Chlorophyll Fluorescence as a Nondestructive Indicator of Broccoli Quality during Storage in Modified-atmosphere Packaging

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Abstract. The objective of this study was to determine if chlorophyll fluorescence could be used as an indicator of anaerobic respiration in broccoli (Brassica oleracea L., Italica group) during modified-atmosphere packaging (MAP). Two types of packages were used, PD-941 bags, which provided optimum MAP conditions for broccoli (3 kPa O2 plus 5 kPa CO2), and PD-961EZ bags, which allowed the CO2 to accumulate (=11 kPa CO2). After 28 days in MAP at 1 °C, the broccoli from both types of bags had similar appearances and weight losses. However, broccoli held in the PD-961EZ bags had developed slight to moderate alcoholic off-odors and had higher ethanol, acetaldehyde, and ethyl acetate content, as compared with broccoli in PD-941 bags. Chlorophyll fluorescence parameters (Fv/Fm, T1/2, Fmd, and Φpot) were lower for broccoli held in the PD-961EZ bags than in PD-941 bags, and these differences increased with storage duration. These results indicate that chlorophyll fluorescence is a reliable, rapid, nondestructive indicator of broccoli quality during MAP, and that it could be used to determine if broccoli has developed off-odors without opening the bag and disrupting the package atmosphere.

The modified-atmosphere packaging (MAP) method has become popular for extending the storage life of broccoli. Reduced O2 and/or elevated CO2 slow respiration, ethylene production, weight loss, and decay, and retard yellowing of broccoli (Anelli et al., 1984; Kasmire et al., 1974; Lebermann et al., 1968; Lipton and Harris, 1974; Makhlof et al., 1989; Wang, 1979). Saltveit (1993) recommends storing broccoli heads in 1 to 2 kPa O2 plus 5 to 10 kPa CO2 to maintain quality at temperatures ranging from 0 to 5 °C.

Maintaining optimum gas concentrations in MAP throughout handling and transport is often difficult. When gas concentrations become extreme, broccoli can develop off-odors and off-flavors via anaerobic respiration, rendering it unmarketable (Barmore, 1987; Gillies et al., 1997). No simple and rapid method is available to determine this without opening the bag and disrupting the atmospheric conditions. The appearance of broccoli heads held in optimum MAP is generally similar to that of those held in unsuitable MAP, although off-odors and/or off-flavors have developed in the latter group (Gillies et al., 1997).

Chlorophyll fluorescence is an effective indicator of low O2 and/or high CO2 stress in apples (Malus domestica Borkh.) (DeEll et al., 1995, 1998). Prange et al. (1997) demonstrated that chlorophyll fluorescence measurements could be taken through glass jars, thus allowing continuous monitoring of low O2 stress in apples.

A few reports are available concerning the use of chlorophyll fluorescence in broccoli. Toivonen (1992) first demonstrated that there was a strong association between chlorophyll fluorescence changes and declines in both respiration and vitamin C content in broccoli. Tian et al. (1996) later showed that chlorophyll fluorescence could be a sensitive indicator of responses of broccoli to hot water treatment, before visual changes are noted. More recently, Toivonen and DeEll (1998) showed that chlorophyll fluorescence is independent of head maturity, suggesting that this technique would be reliable for use.

The objective of our research was to determine if chlorophyll fluorescence could be used as an indicator of broccoli quality during MAP. More specifically, we wished to determine if chlorophyll fluorescence could be used to evaluate the development of anaerobic behavior in broccoli in response to high CO2 concentrations in MAP without opening the package.
eluted, the sample was switched to a second column via an in-line two-way valve. The O<sub>2</sub> and nitrogen were then separated on a 2.44-m × 3.2-mm i.d. stainless steel column packed with 80/100 mesh Molecular Sieves 5A. The gases were quantified with a thermal conductivity detector. The flow rate of the carrier gas (He) was 30 mL·min<sup>−1</sup> and column temperature was isothermal at 55 °C. Calibration was performed using commercially analyzed gas standards. The O<sub>2</sub> co-elutes with argon and thus a correction to account for this was applied (Beveridge and Day, 1991; Supina, 1974).

Quality and defect evaluations. All individual broccoli heads were evaluated after 28 d of storage at 1 °C. Appearance was evaluated using a 1 to 5 scale, with 5 = excellent or having a freshly harvested appearance (e.g., dark green, compact head, no defects); 3 = average (e.g., lighter green, less compact head, few slight defects); and 1 = unmarketable, showing yellowing, loose florets, and major defects. Black speck is a physiological disorder that develops on broccoli stalks (DeEll, 1998), and this was evaluated using a 1 to 5 scale, with 5 = no black speck symptoms; 4 = sunken lesions, little discoloration; 3 = minor black speck development; 2 = obvious symptoms and therefore unmarketable; and 1 = severe black speck, unmarketable. Odor was evaluated also using a 1 to 5 scale, with 5 = strong alcoholic odor; 4 = obvious off-odor; 3 = slight but obvious off-odor, limit of marketability; and 1 = no off-odors. In addition, weight loss was obvious off-odor, limit of marketability; and 1 = no off-odors. In addition, weight loss was similar in both bag types (Table 1). Less black speck appeared to develop on broccoli in PD-961EZ bags than in PD-941 bags, although the difference was nonsignificant (<i>P</i> = 0.15). This was expected since black speck can be controlled by high CO<sub>2</sub> concentrations (DeEll and Toivonen, unpublished data). These results indicate that the visual attributes were similar for broccoli held in both bag types.

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No off-odors had developed in PD-941 bags after 28 d of storage at 1 °C, whereas slight to moderate alcoholic off-odors were evident in PD-961EZ bags (Table 1). Ethanol, acetaldehyde, and ethyl acetate content of the broccoli was also higher in PD-961EZ bags than in PD-941 bags (Table 1). These results show that anaerobic respiration had been induced in the broccoli held in PD-961EZ bags in response to the high CO<sub>2</sub> concentrations. However, this could only be determined after the bags were opened.

Chlorophyll fluorescence measurements both in the dark (F<sub>v</sub>/F<sub>m</sub> and T<sub>1/2</sub>) and in the light [F<sub>md</sub> and Φ<sub>EOF</sub>] for those in PD-961EZ bags (Figs. 2 and 3). These results show that anaerobic respiration had been induced in the broccoli held in PD-961EZ bags in response to the high CO<sub>2</sub> concentrations. However, this could only be determined after the bags were opened.

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film type. A preliminary test showed that chlorophyll fluorescence values of the broccoli heads were not significantly different when measured through either of the two films used (DeEll, unpublished data). Storage duration interacted with bag type, as the differences in fluorescence between the two bag types increased as storage time increased.

Changes in chlorophyll fluorescence may be associated directly with the high CO2 concentrations, as similar fluorescence changes in response to CO2 have been found in maize (Zea mays L.) (Ireland et al., 1984), spinach (Spinacia oleracea L.) (Furbank and Walker, 1986), and barley (Hordeum vulgare L.) leaves (Bukhov et al., 1997). Another possibility is that the ethanol accumulation in the tissue may affect the chlorophyll fluorescence response. Prange et al. (1997) found that Fo increased and Fv/Fm decreased in apples subjected to a stressful low O2 atmosphere (0.07 kPa). DeEll et al. (1999) postulated that this probably resulted from a disassociation of the light harvesting complex (LHC) and the reaction centers of photosystem II in the thylakoid membranes. Such a disassociation would reduce the probability of energy transfer into photosystem II, and thus less of the absorbed energy would be available for excitation energy transfer (lower Fv/Fm) and more would be given off as stray fluorescence (higher Fo). Prange et al. (1997) also found an exponential relationship between increasing ethanol production rate and decreasing Fv/Fm in apples, suggesting that ethanol accumulation in plant tissue and thylakoid membranes reduces the exciton energy transfer of photosystem II.

Ethanol does not accumulate in broccoli until 3 d of storage in PD-961EZ bags when held at 1 °C, but at this point levels accumulate precipitously (Toivonen, unpublished data). The abrupt reduction in T1/2 between 2 and 4 d (Fig. 2), as well as the commencement of reductions in the other fluorescence parameters for broccoli in the PD-961EZ bags, reflects such ethanol accumulation patterns. Ethanol accumulation in plant tissue affects membrane function in anaerobic situations (Toivonen, 1997), and changes in membrane function affect fluorescence (DeEll et al., 1999). Therefore, the fluorescence changes reported in this study are probably linked directly to membrane modifications induced in the tissues by ethanol.

Our results indicate that chlorophyll fluorescence is a good indicator of anaerobic respiration in broccoli during MAP storage. This technique showed that broccoli held in the PD-961EZ bags had developed physiological problems, even though its appearance was not affected (Table 1). Without using chlorophyll fluorescence measurements, the quality of the broccoli could only be determined after the bags were opened, and this would have made it unmarketable.

Results from this study indicate that chlorophyll fluorescence is a rapid, nondestructive technique that can be used to evaluate the quality of broccoli during MAP without breaching the package seal. They also suggest that chlorophyll fluorescence has potential for use as an indicator of quality for any chlorophyll-containing product held in MAP, and this application warrants further research.

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