not the only flavor-related volatiles that are altered in amount by the storage of waxed oranges. Nisperos-Carriedo et al. (1990) compared the effects of five different coatings during the storage of *Pineapple* oranges stored at 21°C for 12 days and found the coated fruit to have increased levels of at least five volatile components, some of them being potentially beneficial to the flavor of the fruit. In a study that attempted to more closely simulate commercial conditions, Baldwin et al. (1995) reported changes in numerous flavor-related volatiles as a result of waxing and storage.

Peel color

Size of tangerine fruit did not have an effect on L* and chroma value of peel color. Hue angle of tangerine fruit No. 6 was higher than that of fruit No. 4. The L*, chroma and hue angle of tangerines coated with Zivdar, Fomesa and non-coated control were not significantly different (Table 3). The results also showed that the L*, chroma and hue angle of tangerine fruit in all treatments slightly decreased during storage, with peel color changing slightly from green to yellow (data not shown). Hue angle is a good estimate of color change from green to yellow (McGuire, 1992). The hue angle decreases as the yellow pigments increase, showing the fruit peel turning to a yellow-orange color. The loss of green color was the most obvious change in tangerine fruit, which was due to the degradation of the chlorophyll molecule and an increase in carotenoid pigments during storage. This degradation was due to the oxidative system, pH change and enzymes like chlorophyllases (Wills et al., 2007).

Chemical compositions

Total soluble solids (TSS). The data pertaining to total soluble solids as affected by fruit size are shown in Table 4. Total soluble solids of tangerine fruit No. 4 (12.7±0.8%) was higher than fruit No. 6 (11.7±0.3%). De Salvador et al. (2006) mentioned that *Red Chief* apple fruit in the size classes from 55 to 65 mm had higher total soluble solids contents than size classes from 70 to 85 mm. Ketsa (1988) reported that the size of *Khieo Wan* tangerine fruit had an influence on total soluble solids contents and total soluble solids decreased as fruit size increased. The granulated fruit (larger in size) of some mandarins (*Nagpur, Kinnow, Kaula, Cleopatra*); sweet oranges (*Mosambi, Blood Red,*

Valencia Late, Malta Blood Red, Jaffa); lemons (*Kagzi Kalan, Eureka, Lisbon*); lime (*Kagzi*); grapefruit (*Duncan, Foster, Marsh*); pummelos (*Local, China, Kaoopan*); and tangelo (*Thornton*) had lower total soluble solids than normal size fruit (Sharma et al., 2006). However, the total soluble solids from Thai tangerine fruit (*Som Khieo Waan*) with different sizes (5.0, 5.5, 6.0, 7.0, 7.5 or more than 7.5 cm) were not significantly different (Jungsakulrujirek and Noomhorm, 1998).

Table 4. Effects of fruit size and coating material on total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, pH and vitamin C content of tangerine fruit stored at room temperature (24±3°C) and 59±6% relative humidity for 10 days.

Treatments	TSS (%)	TA (%)	TSS/TA ratio	pH	Vitamin C content (mg/100 ml juice)
Factor 1: Fruit size					
No. 4	12.66±0.83a	0.66±0.08a	19.49±1.77	3.18±0.08b	21.66±2.87
No. 6	11.73±0.28b	0.56±0.04b	21.18±1.73	3.30±0.06a	21.44±1.59
Factor 2: Coating material					
Zivdar	12.08±0.86	0.60±0.09	20.49±2.46	3.27±0.09	21.34±1.88
Fomesa	12.38±0.83	0.64±0.09	19.69±1.60	3.22±0.08	20.70±3.06
Non-coated	12.12±0.70	0.59±0.07	20.83±1.73	3.23±0.11	22.61±1.44
Factor 1	*	*	ns	*	ns
Factor 2	ns	ns	ns	ns	ns
Factor 1×2	ns	ns	ns	ns	ns

Note: Means with different letters in the same column differ significantly (P<0.05) * = significance, ns = non significance.

No significant differences of total soluble solids were observed between non-coated control and coated fruit after 10 days of storage. Total soluble solids of tangerine fruit in all treatments were relatively constant throughout the storage period (Table 4).

Titratable acidity (TA). Fruit No. 4 had a higher percentage of titratable acidity than fruit No. 6 (0.66±0.08 and 0.56±0.04%, respectively) (Table 4). Similarly, there was an inverse relationship between fruit size and titratable acidity in *Khieo Waan* tangerine (Ketsa, 1988). Sharma et al. (2006) reported that granulated fruit (larger in size) of some mandarins (*Nagpur, Kinnow, Kaula, Cleopatra*); sweet oranges (*Mosambi, Blood Red, Valencia Late, Malta Blood Red, Jaffa*); lemons (*Kagzi Kalan, Eureka, Lisbon*); lime (*Kagzi*); grapefruit (*Duncan, Foster, Marsh*); pummelos (*Local, China, Kaoopan*); and tangelo (*Thornton*) had lower titratable acidity than normal fruit. De Salvador et al. (2006) stated that *Golden Delicious* 'apples in size classes under 80 mm had a higher titratable acidity than fruit in size classes 80-90 mm.

There were no significant differences in titratable acidity among tangerine fruit treated with Fomesa, Zivdar and non-coated control fruit. Titratable acidity of coated and non-coated tangerine fruit decreased with increase in storage duration (Table 4).

TSS/TA ratio. The size and coating treatments had no effect on the TSS/TA ratio of tangerine fruit (Table 4). Pailly et al. (2004) reported that fruit size had no effect on TSS/TA ratios of *Star Ruby* grapefruit during storage at two different air temperatures (6 and 10°C). The TSS/TA ratios of juice from Thai tangerine fruit with different sizes (5.0, 5.5, 6.0, 7.0, 7.5 or more than 7.5 cm) were not significantly different (Jungsakulrujirek and Noomhorm, 1998). Increase of the TSS/TA ratio during storage has been observed in the *Tarocco* blood orange (Schirra and Chessa, 1988), *Hamlin* and *Valencia* orange (Echeverria and Ismail, 1987) and grapefruit (Bruemmer and Roe, 1969). The results also suggested that the ratio of TSS/TA of both coated and non-coated tangerines increased during storage for 13 days (data not shown).

pH. Tangerine fruit size No.6 had a higher pH value (3.3 ± 0.1) than fruit No.4 (3.2 ± 0.1) (Table 3). No significant differences in pH values were found among tangerine fruit coated with Zivdar (3.3 ± 0.1), Fomesa (3.2 ± 0.1) and non-coated control (3.2 ± 0.1). The pH value increased along with storage time in both coated and non-coated fruit (Table 4).

Vitamin C. There were no significant differences in vitamin C content between tangerine fruit No. 4 (21.7 ± 2.9 mg/100 ml juice) and No. 6 (21.4 ± 1.6 mg/100 ml juice) (Table 4). Ketsa (1988) reported that there was no relationship between fruit size and ascorbic acid content in *Khieo Waan* tangerine fruit. There was no significant difference in vitamin C content of coated- and non-coated fruit. Vitamin C content of tangerine fruit in all treatments slightly decreased during storage (Table 4).

CONCLUSION

No significant differences existed between the internal O₂ and CO₂ concentrations, ethanol content in fruit juice, PDC activity, L* and chroma values of peel color, TSS/TA ratio, as well as vitamin C content of small size (No. 4) and large size (No. 6) tangerines. However, the size of tangerine fruit had an effect on hue angle of peel color, pH level, TSS and TA. The weight loss was lower with less off-flavor and a better appearance in larger tangerines than smaller ones. In addition, fruit size No. 6 also possessed a higher ADH activity than fruit size No. 4.

Fruit coated with Zivdar and Fomesa had lower weight loss than non-coated fruit. Coated tangerine fruit with Zivdar beneficially affected the optimal exchange of O₂ and CO₂ gases with a lower level of internal ethanol content as well as contributed to a slower rate of off-flavor than fruit coated with Fomesa. Non-coated fruit had higher internal O₂ and lower internal CO₂ and ethanol content in fruit juice than coated fruit. Non-coated fruit had the best taste and odor during storage. Coating treatments did not affect PDC and ADH activities, skin color, TSS, TA, TSS/TA ratio, pH and vitamin C content.

ACKNOWLEDGEMENTS

This research was supported by the Thailand Research Fund and a grant from the Strategic Scholarships for Frontier Research Network for the Ph.D. Program Thai Doctoral degree from the Office of the Higher Education Commission, Thailand. We also thank the Faculty of Agriculture, Chiang Mai University for their support and Suan Som Sai Thong Changwat Chiang Mai Company Limited for providing *Sai Nam Phueng* tangerine fruit and Nature Bright Company Limited for supplying the Zivdar coating.

REFERENCES

- Ahmad, M., and I. Khan. 1987. Effect of waxing and cellophane lining on chemical quality indices of citrus fruit. *Plants Foods for Human Nutrition* 37: 47-57.
- Amarante, C., and N.H. Banks. 2001. Postharvest physiology and quality of coated fruits and vegetables. *Horticultural Reviews* 26: 161-238.
- Aquino, S.D., M. Angioni, S. Schirru., and M. Agabbio. 2001a. Quality and physiological changes of film packaged Malvasio mandarins during long term storage. *Lebensmittel-Wissenschaft und Technologie* 34: 206-214.
- Aquino, S.D., M.G. Molinu, A. Piga., and M. Agabbio. 2001b. Influence of film wrapping on quality maintenance of Salustiana oranges under shelf life conditions. *Italian Journal of Food Science* 13: 87-100.
- Baldwin, E.A., M.O. Nisperos-Carriedo., and R.A. Baker. 1995a. Edible coatings for lightly processed fruits and vegetables. *HortScience* 30: 35-38.
- Baldwin, E.A., M.O. Nisperos-Carriedo., and R.A. Baker. 1995b. Use of edible coatings to preserve quality of lightly (and slightly) processed products. *Critical Reviews in Food Science and Nutrition* 35: 509-524.
- Baldwin, E.A., J.K. Burns, W. Kazokas, J.K. Brecht, R.D. Hagenmaier, R.J. Bender., and E. Pesis. 1999. Effect of two edible coatings with different permeability characteristics on mango (*Mangifera indica* L.) ripening during storage. *Postharvest Biology and Technology* 17: 215-226.
- Ben-Yehoshua, S. 1969. Gas exchange, transpiration and the commercial deterioration of stored fruits. *Journal of the American Society for Horticultural Science*. 94: 524-528.
- Ben-Yehoshua, S. 1987. Transpiration, water stress, and gas exchange, pp. 113-173. In: Weichman, J. (ed.). *Postharvest Physiology of Vegetables*. Marcel Dekker Inc., New York.
- Bonnichsen, R.K., and H. Theorell. 1951. An enzymatic method for the micro determination of ethanol. *Scandinavian Journal of Clinical and Laboratory Investigation* 3: 58.
- Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.
- Bruemmer, J.H., and B. Roe. 1969. Post-harvest treatment of citrus fruit to increase Brix/acid ratio. *Proceedings of the Florida State Horticultural Society* 82: 212-215.

- Burton, W.G. 1985. Post-harvest Physiology of Food Crops. Longman, London. 339 p.
- Cameron, J.W., and R.K. Soost. 1977. Acidity and total soluble solids in Citrus hybrids and advanced crosses involving acidless orange and acidless pummelo. *Journal of the American Society for Horticultural Science* 102: 198-201.
- Chang, L.A., L.K. Hammett., and D.M. Pharr. 1983. Carbon dioxide effects on ethanol production, pyruvate decarboxylase, and alcohol dehydrogenase activities in anaerobic sweet potato roots. *Plant Physiology* 71: 59-62.
- Cohen, E., Y. Shalom., and I. Rosenberger. 1990. Postharvest ethanol buildup and off-flavor in 'Murcott' tangerine fruits. *Journal of the American Society for Horticultural Science* 155: 775-778.
- Davis, P.L., and R.C. Hofmann. 1973. Effects of coating on weight loss and ethanol buildup in juice oranges. *Journal of Agricultural and Food Chemistry* 21: 455-456.
- de Salvador, F.R. Fisichella., and M. Fontanari. 2006. Correlations between fruit size and fruit quality in apple trees with high and standard crop load levels. *Journal of Fruit and Ornamental Plant Research* 14: 113-122.
- Díaz-Pérez, J.C. 1998. Transpiration rates in eggplant fruit as affected by fruit and calyx size. *Postharvest Biology and Technology* 13: 45-49.
- Díaz-Pérez, J.C., M.D. Muy-Rangel., and A.G. Mascorro. 2007. Fruit size and stage of ripeness affect postharvest water loss in bell pepper fruit (*Capsicum annuum* L.). *Journal of the Science of Food and Agriculture* 87: 68-73.
- Echeverria, E., and M. Ismail. 1987. Changes in sugars and acids of citrus fruits during storage. *Proceedings of the Florida State Horticultural Society* 100: 50-52.
- Hagenmaier, R.D. 2000. Evaluation of a polyethylene–candelilla coating for 'Valencia' oranges. *Postharvest Biology and Technology* 19: 147-154.
- Hagenmaier, R.D. 2001. Ethanol content of 'Murcott' tangerines harvested at different times and treated with coatings of different O₂ permeability. *Proceedings of the Florida State Horticultural Society* 114: 170-173.
- Hagenmaier, R.D. 2002. The flavor of mandarin hybrids with different coatings. *Postharvest Biology and Technology* 24: 79-87.
- Hagenmaier, R.D., and P. Shaw. 1991. Permeability of coatings made with emulsified polyethylene wax. *Journal of Agricultural and Food Chemistry* 39: 1705-1708.
- Hagenmaier, R.D., and R.A. Baker. 1993. Cleaning method affects shrinkage rate of citrus fruit. *HortScience* 28: 824-825.
- Hagenmaier, R.D., and R.A. Baker. 1994. Internal gases, ethanol content and gloss of citrus fruit coated with polyethylene wax, carnauba wax, shellac or resin at different application levels. *Proceedings of the Florida State Horticultural Society* 107: 261-265.
- Imahori, Y., K. Matushita, M. Kota, Y. Ueda, M. Ishimaru., and K. Chachin. 2003. Regulation of fermentative metabolism in tomato fruit under low oxygen stress. *Journal of Horticultural Science & Biotechnology* 78: 386-393.

- Issarakraisila, M. 1984. A study on fruit development, harvesting indices and post-harvest changes in Som Khieo-Wan (*Citrus reticulata* Blanco.) and Som Tra (*Citrus sinensis* Osbeck.). M.S. Thesis, Kasetsart University, Thailand.
- Jungsakulrujirek, S., and A. Noomhorm. 1998. Effect of harvesting time and fruit size on titratable acidity, soluble solid and distribution of limonin in Thai tangerine juice. *International Journal of Food Science and Technology* 33: 367-374.
- Kanellis, A.K., T. Solomos., and K.A. Roubelakis-Angelakis. 1991. Suppression of cellulose and polygalacturonase and induction of alcohol dehydrogenase isozymes in avocado fruit mesocarp subjected to low oxygen stress. *Plant Physiology* 96: 269-274.
- Ke, E., and A.A. Kader. 1990. Tolerance of 'Valencia' oranges to controlled atmospheres as determined by physiological responses and quality attributes. *Journal of the American Society for Horticultural Science* 115: 779-783.
- Ke, D., M. Mateos., and A.A. Kader. 1993. Regulation of fermentative metabolism in fruits and vegetables by controlled atmospheres. *Proceeding 6th International Controlled Atmosphere Research Conference, Cornell University, Ithaca, New York*, pp. 63-77.
- Ke, D., E. Yahia, M. Mateos., and A.A. Kader. 1994. Ethanol fermentation of 'Bartlett' pears as influenced by ripening stage and atmospheric composition. *Journal of the American Society for Horticultural Science* 119: 976-982.
- Kenedy, R.A., M.E. Rumpho., and T.C. Fox. 1992. Anaerobic metabolism in plants. *Plant Physiology* 100: 1-6.
- Ketsa, S. 1988. Effects of fruit size on juice content and chemical composition of tangerine. *Journal of Horticultural Science* 63: 171-174.
- Ketsa, S. 1990. Effect of fruit size on weight loss and shelf life of tangerine. *Journal of Horticultural Science* 65: 485-488.
- Koo, R.C.J., T.W. Young, L.R. Reece., and J.W. Kesterson. 1974. Effects of nitrogen, potassium and irrigation on yield and quality of lemon. *Journal of the American Society for Horticultural Science* 99: 289-291.
- Levy, Y., A. Bar-Akiva., and Y. Vaadia. 1974. Influence of irrigation and environmental factors on grapefruit acidity. *Journal of the American Society for Horticultural Science* 103: 73-76.
- McDonald, R.E., and B.M. Hillebrand. 1980. Physical and chemical characteristics of lemons from several countries. *Journal of the American Society for Horticultural Science* 105: 137-141.
- Mannheim, C.H., and T. Soffer. 1996. Permeability of different wax coatings and their effect on citrus fruit quality. *Journal of Agricultural and Food Chemistry* 44: 919-23.
- McGuire, R.G. 1992. Reporting of objective color measurements. *HortScience* 27: 1254-1255.
- Ministry of Fisheries, Crops and Livestock, New Guyana Marketing Corporation, and National Agricultural Research Institute. 2004. Postharvest handling technical series, Tangerine: Postharvest care and market preparation. *Technical Bulletin no. 31*. 19 p.

- Nanos, G.D., R.J. Romani., and A.A. Kader. 1992. Metabolic and other responses of 'Barlett' pear fruit and suspension-cultured 'Passe Crassane' pear fruit cells held in 0.25% O₂. *Journal of the American Society for Horticultural Science* 117: 934-940.
- Nisperos-Carriedo, M.O., P.E. Shaw., and E.A. Baldwin. 1990. Changes in volatile flavor components of Pineapple orange juice as influenced by the application of lipid and composite film. *Journal of Agricultural and Food Chemistry* 38: 1382-1387.
- Pailly, O., G. Tison., and A. Amouroux. 2004. Harvest time and storage conditions of 'Star Ruby' grapefruit (*Citrus paradisi* Macf.) for short distance summer consumption. *Postharvest Biology and Technology* 34: 65-73.
- Porat, R., B. Weiss, L. Cohena, A. Dausa., and A. Biton. 2005. Effects of polyethylene wax content and composition on taste, quality, and emission of off-flavor volatiles in 'Mor' mandarins. *Postharvest Biology and Technology* 38: 262-268.
- Raghav, P.K. and A.K. Gupta. 2000. Quality and shelf life of individually shrink wrapped Kinnow fruit. *Journal of Food Science and Technology* 37: 613-616.
- Ranganna, S. 1986. *Manual of Analysis of Fruit and Vegetable Products*. Tata McGraw-Hill Publishing Company Limited, New Delhi. 634 p.
- Schirra, M., and I. Chessa. 1988. Physiological behaviour of Tarocco orange during cold storage, pp. 1491-1498. In: *Proceedings of the 6th International Citrus Congress*, Tel Aviv, Israel.
- Sharma, R.R., R. Singh., and S.K. Saxena. 2006. Characteristics of citrus fruits in relation to granulation. *Scientia Horticulturae*. 111: 91-96.
- Shi, J.X., R. Porat, R. Goren., and E.E. Goldschmidt. 2005. Physiological responses of 'Murcott' mandarins and 'Star Ruby' grapefruit to anaerobic stress conditions and their relation to fruit taste, quality and emission of off-flavor volatiles. *Postharvest Biology and Technology* 38: 99-105.
- Sites, J.W., and H.J. Reitz. 1950. The variation in individual Valencia oranges from different locations on the tree as a guide to sampling methods and spot-picking for quality. II. Titrable acid and the soluble solids/titrable acid ratio of the juice. *Proceedings of the American Society for Horticultural Science* 55: 73-80.
- Wills, R., B. McGlasson, D. Graham., and D. Joyce. 2007. *Postharvest: An introduction to the physiology and handling of fruit, vegetables and ornamentals*. 5th edition, CABI, Oxfordshire. 227 p.
- Wutscher, H.K., and V.A. Shull. 1972. Performance of 13 citrus cultivars as rootstocks for grapefruit. *Journal of American Society for Horticultural Science* 97: 778-781.