Induction of extended shelf-life of cucumber by polyamines

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ABSTRACT

The adequate potential of food and agriculture is a vital key to ensuring a healthy and secure future for the increasing population. The developed crop improvement program has moderately fulfilled the food demand, but with the growing population, the quality of food production needs to be improved to combat global hunger. Further, the process of preservation must be improved to nullify the issue of deterioration of fruits and vegetables, which are considered highly perishable commodities. The introduction of the postharvest technique gives an immense boost to this shelf-life extension of fruit and promotes sustainable agriculture. There is no doubt that multiple preservatives are available to fulfill these requirements. Nevertheless, a few of them may not be suitable due to their negative residual effects; instead, an exogenous coating that can extend the shelf-life period of fruits and delay the deterioration process is preferred. The core point behind the concept is to check the barrier of moisture, respiratory passage, and invasion of solutes through the skin of the fruit. A perfect coating would extend the shelf life without affecting its quality. In recent years, genetic, transcriptomic, and metabolomic research has revealed that different polyamines regulate tolerance to abiotic stress in complex ways. This chapter looks at polyamines as coatings, their biosynthesis, and how polyamines can be applied to cucurbits to confirm the slowing down of the ripening process and maintain the quality of the fruit. These techniques also provide a wealth of ideas to major vendors involved in the storage and transportation of perishable commodities.

Keywords: Exogenous coating, fruits, Polyamines, postharvest, shelf-life

Abbreviations:

Cadaverin: CAD; Putrescine: PUT; Spermidine: SPD; Spermine: SPM

INTRODUCTION

Polyamines are one of the earliest classes of compounds in biochemistry (Galston, 1991). Although, the tetramine spermine (SPM) was first detected in human spermatozoa over 300 years ago (van Leeuwenhoek, 1678), at least two diamine putrescines (PUT and CAD) were first discovered in putrefying corpses more than 100 years ago (Brieger, 1885). Further studies of PUT, SPM, and spermidine (SPD), the most abundant polyamines, were done in the 1920s, which demonstrated that these compounds are nitrogen-containing, and have a low molecular weight (Dudley *et al.,* 1926, 1927). The current view of PUT and SPD is that they are present in every living cell. SPM is also found in bacterial cells (Pegg and Michael, 2009), contradicting earlier assertions that this tetramine does not occur in prokaryotes. According to Illingworth et al., (2003), A portion of the polyamine biosynthesis pathway was assumed to have been acquired by plants from cyanobacteria. Hence, this is a metabolic route originating from ancient plants that are common to all organisms (Minguet *et al.,* 2008). Polyamines seem to be vital for life, based on a variety of studies. As a result, yeast, bacteria, and plants perish when PUT and/or SPD levels are reduced due to chemical or genetic reasons. (Hamasaki Katagiri *et al.,* 1998; Roberts *et al.,* 2001; Imai *et al.,* 2004; Urano *et al.,* 2005). Despite being viable, SPM deficiency causes various levels of malfunction in organisms. Despite not being necessary, SPM can nevertheless have a significant impact on growth and development (Minguet *et al.,* 2008; Yamaguchi *et al.,* 2007; Wang *et al.,* 2004).

Despite their protonation at normal cellular pH, polyamines are thought of as substances with structural properties due to their ability to bind different ionic macromolecules (DNA, RNA, chromatin, proteins) with their tautomeric shape. But it was later discovered that polyamines also function as regulatory molecules in a variety of essential biological functions, in addition to stabilising macromolecular structures (Alcázar *et al.,* 2006; Kusano *et al.,* 2008)). DNA and protein synthesis, cell proliferation and differentiation, cellular death, and gene expression are some of these processes (Seiler and Raul, 2005). An array of physiological processes in plants involving polyamines have been documented, including embryogenesis, fruit development and ripening, floral

initiation and development, organogenesis, leaf senescence, as well as biotic and abiotic plant stress retorts (Alcázar *et al.,* 2006; Kusano *et al.,* 2008).

Changes in plant polyamine metabolism can be brought on by a variety of abiotic stressors (Bouchereau *et al.,* 1999; Alcázar *et al.,* 2006; Groppa and Benavides, 2008). PUT may make up 1.2 percent of the plant's dry matter or at least 20 percent of its nitrogen in stressed plants as a gauge of how crucial this process is (Galston, 1991). It is still unclear how increased polyamine levels relate to abiotic stress responses (Alcázar *et al.,* 2006; Kusano *et al.,* 2008; Gill and Tuteja, 2010). Global 'omic' techniques have been utilized to uncover genes influencing polyamine production and signaling pathways using the Arabidopsis genome sequence. Analysis of loss and gain of function mutations can provide insight into polyamine processes. Polyamines, such as abscisic acid (ABA), which modulates abiotic stress responses, are thought to operate as cellular signals and interconnect hormonal networks. Polyamines have also made it easier to generate stress-resistant Arabidopsis transgenic plants thanks to breakthroughs in understanding their molecular roles. Even though stress-resistant cultivars using naturally occurring chemicals are at the heart of sustainable agriculture, there are currently several barriers to their application to profitable crops. By further exploiting natural variability, we expect to uncover new avenues for research related to both fundamental and applied plant PT.

A GLIMPSE OF POLYAMINE BIOSYNTHESIS

Research has indicated that polyamine levels are largely controlled by anabolic and catabolic processes in plants in addition to their link with hydroxycinnamic acids. In Figure1, these processes are shown in schematic representation, along with their connections with other metabolic pathways. Put is the catalyst for the initiation of polyamine biosynthesis. In mammals, it is generated from ornithine by enzymes such as ornithine decarboxylase. A different pathway exists for the formation of polyamines in plants and bacteria, in which arginine is decarboxylated by arginine decarboxylase (ADC). The further two steps were catalysed by agmatine iminohydrolase (AIH) and N-carbomylputrescine amidohydrolase (CPA) (Slocum *et al.,* 1984). When Arabidopsis homologous ADC2 is overexpressed, polyamines accumulation is considerably enhanced, suggesting that ADC is the limiting step in PUT biosynthesis (Alcázar *et al.,* 2005). ADC is mainly found in the chloroplasts of the thylakoids of oats (Borrell *et al.,* 1995), whereas it is more commonly found in the nucleus of tobacco tissues (Bortolotti *et al.,* 2004). ADC pathway compartmentalization occurs within cells in plants, which may cause gradient Put concentrations within cells. Polyamine transport genes have been discovered in yeast and E. coli (Igarashi and Kashiwagi, 2000). In plants, however, no transport or shuttle mechanism has yet been discovered. Several recent studies suggest different evolutionary origins of ADC/ODC (ornithine decarboxylase) pathways. Hence, ADC, AIH, and CPA in plants might all descend from cyanobacterial ancestors, while ODC might be a combination of bacterial genes and cyanobacterial endosymbionts from a common ancestor of plants and animals (Illingworth *et al.,* 2003). The PUT thus formed from ADC and/or ODC can successively make a precursor for triamine SPM and SPD. The use of aminopropyl groups can be achieved by using SPM synthases. D-adenosylmethionine, the aminopropyl group donor is formed upon the decarboxylation of SAM (S-adenosylmethionine (Slocum *et al.,* 1984) (**Fig. 1**).

Fig. 1. Glimpse of polyamine biosynthesis and its association with nearby metabolic pathway. The respective alphabets designate individual enzymes. The arrow marks represent the flow of the biosynthetic process. **(A)** glutamate N-acetyltransferase, **(B)** acetyl glutamate kinase, **(C)** N-acetyl--phosphate reductase, **(D)** acetylornithine transaminase, **(E)** acetylornithine deacetylase, **(F)** ornithine-carbamoyl transferase, G: arginosuccinate synthase, **(H)** arginosuccinate lyase, **(I)** arginine decarboxylase, **(J)** agmatine iminohydrolase, **(K)** N-carbamoylputrescine amidohydrolase, **(L)** ornithine decarboxylase, **(M)** SAM synthetase, **(N)** SAM decarboxylase, **(O)** spermidine synthase, **(P)** spermine synthase, **(Q)** thermospermine synthases, **(R)** glutamate decarboxylase, **(S)** diamine oxidase, **(T)** arginase, **(U)**: ornithine aminotransferase.

All living organisms have been traditionally thought of as having an ODC pathway. The ODC gene has been identified in most species of plants examined and the genes encoding ODC have also been identified (Michael *et al.,* 1996; Imanishi *et al.,* 1998). Contrary to what you might expect, the arabidopsis plant does not contain the gene for ODC and the enzyme activity associated with it has not been detected (Hanfrey *et al.,* 2001). Due to this, PUT is only produced in Arabidopsis via the ADC pathway. Despite having duplicates of ADC genes (ADC1 and ADC2), Arabidopsis has only one gene for CPA and AIH (Janowitz *et al.,* 2003; Piotrowski *et al.,* 2003). A single spermidine synthase gene (SPMS) has been identified in the Arabidopsis genome, as well as four SAM decarboxylases (SAMDC 1-4; Urano et al., 2003) and two spermidine decarboxylases (SPDS1 and SPDS2). There has been a discovery of metabolon formed by the cooperative action of three aminopropyltransferases in Arabidopsis (Panicot *et al.,* 2002). The activity of SPDS effectively channels SPD to SPMS, which in turn regulates the synthesis of SPM.

THE CUCURBITACEAE FAMILY
The Cucurbitaceae family consists of approximately 800 species spread across 130 genera (Esteras et al., 2012). Several genera of edible plants have been identified, such as Cucumis, Cucurbita, Lagenaria, Benincasa, Citrullus, Sechium, and Momordica (Lebeda *et al.,* 2007; Rolnik and Olas, 2020). Watermelons, melon, cucumbers, squashes, and pumpkins are among the most cultivated crops worldwide. Researchers found that genotype plays a significant role in modulating antioxidant content between cultivars of Cucurbita spp., Citrullus lanatus (Thunb.) (Matsun) Et Nakai), and Cucumis melo (L.) (Kostecka-Gugała *et al.,* 2020; Yoo *et al.,* 2012; Salehi *et al.,* 2019). Nevertheless, antioxidant content varies with growing conditions and ripening stages. In addition to their major crop species, there are several minor plant species of regional importance in the genus Cucurbita (Esteras *et al.,*

2012; Lebeda *et al.*, 2007). Cucurbita's shoots, leaves, fruits, and seeds are not only full of health advantages but also a great source of nutrients (Yadav *et al.*, 2010; Benariba *et al.*, 2019). The genus has also been recognized as a source of antioxidants, and several species have been used in traditional medicine (Caili *et al.*, 2006). Phytosterols and carotenoids are among the compounds present in their fruits, including β-cryptoxanthin, α- and β-carotene, zeaxanthin, and lutein (Boiteux *et al.*, 2007; Rodriguez-Amaya *et al.*, 2008). Carotenoids such as β-carotene and α-carotene dominate, respectively containing 61.6 g/g dry weight and 29.3 g/g dry weight. Cultivars differed in their content of α-carotene and β-carotene, as well as in their maturation state and extraction method. Carotenoid concentration for mature fruits of *C. maxima* varied from 63.7 mg/100g (acetone) to 132.2 mg/100g (ethanol/olive oil) dry weight due to a combination of cultivars and extraction techniques (Shi *et al.*, 2013; Wang *et al.*, 2011). A report on pumpkins (*C. maxima* and *C. pepo*) found that the total carotenoid content ranged from 5 to 160 mg/100 g dry weight (Nawirska *et al.*, 2009). In addition, homogeneous antioxidant capacities of *C. pepo*, *C. moschata*, *C. maxima*, and *C. ficifolia* were compared, indicating substantial intraspecies variation within the genus. There was a high level of phenolic compounds in the fruit of the Japanese *C. moschata* 'Kogigu' (70.8 mg/100 g fresh weight, FW). The antiradical and anti-oxidant properties of additional widespread cultivar, Hokkaido, were the most impressive. Indomatrone and Bambino - The highest total polyphenol content was found in the cultivars *C. maxima*, which had values of 50.4 and 41.6 mg/100 g FW of chlorogenic equivalents (CAE), respectively. Additionally, the 'Indomatrone' fruit contains more salicylic acid (2.56 mg/100 g FW) than any other fruit. It was found that the phenolic content of *C. pepo* varied from variety to variety, with Kamo having the highest (51.5 CAE/100 g FW), and Sweet Dumpling having the lowest (48.1 CAE/100 g FW). A total phenol content of 20.6 CAE/100 g FW was detected in the 'Angel Hair' of *C. ficifolia* (Kostecka-Gugała *et al.*, 2020).

POSTHARVEST STUDIES IN CUCURBITS

Several species of the Curcurbitaceae family contribute to the human diet, including some widely cultivated and wild species including cucumbers, melons, and squashes. Few postharvest studies of cucurbits are mentioned below:

Coccinia grandis:

Ivy gourds are beneficial and provide a respectable source of calcium, protein, and fiber as well as vitamins and β carotene (Suresh-Babu and Rajan, 2001). It possesses medicinal values such as antioxidant properties, anti-hyperglycemic, and antihyperlipidemic (Attanayake *et al.,* 2016). The untreated ivy gourd may sustain for a period of 5 days as shown in **Fig. 2**. Guar gum and alginate coating have been applied to extend the shelf-life of ivy gourd for a period of 9 days after post-harvest (Gundewadi *et al.,* 2017). This is the only postharvest study on this vegetable.

Cucumis sativus:

Cucumber is prominently used as salad. Its demand in daily life as well in the hotel industry is impeccable. Because cucumbers are perishable, they cannot be stored for more than a month (Todd *et al.*, 2000). The general survival period of cucumber (without any coating) is 6 days after its harvest (Figure2). Therefore, its shelf-life extension is of utmost essential for vendors along with the users. A study on the shelf-life extension of cucumber gives an indication of the need for certain edible coatings (beeswax) along with Equilibrium Modified Atmosphere storage conditions can able to store it for two weeks (Hima *et al.*, 2018). Amer and Azam (2018) use the hot water treatment prior to refrigerated conditions. This treatment, however, can only extend the cucumber's shelf life by 9 days. Using corn starch, tulsi aqueous extract (1.5% &2%), and Ziziphus mauritiana leaf extract, Bakliwal et al., (2019) improved cucumber shelf-life to 21 days by treating the cucumbers with herbal edible coating. Patel and Panigrahi (2019) suggest that cucumbers can be extended in shelf-life for a period of 30 days by treating them with 1.5% starch plus 2.5% D-glucose. This coating lowers the respiration rate, resulting in longer shelf life. A recent study (Kaur *et al.*, 2021) on cucumber post-harvest can result in 21 days, similar to Bakliwal *et al.*, (2019). After the LDPE packing, they installed the cucumber evaporative cool shop.

Lagenaria siceraria:

The bottle gourd, or *Lagenaria siceraria* (Mol.) Standl. is one of the most significant and popular vegetables in India. In general, fruits *of L. siceraria* contain all of the essential elements for human health and well-being (Kubde *et al.*, 2010). The authors of Azcon *et al.*, (1975) attributed the physiological and biochemical changes in bottle gourd fruit to the effects of different sources of nutrients, such as organic/inorganic and integrated. In *L. siceraria* fruits, post-harvest losses are caused by microbial spoilage, improper packaging, and handling during transport (Habib-ur-Rahaman, 2003). As part of their experiments, Singh *et al.*, (2010) presented a post-harvest study on L. siceraria with LDPE as packaging material and succeeded in extending its life up to 12 days. In contrast to this study, **Fig. 2** provides an idea of the shelf-life of control *L. siceraria* is 14 days. However, the treatment of *L. siceraria* fruits with gallic acid and calcium chloride (in 1:1, 2:2, 3, and 4:4 μM) can enhance their shelf life to 21 days (Suthar *et al.*, 2021). In their study, the main aim was to examine the viability, sensory evaluation,

antioxidant enzyme activities, and vitamin C content of *L. siceraria* fruits after 21 days of storage at room temperature under a calcium chloride-gallic acid combination. Among the treatments, the 2:2 μM CG combination was superior in every way. By delaying the ripening process, the combination of calcium chloride and gallic acid proved advantageous.

Luffa aegyptiaca:

The sponge gourd is considered to provide a soothing effect to the stomach, a stimulant to the genital parts along with useful for the intestine (Porterfield, 1995). As per **Fig. 2** (our observation), it survives for a period of 9 days after harvest and without any treatment. However, Han *et al.,* (2013), Sponge gourds' shelf life have only been increased by 10 days. It needs to be looked into again, possibly using different coatings.

***Momordica charantia*:**

It is a rich source of minerals and vitamin C alongside its anti-diabetic features. The untreated fruits can survive for a maximum period of 14 days (**Fig. 2**)., Although processed bitter gourds were the subject of chemical treatments studies (Devi *et al.*, 2019; Srilatha *et al.*, 2021), there were no instances of postharvest removal of the entire fruit.

***Trichosanthes dioica*:**

As a vegetable, a pointed gourd is undeniably useful. But it has certain medicinal values that can target skin disease, alopecia, epilepsy, and diabetes (Adiga *et al.,* 2010). By applying a wax coating, Patel *et al.,* (2013) were able to extend the pointed gourd's shelf life for a duration of 6 days. This is only a 1-day extension from the untreated pointed gourd (**Fig. 2**).

POLYAMINES AS COATING:

Polyamines such as putrescene, spermine, and spermidine are considered to possess antisenescence features (Kumar *et al.,* 1997). However, the level of these polyamines starts decreasing during the aging process of fruits. This leads to a drastic reduction in texture and directly to the shelf-life of fruits. The successful use of polyamines as the exogenous coating was reported observed in the extension of shelf-life in fruits such as apricot (Martínez-Romero *et al.,* 2002); plum (Pérez-Vicente *et al.,* 2002), mango (Malik and Sing, 2005), strawberry (Khosroshahi, Esna-Ashari, & Ershadi, 2007); and capsicum (Patel *et al.,* 2019). In low-temperature conditions, the accumulation of putrescene is believed to lessen chilling injury, perhaps through polyamines (Kramer *et al.,* 1989). Low temperature acclimated citrus plants show lower SPD and SPM as well as a uniform and substantial increase in PUT (McDonald and Kushad, 1986).
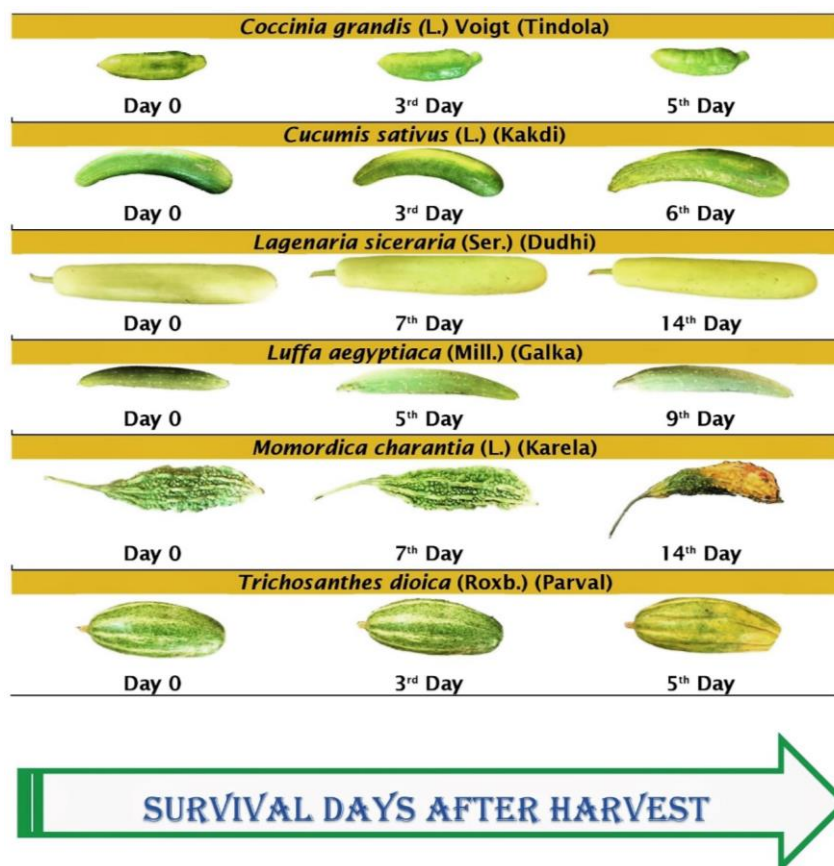

Fig. 2. Survival period of a few Cucurbits.

CONCLUSION:

The above review gives an idea of the benefits of using polyamines for the shelf-life extension of fruits and vegetables. Polyamines have yet to be documented as an exogenous cucumber coating for extending postharvest shelf life.Thus, the polyamines such as PUT, SPD, and SPM can be applied in different compositions and may amalgamate with others to target the post-harvest studies, particularly in cucumbers. As a result of the foregoing studies, it may be prudent to employ a plant-based substance, such as polyamines, as a coating to lengthen the shelf life of vegetables and fruits.

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