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The Application of Stress Treatments to Prevent the Development of Chilling Injury

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ABSTRACT

The control of temperature is the most important tool to avoid postharvest losses during the commercialization of fruits and vegetables. However, one problem usually found during the cold storage of commodities of tropical and subtropical origin is the development of physiological disorders collectively known as chilling injury. Although chilling injury can be prevented by maintaining the commodity at temperatures above the critical threshold, these temperatures cause a significant reduction in the shelf life. Different mechanisms have been proposed to explain both, the physical and biochemical bases of this phenomenon. Over the last years, the theory that oxidative stress may be involved in chilling injury has gained importance. According to this theory, an oxidative unbalance causing an excessive production of reactive oxygen species (ROS) would be the responsible for initiating the damage. Due to consumer concerns about the presence of chemicals in food, the use of physical techniques to avoid the development of chilling injury has been considered in many studies. According to this approach, the exposure of fruit to a stress may be used as a strategy to protect the commodity from the subsequent stress represented by the cold storage. From a biochemical point of view, evidence has been found that links this protective effect with the synthesis of a group of proteins known as heat shock proteins (HSP). The present review discusses the application of different stress treatments and the link between treatment application and HSP production. It is also discussed the role played by these protein in the protection and their potential use as biochemical markers to optimize the application of stress treatments.

Keywords: anaerobic treatments, cold treatments, oxidative stress, heat shock, heat treatments, ROS

Abbreviations: ACC, 1-aminocyclopropane-1-carboxilic acid; **ADH**, alcohol dehydrogenase; **APX**, ascorbate preoxidase; **ATP**, adenosine-5'-triphosphate; **CAT**, catalase; **CI**, chilling injury; **DDT**, dichloro-diphenyl-trichloroethane; **DNA**, deoxyribonucleic acid; **H_2O_2**, hydrogen peroxide; **HSP**, heat shock protein; **HSP60**, heat shock protein 60 kDa; **HSP70**, heat shock protein 70 kDa; **HSP90**, heat shock protein 90 kDa; **HSP100**, heat shock protein 100 kDa; **mRNA**, messenger ribonucleic acid; **PCB**, polychlorinated biphenyl; **PDC**, pyruvate decarboxylase; **ROS**, reactive oxygen species; **sHSP**, small heat shock protein; **SOD**, superoxide dismutase

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INTRODUCTION

It is well known that the best way to prevent the development of CI of sensitive fruits is by avoiding the exposure at temperatures below the critical threshold (Wills *et al.* 1998). However, this condition can accelerate the ripening process, reducing noticeably the shelf life of the produce. Therefore, the development of techniques that allow the storage at temperatures below that threshold, and prevent at the same time the development of physiological damage, constitute nowadays one of the main challenges in postharvest technology (Lurie and Sabehat 1997).

Different approaches have been traditionally used to alleviate the development of CI, which can be classified into: temperature conditioning, intermittent warming, use of controlled atmospheres, application of growth regulators,

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application of waxing and other coverage, and modified atmosphere packaging (Wang 1993). A more recent strategy is the development of transgenic varieties tolerant to CI, for which the genes involved in the tolerance should be clearly identified (Petersen and Reid 1990; Malik *et al.* 1999; Nautiyal *et al.* 2005).

Owing to consumers concerns about the effect of chemicals on health, many efforts have been put on the development of non chemical techniques to improve the preservation of foods. Among the strategies explored, it was found that the exposure of plant tissues to a certain level of abiotic stress can generate protection not only against higher levels of the same stress, but also against other types of stress. By applying this concept to postharvest technology, it can be hypothesized that the controlled exposure of chilling-sensitive fruits to a particular stress (stress treatment) can protect them from another stress, which is represented by the exposure to low temperatures (cold storage), preventing then the development of CI.

CHILLING INJURY AND OXIDATIVE STRESS

Two main theories have been proposed to explain the development of CI in plants: one of them postulates that low temperatures induce changes in the physical properties of membranes, leading to malfunction of these structures (Steponkus 1984; Wills *et al.* 1998). The other theory proposes that CI is mainly originated in the dissociation of enzymes and other proteins, resulting in the modification of enzymatic kinetic and in structural alterations in certain proteins such as tubulins (Graham and Patterson 1982). Over the last years, an increasing number of evidences have linked the development of CI to the oxidative damage. Oxidative stress is defined as the cellular state where the balance between pro-oxidants and antioxidants has been altered. This unbalance is provoked either by an excessive production of the so-called reactive oxygen species (ROS), or by a deficiency in the antioxidant mechanisms, a situation that eventually leads to cellular damage (Scandalios 1993).

The term ROS includes partially reduced forms of atmospheric oxygen originated in the excitation of this molecule to render singlet oxygen, and oxygen molecules to which 1, 2, or 3 electrons has been transferred to produce respectively superoxide anion, hydrogen peroxide (H_2O_2), or hydroxyl radical. In contrast to atmospheric oxygen, ROS are able to oxidize almost unrestrictedly different cellular compounds, eventually leading to the cell destruction (Wojtaszek 1997; Grassman *et al.* 2002). Among the alterations caused by oxidative stress, it can be mentioned the formation of DNA-DNA or DNA-protein adducts, chemical changes in proteins, oxidation of aromatic rings, changes in peptide bonds, production of carbonilic derivatives, and alterations in lipids (Scandalios 2002).

Different potential sources of ROS can be found in plants. In some cases, these chemical species are formed by reactions originated in the normal metabolism, such as photosynthesis and respiration, while in other; they are initiated in processes exacerbated by biotic or abiotic stresses (Mittler 2002). The main scavenging mechanisms found in plants to eliminate ROS include the synthesis of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), and the overproduction of antioxidants such as α-tocopherol, β-carotene, ascorbate and glutation (Zhang *et al.* 1995; De Gara *et al.* 2003).

Evidences of the link between oxidative stress and chilling injury

Among the findings that link oxidative stress to CI, Wise and Naylor (1987) demonstrated that the exposure of cucumbers to low temperatures induced the peroxidation of lipid and the depletion of antioxidants. In another research, it was reported that mitochondria isolated from peppers susceptible to chilling produced larger amounts of superoxide anions in comparison to chilling tolerant species (Purvis *et al.* 1995). In addition, Prasad *et al.* (1994) found high levels of H_2O_2 accumulated in maize seedlings exposed to 4°C. From these and other studies, it was inferred that the capacity of plants to withstand chilling conditions is closely related to their ability to reduce or inactivate ROS, by increasing the activity of the defence system (Ju *et al.* 1994). More specifically in the fruit field, Sala (1998) reported that the activities of CAT, APX and glutatione reductase in refrigerated mandarins were higher in chilling-tolerant than in -susceptible varieties, this finding suggesting the occurrence of a more efficient antioxidant system in the former. Later, Sala and Lafuente (2000) found that the application of heat treatments conferred chilling tolerance to mandarins, and this tolerance was correlated with an increased activity of CAT. In addition, the application of CAT inhibitors induced symptoms similar to CI. Results obtained by applying genetic techniques indicated that transgenic tomatoes expressing an antisense gene for CAT, where levels of this enzyme were low and endogen concentrations of H_2O_2 high, were highly susceptible to chilling exposure (Kerdnaimongkol and Woodson 1999). Although the possibility of generating transgenic plants with high CAT levels emerges as a promising solution for CI, it has been shown that these plants were, at the same time, highly susceptible to pathogen attacks (Polidoros *et al.* 2001).

STRESS TREATMENTS

Although plants are able to adapt to a wide range of environmental stimuli, their optimal development can be attained only within a narrow range of external conditions. The exposure to any condition outside this limit is referred to as *stress*. Therefore, the term *stress* can be defined as the factor, or sequence of external factors able to alter the normal physiological processes of a particular organism (Kays 1991). In the case of fruits, practically any technique of preservation would impose a certain level of stress to tissues and provoke different physiological disorders (Shewfelt and Prussia 1993). Among the different types of stress underwent by fruits during the postharvest period, it can be mentioned: extreme temperatures, extreme humidity, microbial attacks, variation in the atmosphere composition, and mechanical damage (Kays 1991). The response of tissues to stress is variable, and depends on different factors such as genetic, growing conditions, endogenous mechanisms of defence, harvest maturity, and severity/duration of the exposure (Shewfelt 1993).

As mentioned above, the exposure of fruits to a stress of limited intensity can potentially render a reversible response able to maintain the overall quality of produce during the postharvest life. However, if not adequately monitored and controlled, the response can become irreversible, a situation that may have devastating effects on fruit quality. Therefore, the successful application of stress treatments in postharvest will strongly depend on the possibility of accurately establishing this region of reversibility With this aim, it would be important to find a biochemical marker able to reflect accurately the stress underwent by the tissues. Considering that quality changes are the external expression of cellular and molecular processes, markers associated to the defence mechanism can be suitable candidates to help distinguish between a reversible and a permanent damage caused by a stress (Shewfelt 1993).

HEAT SHOCK TREATMENTS

Among the different stress treatments that can be potentially applied to fruits, heat treatment represents the one to which more effort has been devoted to. Because in the application of this kind of stress fruits are transiently exposed to a high temperature condition, these treatments are generally referred to as *heat shock treatments*. Several authors have reported on the relationship between the response to heat shock treatments and the protection against the potential damage caused by high temperatures (Paull and Chen 1990;

Ali and Banu 1991). According to Schoffl *et al.* (1998), heat stress induces a cellular response able to protect not only the cell itself, but also the whole organism from a severe damage. In the vast majority of plants, animals and prokaryotes studied so far, it was found that a transient increase of 5 to 10°C above the normal temperature for periods ranging from 15 min to some hours, induced the synthesis of a group of proteins normally absent, or present at a low levels in non-stressed cells, which were termed *heat shock proteins* (HSPs) (Brodl 1989). As a part of the same physiological response, there is also a noticeable decrease in the concentration of other proteins which are synthesised at normal temperatures. This is believed to be a strategy to redirect the whole metabolic energy towards the synthesis of HSPs (Key *et al.* 1981). Interestingly, a correlation was established between the synthesis of HSPs and the acquisition of thermotolerance, a condition that allows the cells to withstand an otherwise lethal heat exposure. A strong evidence in this way was the demonstration that certain stress proteins induced by a heat shock were present in thermotolerant varieties, but absent in thermolabile ones (Krishnan *et al.* 1989).

Heat shock proteins

HSPs are generally associated to chaperones. The term chaperones was initially used for proteins participating in the folding of others proteins, but that are not a component of the final protein complex. More recently, chaperones were defined as proteins able to bind and stabilize certain structures otherwise unstable, assisting in the correct function of other proteins (Miernyk 1997). It is important to denote that most proteins showing this function were indeed HSPs (Wang *et al*. 2004).

HSPs are involved in the regulation of a wide variety of cellular processes such as the transport of macromolecules through membranes, the correct assembly of oligomeric proteins, the dissociation of misfolded proteins, and the degradation of denatured proteins (Trofimova *et al.* 1999). According to Ali and Banu (1991), HSPs synthesis can also be triggered by stresses other than heat, for instance presence of heavy metals, non ionic detergents, low pH, biotic stress, tissue damage, and genetic lesion. These proteins are encoded by multigene superfamilies, yet not all members are regulated by high temperatures (Vierling 1991). They also participate in homeostasis-related processes of non stressed cells, especially those associated to protein functionality. Their synthesis is generally induced by the application of treatments able to provoke protein damage, and the concentrations attained are usually proportional to the intensity of the applied stress (Guidi 2008). These proteins have the ability to neutralize proteotoxic effects by preventing protein denaturation, maintaining the folded state, and repairing abnormalities (Soto *et al*. 1999; Bierkens 2000).

Five families of HSP/chaperones are presently identified: HSP70s, chaperonins (HSP60s), HSP90s, HSP100s, and small HSPs (sHSPs). Although the participation of HSPs in cellular processes has been exhaustively described in different organisms, their role in plants is poorly understood. It is believed that, in addition to their participation in the acquired tolerance to stress, these proteins act synergistically with other mechanisms and/or in association with other cellular components to diminish cellular damage (Wang *et al.* 2004). Since the most important HSPs families in plants are HSP70s and sHSPs, these groups will be described in more detail.

HSP70s family

Proteins belonging to HSP70s constitute the cellular machinery that assist in a wide range of protein folding processes in almost all cellular compartments. They have the essential function of preventing protein aggregation and helping in the refolding of non-native proteins, under both normal and stress conditions. Besides this, members of this family are also involved in processes like hydrolysis of unstable proteins, which are tagged to be processed in lysosomes or proteosomes (McClellan and Frydman 2001). Another significant function of HSP70s is their participation in the importation and translocation of proteins (Chirico *et al.* 1988). The overexpression of HSP70s genes has been correlated with the acquisition of thermotolerance and with the augmented tolerance to saline and hydric stress. It seems that they play an important regulatory function by stimulating other stress-related genes. Interestingly, more than half of the polypeptides being synthesized in ribosomes are associated to HSP70s, probably to prevent misfolding. The main function of these proteins is to assist in the protein folding process by suppressing unproductive secondary reactions, thus allowing the correct expression of the information contained in the primary sequence of proteins (Ali and Banu 1991; Miernyk 1997).

Small heat shock proteins family

Small heat shock proteins have molecular weights ranging between 15 and 40 kDa, and constitute the most diverse family in plants in terms of sequence identity, cellular location and function. These proteins can be synthesized in prokaryotes and eukaryotes in response to heat and other types of stresses. In addition, they are also associated to certain developmental stages of plant (Vierling 1991). Plant sHSPs are codified by 6 families of nuclear multigenes, each one being destined to a particular cellular compartment (Wang *et al*. 2004). Their high level of diversification probably reflects the molecular adaptation to stress conditions that are unique to plants, given that they have to withstand a wide range of environmental strains such as heat, cold, high salinity, oxidative stress (Sabehat *et al.* 1998), drought and mechanical injury (Heikkila *et al.* 1984).

One important issue to be unveiled is the precise mechanism of action of HSPs. It is known that denatured proteins have an important tendency to aggregate because some of the hydrophobic residues, normally buried, are exposed on their surfaces. Therefore, the capacity of sHSPs to bind hydrophobic regions can probably help refold denatured enzymes allowing them to recover their lost activity (Lee *et al.* 1995). Apparently, sHSPs would act synergistically with other HSPs. They are believed to prevent the aggregation of proteins in an ATP-independent manner, whereas other HSPs (such as HSP70s) would be in charge of the refolding process in an ATP-dependent manner (Lee and Vierling 2000). The mechanism of interaction between different HSPs, or between HSPs and other defence mechanisms constitute a complex field of research that is far from being fully understood.

Use of HSP with monitoring purposes

Although the study of HSPs is generally considered as a topic of basic research, several authors have focussed their studies on the potential use of HSPs for practical purposes. Considering that biochemical changes usually constitute the first detectable cellular response against environmental strains, the analysis of HSPs has been proposed as a mean to monitor environmental pollution. Two important advantages are, their rapid accumulation after the exposure to a stress condition, and their persistence at high concentrations even after the stress condition has ceased (Bierkens 2000). Dunlap and Matsumura (1997) identified a highly conserved amino acid sequence among HSP70s from different species. By using antibodies raised against this sequence, these authors were able to detect an increase in HSPs in both, animal and plant species submitted to physical stress or exposed to chemical contaminants such as PCB, DDT or lindane.

From the postharvest point of view, the use of HSPs as biochemical markers constitutes a challenge. Some of the potential applications are: the assessment of the intensity of heat treatment, and the monitoring of the protection conferred by these treatments during the cold storage. In this way, the accumulation of sHSPs has been correlated with the protection exerted by heat treatments against CI (Sabehat *et al.* 1996; 1998; Sunn *et al.* 2002). Polenta *et al.* (2007) isolated and characterized the main sHSPs induced in tomato fruit by the application of heat treatments. In this study, sHSPs accumulation was quantitatively correlated with CI protection. Interestingly, the same antibody was also able to detect the accumulation of sHSPs in grapefruit, for which an immunological method able to analyse this parameter can probably provide with a tool to monitor heat treatment application in different commodities.

Technological aspects of heat treatments

Heat treatments were initially applied in postharvest to eliminate larvae of insects, or as an alternative for fruit disinfestation (Klein and Lurie 1992). Over the last years, several authors have reported on the use of heat treatments to preserve quality and extent the shelf life of fruits and vegetables (Sabehat *et al.* 1996; Lurie 1998). The technological application of heat treatments is usually in the form of hot water, vapour, or forced air (Erkan *et al.* 2005). In the case of hot water, the application can be accomplish by immersion, rinsing and brushing. When compared to air treatment, water treatments are easier to apply, shorter, and more efficient in the heat transfer. Their main effects are the inhibition of ripening, the prevention of CI, and the induction of resistance against pathogens (Fallik 2004). One advantage is the feasibility of combining these treatments with the application of fungicides (Schirra and Ben-Yehoshua 1999). However, the higher temperature required for them to be effective may induce, in some cases, heat-related damage (Porat *et al.* 2000). Fallik *et al.* (1999) developed a variant termed *hot water brushing*, in which the surface of fruit is brushed to eliminate fungicides and spores, and fruit are then submitted to a hot water for 10 to 25 seconds, at temperatures between 48 and 63°C. Other forms of application mentioned are: vapour treatments, which was specifically developed for insect control; and air treatments, which have been used for both, insect and pathogen control, or experimentally in studies on fruit response to high temperatures (Lurie 1998). Budde *et al.* (2006) found that the form of application of these treatments has a significant impact on their effectiveness. The authors found that long air heat treatments applied to peaches greatly affected fruit quality by diminishing total acidity and increasing red pigments in flesh and peel. On the other hand, ethylene production was increased in air treatments, but did not change in fruit treated by short-term immersion treatments. Apparently, changes induced by immersion treatments are less dramatic, avoiding some negative effects on quality. Civello *et al.* (1997) reported that heat treatments helped preserve the overall quality of refrigerated strawberries, and increased HSPs concentration. However, these authors warned against negative effects on quality, which were detected in fruit submitted to some time-temperature combinations. Polenta *et al.* (2006) also found that treatment effectiveness in tomatoes was highly dependent on its intensity.

The most common changes caused by thermal treatments on fruits are: increased softening, alteration of the respiratory rate, decreased ethylene production, and alteration of volatiles profiles (Paull and Chen 2000). Heat treatments usually affect differentially some physiological processes that normally occur at the same time during fruit ripening. Generally, processes requiring *de novo* synthesis of proteins are inhibited, while those for which protein synthesis is not a condition are accelerated (Lurie and Klein 1990). Evidence indicates that one of the main effects of high temperatures is the inhibition of ripening-related genes. For instance, Picton and Grierson (1988) reported that the exposure of tomatoes to 35°C induced a marked decrease in the level of polygalacturonase- and ethylene-related transcripts. Lurie *et al.* (1996) detected, in fruit exposed to 38°C, a decline in the levels of mRNA corresponding to ACC oxidase and phytoene synthase.

The application of heat treatments in postharvest technology is not restricted to fruits. Gómez *et al.* (2008) extended the postharvest life of spinach leaves by applying moderate heat treatments. Although HSPs concentration was increased in treated leaves, the authors found evidence that the protective mechanism would also involve the adaptive response to oxidative stress. Similarly to the case of CI, oxidative stress seems to play a central role in most of the protective and damaging mechanisms in plants (Kotak *et al.* 2007).

Temperature is one of the most important parameters to be defined for treatment application, which should be high enough to induce the response without causing damaging effects. Paull and Chen (2000) explain that high temperatures induce two types of response in fruits: one of them evidenced at temperatures below 42°C, which consists in the acquisition of chilling tolerance, reduction in the ripening rate and development of quality alterations. The second type of response is evidenced at temperatures above 45°C, and can provoke lethal damages. The temperature considered to be lethal is highly dependent on the type of commodity, having been established 45°C for tomatoes, 63°C for grapes, and 50°C for apples.

One interesting emerging approach is the combination of heat treatments with other technologies to prevent negative side-effects. In this way, Murray *et al.* (2007) were successful in improving the quality of peaches refrigerated for up to 4 weeks by applying a combination of pre-storage heat treatment followed by controlled atmosphere storage. In this research, the application of both treatments prevented the development of CI, while quality attributes such as softening, juiciness and flesh colour were similar to untreated fruit that were left to ripe normally. The most remarkable finding was that the combined treatment prevented the fruit from undergoing a severe flesh reddening, a defect found when the peach variety the authors worked with was submitted to heat treatment alone.

Although the potential use of heat treatments emerges as a promising alternative to prevent chilling injury, the adoption by the industry is still slow, even though the cost to implement this technology is affordable (Ferguson *et al.* 2000).

ANAEROBIC STRESS TREATMENTS

The application of anaerobic treatments would be especially convenient in developing countries, where refrigeration is still inadequate. Among the potential benefits, it can be mentioned the low cost and the non-chemical nature (Fallik *et al.* 2003). For the effective application of anaerobic treatments, it is essential to have an adequate understanding of the effects of anaerobiosis on plant tissues. It is well known that the oxygen availability of fruits and vegetables is strongly related to the respiratory rate. As the internal concentration of oxygen decreases, so does the respiration until the oxygen concentration attains a value beyond which a fermentative metabolism is induced. This particular value is referred to as *extinction point* or *critical concentration* (Wills *et al.* 1998). Fruits submitted to anaerobic conditions increase the levels of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH), both enzymes related to the fermentative metabolism. This fact reflects, from a biochemical point of view, the change of metabolism from aerobic to anaerobic (Kato-Noguchi and Watada 1997). Nanos *et al.* (1992) found that pears submitted to anaerobiosis had high levels of PDC and ADH. These augmented concentrations helped tolerate the high levels of ethanol and acetaldehyde normally produced during fruit ripening. In another experiment, anaerobic treatments applied to avocado before cold storage, delayed fruit ripening, prevented the development of chilling injury, and extended its postharvest life (Pesis *et al.* 1993). Similarly to heat treatments, the exposure to anaerobic stress has been related to the overexpression of HSPs (Neumann *et al.* 1994; Bierkens 2000), whose presence can partially explain the effectiveness of the treatments. Another parallelism between heat and anaerobic treatments is that the effectiveness of both treatments is highly dependent on the appropriate treatment extent. Thus, in tomato fruit submitted to 24-h anaerobic treatment, the growth of *Botrytis cinerea* was inhibited, but when the exposure was extended to 48 hours, treated fruits showed accelerated decay and development of off-flavors (Fallik *et al.* 2003). Among different types of fruits, Polenta *et al.* (2005) state that peach is especially suitable for the application of this technology, which proved effective to delay ripening and prevent the development of CI. These authors highlight the requirement of an effective control of the treatment intensity, and propose ethanol accumulation as the monitoring parameter, considering that it reflects the intensity of the anaerobic exposition, and also correlates well with fruit tolerance and sensory perception by consumers.

Potential damage caused by anaerobiosis

Although anaerobic stress applied at sublethal levels offers the benefit of preventing the development of physiological disorders in fruits (Fallik *et al.* 2003), potential damages can be developed if this technology is not properly used. The tolerance of fruits to low oxygen concentrations depends on the nature, size, the developmental stage of the commodity, and the ability of tissues to metabolise the ethanol accumulated when the situation returns to normal. Interestingly, if the damage underwent has not been severe, plant tissues have the capacity of recycling the metabolites and, as a result, the damage can be restricted (Kays 1991). In addition, there is a considerable variation among different cultivars regarding the tolerance to anaerobiosis (Chang *et al.* 1982). The extent of the damage is highly dependent on the duration of the exposure. The longer the exposure, the higher the accumulation of the metabolites generated by the stress, and therefore the more severe the damage. It was found that low concentrations of ethanol inhibited the ripening of tomatoes (Salveit and Sharaf 1992). However, if the ethanol concentration is high, additional detrimental effects can be found such as pitting, irregular ripening, and development of off-flavor (Ratanachinakorn *et al.* 1999). It is still not clear whether the damage is provoked by the toxicity of the fermentative metabolites such as lactic acid, acetaldehyde and ethanol, or it is just a consequence of the shortage of energy caused by the change of metabolism (Peppelenbos and Oosterhaven 1998). The presence of acetaldehyde has been related to physiological disorders of apples, such as superficial scald and browning (Smagula and Bramlage 1977).

One aspect that has raised interest lately is the participation of ROS in the damage caused by low oxygen concentrations. It was reported that, after the exposure of fruits to anaerobic environments, the re-exposure to normal atmospheric levels induces a sharp increase of the concentration of ROS. For low levels of accumulation, this situation can be reverted by endogenous antioxidants already present in the tissues, while for long exposures after which high concentrations of ROS are attained, a rapid and important synthesis of SOD and CAT also occurs (Garnczarska and Bednarski 2004).

COLD STRESS TREATMENTS

The basis of the application of cold treatments to prevent the development of CI relies on the ability of plant tissues to acclimatize to extreme temperatures by generating an adaptive biochemical response. According to Neven *et al.* (1992), the exposure of plants to low temperatures elicits different biochemical processes that increase their tolerance to this condition. Glatz *et al.* (1999) postulate the existence of a cellular mechanism which is triggered in response to stress. According to these authors, cell membranes are able to detect extreme environmental changes and activate stress resistance genes. The first event is the drop of the environmental temperature, which leads to changes in the fluidity of membranes. The next event is the overexpression of genes codifying for the enzyme desaturase, which act on the fatty acids of the membranes to recover their fluidity. The increased fluidity, together with the protection exerted on these structures by HSP, would help recuperate their functionality. The acclimation response also involves different processes such as the synthesis of specific proteins, and changes in the structure and function of enzymes involved in central reactions of the basal metabolism. Proteins synthesized during a cold stress are of the type of dehydrins and HSPs (Sung *et al.* 2003). These proteins are synthesized in response to the presence of denatured proteins sensitive to cold, which are formed in the range of temperatures between the freezing point and the threshold temperature for the development of CI (Neven *et al.* 1992). Similarly to heat shock, the function of these newly-synthesised proteins is the stabilization of macromolecules and others cellular structures (Bravo *et al.* 1999). Although some elements of the response are common for all stresses, some changes are specific for each particular type. For instance, some proteins belonging to the HSP70 family are actively synthesized during cold acclimation, but not during hydric or heat stress. On the contrary, other HSPs are induced irrespective of the type of stress (Vierling 1991; Neven *et al.* 1992). These *cold stress proteins* are believed to participate in the maintenance of cellular homeostasis and protein synthesis, which are important elements towards the cold acclimation (Anderson *et al.* 1994). Kadyrzhanova *et al.* (1998) cloned two tomato genes codifying for sHSPs, whose presence was related to the acquisition of cold tolerance. These genes are also induced by high temperatures, but not by other stresses such as anaerobiosis. The physiological and quality changes induced by cold treatments are the consequence of complex regulatory changes at molecular level. Interestingly, most of the tomato mRNAs corresponding to genes involved in the ripening process decrease sharply when fruits are stored at 4°C, but rise after the transfer to 24°C (Watkins *et al.* 1990).

Technological aspects of cold treatments

The application of cold treatments to fruits can overcome some drawbacks associated to heat and anaerobic treatments (Woolf *et al.* 2003). Among them, it can be mentioned the development of external damage, alterations of quality detected after ripening, and logistic problems linked to the application itself. By applying cold treatments, these authors prevented the development of epidermal damage in Haas avocado after cold storage. In another successful research, Inaba and Crandall (1986) were able to delay the colour development in cold-treated tomatoes after re-exposure to room temperature. Hofman *et al.* (2003) proposed the use of cold acclimation as a pre-treatment previous to low temperature quarantine treatments. These treatments are required by many countries to minimize the risk of insect transmission in international trade. By applying this pre-treatment, it was possible to eliminate epidermal damages and improve the overall quality of avocado after a quarantine treatment of 16 days at 1°C.

CONCLUDING REMARKS

The prevention of chilling injury is one of the most important research areas in postharvest technology. Due to consumer concerns regarding the use of chemicals in food, the application of physical techniques emerges as an innovative approach. However, considering that the exposure to a stress condition beyond a certain level induce changes to tissues that may become permanent, there is a need to establish accurately the limit of reversibility of the treatment. With this aim, HSPs offer an interesting prospective for their potential use as biochemical marker to optimize treatments application. The development of studies unveiling the precise role of these proteins, together with the description of other cellular mechanisms of protection against oxidative stress, will help understand the whole process. An important effort is yet necessary to meet the final goal, which is the adoption of these novel technologies.

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