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Antioxidant potential, anti-proliferative activities, and phenolic content in water-soluble fractions of some commonly consumed vegetables: Effects of thermal treatment

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Abstract

Thermal treatments associated with food processing can alter the phenolic content of vegetables; yet, the biological properties associated with altered phenolic content have not been well delineated. We assessed the effects of various thermal treatments on total phenolic content, antioxidant and anti-proliferative activities of water-soluble fractions from six commonly consumed vegetables. Phenolic content in the water-soluble fraction of the tested vegetables was in the order of spinach > 'komatsuna' > 'haruna' > 'chingensai' > white cabbage > Chinese cabbage. Total antiradical activity against the DPPH radical was in the order of 'komatsuna' > spinach > 'haruna' > 'chingensai' > white cabbage > Chinese cabbage. Antiradical activity against hydroxyl radicals (deoxyribose assay) was highest for spinach and white cabbage. White cabbage extract showed the highest anti-proliferative activity in HL 60 cells. Normal cooking temperatures detrimentally affected phenolic content as well as antiradical and anti-proliferative activities of juice from most of the vegetables tested. However, mild heating of vegetable juices (50 °C, 10–30 min) preserved 80–100% of phenolic content, and both antioxidant activity and cell proliferation inhibition activities. The degree of thermal processing affects not only the content of phenolic compounds in vegetables but also beneficial biological effects associated with these compounds.

Keywords: Green vegetables; Thermal effect; Phenolic content; Antioxidant; Cell proliferation inhibition; HL-60

1. Introduction

Diets rich in fruits and vegetables may be protective against chronic diseases such as cancer and heart disease (Rimm et al., 1996; Steinmetz & Potter, 1996; van't Veer, Jansen, Klerk, & Kok, 2000). These protective effects are generally attributed to the presence of various functional components, such as phenolic compounds, vitamin C, vitamin E, provitamins, minerals, and fiber. Many of these compounds have bioactive mechanisms for effectively scavenging reactive oxygen species (ROS) and reducing cell proliferation in cancer cell lines (Kampa et al., 2000; Meyer et al., 2005). Oxidative stress appears to be the critical factor in the pathogenesis of many diseases because ROS have the ability to damage macromolecules like DNA, protein and lipids (Baskin & Salem, 1997).

To minimize the harmful effects of oxidative stress in the human body, it is necessary to supply adequate amounts of ROS-scavengers, and fruits and vegetables are considered to be the major contributors of ROS-scavenging antioxidants. However, various factors that occur during the processing of vegetables affect nutrient quality and degrade phenolic constituents and other antioxidants. Recently, even mild thermal processing techniques such as blanching have raised concern regarding the impact on functional components, yet thermal processing is an important method of preserving foods, and for maintaining functional attributes including texture, flavor, and color. The challenge is to identify thermal technologies that are

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effective in preserving foods, yet maximize nutritional integrity. Thus, studying the effects of mild thermal treatments on the activity of major functional components in vegetables is important.

The vegetables spinach (*Spinacia oleracea* L.), 'komatsuna' (*Brassica rapa* L. nothovar), 'haruna' (*Brassica campestris* L. *Pekinensis* group var. dentata), 'chingensai' (*Brassica campestris* L. *Chinensis* group var. utilis), white cabbage (*Brassica oleracea* L. var. *capitata*), Chinese cabbage (*Brassica campestris* L. var.*pekinensis*) are among the most popular leafy vegetables in Japan as well as in many other countries. These leafy vegetables are generally cooked before being consumed. Losses of antioxidant components from some vegetables at home cooking temperatures have been reported (Ismail & Lee, 2004; Papetti, Daglia, & Gazzani, 2002), but few studies have investigated the extent of loss when vegetables are exposed to lower thermal environments. We measured the total phenolics, total antioxidant and cell proliferation inhibition activity of selected water-soluble fractions in spinach, 'komatsuna', 'haruna', 'chingensai', white cabbage and Chinese cabbage. The investigation was further extended to determine the effects of various thermal treatments on the biological activities of the water-soluble fractions of the vegetables.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu reagent (ICN Biomedicals Inc., Ohio, USA), chlorogenic acid, ethylenediaminetetraacetic acid (EDTA), sodium bicarbonate (Nacalai Tesque Inc., Kyoto, Japan). Gallic acid, 2-(*N*-morpholino)ethanesulfonic acid (MES) and 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma–Aldrich (St. Louis, MO). RPMI 1640 medium and fetal bovine serum (FBS) were purchased from Gibco Life Technologies (Grand Island, NY). All other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan) unless otherwise stated.

2.2. Vegetables

Spinach (*Spinacia oleracea* L.), 'komatsuna' (*Brassica rapa* L. nothovar), 'haruna' (*Brassica campestris* L. *pekinensis* group var. *dentata*), 'chingensai' (*Brassica campestris* L. *Chinensis* group var. utilis), white cabbage (*Brassica oleracea* L. var. *capitata* L), Chinese cabbage (*Brassica campestris* L. var.*pekinensis*), were collected directly from farms and a wholesale market at Tsuchiura, Ibaraki, Japan. The vegetables were cleaned under running tap water and excess water was drained off.

2.3. Sample preparation

Vegetable extracts were prepared as previously described (Racchi et al., 2002) with minor modifications. Briefly, the vegetables were cut into small pieces (3 or 4 cm × 0.5 or 1 cm), homogenized in a cooled (8–10 °C) blender (MK-K56, National, Osaka, Japan) and then squeezed manually with fresh cotton to extract the juices from each vegetable. During the extractions, temperature was below 10 °C. The collected juices were immediately centrifuged at 5000*g* for 10 min at 4 °C, the supernatant was filtered through filter paper (Whatman No. 4) to remove floating materials, and the volume measured. The vegetable residues were weighed and frozen, immediately. Each juice was further filtered by Millipore membranes of cellulose acetate/cellulose nitrate mixed esters (0.45 μm) then subdivided into four batches. One batch was held on ice for 30 min. The other three were immediately subjected to thermal treatment at 50 °C, 75 °C and 100 °C for 10 and 30 min each. All vegetable juices were further centrifuged at 3000*g* for 5 min before analysis. Samples were prepared from at least three different vegetable batches collected from farms or retailers.

2.4. Freeze drying

The fresh cut vegetables and the vegetable residues were separately frozen at −30 °C in a freezer for 6 h. The samples, after uniform freezing, were freeze-dried for 72 h using a freeze-dryer (FDU-830, Eyela, Tokyo) in which the pressure was always kept below the triple point pressure (600 Pa), and mostly below 5 Pa. After removing the water by freeze-drying process (Mayland, Flath, & Shewmaker, 1997), the dried samples were weighed, and water contents of the samples were calculated using following formula:

$$\text{Water content } (\%) = (\text{fresh-weight} - \text{dry-weight})/\text{fresh weight} \times 100$$

2.5. Determination of total phenolic content

The content of total phenolics was measured spectrophotometrically using the Folin-Ciocalteu colorimetric method reported previously (Singleton, Orthofer, & Lamuela-Raventos, 1999). All vegetable juices were diluted with distilled water to obtain readings within the standard curve ranges of 0.0–800.0 μg of gallic acid/ml. Briefly, 125 μl of the standard gallic acid solution or diluted vegetable extract, 500 μl of distilled water and 125 μl of Folin-Ciocalteu reagent were mixed and held at room temperature for 6 min. Then, 1.25 ml of a 7% sodium carbonate aqueous solution was added and brought to a final volume to 3 ml with water. The mixtures were allowed to stand at room temperature for 90 min before the absorbance was measured at 760 nm using a Shimadzu spectrophotometer (UV mini-1240, Shimadzu, Osaka, Japan). Values were expressed as micrograms of gallic acid equivalents (GAE) per ml of vegetable juice.

2.6. DPPH assay

The antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical (Lim, Hu, & Kitts, 2001). A 40 μl aliquot of vegetable juice (sample) or a 40 μl aliquot of 50 mM MES buffer (pH 6.4) (control sample) was added to 3.9 ml of 60 μM DPPH in 50% ethanol/MES (v/v) solution. The decrease in absorbance was determined at 515 nm when the reaction reached a plateau (after 20 min of reaction). The percent of antiradical activity (ARA) against DPPH was calculated according to the following equation:

$$\text{ARA}\% = (\text{Absorbance of control} - \text{Absorbance of sample})/\text{absorbance of control} \times 100$$

The scavenger activity was also determined for a methanolic solution of chlorogenic acid (CA) at concentrations of 125, 250 and 500 μg/ml.

2.7. Deoxyribose assay

The scavenger activity of the vegetable juices, based on the inhibition of deoxyribose degradation caused by the attack of hydroxyl radicals, was evaluated using the Aruoma (1994) method with some modifications. The hydroxyl radical was induced in the system by the Fenton reaction. In a final volume of 1.2 ml, the reaction mixture contained $FeCl_3$ (25 μM) premixed with EDTA (100 μM) in KH_2PO_4/KOH buffer (pH 7.4); 2-deoxy-D-ribose (2.8 mM); H_2O_2 (2.8 mM); ascorbic acid as the promoter of the reaction reducing Fe(III) to Fe(II) (100 μM); and 12 μl vegetable juice (sample); or 12 μl of KH_2PO_4/KOH buffer (control sample). Both samples were placed in a water bath at 37 °C for 1 h, and then 1 ml of 1% thiobarbituric acid and 1 ml of 2.8% trichloroacetic acid were added. The reaction mixtures were heated in a water bath at 80 °C for 20 min, kept in ice for 5 min, and then centrifuged for 5 min at 3000 rpm to separate the particles. The absorbance values of the sample supernatant and the control sample were read in a spectrophotometer at 532 nm against the relative solutions prepared as described, but without ascorbic acid, to correct for interference (i.e., juice color and thiobarbituric acid-reactive substances (TBARS) that might naturally occur in vegetable juices).

The scavenger activity was expressed as the percentage inhibitory activity (IA%) of degradation of deoxyribose in the presence of the vegetable juice (sample), relative to the control sample (without the vegetable juice), using the following equation:

$$\text{IA}\% = 100 - (\text{absorbance/absorbance control sample}) \times 100.$$

The scavenger activity was also determined for an aqueous solution of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), which was assayed at final concentrations of 62.5, 325, and 750 μM.

2.8. Cell viability assay

For determining cell viability, vegetable juices were freeze dried and then brought to original juice volume with phosphate buffer saline (PBS). The trypan blue assay (Algan, Stobbe, Helt, Hanks, & Chapman, 1996) was used to evaluate cell viability. Briefly, HL-60 (No. JCRB 0085, Health Science Research Resources Bank, Osaka, Japan) were grown in RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum (FBS). Cells were grown at 37 °C in a humidified incubator (model SCI-165D, Astec Co. Ltd., Tokyo, Japan) with 5% CO_2 and 95% air, and used for assays during the exponential growth phase. Cell concentrations of 20×10^4 cells/well in 0.9 ml of the growth media were placed in each well of a 24-well flat-bottom plate. Then 100 μl of the samples were added to the cultures. Control cultures received an equal amount of PBS. After 24 h of incubation, viable cells were counted using an improved Neubauer hemacytometer (American Optical Scientific Instrument Division, Buffalo) and trypan blue dye.

2.9. Statistical analysis

Statistical analysis was conducted using one-way ANOVA followed by Student–Newman–Keuls post-hoc test for multiple comparisons. A P-value <0.05 was considered statistically significant. Data are expressed as means ± standard deviation (SD) of at least three independent measurements.

3. Results and discussion

It has long been perceived that thermally processed foods have lower nutritional value than fresh commodities because of the decline of vitamins and loss of some physiochemical characteristics. Today, consumers have an increasing concern regarding safety and quality of foods, and as a result, many consumers look for minimally processed foods that preserve the quality of the food during processing. To develop an effective thermal technology for producing foods with very high organoleptic and nutritional qualities, extensive investigation is needed pertaining to functional changes of foods treated under a range of thermal conditions.

This study identified effects of various thermal treatments on the total phenolic content, antioxidant potential and cell proliferation inhibitory activity in selected water-soluble fractions of six vegetables. To assess the effects of thermal treatment to which most vegetables are exposed in home cooking, and to compare the effect of mild heating on the components, samples were analyzed following treatment at 50 °C, 75 °C and 100 °C for 10 and 30 min each.

The characteristics of the vegetable juices, including the raw juice volume obtained from 100 g of each fresh vegetable are shown in Table 1. The pH of the juices following the time and temperature conditions tested are reported in Tables 2 and 3. Chingensai had the highest water content followed by 'komatsuna' > Chinese cabbage > 'haruna' > white cabbage and spinach. Overall, thermal treatment caused a decrease in the pH of the juice of all vegetables tested, indicating that the compounds generated during heating process have acidic properties. The products may be generated due to oxidation of phenolic compounds or additional unknown reactions. Further work is needed to understand the mechanism responsible for the decline in the heated vegetable extracts. This observation is consistent with a previous report that showed thermal treatment may cause a decrease in the pH of vegetable juice (Racchi et al., 2002).

The total phenolic contents of six selected vegetables are shown in Fig. 1. The means of total phenolic contents in spinach, 'komatsuna', 'haruna', 'chingensai', white cabbage and Chinese cabbage were 1510 ± 156, 1111 ± 195.6, 778 ± 170.6, 633.3 ± 96.95, 623.3 ± 44.5 and 543.3 ± 84 μg GAE/ml of sample, respectively. Data showed that spinach had the highest amount of phenolic content followed 'komatsuna', 'haruna', 'chingensai', white cabbage and Chinese cabbage.

We assessed the effect of thermal treatment on total phenolic content relative to fresh, raw juice with the total phenolic content of the raw juice set at 100%. Thermal treatment at the six time–temperature combinations influenced the contents of phenolic compounds in all vegetable juices (Tables 4 and 5). Cooking samples at 100 °C for 10 or 30 min decreased phenolic contents below 60% (50–60%) of the value of raw juices for spinach ($p < 0.001$), 'komatsuna' ($p < 0.05$), and 'haruna' ($p < 0.01$). Heating at 75 °C produced similar results. When juices were heated at 50 °C, however, over 80% (78–92%) of phenolic components were preserved, and changes were not significant for 'komatsuna', 'haruna', 'chingensai', white cabbage and Chinese cabbage. Total phenolic concentrations in spinach juice were about 48 ± 5% lower ($p < 0.001$) when the juice was heated at 75 or 100 °C for 10–30 min. The value was 20 ± 6% lower ($p < 0.01$) than that of raw juice when treated at 50 ° for 10–30 min. Among the tested vegetables,

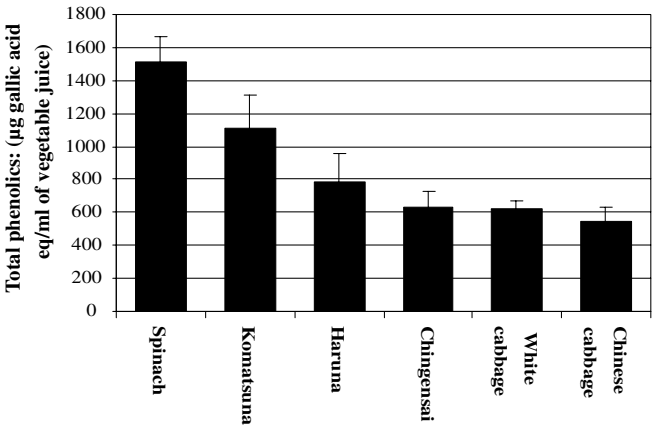

Fig. 1. Total phenolic contents (μg GAE/ml) in the tested vegetable juices (raw). Coefficient of variations percentage (CV%) for the samples are 10.4 (spinach) 17.6 (komatsuna), 21.7 (haruna), 15.3 (chingensai), 6.8 (white cabbage), 15.7 (Chinese cabbage). Data points represent means ± SD of three independent determinations.

Table 1
Index of selected vegetables for water content and juice collected

Vegetable	Water content			Juice collected (ml/100 g vegetable)		
	Mean	SD (±)	CV (%)	Mean	SD	CV (%)
Spinach	93.6	0.76	0.81	58.5	1.7	2.9
Komatsuna	95.38	0.56	0.58	66.6	0.15	0.22
Haruna	95	0.66	0.69	70.75	1.1	1.55
Chingensai	96.4	0.28	0.29	76.5	1.4	1.83
White cabbage	93.7	0.37	0.39	73.5	1.7	2.31
Chinese cabbage	95.12	0.46	0.48	74.1	1.1	1.48

Values represent mean ± standard deviations (SD) of three independent determinations. CV = coefficient of variation.

Table 2
Thermal effect on pH of the vegetable juices

Treatment conditions	Spinach			Komatsuna			Chingensai		
	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)
Raw (control)	6.32	0.11	1.74	6.45	0.13	2.02	6.39	0.13	2.03
50 °C, 10 min	6.15	0.12	1.95	6.31	0.12	1.90	6.19	0.12	1.94
50 °C, 30 min	6.18	0.14	2.27	6.27	0.14	2.23	6.11	0.14	2.29
75 °C, 10 min	6.22	0.15	2.41	6.05	0.13	2.15	5.90	0.15	2.54
75 °C, 30 min	6.16	0.12	1.95	5.71	0.12	2.10	5.52	0.10	1.81
100 °C, 10 min	6.20	0.11	1.77	5.60	0.11	1.96	5.48	0.12	2.19
100 °C, 30 min	6.23	0.11	1.77	5.55	0.11	1.98	5.40	0.14	2.59

Values represent mean ± standard deviations (SD) of three independent determinations. CV = coefficient of variations.

Table 3
Thermal effect on pH of the vegetable juices

Treatment conditions	White cabbage			Chinese cabbage			Haruna		
	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)
Raw (control)	6.50	0.10	1.54	6.41	0.13	2.03	6.39	0.11	1.72
50 °C, 10 min	6.31	0.12	1.90	6.25	0.17	2.72	6.31	0.12	1.90
50 °C, 30 min	6.21	0.13	2.09	6.21	0.19	3.06	6.26	0.16	2.56
75 °C, 10 min	6.26	0.08	1.28	6.08	0.15	2.47	6.22	0.20	3.22
75 °C, 30 min	6.19	0.12	1.94	6.05	0.12	1.98	6.15	0.21	3.41
100 °C, 10 min	6.24	0.13	2.08	6.02	0.08	1.33	6.09	0.15	2.46
100 °C, 30 min	6.13	0.10	1.63	5.88	0.10	1.70	6.02	0.12	1.99

Values represent mean ± standard deviations (SD) of three independent determinations. CV = coefficient of variations.

Table 4
Thermal effect on total phenolic contents (GAE) in the vegetable extracts

Treatment conditions	Spinach			Komatsuna			Haruna		
	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)
Raw (control)	100	10.1	10.1	100	17.9	17.9	100	15.4	15.4
50 °C, 10 min	82.7[b]	5.6	6.8	89.2	15.4	17.3	92.5	15.9	17.2
50 °C, 30 min	78.8[b]	6.7	8.5	86.8	18.4	21.2	86.6	11.2	12.9
75 °C, 10 min	55[c]	4.5	8.2	60.4[a]	12.9	21.4	68.1[a]	7.8	11.5
75 °C, 30 min	49.8[c]	6.3	12.7	53[a]	12.5	23.6	63.7[a]	8.6	13.5
100 °C, 10 min	52.2[c]	5.2	10	51.7[a]	14	27.1	59.6[b]	8.9	14.9
100 °C, 30 min	50.8[c]	4.2	8.3	53.6[a]	10.3	19.2	60.6[b]	11.4	18.8

Phenolic contents in raw extracts (control) were set to 100%.
Values represent mean ± standard deviations (SD) of three independent determinations. CV = coefficient of variations. Within same column, means followed by different letters 'a' ($P < 0.05$), 'b' ($P < 0.01$), and 'c' ($P < 0.001$) show statistical differences from the control group.

Table 5
Thermal effect on total phenolic contents (GAE) in the vegetable extracts

Treatment conditions	Chingensai			White cabbage			Chinese cabbage		
	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)
Raw (control)	100	15.5	15.5	100	8.5	8.5	100	9.6	9.6
50 °C, 10 min	96.1	13.2	13.7	95.5	5.2	5.4	97.1	8.9	9.2
50 °C, 75 min	88.1	11.8	13.4	92.5	4	4.3	91.8	8.5	9.3
75 °C, 10 min	75.1	9.8	13.0	90	4.5	5	84	7.4	8.8
75 °C, 30 min	68.4[a]	11.1	16.2	78[b]	6.5	8.3	78.9[a]	6.9	8.7
100 °C, 10 min	67.4[a]	11.1	16.5	77.7[b]	5.9	7.6	76.3[a]	4.5	5.9
100 °C, 30 min	65.6[a]	10.7	16.3	78.7[b]	4.1	5.2	73.7[b]	4.3	5.8

Phenolic contents in raw extracts (control) were set to 100%.
Values represent mean ± standard deviations (SD) of three independent determinations. CV = coefficient of variations. Within same column, means followed by letters 'a' ($P < 0.05$), 'b' ($P < 0.01$), and 'c' ($P < 0.001$) show statistical differences from the control group.

white cabbage and Chinese cabbage showed better resistance towards thermal treatment, whereas spinach appeared to become less resilient towards thermal treatment.

Data obtained form the DPPH assay (Fig. 2) indicated that the raw juices of spinach and 'komatsuna' had ARA values of 71 ± 9.5 and 70 ± 10.4%, respectively. Haruna, 'chingensai', white cabbage and Chinese cabbage reached ARA values of 52 ± 7.9, 36 ± 6.3, 32 ± 2.7, and 8 ± 1%, respectively. In this assay, therefore, the 'komatsuna' and spinach had activities similar to that shown by a 500 μg/ml chlorogenic acid solution with ARA values of 72 ± 3.4%, whereas the 'chingensai' and white cabbage showed ARA values closer to those produced by 250 μg/ml of chlorogenic acid solution, with ARA values of 36 ± 5.4%. Chinese cabbage produced an ARA% value below that associated with 125 μg/ml chlorogenic acid. Thermal treatments at 75 °C and 100 °C resulted in decreases in anti-DPPH radical activity to 55–65% ($p < 0.01$) of the activity of raw juices of spinach, 'komatsuna', and 'chingensai' (Table 6). These decreases parallel the decrease of their phenolic contents. For 'haruna' and 'chingensai' the values were 65–75% of the raw juices. White cabbage juice showed greater resistance to thermal treatments as measured by anti-DPPH activity (Table 7).

In the deoxy ribose assay, the raw juice of spinach and white cabbage showed the highest deoxy ribose degradation inhibitory activity (IA) among the tested vegetables (Fig. 3). The values were 41.6 ± 4.2 and 40 ± 7.2%, respectively. These values are higher than those obtained with 375 μM solution of Trolox, a water-soluble analogue of vitamin E. Komatsuna and 'haruna' reached IA values of

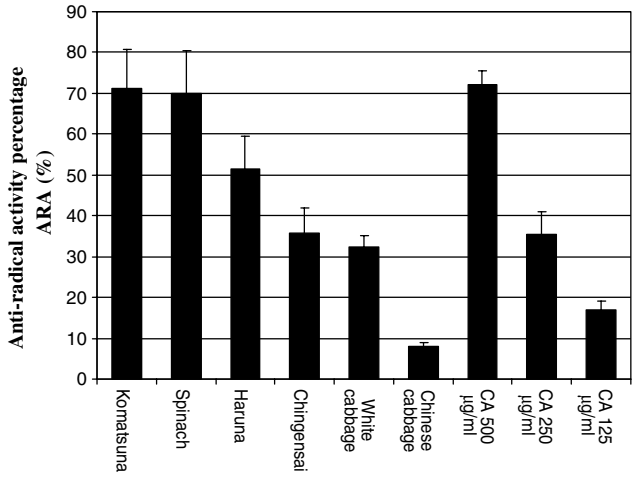

Fig. 2. Antiradical activity percentage (ARA%) of tested vegetable juices (raw) and of 500, 250 and 125 μg/ml of chlorogenic acid (CA) solutions in DPPH assay. Coefficient of variations percentage (CV%) for the samples are 14.9 (spinach) 13.4 (komatsuna), 17.7 (haruna), 8.5 (chingensai), 12.5 (white cabbage), 4.8 (Chinese cabbage). Data points represent means ± SD of three independent determinations.

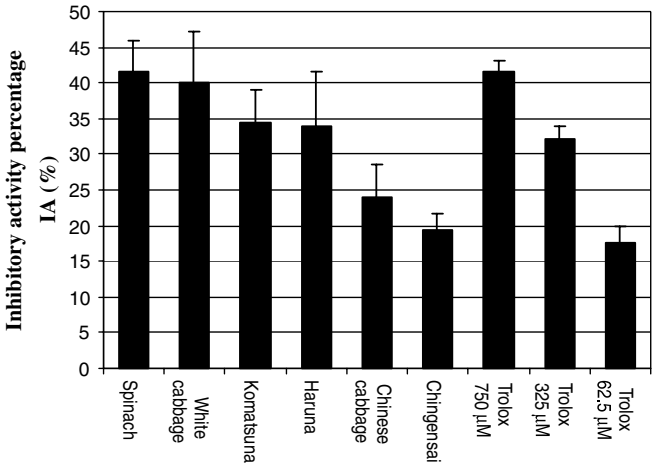

Fig. 3. Inhibitory activity percentage (IA%) of tested vegetable juices (raw) and of 750, 325 and 62.5 μM of Trolox solutions against hydroxyl radical in the deoxyribose assay. Coefficient of variations percentage (CV%) for the samples are 10 (spinach), 13.8 (komatsuna), 22.2 (haruna), 11.9 (chingensai), 18 (white cabbage), 19.1 (Chinese cabbage). Data points represent means ± SD of three independent determinations.

34.5 ± 4.7 and 33.6 ± 7.5%, respectively. The Chinese cabbage and 'chingensai' produced lower anti-hydroxyl radical activity of IA values 24.5 ± 4.5% and 19.3 ± 2.3%, respectively. Thermal treatments had a strong effect on values for spinach IA% (Table 8). When spinach juice was heated at cooking temperatures, IA% values decreased by 70–75% ($p < 0.001$). A moderate effect ($p < 0.05$) was observed on 'haruna', 'komatsuna' and white cabbage IA% values. Thermal treatment at 75 °C or at 100 °C reduced the value to between 50% and 60% that of raw juices (Table 8). This

Table 6
Thermal effect on anti-DPPH activity in the vegetable extracts

Treatment conditions	Spinach			Komatsuna			Chingensai		
	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)
Raw (control)	100	15	15	100	13.3	13.3	100	15.5	15.5
50 °C, 10 min	73.8[a]	14	19	87.2	13.1	15	96	13.1	13.6
50 °C, 30 min	68.7[b]	10.4	15.1	83.2	14.3	17.2	87.9	11.84	13.6
75 °C, 10 min	55[b]	9.4	17.1	63.3[b]	9.5	15	75.1[b]	9.8	13
75 °C, 30 min	58[b]	7.5	12.9	62[b]	7.7	12.4	68.5[b]	11.1	16.2
100 °C, 10 min	60[b]	9.8	16.3	65.4[b]	8.3	12.7	67.4[b]	11.1	16.6
100 °C, 30 min	54.6[b]	9.1	16.7	63.1[b]	9.7	15.3	65.6[b]	10.7	16.3

The activities in raw extracts (control) were set to 100%.
Values represent mean ± standard deviations (SD) of three independent determinations. CV = coefficient of variations. Within same column, means followed by letters 'a' ($P < 0.05$) and 'b' ($P < 0.01$) show statistical differences from the control group.

Table 7
Thermal effect on anti-DPPH activity in the vegetable extracts

Treatment conditions	Haruna			Chinese cabbage			White cabbage		
	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)
Raw (control)	100	15.3	15.3	100	20	20	100	15.6	15.6
50 °C, 10 min	94	15.1	16.1	95.8	19	19.8	97.9	15.4	15.7
50 °C, 30 min	87.1	12.3	14.1	85	15	17.6	93.4	15.5	16.6
75 °C, 10 min	76.2[a]	9.9	13	72.3	13.6	18.8	95.2	13.8	14.5
75 °C, 30 min	70.8[a]	7.7	10.9	63.2[a]	9.1	14.4	90.6	8.6	9.5
100 °C, 10 min	73.8[a]	6.5	8.8	57.3[a]	9.1	15.9	95.4	12.2	12.8
100 °C, 30 min	72.5[a]	6.5	9	54.9[a]	8.4	15	90.7	16.8	18.5

The activities in raw extracts (control) were set to 100%.
Values represent mean ± standard deviations (SD) of three independent determinations. CV = coefficient of variations. Within same column, means followed by letter 'a' ($P < 0.05$) show statistical differences from the control group.

Table 8
Thermal effect on inhibitory activity against hydroxyl radical in the vegetable extracts

Treatment conditions	Spinach			Komatsuna			Haruna			White cabbage		
	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)
Raw (control)	100	12	12	100	16.8	16.8	100	23	23	100	12.2	12.2
50 °C, 10 min	99	12.9	13	106	17.5	16.5	104	24.5	23.6	103	17.6	17.1
50 °C, 30 min	78.6	16.7	21.	97.5	24.1	24.7	95	22.6	23.8	96.2	21	21.8
75 °C, 10 min	34[c]	11	32.4	69.6[a]	13.6	19.5	69.8	15.1	21.6	90.5	15.7	17.3
75 °C, 30 min	31[c]	8.8	28.4	70.5[a]	10.9	15.5	53.4[a]	12	22.5	66.2[a]	5.5	8.3
100 °C, 10 min	26.1[c]	9.4	36	59.3[a]	4	6.7	50[a]	10.9	21.8	63.5[a]	5	7.9
100 °C, 30 min	24.7[c]	10.1	40.9	58.8[a]	5.4	9.2	45.9[a]	7.8	17	57.7[a]	3.7	6.4

The activities in raw extracts (control) were set to 100%.
Values represent mean ± standard deviations (SD) of three independent determinations. CV = coefficient of variations. Within same column, means followed by different letters 'a' ($P < 0.05$) and 'c' ($P < 0.001$) show statistical differences from the control group.

study also indicated that heating juice at 50 °C had minimal effect on the IA% values in all vegetables tested.

The activities of the samples on the viability of HL-60 cells are summarized in Fig. 4. Among the tested vegetables white cabbage showed potent anti-proliferative activity with cell viability less than 1% of the control under these experimental conditions. Komatsuna had the second highest activity with cell viability 20.5 ± 7% of the control. This value for 'chingensai' 'haruna', Chinese cabbage and spinach were 27.1 ± 3.4%, 28.4 ± 3.2%, 32.1 ± 2% and 65.4 ± 7.2%, respectively. For these assays we used a sample dilution of 1/10 in the culture media. In a preliminary study we found that, with the exception of white cabbage, samples diluted 1/20 or more had little effect on cell viability compared to control cells. Thermal treatment of raw juices increased the viability of HL-60 cells in comparison to that of the raw juice (Tables 9 and 10). Heat treatment at 100 °C for 10–30 min resulted in an 80% decrease in proliferation inhibition activity in spinach, 75% in 'komatsuna', 75% in 'haruna', 85% in 'chingensai' 60% in white cabbage and 70% in Chinese cabbage to their respective crude juices (100%). However, when exposed to mild heating, such as heating at 50 °C for 10–30 min, juices exhibited proliferation inhibition activity of about 90% of the control juices.

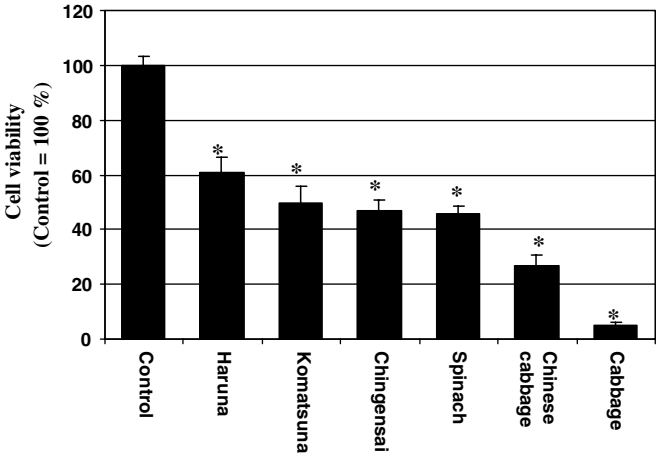

Fig. 4. Anti-proliferative activity of tested vegetable juices (raw) against the proliferation of HL-60 cells. Coefficient of variations percentage (CV%) for the samples are 8.9 (control), 11 (spinach), 6.4 (Chinese cabbage), 11.3 (haruna), 11.6 (chingensai), 34.8 (komatsuna), 74.5 (white cabbage). Anti-proliferative activity was determined by counting viable cells using trypan blue dye. Data points represent means ± SD of three independent determinations. Bars with asterisk '*' ($P < 0.001$) show statistical differences from the control group.

In this study the highest level of total phenolics was consistently found in raw juice, followed by juices heated at 50 °C, and then 75 and 100 °C. Heating juices at 75 °C for a longer period or at 100 °C for more than 10 min caused similar changes in phenolic content which are statistically significant in comparison to the raw juices. The effect, however, is minimal when vegetables are heated at milder conditions. Thermal conditions that occur during air drying, and home cooking are known to affect the phenolic content of some fruits and vegetable (Asami, Hong, Barrett, & Mitchell, 2003; Gorinstein et al., 2005). This study also shows that normal cooking conditions strongly affect the antioxidant activity of the green vegetables tested with the exception of white cabbage. The antioxidant activity of vegetables like spinach, 'komatsuna', 'haruna', 'chingensai' showed great sensitivity to cooking temperature. The results indicated that the thermo-labile components were responsible for the majority of the antioxidant activity in the juices. In contrast, white cabbage was relatively insensitive to thermal treatment. From this study, we found a relationship ($R = 0.94$) between total phenol content and anti-DPPH radical activity in vegetable juices. The degradation of phenolic compounds in response to thermal treatment shows somewhat resemblance with the behavior of the components responsible for antioxidant activity, in the tested vegetable juices.

HL-60 cells, a human promyelocytic leukemia cell line, has been used in many studies to investigate *in vitro* anti-proliferation activity of plant extracts. In this study, we used the HL-60 cell line as a model to study the inhibitory effect of vegetable extracts against tumor promotion or cancer cell proliferation. We found that all the samples prepared from vegetable extracts exhibited anti-proliferative effects on the viability of HL-60 cells, with white cabbage having the greatest activity. The activity was, however, insignificant except for white cabbage when the samples were diluted over 20 times in culture media. The results suggested that inhibition of human leukemia cells could

Table 9
Thermal effect on anti-proliferative activity in the vegetable extracts

Treatment conditions	Spinach			Komatsuna			Haruna		
	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)	Mean	SD (±)	CV (%)
Raw (control)	100.0	5.5	5.5	100.0	13.6	13.6	100	15	15
50 °C, 10 min	87.2	6.4	7.3	97.0	7.2	7.5	95	9	9.5
50 °C, 30 min	82.0	10.8	13.2	86.4	7.6	8.8	91	13	14.2
75 °C, 10 min	69.1[a]	8.3	11.9	68.5[a]	6.0	8.7	66[b]	8	12.1
75 °C, 30 min	43.4[c]	5.4	12.4	34.2[c]	6.4	18.7	42[b]	9	21.4
100 °C, 10 min	25.1[c]	6.8	27.2	27.0[c]	12.4	45.8	25[c]	9	36
100 °C, 30 min	21.6[c]	9.7	45.0	14.2[c]	5.5	38.9	23.5[c]	9	38.3

Anti-proliferative activities in raw extracts (control) were set to 100%.
Values represent mean ± standard deviations (SD) of three independent determinations. CV = coefficient of variations. Within same column, means followed by letters 'a' ($P < 0.05$), 'b' ($P < 0.01$), and 'c' ($P < 0.001$) show statistical differences from the control group.

Table 10
Thermal effect on anti-proliferative activity in the vegetable extracts

	Chingensai			White cabbage			Chinese cabbage		
	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)
Raw	100.0	8.2	8.2	100.0	2.6	2.6	100.0	5.5	5.5
50 °C, 10 min	95.4	5.3	5.6	92.7	5.6	6.0	92.1	6.5	7.1
50 °C, 30 min	78.9[b]	7.3	9.2	91.0	7.4	8.2	91.6	4.2	4.6
75 °C, 10 min	50.0[c]	9.6	19.2	56.5[c]	5.1	9.1	82.0[a]	5.2	6.3
75 °C, 30 min	33.5[c]	5.5	16.5	48.1[c]	3.1	6.4	60.9[c]	3.5	5.8
100 °C, 10 min	25.7[c]	12.4	48.1	41.0[c]	2.6	6.4	48.1[c]	7.9	16.4
100 °C, 30 min	23.7[c]	10.2	43.2	41.5[c]	3.1	7.4	30.7[c]	7.2	23.6

Anti-proliferative activities in raw extracts (control) were set to 100%.
Values represent mean ± standard deviations (SD) of three independent determinations. CV = coefficient of variations. Within same column, means followed by letters 'a' ($P < 0.05$), 'b' ($P < 0.01$), and 'c' ($P < 0.001$) show statistical differences from the control group.

not be explained solely by the phenolic contents. Therefore, it is assumed that unique substances in each vegetable are responsible to their anti-proliferative activities. For example, cruciferous vegetables like cabbage and cauliflower contain isothiocyanates, which have been shown to exhibit anticancer properties (Garikapaty et al., 2005; Jakubikova, Bao, & Sedlak, 2005). However, the vegetables like spinach which contain substantial amount of vitamin C, flavonoids, and carotenoids, have also been shown to inhibit cancer cell proliferation (Lomnitski, Bergman, Nyska, Ben-Shaul, & Grossman, 2003; Kuriyama et al., 2005). Results obtained from the thermally treated juices showed that cooking temperature strongly affects the cell proliferation inhibition activity in vegetable juices. White cabbage and Chinese cabbage showed a little more resistance to thermal treatment in terms of its thermal stability. Thus, it is speculated that cytotoxic components in dark green vegetables like spinach, 'komatsuna', and 'chingensai' are different from those of white cabbage or Chinese cabbage, which seem to be more stable towards thermal treatment.

In conclusion, this study shows that commonly consumed vegetables contain water-soluble components that posses antioxidant activity, based on two assays, and anti-proliferative activity against the HL-60 cell line. Heating vegetable juices at cooking temperatures strongly affects their phenolic contents and their antioxidant and anti-proliferative activities. The functional components of water-soluble fractions, when heated at mild thermal conditions, are preserved and these components are antioxidant and anti-proliferative in nature. Although, heating vegetables at mild thermal condition may not enough to inactivate microbes and enzymes to obtain safe and stable products, on an industrial scale, non-thermal processing techniques such as high pressure (HP), radiation or electric pulse can be combined with mild thermal treatment to ensure safety, quality and stability of the food products. And, the results obtained in this study may be useful to food manufacturers in evaluating thermal processing technology for food quality and to consumers who wish to adopt cooking techniques that are consistent with preserving the ability of vegetables to deliver their maximum health benefits.

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