Fresh-cut kale quality and shelf-life in relation to leaf maturity and storage temperature

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Abstract
Kale (Brassica oleracea var. acephala) is a very nutritious leafy vegetable and its consumption in fresh-cut salads has increased in recent years. Kale leaves may be harvested at different stages of maturity, resulting in a heterogeneity that may be detrimental to fresh-cut salad quality and shelf-life. Changes in composition and visual parameters were investigated in fresh-cut kale leaves (‘Lacinato’) harvested at three maturity stages used commercially based on leaf position and size (immature, mature, overmature), two temperatures (0 and 5°C) and five periods of storage (0, 14, 21, and 28 days; up to 42 days at 0°C). Product was cut manually into 2 cm strips, washed in chlorinated water, manually centrifuged, and packaged in unsealed LDPE bags. Total chlorophyll content decreased during storage, with the lowest concentrations found in pieces from overmature leaves at 5°C, while the total carotenoid content did not vary among the conditions studied. Ammonia content, an indicator of protein breakdown and senescence, remained low for pieces from all maturity stages stored at 0°C up to 42 days, was intermediate in immature cut leaves at 5°C, and increased dramatically in pieces from mature and overmature leaves at 5°C between 21 and 28 days. Objective color values as well as marketability indicators (off-odors, overall visual quality, yellowing, decay, cut-end browning) exhibited significant differences in response to the postharvest conditions studied. In general, the loss of composition and visual quality of fresh-cut kale leaves increased with increasing temperature, days of storage, and leaf maturity.

Keywords: visual appearance, senescence indicators, ammonia, malondialdehyde

INTRODUCTION
Kale (Brassica oleracea var. acephala) is a very nutritious leafy vegetable and its consumption in fresh-cut salads has increased recently, although kale consumption is low compared with other leafy greens such as spinach or lettuce (USDA, 2012). Glucosinolates, ascorbic acid, carotenes, α-tocopherol and phenolic compounds are among the chemical components that contribute to the high antioxidant capacity and high nutritional value of this leafy product (Soengas et al., 2012) and these are affected by leaf maturity (Korus, 2011). Leaf senescence involves the loss of chlorophyll and other constituents, membrane deterioration, and nutrient recycling (Guiboileau et al., 2010; Hörtensteiner, 2006) and is largely controlled by temperature in harvested products (Koukounaras, 2009).

Commercially grown kale is harvested over a period of time, and each harvest may consist of leaves at different stages of development or maturity (Albornoz, 2014). Fresh-cut products should be stored at temperatures <5°C (Cantwell and Suslow, 2002). The main objective of this study was to determine whether leafy maturity affected the quality and shelf-life of fresh-cut kale when stored within the recommended temperature range. Another objective was to determine whether ammonia (Cantwell et al., 2010) and malondialdehyde (MDA) (Dai et al., 2011) were useful indicators of quality and senescence for fresh-cut kale.

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MATERIALS AND METHODS

Leaves of kale cultivar ‘Lacinato’ were harvested at three maturity stages from a commercial field near Oxnard, CA, USA, placed in unsealed plastic bags, transported in coolers with ice, and held at 0°C overnight. Maturity stage criteria were based mainly on leaf size (Table 1). Immature leaves were the youngest, and had not reached full expansion. Mature leaves were larger, but had not reached full expansion. Overmature leaves were fully expanded, with some presenting slight signs of leaf senescence (Guiboileau et al., 2010).

Table 1. Parameters used to specify maturity of leaves of kale ‘Lacinato’.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Immature</th>
<th>Mature</th>
<th>Overmature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>&lt;20</td>
<td>20-30</td>
<td>&gt;35</td>
</tr>
<tr>
<td>Width (cm)</td>
<td>&lt;4</td>
<td>4-5</td>
<td>&gt;5</td>
</tr>
</tbody>
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For fresh-cut preparation (Albornoz, 2014), leaves were cut into strips of about 1 cm across the midrib with a stainless steel knife, washed in chlorinated water (5:1 ratio of solution to product weight; 50 µL L⁻¹ sodium hypochlorite solution adjusted to pH 7.0), shaken, drained on clean towels, placed in unsealed polyethylene bags on trays, overwrapped with a larger bag to prevent water loss and stored at 5°C. One bag was equivalent to one replication and contained cut product from four to six leaves. Separate samples were used for measurement of respiration by determination of CO₂ production on an infrared analyzer.

Product was sampled periodically for marketability analysis (off-odors, overall visual quality, yellowing, decay/deterioration, and discoloration/browning), composition (chlorophyll and carotenoids, ammonia, MDA) and objective color measurements. At 0°C, product was sampled at 0, 14, 28, and 42 days; at 5°C product was evaluated at 0, 14, 21, and 28 days.

Objective color values were measured with a reflectance colorimeter (Konica Minolta Chroma Meter CR-300) and hue values are reported. Subjective evaluations were performed using hedonic scales. Bags were removed from storage and immediately evaluated at ambient temperature. All product in each bag received one score per quality parameter. Off-odors were scored on a 1 to 5 scale, where 1 = none, 2 = slight, 3 = moderate, 4 = moderately severe and 5 = severe. Overall visual quality was evaluated on a 9 to 1 scale, where 9 = excellent, fresh appearance, 7 = good, 5 = fair (limit of marketability), 3 = poor (usable but not salable), and 1 = unusable. Intermediate numbers were assigned where appropriate. Yellowing was scored on a 1 to 5 scale, where 1 = none; 2 = slight loss of greenness or slight yellowing (<2% affected); 3 = moderate loss of greenness/yellowing (<5% affected); 4 = moderately severe (5-15% affected); 5 = severe, unusable (>15% of leaf surfaces with yellowing). Decay or visible deterioration was scored on a 1 to 5 scale, where 1 = none; 2 = slight decay (<2% affected); 3 = moderate decay, (<5% affected); 4 = moderately severe (5-15% affected); 5 = severe, unusable (>15% with decay). Discoloration and/or cut-edge browning was scored on a 1 to 5 scale, where 1 = none; 2 = slight (<2% affected); 3 = moderate (>5% affected); 4 = moderately severe (>15% affected); 5 = severe, unusable (>15% with discoloration).

For compositional analyses, midribs were removed, leaf pieces were finely chopped with a sharp stainless steel knife, and 4 g subsamples were frozen at -20°C. Chlorophyll and carotenoid samples were homogenized (Ultra Max T25 Basic homogenizer by Ika-Werke) for 1.5 min at 17,500 rpm, adding 12 ml acetone 80% (10 mg MgCO₃ in 1000 ml 80% acetone), and absorbance of centrifuged extracts was measured at 663.2, 646.8 and 470 nm (Shimadzu spectrophotometer UV-1700) as described by Lichtenthaler (1987). Ammonia was measured on water extracts and concentrations were determined by a color reaction (measured at 635 nm) in phenol containing nitroprusside and alkaline hydrochlorite and expressed in mg NH₄/g fresh weight, as described by Beecher and Whitten (1970) and Weatherburn (1967). MDA was determined as described by Heath and Packer (1968) and
The experiment was conducted in a completely randomized factorial design with three replications. Data are expressed as means ± standard error (SEM).

RESULTS AND DISCUSSION

Respiration rates were significantly higher at 5°C than at 0°C. Immature leaves had the highest respiration rates at both temperatures, while respiration rates of mature and overmature leaves were similar at each temperature (Figure 1). Average respiration rates for immature, mature, and overmature leaves from days 2 to 28 at 0°C were 6.9, 4.4, and 4.9 µL CO₂ g⁻¹ h⁻¹, respectively. Corresponding values from days 2 to 20 at 5°C were respectively 10.2, 8.7, and 8.1 µL CO₂ g⁻¹ h⁻¹.

![Figure 1](image)

Figure 1. Respiration rates of fresh-cut kale leaves harvested at three stages of maturity and stored at 0°C (A) or 5°C (B). Data are means of three replicates ± SEM.

Fresh-cut kale stored at 0°C had superior visual quality attributes (Albornoz, 2014) and marketability scores (Figure 2) than fresh-cut leaves stored at 5°C. Differences in performance due to maturity were evident at 5°C, starting at 21 days of storage. Decay/deterioration and discoloration scores increased with time, as did yellowing scores (Figure 3), and all contributed to the overall loss of visual quality (Figure 2).
Chlorophyll content decreased over time at 5°C and, on day 28, was different due to maturity stage, while at 0°C there were no significant changes (Figure 4a, b). Changes in carotenoids were minimal (data not shown) at both 0 and 5°C and among maturity stages (data not shown). Chlorophyll changes were consistent with yellowing scores and hue values at 5°C, but the relationships were not as consistent with storage at 0°C (Figures 3 and 4).
Figure 3. Yellowing score (A, B) and hue color value (C, D) of fresh-cut kale leaves harvested at three stages of maturity and stored at 0°C for up to 42 days (A, C) or at 5°C for up to 28 days (B, D) in unsealed plastic bags. Data are means of three replicates ± SEM.

As a marker for oxidative stress and lipid peroxidation, MDA exhibited no significant differences as a result of temperature or time. This differed from the increases found by Dai et al. (2011) after harvest of millet leaves. However, the immature kale leaves had significantly lower MDA content than mature and overmature leaves throughout the experiment (Figure 4c, d).

As an indicator of senescence and stress, ammonia content increased over time at both temperatures (Figure 4e, f). At 5°C, there were large increases in ammonia in the mature and overmature leaves, consistent with the time course of visual quality changes. There was little increase in ammonia at 0°C, and the concentrations in the immature leaves were lower than those in mature and overmature leaves throughout the experiment. The increases in ammonia concentrations are also consistent with other recent postharvest studies on leafy green products (Cantwell et al., 2010).
CONCLUSIONS

Leaf maturity at harvest had a significant impact on the postharvest performance of fresh-cut kale leaves, especially at 5°C. Compositional analyses were consistent with the marketability evaluations, with more rapid fresh-cut product deterioration and senescence at 5°C and minimal changes at 0°C. This was also consistent with respiration rates being directly proportional to storage temperature but inversely proportional to shelf-life.
Ammonia, but not MDA, was a good indicator of quality and deterioration. MDA did reflect differences in leaf maturity.

ACKNOWLEDGEMENTS
We thank Dr. Gyunghoon Hong for his valuable contributions. This research was partially supported by a Jastro Research Award from UC Davis.

Literature cited