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Postharvest Biology and Technology Book Series

Emerging Postharvest Treatment of Fruits and Vegetables

Kalyan Barman
Swati Sharma
Mohammed Wasim Siddiqui
Editors

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EMERGING POSTHARVEST TREATMENT OF FRUITS AND VEGETABLES

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Postharvest Biology and Technology

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Edited by
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Recently, Dr. Siddiqui was conferred with several awards, including the Best Citizen of India Award (2016); Bharat Jyoti Award (2016); Best Young Researcher Award (2015) by GRABS Educational Trust, Chennai, India; and the Young Scientist Award (2015) by the Venus International Foundation, Chennai, India. He was also a recipient of the Young Achiever Award (2014) for the outstanding research work by the Society for Advancement of Human and Nature (SADHNA), Nauni, Himachal Pradesh, India, where he is an Honorary Board Member and Life Time Author. He has been an active member of the organizing committees of several national and international seminars, conferences, and summits. He is a key member in establishing the World Food Preservation Center (WFPC), LLC, USA. Presently, he is an active associate and supporter of WFPC, LLC, USA. Considering his outstanding contribution in science and technology, his biography has been published in *Asia Pacific Who's Who* and *The Honored Best Citizens of India*.

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LIST OF ABBREVIATIONS

1-MCP	1-methylcyclopropene
6-BAP	6-benzylaminopurine
ACC	1-aminocyclopropane-1-carboxylic
ACO	ACC oxidase
ACS	ACC synthase
AEW	alkaline electrolyzed water
AOX	alternative oxidase
APX	ascorbate peroxidase
ASC	ascorbate
ATP	adenosine triphosphate
BI	browning index
BRs	brassinosteroids
BS	black splendor
CA	controlled atmosphere
CAT	catalase
CCO	cytochrome C oxidase
CHI	chitinase
CI	chilling injury
CRM	confocal Raman microspectrometry
CYS	cysteine
DETANO	diethylenetriamine/nitric oxide
DHAR	dehydroascorbate reductase
EOs	essential oils
ETDA	ethylenediamine tetraacetic acid
ETR	ethylene receptors
EW	electrolyzed water
FAD	fatty acid desaturase
FDA	Food and Drug Administration
FT	Fourier transform
FW	fresh weight
GABA	γ-aminobutyric acid
GLU	β-1,3-glucanase
GPAT	glycerol-3-phosphate acyltransferase
GPX	glutathione peroxidase

GR	glutathione reductase
GRAS	generally recognized as safe
GSH	glutathione
GSSG	oxidized glutathione
GST	glutathione-S-transferase
HAT	hot air treatment
HPP	high-pressure processing
HSPs	heat shock proteins
HWB	hot-water brushing
HWRB	hot water rinsing and brushing
HWT	hot water treatment
JA	jasmonic acid
LOX	lipoxygenase
MA	modified atmosphere
MAP	modified atmosphere packaging
MC	methylcellulose
MDHAR	monodehydroascorbate reductase
MeJA	methyl jasmonate
MMT	montmorillonite
MNV	murine norovirus
NAI	negative air ions
NEW	neutral electrolyzed water
NO	nitric oxide
ODC	ornithine decarboxylase
OP	oxygen permeability
ORP	oxidation–reduction potential
OTR	oxygen transmission rate
PAL	phenylalanine ammonia lyase
PAs	polyamines
PBN	*N*-*tert*-butyl-*a*-phenylnitrone
PDH	proline dehydrogenase
PDJ	*n*-propyl dihydrojasmonate
PG	polygalacturonase
pI	isoelectric point
PO	oxygen permeance
POD	peroxidase
PPO	polyphenol oxidase
PR	pathogenesis related
RF	radiofrequency

RH	relative humidity
ROS	reactive oxygen species
SA	salicylic acid
SAM	*S*-adenosyl methionine
SAR	systemic acquired resistance
SDH	succinic dehydrogenase
SERS	surface-enhanced Raman scattering
SiOx	silicon oxide
SNAP	*S*-nitroso-*N*-acetylpenicillamine
SNP	sodium nitroprusside
SOD	superoxide dismutase
SPIs	soy protein isolates
SS	soluble solids
SSC	soluble solid concentration
TA	titratable acidity
TBZ	thiobendazole
TIL	temperature-induced lipocalins
TS	tensile strength
TSD	type II Sk2 dehydrin
TSS	total soluble solids
UV	ultraviolet
WP	whey protein
WVP	water vapor permeability
WVTR	water vapor transmission rate

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As we know, preserving the quality of fresh produce has long been a challenging task. In the past, several approaches were in use for the postharvest management of fresh produce, but due to continuous advancement in technology, the increased health consciousness of consumers, and environmental concerns, these approaches have been modified and enhanced to address these issues and concerns.

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We look forward to hearing from you soon.

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PREFACE

The ultimate aim of science is to achieve better understanding of the world we live in and to improve the living standards for human beings. Agricultural science, in particular, has evolved enormously with the advancement in human civilization. The postharvest preservation and storage practices for utilization of agricultural produce date back to the time of initialization of human settlements and horticultural produce and crop cultivation.

Postharvest technology of horticultural crops has undergone many changes with the changing times. The discipline has emerged from simple and important technologies like drying and fermentation to the modern-day innovative and highly effective postharvest treatments encompassing irradiation, biological control using microbial treatments, application of biosensors to evaluate the different stages, and applications that address diseases to the issues of residues of harmful agrochemicals, plant genetic resources, and other pesticides.

Postharvest science is evolving continuously. The research impact of safe and simple postharvest grassroot technology has the potential to save agricultural produce and provide food for millions of hungry people worldwide. Endeavors are being made by scientists involved in postharvest research for maintenance of the quality and safety of fresh horticultural produce to enhance the postharvest life and to extend the availability of the produce in both time and space. There is demand for the development and application of adequate technologies for preservation of the perishable food products, particularly fresh fruits and vegetables.

It has been proven that the consumption of fruits and vegetables is very important for the prevention of many life-threatening diseases, like cancer, diabetes, cardiovascular diseases, etc., and to maintain good health. However, the rapid loss in quality of the harvested fruits and vegetables and high percentage of spoilage drastically lower their availability and result in appreciable increase in costs. The postharvest loss in developing countries reaches ever-escalating heights of 25–40% in different fresh produce at different stages of handling. Consequently, the burden of huge nutritional and economical losses falls upon all the growers, traders, as well as consumers.

This present book has been designed with the main consideration to serve as a consortium of information on new postharvest treatments that are

used for postharvest quality maintenance and extension of storage life of fresh and minimally processed produce. It has been framed to act as a reference guide as well as to formulate and provide further new thrust areas for research to find new, easy to apply, and effective techniques for reduction in postharvest losses and enhancing nutritional security.

The present book is divided into 12 chapters, all of which cover exhaustively the most significant postharvest treatments employed for maintenance of the quality, safety, visual acceptability, and availability of the fruit and vegetables and their minimally processed products for a longer duration after harvest.

The postharvest storage of tropical and subtropical fruits at low temperature is limited due to the occurrence of chilling injury. This results in serious reduction of the quality as well as the shelf-life of such produce. This issue is addressed in Chapter 1 in the form of the various treatments to alleviate chilling injury during postharvest storage at low temperatures.

Heat and calcium treatments for enhancing storability of fruits and vegetables are detailed in Chapters 2 and 3. Chapters 4 and 5 elaborate on the significance and mechanisms of action of methyl jasmonate and nitric oxide as postharvest treatments for preservation of the quality of fresh produce. The potential applications of nanotechnology in postharvest field are discussed in Chapter 6.

The basic concepts as well as applications of biologically safe, effective, and promising technologies like biological control are discussed in Chapter 7. Chapter 8 discusses in detail the influences of ozone treatments on the postharvest quality of horticultural produce. Chapter 9 describes comprehensively the advances in edible coatings and films for fresh fruits and vegetables. The present times of busy schedules of the family members and increasing number of working women demand convenience and ready-to-eat horticultural food products. Hence, Chapter 10 has been framed to deal elaborately with the various postharvest treatments to reduce browning in minimally processed products. Chapter 11 focuses on natural antimicrobial agents of plant origin, essential oils and plant extracts, their source, and antimicrobial activity so as to adjudge their probable postharvest application to the fruit and vegetable industry. Chapter 12 gives insight into the role of polyamines in delaying ripening and senescence of fruits and vegetables.

We hope that this book will contribute immensely as essential reference reading for the students, teachers, professors, scientists, and entrepreneurs engaged in fresh horticultural produce handling related to this field. It will also lead to further progress in various postharvest technologies for

maintenance of nutritional, visual, and sensory acceptance of the fresh horticultural produce. The compilation of the significant research studies associated with each chapter of this book will result in giving a focused direction of the present status of research activities and thrust areas for future studies.

The editors will appreciate suggestions and constructive comments for improvement for future works from the readers.

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CHAPTER 1

POSTHARVEST TREATMENTS TO ALLEVIATE CHILLING INJURY IN FRUITS AND VEGETABLES

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ABSTRACT

Fresh fruits and vegetables are stored at low temperature to delay ripening, senescence, thereby extend their shelf life. But, most tropical and subtropical origin horticultural crops develop chilling injury during storage at low temperature. In response to chilling stress, different physiological and biochemical alterations leading to cellular dysfunctions take place in chilling sensitive produce. These alterations and dysfunctions cause development of a variety of internal and external chilling injury symptoms. In this chapter, different postharvest technologies using physical and chemical treatments such as prestorage temperature conditioning, intermittent warming, ultraviolet radiation and salicylic acid, nitric oxide, methyl jasmonate, polyamines, brassinosteroid, etc. in alleviating chilling injury of fruits and vegetables and their mechanism of action have been discussed.

1.1 INTRODUCTION

Low-temperature storage has been widely used as main strategy to prolong shelf life and preserve quality of fresh fruits and vegetables. Storage at low temperature reduces rate of metabolic activity, delay ripening and senescence, and incidence of decay-causing microorganisms (McGlasson et al., 1979). However, fruits and vegetables of tropical and subtropical origin are sensitive to low temperature during postharvest storage, negatively affecting their quality. The occurrence of chilling injury (CI) is manifested at temperatures above 0°C, depending upon the origin and nature of crop. On the basis of incidence and severity of CI in produce, fruits and vegetables can be classified into three categories, namely, (1) chilling resistant, (2) chilling sensitive, and (3) slightly chilling sensitive (Wang, 1994a). The postharvest life of fruits and vegetables under group 1 is inversely proportional to the storage temperature, the lower being the temperature the higher will be the shelf life provided the temperature is not below the freezing point. Shelf life of fruits and vegetables under group 2 increases with decrease in storage temperature up to a certain limit called critical temperature thereafter decreases with lowering the temperature. The critical temperature of commodities under group 2 in which most of the crops of tropical and subtropical origin belong generally ranged between 10°C and 13°C. The critical temperature of commodities belonging to the group 3 is lower than group 2 and often ranged between 3°C and 4°C. The occurrence of CI in harvested horticultural produce varies among species, variety of a single species, and even the climatic condition in which the crop is grown (Lyons, 1973).

1.2 SYMPTOMS OF CHILLING INJURY

The symptoms of CI vary with commodities. The most common symptoms of CI in tropical and subtropical horticultural produce are surface pitting, discoloration of peel, sunken lesions, internal breakdown, water-soaked appearance, lenticels spotting, shriveling, incomplete or impaired ripening, poor color development, off-flavor development, and increased susceptibility to microbial attack (Hardenburg et al., 1986). The development of symptoms depends not only on species or cultivars but also on maturity stage, type of tissue, and storage environment such as relative humidity. Among the symptoms, pitting on the produce surface is the most common symptom which takes place during CI in many tropical and subtropical fruits and vegetables. This is found in crops such as papaya, pomegranate, citrus,

cucumber, sweet pepper, okra, melon, eggplant, and sweet potato. Due to the onset of CI, some produce such as mango, banana, papaya, avocado, melon, sapodilla, and tomato do not ripen properly or completely fail to ripen. Some symptoms of CI are very specific to certain commodities such as brown streaking of vascular tissues in banana, membranous staining in lemon, and mahogany browning of potato (Wang, 1994a). Likewise, internal discoloration occurs in pineapple, avocado, taro, and sweet potato. The symptoms at microscopic level include swelling and disorganization of the mitochondria and chloroplast in which dilation of thylakoid and unstacking of grana occurs. Lipid droplets accumulate inside the chloroplast, nuclear chromatin condenses, and reduction in size and number of starch granules takes place in the cell (Sevillano et al., 2009).

1.3 FACTORS AFFECTING PRODUCE SUSCEPTIBILITY TO CHILLING INJURY

The development of CI in a specific commodity depends upon several factors such as produce origin, genetic makeup, maturity stage, chemical composition of the produce tissue, and the storage environment such as temperature, relative humidity, atmospheric composition, and light. The origin and genetic makeup of a commodity determines the resistance or sensitivity of the product toward CI (Patterson and Reid, 1990). For example, produce originated in the temperate region are resistant to CI while those from tropical and subtropical region are sensitive to it. However, the critical threshold temperature below which CI takes place in commodities varies from crop-to-crop and with stage of maturity. Fruits and vegetables such as mango, papaya, avocado, melon, and tomato are more sensitive to CI at immature stage compared to maturity (Paull, 1990). Likewise, CI is also affected by the chemical composition of the produce tissue exposed to chilling temperature. It has been reported that the commodities resistant to CI tend to have higher degree of unsaturations in the fatty acids of membrane lipids than those of the chilling sensitive tissues (Tabacchi et al., 1979). Higher level of reducing sugars and proline content in the produce tissue also induce resistance toward CI (Purvis, 1981; Purvis and Grierson, 1982). Apart from these, several other mechanisms induce chilling tolerance in fruits and vegetables (Fig. 1.1). Among the environmental factors, temperature plays the predominant role in inducing CI. The prevalence of low relative humidity in the storage environment also promotes CI while its severity reduces at high relative humidity.

1.4 RESPONSE OF COMMODITIES TO CHILLING TEMPERATURE

Fruits and vegetables of tropical and subtropical origin when exposed to low temperature below their critical temperature initially cause primary responses including alteration in cell membrane conformation and structure, thereby affecting its membrane permeability with an increase in electrolyte leakage and lipid phase transition (Raison and Orr, 1990; Lyons and Raison, 1970). The changes in composition of membrane lipid including lipid peroxidation, increase in fatty acid saturation index, sterol:phospholipid ratio, and degradation of phospholipids and galactolipids takes place during storage at chilling temperature (Parkin and Kuo, 1989; Whitaker, 1992; Matsuo et al., 1992). As a result, decrease in fluidity and functionality of cell membrane and its associated proteins occur. It has been reported that higher adaptation of a commodity to chilling temperature is mainly attributed to increase in proportion of unsaturated fatty acids by inducing activity of fatty acid desaturase (FAD, EC 1.14.19.1–3) and some specific isoforms of glycerol-3-phosphate acyltransferase (GPAT, EC 2.3.1.15) thereby reducing the fluidity of membranes (Vigh et al., 1998; Murata et al., 1992). Storage of produce below the critical temperature thus leads to reorganization of membrane lipids from liquid crystalline into a rigid solid gel state. Exposure at this temperature for a sufficiently long time leads to loss of membrane elasticity which causes alternation in functionality of membrane proteins and membrane rupture that leads to leakage of water, electrolytes, and other metabolites from the cell (Sevillano et al., 2009). As a consequence of above primary responses, a cascade of secondary responses develops such as leakage of electrolytes, loss of turgidity and metabolic energy, and finally death of cell (Lyons, 1973).

In addition to the above direct effect of chilling temperature on membrane lipids, low temperature also causes a secondary response causing an increase in the level of reactive oxygen species (ROS) leading to oxidative stress in the commodity. ROS such as singlet oxygen, superoxide radical, hydrogen peroxide, hydroxyl radical, etc. oxidize different cellular components and thereby damage the cells (Sevillano et al., 2009). Due to oxidative stress death of cell occurs due to disintegration of cell membrane by lipid peroxidation, protein oxidation, inhibition of enzyme activity, and damage to DNA and RNA (Scandalios, 1993; Mittler, 2002). Plants protect themselves from this oxidative stress by two ways: first is by activation of ROS avoidance genes such as alternative oxidase and the second is by inducing the activity genes for ROS scavengers such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase,

glutathione-S-transferase, monodehydroascorbate reductase, dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Möller, 2001).

1.5 POSTHARVEST TREATMENTS TO ALLEVIATE CHILLING INJURY

1.5.1 TEMPERATURE CONDITIONING

Prestorage temperature of the commodity can significantly affect the susceptibility of tropical and subtropical produce toward CI (Hatton, 1990; Paull, 1990). Exposure of chilling sensitive produce before storage to a temperature slightly above the critical threshold temperature have been found to increase tolerance of produce to CI during low-temperature storage. Such low-temperature conditioning delays the development of CI symptoms by inducing some physiological and biochemical modifications. These includes increase in sugar, starch, and proline content, maintaining higher content of membrane phospholipids, polyamines (PAs), squalene and long-chain aldehydes, and an increase in ratio of unsaturated to saturated fatty acids. The susceptible produce is exposed to the conditioning temperature either directly in a single step or gradually by multiple steps while, the later process is more effective in alleviating CI. For example, in eggplant, temperature conditioning at 15°C for 2 days followed by 10°C for 1 day was more effective in reducing CI than at 15°C for 2 days alone, during storage at 6.5°C (Nakamura et al., 1985). Alleviation of CI by low-temperature conditioning have been found effective in fruits such as mango, papaya, lime, lemon, and grapefruit and vegetables such as tomato, cucumber, eggplant, sweet pepper, sweet potato, watermelon, and zucchini squash (Hatton, 1990) (Table 1.1).

Unlike low-temperature conditioning, exposure of produce to a high temperature above 35°C for a short duration also reduce incidence of CI. The beneficial effect of prestorage high-temperature conditioning against CI was first reported in grapefruit by Brooks and McColloch in 1936. They reported that conditioning at 38°C for 17–22 h reduces skin pitting of grapefruit during storage at 4.5°C. High-temperature conditioning can be done either by hot water dipping, hot water rinsing and brushing, hot forced air or water vapor. High temperature induces expression of stress genes that encode heat-shock proteins (HSPs) and other stress proteins, reduces chromatin condensation and DNA breakdown, and suppresses oxidative activity. These HSPs exhibit protective role against CI by molecular chaperone activity that constitutes of (1) identifying and binding with unfolded

proteins so as to correctly complete their folding, (2) preventing aggregation of proteins, and (3) facilitating renaturation of aggregated proteins (Aghdam and Bodbodak, 2014). The temperature conditioning induced the expression of various genes that encode enzymes which modify the membrane lipid composition (Sapitnitskaya et al., 2006). Exposure of commodity to high temperature has also been reported to increase desaturation index of membrane lipids thereby membranes become more fluid and leakage of electrolytes become lower during storage at low temperature (Lurie, 1998). Besides this, high temperature also causes an increase in antioxidant activity and PA levels in produce during low-temperature storage (Ghasemnezhad et al., 2008; Mirdehghan et al., 2007a). Heat treatment of peach fruit before storage at low temperature reduced CI by increasing activities of antioxidant enzymes (SOD, CAT, APX, and GR) and decreasing lipoxygenase (LOX) activity, which led to reduction of oxidative stress by decreasing accumulation of H_2O_2 and O_2^- contents (Cao et al., 2010). Reduced accumulation of H_2O_2 lowers membrane unsaturated fatty acids peroxidation, maintains higher unsaturated fatty acids:saturated fatty acids ratio and reduces lignin synthesis thereby maintains higher membrane integrity (Hodges et al., 2004; Shao and Tu, 2013). Alteration of phenylalanine ammonialyase (PAL) and polyphenol oxidase enzymes in response to high temperature treatment has also been associated with mitigating the effect of CI in banana (Chen et al., 2008) and loquat (Shao and Tu, 2013). In addition to this, enhanced sugar metabolism in response to high-temperature conditioning also plays pivotal role mitigating CI in fruits and vegetables. Hot air treatment of loquat fruit increased reducing sugars (glucose and fructose) content and decreased sucrose content during cold storage by enhancing activities of acid invertase, neutral invertase, sucrose synthase, and sucrose-phosphate synthase enzymes (Shao et al., 2013; Li et al., 2011). The higher content of glucose in fruit led to increase in ascorbic acid and glutathione content, increased activities of APX and GR, activation of AA/GSH cycle, thereby mitigating oxidative stress (Aghdam and Bodbodak, 2014). In tomato, Zhang et al. (2013a) reported that hot air treatment alleviate CI by increasing arginase activity. Arginase catalyzes conversion of arginine to ornithine which causes synthesis of PAs (putrescine, spermidine, and spermine) and proline that provide chilling tolerance to the chilling sensitive fruits and vegetables (Zhang et al., 2011; Shang et al., 2011). High-temperature conditioning has been found beneficial in alleviating CI in mango, citrus, pomegranate, grape, avocado, persimmon, tomato, sweet pepper, cucumber, zucchini squash, etc. (Table 1.2). However, in some cases the beneficial effect of high-temperature

conditioning to alleviate CI was found to be cultivar specific. For example, high-temperature conditioning of tomato cv. Rutgers prior to cold storage did not show beneficial effect (Whitaker, 1994). Several factors such as time and temperature of exposure of commodity to high temperature, size, and shape affect the effectiveness of the treatment to alleviate CI.

TABLE 1.1 Effect of Low-temperature Conditioning in Alleviating CI.

Crop	Conditioning temperature	Storage temperature	Reported results	References
Loquat	5°C for 6 days	0°C for 54 days	Alleviated CI by maintaining higher phenol content	Cai et al. (2006)
Mango	0°C for 4 h, and then 20°C for 20 h	2°C 85–95% RH for 12 days	Increased chilling tolerance by enhancing activities of CAT, APX, and glutathione and phenolic content	Zhao et al. (2006)
Zucchini squash	15°C for 2 days	5°C for 14 days	Reduced CI by increasing APX, AFR, DHAR enzyme activities	Wang (1996, 1994c)

AFR, ascorbate free radical; APX, ascorbate peroxidase; CAT, catalase; CI, chilling injury; DHAR, dehydroascorbate reductase; RH, relative humidity.

TABLE 1.2 Effect of High-temperature Conditioning in Alleviating Chilling Injury.

Crop	Treatment	Storage condition	References
Alleviate chilling injury by increasing antioxidant enzyme activity			
Banana	Hot water (52°C for 3 min)	7°C for 10 days	Wang et al. (2012a)
Orange	Hot water (41°C for 20 min)	1°C for 20 days	Bassal and El-Hamahmy (2011)
Lemon	Hot water (53°C for 3 min)	1.5°C for 56 days	Safizadeh et al. (2007)
Mandarin	Hot water (50°C for 2 min)	2°C for 56 days	Ghasemnezhad et al. (2008)
Peach	Hot air (38°C for 12 h)	0°C for 35 days	Cao et al. (2010)
Loquat	Hot air (38°C for 36 h)	4°C for 28 days	Shao and Tu (2013)
	Hot air (45 °C for 3 h)	5°C for 35 days	Shao et al. (2013)
Alleviate chilling injury by accumulation of HSPs			
Grapefruit	Hot water (62°C for 20 s)	2°C for 56 days	Rozenzvieg et al. (2004)

TABLE 1.2 *(Continued)*

Crop	Treatment	Storage condition	References
Banana	Hot air (38°C for 3 days)	8°C for 12 days	He et al. (2012)
Cherimoya	Hot air (55°C for 5 h)	20°C for 5 days	Sevillano et al. (2010)
Grape	Hot air (38°C for 10 h)	−2°C for 3 days	Zhang et al. (2005)
Tomato	Hot air (38°C for 3 days)	2°C for 21 days	Lurie et al. (1996), Sabehat et al. (1998a, 1998b)
Reduce chilling injury by reducing electrolyte leakage and increasing membrane integrity			
Pomegranate	Hot water (45°C for 4 min)	2°C for 90 days	Mirdehghan et al. (2007a)
Loquat	Hot air (38°C for 5 h)	1°C for 35 days	Rui et al. (2010)
	Hot air (38°C for 36 h)	4°C for 28 days	Shao and Tu (2013)
	Hot air (45°C for 3 h)	5°C for 35 days	Shao et al. (2013)
Cucumber	Hot air (37°C for 24 h)	2°C for 9 days	Mao et al. (2007a, 2007b)
Improve chilling tolerance by increasing arginase activity, polyamine, and proline content			
Pomegranate	Hot water (45°C for 4 min)	2°C for 90 days	Mirdehghan et al. (2007a)
Tomato	Hot air (38°C for 16 h)	2°C for 28 days	Zhang et al. (2013b, 2013c)
	Hot air (38°C for 12 h)		
Pepper	Hot water (53°C for 4 min)	8°C for 28 days	González-Aguilar et al. (2000)
Reduce chilling injury by altering PAL and PPO activity			
Banana	Hot air (38°C for 2 days)	8°C for 12 days	Chen et al. (2008)
Loquat	Hot air (38°C for 36 h)	4°C for 28 days	Shao and Tu (2013)
Alleviate chilling injury by enhancing sugar metabolism			
Pomegranate	Hot water (45°C for 4 min)	2°C for 90 days	Mirdehghan et al. (2006)
Peach	Hot air (39°C for 3 days)	20°C for 7 days	Lara et al. (2009)
Loquat	Hot air (45°C for 3 h)	5°C for 35 days	Shao et al. (2013)

HSPs, heat-shock proteins; PAL, phenylalanine ammonialyase; PPO, polyphenol oxidase.

1.5.2 INTERMITTENT WARMING

In this method, intermittent warming of produce for a short time is performed for one or more periods during storage at low temperature. This interruption in low temperature facilitates to alleviate CI and extend storage life of chilling-sensitive produce, provided the treatment is applied before the CI becomes irreversible. In this method, the time of treatment application and early detection of CI is very important which otherwise proceeds beyond recovery and warming of produce will accelerate degradation. In Israel, intermittent warming is commercially practiced to reduce CI in lemon fruit (Cohen, 1988). "Eureka" and "Villa Franca" lemons can be stored successfully for up to 6 months at 2°C by warming the fruit at 13°C for 7 days at every 21 days interval. Likewise, application of intermittent warming was also found beneficial in reducing CI in sweet pepper, cucumber, and zucchini squash (Kramer and Wang, 1989; Wang and Baker, 1979). During intermittent warming when the produce is exposed to high temperature, it induces higher metabolic activities which cause the tissue to metabolize excess intermediate products, thereby replenish deficiencies that developed during chilling. Exposure of produce to high temperature for a short period causes warming of chilled tissues which helps to repair damage to cell membranes or organelles and increases the synthesis of polyunsaturated fatty acids (Lyons and Breidenbach, 1987). Intermittent warming have been found effective in alleviating CI in several fruits and vegetables such as peach, nectarine, lemon, grapefruit, tomato, cucumber, sweet pepper, zucchini squash, etc. (Forney and Lipton, 1990).

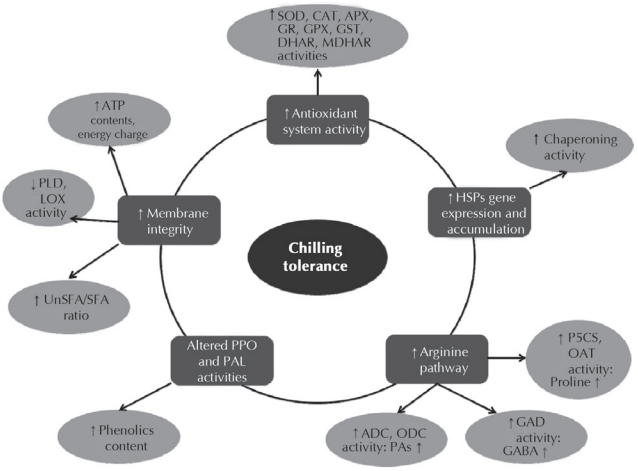

FIGURE 1.1 Mechanism of chilling tolerance in fruits and vegetables.

1.5.3 SALICYLIC ACID

Salicylic acid (SA) is an endogenous signaling molecule that belongs to the group of phenolic compounds. It is present ubiquitously throughout the plant kingdom and is involved in regulating several plant developmental processes such as photosynthesis, respiration, transpiration, stomatal closure, cell growth, ion uptake and transport, senescence-associated genes expression, etc. (Klessig and Malamy, 1994; Clarke et al., 2004; Morris et al., 2000; Rajjou et al., 2006; Harper and Balke, 1981; Khan et al., 2003). SA has been reported to play a significant role in modulating the response of plants to various biotic and abiotic stresses (Asghari and Aghdam, 2010). It is involved in activating local and systemic disease resistance in response to the pathogen attack (Alverez, 2000) and also modulates plant response to various abiotic stresses such as drought, salinity, heat shock, chilling stress, and UV light (Ding and Wang, 2003; Ding et al., 2001).

Postharvest treatment of fruits and vegetables with SA has proved its effectiveness in alleviating CI during their low-temperature storage (Table 1.3). One of the important mechanisms of SA in reducing CI is induced expression of ROS avoidance genes and ROS scavenging genes such as SOD, CAT, APX, etc. (Asghari and Aghdam, 2010). Likewise, it also induces synthesis and further accumulation of HSPs conferring protection against CI (Tian et al., 2007). In peach fruit, SA treatment alleviated CI during storage at 0°C by enhancing activities of APX, GR and increasing reduced-to-oxidized ascorbate ratio (AsA/DHAsA) and reduced-to-oxidized glutathione ratio (GSH/GSSG). This also increased accumulation of heat-shock proteins (HSP101 and HSP73) in the cells (Wang et al., 2006). Luo et al. (2011) reported that SA alleviates CI in plum by increasing accumulation of PAs and reducing electrolyte leakage and MDA accumulation. This was also found by Aghdam et al. (2012a) and Cao et al. (2009) in tomato and cucumber, respectively. Postharvest immersion treatment of pomegranate fruit in 2.0 mM SA solution is found to be highly effective in reducing CI and electrolyte leakage during low-temperature storage (Sayyari et al., 2009). Barman and Asrey (2014) also found it effective in alleviating CI in mango during storage at 8°C.

TABLE 1.3 Effect of Salicylic Acid in Inducing Chilling Tolerance.

Crop	SA treatment	Storage conditions	Reported results	References
Lemon	SA 2.0 mM	−0.5, 2, or 4.5°C for up to 28 days + 7 days at 23°C	Enhanced chilling tolerance by increasing total phenolics and PAL activity and decreasing activity of POD	Siboza et al. (2014)
Loquat	Acetyl SA 1.0 mM L^{-1}	5°C for up to 39 days + 5 days at 20°C	Reduced CI by impairing accumulation of superoxide free radicals	Cai et al. (2006)
Mango	SA 2.0 mM L^{-1}	5°C for up to 30 days + 5 days at 25°C	Alleviated CI by inhibiting O_2^- accumulation, delayed decrease of H_2O_2, and higher reducing status of ascorbate and glutathione.	Ding et al. (2007)
Mango	SA 2.0 mM	8°C for up to 30 days + 3 days at 25°C	Reduced CI by inducing expression of ROS avoidance and scavenging genes and accumulation of HSPs	Barman and Asrey (2014)
Peach	SA 1.0 mM	0°C for up to 28 days + 3 days at 20°C	Suppressed CI by inducing antioxidant systems and HSPs	Wang et al. (2006)
Plum	SA 1.5 mM	1°C for up to 60 days + 3 days at 20°C	Reduced CI symptom by delayed activities of PPO, POD, and increased accumulation of polyamines	Luo et al. (2011)
Pomegranate	Acetyl SA 1.0 mM	2°C for up to 84 days + 4 days at 20°C	Alleviated CI by increasing antioxidant capacity	Sayyari et al. (2011a)
Pomegranate	SA 2.0 mM	2°C for up to 3 months + 3 days at 20°C	Reduced CI symptoms during storage	Sayyari et al. (2009)
Tomato	SA 2.0 mM	1°C for up to 3 weeks + 3 days at 20°C	Reduced activity of phospholipase and lipoxygenase thereby reduced CI	Aghdam et al. (2012a, 2014)
Cucumber	Chitosan-g-SA conjugate (0.57% w/v)	2°C for up to 12 days + 2 days at 20°C	Alleviated CI by increased endogenous SA concentrations and antioxidant enzyme activities including SOD, CAT, APX, and GR	Zhang et al. (2015)

APX, ascorbate peroxidase; CAT, catalase; CI, chilling injury; GR, glutathione reductase; HSPs, heat-shock proteins; PAL, phenylalanine ammonialyase; POD, peroxidase; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase.

1.5.4 NITRIC OXIDE

Nitric oxide (NO) is a highly reactive, free-radical gas that acts as a multifunctional signaling molecule in regulating several physiological processes in plants (Wendehenne et al., 2001). It is involved in plant growth and development starting from germination, root development, stomatal closure, flowering, fruit ripening, reproduction, and senescence (Beligni and Lamattina, 2001; Correa-Aragunde et al., 2004; Neil et al., 2003; He et al., 2004; Leshem, 2000; Prado et al., 2004; Leshem et al., 1998). NO also reported to induce resistance against various biotic and abiotic stresses (Manjunatha et al., 2010). Nitric oxide can be applied either by fumigation or by immersing the fruits and vegetables in sodium nitroprusside (SNP) solution, a donor of NO.

CI in fruits and vegetables during low-temperature storage is associated with generation of ROS, which induces oxidative stress. NO acts as a reaction cascade breaker and prevents damages due to CI (Farias-Eisner et al., 1996; Durzan and Pedroso, 2002). In addition to this, NO reverses the effect of ROS directly, either by suppressing the activities of ROS enzymes or relevant signaling cascade in a tightly coordinated manner (Clark et al., 2000). It is found that application of NO upregulates the activities of SOD, CAT, and peroxidase (POD) enzymes, which are involved in removal of ROS (Flores et al., 2008; Zhu et al., 2008). Fumigation of banana (Wu et al., 2014) and cucumber (Yang et al., 2011a) with NO has been reported to induce chilling resistance of these fruits by increasing the activities of antioxidant enzymes such as SOD, CAT, POD, and APX thereby decreasing the accumulation of ROS. Likewise, application of NO has been found to alleviate CI in many fruit crops such as peach, mango, plum, and loquat during their low-temperature storage (Singh et al., 2009; Zhu et al., 2010; Xu et al., 2012; Aghdam and Bodbodak, 2013) (Table 1.4).

TABLE 1.4 Effect of Nitric Oxide in Alleviating Chilling Injury.

Crop	NO treatment	Storage temperature (°C)	Reported results	References
Banana	60 μL L^{-1} NO fumigation	7	Improved chilling-resistance by increasing antioxidant enzyme activities (SOD, CAT, POD, and APX) and related gene expression, and decreasing accumulation of ROS	Wu et al. (2014)

TABLE 1.4 *(Continued)*

Crop	NO treatment	Storage temperature (°C)	Reported results	References
Peach	15 μL L^{-1} NO fumigation	5	Alleviated chilling injury by protecting cell membrane and cellular integrity	Zhu et al. (2010)
Loquat	NO-specific scavengers and NO synthase inhibitors	1	Chilling tolerance can be enhanced by elevating endogenous accumulation of NO	Xu et al. (2012)
Mango	1.5 mM SNP immersion	8	Reduced chilling injury and electrolyte leakage	Barman et al. (2014)
Mango	10, 20, and 40 μL L^{-1} NO fumigation	5	Alleviated chilling injury by reducing oxidative stress	Zaharah and Singh (2011)
Plum	10 μL L^{-1} NO fumigation	0	Reduced chilling injury by inhibition of ethylene production	Singh et al. (2009)
Tomato	0.02 mM SNP immersion	2	Reduced chilling injury by inducing NO accumulation and expression of LeCBF1	Zhao et al. (2011)
Cucumber	25 μL L^{-1} NO fumigation	2	Improved chilling-resistance by increasing activities of SOD, CAT, POD, and APX enzymes and decreasing accumulation of ROS	Yang et al. (2011a)

APX, ascorbate peroxidase; CAT, catalase; NO, nitric oxide; POD, peroxidase; ROS, reactive oxygen species; SNP, sodium nitroprusside; SOD, superoxide dismutase.

1.5.5 POLYAMINES

PAs are low molecular weight small aliphatic amines that are ubiquitous in all living organisms. The most common PAs found in every plant cells are putrescine (Put), spermidine (Spd) and spermine (Spm), the concentration

of which depends greatly on environmental conditions, especially stress (Galston and Sawhney, 1990). In plants, PAs have been implicated in a wide range of biological processes including growth, development, and responses to abiotic stresses (Evans and Malmberg, 1989; Flores et al., 1989; Galston and Sawhney, 1990). They are mainly localized in vacuoles, mitochondria, and chloroplast of the cells (Slocum, 1991).

Fruits and vegetables when exposed to chilling temperature, changes in cell membrane lipid take place from liquid-crystalline to solid-gel state that lead to increase in membrane permeability and leakage of ions (Stanley, 1991; Gómez-Galindo et al., 2004). PAs play a very crucial role in alleviating CI of fruits and vegetables (Table 1.5). Exogenous application of PAs is reported to induce cold acclimation thereby protecting the produce from CI by increasing the level of endogenous PAs that lead to maintenance of membrane fluidity and reducing electrolyte leakage during storage at low temperature (Mirdehghan et al., 2007a). The alleviation of CI by PAs is attributed to protecting the membrane lipids from being conversion in physical state and its antioxidant property, which prevents lipid peroxidation of cell membrane (Mirdehghan et al., 2007a; Barman et al., 2011). Due to polycationic nature at physiological pH, PAs bind with negatively-charged macromolecules such as phospholipids, proteins, and nucleic acid thereby stabilizing the cell membranes under chilling stress conditions (Smith, 1985). Moreover, PAs have also been reported to have antioxidant properties which are helpful in removal of ROS generated during CI. The antioxidant property of exogenously applied PAs is reported due to its ability to reduce level of hydrogen peroxide, malondialdehyde content, and increase antioxidant enzyme activity (SOD, POD, and CAT) in plant (Nayyar and Chander, 2004). Further, it has been reported that ethylene biosynthesis in plants sensitive to low temperature increases along with development of CI due to increase in precursors of ethylene biosynthesis and enzyme activities involved in ethylene biosynthesis (Concellón et al., 2005; Lederman et al., 1997). The PAs and ethylene use the same precursor SAM (*S*-adenosyl methionine) for their biosynthesis. Therefore, an increase in PA levels affects the level of ethylene production. However, increase in ethylene production with the onset of CI cannot be generalized in all the chilling sensitive plants. For example, in some commodity beneficial effect of ethylene against CI has also been found. In banana, postharvest application of propylene, an ethylene analogue induced resistance toward development of CI.

TABLE 1.5 Effects of Polyamines in Inducing Chilling Tolerance.

Crop	PA treatment	Storage temperature (°C)	Reported results	References
Apricot	Put and Spd (1.0 mM)	1	Alleviated CI by increasing antioxidant enzyme activity (SOD, POD, and CAT)	Saba et al. (2012)
Pomegranate	Put and Spd (1.0 mM) by pressure-infiltration and immersion	2	Reduced CI by inducing cold acclimation	Mirdehghan et al. (2007b, 2007c)
Pomegranate	Put (2.0 mM) by immersion	3	Reduced CI due to antisenescence property of Put	Barman et al. (2011)
Zucchini squash	Put, Spd, and Spm (0.1, 0.25, 0.5, 2.0, and 4.0 mM) by infiltration	2	Alleviated CI by stabilizing and protecting cell membranes	Martînez-Téllez et al. (2002)

CAT, catalase; CI, chilling injury; PA, polyamine; POD, peroxidase; Put, putrescine; SOD, superoxide dismutase; Spd, spermidine; Spm, spermine.

1.5.6 METHYL JASMONATE

Jasmonates are the class of endogenous plant growth regulators, play an important role in plant growth, development, fruit ripening and responses to biotic and abiotic stresses (Creelman and Mullet, 1997). Among the jasmonates, methyl jasmonate (MeJA) has received much attention owing to its ability to enhance chilling tolerance to harvested fruits and vegetables (Li et al., 2012a). Exogenous application of MeJA has been found to alleviate CI by the following mechanisms: (1) increasing membrane integrity, (2) increasing expression and accumulation of HSPs, (3) enhancing antioxidant system, (4) enhancing arginine pathway, and (5) altering PPO and PAL activities (Table 1.6). In general, higher levels of unsaturated fatty acids (linolenic acid and linoleic acid), increased fatty acid desaturase enzymes activity causing increase in membrane saturation degree and its fluidity are responsible for increased chilling tolerance of fruits and vegetables (Hernández et al., 2011). Increase in membrane fluidity decreases the affinity of membrane toward phase change of membrane lipids from liquid crystalline to solid gel state (Los and Murata, 2004). Membrane lipid peroxidation is also carried out by the oxidation of unSFA by the enzyme LOX or ROS (Aghdam and