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Review

# Postharvest heat disinfestation treatments of mango fruit

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**Abstract**

Postharvest heat disinfestation treatments have emerged over the past decade as viable non-chemical control methods for fruit flies in mango fruit around the world. The physiological responses of mango fruit both during and following a heat treatment determine the eventual eating quality of the fruit. This review describes the methods used to heat treat mango varieties for insect disinfestation. The physiological effects of heat treatments, particularly pretreatment conditioning and hot water treatments, on the fruit are covered in detail. 

*Keywords:* Mango; Disinfestation; Hot water; Hot air; Conditioning; Heat tolerance; Damage; Ripening

## 1. Mango

### *1.1. Importance and distribution*

The mango (*Mangifera indica* Linn.) is a member of the family Anacardiacae (Lizada, 1993). Known for its unique flavour and attractive appearance, the

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mango fruit is a valued source of export income for the producing countries (Medlicott et al., 1986a; Mitra and Baldwin, 1997). Botanically, the mango fruit is a large, fleshy drupe, and as such the pericarp is divided into three layers: a thin outer skin, the epicarp; an edible fleshy middle layer, the mesocarp; and an inner hard shell, the endocarp, which surrounds the single seed (Hulme, 1971; Mukherjee, 1997). Ripe mangoes are considered an excellent source of vitamins C, B1 and B2 and provitamin A (Mukherjee, 1997). Producing 9.64 million tonnes of fruit from an area of 1.17 million hectare, India is the single largest producer of mangoes with approximately 66% of the world's mango production. Traditionally, the major mango exporting countries are India, the Philippines, Thailand, and Mexico. World mango production has, however, increased by nearly 50% between 1971 and 1993 (FAO Production Yearbook, 1993), with most of the new production occurring in South and Central America, Africa and Australia (Mukherjee, 1997). It is expected that the volume of fresh mango in world trade will increase in the coming years. World trade involves a few of the numerous mango varieties, and the most common are 'Alphonso' (India), 'Carabao' (the Philippines), 'Haden', 'Keitt' and 'Manila' (Mexico) and 'Tommy Atkins', 'Haden' and 'Keitt' (Florida). Fruit size, shape, colour and flavour are distinctive for each of these varieties.

The Australian mango industry is an example of the trends occurring in other countries looking to export. Production in Australia is concentrated in the northern regions of the country (12–25°S). In the 1996/1997 season, 32 403 t of fruit were produced, worth an estimated $ 70 million from over 1.3 million trees (DPI-QHI, 1998). The industry is currently dominated by one variety, 'Kensington', which accounts for over 85% of production. 'Kensington' fruit are ovate to slightly oblong in shape, 350–750 g in size and when ripe have a yellow skin colour, sometimes tinged with an orange–red blush (Knight, 1997). Ripe fruit have yellow flesh and the endocarp is pale yellow. Production is expected to increase by up to 30% in the next 5 years with the maturation of trees under cultivation. Over half of the new plantings are comprised of varieties, such as 'Keitt', 'R2E2', 'Palmer' and 'Nam Doc Mai'. At present, only 10% of Australian grown mangoes are exported, mainly to Hong Kong, Singapore, Malaysia, Japan and the Middle East, but this percentage is expected to increase as market impediments, like the risk of fruit fly introduction, are removed.

### *1.2. Postharvest characteristics of fresh mango fruit*

World trade in fresh mango fruit is restricted by the highly perishable nature of this climacteric fruit (Lizada, 1993; Mitra and Baldwin, 1997), that displays a characteristic peak of respiratory activity during ripening (Tucker, 1993). The respiratory climacteric may correspond to optimum eating ripeness, or may

precede or postdate this according to fruit type. Mango fruit have high respiration rates during ripening and are considered to have a short storage life (Mitra and Baldwin, 1997). Under tropical conditions, green, physiologically mature mango fruits ripen within 6–7 days of harvest at 20–25°C and become overripe and spoiled within 15 days after harvest. Mango fruit are sensitive to physical injury and susceptible to postharvest disease and chilling injury (Lizada, 1993; Mitra and Baldwin, 1997). Postharvest technologies developed for mango fruit have tended to concentrate on quarantine, disease control, packaging and long-term storage so that expansion can occur in the marketing of the fresh fruit.

### *1.3. Disinfestation of fresh mango fruit*

Mango fruit are hosts for Tephritid fruit flies that are considered a quarantine risk by many importing countries. Depending on the country of origin, the mango fruit may be infested by one or more species of fruit flies. In Australia, for example, the mangoes may be infested by the endemic Queensland fruit fly (*Bactrocera tryoni* (Froggatt)), the introduced Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)), and the Papaya fruit fly (*Bactrocera papayae* (Drew and Hancock)) (Corcoran and Peterson, 1996; Heather et al., 1997). For fresh mango fruit to be accepted by many importing markets, the fruit must be treated to ensure that it is free of fruit flies. Termed disinfestation treatments, formerly these treatments were often chemical fumigation of fruit (Paull and McDonald, 1994), but now have been replaced by chemical-free disinfestation treatments. In general, chemical-free disinfestation treatments that kill the egg and larval stages of the fruit flies in the fresh fruit involve heating the fruit to a specific core temperature and maintaining the elevated core temperature for a defined period of time. To be accepted by quarantine authorities in many countries, disinfestation treatments must meet a prescribed degree of statistical probability that the treatment will kill over 99.9968%. This Probit value infers that not more than three individuals from a population of 100 000 will survive a quarantine treatment (Paull and Armstrong, 1994). For example, Japan uses a variation of the Probit 9 concept that requires no survivors from a treated population of 30 000 target pests (Kuo et al., 1987; Unahawutti et al., 1986; Paull and Armstrong, 1994).

### *1.4. Methods of heat disinfestation for mango fruit*

Heat disinfestation of mango fruit requires the fruit to be heated to a specified core temperature for a defined period (Paull and McDonald, 1994). Fruit are heated via energy transfer from a heating medium, which is either air or water (Jordan, 1993). Currently, there are three methods in use to heat mango fruit. They are vapour heat treatment (VHT), forced hot-air treatment (FHAT) and hot water immersion treatment (HWT).

*1.4.1. Vapour heat treatment (VHT)*

VHT is also referred to as high humidity air heating. This process involves heating air that is nearly saturated with moisture and passing the air stream across the fruit. When the temperature of the mango fruit is at or below the dew point, condensation of atmospheric moisture occurs on the surface of the fruit. In this way, fruit are heated by conductive energy transfer. The heat from the fruit surface is transferred toward the fruit centre (Jordan, 1993). Commercial facilities operate in Okinawa, the Philippines, Thailand, the United States and Australia, and protocols are being used for mangoes (Sunagawa et al., 1987; Merino et al., 1985; Unahawutti et al., 1986; Armstrong, 1996; Heather et al., 1997). The VHT disinfestation protocols accepted for mango access to the high-value markets in Japan include: 46°C fruit core temperature held for 10 min for Philippine 'Carabao' mango; 46.5°C fruit core temperature held for 30 min for Taiwanese 'Irwin' and 'Haden' mangoes; 46.5°C fruit core temperature held for 10 min for Thailand 'Nang Klang Wun' mangoes and 47°C fruit core temperature held for 10 min for 'Nam Doc Mai', 'Pimsen Dang' and 'Rad' mangoes. The protocol accepted by the Japanese authorities for entry of Australian 'Kensington' mangoes into Japan is a fruit core temperature of 47°C held for 15 min (Heather et al., 1997). Mexican 'Manila' mangoes are allowed entry into the USA with a 43.3°C 6 h treatment (Anonymous, 1994; Kitigawa, 1994; Johnson and Heather, 1995).

*1.4.2. Forced hot-air heating (FHAT)*

With forced hot-air heating, also known as non-condensing air heating, the heating of fruit is carried out by passing air held at a specified temperature through a bed of fruit. The heat moves from the warm air to the cooler fruit by conduction via the skin. Heat rapidly transfers toward the centre of the fruit. However, the transfer from the air to the skin is considerably slower than the transfer rate from the skin to the centre of the fruit (Jordan, 1993). The difference between VHT and FHAT is that the fruit surfaces remain dry during a forced air treatment compared to condensation on the fruit surface during VHT (Armstrong, 1996). In an FHAT, the relative humidity of the air passed across the fruit can be as low as 30% and may fluctuate during treatment, with heat transfer by convection only (Hallman and Armstrong, 1994). If the relative humidity is too low, fruit weight loss and shrivelling may occur. Therefore, to preserve fruit quality, the control of relative humidity during FHAT is important (Williamson and Winkelman, 1994). Quarantine treatments using this technology have been developed in the United States for mango (Mangan and Ingle, 1992; Animal and Plant Health Inspection Service, 1996). However, commercial usage of FHAT currently occurs only for papaya grown in Hawaii and exported to mainland United States, and in the Cook Islands where papaya is treated before being exported to New Zealand (Armstrong, 1996).

*1.4.3. Hot water immersion treatment (HWT)*

Hot water immersion quarantine treatments have been developed to disinfest mangoes of fruit flies (Sharp et al., 1988, 1989a,b,c; Sharp and Picho-Martinez, 1990; Sharp and Spalding, 1984; Sharp, 1986, 1989; Segarra-Carmona et al., 1990; Nascimento et al., 1992). With hot water immersion, the heat transfer occurs from the water to the skin of the fruit, and from the skin through the flesh to the centre. The water to skin heat transfer is faster than the skin to centre transfer. The rate of heating of the skin and outer mesocarp of the fruit is substantially faster when fruit are immersed in hot water than when air of the same temperature is passed over the fruit (Couey, 1989; Stewart et al., 1990; Jordan, 1993).

Compared with VHT and FHAT, hot water immersion has a number of advantages which include: relative ease of use by horticultural industries, short treatment time, reliable and accurate monitoring of fruit and water temperatures, plus the added benefits of killing surface decay organisms and cleaning the fruit surface of plant exudates (Sharp, 1994). Another important advantage of hot water immersion technology from an economic point of view is that the cost of a typical commercial system is approximately 10% that of a commercial VHT system (Jordan, 1993).

The use of HWT of mango fruit on a commercial scale has been more widespread in the USA and Central America. Hot water dips for quarantine purposes usually consist of immersing fruits in water held at 43–46°C, as temperatures above 46°C tend to produce excessive damage (Sharp, 1994). In Central America, the commercial use of HWT for treating mangoes is widespread. Only a single dipping temperature (46.1°C) is used, either in a batch process or continuously for 65–90 min depending on fruit size. Disinfestation protocols using hot water immersion are approved for the entry of flat, elongated and round mango varieties from Mexico, Puerto Rico, US Virgin Islands, West Indies, Panama, and South America into the USA. Typically, cultivars with elongated fruit of less than 375 g in weight are heated to 46.1°C for less than 65 min, while cultivars with elongated fruit weighing 375–570 g are heated to 46.1°C and held at this temperature for 75 min (Anonymous, 1994). There has been evidence that both these treatments can induce quite high levels of fruit skin damage, and this is dealt with by a final sorting of fruit before entry to USA (Jordan, 1993). The type of equipment used in Mexico for HWT of mango fruit ranges from bulk dip systems to complex automated continuous systems.

Currently in Australia, there is a commercial need for a more cost-effective alternative to VHT to allow for expansion of fresh mango fruit exports into new importing countries. There is currently no commercial use of HWT technology for fresh mango fruit disinfestation, because the high temperatures necessary to kill all stages of the life cycles of the fruit fly species have, to date, proven injurious to the fruit (Jacobi and Wong, 1992).

## 2. Mango fruit physiological responses to heat treatment

### *2.1. Fruit heat tolerance*

For insect disinfestation purposes, it is possible to determine a temperature and duration combination that is lethal to all stages of the pest life cycle. However, the fruit sets the upper temperature limit of the disinfestation treatment beyond which irreversible injury occurs. Fruit heat tolerance varies with a number of factors including species, genotypic variability within species, stage of fruit maturity, fruit size, exposure to different environmental and/or preharvest factors, the type of heat treatment applied, and whether postharvest conditioning treatments have been given before a heat treatment.

### *2.2. Mango fruit damage caused by hot water immersion*

Transferring harvested fruit from ambient growth temperatures to an elevated temperature induces stress. The severity of the induced stress is determined by both the temperature differential and the duration of the exposure (Paull, 1994). For mango fruit, immersion in hot water at 42–49°C has been reported to induce a range of external and internal heat injuries in a number of cultivars (Table 1). Skin damage, including skin scalding, lenticel damage, and cavitation are commonly reported, together with the retention of unripe, starchy areas in the mesocarp as the fruit ripen. The severity and incidence depends upon mango variety, method of heat application and the level of stress suffered by the tissue.

### *2.3. The effect of heat on fruit ripening*

The effect of heat treatments on fruit ripening can be variable, but can be categorised as either inhibiting, promoting or disrupting fruit ripening. Lurie (1998) stated that the response of a particular fruit or vegetable to heat will result from a number of factors including, preharvest environmental conditions, the physiological age of the commodity at the time of harvest, the temperature and duration of the heat treatment, the post-treatment storage conditions and whether the heat treatment damaged the commodity. Disparate ripening responses to heat treatment may also be associated with cultivar differences within a fruit species.

#### *2.3.1. Mango fruit ripening promoted by heat treatment*

Mango is generally harvested green mature and during the ripening process, the skin develops yellow or red pigments. Heat treatments have been reported to accelerate the yellowing of the fruit skin (Table 2), and in some cases, improve the uniformity of colour development (Ledger, 1995). These treatments have also been found to accelerate fruit softening of a number of mango cultivars (Table 2).

Table 1
Injuries to mango cultivars induced by disinfestation treatments, applied as hot water, vapour heat or forced hot-air treatments

| Mango cultivar | Heat injury | Heat treatment | Reference |
|---|---|---|---|
| 'Tommy Atkins' | Darkened lenticels | HWT 46°C for 120 min or HWT 49°C for 60 min | Spalding et al. (1988) |
| 'Keitt' | Darkened lenticels | HWT 46°C for 90 min or HWT 49°C for 60 min | Spalding et al. (1988) |
| 'Kensington' | Skin scalding; uneven skin colour development with ripening; starch retention in the form of layers and spots in ripe fruit; internal cavities | HWT 48°C for 7.5–30 min | Jacobi and Wong (1992) |
| 'Kensington', 'Irwin', 'Haden', 'Tommy Atkins', 'Strawberry' | Skin scalding | HWT 42–48°C for 30–90 min | Smith and Chin (1989) |
| 'Kensington' | Internal cavities; starchy regions retained in ripened mesocarp | HWT 47°C for 25 min | Joyce et al. (1993) |
| 'Keitt' | Internal cavities formed near the seed surrounded by hard unripe tissue | VHT 46°C for 3–4 h or VHT 48°C for 5 h | Mitcham and McDonald (1993) |
| 'Tommy Atkins' | Peel pitting | FHAT 51.5°C for 125 min | Miller et al. (1991) |
| 'Carabao' | IB in inner mesocarp of ripe fruit; white, starchy, tough lesions; fermented odour | VHT 46°C for 10 min | Esguerra et al. (1990) |

Table 2
Heat treatments that induced accelerated ripening of cultivars following heat treatments, applied as HWT, FHAT and VHT

| Ripening response | Cultivar | Heat treatment | Reference |
|---|---|---|---|
| Yellowing of skin | 'Tommy Atkins', 'Keitt' | HWT 46°C for 60–120 min or HWT 49°C for 60 min | Spalding et al. (1988) |
| Yellowing of skin | 'Tommy Atkins', 'Keitt' | Conditioning 38°C for 48 h | Pesis et al. (1997) |
| Yellowing of skin | 'Carabao' | VHT 46°C for 10 min | Esguerra and Lizada (1990) |
| Yellowing of skin, uniformity of colour | 'Keitt' | HWT 46.1–46.7°C for 20–60 min | Segarra-Carmona et al. (1990) |
| Yellowing of skin, uniformity of colour, fruit softening | 'Tommy Atkins', 'Keitt', 'Palmer' | FHAT 46°C for 3.25 h or FHAT 48°C for 2.5 h or HWT 44°C core for 25 min | McGuire (1991) |
| Yellowing of skin, uniformity of colour, fruit softening | 'Kensington' | VHT and HWT 47°C for 7.5–30 min or VHT 46.5°C for 10 min or VHT 47°C for 15 min | Jacobi and Wong (1992), Jacobi et al. (1995), Jacobi and Giles (1997) |
| Fruit softening | 'Tommy Atkins' | FHAT 51.5°C for 125 min | Miller et al. (1991) |
| Increased respiration rate | 'Keitt', 'Tommy Atkins' | FHAT 46°C for 3–4 h or FHAT 48°C for 5 h | Mitcham and McDonald (1993) |
| Increased respiration rate | 'Carabao' | VHT 46°C for 10 min | Esguerra and Lizada (1990) |

The mechanisms by which heat treatments accelerate mango fruit ripening have not as yet been described, but are most probably associated with accelerated synthesis of carotenoids, degradation of chlorophyll (by chlorophyllase), and synthesis of cell wall degrading enzymes such as polygalacturonase.

There is general agreement in the literature that mango fruit not damaged by treatment with either hot water or hot air, have comparable organoleptic qualities of flavour, aroma, or altered pH, total soluble solids, and titratable acidity levels to those of untreated fruit (Sharp and Spalding, 1984; Merino et al., 1985; Unahawutti et al., 1986; Diaz et al., 1988; Sharp et al., 1988; Spalding et al., 1988; Smith and Chin, 1989; Esguerra and Lizada, 1990; Miller et al., 1991; Jacobi and Wong, 1992; Mangan and Ingle, 1992; Jacobi et al., 1995; Jacobi and Giles, 1997). In summary, when heat treatments do not cause injury to mango fruit, the marketability of the fruit is improved by the acceleration of certain ripening processes, such as increased skin yellowness and uniformity of ripe skin colour.

### *2.3.2. Mango fruit ripening inhibited by heat treatment*

Some heat treatments can also delay or inhibit ripening in certain mango varieties. 'Tommy Atkins' fruit given a VHT at 50°C for up to 240 min had a reduction in ACC oxidase activity, colour development and softening in the inner mesocarp tissue (Mitcham and McDonald, 1997). However, 3 days after treatment, the ACC oxidase activity recovered in most treated fruit. It was concluded that mango fruit appear to recover from many of the effects of heat stress associated with VHT.

## *2.4. Intra-specific variation in heat tolerance*

Variation in the heat tolerances of different cultivars of mango have been compared and cultivars ranked for ability to resist heat-induced injury (Table 3). Differences may be due to anatomical differences (skin or cuticle thickness), or differences in the skin composition. There have been no comparative studies relating the level of heat tolerance of different cultivars to differences in fruit anatomical structure.

## *2.5. The effect of fruit maturity on heat tolerance*

Fruit are defined as being physiologically mature when they can be detached from the parent plant and still continue to ripen and fully realise the characteristic eating qualities of that fruit species (Beever and Hopkirk, 1990). Johnson et al. (1997) defined a fully mature mango fruit as one that has produced a fully developed seed and has reached its full physiological potential for size and dry matter accumulation within the constraints of the growth environment. Reliable

Table 3
The ranking of heat tolerance between different cultivars of mango[a]

| Mango cultivar (listed most heat tolerant to least heat tolerant) | Heat treatment | Reference |
|---|---|---|
| 'Tommy Atkins', 'Keitt' | FHAT 51.5°C for 125 min | McDonald and Miller (1994) |
| 'Haden', 'Davis Haden', 'Pahiri', 'Alphonso' | HWT 51.7–52.2°C for 15–20 min | Pennock and Maldonaldo (1962) |
| 'Zill', 'Haden', 'Sensation', 'Kent', 'Keitt' | HWT 49–63°C for 0.5–15 min | Smoot and Segall (1963) |
| 'Irwin', 'Kensington', 'Haden', 'Strawberry' | HWT 48° for up to 90 min | Smith and Chin (1989) |

[a] Cultivars are listed in order of decreasing heat tolerance. HWT: hot water treatment, FHAT: forced hot-air treatment, and VHT: vapour heat treatment.

mango fruit harvest maturity indices have been sought to predict subsequent fruit quality (Peacock et al., 1986; Medlicott et al., 1988) and determine responses to postharvest treatments (Esguerra and Lizada, 1990). The period of rapid growth within the sigmoidal pattern of fruit development of mango is characterised by an increase in alcohol-insoluble solids, principally starch (Mendoza et al., 1972; Tandon and Kalra, 1984). This increase in dry matter has been used as a recommended maturity index for the 'Kensington' mango variety in Australia (Baker, 1986). The maturity standard for 'Kensington' requires that mature fruit attain a minimum 14% dry matter and have an internal flesh colour corresponding to 27 on the Hunter ''b'' scale or Stage 3 on a mango picking guide (Holmes et al., 1990; Baker, 1986; Anonymous, 1993; Crane et al., 1997). A two-factor maturity index was developed for Australian mangoes as total solids or dry matter used alone are not completely accurate in all instances. The failure is due to the relationship between dry matter and eating quality being affected by growing conditions (Peacock et al., 1989). The carbohydrate metabolism of the mango fruit has a major impact on its physiological maturity, since during fruit development non-structural carbohydrates such as starch and sugars accumulate. The level of starch and sugars determines eating quality of the fruit.

### *2.5.1. Immature mango fruit have lower heat tolerance*

Fruit maturity has been identified as a critical factor in determining the level of fruit heat tolerance. A commonly reported response for a range of mango cultivars is that immature mango fruit are more susceptible to heat injury than mature fruit. For example, 'Keitt' mangoes picked immature, when the pulp was a light yellow colour, and heated by immersion in hot water ranging in temperature from 46.1 to 46.7°C for 20–60 min suffered skin shrivelling and pitting (Segarra-Carmona et al., 1990). In the Philippines, immature 'Carabao' fruit (float in 1% salt solution) exhibit internal breakdown (IB) after a VHT to a

46°C fruit core temperature for 10 min (Esguerra and Lizada, 1990; Esguerra et al., 1990). Characteristically, IB is recognised by the presence of spongy white starchy tissue in the inner portion of the mesocarp and is sometimes accompanied by a fermented odour. No external injury symptoms are visible on IB-affected fruit.

Evidence is accumulating that in mango fruit there is a link between heat-induced disruption to carbohydrate metabolism and the physiological stage of the fruit at the time of the heat treatment. A heat-induced internal injury that is common to all mango cultivars is the occurrence of unripe starchy areas within the fruit mesocarp tissues. This disorder has been recorded in a range of varieties including: 'Carabao' (Esguerra and Lizada, 1990; Esguerra et al., 1990), 'Alphonso' (Katrodia, 1988; Katrodia and Rane, 1988; Katrodia and Sheth, 1988), 'Keitt' (Mitcham and McDonald, 1993), and 'Kensington' (Jacobi and Wong, 1992; Joyce et al., 1993; Jacobi et al., 1995). The presence of unhydrolysed starch in mango fruit has been associated with the occurrence of IB. Katrodia (1988) described the IB disorder as one where, unhydrolysed starch remains in the fruit pulp in fruit at pre- and post-harvest stages. Joyce et al. (1993) suggested that the starchy regions within ripe mesocarp tissues represented a differential effect of heat on starch degradation, a process particularly sensitive to heat injury. Further investigations of carbohydrate metabolism of mango fruit are required in order to understand the processes involved in the retention of starch in mango fruit following heating.

### *2.5.2. The carbohydrate metabolism of mango fruit*

Mango fruit accumulate starch rapidly as the fruit approaches the green mature stage (Subramanyam et al., 1976; Tandon and Kalra, 1983; Tandon et al., 1985). This accumulation is accompanied by an increase in amylase activity (Fuchs et al., 1980; Tandon and Kalra, 1983). However, together with the increase in amylase activity, the mesocarp of unripe 'Haden' and 'Alphonso' fruit also contains an alpha-amylase inhibitor (Mattoo and Modi, 1970; Fuchs et al., 1980). Lizada (1993) suggested that the presence of this inhibitor might account for starch accumulation despite the increase in alpha-amylase activity during fruit maturation. In ripe fruit, the alpha-amylase inhibitor is undetectable (Mattoo and Modi, 1969; Kamath et al., 1987). Unripe bananas (*Musa acuminata* (Colla)) also contain compounds that inhibit the activity of catalase and amylase enzymes. The compounds in bananas are similar to those in mango in that they are heat-labile and proteinaceous in nature (Mattoo and Modi, 1970). Accumulated starch is hydrolysed during ripening and is accompanied by the disappearance of starch granules and a reduction in starch levels (Mattoo and Modi, 1969; Lakshminarayana et al., 1970; Subramanyam et al., 1976; Morga et al., 1979; Fuchs et al., 1980; Kalra and Tandon, 1983; Tandon et al., 1985; Medlicott et al., 1986b; Selvaraj et al., 1989; Parikh et al., 1990; Gomez-Lim, 1997). In Philippine

'Carabao' mangoes, starch levels in green unripe fruit are around 11%, while ripe fruit contain only 0.35% starch (Morga et al., 1979).

Alpha-amylase activity has been reported to increase at least fourfold during ripening of Indian mango cultivars (Mattoo et al., 1975; Majmudar et al., 1981). Increasing alpha-amylase activity parallels an increase in starch hydrolysis (Fuchs et al., 1980). As a consequence of starch hydrolysis, total sugars in mango fruit increase during ripening, mainly as sucrose, glucose and fructose (Vazquez-Salinas and Lakshminarayana, 1985; Selvaraj et al., 1989; Castrillo et al., 1992). However, sucrose predominates during later stages of mango fruit ripening (Subramanyam et al., 1976; Fuchs et al., 1980; Tandon and Kalra, 1983; Vazquez-Salinas and Lakshminarayana, 1985; Medlicott and Thompson, 1985; Selvaraj et al., 1989; Castrillo et al., 1992). Measurements have shown that this sugar constitutes more than 60–75% of the total sugars in ripe fruit (Vazquez-Salinas and Lakshminarayana, 1985; Castrillo et al., 1992).

*2.5.3. The effect of heat on carbohydrate metabolism of mango fruit*

There are several reports that indicate that when mango fruit sustains sudden heat-induced damage, starch metabolism in the ripening fruit is disrupted. The IB disorder of 'Carabao' mango fruit grown in the Philippines and treated with a VHT of 46°C for 10 min is characterised by the presence of starchy areas in the fruit mesocarp (Esguerra et al., 1990). Neuvo et al. (1984) found in 'Carabao' mesocarp parenchyma cells that had IB disorder have an average of 18 starch granules per cell compared with only 2 granules per cell in adjacent healthy tissues. Hot water-damaged mesocarp tissue of 'Kensington' mango has been found to contain significantly higher levels of starch than non-damaged tissue (Jacobi et al., 1995). 'Kensington' fruit immersed in 46°C water to maintain a 45°C fruit core temperature for 30 min and then ripened at 22°C had starch levels of approximately 15 mg/g fresh weight when ripe. In contrast, when fruit were conditioned at 40°C for up to 24 h prior to the 46°C HWT, starch levels in ripe fruit were less than 5 mg/g fresh weight. To date, no enzyme studies have been reported for 'Kensington' mango fruit to explain the disruption to starch metabolism in heat-treated fruit. Within spongy mesocarp tissue of heat injured 'Alphonso' mango fruit, starch remained unhydrolysed and the starch levels were 7.98% (Katrodia et al., 1988), while starch was hydrolysed and starch levels declined to 0.25% in healthy ripe tissue. Amylase activity in healthy tissue was 3.21 mg maltose/h/mg protein at 37°C compared with 0.50 in heat-damaged tissue. Katrodia et al. (1988) suggested that the temperatures are not sufficiently stressful to cause protein denaturation, but are sufficient to reduce enzyme activities.

Therefore, the mechanisms of disruption to carbohydrate metabolism in heat-treated mango remain largely unknown. There is a huge scope for research into this whole area of fruit biochemistry with the knowledge that may emerge

benefiting the eventual development of optimum heat treatments for minimum disruption to fruit physiology and maximum quality retention.

### 2.6. Conditioning fruit prior to heat treatment increases fruit heat tolerance

Conditioning a commodity before a subsequent heat or cold quarantine treatment has been found to be beneficial in reducing and even eliminating stress-induced injury. Temperature conditioning is a process of whereby a commodity is exposed to a higher than normal recommended storage temperature for a specified period (McDonald and Miller, 1994). Conditioning fresh fruit before applying postharvest treatments has been shown to have the potential to protect the product against physiological, storage-induced disorders, reversible inhibition of fruit ripening, as well as enhancing the natural resistance of fruit to pathogen infection (Klein and Lurie, 1992).

#### 2.6.1. Effect of conditioning treatments on mango fruit ripening

Conditioning mango fruit at 34–38°C applied for up to 3 days have been reported to accelerate some ripening processes and to delay others. In the 6 days following the conditioning of 'Keitt' mangoes at 38°C for up to 48 h, conditioned fruit were softer, had yellower skins, and higher total soluble solids levels than untreated fruit (McCollum et al., 1993). Similarly, increased skin colour development occurred in 'Tommy Atkins' mangoes conditioned at 38°C for 48 h (Pesis et al., 1997), and total soluble solids content and softening of 'Tommy Atkins' fruit intermittently warmed to 34°C over a 48 h period were greater than that of non-conditioned fruit (Nyanjage et al., 1998). 'Nam Dok Mai' mangoes conditioned at 38°C for 3 days had inhibited ethylene biosynthesis during the heat treatment, but resumed following fruit removal from 38°C and subsequently fruit displayed an ethylene ripening peak (Ketsa et al., 1999). This ethylene inhibition was due to inhibition of both 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-carboxylic acid oxidase (ACO). Following heat treatment, ACO recovered full activity, while ACS activity partially recovered to achieve an ethylene peak.

#### 2.6.2. Conditioning fruit increases the resistance to injury from heat disinfestation treatments

Conditioning treatments at 32–42°C applied before heat disinfestation treatments, have been shown to reduce or prevent high temperature induced damage to fruit. This observation has been reported for many different fruit species (Klein and Lurie, 1992). In mango, when 'Kensington' fruit are disinfested with water by raising the fruit core temperature to 47°C for 15 min, heat-induced injuries may occur (Jacobi and Wong, 1992). However, conditioning the fruit with air to a 37°C fruit core temperature for at least 12 h reduces the

incidence of injuries (Joyce and Shorter, 1994). Raising the temperature of the conditioning treatment from 38 to 40°C prior to HWT to a 45°C fruit core temperature held for 30 min was subsequently found to completely eliminate HWT-induced fruit injuries (Jacobi et al., 1995).

*2.6.2.1. Conditioning treatments and the production of heat shock proteins*. Heat shock proteins (HSPs) have been measured in fruit conditioned using temperatures in the range 38–40°C. Changes in the gene expression for HSP17 and HSP70 have been reported in papaya (*Carica papaya* L.), tomato (*Lycopersicon esculentum* Mill.) and avocado (*Persea americana* Mill.) fruit in response to the application of conditioning treatments, and the accumulation of these HSPs correlated with increased heat tolerance (Fender and O'Connell, 1990; Paull and Chen, 1990; Lurie et al., 1993; Woolf et al., 1995; Lurie et al., 1996). Despite establishing that HSP production in fruit increases during heat conditioning, the mechanism by which HSP17 and HSP70 families protect fruit cells from heat-induced damage is not known. It could be expected that the reduction in heat injuries observed when mango fruit are conditioned is correlated with production of HSPs. However, the optimum conditions for HSP production and the persistence of HSPs in mango tissue are unknown.

## *2.7. The effect of preharvest factors and environment on fruit heat tolerance*

The physiological responses of cultivars of different fruit species to heat treatments can be modified by season and growing location (Paull, 1990; Shellie and Mangan, 1994). The reason for the variation in response between production regions may arise from the combination of a number of factors operating within each environment such as climate, soil type, season, production practices (such as irrigation and fertilisation) of individual growers, and harvest practices used.

Rainfall, especially at or immediately before the time of fruit harvest has been observed to have an adverse influence on the response of fruit to a postharvest heat treatment. Johnson et al. (1992) and Cooke and Johnson (1994) found that 'Kensington' mangoes, if harvested in wet conditions, were more susceptible to heat damage manifested as skin browning when fruit were dipped for 5 min in benomyl solution held at 52°C. 'Kensington' mango fruit have also shown increased skin browning and internal starch layer and starch spot injuries after being harvested several days following a prolonged period of heavy rain in North Queensland and then being given a VHT to 46.5°C fruit core temperature for 10 min (Jacobi and Wong, 1993). Similarly, Jacobi and Giles (1997) observed an increased severity of skin browning injury on 'Kensington' mango fruit harvested following a 3-day period of rainy weather and then given a VHT to 47°C fruit core temperature held for 15 min approximately 24 h later.

Extreme preharvest temperatures have also been reported to influence the heat tolerance of mango fruit. The IB disorder has been associated with preharvest heat stress of Indian-grown 'Alphonso' mangoes (Katrodia, 1988). The fruit show unripe, hard, rubbery regions within the mesocarp tissue when the fruit is fully ripe. In IB damaged mesocarp, the tissue is pale in colour, lacking in juice and very acidic. Katrodia and Rane (1988) proposed that the cause of IB damage is due to excessive convective heat fluxes from the soil that are received at the distal end of the fruit and then diverted to either side of the fruit. Convective heat fluxes from the soil have been found to raise fruit core temperatures to 48.5°C and to result in IB (Katrodia and Sheth, 1988). The temperature of bare soil in Indian orchards has been recorded as high as 52.4°C, but when the orchards were mulched with straw and dry mango leaves, the soil temperature was reduced and correspondingly the incidence of IB was reduced (Katrodia and Sheth, 1988).

### *2.8. Detecting heat stressed fruit*

The development of visible fruit damage is an expression of stress-induced impairment of metabolic activity (Couey, 1989). The severity of a temperature stress to a tissue is a function of both the temperature and the duration at that temperature. Following the temperature stress, the development of injuries is a time-dependent process. A method to identify and quantify the level of metabolic damage prior to the physical expression of injury would be useful for both research and commercial purposes. Application of the method could be used to sort fruit that have been stressed, but are yet to develop visible symptoms, from non-stressed fruit. Several techniques are available to measure the stress response of fruit. Some methods are destructive, such as electrolyte leakage, and therefore, may not be beneficial in a commercial fruit handling system.

#### *2.8.1. Nuclear magnetic resonance*

Nuclear magnetic resonance (NMR) imaging is a non-destructive means of examining fruit for IB defects and is based on the distribution and magnetic environment of water in soft tissues (Clarke et al., 1997). NMR has been used successfully to reveal a number of internal disorders in fruit including watercore in apples (Wang et al., 1988), core breakdown in pears (Wang and Wang, 1989), and internal heat injury of mango and papaya fruit following heat disinfestation treatments (Joyce et al., 1993; Suzuki et al., 1994). There are currently no practical examples of NMR sensors being used on-line for sorting edible commodities. Part of the reason lies in the fact that fruit and vegetables are relatively high volume, low value items, while NMR protocols are slow and costly (Clarke et al., 1997). In contrast, assaying chloroplast

activity non-destructively by means of chlorophyll fluorescence (CF) techniques has proven to be a rapid method of monitoring plant response to stress.

*2.8.2. Chlorophyll fluorescence*

CF has long been used as a research tool in plant photosynthetic studies (Smillie and Hetherington, 1983; Smillie et al., 1987). More recently, CF emitted from chlorophyll a has been used as a means of rapidly and non-destructively monitoring plant response to environmental stresses (Smillie, 1979; Smillie and Hetherington, 1983) and methods to screen plants for tolerance to environmental stress were developed (Smillie and Gibbons, 1981; Smillie and Hetherington, 1983, 1990; Smillie et al., 1987).

The recording of CF parameters from chlorophyll-containing plant tissues is extremely rapid. Baseline CF measurements ($F_0$), recorded when photosystem II receptors are fully oxidised are measured in less than 0.1 s, and measurements of the maximal fluorescence emission ($F_{max}$) from tissue, determined when all acceptors are fully reduced, generally takes less than 3 s (Bolhar-Nordenkampf et al., 1989; Krause and Weis, 1991). When measuring the level of heat tolerance of green tissue, heat-induced changes in the level of $F_0$ have been used on the basis that heat-induced damage causes structural alterations at the PS II pigment level and that the rate of alteration occurs more rapidly in heat-sensitive than in heat-tolerant tissue (Krause and Weis, 1984). Thermal damage of PS II is characterised by a drastic increase in $F_0$ (Krause and Santarius, 1975; Schreiber and Berry, 1977; Schreiber and Armond, 1978) and a heat-induced decrease in the variable component of fluorescence, $F_V$ $(F_{max} - F_0)$ (Krause and Santarius, 1975; Schreiber and Berry, 1977; Schreiber and Armond, 1978; Weis, 1982). Typically, when using the new generation of fluorometer, the modulated light fluorometer, plant heat tolerance is assayed by measuring the extent of the decrease in the $F_V/F_M$ ratio induced by treating the tissue with a standardised heat treatment applied for a specified period.

In studies on the effects of high temperature disinfestation treatments on the CF of 'Kensington' mango fruit by Joyce and Shorter (1994) and Jacobi et al. (1995), fruit immersed in hot water to a fruit core temperature of either 47°C held for 25 min or 45°C for 30 min, or given a pretreatment of 37°C for up to 19 h or 38–40°C for 8 h before HWT. A significant increase in $F_0$ was recorded after HWT and this declined with time after treatment. The $F_V/F_M$ ratio declined after HWT, reflecting a sustained reduction in $F_V$. These changes in CF reflected heat-induced injuries to the fruit peel caused by the HWT, which included translucence, shrivelling, dimples and brown discolouration. The pretreatment did not influence either of the CF parameters, even though it did reduce the visible injury to the fruit. Therefore, the results of the mango studies revealed that CF detected changes in the fruit skin caused by the heat injury, but could not

detect the amelioration provided by the pretreatments. It may be possible that a certain change in CF does not necessarily indicate damage in a tolerant fruit.

### 2.9. Final conclusions

Heat treatments have been validated by many countries as viable non-chemical disinfestation treatments for many mango cultivars around the world. However, to date, no single heat disinfestation treatment has been found to be universally acceptable to all mango cultivars, and the difference in response between mango cultivars has not been fully explained. Despite the heat tolerance studies already conducted, there remains a lack of understanding at a more basic level of the physiological and biochemical responses of mango fruit to temperature stress. Knowledge of the physiology and biochemistry would provide a solid foundation of information to possibly predict mango fruit responses to heat treatment and, therefore, enable the development of effective strategies for heat treatment and marketing of this fruit throughout the world.

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