論文内容の要旨

生物・環境工学専攻
平成 27 年度博士課程入学

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論文題目　Influence of modified atmosphere on the induction and activity of respiratory enzymes in broccoli florets
(調整気相がブロッコリー花蕾に含まれる呼吸酵素の誘導量と活性に及ぼす影響)

1. Introduction

Modified atmosphere packaging (MAP) and control atmosphere (CA) storage were recognized as two of efficient methods to prolong the shelf life by inhibiting O_2 uptake rate of horticultural products. O_2 uptake rate is strongly correlate to the deterioration in the plant, which is driven by respiratory enzymes – alternative oxidase (AOX) and cytochrome *c* oxidase (COX). Both of these respiratory enzymes are located in electron transport chain (ETC) in the membrane of mitochondria. AOX is known having function on stress tolerance, and COX is operated to ATP synthesis. However, changing of these two respiratory enzymes under different atmospheric conditions was not clear. In this study, we will reveal the effect of different atmospheric conditions on two respiratory enzymes by measuring respiratory enzyme protein amounts and activities. Also, we will investigate the quality during storage by determining O_2 uptake rate, weight loss, bud color, and ascorbic acid content.

2. Materials and Methods

(1) Sample reparations and packaging methods: Twelve fresh heads of broccoli (*Brassica oleracea* L. var. *italica*, "Pixel" cultivar) were harvested one day before storage. 15-g samples of broccoli florets were randomly cut and sealed into micro-perforated pouches with different O_2 transmission rates to modify the in-package gas composition as Control (O_2≈21%, CO_2≈0%) and MAP (O_2≈5.9%, CO_2≈10.2%). In CA storage, broccoli florets were placed in a 7-L acrylic jar under a continuous stream of modified atmosphere composed of 5.9% O_2, 10.2% CO_2, and 83.9% N_2. All broccoli florets were stored in the dark at 15 or 25°C.

(2) In-package gas composition and O_2 uptake rate analysis: O_2 and CO_2 concentrations in the headspace of each pouch were measured using a gas analyzer (CheckMate 3, Dansensor A/S, Ringsted, Denmark). The headspace volume was measured by the water displacement method (Makino et al., 1996).

(3) Weight loss: [(Fresh broccoli weight) – (weight of broccoli after storage)]×100% / (fresh broccoli

weight)

(4) Color space value of buds: Bud colors were measured using a CM-700d colorimeter (Konica Minolta Inc., Osaka, Japan). Result of *L*a*b** was used to calculate *–a*/b** as an index for evaluating green color (Makino et al., 2016).

(5) Ascorbic acid value: Crushed broccoli sample powder (0.5 g) was mixed in a 10-ml, 2.5 % meta-phosphoric acid solution. The test strip was immersed with centrifuged supernatant and read with reflectometer (RQflex 10, Merck KGaA, Gramany) (Drogoudi and Tsipouridis, 2007).

(6) Determinations of AOX and COX protein amounts: AOX and COX protein amounts were measured by SDS-page (Noguchi et al., 2005)

(7) Determination of O_2 Isotope Discrimination: AOX to COX activity, referred as *D*, can be measured by O_2 isotope ratio, based on the different O_2 isotopes utilities between AOX and COX (Guy et al., 1989). Sample gas was trapped by gas chromatograph (Shimadzu GC-8A, Kyoto) (Noguchi et al., 2001) and analyzed by isotope ratio mass spectrometer (Isoprime 100, Isoprime Ltd, Manchester, UK) (Fourel et al., 2011).

3. Results and Discussion

In this study, in-package gas composition in Control was at the same level with atmosphere; atmospheric condition in MAP storage was changing from normoxia to hypoxia during storage; CA storage was in a stable hypoxic condition (Figure 1A, 1B)

At 25°C, broccoli florets stored in Control showing the characteristics which leading to deterioration: uninhibited O_2 uptake rates, higher weight loss, rapid loss of ascorbic acid content, and earlier bud yellowing than those stored in hypoxia (MAP and CA storage). Broccoli florets stored at 15°C in Control also showed earlier deterioration than those in hypoxia. However, low temperature inhibited O_2 uptake rate of broccoli stored in three types of atmospheric conditions, which delaying the deterioration rate in broccoli than those stored at 25°C. Hypoxic condition was proved to prevent the deterioration during storage. Moreover, low temperature may alleviate the effect of variant atmospheric conditions on broccoli florets.

AOX and COX amounts in broccoli before storage were set as 1-fold. Relative AOX protein amounts were significant increasing in Control and MAP at 25°C after 50.5 h storage (Figure 2A). However, relative COX amounts were more stable than AOX during storage. At 32.5 h storage time, broccoli stored in Control had higher relative COX amount than those in CA storage (Figure 3A). It may relate to COX shows higher transcription in normoxia than in hypoxia (Fontanesi et al., 2006). The declined relative COX amount at 50.5 h storage in Control may cause by tissue aging (Millar et al., 1998). At 15°C, relative AOX amounts were all retarded at three atmospheric conditions (Figure 2B). It may because of the inhibited O_2 uptake rate at low temperature. Relative COX amounts were gradually decreasing during storage (Figure 3B). At 150 h storage time, lowest relative COX amount was observed in Control. It may cause by senescence in the broccoli during storage.

D-value was measured by different O_2 isotope utilities between AOX and COX. At 25°C, no significant different was showed in Control during storage (Figure 4A). Lowest *D*-value was observed in CA storage at 32.5 h storage period. Previous research indicated that COX activity may be steadily maintained to continue respiratory ATP production, even under hypoxic conditions (Smialek et al., 1970). Increasing *D*-value was found in MAP at 50.5 h storage period. Inhibited ETC under hypoxia may lead the accumulation of organic acids and pyruvate, which can be used as an activator for AOX (Wagner, 1995). Moreover, another factor may be the decreasing COX protein activity due to aging during storage (Navarro and Boveris, 2004). At 15°C, *D*-value of Control and CA storage had similar tendency – increasing during 0 to 50.5 h storage, and decreasing from 50.5 to 150 h storage (Figure 4B). *D*-values of broccoli florets stored in Control and CA storage at 50.5 h storage were both higher than the fresh one. It may relate to the sudden temperature changing – broccoli florets were moved and stored at 15°C incubator. Under low temperature, decomposition of substrates was delayed. Those accumulated substrates may become the activator for AOX protein, which stimulate AOX activity during storage. No differences were observed between normoxia and hypoxia in this study. It suggested that *D*-values had similar tendency under stable in-package atmospheric condition.

To conclude, increasing relative AOX protein amount was correlate to the deterioration in broccoli florets. Relative COX protein amount was more stable than AOX. Encouraged *D*-value may be induced when the environmental conditions were changing. Both temperature and in-package gas composition can affect respiratory enzyme during storage. Besides, low temperature can alleviate the effect of in-package gas composition on respiratory enzyme.

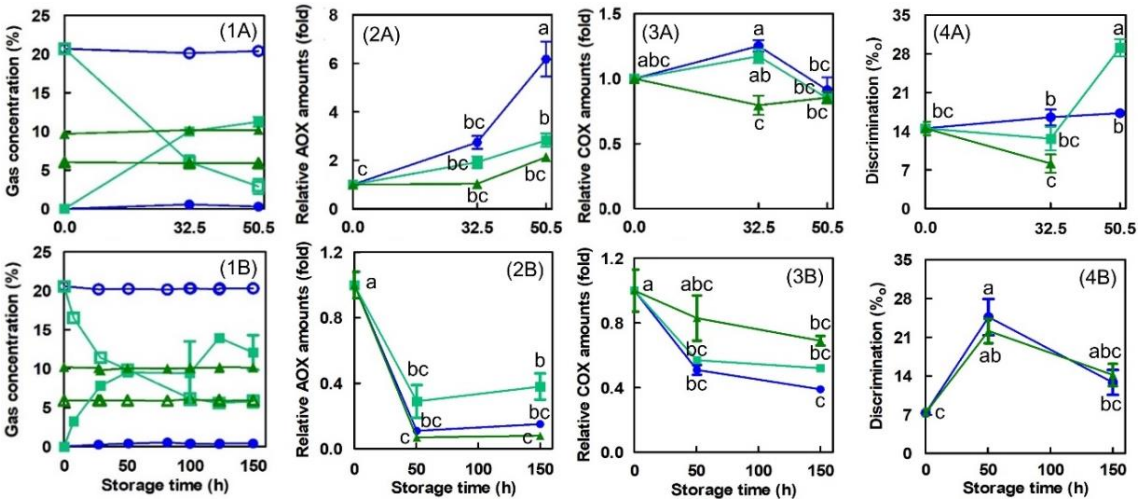

Figure. In-package O_2 concentration in normoxia (○), MAP (□), and CA storage(△); in-package CO_2 concentration in normoxia (●), MAP (■), and CA storage(▲) at 25°C (1A) and 15°C (1B). Broccoli stored in normoxia (●), MAP (■), and CA storage (▲): relative AOX protein amount in broccoli florets at 25°C (2A) and 15°C (2B); relative COX protein amount in broccoli florets at 25°C (3A) and 15°C (3B); and O_2 isotope discrimination in broccoli florets at 25°C (4A) and 15°C (4B).

4. References

(1) Drogoudi and Tsipouridis. Sci. Hortic. 2007, 115, 34-39.

(2) Fontanesi et al. Am. J. Physiol. Cell Physiol. 2006, 291(6), C1129-C1147.

(3) Fourel et al. Mass Sp. 2011, 25(19), 2691-2696.

(4) Guy et al. Planta. 1989, 177(4), 483-491.

(5) Makino et al. Trans. ASAE. 1996, 39(3), 1067-1073.

(6) Makino, et al. Jpn. J. Food Eng. 2016, 17(4), 107-113.

(7) Millar et al. Plant Physiol. 1998, 117(3), 1083-1093.

(8) Navarro and Boveris. Comp. Physiol. 2004, 287(5), R1244-R1249.

(9) Noguchi et al. Funct. Plant Biol. 2001, 28(1), 27-35.

(10) Noguchi et al. Plant Cell Environ. 2005, 28(6), 760-771.

(11) Wagner et al. FEBS Lett. 1995, 368(2), 339-342.