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High-Throughput Antioxidant Profiling in Vegetables by Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry

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ABSTRACT

Vegetables are an important source of dietary antioxidants. Electrospray ionisation Fourier-transform ion cyclotron resonance mass spectrometry was applied to study the antioxidant profiles of beans, broccoli, cabbage, endive, peas and 3 varieties of lettuces. Metabolite extracts from vegetables were analysed in positive and negative ion mode in the *m/z* range <1000. Mass peaks were assigned by comparing measured and theoretical molecular masses with an accuracy of 0.5 mDa using a metabolite reference database. More than 300 mass peaks were identified, and 100 of them were assigned as potential antioxidant compounds. Antioxidants were grouped into 7 metabolic categories. Phenolic compounds were the most numerous group of antioxidants (45-62%), followed by organic acids (12-24%), amino acids and amines (11-17%), pyridines and purines (5-10%), organosulphur compounds, terpenoids, and alkaloids (<5%). Phenolic compounds included flavonoids, phenolic acids, lignans, coumarins, stilbenes, phenylpropanoid glycosides and quinones. Flavonoids and phenolic acids accounted for more than half of all phenolic compounds. About 20% of antioxidants were found in all analysed vegetables, while 16% of compounds were variety-specific. Hierarchical cluster analysis revealed similarities in antioxidant profiles between cabbage and broccoli, beans and peas and lettuces and endive. With respect to occurrence in vegetable species, antioxidants can be classified as ubiquitous (appearance frequency >90%), common (appearance frequency between 10% and 90%), and rare (appearance frequency <10%). The results of this study demonstrate that ubiquitous antioxidants include primary metabolites and key compounds in secondary metabolic pathways, while rare antioxidants represent the end products of secondary metabolism.

Keywords: bean, broccoli, cabbage, endive, FTICRMS, lettuce, metabolic profiling, pea
Abbreviation: FTICRMS, Fourier-transform ion cyclotron resonance mass spectrometry

INTRODUCTION

Dietary antioxidants are defined as food compounds which significantly decrease the adverse effects of reactive oxygen species, reactive nitrogen species, or both on the normal physiological function in humans (SEDRI 1998). Vegetables are a rich source of dietary antioxidants, and links between vegetable consumption and a lower risk of coronary heart diseases, diabetes, cataract, degenerative diseases and cancer have been extensively reported (Goldberg 2003; Valko *et al*. 2007). Antioxidants are the products of primary and secondary plant metabolism. Primary metabolites participate in growth, respiration, photosynthesis, hormone and protein synthesis. Primary metabolites with antioxidant properties include many vitamins and organic acids. Secondary metabolites determine the colour of vegetables, protect plants against herbivores, microorganisms and UV radiation, as well as attract pollinators and seed-dispersing animals. Secondary metabolites with antioxidant properties include phenolic compounds (e.g. flavonoids and phenolic acids), terpenoids (e.g. carotenoids and tocopherols), and alkaloids (e.g. caffeine) (Crozier *et al*. 2006; Hounsome *et al.* 2008).

The most characterised antioxidants with respect to their biological activity and occurrence in vegetables are vitamins, carotenoids and flavonoids (Kaur and Kapoor 2001). Vegetables contain vitamins C, E and B. Ascorbic acid is the major form of vitamin C in vegetables, while dehydroascorbic acid represents less than 10% of total vitamin C content (Wills *et al*. 1984). The vitamin B complex of plants includes vitamins B1 (thiamine and its phosphates), B2 (riboflavin and riboflavin 5-phosphate), B3 (nicotinic acid and nicotinamide), B5 (pantothenic acid), B6 (pyridoxal, pyridoxamine, pyridoxine and their 5'-phosphates), B7 (biotin) and B9 (folic acid). Tocopherols and tocotrienols, known as vitamin E, have been reported in many leafy vegetables, tomatoes, and turnip. Plant carotenoids (α-carotenes, β-carotenes, xanthophylls, lycopene) are orange, yellow, and red lipid-soluble pigments, found in high amounts in tomatoes, carrots and sweet peppers. Carotenoids are essential precursors of vitamin A, which can be produced within the human body from α-carotene and β-carotene (Granado *et al*. 1992; Crozier *et al.* 2006). Flavonoids include ~4,000 phenolic compounds, such as flavonols, flavones, isoflavones, chalcones and anthocyanidins. Flavonols quercetin, kaempferol and isorhamnetin have been found in onions, leeks, endives and broccoli; flavones apigenin, luteolin and chrysoeriol in parsley, thyme and celery; isoflavones in soy; chalcones and dihydrochalcones in tomatoes. Anthocyanidins cyanidin, delphinidin and malvidin have been identified in red cabbage, radish and red lettuces (Hollman and Katan 1999; Yao *et al.* 2004). In addition to the above compounds, antioxidants have been found among other groups of plant metabolites, such as organic acids, amino acids, polyamines, pyridines and organosulphur compounds (see **Table 1** for references).

Over the past decade, a significant amount of information has been accumulated on the identification and characterization of plant metabolites. Developments in mass spectrometry, nuclear magnetic resonance and infrared spectrometry enabled the characterization of plant metabolomes. Statistical tools (e.g. hierarchical cluster analysis, principal component analysis and self-organizing map analysis) were

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applied to compare large data sets. This analysis allowed the identification of compounds (or groups of compounds), which determine the differences between genotypes or phenotypes. Using this approach, the metabolic profiles of potato, tomato, and lettuce have been characterized (Roessner *et al.* 2001; Garratt *et al.* 2005; Schauer *et al.* 2005).

In this study, we applied Fourier-transform ion cyclotron resonance mass spectrometry (FTICRMS) to study antioxidant profiles in beans, peas, broccoli, cabbage, endive and lettuces. A major advantage of FTICRMS over other techniques is its high mass resolution and accuracy, which allows identification of organic compounds without their preliminary separation. The combination of FTICRMS with the low-fragmentation electrospray ionization technique provides an efficient research tool for the characterisation of small biomolecules. Recently, this technique has been successfully applied for metabolic profiling in *Arabidopsis thaliana* seedlings (Ohta *et al.* 2007; Takahashi *et al*. 2008). Development of software for FTICRMS data analysis and metabolite reference database (Shinbo *et al*. 2006) enabled characterisation of metabolic profiles in plants under dark/light transitions and herbicide treatments (Oikawa *et al.* 2006; Ohta *et al.* 2007).

In our recent study we applied FTICRMS to characterise antioxidant compounds in white cabbage during 6 months of commercial storage (Hounsome *et al.* 2009). Identified compounds included 8 vitamins, 15 flavonoids and 11 phenolic acids. We suggested that changes in antioxidant compounds in cabbage during long-term storage reflect several ongoing processes, such as postharvest senescence, biennial cycle and response to fungal infection.

In this study we demonstrate the application of the electrospray ionization FTICRMS for screening antioxidant compounds in 8 vegetable varieties. We compared antioxidant profiles in beans, broccoli, cabbage, endive, peas and 3 varieties of lettuces, and classified antioxidants with respect to their occurrence in vegetables. This project was conducted as a part of the Research Councils' Rural Economy and Land Use Programme (http://www.relu.ac.uk/) to investigate different aspects of vegetable consumption in the UK from a consumer perspective. Samples were kindly provided by 3 major UK commercial growers who supply vegetables to the UK supermarket chains.

MATERIALS AND METHODS

Plant material and sample preparation

Analysed vegetables included beans (*Phaseolus vulgaris* L. cv. 'Fontana'), broccoli (*Brassica oleracea* L. var. *italica* cv. 'Monaco'), white cabbage (*Brassica oleracea* L. var. *capitata* L. *alba* DC. cv. 'Bison'), endive (*Chicorium endivia* L. var. *crispum* cv. 'Glory'), peas (*Pisum sativum* L. cv. 'Ambassador') and three varieties of lettuces (*Lactuca sativa* L.): cos (var. *longifolia* cv. 'Frisco'), iceberg (var. *capitata* cv. 'Diamond') and red Batavia (cv. 'Sovereign'). Due to the pragmatic nature of this project, plant samples were obtained from 3 major UK commercial growers. Names of companies are not mentioned to maintain commercial confidentiality. Vegetables were grown in a conventional agricultural system during the 2006 growth season in different fields and collected at different times of the year as part of commercial operations. Vegetables were randomly sampled from different batches shortly before their dispatch to supermarkets. Broccoli, cabbage, endive and lettuces were sampled as whole vegetables (broccoli n=4, cabbage n=10, endive n=4, cos lettuce n=4, iceberg lettuce n=4, red Batavia lettuce n=4). Beans and peas were sampled in pods, each sample batch (beans n=6, peas n=4) was between 200-300 g. Vegetables were transported refrigerated to the laboratory and processed on the same day upon arrival. Edible parts of vegetables were used for the analysis. As whole lettuces, cabbages and broccoli florets were too large to process, a standard sampling procedure was established to ensure compatibility between samples. Vegetable tissues (100-150 g) were washed with deionised water and homogenised in liquid nitrogen. Frozen powder (0.25 g) was resuspended in 1 mL 50% methanol solution. Samples were centrifuged for 10 min at 2000 × *g*. Supernatant was diluted 1: 100 using solutions containing 50% methanol and 0.1% formic acid for analysis in positive ion mode and 50% acetonitrile for analysis in negative ion mode. Acid hydrolysis was performed as described by Hertog and others (1982) to convert glycosylated forms of vitamins and flavonoids. For this purpose vegetable extracts were incubated at 80°C for 2 h in 1.2 M HCl and 50% methanol. Processed samples were stored at -20°C and analysed within 1-2 weeks. No significant changes in mass spectra were observed during 6 months of sample storage.

FTICRMS measurements

Mass spectra were obtained using the Bruker Apex IV FT-ICR mass spectrometer (Bruker Daltonics Inc. Billerica, MA, USA), equipped with 160 mm bore 4.7 Tesla actively shielded magnet (Magnex Scientific Ltd, Oxford, UK) and an Apollo electrospray ionization source. Samples were directly injected into the mass spectrometer using a Cole Parmer series 74900 syringe pump (Cole Parmer Instrument Co., London, UK) at a flow rate of 120 µL/h. Ions were generated in positive and negative ion mode at a nebulizer N_2 gas pressure of 3.5×10^5 Pa. and a dry gas temperature of 250°C. Source voltages on the capillary entrance were -4500 V and 4300 V, on the spray shield -3900 V and 4000 V, and on the capillary exit 78 V and -70 V for positive and negative modes, respectively. Ions were accumulated for 0.3 seconds in the external hexapole ion guide. The potential on the front trapping plate was 0.84 V and -1 V, on the back plate 0.8 V and -1.1 V, and on the analyser entrance -4 V and 8 V for positive and negative modes, respectively. The data were acquired using apexControl 1.0 (Compass™ software suite, Bruker Daltonics Inc.). The scan range was set to *m/z* 50-1000. One hundred scans were accumulated to obtain each mass spectrum. The mass scale was calibrated using external standards, containing 0.5 µg/mL caffeine, diphenhydramine, sulphametazine, oxybutynin, terfenadine and reserpine. Internal calibration was performed by the addition to the sample of 1 µM ^{15}N-labelled amino acids glycine and tyrosine (98% enrichment, Cambridge Isotope Laboratories, Inc. UK).

Data analysis

Mass spectra were analysed using DataAnalysis 3.3 (Compass™ software suite, Bruker Daltonics Inc.). Signal-to-noise threshold cut-off was set to 4. ^{13}C peaks were excluded from mass analysis. Peak list files were screened through the KNApSAcK metabolite reference database (http://kanaya.naist.jp/KNApSAcK/), containing ~23,000 metabolites. Compounds were identified by comparing the measured and theoretical exact molecular masses with an accuracy of ±0.5 mDa. Mass lists were screened for 3 ion species $(M+H)^+$, $(M+K)^+$ and $(M+Na)^+$ in positive ion mode, and for one ion $(M-H)^-$ in negative ion mode. Each list consisted of 1500 to 3000 ion masses. In total, 40 vegetables representing 8 varieties were analysed in 3 replications in both positive and negative ion mode. The complete dataset contained 240 peak lists (120 samples in 2 ion modes). Hierarchical cluster analysis was performed using GeneSight 3.5 (BioDiscovery Inc.) on identified masses.

RESULTS

Analysis of the FTICRMS spectra of vegetables in the m/z < 1000 revealed ~3000 mass peaks in positive ion mode and ~1500 in negative. A mass accuracy of < 2 ppm was routinely achieved under our experimental conditions. More than 300 mass peaks were assigned by comparing measured and theoretical molecular masses with a precision of 0.5 mDa. One hundred peaks were assigned as antioxidant compounds. Although the screening of mass spectra with a precision of 0.5 mDa in most cases resulted in just one empirical formula, the database search often produced several structurally similar antioxidant compounds. **Table 1** summarises the information on antioxidants found in beans, peas, broccoli, cabbage, endive and lettuces. Only metabolites with reported antioxidant activities were included in this study.

The major metabolic classes of antioxidants found in

Table 1 List of antioxidant compounds found in vegetables.

Exact mass	Molecular formula	Metabolic category	Assigned compound	Reference	Vegetables
88.0160	$C_3H_4O_3$	organic acid	pyruvic acid	Mallet and Sun 2003	beans, cabbage
88.1000	$C_4H_{12}N_2$	polyamine	putrescine	Das and Misra 2004	beans, broccoli, cabbage, cos, endive, iceberg, red Batavia
90.0317	$C_3H_6O_3$	organic acid	lactic acid	Groussard *et al.* 2000	beans, broccoli, cabbage
102.0317	$C_4H_6O_3$	organic acid	acetoacetic acid	Mallet and Sun 2003	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
110.0368	$C_6H_6O_2$	phenolic	catechol, hydroquinone, resorcinol	Arts *et al.* 2003	beans, cabbage, cos
118.0266	$C_4H_6O_4$	organic acid	succinic acid	Naylin *et al.* 2006	beans, cos
122.0368	$C_7H_6O_2$	phenolic	benzoic acid	Weitzman and Stossel 1982	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
122.0480	$C_6H_6N_2O$	pyridine	nicotinamide (vitamin B_3)	Crowley *et al.* 2000	cos, endive, iceberg, peas
123.0320	C_6H5NO_2	pyridine	nicotinic acid (vitamin B_3)	Crowley *et al*. 2000	beans, broccoli, cabbage, peas
124.0524	$C_7H_8O_2$	phenolic	guaiacol, orcinol, p-hydroxybenzyl alcohol	Dizhbite *et al.* 2004 Hladyszowski *et al.* 1998 Liu and Mori 1993	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
130.1218	$C_5H_{14}N_4$	amine	agmatine	Lee *et al.* 2003	broccoli, cabbage, endive, peas
132.0575	C_9H_8O	phenolic	cinnamaldehyde	Kim *et al.* 2007	beans, broccoli, cabbage, cos, endive, iceberg, peas
134.0215	$C_4H_6O_5$	organic acid	malic acid	Puntel *et al.* 2007	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
135.0354	$C_4H_9NO_2S$	amino acid	homocysteine	Meucci and Mele 1997	beans, cabbage, cos, endive, iceberg, red Batavia
136.0524	$C_8H_8O_2$	phenolic	phenylacetic acid	Kim and Lee 2004	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
136.1252	$C_{10}H_{16}$	terpenoid	limonene	Gerhäuser *et al.* 2003	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
137.0841	$C_8H_{11}NO$	amine	tyramine	Yen and Hsieh 1997	beans, cos
138.0317	$C_7H_6O_3$	phenolic	hydroxybenzoic acid	Gadow *et al.* 1997	peas
145.1579	$C_7H_{19}N_3$	polyamine	spermidine	Das and Misra 2004	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
146.0224	$C_6H_{10}S_2$	organosulphur	diallyl disulfide	Koh *et al.* 2005	cabbage, peas
146.0368	$C_9H_6O_2$	phenolic	coumarin	Kostova 2006	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
146.0579	$C_6H_{10}O_4$	organic acid	adipic acid	Papadopoulos *et al.* 2001	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
148.0524	$C_9H_8O_2$	phenolic	cinnamic acid	Kanski *et al.* 2002	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
149.0510	$C_5H_{11}NO_2S$	amino acid	methionine	Meucci and Mele 1997	beans, broccoli, cabbage, cos, iceberg, endive, peas
150.0164	$C_4H_6O_6$	organic acid	tartaric acid	Papadopoulos *et al.* 2001	cos, endive, iceberg, red Batavia
150.0537	$C_6H_{14}S_2$	organosulphur	dipropyl disulfide	Munday *et al.* 2003	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
150.0681	$C_9H_{10}O_2$	phenolic	coumaryl alcohol	Ly *et al.* 2003	beans, broccoli, cabbage, cos, endive, peas, red Batavia
152.0473	$C_8H_8O_3$	phenolic	vanillin, 3,4- dihydroxyacetophenone	Kamat *et al.* 2000	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
154.0266	$C_7H_6O_4$	phenolic	2,3-dihydroxybenzoic acid, gentisic acid, protocatechuic acid	Sha and Schacht 2000 Ashidate *et al.* 2005 Saito and Kawabata 2004	beans, endive
154.1358	$C_{10}H_{18}O$	terpenoid	geraniol	Choi *et al.* 2000	cabbage, peas
164.0473	$C_9H_8O_3$	phenolic	coumaric acid	Kikuzaki *et al.* 2002	peas, red Batavia
164.0685	$C_6H_{12}O_5$	phenolic	quercitol	Orthen *et al.* 1994	beans, broccoli, cabbage, peas, red Batavia
164.0837	$C_{10}H_{12}O_2$	phenolic	eugenol	Reddy and Lokesh 1992	beans, cabbage, cos, endive, iceberg, peas, red Batavia
166.0491	$C_6H_6N_4O_2$	purine	methylxanthine	Geraets *et al.* 2006	broccoli, cabbage, cos, peas, red Batavia
166.0630	$C_9H_{10}O_3$	phenolic	apocynin, paeonol	Ben-Shaul *et al.* 2001 Hsieh *et al.* 2006	broccoli, cabbage
168.0423	$C_8H_8O_4$	phenolic	vanillic acid	Mansouri *et al.* 2005	cabbage, red Batavia
168.0899	$C_8H_{12}N_2O_2$	pyridine	pyridoxamine (vitamin B_6)	Jain and Lim 2001	beans, cabbage, cos, endive, red Batavia
170.0215	$C_7H_6O_5$	phenolic	gallic acid	Yilmaz and Toledo 2004	cabbage, endive
174.0164	$C_6H_6O_6$	organic acid	aconitic acid, dehydroascorbic acid	Sousa *et al.* 2007 Otero *et al.* 1997	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
174.0528	$C_7H_{10}O_5$	organic acid	shikimic acid	Sousa *et al.* 2007	cabbage, peas
176.0321	$C_6H_8O_6$	organic acid	ascorbic acid	Otero *et al.* 1997	broccoli, cabbage
177.0460	$C_6H_{11}NO_3S$	organosulphur	alliin	Chung 2006	beans, cabbage
179.0794	$C_6H_{13}NO_5$	amine	glucosamine	Yan 2007	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
180.0423	$C_9H_8O_4$	phenolic	caffeic acid	Gülçin 2006	beans, broccoli, cabbage, cos, endive, iceberg, red Batavia

Table 1 (Cont.)

Exact mass	Molecular formula	Metabolic category	Assigned compound	Reference	Vegetables
180.0786	$C_{10}H_{12}O_3$	phenolic	coniferyl alcohol	Barclay *et al.* 1997	beans, cabbage, cos, endive, iceberg, peas, red Batavia
181.0739	$C_9H_{11}NO_3$	amino acid	tyrosine	Meucci and Mele 1997	beans, cabbage, endive, iceberg, red Batavia
182.0579	$C_9H_{10}O_4$	phenolic	dihydrocaffeic acid, 4-hydroxy-3,5-dimethoxybenzaldehyde	Huang *et al.* 2004 Zheng *et al.* 2008	beans, cabbage, cos, endive, iceberg, peas, red Batavia
192.0270	$C_6H_8O_7$	organic acid	citric acid, isocitrate	Papadopoulos *et al.* 2001	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
192.0423	$C_{10}H_8O_4$	phenolic	scopoletin	Shaw *et al.* 2003	cabbage
194.0804	$C_8H_{10}N_4O_2$	alkaloid	caffeine	Devasagayam *et al.* 1996	beans, cabbage, cos, endive, iceberg, peas, red Batavia
196.0610	$C_9H_{10}O_4$	phenolic	veratric acid	Miyazawa *et al.* 2003	beans, cabbage,
198.0528	$C_9H_{10}O_5$	phenolic	syringic acid	Schmeda-Hirschmann *et al.* 2004	beans, cabbage, cos, iceberg
208.0372	$C_{10}H_8O_5$	phenolic	fraxetin	Fernandez-Puntero *et al.* 2001	broccoli, cos, red Batavia
212.0837	$C_{14}H_{12}O_2$	phenolic	pinosylvin	Stojanović and Brede 2002	beans, cabbage, cos, endive, iceberg, peas, red Batavia
214.0994	$C_{14}H_{14}O_2$	phenolic	dihydropinosylvin	Cai *et al.* 2003	broccoli, cabbage, cos, iceberg, red Batavia
216.0423	$C_{12}H_8O_4$	phenolic	bergapten, xanthotoxin,	Yu *et al.* 2005 Fylaktakidou *et al.* 2004	beans, broccoli, cabbage, cos, endive, iceberg, peas, red Batavia
220.0372	$C_{11}H_8O_5$	phenolic	purpurogallin	Sugiyama *et al.* 1993	cabbage, cos, iceberg
222.0674	$C_7H_{14}N_2O_4S$	amino acid	cystationine	Wada *et al.* 1996	beans, cabbage, cos, endive, iceberg, peas, red Batavia
222.0892	$C_{12}H_{14}O_4$	terpenoid	apiole	Singh *et al.* 2005	cos, endive, red Batavia
226.0994	$C_{15}H_{14}O_2$	phenolic	pinosylvin methyl ether	Pietarinen *et al.* 2006	beans, cabbage, cos, endive, iceberg, peas, red Batavia
228.0423	$C_{13}H_8O_4$	phenolic	euxanthone	Lin *et al.* 2005	beans, peas
228.0786	$C_{14}H_{12}O_3$	phenolic	resveratrol, 5,6-dehydrokawain	Cai *et al.* 2003 Habsah *et al.* 2003	beans, cos
228.1150	$C_{15}H_{16}O_2$	phenolic	dihydropinosylvin-monomethyl ether	Pietarinen *et al.* 2006	beans, endive
238.0630	$C_{15}H_{10}O_3$	phenolic	3-hydroxyflavone, 5-hydroxyflavone	Lemańska *et al.* 2001	beans, cabbage, cos, endive, iceberg, peas, red Batavia
240.0786	$C_{15}H_{12}O_3$	phenolic	flavidin, 2,4-dihydroxychalcone, 7-hydroxyflavanone	Jayaprakasha *et al.* 2004 Calliste *et al.* 2001 Ávila *et al.* 2001	beans, cabbage, endive, iceberg, peas, red Batavia
242.0943	$C_{15}H_{14}O_3$	phenolic	equol	Arora *et al.* 1998	beans, cabbage, cos, endive, iceberg, peas, red Batavia
244.0372	$C_{13}H_8O_5$	phenolic	gentisein, 1,2,5-trihydroxyxanthone	Lin *et al.* 2005 Minami *et al.* 1994	beans, cos, endive, iceberg, red Batavia
244.0736	$C_{14}H_{12}O_4$	phenolic	oxyresveratrol, piceatannol	Lorenz *et al.* 2003 Ovesna *et al.* 2006	beans, cabbage, iceberg
249.0402	$C_8H_{12}NO_6P$	pyridine	pyridoxine 5-phosphate (vitamin B_6)	Jain and Lim 2001	beans, cabbage, cos, endive, iceberg, red Batavia
250.1317	$C_{13}H_{18}N_2O_3$	phenolic	caffeoylputrescine	Drolet *et al.* 1986	cabbage, cos, peas
252.1150	$C_{17}H_{16}O_2$	phenolic	hinokiresinol	Song *et al.* 2007	beans, cabbage, cos, endive, iceberg, peas, red Batavia
254.0579	$C_{15}H_{10}O_4$	phenolic	chrysin, rubiadin	Harris *et al.* 2006 Tripathi *et al.* 1997	beans, cabbage, peas
256.0372	$C_{14}H_8O_5$	phenolic	purpurin	Watanabe *et al.* 1993	red Batavia
266.1307	$C_{18}H_{18}O_2$	phenolic	honokiol, magnolol	Park *et al.* 2003 Chen *et al.* 2001	beans, cabbage, endive, iceberg, peas, red Batavia
271.0606	$C_{15}H_{11}O_5$	phenolic	pelargonidin	Pietta 2000	cabbage
272.0685	$C_{15}H_{12}O_5$	phenolic	butein, butin, naringenin chalcone	Chen *et al.* 2006 Zhang *et al.* 2008 Calliste *et al.* 2001	beans, cabbage
272.0896	$C_{12}H_{16}O_7$	phenolic	arbutin	Myagmar *et al.* 2004	cabbage
286.0477	$C_{15}H_{10}O_6$	phenolic	kaempferol, luteolin	Brown *et al.* 1998	peas
286.0841	$C_{16}H_{14}O_5$	phenolic	brazilin	Choi *et al.* 2007	beans, cabbage
287.0556	$C_{15}H_{11}O_6$	phenolic	cyanidin	Meyer *et al.* 1998	cabbage
290.0790	$C_{15}H_{14}O_6$	phenolic	catechin, epicatechin	Yilmaz and Toledo 2004	peas
292.1099	$C_{19}H_{16}O_3$	phenolic	purpuritenin A	Maurya and Yadav 2005	beans, red Batavia
304.0583	$C_{15}H_{12}O_7$	phenolic	dihydroquercetin	Dok-Go *et al.* 2003	beans
306.0740	$C_{15}H_{14}O_7$	phenolic	gallocatechin, epigallocatechin	Rice-Evans *et al.* 1997	cabbage
307.0838	$C_{10}H_{17}N_3O_6S$	organosulphur	glutathione	Noctor and Foyer 1998	cabbage
326.1518	$C_{20}H_{22}O_4$	phenolic	licarin A	Yu *et al.* 2000	cos, iceberg
327.1471	$C_{19}H_{21}NO_4$	alkaloid	boldine	Jang *et al.* 2000	beans, cabbage, cos, iceberg

Table 1 (Cont.)

Exact mass	Molecular formula	Metabolic category	Assigned compound	Reference	Vegetables
328.0947	$C_{18}H_{16}O_6$	phenolic	salvigenin	Lu and Foo 2002	beans
328.1311	$C_{19}H_{20}O_5$	phenolic	decursin	Kang and Kim 2007	iceberg
338.1154	$C_{20}H_{18}O_5$	phenolic	lupiwighteone, wighteone	Erasto *et al.* 2004	beans
342.1315	$C_{16}H_{22}O_8$	phenolic	coniferin	Kayano *et al.* 2004	peas
344.0896	$C_{18}H_{16}O_7$	phenolic	cirsilineol, nevadensin, penduletin, usnic acid	Kelm *et al.* 2000 Ganapaty *et al.* 2007 Hajdú *et al.* 2007 Odabasoglu *et al.* 2006	cabbage
360.1573	$C_{20}H_{24}O_6$	phenolic	lariciresinol	Pietarinen *et al.* 2006	beans, cabbage
372.1420	$C_{17}H_{24}O_9$	phenolic	syringin	Es-Safi *et al.* 2007	cos, iceberg
388.0907	$C_{18}H_{16}N_2O_8$	phenolic	betanidin	Kanner *et al.* 2001	cabbage
388.1158	$C_{20}H_{20}O_8$	phenolic	artemetin	Dugas *et al.* 2000	cabbage
402.1315	$C_{21}H_{22}O_8$	phenolic	nobiletin	Murakami *et al.* 2000	cos, red Batavia
420.1784	$C_{22}H_{28}O_8$	phenolic	lyoniresinol	Tomosaka *et al.* 2008	peas
462.0798	$C_{21}H_{18}O_{12}$	phenolic	kaempferol glucuronide, luteolin glucuronide, scutellarein glucuronide	Plumb *et al.* 1999 Lu and Foo 2001 Sanz *et al.* 1994	endive
578.1424	$C_{30}H_{26}O_{12}$	phenolic	apigenin 7-rutinoside, procyanidin B1-B8	Wang *et al.* 2003 Teissedre *et al.* 1996	beans

vegetables included phenolics, terpenoids, organic acids, amino acids and amines, pyridines and purines, alkaloids and organosulphur compounds. Phenolic compounds represented the most numerous group of antioxidants (45-62%), followed by organic acids (12-24%), amino acids and amines (11-17%), pyridines and purines (5-10%), organosulphur compounds, terpenoids and alkaloids (<5%) (**Fig. 1**). Phenolic compounds included flavonoids (e.g. naringenin, cyanidin, quercitol), phenolic acids (e.g. benzoic, cinnamic, caffeic), lignans (e.g. hinokiresinol, lariciresinol, lyoniresinol), coumarins (e.g. decursin, fraxetin, xanthotoxin), stilbenes (e.g. resveratrol, oxyresveratrol, pinosylvin), phenylpropanoid gycosides (syringin, coniferin), quinones (arbutin, purpurin), etc. (**Table 1**). Flavonoids, the most numerous group of phenolics, were presented flavanols (catechin, gallocatechin), flavanones (naringenin, butin), flavones (artemetin, chrysin), isoflavones (gentisein, equol), flavonols (kaempferol) and anthocyanidins (cyanidin, pelargonidin). Phenolic acids included benzoic acid and its derivatives (dimethoxybenzoic, syringic, gallic, vanillic, veratric acids), cinnamic acid and its derivatives (caffeic, coumaric, rosmarinic acids) and phenylacetic acid (**Table 1**). Flavonoids and phenolic acids accounted for more than half of all phenolic compounds. About 12% of phenolics (e.g. benzoic acid, cinnamic acid, coniferyl alcohol, coumarin and vanillin) were present in all tested vegetables.

Non-phenolic antioxidants represented 38-55% of total number of assigned compounds (**Fig. 1**). These antioxidants included organic acids (acetoacetic, aconitic, adipic, ascorbic, citric, lactic, malic, pyruvic, shikimic, succinic, tartaric), amino acids (cystationine, homocysteine, methionine, tyrosine), amines (agmatine, tyramine), polyamines (putrescine, spermidine), pyridines (nicotinamide, nicotinic acid, pyridoxamine, pyridoxine 5-phosphate), organosulphur compounds (alliin, diallyl disulfide, dipropyl disulfide, glutathione), terpenoids (apiole, geraniol, limonene) and alkaloids (boldine, caffeine) (**Table 1**). About 30% of non-phenolic antioxidants were present in all vegetables. These were organic acids (acetoacetic, adipic, citric, malic), amines (putrescine, spermidine, glucosamine), amino acids (cystationine, tyrosine) and pyridines (pyridoxine 5-phosphate and pyridoxamine).

To classify vegetables with respect to their antioxidant composition, we performed hierarchical cluster analysis on mass datasets (**Fig. 2**). Vegetables were clustered into 3 major subgroups: i) cabbage and broccoli, ii) beans and peas and iii) endive and lettuces. Analysis demonstrated higher similarity of antioxidant profiles in endive and lettuces (short dendrogram arms), which reflects the close systematic relationship between these species. Lower similarity

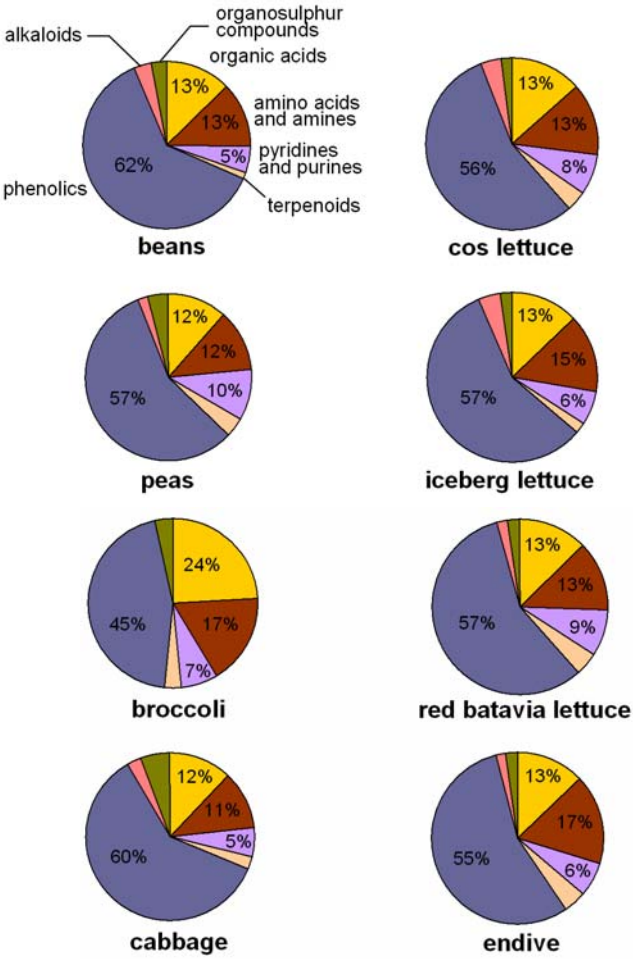

Fig. 1 Ratios between metabolic classes of antioxidants in vegetables. The numbers refer to percentage of compounds of each class.

(longer dendrogram arms) between beans and peas may reflect interspecies differences. Low similarity between cabbage and broccoli may result from different edible organs used for analysis (florets in broccoli and leaves in cabbage).

To characterise the distribution of antioxidants in vegetables we performed frequency analysis on all samples used in this study (**Fig. 3**). Analysing frequencies is important because of variation in the metabolite composition of samples taken from the same plants. Because different numbers

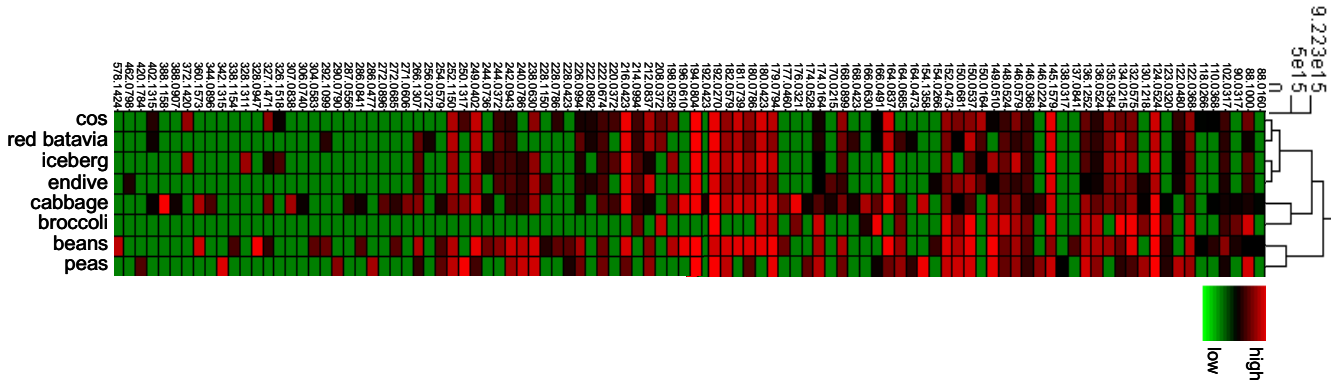

Fig. 2 Hierarchical cluster analysis of antioxidant profiles in vegetables. The dataset includes antioxidant compounds found in positive and negative ion modes (**Table 1**). Clustering was performed along the columns using the Euclidean distance and division cluster linkage method. The colours indicate global variation in relative peak intensities. Peak intensities were normalized with respect to internal calibrants and averaged for each variety to reduce the dataset size (originally 120 columns).

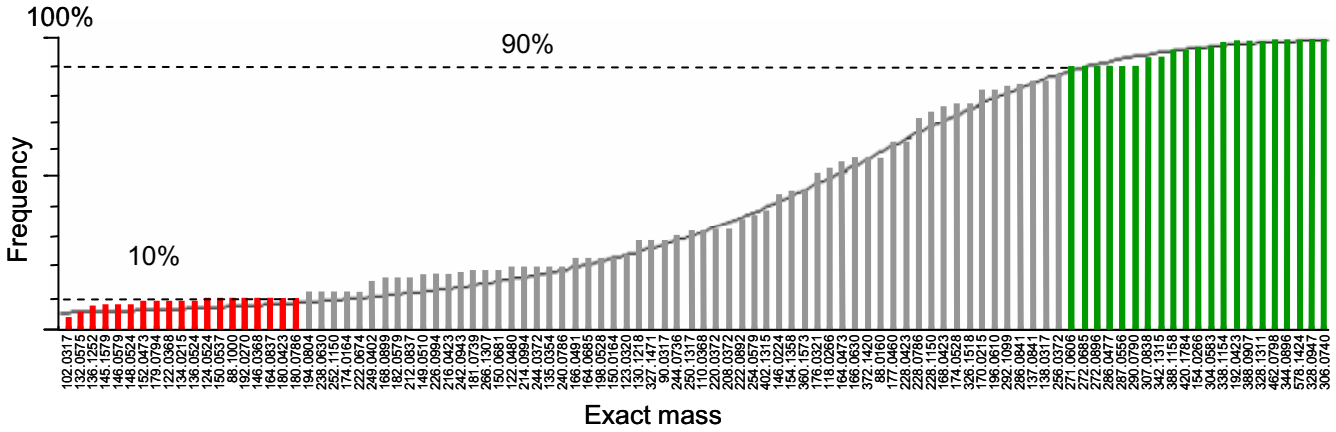

Fig. 3 Frequencies of antioxidant appearance in vegetables. Bars represent summed weighted frequencies of antioxidant compounds from 40 vegetable samples, fitted by sigmoidal function.

of samples were taken from different vegetables, the sums of weighted frequencies for each variety were calculated. Frequencies were plotted in ascending order and fitted by sigmoidal (Boltzmann) function. Three groups of compounds were derived from this analysis: 1) compounds appearing with frequency ≥90%; 2) compounds with frequency between 10 and 90%; and 3) compounds with frequency ≤ 10%. The first group of antioxidants, which we called ubiquitous, consists of 21% of all assigned antioxidants. It includes compounds from different metabolic classes, such as organic acids (acetoacetic, adipic, citric, malic), amines (putrescine, spermidine, glucosamine), pyridines (pyridoxine 5-phosphate and pyridoxamine), terpenoids (limonene), organosulfur compounds (dipropyl disulfide) and phenolic compounds (cinnamic acid, cinnamaldehyde, coumarin, vanillin, hinokiresinol, etc.). The second group, called common antioxidants, contains 60% of all compounds, representing all 7 metabolic categories. The third group (rare antioxidants) accounts for 19% of assigned compounds and contains only phenolic metabolites, such as flavonoids (e.g. gallocatechin, salvigenin, betanidin) and coumarins (decursin and scopoletin). Our analysis demonstrates that ubiquitous antioxidants include primary metabolites, as well as key compounds in secondary metabolic pathways, while rare antioxidants represent the end products of secondary metabolism.

DISCUSSION

Fourier-transform ion cyclotron resonance mass spectrometry has recently been proved to provide a powerful tool for plant metabolomics and metabolic profiling (Oikawa *et al.* 2006, Ohta *et al.* 2007; Takahashi *et al.* 2008). This mass instrument offers sufficient mass resolution and mass accuracy for identifying compounds, present in complex plant samples, which usually require preliminary chromatographic separation. Progress in the application of this technology was limited by the high running cost of FTICR mass spectrometer and the absence of information resources for identification of plant metabolites. The latter was successfully addressed by developing a comprehensive internet-based reference database KNApSAcK (Shinbo *et al.* 2006), containing information about ~23000 plant metabolites (http://kanaya.naist.jp/KNApSAcK/). Examples of FTICR-MS application for metabolomic analysis and metabolite profiling in plants are presented in papers by Aharoni *et al.* (2002), Mungur *et al.* (2005), Oikawa *et al.* (2006), Ohta *et al.* (2007), Takahashi *et al.* (2008).

The purpose of this study was to apply the FTICRMS for profiling antioxidant compounds in vegetables. Although FTICRMS provides a fast and efficient tool for screening plant metabolites, some limitations of this approach should be mentioned. 1. As shown in **Table 1**, the empirical formula derived from mass analysis can be assigned to several structurally similar antioxidants. 2. Although quantitative measurements of metabolites using FTICRMS have been previously demonstrated (Bristow *et al.* 2005), quantification of all compounds in complex metabolite mixtures is not yet possible. This problem is thought to arise from several effects, such as ion suppression and different ionization of molecules. It has been shown that ion suppression can be minimised by diluting the samples and accumulating a large number of mass scans (Kujawinski *et al.* 2002). Therefore, in our study we used very diluted samples and hundreds of mass scans to produce one spectrum. It should be mentioned that since many antioxidants can be oxidised during sample processing, factors such as temperature and pH should be carefully considered when selecting the meta-

bolite isolation procedure.

Antioxidants are chemical compounds which delay the start or slow the rate of free radical formation. These compounds are characterised by the ability to donate the hydrogen atom or electron/proton, or ability to chelate metal ions, involved in formation of reactive oxygen species, or both (donating and chelating). In most cases, antioxidant action involves a combination of different mechanisms, therefore antioxidant properties cannot be attributed to a certain class of chemical compounds or to certain functional groups in these compounds. Our study demonstrates that antioxidants occur among different classes of plant metabolites such as organic acids, phenolic acids, sulphur-containing amino acids, polyamines, pyridines, flavonoids, lignans, coumarins, stilbenes and alkaloids. Although ascorbic acid, carotenoids and tocopherols are often cited as the 'major' antioxidants in plants, the largest group of antioxidant compounds in vegetables are represented by phenolics (flavonoids, phenolic acids, lignans, coumarins, stilbenes, phenylpropanoid gycosides and quinines). The second large group of antioxidants in vegetables are organic acids (**Fig. 1**). Ascorbic acid, which belongs to this group, is, however, less abundant in vegetables than adipic, citric and malic acids (**Table 1**). Although some antioxidants, such as ascorbic acid and glutathione, are ubiquitous in growing plants (Noctor and Foyer 1998), these compounds seem to be unstable during the postharvest storage of vegetables. For example, ascorbic acid content in broccoli was shown to decrease by up to 70 %, depending on storage duration, temperature and packaging (Podşedek 2007). Loss of antioxidant compounds in vegetables is associated with postharvest senescence induced by wounding and disruption of energy, nutrient and hormone supply. This involves production of ethylene and free radicals, which trigger lipid peroxidation and degradation of chlorophyll and proteins. Antioxidants are involved in scavenging reactive oxygen species produced during senescence, therefore their levels in vegetables often decrease upon harvesting. Postharvest stability of antioxidants varies among plant species. Cruciferous vegetables retain ascorbic acid better than other vegetables (Lee and Kader, 2000). Our previous study has demonstrated that white cabbage retained about 50% of ascorbic acid after 6 months of storage (Hounsome *et al.* 2009). In agreement with literature reports, we found cabbage and broccoli to be a good source of ascorbic acid. The fact that ascorbic acid was not found in other vegetables most likely reflects the loss of this compound during the postharvest period, rather than problems with FTICRMS detection.

Some compounds, such as sulphur-containing amino acids and polyamines are usually not mentioned in literature as dietary antioxidants. However, these compounds are highly abundant in plant tissues, and play an important role in antioxidant defence system in humans (Løvaas 1997; Meucci and Mele 1997). Amino acids homocysteine, methionine, tyrosine and cystationine, polyamines putrescine and spermidine, and sugar amine glucosamine were found in almost all tested vegetables (**Table 1**). Another group of plant metabolites, organosulphur compounds, are mainly known due to glucosinolates, which do not possess antioxidant properties. In this study we found several organosulphur compounds with reported antioxidant activities: diallyl disulfide, dipropyl disulfide, alliin and glutathione (**Table 1**). In addition to organosulphurs, two other non-phenolic antioxidants, alkaloid caffeine and terpenoid limonene were found in all samples.

In recent years the focus of research has shifted from using antioxidant supplements towards a balanced combination of antioxidants provided by diet (Liu 2003). Despite the large amount of information on the beneficial effects of antioxidants, randomised clinical trials have failed to show any consistent benefit from the use of antioxidant supplements on cardiovascular disease or cancer risk, with some trials even suggesting possible harm in certain subgroups (Stanner *et al.* 2004). These trials have usually involved the administration of single antioxidant nutrients given at relatively high doses. A number of studies have demonstrated complex interactions between antioxidant compounds, known as synergistic effect. It has been shown that mixtures of antioxidants have higher antioxidant activity than individual antioxidants. For example, mixtures of carotenoids were more effective against oxidative damage than the single compounds (Stahl *et al*. 1998). Synergistic interactions have been also demonstrated between phenolic acids, beta-carotene, and ascorbic acid, as well between flavonoids and tocopherol (Trombino *et al.* 2004; Marinova *et al.* 2008). Although the mechanism of these interactions is not well understood, the data demonstrate the importance of complex assessment of antioxidant compounds in human foods. In this respect metabolite profiling can provide valuable information about antioxidant composition and distribution in vegetables.This study attempts to provide an overview of the whole range of antioxidants present in vegetables. FTICRMS is a powerful tool for high-throughput antioxidant profiling in plants. However, further development of metabolite identification and quantification is essential for comprehensive analysis of plant antioxidants.

CONCLUSIONS

In this study we analysed antioxidants profiles in 8 vegetables: beans, broccoli, cabbage, endive, peas and 3 varieties of lettuces using Fourier-transform ion cyclotron resonance mass spectrometry. Our data demonstrates that:

1. Electrospray ionisation FTICRMS provides a powerful tool for antioxidant profiling in plants;
2. Antioxidant compounds are found among different classes of plant metabolites, such as phenolics, organic acids, amino acids and amines, pyridines and purines, terpenoids, alkaloids and organosulphur compounds;
3. Phenolic compounds are the most numerous metabolic group, which accounts for 45-62% of all antioxidant compounds, followed by organic acids (12-24%), amino acids and amines (11-17%), pyridines and purines (5-10%), organosulphur compounds, terpenoids, and alkaloids (<5%)
4. With respect to occurrence in vegetables, antioxidants can be classified as ubiquitous (appearance frequency >90%), common (appearance frequency between 10% and 90%), and rare (appearance frequency <10%).

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REFERENCES

Aharoni A, Ric de Vos CH, Verhoeven HA, Maliepaard CA, Kruppa G, Bino R, Goodenowe DB (2002) Nontargeted metabolome analysis by use of Fourier Transform Ion Cyclotron Mass Spectrometry. *OMICS* **6**, 217-234

Arora A, Nair MG, Strasburg GM (1998) Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. *Archives of Biochemistry and Biophysics* **356**, 133-141

Arts MJTJ, Dallinga JS, Voss H-P, Haenen GRMM, Bast A (2003) A critical appraisal of the use of the antioxidant capacity (TEAC) assay in defining optimal antioxidant structures. *Food Chemistry* **80**, 409-414

Ashidate K, Kawamura M, Mimura D, Tohda H, Miyazaki S, Teramoto T, Yamamoto Y, Hirata Y (2005) Gentisic acid, an aspirin metabolite, inhibits oxidation of low-density lipoprotein and the formation of cholesterol ester hydroperoxides in human plasma. *European Journal of Pharmacology* **513**,

173-179

Ávila V, Bertolotti SG, Criado S, Pappano N, Debattista N, García NA (2001) Antioxidant properties of natural flavonoids: quenching and generation of singlet molecular oxygen. *International Journal of Food Science and Technology* **36**, 25-33

Barclay LRC, Xi F, Norris JQ (1997) Antioxidant properties of phenolic lignin model compounds. *Journal of Wood Chemistry and Technology* **17**, 73-90

Ben-Shaul V, Lomnitski L, Nyska A, Zurovsky Y, Bergman M, Grossman S (2001) The effect of natural antioxidants, NAO and apocynin, on oxidative stress in the rat heart following LPS challenge. *Toxicology Letters* **123**, 1-10

Bristow T, Stokes P, O'Connor G (2005) Quantitative Fourier transform ion cyclotron resonance mass spectrometry - the determination of creatinine by isotope dilution mass spectrometry. *Rapid Communication in Mass Spectrometry* **19**, 375

Brown JE, Khodr H, Hider RC, Rice-Evans CA (1998) Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties *Biochemical Journal* **330**, 1173-1178

Cai Y-J, Fang J-G, Ma L-P, Yang L, Liu Z-L (2003) Inhibition of free radical-induced peroxidation of rat liver microsomes by resveratrol and its analogues. *Biochimica et Biophysica Acta – Molecular Basis of Disease* **1637**, 31-38

Calliste CA, Le Bail JC, Trouillas P, Pouget C, Habrioux G, Chulia AJ, Duroux JL (2001) Chalcones: structural requirements for antioxidant, estrogenic and antiproliferative activities. *Anticancer Research* **21**, 3949-3956

Chen WJ, Song JR, Guo P, Wen ZY (2006) Butein, a more effective antioxidant than α-tocopherol. *Journal of Molecular Structure: THEOCHEM* **763**, 161-164

Choi B-M, Lee J-A, Gao SS, Eun SY, Kim Y-S, Ryu S-Y, Choi Y-H, Park R, Kwon DY, Kim B-R (2007) Brazilin and the extract from *Caesalpinia sappan* L. protect oxidative injury through the expression of heme oxygenase-1. *BioFactors* **30**, 149-157

Choi H-S, Song HS, Ukeda H, Sawamura M (2000) Radical-scavenging activities of citrus essential oils and their components: detection using 1,1-diphenyl-2-picrylhydrazyl. *Journal of Agricultural and Food Chemistry* **48**, 4156-4161

Chung LY (2006) The antioxidant properties of garlic compounds: allyl cysteine, alliin, allicin, and allyl disulfide. *Journal of Medicinal Food* **9**, 205-213

Crowley CL, Payne CM, Bernstein H, Berstein C, Roe D (2000) The NAD^+ precursors, nicotinic acid and nicotinamide protect cells against apoptosis induced by a multiple stress inducer, deoxycholate. *Cell Death and Differentiation* **7**, 314-326

Crozier A, Clifford M, Ashihara H (2006) *Plant Secondary Metabolites: Occurrence Structure and Role in the Human Diet*, Blackwell Publishing Ltd., Oxford, UK, 384 pp

Das KC, Misra HP (2004) Hydroxl radical scavenging and single outlet oxygen quenching properties of polyamines. *Molecular and Cellular Biochemistry* **262**, 127-133

Devasagayam TPA, Kamat JP, Mohan H, Kesavan PC (1996) Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species. *Biochimica et Biophysica Acta – Biomembranes* **1282**, 63-70

Dizhbite T, Telysheva G, Jurkjane V, Viesturs U (2004) Characterization of the radical scavenging activity of lignins-natural antioxidants. *Bioresource Technology* **95**, 309-317

Dok-Go H, Lee KH, Kim HJ, Lee EH, Lee J, Song YS, Lee Y-H, Jin C, Lee YS, Cho J (2003) Neuroprotective effects of antioxidative flavonoids, quercetin, (+)-dihydroquercetin and quercetin 3-methyl ether, isolated from *Opuntia ficus-indica* var. saboten. *Brain Research* **965**, 130-136

Drolet G, Dumbroff EB, Legge RL, Thompson JE (1986) Radical scavenging properties of polyamines. *Phytochemistry* **25**, 367-371

Dugas AJ Jr, Castaneda-Acosta J, Bonin GC, Price KL, Fischer, NH, Winston GW (2000) Evaluation of the total peroxyl radical-scavenging capacity of flavonoids: structure-activity relationships. *Journal of Natural Products* **63**, 327-331

Erasto P, Bojase-Moleta G, Majinda RRT (2004) Antimicrobial and antioxidant flavonoids from the roots wood of *Bolusanthus spesiosus*. *Phytochemistry* **65**, 875-880

Es-Safi N-E, Kollmann A, Khlifi S, Ducrot PH (2007) Antioxidative effect of compounds isolated from *Globularia alypum* L. structure–activity relationship. *LWT-Food Science and Technology* **40**, 1246-1252

Fernández-Puntero B, Barroso I, Iglesias I, Benedí J, Villar A (2001) Antioxidant activity of fraxetin: *In vivo* and *ex vivo* parameters in normal situation versus induced stress. *Biological and Pharmaceutical Bulletin* **24**, 777-784

Fylaktakidou KC, Hadjipavlou-Litina DJ, Litinas KE, Nicolaides DN (2004) Natural and synthetic coumarin derivatives with anti-inflammatory / antioxidant activities. *Current Pharmaceutical Design* **10**, 3813-3833

Gadow A, Joubert E, Hansmann CF (1997) Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), alpha-tocopherol, BHT, and BHA. *Journal of Agricultural and Food Chemistry* **45**, 632-638

Ganapaty S, Chandrashekhar VM, Chitme HR, Narsu ML (2007) Free radical scavenging activity of gossypin and nevadensin: An *in-vitro* evaluation. *Indian Journal of Pharmacology* **39**, 281-283

Garratt LC, Linforth R, Taylor AJ, Lowe KC, Power JB, Davey MR (2005) Metabolite fingerprinting in transgenic lettuce. *Plant Biotechnology Journal* **3**, 165-174

Geraets L, Moonen HJJ, Wouters EFM, Bast A, Hageman GJ (2006) Caffeine metabolites are inhibitors of the nuclear enzyme poly(ADP-ribose)polymerase-1 at physiological concentrations. *Biochemical Pharmacology* **72**, 902-910

Gerhäuser C, Klimo K, Heiss E, Neumann I, Gamal-Eldeen A, Knauft J, Liu G-Y, Sitthimonchai S, Frank N (2003) Mechanism-based *in vitro* screening of potential cancer chemopreventive agents. *Mutation Research* **523-524**, 163-172

Goldberg G (2003) Plants: Diet and Health. The report of a British Nutrition Foundation Task Force. Blackwell Publishing Ltd., Oxford, UK, 347 pp

Granado F, Olmedilla B, Blanco I, Rojas-Hidalgo E (1992) Carotenoid composition in raw and cooked Spanish vegetables. *Journal of Agricultural and Food Chemistry* **40**, 2135-2140

Groussard C, Morel I, Chevanne M, Monnier M, Cillard J, Delamarche A (2000) Free radical scavenging and antioxidant effects of lactate ion: an *in vitro* study. *Journal of Applied Physiology* **89**, 169-175

Gülçin I (2006) Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). *Toxicology* **217**, 213-220

Habsah M, Lajis NH, Ali AM, Sukari MA, Hin YY, Kikuzaki H, Nakatani N (2003) The antioxidative components from *Alpinia nutans*. *Pharmaceutical Biology* **41**, 7-9

Hajdu Z, Hohmann J, Forgo P, Martinek T, Dervarics M, Zupko I, Falkay G, Cossuta D, Mathe I (2007) Diterpenoids and flavonoids from the fruits of *Vitex agnus-castus* and antioxidant activity of the fruit extracts and their constituents. *Phytotherapy Research* **21**, 391-394

Harris GK, Qian Y, Leonard SS, Sbarra DC, Shi X (2006) Luteolin and chrysin differentially inhibit cyclooxygenase-2 expression and scavenge reactive oxygen species but similarly inhibit prostaglandin-E2 formation in RAW 264.7 cells. *Journal of Nutrition* **136**, 1517-1521

Hertog MGL, Hollman PCH, Dini P, Venema DP (1982) Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry* **40**, 1591-1598

Hladyszowski J, Zubik L, Kozubek A (1998) Quantum mechanical and experimental oxidation studies of pentadecylresorcinol, olivetol, orcinol and resorcinol. *Free Radical Research* **28**, 359-368

Hollman PCH, Katan MB (1999) Dietary flavonoids: intake health effects and bioavailability. *Food and Chemical Toxicology* **37**, 937-942

Hounsome N, Hounsome B, Tomos D, Edwards-Jones G (2008) Plant metabolites and nutritional quality of vegetables. *Journal of Food Science* **73**, R48-R65

Hounsome N, Hounsome B, Tomos D, Edwards-Jones G (2009) Changes in antioxidant compounds in white cabbage during winter storage. *Postharvest Biology and Technology* **52**, 173-179

Hsieh C-L, Cheng C-Y, Tsai T-H, Lin I-h, Liu C-H, Chiang S-Y, Lin J-G, Lao C-J, Tang N-Y (2005) Paeonol reduced cerebral infarction involving the superoxide anion and microglia activation in ischemia-reperfusion injured rats. *Journal of Ethnopharmacology* **106**, 208-215

Huang J, de Paulis T, May JM (2004) Antioxidant effects of dihydrocaffeic acid in human EA.hy926 endothelial cells. *The Journal of Nutritional Biochemistry* **15**, 722-729

Jain SK, Lim G (2001) Pyridoxine and pyridoxamine inhibits superoxide radicals and prevents lipid peroxidation, protein glycosylation, and $(Na^+ + K^+)$-ATPase activity reduction in high glucose-treated human erythrocytes. *Free Radical Biology and Medicine* **30**, 232-237

Jang YY, Song JH, Shin YK, Han ES, Lee CS (2000) Protective effect of boldine on oxidative mitochondrial damage in streptozotocin-induced diabetic rats *Pharmacological Research* **42**, 361-371

Jayaprakasha GK, L Jaganmohan Rao, Sakariah KK (2004) Antioxidant activities of flavidin in different *in vitro* model systems. *Bioorganic and Medicinal Chemistry* **12**, 5141-5146

Kamat JP, Ghosh A, Devasagayam TPA (2000) Vanillin as an antioxidant in rat liver mitochondria: Inhibition of protein oxidation and lipid peroxidation induced by photosensitization. *Molecular and Cellular Biochemistry* **209**, 47-53

Kang SY, Kim YC (2007) Decursinol and decursin protect primary cultured rat cortical cells from glutamate-induced neurotoxicity. *Journal of Pharmacy and Pharmacology* **59**, 863-870

Kanner J, Harel S, Granit R (2001) Betalains - a new class of dietary cationized antioxidants. *Journal of Agricultural and Food Chemistry* **49**, 5178-5185

Kanski J, Aksenova M, Stoyanova A, Butterfield DA (2002) Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal. *The Journal of Nutritional Biochemistry* **13**, 273-281

Kaur C, Kapoor HC (2001) Antioxidants in fruits and vegetables – the millenium's health. *International Journal of Food Science and Technology* **36**, 703-725

Kayano S, Kikuzaki H, Ikami T, Suzuki T, Mitani T, Nakatani N (2004) A new bipyrrole and some phenolic constituents in prunes (*Prunus domestica* L.) and their oxygen radical absorbance capacity (ORAC). *Bioscience, Biotechnology and Biochemistry* **68**, 942-944

Kelm MA, Nair MG, Strasburg GM, DeWitt DL (2000) Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. *Phytomedicine* **7**, 7-13

Kikuzaki H, Hisamoto M, Hirose K, Akiyama K, Taniguchi H (2002) Antioxidant properties of ferulic acid and its related compounds. *Journal of Agricultural and Food Chemistry* **50**, 2161-2168

Kim DH, Kim CH, Kim M-S, Kim JY, Jung KJ, Chung JH, An WG, Lee JW, Yu BP, Chung HY (2007) Suppression of age-related inflammatory NF-κB activation by cinnamaldehyde. *Biogerontology* **8**, 545-554

Kim D-O, Lee CY (2004) Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. *Critical Reviews in Food Science and Nutrition* **44**, 253-273

Koh S-H, Kwon H, Park KH, Ko JK, Kim JH, Hwang MS, Yum YN, Kim O-H, Kim J, Kim H-T, Do B-R, Kim KS, Kim H, Roh H, Yu H-J, Jung HK, Kim SH (2005) Protective effect of diallyl disulfide on oxidative stress-injured neuronally differentiated PC 12 cells. *Molecular Brain Research* **133**, 176-186

Kostova I (2005) Synthetic and natural coumarins as antioxidants. *Mini Reviews in Medicinal Chemistry* **6**, 365-374

Kujawinski EB (2002) Electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS): Characterization of complex environmental mixtures. *Environmental Forensics* **3**, 207-216

Lee GT, Ha H, Lee HC, Cho YD (2003) Agmatine reduces hydrogen peroxide in mesangial cells under high glucose conditions. *Journal of Biochemistry and Molecular Biology* **36**, 251-257

Lee SK, Kader AA (2000) Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology* **20**, 207-220

Lemańska K, Szymusiak H, Tyrakowska B, Zieliński R, Soffers AEMF, Rietjens IMC (2001) The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. *Free Radical Biology and Medicine* **31**, 869-881

Lin LL, Huang F, Chen SB, Yang DJ, Chen SL, Yang JS, Xiao PG (2005) Xanthones from the roots of *Polygala caudata* and their antioxidation and vasodilatation activities *in vitro*. *Planta Medica* **71**, 372-375

Liu J, Mori A (1993) Antioxidant and pro-oxidant activities of *p*-hydroxybenzyl alcohol and vanillin: Effects on free radicals, brain peroxidation and degradation of benzoate, deoxyribose, amino acids and DNA. *Neuropharmacology* **32**, 659-669

Liu RH (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition* **78**, 517S-520S

Lorenz P, Roychowdhury S, Engelmann M, Wolf G, Horn TFW (2003) Oxyresveratrol and resveratrol are potent antioxidants and free radical scavengers: effect on nitrosative and oxidative stress derived from microglial cells. *Nitric Oxide* **9**, 64-76

Løvaas E (1997) Antioxidative and metal-chelating effects of polyamines. In: Sies H (Ed) *Antioxidants in Disease Mechanisms and Therapy: Antioxidants in Disease Mechanisms and Therapeutic Strategies*, Academic Press, San Diego, pp 119-150

Lu Y, Foo LY (2001) Antioxidant activities of polyphenols from sage (*Salvia officinalis*). *Food Chemistry* **75**, 197-202

Lu Y, Foo LY (2002) Polyphenolics of *Salvia* - a review. *Phytochemistry* **59**, 117-140

Ly TN, Shimoyamada M, Kato K, Yamauchi R (2003) Isolation and characterization of some antioxidative compounds from the rhizomes of smaller Galanga (*Alpinia officinarum* Hance). *Journal of Agricultural and Food Chemistry* **51**, 4924-4929

Mallet TR, Sun J (2003) Antioxidant properties of myocardial fuels. *Molecular and Cellular Biochemistry* **253**, 103-111

Mansouri A, Makris DP, Kefalas P (2005) Determination of hydrogen peroxide scavenging activity of cinnamic and benzoic acids employing a highly sensitive peroxyoxalate chemiluminescence-based assay: Structure-activity relationships. *Journal of Pharmaceutical and Biomedical Analysis* **39**, 22-26

Marinova E, Toneva A, Yanishlieva N (2008) Synergistic antioxidant effect of α-tocopherol and myricetin on the autoxidation of triacylglycerols of sunflower oil. *Food Chemistry* **106**, 628-633

Maurya R, Yadav PP (2005) Furanoflavonoids: an overview. *Natural Product Reports* **22**, 400-424

Meucci E, Mele MC (1997) Amino acids and plasma antioxidant capacity. *Amino Acids* **12**, 373-377

Meyer AS, Heinonen M, Frankel EN (1998) Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation. *Food Chemistry* **61**, 71-75

Minami H, Kinoshita M, Fukuyama Y, Kodama M, Yoshizawa T, Sugiura M, Nakagawa K, Tago H (1994) Antioxidant xanthones from *Garcinia subelliptica*. *Phytochemistry* **36**, 501-506

Miyazawa M, Oshima T, Tokura M, Hisama M (2003) Suppression of chemical mutagens-induced SOS response by phenolic acids from black rice bran using *Salmonella typhimurium* TA1535/pSK1002 *umu* Test. *Journal of Oleo Science* **52**, 471-481

Munday R, Munday JS, Nunday CM (2003) Comparative effects of mono-, di-, tri-, and tetrasulfides derived from plants of the *Allium* family: Redox cycling *in vitro* and hemolytic activity and phase 2 enzyme induction *in vivo*. *Free Radical Biology and Medicine* **34**, 1200-1211

Mungur R, Glass ADM, Goodenow DB, Lightfoot DA (2005) Metabolite fingerprinting in transgenic *Nicotiana tabacum* altered by the *Escherichia coli* glutamate dehydrogenase gene. *Journal of Biomedicine and Biotechnology* **2**, 198-214

Murakami A, Nakamura Y , Torikai K, Tanaka T, Koshiba T, Koshimizu K, Kuwahara S, Takahashi Y, Ogawa K, Yano M, Tokuda H, Nishino H, Mimaki Y, Sashida Y, Kitanaka S, Ohigashi H (2000) Inhibitory effect of citrus nobiletin on phorbol ester-induced skin inflammation, oxidative stress and tumor-promotion in mice. *Cancer Research* **50**, 5059-5066

Myagmar B, Shinno E, Ichiba T, Aniya Y (2004) Antioxidant activity of medicinal herb *Rhodococcum vitis-idaea* on galactosamine-induced liver injury in rats. *Phytomedicine* **11**, 416-423

Naylin N, Suganuma T, Taing O (2006) Antioxidant activities of compounds isolated from fermented broth of *Zygosaccharomyces rouxii*. *Food Biotechnology* **20**, 131-141

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**, 249-279

Odabasoglu F, Cakir A, Suleyman H, Aslan A, Bayira Y, Halicia M, Kazaze C (2006) Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. *Journal of Ethnopharmacology* **103**, 59-65

Ohta D, Shibata D, Kanaya S (2007) Metabolic profiling using Fourier-transform ion-cyclotron-resonance mass spectrometry. *Analytical and Bioanalytical Chemistry* **389**, 1469-1475

Oikawa A, Nakamura Y, Ogura T, Kimura A, Suzuki H, Sakurai N, Shinbo Y, Shibata D, Kanaya S, Ohta D (2006) Clarification of pathway-specific inhibition by Fourier transform ion cyclotron resonance/mass spectrometry-based metabolic phenotyping studies. *Plant Physiology* **142**, 398-413

Orthen B, Popp M, Smirnoff N (1994) Hydroxyl radical scavenging properties of cyclitols. *Proceedings of the Royal Society of Edinburgh. Section B: Biology* **102**, 269-272

Otero P, Viana M, Herrera E, Bonet B (1997) Antioxidant and prooxidant effects of ascorbic acid, dehydroascorbic acid and flavonoids on LDL submitted to different degrees of oxidation. *Free Radical Research* **27**, 619-626

Ovesná Z, Kozics K, Bader Y, Saiko P, Handler N, Erker T, Szekeres T (2006) Antioxidant activity of resveratrol, piceatannol and 3,3',4,4',5,5'-hexahydroxy-trans-stilbene in three leukemia cell lines. *Oncology Reports* **16**, 617-624

Papadopoulos K, Triantis T, Dimotikali D, Nikokavouras J (2001) Evaluation of food antioxidant activity by photostorage chemiluminescence. *Analytica Chimica Acta* **433**, 263-268

Park EJ, Zhao YZ, Na M, Bae K, Kim YH, Lee BH, Sohn DH (2003) Protective effects of honokiol and magnolol on tertiary butyl hydroperoxide- or D-galactosamine-induced toxicity in rat primary hepatocytes. *Planta Medica* **69**, 33-37

Pietarinen SP, Willför SM, Ahotupa MO, Hemming JE, Holmbom BR (2006) Knotwood and bark extracts: strong antioxidants from waste materials. *Journal of Wood Science* **52**, 436-444

Pietta PG (2000) Flavonoids as antioxidants. *Journal of Natural Products* **63**, 1035-1042

Plumb GW, Price KR, Williamson G (1999) Antioxidant properties of flavonol glycosides from green beans. *Redox Report* **4**, 123-127

Podsędek A (2007) Natural antioxidants and antioxidant capacity of *Brassica* vegetables: A review. *LWT-Food Science and Technology* **40**, 1-11

Puntel RL, Roos DH, Grotto D, Garcia SC, Nogueira CW, Rocha JBT (2007) Antioxidant properties of Krebs cycle intermediates against malonate pro-oxidant activity in vitro: A comparative study using the colorimetric method and HPLC analysis to determine malondialdehyde in rat brain homogenates. *Life Sciences* **81**, 51-62

Reddy ACP, Lokesh BR (1992) Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Molecular and Cellular Biochemistry* **111**, 117-124

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

Roessner U, Luedemann A, Brust D, Fiehn O, Linke T, Willmitzer L, Fernie AR (2001) Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. *The Plant Cell* **13**, 11-29

Saito S, Kawabata J (2004) Synergistic effects of thiols and amines on antiradical efficiency of protocatechuic acid. *Journal of Agricultural and Food Chemistry* **52**, 8163-8168

Sanz MJ, Ferrandiz ML, CejudoM, Terencio MC, Gil B, Bustos G, Ubeda A, Gunasegaran R, Alcaraz MJ (1994) Influence of a series of natural flavonoids on free radical generating systems and oxidative stress. *Xenobiotica* **24**, 689-699

Schauer N, Zamir D, Fernie AR (2005) Metabolic profiling of leaves and fruit of wild species tomato: a survey of the *Solanum lycopersicum complex*. *Journal of Experimental Botany* **56**, 297-307

Schmeda-Hirschmann G, Tapia A, Theoduloz C, Rodríguez J, López S,

Feresin GE (2004) Free radical scavengers and antioxidants from *Tagetes mendocina*. *Zeitschrift für Naturforschung C* **59c**, 345-353

SEDRI, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (1998) *Dietary Reference Intakes: Proposed Definition and Plan for Review of Dietary Antioxidants and Related Compounds*, National Academy Press, Washington, DC, 24 pp

Sha SH, Schacht J (2000) Antioxidants attenuate gentamicin-induced free radical formation *in vitro* and ototoxicity *in vivo*: D-methionine is a potential protectant. *Hearing Research* **142**, 34-40

Shaw C-Y, Chen C-H, Hsu C-C, Chen C-C, Tsai Y-C (2003) Antioxidant properties of scopoletin isolated from *Sinomonium acutum*. *Phytotherapy Research* **17**, 823-825

Shinbo Y, Nakamura Y, Altaf-Ul-Amin M, Asahi H, Kurokawa K, Arita M, Saito K, Ohta D, Shibata D, Kanaya S (2006) KNApSAcK: A comprehensive species-metabolite relationship database. *Biotechnology in Agriculture and Forestry* **57**, 165-181

Singh G, Maurya S, de Lampasona MP, Catalan C (2005) Chemical constituents, antimicrobial investigations, and antioxidative potentials of *Anethum graveolens* L. essential oil and acetone extract. *Journal of Food Science* **70**, M208-M215

Song MC, Yang HJ, Bang MH, Kim DK, Jeong TS, Kim JP, Baek NI (2007) Antioxidant and antiatherogenic activity of *cis*-hinokiresinol from *Trapa pseudoincisa*. *Archives of Pharmacal Research* **30**, 1392-1397

Sousa C, Lopes G, Pereira DM, Taveira M, Valentão P, Seabra RM, Pereira JA, Baptista P, Ferreres F, Andrade PB (2007) Screening of antioxidant compounds during sprouting of *Brassica oleracea* L. var. costata DC. *Combinational Chemistry and High Input Screening* **10**, 377-386

Stahl W, Junghans A, de Boer B, Driomina ES, Briviba K, Sies H (1998) Carotenoid mixtures protect multilamellar liposomes against oxidative damage: synergistic effects of lycopene and lutein. *FEBS Letters* **427**, 305-308

Stanner SA, Hughes J, Kelly CNM, Buttriss J (2004) A review of the epidemiological evidence for the 'antioxidant hypothesis'. *Public Health Nutrition* **7**, 407-422

Stojanović S, Brede O (2002) Elementary reactions of the antioxidant action of *trans*-stilbene derivatives: resveratrol, pinosylvin and 4-hydroxystilbene. *Physical Chemistry Chemical Physics* **4**, 757-764

Sugiyama H, Fung KP, Wu TW (1993) Purpurogallin as an antioxidant protector of human erythrocytes against lysis by peroxyl radicals. *Life Sciences* **53**, PL39-43

Takahashi H, Kai K, Shinbo Y, Tanaka K, Ohta D, Oshima T, Altaf-Ul-Amin M, Kurokawa K, Ogasawara N, Kanaya S (2008) Metabolomics approach for determining growth-specific metabolites based on Fourier transform ion cyclotron resonance mass spectrometry. *Analytical and Bioanalytical Chemistry* **391**, 2769-2782

Teissedre PL, Frankel EN, Waterhouse AL, Peleg H, German JB (1996) Inhibition *of in vitro* human LDL oxidation by phenolic antioxidants from grapes and wines. *Journal of the Science of Food and Agriculture* **70**, 55-61

Tomosaka H, Chin YW, Salim AA, Keller WJ, Chai H, Kinghorn AD (2008) Antioxidant and cytoprotective compounds from *Berberis vulgaris* (barberry). *Phytotherapy Research* **22**, 979-981

Tripathi YB, Sharma M, Manickam M (1997) Rubiadin, a new antioxidant from *Rubia cordifolia*. *Indian Journal of Biochemistry and Biophysics* **34**, 302-306

Trombino S, Serini S, Di Nicuolo F, Celleno L, Andò S, Picci N, Calviello G, Palozza P (2004) Antioxidant effect of ferulic acid in isolated membranes and intact cells: synergistic interactions with alpha-tocopherol, beta-carotene, and ascorbic acid. *Journal of Agricultural and Food Chemistry* **52**, 2411-2420

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazura M, Telserd J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology* **39**, 44-84

Wada K, Kamisaki Y, Nakamoto K, Itoh T (1996) Effect of cystathionine as a scavenger of superoxide generated from human leukocytes or derived from xanthine oxidase in vitro. *European Journal of Pharmacology* **296**, 335-340

Wang M, Simon JE, Aviles IF, He K, Zheng QY, Tadmor Y (2003) Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). *Journal of Agricultural and Food Chemistry* **51**, 601-608

Watanabe N, Yamamoto K, Ihshikawa H, Yagi A (1993) New chlorophyll-A-related compounds isolated as antioxidants from marine bivalves. *Journal of Natural Products* **56**, 305-317

Weitzman SA, Stossel TP (1982) Effects of oxygen radical scavengers and antioxidants on phagocyte-induced mutagenesis. *The Journal of Immunology* **128**, 2770-2772

Wills RBH, Wimalasiri P, Greenfield H (1984) Dehydroascorbic acid levels in fresh fruit and vegetables in relation to total vitamin C activity *Journal of Agricultural and Food Chemistry* **32**, 836-838

Yan Y, Liu W-S, Han B-Q, Wang C-H, Fu C-W, Liu B, Chen L-H (2006) The antioxidative and immunostimulating properties of D-glucosamine. *International Immunopharmacology* **7**, 29-35

Yao LH, Jiang YM, Shi J, Tomás-Barberán FA, Datta N, Singanusong R, Chen SS (2004) Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition* **59**, 113-122

Yen GC, Hsieh CL (1997) Antioxidant effects of dopamine and related compound. *Bioscience, Biotechnology, and Biochemistry* **61**, 1646-1649

Yilmaz Y, Toledo RT (2004) Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. *Journal of Agricultural and Food Chemistry* **52**, 255-260

Yu J, Wang L, Walzem RL, Miller EG, Pike LM, Patil BS (2005) Antioxidant activity of citrus limonoids, flavonoids, and coumarins. *Journal of Agricultural and Food Chemistry* **53**, 2009-2014

Yu YU, Kang SY, Park HY, Sung SH, Lee EJ, Kim SY, Kim YC (2000) Antioxidant lignans from *Machilus thunbergii* protect CCl_4-injured primary cultures of rat hepatocytes. *Journal of Pharmacy and Pharmacology* **52**, 1163-1169

Zhang R, Chae S, Kang KA, Piao MJ, Ko DO, Wang ZH, Park DB, Park JW, You HJ, Hyun JW (2008) Protective effect of butin against hydrogen peroxide-induced apoptosis by scavenging reactive oxygen species and activating antioxidant enzymes. *Molecular and Cellular Biochemistry* **318**, 33-42

Zheng H, Chen Z-W, Wang L, Wang S-Y, Yan Y-Q, Wu K, Xu Q-Z, Zhang S-M, Zhou P-K (2007) Radioprotection of 4-hydroxy-3,5-dimethoxybenzaldehyde (VND3207) in culture cells is associated with minimizing DNA damage and activating Akt. *European Journal of Pharmaceutical Sciences* **33**, 52-59