Misting Affects Market Quality and Enzyme Activity of Broccoli During Retail Storage

M.M. BARTH, A.K. PERRY, S.J. SCHMIDT, and B.P. KLEIN

ABSTRACT

Vitamin C, enzyme activities, color and texture changed in misted and nonmisted broccoli at 0, 24, 48 and 72 hr intervals during display cabinet storage (18°C). Reduced ascorbic acid (RAA), ascorbate oxidase and peroxidase activities, total chlorophyll extractions, Hunterlab color and Instron texture measurements were followed. Misting significantly enhanced RAA ($p=0.0001$), and retention of chlorophyll ($p=0.0001$), and green color as measured by hue angle ($p=0.0021$) during 72 hr storage. RAA degradation in misted and nonmisted broccoli and total chlorophyll degradation in nonmisted samples followed first order kinetics. Peroxidase activity was significantly greater in both the nonmisted broccoli floret/stem ($p=0.0221$) and stalk tissue ($p=0.0079$). No significant differences were found between misted and nonmisted broccoli for ascorbate oxidase activity ($p=0.9426$) or Instron shear values ($p=0.3652$).

Key Words: ascorbic acid, texture, peroxidase, chlorophyll, broccoli

INTRODUCTION

NUTRIENT and market quality retention in vegetables is affected by many factors including postharvest processing, storage time and conditions, such as temperature, relative humidity, light, and atmosphere (Fisher and Van Duyne, 1952; Ezell and Wilcox; 1959; Klein and Perry, 1982; Kailasapathy and Koneshan, 1986; Weichmann, 1986; Berrang et al., 1990). Ascorbic acid (vitamin C) is a labile essential nutrient in the human diet (Hellman and Burns, 1958; Food & Nutrition Board, 1989). Thus vitamin C retention is often measured when evaluating postharvest storage effects on nutrients in vegetables (Zepplin and Elvehjem, 1944; McCombs, 1957; Klein and Perry, 1982; Sumner et al., 1983; Hudson et al., 1986; Klein, 1987; Vanderslice et al., 1990; Wu et al., 1992).

Color is the major quality attribute of vegetables considered to have the most impact upon consumer selection of produce (Francis and Clydesdale, 1975). For green vegetables, chlorophyll content is associated with greenness. Retention of green color by vegetables after processing has been assessed as a measure of quality (Sweeney and Martin, 1958). Texture is also an important quality attribute of horticultural crops (Ilker and Szczesniak, 1990). Many factors have been shown to affect green color and texture retention in processed vegetables, such as temperature, relative humidity, pH, blanching conditions, and atmospheric composition (Ezell and Wilcox, 1959; Groeschel et al., 1966; Lipton, 1975; Wang and Hruska, 1977; Wang, 1979; Bourne, 1982; Shewfelt et al., 1983; Faboya, 1985; Kader, 1986; Perrin and Gaye, 1986; Rij and Ross, 1987; Rushing, 1990).

In an earlier study, we reported that misting of broccoli spears resulted in enhanced ascorbic acid retention during display cabinet storage for 72 hr (Barth et al., 1990). About 90% of the major retail supermarket chains mist vegetables. However, the effects of misting on quality attributes such as color, texture and enzyme activities have not been clearly assessed. The objective of our current study was to determine the effects of misting on market quality and enzyme activities in broccoli over time (0, 24, 48, 72 hr) during display cabinet storage (18 ± 4°C) based on changes in chemical and physical factors and enzyme activities.

Authors Perry, Schmidt, and Klein are with the Division of Foods & Nutrition, University of Illinois at Urbana-Champaign, 905 S. Goodwin Ave., 386 Bevier Hall, Urbana, IL 61801. Author Barth's current address: Dept. of Nutrition & Food Science, Univ. of Kentucky, 121 Funkhouser Building, Lexington, KY 40506-0054.

MATERIALS & METHODS

Broccoli

Broccoli (cv. Green Duke), grown in California, was obtained from a local wholesale distributor in Urbana, IL in October, 1989 and February, 1990.

Experiment Design

Upon delivery, random distribution of 22 broccoli bunches was made to the misted and nonmisted sections of a refrigerated produce display cabinet (Corrigan Misting System, Northbrook, IL). Misting intervals were set at 4 sec every 4 min, providing a total of 43.5 mL water/4 min. Broccoli samples were stored in the display cabinet, maintained at 18 ± 4°C, over 72 hr. Room temperature was 24 ± 4°C, and relative humidity 35%. The temperature of the misted section of the case was about 4°C lower than the nonmisted section due to evaporative cooling. The relative humidities were misted 56% and nonmisted 42%. Relative humidity was measured using a Psychro-Dyne hygrometer (Environmental Tectonics Corp., Southampton, PA). The room was equipped with fluorescent lights, kept on throughout the study to simulate usual market conditions.

Sampling

At each time interval (0, 24, 48 and 72 hr), a composite of one floret with 2 in of stem from the right side of each broccoli bunch was removed. After broccoli bunch was sampled, it was rotated one quarter turn to the right. Misted samples were blotted dry with a cloth towel. Both misted and nonmisted samples were ground at speed control #2 using a Kitchen-Aid K-5A grinder (Hobart Manufacturing, Troy, Ohio). Ground broccoli samples were then used for moisture, RAA, ascorbate oxidase, peroxidase, total chlorophyll, and Hunterlab color difference measurements. Peroxidase activity of broccoli stalk tissue was also determined. Instron shear values of stalks were measured.

Moisture content

Ground broccoli (≈5g) was used for each moisture determination (AOAC, 1980). Percent solids was calculated and used to express RAA and total chlorophyll contents on a dry weight basis.

Reduced ascorbic acid

Reduced ascorbic acid (RAA) was determined by the titrimetric assay described by Pelletier (1985). Ground broccoli (20g) was extracted with 100 mL 6% metaphosphoric acid for 3 min using a Tekmar tissue homogenizer (Tekmar Corp., Cincinatti, OH), made up to 250 mL volume with 6% metaphosphoric acid, and filtered through Whatman #42 filter paper. Aliquots (5 mL each) of the filtrate were titrated with 2,6-dichloroindophenol. RAA content was determined on wet and dry bases. Percent RAA retention was also calculated.

Table 1—Mean percent moisture content (wet weight basis) and reduced ascorbic acid (RAA) content and percent RAA retention (dry weight basis) in misted (M) and nonmisted (NM) broccoli over 72 hr storage[z][a][b][c][d]

Storage Interval (hr)	% Moisture		RAA (mg/g)		% RAA retention	
	M	NM	M	NM	M	NM
0	86.8 ± 1.48		5.6 ± 0.29		100%	
24	86.5 ± 1.10	83.9 ± 0.59	5.6 ± 0.46	4.4 ± 0.21	101.0 ± 12.94	79.2 ± 7.53
48	86.1 ± 1.36	84.0 ± 0.97	4.3 ± 0.29	2.8 ± 0.06	76.5 ± 5.19	50.0 ± 3.06
72	85.0 ± 0.72	80.2 ± 0.52	3.6 ± 0.13	2.1 ± 0.27	64.3 ± 1.15	38.7 ± 7.06

[z] Values shown are means ± standard deviations, n=3.
[a] 95% confidence interval for % moisture content = ±1.91%.
[b] 95% confidence interval for RAA content (dry wt) = ±0.49 mg/g.
[c] 95% confidence interval for percent RAA retention (dry wt) = ±10.93%.
[d] Mean initial RAA content (wet wt) = 73.9 mg/100 g.

Enzyme activities

Ascorbate oxidase activity was determined by an adaptation of a spectrophotometric assay (Vines and Oberbacher, 1963). Ground broccoli (20g) was homogenized with 50 mL 0.1M phosphate buffer (pH= 6.5) as above, and the filtrate was used for enzyme assay. The assay was carried out in disposable, 5 mL UV cuvettes with a 1 cm light path at 25°C, using a Beckman Model 25 spectrophotometer (Beckman Instruments, Fullerton, CA). Ascorbic acid, 0.5 μM quantities in 3 mL of 0.1M phosphate buffer (pH= 5.6) was added to both the reaction and reference cuvettes. Samples of the vegetable filtrates (0.1 mL) were added to the sample cells and the cuvettes were inverted once prior to spectrophotometric reading. Change in absorbance at 265 nm was recorded at 1 min intervals for 3 min. Enzyme activity was expressed as change in absorbance/min (at 265 nm)/gram vegetable tissue.

Peroxidase activity was also determined by a spectrophotometric assay (Hemeda and Klein, 1990) for both floret/stem and stalk tissue. Absorbance readings were made at 1 min intervals for 2 min. Enzyme activity was expressed as change in absorbance/min (at 470 nm)/g vegetable tissue. One unit of activity was defined as a change in absorbance of 0.001/min for both peroxidase and ascorbate oxidase enzymes. Percent enzyme activity (relative to initial values) was then calculated for both enzymes.

Total chlorophyll

Total chlorophyll content of broccoli samples was determined using an adaptation of the spectrophotometric assay of Anderson and Boardman (1964). Ground broccoli tissue (9g) was extracted in 54 mL acetone:3 mL 0.1N NH_3OH solution by homogenization using an homogenizer at 60 rpm for 1 min under cold conditions (5°C). Homogenate was stored in the dark prior to centrifugation. The homogenate was centrifuged in 50 mL tubes for 20 min at 5°C. The supernatant was decanted and aliquots transferred to 5 mL quartz cuvettes (1 cm light path) prior to reading absorbance at 700, 663, 645, and 626 nm. Total chlorophyll was expressed as μg total chlorophyll/g vegetable tissue on wet and dry bases. Percent total chlorophyll retention was also calculated.

Hunterlab color

The color of ground broccoli samples was evaluated using a Hunterlab Color Difference Meter (Hunterlab, Fairfax, VA) Model 25. The instrument was calibrated with a standard green tile CG-6625 (L-28.01, a= −25.8, b= +5.6) using a D-65 incandescent lamp. Ground broccoli (≈20g) was packed into a cylindrical, clear lucite cup and L, a, b values were measured. Hunter a and b values were used to compute hue angle ($\tan^{-1}$b/a) values (Shewfelt et al., 1984).

Instron texture

Shear values of broccoli stalks were determined using the Instron Universal Testing Machine (Instron Corp., Canton, MA), Model 1132 with a Warner-Bratzler blade attachment. A 20 kg load cell was used with crosshead and chart speeds of 20 cm/min. For each measurement a uniform 10.0 cm stalk, (2 cm diam) was placed on the Instron platform, perpendicular to the path of the Warner-Bratzler blade and deformation measurements were made.

Statistical analysis

Three replications of the study were performed and duplicate or triplicate readings were made for each attribute. Significance was determined by a two-way analysis of variance (ANOVA) at the 95% confidence level. Sources of variance were misted or nonmisted and storage time. The 95% confidence intervals were computed to determine differences, if significance was found by two-way analysis of variance for an attribute.

RESULTS & DISCUSSION

Moisture

Moisture content in misted vs nonmisted broccoli samples over 72 hr storage (Table 1) showed mean initial moisture (wet basis) was 86.8%. After 72 hr storage, the mean moisture contents were: misted 85% and nonmisted 80.2%. Mean percent moisture content was significantly greater ($p \leq 0.05$) in misted broccoli samples at 24, 48 and 72 hr storage intervals, confirming earlier reports (Barth et al., 1990).

Reduced ascorbic acid retention

The mean initial reduced ascorbic acid (RAA) content in the broccoli samples on a wet basis (73.9 mg/100g) was similar to that reported by Wills et al. (1983) and Barth et al. (1990). Mean RAA content and percent RAA retention (Table 1) were significantly greater in misted broccoli samples calculated on a dry weight basis (p=.0001). Mean percent RAA retained in misted and nonmisted samples after 72 hr storage was 64.3% and 38.7%, respectively. The mean RAA content and percent RAA retained were significantly greater ($p \leq 0.05$) in misted broccoli samples at 24, 48 and 72 hr storage intervals.

Degradation of RAA exhibited first order kinetic behavior in misted and nonmisted broccoli samples over 72 hr storage. The first order reaction rate constants, k, for the misted and nonmisted broccoli samples were 6.65×10^{-3}/hr and 13.84×10^{-3}/hr, respectively. In a previous study, Barth et al. (1990), reported total ascorbic acid (TAA) degradation in misted and nonmisted broccoli followed first order reaction kinetics over 72 hr storage (16 ± 4°C). They reported rate constants at 4.30×10^{-3}/hr for misted and 7.64×10^{-3}/hr for nonmisted broccoli.

Enzyme activities

Ascorbate oxidase is the primary enzyme hypothesized to be responsible for enzymatic degradation of reduced ascorbic acid to dehydroascorbic acid (McCombs, 1957). No significant difference was found in ascorbate oxidase activity (p=.9426) between misted and nonmisted samples (Table 2). Ascorbate oxidase activity apparently did not significantly influence ascorbic acid degradation. Factors possibly contributing to the observed increased rate of ascorbic acid degradation in nonmisted broccoli samples include lower relative humidity and higher temperature in the nonmisted section of the display case. Evaporative cooling effects of misting, made the nonmisted section of the display cabinet about 4°C warmer than the misted

Table 2—Mean percent ascorbate oxidase and peroxidase activities in misted (M) and nonmisted (NM) broccoli over 72 hr storage[zabcde]

Storage Interval (hr)	% Ascorbate oxidase activity (floret/stem)		% Peroxidase activity (floret/stem)		% Peroxidase activity (stalk)	
	M	NM	M	NM	M	NM
0	100%		100%		100%	
24	94.8 ± 12.82	94.0 ± 5.34	114.1 ± 19.36	146.9 ± 36.78	134.3 ± 23.60	165.5 ± 19.51
48	83.7 ± 10.35	75.2 ± 9.00	116.1 ± 32.72	145.6 ± 28.91	134.2 ± 39.02	206.1 ± 67.57
72	86.3 ± 24.37	93.1 ± 28.12	109.7 ± 30.12	187.8 ± 69.38	159.3 ± 28.10	228.0 ± 14.30

[z] Values shown are means ± standard deviations, n=3.
[a] 95% confidence interval for % peroxidase activity (floret/stem) = ±59.89%.
[b] 95% confidence interval for % peroxidase activity (stalk) = ±55.45%.
[c] Mean initial ascorbate oxidase activity (floret/stem) = 1.9 units/min/g veg.
[d] Mean initial peroxidase activity (floret/stem) = 14.7 units/min/g veg.
[e] Mean initial peroxidase activity (stalk) = 10.6 units/min/g veg.

Table 3—Mean total chlorophyll content (wet and dry weights basis) and percent chlorophyll retention (dry weight basis) in misted (M) and nonmisted (NM) broccoli over 72 hr storage[zabc]

Storage Interval (hr)	Mean total chlorophyll content (μg/g)				% Total chlorophyll retention	
	M	NM	M	NM	M	NM
	(wet wt)		(dry wt)		(dry wt)	
0	107.1 ± 9.56		10.3 ± 0.48		100%	
24	115.4 ± 10.56	91.7 ± 7.78	11.3 ± 0.92	7.3 ± 0.85	109.7 ± 9.13	71.1 ± 11.47
48	95.1 ± 7.56	59.9 ± 12.93	9.9 ± 0.14	4.1 ± 0.69	96.3 ± 4.06	40.4 ± 6.93
72	91.8 ± 7.86	42.1 ± 13.17	9.4 ± 0.01	2.6 ±0.71	91.4 ± 4.28	25.6 ± 6.74

[z] Values shown are means ± standard deviations, n=3.
[a] 95% confidence interval for total chlorophyll content (wet wt) = ±17.67 μg/g veg.
[b] 95% confidence interval for total chlorophyll content (dry wt) = ±1.07 μg/g veg.
[c] 95% confidence interval for % total chlorophyll (dry wt) = ±11.47%.

section. Increased ascorbic acid losses in vegetable tissue have been demonstrated during low humidity, elevated temperature postharvest storage (Ezell and Wilcox, 1959).

Peroxidase activity in the nonmisted floret/stem and in both misted and nonmisted stalk (Table 2) increased over 72 hr storage. However, peroxidase activity and percent peroxidase activity were significantly greater in nonmisted broccoli samples for both floret/stem ($p=0.0221$) and stalk tissue ($p=0.0079$). Peroxidase activity was significantly lower ($p\leq0.05$) in misted floret/stem at 72 hr and in misted stalk tissue at 48, and 72 hr. There may be a relationship between increased peroxidase activity and observed ascorbic acid losses in nonmisted tissue (Blundstone et al., 1971). Generally, peroxidase activity was greater in broccoli stalk than in floret/stem tissue after 72 hr storage.

Total chlorophyll retention

Total chlorophyll content, on both wet and dry basis and percent total chlorophyll retention, dry weight basis, (Table 3) showed misting enhanced total chlorophyll retention over 72 hr ($p=0.0001$). Percent total chlorophyll loss in misted samples was minimal (8.6%) after 72 hr storage, whereas in nonmisted samples the loss after 72 h was 74.4%. Total chlorophyll retained was significantly greater ($p\leq0.05$) in misted vs. nonmisted broccoli samples at 24, 48 and 72 hr storage intervals on both wet and dry weight bases. Within the nonmisted group, chlorophyll retention was significantly lower ($p\leq0.05$) at each interval after 0 time.

Chlorophyll degradation in nonmisted broccoli over 72 hr storage exhibited first order kinetics. The first order reaction rate constant, k, for the nonmisted broccoli samples was 1.92×10^{-2}/hr. Chlorophyll degradation has been shown to be affected by many factors including temperature and relative humidity (Shewfelt et al., 1983; Perrin and Gaye, 1986; Rij and Ross, 1987). However, the mechanism of chlorophyll degradation is not well understood (Hendry et al., 1987).

Color retention

In Hunterlab instrument measurements color values are lightness to darkness (L), red to green (±a), and blue to yellow (±b). Hue angle ($\tan^{-1}b/a$) represents greenness. The misted samples were significantly greener than the nonmisted samples over the 72 hr sampling period ($p=0.0021$), as measured by hue angle values (Fig. 1). Green color retention was significantly greater in the misted than in nonmisted broccoli samples at each storage interval ($p<0.05$). There was no significant difference in 'L' values between misted and nonmisted samples ($p=0.7134$). The 'L' values remained near their initial values for both groups. Generally, results from total chlorophyll determination and Hunterlab hue angle values showed that green color was better retained in the misted broccoli samples.

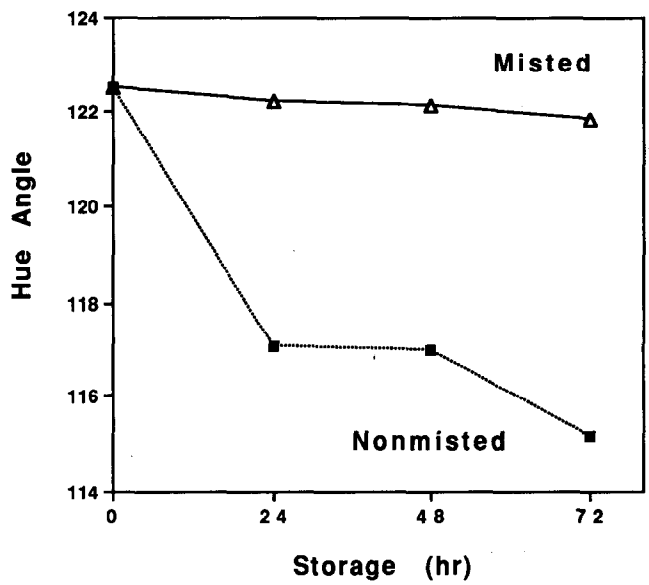

Fig. 1—Hunterlab hue angle values ($\tan^{-1}b/a$) in misted and nonmisted broccoli over 72 hr storage[z]. ([z] 95 confidence interval = ± 4.89)

Texture

Texture measures with the Instron Universal Testing Machine showed shear values between groups were not significantly different ($p = 0.3652$).

CONCLUSIONS

MISTING of broccoli under market display conditions improved reduced ascorbic acid, chlorophyll and green color retention, thus improving market quality.

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