

RESEARCH ARTICLE

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, was 5.72 $\mu\text{mol TE/g fw}$ (Trolox equivalent). The green variety had the highest DPPH (free radical scavenging activity) antioxidant activity, which was 91.2 $\mu\text{mol TE/g fw}$. The red variety had the highest FRAP (ferric reducing antioxidant power) antioxidant activity, which was 80.8 $\mu\text{mol TE/g fw}$. 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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Introduction

Cabbage (*Brassica oleracea* L. var. capitata) is one of the most important vegetables grown worldwide. It belongs to the family Cruciferae, which includes broccoli, cauliflower, and kale. The different cultivated types of cabbage show great variation in respect of size, shape and color of leaves as well as the texture of the head (Singh et al., 2006). Approximately 6.3 kg of *Brassica* vegetables are consumed per person annually (Jordbruksverket, 2003). Cabbage is consumed either raw or processed in different ways, e.g., boiled or, fermented or, used in salads. Due to its antioxidant, anti-inflammatory and antibacterial properties, cabbage has widespread use in traditional medicine, in alleviation of symptoms associated with gastrointestinal disorders (gastritis, peptic and duodenal ulcers, irritable bowel syndrome) as well as in treatment of minor cuts and wounds and mastitis. Fresh cabbage juice, prepared either separately or mixed with other vegetables such as carrot and celery, is often included in many commercial weight-loss diets (Samec, 2011), diets that improve the bioavailable content of nonheme iron (Chiplonkar et al., 1999), as well as alternative therapies for cancer patients (Maritess et al., 2005). Clinical research has shown positive effects of cabbage consumption in

healing peptic ulcers (Cheney, 1949), and facilitating the reduction of serum LDL levels (Suido et al., 2002). Chemical components analysis has shown that the main constituents of cabbage are carbohydrates, comprising nearly 90% of the dry weight, where approximately one third is dietary fiber and two thirds are low-molecular-weight carbohydrates (LMWC). Other characteristic components are glucosinolates (Wennberg et al., 2006). Interest in the role of free radical scavenging-antioxidants in human health has prompted research in the fields of horticulture and food science to assess the antioxidant phytochemicals in fruits and vegetables. Some studies have been conducted to quantify the phenolic compounds, carotenoids, vitamin C, and antioxidant potential (Nilsson et al., 2006; Kusznierevicz et al., 2008). The antioxidant properties were tested in many studies by using different approaches (Liu et al., 2008; Zanfini et al., 2010). The content of antioxidants depends on a lot of factors, especially on cultivars, stage of maturity and growing conditions (Hart and Scott, 1995). These antioxidants exist in nature in combination, and in combination they certainly cooperate on total antioxidant activity. The functional quality and antioxidant constituents of cabbage heads are strongly influenced by environmental factors and genetics. The ferric reducing antioxidant power (FRAP),

Department of Nutrition and Food Hygiene, School of Public Health, Harbin Medical University, Harbin, Heilongjiang, China
*For correspondence: sun2002changhao@yahoo.com

Trolox equivalent antioxidant capacity (TEAC) and Free radical scavenging activity (DPPH) assays are the three most frequently used for assessing the antioxidant activities (Magalhaes et al., 2008).

Different botanical compounds gained attention as therapeutic agents that relieve pain and inflammation (Hernandez-Ortega et al., 2013).

The present study, therefore aims to determine the total antioxidant, flavonoid and phenol levels, and measure the antioxidant and anti-inflammatory activities such as (FRAP), (TEAC), (DPPH), Cell viability (MTT) and NO production. Results from this study will aid the program of selecting unique cabbage varieties for improved nutritional value and will provide insight into the mechanisms of the antioxidant and anti-inflammatory.

Materials and Methods

Chemicals and cells

Ascorbic acid, ABTS: 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP, Quercetin, Trolox and Folin-Ciocalteu 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 $10 * 10 * 10 \text{ mm}^3$ 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 grounded to powder, packed in N_2 -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 $\sim 3000 \text{ g}$ 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

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% Na_2CO_3 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 (AlCl_3), 6 μl of 1mol/l potassium acetate ($\text{CH}_3\text{CO}_2\text{K}$) 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).

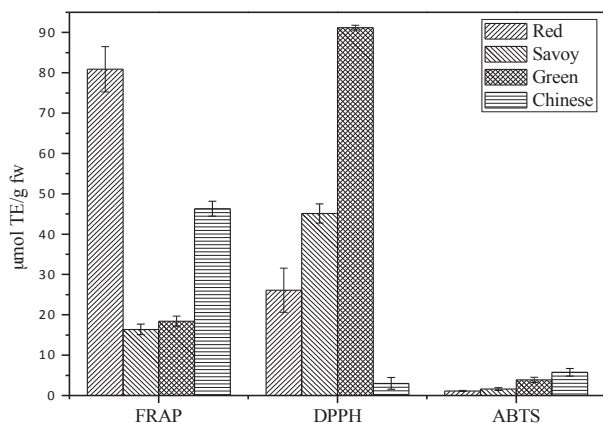
Antioxidant activity determination

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 $\lambda = 734 \text{ nm}$. Sample extract (10 μl) was added to 90 μl of ABTS⁺ solution and absorbance readings at 734 nm were taken at 30°C for exactly 10 min after initial mixing. The percentage inhibition of ABTS⁺ of the test sample and known solutions of Trolox were calculated by the following formula: % Inhibition = $100 \times (A_0 - A) / A_0$ where A_0 was the beginning absorbance at 734 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the test sample at 734 nm. The calibration curve between % inhibition and known solutions of Trolox (100-2000 μM) was then established. The radical-scavenging activities of the test samples were expressed as micromole Trolox equivalent per gram based on the freeze-dried weight ($\mu\text{mol TE/g fw}$).

Table 1. Total Antioxidant, Phenol and Flavonoid Contents of Selected Cabbage Varieties

	Total Antioxidant $\mu\text{g AAE/mg}$	Total Phenol mM (TEAC)	Total Flavonoid mg Qc/g
Red	10.6 \pm 0.61a	32.7 \pm 0.66a	74.2 \pm 6.77a
Savoy	9.34 \pm 0.38b	17.2 \pm 0.68c	51.1 \pm 5.89bc
Green	8.73 \pm 0.04b	16.5 \pm 0.41c	39.9 \pm 7.54c
Chinese	9.15 \pm 0.68b	22.2 \pm 0.73b	57.1 \pm 8.96b

*Values are the average of three individual samples each analyzed in triplicate \pm 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 ($\mu\text{mol TE/g fw}$).**

Values are the average of three individual samples each analyzed in duplicate \pm 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 and known solutions of Trolox were calculated by the following formula: % Inhibition = $100 \times (A_0 - A) / A_0$ where A_0 was the beginning absorbance at 517 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the sample extract at 517 nm. Methanol (95%) was used as a blank. Results were expressed as $\mu\text{mol TE/g fw}$.

Determination of ferric reducing antioxidant power (FRAP) assay: FRAP was performed according to the procedure (Re et al., 1999). The FRAP reagent included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM FeCl_3 in the ratio 10:1:1 (v:v:v). Three ml of the FRAP reagent was mixed with 100 μl of sample extract in a test tube and vortexed and incubated at 37°C for 30 min in a water bath. Reduction of the ferric-tripyridyltriazine to the ferrous complex formed an intense blue color which was measured at 593 nm at the end of 4 min. Results were expressed in terms of $\mu\text{mol TE/g fw}$.

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 (1×10^5 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 NO_2 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% H_3PO_4). 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 $\mu\text{g AAE/mg fw}$ in Green to 10.59 $\mu\text{g AAE/mg fw}$ in Red heads. In comparison with our cabbage varieties, total antioxidant value was lower than that cabbage value used in other study (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

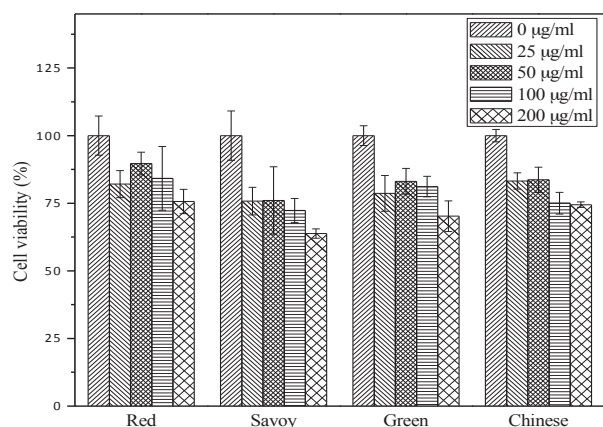


Figure 2. Cell Viability is Expressed as (%). Values are the average of three individual samples each analyzed in duplicate \pm standard deviation. Different uppercase superscript letters respectively indicate significant difference ($p < 0.05$) analyzed by Duncan's multiple-range test

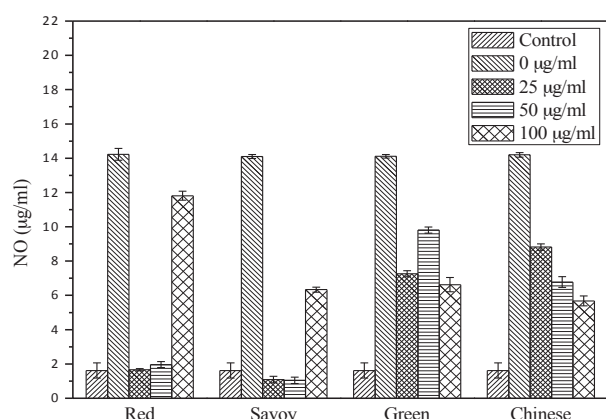


Figure 3. NO Production by RAW 264.7 Cells. Values are expressed as $\mu\text{g/ml}$ and presented as mean \pm standard deviation of three individual experiments. The asterisk indicates treatment group which produces statistically significantly ($p < 0.05$) different cytokine induction when compared to the control group (no treated cells)

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 $\mu\text{mol TE/g fw}$ in (Figure 1). The antioxidant activity measured by ABTS⁺ assay was between 1.11 $\mu\text{mol TE/g fw}$ for Red and 5.72 $\mu\text{mol TE/g fw}$ 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 $\mu\text{mol TE/g fw}$ to 8.13 $\mu\text{mol TE/g fw}$ as the mean value of Trolox equivalent antioxidant capacity for these varieties, respectively. The results obtained by DPPH assay were between 3.01 $\mu\text{mol TE/g fw}$ for Chinese Variety and 91.19 $\mu\text{mol TE/g fw}$ for Green varieties. In comparison with

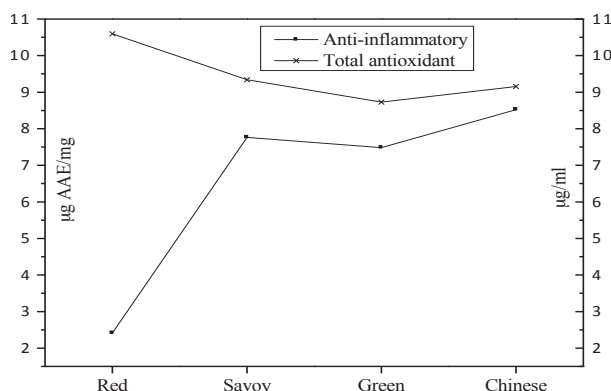


Figure 4. Correlation Between Antioxidant and Anti-inflammatory Activities. Two independent trials were carried out in triplicate

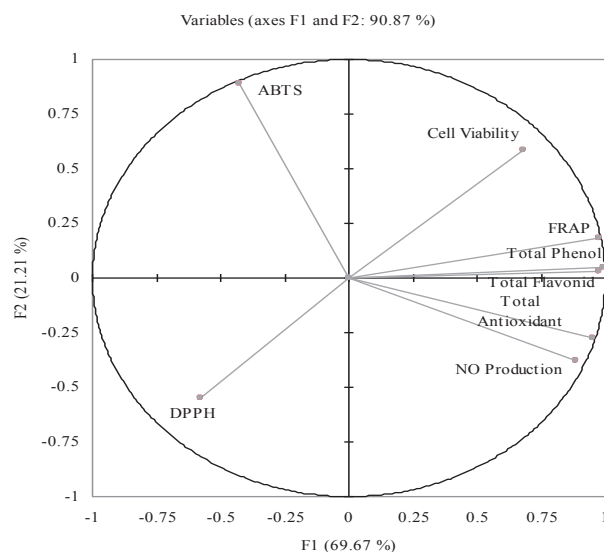


Figure 5. Plots of the Scores for Antioxidant and Anti-inflammatory Activities Content

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 $\mu\text{mol TE/g fw}$ for Savoy and 80.87 $\mu\text{mol TE/g fw}$ 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 $\mu\text{g extract/ml}$ 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

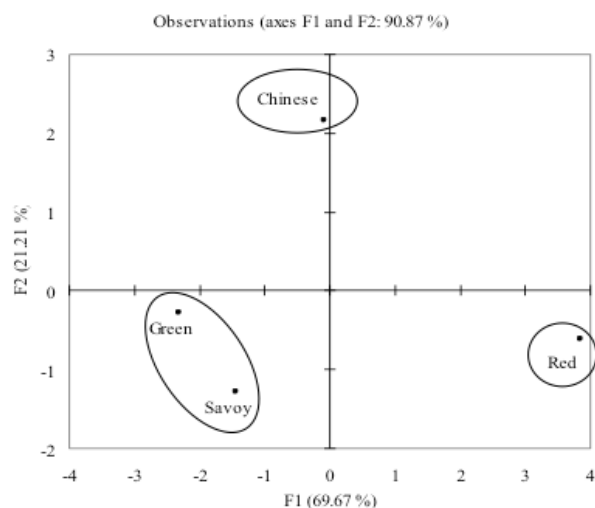


Figure 6. Plots of the x- loadings Antioxidant and Anti-inflammatory Activities Content

Table 2. Discriminate Variables Factors of Principal Components Analysis

	F1	F2
Eigen Value	5.67	1.7
Variability (%)	69.7	21.2
Cumulative (%)	69.7	90.9
Total Antioxidant	+16.4	-
Total Phenol	+17.8	-
Total Flavonoid	+17.1	-
FRAP	+17.1	-
DPPH	-	-17.7
ABTS	-	+46.9
Cell Viability	+14.0	-
NO Production	-	+20.3

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-donating 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 $\mu\text{g}/\text{ml}$ (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 $\mu\text{g}/\text{ml}$ and 7.77 $\mu\text{g}/\text{ml}$ fw, respectively. The highest antioxidant level in Red cabbage 10.59 μg AAE/mg fw produced the lowest anti-inflammatory activity 2.42 $\mu\text{g}/\text{ml}$.

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 summery, 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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