Heat treatment and fruit ripening

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Abstract

Postharvest heat treatments lead to an alteration of gene expression and fruit ripening can sometimes be either delayed or disrupted. The extent of the alternation of fruit ripening is a function of the exposure temperature and duration and how quickly the commodity is cooled following the heat treatment. The most commonly measured components of fruit ripening affected by heat treatments include fruit softening, membrane and flavor changes, respiration rate, ethylene production, and volatile production. Cell wall degrading enzymes and ethylene production are frequently the most disrupted and are sometimes not produced or their appearance is delayed following heating. Other processes associated with ripening are not altered to the same extent or soon recover. Fruit sensitivity to heat treatments is modified by preharvest weather conditions, cultivar, rate of heating, and subsequent storage conditions. The amount of sensitivity or tolerance to heat stress of a commodity is related to the level of heat protective proteins at harvest and the postharvest production of heat shock proteins. Two types of heat responses are seen. The first is a normal cellular response (< 42°C) that can lead to reduced chilling sensitivity, delayed or slowed ripening and a modification of quality. The second occurs near the threshold for damage (> 45°C and is modified by the pre-stress environmental conditions, the cellular response to stress and cellular recovery. Loss of membrane integrity appears to be an effect and not a cause of injury. The site of the injury lesion is still unknown and could be associated with transcription, translation and cellular recovery capacity after an injury threshold has been exceeded. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Heat treatments (thermotherapy) have been used for over a century to free plant materials from pathogens, the temperature and exposure duration having been determined empirically (Grondeau and Samson, 1994). Postharvest heat treatments of fruit are used for insect disinfection, disease control, to modify fruit responses to other stresses and maintain fruit quality during storage (Armstrong et al., 1989; Couey, 1989; Klein and Lurie, 1990; Paull, 1994; Paull and McDonald, 1994; Lurie, 1998; McDonald et al., 1999). Postharvest exposure to temperatures <
40–42°C often increases storage life and improves the flavor of a number of fruit (Barber and Sharpe, 1971; Liu, 1978; Lurie, 1998; Shellie and Mangan, 1998). The symptoms that occur at higher temperatures (> 45°C) are skin scald, and failure of the fruit to soften fully, or softening at a reduced rate (Jones, 1942; Akamine and Seo, 1978; Chan et al., 1981; Kerbel et al., 1985; Couey, 1989; Paull and Chen, 1990).

There are many hypotheses to explain heat injury (Crisan, 1973; Alexandrov, 1977; Levitt, 1980). Most of the concepts and experimental evidence involve protein denaturation, disruption of protein synthesis and loss of membrane integrity. Protein denaturation at lethal temperatures is regarded as non-reversible, while lower temperatures can lead to a reversible inactivation (Brandts, 1967; Alexandrov, 1977; Bernstam, 1978). However, the temperatures and exposure times involved in protein denaturation in vitro are considerably above those used in postharvest treatments and may not apply to the same protein in vivo. Less than lethal temperatures and duration can lead to short-term reversible disruption of transcription and translation steps in protein synthesis (Bernstam, 1978; Vierling, 1991; Lurie et al., 1996b).

Though much is known about the changes that follow heat treatment of fruit, how this leads to cellular death is unanswered. The answer may be different for chronic versus acute exposure to extreme temperature. Other factors that influence the temperature responses include species and variety under test and pretreatment heat exposure history. This complexity has led to some difficulty in interpretation of the published studies. The need is to determine the underlying physiological changes that are possibly reversible, and determine when the cellular disruption exceeds the cell’s ability for repair and recovery.

2. Heat responses and criteria

The difficulty faced in evaluation of heat tolerance limits for fruit ripening is in selection of criteria. The criteria (cytological disturbance, metabolic depression and necrosis) are dependent upon the temperature chosen and exposure time. However, overlaying these criteria are cellular protection mechanisms, ability to repair damage and the mechanism by which these are influenced by heat stress. Tied closely to these criteria is the timing of observations.

Cytological changes are well suited for small tissues but may not reflect the situation in larger plant organs such as a fruit. At the other extreme is the most often measured criterion, necrosis. Though necrosis is an integrative measure, its development reflects a cascading effect that may take days to develop, then remains constant. Tissue necrosis is often associated with polyphenoloxidase (PPO) activity, although, PPO expression and activity are also affected by heat treatments. In leaf tissue, heat treatments from 45 to 55°C for < 1 min significantly decrease PPO activity along with the synthesis of phenolic compounds (Loaiza-Velarde et al., 1997). Since numerous stresses can lead to the oxidation of polyphenols, this metabolic response provides little information on methods to minimize damage or an explanation of the initial events during and immediately following heat stress.

3. Temperature stress

The transfer of plants to an elevated temperature produces stress. The severity of stress is primarily determined by the temperature differential and the duration of exposure (Lurie, 1998). Other factors such as rapidity of the change in temperature and the previous growing conditions are also important. The effect of exposure time, although recognized by many (Alexandrov, 1977; Blum, 1987) is now being investigated in more detail (Burmeister et al., 1997; Porat et al., 2000). The influence of heat on postharvest fruit ripening is dependent upon (i) level of field-induced thermotolerance, (ii) cultivar, (iii) fruit size and morphological characteristics, (iv) physiological state (stage of ripeness), (v) heat transfer rate and energy balance (thermal difference, heat capacity and relative humidity), (vi) final temperature, and (vii) the duration of exposure at different temperatures. The color of fruit also influences the pre-
harvest thermal history of a fruit. Surface temperatures of dark green fruit may reach 24°C above ambient air temperatures while light-colored fruit may only be 10–12°C above ambient (Barber and Sharpe, 1971). The factors involved in field-induced thermotolerance have been reviewed by Woolf and Ferguson (2000) in this issue. The role of cultivar in heat responses has been less well studied, though differences are found for grapefruit (Miller and McDonald, 1991), and observations suggest ‘Kapoho’ papaya is more susceptible than ‘Sunrise’ (Paull, 1995), while no difference was found for two apples varieties (Klein and Lurie, 1990).

Fruit size, shape, and morphology, play a significant role by affecting the uniformity of tissue heating. Uniformity of heating is related to the rate of heat conduction, which in plant tissue is low, being similar to water. For apple fruit the thermal conductivity is $< 0.5 \text{W m}^{-2}\text{K}^{-1}$ and as for many fruit, is less than water $0.6 \text{W m}^{-2}\text{K}^{-1}$ (Sweat, 1974). The time constant to heat the fruit center will normally be longer than the calculated time (Table 1) and is measurably longer than the surface. For example it is less than a few minutes for small fruit and 300 min for a jack fruit. The time constant is further reduced at higher wind speeds that reduce the boundary layer thickness, thereby affecting heat transfer. If water is used as the heat transfer medium, the boundary layer is reduced even further and the greater heat capacity of water increases the rate of heat transfer. When both water and air heating is used, the mass of fruit tissue is not uniformly treated, there being a gradient from surface to the center in both temperature and duration. These differences are crucial in commercial heat treatments and reflect a need to uniformly heat a mass of fruit.

Another factor may be involved that overlies the field induction of thermotolerance. Ferguson et al. (1998) have shown that the development of thermotolerance associated with heat shock protein (HSP) in apple fruit follows a daily cycle. The high levels of HSPs in shaded fruit are maintained over the night period and drop after the onset of daytime. The minimum for HSP-70 message occurs about midday. Part of the cycle of HSP RNA levels reflects the lag in response shown in fruit due to buffering of thermal changes as the highest fruit temperatures are achieved about 15:00 h. The same loss kinetics is found for papayas where HSP RNAs decline 8–24 h after the HS induction treatment (Paull and Chen, 1990). However, this may also reflect diurnal rhythms. Piechulla and Gruissem (1987) have shown that such rhythms in gene expression do occur in developing tomato fruits. As found for chilling injury (Patterson et al., 1978), a circadian rhythm in thermotolerance may exist, aside from the diurnal ambient temperature changes. Some crop plants are less injured by a 5 min exposure to 50°C during noon than during the rest of the day (Laude, 1939). Other plants are less injured by 46°C, if treated during the dark period (Schwemmle and Lange, 1959).

Table 1
Thermal constant as a measure of how closely tissue temperature tracks air temperature for fruit treated as simple geometric shapes at 20°C

<table>
<thead>
<tr>
<th>Fruit size</th>
<th>Volume/area (cm²)</th>
<th>Wind speed (m s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (cm)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Rowan berry</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Crab apple</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Jack fruit</td>
<td>60</td>
<td>10</td>
</tr>
</tbody>
</table>

* Values are for non-transpiring organs (after, Monteith 1981).
A short exposure to near-lethal temperature is a ‘crisis’ situation that relates to survival and the capacity to recover, while longer exposure to lower temperatures is more difficult to quantify. Growth and metabolism can also be adversely affected below such ‘crisis’ temperatures. For example, avocado fruits stored at 30 and 34°C ripened abnormally, showed considerable surface pitting, and poor flavor. Ethylene treatment increases the threshold temperature for injury in avocado (Lee and Young, 1984). Exposure of pears (Maxie et al., 1974), and tomatoes (Ogura et al., 1976; Yoshida et al., 1984; Biggs et al., 1988; Picton and Grierson, 1988; Lurie and Klein, 1992) to 30°C for 48 h or more results in a delay of various aspects of ripening such as pigmentation, softening, and by a marked short-term decline in ethylene production (Maxie et al., 1974; Yoshida et al., 1984; Biggs et al., 1988; Picton and Grierson, 1988; An and Paull, 1990), and reduced volatiles (McDonald et al., 1999).

4. Response to heat treatment

4.1. Cellular manifestations

The gross cytological changes that follow high temperature exposure (> 45°C) have been described (Belehradek, 1957) and include coagulation of the cytoplasm, cytolysis, nuclear changes and altered mitosis. Protoplasmic streaming is inhibited, and there is increased protoplasmic viscosity and a loss of membrane permeability (Alexandrov, 1977). Lower temperatures (< 40°C) have no observed effects on cytology (Cheng et al., 1988). Heating fruit also changes the cuticle structure by reducing the number of cracks in apples (Lurie et al., 1996a), cuticle permeability in peaches (Phillips and Austin, 1982), and enhanced water loss in grapefruit (Porat et al., 2000), though no similar change is found for oranges (Williams et al., 1994).

Cellular membranes have been stressed with respect to heat injury (Alexandrov, 1977), and the role of membranes in cell death induced by heat has been reviewed (Blum, 1987). A sigmoidal curve describes electrolyte leakage by tomato discs induced by increasing temperature (Inaba and Crandall, 1988). Critical exposures times for leakage were 34 min at 55°C, 105 min at 50°C and 166 min at 45°C. The tomatoes did not, however, exhibit visible heat injury. A 50% higher electrolyte leakage was found from apple fruit discs isolated after being held at 38°C for 2 days (Lurie and Klein, 1990); after the disks were transferred to 20°C, leakage declined within 2 days, to control levels. However, little change has been reported in the first few hours of exposure, suggesting membrane changes may be an effect, not a cause of damage.

Much of the work on membrane lipids has been on bulk cellular extracts, and its relevance to the in vivo situation has been questioned (Blum, 1987). Membrane lipid saturation is not correlated with heat tolerance in some systems (Gombos et al., 1994). Phospholipid content is higher in heated apple fruit, is more saturated, and the membrane micro-viscosity and sterols are increased (Lurie et al., 1995). Transgenic tobacco plants in which the chloroplast omega-3-fatty acid
desaturase was silenced have lower levels of trienoic fatty acids and are better able to acclimate to higher temperature (Murakami et al., 2000). The resistance in these transgenic plants to heat is not transient and is unlike the protection conferred by induction of HSPs. The tentative conclusion is that the loss of cellular membrane integrity with increasing temperatures is gradual, with repair and reversibility possible until a lethal cell temperature is reached. The acclimation to high temperature stress does involve the membranes and develops following a number of days of exposure to elevated temperatures.

4.2. Physiological manifestations

4.2.1. Respiration

The respiration rate of ripening fruit is initially increased by exposure to higher temperatures (Jones, 1939; Akamine, 1966; Maxie et al., 1974; Ogura et al., 1976; Inaba and Chachin, 1988, 1989; Klein and Lurie, 1990; Lurie and Klein, 1991; Mitcham and McDonald, 1993). The response to temperature varies with the physiological age of the tissue. The temperature coefficient (Q_{10}) of apple fruit declines from 2.8 in young fruit to 1.6 in fruit 6 weeks later over the range 10–30°C (Jones, 1981). Though misleading if Q_{10} is applied outside the range tested, the difference in physiological age is of significance. After the heat treatment, the respiration rate declines to near or below the level of the non-heated control. Twelve days or more at ca. 33°C results in the suppression of respiration in tomato fruit and this action is not completely reversed after returning the tomato fruit to ambient temperatures (Ogura et al., 1976; Inaba and Chachin, 1988). Heat treatments do have an impact on the subsequent climacteric respiratory rise. The increase between the initial preclimacteric level and the climacteric peak in avocado fruit was 250% at 25°C, and only 30% at 30°C (Biale and Young, 1971). During heat treatments at 43–48°C, ripening fruit initially show an elevated respiration rate in papayas (Jones, 1939; Paull and Chen, 1990) and mangoes (Mitcham and McDonald, 1993) that declines to the same level or below of fruit that received no heat treatment. The extent and timing of the initial peak in papayas depend upon the relative humidity of the heated air (Jones, 1939). The heat-treated fruit climacteric peak is delayed about 2 days (Mitcham and McDonald, 1993). Similarly in apples, the respiration is lower upon return of heated fruit to ambient temperatures compared to non-heated fruit (Klein and Lurie, 1990).

The respiratory control ratio is higher in tomato mitochondria isolated from fruit stressed for 3 days at 37°C (Cheng et al., 1988) and mitochondrial Ca^{2+}-ATPase activity is also very significantly reduced by the same stress. There is a shift with chronic heat exposure to the cyanide insensitive pathway (Inaba and Chachin, 1989). The shift to alternative oxidative phosphorylation may reflect disruption of the thermoregulatory role of this pathway in order to maintain cellular stasis (Breidenbach et al., 1997).

4.2.2. Ethylene

Ethylene synthesis is reversibly inhibited at higher temperatures (Biale, 1960; Field, 1984). Fruit exposed to long periods at high temperature quickly recover their ability for ethylene synthesis (Ogura et al., 1976; Biggs et al., 1988; Dunlap et al., 1990). The conversion of ACC to C_2H_4 is apparently highly susceptible to heat damage above 30°C (Yu et al., 1980). There is a rapid loss (75%) of ACC oxidase in papaya and other fruits exposed for short periods to temperatures >40°C (Chan, 1986a,b; Dunlap et al., 1990; Klein and Lurie, 1990; Paull and Chen, 1990; Ketsa et al., 1999). The heat inactivation of ACC oxidase in papaya and cucumber is biphasic with both phases following first order kinetics (Chan, 1986a,b). The heat resistant ACC oxidase is about 25% of total ACC oxidase activity in papayas. Full recovery of ACC oxidase activity occurs within 3 days of removal of heat in papaya (Paull and Chen, 1990), apples (Klein and Lurie, 1990), muskmelon (Dunlap et al., 1990) and mango (Ketsa et al., 1999). Protein loss in more likely due to a decrease in ACC oxidase mRNA and hence synthesis (Lurie et al., 1996b), rather than denaturation. The recovery of ethylene production ability requires protein synthesis (Biggs et al., 1988), suggesting low level production of ethyl-
ene-related mRNA during heat treatment (Picton and Grierson, 1988), or reactivation of pre-existing mRNA and/or proteins.

Temperatures > 35°C cause an accumulation in endogenous ACC content in apple and tomato tissue (Yu et al., 1980; Atta Aly, 1992) and reduced ethylene production in both fruits (Biggs et al., 1988; Klein, 1989). The accumulation of ACC does not occur in fruit subjected to higher temperatures or for longer duration. ACC synthase is therefore regarded as less sensitive to loss due to heat stress than ACC oxidase in tomato and apple (Klein, 1989; Atta Aly, 1992). ACC synthase activity is lost in mangoes and only partially recovers on return to ambient temperatures (Ketsa et al., 1999). The differences in response between the oxidase and the synthase may be related to differences in turnover rates.

Wound-induced ethylene synthesis is less sensitive to heat stress inactivation than climacteric-related ethylene synthesis (Biggs et al., 1988). Pears, tomatoes and bananas do not respond to exogenous ethylene during heat treatment (Maxie et al., 1974; Seymour et al., 1987; Yang et al., 1990), and it has been suggested (Yang et al., 1990; Lurie, 1998) that this loss of sensitivity is associated with inactivation of ethylene receptors. Result with the ethylene receptor inhibitor, 1-methycyclopropene, suggest that recovery of ethylene sensitivity in plum and banana occurs in 4–5 days (Abdi et al., 1998; Golding et al., 1998), which does not agree with the recovery time found for tomatoes heated for 12 h (Biggs et al., 1988). Disruption of ethylene synthesis may mediate heat-induced ripening inhibition (Lurie, 1998), but its effect is more likely to be part of an injury response, related possibly to recovery of ethylene synthesis and membrane disruption. Heat treatments that cause injury do engender a burst of 'wound' ethylene 1–2 days after treatment that is followed by a subsequent advanced ethylene climacteric peak four days later, and sooner than the peak in papaya fruit not subject to an injurious heat treatment (Paull and Chen, 1990).

4.2.3. Softening and cell wall metabolism

Flesh softening is often slowed following exposure to 38–40°C (Table 2), even if the treatment is applied for an extended period (4 days) before storage (Klein and Lurie, 1990; Lurie and Nussinovitch, 1996). Following disinfection temperatures of 45–50°C softening is faster (Mitcham and McDonald, 1992; Shellie and Man- gan, 1994) or disrupted (Paull and Chen, 1990). Exposure of apples to 38°C for 4 days resulted in fruit with less soluble and more insoluble pectin (Klein et al., 1990; Ben-Shalom et al., 1993, 1996). Tomatoes had less soluble polyuronides, and had less galactose and arabinose loss after 96 h at 40°C (Mitcham and McDonald, 1992). Heat-injured papaya fruit that fail to soften, also show little change in soluble pectin amounts or molecular weights during ripening (Qiu et al., 1995). The softening disruption has been ascribed to reduction of cell wall hydrolytic enzymes. Though heat disruption of cell wall breakdown has been proposed as the cause of delayed or poor softening, the actual enzyme having the central role in softening has not been determined (Lashbrook et al., 1998; Rose et al., 1998). The findings with different cell wall degrading enzymes do show that the disruption may be associated with mRNA synthesis and stability, or protein synthesis and degradation.

In the range 35–60°C, considerable (87%) loss of activity of purified glucanase from tomato fruit can occur (Hinton and Pressey, 1980), and 50% glucanase activity is lost by exposure to 50°C for 5 min (Pressey, 1983). Tomato endo-mannase and galactosidase are rapidly lost and the activity returns 4 and 14 days, respectively, after heating at 40°C for 2 days (Sozzi et al., 1996). The role of these enzymes in fruit softening is unclear. Similarly, pectinesterase is suppressed in tomatoes held at 33°C (Ogura et al., 1975), though there is little difference in the degree of apple pectin esterification after treatment at 38°C for 4 days (Klein et al., 1995).

The failure of papaya to recover the ability to soften (Paull and Chen, 1990) could be related to an aspect of papaya ripening where the mRNAs for cell wall degrading enzymes are produced for only a short period during a specific stage of ripening (Paull and Chen, 1983). The inner mesocarp exhibits noticeable carotenoid development when the skin is <10% yellow while the outer
Table 2
Response of fruits to various exposure times and temperatures with respect to the development of phytotoxic symptoms, softening, skin color development, sugar and acidity

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Method of heating</th>
<th>Exposure time</th>
<th>Exposure temperature (°C)</th>
<th>Phytotoxic symptoms</th>
<th>Softening</th>
<th>Color development</th>
<th>Sugar and acidity</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applesb</td>
<td>HA</td>
<td>96 h</td>
<td>43</td>
<td>Y</td>
<td>Firmer</td>
<td>Increased</td>
<td>Decreased acidity</td>
<td>Klein and Lurie (1990)</td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td>96 h</td>
<td>45</td>
<td>Y</td>
<td>Firmer</td>
<td>Increased</td>
<td>Lower acidity</td>
<td>Liu (1978)</td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td>96 h</td>
<td>38</td>
<td>N</td>
<td></td>
<td>Increased</td>
<td>Sweeter</td>
<td>Lurie and Nussinovitch (1996)</td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td>24h</td>
<td>46</td>
<td>N</td>
<td>Firmer</td>
<td>--</td>
<td>Sweeter</td>
<td>Klein and Lurie (1992)</td>
</tr>
<tr>
<td>Bananas mature green</td>
<td>HW</td>
<td>45 min</td>
<td>50</td>
<td>N</td>
<td></td>
<td>Delayed</td>
<td></td>
<td>Armstrong (1982)</td>
</tr>
<tr>
<td>Mangoes immature</td>
<td>HA</td>
<td>10 min</td>
<td>46</td>
<td>N</td>
<td></td>
<td>Slower</td>
<td>Increased Sugar</td>
<td>Liu (1978)</td>
</tr>
<tr>
<td>Mature</td>
<td>HA</td>
<td>10 min</td>
<td>46.5</td>
<td>N</td>
<td></td>
<td>Faster</td>
<td>Decreased acidity</td>
<td>Jacobi and Giles (1997)</td>
</tr>
<tr>
<td></td>
<td>VH</td>
<td>15 min</td>
<td>46.5</td>
<td>N</td>
<td></td>
<td>None</td>
<td>None</td>
<td>Jacobi et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>VH</td>
<td>30 min</td>
<td>47</td>
<td>Y</td>
<td></td>
<td>Increased</td>
<td>None</td>
<td>Jacobi et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>VH</td>
<td>4.5 h</td>
<td>46</td>
<td>N</td>
<td></td>
<td></td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Oranges</td>
<td>HW</td>
<td>42 min</td>
<td>45</td>
<td>N</td>
<td>Firmer</td>
<td>Increased</td>
<td>Higher sugar/acid ratio</td>
<td>Mangle and Mangan (1998)</td>
</tr>
<tr>
<td>Papaya mature green</td>
<td>HW</td>
<td>60 min</td>
<td>47.5</td>
<td>Y</td>
<td>Firmer</td>
<td>Delayed</td>
<td>Reduced acidity</td>
<td>Williams et al. (1994)</td>
</tr>
<tr>
<td>Peaches</td>
<td>HW</td>
<td>40 min</td>
<td>40</td>
<td>Y</td>
<td></td>
<td></td>
<td>Paull and Chen (1990)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td>48 h</td>
<td>46</td>
<td>Y</td>
<td></td>
<td>Increased</td>
<td>Reduced acidity</td>
<td>Kerbel et al. (1985)</td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td>7 days</td>
<td>38</td>
<td>Y</td>
<td></td>
<td>--</td>
<td>Sweeter</td>
<td>Kerbel et al. (1985)</td>
</tr>
<tr>
<td>Pears</td>
<td>HA</td>
<td>72 h</td>
<td>38</td>
<td>N</td>
<td>Firmer</td>
<td>Increased</td>
<td>Higher sugar</td>
<td>Lurie and Klein (1992)</td>
</tr>
<tr>
<td>Tomatoes mature green</td>
<td>HA</td>
<td>48 h</td>
<td>40</td>
<td>N</td>
<td></td>
<td>Delayed</td>
<td></td>
<td>Sozzi et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td>96 h</td>
<td>40</td>
<td>N</td>
<td></td>
<td>Delayed</td>
<td></td>
<td>Mitcham and McDonald (1992), Cheng et al. (1988)**</td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td>72 h</td>
<td>37</td>
<td>N</td>
<td></td>
<td>Firmer</td>
<td></td>
<td>Inaba and Chachin (1989)</td>
</tr>
</tbody>
</table>

*a* Selected recent references — for earlier references refer to Paull (1990).

*b* Damage to fruit is very dependent upon cultivar, size, shape, maturity and handling.

*c* Phytotoxicity: Y—Yes; N—No.

*d* Method of heating: HW-Hot Water; HA-Hot Air; VH-Vapour Heat.
mesocarp shows no carotenoids (Paull and Chen, 1983). This suggests that the inner mesocarp is at a more advanced stage of ripening and at a stage which may be more susceptible to heat stress than the less mature outer region (Paull and Chen, 1990). Papaya cell wall polygalacturonase and xylanase production occurs over a short period during ripening that contrasts with the continual production of polygalacturonase in tomatoes (Grierson, 1986) and cellulase in avocado (Christoffersen et al., 1982).

Chan et al. (1981) observed that papaya fruit with 25 or 50% of the skin yellow had less polygalacturonase than fruit at the color-break stage after 65 min exposure to 46°C. A similar reduction was found for tomatoes held for several days at 33°C (Ogura et al., 1975). These results could be due to either denaturation or disruption of polygalacturonase synthesis. Since polygalacturonase shows first order heat denaturation kinetics and is 60% denatured by 20 min at 70°C (Chan and Tam, 1982), exposure of fruit to 49°C would be expected to induce minimal denaturation in papaya. The failure to soften, therefore, is possibly attributable to suppression of the mRNA for wall softening enzymes, as found in tomatoes (Picton and Grierson, 1988). The loss of polygalacturonase mRNA in tomatoes is not overcome by exogenous ethylene (Picton and Grierson, 1988). Polygalacturonase activity, however, does return after a 6 day lag when returned to 25°C (Ogura et al., 1975; Yoshida et al., 1984). A similar reversibility in fruit softening has been found in apples held at 38°C for 4 days (Lurie and Klein, 1990).

4.2.4. Chlorophyll and carotenoid metabolism

Heating leaves to 44°C leads to loss of chloroplast activity, especially electron transport by photosystem II (Berry et al., 1975). An associated characteristic, delayed light emission, declines in papaya skin with first order kinetics when fruit are heated between 42 and 48°C (Chan and Forbus, 1988). At higher temperatures (49–51°C), heat inactivation is biphasic.

Above 25°C, bananas (Burg, 1962; Blackbourn et al., 1989) fail to degreen, while above 30°C papaya fruit fail to ripen normally, with the pulp becoming soft and watery, although the skin chlorophyll changes are not affected (An and Paull, 1990). Degreening is accelerated in apples (Liu, 1978; Klein et al., 1990; Lurie and Klein, 1990), plantain and tomato (Seymour et al., 1987; Lurie and Klein, 1991) at 35–40°C for 4 days and delayed with a shorter treatment of 2 days at 40°C in tomato (Sozzi et al., 1996). The difference in tomato response suggests that a threshold duration has been exceeded. This is supported from studies with maple leaves (McCain et al., 1989) where the damage occurred over a narrow range from 53 to 57°C. In soybean leaves, 1 min at 53°C causes no damage, while at 54°C chlorosis occurs, and necrosis at greater than at 55°C (Coleman et al., 1988). Alternatively, at all temperatures, dehydration at the higher temperature alters the degreening rate.

Carotenoid synthesis is inhibited by temperatures >30°C (Goodwin and Jamikorn, 1952; Cheng et al., 1988; Lurie et al., 1996b; Sozzi et al., 1996). The inhibition of lycopene synthesis in tomatoes (Cheng et al., 1988; Lurie et al., 1996b; Sozzi et al., 1996) is due to inhibition of mRNA transcription of lycopene synthesis that recovers after removal of the heat.

4.2.5. Flavor and volatile production

The impact on flavor varies with species, temperature and duration of the heat treatment. Avocado flavor is adversely affected by treatment at 43°C for longer than 5 h (Kerbel et al., 1987). Juice from heat-treated grapefruit (46°C — 5 h or 48°C — 3 h) from early and mid-season fruit was not different from unheated fruit (McGuire and Reeder, 1992), while juices from heated late-season fruit or grapefruit placed in a constant temperature water bath instead of gradual heating were judged significantly inferior (McGuire, 1991). The inferiority may be due to damaged oil glands, especially when water was used as the heat treatment medium rather than air, as for ‘Navel’ and ‘Valencia’ oranges treated at 46°C (Shellie and Mangan, 1994, 1998). The free juice content in peaches is less, as the duration of the heat treatment increases from 24 to 48 h at 41–46°C (Lay-Yee and Rose, 1994). However, no difference in eating quality is found for mango sub-
ject ed to a high humidity heat treatment (Jacobi et al., 1995; Jacobi and Giles, 1997).

The sugar to acid ratio is often a measure used to determine the effect of heat treatments on quality (Table 2). At lower treatment temperatures (38°C, 4 days), apples have a higher ratio immediately following treatment due mainly to reduced acid levels (Liu, 1978; Porritt and Lidster, 1978; Klein and Lurie, 1990). Titratable acidity is also reduced in nectarines subjected to treatments of 41–46°C for 24–48 h (Lay-Yee and Rose, 1994), a similar reduction occurring in strawberry acidity (Garacia et al., 1995). There is generally no significant effect on soluble solids. Mango soluble solids are not affected by an insect vapor heat treatment (Jacobi et al., 1995; Jacobi and Giles, 1997). Grapefruit (46–50°C for up to 7 days) and oranges (46–47.5°C plus storage) showed a similar effect: reduced acidity, no effect on soluble solids and a higher sugar/acid ratio (McGuire, 1991; Miller and McDonald, 1991; McGuire and Reeder, 1992; Shellie and Mangan, 1998). Heat treatments, either water or hot air (38–48°C for 1 h to 3 days) have no effect on tomato soluble solids or acidity (Lurie and Klein, 1991; Lurie and Sabehat, 1997; McDonald et al., 1999). The variable effects of heat treatments on sugars and acidity, most often titratability acidity, depends upon the temperature used and duration.

Apple volatile production, though enhanced during a 38°C treatment is immediately inhibited after heat treatments and recovers slowly (Fallik et al., 1997). Tomato volatile flavor level and profile is altered by temperature treatments of 1 h increasing from 38–48°C; two components increased and five decreased, out of the 15 analyzed (McDonald et al., 1996, 1999).

4.2.6. Protein metabolism

Exposure of ripening pear fruit to 40°C leads to a disruption of protein synthesis via a loss of polysomes (Romani and French, 1977). This response is readily reversible and establishes a close correlation between the protein synthesizing machinery and the progression of ripening. Apples held at 38°C for 4 days had reduced S35-methionine incorporation into proteins (Lurie and Klein, 1990). HSP synthesis in pear cells is also greater at 39°C than above 40°C, when the treatment is extended for up to 8 h (Ferguson et al., 1994). Papaya fruit protein synthesis changes following exposure to 38°C for 2 h and there is no accumulation in the control fruit held at 22°C for 10 h. Translated polysomal RNA confirmed that new polypeptides are synthesized following heat shock induction (Paull and Chen, 1990). Thermotolerance decreases with continued exposure of fruit to 42°C (Paull and Chen, 1990) suggesting that 42°C is the limit for induction of HS tolerance and protein degradation is enhanced, as found for pear cells (Ferguson et al., 1994). Ubiquitin may not be involved in specific protein degradation above 40°C. Continued exposure to 42°C, though still allowing HS polypeptide synthesis, may also cause damage. These results agree with those for tomato fruit treated for long periods at 35°C (Picton and Grierson, 1988). Apple held for 4 days at 38°C had a change in protein synthesis profiles (Lurie and Klein, 1990). The new protein bands on electrophoresis gels at the low molecular weight (14–22 kD) and high molecular weight (68 and 92 kD) ranges (Lurie and Klein, 1990) correspond with HSP positions and agree with the results obtained with papaya (Paull and Chen, 1990).

Thermal injury (46°C, 65–90 min) to papaya fruit softening during ripening is correlated with a 90% decrease in normal polygalacturonase levels (Chan et al., 1981), the injury being more severe in riper fruit. These researchers also noted the same conditions as those observed in our laboratory (Paull and Chen, 1990) of heated fruit showing delayed ripening and leaving a 1–1.5 cm thick area of hard tissue. Partial recovery to 25% of normal polygalacturonase levels occurred after 6 days at 24°C (Chan et al., 1981). The conclusion from this work was that transcription is disrupted by heat. This disruption could possibly occur by mRNA being released from the ribosomes at 40°C (Romani and French, 1977; Stuger et al., 1999). Protein degradation, particularly of rate limiting enzymes, may continue at a higher rate due to the higher temperature (Vierstra, 1994; Callis, 1995). This degradation could lead, if a threshold was exceeded, to disruption of metabolic fluxes through pathways.
4.2.7. Nucleic acid metabolism

Synthesis of ripening-specific mRNAs and new enzymes is induced during ripening (Christoffersen et al., 1982; Tucker and Grierson, 1982; Spiers et al., 1984; Grierson, 1986). Elevated stress temperatures may lead to inhibition and/or reduced expression of softening and other ripening-related mRNA (Picton and Grierson, 1988). Transcriptional regulation is probably modified by mRNA stability. Post-translational modification may also be involved in regulating the overall accumulation of cell wall degrading enzymes (Biggs and Handa, 1988). The inhibition of ripening above 30°C has been ascribed to disruption of the enzymes involved in ethylene synthesis (Maxie et al., 1974; Ogura et al., 1975; Lurie, 1998), loss of cell wall degrading enzymes (Chan et al., 1981), and to suppression of ripening-related mRNA synthesis (Picton and Grierson, 1988). Heat-stressed cells re-program their translational machinery in favor of HSPs (Nover, 1991). Translation of other transcripts is almost completely suppressed. However, most untranslated messages are not degraded (Nover et al., 1989). These messages may be secreted into storage particles within small molecular weight HSPs that appear after prolonged stress (Stuger et al., 1999).

The failure of heated fruit to soften suggests (a) a failure to transcribe, (b) a loss of mRNAs coding for wall softening enzymes, or (c) a failure to translate mRNAs. In addition, heating could activate enzymes or enhance enzyme turnover leading to cellular damage (Swartz et al., 1956; Callis, 1995) directly, or indirectly by destroying cellular compartmentation.

5. Mechanism of responses to high temperature

HSPs produced in response to high temperature are believed to prevent irreversible protein denaturation that would be detrimental to the cell (Parsell and Linquist, 1993) and this activity may be enhanced in plants by small HSPs (Lee and Vierling, 2000). HSPs and associated thermotolerance have been reported for field-grown cotton leaves (Burke et al., 1985), soybean leaves (Kimpel and Key, 1985), sorghum (Ougham and Stod- dart, 1986), papaya fruit (Paull and Chen, 1990) and apples (Klein and Lurie, 1990; Ferguson et al., 1998). These proteins are produced within 30 min after exposure to temperatures in the range 34–42°C (Kanabus et al., 1984). The lag period for induction for HS response is slower than other stress responses (Trewavas, 1999), and there is a synergistic interaction with ABA (Xiong et al., 1999). The decay of HSPs occurs, with a corresponding loss in thermotolerance. This phenomenon appears to confer a temporary, acquired heat resistance to sub-lethal temperatures (Altschuler and Mascarenhas, 1982). There is a fundamental role for HSPs in cellular function during high temperature stress (Vierling, 1991; Queitsch et al., 2000). Arabidopsis plants expressing less than usual amounts of HSP101, as a result of mutation, antisense or co-suppression, grow at normal rates but have severely diminished capacity to acquire heat tolerance after mild conditioning pretreatments (Queitsch et al., 2000; Hong and Vierling, 2000). The smaller MW HSPs protect cellular proteins from thermal aggregation (Vierling, 1997) and protein folding activity and HS-induced protein aggregation preventive activity are integrated (Lee and Vierling, 2000). Other HSPs have unique functions, such as the reactivation of proteins that have already aggregated or promote the degradation of misfolded proteins. Different mechanisms of tolerance seem to be induced by sudden HS and that acquired by sustained growth at a moderately high temperature (Wu and Wallner, 1984). Membrane fatty acid desaturation plays a role in acclimation to higher temperatures (Murakami et al., 2000).

HS may induce reactive oxygen intermediates that can cause membrane and protein damage. The creation of oxygen radical scavengers, such as superoxide dismutases, peroxidases, and catalases, is induced by HS (Holmberg and Bulow, 1998). HS also induces ascorbate peroxidase in tomato associated with a HS cis-element in the promoter (Storozhenko et al., 1998). The reported effect of reactive oxygen is supported by HSPs being produced at higher temperature when the temperature is raised slowly (3°C h⁻¹) than when the temperature is rapidly increased (Altschuler and Mascarenhas, 1982). The slower temperature in-
crease allows adaptation, and emphasizes the importance of the heat-stress profile (final temperature and duration). It has also been suggested that the amount of free iron present in a cell reduces oxidative damage, as transgenic tobacco plants expressing alfalfa ferritin show reduced damage to abiotic and pathogenic stress (Deak et al., 1999). Selections of mutant plants have also shown that a number of different types of thermostability may exist (Mullarkey and Jones, 2000).

Many procedures have been empirically developed to reduce the injury caused by heat treatment. The disruption of papaya fruit softening by the injurious heat treatment is reduced or prevented by a pretreatment at 42°C for 1h (Fig. 1A) or varying the pretreatment time at 38°C (Fig. 1B), these treatments being followed by 3 h at 22°C (Paull and Chen, 1990). The greater damage to papaya heated at 38°C for longer than 2 h may be due to fruit undergoing ripening and riper fruit are more sensitive to damage than mature green fruit. The 8 h approach time to 44°C for the papaya vapor heat treatment (Seo et al., 1974) is designed to reduce subsequent injury when the fruit is exposed to 44°C for 8.75 h. This vapor heat treatment approach time would be expected to provide the conditions necessary to develop HSP and associated thermotolerance. The rapid rise of temperature to 49°C using vapor heat always damages ‘Valencia’ oranges and grapefruit (Sinclair and Lindgren, 1955), but these fruit are tolerant when heated for 8 h at 43°C, before the 49°C treatment. Cucumber can be preconditioned by a 24 h treatment at 32.5°C to tolerate subsequent exposure for 50 min to hot water at 46°C (Chan and Linse, 1989). Though 32.5°C is lower than the temperature normally used to induce HSP, the longer (24 h) duration would probably induce HSPs and thermotolerance. An understanding of the conditions necessary to induce HSPs and thermotolerance are essential to optimize heat treatments used for insect disinfestation and disease control.

6. Conclusions

Changes in fruit ripening during and following heat treatments can be divided broadly into two types (Table 3). The first type of response is the normal stress cellular responses that lead to modification of chilling sensitivity, delayed or slowed ripening and some slight modification of quality. Preharvest conditions, species, cultivar, the stage of ripening, temperature and its duration and

Fig. 1. Effect of pretreatment temperature for one hour (A) and pretreatment time at 38°C (B) in hot water on the development of internal abnormalities in papaya flesh. The internal abnormalities are due to a failure of the flesh to soften, the procedures used are described in Paull and Chen (1990). After pretreatment, fruit were held for 3 h at 22°C before exposure to 70 min at 49°C.
Table 3
Proposed impact of two levels of heat stress, <40–42°C, and >45°C, on the immediate effects on plant metabolism including heat shock (HS) protein synthesis and upon return to ambient temperatures

<table>
<thead>
<tr>
<th>Heat stress (°C)</th>
<th>Immediate effects</th>
<th>Return to ambient temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiration</td>
<td>Increased</td>
<td>Returns to control levels</td>
</tr>
<tr>
<td>Ethylene</td>
<td>Decreased</td>
<td>Recover slowly</td>
</tr>
<tr>
<td>Transcription</td>
<td>HS-mRNA produced</td>
<td>No longer transcribed</td>
</tr>
<tr>
<td></td>
<td>Other mRNA</td>
<td></td>
</tr>
<tr>
<td>Translation</td>
<td>HS proteins</td>
<td>Decline over 18 h</td>
</tr>
<tr>
<td>mRNA</td>
<td>Sequestered</td>
<td>Returns for translation</td>
</tr>
<tr>
<td>Protein</td>
<td>Degradation</td>
<td>Returns to normal degradation rate</td>
</tr>
<tr>
<td>Reactive oxygen</td>
<td>Increases</td>
<td>Returns to normal levels</td>
</tr>
<tr>
<td></td>
<td>damage to proteins and membranes</td>
<td></td>
</tr>
<tr>
<td>&gt;45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiration</td>
<td>Increased</td>
<td>Returns to control levels</td>
</tr>
<tr>
<td>Ethylene</td>
<td>Decreased</td>
<td>Very slowly returns over days</td>
</tr>
<tr>
<td>Transcription</td>
<td>HS mRNAs poorly inducted</td>
<td>No longer transcribed</td>
</tr>
<tr>
<td></td>
<td>mRNA synthesis</td>
<td>Returns depending upon stress</td>
</tr>
<tr>
<td>Translation</td>
<td>Polysomes lost</td>
<td>Returns depending upon stress</td>
</tr>
<tr>
<td>Protein</td>
<td>Degradation</td>
<td>Returns depending upon stress</td>
</tr>
<tr>
<td></td>
<td>especially rate limiting enzymes may have higher turnover rates</td>
<td></td>
</tr>
<tr>
<td>Reactive oxygen</td>
<td>Increases</td>
<td>If threshold for damage is exceeded, leads to necrosis</td>
</tr>
<tr>
<td></td>
<td>damage to proteins and membranes</td>
<td></td>
</tr>
</tbody>
</table>

* Necrosis is defined as cell death due to environmental stress beyond a threshold level for which the cells’ cellular machinery is unable to maintain integrity or recover during or following removal of the stress.

Fig. 2. Proposed model for heat injury in fruit. Injury is associated with disruption of normal cellular metabolism especially transcription and translation and the effect of rate limiting enzymes causing unbalanced metabolic fluxes beyond an injury threshold leading to necrosis.
possibly diurnal variations are modifying factors. The second type of response occurs when the stress exceeds a threshold and the cells’ ability to recover is lost (Fig. 2). This response is associated with the disruption of various aspects of ripening. The site of the lesion may be associated with either or both transcription and translation and a cellular threshold of metabolic disruption.

There are similarly two areas in need of answers, and an understanding of these should enable us to develop better approaches to avoiding injury and maintaining fruit quality. The first area is the role of adaptation including the effect of HSP, fruit physiological status and cultivar differences. The second area focuses on the heat lesion. The question evolves around the impact of heat treatments on transcription, translation, enzyme turnover and cellular recovery capacity.

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References


