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Effect of polyamines on storability and quality of pomegranate fruit (*Punica granatum* L.) cv. Bhagwa

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ABSTRACT

Pomegranate cv. Bhagawa fruits harvested at adequate stage of maturity were dipped in aqueous solutions containing various concentrations of the polyamines putrescine (1mM, 2mM and 3mM) and spermidine (0.5mM, 1mM and 1.5mM), along with Tween-20 as a surfactant, for 5 minutes. The fruits were then stored at 5°C and 8°C temperature with under 90-95% relative humidity. Polyamine-treated fruits showed reduced chilling-injury, weight loss and respiration rate during storage at these 5°C and 8°C temperatures. An increasing trend in total soluble solids (TSS) content, and a decreasing trend in acidity were found in polyamine-treated fruits during storage at 5°C and 8°C temperature. Maximum reduction in chilling-injury was obtained with putrescine (2mM) at both the storage temperatures. Control fruits stored at 5°C and 8°C temperature rapidly developed chilling-injury developed symptoms of brown discoloration of skin and weight-loss in pomegranate fruits.

Key words: Pomegranate, polyamines, shelf-life, storability, chilling injury

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a widely grown fruit crop in almost all the tropical and subtropical countries. It is classified as a non-climacteric fruit, and, in spite of the low respiration rate reported (Ben-Arie *et al*, 1984), it is a highly perishable commodity. Pomegranate, when stored at room temperature, suffers reduction in shelf-life by accelerated desiccation and decay, which makes it necessary to store fruits at low temperatures. However, when stored below 5°C, pomegranate fruits develop chilling-injury (CI), resulting in reduced internal and external fruit quality (Mirdehghan and Rahemi, 2005). To reduce occurrence of CI, several technologies have been tested, including chemical treatment with thiabendazole (McDonald *et al*, 1991), controlled and modified atmosphere storage, intermittent warming (Artés *et al*, 1996), shrink-film wrapping and coatings (Nanda *et al*, 2001). Polyamines (PAs) are low molecular-weight, small, aliphatic amines ubiquitous in living organisms, and have been implicated in a wide range of biological processes, including plant growth, development and response to stress (Smith, 1985). The most common PAs, putrescine (PUT), spermidine (SPD) and spermine (SPM) are found in every plant cell. It is believed that PAs have anti-senescence function (Kumar *et al*, 1997), but their levels usually decrease during ripening in most fruits. This general diminution affects textural attributes of the fruit and its shelf-life. Thus, exogenous application of PAs has been reported to enhance shelf-life and textural attributes in fruits like plum (Pérez-Vicente *et al*., 2002) and mango (Malik and Singh, 2005). Scanty information is available on the effect of polyamines on extending shelf-life, alleviating CI, and maintaining quality attributes of pomegranate fruits. In view of the importance of pomegranate cv. Bhagwa, and problems faced by growers/traders in cold-storing, this experiment aimed to study the effect of polyamines on storage-life and quality attributes of pomegranate fruits under low-temperature storage.

MATERIAL AND METHODS

Sample preparation: Pomegranate cv. Bhagawa fruits were procured from a commercial orchard in Solapur (Maharashtra). Fruits were harvested at commercial maturity stage and transported immediately to the laboratory. Uniform-sized fruits, free from sunburn, cracks or bruises were selected. The experiment was conducted in Completely

Randomized Design, including seven treatments, viz., T_1–1mM putrescine, T_2–2mM putrescine, T_3–4mM putrescine, T_4–0.5mM spermidine, T_5–1mM spermidine, T_6–1.5mM spermidine and T_o–Control, with three replications. Fruits were treated with various concentrations of putrescine (PUT) (1mM, 2mM and 3mM) and spermidine (SPD) (0.5mM, 1mM and 1.5mM), along with Tween-20 as a surfactant, for 5 min and washed in distilled water (Control). After treatment, fruits were air-dried and kept in ventilated, corrugated fiber-board boxes. Fruits packed in boxes were kept in the laboratory at room temperature, and at low temperatures of 5°C and 8°C. After 15, 30, 45, 60 and 75 days of storage, fruits from each treatment were sampled. Fruit peel was carefully cut at the equatorial zone with sharp knives and arils were taken out from which juice was extracted, manually, for further analysis.

Weight-loss in fruits was determined during storage at different sampling intervals of 15, 30, 45, 60 and 75 days after treatments and expressed as percentage. Respiration rate was measured using auto gas analyzer (Model: Checkmate 9900 O_2/CO_2, PBI Dansensor, Denmark). Respiration rate was expressed in milliliters of CO_2 released per kg of fruit per hour (mL CO_2 kg^{-1} h^{-1}). Pomegranate fruits for studies on chilling-injury were rated on a scale of 0–4 (Wild and Hood, 1989). For juice recovery, arils were removed from the fruit and weighed using an electronic balance. Juice was extracted by a hydraulic juice press and weighted. Juice recovery was expressed as percentage of total aril weight at the time of measurement. Total soluble solids content was determined using Erma hand refractometer at 20°C and results expressed as percentage. Titrable acidity was estimated as per Ranganna (1986). Data were subjected to ANOVA in Completely Randomized Design, and, the means were separated by LSD test.

RESULTS AND DISCUSSION

Effect of polyamines on physiological changes in fresh pomegranate fruit

Physiological loss of weight: Physiological loss in weight was found to increase with advancement in storage period at room temperature. All the treatments led to loss in fruit weight during the entire storage period up to 75 days (Table 1). At 30 days of storage, highest (18.5%) physiological loss in weight was found in Control treatment T_0, while, the lowest was recorded in treatments T_1 and T_5 (8.8 and 9.0%, respectively) in fruit stored at room temperature. At 5^0C, the highest percentage of weight loss (8.9%) was recorded in Control fruits, and the lowest (2.22%) in treatment T_1. At 30 days of storage, a similar trend in physiological loss of weight was observed at 8^0C storage. At 30 days of storage, end of the shelf-life of fruit was observed in Control treatment T_0. At 75 days of storage, the lowest (11.00%) physiological loss in weight was recorded in treatment T_1, followed by that in treatment T_5 (11.12%) at 5^0C. At 75 days of storage, a similar trend of physiological loss in weight was observed at 8^0C. Loss of weight in the stored pomegranate fruit is mainly due to transpiration of water from the fruit, and is apparent as shrivelling. Loss in weight was found reduced with application of PUT. Lower weight-loss in PUT treated fruits can be attributed to stabilization or consolidation of cell integrity and permeability of tissues, and amelioration of CI. The CI induces tissue disruption and the connection between fruit skin and the external atmosphere, allowing transfer of water vapour. Besides this, lower respiration rate in PUT treated fruits may also contribute to lower rate of weight-loss (Valero *et al*, 1998). Elyatem and Kader (1984) also established a strong relation between respiration rate in pomegranate and loss in weight.

Table 1. Effect of polyamines on physiological loss in weight (PLW %) in pomegranate fruit stored at room temperature and low temperature

Treatment	Storage period (days)											
	Room temperature		5°C temperature					8°C temperature				
	15	30	15	30	45	60	75	15	30	45	60	75
T_1	2.18	8.8	1.35	2.21	6.12	9.84	11.00	1.40	2.40	6.19	9.84	11.07
T_2	3.31	11.23	2.22	3.70	9.10	12.16	14.13	2.29	3.82	9.13	12.17	14.21
T_3	2.91	12.23	2.74	3.97	8.90	11.44	13.86	2.91	3.94	8.90	11.49	14.07
T_4	2.87	11.80	2.83	3.98	9.12	12.20	14.16	2.86	4.01	9.16	12.21	14.24
T_5	2.27	9.00	1.41	2.74	7.14	10.04	11.12	1.64	2.80	7.16	10.08	11.26
T_6	2.92	12.00	2.87	4.01	8.89	11.05	13.92	2.90	3.98	9.10	11.85	14.04
T_0	9.34	18.50	4.30	8.90	00	00	00	4.42	9.09	00	00	00
SE±	0.36	0.12	0.026	0.157	0.03	0.06	0.03	0.01	0.08	0.01	0.01	0.04
CD at 5%	1.11	0.38	0.08	0.476	0.09	0.09	0.09	0.03	0.26	0.04	0.03	0.12

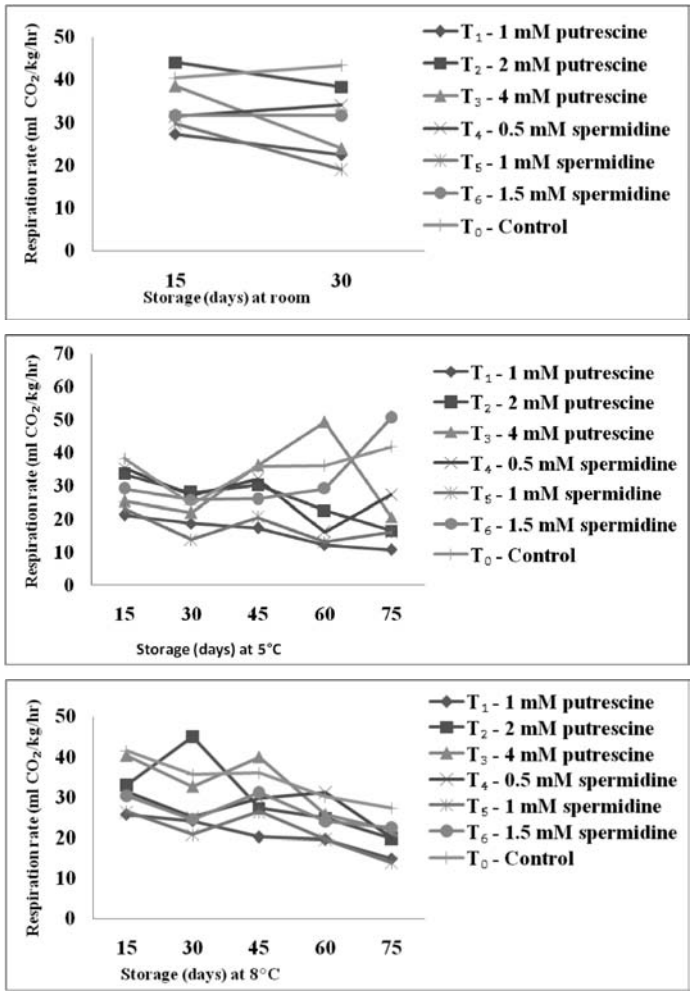

Fig. 1 Effect of polyamines on respiration rate in pomegranate fruit stored at room temperature and low temperature

Respiration rate: Respiration rate of fruit increased with advancement in storage period under all treatments tested (Fig. 1). Up to 15 days of storage, no significant difference in respiration rate was seen in fruits treated with PUT and SPD, at room temperature, 5°C and 8°C. However, a marked difference was recorded in respiration rate at 45 and 60 days of storage under all the treatments used. At 60 days of storage, highest respiration rate (41.80mL CO_2 kg^{-1} h^{-1}) was recorded under Control, while, the lowest (10.76mL CO_2 kg^{-1} h^{-1}) in T_1 treatment at 5°C. Comparatively higher respiration rate in the Control fruits was mainly due to CI. Chilling injury is known to abrade cell membrane and other cell organelles, which leads to higher cell-respiration rate. The above findings are in agreement with MacRae (1987) in persimmon.

Chilling injury: CI developed in pomegranate fruits from 45th day of storage at 5°C and 8°C (Table 2). Symptoms appeared as skin-browning, and its intensity increased with storage duration prolonged to 75 days. Highest CI was recorded in the Control fruits (40.00%). However, application of PUT and SPD led to significant reduction in CI and skin-browning. At the end of the experiment, fruits treated with T_1 showed 55% lower symptoms of CI compared to the Control fruits. Chilling injury could be considerably reduced by the sole application of PUT or SPD. In addition, adaptation or cold acclimation has been proposed to cause an increase in the proportion of unsaturated membrane lipid, and, this is considered to be a critical factor for maintenance of cellular integrity under chilling conditions (Campos *et al*, 2003). Here, the Control fruits failed at cold-acclimation/adaptation, thus leading to CI. Polyamines play a very significant role in alleviating chilling injury symptoms in fruits. When polyamines are applied exogenously, they seem to induce cold-acclimation, which could help maintain membrane fluidity at low temperatures, and in thus, responsible for reducing electrolyte-leakage and skin-browning. Polyamines, due to their antioxidant property, prevent mainly lipid peroxidation, thus protecting membrane lipids from conversion in physical state (Mirdehghan *et al*, 2007).

Table 2. Effect of polyamines on chilling injury (%) in pomegranate fruit stored at room temperature and low temperature

Treatment	Storage period (days)							
	5°C temperature				8°C temperature			
	30	45	60	75	30	45	60	75
T_1	00	-	14.06 (16.97)	21.20 (12.23)	00	27.20 (15.78)	29.20 (16.98)	39.22 (23.08)
T_2	00	21.73 (12.55)	29.20 (8.085)	39.40 (23.20)	00	30.20 (17.75)	34.06 (19.91)	45.50 (27.06)
T_3	00	19.22 (11.08)	31.05 (18.08)	37.06 (21.75)	00	30.32 (17.68)	32.22 (18.79)	45.30 (26.93)
T_4	00	24.66 (14.29)	32.33 (18.86)	39.33 (22.86)	00	30.22 (17.52)	32.06 (18.70)	44.19 (26.22)
T_5	00	-	18.30 (18.54)	26.60 (15.42)	00	28.30 (16.44)	30.10 (17.51)	41.03 (24.22)
T_6	00	17.32 (19.76)	18.40 (10.60)	39.06 (22.99)	00	-	34.20 (20.00)	44.07 (26.23)
T_0	00	28.30	35.00	40.00	00	30.25	36.00	46.00
SE±		0.75	0.011	0.015		0.010	0.029	0.37
CD at 5%		2.28	0.034	0.046		0.031	0.088	0.14

*Figures in parentheses indicate transformed value

Juice recovery: Juice recovery decreased in all the treatments (Fig. 2). However, the decline was much higher in Control (arils from untreated fruit) compared to treatment with PUT and SPD. At 30 days of storage, this trend was found to be reverse, where, juice recovery increased in Control fruits. But, this surge was observed much later, at 45 days of storage in PUT- and SPD- treated fruits. Regardless of the treatment, juice recovery depleted after 45 days of storage. However PUT treatment proved to be better over the Control. In the present investigation, application of PUT and SPD gave positive results going by the higher juice recovery over Control. Owing to its anti-senescence property, PUT retards respiration rate and activities of enzymes responsible for cell-wall degradation. Further, the role of PAs in reducing CI and associated activities of cell-wall degrading enzymes have been reported by several workers (Fernández-Trujillo *et al*, 1998). Thus, in the Control pomegranate fruits, increase in juice recovery after 30 days of storage may be attributed to CI-mediated activity of cell-wall degrading enzymes such as pectinmethylesterase and polygalacturonase.

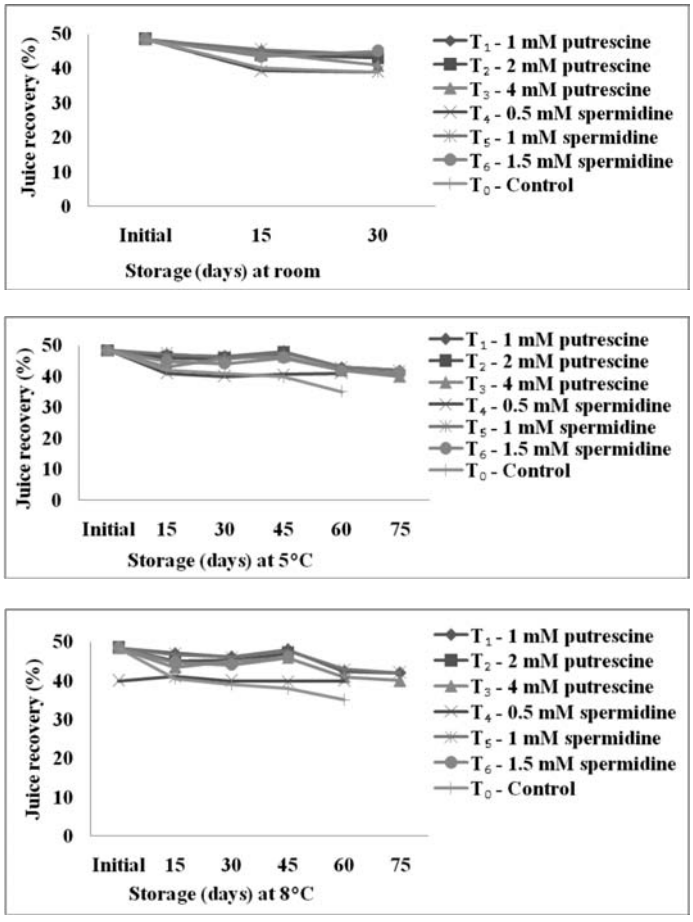

Fig. 2. Effect of polyamines on juice recovery in pomegranate fruit stored at room temperature and low temperature

Effect of polyamines on chemical composition of fresh pomegranate fruit

Total soluble solids (TSS): The total soluble solids increased with increase in storage period (Table 3). At 15 days of storage, the lowest (15.37%) TSS was recorded in treatment T_4, at room temperature. The highest (15.49%) TSS was recorded in treatment T_1, followed by that in treatment T_5 (15.47%). At 60 days of storage, a similar trend was observed too. At 75 days of storage, highest TSS was recorded in Control treatment T_0 (17.01%), at 5^0C, while, the lowest was recorded in treatment T_4 (16.17%), followed by treatment T_1 (16.18%).

Titrable acidity: Titrable acidity decreased with increase in storage period (Table 4). At 15 days of storage, highest (0.61%) titrable acidity was recorded in Control treatment T_0. The lowest (0.36%) titrable acidity was recorded in treatment T_1, followed by treatment T_5 (0.37%). At 60 days of storage, a similar trend was observed. At 75 days of storage, highest titrable acidity was recorded in Control treatment T_0 (0.39%), while, lowest titrable acidity was recorded in treatment T_1 (0.29%), followed by treatment T_5 (0.33%). Previous work on plum (Serrano *et al*, 2003)

Table 3. Effect of polyamines on total soluble solids (%) in pomegranate fruit stored at room temperature and low temperature

Treatment	Storage period (days)														
	Room temperature			5^0C temperature						8^0C temperature					
	0	15	30	0	15	30	45	60	75	0	15	30	45	60	75
T_1	15	15.49	15.74	15	15.5	15.76	16.21	16.41	16.18	15	15.50	15.71	16.17	16.37	16.37
T_2	15	15.37	15.73	15	15.42	15.62	15.84	15.95	16.16	15	15.42	15.60	15.81	15.90	15.90
T_3	15	15.36	15.72	15	15.41	15.58	15.64	15.90	16.15	15	15.41	15.62	15.71	15.81	15.81
T_4	15	15.37	15.70	15	15.41	15.68	15.54	15.85	16.10	15	15.41	15.56	15.61	15.71	15.75
T_5	15	15.47	15.77	15	15.46	15.71	16.14	16.35	16.17	15	15.46	15.66	16.12	16.35	16.35
T_6	15	15.39	15.70	15	15.40	15.70	16.17	16.20	16.07	15	15.40	15.65	16.11	16.16	16.16
T_0	15	15.42	15.60	15	15.39	15.56	16.23	16.50	17.01	15	15.39	15.53	16.22	16.47	16.60
SE±		0.007	0.005	0	0.005	0.008	0.005	0.005	0.005	0	0.005	0.01	0.006	0.005	0.005
CD at 5%		0.023	0.015	0	0.015	0.026	0.017	0.17	0.17	0	0.015	0.03	0.019	0.017	0.017

Table 4. Effect of polyamines on titrable acidity (%) in pomegranate fruit stored at room temperature and low temperature

Treatment	Storage period (days)														
	Room temperature			5°C temperature						8°C temperature					
	0	15	30	0	15	30	45	60	75	0	15	30	45	60	75
T_1	0.36	0.36	0.36	0.36	0.32	0.31	0.30	0.30	0.29	0.36	0.34	0.33	0.33	0.32	0.30
T_2	0.36	0.40	0.39	0.36	0.37	0.36	0.35	0.35	0.33	0.36	0.36	0.35	0.34	0.33	0.32
T_3	0.36	0.51	0.50	0.36	0.36	0.36	0.35	0.35	0.34	0.36	0.70	0.36	0.35	0.34	0.34
T_4	0.36	0.56	0.55	0.36	0.37	0.40	0.39	0.39	0.38	0.36	0.43	0.42	0.41	0.40	0.35
T_5	0.36	0.37	0.36	0.36	0.36	0.35	0.34	0.34	0.33	0.36	0.30	0.35	0.34	0.30	0.32
T_6	0.36	0.46	0.45	0.36	0.41	0.36	0.35	0.35	0.35	0.36	0.36	0.36	0.35	0.34	0.31
T_0	0.36	0.61	0.58	0.36	0.42	0.41	0.40	0.40	0.39	0.36	0.38	0.37	0.36	0.36	0.36
SE±	—	0.008	0.008	—	0.007	0.006	0.007	0.006	0.006	—	0.007	0.007	0.007	0.007	0.003
CD at 5 %	—	0.027	0.0027	—	0.02	0.02	0.022	0.02	0.02	—	0.021	0.022	0.0023	0.024	0.012

and pomegranate (Mustapha *et al*, 1995) also confirm these findings.

CONCLUSION

Exogenous application of polyamines like putrescine and spermidine showed improvement in storability of pomegranate at 5°C, which otherwise would lead to chilling injury and compromised quality. Treatment with putrescine reduced respiration rate and physiological loss of weight, and enhanced total soluble solids content, amount of reducing sugars, and decreased acidity of the fruit. Thus, shelf-life can be extended in pomegranate fruits stored at low temperatures (5°C) for upto 75 days. As demonstrated by us, application of 1mM putrericine could be effective in alleviating chilling injury symptoms during long duration, low-temperature storage of pomegranate fruits. However, further studies are necessary on combined use of putrescine with other treatments in alleviating chilling injury and possible mechanisms of action for increasing post-harvest life of the fruit.

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