HortScience 35(7):1308–1311. 2000.

Processing Line Effects on Storage Attributes of Fresh-cut Spinach Leaves

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Additional index words. chlorophyll loss, lipid peroxidation, *Spinacia oleracea*, storage quality

Abstract. **The degree of damage that may occur through harvesting and packing represents one of the major factors that can affect quality of fresh-cut produce. The purpose of this study was to examine the effects of different steps in a representative fresh-cut processing line on storage quality of spinach (*Spinacia oleracea* L.). To this end, spinach leaves were removed at successive points on the line: 1) before entry into the line (control); 2) after a shaking procedure but before initial rinsing with 10 °C water + 5 mg·L^{-1} chlorine dioxide; 3) after centrifugal drying; and 4) after commercial packaging. After removal from the different points in the line, the spinach samples were stored at 10 °C for 16 days, during which time malondialdehyde (MDA) concentration (lipid peroxidation assay), electrolyte leakage (membrane leakiness), chlorophyll content (a, b, and total), and color attributes (L, saturation, hue angle) were measured. Both lipid peroxidation and electrolyte leakage increased with time of storage and with stage of procesing. Electrolyte leakage increased most in material removed after the shaking procedure, but prior to hydrocooling. Overall total chlorophyll loss during storage did not change with time of removal from the processing line, although overall chlorophyll b content decreased in stored material 8 days following centrifugal drying and packaging. A more rapid loss in chlorophyll a relative to chlorophyll b over the first 8 days of storage was reflected in hue angle measurements regardless of the point of removal. The processing line under study, thus had both beneficial and detrimental effects on storage quality of spinach. Detrimental effects associated with centrifugal drying and packaging procedures could be modified to improve quality.**

One of the major factors that can affect quality of fresh-cut produce is the degree of damage that may occur during harvesting and packing (Ahvenainen, 1996; Shewfelt and Prussia, 1993). Mechanical injuries can be caused by such actions as cutting, impact, compression, abrasion, puncturing, and tearing. The surface of the commodity is often exposed to air and to possible contamination with bacteria, yeasts, and molds as a result of mechanical damage. Improper temperature during handling can exacerbate microorganism colonization and growth rates. Estimated crop losses between the field and the consumer range from 10% to 25% (Coursey, 1983).

The process of harvesting, washing, and bagging spinach is similar to that for other leafy vegetables. Spinach leaves harvested from the field are first placed on a perforated shaking platform to facilitate the removal of soil and debris. Leaves are then dropped into 10 °C water to remove field heat and particulate matter left on the leaves after the shaker and to inhibit microbial activity. Following the initial rinse, leaves are passed along a conveyer belt for manual inspection and removal of unwanted material. Leaves are then packed into nylon mesh bags and dipped into another container of circulating water at 10 °C for a final rinse. The bags are then spun to remove water. Following this, the spinach is mechanically packed into bags, stored at ≈2 °C, and is ready for shipment to retail outlets.

There are many physical, visual, and biochemical methods to assess quality of produce. Loss of produce quality because of damage-induced senescence and/or pathogenic establishment is often manifested through loss of cellular integrity. The malondialdehyde (MDA) assay has been used extensively since the 1950s to estimate lipid peroxidation, and thus oxidative stress, in biological systems (Hodges et al., 1999; Sinnhuber et al., 1958), while electrolyte leakage provides a good indicator of the degree of membrane permeability (Lyons et al., 1979; Wismer et al., 1998). Reductions in chlorophyll content have also served to estimate the level of induced or natural senescence (Meir et al., 1995; Philosoph-Hadas et al., 1991). The oxidative degradation of chlorophylls can be evaluated by colorimetry (Setser, 1984).

The purpose of this study was to examine the effects of sequential steps in a fresh-cut processing line on storage quality of spinach in order to identify processing line components contributing to reduction of spinach marketability.

Materials and Methods

Plant material. Six-week-old, field-cultivated spinach leaves were obtained from a local grower (Melvin Farms, Canning, N.S.) on 29 July 1998. Leaves were well-mixed prior to passage through the spinach processing line to represent a uniform composite of that day's harvest. The experiment was repeated again with spinach from a later harvest on 31 Aug. 1998.

Spinach processing line. A local, partly-mechanical spinach processing line (Melvin Farms) was chosen as the subject of the experiment. In this line, spinach leaves directly from the field were first placed on a shaking platform uniformly covered with 2.5-cm-diameter holes for 30 s to facilitate the removal of soil and debris. Leaves were then submersed directly into circulating water (10 °C) for 1 min to remove field heat and particulate matter left on the leaves after the shaker. Following the initial rinse, leaves were passed along a solid conveyer belt for ≈30 s for manual inspection. They were then manually packed into nylon mesh bags and submersed into another container of circulating water at 10 °C + 5 mg·L^{-1} chlorine dioxide for 3 min for a final rinse. After mechanical removal from the rinse tank, the mesh bags were lightly spun (220 g_n) in a centrifugal dryer (model 655; Bock, Toledo, Ohio) for 3.5 min to remove water. Finally, the leaves were manually removed from the mesh bags and placed into a packing machine (model PP-14-14; Pre Pack Machinery, Champlain, Ill.). The entire process was completed in ≈4 min. For this experiment, spinach samples were removed at four successive locations: 1) before transfer onto processing line (control); 2) after the shaker, but before the initial rinse; 3) after centrifugation; and 4) immediately following packaging.

Storage treatment. About eight, 250-g fresh weight (FW) samples of leaves from each of the four treatments were placed in perforated bags. Bags were then sealed and placed on wire shelves in a dark, controlled-temperature chamber (Econaire GR 100; Econaire, Winnipeg, Man.) at 10 °C and 95% relative humidity (RH). Samples were removed from storage on days 0, 1, 2, 4, 8, 12, and 16 and analyzed for MDA and chlorophyll concentra-

Received for publication 26 Oct. 1999. Accepted for publication 15 Feb. 2000. Atlantic Food and Horticulture Research Centre Contribution No. 2206. We thank Richard Melvin of Melvin Farms, Canning, N.S., for supplying spinach and access to their spinach processing line. We also thank Ryan Donovan, David Canton, Prudence Dauphinee, and Tineka Bishop for technical assistance; Ken McRae and Brad Walker for assistance with statistical procedures; and Katherine Sanford and Robert Prange for advice on the preparation of the manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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tion, color and electrolyte leakage. Logistics dictated that day 0 samples could only be analyzed ≈2 h following removal from the processing line.

Lipid peroxidation and chlorophyll analyses. Estimates of lipid peroxidation were assessed spectrophotometrically in four replicate 7.5–10-g FW samples of spinach tissue using a modified TBA-MDA assay, which corrects for compounds other than the TBA-MDA adduct that absorb at 532 nm (Hodges et al., 1999).

Chlorophylls a and b and their pheophytins were determined in four replicate 7.5–10-g FW samples of leaves as described by Wintermans and De Mots (1965).

Electrolyte leakage. Electrolyte leakage was estimated by the modified electrical conductivity procedure of Kuo and Parkin (1989). Four subsamples of eight discs 2 cm in diameter were taken from each sample and placed in 50-mL test tubes containing 35 mL of 0.4 mol·L^{-1} mannitol in deionized water. Samples were equilibrated for 1 h in a 24 °C circulating water bath. Initial electrical conductivity (EC) was read using a conductivity meter (DsPH-3; Presto-Tek, Los Angeles). Samples were then frozen at –20 °C for 24 h and the electrical conductance was read to provide a total EC reading. Percentage of change in EC was calculated for each treatment.

Colorimetry. Leaf color was measured using a HunterLab LabScan 6000 Spectrocolorimeter (Hunter Associates Laboratory, Reston Va.). Each sample (≈10 g FW) was carefully placed into a round (10-cm-diameter), 3-cm deep white plastic cell, and placed at the 4.5-cm viewing port, and two readings of Hunter CIE-L*a*b* reflectance measurements were taken of the top surface; the cell was rotated 90° between readings. The a and b values were converted to hue angle and saturation by $^{-1}\cos[a / \sqrt{(a^2 + b^2)}]$ and $\sqrt{(a^2 + b^2)}$, respectively (Little, 1975). L values are represented by 0 = black and 100 = white, hue angle refers to 180° = green and 90° = yellow, and saturation is a measure of color purity or vividness.

Statistical analyses. All MDA, electrolyte leakage, chlorophyll, and colorimetry results were based on at least three readings each of four samples for each of two harvests. The effects of harvest, storage time, and processing line removal point were analyzed by a three-factor completely randomized analysis of variance and SE values calculated using Genstat 5 (release 4.1).

Results

MDA content (nmol·g^{-1} FW) of all samples increased over the 16-d storage period (Fig. 1A). However, the increase in MDA content was more rapid in tissue subjected to the last two processing stages (postspin and postpackaging). Following storage, leaves subjected to spinning and packing had higher amounts of MDA than leaves that were not, and leaves that passed through the entire processing line exhibited higher levels of MDA than did those removed prior to this point. No differences in MDA content were observed between fresh (control) leaves and those removed from the line after the first step.

As with MDA content, electrolyte leakage (percentage of change in EC) increased with storage time regardless of the point at which leaves were removed from the processing line (Fig. 1B). Unlike MDA content, no differences in EC were observed between control leaves and those sampled after centrifugal drying or after packaging. However, electrolyte leakage from leaves removed after shaking increased after day 8 of storage more than did that from leaves removed from the other points on the processing line. This increase was maintained for the rest of the storage period.

Concentrations of total chlorophyll (mg·g^{-1} FW) at day 0 were greater in spinach that was centrifugally dried and packaged than in that removed after shaking (Fig. 2A). This difference was maintained for the first 2–4 d of storage, after which there were no effects of removal from different points on the processing line; total chlorophyll decreased in all samples after 12 d of storage. Chlorophyll a concentration, representing the majority of the total chlorophyll present, was also initially greater at day 0 in spinach removed from the processing line after centrifugal drying and after packaging than in spinach removed after the shaker (Fig. 2B). Overall chlorophyll a content declined the least during storage in spinach removed after shaking but prior to hydrocooling; the decreases were similar in the material removed from the other points on the processing line. Chlorophyll b content declined only after centrifugal drying and after packaging, and only after 8 d of storage (Fig. 2C); contents were initially lower in leaves removed after shaking than those removed after drying, and changes during storage were nonsignificant for control leaves or leaves removed after shaking.

After 8 d, the leaves were darker in color, as indicated by the decline in L values (Fig. 3A). Control leaves and leaves removed after shaking had lower L values at days 8 and 16 than did those removed later. Saturation values remained fairly constant for the first 8 d of storage, but decreased between days 8 and 12 (Fig. 3B). Saturation was not affected by point of removal. At day 0, leaves removed after centrifugal drying and packaging were greener

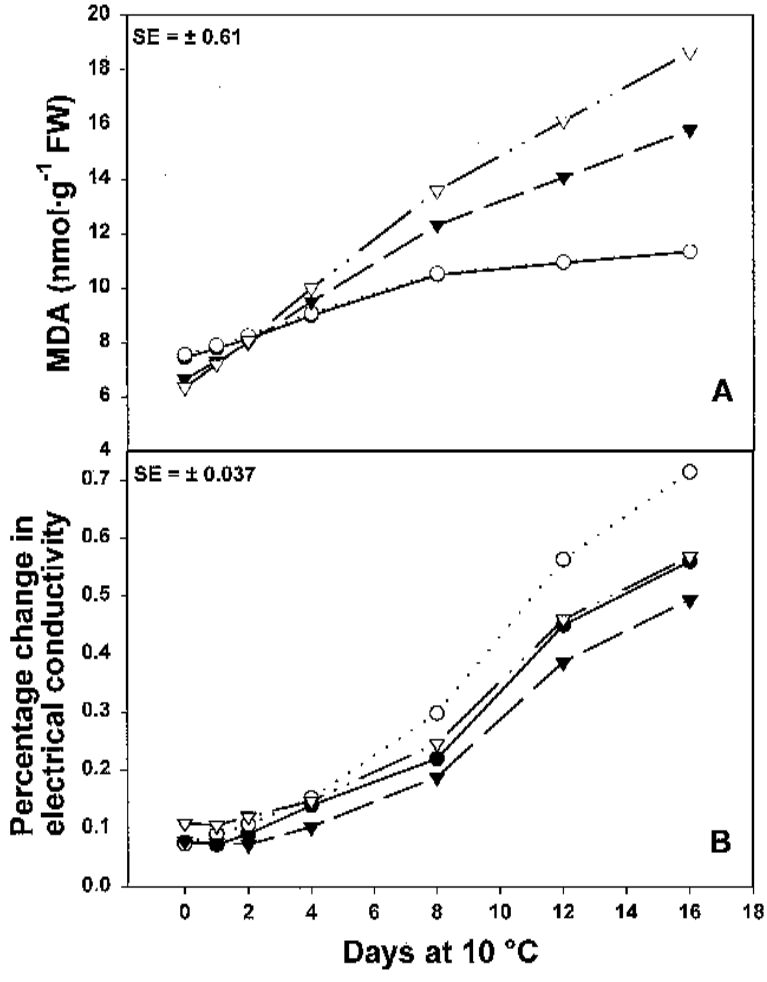

Fig. 1. Changes in (**A**) MDA content (nmol·g^{-1} FW) and (**B**) electrolyte leakage (percentage of change in EC) of spinach over a 16-d period following removal from four different points of a processing line: 1) prior to entry into the line (control) (●); 2) after the shaker but before initial rinse (❍); 3) after centrifugal drying (▼); and 4) after packaging (▽).

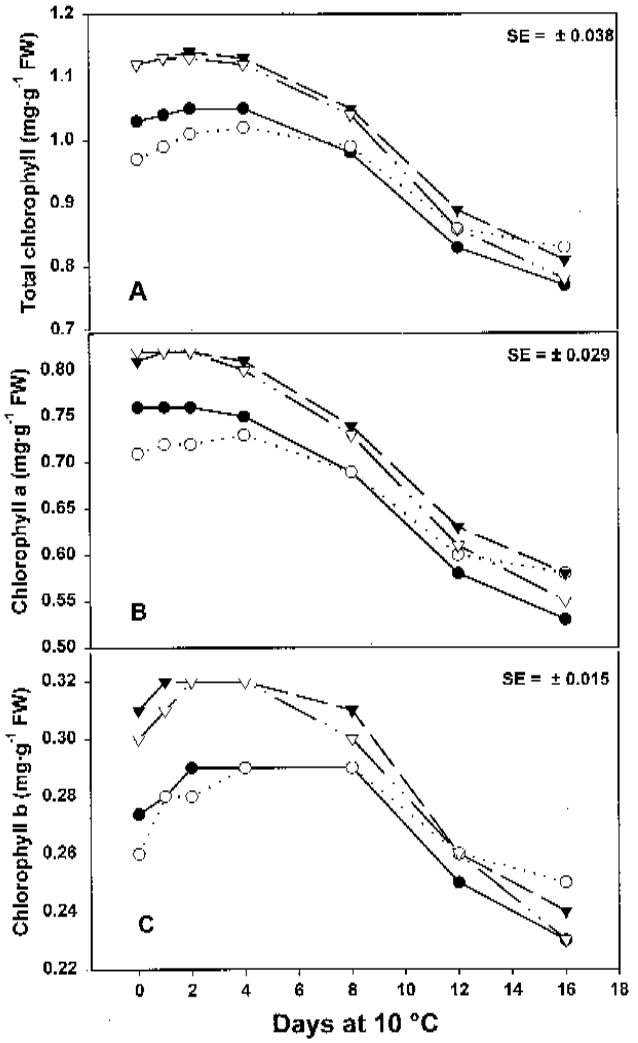

Fig. 2. Change in chlorophyll content (mg·g^{-1} FW) of spinach over a 16 d period following removal from four different points of a processing line. Figure legends as in Fig. 1.

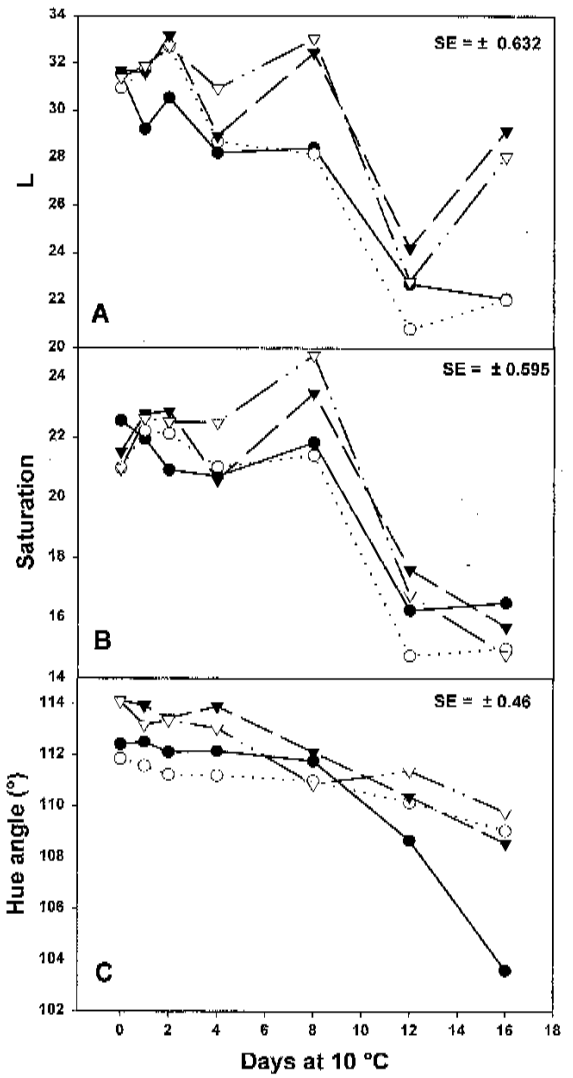

Fig. 3. Change in (**A**) L, (**B**) saturation, and (**C**) hue angle of spinach over a 16-d period following removal from four different points of a processing line. Figure legends as in Fig. 1. L values: 0 = black and 100 = white; saturation: measure of vividness; hue angle values; 180° = green and 90° = yellow.

(higher hue angle) than control leaves or those removed after shaking (Fig. 3C); this difference was maintained for 4 d in storage. Hue angle shifted toward yellow in all material during storage, but this decline was most dramatic in the control leaves following 8 d of storage.

Discussion

Quality of fruits and vegetables gradually decreases following harvest. Although this cannot be prevented, the dynamics of this process can be altered. This degree of alteration varies with preharvest cultural and environmental conditions, harvest techniques, processing routines, storage protocols, and retail distribution and marketing procedures. Mechanical, physical, and/or biochemical damage during fresh-cut processing routines can drastically affect the market life, color, texture, and flavor of a commodity (Watada et al., 1996). Many cells can be ruptured during fresh-cut processing, and intracellular components, such as oxidizing enzymes (e.g., polyphenol oxidase), can be liberated (Ahvenainen, 1996). As well, loss of cellular integrity of a commodity due to fresh-cut processing techniques offers a ready route for microbiol invasion. The term "latent damage" has been coined to describe damage incurred at one step that does not manifest itself until a later stage (Shewfelt, 1986).

Processing lines, such as the one under study, include unit operations designed to prolong the storage life of fresh fruits and vegetables. The shaking procedure is necessary to remove loose foreign materials such as dirt and insects, both of which could damage the final product. Hydrocooling delays the inevitable decline in quality of fresh produce and extends its shelf life (Sargent et al., 1988). It removes field heat rapidly, lowering temperatures to those approximating that of optimal storage, while simultaneously cleaning the product. Moreover, the addition of chlorine to the wash water can reduce microorganism levels during hydrocooling; although chlorine has little residual effect, it may impact future storage potential through reducing initial exposure of fresh-cut produce to inoculum during processing (Ryall and Lipton, 1979). The excess moisture derived from hydrocooling can be removed by centrifugal drying to reduce microbiol growth.

Exclusion from, or removal at successive points on, the processing line had little effect on lipid peroxidation as assessed by MDA content until after day 4. On day 8 and thereafter, lipid peroxidation varied with the position from which samples were removed. Control samples and those taken just prior to hydrocooling exhibited the least increase in lipid peroxidation, whereas those removed after centrifugal drying or after packaging exhibited progressively more lipid oxidation. Hydrocooling is an important procedure in delaying the decline in postharvest quality (Ryall and Lipton, 1979). As the spinach was not removed from the processing line directly after the hydrocooling step, but only after both the hydrocooling and centrifugal processes, the direct influence of hydrocooling on lipid peroxidation could not be assessed. However, the processing steps following hydrocooling

may negate some of its beneficial effects, and certainly contribute to additive lipid peroxidation.

As with MDA content, electrolyte leakage increased over the 16-d storage regime regardless of the point of removal from the processing line. However, in contrast with the MDA concentrations, spinach removed just after shaking exhibited the greatest increase in electrolyte leakage on and after day 12. Content of MDA is a measure of lipid peroxidation while electrolyte leakage assesses membrane leakiness, although lipid peroxidation can also be associated with membrane permeability (Parkin et al., 1989; Thompson et al., 1991), as evinced by the increase in both MDA content and electrolyte leakage as the storage period was extended. The electrolyte leakage results suggest that, unlike lipid peroxidation, further processing after shaking did not increase membrane leakiness. Hydrocooling, by rapidly lowering the temperature, may have delayed the development and rate of membrane leakiness following processing. The possible removal and/or regulation of microbiol contamination by chlorine during the hydrocooling rinse may also have contributed to the reduction of leakiness by reducing microbiol breakdown of the tissues.

Decreases in chlorophyll content are often used to estimate the extent of cellular degradation and/or senescence (Meir et al., 1995; Philosoph-Hadas et al., 1991). The lower contents of chlorophyll a and total chlorophyll on day 0 in leaves sampled after shaking than in leaves sampled after centrifugal drying or packaging could reflect the effects of hydrocooling in reducing chlorophyll loss between sampling and analysis in the latter two samples. Although there was no effect of point of removal on chlorophyll loss during storage, the overall decrease in chlorophyll b content was greater in spinach removed after centrifugal drying and packaging than in tissue removed prior to the centrifugation, perhaps because of their relatively higher initial levels of chlorophyll b.

Relative differences in loss of chlorophyll a and b were reflected in hue angle differences among samples. Chlorophyll a appears as bright blue-green, whereas chlorophyll b is visualized as more yellow-green. The more rapid decrease in chlorophyll a than in chlorophyll b content over the first 8 d of storage led to a shift from green to yellow, manifested as a decrease in hue angle. Hue angle decreases more rapidly in spinach held at abusive temperatures (21 °C) than at 4 °C (Gnanasekharan et al., 1992) and, similarly, delays in cooling of broccoli (*Brassica oleracea* L. Italica Group) result in accelerated yellowing (Brennan and Shewfelt, 1989; Gillies and Toivenen, 1995).

The farther the spinach travels along the processing line, the more the additive damage will induce and enhance lipid peroxidation and chlorophyll b loss during subsequent storage. The centrifugal drying and packaging steps caused highly significant increases and decreases in lipid peroxidation and chlorophyll b concentrations, respectively, during storage. However, these two steps inhibited the development of membrane permeability, as assessed by electrical conductivity, during storage. The processing line under study thus had both beneficial and detrimental effects on selected storage quality characteristics of spinach. We had assumed that the hydrocooling step would be the most important procedure affecting membrane permeability during storage. As accumulated damage was the probable candidate in predisposing spinach to lipid peroxidation and chlorophyll loss during storage, procedures designed to limit the amount of injury in centrifugal drying and packaging steps should lead to enhanced storage quality.

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