Physico-Chemical Characteristics of Chok Anan Mango Fruit after Hot Water Treatment

Phebe Ding* and Salumiah Mijin
Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT
Mango fruit is prone to postharvest disease, especially anthracnose and stem end rot. Hot water dip (HWD) treatment has been used to control postharvest disease in fruit but little information about the response of Chok Anan mango fruit towards HWD treatment. This study was conducted to determine optimum duration of HWD in controlling postharvest diseases. Mature green Chok Anan mango fruit were treated at 26 and 55°C for 5, 15 and 25 min and fruits at ripening stage 1, 3 and 5 were analyzed for peel colour, flesh firmness, soluble solids concentration (SSC), ascorbic acid, pH, titratable acidity, disease incidence and heat induced injury. HWD treatment did not affect peel colour, SSC, ascorbic acid, pH and titratable acidity and induced heat injury to the fruit. Disease incidence of ripening stage 5 (fully ripened) fruit reduced significantly after HWD. In addition, the fruit underwent normal ripening as ripening progressed. It is concluded that combination of 55°C hot water for 5 min can be used as postharvest disinfestation treatment for Chok Anan mango fruit while maintaining physico-chemical characteristics of fruit.

Keywords: Anthracnose, disease incidence, heat injury, postharvest quality, peel colour

INTRODUCTION
Mango (Mangifera indica L.) belongs to family Anacardiaceae. It is originated from India. It has been cultivated in India since 4,000 years ago. It is one of the popular tropical and subtropical fruit as it contains high antioxidants such as ascorbic acid and carotenoids. Under tropical conditions, the fruits ripen rapidly and then follow by senescence. Mango fruits are susceptible to postharvest fungal pathogen infections with anthracnose and stem end rot as their major problems. The symptoms of infection do not develop until the fruit ripened.
Colletotrichum spp. is the most important pathogens that cause this type of infection. Current commercial fungicides such as benomyl and thiabendazole have been widely used to control postharvest pathogens. With the growing consumer awareness about safe food, chemical residues free methods to control postharvest pathogen have been a major focus. One of the safe method used to control postharvest pathogen is by dipping freshly harvested mango fruit in 55°C for 5 to 10 min and this treatment is known as hot water dip (HWD) (Coates et al., 1993). HWD treatment is regards as an effective non-chemical method to control postharvest diseases if combinations of suitable temperatures and exposure times being used and at the same time prevent the quality loss of produce (Lurie, 1998). The effectiveness of hot water dips as fungal pathogen control depends on location of fungal spores that are either on the surface or in the first few cell layers under fruit peel.

HWD treatment has been used to control postharvest disease while maintaining fruit quality in ‘Kensington’ (Jacobi et al., 2000), ‘Keitt’, ‘Kent’ and ‘Tommy Atkins’ (Mansour et al., 2006) mangoes but little information is available for Chok Anan mango fruit. Chok Anan mango is an important and popular variety in Malaysia. However, there is not much of attention given to this variety especially in postharvest treatment. Furthermore, although heat treatments have been used by many countries as non-chemical disinfection treatments, no single heat treatment has been found to be universally acceptable for all mango cultivars (Jacobi et al., 2001). From our literature search, 55°C of hot water for 5 min has been used by Thai to compare efficacy of yeasts antagonists, chitosan or their combination for controlling the severity of anthracnose lesions in Chok Anan mango fruit (Chantrasri et al., 2007). Unfortunately, they did not look into physico-chemical characteristics of Chok Anan mango fruit after hot water treatment. The information on fruit quality after hot water treatment is prerequisite in developing technologies. Therefore, the objective of this study was to determine optimum duration of HWD treatment using 55°C as non-chemical disinfection treatment for Chok Anan mango fruit while maintaining fruit quality.

MATERIALS AND METHODS

Plant Material

Chok Anan mango (Mangifera indica L.) fruits were obtained from Selangor Wholesale Market, Selangor at mature green stage or ripening stage 1. Fruits were selected for uniformity of shape, colour and size (200–300 g) and any blemishes or diseased fruit were discarded. The fruits were divided into two lots.

For the first lot, fruits were dipped in water at 26°C for 5, 15 and 25 min as control while fruits for the second lot were dipped in heated water of 55°C for same durations. After dipping fruit in 26 and 55°C, fruit were showered with ambient temperature water (26°C) for 20 min and then allowed to air dry before the fruits were packed in a carton and gassed with 0.02 ml acetylene generated from calcium carbide (CaC₂)
at the rate of 20 g CaC$_2$/kg fruit. Cartons containing the fruit and CaC$_2$ were placed in a room of 26°C with relative humidity of 75-80% for ripening initiation. After 48 h, the cover of the carton was removed and fruit were allowed to continue ripening until the peel turned full yellow. The fruits of ripening stage 1 (mature green), 3 (50% green – 50% yellow) and 5 (100% yellow) were analyzed. A total of 250 fruits were used for the experiments which repeated four times.

**Selected Quality Characteristics**

**Peel Colour Determination**

Peel colour of Chok Anan mango was determined using a chroma meter (Model CR-300 Minolta Corp., Osaka, Japan). The meters were equipped with a measuring head that has an 8-mm-diameter measuring area and calibrated with a standard white tile. Calibration values were $L^*=97.95$, $a^* = -0.07$ and $b^* = 1.66$ using the illumination (CIE 1976). From a fruit, three readings at equatorial region were recorded and mean value was computed. Measurements were expressed in chromaticity values of $L^*$, $C^*$ and $h^*$.

The $L^*$, indicates the lightness, with values ranging from 0=black to 100=white. The $C^*$ values refer to the vividness of colour were computed from values of $a^*$ and $b^*$ i.e. $C^*=(a^2+b^2)^{1/2}$ which represented the hypotenuse of a right triangle. Hue angle ($h^*$) was calculated as $\tan^{-1} b^*/a^*$.

**Flesh Firmness Determination**

The firmness of the fruit was evaluated using a bishop penetrometer FT 327 (Italy) with an 11-mm-diameter plunger. Forces from constant penetration of the plunger were applied perpendicularly to the 1 cm of peeled mango fruit which was cut from equatorial region of a fruit with a smooth motion in two to three seconds. The readings in kilograms-forces were made at two opposite direction of every slice of the mango fruit and were converted to newton (N).

**Titratable Acidity (TA) Determination**

The TA of the fruit was determined by slicing out 10 g of the Chok Anan mango fruit. Forty milliliters of distilled water was then added to the 10 g of fruit and blended in a high-speed blender (Model MX V2 National) for one min. The macerate was filtered with cotton into a conical flask. After that, 5 ml of filtrate was titrated with 0.1 mol/ml sodium hydroxide. Three drops of 1% phenolphthalein indicator was added into filtrate. The indicator added filtrate was then titrated until it’s turned pink. From the titre, the percentage of citric acid was calculated.

$$\% \text{ Citric acid} = \frac{[\text{ml NaOH} \times 0.1 \text{ ml/weight of sample titrated}] \times 0.64}{\text{weight of sample titrated}}$$

**Soluble Solids Concentration (SSC) Determination**

The SSC of mango fruit were determined using a hand refractometer (Model N1, Atago, Japan). The refractometer was
calibrated with distilled water until the reading reached 0. A drop of the extracted juice from the remainder of TA determination was then placed on the prism glass of refractometer to obtain the %SSC reading. The readings were corrected to a standard temperature of 20°C by adding 0.28% to obtain % SSC at 27°C.

**pH Determination**
The remainder of the juice from the TA determination was used to measure pH using glass electrode pH meter (Model Crison Micro pH 2000). The pH meter was calibrated with buffers at pH 4.0 and 7.0 before being used.

**Ascorbic Acid (AA) Determination**
The AA contained in the Chok Anan mango fruit was determined by using titration method. Ten gram of mango fruit was blended with 3% cold HPO₃. Then five milliliter juice sample was titrated with dye until the juice changed to pink colour. Volume of dye (titre) used was recorded and vitamin C (mg/100 g) was calculated as follows:

Vitamin C (mg/100 g)  
= [Titre (ml) × dye factor × volume made up (ml) × 100] / [Aliquot used for estimation (ml) × weight of sample (g)]

To standardize the dye, 5 ml of standard ascorbic solution was taken and 5 ml of 3% cold HPO₃ was added. The mixture was titrated with the dye solution to a pink colour. The dye factor was determined as follows:

Dye factor  
= μg acid ascorbic / ml of dye (titre)  
= 0.5 / ml dye (titre)

**Disease Incidence Assessments**
Disease incidence was assessed at ripening stages 3 and 5 during fruit ripening. The severity of disease was assessed after treatment according to the percentage of peel area affected by disease per fruit and then the percentage score was related to a 5-point scale where 0= 0%; 1= 1-5%; 2= 6-15%; 3= 16-30% and 4= 31-100% of peel area affected by disease (Ding & Ong, 2010).

**Heat Induced Injury Assessment**
The severity of heat induced injury was assessed after treatment according to the percentage of peel area affected by heat per fruit. The symptom of peel injury induced by hot water treatment included translucence, shriveling, dimples, brown discoloration and decay. Mango fruits were sorted into 5-point scale according to their heat injury severity where 0= 0%; 1= 1-5%; 2= 6-15%; 3= 16-30% and 4= 31-100% (Ding & Ong, 2010).

**Statistical Analysis**
The experimental design was a randomized complete block design with a factorial arrangement of treatments (two water temperatures × three dipping times × three ripening stages) and four replications. Data were analyzed using the analysis of variance (ANOVA) (SAS Institute, Cary, N.C. 1989). When the F values showed significant (P ≤ 0.05) differences, least significance
difference (LSD) test was used to separate the means. Data for the disease incidence and heat induced injury were transformed into $\log_{10}$ prior to analysis.

RESULTS
The peel colour of Chok Anan mango fruit was not significantly affected by interactions with an exception for hue angle where the three factors were substantially interacted among water temperature x dipping time x ripening stage (Table 1). The insignificant effect of peel colour was also extended to main effect except for the ripening stages. The $L'$ and $C'$ values of Chok Anan mango fruit increased significantly as ripening progressed. In contrast, $h^\circ$ values of Chok Anan mango fruit peel showed an opposite trend to $L'$ and $C'$ values with decreasing values as ripening progressed from stage 1 to 5.

There were no significant interactions effects between the factors in flesh firmness of Chok Anan mango fruit (Table 2). However, flesh firmness was affected significantly by water temperature and

### TABLE 1
Effects of two water temperatures, three dipping times and three ripening stages on peel colour ($L'$, $C'$ and $h^\circ$) of Chok Anan mango fruit

<table>
<thead>
<tr>
<th>Factor</th>
<th>Peel colour</th>
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<tbody>
<tr>
<td></td>
<td>$L'$</td>
</tr>
<tr>
<td>Dipping temperature (W), °C</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>62.42</td>
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<tr>
<td>55</td>
<td>63.13</td>
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<tr>
<td>F-test significance</td>
<td>NS</td>
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<td>Dipping time (D), min</td>
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<tr>
<td>5</td>
<td>62.61</td>
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<tr>
<td>15</td>
<td>62.87</td>
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<tr>
<td>25</td>
<td>62.84</td>
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<tr>
<td>F-test significance</td>
<td>NS</td>
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<tr>
<td>Ripening stage (R)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>52.00 c</td>
</tr>
<tr>
<td>3</td>
<td>65.02 b</td>
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<tr>
<td>5</td>
<td>71.30 a</td>
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<td>F-test significance</td>
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<td>Interaction</td>
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<td>W x D</td>
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<tr>
<td>D x R</td>
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<td>W x D x R</td>
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</table>

$L'$ = lightness, $C'$ = chroma and $h^\circ$ = hue angle.

NS, *, ** Non significant or significant or highly significant at $P \leq 0.05$.

2Mean separation within columns and factors followed by the same letter are significantly different by LSD at $P \leq 0.05$. 

ripening stage. Flesh firmness of Chok Anan mango fruit dipped in 55°C was firmer as compared to the control. As fruit ripened, firmness of fruit encountered significant decrease. SSC for Chok Anan mango fruit was not affected by interactions between water temperature and dipping time (Table 2). However, the SSC was significantly affected by ripening stage with 301% increase when fruit ripened. Similar to SSC, AA, pH and TA of Chok Anan mango fruit was not affected by interactions, water temperature and dipping time (Table 2). Disease incidence of Chok Anan mango fruit was affected by significant interaction between water temperature and ripening stage (Table 3). Disease incidence occurred in fruit either dipped in 26 or 55°C (Fig.1).

Nonetheless, fruits treated with 55°C water only started to show disease incidence when at ripening stage five. Control fruit was infected by pathogen as early as ripening stage 3 and became more serious when at
By ripening stage 5, fruits treated with 55°C of hot water had successfully inhibited the occurrence of disease by 94% as compared to the control. Although the disease incidence has been suppressed tremendously by hot water, the heat did not cause any injury to Chok Anan mango fruit as found in Table 3.

**DISCUSSION**

The peel colour, SSC, AA, pH and TA of Chok Anan mango fruit was not affected by water temperatures, dipping times and their interactions (Tables 1 & 2). As ripening stage advanced, the peel colour showed significant increase in lightness and chromaticity with decrease hue values which reflected colour changed from green to yellow. Similar to peel colour, the firmness, SSC, AA, pH and TA of Chok Anan mango fruit also showed significant changes as ripening stage advanced. With the significant changes in these quality characteristics, Chok Anan mango fruit became palatable.

The ability of a fruit to undergo normal ripening is a main concern when using heat
in postharvest chain besides controlling and/or reducing disease incidence (Jacobi et al., 2001; Paull & Chen, 2000). Jacobi et al. (2001) has reviewed different varieties of mangoes response distinctly to various combination of heat temperature and exposure time. They even ranked different cultivars of mango according to its heat tolerance in their review. ‘Irwin’, ‘Kensington’, ‘Haden’ and ‘Strawberry’ mangoes are the least heat tolerant cultivars as compared to ‘Haden’, ‘Davis Haden’, ‘Pahiri’ and ‘Alphonso’ mangoes. Some of the mangoes showed uneven skin development when ripening while some showed fruit softening after heat treatment. However, in the present study, Chok Anan mango fruits did not have these problems after HWD treatment. This indicated that Chok Anan mango could tolerate 55°C hot water for 25 min. It is reported that the influence of heat on postharvest fruit ripening is also dependent on level of field-induced thermotolerance besides differences in cultivar (Paull & Chen, 2000). Chok Anan mango fruit has been exposed to hot and humid field condition throughout its growing season. Therefore, it is expected that this variety of mango could tolerate high postharvest heat treatment compared to other variety of mango planted in subtropical regions. Unfortunately, besides this report, there is no other reports have been found on heat tolerance level of Chok Anan mango fruit whether it could withstand temperature beyond 55°C or not and/or with extended exposure time.

Besides mangoes, there are other species of fruit been treated with hot water which responded differently towards water temperature and exposure time used. Thompson Seedless table grapes dipped in

![Fig.1: Effects of water temperature and ripening stage on disease incidence of Chok Anan mango fruit. Means followed by the different small and capital letters denote significant differences within water temperatures and among ripening stages, respectively (P ≤ 0.05, LSD).](image)

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30, 40 and 50°C hot water for 3 min and stored for 1 month at 1°C, did not show any differences in peel colour among the temperatures used (Karabulut et al., 2004). Litchi dipped in 52°C hot water for 1 min did not show any significant difference from control in terms L* and C* values after 49 days of storage at 5°C (Olessen et al., 2004). SSC of ‘Kent’ mango fruit was higher after treatment with hot water at 50°C for 5 min (Mansour et al., 2006). For sapote mamey fruit, HWD treatment caused lower SSC than control after 4-day of ripening (Diaz-Perez et al., 2001). While in strawberry, SSC was not affected by HWD treatment (Zhang et al., 2007). Similar to the finding of this study, AA and TA of strawberry were not affected by hot water treatment. However, HWD treated sapote mamey has higher TA and lower pH as compared to the control (Diaz-Perez et al., 2001). ‘Hom Thong’ banana treated with 50°C hot water for 10 min only showed significant higher AA than control at day 2 during 10 days of storage at 25°C (Ummarat et al., 2011). While the rest of storage days did not show any differences in AA as compared to the control.

Heat treatment has inhibited softening of Chok Anan mango fruit (Table 2). This indicated that HWD Chok Anan mango fruit took longer time to soften and thus prolong its shelf life. This characteristic is desired by retailers. A contrary finding was reported in HWD ‘Tommy Atkin’, ‘Keitt’, ‘Palmer’ (McGuire, 1991) and ‘Kensington’ mangoes (Jacobi and Giles, 1997). These researchers reported that the softening of mangoes fruits were accelerated after heat treatment. For strawberry, HWD did not affect fruit softening even after 3 days of storage at 20°C (Zhang et al., 2007). The firmness of sapote mamey fruit after HWD at 60°C for 60 min followed by 4-day ripening at 25°C was higher than control (Diaz-Perez et al., 2001). Again, the response of fruit towards HWD treatment depended on variety and species of fruit.

Heat treatment gives to fruit during postharvest handling is a kind of stress. The severity of stress is determined by temperature and exposure time given (Lurie, 1998). Difference responses of fruit to temperature and exposure time are related to the level of heat protective proteins at harvest and postharvest production of heat shock protein (Paull & Chen, 2000). Heat shock protein is believed to act as chaperones that are responsible for protein refolding under stress conditions (Wang et al., 2004). Chok Anan mango fruit could undergo normal colour changes even though has been treated with 55°C water for 25 min. It has been reported that the change of fruit peel colour is associated with enzymatic degradation of green chlorophyll and synthesis of yellow carotenoid (Ding et al., 2007). Enzymes are well known for its heat sensitive, but in this study the enzymatic reaction involved in colour changes of Chok Anan mango fruit was not affected by heat treatment.

However, this was not true for enzymes involved in Chok Anan mango fruit softening. The firmness of hot water treated Chok Anan mango fruit has been retained. Paull & Chen (2000) have summarized
flesh softening is temperature dependent with slow softening at 38-40°C and faster or disrupted after exposure to 45-50°C of heat. This finding may not apply to all types of fruit. The softening of *Musa* AAA Berangan ripened at 37°C was retained while fruit ripened at 25°C showed faster ripening (Ratule *et al*., 2007). They found out banana ripened at 25°C showed a more advanced dissolution of pectin in the middle lamella than fruit ripened at elevated temperature of 37°C. Most probably the dissolution of pectin in heat treated Chok Anan mango fruit is slower than control fruit. Thus, fruit treated with 55°C hot water has higher firmness than control. Hydrolytic enzymes are needed to dissolve cellulose, hemicellulose and pectin in cell wall which lead to softening. However, the actual enzymes having the central role in softening of heat treated fruit have not been determined yet (Paull & Chen, 2000).

Postharvest pathogen of Chok Anan mango fruits were reduced significantly after treating with 55°C hot water (Table 3). On top of this, the heat used in present study did not cause any injury to fruit. The non-significant effect in heat induced injury to fruit tissue caused by water temperature, dipping time, ripening stage and their interactions, again showed that 55°C can be used to treat Chok Anan mango fruit. Since varying dipping time from 5 to 25 min did not affect the efficacy of heat treatment, it is suggested that the combination of hot water 55°C and 5 min dipping time is sufficient to be used to control postharvest pathogens in Chok Anan mango fruit while retaining fruit quality.

Heat treatments, as one of the disinfestation methods, have been widely used to control disease in mango industry (Jaboci *et al*., 2001). The treatments require mango fruit to be heated to a specific core temperature for a defined period (Paull & McDonald, 1994). Heat is transferred via energy from a heating medium, which is water in this study. After treating Chok Anan mango fruit at 55°C for 5, 15 and 25 min, the fruit core temperatures were 40.08, 48.10 and 51.74°C, respectively (unpublished data). Before heat treatment, the initial core temperature of fruit was 26.55°C. The energy of hot water was transferred from peel (exocarp) then into flesh (mesocarp). Along the energy transfer, fungal infection sites that contain mycelium and spores were reached and killed by the heat. After dipping for 25 min, the fruit core temperature was 51.74°C and yet fruit did not show any symptoms of heat injury. Apparently, Chok Anan mango fruit could tolerate core temperature up to 51.74°C.

Another reason for the success in controlling postharvest pathogen by heat treatment is ‘melting’ of the cuticular wax which sealed micro-cracks, stomata and/or lenticels that appeared on fruit surface. The peel of a mature mango fruit is composed by a single layer of epidermis with lenticels and a well-defined cuticle with wax deposition (Muhammad & Ding, 2007). Micro-cracks can easily be found among cuticular platelets of mango fruit. These opening structures are important invasion site for pathogens. As reported in ‘Oroblanco’
grapefruit, ‘Fortune’ mandarins, cactus pears and organically grown grapefruit, after heat treatments, the wax platelets melted and eventually lead to covering and sealing of these openings (Schirra et al., 2000). This provided mechanical barrier against pathogen. Also, germinated spores, conidia and hyphae appeared covered and mummified by molten wax as occurred in cactus pears that were subjected to curing at 37°C for 30 h (Schirra et al., 1999). However, such beneficial effects may be stalled during prolonged shelf life as cracks tend to reappear and damaged stomata may attract hyphae penetration. The melting point of mango wax is 62°C (Panhwar, 2005) and thus the 55°C of hot water used in the present study is able to ‘melt’ some of the wax. The melted wax could have covered and sealed the lenticels and micro-cracks of Chok Anan mango fruit. Therefore, the disease incidence was not present in ripening stage 3 of hot water treated fruit but not in control fruit (Table 3).

The occurrence of disease incidence in ripening stage 5 of hot water treated Chok Anan mango fruit indicated HWD treatment is not able to inhibit the growth of pathogen completely (Table 3). Most probably more than one type and/or species of pathogen exist in the fruit. It has been proven that the sensitivity of pathogens to temperature and exposure time varied according to pathogen species as found in crown rot of banana cv Bungulan (Alvindia, 2012). Generally, the mycelium growth and spore germination of crown rot-causing pathogens was slower at a combination of higher water temperature and longer exposure time. However, the tolerance of fruit to hot water should take into consideration during using high water temperature and long exposure time. It is advisable to use temperatures less than the lethal temperatures and duration so that short-term disruption of transcription and translation steps in protein synthesis can be reversed (Paull & Chen, 2000).

It has been reported that the response of fruit to heat varies with species, genotypes within species, physiological stage or fruit maturity, fruit size and morphological characteristics, exposure to different environmental and/or preharvest factors (such as rainfall, soil type and production practices), the type of heat treatment applied, heat transfer rate and energy balance (thermal difference, heat capacity and relative humidity), final temperature and the duration of exposure at different temperatures, and whether postharvest conditioning treatments have been given before and after a heat treatment (Jacobi et al., 2001; Paull & Chen, 2000). In short, there is no single heat disinfestation treatment has been found to be applied for all mango cultivars including other fruits as a lot of factors involved in determining the success of the treatment.

From the findings of the present study, it can be concluded that postharvest pathogen of Chok Anan mango fruit can be controlled using 55°C hot water for 5 min without having to compromise the postharvest qualities of the fruits with further improvement of firmness. This report provided basic understandings on Chok Anan mango...
fruits response to 55°C hot water dipped for 5, 15 and 25 min. Since there are so many factors affecting the success HWD treatment, knowledge of basic principles of physiological and biochemical responses of mango fruit to temperature stress is essential. In future, a more comprehensive work using different hot water temperatures, type of heat treatment, duration of heat exposure and even different physiological state of fruit should be carried out to provide a solid foundation of information to possibly predict Chok Anan mango fruit response to heat treatment. In order to market this fruit internationally, this information is needed to enable the development of effective strategies for heat treatment since the number of health conscious consumers is growing every year.

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