

# POLYAMINES AS AN ECOFRIENDLY POSTHARVEST TOOL TO MAINTAIN FRUIT QUALITY

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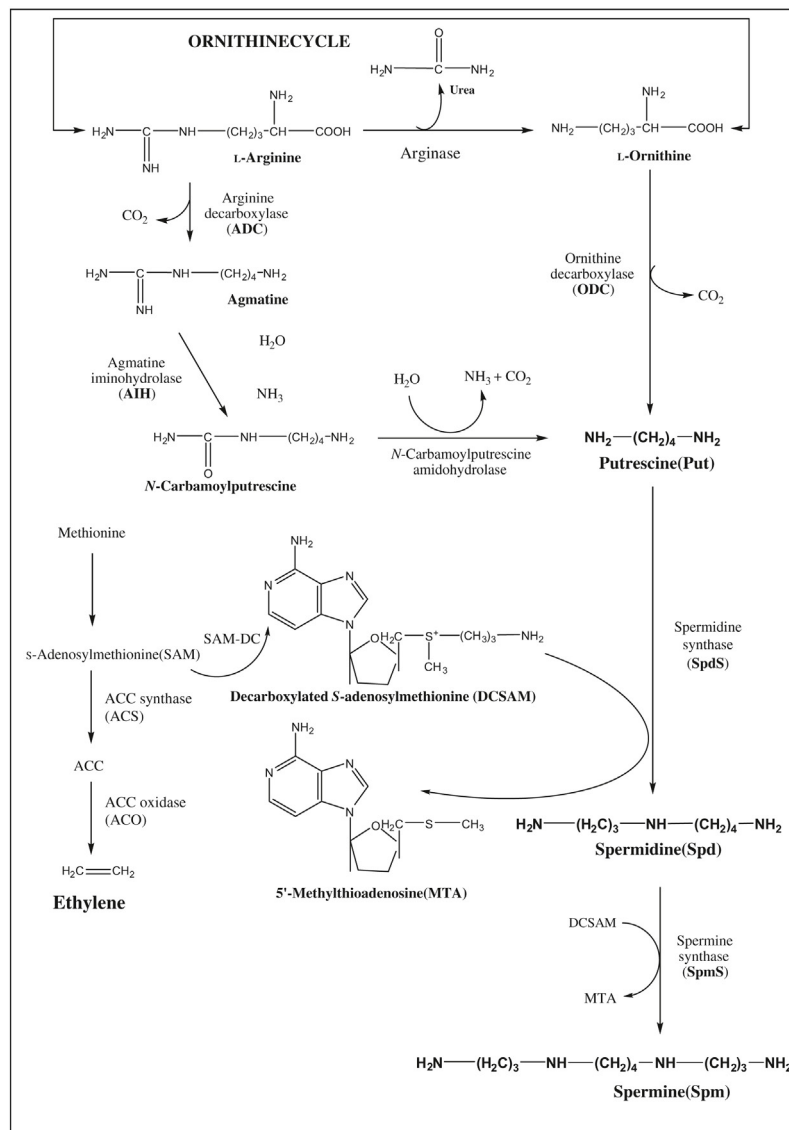
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## 1 Introduction

Polyamines (PAs) are present in all eukaryotic cells (both animal and plant) and have important roles in several biological functions related with cell growth and differentiation. In plant organs, the main PAs are putrescine (Put, 1,4-diaminobutane), spermidine (Spd, *N*-3-aminopropyl-1,4-diaminobutane), and spermine [Spm, bis(*N*-3-aminopropyl)-1,4-diaminobutane]. These PAs are involved in a wide range of growth and developmental process, such as cell division, dormancy breaking, germination, development of flower buds, fruit set, growth and ripening, as well as in plant responses to environmental stresses including chilling injury (CI) (Groppa and Benavides, 2008; Valero and Serrano, 2010; Tiburcio et al., 2014). This chapter will focus on the role of PAs in fruit growth and ripening, with special emphasis on the effects of pre- and postharvest PA treatment on fruit quality attributes, bioactive constituents with antioxidant activity, and tolerance of fruit to CI damages.

## 2 Polyamine Biosynthesis and Regulation in Plant Tissues

The PA biosynthetic pathway in plants is shown in Fig. 7.1, in which the connection with ethylene biosynthesis is also provided. The biosynthesis pathway starts with Put, which is produced by



**Figure 7.1 Polyamine biosynthesis pathway and interconnection with ethylene biosynthesis in plant tissues.**

two alternative pathways, from ornithine in a reaction catalyzed by ornithine decarboxylase (ODC, EC 4.1.1.17) and from arginine as a result of the action of arginine decarboxylase (ADC, EC 4.1.1.19) via agmatine. These two enzymes involved in Put synthesis are differentially compartmentalized, since ADC is located at the chloroplast and ODC at the cytoplasm. In addition, arginase hydrolyzes arginine to urea and ornithine, the latter being

converted into Put by ODC. Put is subsequently converted into Spd and Spm by the addition of two aminopropyl residues from decarboxylated *S*-adenosylmethionine (DCSAM), which is derived from *S*-adenosylmethionine (SAM) by SAM decarboxylase (SAMDC, EC 4.1.1.50). These reactions are sequentially catalyzed by two closely related enzymes, Spd synthase (SpdS, EC 2.5.1.16) and Spm synthase (SpmS, EC 2.5.1.22). There is evidence supporting that the two pathways for Put biosynthesis play different roles in plant development and growth, ADC being related to maturation and response to environmental stresses, and ODC having the main role in cell division. On the other hand, SAM is also a precursor of ethylene via the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS), which is further converted to ethylene by ACC oxidase (ACO). Furthermore, in some plants, the methyl moiety of SAM can be transferred to Put via Put-*N*-methyltransferase, to form *N*-methyl-Put, which serves as a precursor of nicotine and other alkaloids. Thus the multiple essential roles of SAM require regulation of its synthesis, recycling, and distribution to sustain these different pathways, this SAM metabolism being highly compartmentalized and regulated by various feedback loops that also control synthesis of methionine precursors in plastids (Kusano et al., 2007; Groppa and Benavides, 2008; Valero and Serrano, 2010; Sauter et al., 2013).

The intracellular free PA pool depends on its synthesis and also on several metabolic pathways including degradation, conjugation, and transport. Thus, copper containing diamine oxidases catalyze the oxidation of Put, and flavine-containing PA oxidases (PAO) oxidize Spd and Spm, producing 4-aminobutanal, and *N*-(3-aminopropyl)-4-aminobutanal, respectively. Finally, PAs can be conjugated either with small molecules, especially hydroxycinnamic acids to form soluble Pas, or with high molecular mass substances, such as hemicelluloses, lignin, or protein of the cell wall to form cell wall-bound PAs and could serve as a pool of free PAs (Valero et al., 2002; Bianchi et al., 2006; Tiburcio et al., 2014).

In spite of ethylene and PA (Spd and Spm) biosynthesis sharing a common precursor (SAM), they exert opposite effects in fruit ripening and senescence, and the balance between these two opposite growth regulators is crucial to retard or to accelerate both processes. Thus, reduced levels of PAs have been correlated with increased ethylene production, fruit ripening, and senescence, while high endogenous concentrations of PAs are associated with a delay in these processes (Valero et al., 2002; Valero and Serrano, 2010; Tiburcio et al., 2014).

### 3 Role of Endogenous Polyamines in Fruit Development and Ripening

PAs are involved in the overall physiological process from floral development to fruit growth and ripening. Thus, increase in total PAs or in a single PA type accompanies floral development, while inhibition of PA biosynthesis causes a strong decrease in flowering, this inhibitory effect being abolished by applying exogenous Spd. Moreover, PAs are also related to flower genders or fertility as well as to the pollen germination and pollen tube growth and promote flowering of some plants under noninductive conditions. Some mutant plants deficient in PA metabolism demonstrated aberrant morphology in anthers and ovules (Liu et al., 2006a). In addition, a clear relationship has been reported between the concentration of free PAs (mainly Spd and Spm) in the apricot ovary and the ovule development, its viability, and fruit set (Albuquerque et al., 2006). Accordingly, a large accumulation of free PAs occurs concomitantly with flower development in damson plum, being closely related to the onset of ovarian development (De Dios et al., 2006). In addition, fruit set in grape vine was positively influenced by the increase in free and soluble-conjugated Spd in the floral organs (Aziz et al., 2001) and Put directly applied to the flower 2 days before pollination increased fruit set in Japanese pears, due to stimulation of pollen germination and initial pollen tube growth (Franco-Mora et al., 2005b). Similarly, spray treatments of date palm tree at bloom phase with 0.45 mM Put, alone or in combination with 2% potassium citrate, increased fruit set and fruit retention percentages (Abd El-Migeed et al., 2013). In a similar way, fruit retention was increased in mango tree by Put, Spd, and Spm treatments, especially with Spm when applied at full blossom stage (Malik and Singh, 2006). This effect has been ascribed to the increased levels of endogenous PAs in the fruitlets and pedicels making them less prone to abscise, especially during the initial 4–6 weeks of heavy fruitlet abscission, by inhibiting endogenous ethylene biosynthesis, which is the known trigger in abscission. Moreover, PA treatments have also led to improved fruit volume and weight in date (Abd El-Migeed et al., 2013) and apricot (Ali et al., 2010) fruits, due to the PA effects on increasing fruit sink strength and favoring phloem sugar translocation towards them.

In a wide range of fruit species, it has been found that the concentration of PAs is high at the early phase of fruit growth, while a decline occurred as the growth rate decreased, reaching the lowest concentrations at the ripening phase, these PA changes occurring in climacteric fruits such as tomato (Martínez-Madrid et al., 1996; Yahia et al., 2001; Pandey et al., 2015), plum (Zuzunaga et al., 2001),

peach (Liu et al., 2006b), and apricot (Paksasorn et al., 1995) as well as in nonclimacteric ones, such as pepper (Pretel et al., 1995), strawberry (Ponappa and Miller, 1996), and grapevine (Agudelo-Romero et al., 2013). In grapevine the decrease in free and conjugated PA content during grape ripening was due to their catabolism, since it was accompanied by upregulation of genes coding for diamine oxidase (CuAO) and PAO, together with a significant increase in their enzymatic activity and in the hydrogen peroxide content, in spite of an increase in ADC expression (Agudelo-Romero et al., 2013). The high PA concentration soon after full bloom has been related to the high growth rate and active cell division. However, in avocado mesocarp cells continue to divide as long as the fruit remains attached to the tree and nevertheless PAs also decreased during fruit growth (Kushad et al., 1988).

The decrease in PAs at late stages of fruit growth has been regarded as a signal for fruit ripening, although a few exceptions exist. Thus Put increased during ripening in long-keeping tomato (Martínez-Madrid et al., 1996; Yahia et al., 2001) and both Put and Spd levels raised in Golden Japan plum (Zuzunaga et al., 2001), a suppressed climacteric plum phenotype. The high levels of PAs in these mature fruits may be responsible for the long-keeping quality and low ethylene production of these tomato and plum cultivars. Moreover, since ethylene and PAs share their common precursor, it is normally accepted that they compete each other during fruit development and ripening and then diminution in Spd and Spm during fruit ripening may be a consequence of SAM diversion to ACC for ethylene biosynthesis, concomitantly with the increase of Put (Valero et al., 2002). In agreement with this proposal an inverse relationship has been found between PA content and ethylene production and ACC concentration during ripening of seven pear cultivars, ranging from low to moderate and high ethylene production rates at ripening (Franco-Mora et al., 2005a). In addition the genetic modification of tomato fruit by overexpressing human-SAMDC led to fruit with elevated PA levels and reduced levels of ethylene in comparison to wild type, due to lower accumulation of ACS and ACO gene transcript, these transgenic fruits exhibiting delay in on-vine ripening and extended postharvest storage (Madhulatha et al., 2014). Accordingly, transgenic tomato plants overexpressing the mouse ODC gene produced fruit with enhanced levels of Put, Spd, and Spm and reduced ethylene production and respiration rate, the on-vine ripening process being delayed with respect to fruits from untransformed plants (Pandey et al., 2015).

However, increase in Put concentration during ripening has been also found in some climacteric fruits, such as paraguay

(Martínez-Madrid et al., 2000), peach (Liu et al., 2006b), and cherimolla (Escribano and Merodio, 1994) as well as increases in Put, Spd, and Spm in the climacteric damson plum (De Dios et al., 2006). Moreover, the introduction of the yeast *SAMDC* gene into a commercial variety of tomato led to increased levels of Spd and Spm, although these transgenic tomatoes produced more ethylene than did the parental line (Mehta et al., 2002). On the other hand, 1-methylcyclopropene (1-MCP) treatment of apple fruit inhibited the autocatalytic production of ethylene but no evidence for elevated levels of total PAs, Put, Spd, or Spm during apple storage were observed (Deyman et al., 2014), while in 1-MCP-treated tomatoes ethylene production was inhibited and PA content increased (Van de Poel et al., 2013). Thus it seems that the level of the precursor SAM is not generally the limiting factor for ethylene and PA biosynthesis pathways and that both metabolic pathways can operate simultaneously in vivo, at least in some fruit species.

## 4 Effects of Pre- and Postharvest Polyamine Application on Fruit Ripening and Quality Attributes

According to consumers the term “quality” can be defined as a fruit with a perfect shape, size, color, firmness, aroma, and absence of defects such as cuts, bruises, or decay. However, fruits are appreciated not only because of their attractive sensorial properties, but also because of their nutritional and health benefits, due to their antioxidant compound content with the beneficial role in the prevention of degenerative diseases (Serrano et al., 2011; Villa-Rodriguez et al., 2015). The highest quality attributes are reached at the appropriate ripening stage, but fruits deteriorate rapidly after harvest with significant losses in their quality properties (Valero and Serrano, 2013).

Given the opposite effects of PAs and ethylene on retarding or accelerating fruit ripening and senescence, respectively, as commented in the previous section, a great deal of research has been focused on the effect of pre- or postharvest PA treatments on fruit quality parameters related to the ripening process. In this sense several experiments have shown that preharvest treatments with PAs during the fruit growing season can decrease ethylene production and delay the ripening process in a wide range of fruit species. However, most of the research about the effect of PAs on fruit ripening has been performed with postharvest treatments and their effects are similar to those of preharvest treatments, thus there is a

delay in the postharvest ripening process leading to maintenance of fruit quality attributes.

## 4.1 Ethylene Production

Foliar spray treatments of peach trees 19 days before fruit harvest with Put (10 mM), Spd (0.1, 1, and 5 mM), or Spm (2 mM) strongly reduced or even nullified ethylene production during peach on-tree ripening, with Spd being more efficient than Put or Spm (Bregoli et al., 2002). In addition, field applications of 1 mM Spd on peach trees at 41 days after full bloom led to a lower accumulation of *ACO* and *ACS* transcripts at harvest, in accordance with their effect on inhibiting ethylene biosynthesis (Torrighiani et al., 2012). Moreover, it has been reported that *SAMDC* and *ADC* transcript levels were initially depressed in peach fruit by preharvest Spd treatment, as well as *ACO* and *ACS* transcripts, and later, at harvest time, recovered up to control levels, while the effect of Put treatments on *ACO* and *ACS* were still evident at harvest, in which an increase of ethylene receptor mRNA was also found (Ziosi et al., 2006). Similar results regarding the efficacy of PAs on decreasing ethylene production were obtained in nectarine during on-tree fruit ripening (Torrighiani et al., 2004). Moreover, preharvest treatments with PAs have been shown to be also effective in delaying ethylene production during postharvest storage. Thus preharvest treatments of apricot trees with 0.1 mM of Put, Spd, or Spm 20 days before harvest decreased postharvest ethylene production (Paksasorn et al., 1995). Accordingly, preharvest foliar spray treatment of plum trees with Put delayed and inhibited both ethylene production and respiration rate during postharvest storage, these effects being higher as Put concentration increased from 0.1 to 2 mM, and also evident after a 6-week period of cold storage (Khan et al., 2008).

On the other hand, postharvest application of PAs, by immersion or vacuum infiltration, has been reported to inhibit ethylene production in some climacteric fruits, including Kesington Pride mango (Malik and Singh, 2005), Babygold-6 peach (Martínez-Romero et al., 2000), Mauricio apricot (Martínez-Romero et al., 2002), and Black Diamond, Black Star, Santa Rosa, and Angeleno plums (Pérez-Vicente et al., 2002; Serrano et al., 2003; Khan et al., 2008) delaying fruit ripening and extending shelf life. The inhibitory effects of exogenous PAs in ethylene production have been ascribed to both the competitive biosynthesis mechanism between ethylene and PAs and to the inhibition of ACC synthase and ACC oxidase. However, in apples Put treatment did not decrease ethylene production through the normal

course of ripening during storage (Wang et al., 1993). Since ethylene inhibition by Put treatment has been shown to be inversely correlated to the maximum level at the climacteric peak (Valero et al., 2002; Serrano et al., 2003), the failures of PA treatments on inhibiting synthesis of ethylene in some fruits may be due to their high levels of ethylene production. The effect of PA treatments on inhibiting ethylene production in climacteric fruits led to a delay in the evolution of the postharvest ripening process, since in these fruits ethylene is the main responsible hormone regulating this process (Cherian et al., 2014). Nevertheless, the delayed postharvest ripening process as a consequence of PA treatments has been also observed in nonclimacteric fruits, such as Mollar de Elche pomegranate (Mirdehghan et al., 2007a), blueberry (Basiouny, 1996), and table grape (Harindra Champa et al., 2015).

## 4.2 Fruit Quality Parameters

Pre- and postharvest PA treatments have shown to have beneficial effects on fruit quality attributes. Thus foliar spray with Put and Spm to apricot Canino trees increased yield, fruit weight, and fruit volume compared with fruits from control trees. In addition, at harvest time, fruits of treated trees with both Put and Spm had a significantly higher total soluble solids (TSSs) concentration and they were firmer than fruits of control trees, whereas fruit total acidity (TA) was lower in fruits from PA treated trees, showing that PAs could be recommended in cultural practices to enhance the production of apricot tree orchards and improve fruit quality (Ali et al., 2010). Accordingly, Put treatment of date palm tree at bloom stage increased fruit weight, length, diameter, and volume, as well as the content of reducing and nonreducing sugars, and reduced TA and tannin concentration, leading to date fruits with improved quality attributes at harvest (Abd El-Migeed et al., 2013). Moreover, the activation of the Put biosynthetic pathway in tomato fruits from transgenic tomato plants overexpressing the mouse *ODC* gene led to fruit with higher Put, Spd, and Spm content, which also had improved quality traits, such as TSS, TA, and sugar content (Pandey et al., 2015) as well as in transgenic tomatoes overexpressing human-*SAMDC* (Madhulatha et al., 2014). On the other hand, treatments of mango trees with Put (0.5, 1, or 2 mM) 7 days prior to harvest led to higher levels of firmness and TSS and lower fruit rot index after 20 days of storage at 20°C as compared to fruit from nontreated trees (Malik and Singh, 2005), although sugar content was at a lower concentration, probably due to a slower conversion of starch to sugars and delay in the evolution of color development (Malik and Singh, 2006). Accordingly, Put treatments of plum trees



also decreased fruit softening, maintained TA at higher levels, diminished the increase in TSS, and delayed the color evolution during postharvest storage as compared to control fruits, showing a delay in ripening evolution and leading to a net extension of plum shelf life (Khan et al., 2008). Moreover, foliar spray treatments of peach trees 19 days before fruit harvest with Put (10 mM), Spd (0.1, 1, and 5 mM), or Spm (2 mM) markedly slowed down the softening process, while only Spd affected the accumulation of TSS, leading to lower levels at harvest as compared with control fruits (Bregoli et al., 2002). Similar results were obtained in nectarine during on-tree fruit ripening, in which PA treatments decreased flesh softening and acidity losses and increased TSS (Torrighiani et al., 2004).

Accordingly, postharvest Put application markedly slowed softening during ripening at ambient temperature in Angelino plum, this effect being higher as Put concentration increased from 0.1 to 2 mM (Khan et al., 2008) as well as in Black Star, Black Diamond, Golden Japan, and Santa Rosa cultivars (Serrano et al., 2003). Put treatment at 10 mM was also effective in delaying softening in blueberry, while no effect was observed with 1 mM Spd treatment (Bassiouny, 1996). However, in apricot, both Put and Spd were effective in reducing fruit softening during cold storage (Koushesh-saba et al., 2012). Several mechanisms have been postulated to explain the increased fruit firmness after Put treatment. One is supported by decreased activity of cell wall hydrolytic enzymes involved in softening, such as endo- and exo-polygalacturonase (PG), pectin esterase (PE), and pectin methyl esterase (PME). Thus Spm at the dose of 1.0 mM effectively maintained grape berry firmness during long-term cold storage, because the enzymatic activity of PME was effectively repressed (Harindra Champa et al., 2015). Accordingly in peach fruit it has been shown that the effect of PA treatment on reducing fruit softening is due to a strong downregulation of genes responsible for fruit softening, such as those coding for PG and PME (Torrighiani et al., 2012). Other mechanisms involve the PA capacity to cross-link pectic substances in the cell wall, producing rigidification and also blocking the access of such degrading enzymes reducing the rate of softening during storage (Valero and Serrano, 2010 and references cited therein). However, it is also true that exogenous 10 mM Put or 1mM Spd treatments of Redhaven peaches harvested at two different ripening stages failed in maintaining fruit firmness, while positive effects of these treatments were found in nectarines, because Put was taken to a higher extent in nectarines than in peaches (Bregoli et al., 2006).

On the other hand, postharvest Put treatments decreased the weight loss throughout storage in plum cultivars with respect to those observed in control fruits (Serrano et al., 2003), as well as

Put or Spm treatment of table grape, which was attributed to the improved biophysical properties of the berries by means of stabilization and consolidation of both cell integrity and permeability as a consequence of Spm treatment manifested as lower electrolyte leakage during storage (Shiri et al., 2013; Harindra Champa et al., 2015). In table grape, Put treatments were also effective in reducing decay incidence, rachis browning, and berry shattering and cracking during prolonged cold storage (Shiri et al., 2013). Another effect of PA treatments is amelioration of chlorophyll breakdown in several fruits, such as lemon and apricot, which is an indicator of reduced senescence rate (Martínez-Romero et al., 2002; Valero et al., 2002). Also, exogenous PAs retarded chlorophyll loss in muskmelon by reducing the hydrolytic activities acting on chloroplast thylakoid membranes (Lester, 2000). Similarly, Put treatments reduced color change during low-temperature storage in a wide range of plum cultivars, the effect being also attributed to lower chlorophyll degradation and delay in the senescence process (Serrano et al., 2003; Khan et al., 2008). The effects of PAs on retarding color evolution were in the order:  $SPM^{4+} > SPD^{3+} > PUT^{2+}$ , following the order of their available number of cations, which has been argued as the reason for their difference in effectiveness (Valero et al., 2002). In addition, 1-MCP treatments of pepper fruits delayed senescence manifested as lower chlorophyll degradation and weight loss in treated pepper with respect to controls, throughout maintenance of higher Put, Spd, and Spm concentrations (Cao et al., 2012). Finally, most of the reports about PA postharvest treatments have shown to have little or no effect on TSS evolution during fruit postharvest storage, while they significantly delayed the diminution in TA that normally occurs during storage in a wide range of fruits, such as plum, pomegranate, and blueberry (Basiouny, 1996; Mirdehghan et al., 2007a; Khan et al., 2008; Valero and Serrano, 2010).

Taking into account data of the observed parameters relating to fruit quality (firmness, color, TSS, and TA), as well as the visual appearance of the fruits, it could be concluded that PA treatment, either at pre- or postharvest time, delayed the postharvest ripening process, with a net effect on maintaining fruit quality attributes and increasing the fruit shelf life. These effects could be because PA treatments led to increases in endogenous Put and Spd concentrations, as have been shown in lemon (Valero et al., 1997), peach (Martínez-Romero et al., 2000), apricot (Martínez-Romero et al., 2002), plum (Pérez-Vicente et al., 2002; Serrano et al., 2003), and pomegranate (Mirdehghan et al., 2007a), the increased PA concentration being evident after treatment and remaining during postharvest storage.

### 4.3 Bioactive Constituents With Antioxidant Activity

Fruits and vegetables contain a wide range of phytochemical compounds that exhibit antioxidant activity, the most common being phenolics, including anthocyanins, carotenoids, vitamins (C and E), and glucosinolates (Valero and Serrano, 2013), which are related with the protective effects of fruit consumption against several chronic diseases associated with aging including atherosclerosis, cardiovascular diseases, several types of cancer, cataracts, blood pressure increase, ulcers, neurodegenerative diseases, brain and immune dysfunction, and even against bacterial and viral diseases (Martin et al., 2013; Nile and Park, 2014). There is little information about the effect of PA treatment on the concentration of bioactive compounds in fruits. The first evidence of the *in vivo* role of PAs in the fruit content of bioactive compounds was obtained with transgenic tomatoes having the yeast *SAMDC* gene, in which Spd and Spm concentrations in ripe fruits were higher than in controls and these red ripe transgenic tomatoes accumulated threefold more lycopene than did the red fruits from the parental lines (Mehta et al., 2002). Accordingly, tomato plants overexpressing the mouse *ODC* gene produced fruits with higher PA concentration as compared with fruits from untransformed plants, these transgenic fruits having also higher concentration of lycopene and ascorbic acid (Pandey et al., 2015). Similarly, higher levels of PAs as well as ascorbic acid and lycopene were found in transgenic tomatoes overexpressing the human *SAMDC* gene, the higher lycopene accumulation being attributed to elevated levels of lycopene gene transcripts in transgenic tomatoes, while the higher ascorbic acid content could be due to the lower ethylene production of these tomatoes, since it is used as a cofactor for the ACO enzyme (Madhulatha et al., 2014). Moreover, treatments with Put, Spd, or Spm at concentrations of 0.01, 0.1, and 1 mM of mango trees by foliar spraying at final fruit set stage led to fruits with significantly higher total carotenoids in the pulp at harvest time as compared with fruits from control trees, the maximum increase being observed with Put treatments (95%) followed by Spd (33%) (Malik and Singh, 2006). This is of special significance as carotenoids including lycopene as well as ascorbic acid are bioactive compounds with high antioxidant activity and beneficial effects for human health (Friedman, 2013; Martin et al., 2013; Nile and Park, 2014).

On the other hand, it has been reported that in Mollar de Elche pomegranate arils, the application of 1 mM of Put or Spd, either by pressure infiltration or immersion, was effective in maintaining the concentration of total anthocyanins at higher levels than in

control fruits during storage, these effects being similar in Put- and Spd-treated fruits, independently of the method of application (Mirdehghan et al., 2007c). Accordingly, Mridula pomegranate treated with 2 mM Put by the immersion method retained higher anthocyanin, ascorbic acid, and tannin concentrations and antioxidant activity than control fruits, the effects being increased when Put was applied in combination with carnauba wax (Barman et al., 2014). In grape berries, total anthocyanin content showed an increasing trend up to 45 days and then declined rapidly during next 30 days of storage, while in Spm-treated berries at 0.5, 1, and 1.5 mM anthocyanin concentration was increased in a dose-dependent manner (Harindra Champa et al., 2015). Delaying of fruit skin color degradation when treated with Spm has been also reported in mangos by Malik and Singh (2005, 2006). The mechanism by which Put and Spd induce these effects is still unknown, although they may be related to their antisenescence effects, which are allied to the suppression of membrane lipid peroxidation and maintenance of the integrity of membranes (Lester, 2000; Valero and Serrano, 2010). However, pre- or postharvest treatments of plums with Put led to reduced levels of total antioxidants after postharvest storage and were lower in treated plum than in controls (Khan et al., 2008), which could be due to the effect of Put on delaying the ripening process, since the bioactive compounds with antioxidant activity have been reported to increase during plum fruit ripening on trees and during postharvest storage (Díaz-Mula et al., 2009).

With respect to the effect of PA treatment on phenolic compounds, different results have been obtained, depending of the applied PA, concentration, and fruit species. Thus table grape Spm treatment at 0.5 and 1 mM retained higher total phenol content (TPC) over the control, while Spm at 1.5 mM resulted in significantly lower TPC (Harindra Champa et al., 2015). On the other hand, higher TPC as a consequence of Put and Spd treatments has been reported in pomegranate (Mirdehghan et al., 2007c). Accordingly, Put at concentrations of 1 and 2 mM applied as dipping treatment to table grape led to increased TPC, catechin, total quercetin, and antioxidant activity as compared with control berries (Shiri et al., 2013). On the contrary, Koushesh-saba et al. (2012) observed lower TPC in Put- and Spd-treated apricots than in controls. Thus the effects of PAs on TPC remain elusive.

On the other hand, the enhancement of hydrophilic total antioxidant activity found in pomegranate arils after PA treatments could be attributed to the PA capacity acting as effective scavengers of free radicals, and even to their role on the superoxide dismutase (SOD)/ascorbate–glutathione cycle (Mirdehghan

*et al.*, 2007c). On the contrary, pre- and postharvest Put application to Angelino plum led to a linear reduction in the levels of ascorbic acid, carotenoids, and TAA during postharvest storage, which were more pronounced with increased concentrations of Put and storage periods, these effects being ascribed to increased ascorbate oxidase activity (Khan *et al.*, 2008), according to a previous report on pepper and tomato (Yahia *et al.*, 2001). Thus more research is needed to clarify the effects of PA treatments on fruit functional compounds.

## 5 Polyamines and Chilling Injury

Storage at low temperature is widely used as a postharvest tool to delay fruit ripening and senescence processes and to maintain quality attributes. However, many tropical and subtropical fruits suffer physiological alterations known as chilling injury (CI) when stored at low but nonchilling temperatures, usually below 10–12°C depending on commodities. Chilling symptoms mainly develop during shelf life after removing fruits from low-temperature storage and are manifested as surface pitting and higher susceptibility to decay in pepper, zucchini, or pomegranate (Serrano *et al.*, 1997, 1998; Mirdehghan *et al.*, 2007b), flesh browning and mealiness in apricot, peach, and nectarines (Valero *et al.*, 1997; Lurie and Crisosto, 2005), flesh browning and translucency in plums (Luo *et al.*, 2011), or flesh browning and water-soaked appearance in bamboo shoots (Luo *et al.*, 2012). Other reported CI symptoms are failure of fruit to ripen, or uneven or slow ripening, accelerated senescence and ethylene production, shortened storage or shelf life, compositional changes affecting flavor and texture, loss of growth or sprouting capability, wilting, and increased decay due to leakage of plant metabolites, which encourage growth of microorganisms, especially fungi (Valero and Serrano, 2010). Development of these chilling disorders reduces fruit quality and consumer acceptance, and then the onset of CI symptoms becomes an economically important postharvest problem that determines the postharvest storage potential of the fruit. In this sense, scientific researchers are focused on developing new technologies based on natural, safe, and environmentally friendly compounds to prevent or alleviate CI symptoms, satisfying the consumer's concerns about chemical residues in fruits and vegetables along with legal restriction on the use of synthetic chemicals.

Cell membranes are the first cell structures affected by CI, which change from a flexible liquid-crystalline phase to a solid-gel structure at chilling temperatures, leading to losses of the cell membrane semipermeability and functionality (Rui *et al.*, 2010).

In addition, disorganization of mitochondria and chloroplast occurs, which sets off a cascade of secondary reactions, including ethylene production, increased respiration, reduced photosynthesis, and interference with energy production, accumulation of toxic compounds, such as ethanol and acetaldehyde, and altered cellular structure (Kratsch and Wise, 2000). In pomegranate skin it has been found that membrane lipid composition changes during storage, with losses of saturated and unsaturated fatty acids and reduction in the ratio of unsaturated/saturated fatty acids, affecting membrane permeability and causing leakage of intracellular water, ions, and metabolites, which can be monitored by determining electrolyte leakage (EL) (Mirdehghan et al., 2007b). Thus EL is a measurement of loss of semipermeability of cell membranes, which increases as a consequence of membrane damage, and has been widely used as an indicator of CI (Mirdehghan et al., 2007b; Rui et al., 2010; Sayyari et al., 2009). Another indicator of the structural integrity of the plant membranes is malondialdehyde (MDA), which is a secondary end product of the oxidation of the membrane polyunsaturated fatty acid, and increases in chilling injured fruit and vegetable tissues (Luo et al., 2011, 2012; Palma et al., 2015). In addition, increases in phospholipase-D and lipoxigenase activities, responsible for the degradation of unsaturated fatty acids, reduced cell membrane integrity and therefore increased CI impact (Aghdam et al., 2015). Membrane lipid peroxidation can be also stimulated by radical oxygen species (ROS) generated as a consequence of chilling stress (Sevillano et al., 2009).

PAs, as polycationic molecules at physiological pH, can bind strongly to anionic components of the cell membranes, such as phospholipids, leading to stabilization of the bilayer surface. Then, as the maintenance of membrane stability at low temperature is an important factor for plant resistance to cold stress and given the relationship between PAs and membrane protection, the possible role of PAs on protecting fruit tissues against CI is of great interest (Mirdehghan et al., 2007a, 2007b; Groppa and Benavides, 2008; Zhang et al., 2010). In addition, PAs exhibit antioxidant activity by scavenging ROS, leading to enhanced membrane stability and integrity under CI stress (Hussain et al., 2011). In this sense, increases in Put concentration have been found in several fruits suffering CI, such as lemon, orange, lime, grapefruit, pepper, tomato, peach, pepino, and zucchini, among others (Serrano et al., 1997, 1998; Martínez-Romero et al., 2003; González-Aguilar et al., 2000; Shang et al., 2011; Zhang et al., 2013a). In addition, in peach fruit it was shown that wooliness was associated with an increase in Spd concentration (Valero et al., 1997). Moreover, increases in Spd concentration have been also reported in

pomegranate skin in storage, being associated with enhanced CI symptoms (Mirdehghan et al., 2007b). Accordingly, Put and Spd concentrations increased in bamboo shoots in storage at 1°C and they were correlated with CI incidence (Luo et al., 2012). Such results support the proposal that accumulation of Put and/or Spd or Spm in tissues seems to be a general response of fruit to chilling temperatures, although they do not indicate whether the increase in Put or Spd is a protective response to CI or whether Put or Spd themselves are the result of the stress-induced injury.

In this sense, it has been shown that prestorage treatments that reduce CI are related with increased PA concentration in fruit tissues. Thus CO<sub>2</sub> treatments of zucchini before storage reduced CI throughout increases in PA levels (Serrano et al., 1998). Accordingly, pretreatment at 25°C for 2 days before cold storage reduced CI in peach by elevating all the three PA levels (Xu et al., 2005). In Fortune mandarins, temperature pretreatments for 3 days above 20°C increased progressively both Put and Spd levels in flavedo, as did temperature treatment, and reduced CI (González-Aguilar et al., 2000). Heat treatments have been also effective on decreasing CI by increasing PA concentration in a wide range of fruits (Aghdam and Bodbodak, 2014). Likewise, in plum fruits, CI symptoms were reduced by prestorage treatment at 45 and 50°C for 35 and 30 min, respectively, which also maintained increased PA levels (Abu-Kpawoh et al., 2002). Accordingly, heat treatment of pomegranate fruit led to a decrease in CI and an increase in Put and Spd concentrations in the skin during cold storage (Mirdehghan et al., 2007b). Additionally, the levels of sugars (glucose and fructose), organic acids (malic, citric, and oxalic acids), total phenolics, ascorbic acid, and anthocyanin remained also at higher concentrations in arils from treated fruits, showing that with this simple and noncontaminant technology, the functional and nutritive properties, after long periods of storage, could then be even greater than in recently harvested fruits (Mirdehghan et al., 2006). The induction of resistance to CI by heat treatment associated with increased Put concentration has also been reported in peach (Cao et al., 2010). On the other hand, tomato hot air treatment at 38°C for 12 h before storage at 2°C reduced CI symptoms and enhanced the accumulation of endogenous Put, due to the activation of arginine catabolism, by enhancing transcript levels of ADC, ODC, OAT, and arginase (Zhang et al., 2013a). Similarly, arginine treatment of tomato fruit reduced CI and enhanced accumulation of PAs, especially Put, as well as proline and nitric oxide concentrations, which resulted from the increased activities of these arginine catabolic enzymes (Zhang et al., 2013b). As stated in earlier, previous studies showed that ODC plays a main role in cell division and ADC in

plant responses to stress, although these results provide evidence that both ADC and ODC pathways may account for PA accumulation under chilling conditions.

On the other hand, UV-C irradiation (for 3, 5, or 10 min) of peach fruits before storage at 5°C significantly reduced CI after 14 and 21 days of cold storage + 7 days at 20°C, this effect being related with higher accumulation of Spd and Spm and with the additional benefit of reducing fruit decay and softening (Gonzalez-Aguilar *et al.*, 2004). Other natural compounds, such as salicylic acid (SA), acetyl salicylic acid (ASA), methyl salicylate (MeSa), and methyl jasmonate (MeJa), have been reported as easy to apply treatments for alleviating CI in fruits, vegetables, and even in cut flowers (Asghari and Aghdam, 2010; Aghdam and Bodbodak, 2013). For instance, Zhang *et al.* (2011) observed that tomato postharvest treatment with 0.05 mM MeSa for 12 h alleviated CI by increasing gene expression and enzymatic activity of arginase, ODC, and ADC, leading to higher Put, Spd, and Spm concentration in MeSa-treated fruit than in controls. Similar results have been found in cherry tomato after treatment with 0.05 mM MeJa for 12 h before storage at chilling temperature (Zhang *et al.*, 2012). Accordingly, SA treatment (at 1.5 mM for 10 min) significantly reduced CI in plums and this event was associated with enhanced endogenous Put and Spd concentrations and led to reduction of MDA, indicating an improvement of cell membrane integrity, due to protection of cell membrane lipids from peroxidation (Luo *et al.*, 2011). SA treatment led also to reduction in both respiration rate and ethylene production, with the additional benefit of delaying the postharvest ripening process of this climacteric fruit. SA (applied at 1 mM for 5 min) was also effective in reducing CI in peaches by increasing endogenous PA content (Put, Spd, and Spm), this effect being higher when SA treatment was combined with hot air (38°C for 12 h) treatment (Cao *et al.*, 2010). In bamboo shoots, SA treatments (by dipping in 1 mM SA for 15 min) reduced CI symptoms, EL, MDA content, and disease incidence, while Put, Spd, and Spm increased in storage, these increases being higher than in control shoots (Luo *et al.*, 2012). SA (at 0.7, 1.4, or 2 mM) treatments applied by dipping for 10 min in pomegranate fruit were highly effective on reducing CI on the husk, although the role of PAs on these effects has not yet been elucidated (Sayyari *et al.*, 2009).

Since most of these treatments, which showed beneficial effects in alleviating chilling injury, were accompanied by increases in PAs, a particular role for endogenous PAs in increasing fruit tolerance to cold stress could be proposed. Thus PAs could work as free radical scavengers, stabilizing membranes by means of ionic interactions to provide protection against chilling stress, this effect



being greater as the number of positive charges per molecules is increased, that is, Spm > Spd > Put.

This hypothesis is supported by the fact that exogenous PA treatments after harvest but before cold storage decreased CI in chilling sensitive fruits, such as apple (Kramer et al., 1991), mango (Kondo et al., 2003; Nair and Singh, 2004), and zucchini (Martínez-Téllez et al., 2002). In zucchini fruits, Put treatment was more effective than those of Spd or Spm and its effects on improving fruit chilling tolerance have been attributed to enhancement of betaine and proline concentrations, which can act not only as osmoprotectants but also as a membrane stabilizer contributing to the stabilization and integrity of cellular membranes under chilling stress (Palma et al., 2015). Accordingly, CI was reduced in apricot fruits by Put and Spd treatments before storage at chilling temperatures, by increasing the activity of antioxidant enzymes, such as SOD, CAT, and peroxidase, which are involved in protecting plants from damage caused by ROS at low temperature (Koushesh-saba et al., 2012). Moreover, prestorage treatments of pomegranate with Put or Spd (1 mM), by immersion or pressure infiltration, decreased significantly the occurrence of CI after cold storage at chilling temperatures, which was related to increases in Put and Spd concentrations in the skin, these concentrations being three- and twofold higher, respectively, in treated than in control fruits (Mirdehghan et al., 2007a). This evidence suggests an activation of the PA biosynthesis pathway, with part of the exogenous Put being used to transform to Spd using DCSAM, while the increased concentration of Put after Spd treatment could be attributed to an upregulation of ADC, a key enzyme of one of the routes for Put biosynthesis. These treatments were also effective at maintaining the concentration of ascorbic acid, total phenolics, and total anthocyanins in the arils at higher levels than in control fruits, as well as the TAA, leading to increases in the health beneficial effects of fruit consumption (Mirdehghan et al., 2007c). In addition, 20 mM Spm spray treatment of whole branches of apple tree decreased low-temperature injuries of apple fruitlets, such as splitting and spotting, which are related to an increase in Put and Spd concentrations (Yoshikawa et al., 2007).

Thus the results presented here support the hypothesis that the PA treatments could induce acclimation of fruits to low temperature, and in turn protect them from CI. In this sense, PAs may be involved in reducing CI due to their ability to preserve membrane integrity, both by lowering the membrane phase transition temperature fluidity and by retarding lipid peroxidation, resulting in increased cell viability, due to their membrane-binding capacity and/or antioxidant properties. Thus the increases of PAs occurring

in chilling injured fruits could be a natural defense mechanism of fruit tissues against this stress, although this effect itself may not be totally accurate if the increase in PAs is not high enough.

## 6 Concluding Remarks and Future Trends

The results of this chapter provide evidence for the numerous beneficial effects of the exogenous PA treatments, both at pre- and postharvest time in fruit quality attributes including their concentration in antioxidant compounds. However, commercial application is nowadays limited, since no specific regulations exist regarding the use of exogenous PAs in Europe. Nevertheless, in the United States a patent was filed many years ago (Law et al., 1988) for the use of PAs as a method of extending shelf life and enhancing the quality of fruits. Taking into account that PAs are naturally occurring molecules their application as pre- or postharvest treatment could be considered as an environmentally compatible tool as they can be metabolized by fruit cells. In addition, it should be pointed out that although exogenous application of PAs enhances their endogenous levels, the concentrations remain far lower than the toxic ones. Finally, more extensive metabolic profiling is needed in order to gain deeper insight into the nutritional attributes of PA-enriched/-treated fruit. We are only now beginning to understand their role in growth, development, and senescence through molecular genetics and modern biochemical approaches, and the elucidation of PA roles in modulating pre- and postharvest biology will contribute to the development of functional foods using modern biotechnology. Then, modern agriculture, which is searching for effective biological molecules with well-known metabolic effects but without toxicological effects, may have the answer in PAs.

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