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Color, Ethylene Production, Respiration, and Compositional Changes in Broccoli Dipped in Hot Water

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Abstract. Color, ethylene production and respiration of broccoli (*Brassica oleracea* L. var. italica) dipped in hot water (45 °C, 10 minutes; 47 °C , 7.5 minutes; and 20 °C, 10 minutes as control) were measured. Hot-water treatment (HWT) delayed yellowing. Compared to the control, ethylene production and respiration in broccoli dipped at 45 °C decreased but recovered, and rates of both were enhanced after 24 and 48 hours, respectively, at 20 °C in darkness. There was no recovery of ethylene production or respiration in broccoli dipped at 47 °C. Following HWT of 47 °C for 7.5 minutes, respiration, starch, sucrose, and soluble protein content of florets and stems decreased dramatically during the first 10 to 24 hours after harvest. At the same time, fructose contents in florets and stems increased. Glucose increased in the florets but decreased within 24 hours in stems. Thereafter, glucose and fructose in florets and stems decreased. Sucrose content in florets and stems increased dramatically within a short period of treatment (<10 hours) and then declined. Protein in HWT florets and stems decreased during the first 24 hours and then increased until 72 hours. Ammonia content was lower in HWT broccoli during the first 24 hours and then increased above the level in the controls.

Harvesting and handling cause a series of stresses to broccoli, including wounding, separation from nutrient and hormone source, and dehydration. As an immature tissue, harvested broccoli is unable to maintain metabolic homeostasis and senescences rapidly (Huber, 1987; King and Morris, 1994a, 1994b), thus lowering its postharvest quality. The most obvious characteristic of postharvest senescence in broccoli is floret yellowing (King and Morris, 1994a; Rushing, 1990; Tian et al., 1994). Ethylene plays an important role in broccoli yellowing (Tian et al., 1994), but earlier physiological changes that occur in broccoli during the first 6 to 24 h after harvest are loss of sugars, proteins, and organic acids (King and Morris, 1994b).

Heat treatment can delay chlorophyll degradation and the consequent yellowing of green tissue (Klein and Lurie, 1991; Paull, 1990). Previous work has indicated that postharvest hot-water dipping efficiently prevents broccoli degreening (Forney, 1995; Kazami et al., 1991a, 1991b; Tian et al., 1996). Different optimum treatments (combinations of temperature and duration) have been reported. Broccoli ('Dominator') dipped at 45 °C for 14 min maintained its green color for 3 d at 20 °C in darkness, but florets yellowed subsequently (Kazami et al., 1991a). Forney (1995) found immersion of broccoli ('Cruiser' or 'Paragon') in 50 °C water for 2 min to reduce yellowing effectively. In our previous research, broccoli ('Shogun') dipped at 47 °C for 7.5 min showed no significant color change during 5 d of storage at 20 °C in darkness (Tian et al., 1996).

To further understand how heat treatment influences quality in broccoli, we investigated the effects of hot-water treatment (HWT) on ethylene production, respiration, and compositional changes.

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Materials and Methods

Plant materials. Broccoli (var. *italica*, 'Shogun') was harvested from commercial gardens in Levin, New Zealand, during June and Aug. 1994. Heads were brought to the Levin Research Center within half an hour. Floret groups were excised and handled in the laboratory as previously described (Tian et al., 1994). A floret group is defined as all the florets on the secondary branchlets of the main broccoli stem. The stems are defined as the secondary branchlets on which the floret group is attached.

Color, ethylene production, and respiration measurements. After harvest, 45 broccoli floret groups were cut from seven heads. Floret groups (15 groups per treatment) were dipped in water at 20 °C for 10 min (control), 45 °C for 10 min, or 47 °C for 7.5 min before measuring color (hue angle). Floret groups were placed in three 600-mL jars per treatment (five groups per jar) with a continuous flow of humidified air (flow rate = 10 mL·min^{-1}) at 20 °C in darkness. Color (hue angle), ethylene production, and respiration rate were measured at timed intervals after harvest as previously described (Tian et al., 1994).

Compositional analyses. Ninety floret groups were cut from 20 broccoli heads. Floret groups (45 groups per treatment) were dipped in water at 20 or 47 °C for 7.5 min., placed in three 20 × 15-cm plastic bags per treatment per sample time (five groups per bag), and stored at 20 °C in darkness. Each bag had 12 holes (0.5 cm in diameter) for maintaining high humidity and low CO_2 concentration (not detectable by gas chromatography). Samples (5 g fresh mass, n = 3) for analyses of sugars, starch, proteins, and ammonia were taken at 0, 1, 5, 7, 10, 24, 48, and 72 h after treatment. At each sampling time, floret groups were divided into florets and stems (0.5 cm beneath floret), immediately frozen in liquid N, stored at –75 °C, and later freeze-dried, and pulverized.

Sucrose, fructose, and glucose concentrations were measured in 62.5% methanol extracts of freeze-dried powder using sugar test kits (Boehringer, Mannheim, Germany). Starch in the powders was first solubilized with dimethysulphoxide and quantified with starch kits (Boehringer).

To measure soluble protein content, freeze-dried material (10 mg) was extracted on ice in 1 mL extraction buffer consisting of (in mM) 50 Tris-HCl (pH 7.6), 10 $MgSO_4$, and 1 dithiothreitol for 15 min. After centrifugation (15000*g*, 1 to 2 min), 50 L of the supernatant were analyzed for protein using a protein test kit (Boehringer). Ammonia content in extracts of the dried powders was analyzed by the alkaline hypochlorite/phenol nitroprusside reaction (King et al., 1994b).

Results

COLOR, ETHYLENE PRODUCTION, AND RESPIRATION IN BROCCOLI FLORET GROUPS. The color of control and HWT broccoli florets did not change during the first 48 h (Fig. 1A). In the controls, hue angle of florets began to decrease at 48 h, declined >30% at 120 h, and then remained constant from 120 to 144 h. Broccoli floret groups dipped at 47 °C did not change color significantly during 144 h at 20 °C. Hue angle in those dipped at 45 °C did not differ significantly from those dipped at 47 °C until 96 h when hue angle decreased significantly.

There were different patterns of ethylene production between broccoli floret groups in the three treatments (Fig. 1B). In the controls, wound ethylene was found 3 h after cutting. Ethylene production increased again after 8 h and was maintained at the same level until 72 h before decreasing to the lower level (<0.2 $nL \cdot g^{-1} \cdot h^{-1}$) at 120 h. Ethylene production in HWT broccoli decreased immediately after treatments, and no sign of wound ethylene was detected. Ethylene production in broccoli dipped at 47 °C for 7.5 min was negligible after 24 h but increased in those dipped at 45 °C after 24 h. At 144 h, ethylene production in floret groups dipped at 45 °C was >7-fold higher than in the control.

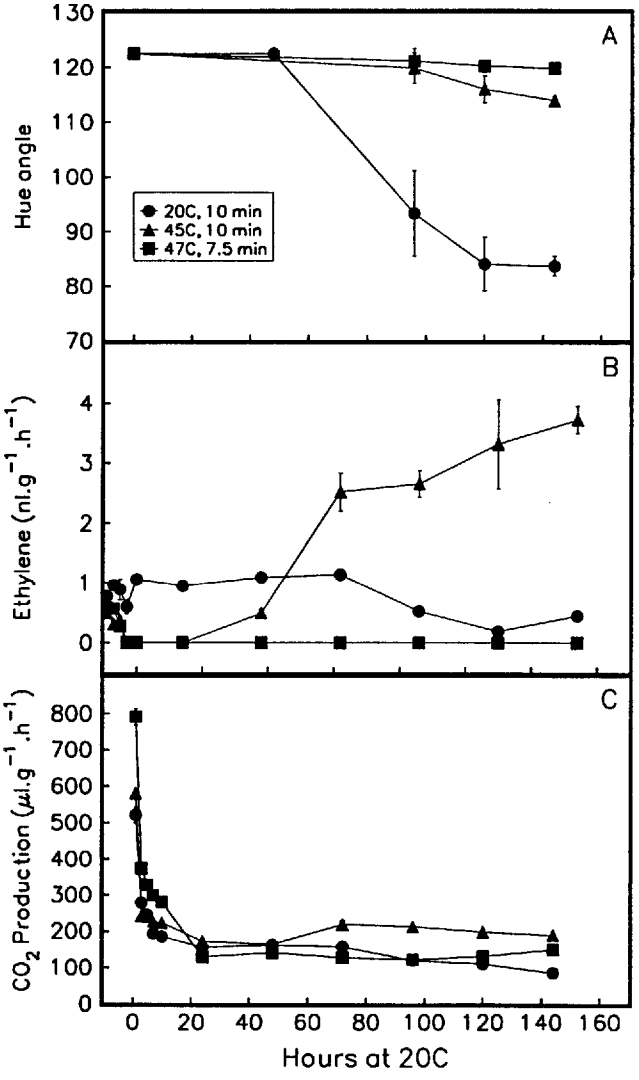

Fig. 1. (**A**) Color, (**B**) ethylene production, and (**C**) respiration rate in excised broccoli floret groups stored at 20 °C in darkness after treatment with 20, 45, or 47 °C water. Ethylene production and respiration rate are calculated on a fresh-mass basis. Error bars represent SE of the means, n = 3.

Respiration rate was high immediately after HWT but then decreased dramatically within 12 h and was relatively stable thereafter (Fig. 1C). Respiration rates immediately after treatment were temperature dependent. After 48 h, respiration in floret groups treated at 45 °C increased and remained slightly higher than in the other two treatments.

CARBOHYDRATE CONTENT. In control florets, sucrose content was initially higher than glucose or fructose (Fig. 2), but decreased rapidly. Sucrose content decreased >30% within 10 h after harvest and remained at low levels for up to 72 h (Fig. 2A). Patterns of change in glucose and fructose content were similar but differed from changes in sucrose (Fig. 2B and C). Contents of glucose and fructose increased within 1 h after harvest and then declined by almost half at 3 h. Thereafter, they increased dramatically and reached a maximum at 10 h before decreasing slowly.

Sucrose content in HWT broccoli florets was higher than in the controls (Fig. 2A). Sucrose content increased immediately after treatment, reached a peak at 5 h, decreased rapidly until 24 h, then held constant during the remainder of the experiment. There was no significant differences between the HWT and control in fructose (Fig. 2B) and glucose (Fig. 2C) contents during 10 and 24 h after harvest, respectively. After 24 h, contents of glucose and fructose in HWT florets declined rapidly until 48 h and then more slowly until 144 h. Glucose content was slightly higher than fructose during the entire test period.

There was a similar trend in sucrose content of stems and florets (Fig. 2A and D). In stems, sucrose content was lower than fructose and glucose contents (Fig. 2 D–F). Interestingly, the pattern of changes in glucose and fructose content differed between stems and florets (Fig. 2B, C, E, and F). Fructose content increased immediately after harvest and reached a maximum at 10 h. Thereafter, it decreased 52% until 48 h, then remained constant until 72 h (Fig. 2E). Glucose content in stems decreased gradually (Fig. 2F) in the controls and was <50% of the initial level at 48 h.

Sucrose content in HWT stems changed in a similar way to that in the florets (Fig. 2D). Sucrose in HWT stems was higher than in the controls from a few hours after the treatment until 72 h. HWT reduced the fructose content in stems immediately after treatment (Fig. 2E), and after 48 h, the fructose was half that of the controls. Glucose content in HWT stems was slightly lower than in the controls but the difference became significant after 24 h.

In florets, starch degraded immediately after harvest (Fig. 3A). After 24 h, starch content had decreased by 86%. Only small losses occurred from 24 to 72 h. Starch contents were several fold lower in stems than in florets (Fig. 3B). Starch content of control and HWT florets was unchanged until 7 h after HWT but fell more quickly thereafter than in control florets. There was no significant difference between starch contents of the control and HWT florets at 48 h. After HWT, starch content of stems dropped immediately and was maintained at lower levels than in the controls until 24 h. There was no significant difference between control and HWT from 24 to 48 h. Interestingly, starch content of HWT recovered after 48 h and was 3-times higher than that of the control at 72 h (Fig. 3B).

Soluble protein content. Soluble protein content of control florets decreased 45% after 7 h and then increased slightly at 24 h (Fig. 4A). After 24 h, protein content decreased slowly and was ≈10% of the initial level at 72 h. Compared with the control, soluble protein content in HWT florets decreased faster in the first 7 h. Thereafter it recovered gradually and was higher than the control at 48 and 72 h.

Soluble protein content in stems was lower than in florets (Fig. 4B). Similar trends to the changes in the florets were observed. Protein content in control stems decreased by 25% within 7 h and was maintained at a similar level up to 72 h. After HWT, protein content in stems decreased faster than in the controls. It increased again after 7 h, reached the same level as in the controls at 24 and 48 h, and was greater than in the controls at 72 h.

Ammonia contents. Florets and stems had low ammonia contents (<1 mg·g^{-1}) (Fig. 5). In florets and stems, the pattern of ammonia accumulation was similar with a dramatic increase between 7 to 12 h after treatment. In the control florets, ammonia content at 72 h reached levels 7-times higher than the initial level (Fig. 5A). Ammonia content in HWT florets accumulated to the same levels within 48 h and remained high after 72 h.

Ammonia content of stems changed in a similar way to that in the florets in the first 24 h (Fig. 5B) but then decreased. The increase in ammonia production after 7 h was less in HWT stems than in controls. Ammonia content levels in HWT stems did not decrease subsequently. At 72 h, levels were 3-times greater than in the control stems.

Discussion

In this work, wound-induced ethylene appeared within 3 to 5 h after harvest in control florets but was not detectable in HWT floret groups in which color loss was delayed. Thus, wound-induced ethylene may be involved in increasing the sensitivity of floret sepals to ethylene. There was a close relationship between ethylene production and degreening in HWT broccoli (Fig. 1). After HWT, ethylene production declined immediately, and yellowing was delayed. After 24 h at 20 °C in darkness, ethylene production had not only recovered in broccoli dipped at 45 °C but also increased dramatically thereafter (Fig. 1), and color (hue angle) began to decline at 72 h. These results indicated that 1) ethylene played an important role in broccoli yellowing and 2) after HWT (45 °C), enzymes that are involved in ethylene biosynthesis and chlorophyll loss were initially inactivated and then recovered at different times and rates. Endogenous ethylene may, therefore, play an important role in regulation of gene expression of chlorophyllase, which is the key enzyme catalyzing chlorophyll degradation. Similar observations of the recovery of ethylene

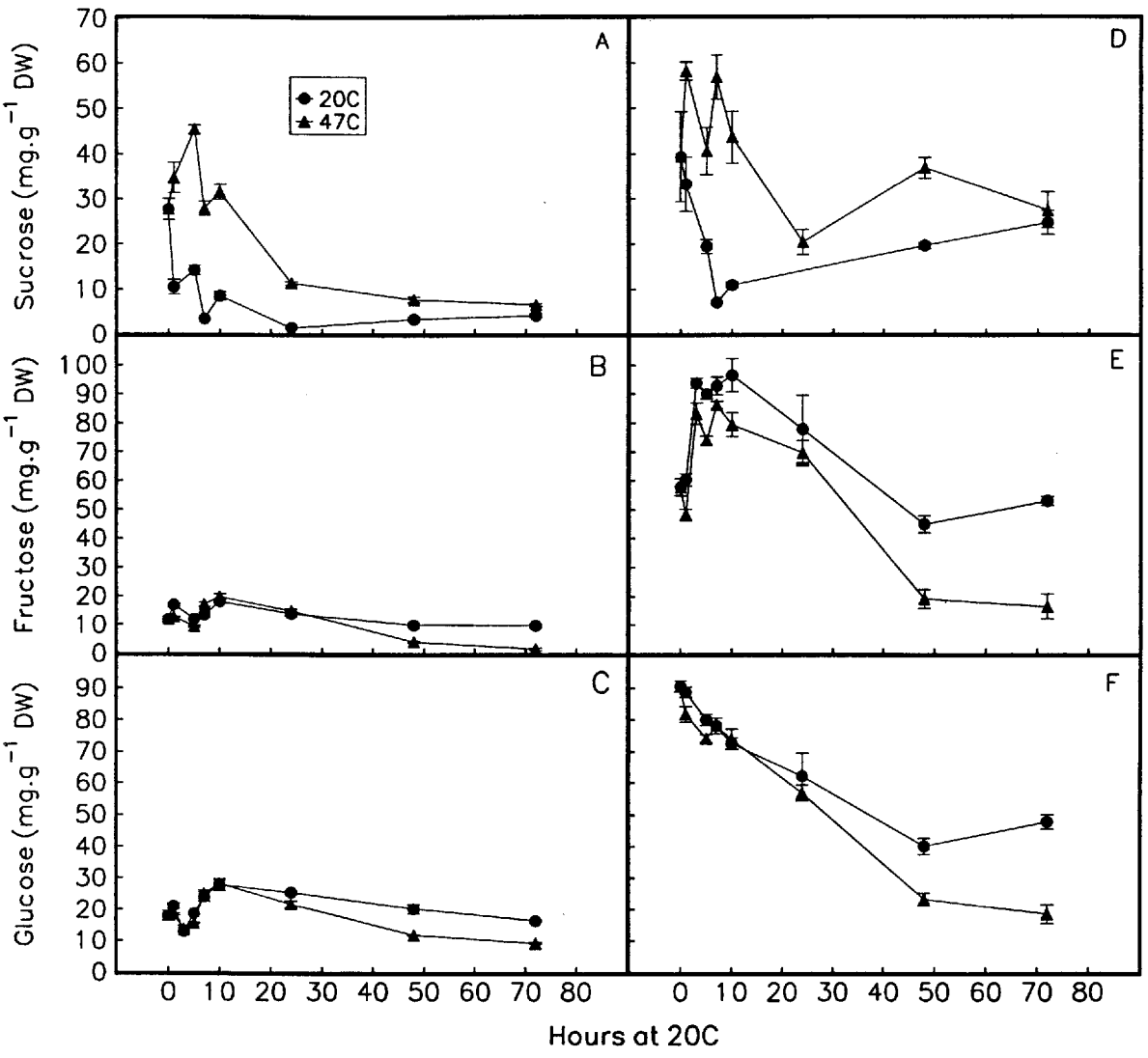

Fig. 2. Changes in contents of sucrose, glucose, and fructose in excised (**A, B,** and **C**, respectively) broccoli florets and (**D, E,** and **F**, respectively) stems stored at 20 °C in darkness after treatment with 20 or 47 °C water. Error bars represent SE of the means, n = 3.

production in heated broccoli (Kazami et al., 1991a) and netted muskmelon (Dunlap et al, 1990) have been reported in which the recovery was due to the conversion of ACC to ethylene via ACC oxidase. Previous results indicated that increased sensitivity to ethylene played an important role in broccoli yellowing (Tian et al., 1994). Thus, recovery of ethylene production might also induce synthesis of new ethylene receptor.

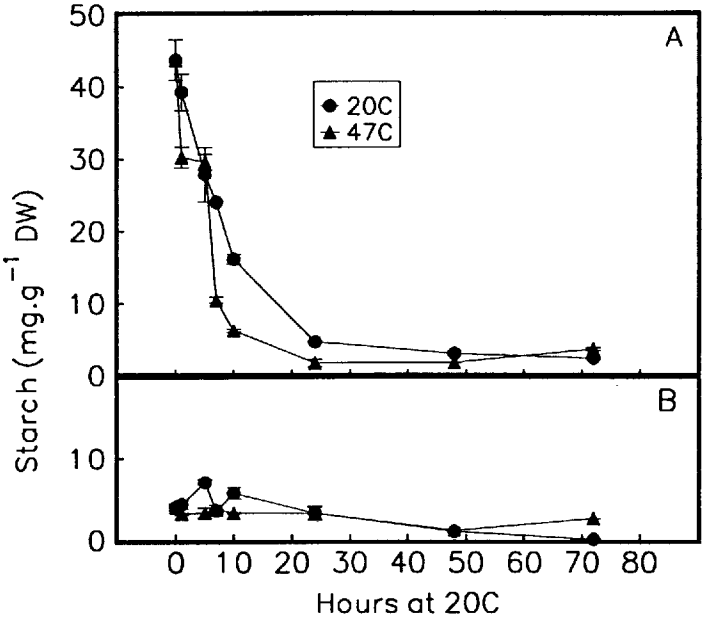

Fig. 3. Changes in starch contents in excised broccoli (**A**) florets and (**B**) stems stored at 20 °C in darkness after treatment with 20 or 47 °C water. Error bars represent SE of the means, n = 3.

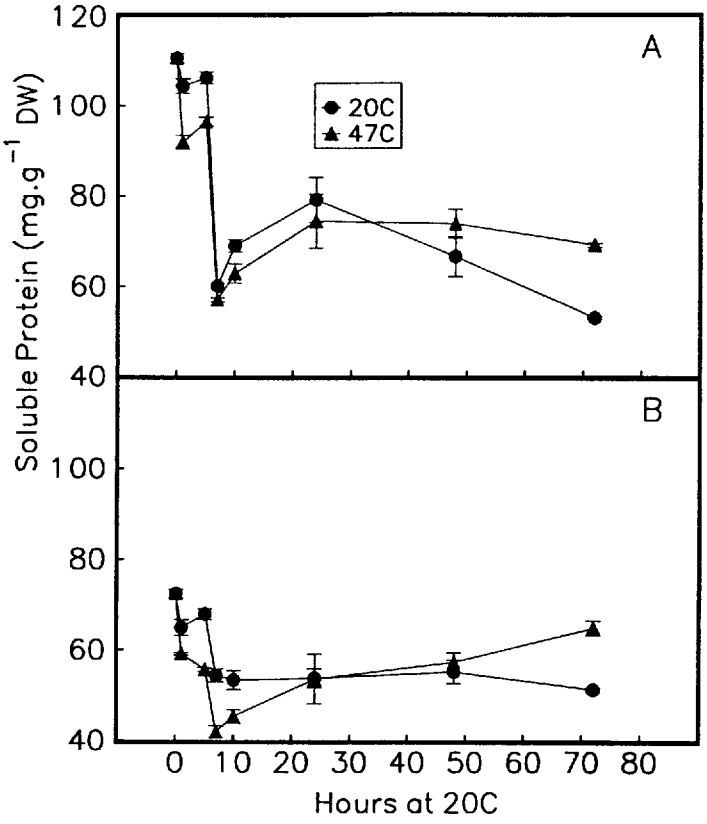

Fig. 4. Changes in soluble protein contents in excised broccoli (**A**) florets and (**B**) stems stored at 20 °C in darkness after treatment with 20 or 47 °C water. Error bars represent SE of the mean, n = 3.

In broccoli dipped at 47 °C, neither recovery of ethylene production and respiration nor color change were observed during the entire test period. High temperature (47 °C) may have damaged ethylene receptors and ethylene synthetic enzymes and prevented biosynthesis of new proteins, such as heat-shock proteins. Some of the high molecular mass heat-shock proteins (hsps) have functions as chaperones and can minimize high-temperature stress damage by associating with partially denatured proteins, preventing breakdown or aggregation (Ferguson et al., 1994). It is possible that more hsps were synthesized in broccoli dipped at 45 than at 47 °C, enhancing recovered enzyme activity and RNA production for enzymes involved in ethylene biosynthesis, respiration, and chlorophyll degradation.

Because 47 °C treatment was more effective in maintaining green color, compositional changes in broccoli treated at this temperature were investigated. Contents of total soluble sugars in stems were greater than in florets (Fig. 2). Rapid reductions in respiration rate and sucrose contents in broccoli were similar to those observed in other immature tissues, such as asparagus spear tips and excised maize root tips (Irving and Hurst, 1993; King et al., 1990, 1993; Saglio and Pradet, 1980). HWT induced an immediate increase of sucrose in florets and stems and reduced the glucose and fructose content after 12 and 24 h, respectively. Patterns of glucose changes in the stems were different from other sugars. Generally, sucrose degraded to equal molar amounts of glucose and fructose. Thus, sucrose decreased and fructose increased simultaneously. Glucose contents were greater than fructose contents in florets. The faster decrease in the stems may indicate that glucose is metabolized or transferred from stems to florets but that fructose is not initially. Our results support the hypothesis that some sugars from middle and base sections of broccoli stalks might be translocated to floral sections to help maintain the floral sugar pool and support the high respiration demand of florets (King and Morris, 1994a).

Sugars, such as glucose, fructose, and sucrose, may stimulate ethylene production and ACC oxidase activity in fruits (Tian, 1990) and vegetables (Meir et al., 1985; Riov and Yang, 1982). Early changes of sugars in broccoli florets and stems may contribute to the increase in ethylene production that enhances the sensitivity of the floret sepals to ethylene.

Starch content of broccoli florets and stems was <50 and 10 mg·g^{-1} dry mass, respectively (Fig. 3). Early starch degradation may be explained by the conversion of starch to glucose in florets during the first 24 h that contributed toward maintaining the floral sugar pool (King and Morris, 1994a). Compared with the control, starch levels declined faster in HWT broccoli florets during 7 to 24 h. This may be due to the inhibition of starch synthase by the treatment (Denyer et al., 1994; Keeling et al., 1994). Thereafter, starch was maintained at a similar level to that in the control. Interestingly, starch content in HWT stems started to increase after 48 h and reached double the amount in the controls at 72 h. A similar result was reported for wheat and maize (Keeling et al., 1994). These authors demonstrated that synthesis and activity of soluble starch synthase were inhibited by heat initially but then enzyme activity recovered later. The increase in starch concentration in HWT broccoli florets also was noticeable between 24 and 72 h.

Protein content was greater and declined faster in florets than in stems (Fig. 4), results similar to those of King and Morris (1994b). The relationship between decline in soluble protein concentration

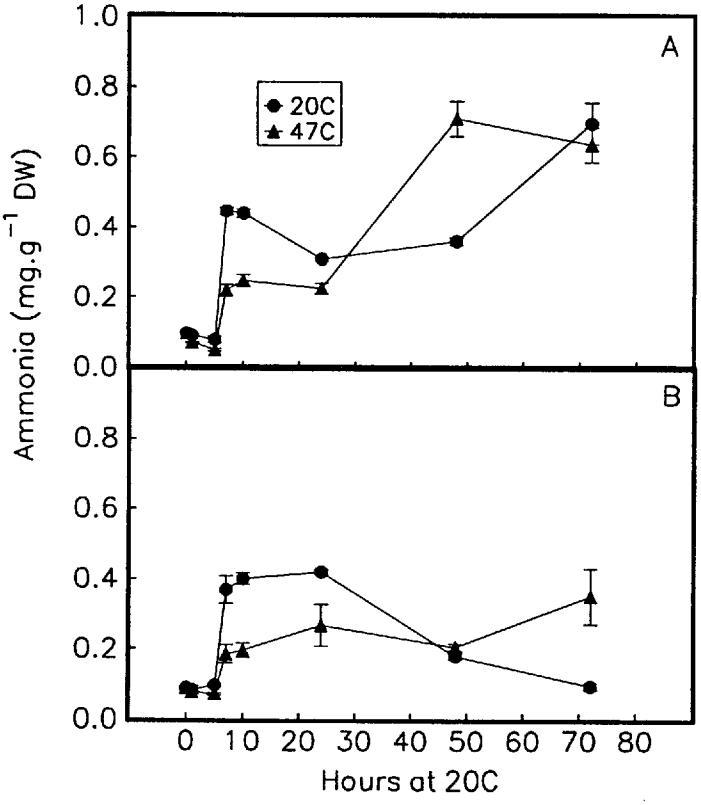

Fig. 5. Changes in ammonia contents in excised broccoli (**A**) florets and (**B**) stems stored at 20 °C in darkness after treatment with 20 or 47 °C water. Error bars represent SE of the means, n = 3.

and increase in ammonia was not clear. For the first 7 h after HWT, there was a massive decline in soluble protein content in the florets but little change in ammonia. The subsequent increase in the protein content until 24 h is matched by an increase in ammonia. One might have expected degradation of heat-damaged proteins and catabolism of the released amino acids to have been accompanied by ammonia accumulation. Perhaps, the soluble protein contents we have measured have been affected by differential release of insoluble (bound) protein and the subsequent catabolism of this protein. Further work is required to clarify the relationship between protein changes and ammonia accumulation in HWT broccoli.

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