Dry Matter Content and Stability of Carotenoids in Kale and Spinach During Drying

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Abstract. Drying of spinach (Spinacia oleracea L.) and kale (Brassica oleracea L. var. acephala D.C.) is required to determine percentage of dry matter (%DM) and pigment concentration of fresh leaves. ‘Melody’ spinach and ‘Winterbor’ kale were greenhouse-grown in hydroponic nutrient solutions containing 13 or 105 mg L−1 N. Using vacuum freeze dryers and convection ovens, plant tissues were dried for 120 h at five different temperature treatments: 1) freeze drying at −25 °C; 2) freeze drying at 0 °C; 3) vacuum drying at +25 °C; 4) oven drying at +50 °C; and 5) oven drying at +75 °C. Spinach leaf tissue %DM was affected, but kale %DM was unaffected by drying temperature. Spinach and kale leaf tissue %DM were both affected by N level. The high N spinach decreased from 7.3 to 6.4%DM when drying temperature increased from +25 to +75 °C. The low N spinach decreased from 12.7 to 9.6%DM as the drying temperature increased from −25 to +50 °C. Kale averaged from 14.8%DM for the high N treatment and from 21.8%DM for the low N treatment. However, drying temperature did not have a significant impact on measured %DM in kale. Leaf, β-carotene, and chlorophyll levels for both spinach and kale leaf tissue were affected by drying temperature. Measured concentrations of all pigments decreased over 70% as the drying temperature increased from −25 to 75 °C. The largest pigment fresh and dry weight concentrations for spinach and kale were measured at drying temperatures below +25 °C. The spinach and kale samples dried between −25 and +25 °C were not significantly different from each other in %DM or pigment concentration measured on a dry or fresh weight basis. Thus, drying leaf tissue for accurate pigment analysis requires temperatures below +25 °C using vacuum or freeze drying technology.

Determination of carotenoid concentrations in plant tissue requires dried samples for analysis (Kopsell et al., 2004; Tai and Chen, 2000). However, the plant growth environment can have a significant impact on the water content of the harvested plant (Lefsrud et al., 2005, 2006). A number of methods are used to remove tissue moisture before analysis, including convection oven drying, freeze drying, vacuum drying, air drying, and microwave drying (Diaz-Maroto et al., 2002a, 2002b, 2004; Ingram et al., 2000; Karathanos, 1999; Litvin et al., 1998; Stegen et al., 1998). To determine the effect of drying temperature on plant samples, Stegen et al. (1998) compared the water content of a number of plants using microwave, oven, and vacuum drying (performed at 22 °C). Alfalfa (Medicago sativa L.), onion (Allium cepa L.), corn (Zea mays L.), and potato (Solanum tuberosum L.) had a final moisture content that was not significantly different among the three drying methods, but bean (Pisum sativum L.), bean shell, and garlic (Allium sativum L.) were significantly different. The microwave oven and vacuum drying of the latter three plant samples did not significantly affect the moisture measurement, but oven drying resulted in a significant change in the measured moisture content.

Moisture content within biological samples changes during drying and can result in the release of organic compounds, volatile organic compounds (VOCs), destruction of pigments, and changes in chemical composition. The loss of VOC through drying of a wide variety of plants has been reported (Diaz-Maroto et al., 2002a, 2002b, 2004; Ingram et al., 2000). However, the published literature on release of organic compounds not listed as VOC is limited. At temperatures greater than 65 °C, Karathanos (1999) reported that decomposition of low-molecular-weight sugars (specifically fructose), in addition to the evaporation of water, could occur within the sample. An ASABE standard (ASABE, 2007) recommends that for further analytical analysis of plant forages, drying temperatures less than 65 °C should be used. Diaz-Maroto et al. (2002a) found that oven drying and air drying bay leaf (Laurus nobilis L.) did not affect the aroma of the fresh herb; however, freeze drying resulted in a reduction. Diaz-Maroto et al. (2002b) reported that oven drying and freeze drying of parsley (Petroselinum crispum L.) resulted in a decrease in VOC within the sample, but freeze drying resulted in few losses. Oven-dried and freeze-dried basil (Ocimum basilicum L.) had a decrease in VOC, but air-dried basil was not different compared with a fresh sample (Diaz-Maroto et al., 2004).

Carotenoids and other plant compounds can also be affected by drying temperature. A significant decrease in lutein and β-carotene was measured in daylily (Hemerocallis diasticha Donn ex Sweet) when oven dried at 48 °C compared with −53 °C freeze drying (Tai and Chen, 2000). Cinar (2004) reported that freeze drying reduced the losses of carotenoids during storage in orange peel [Citrus sinensis (L.) Osbeck], potato, and carrot (Daucus carota L.). Alomar et al. (1999) reported that oven drying of silage resulted in a reduction in crude protein and an increase in crude fiber, neutral detergent fiber, and acid detergent fiber when compared with freeze drying. Carotenoids are lipid-soluble, yellow, orange, and red pigments produced by plants, algae, and bacteria. In higher plants, carotenoids function in photoprotection as light-harvesting antennae pigments and free radical scavengers (Miki, 1991; Taiz and Zeiger, 1998; Tracewell et al., 2001). Two important dietary carotenoids in human health maintenance are lutein and β-carotene. Increased intake of lutein and β-carotene has been associated with reduced risk of lung cancer and chronic eye diseases such as cataracts and age-related macular degeneration (Ames et al., 1995; Landrum and Bone, 2001; Le Marchand et al., 1993; Sema and Dagnew, 2003). Increasing the lutein and β-carotene concentrations in vegetable crops through cultural management techniques would be beneficial to the health status of consumers. Kale (Brassica oleracea L. var. acephala D.C.) ranks highest and spinach (Spinacia

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oleracea L.) ranks second among vegetable crops for lutein and β-carotene content (Holden et al., 1999; U.S. Dept. Agr., 2002). However, kale has low consumption rates with per capita fresh intake of less than 0.33 kg/year in the United States (Lucier and Plummer, 2003). Spinach has one of the highest consumption rates among green, leafy vegetables in the United States with per capita intakes of 0.73, 0.09, and 0.36 kg/year for fresh, canned, and frozen markets, respectively (Lucier and Plummer, 2003).

With increased interest in dried plant material for human health within supplemental markets, the purpose of this study was to determine the effect of drying conditions on water content, measured dry matter content, and stability of pigments (carotenoids and chlorophylls) in kale and spinach.

Material and Methods

Plant culture

‘Melody’ spinach and ‘Winterbor’ kale (Johnny’s Selected Seed, Winslow, ME) were seeded on 22 Feb. 2005 into rockwool growing cubes (Grodan A/S, Dk-2640, Hedehusen, Denmark). The seeds were germinated and grown in a greenhouse (22 °C day/14 °C night) under natural lighting conditions (Knoxville, TN, lat. 35°55′N). Plants were grown according to the method described in Lefsrud et al. (2007). Peter’s 20N–6.9P–

b 4.6 mm i.d., 5-cm spacing in each container lid. A HPLC unit with photodiode array detection was counted for by the interaction between N treatment and drying temperature (P ≤ 0.01). The measured %DM for spinach was influenced by drying temperature (P = 0.011). No significant variation was accounted for by the interaction between N treatment level and drying temperature (P = 0.081). Average %DM for the high N treatment increased from 7.0% for the high N treatment to 10.7% for the low N treatment (Table 1; Fig. 1). The average %DM for the high N spinach treatment varied among the drying temperature treatments and resulted in an increase from 6.4% for the +75 °C convection oven to 7.3% for the +25 °C vacuum dryer. The average %DM for the low N spinach treatment increased from 9.6% for the +50 °C convection oven to 12.7% for the +25 °C freeze dryer. Spinach leaf tissue %DM decreased linearly with increases in drying temperature for both the high N [%DM = 7.2 to 0.008 (T), r² = 0.27, P = 0.05] and low N treatments [%DM = 11.4 to 0.02 (T), r² = 0.49, P = 0.004].

Results

The moisture content of the tissue samples did not change significantly between the first 100 h of drying when compared with the final 20 h. Equilibrium moisture content was confirmed for all drying methods using this two-step drying process.

Spinach biomass accumulation. The measured percent dry matter (%DM) for spinach was influenced by N treatment level (P ≤ 0.01). The measured %DM for spinach was influenced by drying temperature (P = 0.011). No significant variation was accounted for by the interaction between N treatment level and drying temperature (P = 0.081). Average %DM for the high N treatment increased from 7.0% for the high N treatment to 10.7% for the low N treatment (Table 1; Fig. 1). The average %DM for the high N spinach treatment varied among the drying temperature treatments and resulted in an increase from 6.4% for the +75 °C convection oven to 7.3% for the +25 °C vacuum dryer. The average %DM for the low N spinach treatment increased from 9.6% for the +50 °C convection oven to 12.7% for the +25 °C freeze dryer. Spinach leaf tissue %DM decreased linearly with increases in drying temperature for both the high N [%DM = 7.2 to 0.008 (T), r² = 0.27, P = 0.05] and low N treatments [%DM = 11.4 to 0.02 (T), r² = 0.49, P = 0.004].

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Spinach carotenoid and chlorophyll pigment accumulation. Maximum spinach tissue lutein accumulation expressed on a fresh weight (FM) basis was 9.8 mg/100 g\(^{-1}\) for the low N treatment at the –25 °C drying temperature (Table 1). Spinach leaf tissue lutein concentrations responded to drying temperature (P ≤ 0.001) but not to N treatment (P = 0.273) nor their interaction (P = 0.0203). Spinach leaf tissue lutein decreased linearly with increases in drying temperature for both the high N [lutein FM = 8.4 to 0.07 (T), r\(^2\) = 0.84, P ≤ 0.001] and low N level [lutein FM = 8.1 to 0.07 (T), r\(^2\) = 0.76, P ≤ 0.001].

Maximum spinach tissue β-carotene accumulation expressed on a FM basis was 4.9 mg/100 g\(^{-1}\) under the high N treatment at the –25 °C drying temperature (Table 1). Spinach leaf tissue β-carotene concentrations responded to drying temperature (P ≤ 0.001) and to N treatment (P ≤ 0.001), but not to their interaction (P = 0.06). Spinach leaf tissue β-carotene decreased linearly with increases in drying temperature for both the high N [β-carotene FM = 4.3 to 0.04 (T), r\(^2\) = 0.89, P ≤ 0.001] and low N level [β-carotene FM = 3.3 to 0.03 (T), r\(^2\) = 0.66, P ≤ 0.001].

Spinach leaf tissue Chl a FM concentrations responded to drying temperature (P ≤ 0.001), N treatment (P ≤ 0.01), and to the interaction between N treatment and drying temperature (P ≤ 0.001). Spinach leaf tissue Chl a decreased linearly with increases in drying temperature for both the high N [Chl a FM = 7.93 to 1.0 (T), r\(^2\) = 0.83, P = 0.001] and low N treatments [Chl a FM = 57.7 to 0.8 (T), r\(^2\) = 0.78, P ≤ 0.001; Table 1]. Spinach leaf tissue Chl b also decreased linearly with increases in drying temperature for both the high N [Chl b FM = 28.7 to 0.4 (T), r\(^2\) = 0.82, P ≤ 0.001] and low N treatments [Chl b FM = 21.6 to 0.3 (T), r\(^2\) = 0.77, P ≤ 0.001; Table 1].

Maximum spinach tissue lutein accumulation expressed on a dry weight (DM) basis was 1.3 mg g\(^{-1}\) for the high N treatment and the –25 °C drying temperature (Table 2). Spinach leaf tissue lutein concentrations responded to drying temperature (P ≤ 0.001), N treatment (P ≤ 0.001), and to the interaction between N treatment and drying temperature (P = 0.008). Spinach leaf tissue lutein decreased linearly with increases in drying temperature for both the high N [lutein DM = 1.16 to 0.01 (T), r\(^2\) = 0.84, P ≤ 0.001] and low N treatment [lutein DM = 0.70 to 0.01 (T), r\(^2\) = 0.78, P ≤ 0.001].

Spinach leaf tissue DM Chl a and b concentrations responded to drying temperature (P ≤ 0.001), N treatment (P ≤ 0.001), and to the interaction between N treatment and drying temperature (P ≤ 0.001). Spinach leaf tissue Chl a decreased linearly with increases in drying temperature for both the high N [Chl a FM = 11.0 to 0.1 (T), r\(^2\) = 0.83, P ≤ 0.001] and low N treatments [Chl a DM = 4.9 to 0.07 (T), r\(^2\) = 0.80, P ≤ 0.001; Table 2]. Spinach leaf tissue Chl b decreased linearly with increases in drying temperature for both the high N [Chl b DM = 4.0 to 0.05 (T), r\(^2\) = 0.82, P ≤ 0.001] and low N treatments [Chl b = 1.9 to 0.07 (T), r\(^2\) = 0.79, P ≤ 0.001; Table 2].

Kale biomass accumulation. Percent dry matter for kale was influenced by N treatment level (P ≤ 0.01). No significant influence was measured for the kale drying temperature (P = 0.63) or for the interaction between N treatment and drying temperature (P = 0.90). Average %DM for the kale increased from 14.8% for the high N treatment to 21.8% for the low N treatment (Table 3). The average %DM for the high N kale treatment increased from 13.2% for the +75 °C convection oven to 15.9% for the +25 °C vacuum dryer. The average %DM for the low N kale treatment increased from 21.4% for the –25 °C freeze dryer to 22.5% for the +25 °C vacuum dryer. No trend was measured for the kale %DM for either the high or low N treatment.
The largest lutein accumulation was with the high N treatment and the \( Chl_a \) treatment (Table 3). Kale leaf tissue \( Chl_b \) decreased linearly with increases in drying temperature for both the high N \[ \text{Chl}_b \text{FM} = 5.4 \pm 0.03 (T), r^2 = 0.42, P \leq 0.001 \] and low N treatments \[ \text{Chl}_b \text{FM} = 2.9 \pm 0.3 (T), r^2 = 0.84, P \leq 0.001 \].

The kale maximum lutein DM was 0.84 mg g\(^{-1}\) with the high N treatment and the \(-25^\circ\)C drying temperature (Table 4). Kale leaf tissue lutein concentrations responded to N treatment \((P \leq 0.001)\), drying temperature \((P \leq 0.001)\), and to the interaction between N treatment and drying temperature \((P = 0.003)\). Kale leaf tissue lutein decreased linearly with increases in drying temperature for both the high N \[ \text{Chl}_b \text{FM} = 5.4 \pm 0.03 (T), r^2 = 0.42, P \leq 0.001 \] and low N treatments \[ \text{Chl}_b \text{FM} = 2.9 \pm 0.3 (T), r^2 = 0.84, P \leq 0.001 \].

The kale maximum \( \beta \)-carotene DM was 0.37 mg g\(^{-1}\) with the high N treatment and the \(-25^\circ\)C drying temperature (Table 4). Kale leaf tissue \( \beta \)-carotene concentrations responded to N treatment \((P \leq 0.001)\), drying temperature \((P \leq 0.001)\), and to the interaction between N treatment and drying temperature \((P \leq 0.001)\). Kale leaf tissue \( \beta \)-carotene decreased linearly with increases in drying temperature for both the high N \[ \beta \text{-carotene FM} = 0.34 \pm 0.02 (T), r^2 = 0.74, P \leq 0.001 \] and low N treatments \[ \beta \text{-carotene FM} = 0.14 \pm 0.01 (T), r^2 = 0.61, P \leq 0.001 \].

Kale leaf tissue DM \( Chl_a \) and \( b \) concentrations responded to N treatment \((P \leq 0.001)\), drying temperature \((P \leq 0.001)\), and to the interaction between N treatment and drying temperature \((P \leq 0.001)\). Kale leaf tissue \( Chl_a \) decreased linearly with increases in drying temperature for both the high N \[ Chl_a \text{FM} = 6.3 \pm 0.05 (T), r^2 = 0.67, P \leq 0.001 \] and low N treatments \[ Chl_a \text{FM} = 3.1 \pm 0.03 (T), r^2 = 0.87, P \leq 0.001 \].

**Discussion**

A consistent drying time of 120 h was determined for this study based on drying results of the \(-50^\circ\)C freeze drying procedure. This method was based on the ASABE Standard (ASABE, 2007) for drying of forages, which requires a 25-g sample be dried at 60 \(^\circ\)C for 72 h. For further chemical analysis of the samples, the ASABE standard does not recommend drying at temperatures above 65 \(^\circ\)C. From observations and analytical measurement, a minimum of 100 h was required for the \(-50^\circ\)C freeze-dried samples and was confirmed after another 20 h. To maintain a consistent drying time and reduce possible loss of organic compounds and VOC, the drying time was standardized across the experiment. From observations
Table 4. Mean pigment concentrations* expressed on a dry weight (DM) basis in the leaf tissues of ‘Winterbor’ kale grown with two nitrogen levels and five drying temperatures.

| Drying Temperature (°C) | Lutein (µg g⁻¹ DM) | β-carotene (µg g⁻¹ DM) | Chl a* | Chl b* |
|------------------------|-------------------|-----------------------|--------|--------|
| +75                    | 0.15 ± 0.02       | 0.10 ± 0.01           | 0.5 ± 0.2 | 0.4 ± 0.2 |
| +50                    | 0.68 ± 0.07       | 0.25 ± 0.03           | 2.9 ± 0.5 | 2.2 ± 0.5 |
| +25                    | 0.83 ± 0.04       | 0.34 ± 0.02           | 8.0 ± 0.6 | 3.0 ± 0.2 |
| 0                      | 0.80 ± 0.06       | 0.33 ± 0.03           | 8.2 ± 0.9 | 3.1 ± 0.4 |
| -25                    | 0.84 ± 0.03       | 0.37 ± 0.01           | 8.0 ± 0.6 | 3.0 ± 0.2 |

*Mean composition of leaf tissue (pooled six-plant subsample) of three replications ± se.

**Chl a = chlorophyll a; Chl b = chlorophyll b.

*Significant at P ≤ 0.001.

and previous experiments, drying time of the samples in the convection oven (+50 °C) required at least 72 h to reach moisture equilibrium. From previous personal experience, no differences were noticed when measuring pigment concentrations between freeze-dried samples between 0 and –50 °C. However, no tests were performed on samples dried in the convection oven until this experiment. The stability of these pigments (lutein, β-carotene, and chlorophyll) has been reported to be very stable in darkness for 2 weeks below +45 °C with measurable changes only occurring after 4 weeks (Daood et al., 2006; Tang and Chen, 2000).

Tissue N and chlorophyll content has been correlated in a number of studies with lower N levels resulting in less chlorophyll production. Under limited N, both Chl a and b pigments are reduced, resulting in potential leaf tissue chlorosis (Taiz and Zeiger, 1998). In the current study, the spinach chlorophyll FM concentrations decreased 17% and 11% and the kale decreased 32% and 26% for Chl a and b, respectively, with decreased N.

Lefsrud et al. (2007) reported that ‘Melody’ spinach tissue lutein, as a function of FM, did not change in response to increasing N treatment concentrations. However, when lutein was measured on a DM basis for ‘Melody’ spinach, lutein increased 80% as the N level was increased by a factor of eight times. Lefsrud et al. (2007) reported that %DM was negatively correlated with N concentration when growing spinach in nutrient solution culture. In that study, spinach %DM varied from 9.6% at 13 mg L⁻¹ N to 6.9% at 105 mg L⁻¹ N when the leaf tissue of the plant was oven dried at +50 °C. In the current study, the %DM of the spinach samples varied from 6.4% to 12.7% for the high and low N treatments, respectively. The %DM for the kale varied from 13.2% to 22.5% for the high and low N treatments, respectively. It has been reported for many years that plants with elevated N fertilizer generally possess unusually high water content (Pearsall and Ewing, 1929). This increased water content in addition to increases in the accumulation of other organic compounds (Grunwald et al., 1977; Lefsrud et al., 2007) can affect the loss of organic compounds during drying. The loss of these organic compounds during drying can result in a discrepancy in the calculated pigment concentrations based on either the FM or DM calculations and has been attributed to a dilution effect (Lefsrud et al., 2006, 2007).

Karathanos (1999) and Stegen et al. (1998) reported decreases in %DM when oven drying temperatures were greater than +65 °C. Karathanos (1999) suggested that the decrease in %DM was the result of decomposition of low-molecular-weight sugars during water evaporation. Other researchers working on plants with a high content of essential oils report the loss of VOC as drying temperature increases (Diaz-Maroto et al., 2002a, 2002b, 2004). These results suggest that changes in %DM resulting from drying temperature and method can be expected in plants with a high content of essential oils, but may also occur with plants without high contents of essential oils.

Lutein, β-carotene, and chlorophyll levels for both spinach and kale leaf tissues were affected by drying temperature in the current study. Concentrations of all four pigments decreased ~70% as the drying temperature increased from –25 to +75 °C. Tai and Chen (2000) reported that oven drying resulted in concentration decreases of 42% for lutein and 48% for β-carotene when compared with freeze drying. Daood et al. (2006) did not report any changes in lutein concentration in freeze-dried samples (Capsicum annuum L.) were dried at temperatures less than 50 °C. King et al. (2001) reported that chlorophyll concentrations of vacuum-dried spinach samples (condenser at –50 °C) remained higher than spinach that was dried at +1 °C. In the current study, the largest spinach pigment FM and DM levels were measured at the –25 °C freeze dryer treatment. The largest kale pigment FM and DM levels were split between the –25 °C freeze dryer and +25 °C vacuum dryer treatments. Comparing data from samples dried between –25 and +25 °C resulted in no statistical difference between these drying temperatures. However, a linear decrease was observed in data for drying temperature of +25, +50, and +75 °C. The FM spinach β-carotene did not follow this trend and decreased as drying temperature increased from –25 to +25 °C. To limit possible degradation of pigments within kale and spinach tissue, samples should be dried at temperatures below +25 °C. Hence, the use of vacuum or freeze drying technology is suggested.

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