The Effect of Post-Harvest and Packaging Treatments on Glucoraphanin Concentration in Broccoli (Brassica oleracea var. italica)

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The effects of post-harvest and packaging treatments on glucoraphanin (4-methylsulfinylbutyl glucosinolate), the glucosinolate precursor of anticancer isothiocyanate sulforaphane [4-methylsulfinylbutyl isothiocyanate], were examined in broccoli (Brassica oleracea var. italica) during storage times. The results showed that at 20 °C, 55% loss of glucoraphanin concentration occurred in broccoli stored in open boxes during the first 3 days of the treatment and 56% loss was found in broccoli stored in plastic bags by day 7. Under both air and controlled atmosphere (CA) storage, glucoraphanin concentration appeared to fluctuate slightly during 25 days of storage and the concentrations under CA was significantly higher than those stored under air treatment. In modified atmosphere packaging (MAP) treatments, glucoraphanin concentration in air control packaging decreased significantly whereas there were no significant changes in glucoraphanin concentration in MAP with no holes at 4 °C and two microholes at 20 °C for up to 10 days. Decreases in glucoraphanin concentration occurred when the broccoli heads deteriorated. In the present study, the best method for preserving glucoraphanin concentration in broccoli heads after harvest was storage of broccoli in MAP and refrigeration at 4 °C. This condition maintained the glucoraphanin concentration for at least 10 days and also maintained the visual quality of the broccoli heads.

KEYWORDS: Glucoraphanin; broccoli; post-harvest; controlled atmosphere (CA); modified atmosphere packaging (MAP)

INTRODUCTION

The consumption of cruciferous vegetables, such as broccoli, cabbage, cauliflower, Brussels sprouts, and Chinese broccoli, may be important for cancer prevention since they are rich in sulfur-containing glycosides called glucosinolates (1, 2). When plant cell tissues are damaged, as occurs during cutting or chewing, the enzyme myrosinase (thioglucoside glucohydrolase, EC 3:2:3:1) initiates rapid hydrolysis of glucosinolates to yield glucose, sulfate, and either isothiocyanates, thiocyanates, nitriles, or oxazolindine-2-thiones (3). These hydrolysis products contribute to the characteristic flavors and odors of Brassica vegetables (1).

Broccoli (B. oleracea var. italica) contained high levels of glucoraphanin, a glucosinolate precursor of the isothiocynate sulforaphane which has been shown to effectively reduce developing mammary tumors by inducing phase II enzymes in the body (4, 5, 6, 7).

There is limited information available relating to glucosinolate metabolism in Brassica vegetables after harvest. Changes in the breakdown products of glucosinolates such as volatile isothiocyanates, thiocyanate ion, and goitrin have been measured in cabbage during refrigerated storage and controlled atmosphere (CA) storage (8, 9). During refrigerated air storage (RS), the concentration of volatile isothiocyanates, thiocyanate ion, and goitrin declined during storage and was associated with a decrease in the quality of the cabbage. Similar results were observed under CA storage except that the cabbage had more volatile isothiocyanates and goitrin during the early storage period and the content declined at a higher rate toward the end of storage (214 days) (9).
Hansen et al. (10) studied the glucosinolate content of broccoli stored for 7 days at 10 °C under air and CA conditions (0.5% O₂, 0.5% O₂ + 20% CO₂, and 20% CO₂) and concluded that CA treatment and storage time had no significant effect on the relative content of methylsulfinylalkyl glucosinolates (glucoiberin and glucoraphanin) and 3-indolylmethyl glucosinolates (glucobrassicin, neoglucobrassicin, and 4-methoxyglucobrassicin). In contrast, sulforaphane, a breakdown product from glucoraphanin, decreased after storage of broccoli was held in perforated polyethylene vegetable bags at 4 °C for 21 days after harvest (11). However, Rodrigues and Rosa (12) suggested that refrigeration at 4 °C and freezing were the best preservation processes for maintaining a high content of glucosinolates in broccoli.

The modification of CO₂ and O₂ levels to produce the desired atmosphere under CA storage and modified atmosphere packaging (MAP) has been shown to influence the quality of broccoli (8, 13, 14, 15, 16). Lipton and Harris (17) reported that for broccoli shipped over long distances or stored prior to processing, lowering O₂ to 0.5–1% or increasing CO₂ to 10% helped to retard deterioration when the temperature could be maintained near 0 °C. However, Makhlof et al. (13) reported that 6% CO₂ and 2.5% O₂ was the optimum atmosphere for long-term storage (greater than 3 weeks), which maintained broccoli quality while avoiding physiological injury. Moreover, Barth and Zhuang (18) reported that MAP results in the best retention of antioxidants and vitamins in lightly processed broccoli florets during storage. Storage of broccoli in different types of poly(vinyl chloride) film wraps was also found to extend shelf life and maintain the quality of broccoli by delaying yellowing and reducing the loss of chlorophyll (15, 19). Thus, the storage conditions and packaging, which can maintain the quality of broccoli with respect to odor, flavor, and nutritional value, may also affect glucosinolate content.

It is very important to determine the best method to maintain this beneficial glucosinolate (glucoraphanin) in broccoli during transportation to the consumers. Thus, the objective of this work was to investigate the effects of storage conditions on glucoraphanin concentration in broccoli after harvest. The effects of storage temperature, storage under air or controlled atmosphere conditions, as well as storage in modified atmosphere packaging (MAP) were examined.

MATERIALS AND METHODS

Broccoli. Broccoli heads (genotype Marathon) were freshly harvested from the field (Favero Gardens, Cranbourne, Victoria) in the morning of the experiment, immediately placed on ice and transported in expanded polystyrene boxes to the post-harvest laboratory at the Institute for Horticultural Development (Knoxfield, Victoria). Individual head sizes ranged from 10 to 20 cm in diameter. All broccoli heads were stored at 4 °C until they were allocated to the experimental treatments.

Storage Conditions. Experiment 1: Effect of Temperature and Packaging.

Broccoli heads were divided into two groups:

- **Treatment A**: Broccoli heads were placed in perforated low-density polyethylene (LDPE) bags (40 µm thick, 27 cm × 38 cm) with two macroholes, 8.8-mm diameter, one on each side of the bag to minimize moisture loss and create a high humidity storage environment.
- **Treatment B**: Broccoli heads were stored in open boxes (55 cm × 28 cm) at ambient humidity.

Broccoli heads were then separated into two groups and stored in dark storage rooms at two different temperature (4 °C and 20 °C). There were four replicates per packaging treatment at each sampling time and four heads per replicate. The sampling times were 3 and 7 days after harvest.

Experiment 2: Effect of Air and Controlled Atmosphere Storage at 4 °C.

Broccoli heads were placed in 40-µm-thick perforated LDPE bags (27 cm × 38 cm) with the same size of macroholes as experiment 1 to minimize moisture loss. The broccoli was stored in controlled atmosphere units (150 liter chambers). There were four broccoli heads in each of four bags for each unit. There were four units in each storage atmosphere. Each unit contained an inlet and outlet tubing to permit continuous flow of the gas (controlled composition and flow rate) through the units. There were two storage atmospheres, a control (21% O₂ and 0.03% CO₂) and an atmosphere containing 1.5% O₂ and 6% CO₂ (CA) with the balance as nitrogen (N₂). The relative humidity in the chambers was between 80 and 95%.

The pressure of CO₂ through the chamber was 120 Kpi and for N₂ and air was 240 Kpi. The CA was identified from previous work, which indicated that 1–2% O₂ and 5–10% CO₂ was optimal in retarding the deterioration of broccoli and cabbage (9, 13, 17, 20, 21). In addition, Thompson (22) suggested that controlled atmosphere might not be effective at room temperature; therefore, both storage conditions (control and CA) were operated in a cool dark room at 4 °C. Broccoli samples were taken from each unit at 3, 10, 15, and 25 days after harvest in each storage atmosphere and assessed for glucoraphanin concentration.

Experiment 3: Effect of Modified Atmosphere Packaging (MAP).

Broccoli heads were divided into four groups:

- **Treatment A**: Broccoli heads were placed in LDPE bags (27 cm × 38 cm) without holes (MAP) and stored in a cool dark room at 4 °C.
- **Treatment B**: Broccoli heads were placed in LDPE bags with four macroholes (8.8-mm diameter, two on each side of the bag) as a standard air control treatment to enable equilibration of atmospheres within and outside the packaging with maintenance of high humidity and then stored in a dark room at 4 °C.
- **Treatment C**: Broccoli heads were placed in LDPE bags with two microholes of approximately 750 µm in diameter on each side of the bag (MAP) and stored in a cool dark room at 20 °C.
- **Treatment D**: Broccoli heads were treated with the same method as in treatment B but stored in a dark room at 20 °C (a standard air control treatment).

There were four replicates per treatment at each sampling time and two heads per replicate in this experiment. The sampling times were 3, 7, and 10 days after harvest.

The atmospheres within the bags were measured for CO₂ and O₂ 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 concentrations.

Extraction of Glucoraphanin. A small floret (3-cm diameter with 2-cm length of stem tissues) was cut from the center of each head and at the same position in each replicate (total four florets from four broccoli heads in experiments 1 and 2). In experiment 3, there were two heads per replicate and two florets were cut from the center of each head (total four florets from two broccoli heads). The total weight for all four florets was between 25 and 35 g. Four florets were immediately plunged into 65 mL boiling water and extracted for glucoraphanin following the previous method developed by Rangkadioklak et al. (23). The plant residues were re-extracted twice with 15 mL of boiling water and the volume adjusted to 100 mL with distilled water. Then, 1.0 mL of each extract was transferred into 10.0-mL volumetric flasks and the volume adjusted with distilled water. All samples were filtered through a 0.2-µm aqueous filter membrane. Extracts were stored at −15 °C until analyzed by HPLC.

Reverse-Phase Paired-Ion Chromatography of Glucoraphanin. Plant extracts were analyzed, using glucoraphanin as external standard, by HPLC method previously described (23) on µ-Bondapak C18 column (Waters, Melford, MA) at 230-nm detection. Five millimoles of tetramethylammonium bromide (97%) with methanol (3%) was used as a mobile phase at a flow rate of 1.4 mL/min. Standard glucoraphanin was extracted and purified from broccoli following the method from Prestera et al. (24). The percentage purity of standard glucoraphanin was quantified using reference glucoraphanin standard and also identified by LC/EC-MS method at the Institute of Food Research, U.K.
Concentration of glucoraphanin was expressed in μmol g⁻¹ dry weight. To determine fresh weight/dry weight ratios, another set of florets (4 florets per replication) from the same broccoli heads as the heads used for extraction were weighed, dried at 80 °C for 48 h, and re-weighed.

**Visual Color Rating Scale.** Color of broccoli heads was visually rated using color rating scales 1–5 described by Tomkins et al. (16). Visual color rating scales were 1 = dark green, 2 = trace yellow (10% yellow), 3 = slightly yellow (25% yellow), 4 = medium yellow (50% yellow), 5 = completely yellow (100%).

**Statistical Analysis.** Analysis of variance (ANOVA) was performed using general linear model test on glucoraphanin concentration data between treatments and days after harvest. Mean separation was determined by least significant differences (Fisher’s LSD) at P = 0.05.

**RESULTS AND DISCUSSION**

**Glucoraphanin Concentration of Broccoli Stored under Different Temperature and Packaging Conditions.** At 20 °C, glucoraphanin concentration in broccoli stored in plastic bags had decreased significantly by day 7 (56% loss) while glucoraphanin concentration in broccoli stored in open boxes declined significantly during the first 3 days of the treatment (55% loss) (Figure 1). In contrast, there were no significant differences in glucoraphanin concentration in broccoli stored in plastic bags and open boxes during 7 days of storage. Broccoli stored at 4 °C in both plastic bags and open boxes retained its green color and still appeared fresh after 7 days of storage. At 20 °C, broccoli stored in plastic bags showed a trace of yellow in the heads but looked reasonably fresh on day 3. By day 7, broccoli had deteriorated with the development of mold on the heads and there was also the production of an unpleasant odor. At 20 °C, broccoli stored in open boxes was slightly yellow by day 3 and completely yellow by day 7.

A large decrease in glucoraphanin concentration was observed in broccoli stored in both plastic bags and open boxes at 20 °C, which coincided with a rapid loss in the visual quality of the broccoli. At the high temperature and in open boxes, plant cells are most likely to rapidly become damaged resulting in changes to the physical properties (such as color and odor) of the broccoli heads. Loss of cellular integrity, that is, bursting vacuoles, most likely allowed the enzyme myrosinase to come in contact with glucosinolates including glucoraphanin. Myrosinase hydrolysis of the glucoraphanin to other compounds probably explained the rapid decrease in glucoraphanin concentration that occurred by day 3. In the plastic bag treatments, the glucoraphanin concentration remained stable until day 3 as the broccoli heads maintained their green color and freshness. However, after day 3, the oxygen in the bags reached zero, which resulted in a switch to anaerobic respiration and resulted in cell death and deterioration of broccoli heads. The cell deterioration most likely activated myrosinase activity, and thus the glucoraphanin concentration declined rapidly by day 7. These results agreed with the finding of Rodrigues and Rosa (12) who reported that glucoraphanin levels declined 82% when fresh broccoli was left at room temperature (20 °C) for 5 days. However, they found that glucoraphanin levels decreased by 31% in the principal inflorescence when stored at 4 °C but no significant decrease in the concentration in the secondary inflorescence of broccoli kept in refrigeration at 4 °C for 5 days. The results obtained with the secondary inflorescence agreed with the results reported in this study where there were no significant changes in glucoraphanin concentration in broccoli stored at 4 °C in air (open boxes).

The present results indicated that storage of broccoli without packaging at room temperature (20 °C) should be avoided to preserve glucoraphanin concentration. The best method to maintain the glucoraphanin and the visual quality of broccoli is storage in plastic bags at low temperature (4 °C).

**Glucoraphanin Concentration of Broccoli Stored under Air and Controlled Atmosphere Storage Conditions.** Glucoraphanin concentration in broccoli heads appeared to fluctuate slightly during 25 days of storage under both air and CA (1.5% O₂ + 6% CO₂) storage at 4 °C (Figure 2). However, there was no significant change in glucoraphanin concentration in broccoli stored in air or CA during the storage time although the concentration of glucoraphanin was significantly higher in the broccoli under CA. In the CA storage treatment, the broccoli heads maintained their green color and freshness up to 25 days while yellowing (trace yellow) was observed in broccoli heads stored under air by day 25.

These results agreed with those of Hansen et al. (10) who reported that the total glucosinolate content of broccoli stored in air increased from day 0 to day 7 followed by a slight decline by day 9 as yellowing of the heads was observed. Under CA storage, they reported that total glucosinolate content increased 21% under 0.5% O₂ + 20% CO₂ and decreased 15% in broccoli stored under 20% CO₂ without O₂ while there was no change for broccoli stored under 0.5% O₂ without CO₂. From their few observations, no significant differences were found between the relative content of methylsulfinylalkyl glucosinolates (glucobrin and glucoraphanin) in both CA and air treatments and storage time.
Berard and Chong (9) reported changes in the content of glucosinolate breakdown products (volatile isothiocyanates, thiocyanate ion, and goitrin) in cabbage stored at 1 °C in air and CA (2.5% O2 and 5% CO2). The content either fluctuated or increased slowly during the first 122 days and then increased more rapidly until the end of storage (214 days). The increase coincided with increasing senescence of the cabbage heads. However, there was no significant change in volatile isothiocyanates during the storage time. Glucoraphanin concentration in broccoli heads, stored under the CA condition used in this study, was higher than that stored under air. In contrast, the results reported by Hansen et al. (10) showed that the highest glucoraphanin concentration was found in broccoli stored in air followed by that stored in 0.5% O2 + 20% CO2, 0.5% O2, and 20% CO2.

The results of this study showed that storage conditions affect the level of glucoraphanin in broccoli and therefore, its nutritional value. The levels of glucoraphanin, and hence its breakdown product sulforaphane, will affect the subsequent anticarcinogenic activity in the broccoli florets (6, 25). The CA (1.5% O2 + 6% CO2) condition maintained glucoraphanin concentration and visual quality of broccoli up to 25 days after harvest. Makhlouf et al. (13, 14) indicated 6% CO2 was considered tolerable for broccoli while 10% CO2 was observed to be critical for the induction of physiological disorders (development of “off-odors” and damage to the product). Low O2 delayed yellowing but caused stress and lead to the development of undesirable odors at 0.25% O2 or less (17). The results from Bastrash et al. (26) suggested that atmosphere consisting of 6% CO2 with 1–2% O2 resulted in extended storage of broccoli florets from 5 to 7 weeks. However, this extension in storage also depended on the temperature and the length of the storage time. Nevertheless, further studies on CA storage conditions, particularly the modification of % O2 and % CO2 levels should also be investigated to identify the optimum conditions for the preservation of glucoraphanin concentration in broccoli after harvest.

Effect of MAP on Glucoraphanin Concentration. Glucoraphanin concentration decreased by 30% in broccoli stored in air control packaging at 4 °C during the first 3 days and then slightly increased at the end of storage time (Figure 3). A large decrease in glucoraphanin concentration occurred in broccoli stored in air control packaging at 20 °C with 48% loss at day 3 and 64% loss at day 10. In contrast, there were no significant changes in glucoraphanin concentration in broccoli heads stored for up to 10 days in MAP with no holes at 4 °C and two microholes at 20 °C. This is the first report demonstrating the stability of glucoraphanin in broccoli stored in MAP. There is one report on the fate of sulforaphane, the breakdown product of glucoraphanin, in fresh broccoli stored in perforated polyethylene Ziploc vegetable bags (~2.5 microholes/cm2) at 4 °C for 21 days by Howard et al. (11). Their results showed that the sulforaphane content of fresh broccoli decreased by 33% at 7 days after harvest in the 1994 crop and 45% in the 1995 crop at 9 days after harvest. These two studies detected different compounds (glucoraphanin and sulforaphane) and also showed that different types of perforated polyethylene film had different effects in preserving broccoli. Moreover, the differences in genotypes of broccoli or environment where broccoli was grown in these two studies and the factors that regulated hydrolysis products of glucosinolates such as myrosinase activity and hydrolysis conditions may also explain the differences of these two results.

After packaging, most of the green color and freshness of the heads was maintained in both packaging treatments (MAP and air control) at 4 °C for up to 10 days (Table 1). In the MAP treatment at 20 °C, most of the green color was maintained for 7 days while in the control packaging, 30–40% of the florets were yellow by day 3 (one or two > 40%), 60–80% yellow by day 7 (with one broccoli completely yellow), and almost all completely yellow by day 10. These results agreed with Tomkins et al. (16), Barth and Zhuang (18), and Rij and Ross (19), who...
reported broccoli heads remained green after packaging with different types of polymeric films. However, at 20 °C in the present study, condensation formed inside the bags at day 7 and the free water in MAP and control packaging encouraged the development of soft rots and unpleasant odors.

Changes in O2 and CO2 in the MAP at both temperatures were rapid (Figure 4). At 20 °C, the O2 level in the MAP with microholes (MAP) dropped rapidly to 0.4% by day 7 and 0% by day 10. At the same time, the CO2 level rose steadily to 21% after 10 days. At 4 °C, the changes in O2 and CO2 inside the MAP (with no holes) was also rapid but not to the level observed at 20 °C. The CO2 rose steadily to 15% after 10 days while O2 dropped to 3% by day 7 and 0.2% by day 10. The O2 and CO2 levels in the air control treatment at both 4 °C and 20 °C were at the same level as the atmosphere in the storage rooms (21% O2 and 0.03% CO2).

Atmosphere modification using the microhole packaging at 20 °C (increase in % CO2 and decrease in % O2) indicated that the broccoli heads eventually switched to anaerobic respiration, which led to the production of off-odors and tissue death (as observed at the end of storage time). The present results also indicated that decreases in glucoraphanin concentration occurred when the broccoli deteriorated. This is most likely due to loss of cellular integrity that allows myrosinase to come in contact with glucoraphanin. Thompson (22) suggested that microhole packaging maintained a high humidity around the produce but it may be less effective in delaying fruit ripening. However, atmosphere modification (% O2 and % CO2) was also rapid in the MAP with no holes at 4 °C but slower than MAP with the microholes at 20 °C. Therefore, this packaging created an adequate atmosphere that maintained the quality of broccoli and also the glucoraphanin concentration for at least 10 days. However, further work is required to study the effect of different types of MAP (such as modification of the types of perforated films: thickness, size of the punched holes, or number of the holes in the films) on glucoraphanin or other glucosinolates in broccoli.

The method, which can preserve the quality and freshness of broccoli, should also maintain the concentration of glucoraphanin in broccoli after harvest. The best method for preserving glucoraphanin concentration in broccoli heads after harvest was storage of broccoli in MAP and refrigeration at 4 °C. This condition maintained the glucoraphanin concentration for at least 10 days and also maintained the visual quality of the broccoli heads. Broccoli heads could only be stored without packaging at 4 °C for a week without loss of the glucoraphanin concentration. Controlled atmosphere storage, which is the condition in which broccoli is stored before transportation to the consumers, was the best storage condition to maintain glucoraphanin concentration up to 25 days after harvest.

In this study, only the effects of post-harvest and packaging treatments on glucoraphanin concentration were measured since glucoraphanin is the precursor of sulforaphane, the beneficial anticancer compound. The decrease in glucoraphanin concentrations during storage was most likely related to myrosinase activity when broccoli deteriorated. Further studies should measure the activity of myrosinase, which has the important role in changes in glucoraphanin concentrations, and also the concentrations of hydrolysis products of glucoraphanin especially sulforaphane. Consumers will have more health benefits from eating fresh, unspoiled broccoli that has been properly stored, or as in sprouts, where the myrosinase enzyme has been inactivated through the process of cooking or preservation. Glucosinolates pass through the gastrointestinal tract where myrosinase enzyme from intestinal microflora hydrolyzes the intact glucosinolates to release the beneficial hydrolysis products (27). In addition, Michaelson et al. (28) also indicated that there was in vivo absorption of intact glucosinolates in upper gastrointestinal tract and degradation and transformation of the remaining glucosinolates in distal gastrointestinal tracts. Degradation of glucoraphanin during storage before consumption may lead to less production of sulforaphane in the body for cancer prevention.

**LITERATURE CITED**


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