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Heating the ends of leaves cut during coring of whole heads of lettuce reduces subsequent phenolic accumulation and tissue browning

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Abstract

Removal of the central axis or core of head lettuce harvested for the fresh-cut market (i.e., coring) is done in the field to reduce transit weight and wastage at the processing plant. Wounding done to the base of the leaves during coring induces an increase in phenolic metabolism and subsequent browning of the injured and adjacent leaf tissue. Applying a moist heated surface (e.g., 55 °C) to the tissue freshly exposed by coring for 10–15 s significantly reduced phenolic accumulation and subsequent browning. This inhibition persisted for 6 days at 10 °C and was not accompanied by increased decay of the treated tissue. Wetting the heated surface with an inhibitor of wound-induced phenolic metabolism (e.g., C6 or C8 mono-carboxylates) reduced the accumulation of wound-induced phenolic compounds at marginal or non-heat-shock inducing temperatures, but not at heat-shock inducing temperatures that significantly reduced the wound response by themselves. Heat-shocking the freshly exposed tissue during coring may be an effective method to reduce subsequent browning of the injured and adjacent lettuce leaf tissue.

Keywords: Abiotic stress; Carboxylic acids; Field coring; Fresh-cut; Phenolic metabolism; Wound signal; Wounding

1. Introduction

Head lettuce is often cored in the field during harvest to lessen the weight shipped to fresh-cut facilities and the refuse produced during salad preparation. This coring procedure produces injuries at the cut ends of the leaves. Wounding lettuce leaves induces a wound response that results in the accumulation of phenolic compounds that participate in subsequent tissue browning (Bolin and Huxsoll, 1991; Cantos et al., 2001; Brecht et al., 2004; Pereyra et al., 2005). Wounding produces a signal that propagates from the site of injury and induces the de novo synthesis of phenylalanine ammonia lyase (PAL, EC 4.3.1.5), and the synthesis and accumulation of soluble phenolic compounds (e.g., chlorogenic acid) in wounded and adjacent non-wounded tissue (Brecht et al., 2004; Campos-Vargas et al., 2004; Tomás-Barberán et al., 1997b). This sequence of biochemical steps may include other enzymes and substrates (e.g., endogenous antioxidants) in different lettuce cultivars (Cantos et al., 2001; Degl'Innocenti et al., 2005; Tavarini et al., 2007).

The wound response and subsequent tissue browning of lettuce leaves can be reduced by interfering with the synthesis and/or propagation of the wound signal (Choi et al., 2005), by storage in a reduced oxygen and elevated carbon dioxide atmosphere (Mateos et al., 1993), or by applying chemicals (e.g., carboxylic acids) that prevent the accumulation or oxidation of the wound-induced phenolic compounds (Tomás-Barberán et al., 1997a; Saltveit et al., 2005b). In addition, a brief heat shock (e.g., 45 °C for 90 s) effectively reduces phenolic accumulation and tissue browning by altering the translation of wound-induced PAL mRNA (Campos-Vargas et al., 2004; Murata et al., 2004). Above a threshold of activity and below a lethal maximum temperature, the inhibitory effect of a heat-shock treatment increases with increasing duration of exposure and temperature.

This paper reports that applying a heat-shock treatment during, or immediately after the coring procedure reduces wound-induced accumulation of phenolic compounds and subsequent tissue browning. Research was done to see if the duration of heat shock could be reduced so that it could be used as part of the coring operation in the harvest of lettuce destined for fresh-cut processing. The interaction between an effective heat-shock treatment and a chemical inhibitor of tissue browning was studied to see if their combined effects could

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be used to reduce duration or temperature of the heat-shock application.

2. Materials and methods

2.1. Plant material

Iceberg and Romaine lettuce (*Lactuca sativa* L.) were purchased from local commercial sources, and stored in the lab at 2.5 °C until used. In preliminary experiments, the top half of the head was cut away and the leaves carefully removed. Green leaf tissue was cut from the mid-rib and the mid-rib segments were cut into 2-cm sections starting 2 cm from the cut base of the leaf. Whole heads of Iceberg lettuce were also cored with a sharp hemispherical tool (i.e., a sharpened metal 5 cm diameter ice-cream scoop).

2.2. Heat treatment

The freshly exposed cut surface of the mid-rib segment was pressed against a heated surface for a proscribed period of time. The surface was that of metal, glass, or plastic containers holding heated water. A thermocouple was positioned on the surface upon which the tissue was pressed to accurately measure the treatment temperature. Later the surface was that of wet filter paper overlaying two layers of wet capillary cloth that was heated from below with an electric heater. The heater was adjusted to produce a range of desired temperatures in the wet capillary cloth. A thermocouple was positioned on the surface of the capillary cloth and overlaid with a piece of clean filter paper between each treatment. The freshly excised surface was pressed against the filter paper directly over the thermocouple for a specified time. The temperature was recorded and the 20-mm segment was placed in a 20 mm × 100 mm diameter plastic Petri dishes. The dishes were put into a wet paper towel lined plastic tub, loosely covered with aluminum foil and put into a 10 °C incubator.

A spherical piece of chrome plated steel (i.e., a 5 cm diameter trailer hitch) with a diameter equal to that of the sharpened metal ice-cream scoop was used to apply the heat treatment. The metal sphere was heated by holding it in a heated water bath at the proscribed temperature until the desired temperature was reached. Its temperature was measured with a thermocouple attached to its surface. In some experiments, a piece of cloth (i.e., a baby sock) was stretched over and covered the metal's surface and a mono-carboxylate solution was used as the heating solution. All solutions were made in 25 mM potassium phosphate buffer at pH 7.0, and the maximum concentration of the solutions (plus carboxylates) did not exceed 55 mM. This concentration was below the range of hypertonic solutions that reduce wound-induced increases in phenolic content in fresh-cut lettuce (Kang and Saltveit, 2003). The covered heated metal sphere was firmly held in the hole made in freshly cored Iceberg lettuce with the ice-cream scoop for a specified period of time. The heads were put in plastic tubs lined with wet paper towels, the tubs covered with aluminum foil and held for either 2 or 10 days at 10 °C before tissue samples were excised from the cored areas for measurement of phenolic content.

2.3. Measurement of phenolic content

Each 20-mm mid-rib segment was cut transversely into two sections, one with the heat-treated end and the other with the non-heat-treated end that served as the control. In experiments with whole heads, thin sections of tissue (i.e., 2-mm thick) were removed from the surface of the excised cored. Around 3.0 ± 0.05 g of tissue was put into 50 mL plastic centrifuge tubes along with 20 mL of methanol. The tissue was ground, and the absorbance of a clarified aliquot was measured at 320 nm (Loaiza-Velarde et al., 1997) and expressed as absorbance per gram fresh weight (Abs 320 nm/g FW).

2.4. Statistical design

Each experiment had at least three replicates of each treatment and was repeated five times with similar results. The temperature could not be exactly controlled in some of the heat treatments, so tests were done until sufficient numbers of observations were made within ±1 °C of the desired temperature.

3. Results and discussion

3.1. Heating with dry surfaces

Pressing one end of a 20-mm long freshly cut Romaine or Iceberg lettuce mid-rib tissue segment against a dry heated metal surface did not reduce subsequent tissue browning (Fig. 1). In fact, tissue heated above 50 °C had higher levels of phenolic compounds (Abs 320 nm/g FW) than did the opposite end of the mid-rib segment that was not pressed against the heated metal surface. The surface of heated tissue browned extensively, and tissue collapse allowed the vascular bundles to protrude from the surface. When segments were placed in plastic Petri dishes immediately after the heat treatments, condensation of water vapor was observed on the top lid of the Petri dish directly above the heated end of the segment. This loss of water may have exacerbated the damage done to the tissue by heating. To

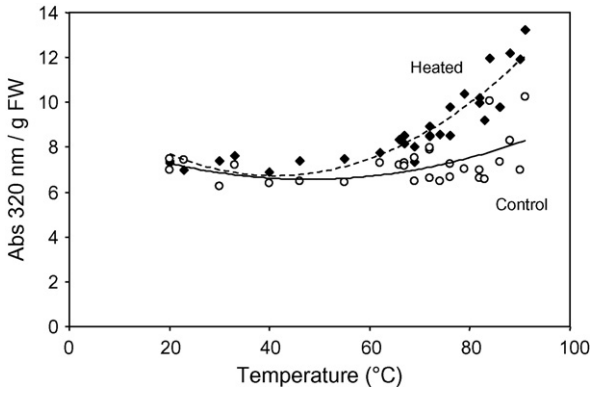

Fig. 1. Phenolic content of Iceberg lettuce mid-rib segments subjected to dry heat treatments. One cut end of a 20-mm mid-rib segment was pressed against the side of a metal can containing water at the respective temperature (heated), while the other cut end was non-treated (control). Phenolic compounds were extracted after 48 h at 10 °C and analyzed as described in Section 2.

eliminate water loss as a confounding factor in tissue browning, the heated segments were placed in 20 °C water for 10 min immediately after treatment to reduce water loss accompanying heating the tissue. While this reduced desiccation of surface tissue, it did not increase the efficacy of the treatment in reducing tissue browning (data not shown).

Replacing the heated metal surface with stainless steel, galvanized steel, aluminum, glass, or plastic did not significantly alter the results (data not shown). Likewise, using a rough glass, metal, or plastic surface instead of a smooth surface, or altering the force applied to the tissue segment from light to an excessive force that in itself damaged the tissue, did not reduce phenolic accumulation and subsequent tissue browning at the temperatures tested (data not shown).

3.2. Heating with wet surfaces

In contrast to the damage caused by heating mid-rib segments with a dry surface, heating the tissue segments with wet surfaces resulted in less wound-induced phenolic accumulation and tissue browning (Fig. 2). Temperature treatments appear to have two affects on the accumulation of phenolic compounds in excised lettuce tissue. At temperatures from 40 to 55 °C, the concentration of wound-induced phenolic compounds decreased as the temperature of the treatment increased (Fig. 2A). Since the tissue was little damaged by the heat treatment itself, extending the duration of the treatment further suppressed phenolic accumulation until a minimum had been reached after which increasing the duration had no effect (Fig. 2B).

In contrast, at temperatures above 55 °C, the suppression of phenolic accumulation by the heat treatments appears to have been superseded by the damage done by the treatment itself, so that phenolic content actually increased as the duration of the treatment increased (Fig. 2A and B). Phenolic accumulation was stimulated when tissue were exposed to temperatures above 55–60 °C for longer than 45 s.

A more extensive study of phenolic accumulation in mid-rib segments exposed to 45–88 °C for 5–20 s produced a family of quadratic curves when the data were expressed as a percent reduction in phenolic content (Fig. 3A). The first derivative of the polynomial equations fitted to these data gave the optimal temperature for each duration of treatment (i.e., 5 s at 68.3 °C, 10 s at 65.0 °C, 15 s at 60.3 °C, or 20 s at 57.1 °C), and the optimal inhibition of phenolic content for that temperature (Fig. 3B). The relationship between the seconds of heat shock and the opti-

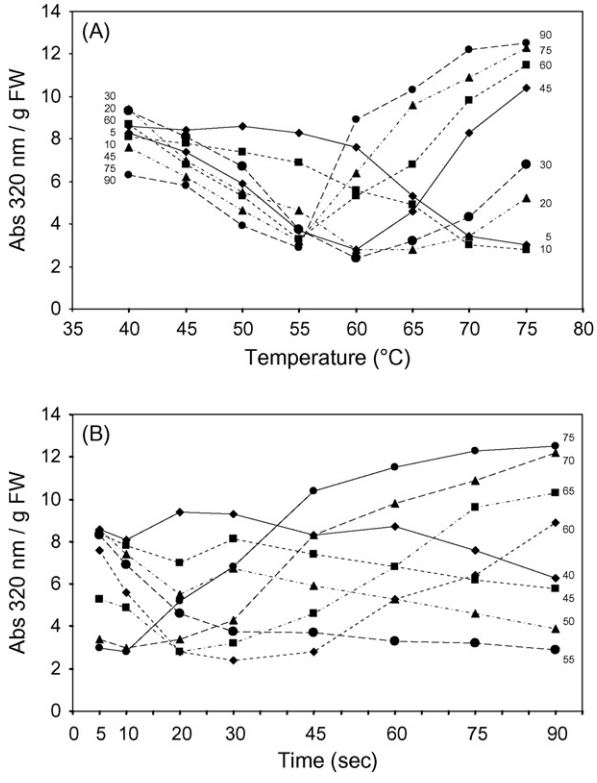

Fig. 2. Phenolic content of Iceberg lettuce mid-rib segments subjected to wet heat treatments. One cut end of a 20-mm mid-rib segment was pressed against a heated moist pad for 0–90 s, while the other cut end served as the non-heated control. Phenolic compounds were extracted after 48 h at 10 °C and analyzed as described in Section 2. Data presented as changes in phenolic content (Abs 320 nm/g FW) with duration and temperature of treatment. Data are presented for increasing temperature (A), or for increasing duration of treatment (B) to facilitate recognition of patterns with duration or with temperature, respectively.

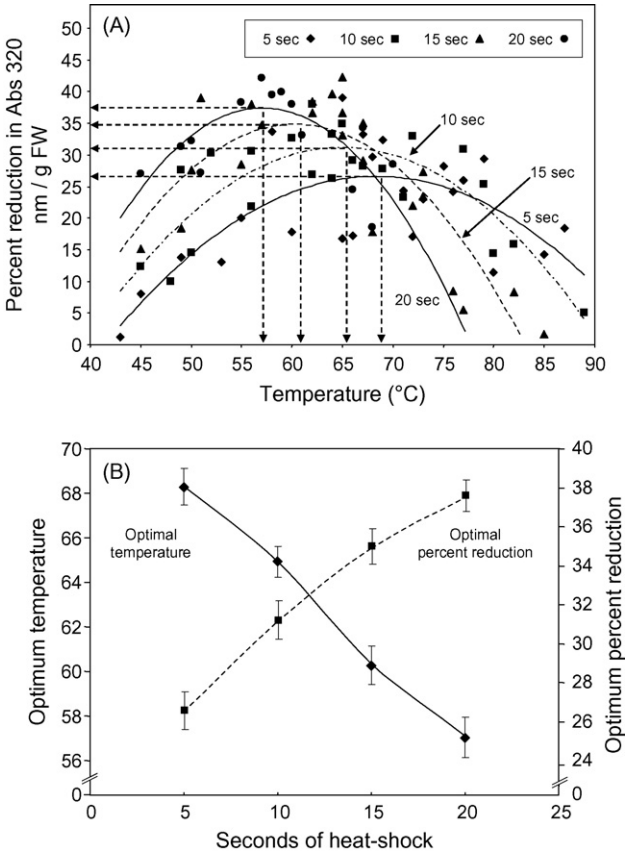

Fig. 3. Phenolic content of Iceberg lettuce mid-rib segments subjected to wet heat treatments. One cut end of a 20-mm mid-rib segment was pressed against a heated moist pad for 0–90 s, while the other cut end served as the non-heated control. Phenolic compounds were extracted after 48 h at 10 °C and analyzed as described in Section 2. Data presented as percent reduction in phenolic content between heated and non-heated ends of the mid-rib segment (A) and optimum temperature and percent reduction for specific heat-shock durations (B). Vertical bar represents the standard deviation about that mean.

mum temperature was linear ($R^2 > 0.95$) and negative, with the optimum temperature falling from 68.3 to 57.1 °C as the duration of heat shock increased from 5 to 20 s. The relationship between the seconds of heat shock and the optimum percent reduction in phenolic content was also linear ($R^2 > 0.95$), but the relation was positive with the optimum reduction in accumulated phenolic content increasing from 26.5 to 37.5% as the duration of heat shock increased from 5 to 20 s. As the duration of heat shock increased, the optimal temperature decreased while the optimum percent reduction in accumulated phenolic content increased (Fig. 3B). For example, a 5 s heat treatment at 68 °C reduced phenolic content 26.5%, while a 20 s heat treatment at 57 °C reduced phenolic content 37.7%. Each polynomial curve had a relative broad apex so a fluctuation of a few degrees from the optimum temperature produced similar reductions in phenolic content (Fig. 3A).

3.3. Heating the core of freshly cored Iceberg lettuce

Lettuce purchased from local commercial sources was allowed to reach room temperature (ca. 20 °C) before being cored with the sharpened ice-cream scoop. Tissue initially removed during coring had fairly consistent levels of phenolic compounds (5.3 ± 1.1 Abs 320 nm/g FW) (Fig. 4). The heated and covered metal ball was then pressed into the hole made by coring the lettuce and held in close contact with the freshly cut tissue for a specified length of time. Tissue treated at 55 °C for 5–15 s, or 60 °C for 5–10 s, or 65 °C for 5 s had phenolic contents similar to initial levels. In contrast, tissue treated at 60 °C for 15 s, 65 °C for 10 or 15 s, or 70 °C for 5–15 s had phenolic contents that were from 65 to 90% higher than initial levels.

As we previously saw with excised mid-rib segments, heat-shock treatments applied to whole heads of cored lettuce appears

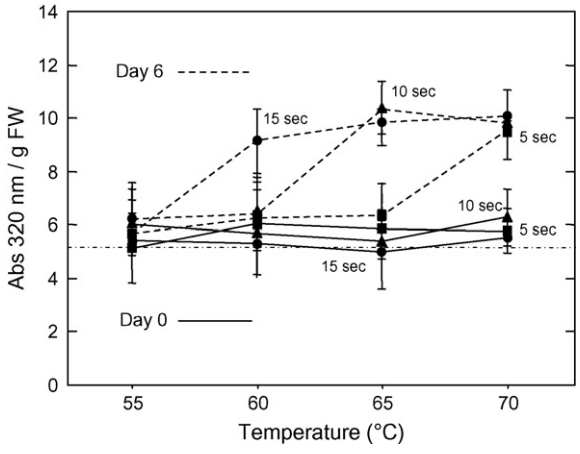

Fig. 4. Phenolic content of tissue adjacent to the cavity of cored Iceberg lettuce. The core was removed (day 0) and a heated metal sphere covered with a wet baby sock was inserted into the core. The metal ball was heated to 55, 60, 65 or 70 °C, and applied to the tissue for 5, 10, or 15 s. The horizontal dash/dot line represents the initial phenolic content of the non-treated tissue. The heads were held at 10 °C for 0 days (day 0, solid lines), or 6 days (day 6, dashed lines) before mid-rib tissue was excised from the ends of leaves in the core. Phenolic compounds were extracted and analyzed as described in Section 2. Vertical bar represents the standard deviation about that mean.

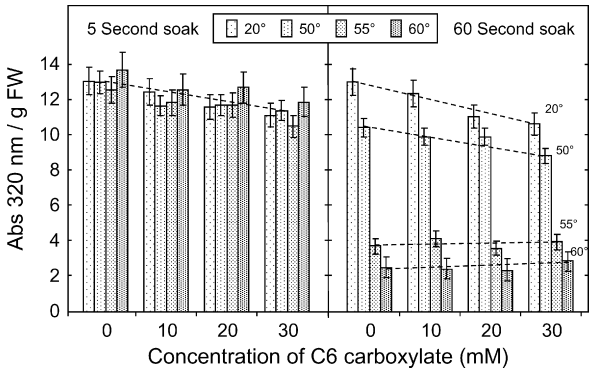

Fig. 5. Phenolic content of Iceberg lettuce mid-rib segments subjected to heat treatments and immersion in carboxylate solution. The 5-mm mid-rib segments were immersed in 100 mL of a 0, 10, 20, or 30 mM hexaonate solution (pH 7.0) at 20, 50, 55, or 60 °C for 5 or 60 s. Phenolic compounds were extracted after 48 h at 10 °C and analyzed as described in Section 2. Dashed lines are linear regression lines for all the temperature treatments for the 5 s soak, or for individual temperatures for the 60 s soak. Vertical bar represents the standard deviation about that mean.

to have two effects on phenolic metabolism. The beneficial effect is to reduce the accumulation of phenolic compounds that contribute to subsequent tissue browning, while the deleterious effect is to stimulate additional tissue browning. The components of these two opposing responses are induced by different combinations of temperature and duration of exposure (Fig. 4). The optimum reduction in subsequent browning in heat-treated fresh-cut lettuce is a compromise between levels and durations of heating that stimulates and suppresses phenolic metabolism. The exact combination of temperature and duration may vary with cultivar and other pre-harvest factors.

3.4. Combining heating and inhibitors of tissue browning

Increasing concentrations of heated mono-carboxylate solutions produced significant, though slight (0.058 Abs/mM) reductions in wound-induced phenolic accumulation when the temperature treatment did not induce a heat-shock response (i.e., all 5 s dips and 60 s dips at 20 and 50 °C in 0, 10, 20 or 30 mM hexaonate) (Fig. 5). There were similar declines of 0.057 and 0.049 Abs/mM with concentration for all 5 s dips and the 60 s dip at 20 °C, and the 60 s dip at 50 °C, respectively. The average phenolic content was 12.0 and 10.1 Abs/g for these two groups of treatments. The 60 s dip at 55 and 60 °C had far lower phenolic content of 3.9 and 2.4 Abs/g, respectively, but there was no significant decline with increasing C6 concentration. Similar results were seen with C8 mono-carboxylate solutions (data not shown). There was no effect of increasing concentration of either mono-carboxylate when the time × temperature treatment was sufficient to induce a heat-shock response.

4. Conclusion

Heat-shock treatments may reduce wound-induced increases in phenolic metabolism by interfering with the translation of wound-induced PAL mRNA into the PAL protein (Campos-Vargas et al., 2004). The inability of optimal heat-shock

treatments to completely suppress the increase in wound-induced phenolic metabolism (Fig. 3B), even if administered coincident with wounding, suggests that a portion of the wound response is immune to this type of inhibition. Apparently, there is some component of wound-induced increases in phenolic metabolism and phenolic content (e.g., PAL mRNA translation into functional PAL or activation of latent PAL activity) that is not suppressed by heat-shock treatments.

Chemical inhibitors were applied to see if they could target this refractory component of the wound response. Application of chemical inhibitors (e.g., *n*-alcohols, mono-carboxylates) immediately after wounding can reduce wound-induced phenolic accumulation to initial levels and prevent subsequent browning in wounded lettuce (Saltveit et al., 2005a,b). However, the upper range of these effective concentrations also produces increased ion leakage which reduced shelf-life. While chemical inhibitors were effective in reducing wound-induced phenolic accumulation when combined with brief (e.g., 10 s) heat-shock temperatures (e.g., 45–60 °C), they were not additive or synergistic in reducing phenolic content in tissue subjected to longer durations of the heat-shock treatment (Fig. 5). A brief (e.g., 5–10 s) heat-shock treatment (e.g., 70–60 °C) applied to freshly exposed tissue during coring could be an effective method to reduce subsequent browning of the injured and adjacent lettuce leaf tissue.

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