

Changes in Color and Chlorophyll Fluorescence of Broccoli Florets following Hot Water Treatment

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Abstract. Hot water treatments (HWTs), at a range of temperatures (43 to 55C) and durations (10 sec to 30 min), were applied to floret groups of 'Shogun' broccoli (*Brassica oleracea* L. var *italica*) directly after harvest. Floret groups were then stored at 20C in the dark for 3 days. A range of optimal treatments was found in which yellowing was markedly reduced, and heat damage (water soaking and decay) did not occur. Chlorophyll fluorescence measurements indicated that in the optimum treatment that prevented yellowing the F_v/F_m ratio following HWT decreased immediately and was maintained at a constant level for the next 3 days. A further experiment examined the effect of HWT durations up to 20 min at 47C on fluorescence and yellowing. Longer durations of HWTs (>5 min) progressively reduced yellowing and the F_v/F_m ratio. From these three experiments a HWT of 47C for 7.5 min was selected as the best treatment. This treatment consistently reduced yellowing for up to 5 days. A decrease in the F_v/F_m ratio may be a useful indicator of broccoli florets response to hot water treatments.

The shelf life and market potential of cut broccoli heads is limited by rapid yellowing of the florets (King and Morris, 1994; Rushing, 1990; Tian et al., 1994). Postharvest heat treatments are known to delay chlorophyll loss in green tissue (Klein and Lurie, 1991; Paull, 1990). One recent study showed that broccoli (cv. Dominator) treated at 45C for 14 min retained 81% of its chlorophyll for 5 days postharvest (Kazami et al., 1991a). Previous work with New Zealand broccoli showed that cultivars such as Shogun, Green Beauty, and Green Belt differ in their postharvest behavior, particularly in respiration rate, ethylene production, and yellowing rate (King and Morris, 1994; Tian et al., 1995).

Measurement of chlorophyll fluorescence provides a nondestructive assessment of loss of chloroplast function, and has been used as an indicator of stress response and senescence in a range of harvested plant tissues and organs (Krause and Weis, 1984; Smillie et al., 1987). Toivonen (1992) showed that a decrease in variable fluorescence (F_v) in broccoli florets is highly correlated with a reduction in respiration rate and ascorbic acid content during 4 to 24 days of storage at 1C, suggesting that fluorescence may be a reliable indicator of broccoli senescence at low temperature.

In the present work, we studied the effects of HWTs on yellowing of 'Shogun' broccoli florets. We used an extensive range of HWTs to define the optimum heat treatments for reducing broccoli yellowing. We followed this with studies of the relationship between color maintenance and fluorescence measurement.

Material and Methods

Plant material. 'Shogun' broccoli was used in all experiments. For investigation of yellowing and heat damage of HWT, broccoli

heads were harvested from local gardens in Auckland, New Zealand, during February, 1994 (summer), and transported to the Mt. Albert Research Centre, Auckland, in less than 2 h. For testing the relationship between yellowing and chlorophyll fluorescence, heads were harvested from local gardens in Levin during March and August, 1994 (early autumn and winter, respectively), and transported to the laboratory in less than 0.5 h. Floret groups were excised and handled in the laboratory as described previously (Tian et al., 1994). In all cases, five floret groups were randomly assigned to each HWT.

Hot water treatment. For measuring yellowing after HWT, florets were treated in mesh cages in water baths, consisting of 80-liter fiberglass tubs and Grant water bath temperature controller units $\pm 0.1C$. (1.4-kW heater; model GRAVF; Cambridge, U.K.) with the stirrer blade removed. Uniform water circulation and temperature within the baths was achieved by pumping water (model UPS 20-60B 150; Grundfos pumps, Copenhagen, Denmark) through perforated PVC tubing (22 mm i.d.) arranged in a grid pattern in the base of the baths. This system resulted in water temperatures varying by no more than $\pm 0.2C$ throughout the baths and over time. For measuring chlorophyll fluorescence after HWT floret groups were placed in mesh bags and dipped in a 47-liter water bath, $\pm 0.1C$. (Julabo-20B; Cambridge, U.K.)

Chlorophyll fluorescence. Measurement of chlorophyll fluorescence of green tissues is a nondestructive method used to detect F_o (initial fluorescence), F_m (maximum fluorescence) and calculate F_v/F_m (a ratio of variable fluorescence to maximum fluorescence), where $F_v = F_m - F_o$. The ratio of F_v/F_m is a measure of the photochemical photon yield in photosystem II (Krause and Weis, 1984; Lavorel and Etienne, 1977).

To measure chlorophyll fluorescence, florets were placed in five chambers/treatment (5 to 8 florets/chamber) in the dark at 20C for dark adaptation. After 40 min, chlorophyll fluorescence was measured using a plant efficiency analyzer (Hansatech, U.K.) with a sensor containing both a custom array of red light-emitting diodes providing saturating light levels for accurate F_m determination, and a fast response, low noise fluorescence detector for determination of F_o .

Experimental design. To define an optimum HWT for reducing yellowing of broccoli florets, 225 floret groups were cut from 50

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heads and treated in hot water at a range of temperatures from 43 to 55C for six durations from 10 sec to 30 min. Florets were then stored at 20 ± 0.5C in the dark for 72 h at 97% relative humidity. Color (a decrease in hue angle indicated a change from green to yellow) of floret groups was measured using a chromameter (model CR-200; Minolta, Japan) at harvest and 72 h after storage according to Tian et al. (1994). Samples were examined for heat damage after 72 h storage and rated on a scale of 0 to 3: 0, no damage; 1, water soaking of <20% of floret groups; 2, water soaking of 20% to 50% and <20% rotten; and 3, >50% rotten.

The effects of HWT on chlorophyll fluorescence were first examined by selecting four treatments (5 floret groups/treatment) that represented control (20C for 1 min), noneffective at maintaining green color (45C for 1 min), effective at maintaining green color (47C for 7.5 min), and heat-damaged (53C for 3 min) treatments. Following HWT, floret fluorescence and hue angle were determined immediately (<0.5 h) and at selected intervals for up to 72 h at 20C in the dark.

To investigate the relationships between HWT duration and chlorophyll fluorescence, florets were treated in water at 47C for 1, 3, 5, 7.5, 12, or 20 min. A control treatment of 20C for 10 min was also used, and floret groups in all treatments were then stored at 20C in the dark. Color and fluorescence measurements were made during 144 h at 20C in the dark.

Statistical analysis. Contour plots are an effective means of presenting results from factorial experiments (Fig. 1). They show the effect of temperature and duration on a measured factor using the local regression methods implemented by the S-Plus loess function and result in contour lines (bold-solid lines with large numbers) with the mean value for each treatment shown at each point with bold, italicized moderate sized numbers. The decrease in hue angle (Fig. 1a) was calculated as the percent decrease from the mean initial values, and 95% confidence intervals are presented on either side of the contour lines (broken lines with small lighter numbers). Because heat damage was rated on a treatment basis, no measure of variability was made, and 95% confidence intervals could not be calculated (Fig. 1b).

Statistical analyses of data from chlorophyll fluorescence were carried out using SAS (Cary, N.C.) to calculate analysis of variance, means with corresponding standard error, and least significant difference for multiple comparisons.

Results

Increasing yellowing (percent decrease in hue angle, Fig. 1a) and heat damage (Fig. 1b) of hot water treated broccoli occurred during storage after HWT. Florets treated at 20C and held under high RH for 3 days at 20C in the dark exhibited an average decrease in hue angle of between 20.1% and 22.3% (Fig. 1a). Hot water treatments for short durations at low temperatures (1 min at 45 and 47C) tended to have no effect on the decrease in hue angle. However, at high temperatures (51C), short durations were effective. With lower temperatures, as the duration of HWT increased, the decrease in hue angle was reduced to levels less than 5% (20 min at 45C and 7.5 min at 47C). Longer duration HWTs at temperatures >49C (7.5 min at 51C, and 2 min at 55C) resulted in some decrease in hue angle, but were not as effective as moderate durations. Hot water treatments resulting in the smallest hue angle decrease for each temperature were 30 min at 43C; 20 min at 45C; 7.5 to 17.5 min at 47C; 5 min at 49C; 1 min at 51C; 30 sec at 53C; and 10 sec at 55C (Fig. 1a, between the two 5% contour lines).

Heat damage of broccoli florets occurred after many of the HWTs and increased with longer treatment duration and higher temperature

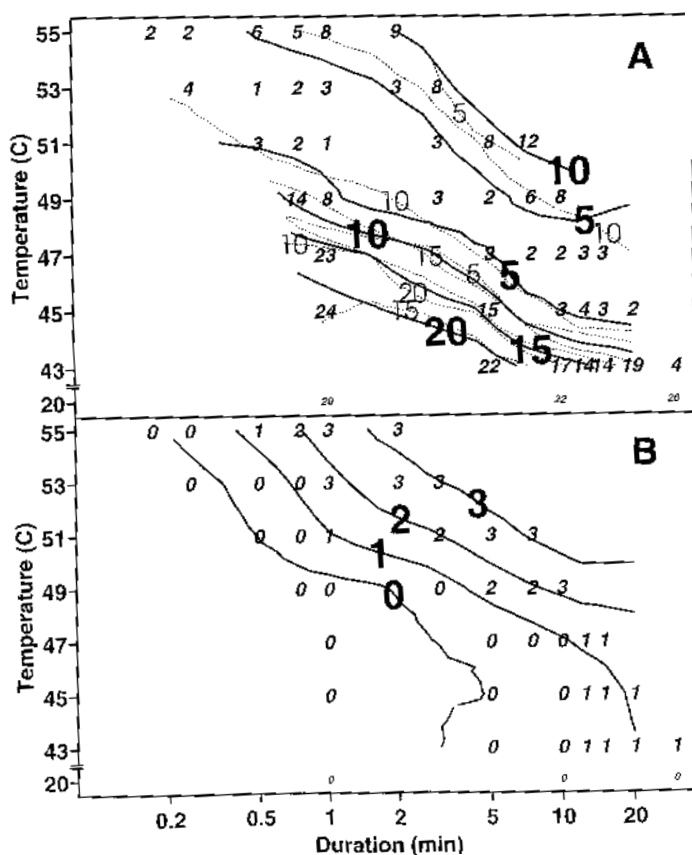


Fig. 1. Contour plots of decrease (%) of hue angle (a) and heat damage (b) in broccoli florets after 3 days at 20C in the dark, following a range of hot water treatments. Bold lines (with large numbers) represent fitted contour lines, and broken lines on either side (small non-bold numbers) represent 95% confidence intervals. Small, bold italicized numbers represent the mean values for each treatment combination (Numbers rounded up to zero decimal points). See Materials and Methods for statistical analysis details.

(Fig. 1b). Optimum treatments were defined as those in which the decrease in hue angle was <5% and no heat damage was observed. These were 7.5 and 10 min at 47C; 3 min at 49C; 15, 30 and 45 sec at 51C; 15 sec at 53C; and 10 sec at 55C (Fig. 1 a and b).

Four HWTs were selected to examine the effect on chlorophyll fluorescence; 20C for 1 min, 45C for 1 min, 47C for 7.5 min, and 53C for 3 min. Immediately following HWT, F_0 increased and F_v decreased with increasing temperature duration combinations

Table 1. Effect of hot water treatments on chlorophyll fluorescence (F_0 , F_v , F_v/F_m) and color change (hue angle) of broccoli florets immediately after treatment (< 0.5 h) and after 72 h at 20C in darkness.

	Days	20C	45C	47C	53C
	at 20C	1 min	1 min	7.5 min	3 min
F_0	0	3.58 ^{Ac}	4.06 ^{Ac}	8.26 ^{Ab}	11.0 ^{Aa}
	3	2.29 ^{Bc}	2.67 ^{Bc}	6.59 ^{Bb}	8.10 ^{Ba}
F_v	0	14.94 ^{Aa}	13.69 ^{Aa}	2.02 ^{Ab}	1.58 ^{Ac}
	3	5.39 ^{Ba}	5.83 ^{Ba}	1.67 ^{Bb}	0.38 ^{Bc}
F_v/F_m	0	0.81 ^{Aa}	0.77 ^{Aa}	0.20 ^{Ab}	0.13 ^{Ac}
	3	0.70 ^{Ba}	0.68 ^{Ba}	0.20 ^{Ab}	0.04 ^{Bc}
Hue angle	0	124.3 ^{Aa}	123.5 ^{Aa}	122.6 ^{Aa}	124.9 ^{Aa}
	3	99.0 ^{Bc}	95.3 ^{Bc}	121.4 ^{Aa}	114.3 ^{Bb}

Different characters (upper case) within the same column and the different characters (lower case) within the same line represent significant differences at the 5% level (LSD).

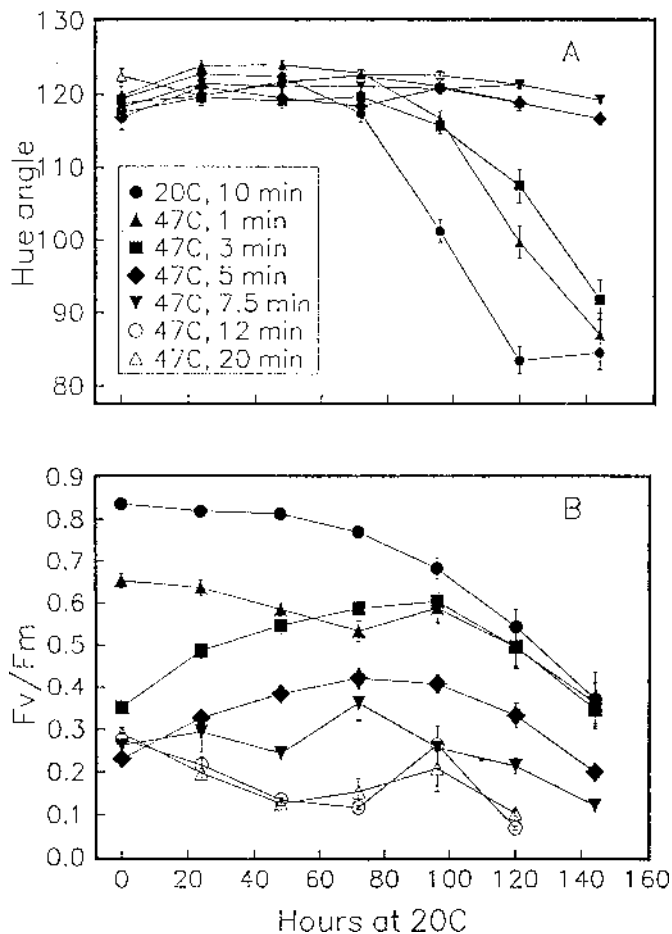


Fig. 2. Changes in hue angle (a) and in F_v/F_m ratio (b) of broccoli florets treated at 47C for various durations before storage at 20C in the dark for up to 144 h. Vertical bars represent \pm SE of the mean.

(Table 1). Color (hue angle) was similar among treatments immediately after HWT, but changed during storage in all but the 7.5 min at 47C treatment (Table 1). Immediately following treatment, the F_v/F_m ratio was unchanged with the 45C for 1 min HWT, but significantly decreased in florets treated at 47 and 53C. The changes in F_v/F_m were due to both an increase in F_o and a decrease in F_m , but especially to the latter (Table 1).

During 72 h in the dark, F_v/F_m decreased in all treatments except 47C for 7.5 min (Table 1). Floret yellowing from the four treatments followed a similar trend to changes in F_v/F_m . Following 20, 45, and 53C treatments, hue angle decreased by 20.4%, 22.9%, and 8.5%, respectively. The least decrease (10%) was found following the 47C treatment (Table 1). Heat damage was observed only in florets treated at 53C for 3 min. The F_v/F_m decrease in response to damaging HWTs was observed in the first 24 h after HWT (data not shown); a similar decrease also occurred in florets at 47C for longer durations (Fig. 2b).

A closer examination of the effect of HWT duration on yellowing and F_v/F_m of broccoli florets was carried out on florets treated at 47C (Fig. 2 a and b). In control florets (20C for 10 min), the decrease in hue angle commenced 48 h after treatment, reaching a minimum value at 120 h (Fig. 2a). A similar but smaller decrease was observed following HWTs of 1 and 3 min at 47C. Hot water treatments of 5 min and longer resulted in little change in hue angle; HWTs of 12 and 20 min resulted in a slight hue angle decrease, but caused heat damage. No measurements were made

after 120 h. A HWT of 47C for 7.5 min was the most effective at minimizing yellowing over the 144 h period.

The F_v/F_m ratio of control florets decreased progressively following treatment (Fig. 2b). Hot water treatments of 47C for 1 or 3 min resulted in decreased F_v/F_m immediately after treatment, although after 120 h there was little difference between these and the control treatments. In HWTs that resulted in heat damage (47C for 12 and 20 min), F_v/F_m values were significantly reduced immediately after treatment (< 0.3), then dropped continuously to about 0.1 at 120 h, at which point the florets had decayed. F_v/F_m decreased after 3 and 5 min HWTs, exhibited modest recovery up to 96 and 72 h respectively, and then declined. The F_v/F_m value for florets from the 7.5 min treatment dropped after treatment but remained stable up to 120 h.

Discussion

There is a range of HWTs that reduce yellowing of broccoli florets, and a number of these do not result in heat damage. Some of the treatments, such as 55C for 15 sec, require highly accurate temperature/time control. However, lower temperatures could be used over a wider range of durations while still being optimal. Such a treatment, for example, 47C for 7.5 min, was selected for chlorophyll fluorescence studies. This treatment resulted in hue angle decreases of less than 2.5% after 72 to 120 h (Figs. 1 and 2; respectively).

Our factorial approach identified a range of optimal treatments compared to those previously found (Kazami et al., 1991a, 1991b). However, the HWTs in which we have most confidence are similar to that found for the cultivar 'Dominador' (Kazami et al., 1991a, 1991b). These results, and the fact that our experiments used broccoli harvested from two regions over three seasons, suggest there may be little genetic or environmental influence on the heat response.

The heat damage observed in broccoli florets was largely due to water soaking, and in extreme cases allowed infection to occur. This mostly occurred at higher temperatures and longer durations (Fig. 1b) where hue angle also decreased. It is likely that membrane leakage and cell and chlorophyll breakdown are the major manifestations of heat damage in broccoli. However, optimal treatments have resulted in better control of yellowing than previously observed (Kazami et al., 1991a) suggesting there may be commercial applications for HWTs.

Immediately after treatment, the major increase in F_o and reductions in F_v and the F_v/F_m ratio occur at temperatures at which yellowing was most effectively reduced (Fig. 2 a and b; Table 1). The F_v/F_m ratio is independent of chlorophyll concentration in non-senescent tissue and is a rapid and accurate measure of the efficiency of the photosynthetic mechanism (Krause and Weis, 1984). The F_v/F_m ratio is a sensitive tool for testing heat damage to the System II reaction center (Havaux, 1993; Michalski and Wetter, 1994). We were therefore interested in the use of F_v/F_m as a measure of the potential of a HWT to maintain green color during postharvest life. Reduction in F_v/F_m in 20C control treatments correlated well with reduction of yellowing (Fig. 2 a and b). This could be interpreted as an interaction between fluorescence yield and chlorophyll concentration. Reduction of the F_v/F_m ratio immediately after HWT did not correlate with overall floret quality. However, a continuing decline of F_v/F_m between 0 and 48 h may reflect heat damage to florets (47C for 12 and 20 min). This decline was not apparent in the optimal treatment (47C for 7.5 min).

In measurements of chlorophyll fluorescence, F_v reflects the functioning of photosystem II, and F_o represents the probability of

exciting energy transfer between PS II antenna chlorophyll a, and the PS II reaction center (Armond et al., 1980; Gounaris et al., 1984; Havaux, 1992). The more than threefold decrease in F_v observed immediately following HWT most likely reflected inactivation of the photosystem II reaction center. The increase in F_o may reflect the perturbation of energy transfer mentioned above (Sundry et al., 1986). Since the F_v/F_m ratio reflects the quantum efficiency of the photosynthetic system, its decrease in broccoli following HWT also may indicate damage to the photosystem II reaction centers and subsequent dissociation of the pigment-protein complex from the central core of the light-harvesting apparatus (Gounaris et al., 1984; Havaux, 1993; Tekeuchi and Thornber, 1994).

While F_v/F_m decreased immediately after treatment, in some treatments the ratio recovered during storage at 20C (Fig. 2b). This may be explained by repair or recovery of the photosystem II reaction center which is necessary to restore electron flow (Mattoo et al., 1984).

Green tissue yellowing is generally caused by chlorophyll loss. Chlorophyllase is a key enzyme in chlorophyll breakdown (Holden, 1961). Thus, there is a need to investigate the potential relationship between changes in chlorophyllase activity and decreased F_v/F_m ratio in broccoli after hot water treatment.

In conclusion, chlorophyll fluorescence is clearly affected by heat treatments of plant tissue, and it may also have the potential to discriminate between the beneficial effects of heat on maintaining green color (HWT of 47C for 7.5 min) and treatments which result in excessive damage (47C for 20 min). Broccoli florets yellowing can be significantly reduced by HWTs. Lower F_v/F_m ratios were associated with maintenance of green color.

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