Changes in the Radical-scavenging Activity of Shredded Vegetables During Storage

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In this study, we examined changes in radical-scavenging activity, as well as in ascorbic acid content, total phenol content and color, in 6 kinds of shredded vegetables during storage. Radical-scavenging activity, ascorbic acid content and total phenol content of red cabbage were initially higher than those of the other vegetables. In shredded vegetables stored at 10°C for 7 days under air or under nitrogen gas, radical-scavenging activity remained unchanged, but ascorbic acid in green pepper decreased in the first day and remained unchanged thereafter. Total phenolics also remained relatively constant in shredded vegetables during storage.

Keywords: Radical-scavenging activity, Ascorbic acid, Polyphenol, Storage, Shredded vegetables

Introduction

In recent years, the role of natural dietary antioxidants in disease prevention has been the focus of numerous investigations. Antioxidants inhibit free radical reactions and may therefore protect cells against oxidative damages. Several studies indicate that antioxidants found at high levels in fruits and vegetables may aid in the prevention of cancer and cardiovascular disease (Phillips et al., 1993; Slattery et al., 2000; Pryor, 2000; Xing et al., 2001). These antioxidants include carotenoids, ascorbic acid, tocopherols, and phenolics. Among phenolics, flavonoids are potent in vitro antioxidants. Flavonoids include different groups of flavones, isoflavones, flavonols, flavanones, catechins, and anthocyanins (Shahidi et al., 2006; Wang and Mazza, 2002).

Functions of diverse phenolic antioxidants in the diet have been discussed by many authors (Viña and Chaves, 2006; Cao et al., 1996; Vinson et al., 1994). In view of these findings, an increase in the consumption of fruits and vegetables has been suggested to explicitly corroborate the beneficial effects of antioxidant phytochemicals (Pryor, 2002).

In recent years, there has also been an increasing demand for fresh-cut vegetables and fruits, mainly because of their convenience as ready-to-eat products, as well as the health benefits associated with their consumption (Doll, 1990; Rimm et al., 1996). Fresh-cut vegetable has been one of the commodities with higher request rates by salad bars and fast-food services. Unfortunately, the damage inflicted to plant tissues by shredding or slicing accelerates reactions that lead to quality defects such as browning of cut edges, discoloration and reduced turgidity (King et al., 1991). Other unit operations applied during processing, including washing, drying, packaging and storage, also affect the extent and rate of physiological reactions and microbiological processes that influence the development of quality defects (Bolin et al., 1977). A more thorough understanding of these effects could lead to improved processing schemes for quality retention.

The aim of the present study is to evaluate of the effect of fresh cutting and cold storage under air or nitrogen gas on the radical-scavenging activity in 6 vegetables (red cabbage, white cabbage, lettuce, green pepper, Japanese radish and carrot). In addition, the changes in the total phenol content, ascorbic acid content and color of these vegetables during storage were also determined. The paper will provide important information towards the selection and characterization of healthier fresh-cut produce.

Materials and Methods

Reagents 1,1-Diphenyl-2-picrylhydrazyl (DPPH), L-ascorbic acid, tris(hydroxymethyl)aminomethane (Tris),
2,4-dinitrophenylhydrazine, 2,6-dichloroindophenol, Folin-Ciocalteu reagent, sodium carbonate, triethylamine and gallic acid were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Metaphosphoric acid, stannous chloride, ethyl acetate, sodium chloride, ethanol were purchased from Wako Pure Chemical Industries, Osaka, Japan. Acetonitrile, n-hexane, 2-propanol, dichloromethane and methanol (HPLC grade) were also from Wako Pure Chemical Industries. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA).

The water used in this work was purified with a Milli-Q-Labo device (Millipore Japan, Tokyo, Japan).

Preparation of Samples  Red cabbage (Brassica oleracea var. capitata f. rubra), white cabbage (Brassica oleracea L. var. capitata L.), lettuce (Lactuca sativa L.), green pepper (Capsicum annuum L.), Japanese radish (Raphanus sativus L.) and carrot (Daucus carota L.) were purchased from a local market in Nara, Japan, on the day of the experiment. The heads of red and white cabbages were removed and the internal leaves were used for sampling. Japanese radish and carrot were cleaned with tap water, topped and tailed using a ceramic knife, and trimmed and peeled using a ceramic hand peeler. Green peppers were cleaned with tap water, followed by the removal of stem and seeds. Vegetables, except lettuce, were then shredded by a food processor with a titanium blade (MK-K78, Panasonic, Osaka, Japan) to 1-mm width; lettuce was cut into edible portions of 2 × 2 cm.

Packaging  Shredded vegetables were packaged in 100-g lots in 15-μm oriented nylon/60-μm polyethylene bags (0.03 ×300×350 mm) (Asahi Kasei Pax, Tokyo, Japan). The bags were then flushed with nitrogen gas or air, and sealed by a plastic film sealer (FR200A, Mikuni, Tokyo, Japan). Bags with vegetables were stored in a temperature-controlled refrigerator at 10°C for 7 days. Stored vegetables were analyzed on day-0, -1, -3, and -7. For each analysis, the vegetable (35 g) was rapidly frozen in liquid nitrogen and then freeze-dried for 24 h using a freeze-dryer (VD-400F, Taitec, Saitama, Japan). The freeze-dried vegetable was ground into fine powder using a food grinder (IFM-300DG, Iwatani, Tokyo, Japan). The freeze-dried powder was dried by flushing with nitrogen gas. The obtained residue was dissolved in 2 ml of 90% methanol and filtered through a 0.45-μm filter (Cosmonice Filter W, 13 mm, Nacalai Tesque Inc., Kyoto, Japan). The resulting solution was used for the measurement of the radical-scavenging activity, total phenol content, and ascorbic acid content after appropriate dilution.

Color  The color values (L*, a*, and b*) of vegetables were obtained with each vegetable powder using a color meter (NE-2000, Nippon Denshoku Industries, Tokyo, Japan). Four replicated results were evaluated for each vegetable. A decrease in L* value indicates a loss of brightness. The increase of a* value indicates browning, whereas the increase of b* value indicates yellowing or discoloration (Gonzalez-Aguilar et al., 2000).

Determination of DPPH Radical-Scavenging Activity  DPPH radical-scavenging activity was determined by the DPPH-HPLC method (Yamaguchi et al., 1998). An aliquot of the sample solution (200 µL) was mixed with 800 µL of 100 mM Tris-HCl buffer (pH 7.4) and added to 1 mL of 500 µM DPPH in ethanol. The mixture was vigorously shaken and left to stand for 20 min at room temperature in the dark. The reaction mixture was then subjected to an HPLC analysis.

The HPLC analysis was carried out using a Shimadzu LC-10AD pump (Shimadzu, Kyoto, Japan), a Rheodyne injector fitted with a 20-µL loop and a Shimadzu SPD-10AV UV-VIS detector set at 517 nm at ambient temperature. The column used was a TSKgel Octyl-80Ts (4.6×150 mm, Tosoh, Tokyo, Japan). The mobile phase consisted of methanol-water (70:30, v/v), and the flow rate was 1 mL/min. Trolox was used as the control standard and 200 µL of Trolox solution (final concentration of 50 µM) in ethanol was similarly assayed for each run.

The DPPH radical-scavenging activity was evaluated from the difference in decrease of the peak area of the DPPH radical detected at 517 nm. The activity was expressed as µmol of Trolox equivalent in 100 g of each fresh vegetable.

Measurement of Ascorbic Acid Content in Vegetables  Since dehydroascorbic acid has no radical-scavenging activity toward DPPH and hydroxyl radicals (Yamaguchi et al., 2001), we measured only ascorbic acid content. Ascorbic acid content was determined by HPLC according to the method of Kishida et al. (1992) as follows: briefly, each sample solution (100 µL) was mixed with or without 50 µL of 0.2% 2,6-dichloroindophenol; 50 µL of 1% stannous chloride in 5% metaphosphoric acid (50 µL) and 120 µL of 2% 2,4-dinitrophenylhydrazine in 4.5 M sulfuric acid were then added to the solution. The mixture was incubated in a water bath for 3 h at 37°C, and then ethyl acetate (1 mL) and water (1 mL) were added. After shaking and centrifuging (1,500×g, 4°C) for 5 min, 300 µL of the ethyl acetate layer was taken out and dried by flushing nitrogen gas. The residue was dissolved in 200 µL of acetonitrile and applied to HPLC analysis.
The HPLC analysis was carried out on a Cosmosil 5C18-AR-II column (4.6×250 mm, Nacalai Tesque) using a detector set at 505 nm. The mobile phase was acetonitrile-water (50:50, v/v) adjusted to pH 3.5 with 0.1% triethylamine and phosphoric acid. The flow rate was 1 mL/min.

The ascorbic acid content was calculated by subtracting the value of sample mixed without 2,6-dichloroindophenol from that with 2,6-dichloroindophenol. The data were expressed as mg per 100 g of each fresh vegetable.

**Determination of Total Phenol Content** Total phenol content was measured according to the method of Singleton and Rossi (1965). The sample solution (200 mL) was added to 800 mL of 7.5% sodium carbonate, and 1 mL of Folin-Ciocalteu reagent was added to the mixture, which was then left to stand for 30 min. The absorbance was measured at 765 nm using a Shimadzu UV-2100PC UV-VIS spectrophotometer. The total phenol content was expressed as μmol of gallic acid equivalent per 100 g of each fresh vegetable.

**Statistical Analysis** All data represent the means of three or four replicates. Student’s t-test was accomplished using Microsoft Excel 2000. Differences at p<0.05 were considered to be significantly different.

**Results and Discussion**

**Radical-Scavenging Activity, Ascorbic Acid Contents and Total Phenol Contents of Fresh Vegetables** Table 1 shows the radical-scavenging activity, ascorbic acid content, and total phenol content of fresh red cabbage, white cabbage, green pepper, lettuce, Japanese radish, and carrot. The highest radical-scavenging activity was found in red cabbage (1051 μmol Trolox eq./100 g fresh weight), followed by green pepper (600), white cabbage (243), lettuce (106), and Japanese radish (98). Carrot had the lowest activity (50). In a previous study on the antioxidant activity of vegetables, Reyes et al. (2007) reported that red cabbage had the highest radical scavenging activity among the vegetables examined, while white potato, white cabbage and radish had moderate activity, and lettuce and carrot had relatively low activity. Our result is in agreement, with the exception of lettuce. As shown in Table 1, total phenol content in red cabbage was the highest (546 μmol gallic acid eq./100 g fresh weight), followed by green pepper (306), carrot (185), Japanese radish (132), white cabbage (118), and finally lettuce (89), the lowest among the vegetables examined.

Ascorbic acid content of green pepper was the highest (74 mg/100 g fresh weight), followed by red cabbage (47), white cabbage (30), lettuce (4) Japanese radish (4), and carrot (1) (Table 1). Ascorbic acid contents of 8 vegetables reported by Yamaguchi et al. (2007) were comparable with the results of the present study. The contribution of ascorbic acid to the radical-scavenging activity in the vegetables ranged from 12% (carrot) to 78% (green pepper). Ascorbic acid was found to be the main antioxidant component in green pepper and white cabbage. Therefore, the major portion of radical-scavenging activity of the other vegetables seems to be due to other compounds such as phenolic compounds (flavonoids and phenolic acids), sulfur compound and chlorophyll (Endo et al., 1985). Many phenolic compounds have been isolated from vegetables and their antioxidant activity has been evaluated (Rice-Evans et al., 1996; Viña and Chaves, 2006).

**Changes in the Color of Shredded Vegetables During Storage** Table 2 shows the changes in L*, a* and b* values of the shredded vegetables after 7-day storage at 10 °C under air or under nitrogen gas. The L* value did not change during storage of shredded vegetables. The a* value of red cabbage decreased to 58-75% during 7-day storage under air or
under nitrogen gas. The \( b^* \) value of red cabbage decreased to 48\% during 7-day storage under air, but it remained unchanged under nitrogen gas. In the case of white cabbage, the \( a^* \) value decreased during storage regardless of air or nitrogen. However, the color values of the other vegetables did not change during storage.

The pigments in red cabbage are mainly cyanidin-based anthocyanins such as cyanidin 3,5-diglucoside and cyanidin 3-sophoroside, which are major contributors to radical-scavenging activity of red cabbage (Stintzing et al., 2002). Structural changes in these anthocyanins cause decolorization and browning by deglycosidation and enzymatic oxidation, followed by polymerization and brown pigment formation. Deglycosidation forms aglycon type of polyphenols, which decreases color but increases phenolic OH. The loss of color in red cabbage may be due to deglycosidation of anthocya-

**Effect of Storage on Radical-Scavenging Activity of Shredded Vegetables** Table 3 shows the changes in the radical-scavenging activity during storage of shredded vegetables under air or under nitrogen gas. The percent of radical-scavenging activity remaining in vegetables after 7-day storage under air ranged from 82\% (green pepper) to 105\% (red cabbage), with an average of 96\%. In the case of storage under nitrogen gas, activity remaining ranged from 82\% (green pepper) to 102\% (carrot), with an average of 91\%. Retention of radical-scavenging activity during storage was little affected by oxygen in the air. Alasalvar et al. (2005) reported that antioxidant activity of orange carrots remained very stable in air and under MAP (95\% oxygen + 5\% carbon dioxide, 90\% nitrogen + 5\% oxygen + 5\% carbon dioxide) as measured on the basis of an oxygen-radical absorbance
capacity assay. Ou et al. (2002) reported that the oxygen radical absorption capacity (ORAC) and fluorescence recovery after photobleaching (FRAP) values of vegetables are not only dependent on species but are also highly dependent on geographical origin and harvest time.

**Effect of Storage on Total Phenol Content of Shredded Vegetables** Table 4 shows the changes in the total phenol content of shredded vegetables stored at 10 °C for 7 days under air or under nitrogen gas. The percent of total phenolics remaining ranged from 83% (lettuce) to 123% (white cabbage), with an average of 99%. In the case of red and white cabbages, the total phenol content significantly increased during 7-day storage (p<0.05) regardless of air or nitrogen, but that of lettuce significantly decreased after 7-day storage under air (p<0.05). In the other vegeta-
bles, the total phenolic content did not change significantly during storage under air or under nitrogen gas. The apparent increase of polyphenols in cabbages may be due to deglycosidation of anthocyanins.

**Effect of Storage on Ascorbic Acid Content of Shredded Vegetables** Table 5 shows the changes in the ascorbic acid content of shredded vegetables during storage at 10°C for 7 days under air or nitrogen gas. The percent of ascorbic acid in vegetables remaining after 7-day storage under air ranged from 45% (Japanese radish) to 97% (cabbage). In case of storage under nitrogen gas, the percent remained ranged from 74% (green pepper) to 95% (red cabbage). Since the ascorbic acid content of lettuce and carrot was low, the change of its content was omitted from discussion. In the case of green pepper, the ascorbic acid content decreased to 65-74% during 7-day storage under air or under nitrogen gas (p<0.05). In red and white cabbages, however, the ascorbic acid content did not decrease during storage.

**Conclusion**

The results of the present study clearly show that the radical-scavenging activity in red cabbage, white cabbage, lettuce, bell pepper, Japanese radish and carrot remains unchanged during storage at 10°C under air or nitrogen gas. Oxygen in the air did not affect the shelf life of shredded vegetables except red cabbage, the color of which turned brown during storage under air. Therefore, it is concluded that the presence of oxygen in the package under air does not significantly damage the appearance and the nutraceutical properties of shredded vegetables during storage.

**References**


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**Table 5.** Changes in the ascorbic acid content of the shredded vegetables stored at 10°C for 7 days under air or under nitrogen gas

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Package</th>
<th>Ascorbic acid content (mg/100g fresh weight)</th>
<th>Remaining ascorbate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Storage period (days)</td>
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<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Red cabbage</td>
<td>Air</td>
<td>47.2 ± 1.7 b)</td>
<td>97</td>
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<td></td>
<td>Nitrogen</td>
<td>41.6 ± 0.2</td>
<td>65</td>
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<td></td>
<td>56.7 ± 5.0</td>
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<td></td>
<td></td>
<td>45.8 ± 1.2</td>
<td>95</td>
</tr>
<tr>
<td>White cabbage</td>
<td>Air</td>
<td>29.5 ± 2.7</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Nitrogen</td>
<td>25.8 ± 0.4</td>
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<td></td>
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<td>26.7 ± 0.7</td>
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<td></td>
<td></td>
<td>28.5 ± 0.7</td>
<td>50</td>
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<tr>
<td>lettuce</td>
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<td>3.8 ± 0.3</td>
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<tr>
<td></td>
<td>Nitrogen</td>
<td>1.4 ± 0.2</td>
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<td></td>
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<td>1.1 ± 0.5</td>
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<td></td>
<td></td>
<td>0.3 ± 0.2</td>
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<tr>
<td>Green pepper</td>
<td>Air</td>
<td>73.8 ± 2.6</td>
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<td></td>
<td>Nitrogen</td>
<td>46.9 ± 3.5</td>
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<td></td>
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<td>50.8 ± 3.2</td>
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<td></td>
<td></td>
<td>54.5 ± 1.8</td>
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<tr>
<td>Japanese radish</td>
<td>Air</td>
<td>4.0 ± 0.7</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Nitrogen</td>
<td>2.8 ± 0.3</td>
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<tr>
<td></td>
<td></td>
<td>2.0 ± 0.9</td>
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<tr>
<td></td>
<td></td>
<td>1.8 ± 0.3</td>
<td>45</td>
</tr>
<tr>
<td>Carrot</td>
<td>Air</td>
<td>1.0 ± 0.5</td>
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<tr>
<td></td>
<td>Nitrogen</td>
<td>1.7 ± 0.0</td>
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<td>0.3 ± 0.6</td>
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<td>1.3 ± 0.5</td>
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a) The percentage of ascorbic acid remaining after 7-day storage.

b) The values are the means±SD for three determinations.


