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Textural change and antioxidant properties of broccoli under different cooking treatments

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Abstract

Broccoli (Brassica oleracea L var italica Plenck) was used as a testing material and Sampled in four groups, including fresh, precooked (50 °C, 10 min), cooked (boiling, 8 min), and precooked followed by cooking (precooked + cooked), to investigate the effect of the cooking treatment on the textural change in the vegetable. After freeze-drying and extraction by the use of methanol, the antioxidant properties of the extracts from the four groups were estimated and compared with those of a-tocopherol and butylated hydroxyanisole (BHA). The antioxidant properties include reducing power, ferrous ion chelating power, α , α -diphenyl- β -picrylhydrazyl (DPPH) radical-scavenging activity and inhibitory effect against lipid peroxidation. The data indicated that, after cooking in boiling water for 8 min, the broccoli tissue had a 51% relative peak force of the fresh tissue, whereas the tissue, after precooking at 50 °C for 10 min, and the tissue, after precooking + cooking, had relative peak forces of 172% and 119%, respectively, of the fresh tissue. These results revealed that cooked tissue with precooking softens more slowly than that without precooking; that is, precooked tissue can show a higher resistance to softening during cooking. The extracts from the precooked, cooked, and precooked + cooked broccoli exhibited high reducing powers. At a sample-to-solvent (mg/ml) ratio of 20 mg/ml, these extracts had $1.5 \sim 1.7$ times as high reducing powers as those of α -tocopherol and BHA. These four extracts showed high ferrous ion chelating power, and the extracts from fresh and precooked broccoli had the highest, namely 90.5% at a sample-to-solvent ratio of 2 mg/ml. These extracts also exhibited high DPPH radical-scavenging activity, at 96.8, 97.3, 98.6 and 97.9%, respectively, for the fresh, precooked, cooked, and precooked + cooked samples, at a sample-to-solvent ratio of 20 mg/ml. The four extracts exhibited lower inhibitory effects against the peroxidation of a linoleic acid emulsion system than did α -tocopherol and BHA. $© 2004 Elsevier Ltd. All rights reserved.$

Keywords: Broccoli; Texture; Antioxidant properties; Reducing power; Ferrous ion chelating power; DPPH radical-scavenging activity

1. Introduction

Free radicals and singlet oxygen are recognized as major factors causing various diseases, such as cancer, cardiovascular disorders, and diabetes. Therefore, the health maintenance function of antioxidant components in various foods has received much attention in recent years. Vegetables are important to the human diet, for many studies have shown that a close relationship exists between cancer-inhibiting and the intake of vegetables (Byers & Guerrero, 1995; Krinsky, 1990; Meyskens & Manetta, 1995; Sies & Krinsky, 1995; Zhang, Talalay, Cho, & Posner, 1992). The cancer preventing action of vegetables is supposed to reside in the fact that vegetables contain, not only abundant nutritional antioxidants, such as vitamins C and E , and β -carotene, but also a great quantity of non-nutritional antioxidants, such as flavonoids, flavones, and other polyphenolic compounds.

Several studies have analyzed the antioxidant potential of a wide variety of vegetables (Furuta, Nishiba, & Suda, 1997; Gazzani, Papetti, Massolini, & Daglia, 1998; Hertog, Hollman, & Katan, 1992; Vinson, Hao, Su, & Zubik, 1998). Some studies have indicated that a frequent intake of cruciferous vegetables, such as broccoli, cauliflower, leaf mustard, cabbage, Chinese broccoli, and turnip, can protect against cancer (De Long, Prochaska, & Talalay, 1986; Guo, Lee, Chiang, Lin & Chang, 2001; Prochaska, Santamaria, & Talalay, 1992). Corresponding author. The search has focussed only on the theorem and the However, most research has focussed only on the

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antioxidant properties of fresh tissues of vegetables. The effect of cooking treatments on the antioxidant properties of vegetables has seldom been reported.

Variation in cooking treatment can profoundly affect both the texture and the nutritional value of vegetables. Many studies have revealed that most vegetables precooked at a moderate temperature $50-80$ °C for a suitable period of time and subsequently cooked in boiling water showed greater firmness than those cooked directly without precooking (Chang & Chang, 1992; Chang, Tsai, & Chang, 1995; Lii & Chang, 1987; Shiau & Chang, 1986; Tseng & Chang, 1988; Wu & Chang, 1990). This firming effect of precooking has been attributed to the action of pectinesterase on the cell-wall materials, particularly pectic substances, which result in de-esterification of pectin molecules and the subsequent formation of calcium bridges between free carboxyl groups of adjacent pectin molecules (Bartolome & Hoff, 1972; Chang & Chang, 1992; Hoogzand & Doesburg, 1961; Hsu, Deshpande, & Desrosier, 1965; Lee, Bourne, & Van Buren, 1979). Also, many studies have shown that a loss of the vitamins in vegetables and fruits during cooking varied with the cooking treatment (Beadle, Greenwood, & Kraybill, 1943; Bender, 1966; Chen & George, 1981; Lee, Kirk, Bedford, & Heldman, 1977). In this study, broccoli was used to investigate the effect of cooking treatments on its texture and antioxidant properties.

2. Materials and methods

2.1. Samples

Fresh broccoli (Brassica oleracea L var italica Plenca), purchased from a supermarket in Chang-Hua, Taiwan, was cleaned, and the leaves and inedible stems were removed. The samples were cooked by different methods, including precooking at 50 \degree C for 10 min, cooking in boiling water for 8 min, and precooking, followed by cooking (precooking + cooking). All the treated samples were freeze-dried, ground to powder, separately packed in plastic bags, and kept at 4° C for later use.

2.2. Chemicals

Potassium ferricyanide $(K_3Fe(CN)_6)$, 98.0% pure, and ferric chloride $(FeCl₃ · 6H₂O)$, 97.0% pure, were purchased from Katayama Chemical Co., Ltd., Japan; butylated hydroxyanisole (BHA), $>90\%$ pure, α -tocopherol, 95% pure, and ferrous chloride $FeCl_2 \cdot 4H_2O$, >99% pure, were obtained from Sigma Chemical Co., USA; α , α -diphenyl- β -picrylhydrazyl free radical (DPPH, TCI-GR) and 3-(2-Pyridyl)-5,6-di(p-sulfophenyl)-l,2,4-triazine, disodium salt (ferrozine) (TCI-GR) were obtained from Tokyo Chemical Inc., Japan.

2.3. Cooking

The fresh broccoli samples were precooked and/or cooked in distilled water, respectively, at 50, 60, 70, 80 $\rm{^{\circ}C}$ and at boiling point for 10, 20, 30, 40, 50 and 60 min, respectively. The samples, after precooking and/or cooking treatment, were quickly cooled to room temperature before texture measurement.

2.4. Texture measurement

A rheometer (Sun CR-200d, Sun Scientific Co., Japan), mounted with a plunger (adaptor No. 9) was used to measure the firmness of the stems of the broccoli samples. The plunger moved downward to the flat base, on which the sample was placed, at a speed of 50 mm/ min to measure the puncture force at a depth of 5 mm from the sample surface. The location of the stem where the puncture plunger touched is at the middle point of the stem between the flower head and the end of the stem. For every sample, four measurements were taken for each of four pieces thereof; then, the average of sixteen measured values was expressed as relative firmness in relation to the fresh sample at 100.

2.5. Preparation of sample extracts

The dry broccoli samples (100–1000 mg) were weighed, and to each, 50 ml of methanol were added. The ratios of sample weight to solvent volume were 2, 4, 8, 12, 16, and 20 mg/ml, respectively. The sample suspension in methanol was stirred in a magnetic stirrer for 1 h at a room temperature and then vacuum-filtered to obtain the methanol extract, which was used for testing antioxidant activities.

2.6. Test for reducing power

The method developed by Oyaizu (1986) for a reducing power test was used. The broccoli extracts (10 ml), together with α -tocopherol and BHA methanolic solutions, were spiked with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide and kept in a 50 C water-bath for 20 min. The mixture was then cooled rapidly, spiked with 2.5 ml of 10% trichloroacetic acid, and centrifuged at 3000 rpm for 10 min. The supernatant (5 ml) was then mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride. Absorbance at 700 nm was then measured after reaction for 10 min.The higher the absorbance, the stronger was the reducing power.

2.7. Test for ferrous ion chelating power

The method developed by Decker and Welch (1990) was adopted. Five ml of the test solutions, including broccoli extract, a-tocopherol, and BHA solutions, were spiked with 0.1 ml of 2 mM FeCl₂ and 0.2 ml of 5 mM ferrozine solution. After reaction for 10 min, the absorbance (at 562 nm) of the resulting solutions was recorded. The higher the ferrous ion chelating ability of the test sample, the lower was the resulting absorbance. The percentage of ferrous ion chelating ability, was expressed by [1-(test sample absorbance/blank sample absorbance)] \times 100.

2.8. Test for DPPH radical-scavenging activity

The method developed by Shimada, Fujikawa, Yahara, and Nakamura (1992) was used to test the DPPH radical-scavenging activity. Five ml of the broccoli extract, along with 5 ml of a-tocopherol and BHA methanolic solutions, was mixed with 1 ml of freshly prepared 1 mM DPPH methanolic solution. The solutions were then left standing for 30 min prior to being spectrophotometrically measured at 517 nm. The lower the absorbance, at 517 nm, the higher was DPPH scavenging activity. The percentage of the DPPH scavenging activity was expressed by [1-(test sample absorbance/blank sample absorbance)] \times 100.

2.9. Test of anti-peroxidation activity

The anti-peroxidation activity was assayed by using a linoleic acid emulsion system. A sample (0.5 ml) plus 2 ml of 0.2 M sodium phosphate buffer (pH 7.0) was mixed with 0.02 M linoleic acid emulsion (2.5 ml), and subsequently incubated at 60° C. According to the thiocyanate (FTC) method (Mitsuda, Yasumoto, & Iwami, 1966), the degree of oxidation was measured by reading the absorbance (at 500 nm) of peroxides coloured with FeCl₂ and ammonium thiocyanate. A control test was performed with linoleic acid but without a sample solution.

2.10. Statistical analysis

A student t test was conducted to compare the data, for which all tests were considered statistically significantly at $P < 0.05$.

3. Results and discussion

3.1. Textural changes in broccoli during different cooking treatments

In the study of vegetable texture, an objective and numerically appropriate method, for texture measurement and comparison between samples, is needed. In this study, we used a rheometer to measure the texture and proceeded to a fractural analysis. Fig. 1 shows the rheological values and profiles of the broccoli samples

Peak force (g) :	829.0	1480.0	412.0	939.0
2 mm depth (g):	697.0	1438.0	359.0	932.0
4 mm depth (g):	137.0	967.0	121.0	561.0
Brittleness breakdown (g):	827.0	1479.0	410.0	938.0
Yield stress (g/mm^2) :	3316.0	5290.0	1648.0	3756.0
Peak force				

Fig. 1. Rheological values and profiles obtained for broccoli samples subjected to different cooking treatments in distilled water, as measured using a rheometer. Cooking treatments: A, fresh; B, 50 $^{\circ}$ C, 10 min; C, boiling, 8 min; D, 50 \degree C, 10 min + boiling, 8 min.

 \overline{C}

D

 \overline{B}

 \overline{A}

under different cooking treatments. From the rheological values, it may be observed that the sample directly cooked in boiling water for 8 min had the lowest peak force value (412.0 g), and the sample cooked at 50 \degree C for 10 min had the highest value (1480.0 g). It may also be observed that the peak force is apparently positively correlated with the brittleness value. Therefore, peak force was used as the texture index for the broccoli samples in this study.

Fig. 2 shows the textural changes in the broccoli samples during 1 h of cooking in distilled water at different temperatures. At 80 $^{\circ}$ C, and at the boiling point, the relative peak force of the samples decreased rapidly with increasing cooking time. However, when the samples were cooked at 50, 60, and 70 \degree C for 10 min, the respective relative peak forces increased. The samples cooked at 50 and 60 \degree C for 10 min had 1.7 times the relative peak force of fresh tissue. These results reveal that a more appropriate cooking treatment can increase the relative peak force of vegetable tissue and increase its brittleness, which is preferred by consumers.

50 °C 180 $60 °C$ 70 °C 160 80 °C 140 hoilin Relative peak force (%) $12C$ 100 80 60 4^c $\overline{2}$ \mathbf{o} Ω $10¹⁰$ $\overline{20}$ 30 Δ 0 50 60 Cooking time (min)

Fig. 2. Changes in relative peak force of broccoli samples during 1 h of cooking in distilled water at different temperatures.

Fig. 3. Relative peak force of broccoli samples with precooking (0–60 min) in water (50–70 °C), followed by cooking (8 min) in boiling water.

Fig. 3 displays the textural changes in the broccoli samples during precooking $(0 \sim 60$ min) in water $(50 \sim 70$ °C), followed by cooking (8 min) in boiling water. The results indicate that broccoli precooked at 50, 60, or 70 \degree C for 10 to 60 min and subsequently cooked in boiling water shows greater peak force than that directly cooked without precooking. Particularly, the broccoli sample precooked at 50 \degree C for 10 min and then cooked in boiling water for 8 min had a relative peak force of 119%, which is much greater than that (51%) of the sample directly cooked in boiling water without precooking.

The results of the aforementioned tests reveal that a precooking treatment can maintain the texture of broccoli and increase its resistance to softening during cooking in boiling water. Many studies indicate that this firming effect of precooking can be attributed to the action of pectin-esterase on the cell-wall materials, particularly pectic substances, thereby resulting in deesterification of pectin molecules and the subsequent formation of calcium bridges between free carboxyl groups of adjacent molecules (Bartolome & Hoff, 1972; Chang & Chang, 1992; Hoogzand & Doesburg, 1961; Hsu et al., 1965; Lee et al., 1979).

On the basis of the results of the experiment investigating the effect of cooking treatments on the textural change in the broccoli samples, the precooking and cooking conditions were set at 50 $^{\circ}$ C, 10 min and boiling, 8 min, respectively, and these prescriptions were used to prepare the samples for the following antioxidant properties test.

3.2. Antioxidant properties of methanolic extracts from broccoli samples after different cooking treatments

3.2.1. Reducing power

The reducing powers of the methanolic extracts from the broccoli after different treatments are shown in

Fig. 4. Reducing power of the methanolic extracts from broccoli samples with different cooking treatments. Precooked: 50 $^{\circ}$ C, 10 min; cooked: boiling, 8 min.

Fig. 4. All four groups showed high reducing powers. At a sample-to-solvent ratio of 8 mg/ml, with the exception of the extract from fresh broccoli which exhibited a reducing power comparable to those of BHA and α -tocopherol, the extracts from precooked, cooked, and precooked + cooked broccoli exhibited higher reducing powers than those of BHA and α -tocopherol. When the sample-to-solvent ratio was increased to 20 mg/ml, the same extracts exhibited 1.5–1.7 times the reducing power of BHA and a-tocopherol. From the foregoing results, it may be observed that the broccoli with precooking and/ or cooking treatments exhibited higher reducing powers than the fresh sample. This may be attributed to the fact that the precooking and/or cooking treatments bruised the tissue and exposed the antioxidant components, thereby resulting in a higher reducing power.

It has been reported that the methanolic extracts of several edible plants traditionally consumed by the Chinese, such as Jew's ear, lotus seed, and Job's tears, exhibit less reducing power than α -tocopherol and BHA (Liu, Chang, & Yen, 1999). Our study showed that the methanolic extracts of broccoli after precooking and/or cooking treatments had higher reducing powers than α tocopherol and BHA at a sample-to-solvent ratio ranging from 8 to 20 mg/ml. These results suggest that broccoli, after precooking and/or cooking treatments, possesses higher reducing power than several edible plants traditionally consumed by Chinese people.

3.2.2. Ferrous ion chelating power

Fig. 5 depicts the ferrous ion chelating power of the methanolic extracts from the broccoli samples after different cooking treatments. At a sample-to-solvent ratio of 2 mg/ml, all four groups of samples exhibited their highest ferrous ion chelating powers, ranging from 79% to 90%. This may result from the equilibrium between the chelating and the releasing of ferrous ion by

Fig. 5. Ferrous ion chelating power of the methanolic extracts from broccoli samples with different cooking treatments. Precooked: 50 \degree C, 10 min; cooked: boiling, 8 min.

the antioxidants of the extract. Among these four samples, the extracts from fresh and precooked broccoli had the highest ferrous ion chelating ability, at around 90%; the extract from precooked + cooked broccoli had a ferrous ion chelating power of 82.5%; the extract from cooked broccoli had the lowest ferrous ion chelating power, at 79.0%.

Methanolic extract from broccoli at 2 mg/ml can have more than 79.0% ferrous ion chelating power. When using a soybean sprout extract, a concentration of 3 mg/ ml was required to obtain the same level of ferrous ion chelating ability (Wong & Yen, 1997). The methanolic extracts from mungbean sprouts and radish sprouts exhibited chelating abilities of only 60% and 40%, respectively, at a concentration of 3 mg/ml. The concentration unit used in this study is expressed by the ratio of crude-sample weight to solvent volume, instead of the extract weight to solvent volume that is used in the study of Wong and Yen (1997). On the basis of the differences between the two concentration definitions, broccoli, after different cooking treatments, is estimated to possess a higher ferrous ion chelating ability than soybean, mungbean or radish sprouts, in which the concentration is expressed as the ratio of extractweight to solvent volume.

3.2.3. DPPH radical-scavenging activity

The DPPH radical-scavenging activities of broccoli after different cooking treatments, along with a-tocopherol and BHA, are presented in Fig. 6. At a concentration of 2 mg/ml, a-tocopherol and BHA possessed the highest DPPH radical-scavenging activities at 89% and 97%, respectively. The extract from fresh broccoli exhibited a scavenging activity of 71%; the extracts from cooked and precooked + cooked samples exhibited a scavenging activity of 50%. The extract from the precooked sample showed the lowest scavenging activity of 31%. This ac-

Fig. 6. DPPH radical-scavenging activity of the methanolic extracts from broccoli sample with different cooking treatments. Precooked: 50 C, 10 min; cooked boiling, 8 min.

tivity increased with an increase in the concentrations of the samples from 2 to 12 mg/ml, being identical to the results reported by Guo, Lee, Chiang, Lin, and Chang (2001). All the extracts from samples at 8 mg/ml could reach a scavenging activity comparable to BHA.

3.2.4. Anti-peroxidation effect using ferric thiocyanate method

From the results of the antioxidant properties shown in Figs. 4–6, it may be observed that the antioxidant properties of broccoli, under different cooking treatments, can reach maximum values when the sampleto-solvent ratio is increased to 12 mg/ml. Therefore, the sample prepared by using a ratio of 12 mg/ml was selected for the anti-peroxidation effect test.

Fig. 7 presents the anti-peroxidation activity, measured by the ferric thiocyanate method at 60 \degree C, of the

Fig. 7. Anti-peroxidation activity, measured by the ferric thiocynate method at 60 °C, of methanolic extracts from broccoli samples after different cooking treatments. Precooked: 50 °C, 10 min; cooked: boiling, 8 min.

extracts from broccoli samples after different cooking treatments. After incubating for 8 h, the absorbances of the control, α -tocopherol, and BHA were 0.67, 0.23, and 0.23, respectively, whereas those of the fresh, precooked, cooked, and precooked + cooked broccoli extracts were 0.57, 0.65, 0.49, and 0.57, respectively. These results reveal that BHA and a-tocopherol still exhibit antiperoxidation activity, even after incubation at 60 \degree C for 8 h, being similar to the results reported by Liu et al. (1999). Among the extracts from broccoli under different cooking treatments, the extract from the cooked sample had the highest anti-peroxidation activity, which was 0.41 times as high as those of BHA and α -tocopherol. The extracts from fresh and precooked + cooked broccoli had 0.23 times the anti-peroxidation activity, being as high as those of BHA and α -tocopherol. The extract from precooked broccoli had almost no anti-peroxidation activity. These results also reveal that all the extracts from the samples, after different cooking treatments, exhibited lower anti-peroxidation activity than BHA or a-tocopherol in a linoleic acid emulsion system.

4. Conclusion

The broccoli sample precooked at 50 \degree C in distilled water for 10 min showed a greater peak force, which was positively correlated with brittleness; therefore, this type can be more preferable and acceptable to consumers. In addition, the broccoli samples, after different cooking treatments, still showed high reducing power, ferrous ion chelating power, and DPPH radical-scavenging activity. This study indicated that a precooking and/or cooking treatment had no profound effect on the antioxidant properties of broccoli.

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