

Progress in heat treatments

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Purpose of review: Increasing legislative regulations and negative public opinion of the use of synthetic fungicides and insecticides in fresh fruits and vegetables, together with the growth of organic markets, have increased research interest in postharvest heat treatments. These methods, effective in controlling fungal diseases and pest infestation, have revealed additional benefits on fresh produce such as extending shelf life, improving chilling tolerance, and in some cases the nutritional quality. This article describes the most relevant studies in the recent literature and their implication for the fruits and vegetables industry.

Main findings: Appropriate time-temperature combinations may reduce chilling injury and control decay, without impairing ripening or physico-chemical quality. The induced mechanisms could be enhanced and extended by combination with additional substances and/or beneficial organisms such as methyl jasmonate, ethanol and/or yeast antagonists. However, recent studies have shown that heat treatments can seriously damage fruit sensory quality, causing deterioration of flavor and aroma, and therefore, consumer rejection. Further research is needed to successfully integrate heat treatments into the commercial postharvest chains.

Limitations/implications: Heat treatments could be used to maintain the physico-chemical and nutritional quality of fruits and vegetables, but special attention must be given to adverse effects on sensory quality. Development of heat treatment protocols that maintain natural flavors and aromas is needed to successfully incorporate these applications into commercial practice.

Directions for future research: Combining heat with other treatments such as ethylene-action inhibitors, edible coatings, biological control agents, controlled atmospheres and/or appropriate packaging that could reduce decay, chilling injury and softening while avoiding heat damage, should be further investigated. Identification of biological markers to facilitate the selection of adequate heat protocols that are not injurious would also be highly beneficial.

Keywords: flavor; high temperature; quality; stress protection

Abbreviations

1-MCP	1-Methylcyclopropene
MeJa	Methyl Jasmonate
TA	Titratable Acidity
TSS	Total Soluble Solids

Introduction

Heat treatments have been used commercially to control fungal diseases and pest infestation since the first decades of the 20th century, when the effectiveness of hot water (44–48°C) in controlling molds in citrus was reported. Since then, multiple additional benefits such as chilling tolerance, extension of shelf life and preservation of fruit quality have been revealed. The increasing concern about the use of synthetic fungicides, perceived as harmful to human health and to the environment, is contributing to the growing interest in the development of environmentally-friendly methods for postharvest management. Apart from reducing the dependence on agrochemicals, heat treatments possess the appealing advantage of being relatively simple to apply, as they can sometimes be incorporated into packinghouse sorting lines. Perceived as safe and friendly, there is a collective interest in these methods from the fresh fruit and vegetable industry; organic markets and produce exporters could particularly benefit from these techniques.

Heat is generally applied as a short treatment preceding cold storage. There are five main types of application: 1) forced air, 2)

vapor, 3) water baths, 4) water rinsing and 5) water rinsing with brushing. The protocol for each type varies depending on the fruit species, cultivar, size, harvest maturity and growing conditions. Because of the known deleterious effects of excessive heat on cell physiology, temperatures too high and/or times too long could easily cause damage to the commodity. Treatment effects are localized rather than systemic; uniform coverage is necessary to attain optimal benefits as indicated by Lu *et al.* [1**], who pointed out the significant differences in color, total soluble solids (TSS), titratable acidity (TA), pitting on the stem scar side, discolorations and diseases after cold storage at 4°C between one half of a tomato exposed to a heated air-flow at 39°C and the other half at room temperature. The delay between heat treatment and cold storage is another important factor. Wang *et al.* [2**] investigated a 0.5–24 h delay between hot water immersion at 52°C for 3 min and cold storage (7°C) in banana, concluding that the heated fruit transferred to cold storage after a delay of less than 6 h exhibited significantly better quality than the bananas transferred to cold more than 6 h after heating.

This review discusses the most relevant scientific works regarding heat treatment of horticultural products published in the last five years aiming to be a guide for future research studies. Other aspects of the heat treatment research, such as food safety, fresh-cut products or gene expression effects, will be covered in other papers.

Protection from chilling injury

Low-temperature storage is the most broadly used technique to extend the postharvest life of horticultural products. However, most tropical and subtropical fruits and vegetables are subject to chilling injury if stored at low temperatures. Metabolic dysfunctions induced at low temperatures impair ripening, lack of flavor

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and aroma, pitting, skin discoloration, tissue decomposition, internal or surface browning, membrane leakiness, development of a woolly, grainy or dry texture, lower resistance to mechanical injury, and increased susceptibility to microbial infections. These symptoms are usually manifested days to weeks after cold storage during distribution and retail.

Finding the optimal temperature/time combination is the key for success in the application of a heat treatment. Optimal exposure to heat induces responses that can protect the commodity from the cold. If the heat tolerance of the commodity is surpassed, the treatment could be more harmful than the chilling injury itself. Luengwilai *et al.* [3] applied hot water from 30 to 50°C for 3 to 9 min to mature green tomatoes of different sizes, prior to 2.5°C storage. Temperatures higher than 40°C damaged the fruits, while 40°C for 7 min was the most effective combination. The treated fruit showed good visual quality and advanced ripening scores after storage. Using the same treatment protocol, Luengwilai *et al.* [4**], found that arabinose, fructose-6-phosphate, valine and shikimic acid were the main metabolites associated with the chilling tolerance expressed by the heat-treated tomatoes. In the case of kiwifruit, Ma *et al.* [5] applied hot water for 10 min at temperatures ranging from 35 to 55°C before 0°C storage for 90 days. Treatment at 55°C produced severe fruit injury, while 35 and 45°C treatments improved fruit quality, decreasing malondialdehyde content, lipoxigenase activity and ethylene production.

Studies have shown that defense mechanisms against chilling injury can be successfully enhanced by the plant regulator methyl jasmonate (MeJa). MeJa plays important roles in responses to environmental stresses. Jin *et al.* [6*] combined treatment of peaches with hot air at 38°C for 12 h with 1 mol/L MeJa prior to storage at 0°C for 3-5 weeks. The treatments were shown to induce phenylalanine ammonia lyase, superoxide dismutase and polygalacturonase. The hot air treatment caused severe mealiness in peaches without MeJa, but chilling injury was counteracted by MeJa treatments.

Cold quarantine treatments, which require the exposure of fruit to near-freezing temperatures (1-2°C) for a period of 14-18 days is a common practice used for insect disinfestation of citrus fruit as required by regulatory agencies of many importing countries. However, citrus are very susceptible to chilling injury, so recent research has focused on investigating the physiological mechanisms involved in heat-induced cold tolerance. Considering diverse combinations of temperature-time, Bassal *et al.* [7*] found that hot water immersion of 'Navel' and 'Valencia' oranges at 41°C for 20 min reduced chilling injury by up to 16%, decreased weight loss and increased or maintained juice percentage, SSC/TA ratio and ascorbic acid content, in correlation with higher levels of phenolics, catalase and peroxidase. Lafuente *et al.* [8] studied the effect of hot air at 37°C for 1-2 days on tolerance of 'Fortune' mandarins to a single (16 days) or double (32 days) quarantine treatment at 1.5°C followed by 4 days at 20°C. No off-flavors or changes were found in the most abundant flavonoids, vitamin C or antioxidant capacity, weight loss, firmness, color or acidity. Holland *et al.* [9] stated that chilling damage was overridden for up to 52 days in mandarins pretreated for 3 days at 37°C, specifying that the beneficial effect was likely related to its effect in maintaining normal water soluble pectin levels and increasing putative sites for calcium bridge formation within the cell wall.

Reduction of decay

Decay is one of the main factors limiting the storage and shelf life of fresh horticultural products. Current management of postharvest diseases relies mainly on the use of synthetic compounds. However, development of fungicide resistance, tighter regulations and public concern over their potential impact on the environment

and human health, have led to the search for new strategies for disease management. Heat applications have been proven to be highly effective against decay. One recent example is the study by Karabulut *et al.* [10], who tested the feasibility of hot water immersion, with temperatures from 24 to 70°C for control of *Monilia fruticola* in stone fruit. Treatments at 60°C for 60 s reduced the incidence of brown rot from 80% to less than 2% in plums, while in nectarines decay incidence decreased from 100% to less than 5% on fruit stored at 20°C and from 73 to 28% on cold-stored fruit. However, fruit quality was not investigated in this study.

Heat treatments control fruit decay via three mechanisms: 1) direct germicidal effect on pathogens, 2) inducing defense mechanisms in plant host and 3) melting and spreading the distribution of cuticular waxes on the fruit surface (occluding and sealing open stomata, wounds and microcracks), thereby limiting the sites of pathogen penetration. The three strategies have been recently studied in diverse commodities. Liu *et al.* [11**], treated peaches with hot water at 40°C for 10 min, and indicated that decay control was due to a direct effect of the heat on *Monilia fruticola*, associated with an increase in intracellular reactive oxygen species, mitochondrial dysfunction and a decrease in ATP; and on the host, by enhancing the defense-related enzyme phenylalanine ammonia lyase. A later study by Li *et al.* [12], working with papaya, showed that hot water at 54°C for 4 min controlled *Colletotrichum gloeosporioides* in the fruit peel by inducing the local expression of defense-related proteins. In addition, heat melted the fruit wax, creating a mechanical barrier against pathogen penetration. Similar results were obtained by Yuan *et al.* [13**] who studied the effects of hot water dipping at 53°C for 3 min in muskmelon. The treatments reduced decay caused by *Trichothecium roseum*, *Alternaria alternata*, *Fusarium* spp and *Rhizopus stolonifer*. The treatment cleaned the surface of the fruit, melted the epicuticular waxes, covered and sealed stomata, and also enhanced the activities of the defense-related enzymes phenylalanine ammonia lyase, cinnamate-4-hydroxylase, 4-coumarate: CoA ligase, polyphenoloxidase and peroxidase. In addition it increased the antifungal compounds, cinnamic, coumaric, caffeic and ferulic acids. The heat treatment also resulted in higher levels of phenolic compounds, flavonoids, lignin and hydroxyproline-rich glycoproteins, and maintained fruit firmness by suppressing the activities of cell-wall degrading enzymes. Jing *et al.* [14] obtained very encouraging results in strawberry with hot water rinsing and brushing at 55-60°C for 20 s; fruit was not damaged but heat clearly redistributed the epicuticular wax layer preventing pathogen penetration. After cold storage, treated fruits showed lower decay incidence (0-22.2%) as compared with 58.6% in control fruits.

One drawback of these treatments is that some of the effects contributing to decay control may not persist long-term. In addition, there is little effect when inoculation occurs after heating. To overcome these flaws, heat treatments have been evaluated in combination with additional substances and/or beneficial organisms. One of the recent examples of adding non-toxic substances to the heat treatment comes from Gutierrez-Martinez *et al.* [15**]. Working with mango, complete control of *Pestalotia mangiferae* and *Curvularia lunata* was achieved by treatment with 300 ml/L ethanol and hot water rinsing at 50°C for 60 s, while fruit ripened normally. Heat-shock has been reported to enhance the efficacy of some biocontrol agents, although the physiological and biochemical mechanisms are not understood [16]. Hot air at 38°C for 36 h was combined with the antagonist *Pichia guilliermondii* to control anthracnose in loquat. After 10 days of storage, the decay index was 20, 6.67, 7.78 and 3.33 in control fruit, and fruit treated with heat, biological control, and the combination, respectively. Zhong *et al.* [17**] combined hot recirculating water at 42°C for 40 min with two antagonistic yeasts, *Candida guilliermondii* and *Pichia mem-*

branaefaciens, to control *Botrytis cinerea* on tomato. Treatment with hot water *C. guilliermondii* or *P. membranaefaciens*, as stand-alone treatments, reduced disease incidence from 81.7% in the control to 61.7%, 40.0% or 45.0%, respectively. The synergistic effect of the combined treatment decreased the disease incidence to 21.7% for *C. guilliermondii* and 26.7% for *P. membranaefaciens*. Hong *et al.* [18] applied hot water at 42°C for 2 min to mandarins together with *Bacillus amyloliquefaciens* HF-01 and sodium bicarbonate, reducing decay associated with *Penicillium digitatum*, *P. italicum* and *Geotrichum citri-aurantii* by more than 80% compared to the control, showing a better performance than treatments with the fungicide Imazalil.

Effect on physico-chemical and nutritional quality

Fresh fruits and vegetables are living tissues that are subject to biological activity before and after harvest. Some activities are desirable, and result in adequate firmness, color and biochemical traits; most of them are not and lead to breakdown and death of plant cells. These processes cannot be stopped, but they can be slowed within certain limits. Either for short-term storage, as a mere link between harvest and market, or long-term storage, to extend marketing beyond the end of the season, techniques that slow ripening and delay senescence are indispensable tools to maintain fruit quality. Heat treatments have been proven to modify quality traits; an adequate combination of temperature-time could benefit ripening and postharvest quality.

Chen *et al.* [19] applied hot air at 40°C for 2 days prior to 40 days storage at 10°C, and found higher concentrations of fructose and glucose and lower citric acid, which is generally associated with better flavor quality. Banana quality has also shown a positive response to heat treatment. Ummarat *et al.* [20*] applied hot water at 50°C for 10 min. Hot water treated 'Gros Michel' fruit had a delay in degreening and maintained higher pulp firmness compared to untreated fruit. The treatment increased free phenolics and flavonoids during storage. More recently, Amin *et al.* [21] treated two different varieties of banana, 'Bari Kola' and 'Sabri Cola', with six different combinations of hot water temperatures and times. The bananas treated with combinations of 53°C for 9 min or 55°C for 7 min obtained higher lightness (L^*), TSS, total sugars, acidity and β -carotene than untreated fruit; however, the vitamin C content of the treated bananas was reduced. At temperatures higher than 57°C for 7-9 min, fruit peel was damaged.

With papaya, Chavez-Sanchez *et al.* [22] found that neither quality nor softening were significantly affected by treatment at 55°C for 3 min. However, applications for longer than 3 min led to irregular skin color while maintaining pulp quality. The authors suggested that these longer treatments could be appropriate when the papaya fruit is destined for processing outlets, since there was a significant delay in decay development.

Ornelas-Paz *et al.* [23] were interested in high-humidity hot-air treatments. Using mango as the model fruit, the authors applied dry (50%) or humid (95%) hot air at 43°C for 220 min, followed by cooling and ripening at 20°C for 9 days. During the first days of storage, moist air-treated fruit showed slower color development of skin and mesocarp, softening, weight loss and β -carotene accumulation than dry heat-treated fruit; however, at the end of the storage, there were no differences between fruit treated with different humidity levels. Li *et al.* [24] associated images obtained with scanning electron microscopy with the postharvest quality of different apple cultivars after applying forced-air heat at 45 to 60°C for 3 h. 'Red Fuji' apples subjected to heat at 45°C maintained the highest total phenolics content and antioxidant capacity, while 'Golden Delicious' apples were more sensitive to heat treatment

based on their loss of TA. These differences in quality were related to changes in the microstructure of heated fruit.

Impacts on sensory quality

Worldwide efforts of scientists and producers are devoted to meeting consumer demand for produce with excellent quality traits. Initial purchases are based on appearance and freshness, but flavor enjoyment is essential for repeat purchases. In the past, most researchers assumed that physico-chemical parameters such as TSS, TA, firmness and volatile compounds were good indicators of fruit ripeness and taste. However, scientists are becoming more aware of the importance of sensory analysis with trained panels or consumers, particularly to measure parameters that are difficult to measure with routine chemical analyses. The influence of heat treatments on the sensory quality of fruit has hardly been studied; but recent postharvest research is giving priority attention to these studies.

Kamdee *et al.* [25**] applied hot air at 42°C for 6-24 h to 'Sucrier' bananas with the purpose of inhibiting peel spotting. The treatment for 24 h was highly effective without impairing ripening, but it reduced taste and odor to unacceptable levels, as determined by a sensory panel. An 18 h treatment not only inhibited peel spotting reasonably well, but also maintained acceptable fruit flavor.

Heat treatment effects on sensory traits can also be temporary, as demonstrated by Jemric *et al.* [26], who presented an exhaustive analysis of 'Venus' nectarines treated with hot water immersion at 48°C for 6-12 min followed by storage at 0°C for 2 and 4 weeks. After 2 weeks, nectarines heated for 12 min achieved better sensory scores for firmness, texture, sugar/acidity ratio, aroma, taste and general appearance. However, after 4 weeks, while there were no significant differences between control and heat treated fruit in weight loss, TSS and TA, control fruits received higher sensory scores except for aroma, which remained higher for fruits heated for 12 min.

In some works heat treatments applied in conjunction with other treatments have been shown to prevent some of the negative effects on sensory traits. Moradinezhad *et al.* [27**] combined hot water immersion at 50°C for 3 min with intermittent warming, salicylic acid, and calcium chloride to improve the postharvest life of 'Shishe-Kab' pomegranate fruit for 22 weeks at chilling temperatures. Hot water improved fruit sensory profiles compared with untreated controls, although the taste and juiciness were still unacceptable. Interestingly sensory evaluation was more satisfactory when heated fruit were also treated with salicylic acid or salicylic acid and calcium chloride.

'Gala' apples are very popular worldwide because of their good sensory qualities. Hot air treatment has been used to delay softening of this commodity, but it may result in loss of peel green color, TA and weight. To avoid these issues, Shao *et al.* [28**] studied the combination of storage at 38°C for 4 days with a chitosan coating. The combination suppressed ethylene production and respiration rate and also inhibited fungal development. Fruits heat-treated before coating showed the highest consumer acceptance after storage. However, severe heat damage was observed on the fruit treated after coating formation.

To eliminate the risk of entry of insect pests associated with fruit, importing countries' may require insect disinfestation treatments. Although chemical fumigation has been used for years, legislative pressure is encouraging the use of heat treatments. The combinations of temperature-time for disinfestation protocols is determined by the lethality to all stages of the pest life cycle that might

be found on the produce, and insect pest disinfestations are usually more severe than those applied to control decay or chilling injury. To understand how these treatments affect fruit flavor has been the goal of recent studies. Obenland *et al.* [29**] used hot air at 44°C for 100 min, the APHIS T103-b-1 treatment protocol to disinfest Navel oranges from *Anastrepha* spp. (fruit flies), and studied the changes in sensory quality and volatile composition. Hot air treatment caused a significant loss in flavor quality that was linked to a decrease in fresh flavor, sweetness and/or flavor richness of the fruit, in spite of an increase in TSS/TA. The authors hypothesized that off-flavors were acting in the treated fruit by reducing sweetness. Waxing the fruit before treatment greatly increased the negative effects of the heat treatment on flavor, although waxing after treatment did not. It was hypothesized that wax may act to restrict the exit of volatile compounds from the fruit that form quickly in response to heat treatment and cause off-flavor. The concentration of four esters was considerably enhanced by the heat treatment, and two of them, ethyl hexanoate and ethyl butanoate, may contribute to the loss in flavor quality. Knowing that aroma volatiles can have a decisive influence on consumer response, Singh *et al.* [30] studied the consequences of vapor heat treatments on the aroma volatile profile of mango, by applying 46.5-48°C for 45-20 min, respectively, followed by storage at 26°C for 8 days. Hot vapor accelerated fruit ripening, as evident by skin color and flesh firmness, and fruit also lacked characteristic aroma of 'Chausa' mango, as indicated by a sensory panel. The treatment also altered volatile composition. At the ripe stage, the heat-treated fruit showed lower concentrations of total monoterpenes, especially α -terpinolene, and total esters, which are identified with the eating-ripe stage in mango.

Commercial heat treatment applications

In some cases the industry has found obstacles to incorporate heat treatments on commercial settings. A primary limitation is the sensitivity of fruits and vegetables to the temperatures required for effective treatments. Product heat tolerance varies depending on species, cultivars, maturity, size, environmental and/or preharvest factors, and method of application. In addition, each treatment should be specific to purposes, markets, legislations and postharvest conditions. Even with the appropriate temperature, time and method, and considering the complete postharvest chain, uniformity in the results is difficult to achieve since fresh fruit and vegetables are morphologically and physiologically heterogeneous. The risk of injury to the fruit, especially in high volume commercial applications where absolute control of treatment conditions is needed, is challenging. Recent research, working in close collaboration with the industry, is trying to resolve some of these issues.

Mango treatments with hot water rinsing and brushing were developed a few years ago. This treatment includes rolling the mangos over brushes to be exposed to pressurized recycled hot water (48 or 63°C for 10-25 s, depending on the cultivar). However, the high susceptibility shown by some new mango varieties to lenticel discoloration and to *Alternaria* side rots and *Phomopsis* stem-end rot led Feygenberg *et al.* [31*] to re-evaluate all postharvest treatments, from the orchard to the packinghouse. Apart from adding water and/or sodium hydroxide (0.1-2.0% for 30 s) washes in the orchard, the hot-water spray was set at 55°C and applied over stainless-steel rollers, without brushing. Results with 'Keitt' and 'Shelly' mangos showed that using rollers instead of brushes they were able to reduce the incidence of red discoloration by 50-70% and black discoloration by 50-60%. However, stainless-steel rollers did not reduce the incidence of stem-end decay adequately, and fungicide treatments would be required to resolve this issue.

The USA is the world's leading importer of mangos and requires hot air or water quarantine treatments prior to entry of fruit from

fruit fly infested areas. Hot water treated mangos show an accelerated rate of softening as compared to untreated fruit. 1-Methylcyclopropene (1-MCP), an inhibitor of ethylene action, has been reported to delay fruit softening, reduce the rate of respiration and weight loss, and to increase the ascorbic acid content during mango storage. Ngamchuachit *et al.* [32**] aimed to identify the optimal 1-MCP treatment and the effect of hot water treatment on its efficacy, since combining both treatments could be very favorable for long distance distributions. Hot water treated mangos softened and the skin yellowed more quickly compared to the control. 1-MCP treatment alone resulted in the slowest ripening while the combined treatment had intermediate rates of softening. In addition, application of 1-MCP before the hot water treatment resulted in a significant reduction in softening compared to fruit treated with hot water followed by 1-MCP.

Polyethylene bag packaging and film wrapping reduce water loss and fruit softening. The potential of joining heat treatments with polyethylene packaging in sweet bell pepper was addressed by Fallik *et al.* [33], with the objective of increasing this product's feasibility to be exported at lower temperatures while avoiding chilling injury and maintaining quality. Combinations of hot water rinsing and brushing (55°C for 15 s), hot water dip (52°C for 2 min), curing (incubation at 44°C for 8.5 h) and individual shrink packaging (Cryovac® D-940) were evaluated. Hot water rinsing and brushing, in combination with individual shrink packaging, reduced chilling injury nearly completely, while maintaining good overall quality after 21 d at 1.5°C. The addition of individual pepper shrink packaging was assumed to reduce water loss, reducing one of the symptoms of chilling injury, but the effect of packaging alone was not tested. This study was complemented by Ilić *et al.* [34*], who focused on understanding the development of cold tolerance by analyzing the secondary metabolite pathway. Hot water at 55°C for 12 s and low temperatures (2°C) led to higher phenolic metabolism and antioxidant capacity compared to the control, while no significant differences were observed in carotenoid content before and after heat treatment and cold storage.

Conclusion

Heat treatments could be applied as part of an integrated, pesticide-free alternative for the control of insects, decay and chilling injury by the fruit and vegetable industry in general and undoubtedly by the organic industry. Further research is necessary to understand the physiological processes occurring in internal and external tissues of fruits and vegetables during and after heat treatment. Only by understanding, controlling and predicting the response of individual commodities to these treatments researchers and the industry can effectively utilize heat treatments to consistently supply high quality fruits and vegetables to the consumer. In this respect, one of the main goals of future research should be to minimize the adverse effects of heat treatments on the flavor quality of fresh fruits and vegetables, which has been overlooked for many years. Combination of heat treatments with other postharvest technologies (such as ethylene suppressors, plant growth regulators, edible coatings, biological control agents and adequate packaging) could not only maintain, but also improve the sensory quality of the commodity. Special attention should be given to the order of application of combined treatments, as commodity response can be very different. Further studies of incorporation of adequate heat treatments on commercial packing line settings would be useful. This may help to provide the industry with the treatment parameters that could maximize the benefits of heat treatments from a biochemical, nutritional and sensory perspective. The identification of biological markers to facilitate the selection of adequate heat protocols that are not injurious would also be highly beneficial.

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Papers of interest have been highlighted as:

*Marginal importance

** Essential reading

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- * A technology for prolonged storage of sweet pepper fruit at low temperature based on physical treatments and packaging materials was developed. Fruit treated with hot water and stored sealed individually in shrink packaging showed reduced weight loss, softening, decay and chilling injury, while nutritional quality was preserved.