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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(12): 2815-2819

www.thepharmajournal.com
Received: 02-10-2023
Accepted: 04-11-2023

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A comparative study on postharvest treatments to extend shelf life of broccoli var. Aishwarya in refrigerated condition

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DOI: https://dx.doi.org/10.22271/tpi.2023.v12.i12Saj.25306

Abstract
Broccoli, due to high perishable nature with high respiratory rate, has a short shelf life of two days once harvested. This crop is gaining importance since it is considered as an anticancerous food. Due to high perishability, the shelf life of this crop is also very short. Postharvest treatments were done with 25 ppm and 50 ppm of Benzyl adenine (BA), hot water at 45 °C and 48 °C, 500 ppm and 1000 ppm of Salicylic acid (SA), 50 ppm and 100 ppm of calcium hypochlorite and control treatments were performed in freshly harvested broccoli, and stored in 150 gauge polyethylene bags and stored in refrigerated condition (5±1 ºC, 90-95% RH) to carry out the research work on the extension of shelf life of broccoli by studying the various changes in physicochemical properties *viz*; physiological loss in weight, ascorbic acid, chlorophyll, total soluble solids content along with sensory analysis for smell, texture, browning as well as marketability of the crop during the storage period. Fresh broccoli treated with Benzyl adenine (BA) gave the highest score for all the parameters taken into consideration during the storage study. Further research work needs to be conducted to study the consumption effect of BA on human health.

Keywords: Aishwarya, ascorbic acid, chlorophyll, blanching, physiological loss in weight, sensory analysis

Introduction
Broccoli is an exotic crop to India. It originated in the Mediterranean area and Asia Minor. It has been very popular in Italy since the Roman Empire. The name was derived from the Latin word brachium which means branch or arm, which seem like a tree-like shape reflection which is a compact combination of florets joined by small stems to a larger stalk. It is famous due to its delicious taste, flavour and high in nutritious. It is highly valued due to high content of antioxidants, anticarcinogenic compounds, vitamins (Nestle, 1998) [10] as well as health promoting phytochemicals (Yuan *et al.*, 2010) [23]. Bitter taste of broccoli has properties beneficial for anticarcinogens (Verhoeven *et al*., 1977) [21].
Harvesting is done when heads are young, tender and when the flower is compact and tight. Generally it is harvested with the stalk (15 cm stem). The edible part of broccoli is the immature inflorescence or head, while the floret tissue is also consumed.
Loss of broccoli quality when stored is due to wilt, yellow buds and florets, opened or loose head along with decay (Toivonen and Forney, 2004) [18]. Due to high perishable nature, bruising problem occurs during handling practices. Storage of vegetables in different condition of temperature, relative humidity, light intensity and surrounding atmosphere composition affects the colour and texture of vegetable. Consumers prefer green-coloured florets of broccoli. Adjacent floret tissue shading leads to the senescence with yellow bead and yellow-light green marginal areas of floret (Cantwell and Suslow,1999) [3]. Broccoli in markets arrived generally one or two days of harvest due to which the quality begins to deteriorate. Giving importance to the short shelf life and nutritional value of this crop, a research work was carried out by performing various post-harvest treatments in fresh broccoli for shelf life extension to maintaining quality during the storage period by regular physico chemical analysis.

Materials and Methods
The research work was carried out to study the effect of different postharvest treatments on storability. Tender and dark green colour broccoli heads with unopened florets of cv. Aishwarya were collected from the Horticultural Research Station, Mondouri and taken to the laboratory packed in plastic crates followed by room cooling for few hours and trimmed.

The samples were treated in solutions formulated as stated below

Detail of Treatment
T_1 - 50 ppm Benzyl adenine (BA) for 10 min
T_2 - 25 ppm Benzyl adenine (BA) for 10 min
T_3 - Hot water treatment (HWT) 48 ˚C for 4 min
T_4 - Hot water treatment (HWT) 45 ˚C for 4 min
T_5 – 1000 ppm Salicylic acid (SA) for 10 min
T_6 – 500 ppm Salicylic acid (SA) for 10 min
T_7 - 100 ppm Calcium hypochlorite for 10 min
T_8 - 50 ppm Calcium hypochlorite for 10 min
T_9 -Control

Method of treatment
The solution for the various treatments were freshly prepared. Different concentration of BA, SA and calcium hypochlorite solution were prepared separately in methanol and add distilled water to made up the volume. The freshly harvested broccoli heads variety Aishwarya from Horticultural Research Station, Mondouri were dipped for the required duration followed by air surface drying for few minutes in open air after which packaging was done in 150 gauge polyethylene (PP) bags and kept in refrigerated condition (5±1 ºC, 90-95% RH).

Replication Number: 3

Statistical design: Completely randomized design

Observations recorded
PLW (%), chlorophyll (µg/g), TSS (ºB), yellowing (%), marketability (%), ascorbic acid (mg/100 g), along with sensory evaluation for smell, texture and browning (5 point Hedonic scale), were estimated following standard procedure for analysis at regular 5 days intervals from the day of harvest.

Physico-chemical analysis
Physiological loss in weight (PLW): It is sum total % loss in weight, in respect to the initial weight (before storage) with weight loss recorded at the time of periodical sampling during storage.

$$\text{PLW} = \frac{\text{initial weight} - \text{finalweight}}{\text{initial weight}} \times 100$$

Ascorbic acid
Ascorbic acid was determined using 2, 6- indophenols (2, 6-Dichlorophenol-Indophenol Visual Titration Method) which will get reduced to a colourless leuco-base and converts to dehydroascorbic acid. Though it is a blue coloured compound,due to reaction with ascorbic acid, there is pink colour appearance at the end point is the appearance of pink In acid medium, the dye is pink coloured (Ranganna, 2017) [13].

$$\text{Ascorbic acid} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot of extract taken for estimation} \times \text{Wt: or vol: of the sample taken for estimation}} \times 100$$

Chlorophyll: In spectrophotometer, a known amount of tissue sample by weight is taken and chlorophyll is extracted in 80% acetone until the residue become colourless and made upto known volume with 80% acetone. Optical Density (OD) value is then measured at 660nm and 642.5 nm wavelength in a colorimeter against blank (A.O.A.C,1990) [2]. The amount of chlorophyll can be calculated by using the formula as stated below:

Total chlorophyll (a+b), µg/ ml = (7.12 × OD at 660 nm) + (16.8 × OD at 642.5 nm)

Total soluble solids (TSS) °Brix: For determining the TSS (Erma Hand Refractometer I.S.O 2173), sample juice is dropped on the prism and it reads the percentage of dry substance in it directly (Varnam and Sutherland, 1994) [20].

Sensory evaluation: Sensory characters *viz*., smell, texture, and browning were evaluated. The scoring of sensory value was marked as 1 to 5 point hedonic scale as follows:

Smell
No off odour = 5
Little off odour = 4
Moderate off odour = 3
Strong off odour = 2
Extreme off odour = 1

Texture
Crispy = 5
Little rubbery = 4
Rubbery = 3
Soft = 2
Extremely soft = 1

Browning
Zero browning = 5
Little browning = 4
some browning = 3
Very much browning = 2
Adverse browning = 1

Marketability (%)
It was evaluated on the last day during the 15 days of storage period with relation to the quality *viz*; texture or crispiness, flavour, colour, and defects like yellowing, decay and browning). Value ranging upto 50% was considered to be marketable.

Results and Discussions
PLW (%): PLW increased gradually with prolong storage. Maximum loss in weight was 4.10 to 6.25% in broccoli treated with hot water at 45 ˚C (T_4) on 5 to 10 days of storage while the least loss in weight 0.13,0.63 and 1.83 in heads dipped in BA 50 ppm (T_1) on day 5, 10 and 15. On day 15, the control treatment showed the maximum loss in weight (10.33%) at the end of the storage study.
Wasim *et al*. (2011) [22] showed retension of green colour and weight reduction with benzyl amunopurine treatment at storage of temperature 6±1 ºC. Senescence in the post-harvest stage is regulated by the change in transpiration and respiration rate, sugar content, ethylene emission whereas cytokinin can decrease the senescence symptoms of ethylene.
Loss of moisture from broccoli treated with calcium was lower than the control during the storage study. Treatment with calcium leads to stabilization of cell membrane firmness

with reduction in moisture loss leading to higher cell turgor pressure (Mignani *et al*., 1995; Picchioni *et al*., 1998) [9, 11]. Reduction in PLW of packed broccoli was observed compared to treatment with chemicals applied alone (Singh *et al.*, 2013) [17].

Total soluble solids (ºB): Table1 showed that post-harvest treatments gave significant difference on TSS of broccoli where it followed a decreasing trend except in BA 50 ppm treated heads where there was an increasing trend till day 5 and then decreased further. Maximum TSS (7.90 to 7.97ºB) was recorded in broccoli dipped in BA 50 ppm on day 0 and 5 after storage while minimum was recorded in 7.00, 6.34 and 5.00 in heads dipped in hot water at 48 ˚C. The maximum value was 7.50 and 7.33ºB in broccoli treated with 100 ppm calcium hypochlorite (T_7) and SA1000 ppm treated broccoli (T_5) on day 10 15 of storage and the least was 5.57ºB in control (T_9) on day 15.

Slow increase in TSS during long storage in film covered peach fruits might be due to delayed in ripening with senescence processes which lead to slow conversion of starch to sugars. According to Sabir (2012) [12] soluble solids content of florets was 5.9% at the beginning of storage which increased along with storage accompanying with the increase course in weight loss. Generally, soluble solids also increase with time due to continued anabolic processes as well as catabolic process like cell wall polysaccharides degradation (Puerta and Cisneros, 2011) [12]. Gautam *et al*. (2003) [7] gave evidence that fruits exposed to postharvest treatments with Bavistin , wax emulsion coating and naphthalene acetic acid followed by storage under cold storage conditions showed little amount of TSS and ascorbic acid compared to the control i.e., without any treatment.

Marketability (%)

All the broccoli samples were marketable during the period of storage study while marketability were high broccoli treated with BA 50 ppm (90%) followed by those treated with BA 25 ppm (88%), SA 1000 ppm (85%), SA 500 ppm (84%). At the same time, the untreated also recorded 70% marketability on the same day (Table1).

Ascorbic acid (mg/100 g): Table 2 showed the effect of various postharvest treatments in broccoli with significant difference in amount of ascorbic acid of broccoli during the storage period. Highest value of ascorbic acid content was recorded (125.52, 115.50 and 105.34 mg/100 g) throughout the storage period in the heads treated with BA 50 ppm while the lowest content was in control broccoli heads (T_9) with 89 to 73 mg/g.

Table 1: Post harvest treatment effect on physiological loss in weight (%), marketability and total soluble solids content (ºB) of broccoli var. Aishwarya in refrigerated condition

Treatment	Physiological loss in weight (%)			Marketability (%) on 15th day	Total soluble solids(ºB)			
	Days in storage				Days in storage			
	5	10	15		At harvest	5	10	15
T_1 (BA 50 ppm)	0.13(1.65)	0.63(4.55)	1.83(7.76)	90	7.45	7.97	7.00	6.63
T_2 (BA 25 ppm)	0.87(5.33)	2.84(9.70)	5.03(12.96)	88	7.45	7.03	6.77	6.53
T_3 (HWT at 48 ˚C)	2.37(8.85)	3.60(10.93)	5.87(14.00)	75	7.45	6.34	5.00	6.70
T_4 (HWT at 45 ˚C)	4.10(11.68)	6.35(14.58)	8.87(17.28)	75	7.45	6.77	6.00	5.97
T_5 (SA1000 ppm)	0.53(4.17)	1.23(6.37)	2.03(8.18)	85	7.45	7.17	7.00	7.33
T_6 (SA 500 ppm)	1.40(6.78)	2.62(9.28)	2.47(8.88)	84	7.45	6.83	5.67	5.63
T_7 (100 ppm calcium hypochlorite)	0.50(4.05)	1.30(6.55)	2.97(9.91)	80	7.45	7.27	7.50	7.03
T_8 (50 ppm Calcium hypochlorite)	0.77(5.00)	1.67(7.42)	3.77(11.19)	78	7.45	7.43	6.60	6.70
T_9 (control)	2.67(9.36)	5.30(13.30)	10.33(18.74)	70	7.45	7.17	7.07	5.57
SEm ±	0.13	0.18	0.36		0.0	0.19	0.19	0.16
CD(P=0.05)	0.38	0.53	1.08		NS	0.56	0.56	0.49

Note: Angular transformed data is represented within brackets

Leja *et al.* (2001) [8] demonstrated the combined effect of polymeric foil with low temperature lowered the loss of ascorbic acid. In support of that, Ahmad *et al.* (2013) [1] showed that storing fruits at 4±1 ºC showed a lower rate of physico chemical changes in comparision to ambient conditions (18-32 ºC, RH 45-65%). Rybarczyk *et al*. (2014) [14] gave evidence that elevated pre-storage period, temperature of presto rage and storage temperature also lowered vitamin C broccoli flower buds slower vitamin C loss. Storage temperature influence ascorbic acid in broccoli flower buds with minor extent by temperature of pre-storage. Storage temperature adversely vitamin C content of broccoli while larger amount was found in broccoli at 10 ºC compared to storage at 18 ºC. Postharvest treatments with Calcium resulted in reduction of respiration rate in climacteric and non-climacteric fruits (Saftner *et al*., 1998; Tsantili *et al*., 2002) [16, 19].

Chlorophyll content (µg/g): Table 2 showed the influence of post-harvest treatment on broccoli on chlorophyll content of broccoli during the storage study. Amount of chlorophyll degraded progressively during the storage period. The highest chlorophyll content (2.93.33, 280, 273.33 and 264.50 µg/g) was in broccoli treated with (T_1) during the storage period while the lowest value (205 µg/g) was recorded in control broccoli (T_9) on the last day of storage.

Clarke *et al.* (1994) [5] reported that BA application delay initiation of chlorophyll degradation and emission of ammonia in broccoli. BA application to broccoli showered down chlorophyll degradation while stimulating carpel growth. In broccoli, treatment with cytokinin after harvest slows down degradation of chlorophyll in florets.

Higher chlorophyll retention in BA 50 ppm is similar with the finding of Costa *et al*. (2005) [6] where the exogenous BAP application significantly delay chlorophyll degradation and florets yellowing. Higher retention of chlorophyll in BA treatment can be correlated with Wasim *et al.* (2011) [22] where BA significantly delayed yellowing and chlorophyll degradation with higher ascorbic acid retention in respect to control florets, stored at 6±1 ºC.

Table 2: Post harvest treatments effect on ascorbic acid (mg/g) and chlorophyll content (µg/g) of broccoli var. Aishwarya in refrigerated condition

Treatment	Ascorbic acid (mg/100g)				Chlorophyll (µg/g)			
	Days in storage				Days in storage			
	At harvest	5	10	15	At harvest	1	2	3
T_1 (BA 50 ppm)	129	125.5	115.50	105.34	293	280.00	273.33	264.50
T_2 (BA 25 ppm)		116.67	114.00	104.67		270.00	266.67	256.67
T_3 (HWT at 48˚C)		91.65	95.00	92.33		236.67	243.33	226.67
T_4 (HWT at 45˚C)		94.31	92.67	88.33		266.67	260	244.43
T_5 (SA1000 ppm)		93.59	88.33	83.67		283.33	276.67	261.00
T_6 (SA 500 ppm)		104.00	97.50	83.60		263.33	243.33	221.33
T_7 (100 ppm Calcium hypochlorite)		103.33	100.00	79.67		236.67	230.00	224.10
T_8 (50 ppm Calcium hypochlorite)		111.33	100.00	93.61		256.67	246.67	230.00
T_9 (Control)		110.00	89.00	73.33		270.33	216.67	205.00
SEm±		3.07	3.52	1.94		11.54	10.92	2.83
CD(P=0.05)		9.19	10.55	5.82		32.43	23.11	8.49

Sensory evaluation

Smell

Table 3 showed the decreasing trend of smell score during the storage period. The highest score (4.83,4.63,4.40) was reported in broccoli treated with BA 50 ppm while the least was recorded (4.47,3.97,3.60) in control head i.e. without any post-harvest treatments on day 5, 10 and 15 days of storage. No extreme odour was noted in any of the treatments during the storage study.

Best smell score was maintained in T_1 (BA 50 ppm) and T_2 (BA 25 ppm) at the end of the period of study while the least score was control.

Texture

Texture score did not vary significantly in Table 3 due to various post-harvest treatments during the period of study. Highest texture score (4.80, 4.67 and 4.57) was recorded for broccoli treated with BA 50 ppm throughout the storage period while the minimum (4.70, 4.47, 4.27) was recorded in the control on 5, 10 and 15 days of storage. Texture score followed a decreasing trend since there was continued loss of moisture through respiration and transpiration through the storage period. All the samples had good texture irrespective of the treatment given. However T_1 (BA 50 ppm) and T_1 (BA 50 ppm) gave the best texture and the least in T_9 (control).

Visual quality of broccoli florets started lossing during the 28 days' duration and the loss highest in control i.e., without any packaging. Modified atmosphere packaging significantly preserved the visual quality of florets (Sabir, 2012) [15]

Browning

Table 3 represented the effect of postharvest treatments on browning during the storage period. The maximum score (4.90, 4.70, 4.13) was noted in broccoli dipped in BA 50 ppm while the minimum (4.57, 4 .17, 3.30) was recorded in the control i.e. with no chemical treatment (T_9). Enzymatic browning is responsible for quality loss during storage study. Browning score followed decreasing trend during the storage period. Combined effect of modified atmosphere packaging and low temperature slows down respiration and senescence in green leafy vegetables and extended storage life (Chiesa *et al*., 2004) [4]. Changes in browning among the treatment were not significant though changes do occur among them. The maximum browning score was obtained from broccoli with BA 50 ppm followed by hot water treatment, Salicylic acid treatment and calcium hypochlorite.

Table 3: Effect of post-harvest treatments on sensory characteristics of broccoli var. Aishwarya in refrigerated condition

Treatment	Sensory evaluation of Smell				Texture				Browning			
	Days in storage (DAS)				Days in storage (DAS)				Days in storage (DAS)			
	At harvest	5	10	15	At harvest	5	10	15	At harvest	5	10	15
T_1 (BA 50 ppm)	5	4.83	4.63	4.40	5	4.80	4.67	4.57	5	4.90	4.70	4.13
T_2 (BA 25 ppm)	5	4.80	4.63	4.40	5	4.83	4.63	4.37	5	4.87	4.63	4.10
T_3 (HWT at 48 ˚C)	5	4.77	4.50	4.17	5	4.80	4.50	4.37	5	3.5	4.07	3.93
T_4 (HWT at 45 ˚C)	5	4.87	4.60	4.30	5	4.80	4.53	4.47	5	4.87	4.40	3.63
T_5 (SA1000 ppm)	5	4.87	4.53	4.10	5	4.73	4.60	4.37	5	4.83	4.27	3.53
T_6 (SA 500 ppm)	5	4.56	4.30	3.97	5	4.77	4.60	4.40	5	4.87	4.40	3.47
T_7 (100 ppm Calcium hypochlorite)	5	4.57	4.13	3.70	5	4.77	4.57	4.30	5	4.73	4.43	3.43
T_8 (50 ppm Calcium hypochlorite)	5	4.60	4.30	4.03	5	4.83	4.67	4.47	5	4.83	4.50	3.30
T_9 (Control)	5	4.47	3.97	3.60	5	4.70	4.47	4.27	5	4.57	4.17	3.30
SEm±	0.0	0.10	0.12	0.15	0.0	0.07	0.09	0.08	0.0	0.45	0.08	0.12
CD(P=0.05)	NS	0.13	0.16	0.24	NS	NS	NS	NS	NS	NS	NS	NS

*Score for smell:5 = no off-odour; 4 = slight off odour; 3 = moderately off odour; 2 = severe off-odour;1 = extreme off-odour Score for texture: 5 = crispy; 4 = little rubbery; 3 = rubbery; 2 = soft; 1 = extremely soft Score for browning: 5 = no browning; 4 = some browning; 3 = moderate browning; 2 = very much browning; 1 = extreme browning, NS= non significance

Conclusion

Combination effect of packaging and refrigerated condition helps in increasing the shelf life of fresh broccoli after exposing to various postharvest treatments. Problem of yellowing can be prevented by the combined method of refrigeration, packaging and post-harvest treatments. On the last day of storage (15th day), the maximum quality score of broccoli was recorded in BA treated broccoli in the given condition. Higher concentration of BA (50 ppm) gave better result than BA (25 ppm). Studies on the effect of BA on

consumer health need to be given attention since this chemical will prove to be the best treatment for the longevity of shelf life of fresh green leafy vegetables.

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