



Quality Loss in Minimally Processed Swiss Chard Related to Amount of Damaged Area

S. I. Roura^a, L. A. Davidovich^b and C. E. del Valle^{b*}

Grupo de Investigación en Ingeniería en Alimentos, Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad Nacional de Mar del Plata, Juan B. Justo 4302, 7600 Mar del Plata (Argentina)

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Whole leaves of Swiss chard and leaves cut into 2, 3 and 4 cm wide strips were stored at 4 °C and 98% relative humidity for 11 d. Water, weight and chlorophyll concentration decreased continuously during storage, with losses depending on the degree of processing injury and relating to the damaged area per unit volume rather than to exposed area. Titratable acids and soluble solids contents presented greater decreases in the first 3 d of storage; thereafter their evolution did not show a definite tendency.

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Introduction

Swiss chard (*Beta vulgaris*, type *cycla*) is a leafy vegetable highly appreciated in Argentine cuisine for its nutritional properties and year round supply. Although it is similar to spinach and both products are used interchangeably in most food preparations, consumers favour Swiss chard for its lower price. Quality of whole, fresh Swiss chard leaves is highly dependent on the temperature and humidity of the storage atmosphere. Storage at low temperature (4 °C) and high levels of relative humidity (RH: > 87%) are necessary to delay weight, water and chlorophyll loss, and to help maintain sensory attributes (1). Spoilage of leafy vegetables is associated with high respiration and water loss rates (2). Texture of green vegetables usually becomes unacceptable when they lose about 2% of their water content (3). Water loss is a major cause of deterioration because it results not only in direct quantitative loss (loss of salable weight), but also in loss in appearance (wilting and shriveling), textural quality (softening, flaccidity, limpness, loss of crispness and juiciness) and nutritional quality (2). Retention of green colour is an obvious indicator of the quality of leafy vegetables and is considered to have great impact on consumer selection (4, 5). For green vegetables,

chlorophyll content is associated with greenness. Market quality retention in vegetables is affected by many factors, including postharvest processing, storage time and conditions such as temperature, RH, light and composition of the atmosphere (6, 7). Fresh-cut vegetables are generally much more perishable than intact products. The changes that occur during senescence of these products are induced or enhanced by the physical action of processing. They take place particularly in tissues adjacent to those that are damaged by the cutting action, when acids and hydrolyzing enzymes of the vacuoles are released (8, 9). Bolin and Huxsoll reported that size reduction, required for preparing salad lettuce, shortens storage life of cut lettuce compared to uncut lettuce (10). They found that thin 3-mm slices of shredded lettuce respired more rapidly and had a shorter shelf life than salad-cut lettuce. Cutting lettuce leaves causes a rupturing of the cells resulting in an exudation of cellular fluids. This causes acceleration in the physiological breakdown of the lettuce and a shortened storage life. The effect of piece size was also noticed when lettuce was shredded 1 mm and 3 mm thick (11).

Although there is information on the storage conditions for whole chard leaves (1, 2) and on the effect of processing on the storage life of leafy vegetables (10, 11), no information was found on the effect of cutting on chard. Moreover, the effect of the piece size on the rate of deterioration of processed leafy vegetables has received little attention. The purpose of the present work was to investigate how the piece size of cut chard affects its storage life. To assess changes in the quality of chard,

^a Affiliated to the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

^b Affiliated to Universidad Nacional de Mar del Plata, Argentina

*To whom correspondence should be addressed. Fax: 054 23 810046; E-mail: cdvalle@fi.mdp.edu.ar

chlorophyll content, moisture and weight loss, pH, titratable acidity and soluble solid content were analysed for whole and cut chard.

Materials and Methods

Raw material and sample preparation

Fresh Swiss chard (*Beta vulgaris*, type *cycla*) was obtained from local producers. Leaves were received at our laboratory 40–60 min after harvest. After sorting for integrity, uniformity of colour and size, chard leaves were separated into four lots. In one of them (A) chard leaves were left whole; in the other lots chard leaves were cut into 4 cm (B), 3 cm (C) and 2 cm (D) wide strips. Cuts perpendicular to the midrib were made with a sharp knife. For any strip the volume is $l \times w \times \delta$ where l is the length, w is the width and δ is the thickness. The damaged area is $2 \times l \times \delta$. Therefore, the damage area per unit volume is $2/w$, resulting in values of 0, 0.5, 0.67 and 1 cm^{-1} for lots A, B, C and D, respectively. Immediately after cutting, samples were soaked in a water-chlorine solution (0.2 g of $\text{Cl}_2/1000 \text{ g H}_2\text{O}$) at $1\text{--}3^\circ\text{C}$ for 2 min. Surface moisture was removed with a manual salad centrifuge.

Sample storage

Holding boxes, with overall dimensions of $0.4 \times 0.3 \times 0.3 \text{ m}$, were made of heavy-duty, 0.60 cm thick, transparent acrylic. To create an atmosphere of ca. 98% RH, a beaker with saturated potassium sulphate solution in equilibrium with unsolved potassium sulphate was placed in each box. Boxes were stored at $4 \pm 1^\circ\text{C}$. Chard samples were placed in two layers on $0.29 \times 0.29 \text{ m}$ plastic mesh frames. This disposition was chosen to allow unrestricted interaction between the samples and the holding atmosphere, so that the effect of the damaged area would not be masked by protecting holding surfaces or adjacent chard layers. Twelve frames were placed in each holding box.

Quality evaluation

A set of three frames from each damage condition was weighed throughout the experiment to determine weight loss.

At each storage time, assessed samples were removed for the different analyses. For moisture and chlorophyll determinations, stems were removed and the green tissue was ground with a home food grinder (BGH, 390094, Argentina). Moisture was determined by the weight lost by 10 g samples after 24 h at 80°C (12). Total chlorophyll content was determined using an adaption of the spectrophotometric assay described by Barth *et al.* (13). Ground chard tissue (3 g) was extracted in 18 mL acetone: 1 mL 0.1 mol/L NH_4OH solution using a homogenizer at 60 rpm for 1 min under cold conditions (5°C). Homogenates were stored in the dark prior to centrifugation. Homogenate was centrifuged under $1000 \times g$ in 30 mL tubes for 5 min at 5°C . Supernatant was decanted

and aliquots were transferred to 4 mL cuvettes (1 cm light path) prior to reading absorbance at 642.5 and 660 nm in a spectrophotometer (Shimadzu Corporation, UV-1601 PC UV-Visible, Kyoto, Japan). Total chlorophyll was expressed as mg of total chlorophyll/100 g of vegetable sample on a wet basis.

The juice of 30 g of Swiss chard was obtained with a home juice extractor (BGH, 390050, Argentina) and centrifuged at $1000 \times g$ for 5 min. Juice samples were diluted (1:1) with distilled water. The pH of the diluted samples was measured with a benchtop conductivity/pH meter (Jenco Electronics Ltd, Model 1671, Taiwan). The diluted samples were also titrated to pH 8.1 with 0.1 mol/L NaOH (14). Titratable acidity was calculated as g malic acid/100 g sample.

The soluble solids content in juice samples was determined in triplicate using an Abbe refractometer (Atago Co. Ltd., 976440 Tokyo, Japan) (15).

All assays were performed in triplicate.

Results and Discussion

Since water content is critical for leafy vegetables, we evaluated water losses during storage of chard. Water content in samples during storage is presented in **Fig. 1**. Although the rate of loss appears to change for the practical range of storage times, a constant rate, represented by a straight line, is assumed. Greater water losses were always related to samples with higher damaged area per unit volume. In the respiration process, water is produced and retained by the food (16), so the respiration increase associated with the physical damage would not be responsible for the losses observed. In addition, changes in water content would not be principally attributable to dehydration because samples were stored under an atmosphere close to saturation. Nevertheless, it is known that transpiration rate is influenced not only by environmental factors but also by commodity factors such as surface-to-volume ratio and surface injuries (2). Water loss of chard lots can be attributed to: a) evaporation of a moisture layer that persists on the vegetable surface after washing; b) leakage of cellular fluids caused by mechanical damage (8); and c) increase of permeability of cell membranes enhanced by ethylene (8). The first of these factors is dependent on the exposed area per volume unit, while the leakage of cellular fluids and the permeability increase are related to the damage area per volume unit. Increases in the damaged area due to processing affect the organized surface on the tissue, accelerating the rate of water loss from the vegetable (17). In our experimental system, the damaged area is a small fraction of the overall exposed area. Therefore the differences between lots suggest that water loss could be attributed to cellular fluid leakage due to direct mechanical damage or to enhanced cell membrane permeability. The slopes of the tendency lines in **Fig. 1** represent the different water loss rates. In **Fig. 2**, these slopes were plotted against the corresponding damaged areas per unit volume calculated as indicated in the Materials and Methods section. The high correlation found between

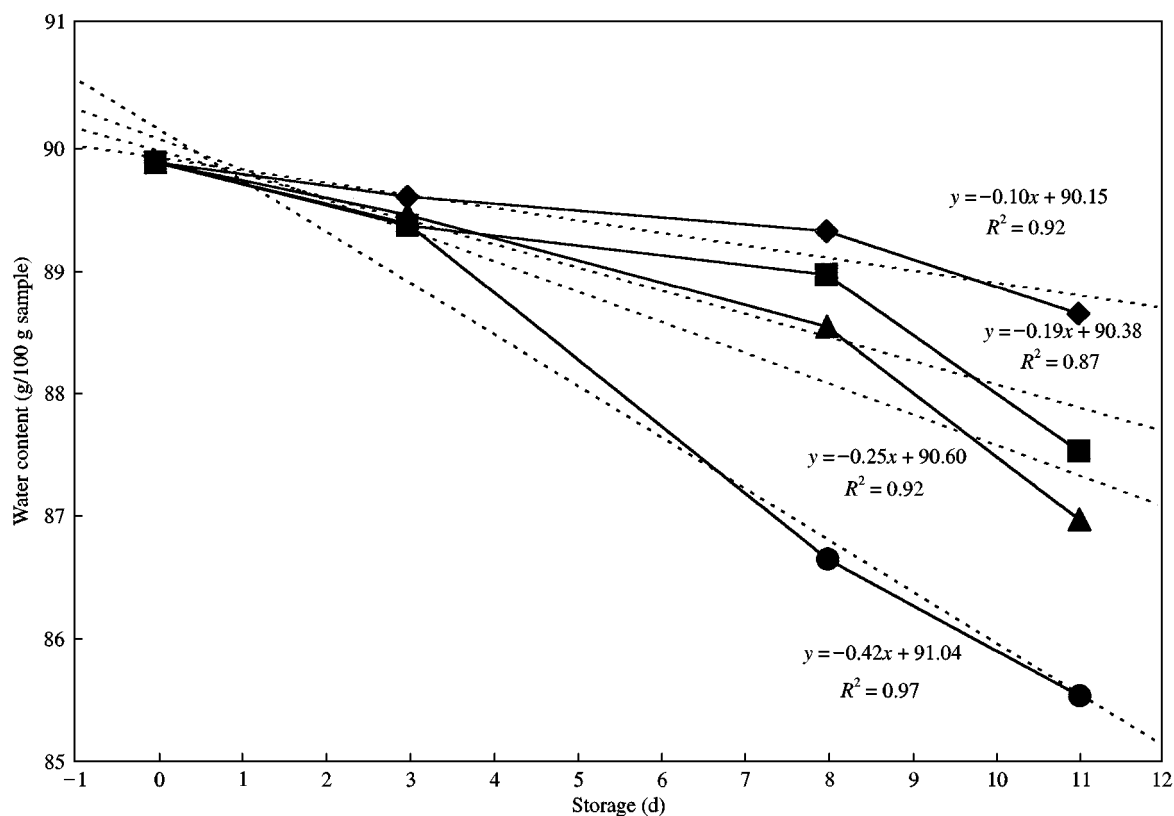


Fig. 1 Water content during storage of Swiss chard leaves with 0, 0.5, 0.67 and 1.0 damaged area per unit volume (cm^{-1}). Dashed straight lines represent tendency lines. (\blacklozenge) = 0; (\blacksquare) = 0.5; (\blacktriangle) = 0.67; (\bullet) = 1

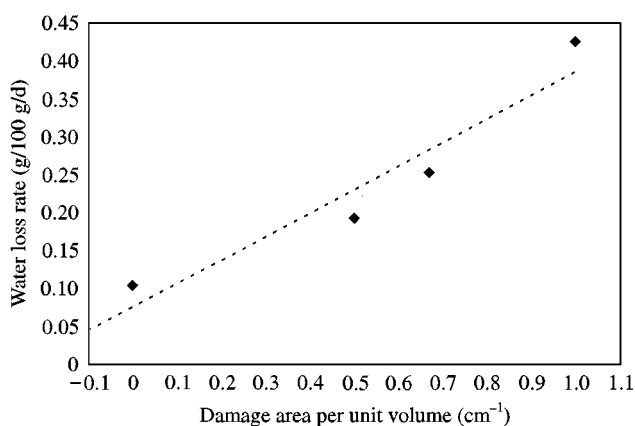


Fig. 2 Water loss rates (obtained from slopes of tendency lines in **Fig. 1**) vs. damaged area per unit volume (cm^{-1}). $y = 0.32x + 0.08$; $R^2 = 0.91$

water loss rates and damage area per unit volume would indicate that water loss is mainly associated with cellular fluid leaks due to mechanical damage.

Minimally processed leafy vegetables present important weight losses that are related to quality degradation of the product and, since they represent a loss in the salable weight, they are extremely important from a commercial point of view. The weight loss, as a percentage of initial weight, is presented in **Fig. 3** for the different lots. At all times and for all samples, the water loss (**Fig. 2**) does not account for the weight loss observed. When commodities are cut or otherwise damaged mechanically, they start respiring more rapidly, and ethylene production is stimu-

lated. Also the processes by which stored food reserves (sugars) are converted first to organic acids, then to more simple carbon compounds, are enhanced (2). Therefore, the weight loss presented in **Fig. 3** that cannot be explained in terms of water loss could be attributed to consumption of constituents through metabolic processes. Accordingly, the greatest weight loss occurred in the first days of storage when the decrease in soluble solids content, indicative of metabolic processes, was greater (**Table 1**). For equal storage time, the greater weight loss was associated with the samples with the most damaged area per unit volume. This would not only corroborate that mechanical damage accelerates these metabolic processes but would also indicate that this acceleration is related to the intensity of the processing injuries. Several authors have found that different metabolic processes are affected by the amount of processing damage. In cut green bananas, the rate of ethylene increase was dependent on the thickness of the slices (18). Carrots cut into slices and sticks or shredded were found to present different respiratory rates (19). Dividing broccoli heads into florets resulted in enhanced respiration throughout storage and increased ethylene production due to differences in gas exchange area, which was greater for the florets (12).

In **Fig. 3**, all samples showed important weight loss in the first days of storage and a tendency to stabilize after some time. The physiological response due to wounding is usually short term. The stimulation of ethylene production by stress typically occurs within a lag of 10–30 min and subsides later, after reaching a peak within several

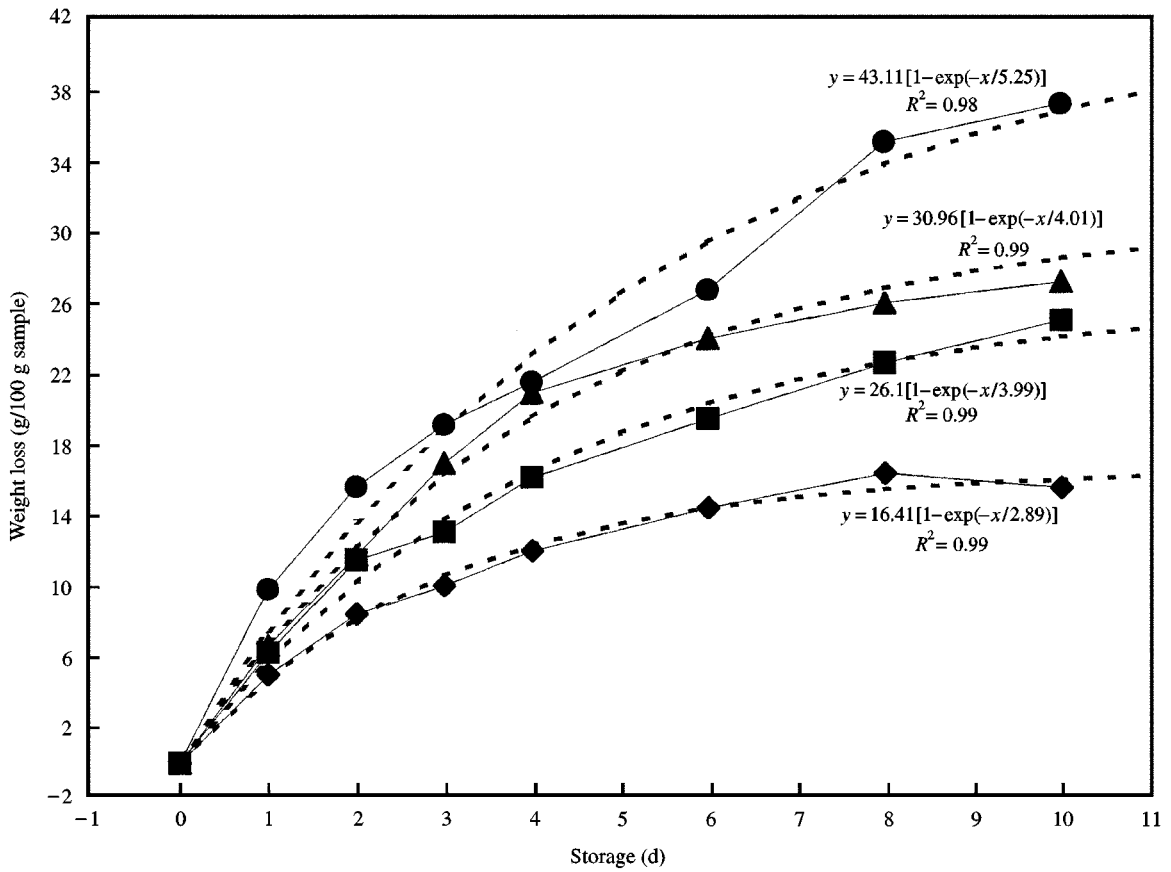


Fig. 3 Weight loss during storage of Swiss chard leaves with 0, 0.5, 0.67 and 1.0 damaged area per unit volume (cm^{-1}). Dashed lines represent tendency lines of the form $y = A[1 - \exp(-x/\tau)]$, where A is the stationary weight loss and τ is the time constant. (—◆—) = 0; (—■—) = 0.5; (—▲—) = 0.67; (—●—) = 1

Table 1 Soluble solids content ($^{\circ}\text{Brix}$) and titratable acidity (% malic acid) during storage of Swiss chard leaves with different damage area per unit volume (cm^{-1}). Means were calculated for three replicates

Days of storage	Titratable acidity % malic acid				Soluble solids content $^{\circ}\text{Brix}$			
	0	0.5	0.67	1	0	0.5	0.67	1
0	0.36 a ^a ± 0.05	0.36 a ± 0.05	0.36 a ± 0.05	0.36 a ± 0.05	7.02 a ± 0.08	7.02 a ± 0.08	7.02 a ± 0.08	7.02 a ± 0.08
3	0.27 a + 0.04	0.26 a ± 0.01	0.25 a ± 0.01	0.23 b ± 0.01	5.78 b ± 0.26	5.68 b ± 0.38	5.05 b ± 0.05	4.37 b ± 0.02
8	0.26 a ± 0.05	0.24 b ± 0.03	0.20 a ± 0.03	0.15 b ± 0.07	5.79 b ± 0.17	5.39 b ± 0.23	4.73 b ± 0.75	3.79 c ± 0.01
11	0.26 a ± 0.05	0.25 b ± 0.01	0.17 b ± 0.03	0.16 b ± 0.03	5.32 c ± 0.04	4.48 c ± 0.01	5.1 b ± 0.12	4.4 b ± 0.01

^aIn each column means followed by different letters are statistically different at the $P < 0.05$ level

hours (12). For tomato cut into small disks, Lee *et al.* (1970) reported an ethylene production 20 times higher than for whole fruit that was noted 15–20 min after cutting (20). The respiration rate of peeled and sliced kiwifruit at 20°C is twice that of whole fruit and this elevated rate is sustained for a 36 h period (8). The important decreases in weight observed during the first days in chard (Fig. 3) suggest a short-term physiological response with increases in the respiration rate and ethylene production. These high initial metabolic rates would not be

sustained because of a depletion of the reserves and a reduction in respiration rate and ethylene production associated with low storage temperatures. An alternative and not exclusive explanation could be related to wound healing on the cut surfaces slowing water loss and restricting gas exchange.

Makhlouf *et al.* suggest that decreases in the respiratory activity and ethylene production of broccoli are due to the effect of low temperature, known to inhibit ethylene production (21).

To fit the data in **Fig. 3** we used the equation:

$$W = A \cdot [1 - \exp(-t/\tau)] \quad \text{Eqn [1]}$$

that represents the evolution of a first order system between the stationary states (22). In Eqn [1], W is the weight loss, A is the stabilization value, t is time and τ is the time constant, indicative of rate of change. The values for A and τ for each lot were found by minimizing the sum of squared differences between experimental values and values predicted by Eqn [1] by a direct search method.

In **Fig. 4a, b**, the values of A and τ are plotted against the corresponding damaged areas per unit volume. The correlation coefficient (R^2) that represents the fraction of the sum of squares of deviation removed by the tendency line (23) was above 0.95 in all cases. Results would indicate that the values at which weight loss tends to stabilize and the rates at which these values are approached, are directly related to the damaged area per unit volume. Again, as with water loss, the differences in the rate of weight loss due to processing is related to damage area rather than to exposed area.

Changes in chlorophyll content correspond to changes in colour which, in turn, are indicative of changes in quality. Chlorophyll content during storage of whole and sliced chard leaves is shown in **Fig. 5**. For the practical range of storage times, the rate of chlorophyll loss was considered uniform, represented by a straight line, the slope of which represents the rate of chlorophyll degradation. **Figure 6** shows the slopes against the damaged area per unit

volume. Results would indicate that the rate of chlorophyll degradation are directly related to the damaged area.

In spinach, degradation of chlorophyll was hastened by exposure to 10 mg/kg ethylene (8). Destruction of chlorophyll by ethylene has been reported to be due to increased chlorophyllase activity (24, 25). An effect of slicing on the rate of respiration and ethylene production was informed in several fruits and vegetables. Therefore, the dependence of chlorophyll degradation with damaged area in Swiss chard leaves could be related to higher ethylene production induced by higher metabolic rates. Yellowing induced by 4 mg/kg ethylene has also been reported for Brussels sprouts, broccoli, cauliflower and cabbage (26).

The energy sources available in the tissues of freshly harvested vegetables are free amino acids and carbohydrates (27). Organic acids are utilized quickly during respiration compared to other compounds (15). Soluble solids of green vegetables also include sugars, which are consumed in the respiratory process. Bolin and Huxsoll reported a decline in the solids soluble values for salad cut lettuce, attributed to carbohydrate utilization via respiration (10).

Both titratable acidity and soluble solids of chard leaves presented their major decreases during the first 3 d of storage, and the largest decreases were associated with the samples with the greatest damaged area per unit volume (**Table 1**). However, the differences among samples were not significant. After 3 d of storage, the evolution of titratable acidity and soluble solids did not show a definite tendency that could be related to the intensity of damage. Kim *et al.*, working with minimally processed apple slices under cold storage, also reported that acidity decreased rapidly in the first three days and attributed the rapid decrease to increased respiration following tissue damage (15). These results suggest that the respiratory process is intense during the first days of storage and then subsides and that it is then affected by the harvest and processing damage. Moreover, this fact would also correspond to the greater weight loss in the first days of storage and to larger losses in the more highly injured samples (**Fig. 3**). The consumption of acids and soluble solids are directly related to metabolic processes. Although the transfer of material may play an important part in providing supplies and eliminating products for the metabolic process, metabolism is a complex phenomenon involving enzymatic activity and substrate availability. This would explain why the decreases in these constituents could not be directly related to the amount of damaged area when the whole storage is considered.

Conclusions

Decreases in weight and in water and chlorophyll content during storage of Swiss chard leaves are affected by the intensity of the processing injuries. Since the damaged area is a small fraction of the overall exposed area, it is concluded that the different rates of decrease between samples is related to damaged area.

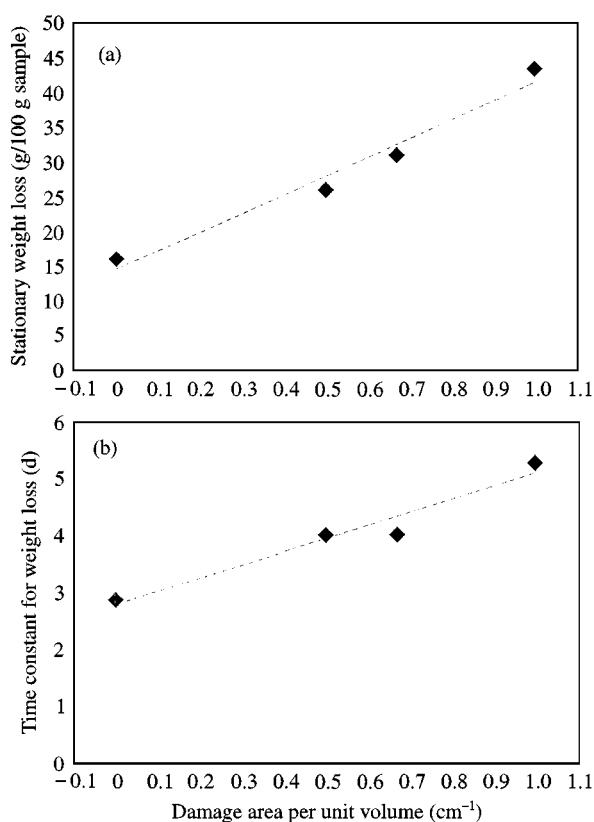


Fig. 4 (a) final weight loss approached by tendency lines of **Fig. 3** vs. damaged area per unit volume (cm^{-1}). $y = 26.17x + 14.94$; $R^2 = 0.97$

(b) time constant from the tendency lines of **Fig. 3** vs. damaged area per unit volume (cm^{-1}). $y = 2.24x + 2.82$; $R^2 = 0.95$

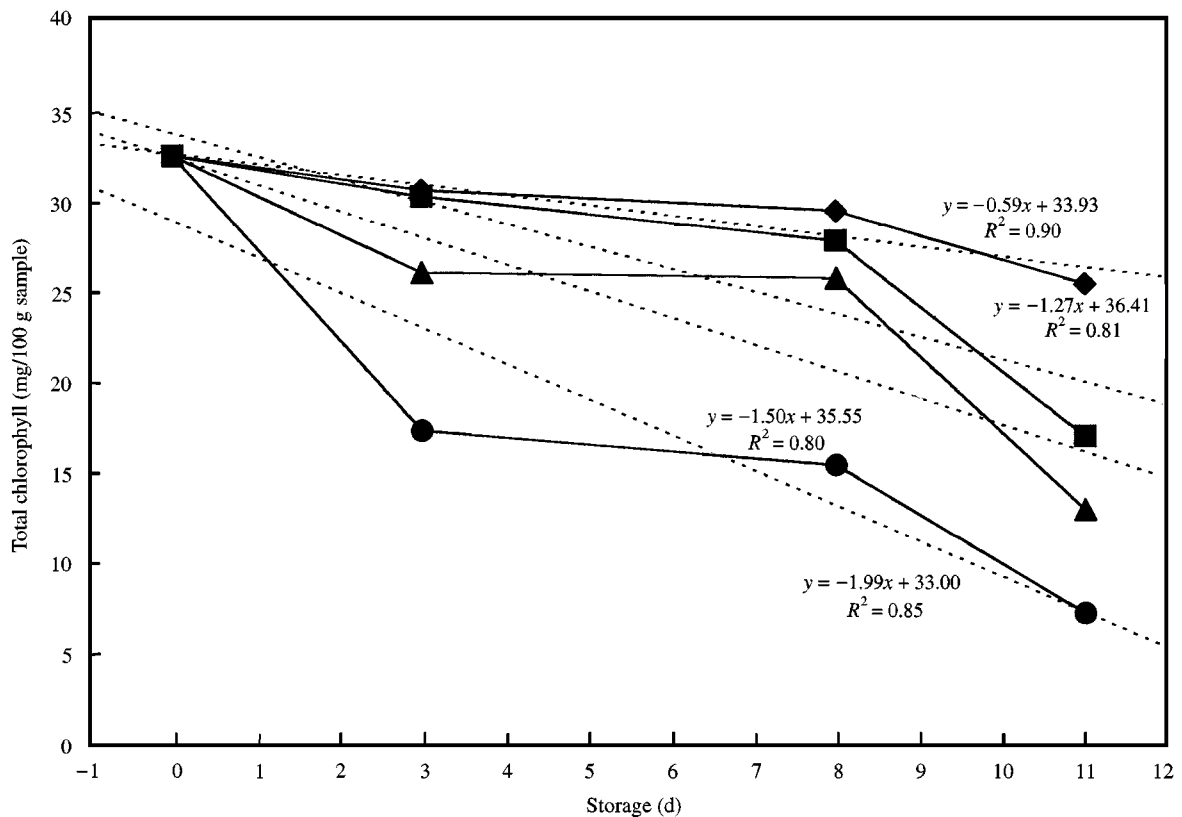


Fig. 5 Total chlorophyll content during storage of Swiss chard leaves with 0, 0.5, 0.67 and 1.0 damaged area per unit volume (cm^{-1}). Dashed straight lines represent tendency lines. (\blacklozenge) = 0; (\blacksquare) = 0.5; (\blacktriangle) = 0.67; (\bullet) = 1

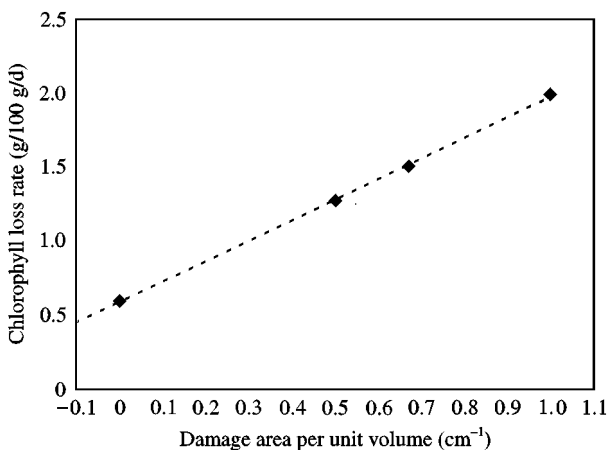


Fig. 6 Chlorophyll loss rates (obtained from slopes of tendency lines in Fig. 5) vs. damaged area per unit volume (cm^{-1}). $y = 1.39x + 0.58$; $R^2 = 0.99$

Mathematical expressions are given that provide a means of estimating decreases in weight and water and chlorophyll content during storage when the damaged area per unit volume is known. Although titratable acids and soluble solids presented important decreases during the first days of storage, these changes did not present a definite relation to damage.

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