Effect of postharvest treatments with polyamines on physiological and biochemical attributes of kiwifruit (Actinidia deliciosa) cv. Allison

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Abstract — Introduction. Kiwifruit is a typical climacteric fruit, which grows profitably well in sub-tempereated climatic conditions. The major problem with kiwifruit is that it does not ripen for about a week at room temperature but after this period there is a sudden rise in the respiration rate and fruits remain in edible condition for only a few days, giving it a very short shelf life in ambient conditions. Materials and methods. Kiwifruits were treated with different concentrations of spermine [(0.5, 1.0 and 1.5) mM] and spermidine [(1.0, 1.5 and 2.0) mM] by the immersion method; biochemical and physiological analyses were carried out, then fruit were stored in ambient conditions for 15 days. Results and discussion. Polyamine-treated fruits did not show any amount of ethylene evolution up to the 6th day of storage as compared with those of control, in which it started after the 3rd day of storage. The respiration rate was also the lowest in treated fruit. Similarly, polygalacturonase and lipoxygenase activities rose rapidly in control fruits in comparison with polyamine-treated fruits. Conclusion. The doses of spermine at 1.5 mM and spermidine at 2.0 mM showed the best results in extending the shelf life of kiwifruits when stored in ambient conditions [(22 ± 4) °C, RH: (65 ± 5)%].
1. Introduction

Kiwifruit (*Actinidia deliciosa*) is a very important fruit, as it has a sweet-sour taste, high nutritional value and a unique appearance. It is an excellent source of potassium, vitamin C and vitamin E and has low sugar content. Hence, it is an ideal fruit for diabetic, hypertension and asthma patients [1]. In India, it was introduced in the early sixties but its importance was realised only in recent years and now its area is expanding day by day.

Kiwifruit is a high-value crop but its popularity has not been realised, primarily because its fruits have the problem of premature ripening and flesh softening and it remains in a marketable stage for only a few days [2]. Fruit softening is caused by several physiological and biochemical changes taking place in the fruit after harvesting and during handling, storage or transportation. So, with this concern, there is a need for developing treatments or procedures which reduce its softening by delaying ripening or interfering with physiological and biochemical changes, especially enzymatic activities, to increase its shelf life, which would be very useful for extending its availability in the market.

Different techniques are used to extend the shelf life of fruits in different countries. However, in recent years, scientists the world over have used 1-MCP [3, 4] and polyamines (PAs) for extending the shelf life of some fruits [5, 6]. Polyamines are low-molecular-weight small aliphatic amines that are used in manipulating a wide range of biological processes in plants and in post-harvest management of horticultural crops [7]. The most common polyamines are putrescine (PUT), spermidine (SPD) and spermine (SPM). Polyamines can delay senescence in fruits by inhibiting 1-amino-cyclopropane-1-carboxylate (ACC) synthesis [8], which retards the evolution of ethylene, a ripening hormone. Any technique or method which inhibits or reduces the ethylene evolution is regarded as being quite useful for extending the shelf life [3, 6]. Thus, use of polyamines can be an effective tool for delaying ripening and fruit softening by inhibiting activities of deteriorative enzymes such as polygalacturonase (PG) and lipooxygenase (LOX), which would increase the shelf life of perishable produce. However, to our knowledge, polyamines have not yet been used for regulation of ripening in kiwifruit, which is urgently required for obtaining the useful benefits of polyamines. Thus, the major objective of our study was to investigate the effect of different concentrations of the polyamines spermidine and spermine on several physiological and biochemical attributes of kiwifruit, which are indicators of fruit softening, ripening and overall fruit quality. The outcome of our studies will be of immense importance for the kiwifruit growers on one hand, and consumers on the other hand.

2. Materials and methods

2.1. Fruit material

Kiwifruits cv. Allison were procured from the fruit orchard of Dr. Y.S. Parmar [Univ. Hortic. For. Solan (H.P.), Indial. The fruits were harvested at appropriate maturity (TSS: 6.2 °Brix). The fruits were sorted, graded, packed in corrugated fibreboard boxes cushioned with paper shavings, and then transported to the Division of Postharvest Technology at the Indian Agricultural Research Institute (IARI), New Delhi. In the laboratory, fruits were again sorted to remove bruised and defective ones. They were then treated with different concentrations of spermidine (SPD at 1 mM, SPD at 1.5 mM and SPD at 2.0 mM) and spermine (SPM at 0.5 mM, SPM at 1.0 mM and SPM at 1.5 mM).

2.2. Treatment of fruits with polyamines

Fruits of ‘Allison’ kiwifruit were divided into four lots and each lot contained 120 fruits, which was replicated three times. Different concentrations of spermidine and spermine were prepared by weighing the known amount of the respective polyamines and dissolving them in distilled water. The fruits
were dipped in individual solution for 2 min and after removing from the solution fruits were air-dried under a fan. The fruits in the control were dipped in distilled water for 2 min. The treated as well as untreated fruits were stored at room temperature [(22 ± 4) °C and 65–70% R.H.] for 15 days. Different physiological (respiration and ethylene evolution rate) and biochemical (total phenol, antioxidant activity, polygalacturonase and lipoxygenase activity) parameters of these fruits were monitored at 3-day intervals during storage.

2.3. Ethylene evolution and respiration rates

Ethylene production and respiration rates were measured using the static headspace technique. Two fruits from each replication were selected at random and enclosed in a hermetically sealed container (1,000 mL), fitted with a silicon rubber septum, for 1 h or less. The concentrations of O₂ and CO₂ were recorded in the headspace of the container using a headspace gas analyser (Model: Checkmate 9900 O₂/CO₂, PBI Dansensor, Denmark) and expressed as mL CO₂·kg⁻¹·h⁻¹. To determine ethylene, one mL of the headspace atmosphere of the container was withdrawn with a gas-tight syringe and injected into a gas chromatograph (Model HP 5890, Hewlett Packard, USA) which was calibrated using standard ethylene gas (Laser Gases, New Delhi). The gas chromatograph was equipped with a Porapak-N (80–100 mesh) column and a flame ionisation detector (FID). Nitrogen was used as the carrier gas at a flow rate of 50 mL·min⁻¹, while hydrogen and air were the fuel gases at flow rates of 25 and 250 mL·min⁻¹, respectively. The temperatures in the injector, column and detector were maintained at (110, 60 and 275) °C, respectively, and the rate of ethylene evolution was expressed as µL·kg⁻¹·h⁻¹.

2.4. Total phenols

The total phenolic content of the fruit extracts was determined by the Singleton and Ross method [9] with some modifications. Five g of fruit extract were crushed in 10 mL of 80% ethanol and then centrifuged. The homogenate was centrifuged at 15,000 g for 20 min at 24 °C and the supernatant was used for assay of total phenols. An aliquot of 0.5 mL of the sample was added to 2.5 mL of 0.2 N Folin-Gioclateau (FC) reagent and left for 5 min. Two mL of 20% of Na₂CO₃ was then added and the total volume made up to 25 mL using 80% ethanol. The above solution was then incubated in a boiling water bath for 15 min until it became blue-black. Absorbance was measured at 760 nm using a 1-cm cuvette in a Perkin-Elmer UV-VIS lambda 25 spectrophotometer. Gallic acid (0–800 mg·L⁻¹) was used to produce a standard calibration curve. The total phenolic content was expressed in mg of gallic acid equivalents (GAE) per 100 g of extract.

2.5. Antioxidant activity

Antioxidant activity in kiwifruit was determined by following the CUPric Reducing Antioxidant Capacity (CUPRAC) method, which was standardised by Apak et al. [10]. To determine antioxidant activity, copper (II) chloride solution, a neocuproine alcoholic solution, and an ammonium aqueous buffer (pH 7) were mixed and then measurements of the developed colour were taken after 30 min in a spectrophotometer at absorbance at 450 nm. To a test tube, 1 mL each of copper (II) chloride solution (10⁻² M), neocuproine solution (Nc) of 7.5×10⁻³ M, and ammonium acetate (NH₄Ac) buffer (pH 7) solution were added. Antioxidant sample (or standard) solution (0.1 mL) and H₂O (1.0 mL) were added to the initial mixture so as to make the final volume 4.1 mL. The tubes were then capped and after 1 h, the absorbance at 450 nm was recorded against a reagent blank. The standard calibration curve of each antioxidant compound was constructed as absorbance versus concentration. The molar absorptivity of the CUPRAC method for each antioxidant was found from the slope of the calibration line concerned and the antioxidant activity was expressed as µmol Trolox·g⁻¹.
2.6. Polygalacturonase activity

Polygalacturonase (PG) activity in kiwifruits was measured following the method of Lazan et al. [12] with minor modifications. One gram of kiwi pulp was weighed and homogenised in 10 mL sodium acetate buffer (0.2 M, pH 6.0) with a pinch of Na₂S₂O₄ and polyvinylpyrrolidone (PVP) in a chilled mortar. The homogenate was centrifuged at 15000 g for 20 min at 4 °C and the supernatant was used for the assay of polygalacturonase (PG) activity. The PG enzyme assay mixture consisted of 0.45 g of pectin and 0.1 g casein dissolved in 0.4% sodium acetate buffer (pH 3.8) and then the solution was diluted to 100 mL with 0.4% sodium acetate buffer (pH 3.8). To measure the PG enzyme activity, 0.2 mL of enzyme extract was added to 2 mL of assay mixture and incubated at 37 °C for 2 h. From this incubated mixture, 0.05 mL was added to 1 mL 5% phenol, followed by 5 mL of 96% H₂SO₄, which was poured over the mixture and allowed to react for 15 min. The content was diluted with 5 mL distilled water, thoroughly mixed and cooled to room temperature. The absorbance was recorded at 490 nm in a spectrophotometer (double beam UV-VIS spectrophotometer UV5704SS). The blank was prepared by adding distilled water instead of enzyme extract to the assay mixture. The reagents used were sodium acetate buffer (0.2 M, pH 6.0), 0.4% sodium acetate buffer (pH 3.8) and 5% phenol solution. Polygalacturonase activity was expressed as µg galacturonic acid fresh weight·g –1·h–1 (OD at 288.07 nm).

2.7. Lipoxygenase activity

Lipoxygenase (LOX) activity in kiwifruits was measured following the method of Axelrod et al. [11] with minor modifications. Preparation of crude enzyme extract: one g of kiwifruit pulp was weighed and homogenised in 10 mL of EDTA which was iced-cooled in a prechilled pestle and mortar. The homogenate was centrifuged at 15000 g for 20 min at 4 °C and the supernatant was used for assay of lipoxygenase activity. Enzyme assay was carried out; firstly, 50 µL of enzyme extract were added to 2.975 mL of substrate solution in a cuvette and readings were recorded for 3 min at 30-sec intervals. Absorbance was recorded at 234 nm in a spectrophotometer (double beam UV-VIS spectrophotometer UV5704SS). The blank was prepared by using a substrate solution. Before this, a substrate was prepared by taking 35 µL Tween-20 in a beaker. To this, linoleic acid was added and 0.2 M NaOH was added to dissolve it completely. Once all of the linoleic acid was dissolved, the final volume was made up to 100 mL by using 0.1 M phosphate buffer (pH 6.5) and then the pH was brought down to 6.5 by adding 0.2 M HCl. The reagents used were EDTA 0.5 M and phosphate buffer (0.1 M, pH 6.5). Lipoxygenase activity was expressed as µmol·min –1·g–1 fresh weight.

2.8. Statistical design and analysis of data

The experiment was laid out in a factorial CRD design with each treatment consisting of 120 fruits with three replications. The data obtained from the experiments were analysed as per design and the results were compared with ANOVA by calculating the coefficient of deviation (Panse and Sukhatme [13]).

3. Results and discussion

3.1. Effects of polyamines on respiration rate

Respiration rate is a major metabolic process taking place in harvested produce. It is a basic process of life, which is directly related to maturation, handling, transportation and subsequent storage life. In our investigation, we found that the kiwifruit treated with spermine at 1.5 mM showed the lowest respiration rate, significantly followed by spermidine at 2.0 mM (figure 1). The effect of the anti-senescence properties of polyamines and favourable water activity might have lowered the respiration activity in treated fruits. Khan et al. have also
reported that putrescine retarded fruit softening in ‘Angelino’ plums during low-temperature storage [14]. In our studies, the respiration rate increased significantly with the increase in the storage period, which may be due to utilisation of accumulated sugars through respiration, as one theory indicates that there is always accumulation of sugars in the fruit tissue and this has to be reduced by burning of the produced sugars by the process of respiration. Although, as of now, it is not clear as to why there is an increase in sugars with the increase in storage period. Our studies are in line with the findings of Malik and Singh [15] and Khan and Singh [16], who reported that polyamine-treated mango and plum fruits, respectively, exhibited a significantly lower mean respiration rate as compared with controls, and hence they ripened more slowly than untreated fruits.

3.2. Effects of polyamines on ethylene evolution

Being a climacteric fruit, kiwifruit has been reported to be a high ethylene generator [17]. Our investigations indicated that untreated fruits evolved quite a high amount of ethylene from the 3rd day onwards but polyamine-treated fruits showed no evolution of ethylene until the 6th day of storage, with spermine at 1.5 mM being the most effective, followed by spermidine at 2 mM (figure 1). Bouchereau et al. concluded that spermidine is the most effective superoxide radical scavenger, and is considered to be helpful in ethylene synthesis and evolution [18]. Thus, lower ethylene evolution in polyamine-treated fruits may be due to suppression of ethylene evolution. It is well documented that polyamine and ethylene biosynthesis are linked through the common precursor S-adenosylmethionine (SAM), and they use the common precursor SAM for their biosynthesis, but these two molecules show opposite effects in relation to senescence [7, 19]. The ethylene biosynthesis could also be modulated by the *in vivo* biosynthesis of polyamines since ethylene and polyamine biosynthetic pathways share SAM as a common intermediate and could compete for the available SAM during senescence [19]. Further, in untreated fruits, ethylene evolution showed its highest peak (57.5 C2H4 µL·kg⁻¹·h⁻¹) on the 12th day and then it declined thereafter, whereas in polyamine-treated fruits ethylene evolution showed a significantly increasing trend with the increase in storage period but at a slower rate (figure 1). Ethylene evolution increased with increase in storage period, which may be due to increased senescence, which triggered ethylene evolution [20]. Our study corroborates those of other authors who reported reduction in ethylene production following polyamine treatments in peach [20] and apricot [21].
3.3. Effects of polyamines on total phenols

We observed that, in kiwifruit, total phenols decreased with the increase in storage period, and were significantly lower in untreated fruits than in polyamine-treated fruits. Total phenols in kiwifruit cv. Allison decreased from the 3rd day onwards (33.9 mg·100 g–1) up until the 15th day of storage (14.3 mg·100 g–1) (figure 2). This decline in phenolics with advancement of the storage period may be attributed to the activity of polyphenol oxidase (PPO) [22]. The loss in astringency which occurs during ripening is probably connected with increased polymerisation of tannins, as reported by Mellenthin and Wang [23]. Further, fruits receiving spermine at 1.5 mM showed the highest mean total phenols (29.8 mg·100 g–1), significantly followed by those which received spermidine at 2.0 mM (28.9 mg·100 g–1) (figure 2). The slower rate of degradation of phenolics in polyamine-treated fruits apparently indicates that they play an important role in delaying the activity of polyphenol oxidase enzymes due to delay in the respiratory activity of the fruits. Seiler and Raul [5] and Kuasno et al. [6] have reviewed the role of polyamines in plants and reported that they help to maintain or increase the content of phenolic compounds in fruits. Similarly, Mirdehghan et al. have reported that polyamine application maintains functional properties in stored pomegranate arils [24].

3.4. Effects of polyamines on antioxidant capacity

Fruits act as free radical scavengers by donating a pair of electrons and neutralising free radicals, which are oxidising in nature and harm-causing agents. We observed from our studies that the antioxidant capacity in polyamine-treated fruits was better than in untreated fruits, in which we could notice a rapid loss in antioxidant capacity. Antioxidant capacity of plant produce is mainly because of the presence of pigments, vitamins and phenols. These antioxidants are well preserved by polyamines, which are basically anti-senescence agents [5–7]. Thus, it is easily understandable that the higher retention of pigments, vitamins and phenols in treated fruits will certainly contribute towards the higher antioxidant capacity of the treated fruits compared with control. Among the different fruits treated with polyamines, fruits that were pre-treated with spermine at 1.5 mM showed the best antioxidant capacity (31.0 µmol Trolox·g–1); they were equally followed by those pre-treated with spermidine at 2 mM (30.0 µmol Trolox·g–1)
Antioxidant capacity decreased significantly with the increase in storage period (figure 3). This is supported by the study of Connor et al. in blueberry (Vaccinium sp.), who reported a decrease in antioxidant capacity with the increase in storage period [25]. There is a controversial debate in the literature about the influence of vitamin C on the antioxidant capacity of fruit and vegetables [26]. On the other hand, it is also known that fruit with high antioxidant capacity generally contain more antioxidants and most of these antioxidants have been shown to be phenolic compounds, and in particular flavonoids [25, 26]. Tavarini et al. studied and reported correlation studies on antioxidants and phytochemical constituents responsible for antioxidant capacity in kiwifruit, and suggested that, in kiwifruit, vitamin C contributed to antioxidant capacity much more than other antioxidant constituents (phenols or carotenoids) [27]. On the other hand, kiwifruit is characterised by a high content of vitamin C and a small amount of phenolics [28].

3.5. Effects of polyamines on polygalacturonase activity

Polygalacturonase (PG) is an important enzyme responsible for fruit softening. With decreased PG activity, fruit softening is delayed, thereby increasing the shelf or storage life. Our study indicated that untreated fruits exhibited much higher PG activity than polyamine-treated fruits, and fruits receiving spermine at 1.5 mM showed the lowest PG activity, followed by spermidine at 2.0 mM (figure 4). Such effects of polyamines on PG activity can be explained on the basis of their role in the plant system. It is well known that polyamines play an important role in increasing fruit firmness by regulating the enzyme polygalacturonase, which is the key enzyme involved in the hydrolytic cleavage of α-(1→4)-galacturonic linkage [29] and is responsible for pectin disassembly during fruit ripening [30]. The effect of polyamines on maintaining fruit firmness can also be attributed to their cross-linkage to the carboxyl group of the pectic substances in the cell wall, resulting in rigidification, which blocks the access of degrading enzymes (PG) and thereby reduces the rate of fruit softening [31]. Among the different treatments of polyamines, fruits receiving spermine at 1.5 mM showed the lowest PG activity (53.3 µg-galacturonic acid-FW g⁻¹·h⁻¹), followed by spermidine at 2.0 mM (52.3 µg-galacturonic acid-FW g⁻¹·h⁻¹) (figure 4). For the polyamine-treated fruits, polygalacturonase activity increased significantly with storage (108.5 µg-galacturonic acid-FW g⁻¹·h⁻¹) and then declined sharply from the 15th day (42.2 µg-galacturonic acid-FW g⁻¹·h⁻¹) of storage (figure 4). The increase in PG activity with the progressive increase in storage time may be ascribed to decreased fruit firmness and increased softening of the fruits by the loss of membrane integrity [30].
3.6. Effects of polyamines on lipoxygenase activity

Lipoxygenase (LOX) is considered as one of the major enzymes responsible for senescence and membrane deterioration in a number of plant tissues [32]. In our study, interestingly, we observed that the LOX activity in kiwifruit increased from the 3rd day to its peak on the 12th day of storage for the polyamine-treated fruits but, in fruits treated with spermine at 1.5 mM and spermidine at 2.0 mM, it was postponed until the 15th day of storage (figure 4). Among the different treatments of polyamines, fruits receiving spermine at 1.5 mM and spermidine at 2.0 mM showed equal and the lowest LOX activity (4.1 µmol·min⁻¹·g⁻¹ FW), but the other treatments of spermine and spermidine gave intermediate results and showed their respective peaks on the 12th day, showing a slight improvement over their untreated counterparts. Tadolini observed that polyamines act as antioxidants by inhibiting lipid peroxidation, which is accounted for by the ability of polyamines to form a ternary complex with iron and the phospholipid polar heads that may change the susceptibility of Fe²⁺ to auto-oxidation and thus its ability to generate free oxygen radicals [33]. Similarly, Borrell et al. suggested that inhibition of lipid peroxidation may be one of the mechanisms responsible for the anti-senescence effects of the polyamines [34]. The increase in LOX activity with the increase in storage period may be due to the progressive increase in fruit softening.

4. Conclusions

From our studies, it can be concluded that all polyamines had a significant effect on fruit ripening, total phenols, antioxidant capacity and softening over control, with no adverse effect on quality parameters of kiwifruit cv. Allison. Of the polyamines, spermine was much more effective than spermidine. Of the different concentrations of polyamines, spermine at 1.5 mM and spermidine at 2 mM were the most effective doses, which delayed fruit ripening by about 6 days, with a significant and effective increase in some quality parameters over control, thereby increasing the postharvest life of Allison kiwifruit by 6 days. Such fruits were acceptable for up to 15 days under ambient conditions in comparison with 9 days in control.

References

Polyamine treatments influence quality attributes of kiwifruit


Efecto de tratamientos post-cosecha con poliaminas sobre las características fisiológicas y bioquímicas de kiwis (Actinidia deliciosa).

Resumen — Introducción. El kiwi es un fruto típicamente climacético, que crece de manera rentable en condiciones subtempladas. El principal problema de este fruto es que, en temperatura ambiente, no madura durante cerca de una semana, pero, después de este periodo, su índice de respiración crece rápidamente y el fruto sólo queda comestible durante unos días, lo que se traduce por una duración de vida muy corta. Material y métodos. Después de la cosecha, se trataron frutos de kiwi mediante inmersión con diferentes concentraciones de espermina [(0,5, 1.0 y 1,5) Mm] y espermidina [(1,0, 1,5 y 2,0) Mm]; se realizaron análisis bioquímicos y fisiológicos durante el almacenamiento de los frutos en condiciones ambientales durante 15 días. Resultados y discusión. Hasta el 6º día, el índice de etileno desprendido por los kiwis tratados con una poliamina fue nulo, a continuación progresó, mientras que, para los frutos testigo, la evolución empezó desde el 3º día. La respiración de los frutos tratados también fue menor que la de los frutos testigo. Igualmente, las actividades de la poligalacturonasa y de la lipoxigenasa aumentaron rápidamente en los frutos testigo, en relación con los frutos tratados con una poliamina. Conclusión. Las dosis de tratamiento de los frutos con espermina a 1,5 mM y espermidina a 2,0 mM mostraron los mejores resultados; permitieron prolongar las duraciones de vida de los kiwis almacenados en temperatura ambiente [(22 ± 4) °C, HR : (65 ± 5) %].

India / Actinidia deliciosa / frutas / almacenamiento / poliaminas / aptitud para la conservación / maduración en postcosecha / producción de etileno / respiración / poligalacturonasa / lipoxigenasa