Cabbage (Brassica oleracea L. var. capitata) Phytochemicals with Antioxidant and Anti-inflammatory Potential

Sami Rokayya, Chun-Juan Li, Yan Zhao, Ying Li, Chang-Hao Sun*

Abstract

Background: The objective of this study was to investigate antioxidant and anti-inflammatory activity of cabbage phytochemicals. Materials and Methods: Color coordinates were evaluated by colorimetry, and the antioxidant and anti-inflammatory activities were analyzed by spectrophotometer for some common cabbage varieties. Results: Red heads had the highest total antioxidant contents followed by Savoy, Chinese and green heads. The Chinese variety had the highest ABTS (2,2-azino-di-(3-ethylbenzthiazoline-sulfonic acid) antioxidant activity, which was 5.72 μmol TE/g fw (Trolox equivalent). The green variety had the highest DPPH (free radical scavenging activity) antioxidant activity, which was 91.2 μmol TE/g fw. The red variety had the highest FRAP (ferric reducing antioxidant power) antioxidant activity, which was 80.8 μmol TE/g fw. The total phenol amounts were 17.2–32.6 mM trolox equivalent antioxidant capacity (TEAC) and the total flavonoid amounts were 40.0–74.2 mg quercetin per gram. Methanolic extracts of different cabbage heads showed different anti-inflammatory activity values. Chinese, Savoy and green heads had the highest anti-inflammatory activity, while red heads had the lowest. Conclusions: The results suggest that these varieties of cabbage heads could contribute as sources of important antioxidant and anti-inflammatory related to the prevention of chronic diseases associated to oxidative stress, such as in cancer and coronary artery disease.

Keywords: Cabbage head - antioxidant - anti-inflammatory - cell
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stock solution (7 mM) was prepared and 2.45 mM of solution and absorbance readings at 734 + of the test solution, 6 μl of 1 mol/l potassium acetate (2,2-azino- of the test solution were + vacuumed amber before 3 was obtained by diluting the stock solution to 3000 g for 20 min at room temperature. The methanolic extracts were centrifuged at ~3000 g for 20 min. The procedure was repeated twice. The supernatant was pooled and centrifuged at 4°C. The concentrated sample was used as a sample extract for estimation of total antioxidant, phenol, flavonoid and antioxidant activities.

Total antioxidant capacity determination
The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid as a standard (Prasad et al., 2013). An aliquot of 20 μl of sample solution was mixed with 0.2 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results were expressed as μg of ascorbic acid equivalent (AAE) per mg of the dried weight of the sample, as determined from the equation of the standard calibration curve.

Phenolic content determination
The total phenol was estimated spectrophotometrically using Foline-Ciocalteu reagent (Prasad et al., 2013). To the 50 μl of the sample extract 140 μl of deionized water, 5 μl of reagent and 0.1 ml of 2% Na2CO3 solution were added. The mixture was allowed to stand for 30 min and absorption was measured at 750 nm against a reagent blank in SpectraMax M2 (Molecular Devices, USA). Results were expressed as mM trolox equivalent antioxidant capacity (TEAC).

Flavonoid content determination
The flavonoid content was determined on triplicate aliquots of the homogenous cabbage extract (1.5 g) (Ilahy et al., 2011). Thirty-microliter aliquots of the methanolic extract were used for flavonoid determination. Samples were diluted with 90 μl methanol, 6 μl of 10% Aluminum chloride (AlCl3), 6 μl of 1 mol/l potassium acetate (CH3CO2K) were added and finally 170 μl of methanol was added. The absorbance was read at 415 nm after 30 min. Quercetin was used as a standard for calculating the flavonoid content (mg Qe/g of fw).

Antioxidant activity determination
Trolox equivalent antioxidant capacity (TEAC) assay: Antioxidant activity was measured using the ABTS+ decoloration method using radical ABTS+ (2,2-azino-di-(3-ethylbenzothiazoline-sulphonic acid) (Kaur et al., 2013). The ABTS+ stock solution (7 mM) was prepared through the reaction of 7 mM ABTS+ and 2.45 mM of potassium persulphate as the oxidant agent. The working solution of ABTS+ was obtained by diluting the stock solution in methanol to give an absorption of 0.70 ± 0.02 at λ = 734 nm. Sample extract (10 μl) was added to 90 μl methanol, 140 μl deionized water, using Foline-Ciocalteu reagent (Prasad et al., 2013). An aliquot of 20 μl of sample solution was mixed with 0.2 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 734 nm against a blank. The results were expressed as μg of ascorbic acid equivalent (AAE) per mg of the dried weight of the sample, as determined from the equation of the standard calibration curve.

Materials and Methods

Chemicals and cells
Ascorbic acid, ABTS: 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), DPPH: 2,2-diphenyl-1-pirclyhydrazyl, FRAP, Quercetin, Trolox and Folin–Ciocalteau reagents, were from (Sigma Chemical Co., St. Louis, MO, USA). Dulbecco’s modified eagle medium (DMEM) and LPS were purchased from Sigma Inc. (St. Louis, MO, USA). The murine macrophage cell line, RAW 264.7 was purchased from Cell Bank of Institute of Biological Sciences (Shanghai, China).

Sample Preparation
The samples analyzed in the present study were four different cabbage head varieties (Red, Savoy, Green and Chinese). Red – Smooth red leaves, often used for pickling or stewing. Savoy – Characterized by crimped or curly leaves, mild flavor and tender texture. Green – Light to dark green, slightly pointed heads, this is the most commonly grown variety. Chinese – Broad green leaves with white petioles, tightly wrapped in a cylindrical formation and usually forming a compact head.

Cabbage heads were purchased in a local supermarket in Harbin-China at commercial maturity. All the heads were cleaned and cut into cubic of 10 * 10 * 10 mm3 before processing. Freeze-dried (FD) treatment was operated 2h at -80°C then put in freeze drying machine (ALPHA 1-4 LSC, Germany) at -50°C and 0.04 ambr for 48h. Samples were cleaned and cut into cubic of 10 * 10 * 10 mm3 in Harbin-China at commercial maturity. All the heads were cleaned and cut into cubic of 10 * 10 * 10 mm3 before processing. Freeze-dried (FD) treatment was operated 2h at -80°C then put in freeze drying machine (ALPHA 1-4 LSC, Germany) at -50°C and 0.04 ambr for 48h. Samples were ground to powder, packed in N2-vacuumed amber bottles and stored at -80°C until use.

Antioxidant extraction
Cabbage powder (1.5 g) of each sample were extracted with 10 ml of 80% methanol, by stirring and sonicating for 20 min. The methanolic extracts were centrifuged at ~3000 g for 20 min at room temperature. The procedure was repeated twice. The supernatant was pooled and stored at 4°C. The concentrated sample was used as a sample extract for estimation of total antioxidant, phenol, flavonoid and antioxidant activities.

Total antioxidant capacity determination
The total antioxidant capacity of the fractions was expressed as micromole Trolox equivalent per gram based on the freeze-dried weight (μmol TE/g fw).
Table 1. Total Antioxidant, Phenol and Flavonoid Contents of Selected Cabbage Varieties

<table>
<thead>
<tr>
<th>Cabbage Varieties</th>
<th>Total Antioxidant (µg AAE/mg)</th>
<th>Total Phenol (mM TEAC)</th>
<th>Total Flavonoid (mg Qe/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>10.6±0.61a</td>
<td>32.7±0.66a</td>
<td>74.2±6.77a</td>
</tr>
<tr>
<td>Savoy</td>
<td>9.34±0.38b</td>
<td>17.2±0.68c</td>
<td>51.1±5.89bc</td>
</tr>
<tr>
<td>Green</td>
<td>8.73±0.40b</td>
<td>16.5±0.41c</td>
<td>39.9±7.54c</td>
</tr>
<tr>
<td>Chinese</td>
<td>9.15±0.68b</td>
<td>22.2±0.73b</td>
<td>57.1±8.96b</td>
</tr>
</tbody>
</table>

*Values are the average of three individual samples each analyzed in triplicate ± standard deviation. Different uppercase superscript letters respectively indicate significant difference (*p < 0.05) analyzed by Duncan’s multiple-range test.

Figure 1. Antioxidant Activities (ABTS, DPPH and FRAP) Contents are Expressed as (µmol TE/g fw).

Values are the average of three individual samples each analyzed in duplicate ± standard deviation. Different uppercase superscript letters respectively indicate significant difference (*p < 0.05) analyzed by Duncan’s multiple-range test.

Free radical scavenging activity using (DPPH) assay: DPPH assay is based on the measurement of the scavenging ability of antioxidants toward the stable radical DPPH (Brand-Williams et al., 1995). A 0.2 ml aliquot of a 0.0062 mM of DPPH solution, in 20 ml methanol (95%) was added to 0.04 ml of each extract and shaken vigorously. Change in the absorbance of the sample extract was measured at 517 nm after 30 min. The percentage inhibition of DPPH of the test sample was expressed as a percentage of the absorbance of control.

Anti-inflammatory activity

Anti-inflammatory extraction: Extracts were prepared by homogenization of 4 g of freeze-dried sample in 10 ml of methanol 80%, using an Ultra Turrax Digital Homogeniser T-25 (Ika Werke GMBH & Co., Staufen, Germany). Supernatant was collected, and the pellet was extracted twice with 10 ml of methanol. Further, supernatants were combined and filtered using Whatman No. 1 paper. The concentrate was extracted twice, filtered, and evaporated vacuum to dryness. Finally, the residue was dissolved in dimethylsulfoxide (DMSO) solution to give a final concentration of 20 mg/ml.

Cell viability assay

Mitochondrial respiration, an indicator of cell viability, was determined by a mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, treated cells (1x105 cells/ml) were incubated with MTT (5 mg/ml) in 96-well plates for 4h, after which they were solubilized in DMSO (150 µl/well). The extent of the reduction of MTT within the cells was then quantified by measurement of the absorbance at 490 nm (Sladowski et al., 1993).

Measurement of NO production

For determination of the quantity of NO generated, the amount of NOX in the supernatant of the media was measured by the Griess method (Lee et al., 2009).

Briefly, cells were incubated for 24h, after which the cell culture medium (0.2 ml) were added to aqueous extract of cabbage varieties containing the Griess reagents (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride in 5% H3PO4). The NO production was then determined based on the absorbance at 540 nm.

Statistical Analysis

Data from replications of all varieties were subjected to a variance analysis (ANOVA) using SPSS 16.0. The Significant difference between the means was determined by Duncan’s New Multiple Range Test (*p < 0.05). The correlation between all studied parameters was determined by the principal compounds analysis (PCA) using XLSTAT software.

Results

Total antioxidant, phenol and flavonoid contents

In our study, the total antioxidant activity of cabbage varieties was determined by phosphomolybdate method and the results were expressed in (Table 1). All the extracts exhibited high total antioxidant activity values from 8.73 µg AAE/mg fw in Green to 10.59 µg AAE/mg fw in Red heads. In comparison with our cabbage varieties, total antioxidant value was lower than that cabbage value in other studies (Raghu et al., 2011). The antioxidant activity of the plant can be attributed to flavonoids and polyphenolic compounds found in it (Joseph et al., 2010). Total phenol and flavonoid contents were significantly different between the studied cabbage varieties (*p < 0.05). Total phenol contents ranged from 16.47 mM trolox equivalent antioxidant capacity (TEAC) in Green to 32.65 µm AAE/mg fw in Red.
mM trolox equivalent antioxidant capacity (TEAC) in Red heads. Green and Savoy total phenol had similar values, 16.47 mM (TEAC) and 17.23 mM (TEAC), respectively. Flavonoid contents ranged from 39.95 mg QE/g fw to 74.15 mg QE/g fw, Red variety had the highest total flavonoid content 74.15 mg QE/g fw followed by Chinese and Savoy varieties, Green variety had the lowest flavonoid contents 57.05 mg QE/g fw, 51.14 mg QE/g fw and 39.95 mg QE/g fw, respectively.

Antioxidant activity (ABTS, DPPH and FRAP)

Antioxidant activity results of cabbage varieties were expressed as μmol TE/g fw in (Figure 1). The antioxidant activity measured by ABTS+ assay was between 1.11 μmol TE/g fw for Red and 5.72 μmol TE/g fw for Chinese Varieties. (Kusznierewicz et al., 2008) had mentioned five different common varieties of cabbage grown in different locations in Europe. They concluded from 2.92 μmol TE/g fw to 8.13 μmol TE/g fw as the mean value of Trolox equivalent antioxidant capacity for these varieties, respectively. The results obtained by DPPH assay were between 3.01 μmol TE/g fw for Chinese Variety and 91.19 μmol TE/g fw for Green varieties. In comparison with our cabbage varieties, the free radical scavenging activity value was similar to these cabbage values (Kusznierewicz et al., 2008). The values of cabbage varieties obtained by FRAP assay were between 16.39 μmol TE/g fw for Savoy and 80.87 μmol TE/g fw for Red varieties. However, the antioxidant capacity also depends on several other factors including genetics, environmental conditions, production techniques used, date of harvest and post harvest storage conditions (Dumas et al., 2003).

Anti-inflammatory activity

The cytotoxicities of cabbage extracts in LPS-induced macrophages were evaluated in a range 0–200 μg extract/ml using MTT reduction assay after 24h of incubation (Figure 2). Therefore, results indicated that the range of concentrations used in this study to treat the cells did not exert any cytotoxic effect. Analysis of NO production by measuring the nitrite with the Griess reaction revealed that placing unstimulated RAW 264.7 cells in culture medium for 24h produced a basal amount of nitrite. When the cells were incubated with extracts from these varieties after treatment with LPS for 24h, the medium concentration of nitrite increased markedly. Excessive production of NO in macrophages represents a potentially toxic effect which, if not counteracted, causes the onset
or progression of many disease pathologies (Evans et al., 2006). Significant concentration-dependent inhibition of NO production was detected when cells were co treated with LPS and various concentrations of the four variety extracts (Figure 3). Cabbage extracts induced a significant (P < 0.05) dose-dependent suppression of NO production.

All tested extracts showed high anti-inflammatory value in a range 0–100 μg concentration where Chinese, Savoy and Green heads had the highest anti-inflammatory activity; while Red heads had the lowest anti-inflammatory activity value. These results indicate that cabbage has a noticeable effect on scavenging free radicals. This could be attributed to its high content of phenolic compounds. It has been documented that antiradical scavenging activity is related to substitution of hydroxyl groups in the aromatic rings of phenolics, thus contributing to their hydrogen-denoting ability (Yen et al., 2005; Evans et al., 2006). Anti-inflammatory of all the tested extracts showed a little high values. NO production value did not grow equally in a dose dependant manner in all varieties except in Chinese Variety. A different reaction course was found for Red, Savoy and Green. They showed inverse relationship between the anti-inflammatory and the dose dependant manner. The reaction of Red variety with NO production was similar to Savoy variety with NO production. Anti-inflammatory activity has been reported (Murakami et al., 2005; Penas et al., 2012).

Discussion

Correlation between antioxidant and anti-inflammatory activities: Green cabbage produced the lowest antioxidant level 8.73 μg AAE/mg fw with a little high anti-inflammatory activity level 7.49 μg/ml (Figure 4). Chinese cabbage 9.15 μg AAE/mg fw and Savoy cabbage 9.34 μg AAE/mg fw produced a little high antioxidant and the highest anti-inflammatory activity levels 8.52 μg/ml and 7.77 μg/ml fw, respectively. The highest antioxidant level in Red cabbage 10.59 μg AAE/mg fw produced the lowest anti-inflammatory activity 2.42 μg/ml.

Principal component analysis: Antioxidant and anti-inflammatory activities measurements of cabbage heads had been submitted to Principal Component Analysis (PCA) to presence of four subspecies of cabbage heads. From this analysis, the following axes of inertia had been withheld, as seen in (Table 2). The structuring accessions showed 90.87% of total variation. Axes were retained because they expressed 69.67% (axes 1), 21.21% (axes 2). Axes 2 was made positively by Trolox equivalent antioxidant capacity using ABTS+ assay and Cell viability. The inertia was made negatively by free radical scavenging activity using DPPH assay. Data projection on plans as defined by inertia axes of PCA from cabbage head samples showed significant differences between varieties. The (Figure 5 and Figure 6) present the plots of the scores and the correlation loadinds respectively. In fact, when applying principal component analysis it seemed that there was a discriminate structure. Green and Savoy cabbage varieties were grouped together. The Red and Chinese cabbage varieties were individualized.

In summary, several of the cabbage varieties proved to be highly active. Furthermore, the results of this study indicated that the activity of different varieties differed within different growing districts, cultivated using different conditions, or subjected to different post-harvest treatments. Taken together, the results of this study provide an experimental basis for the development of new strategies to produce highly functional plants and food items. Although these results warrant further in-vivo studies, the presented in-vitro data suggest the potential of cabbage to attenuate oxidative stress and inflammation.

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