Note

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℃ 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),

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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) and kept at -80°C until analysis.

Preparation of vegetable extracts The freeze-dried powders of fresh and frozen-stored vegetables (0.05-0.5 g) were extracted with 2 ml of 90% methanol containing 0.5% acetic acid for 1 min, and centrifuged at 1,500×g for 10 min at 4°C. Extractions were repeated three times. Next, the combined extracts were 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, 13mm, Nacalai Tesque Inc., Kyoto, Japan). The resulting solution was used for the measurement of the radical-scavenging ac-

tivity, 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 flashing 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

Table 1. Radical-scavenging activity, total phenol content, and ascorbic acid content in the fresh vegetables.

Vegetables	Radical-scavenging activity	Total phenol content	Ascorbic acid content	Contribution of ascorbic acid to radical-scavenging activity
	(µmol Trolox eq./ 100g fresh weight)	(µmol Gallic acid eq./ 100g fresh weight)	(mg/100g fresh weight)	(%)
Red cabbage	1052 ± 47 [a]	546 ± 50	47 ± 2	28
White cabbage	243 ± 8	118 ± 5	29 ± 3	77
Lettuce	106 ± 4	88 ± 5	4 ± 0	23
Green pepper	600 ± 40	184 ± 10	74 ± 3	78
Japanese radish	98 ± 2	137 ± 15	4 ± 1	26
Carrot	50 ± 16	85 ± 15	1 ± 0	12

[a] Values are the means±SD for three determinations.

Table 2. Changes in the color of the shredded vegetables stored at 10°C for 7 days under air or under nitrogen gas.

	Vegetable	Package	Storage period (days)				Change for 7 days(%)
			0	1	3	7	
L*	Red cabbage	Air	57.6	63.1	63.7	64.0	+11
		Nitrogen		58.7	56.0	58.2	+ 1
	White cabbage	Air	89.9	90.4	88.5	88.1	- 2
		Nitrogen		89.2	90.1	90.0	0
	Lettuce	Air	65.3	67.5	68.1	69.5	+ 6
		Nitrogen		64.0	69.1	69.6	+ 7
	Bell pepper	Air	68.9	74.2	72.0	73.7	+ 7
		Nitrogen		73.3	72.8	71.7	+ 4
	Japanese radish	Air	91.0	90.7	91.6	90.8	0
		Nitrogen		90.3	91.1	91.2	0
	Carrot	Air	77.3	78.1	78.2	78.3	+ 1
		Nitrogen		77.9	77.2	77.3	0
a*	Red cabbage	Air	18.0	13.6	9.3	7.6	-58
		Nitrogen		17.1	15.7	13.2	-27
	White cabbage	Air	- 5.4	- 4.7	- 4.4	- 4.4	-18
		Nitrogen		- 4.7	- 4.2	- 4.1	-24
	Lettuce	Air	- 14.1	- 12.1	- 12.1	- 12.9	- 9
		Nitrogen		- 12.3	- 13.7	- 12.5	-11
	Bell pepper	Air	- 15.6	- 14.2	- 14.2	- 13.7	-12
		Nitrogen		- 14.5	- 14.1	- 14.2	- 9
	Japanese radish	Air	- 4.1	- 4.2	- 3.5	- 3.7	-11
		Nitrogen		- 3.7	- 3.7	- 3.6	-12
	Carrot	Air	24.8	23.5	22.8	22.4	- 9
		Nitrogen		23.6	24.2	23.6	- 5
b*	Red cabbage	Air	- 14.1	- 8.3	- 7.7	- 7.3	-48
		Nitrogen		- 13.5	- 13.3	- 13.3	- 5
	White cabbage	Air	19.1	18.9	18.1	18.8	- 2
		Nitrogen		19.6	16.5	17.2	-10
	Lettuce	Air	32.9	29.7	30.2	29.8	- 9
		Nitrogen		30.9	31.5	30.6	- 7
	Bell pepper	Air	33.8	30.2	30.6	30.3	+10
		Nitrogen		31.2	30.4	31.2	- 8
	Japanese radish	Air	17.1	16.9	16.5	16.3	+ 4
		Nitrogen		17.6	17.2	16.4	+ 4
	Carrot	Air	36.8	34.4	34.6	34.8	+ 6
		Nitrogen		35.1	36.0	35.1	+ 5

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 anthocyanins.

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

Table 3. Changes in the radical-scavenging activity of the shredded vegetables stored at 10°C for 7 days under air or under nitrogen gas.

Vegetables	Package	Radical-scavenging activity (µmol Trolox eq./100g fresh weight)				Remaining activity (%)[a]
		Storage period (days)				
		0	1	3	7	
Red cabbage	Air	1052 ± 47 [b]	1026 ± 86	957 ± 77	1109 ± 106	105
	Nitrogen		1017 ± 97	1099 ± 76	1032 ± 45	98
White cabbage	Air	243 ± 8	254 ± 10	305 ± 33	235 ± 15	97
	Nitrogen		222 ± 3	199 ± 21	223 ± 14	92
Lettuce	Air	106 ± 4	113 ± 5	106 ± 4	102 ± 11	96
	Nitrogen		100 ± 2	94 ± 14	96 ± 9	91
Green pepper	Air	600 ± 40	490 ± 43	462 ± 32	490 ± 68	82
	Nitrogen		548 ± 40	465 ± 33	493 ± 2	82
Japanese radish	Air	137 ± 15	120 ± 1	107 ± 8	129 ± 9	94
	Nitrogen		119 ± 0	133 ± 13	130 ± 8	95
Carrot	Air	85 ± 15	90 ± 10	88 ± 5	87 ± 0	102
	Nitrogen		88 ± 4	81 ± 1	73 ± 5	86

[a] The percentage of radical-scavenging activity remaining after 7-day storage.
[b] The values are the means±SD for three determinations.

Table 4. Changes in the total phenol content of the shredded vegetables stored at 10°C for 7 days under air or under nitrogen gas.

Vegetables	Package	Total phenol content (µmol Gallic acid eq./100g fresh weight)				Remaining phenol (%)[a]
		Storage period (days)				
		0	1	3	7	
Red cabbage	Air	546 ± 50 [b]	628 ± 20	647 ± 59	721 ± 46	132
	Nitrogen		492 ± 2	532 ± 48	633 ± 52	116
White cabbage	Air	118 ± 5	128 ± 7	159 ± 4	132 ± 7	112
	Nitrogen		122 ± 5	119 ± 6	144 ± 3	123
Lettuce	Air	88 ± 5	85 ± 3	79 ± 6	63 ± 4	72
	Nitrogen		87 ± 9	65 ± 7	73 ± 1	83
Green pepper	Air	307 ± 9	276 ± 34	246 ± 13	272 ± 15	89
	Nitrogen		292 ± 15	254 ± 15	289 ± 30	94
Japanese radish	Air	132 ± 15	121 ± 1	114 ± 8	127 ± 8	96
	Nitrogen		119 ± 0	127 ± 13	124 ± 8	94
Carrot	Air	185 ± 10	191 ± 38	203 ± 13	185 ± 15	100
	Nitrogen		105 ± 6	139 ± 0	164 ± 18	88

[a] The percentage of total phenolics remaining after 7-day storage.
[b] The values are the means±SD for three determinations.

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 in vegetables after 7-day storage under air ranged from 72% (lettuce) to 132% (red cabbage), with an average of 100%. In the case of storage 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-

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

| Vegetables | Package | Ascorbic acid content (mg/100g fresh weight) | | | | Remaining ascorbate (%)[a] |
| | | Storage period (days) | | | | |
		0	1	3	7	
Red cabbage	Air	47.2 ± 1.7 [b]	41.6 ± 0.2	56.7 ± 5.0	45.8 ± 1.2	97
	Nitrogen		35.9 ± 2.0	46.5 ± 5.5	45.0 ± 2.6	95
White cabbage	Air	29.5 ± 2.7	25.8 ± 0.4	26.7 ± 0.7	28.5 ± 0.7	97
	Nitrogen		24.4 ± 3.0	32.5 ± 2.0	25.6 ± 1.6	87
Lettuce	Air	3.8 ± 0.3	1.4 ± 0.2	1.1 ± 0.5	0.3 ± 0.2	7
	Nitrogen		1.5 ± 0.1	2.0 ± 0.0	1.5 ± 0.3	39
Green pepper	Air	73.8 ± 2.6	46.9 ± 3.5	50.2 ± 2.1	48.0 ± 1.8	65
	Nitrogen		59.5 ± 8.9	50.8 ± 3.2	54.5 ± 1.9	74
Japanese radish	Air	4.0 ± 0.7	2.8 ± 0.3	2.0 ± 0.9	1.8 ± 0.3	45
	Nitrogen		3.6 ± 0.4	3.4 ± 1.2	3.1 ± 0.1	77
Carrot	Air	1.0 ± 0.5	1.7 ± 0.0	0.3 ± 0.6	1.6 ± 0.3	-
	Nitrogen		0.8 ± 0.2	1.0 ± 0.2	1.3 ± 0.5	-

[a] The percentage of ascorbic acid remaining after 7-day storage.
[b] The values are the means ± SD for three determinations.

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.

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