

Partial characterization of white cabbages (*Brassica oleracea* var. *capitata* f. *alba*) from different regions by glucosinolates, bioactive compounds, total antioxidant activities and proteins

Barbara Kuszniereicz^a, Agnieszka Bartoszek^b, Lidia Wolska^a, Jerzy Drzewiecki^c,
Shela Gorinstein^d, Jacek Namieśnik^a

^a Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology, 11/12 G., Narutowicza Str., 80 952 Gdańsk, Poland

^b Department of Pharmaceutical Technology and Biochemistry, Chemical Faculty, Gdańsk University of Technology, 11/12 G. Narutowicza Str., 80 952 Gdańsk, Poland

^c Plant Breeding and Acclimatization Institute, Radzikow, Poland

^d Department of Medicinal Chemistry and Natural Products, The Hebrew University, Hadassah Medical School, POB 12065, Jerusalem 91120, Israel

Abstract

Glucosinolates (GLS), antioxidative compounds, total radical scavenging activities (TRSAs) and proteins of white cabbage samples derived from different regions of Europe, collected in the spring and autumn, were studied. Glucobrassicin and sinigrin were the dominating GLS in all analyzed cabbage samples. Depending on origin, these two GLS accounted for ~30% to ~70% of the total. The total GLS content ranged from 3.3 to 7.7 $\mu\text{mol/g dw}$ in lyophilized vegetables. Assays based on electron transfer [total phenols by Folin-Ciocalteu reagent (FCR), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH)] were used to compare the TRSAs and the main bioactive compounds in cabbage. Total polyphenols varied from 2.4 to 4.9 GAE/g dw. The TRSAs ranged from 2.7 to 8.2 $\mu\text{mol TE/g dw}$ in ABTS test and from 2.4 to 5.4 $\mu\text{mol TE/g dw}$ in DPPH assay. The maximum amount of polyphenol compounds, antioxidant activity, as well as total GLS content, were recorded in Belgian cabbage harvested in the autumn and the lowest ones were found for Poland 2 cabbage harvested in the spring. In extracted and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) proteins from cabbage leaves only in samples from England and Belgium some differences in patterns were found in the regions of 60 and 97 kDa. The calculated correlations between antioxidative potency and the abundance of bioactive compounds were highly statistically significant. This suggests that TRSA could serve as a means of standardization of natural mixtures, at least in the case of cabbage, necessary to compare results of biological studies carried out for vegetable derived samples.

Keywords: White cabbage; Bioactive compounds; Antioxidant activity; Glucosinolates; Proteins

1. Introduction

Experimental and clinical as well as population studies confirmed the benefits of diet rich in fruits and vegetables in prevention of cardiovascular diseases, cancer, hypertension, diabetes and obesity. In particular, several epidemiological studies report an inverse correlation between consumption of *Brassicaceae* and risk of cancer (Verhoe-

ven, Goldbohm, Poppel, Verhagen, & Brandt, 1996; Heber & Bowerman, 2001; Ambrosone et al., 2004). Cruciferous vegetables such as cabbage are among the most important dietary vegetables consumed in Europe owing to their availability in local markets, cheapness and consumer preference. The mechanism of chemopreventive action of cruciferous vegetables is still not fully clarified, however many animal and human intervention studies suggest that the substances present in these plants, especially glucosinolates (GLS) and products of their decomposition, are able to modulate activity of phase I and II enzymes. GLS

degradation products are believed to act as anticarcinogens by decreasing carcinogen activation through the inhibition of phase I enzymes, while increasing detoxification by induction of the phase II enzymes that affect xenobiotic transformations. These compounds were also shown to inhibit tumor cell growth and to stimulate apoptosis (Prestera, Zhang, Spencer, Wilezak, & Talaly, 1993; Nugon-Baundon & Rabot, 1994; Johnson, 2002).

Though less extensively studied, the protective effects against chronic diseases could also depend on the antioxidant activity of compounds present in cruciferous vegetables. Although some studies have been conducted to determine the antioxidant activity, the content of polyphenols and flavonoids as well as other antioxidants in white cabbage (Heimler, Vignolini, Dini, Vincieri, & Romani, 2006; Singh et al., 2006; Stratil, Klejduš, & Kuban, 2006; Reyes, Villarreal, & Cisneros-Zevallos, 2007) is still not well known and their identification not sufficient.

It seems, however, that such data will be of great value, as current knowledge indicates that the occurrence of at least some of mentioned above diseases as well as aging processes may result from oxidative stress leading to a variety of alterations within the human organism caused by reactive oxygen species (ROS). Oxidative stress occurs when the generation of ROS in a system exceeds the system's ability to neutralize and eliminate them. The imbalance can result from a lack of antioxidant capacity caused by disturbance in production, distribution, or by an overabundance of ROS from an environmental or behavioral stressor. If not controlled properly, the excess of ROS can lead to damage of cellular lipids, proteins or DNA, impairing their normal function. The organism defends itself against ROS by engaging several enzymatic systems and endogenous antioxidants. This natural defence is enhanced by antioxidants delivered with diet. Considering the chemical diversity of the antioxidant compounds present in foods and the interaction occurring among these different molecules, the evaluation of the total antioxidant capacity of foods seems to be a more useful marker than the evaluation of individual compounds (Lotito & Frei, 2004). However, no single method to test the total antioxidant capacity of foods fully considers, at the same time, the activity of all the antioxidants (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002; Yu et al., 2002; Lotito & Frei, 2004). Therefore, in the studies described here we used an array of techniques to estimate antioxidative properties and the types of polyphenols present in cabbage samples studied and compare them with the abundance of GLS, the most important bioactive phytochemicals in cruciferous vegetables. The methods were thus selected to provide also some information on composition of cabbage antioxidants.

The role of all plant proteins is very important in the human metabolism (Krajcovicova-Kudlackova, Dusinska, Valachovicova, Blazicek, & Paukova, 2006). In only two reports isoenzymes and seed storage proteins have been used as genetic biochemical markers for characterization

and cultivar identification in oil seed rape (Gupta & Röbbelen, 1986; Mündges, Köhler, & Friedt, 1990). From the point of the possible importance of the cabbage proteins, as one of the indices for its characterization, isolation and identification was done in the present report.

This study was planned to characterize the white cabbages derived from different regions of Europe by the quantification of the GLS, initial characterization of antioxidant phytochemicals (polyphenols, flavonoids, flavanols and anthocyanins), the antioxidant activities and the protein profiles.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (FCR), sodium dodecyl sulfate (SDS), β -mercaptoethanol (β -ME), acrylamide, polyacrylamide, Coomassie Brilliant Blue R and molecular weight marker (14–205 kDa), sulphatase from *Helix Pomatia*, were purchased from Sigma (Germany), sinigrine from Fluka (Germany), glucotropaeolin from AppliChem (Germany). Deionized water was used throughout.

2.2. Samples

The cabbages (*Brassica oleracea* var. *capitata* f. *alba*) were harvested in the period of autumn 2005 and purchased from supermarkets in Leeds (England), Brussels (Belgium), Hamburg (Germany) and Gdansk (Poland 1). Additionally, the cabbage from Poland harvested in the spring 2005 (Poland 2) was analyzed. The foreign cabbages were transported to Gdansk by airmail. The preparation of cabbage extracts was carried out as soon as they reached the laboratory.

2.3. Preparation of extracts

The shredded cabbages were freeze-dried (Alpha 2–4 Christ) and then ground to powder. The powder was stored at -20°C until extraction of antioxidant phytochemicals. Portions of 1 g of all freeze-dried samples were extracted three times with methanol (4 ml). The extracts portions were combined and centrifuged at $10000 \times g$ for 5 min at room temperature. These extracts were used for determination of antioxidative activity and the content of different polyphenols.

2.4. Determination of antioxidative activity-TRSA

The antioxidative potential of cabbage extracts was determined by two complementary radical scavenging assays (ABTS and DPPH). In both cases, Trolox solution was used to generate the standard line (initial concentration 0–1.6 mmol/l) and TRSA were expressed as Trolox equivalents per g dw.



(1) 2,2'-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt (ABTS^{•+}): ABTS^{•+} radical cation was generated by the interaction of ABTS (7 mmol/l) and K₂S₂O₈ (2.45 mmol/l). The mixture was allowed to stand at room temperature for 12 h to give a dark green solution. This solution was diluted with methanol until the absorbance reached 0.7 at 734 nm (Huang, Ou, & Prior, 2005). One milliliter of the resulting solution was mixed with 10 µl of methanolic extracts from cabbage.

(2) 1,1-Diphenyl-2-picrylhydrazyl method (DPPH): DPPH solution (3.9 ml, 25 mg/l) in methanol was mixed with cabbage extracts (0.1 ml). The reaction progress was monitored at 515 nm until the absorbance was stable (Huang et al., 2005).

2.5. Determination of GLS

GLS content in the samples was assessed by the EU official method (ISO 9167-1), based on the HPLC analysis of desulfo-GLS obtained through the removal of the sulfate group of GLS via catalyzed hydrolysis of sulfate. Briefly, 500 mg sample of freeze-dried material was extracted twice with boiling methanol (3 ml). The known amount of glucotropaeolin was added to each sample just before the first extraction as an internal standard for the HPLC analysis. The extract portions were combined to give a final volume of 6 ml. The extracted GLS were purified on 0.5 ml column filled with 0.5 ml of DEAE-Sephadex A-25 anion-exchange resin (Sigma Aldrich, Germany). The column was washed with 2 ml imidazole formate (6 M) and twice with 1 ml Millipore water and then loaded with 6 ml of each extract. Purified sulphatase (75 µl) was added to the column and it was incubated overnight at room temperature. The second day, the desulfo-GLS were eluted with Millipore water (2 × 1 ml) and finally injected into HPLC. Desulfo-GLS were analyzed using an Agilent Model 1100 HPLC system with a LiChrosphere RP-18e column (250 × 3 mm, 5 µm). Chromatography was performed with 1 ml/min flow rate at 30 °C by eluting with gradient of water (A) and acetonitrile/water (20:80, v/v, B) as follows: isocratically 100% A for 1 min, linear gradient to 25% B for 25 min, and linear gradient to 100% A for 5 min. Elution of desulfo-GLS was monitored at 229 nm. The desulfo-GLS were identified using sinigrin and glucotropaeolin as standards and the standard GLS pattern in rapeseed samples (the certified reference material was BCR-367 R, rapeseed). The total GLS level expressed as µmol/g dw was determined by adding up all individual GLS, as suggested by Linsinger, Kristiansen, Beloufa, Schimmel, and Pauwels (2001) for the certification of the total GLS levels in rapeseed.

2.6. Determination of polyphenols, flavonoids, flavanols and anthocyanins

To determine the total content of polyphenols in cabbage extracts, the FCR was used and the measurement

was performed at 765 nm with gallic acid as the standard. The results were expressed as mg gallic acid equivalent (GAE) per g dw (Singleton, Orthofer, & Lamuela-Raventos, 1999).

Flavonoids (extracted with 5% NaNO₂, 10% AlCl₃ × 6H₂O and 1 M NaOH) were measured at 510 nm and in this case (+)-catechin served as a standard. The flavonoid content was expressed as mg of catechin equivalents (CE) per g dw (Singleton et al., 1999).

The total flavanol content was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method. Cabbage extracts (0.2 ml), diluted 1:100 with MeOH, were transferred to a 1.5-ml Eppendorf tube, and 1 ml of DMACA solution (0.1% in 1 N HCl in MeOH) was added. The mixture was vortexed and allowed to react at room temperature for 10 min. The absorbance at 640 nm was then read against a blank prepared similarly but without DMACA. The concentration of total flavanols was estimated from a calibration curve for catechin. Results were expressed as µg CE per g dw (Arnous, Makris, & Kefalas, 2001).

The total anthocyanins were measured by a pH differential method (Cheng & Breen, 1991). Absorbance was measured in a Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 29 600. Results were expressed as mg of cyanidin-3-glucoside equivalent (CGE) per g dw.

2.7. Protein extraction and electrophoresis

Total proteins from defatted lyophilized cabbage samples (20 mg each) were extracted with 500 µl of sample buffer (0.0625 M Tris-HCl, pH 6.25, containing 2% of SDS, 10% glycerol, 5% of mercaptoethanol (2-ME) and 0.001% bromophenol blue. The protein extracts were allowed to stand overnight at room temperature. Samples were boiled for 5 min, and then centrifuged at 18 000 g for 15 min at 15 °C. Each step was repeated twice.

A Hoeffer SE-600 apparatus (Hoeffer Pharmacia Biotech Inc., San Francisco, CA, USA) was used for SDS-PAGE electrophoresis. The Laemmli (1970) method was used: the resolving gel was 12.7% total acrylamide (T) and 1.3% cross linker (C) and the stacking gel was 6% T and 1.7% C. The gel size was 140 × 160 × 1.5 mm. Supernatants (20 µl) were loaded on gel. The run was carried out at constant current 25 mA per gel. Gels were stained with 0.25% Coomassie Brilliant Blue G-250 in methanol/water/glacial acetic solution (5:5:1 v/v) and destained in 1% solution of Brij 35. The following molecular weight markers (Sigma) were used: myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3 phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa), α-lactalbumin (14 kDa).

2.8. Statistical analyses

The values are means \pm SD of three measurements. Where appropriate, data were tested by two-way ANOVA using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA), followed by Duncan's new multiple range test to assess differences between group means. Differences of $P < 0.05$ were considered significant.

3. Results

White cabbage is the major plant food ingredient in Northern hemisphere, yet most surprisingly its chemical composition as regards chemopreventive non-nutrients has not been thoroughly studied. Especially, the types and content of antioxidative components are only primarily recognized. Therefore, we have undertaken research on antioxidative activity of white cabbage and initial characterization of polyphenolic antioxidants present in this vegetable. The major objective was to study the variation in the contents of these compounds between white cabbages derived from different regions of Europe and the possible interrelationships between different bioactive compounds. In most previous reports only one class of bioactive compounds has been examined in cabbage, that is GLS and their breakdown products. GLS content in cabbage was studied by several authors (Kushad et al., 1999; Johnson, 2002; Ciska & Pathak, 2004; Nilsson et al., 2006; Oerlemans, Barret, Bosch Suades, Verkerk, & Dekker, 2006; Wennberg, Ekvall, Olsson, & Nyman, 2006). They are of particular interest in food research because of alleged anticarcinogenic properties.

Therefore, we compared the abundance of polyphenolic compounds and antioxidative activity with the content and composition of GLS in cabbage samples analyzed.

3.1. Total radical scavenging activity

The results of the determinations of TRSA of cabbage samples examined are summarized in Fig. 1. The values of TE determined by ABTS were slightly higher than those obtained for the same extracts by DPPH method. TE values obtained in the present study are comparable with that published by Stratil et al. (2006). In both methods employed, the cabbage from Belgium displayed the highest antioxidative potential (8.23 $\mu\text{mol/g}$ by ABTS; 5.42 $\mu\text{mol/g}$ by DPPH). There were no significant differences between the TE values for cabbages from England (4.60 $\mu\text{mol/g}$ by ABTS; 3.53 $\mu\text{mol/g}$ by DPPH), Germany (4.65 $\mu\text{mol/g}$ by ABTS; 3.05 $\mu\text{mol/g}$ by DPPH) and Poland (4.90 $\mu\text{mol/g}$ by ABTS; 3.31 $\mu\text{mol/g}$ by DPPH). The lowest ability to scavenge ABTS and DPPH radicals displayed cabbage Poland 2.

3.2. GLS content

The contents of total GLS in cabbage samples are shown in Fig. 1. As can be seen, the total GLS content, similarly

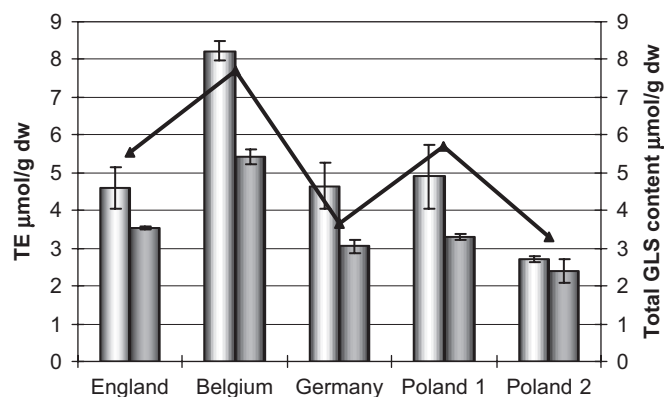


Fig. 1. Total antioxidant activity (TE $\mu\text{mol/g dw}$) of cabbage extracts as determined by ABTS (light grey) and DPPH (dark grey) assays and total content of glucosinolates ($\mu\text{mol/g dw}$) in cabbage samples studied. Results are means \pm SD of three independent measurements.

as antioxidative activity, was significantly higher for cabbage from Belgium compared to the rest of samples from different regions. HPLC profiles of individual GLS and their contents in investigated cabbage samples are presented in Table 1 and Fig. 2, respectively. Glucobrassicin and sinigrin were the dominating GLS in all cabbage samples (Table 1, Fig. 2). Depending on origin, these two GLS accounted for $\sim 30\%$ to $\sim 70\%$ of the total. In the present study the highest content of total GLS was found in Belgian cabbage (7.7 $\mu\text{mol/g dw}$) and was similar to the one observed by Nilsson et al. (2006) who reported the range from 6.40 to 16.3 $\mu\text{mol/g dw}$, while about twice lower than that observed by Kushad et al. (1999) and Wennberg et al. (2006). This discrepancy may stem from the fact that GLS can be hydrolyzed by enzymes present in leaves—myrosinase, but first the enzyme has to be released from plant cells. The lower content of GLS can be caused as a result of loss of a certain part of GLS during shredding of cabbage leaves before lyophilization.

3.3. Correlation

Based on the experimental data, a correlation between antioxidant activities determined by ABTS and DPPH assays was carried out and is presented in Fig. 3. A very good correlation ($R^2 = 0.9628$) was observed between the levels of Trolox equivalents ($\mu\text{mol/g dw}$) determined by ABTS and DPPH scavenging assays (Fig. 3).

3.4. Bioactive compounds and correlation with TRSA

The total contents of polyphenols, anthocyanins, flavanols and flavonoids in cabbage samples summarized in Table 2 were estimated based on reactions with specific substrates or assays dedicated to measurements of antioxidant activity of a particular type of compounds. The variation of total phenolics (from 4.91 to 2.41 mg GAE/g dw), flavonoids (from 1.18 to 2.42 mg CE/g dw), flavanols

Table 1
GLS content ($\mu\text{mol/g dw}$) in cabbages derived from different sources

GLS	England	Belgium	Germany	Poland 1	Poland 2
Glucobrassicin ($\mu\text{mol/g dw}$)	0.26 ± 0.02^b	0.306 ± 0.18^b	0.541 ± 0.10^a	0.075 ± 0.01^d	0.173 ± 0.03^c
Progoitrin ($\mu\text{mol/g dw}$)	0.13 ± 0.002^b	0.29 ± 0.064^a	0.296 ± 0.071^a	0.13 ± 0.004^b	0.13 ± 0.013^b
Epiprogoitrin ($\mu\text{mol/g dw}$)	0.23 ± 0.031^a	0.21 ± 0.015^a	0.122 ± 0.030^b	0.22 ± 0.006^a	0.215 ± 0.010^a
Sinigrin ($\mu\text{mol/g dw}$)	1.54 ± 0.151^c	1.814 ± 0.291^b	0.593 ± 0.130^d	2.01 ± 0.092^a	1.56 ± 0.077^c
Glucorafanin ($\mu\text{mol/g dw}$)	0.007 ± 0.001^d	0.04 ± 0.035^c	0.061 ± 0.020^b	0.054 ± 0.001^a	0.136 ± 0.013^a
Gluconapoleiferin ($\mu\text{mol/g dw}$)	0.03 ± 0.002^b	0.07 ± 0.017^a	0.01 ± 0.001^c	0.03 ± 0.001^b	0.036 ± 0.001^b
Glucoalisin ($\mu\text{mol/g dw}$)	0.009 ± 0.001^a	0.03 ± 0.003^a	0.04 ± 0.003^a	0.02 ± 0.001^a	0.013 ± 0.001^a
Gluconapin ($\mu\text{mol/g dw}$)	0.723 ± 0.005^b	1.43 ± 0.0321^a	1.24 ± 0.210^a	0.43 ± 0.005^c	0.634 ± 0.020^b
4-Hydroxybrassicin ($\mu\text{mol/g dw}$)	0.024 ± 0.005^c	0.08 ± 0.007^a	0.04 ± 0.011^b	0.035 ± 0.001^b	0.02 ± 0.001^c
Glucobrassicinapin ($\mu\text{mol/g dw}$)	0.43 ± 0.021^b	0.515 ± 0.041^a	0.08 ± 0.007^d	0.16 ± 0.006^c	0.11 ± 0.007^c
Glucobrassicin ($\mu\text{mol/g dw}$)	1.82 ± 0.412^a	2.096 ± 0.181^a	0.39 ± 0.045^b	1.717 ± 0.009^a	0.215 ± 0.005^c
Gluconasturcin ($\mu\text{mol/g dw}$)	0.163 ± 0.041^b	0.327 ± 0.062^a	0.14 ± 0.051^b	0.07 ± 0.001^c	0.031 ± 0.008^d
Metoxyglucobrassicin ($\mu\text{mol/g dw}$)	0.117 ± 0.025^b	0.36 ± 0.051^a	0.074 ± 0.010^c	0.296 ± 0.008^a	0.046 ± 0.008^d
Neoglucobrassicin ($\mu\text{mol/g dw}$)	0.07 ± 0.0341^b	0.114 ± 0.012^a	0.02 ± 0.006^c	0.057 ± 0.001^b	0.014 ± 0.001^d

Values are means \pm SD of three measurements. Means in rows with different superscript letters (a–d) in common differ significantly ($P < 0.05$). Samples are designated according to their origin. Poland 1 and Poland 2 refer to vegetables harvested in autumn and spring, respectively.

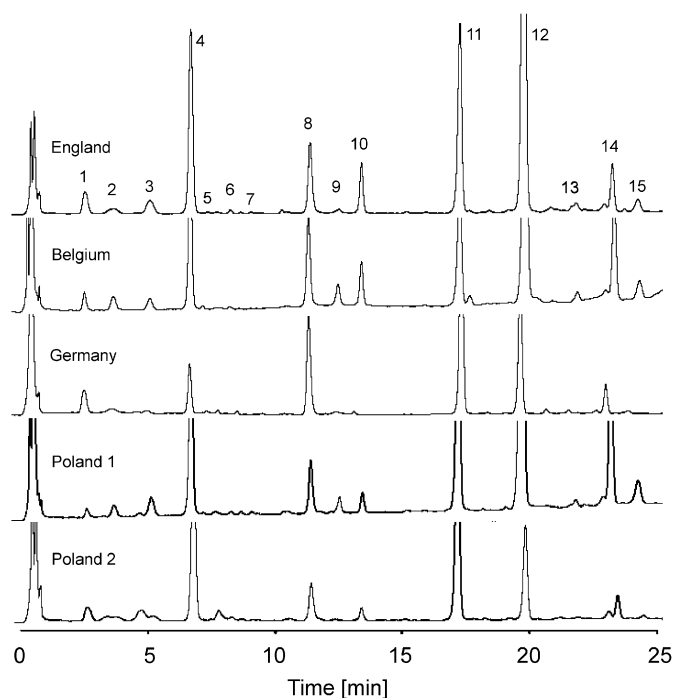


Fig. 2. HPLC fingerprint of GLS in white cabbages from different regions of Europe. (1-desulfoglucobrassicin, 2-desulfoprogoitrin; 3-desulfopropiprogoitrin; 4-desulfosinigrin; 5-desulfoglucorafanin; 6-desulfoglucopoleiferin; 7-desulfoglucobrasicein; 8-desulfoglucobrasicein; 9-desulfoglucobrasicein; 10-desulfoglucobrassicinapin; 11-desulfoglucotrapeolin-internal standard; 12-desulfoglucobrassicin; 13-desulfoglucobrasicein; 14-desulfoglucobrasicein; 15-desulfoglucobrasicein).

(from 2.03 to 4.06 $\mu\text{g CE/g dw}$) and anthocyanins (from 0.022 to 0.038 mg CGE/g dw) determined in the investigated samples was within the range published in other reports. These values must be however regarded as estimates since none of applied assays is fully selective and hence there are some unavoidable overlaps between determined polyphenols with different chemical structures.

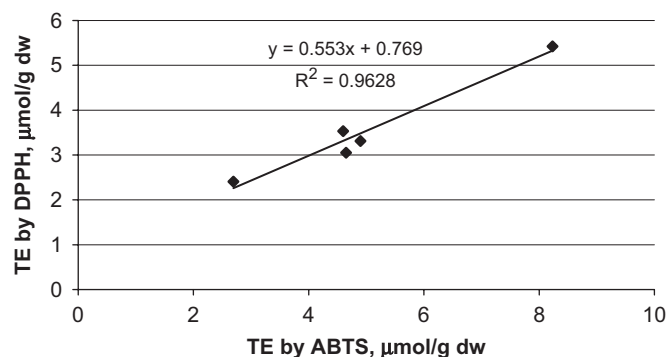


Fig. 3. The correlation between the values of TE ($\mu\text{mol/g dw}$) determined by ABTS and DPPH assays for white cabbage samples. The points are means from three independent measurements.

Again the contents of these compounds in Belgium sample were higher than in other samples analyzed. We observed a significant correlation between antioxidative activity of all cabbage samples and the determined levels of polyphenols with the exception of anthocyanins. The correlations coefficients for the TRSA by ABTS with total phenols, flavonoids and flavanols were 0.8009, 0.8312, 0.8158 and for the TRSA by DPPH with total phenols, flavonoids and flavanols: 0.807; 0.8312, 0.8272, respectively. It means that the correlation was relatively high and nearly equal between the antioxidant activities determined by ABTS and DPPH and the abundance of polyphenols, flavonoids and flavanols. The corresponding correlations for anthocyanins were not statistically significant.

3.5. Protein profiles of cabbage samples

The results of electrophoretic resolution of cabbage proteins are shown in Fig. 4. Cabbage samples England (lane 1) and Belgium (lane 2) differ from each other. The most visible differences were seen in the case of cabbage

Table 2
Estimations of the content of bioactive compounds in cabbages derived from different regions

Indices	Samples				
	England	Belgium	Germany	Poland 1	Poland 2
TP (mg GAE/g)	3.69 ± 0.37 ^b	4.91 ± 0.52 ^a	3.7 ± 0.37 ^b	2.74 ± 0.27 ^c	2.41 ± 0.25 ^c
FL (mg CE/g)	1.82 ± 0.21 ^b	2.42 ± 0.24 ^a	1.82 ± 0.19 ^b	1.41 ± 0.15 ^c	1.18 ± 0.11 ^d
FLA (µg CE/g)	3.05 ± 0.04 ^b	4.06 ± 0.05 ^a	3.01 ± 0.03 ^b	2.3 ± 0.02 ^c	2.03 ± 0.01 ^c
ANTH (mg CGE/g)	0.03 ± 0.003 ^b	0.04 ± 0.004 ^a	0.03 ± 0.003 ^b	0.02 ± 0.002 ^c	nd

Values are means ± SD of three measurements. Means in rows with different superscript letters (a–d) in common differ significantly ($P < 0.05$). Abbreviations: TP: total polyphenols; GAE: gallic acid equivalent; FL: flavonoids; CE: catechin equivalent; FLAV: flavanols; ANTH: anthocyanins; CGE: cyanidin-3-glucoside equivalent; nd: not determined. The calculations were done on dry weight (dw). Samples are designated according to their origin. Poland 1 and Poland 2 refer to vegetables harvested in autumn and spring, respectively.

from England, (lane 1, band ~60 kDa) and from Belgium (lane 2, band ~97 kDa). In Belgium cabbage, the band at 97 kDa (lane 2) is absent, but in other samples at this mobility this band occurs. Patterns of other samples such as Germany (lane 3), Poland 1 (lane 4) and Poland 2 (lane 5) are indistinguishable. Proteins have been separated into numerous components, but showed low polymorphism among the cabbages collected from different regions of Europe. The main differences were observed between the Belgium and other samples. England cabbage differed from the other cabbages in the occurrence of one additional band of 55 kDa (Fig. 4, see one arrow on lane 2). Belgium sample also differed in the absence of 97 kDa band (Fig. 4, see one arrow on lane 2). Germany, Poland 1 and Poland 2 samples are indistinguishable (Fig. 4, lanes 3–5). The main electrophoretic bands occurred between 55 and 100 kDa in all samples with minor differences between the cabbages from other regions. Sample buffer is commonly used for extraction of total proteins from seeds and seedlings in the electrophoretic mobility. It is known also that for cabbage and other species of Brassicaceae vegetables, sample buffer can be used for the same purpose (Curn & Sakova, 1999; Faber, 2000), but such extraction was never applied directly to other parts of cabbage. We have used the buffer extraction for total proteins from cabbage leaves for the first time.

4. Discussion

This study examined the antioxidative properties of the most popular cruciferous vegetable—white cabbage. Despite the high level of consumption around the world and awareness of importance of dietary antioxidants for human health, the antioxidative activity of cabbage has not been thoroughly investigated and consequently little is known about composition of polyphenols, especially that this vegetable is rather a poor source of such compounds. Here we employed two approaches to determine antioxidative capacity of cabbage. Firstly, the total antioxidative activity was assessed. Two methods based on scavenging ABTS or DPPH radicals were used, which are among the most popular spectrophotometric methods for determining the

antioxidant capacity of food samples and chemical compounds. These two stable radical chromogens (the violet DPPH radicals and the blue-green ABTS radical anions) are easy to use, ensure a high level of sensitivity, and allow for analysis of a large number of samples in a timely fashion. According to Stratil et al. (2006), we have chosen Trolox as the most frequently used standard for these two methods. Secondly, an array of assays was employed to specify the types of polyphenolic antioxidants present in cabbage. Moreover, to compare the variability of these indices, the vegetables from different regions were analyzed. The results for cabbages from four European countries, harvested in the period of autumn and additionally, in the case of cabbage from Poland, harvested in spring are summarized in Fig. 1. As can be seen, the total antioxidant activity of cabbage from Belgium as determined by ABTS and DPPH assays was significantly higher than that of the cabbages from other studied regions.

According to our data (Fig. 3), the correlation between results obtained by the ABTS and DPPH assays is highly significant. This corresponds to the data of Awika, Rooney, Wu, Prior, and Cisneros-Zevallos (2003) and Dykes, Rooney, Waniska, and Rooney (2005), who reported that the correlation between DPPH and ABTS methods was significant and corresponded to 0.96 and 0.97, respectively.

In the case of cabbages studied by us, the major contributors to the antioxidant activity were polyphenol compounds. However, it is important to point out that the type of antioxidants is here judged by the indirect assays claimed to be dedicated to the assessment of antioxidative properties of a particular group of phytochemicals. It is known on the other hand, that there are substantial overlaps between these methods and they provide rather indication than identification of the type of compounds.

The levels of polyphenols and the capacity of scavenging DPPH[•] and ABTS^{•+} radicals displayed by cabbage extracts studied were similar to those of Podsedek, Sosnowska, Redzynia, and Anders (2006) who showed that cabbage phenols varied from 21 to 171 mg/100 g (approximately from 2.41 to 19.66 mg/g dw). Similar to our results were reported by Singh et al. (2006) who provided

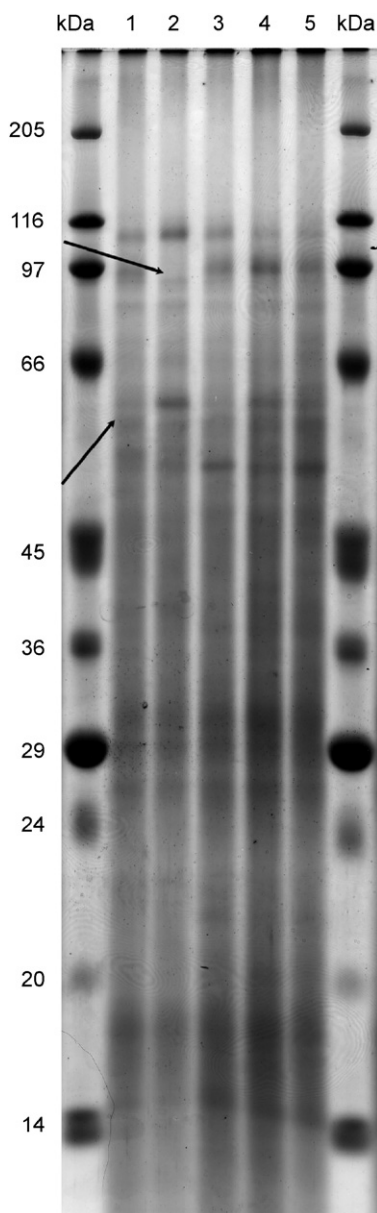


Fig. 4. Comparison of the band intensity of proteins extracted from cabbage samples and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE): kDa, molecular marker [myosin (205 kDa), β -galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3 phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa), α -lactalbumin (14 kDa)]; lanes 1–5 represent cabbage samples from England collected in autumn; Belgium collected in autumn; Germany collected in autumn; Poland 1 collected in autumn; Poland 2 collected in spring, respectively.

estimations of total content of polyphenols for 14 cultivars and the values ranged from 12.58 to 34.41 mg/100 g fresh weight (approximately from 1.44 to 3.96 mg/g dw). Our results also correspond with those of Heimler et al. (2006) who showed that the total phenolic content varied in different samples from 4.30 to 13.80 mg GAE/g dw and the amount of total polyphenols in white cabbage was about 5.31 GAE/g dw and the flavonoids about 1.98 mg CE/g dw.

Our results for cabbage from Belgium showed slightly lower amounts of total polyphenols and higher of flavonoids than the cited ones. The levels of anthocyanins in the investigated samples were low and in sample Poland 2 the amount of the anthocyanins was even not detectable. The only reported results for cabbage (Heo & Lee, 2006) were focused only on red cabbage where anthocyanins appeared to be the major contributors to the antioxidative capacities.

Although, there was a generally very good correlation between antioxidative activity and abundance of polyphenolic compounds in the samples studied, some discrepancies occurred.

The differences in the contents of bioactive compounds and the ABTS and DPPH radical scavenging assays between the samples can be explained by the synergetic effect, that could exist between the individual bioactive compounds, which means that the antioxidant potential may be greater than their sum (Lotito & Frei, 2004). As it was mentioned earlier, there are many methods for total antioxidant determination and each one has its limitations (Yu et al., 2002). Some of these assays give different antioxidant activity trends (Ou et al., 2002), so it is recommended to employ complementary assays for the total antioxidant potential determination, as was done in our experiments.

Another group of bioactive compounds studied were GLS whose content and composition was determined and compared with antioxidative activity of corresponding cabbage samples. The results obtained in this work showed that content of bioactive compounds in the same vegetable may depend on many factors such as geographic region and be associated with climatic conditions. The highest level of bioactive compounds was observed in Belgium cabbage. The lowest antioxidant capacity as well as total GLS content was characteristic for spring cabbage, which means that period of harvest highly affected the level of bioactive compounds. In all samples, the total GLS level paralleled antioxidative potential. We observed a high correlation of TRSA with the total GLS content (Fig. 1). The correlation coefficients between the total antioxidant capacity determined by ABTS and DPPH assays and total GLS in studied cabbages were high and amounted to 0.81 and 0.87, respectively. Such correlation was observed for cabbage and cauliflower by Nilsson et al. (2006). They reported that for white cabbage, sampled in 1999, the total antioxidant capacity was correlated with total GLS ($r = 0.81$). The lowest antioxidant activity as well as total GLS content in Poland 2, representing a spring cabbage may be a result of shorter vegetation period than in autumn vegetables. The autumn cabbages harvested after summer experienced longer exposure on sunlight which substantially affects the synthesis of phytochemicals. Hertog, Hollman, and Katan (1992) reported that three to five times higher flavonoid levels were found in leafy vegetables in summer than in other seasons. The accumulation of plant flavonoids is enhanced in response to

increased light exposure, especially ultraviolet-B rays (Stewart et al., 2000). The water stress during vegetative or pod-filling stage was shown to increase GLS content (Jensen et al., 1996). It is believed that hot dry conditions or water deficit may be related to the increased synthesis of amino acids and sugars, which are the precursors in the biosynthesis of GLS.

The correlation between TRSA and GLS is potentially interesting, because it may reflect a contribution of GLS or their breakdown products or covarying substances to the total antioxidant capacity. Another possible explanation is that the levels of bioactive compounds in an individual plant are interrelated and perhaps depended on the growing conditions that influence their biosynthesis. The significant correlation between GLS content and antioxidative activity demonstrated for all cabbage samples studied, as well as methods applied, suggest that the abundance of bioactive substances in a given cabbage may display similar pattern of variability. This means that the high content of GLS will be accompanied by higher level of antioxidants and perhaps of other non-nutritive phytochemicals. It would imply that a single determination of e.g. TRSA could be used to standardize samples derived from different populations of a given cultivars without necessity of laborious analysis of their actual chemical composition. A problem encountered with natural mixtures while comparing and interpreting especially results of biological experiments has been always the way to unify them and the correlations calculated by us may be very helpful in this regard.

Finally, we have also analyzed cabbages from different sources as regards protein composition. We have noticed relatively low variation of protein patterns; nevertheless on the basis of this pattern, two samples (England and Belgium) were distinguishable. Similarly, Curn and Sakova (1999) detected low levels of protein pattern variation among population of fodder cabbage VV-AR and used protein composition as biochemical markers for detection of homogeneity. In this case, isoenzyme markers as asparate aminotrasferases (AAT) and esterases (EST) were proposed as markers for variation evaluation in the breeding populations. Faber (2000) detected higher level of variation of cruciferins obtained from cabbage seeds of 55 varieties originated from different geographical zones. The few published reports (Curn & Sakova, 1999; Schops, Schierhorn, Schaffner, Mansfeld, & Ulbrich-Hofmann, 2002) described the presence of enzymes in cabbage around 91.7 and 91.9 kDa, but did not show the whole protein profile of cabbages. It seems that protein markers can be suitable for distinguishing samples prepared from cabbage leaves. It should be checked for more numbers of samples from different breeding companies. Nevertheless, we can propose a new biochemical tool of checking of cabbage identity—use of sample buffer and SDS-PAGE for leaves as material. Seeds which are commonly used for this purpose are not necessary.

In conclusion, our findings indicate that GLS, other bioactive compounds and total antioxidant activities can be used as common parameters for characterization of white cabbage samples. Protein profiles can be applied as a new identity feature suitable for biochemical characterization of cabbage samples.

Acknowledgments

The authors are thankful to Mrs. Elena Katrich (Hebrew University of Jerusalem, School of Pharmacy) for technical assistance in determination of bioactive compounds.

The presented research was supported by the Polish State Committee for Scientific Research, Grant no. PBZ-KBN-094/P06/2003/31 and Gdansk University of Technology, Grant no. BW-T.015.

References

- Ambrosone, C. B., McCann, S. E., Freudenheim, J. L., Marshall, J. R., Zhang, Y., & Shields, P. G. (2004). Breast cancer risk in premenopausal women is inversely associated with consumption of broccoli, a source of isothiocyanates, but is not modified by GST genotype. *The Journal of Nutrition*, *134*, 1134–1138.
- Arnous, A., Makris, D. P., & Kefalas, P. (2001). Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. *Journal of Agricultural and Food Chemistry*, *49*, 5736–5742.
- Awika, J. M., Rooney, L. W., Wu, X., Prior, R., & Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *Journal of Agricultural and Food Chemistry*, *51*, 6657–6662.
- Cheng, G. W., & Breen, P. J. (1991). Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *Journal of the American Society of Horticultural Science*, *116*, 865–869.
- Ciska, E., & Pathak, D. R. (2004). Glucosinolate derivatives in stored fermented cabbage. *Journal of Agricultural and Food Chemistry*, *52*, 7938–7943.
- Curn, V., & Sakova, L. (1999). Suitability of different biochemical markers for detection of homogeneity in fodder cabbage [*Brassica oleracea* L. var. *Acephala* (DC.) Alef.]. *Rostlinna Vyroba*, *45*, 45–49.
- Dykes, L., Rooney, L. W., Waniska, R. D., & Rooney, W. L. (2005). Phenolic compounds and antioxidant activity of sorghum grains of varying genotypes. *Journal of Agricultural and Food Chemistry*, *53*, 6813–6818.
- Faber, S. P. (2000). Identifikacija, analiz i registracija sortov, linij I gibrinov kapusty elektroforezom zapasnych belkov I fermentov. In *Identifikacija sortov I registracija genofonda kul' turnych rastenij po belkam semjan* (pp. 133–152). Sankt Petersburg.
- Gupta, S. K., & Röbbelen, G. (1986). Identification of rapeseed (*Brassica napus*) cultivars by electrophoresis. *Plant Breeding*, *96*, 363–370.
- Heber, D., & Bowerman, S. (2001). Applying science to changing dietary patterns. *The Journal of Nutrition*, *131*, 3078S–3081S.
- Heimler, D., Vignolini, P., Dini, M. G., Vincieri, F. F., & Romani, A. (2006). Antiradical activity and polyphenol composition of local *Brassicaceae* edible varieties. *Food Chemistry*, *99*, 464–469.
- Heo, H. J., & Lee, C. Y. (2006). Phenolic phytochemicals in cabbage inhibit amyloid β protein-induced neurotoxicity. *Food Science and Technology*, *39*, 330–336.
- Hertog, M. G. L., Hollman, P. C. H., & Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of Agricultural and Food Chemistry*, *40*, 2379–2383.



- Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53, 1841–1856.
- Jensen, C. R., Mogensen, V. O., Mortensen, G., Fieldsend, J. K., Milford, G. F. J., Anderson, M. N., et al. (1996). Seed glucosinolate, oil, protein content of field grown rape (*Brassica L.*) affected by soil drying and evaporative demand. *Field Crops Research*, 47, 93–105.
- Johnson, I. T. (2002). Glucosinolates in the human diet. Bioavailability and implications for health. *Phytochemistry Review*, 1, 183–188.
- Krajcovicova-Kudlackova, M., Dusinska, M., Valachovicova, M., Blazicek, P., & Paukova, V. (2006). Products of DNA, protein and lipid oxidative damage in relation to vitamin C plasma concentration. *Physiological Research*, 55, 227–231.
- Kushad, M. M., Brown, A. F., Kurilich, A. C., Juvik, J. A., Klein, B. P., Wallig, M. A., et al. (1999). Variation of glucosinolates in vegetable crops of *Brassica oleracea*. *Journal of Agricultural and Food Chemistry*, 47, 1541–1548.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of bacteriophage T4. *Nature*, 227, 680–685.
- Linsinger, T., Kristiansen, N., Beloufa, N., Schimmel, H., & Pauwels, J. (2001). EUR 19764: The certification of the total glucosinolate contents of three rapeseed (colza) materials BCR-190R, -366R and -367R.
- Lotito, S. B., & Frei, B. (2004). Relevance of apple polyphenols as antioxidants in human plasma: Contrasting in vitro and in vivo effects. *Free Radicals in Biology and Medicine*, 36, 201–211.
- Mündges, H., Köhler, W., & Friedt, W. (1990). Identification of rapeseed cultivars (*Brassica napus*) by starch gel electrophoresis of enzymes. *Euphytica*, 45, 179–187.
- Nilsson, J., Olsson, K., Engqvist, G., Ekvall, J., Olsson, M., Nyman, M., et al. (2006). Variation in the content of glucosinolates, hydroxycinnamic acids, carotenoids, total antioxidant capacity and low-molecular-weight carbohydrates in *Brassica* vegetables. *Journal of the Science of Food and Agriculture*, 86, 528–538.
- Nugon-Baundon, L., & Rabot, S. (1994). Glucosinolates and glucosinolate derivatives: Implications for protection against chemical carcinogenesis. *Nutrition Research Review*, 7, 205–231.
- Oerlemans, K., Barret, D. M., Bosch Suades, C., Verkerk, R., & Dekker, M. (2006). Thermal degradation of glucosinolates in red cabbage. *Food Chemistry*, 95, 19–29.
- Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J., & Deemer, E. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *Journal of Agricultural and Food Chemistry*, 50, 3122–3128.
- Podsedek, A., Sosnowska, D., Redzyna, M., & Anders, B. (2006). Antioxidant capacity and content of *Brassica oleracea* dietary antioxidants. *International Journal of Food Science and Technology*, 41(Suppl. 1), 49–58.
- Prestera, T., Zhang, Y., Spencer, S. R., Wilezak, C., & Talaly, P. (1993). The electrophile counterattack response: Protection against neoplasia and toxicity. *Advances in Enzyme Regulation*, 33, 281–296.
- Reyes, L. F., Villarreal, J. E., & Cisneros-Zevallos, L. (2007). The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. *Food Chemistry*, 101, 1254–1262.
- Schops, R., Schierhorn, A., Schaffner, I., Mansfeld, J., & Ulbrich-Hofmann, R. (2002). Identification of phospholipase D from cabbage as N-terminally acetylated PLD2. *Journal of Protein Chemistry*, 21, 407–411.
- Singh, J., Upadhyay, A. K., Bahadur, A., Singh, B., Singh, K. P., & Rai, M. (2006). Antioxidant phytochemicals in cabbage (*Brassica oleracea L. var capitata*). *Scientia Horticulturae*, 108, 233–237.
- Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods of Enzymology*, 299, 152–178.
- Stewart, A. J., Bozonnet, S., Mullen, W., Jenkins, G. I., Lean, M. E. J., & Crozier, A. (2000). Occurrence of flavonols in tomatoes and tomato-based products. *Journal of Agricultural and Food Chemistry*, 48, 2663–2669.
- Stratil, P., Klejdus, B., & Kuban, V. (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables—evaluation of spectrophotometric methods. *Journal of Agricultural and Food Chemistry*, 54, 607–616.
- Verhoeven, D. T., Goldbohm, R. A., Poppel, G., Verhagen, H., & Brandt, P. A. (1996). Epidemiological studies on brassica vegetables and cancer risk. *Cancer Epidemiology Biomarkers & Prevention*, 5, 733–748.
- Wennberg, M., Ekvall, J., Olsson, K., & Nyman, M. (2006). Changes in carbohydrate and glucosinolate composition in white cabbage (*Brassica oleracea var. capitata*) during blanching and treatment with acetic acid. *Food Chemistry*, 95, 226–236.
- Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., & Qian, M. (2002). Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry*, 50, 1619–1624.