

Antioxidant phytochemical and quality changes associated with hot water immersion treatment of mangoes (*Mangifera indica* L.)

Youngmok Kim^a, Angela J. Lounds-Singleton^b, Stephen T. Talcott^{a,*}

^a Department of Nutrition and Food Science, Texas A&M University, 1500 Research Parkway A, College Station, TX 77843-2254, USA

^b Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL 32611-0370, USA

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ABSTRACT

Mangoes are an important tropical fruit crop worldwide and are best noted for their vibrant flesh colour, juicy texture, and sweet flavour, along with important nutrient contributions from their phytochemical constituents. Mangoes imported to the US must be exposed to thermal quarantine treatments, such as irradiation and hot water treatment (HWT), to eradicate invasive pests, yet limited data exist regarding polyphenolic changes to the fruit following hot water immersion treatment. Although the water temperature remains constant, the duration of treatment depends on fruit size. Therefore, these investigations focused on polyphenolic and antioxidant changes to mature, green mangoes following varying times of HWT and their changes during short-term storage. Experimentally, fruit were immersed in 46.1 °C water from 70 to 110 min; half evaluated within 2 h of treatment, while the remainder was evaluated after 4 days of storage at 25 °C for changes in polyphenolics, antioxidant capacity and fruit quality. Free gallic acid and four gallotannins were tentatively identified as the major polyphenolics present by HPLC analysis against authentic standards. Two major polyphenolics in mango, gallic acid and gallotannins, as well as total soluble phenolics, decreased as a result of prolonged HWT, while the antioxidant capacity remained unchanged in all heat-treated mangoes immediately after HWT. However, during 4 days storage, only minor changes were observed in gallic acid and gallotannin concentrations whereas total soluble phenolics and antioxidant capacity in all hot water-treated fruits decreased. The optimum hot water immersion times did not affect the external quality and polyphenolics of mangoes but all heat treatments reduced total soluble phenolics and antioxidant capacity, regardless of the duration of treatment times, during 4 days storage.

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1. Introduction

Mango (*Mangifera indica* L.) is a popular and economically important tropical fruit throughout the world, due to its excellent eating quality (bright colour, sweet taste and luscious flavour) and nutritional composition (vitamins, minerals, fibre, and other phytochemical compounds). Fresh fruit imports to the US, Europe, and Japan have grown, due to increased consumer demand for fresh and processed mango products. In turn, mangoes have become an affordable tropical fruit that has found favour with consumers in a variety of foods and beverages. Mango contains various classes of polyphenols, carotenoids, and ascorbic acid demonstrating different health-promoting properties, mainly from their antioxidant activities (Talcott, Moore, Lounds-Singleton, & Percival, 2005). Gallic acid and gallotannins were the first compounds defined as major polyphenolics present in mango (Saleh & El-Ansari, 1975) and other polyphenols, such as mangiferin,

quercetin, kaempferol, *p*-OH-benzoic acid, *m*-coumaric acid, *p*-coumaric acid and ferulic acid, were recently identified using HPLC-MSⁿ analysis (Schieber, Berardini, & Carle, 2003; Schieber, Ullrich, & Carle, 2000). Phenolic compounds present in mango including gallic acid and gallotannins, were found to naturally decrease during storage, due to ripening, resulting in loss of astringency, which is a characteristic of mango (El Ansari, Reddy, Sastry, & Nayudamma, 1971; Lakshminarayana, Subhadra, & Subramanyam, 1970; Mitra & Baldwin, 1997; Saleh & El-Ansari, 1975).

Mango has been cultivated for about 4,000 years and its production and consumption has gradually increased as its popularity has grown. Originating over 4,000 years ago in India and Burma, its cultivation has spread to Malaysia, Eastern Asia, and Eastern Africa (Mitra & Baldwin, 1997) and now, at least 87 countries grow over 26,286,255 MT per annum (FAO, 2004; Saúco, 2004). Mango production is highest in India, at 41% of the world's production (10,800,000 MT), followed by China, Thailand, Mexico, Pakistan, Indonesia, the Philippines, Nigeria, and Brazil (FAO, 2004); Mexico is known as the leading mango-exporting country (41% of the world market, 102,500 MT), followed by the Philippines (7.8%)

* Corresponding author. Tel.: +979 862 4056; fax: +979 458 3704.

E-mail address: stalcott@tamu.edu (S.T. Talcott).

and Pakistan (7.6%) (Saúco, 2004). The world's largest mango-importing country is the US and the mango market in the US has steadily grown in response to increasing domestic demand. Mangoes are well adapted to tropical and sub-tropical regions of the world but production in the United States (US) is comparatively small, due to climactic limitations. Florida is the only state in the United States where agricultural statistics are reported for mangoes, even though mangoes are also cultivated to various extents in Hawaii, California, Texas, and Puerto Rico (Mossler & Nesheim, 2002). Production volume in the US is insufficient to meet domestic demand, at less than 1% of domestic consumption (FAO, 2004).

A problem for countries that import tropical fruit is the high incidence of invasive insect species from which mangoes are a ready host. Mangoes may host *Ceratitidis capitata* (Mediterranean fruit fly) and *Anastrepha spp.* (Mexican fruit fly), causing infestation risks in the form of adults, larvae, and eggs (Jacobi, Macrae, & Hetherington, 2001; USDA-APHIS, 2002). In the US, all imported mangoes are subjected to quarantine treatment, such as hot water treatment (HWT), hot vapour treatment, or irradiation, to eradicate invasive pests unless they are from pest-free areas approved by APHIS as free of specified pests, such as approved areas in Argentina, Australia, Belize, Brazil, Chile, Ecuador, Guatemala, Mexico, Peru, and Venezuela (USDA-APHIS, 2008). However, the majority of mangoes entering the US have been subjected to a HWT, which has been the subject of several studies investigating the physiological changes in the fruit. HWT has also been shown to decrease the severity of skin disorders, such as darkened lentils and anthracnose (Jacob & Giles, 1997; Kim, Brecht, & Talcott, 2007), while fruit ripening may either be promoted or inhibited, depending on mango variety or external factors such as harvest time, treatment time and temperature (Jacobi et al., 2001; Lurie, 1998).

Mango heat tolerance varies due to a number of factors including origin, species, fruit maturity, shape, size, and weight (Jacobi et al., 2001). The USDA has recognised that inherent differences exist among fruits and thus variable thermal quarantine times are prescribed, ranging from 65 to 110 min at 46.1 °C (USDA-APHIS, 2002). However, no information is available on changes to antioxidant, polyphenolic compounds associated with hot water quarantine treatments conducted at varying times. Since polyphenolics are natural antioxidants in quenching and neutralising free radicals, changes to these compounds following postharvest treatment and ripening is an important link to the potential health benefits of mangoes.

2. Materials and methods

2.1. Fruit preparation

Mangoes (cv. Tommy Atkins) for this study were obtained from Lyons Farms in Homestead, FL, in June 2003 at colour-break stage of ripeness. Harvested fruits were immediately transferred to a storage chamber and stored for 2 days at 14 °C prior to HWT. One hundred and thirty fruits were carefully selected, based on uniformity of colour, firmness and size, and randomly divided into five groups of 26 fruits in each group. The first group was subjected to a laboratory scale fruit heating system (Model HWH-2, Gaffney Engineering, Gainesville, FL) for 70 min (HW70) at 46.1 °C. The average weight for mangoes used in this study was 497.5 g, and a 70 min immersion time was selected with immersion times increased to 90 (HW90) and 110 (HW110), to help understand the implications of polyphenolic changes under prolonged exposure conditions. A fourth group of fruit remained unheated as a control (HW0) and was held immersed in water at 23 °C for 70 min. The fruits in the fifth group were used to measure the temperature

with a thermocouple placed next to the seed kernel and treatment times started when core temperature reached the target value; the fruits used for core temperature measurements were not further used in the study. Following HWT, fruits were held in the air at 23 °C for up to 3 h until core temperatures reached equilibrium. Half of the treated fruits (13 fruits from each group) were immediately peeled and homogenised for chemical analysis, while remaining fruits were held at 25 °C for an additional 4 days of ripening prior to analysis.

Analysis samples were obtained by manually removing mango peel, removing edible flesh from the seed kernel, and homogenising into a fine puree using a laboratory blender (Waring Commercial, Model 31BL91). Purees were then frozen at –20 °C, to facilitate tissue disruption whereby they were thawed at room temperature. A 5 g sample was obtained, treated with 20 µl of pectinase (*Aspergillus aculeatus*, 26,000 units/ml, Sigma Chemical Co., St. Louis, MO), incubated at 35 °C for 3 h, and finally centrifuged until a clear supernatant was obtained from which phytochemical analyses were conducted.

2.2. Chemical analysis

Individual polyphenolics were characterised and quantified by HPLC, as previously described by Talcott, Howard, and Brenes (2000). Clarified juice was filtered through a 0.45 µm PTFE filter (Whatman, Clifton, NJ) and injected into a Waters (Milford, MA) 2695 Alliance chromatography system. Compounds were separated on a Waters Spherisorb ODS 2 column using a gradient elution program. Mobile phases consisted of phase A (98% H₂O and 2% acetic acid) and phase B (68% H₂O, 30% acetonitrile, and 2% acetic acid) run at 0.8 ml/min. Polyphenolics were separated using a gradient elution system that kept mobile phase B at 0% for 5 min and then changed phase B from 0% to 10% in 20 min, 10% to 25% in 30 min, 25% to 50% in 40 min, 50% to 75% in 50 min, 75% to 100% in 60 min, and returned to original conditions in 2 min for the next injection. Polyphenolics were detected and quantified at 280 nm using a Waters 996 photodiode array (PDA) detector against external standards of gallic acid, *p*-OH-benzoic acid, *p*-coumaric acid, ferulic acid and (+)-catechin. Polyphenolics were characterised based on retention time and UV spectral similarities to authentic standards (Sigma Chemical Co., MO).

Total soluble polyphenolics, including contributions from ascorbic acid, were determined at 726 nm using the Folin-Ciocalteu assay, as previously described by Swain and Hillis (1959), with data expressed in mg/l equivalents of gallic acid. Hydrophilic antioxidant capacity was measured using the oxygen radical absorbance capacity (ORAC) assay run according to Talcott and Lee (2002) on a 96-well Molecular Devices (Sunnyvale, CA) fmax[®] fluorescent microplate reader (485 nm excitation and 538 nm emission). The inhibitory properties of the clarified mango juice were evaluated against a peroxy radical with data expressed in µmol Trolox equivalents per ml of juice (TE). Soluble solids content was measured by an Abbe Mark II digital refractometer (Leica Inc, Buffalo, NY).

2.3. Statistical analysis

The means of triplicate analyses of 13 fruit samples from each treatment and sampling time were represented in the dataset. Changes in soluble solids content, phytochemicals, antioxidant capacity, and total soluble phenolics affected by various HWT were analysed by analysis of variance (ANOVA) and mean separation was conducted by the least significance difference (LSD) test ($p < 0.05$) using JMP 5 software (SAS Institute, 2002).

3. Results and discussion

3.1. HWT impacts on fruit external quality and soluble solid content

Mango fruits were subjectively observed for quality changes due to HWT. Heat injury was assessed by visible signs of skin scalding and bruising. After treatment, no visual signs of heat injuries or acceleration in skin colour development were observed, in either untreated or heat-treated fruits over the 4 day storage period. This result was in agreement with previous trials, where HWT of “Tommy Atkins” mangoes at 46.1 °C for 65 min actually decreased the occurrence of stem end rot and anthracnose (Sharp & Spalding, 1984) while HWT on the same variety at 46.1–46.7 °C for 45–65 min had no visible symptoms of heat injury (Sharp, 1986). Even when “Tommy Atkins” mangoes were treated for extended time and temperatures (46.1–46.7 °C, 90 min), no visible heat injury or detrimental effects on fruit quality were observed (Sharp et al., 1989). However, other studies indicated that heat injuries may be induced in mango by abiotic stress when treatment conditions are out of optimum range (Smith & Chin, 1989; Spalding, King, & Sharp, 1988). For example, when “Tommy Atkins” mangoes were immersed into hot water at 46 °C for 120 min or 49 °C for 60 min, darkened lenticels were observed (Spalding et al., 1988) and skin scalding occurred at 42–48 °C for 30–90 min (Smith & Chin, 1989). Therefore, occurrence of heat injury may vary depending on the fruit variety, conditions of treatment, environmental factors, fruit maturity, fruit size, or other pre-harvest conditions (Jacobi et al., 2001).

In this study, the application of HWT at 46.1 °C for 70, 90 and 110 min had no effects on soluble solids content of mango fruits when measured 4 days after HWT. The soluble solids contents were 9.94, 10.48, 9.82 and 10.24 °Brix in HW0, HW70, HW90, and HW110, respectively. Jacobi, Macrae, and Hetherington (2000) also found that mangoes preconditioned with hot air at various temperatures from 22 to 42 °C for 8 h following HWT at 47 °C for 15 min, did not vary in soluble solids content 4 days post-treatment.

3.2. Changes in polyphenolics by HWT

Free gallic acid and four gallotannins were the major polyphenolics tentatively identified in mango and quantified against a standard of gallic acid (Fig. 1). Even though four minor phenolics compounds, *p*-OH-benzoic acid, *p*-coumaric acid, ferulic acid and (+)-catechin were also identified, they were not evaluated in this study, due to their low concentrations in mango (13% of total phenolic concentration). According to Berardini, Carle, and Schieber (2004), the peels and kernels of mango also contain a significant

amount of gallotannins, as determined by HPLC and ESI-MS analyses. However, mango peels and kernels are not considered edible, and are commonly evaluated for exploration of byproduct utilisation. Even though pectinase used in this study may be able to release gallic acid from supernatant during analysis, no evidence of changes in gallic acid by enzyme activity was observed during subsequent storage.

Overall, a significantly lower concentration of gallic acid was observed when extended treatment times (HW90 and HW110) were applied to mangoes (Fig. 2). Following HWT, gallic acid concentration in HW70 was not significantly different from HW0 fruit, but was significantly reduced in HW90 and HW110 by 56% and 66%, respectively. After 4 days storage, no difference was observed in gallic acid concentration at 25 °C in HW0, 70 and 90, but the concentration increased by 40% only in HW110. Total gallotannin concentration (sum of four gallotannins) also decreased in HW90 and HW110 fruits, by 25% and 28%, respectively, after HWT (Fig. 3), especially in relation to the HW70 fruits, which were 42% higher than HW0. Otherwise total gallotannin content was reduced by 12% only in HW70 while others remained unchanged during storage. As shown in Fig. 4, a 30% increase in gallic acid was observed during the 4 day storage while on average the four gallotannins were unaltered. Musingo, Sims, Bates, O’Keefe, and Lamikanra (2001), and Soong and Barlow (2006) both reported an increase in gallic acid following heat treatment and the amount of increase was different depending on the cultivar and heating temperature. In this study, the lack of change in gallic acid and increased gallotannin concentration following HW70 may be explained by enzyme-induced hydrolysis of high molecular weight tannins, not identified under these chromatographic conditions. Berardini et al. (2004) previously showed that mango contains numerous high molecular weight gallotannins that can be broken down into smaller gallotannins. It is also possible that the biosynthesis of gallotannins occurred *via* galloyltransferases present in mangoes (El Ansari et al., 1971), for which the temperature for optimum reaction is 45 °C (Niemetz & Gross, 2001). Otherwise, prolonged treatment times (HW90 and HW110) were a significant factor in lowering the two predominant phenolic compounds present in mangoes. However, there is a lack of information relating to gallic acid and gallotannin stability in fresh mangoes, the mechanism of those two major polyphenolics changes is still not clear.

3.3. Total soluble phenolics and antioxidant capacity changes by HWT

Determination of total soluble phenolics and radical-scavenging capacity has been reliable indices for understanding phytochemical changes in fresh and processed fruits. Total soluble phenolic testing also includes contributions from ascorbic acid, reducing

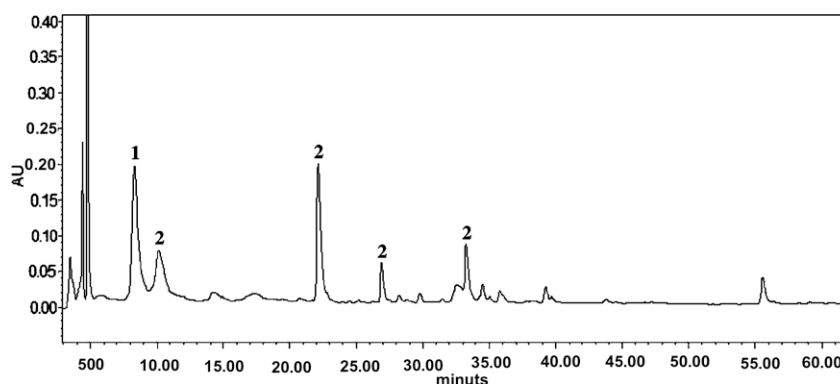


Fig. 1. HPLC chromatogram of polyphenolics, gallic acid and four gallotannins present in mango flesh at Day 0. Peaks were tentatively identified based on retention time and spectral similarities against an authentic standard of gallic acid. Peak assignments: (1) Gallic acid; (2) gallotannin.

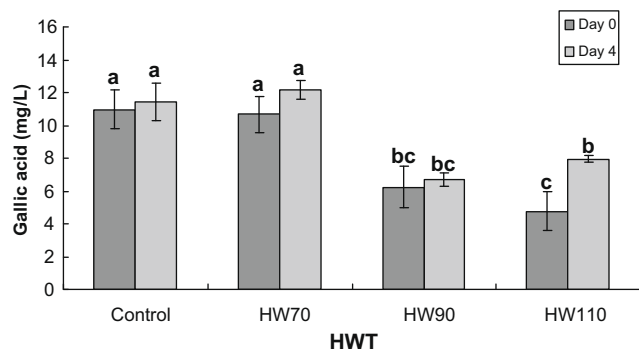


Fig. 2. Changes in average gallic acid concentration (mg/l) on Day 0 and Day 4 as affected by HWT with varying length of HWT of 0 (HW0), 70 (HW70), 90 (HW90), and 110 (HW110) min. Average values and standard error bars of triplicate samples for all treatments are represented.

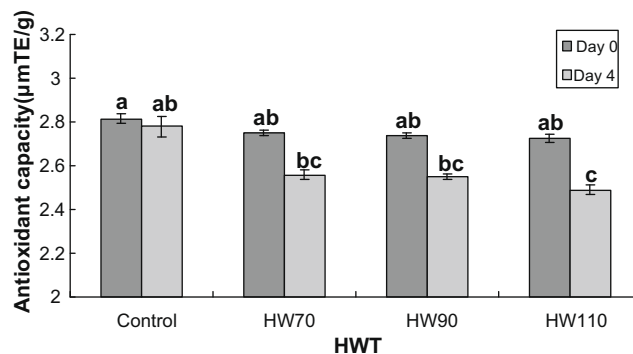


Fig. 5. Changes in total antioxidant capacity ($\mu\text{mol TE/g}$) on Day 0 and Day 4 as affected by four different lengths of HWT of 0 (HW0), 70 (HW70), 90 (HW90), and 110 (HW110) min. Average values and standard error bars of triplicate samples for all treatments are represented. Abbreviation: TE, Trolox equivalents.

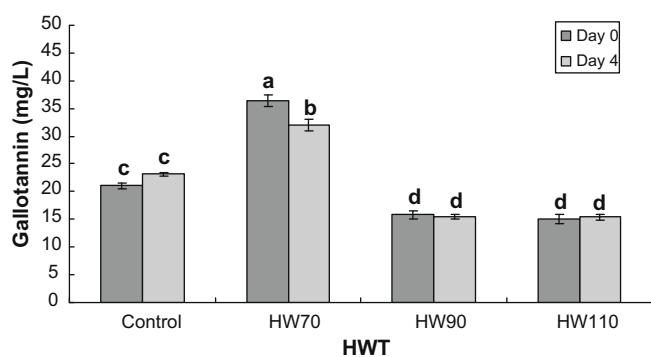


Fig. 3. Changes in total gallotannin concentration (mg/l) on Day 0 and Day 4 as a result of different lengths of HWT of 0 (HW0), 70 (HW70), 90 (HW90), and 110 (HW110) min. Average values and standard error bars of triplicate samples for all treatments are represented.

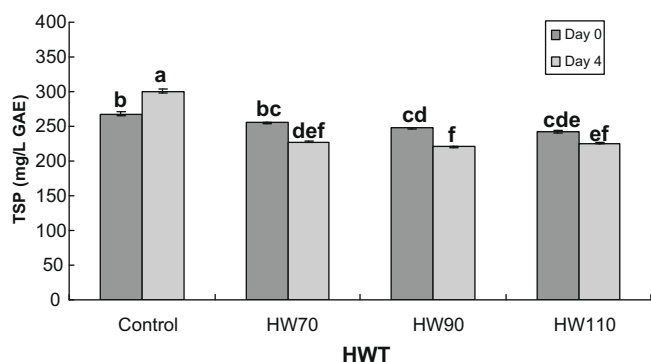


Fig. 4. Changes in TSP (total soluble phenolic) concentration (mg/l GAE) on Day 0 and Day 4 as affected by four different durations of HWT of 0 (HW0), 70 (HW70), 90 (HW90), and 110 (HW110) min. Average values and standard error bars of triplicate samples for all treatments are represented. Abbreviation: GAE, gallic acid equivalents.

sugars, and some soluble proteins, whereas the antioxidant testing employed was only effective for polar compounds with the ability to scavenge peroxy radicals. Total soluble phenolics decreased in response to HWT and prolonged exposure. No difference was observed in HW70 compared to HW0 on Day 1 (Fig. 4). Total soluble phenolics of mangoes were 255, 247 and 242 mg/l gallic acid equivalents (GAE) in HW70, HW90, and HW110, respectively and the control contained total soluble phenolics of 268 mg/l GAE when measured immediately after HWT. During 4-day storage,

total soluble phenolics were reduced by 11, 11 and 7% in HW70, HW90 and HW110 respectively, while total soluble phenolics in HW0 increased by 11%. A gradual increase in total soluble phenolics was reported in mangoes, as starch was converted to simple sugars by amylase activity during storage (Abu-Sarra & Abu-Goukh, 1993; Medlicott & Thompson, 1984; Minessy, Saeed, & El-Rayah, 1984). However, total soluble phenolics in all heat-treated mangoes were reduced in this study during 4 day storage. This can be explained by the fact that heat treatment was previously found to decrease amylase activity by preventing hydrolysis of starch in mango fruits where the enzyme activity was 6.42-fold higher in untreated mangoes than heat-treated (Katrodia, Rane, & Salunkhe, 1988). Likewise, the vitamin C in postharvest crops (Jacobi et al., 2001) was shown to be reduced by oxidation occurring during postharvest handling including heat treatment (Lee & Kader, 2000).

Mangoes contain relatively low hydrophilic antioxidant capacity in relation to other commonly consumed fruits (Wu et al., 2004). The majority of antioxidant capacity of mangoes likely resides with its polyphenolic content, but the observed changes to individual or total polyphenolics in this study were not large enough to result in a significant alteration of antioxidant capacity (Fig. 5). According to Talcott et al. (2005), changes in gallotannin concentration did not impact the antioxidant capacity of heat-treated mangoes during 20 days as observed in this study. Additional antioxidant constituents, not evaluated in this study, may also contribute to radical-scavenging abilities or antioxidant capacity of the lipophilic fraction may also be responsible for this observed change (Talcott et al., 2005). Antioxidant capacity of control mangoes was 2.81 $\mu\text{mol TE/g}$ and hot water-treated fruits showed similar antioxidant capacity of 2.75, 2.73, and 2.72 $\mu\text{mol TE/g}$ in HW70, HW90, and HW110, respectively, with no significant difference when measured right after HWT ($p < 0.05$). However, during 4 day storage, antioxidant capacity was significantly lowered in HW70, HW90, and HW110 compared to the control, whereas antioxidant capacity in HW0 remained unchanged, as observed in total soluble phenolics changes.

4. Conclusion

The optimum conditions for mango quarantine treatment specified by USDA-APHIS were determined based on the minimum temperature and time which can sufficiently destroy the pests found in various mangoes. The average weight of mangoes is used to pre-sort mangoes prior to the treatment but the distribution of individual fruit weights is not taken into account, which causes uneven heating of fruits due to the variability of the fruit weight. In

this study, variable heating times were applied to observe the effect on polyphenolic changes when the fruits are over- or under-heated. The optimum treatment showed no impact on the polyphenolics present in mango and antioxidant capacity while prolonged treatments decreased the polyphenolic concentration. However, the reduction rate of antioxidant capacity in mango was higher in all heat-treated mangoes during storage, even though no significant changes were observed in the polyphenolics. Since most fruits are consumed fresh, loss of antioxidant capacity is not anticipated, due to adverse effects of heat or oxidation caused by heat processing. However, heat-treated mangoes from outside the US may lose their antioxidant capacity by heat treatment or heat-triggered oxidation during storage.

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