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6-Benzylaminopurine improves shelf life, organoleptic quality and health-promoting compounds of fresh-cut broccoli florets

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Fresh-cut broccoli (*Brassica oleracea* var. *italica*) florets were subjected to 6-benzylaminopurine (BAP) treatment by dipping in BAP [0, 5, 10 and 15 ppm] for 10 min and stored at 6±1°C. Treatment with BAP (10 ppm) significantly delayed yellowing and chlorophyll degradation, consequently maintained organoleptic quality during storage. Treated florets had higher retention of health promoting compounds (protein and ascorbic acid) in relation to control. Treatment with 10 ppm BAP could be a useful method to extend shelf life of fresh-cut broccoli florets during storage at 6±1°C at commercial level.

Keywords: 6-Benzylaminopurine, Broccoli, Fresh-cut vegetables

Introduction

Broccoli (*Brassica oleracea* var. *italica*) as a vegetable has high nutritional value due to significant contents of vitamins, antioxidants and anticarcinogenic compounds[1]. Ethylene plays a crucial role in the process of senescence of broccoli florets[2]. Floret yellowing commences between 24 and 48 h after harvest at 20°C and is complete by 96 h[3]. This is a relatively late event in post-harvest senescence of broccoli, and is preceded by major losses of sugars, organic acids and protein[4].

Various techniques, including use of a modified atmosphere (MA) or controlled atmosphere (CA)[5,6], different types of packaging[7,8], treatment with chemicals and cytokinins[9,10], refrigeration[11], heat treatment[12], ethanol vapor treatment[13], heat[14], UV-C[15] and ethylene blocker[1,2] have been investigated during postharvest span of broccoli. In general, quality deterioration (color, flavor and texture) of fresh and fresh-cut products is attributed to the effect of endogenous enzyme, enhanced respiration[16], physical abuse and environmental factors[17]. However, senescence is also strongly regulated by plant hormones. Cytokinins inhibit senescence of leaves and some floral tissues[18]. Exogenous application of cytokinins to plant tissues results in delay in senescence, maintenance of chloroplast activity, decline chlorophyll degradation, production of protein and nucleic acid synthesis and mobilization of nutrients into cytokinin-treated area[18,19]. Lower respiration rates compared to control resulted in extension of shelf-life for cytokinin-treated broccoli[20]. Exposing plant to water stress during floret maturation increased cytokinin content, consequently delayed postharvest yellowing of broccoli florets[21,22]. Influence of cytokinin as 6-benzylaminopurine (BAP) in combination with other methods on postharvest green color retention on broccoli heads and asparagus spears[1,10] showed positive results for quality retention but doses used were too high.

This study analyzes effect of BAP on visual quality, shelf-life, chlorophyll degradation, yellowing, and health promoting compounds of fresh-cut broccoli at 6±1°C.

Experimental Section

Materials

Broccoli cv. Everest heads were obtained from Central Research Center, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya and immediately brought to laboratory and processed. Broccoli florets were dipped in BAP (purity ≥ 98%, National group of Chemical Reagent Co., Ltd., Shanghai, China) solutions [0 (control), 5, 10 and 15 ppm] for 10 min, respectively; water adhering on surfaces was removed with paper towel. Then, treated florets were kept in separate containers. There were three replicates containing 100 g for each

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Table 1—Physical parameters and their scale for evaluating organoleptic quality

S No.	Physical parameters	Scale and scores
1	Dehydration	0 (without), 1 (slight), 2 (more), 3 (severe)
2	Visual mould development	0 (absent), 1 (slightly visible), 2 (10-40% of surface with moulds), 3 (> 40% of surface with moulds)
3	Unpleasant odor	0 (absent), 1 (Slightly presence), 2 (severe)
4	Exuded present	1 (without), 2 (with)
5	Browning	0 (without), 1 (Slightly visible), 2 (10-40% of surface with browning), 3 (> 40% of surface with browning)
6	Yellowing	0 (without), 1 (0-25% of surface with yellowing), 2 (25-75% of surface with yellowing), 3 (> 75% of surface with yellowing)

treatment. All treatments were stored at 6±1°C for 0, 3, 6 and 9 d to appraise quality parameters.

Methods

Organoleptic quality of samples was determined at 5 and 9 d of storage through evaluation of six parameters[23]. Each parameter was scored on a scale with reference to dehydration, visual mould development, exuded present, unpleasant odor, browning and yellowing (Table 1). Each floret was evaluated individually for every parameter. These values were averaged to obtain sensory score of an individual floret. Sensory scores of florets from a tray were averaged to obtain sensory score of an individual tray, which were averaged to obtain sensory score corresponding to samples of a particular treatment and day of storage. A lesser score indicates a better organoleptic quality[23].

Biochemical parameters [total chlorophyll (a+b), ascorbic acid and protein] were estimated on fresh weight basis at 0, 3, 6 and 9 d of storage, using standard methods[24]. To estimate weight loss, trays were weighed after treatments and during storage at 5 and 9 d. Results were expressed as weight loss (%) relative to initial weight. Experiment was designed according to completely randomized design with three replications. Data were analyzed using MSTATC software package.

Results

Organoleptic Quality (OQ) and Weight Loss

All parameters utilized to evaluate loss of OQ of fresh-cut broccoli florets showed an increment during storage at 6±1°C (Table 2), particularly dehydration, degreening and yellowing. Treatment with BAP significantly reduced loss of OQ as compared to control. Value of control sample reached 7.7 after 9 d of storage. However, better performance was detected with higher doses of BAP. Finally, treatment with 10 ppm BAP showed a significant retention in OQ scores (5.08). During storage at 6±1°C, weight loss varied (1.2-1.8% / d) in all samples. No significance differences were detected between 10 and 15 ppm treated sample, but weight loss was significantly higher in control and sample treated with 5 ppm (data not shown).

Table 2—Changes in organoleptic quality (OQ) in control and BAP treated broccoli florets at 0 (initial), 5 and after 9 d of storage at 6±1°C

Treatment	Days in storage		
	0	5	9
Control	0	3.78	7.70
5 ppm	0	3.44	7.06
10 ppm	0	2.58	5.08
15 ppm	0	2.63	5.15
SEm±	-	0.052	0.086
CD at 5%	-	0.119	0.197

Chlorophyll Contents

Chlorophyll (a+ b) contents decreased in all samples during storage (Table 3). However, chlorophyll degradation rate was lower in treated samples than that of control. Control florets lost 71.5% of initial chlorophyll (a+ b) value, while sample treated with 10 and 15 ppm BAP presented loss of 43.5 and 42.4% of initial value, respectively, at last day of storage (9 d).

Ascorbic Acid and Protein Content

Ascorbic acid content of florets was tended to decrease in the course of storage period up to 9 d irrespective of treatments (Table 4). Ascorbic acid retention was differed significantly between control and treated samples. Maximum loss (52%) was observed in control florets; whereas florets treated with higher doses of BAP (10 and 15 ppm) lost only 33% of initial ascorbic acid content.

A decreasing trend in levels of protein contents was observed in all samples, but rate of decreasing protein contents was lower in treated samples in relation to control florets (Table 5). No significant differences between control and sample treated with 5 ppm BAP as

Table 3—Effect of BAP on chlorophyll (a + b) contents (mg/ kg fresh weight) in florets stored during 9 d at 6±1°C

Treatment	Days in storage			
	0	3	6	9
Control	271.7	153.0	104.7	77.0
5 ppm	271.7	181.7	140.3	112.0
10 ppm	271.7	231.3	175.7	153.3
15 ppm	271.7	238.0	183.0	156.0
SEm±	-	5.011	5.147	4.326
CD at 5%	-	11.555	11.870	9.977

Table 4—Effect of BAP on ascorbic acid content (mg/ 100g fresh wt) in florets stored during 9 d at 6±1°C

Treatment	Days in storage			
	0	3	6	9
Control	171.33	135.33	107.67	82.67
5 ppm	171.33	143.67	120.33	99.00
10 ppm	171.33	150.33	133.67	112.00
15 ppm	171.33	153.33	141.33	115.67
SEm±	-	4.921	3.836	3.282
CD at 5%	-	11.349	8.847	7.570

Table 5- Effect of BAP on protein content (g/ kg fresh wt) in florets stored during 9 d at 6±1°C

Treatment	Days in storage			
	0	3	6	9
Control	6.97	4.07	3.70	3.51
5 ppm	6.97	4.50	4.13	3.96
10 ppm	6.97	6.10	5.60	5.10
15 ppm	6.97	6.06	5.80	5.16
SEm±	-	0.134	0.137	0.086
CD at 5%	-	0.309	0.315	0.197

well as between 10 and 15 ppm BAP were found, at 9 d of storage. Florets treated with 10 and 15 ppm BAP has 23% more protein contents than those of control.

Discussion

Broccoli harvesting in immature stage renders florets more vulnerable to senescence hence nutrient and hormone supplies disrupted. Besides, fresh-cut processing operations such as cutting of florets accelerate senescence and consequent tissue damage and deterioration[23]. Treatment of broccoli heads with BAP (100 ppm) can retard senescence related changes[10]. In this study, an attempt has been made to retard postharvest quality changes in fresh-cut broccoli using BAP alone with lower (5, 10 and 15 ppm) concentrations. Shelf life of broccoli florets treated with various concentrations of BAP has significantly improved. Lower concentration (20 ppm) of BAP was also used on green asparagus spears but it was combined with modified atmospheric packaging[1].

Broccoli florets suffer a rapid deterioration after harvest that conducts to a loss of OQ[23]. This includes, among other factors, visual symptoms, production of unpleasant odors, dehydration and growth of pathogens[25]. Loss of water from broccoli florets is one of the most important factors affecting fresh like appearance. This study found that BAP treatment reduces physiological loss in weight and maintains green color; significantly reduces loss of OQ by almost 9 d at 6±1°C. These findings are in accordance with earlier reports[1]. Visual quality was not only directly affected by storage time, but depended on BAP used.

An increment in activities of some enzymes (chlorophyllase and Mg-dechelatase) is responsible for chlorophyll catabolism and as a result yellowing of florets[10]. Funamoto *et al*[12] found ethylene production and respiration directly correlated to increased activity of enzymes and yellowing of broccoli florets. Postharvest increase in ethylene production and respiration has been attributed to wound injuries[26]. Current results showed that exogenous application of BAP significantly delays chlorophyll degradation and yellowing of florets, thereby increased acceptability up to 9 d. These results are supported by Costa *et al*[10], who observed that exogenous cytokinin promotes better preservation of green color of broccoli florets. Zaicovski *et al*[22] showed that concentrations of endogenous cytokinins (zeatin and zeatin riboside) in broccoli florets were directly proportional to water stress and indirectly proportional to broccoli yellowing.

Concentration of vitamin C/ascorbic acid and protein in broccoli generally decreased during storage and loss rate during storage could be lowered by postharvest handling[6,23]. In present study, control florets lost more than half of their initial ascorbic acid after 9 d of storage, while these losses were significantly minimized in florets treated with BAP. Possibly, low respiration rate saved ascorbic acid to be utilized as a source of respiration substrate, thereby increased its retention during storage.

Intense proteolysis occurs during postharvest senescence of broccoli[27], resulting reduction in protein content. Heat treatment may reduce proteolysis[10] but UV- C treatment of broccoli florets did not retard protein loss[23]. In present work, protein contents in both control and treated florets decreased during storage. However, treatment of broccoli florets with higher doses of BAP significantly lowered magnitude of protein loss during postharvest storage. Cytokinin supposed to help in production of protein and nucleic acid synthesis[18]. It is known that BAP retards ethylene production, probably inhibition of ethylene action prevented protein loss in florets. As a consequence, higher protein amount was retained in treated florets after 9 d of storage.

Conclusions

A single treatment with 10 ppm of BAP for 10 min could be used commercially to extend postharvest life of fresh-cut broccoli florets, and to maintain a better nutritional value (higher vitamin and protein contents) and appearance (greenness or fresh-like state).

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