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Effect of modified atmosphere packaging on visual quality and glucosinolates of broccoli florets

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ABSTRACT

Broccoli (*Brassica oleracea* var. *italica*) florets were packaged in polyethylene bags with no holes (M_0), two microholes (M_1), and four macroholes (M_2), and then stored at 4 or 20 °C. The effects of modified atmosphere packaging (MAP) treatments on visual quality and glucosinolate contents were determined by comparing with non-wrapped florets. The results showed that MAP treatments, especially with M_0 and M_1, extended the shelf life and reduced the postharvest deterioration of broccoli florets stored at 4 and 20 °C. All three MAP treatments reduced the decreasing concentration rates of individual, total aliphatic and indole glucosinolates in broccoli florets when compared to those in the control, with M_0 being the most significant, followed by M_1 and M_2 during 23 days of storage at 4 °C or 5 days of storage at 20 °C. Broccoli florets with M_0 treatment maintained the visual quality and glucosinolate contents for 13 days at 4 °C and 3 days at 20 °C.

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1. Introduction

Broccoli is rich in health-promoting phytochemicals, such as glucosinolates, which are a large group of sulphur- and nitrogen-containing secondary metabolites (Poulton & Møller, 1993). These compounds have gained much attention in recent years because of the remarkable anticarcinogenic activity of their major hydrolysis products, isothiocyanates (Mithen, Dekker, Verkerk, Rabot, & Johnson, 2000; Zhang & Talalay, 1994). Glucosinolates are chemically stable until they come into contact with the enzyme myrosinase (β-thioglucoside glucohydrolase, EC 3.2.3.1), which is stored in different compartments of the cells to separate it from glucosinolates. Upon tissue damage, glucosinolates are released from plant vacuoles and are rapidly hydrolysed by myrosinase to glucose and other unstable thiohydroximate-*O*-sulphonate intermediates which, as dictated by chemical conditions, spontaneously rearrange to isothiocyanates, thiocyanates, or nitriles. Usually, production of isothiocyanates is favoured in neutral conditions (Fahey, Zalcmann, & Talalay, 2001). Epidemiological studies have shown that isothiocyanates have protective effects against cancer, particularly bladder, colon and lung cancers.

Compared to the stem, broccoli floret has a higher level of glucoraphanin and the induction ability for phase 2 enzyme (quinone reductase, QR) (Supplementary Table S1; Fahey, Zhang, & Talalay, 1997; Xu, Guo, Yuan, Yuan, & Wang, 2006) as well as a higher content of soluble proteins (Masih, Roginski, Premier, Tomkins, & Ajlouni, 2002). Therefore, broccoli floret is an ideal "ready to eat" minimally processed vegetable with increasing consumer demand. Cooling and controlled atmosphere are usually recommended for the storage of broccoli florets (Izumi, Watada, & Douglas, 1996), which are similar to the storage conditions for broccoli heads (Makhlouf, Castaigne, Arul, Willemort, & Gosseli, 1989). However, cooling facilities and controlled atmosphere are not always available in developing countries, such as China, where high temperatures are often encountered during postharvest storage and handling, transportation, and marketing phases of broccoli production (Jones, Faragher, & Winkler, 2006). Modified atmosphere packaging (MAP), as an alternative method of controlled atmosphere, is simple, economical and also effective in delaying the postharvest deterioration and maintaining the visual quality of broccoli at either low or high temperature (Jones et al., 2006; Rangkadilok et al., 2002; Serrano, Martinez-Romero, Guillen, Castillo, & Valero, 2006).

It has been reported that storage of broccoli heads in MAP and refrigeration at 4 °C could maintain glucoraphanin content and the visual quality for at least 10 days (Rangkadilok et al., 2002). However, after 7 days of storage under MAP (17% O_2 + 3% CO_2) at 1 °C, a reduction of 48% of glucoraphanin content in broccoli heads was observed (Vallejo, Tomas-Barberan, & Garcia-Viguera, 2003). Moreover, Schreiner, Peters, and Krumbein (2006) showed that modified atmosphere, at 8% O_2 and 14% CO_2, could maintain aliphatic and indole glucosinolates in mini broccoli heads for 7 days after an initial decrease in 4 days in the food tray mixed-packaging of mini broccoli and mini cauliflower. Limited information is available for the effects of different storage conditions on broccoli florets. Cooling and controlled atmosphere with a relatively high CO_2 concentration and normal O_2 concentration were effective in maintaining

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the visual quality, glucoraphanin content and QR activity of broccoli florets (Hansen, Møller, Sørensen, & Cantwell, 1995; Xu et al., 2006), but there is no report on the effect of MAP treatments on glucosinolates of broccoli florets. Our former studies showed that packaging of broccoli florets in bags of polyethylene (PE) film, with a thickness of 40 μm, was most effective for extending the shelf life and maintaining the visual quality of broccoli florets (Supplementary Fig. S1; Yuan, 2006). Similarly, Rangkadilok et al. (2002) also reported that packaging of broccoli heads with the same kind of film maintained the visual quality, as well as the glucoraphanin content, for up to 10 days at both 4 and 20 °C. As MAP maintains a high relative humidity (RH), as well as atmosphere modification around the product, it is interesting to know whether the atmosphere (or RH) is beneficial for maintaining the glucosinolate contents and the visual quality of broccoli florets under different types of MAP. In the present study, broccoli florets were placed in bags of polyethylene film (40 μm thick) with no hole or holes of different sizes and numbers, while unwrapped florets stored in open boxes were used as control. The effects of different MAP treatments on the shelf life, visual quality and contents of chemopreventive glucosinolates of broccoli florets were studied during a 23-day storage period at 4 °C or a 5-day storage period at 20 °C.

2. Materials and methods

2.1. Plant materials and modified atmosphere packaging treatments

Broccoli (*Brassica oleracea* var. *italica* cv. Youxiu) heads of prime quality were harvested in the early morning from the greenhouse of Zhejiang University (Hangzhou, China), top-iced and then transported to the postharvest laboratory of the Vegetable Institute, Zhejiang University, within 10 min. Broccoli heads free of visual defects and with a diameter ranging from 13 to 14 cm were chosen, and the inner branches (with florets having stalks of approximately 2 cm) were then cut from these heads for experimentation. The florets were surface-sterilized by washing with a solution of 50 ppm NaOCl for 1 min and dried using a household model spin drier for 2 min. The florets were then divided into two groups and stored in dark rooms at temperatures of 4 or 20 °C. Ten broccoli florets (200–250 g) from each group were placed in a polyethylene bag (40 μm thick, 20 cm × 30 cm). Three types of bags were used: (1) without holes (M_0), (2) with two microholes (750 μm in diameter, one on each side of the bag) (M_1), and (3) with four macroholes (8.8 mm in diameter, two on each side of the bag) (M_2) to enable equilibration of atmospheres inside and outside the bag while maintaining a high humidity. As a control, florets were also directly stored without any wrapping in open boxes at ambient temperature and humidity. There were four replicates per packaging treatment at each sampling time and 10 florets per replicate. Shelf life, visual quality, visual colour rating scale, and weight loss determinations were made in replicates of 10 florets taken daily for florets stored at 20 °C, and at 4 or 5 day intervals for those stored at 4 °C. The treated broccoli florets were used for glucosinolate analysis.

2.2. Determination of shelf life

Shelf life was determined according to our former reports (Wang & KyikyiWin, 2002; Xu et al., 2006). The time for quality to decline to 30% yellowing in florets was assigned as their shelf life.

2.3. Determination of visual quality

Visual quality of florets was scored on a 9 to 1 scale, where 9 refers to excellent and fresh appearance, 7 to good, 5 to fair (limit of marketability), 3 to fair (useable but not saleable), 1 to unusable. Intermediate numbers were assigned where appropriate.

2.4. Determination of visual colour rating scale

Colour of broccoli florets was visually rated using colour rating scales (1–5) as described by Rangkadilok et al. (2002), where 1 refers to dark green, 2 to trace yellow (10% yellow), 3 to slightly yellow (25% yellow), 4 to medium yellow (50% yellow), 5 to completely yellow (100%).

2.5. Determination of weight loss

Weight of individual florets was recorded on the day of harvest and after the different sampling dates. Cumulative weight losses were expressed as percentage loss of original weight on the day of harvest (Serrano et al., 2006).

2.6. Gas analysis

The concentration of CO_2 and O_2 in the PE bags were measured by applying a silicone rubber seal to the outer surface (on the top) of the bag and then using a needle sensor attached to a Gas Analyzer (Novatech Controls 1637) to penetrate the seal on the bag and record the gas concentration.

2.7. Sample preparation and freeze-drying

Freshly harvested and stored broccoli florets were frozen in liquid N_2 and kept in polyethylene bags at −70 °C prior to freeze-drying. Freeze-dried broccoli samples were stored in sealed polyethylene bags at 4 °C prior to analysis. The samples were weighed fresh and re-weighed after being freeze-dried to determine the ratio of fresh weight to dry weight.

2.8. Glucosinolate assay

Glucosinolates were extracted and analysed as previously described with minor modifications (Wang, Grubb, & Abel, 2002; Xu et al., 2006). Freeze-dried samples (25 mg) were boiled in 1 ml of water for 10 min. After recovery of the liquid, the residues were washed with water (1 ml), and the combined aqueous extract was applied to a DEAE-Sephadex A-25 (40 mg) column (pyridine acetate form). The column was washed three times with 20 mM pyridine acetate and twice with water. The glucosinolates were converted into their desulpho analogues by overnight treatment with 100 μl of 0.1% (1.4 units) aryl sulphatase, and the desulphoglucosinolates were eluted with 2 × 0.5 ml of water. HPLC analysis of desulphoglucosinolates was carried out using a Shimadzu (Tokyo, Japan) mode VP liquid chromatograph with a dual wavelength spectrophotometer. Samples (100 μl) were separated at 30 °C on a Waters Spherisorb C18 column (150 × 4.6 mm i.d.; 5 μm particle size) (Milford, MA 01757, USA) using acetonitrile and water at a flow rate of 1.0 ml/min. The procedure employed isocratic elution with 1.5% acetonitrile for the first 5 min, a linear gradient to 20% acetonitrile over the next 15 min, followed by isocratic elution with 20% acetonitrile for the final 10 min. Absorbance was detected at 226 and 280 nm. *Ortho*-nitrophenyl-β-D-galactopyranoside (Sigma) was used as an internal standard for HPLC analysis. Concentrations of individual glucosinolates were determined according to published response factors (Gross, Dalebout, Grubb, & Abel, 2000; Haughn, Davin, Giblin, & Underhill, 1991). The integrated area of the desulpho-4-methylsulphinylbutyl glucosinolate peak was converted to a molar amount under the assumption that this compound had a molar extinction coefficient at 226 nm equal to that of sinigrin (Wang et al., 2002; Xu et al., 2006).

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