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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 O_2 plus 5 kPa CO_2), and PD-961EZ bags, which allowed the CO_2 to accumulate (≈11 kPa CO_2). After 28 days in MAP at 1 °C, the broccoli from both types of bag 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, $T_{1/2}$, Fmd, and Φ_{PSII}) 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 O_2 and/or elevated CO_2 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; Makhlouf et al., 1989; Wang, 1979). Saltveit (1993) recommends storing broccoli heads in 1 to 2 kPa O_2 plus 5 to 10 kPa CO_2 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 O_2 and/or high CO_2 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 O_2 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 CO_2 concentrations in MAP without opening the package.

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Materials and Methods

Plant material and storage. Broccoli, cv. 'Greenbelt', was harvested and cooled in the United States and then imported to Québec by refrigerated truck in June 1998. Upon receipt, the broccoli was 6 d old. Freshly harvested broccoli, cv. 'Emperor', was obtained from a local producer in Québec in July 1998 for the second replication. This broccoli was 1 d old.

Broccoli heads were separated from bunches, and similar heads of uniform size and floret color were selected. Two types of modified-atmosphere packages (Cryovac, Mississauga, Ont.) were used, PD-941 bags designed specially for broccoli and PD-961EZ bags designed for shredded lettuce. Five heads of broccoli were placed in each of seven bags of each type, and all bags were stored at 1 °C. The entire experiment was repeated with the second batch of broccoli to provide two complete replications.

Chlorophyll fluorescence measurements. Chlorophyll fluorescence was measured at 1 °C, using a modulated fluorometer (OS-500; Opti-Sciences Ltd., Tyngsboro, Mass.). Measurements were taken within 1 h after the bags were sealed, and then each day for the first week. Measurements were subsequently taken after 11, 14, 18, 21, 25, and 28 d of storage.

Broccoli heads were dark-adapted for 20 min (Vidaver et al., 1991) prior to the fast actinic test (method 4 on the fluorometer; dark-adapted tissue). A single green 40-W safelight was used to provide a low level of illumination during the fluorescence analysis, to allow the operator to see well enough to handle the broccoli and to operate the instrument. Modulation intensity (660 nm) was set at 100, actinic intensity (peak 670 nm) at 190, and the detector gain at 40, with a run time of 5 s. After taking dark-adapted measurements, the laboratory lights [photosynthetically active radiation (*PAR*) = 4.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$] were turned on and the broccoli was light-adapted for 10 to 15 min. The fluorometer was then switched to the yield test (method 2 on the fluorometer; light-adapted tissue), the modulation intensity changed to 200, and the saturation intensity (35-W halogen lamp) set at 230 for a 0.8-s duration.

On each broccoli head for each test, chlorophyll fluorescence was measured through the bags near the center of the floret mass. The dark-adapted parameters Fv/Fm [Fv = Fm – Fo; Fm = maximum fluorescence (dark), Fo = minimum fluorescence (dark)] and $T_{1/2}$ [half-time (ms) for the rise in Fv], and the light-adapted parameters Fmd [Fm´ – F; Fm´ = maximum fluorescence (light), F = steady-state fluorescence] and Φ_{PSII} [yield = (Fm´ – F)/Fm´] were evaluated (DeEll et al., 1999).

O_2 and CO_2 analyses. The O_2 and CO_2 levels in the package headspace were determined by withdrawing a 2-mL gas sample with a syringe and injecting it into a gas chromatograph (Shimadzu GC-14A, TekScience, Oakville, Ont.), equipped with two columns. The first column was 1.83-m × 3.2-mm i.d. stainless steel packed with 80/100 mesh Porapak Q. When the CO_2 had been

eluted, the sample was switched to a second column via an in-line two-way valve. The O_2 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^{-1} and column temperature was isothermal at 55 °C. Calibration was performed using commercially analyzed gas standards. The O_2 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 off-odor, unmarketable; 3 = slight but obvious off-odor, limit of marketability; and 1 = no off-odors. In addition, weight loss was determined for each bag of five heads.

Acetaldehyde, ethanol, and ethyl acetate determinations. Acetaldehyde, ethanol, and ethyl acetate content was determined by grinding 5 g of broccoli bud tissue in 10 mL of 0.1 M HCl and placing 5 mL of the resultant slurry in a 25-mL vial, which was then sealed with a serum stopper. The vial was incubated in a water bath for 1 h at 37 °C, after which a 0.5-mL sample was withdrawn with a gas-tight syringe. The sample was then analyzed on a gas chromatograph (Model 3700; Varian Canada, Inc., Georgetown, Ont.), fitted with a 2-m × 2-mm i.d. glass column packed with 5% Carbowax 20M on a support of 60/80 mesh Carbopak B. The separation was carried out isothermally at 80 °C and the carrier (He) flow rate was 20 mL·min^{-1}. Standards were made by dissolving known quantities of pure compounds in 0.1 M HCl and then sealing them in 25-mL glass vials with serum stoppers and incubating as described above. The air in the headspace was analyzed and calibration factors calculated.

Statistical analyses. The experimental design was a completely randomized block design, with two complete replications of the experiment. Data were analyzed using the analysis of variance procedure of the statistical program Genstat 5 (Payne, 1993). Both replications were included in the analyses.

Results and Discussion

The atmospheres in the bags stabilized within 4 d of sealing, with O_2 concentrations thereafter averaging 3 kPa and CO_2 concentrations 5 kPa and 11 kPa for PD-941 and PD-961EZ bags, respectively (Fig. 1). The difference in CO_2 concentration was expected, since the Cryrovac PD-941 bag for broccoli is more permeable to CO_2 (19–22 L·m^{-2}·d^{-1}) than is the PD-961EZ bag (6–8 L·m^{-2}·d^{-1}) designed for lettuce (Cryovac, Duncan, S.C.). The recommended CO_2 concentration for MAP of broccoli is 5 to 10 kPa (Saltveit, 1993). Thus, PD-941 bags provided optimum MAP conditions, whereas PD-961EZ allowed the CO_2 concentration to exceed that recommended for broccoli.

After 28 d of MAP, broccoli appearance and weight loss were 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 (P = 0.15). This was expected since black speck can be controlled by high CO_2 concentrations (DeEll and Toivonen, unpublished data). These results indicate that the visual attributes were similar for broccoli held in both bag types.

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 the PD-961EZ bags in response to the high CO_2 concentrations. However, this could only be determined after the bags were opened.

Chlorophyll fluorescence measurements both in the dark (Fv/Fm and $T_{1/2}$) and in the light [Fmd and Φ_{PSII} (fluorescence yield)] were higher for broccoli held in PD-941 bags than for those in PD-961EZ bags (Figs. 2 and 3). These measurements were not influenced by

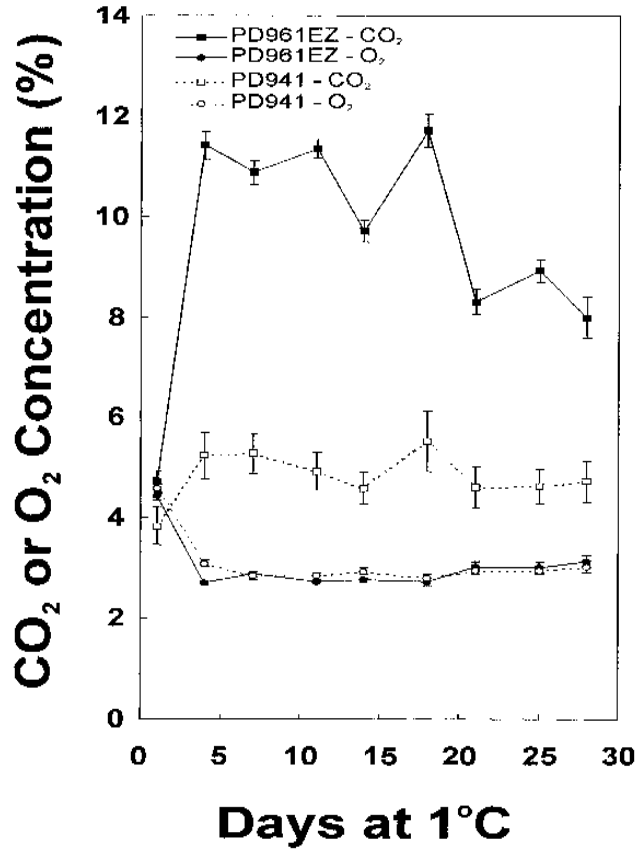

Fig. 1. Effect of bag type used for storing broccoli on CO_2 and O_2 concentrations in the bags during 28 d at 1 °C. The interaction of bag type × storage duration for CO_2 concentration was significant at $P \leq 0.001$ (SEM = 0.413, n = 14, df = 249).

Table 1. Effects of bag type on broccoli characteristics and weight loss after 28 d in MAP at 1 °C.

		Black	Wt		Concentration (μL·L^{-1}) of:		
Bag type	Appearance[z]	speck[z]	loss (%)	Odor[z]	Acetaldehyde	Ethanol	Ethyl acetate
PD-941	3.2	2.7	0.92	1.07	0.18	2.9	0.32
PD-961EZ	3.2	4.4	0.94	3.40	0.47	16.7	0.85
SEM	0.11[y]	0.30[y]	0.068[x]	0.274[x]	0.043[x]	1.54[x]	0.079[x]
Significance	NS	NS	NS	***	***	***	***

[z]1–5 scale. Appearance: 1 = poor color and major defects; 5 = green, no defects. Black speck: 1 = severe; 5 = none. Odor: 1 = no off-odors; 5 = strong alcoholic odor.
[y]n = 70, df = 139.
[x]n = 14, df = 27.
[NS, ***]Nonsignificant at $P \leq 0.05$ or significant at $P < 0.001$.

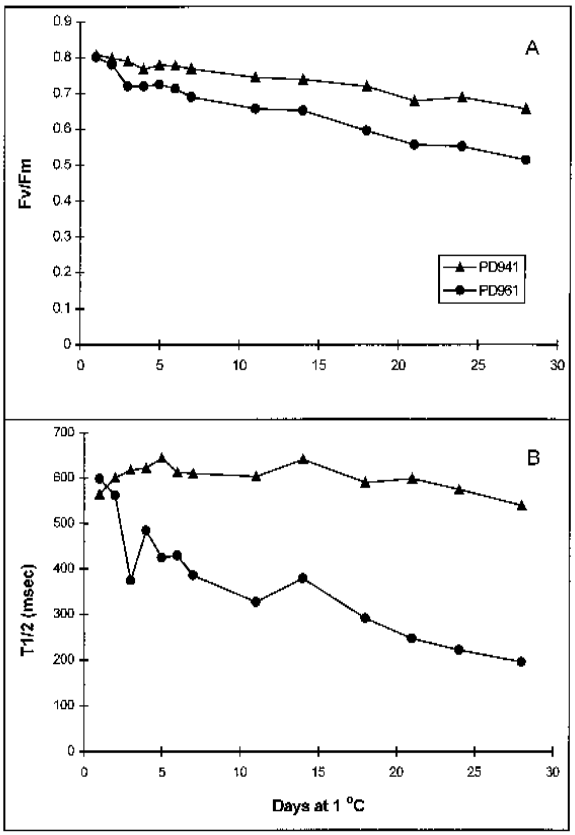

Fig. 2. Dark-adapted chlorophyll fluorescence measurements, Fv/Fm and $T_{1/2}$, of broccoli in PD-941 and PD-961EZ bags during 28 d at 1 °C. Bag type (n = 910, df = 1819) and storage duration (n = 140, df = 1819) were significant at $P \leq 0.001$ for both Fv/Fm and $T_{1/2}$, while the interaction of bag type × storage duration (n =70, df = 1819) was significant at $P \leq 0.001$ for Fv/Fm and $P \leq 0.01$ for $T_{1/2}$ (SEM = 0.015 and 46.22, respectively).

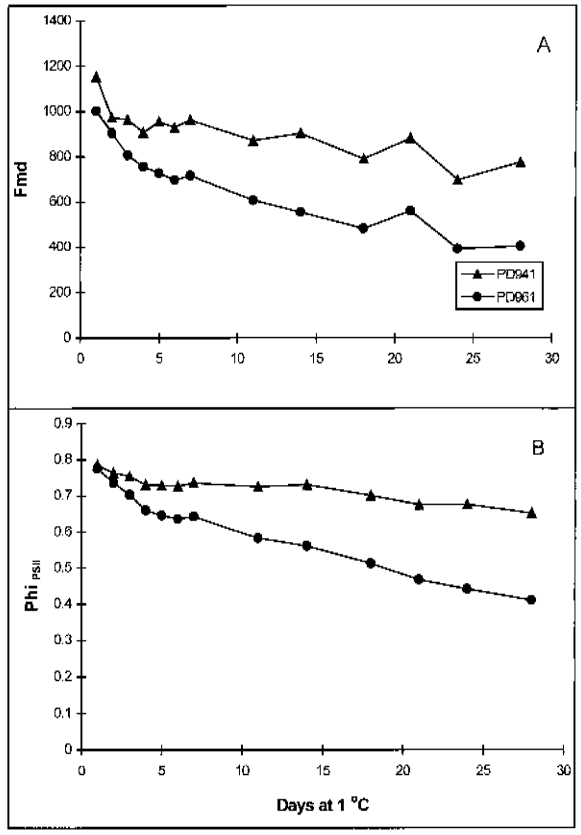

Fig. 3. Light-adapted chlorophyll fluorescence measurements, Fmd and Φ_{PSII}, of broccoli in PD-941 and PD-961EZ bags during 28 d at 1 °C. Bag type (n = 910, df =1819) was significant at $P \leq 0.001$ for Fmd and Φ_{PSII}, storage duration (n =140, df =1819) was significant at $P \leq 0.05$ for Fmd and $P \leq 0.001$ for Φ_{PSII}, and the interaction of bag type × storage duration (n = 70, df =1819) was significant at $P \leq 0.001$ for Φ_{PSII} (SEM = 0.014).

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 CO_2 concentrations, as similar fluorescence changes in response to CO_2 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 O_2 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 exciton 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 $T_{1/2}$ 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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