Available online at www.sciencedirect.com
SCIENCE DIRECT
Food Chemistry 99 (2006) 464–469
Food Chemistry
www.elsevier.com/locate/foodchem

Antiradical activity and polyphenol composition of local *Brassicaceae* edible varieties

Daniela Heimler [a,*], Pamela Vignolini [a], Maria Giulia Dini [a], Franco Francesco Vincieri [b], Annalisa Romani [b]

[a] *Department of Soil Science and Plant Nutrition, University of Florence, P.le delle Cascine 18, 50144 Florence, Italy*
[b] *Department of Pharmaceutical Sciences, U. Schiff 6, 50019 Sesto F.no, Florence, Italy*

Received 16 May 2005; accepted 27 July 2005

Abstract

The antiradical activity, polyphenols, flavonoids and total condensed tannins contents have been determined in the case of seven local edible *Brassicaceae*, i.e. Italian kale, broccoli, Savoy and white cabbage, cauliflower, green cauliflower and Brussels sprouts. Rapid spectrophotometric methods were applied. The results achieved were compared with the quali–quantitative information obtained by HPLC/DAD and HPLC/MS. The polyphenolic compounds detected were: kaempferol and quercetin glycosides and hydroxycinnamic esters. The EC_{50} values ranged from 81.45 to 917.81 mg sample/mg $DPPH^{\cdot}$ and the total phenolic content from 4.30 to 13.80 gallic acid equivalents (mg gallic acid/g sample). The peculiar characteristics of these vegetables can be evaluated and can increase their value as functional food.

Keywords: *Brassicaceae*; Polyphenols; Flavonoids; Antiradical activity; Functional food

1. Introduction

The *Brassicaceae* family includes more than 350 genera and 3500 species, for the majority cool season annuals, characterised by short cycle and wide adaptability; for this reason they are suited for cultivation in different seasons and in a variety of environments.

As regards to the nutritional profile, the *Brassicaceae* have a low caloric value (24–34 kcal/100 g) depending on the low content of protein (1.44–2.82/100 g) and fat (0.12–0.37/100 g) and an average content of fibre of 2.5/100 g. On the contrary, the contents of minerals, vitamins and others phytochemicals such as polyphenols and glucosinolates, sulphur containing compounds, are notable. These vegetables are rich in potassium, calcium, magnesium and phosphorus, vitamins C, E, K and carotenoids (β-carotene, lutein and zeaxanthin).

Many epidemiological studies have correlated the intake of a diet rich in fruits and vegetables with a reduced risk of incidence of chronic diseases, such as cancer and cardiovascular disease. In particular, several epidemiological studies report an inverse correlation between consumption of *Brassicaceae* and risk of cancer (Peto, Doll, Buckley, & Sporn, 1981; Stoewsand, Anderson, & Munson, 1998) probably due to the anticancer action of metabolites of glucosinolates, as demonstrated by some "in vitro" studies (Verhoeven, Verhagen, Goldbohm, Van den Brandt, & Van Poppel, 1997). Nevertheless, we cannot exclude that the protective effect against chronic diseases could also depend on the antioxidant activity of other compounds present in those vegetables, such as vitamin C, polyphenols, vitamin E and carotenoids (Byers & Perry, 1992; Evangelou et al., 1997). A more accurate characterization of antioxidant levels of some varieties of *Brassicaceae* seems, therefore, opportune, on the basis of their cancer preventive properties (Beecher, 1994).

Considering the chemical diversity of the antioxidant compounds present in foods and the interaction occurring

* Corresponding author.
E-mail address: daniela.heimler@unifi.it (D. Heimler).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.07.057

among those different molecules, the evaluation of the total antioxidant capacity of foods seems to be a more useful marker than the evaluation of single compound. However, no single method to test the total antioxidant capacity of foods (TRAP, ORAC, etc.) fully consider, at the same time, the activity of all the antioxidant compounds.

A possible approach could be to consider the antiradical activity together with the polyphenols content.

Plant polyphenols are known to have multifunctional properties such as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers and flavonoids and their derivatives are the largest and most important group of polyphenols. The most important property is their capacity to act as antioxidants protecting the body against reactive oxygen species and may have an additive effect to the endogenous scavenging compounds (Rice-Evans, Miller, & Paganga, 1997). The antioxidative effect is performed through different mechanisms the most general and important of which is direct radical scavenging which depends on the chemical structure of the flavonoids involved (Nijveldt et al., 2001). It is generally accepted that this action leads to afford protection against numerous chronic diseases, including cancer, cardio- and cerebrovascular, ocular and neurological diseases (Block, Patterson, & Subhar, 1992; Youdim & Joseph, 2001).

Brassicaceae are known to contain flavonoids, and especially flavonols (Nielsen, Norbaek, & Olsen, 1998; Price, Casuscelli, Colquhoun, & Rhodes, 1998; Vallejo, Tomas-Barberan, & Garcia-Viguera, 2002) and their antioxidant activity has been assessed in some cases (Chu, Chang, & Hsu, 2000; Kaur & Kapoor, 2002); and correlated to cancer preventive properties (Beecher, 1994).

The aim of this paper is the study of the antiradical activity in correlation to the polyphenolic content of edible parts of some varieties of *Brassica oleracea*, among which also local varieties were considered, which are largely consumed in Italy, i.e. white cabbage, broccoli, Italian kale (black cabbage), Savoy cabbage, cauliflower, green cauliflower, and Brussels sprouts. The comparison of different varieties used in one country is interesting from a nutritional point of view.

2. Experimental

2.1. Sample preparation

The following vegetables were purchased from local markets on February 2004, that is the period in which in Italy cabbages are the most representative vegetables: white cabbage (*B. oleracea* L. var. *capitata* L.), broccoli (*B. oleracea* L. conv. *botrytis* L. var. *italica* Plenk), Italian Kale (*B. oleracea* L. var. *acephala* D C.), Savoy cabbage (*B. oleracea* L. var. *sabauda* L.), green cauliflower (*B. oleracea* L. conv. *botrytis* L. var. *botrytis* cv Verde di Macerata), cauliflower (*B. oleracea* L. conv. *botrytis* L. var. *botrytis* cv Snow ball), and Brussels sprouts (*B. oleracea* L. var. *gemmifera* Zencher). Each experiment was run three times at least; all data are mean values (standard deviation within brackets).The edible part of each vegetable (the florets in the case of broccoli) was frozen in liquid nitrogen and stored at −80 °C before proceeding with the analysis. Frozen tissues were then ground in a mortar with a pestle under liquid nitrogen. A quantity of 1.5 g of tissue was extracted in 20 mL of 70% ethanol (pH 3.2, by formic acid) overnight. This solution was used for the determination of antioxidant activity, total phenolic, and flavonoid contents. For condensed tannins content, the solution was evaporated to dryness under reduced pressure at room temperature by a Rotavapor 144 R, Büchi, (Switzerland) and finally rinsed with a $CH_3CN/CH_3OH/H_2O$ (pH 2, by HCOOH) 60:20:20 mixture to a final volume of 2 mL. For HPLC analysis, the solution was treated as before with the only exception of the addition, before the evaporation, of 40 μL gallic acid (5.88 mM) as internal standard; the concentrated solution was used after an extraction step with *n*-hexane. The extraction yield (95%) was controlled by the addition of gallic acid which is not naturally present in our samples and exhibits a retention time which falls in an empty zone of the chromatogram.

Authentic standards of rutin, clorogenic acid, gallic acid, cathechin, Folin–Ciocalteau reagent, and 1,1-diphenyl-2-picrylhydrazil radical (DPPH) were purchased from Sigma–Aldrich.

All solvents were of HPLC grade purity (BDH Laboratory Supplies, United Kingdom).

2.2. Antioxidant activity

Free radical scavenging activity was evaluated with the DPPH· (1,1-diphenyl-2-picrylhydrazil radical) assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams and Cuvelier (1995) and slightly modified.

Two milliliter of the sample solution, suitably diluted with ethanol, was added to 2 mL of an ethanol solution of DPPH· (0.0025 g/100 mL) and the mixture was allowed to stand. After 20 min, the absorption was measured at 517 nm (LAMBDA 25, Perkin–Elmer spectrophotometer) versus ethanol, as a blank. Each day, a calibration curve of DPPH· was carried out. The antioxidant activity is expressed as EC_{50}, the antioxidant dose required to cause a 50% inhibition (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1998). EC_{50} was calculated plotting the ratio (DPPH·rem): [DPPH· concentration at $t = 20'$]/[DPPH· concentration at $t = 0$] × 100 against the concentration of the antioxidant. EC_{50} is expressed as mg antioxidant/mg DPPH·.

2.3. Total phenolic content

The total phenolic content was determined using the Folin–Ciocalteu method, described by Singleton, Orthofer, and Lamuela-Raventos (1999) and slightly modified according to Dewanto, Wu, Adom, and Liu (2002). To

125 μL of the suitably diluted sample extract, 0.5 mL of deionised water and 125 μL of the Folin–Ciocalteu reagent were added. The mixture was allowed to stand for 6 min and then 1.25 mL of a 7% aqueous Na_2CO_3 solution were added. The final volume was adjusted to 3 mL. The mixture was allowed to stand for 90 min and the absorption was measured at 760 nm against water as a blank. The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/g sample) through the calibration curve of gallic acid. The calibration curve ranged 20–500 μg/mL ($R^2 = 0.9969$).

2.4. Total flavonoid content

The total flavonoid content was determined using a colorimetric method described by Dewanto et al. (2002) and slightly modified in our laboratory. To 0.25 mL of the suitably diluted sample, 75 μL of a 5% $NaNO_2$ solution, 0.150 mL of a freshly prepared 10% $AlCl_3$ solution, and 0.5 mL of 1 M NaOH solution were added. The final volume was adjusted to 2.5 mL with deionised water. The mixture was allowed to stand for 5 min and the absorption was measured at 510 nm against the same mixture, without the sample, as a blank. The amount of total flavonoids is expressed as (+)catechin equivalents (CE, mg (+)catechin/g sample) through the calibration curve of (+)catechin. The calibration curve ranged 10–500 μg/mL ($R^2 = 0.9946$).

2.5. Total condensed tannins

The analysis of condensed tannins (procyanidins) was carried out according to the method of Broadhurst and Jones (1978) and slightly modified in our laboratory. To 50 μL of the suitably diluted sample, 3 mL of a 4% methanol vanillin solution and 1.5 mL of concentrated hydrochloric acid were added. The mixture was allowed to stand for 15 min and the absorption was measured at 500 nm against methanol as a blank. The amount of total condensed tannins is expressed as (+)catechin equivalents (CT, mg (+)catechin/g sample). The calibration curve ranged 50–600 μg/mL ($R^2 = 0.9978$). No absorbance was obtained without vanillin addition.

2.6. HPLC/DAD analysis

Analysis of flavonols and phenolic acids were carried out using a HP 1100L liquid chromatograph equipped with a DAD detector and managed by a HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Flavonols and phenolic acids were separated by using a 50 × 2.2 mm i.d. 3 μm Luna C18 column (Waters) operating at 26 °C, according to the linear solvent gradient system of Table 1 during a 50 min period. UV/Vis spectra were recorded in the 190–600 nm range and the chromatograms were acquired at 260, 280, 305, 330 and 350 nm.

Table 1
The linear solvent gradient system used in HPLC–DAD and HPLC–MS analysis of polyphenols

Time (min)	H_2O/H^+ (%)	CH_3CN (%)
0	98	2
16	87	13
23	87	13
33	75	25
42	0	100
50	0	100

2.7. HPLC/MS analysis

Analyses were performed using a HP 1100L liquid chromatograph linked to a HP 1100 MSD mass spectrometer with an API/electrospray interface (Agilent Technologies, Palo Alto, CA, USA). The mass spectrometer operating conditions were: gas temperature, 350 °C; nitrogen flow rate, 10.0 L/min, nebulizer pressure, 40 psi; quadrupole temperature, 40 °C; and capillary voltage, 3500 V. The mass spectrometer was operated in positive and negative mode at 80–120 eV.

The identity of polyphenols was ascertained using data from HPLC/DAD and HPLC/MS analyses by comparison and combination of their retention times, UV/Vis, and mass spectra with those of authentic standards. Quantification of individual polyphenolic compounds was directly performed by HPLC/DAD using a five-point regression curve ($R^2 \geqslant 0.998$) in the range 0–5 μg on the basis of authentic standards.

3. Results and discussion

Table 2 lists the total phenolic content (expressed as gallic acid equivalents), the flavonoids content (expressed as catechin equivalents), the tannins content (expressed as catechin equivalents) and the antiradical activity (expressed as EC_{50}). Broccoli and Italian kale exhibit the highest content of both total phenolics and flavonoids. The amounts of total phenolics are very similar to those found with the same method by Kaur and Kapoor (2002) in the case of broccoli and Brussels sprouts and by Chun, Smith, Sakawaga, and Lee (2004) for Savoy cabbage. The highest tannins content is shown by cauliflower.

There is a good correlation ($R^2 = 0.974$) between the total phenolics and the flavonoids content.

As regards the EC_{50} values broccoli and Italian kale exhibit the lowest value (from 4 to 23 times lower than what found for all other vegetables).

As regards the correlation between EC_{50} values and phenolics and flavonoids content, the contribution of vitamin C should be taken into account. In some *B. oleracea* subspecies (broccoli, cauliflower, cabbage) the content of vitamin C (an antioxidant molecule which was not evaluated separately in this paper) changed from 27.32 to 74.71 mg/100 g fresh weight (Kurilich et al., 1999). Kurilich, Jeffery, Juvik, Wallig, and Klein (2002) in a paper

Table 2
Total phenolics, flavonoids, and tannins contents; EC_{50} values

Sample	Total phenolics gallic acid/sample (mg/g, dry weight)	Flavonoids (+)-catechin/sample (mg/g, dry weight)	Tannins (+)-catechin/sample (mg/g, dry weight)	EC_{50} sample/DPPH· (mg, dry weight/mg)
White cabbage	5.31 (0.906)	1.98 (0.067)	0.50(0.005)	370.67 (13.576)
Broccoli	12.85 (0.195)	6.71 (0.467)	0.41(0.033)	81.45 (1.919)
Italian kale	13.80 (0.165)	5.09 (0.540)	0.39(0.002)	92.91 (2.651)
Savoy cabbage	4.30 (0.242)	1.35 (0.003)	0.38(0.006)	379.96 (27.442)
Green cauliflower	6.52 (0.772)	2.52 (0.062)	0.48(0.015)	354.47 (20.796)
Cauliflower	5.83 (0.133)	1.69 (0.522)	0.56(0.073)	917.81 (24.114)
Brussels sprouts	8.10 (0.219)	3.07 (0.262)	0.50(0.005)	339.57 (22.848)

Standard deviation within brackets.

concerning different broccoli genotypes did not find any correlation between the antioxidant activity and the flavonoids or vitamin C contents while a correlation was found between the antioxidant activity and the lutein and zeaxanthin contents, which are not extracted under our experimental conditions. On the other side Chun et al. (2004) found good correlation ($R^2 \geqslant 0.95$) between antioxidant activity and total flavonoid and total phenolic contents for raw (red cabbage, green cabbage, Napa cabbage and Savoy cabbage) and processed (sauerkraut products) cabbages using an ABTS radical solution to asses the antioxidant activity. Proteggente et al. (2002) found that total phenolic and vitamin C contents showed a good correlation with TEAC (trolox equivalent antioxidant capacity, determined with the ABTS radical).

The DPPH· method, on a molar base, does not give the same results depending on the standard used (Butkovic, Klasinc, & Bors, 2004) owing to different kinetic in the H atom transfer (Goupy, Dufour, Loonis, & Dangles, 2003); in fact with our method the EC_{50} value (expressed as μmoles of standard/mg DPPH·) is 1.18 for ascorbic acid, 2.80 for kaempferol, 1.15 for quercetin and 2.03 for quercitrin. Comparing the EC_{50} values with the antioxidant activity (Proteggente et al., 2002) expressed as trolox equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP), (ORAC) oxygen radical absorbance

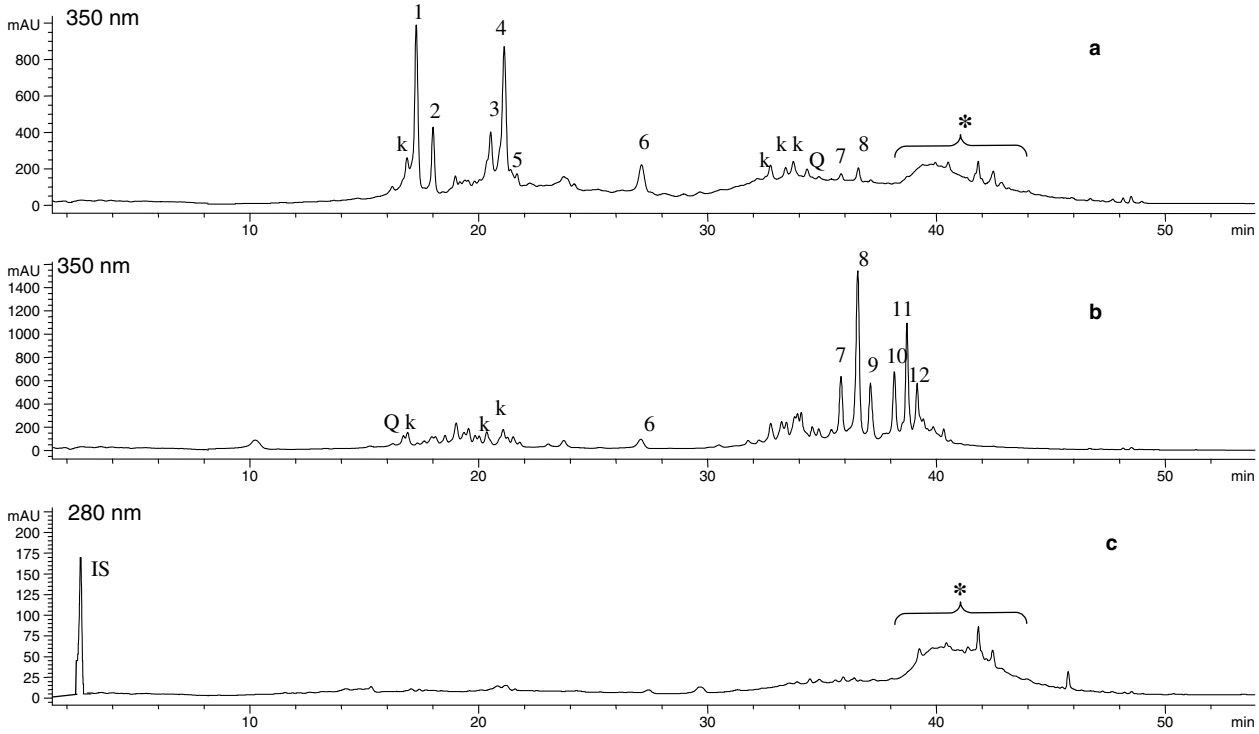

Fig. 1. Chromatographic profiles acquired by HPLC/DAD (350 nm) of the hydroalcholic (ethanol:water 70:30, pH 2) extracts of: (a) Italian kale; (b) Broccoli florets and (c) Brussels sprouts. Polyphenolic compounds: **1**. Kaempferol-3-[2-sinapoylglucopiranosyl (1,2) glucopiranoside]-7-[glucopiranosyl (1,4) glucopiranoside]; **2**. Kaempferol tetraglucoside; **3**. Kaempferol sinapoyl tetraglucoside; **4**. Kaempferol-3-[-2-feruloylglucopiranosyl (1,2) glucopiranoside]-7-[glucopiranosyl (1, 4) glucopiranoside]; **5**. Kaempferol cumaroyl tetraglucoside; **6**. Kaempferol diglucoside; **7**. 1,2-disinapoylgentiobiose; **8**. 1 sinapoyl-2-feruloylgentiobiose; **9**. 1,2-diferuloylgentiobiose; **10**. 1,2,2′-trisinapoyl gentiobiose; **11**. 1,2-disinapoyl-2-feruloylgentiobiose; **12**. 1-sinapoyl-2,2′-diferuloylgentiobiose. *condensed tannins (ipothesis) identified at 280 nm; IS, internal standard (gallic acid); K, kaempferol derivatives, Q, quercetin derivatives; C, caffeic acid derivative (chlorogenic acid).

capacity, the same hierarchy is found for broccoli, cabbage (a mixture of Savoy and white) and cauliflower indicating that the DPPH· method reflects the results obtained with different experimental approaches.

If we consider all vegetables with the exception of cauliflower, a correlation is found between both antioxidant activity and total phenolic content ($R^2 = 0.9388$) and antioxidant activity and flavonoids content ($R^2 = 0.9131$). Cauliflower EC_{50} high value (917.81 mg/mg DPPH·) indicates that the antiradical activity of this vegetable is particularly low, and the difference between cauliflower and green cauliflower, notwithstanding the belongings to the same subspecies, is probably due to the edible part which does not include the green leaves.

As regards polyphenol characterization, as an example, Fig. 1 reports the chromatographic profiles recorded at 350 nm of the extracts of: (a) Italian kale, (b) broccoli and (c) Brussels sprouts. In these extracts, both flavonoids and hydroxycinnamic derivatives were identified by their chromatographic behaviour, UV spectra, MS spectra, retention times in comparison with authentic standards. Among flavonols, we identified kaempferol-3-[2-sinapoylglucopyranosyl (1,2) glucopyranoside]-7-[glucopyranosyl (1,4) glucopyranoside], kaempferol-3-[-2-feruloylglucopyranosyl (1,2) glucopyranoside]-7-[glucopyranosyl (1,4) glucopyranoside] (Nielsen et al., 1998), kaempferol tetraglucoside, kaempferol sinapoyl tetraglucoside, kaempferol cumaroyl tetraglucoside, kaempferol diglucoside (Romani et al., 2003). The predominant hydroxycinnamic acids were identified as 1,2-disinapoylgentiobiose, 1-sinapoyl-2-feruloylgentiobiose, 1,2-diferuloylgentiobiose, 1,2,2′-trisinapoyl gentiobiose, 1,2-disinapoyl-2-feruloylgentiobiose, 1-sinapoyl-2,2′-diferuloylgentiobiose; the pattern found for hydroxycinnamic derivatives in broccoli florets was similar to that described by Vallejo, Tomas-Barberan, and Garcia-Viguera (2003).

Italian kale (black cabbage) polyphenols composition has already been studied in relation to the environment (Romani et al., 2003).

Brussels sprouts as well as all other samples, with the exception of Italian kale and broccoli, exhibit a very poor profile and the attribution of peaks, even if HPLC/DAD and HPLC/MS are employed, is difficult and, overall, the quantitative data are affected by the shape of the chromatogram.

Table 3 lists the quantitative data from HPLC/DAD measurements. There is a quite good correlation ($R^2 = 0.8801$) between total polyphenols from HPLC data and total flavonoids from $AlCl_3$ test (see Table 2). It should be noted that, owing to the shape of chromatograms, all flavonoids have been expressed as rutin content through a calibration curve; this occurrence may affect the results, especially when small amounts are considered.

Our results show how different *Brassicaeae* can be evaluated for their polyphenols content and their antiradical activity giving more information on the peculiar functional characteristics of each food. The importance of the EC_{50} parameter in assessing the antiradical activity of these vegetables can be drawn from its large range of variation (about 100 times) with respect to other tests such as total polyphenols and flavonoids (ranges of variation about 3–5 times).

Table 3
Flavonoids and phenolic acids content (mg/g, dry weight) as obtained from HPLC measurements

Sample	Flavonoids	Phenolic acids	Total polyphenols
White cabbage	2.70	0.07	2.77
Broccoli	3.04	8.69	11.73
Italian kale	11.27	n.d.	11.27
Savoy cabbage	1.02	0.23	1.25
Green cauliflower	2.10	0.07	2.17
Cauliflower	0.29	0.09	0.38
Brussels sprouts	1.12	0.35	1.47

Acknowledgements

The authors express their sincere gratitude to the Cassa di Risparmio di Firenze that contributed to the acquisition of a part of the instrumentation used for this work.

References

Beecher, C. W. W. (1994). Cancer preventive properties of varieties of *Brassica oleracea*: a review. *American Journal of Clinical Nutrition, 59*, 1166–1170.

Block, G., Patterson, B., & Subhar, A. (1992). Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. *Nutrition and Cancer, 18*, 1–29.

Brand-Williams, W., & Cuvelier, M. E. (1995). Use of a free radical method to evaluate the antioxidant activity. *Lebensmittel-Wissenshaft und Technologie, 28*, 25–30.

Broadhurst, R. B., & Jones, W. T. (1978). Analysis of condensed tannins using acified vanillin. *Journal of the Science of Food and Agriculture, 29*, 788–794.

Butkovic, V., Klasinc, L., & Bors, W. (2004). Kinetic study of flavonoid reactions with stable radicals. *Journal of Agricultural and Food Chemistry, 52*, 2816–2820.

Byers, T., & Perry, G. (1992). Dietary carotenes, vitamin C and vitamin E as protective antioxidants in human cancers. *Annual Review of Nutrition, 12*, 139–159.

Chu, Y. H., Chang, C. L., & Hsu, H. F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture, 80*, 561–566.

Chun, O. K., Smith, N., Sakawaga, A., & Lee, C. Y. (2004). Antioxidant properties of raw and processed cabbages. *International Journal of Food Science and Technology, 55*, 191–199.

Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry, 50*, 3010–3014.

Evangelou, A., Kalpouzos, G., Karkabounas, S., Liasko, R., Nonni, A., Stefanou, D., et al. (1997). Dose-related preventive and therapeutic effect of antioxidants-anticarcinogens on experimentally induced malignant tumours in Wirstar rats. *Cancer Letters, 115*, 105–111.

Goupy, P., Dufour, C., Loonis, M., & Dangles, O. (2003). Quantitative kinetic analysis of hydrogen transfer reactions from dietary polyphenols to the DPPH radical. *Journal of Agricultural and Food Chemistry, 51*, 615–622.

Kaur, C., & Kapoor, H. C. (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology, 37*, 153–161.

Kurilich, A. C., Jeffery, E. H., Juvik, J. A., Wallig, M. A., & Klein, B. P. (2002). Antioxidant capacity of different broccoli (*Brassica oleracea*) genotypes using the oxygen radical absorbance capacity (ORAC) assay. *Journal of Agricultural and Food Chemistry, 50*, 5053–5057.

Kurilich, A. C., Tsau, G. J., Brown, A., Howard, L., Klein, B. P., Jeffery, E. H., et al. (1999). Carotene, tocopherol and ascorbate contents in subspecies of *Brassica oleracea*. *Journal of Agricultural and Food Chemistry, 47*, 1576–1581.

Nielsen, J. K., Norbaek, R., & Olsen, C. E. (1998). Kaempferol tetraglucosides from cabbage leaves. *Phytochemistry, 49*, 2171–2176.

Nijveldt, R. J., Van Nood, E., Van Hoorn, D. E. C., Boelens, P. G., Van Norren, K., & Van Leeuwen, P. A. M. (2001). Flavonoids: a review of probable mechanism of action and potential applications. *American Journal of Clinical Nutrition, 74*, 418–425.

Peto, R., Doll, R., Buckley, J., & Sporn, M. (1981). Can dietary β-carotene materially reduce human cancer rates? *Nature, 290*, 201–208.

Price, K. R., Casuscelli, F., Colquhoun, I. J., & Rhodes, M. J. C. (1998). Composition and content of flavonols glycosides in broccoli florets. *Journal of the Science of Food and Agriculture, 77*, 468–472.

Proteggente, A. R., Pannala, A. S., Pagana, G., Van Buren, L., Wagner, E., Wiseman, S., Van De Put, F., & Dacombe, C. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research, 36*, 217–233.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*, 152–159.

Romani, A., Pinelli, P., Galardi, C., Corti, G., Agnelli, A., Vincieri, F. F., et al. (2003). Flavonoids in leaves of black cabbage (*Brassica oleracea* var. acephala DC. subvar. viridis cv. serotina) grown on different soils and at different elevations. *Italian Journal of Food Science, 15*, 197–205.

Sanchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture, 76*, 270–276.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of the Folin–Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Stoewsand, G., Anderson, J., & Munson, L. (1998). Protective effects of dietary Brussels sprouts against mammary carcinogenesis in Sprague-Dawley rats. *Cancer Letters, 39*, 199–207.

Vallejo, F., Tomas-Barberan, F. A., & Garcia-Viguera, C. (2003). Phenolic compound contents in edible parts of broccoli inflorescences after domestic cooking. *Journal of the Science of Food and Agriculture, 83*, 1511–1516.

Vallejo, F., Tomas-Barberan, F. A., & Garcia-Viguera, C. (2002). Potential bioactive compounds in health promotion from broccoli cultivars grown in Spain. *Journal of the Science of Food and Agriculture, 82*, 1293–1297.

Verhoeven, D. T. H., Verhagen, H., Goldbohm, R. A., Van den Brandt, P. A., & Van Poppel, G. A. (1997). Review of mechanism underlying anticarcinogenicity by Brassica vegetables. *Chemico-Biological Interactions, 103*, 79–129.

Youdim, K. A., & Joseph, J. A. A. (2001). A possible emerging role of phytochemicals in improving age-related neurobiological dysfunctions: a multiplicity of effects. *Free Radical Biology and Medicine, 30*, 583–594.