Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 102 (2007) 1389–1393
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*)

Dhan Prakash *, Brahma N. Singh, Garima Upadhyay

Nutraceutical Chemistry, National Botanical Research Institute, Lucknow 226001, India

Received 5 September 2005; accepted 12 June 2006

Abstract

Four (red, violet, white and green) varieties of *Allium cepa* were studied for their total phenolic contents (TPC), antioxidant (AOA) and free radical scavenging activities (FRSA). The TPC varied from 4.6 to 74.1 mg/g GAE, AOA varied from 13.6% to 84.1% and FRSA showed wide range in terms of IC_{50} (inhibitory concentration) from 0.1 to 15.2 mg/ml, EC_{50} (efficient concentration) from 4.3 to 660.8 mg/mg and ARP (antiradical power) from 0.15 to 23.2. The outer dry layers of red and violet varieties showed better inhibition of lipid peroxidation assayed by ammonium thiocyanate than α-tocopherol. The non-site-specific inhibition of hydroxyl radical induced deoxyribose degradation was also higher in the outer dry layers of red and violet varieties than in their middle and inner layers. The outer layers were also potential inhibitors of nitroblue tetrazolium chloride (NBT) reduction caused by superoxide anions. On the other hand the ferrous ion chelating capacity of the red and violet varieties was highest in the inner layers. Specific phenolic composition performed through HPLC and LC–MS/MS showed the presence of gallic acid, ferulic acid, protocatechuic acid, quercetin, and kaempferol. The unutilised outer layers of the red variety were a rich source of quercetin (5110 μg/g) with high AOA, FRSA and also showed significant protection of DNA damage caused by free radicals.

Keywords: *Allium cepa*; Polyphenols; Antiradical power; Reducing power; Free radical scavenging activity; Chelating effect; DNA damage

1. Introduction

Reactive oxygen species (ROS) are produced in the cells by cellular metabolism and other exogenous environmental agents. They are generated by a process known as redox cycling and are catalysed by transition metals, such as Fe^{2+} and Cu^{2+} (Halliwell & Gutteridge, 1999). Overproduction of ROS can damage cellular biomolecules like nucleic acids, proteins, lipids, carbohydrates, proteins and enzymes, resulting in several diseases. Living systems have specific pathways to overcome the adverse affects of various damages. However, sometimes these repair mechanisms fail to keep pace with such deleterious effects (Halliwell, 1995; Nilsson, Stegmark, & Akesson, 2004).

Antioxidants scavenge free radicals and are associated with reduced risk of cancer and cardiovascular diseases. *Allium cepa* L. has been reported to have antimicrobial, antispasmodic, anticholesterolaemic, hypotensive, hypoglycaemic, antiasthmatic, anticancer and antioxidant properties (Challier, Pernau, & Viel, 1998; Dorant, Van Den Breandt, & Goldbohm, 1995; Fukushima, Takada, Hori, & Wanibuchi, 1997; Gazzani, Papetti, Massolini, & Daglia, 1998; NOA; Stajner & Varga, 2003; Yin & Cheng, 1998). Polyphenols, anthocyanins, flavonoids, quercetin, kaempferol and their glycosides have been reported in onions (Crozier, Lean, McDonald, & Black, 1997; Fossen, Pedersen, & Andersen, 1997; Rhodes & Price, 1996; Vinson, 1998).

Huge quantities of onions are consumed all over the world for flavouring various types of food and their outer layers go to waste. Available information on their free

* Corresponding author.
E-mail address: dprakash_in@yahoo.com (D. Prakash).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.06.063

radical scavenging activities is scanty. Therefore, different layers of four onion varieties were investigated for their total phenolic contents, antioxidant and free radical scavenging activities and protection of DNA damage caused by free radicals. Specific phenolic composition using HPLC and MS/MS of various layers was performed.

2. Materials and methods

2.1. Chemicals and materials

Linoleic acid, β-carotene and quercetin were purchased from Acros, Organics, Geel, Belgium; Tween 40, Folin–Ciocalteau's phenol reagent, HPLC solvents and other analytical grade chemicals from E. Merck, Mumbai, India; DPPH, NBT, pBR322 DNA, kaempferol, ferulic, gallic and protocatechuic acids from Sigma–Aldrich, St. Louis; Il. Plant materials collected from experimental field stations of the Institute and also the local market were separated into different layers, chopped, dried, powdered (40-mesh) and stored in polythene bags at 4 °C.

2.2. Chemical analysis

The powdered plant material (1.0 g) was extracted with MeOH:H_2O (1:1, 2×10 ml), overnight at room temperature. Total phenolic contents (TPC) in extract were measured as described by Ragazzi and Veronese (1973) and expressed as mg/g gallic acid equivalent (GAE) on dry weight basis. Antioxidant activity (AOA) was performed by autoxidation of β-carotene and linoleic acid coupled reaction method (Emmons & Peterson, 1999) and expressed as percent of inhibition, relative to control. For other concentration dependent studies, solvent was removed from the extract under reduced pressure and required quantities of the residue thus obtained were dissolved in MeOH. AOA was also determined by using ammonium thiocyanate assay as described by Lee, Kim, Kim, and Jang (2002). Free radical scavenging activity (FRSA) was measured by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution (6×10^{-5} M in methanol) and calculated according to Yen and Duh (1994). The inhibitory concentration (IC_{50}), efficient concentration (EC_{50}) and antiradical power (ARP) were estimated and calculated as described by Kroyer (2004). Reducing capacity of extracts (1.0 mg/ml in methanol) was determined by ferric reducing antioxidant power assay, using quercetin as reference standard (Apati et al., 2003) and expressed as ascorbic acid equivalent (1 mM = 1 ASE). The ASE/ml value is inversely proportional to reducing power. Deoxyribose non-site specific hydroxyl ions scavenging effect was performed as described by Halliwell, Gutteridge, and Aruoma (1987). Ferrous ions chelating capacity was estimated by the method of Decker and Welch (1990). Inhibition of superoxide radical production was assayed using the nitroblue tetrazolium chloride (NBT) method (Nishikimi, Rao, & Yagi, 1972).

2.3. DNA nicking assay

DNA nicking assay was performed using supercoiled pBR322 DNA by the method of Lee et al. (2002). A mixture of 10 μl of plant extracts of different concentrations (20–40 μg/ml) and plasmid DNA (0.5 μg) was incubated for 10 min at room temperature followed by the addition of 10 μl of Fenton's reagent (30 mM H_2O_2, 50 μM ascorbic acid and 80 μM $FeCl_3$). The final volume of the mixture was made up to 20 μl and incubated for 30 min at 37 °C. The DNA was analysed on 1% agarose gel using ethidium bromide staining.

2.4. HPLC analysis

For HPLC analysis, 1 g of dried and powdered plant material was extracted with 50% methanol/water (1×10 ml) for 2 h at room temperature. The plant extract was hydrolysed with 1.2 N HCl by refluxing on a water bath for 1 h (Lin-Chin, Huang, & Wen, 2000). The hydrolysate was processed and subjected to qualitative and quantitative analysis by using a Shimadzu LC-10A (Kyoto, Japan) HPLC system (Bajpai, Pande, Tewari, & Prakash, 2005). Results (μg/g dry wt) were obtained by comparison of peak areas (λ_{max} 254 nm) of the samples with those of standards.

2.5. Qualitative analysis by MS/MS

An API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Ontario, Canada) was used. Analysis were performed on a Turbo ion spray source in negative mode using the following settings: nebuliser gas (N_2) 16, curtain gas (N_2) 12, collision gas (N_2) 1–2 (arbitrary units), focusing potential −400 V, entrance potential −10, declustering potential 25–60 and collision energy 15–35. Full scan acquisition was performed by scanning from *m/z* 150 to 700 at a cycle time of 2 s. MS/MS product ions were produced by collision-associated dissociation of the selected precursor ions in a collision cell. In all the experiments, the first quadrupole was operated at unit resolution. Product ion scan of selected molecules were carried out, in order to confirm the structure of the compounds.

2.6. Statistical analysis

Results are the mean values of three replicates of the same sample. Statistical analysis was performed using analysis of variance.

3. Results and discussion

The total phenolic contents (TPC) and antioxidant activity (AOA) of four (red, violet, white and green) varieties of *A. cepa* are presented in Table 1. The TPC ranged from 4.6 to 74.1 mg/g GAE and AOA from 13.6% to 84.1%. The outer dry layers of the red variety showed the

Table 1
Total phenolic content (TPC) expressed as mg/g gallic acid equivalent (GAE) and antioxidant activity (AOA%) in different varieties and layers of *Allium cepa* on dry weight basis

Variety	Part	TPC (mg/g GAE)	AOA (%)
Red	Outer layers	74.1	84.1
	Middle layers	15.9	50.6
	Inner layers	5.6	15.6
Violet	Outer layers	43.5	73.9
	Middle layers	14.9	36.2
	Inner layers	4.6	14.5
White	Outer layers	7.6	23.4
	Middle layers	6.4	18.1
	Inner layers	5.7	13.6
Green	Outer layers	13.4	55.6
	Middle layers	9.5	26.3
	Inner layers	7.2	15.8
LSD at $P < 0.01$	For layers	0.5	1.7
	For varieties	1.20	1.50

highest TPC (74.1 mg/g GAE) followed by violet onion (43.5 m/g GAE). Their outer layers had the highest contents of TPC followed by a continuous decrease towards the inner part of the bulb. The highest AOA was observed in the outer layer of the red onion (84.1%) followed by violet onion (73.9%) and moderate to low activities in their remaining fleshy portions (Table 1). TPC values of 80.0 and 26.0 mg/g GAE in the dry outer skins of red and yellow onions respectively have been reported (Nuutila, Puupponen-Pimiä, Aarni, & Oksman-Caldentey, 2003).

All four varieties were studied for their free radical scavenging activity (FRSA) and reducing power (Table 2). The FRSA in different samples showed a wide variation of IC_{50} ranging from 0.1 to 15.2 mg/ml, EC_{50} from 4.3 to 660.8 mg/mg DPPH and ARP from 0.15 to 23.20. The outer dry layers of the red onion were found most powerful free radical scavenger compared to the rest of the varieties and standard quercetin. The other layers of the bulb in all varieties showed very low FRSA as evidenced by high IC_{50} (8.7–15.2 mg/ml) and EC_{50} values (371.8–660.8 mg/mg) and low ARP (0.15–0.27) compared to the standard (Table 2).

Table 2
Free radical scavenging activity and reducing power in different varieties and layers of *Allium cepa* on dry weight basis

Variety	Part	IC_{50} (mg/ml)	EC_{50} (mg/mg)	ARP	ASE/ml
Red	Outer layers	0.10	4.3	23.2	9.2
	Middle layers	8.7	372	0.27	13.3
	Inner layers	11.7	508	0.19	28.6
Violet	Outer layers	0.7	30.6	3.26	12.5
	Middle layers	9.8	422	0.23	17.9
	Inner layers	12.7	566	0.17	30.5
White	Outer layers	12.2	530	0.18	9.4
	Middle layers	13.5	587	0.17	10.6
	Inner layers	15.2	661	0.15	12.8
Green	Outer layers	9.1	396	0.25	30.6
	Middle layers	13.1	570	0.17	26.2
	Inner layers	14.2	617	0.16	8.7
LSD at $P < 0.01$	For layers	0.61	10.1	0.81	1.75
	For varieties	0.68	19.7	1.22	1.53
Standard	Quercetin	0.20	8.6	11.6	0.5

IC_{50}(mg/ml of extract), inhibitory concentration; EC_{50}(mg/mg DPPH), efficient concentration; ARP, anti-radical power; ASE/ml, ascorbic acid equivalent (1 mM = 1 ASE) is inversely proportional to reducing power.

The layers of red and violet varieties with high of TPC and AOA were assessed for concentration dependent FRSA, using four different methods (Table 3). The IC_{50} values of lipid peroxidation inhibition measured by ammonium thiocyanate assay in different layers ranged from 1.44 to 4.20 mg/ml (red) and 1.84 to 4.67 mg/ml (violet). The outer layers of both varieties showed better inhibition than α-tocopherol (3.44 mg/ml). The middle and inner fleshy layers showed moderate to low inhibition. The non-site-specific inhibition of hydroxyl radical induced deoxyribose degradation was also higher, as evidenced by low IC_{50} values in outer dry layers of red (0.56 mg/ml) and violet (0.90 mg/ml) varieties, compared to the middle and inner layers. However, BHT standard showed better activity (0.42 mg/ml). The outer dry layers of both red (2.54 mg/ml) and violet (2.96 mg/ml) varieties were similar to BHT (2.85 mg/ml) as potential inhibitors of NBT reduction by superoxide anions. Quercetin had been found to reduce the level of peroxynitrate, an extremely powerful oxidant in the brain, by scavenging superoxide anions (Shutenko et al., 1999). The concentration dependent ferrous ion chelating capacity of red and violet varieties increased from outer to inner layers (Table 3). The most probable reason for the variation of FRSA might be due to variation in the quantities of quercetin in the various layers of different varieties. These results were further supported by HPLC results of the specific phenolic composition (Table 4), which showed high amounts of quercetin in the outer layers of the red variety. The mechanisms of action of quercetin include free radical scavenging, chelation of transition metal ions and inhibition of oxidases (de Groot & Rauen, 1998; Lean et al., 1999; Suzuki, Masahi, Segami, & Ito, 1998).

The four varieties of *A. cepa* were also studied for their specific phenolic composition through HPLC (Table 4) including ferulic acid, gallic acid and protocatechuic acid,

Table 3
Free radical scavenging activity expressed as IC_{50} (mg/ml) in different layers of red and violet varieties of *Allium cepa* on dry weight basis

Variety	Part	*A*	*B*	*C*	*D*
Red	Outer layers	1.44	0.56	2.54	5.96
	Middle layers	2.21	1.31	4.18	5.44
	Inner layers	4.20	1.82	3.40	4.09
Violet	Outer layers	1.84	0.90	2.96	6.38
	Middle layers	2.61	1.38	4.76	5.96
	Inner layers	4.67	2.11	3.81	4.17
LSD at $P < 0.01$	For layers	0.15	0.06	0.11	0.23
	For varieties	0.19	0.08	0.10	0.21

A, hydroxyl radical scavenging activity assayed by ammonium thiocyanate method; *B*, non-site specific inhibition of hydroxyl radical mediated deoxyribose degradation; *C*, inhibition of NBT reduction caused by superoxide anions; *D*, ferrous ion chelating capacity.

Table 4
The composition of total phenols (μg/g dry weight) in different varieties and layers of *Allium cepa*

Variety	Part	FA	GA	PA	Quer	Kaemp
Red	Outer layers	21.4	263	138	5110	481
	Middle layers	39.9	89.7	75.9	1120	44.3
	Inner layers	84.3	71.1	32.4	419	6.5
Violet	Outer layers	13.5	322	94.5	3200	203
	Middle layers	23.9	266	42.6	235	18.9
	Inner layers	45.8	158	13.4	125	11.5
White	Outer layers	42.5	354	18.7	38.6	7.5
	Middle layers	58.6	105	6.4	21.7	4.6
	Inner layers	115.7	84.2	3.1	14.5	3.2
Green	Outer layers	43.5	29.5	40.2	299	15.8
	Middle layers	35.2	21.6	17.9	45.3	6.2
	Inner layers	27.4	9.3	12.8	17.3	5.3
LSD at $P < 0.01$	For layers	4.49	5.26	3.85	4.16	4.20
	For varieties	5.93	11.7	9.13	13.8	3.80

FA, Ferulic acid; GA, Gallic acid; PA, Protocatechuic acid; Quer, Quercetin; Kaemp, Kaempferol.

quercetin and kaempferol. The quantities of ferulic acid varied from 13.5 to 116 μg/g, gallic acid from 9.3 to 354 μg/g, protocatechuic acid from 3.1 to 138 μg/g, quercetin from 14.5 to 5110 μg/g and kaempferol from 3.2 to 481 μg/g. The quantities of gallic acid, protocatechuic acid, quercetin and kaempferol decreased in all varieties from outer dry to inner fleshy layers. The amounts of ferulic acid showed a continuous increase from outer to inner layers in red, violet and white varieties while in green there was a continuous decrease. The outer dry layers of red variety were found to be the richest source of protocatechuic acid (138 μg/g) quercetin (5110 μg/g) and kaempferol (481 μg/g). Highest quantities of ferulic (116 μg/g) and gallic acids (354 μg/g) were in the inner and outer layers of white onion, respectively (Table 4). It has been found that phytochemicals in onions vary greatly due to varietal differences (Bilyk, Cooper, & Sapers, 1984). It has also been reported that yellow, red and pink onions have higher amounts of quercetin than white varieties but colour was not found to be the limiting factor (Patil, Pike, & Yoo, 1995). The outer layers of the bulb go as a waste and can be utilised for the isolation of quercetin that had been reported to provide beneficial effects in human health (Bingham et al., 2003).

The identification of specific polyphenols was further substantiated by MS/MS analysis (Table 5) that showed the deprotonated molecule $[M–H]^-$. Loss of CO_2 was observed for ferulic, gallic and protocatechuic acids, giving the ion $[M–H-44]^-$ as a characteristic ion. Ferulic acid also showed the loss of a CH_3 group, providing $[M-15]^-$ anion radical at *m*/*z* 178 in the product ion scans. The aglycones of quercetin and kaempferol underwent Retro-DielsAlder fragmentation to give an ion at *m*/*z* 151; in the case of kaempferol loss of neutral water molecule gave an ion at *m*/*z* 133 also (Sanchez-Rabaneda et al., 2003).

Table 5
Phenolic compounds identified by MS/MS

Phenols	Ion full scan MS		MS/MS approach
	$[M–H]^-$	Fragments	Product ion scan
Ferulic acid	193	178, 149	193
Gallic acid	169	125	169
Protocatechuic acid	153	109	153
Kaempferol	285	133, 151	285
Quercetin	301	151	301

Hydroxyl radicals generated by the Fenton reaction are known to cause oxidatively induced breaks in DNA strands to yield its open circular or relaxed forms. The concentration dependent (20–40 μg/ml) free radical scavenging effect of 50% MeOH/water extracts of different varieties were studied (Fig. 1) on plasmid DNA damage. The extracts of outer layer of red variety (lanes 7 and 8) showed significant reduction in the formation of nicked DNA and increased native form of DNA. The protection offered by red variety was significantly close to that of 5 U of catalase (lane 3) and 2 U of superoxide dismutase (lane 4). The violet (lanes 5 and 6) variety showed moderate, while green (lanes 9 and 10) and white (lanes 11 and 12) varieties showed comparatively low protection. The red variety with high phenolic content showed better protection compared to the others, indicating that protection was directly proportional to the concentration of TPC. Quercetin effectively protected DNA strand scission from *tert*-butylhydroperoxide (Sestili, Guidarelli, Dacha, & Cantoni, 1998). Therefore, in the red variety presence of high quantities of quercetin might be responsible for better protection of DNA. In biological systems metal binding can occur on DNA leading to partial site-specificity hydroxyl radical formation. Polyphenols are potential protecting agents against the lethal effects of oxidative stress and offer protection of DNA by chelating redox-active transition metal ions.

Onions are widely used all over the world and their outer dry layers go to waste. In present studies it was a found that this portion of red onions was rich source of phenols with promising antioxidant and free radical scavenging activities and ability to provide protection against DNA damage caused by reactive oxygen species. This present study together with previous work suggests the triple synergistic action of phenols in scavenging ROS, repairing DNA radicals and metal chelation (Zhao et al., 2005).

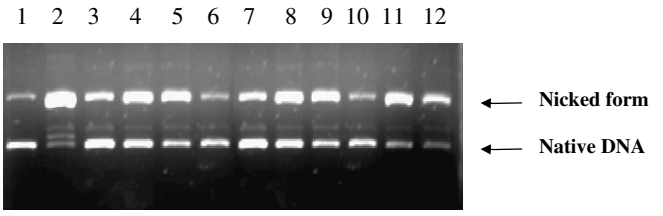

Fig. 1. Inhibitory effects of onion extracts on DNA nicking caused by hydroxy radicals. lane 1, native pBR322 DNA; lane 2, Fenton's reagent + DNA; lane 3, Fenton's reagent + DNA + 5 U Catalase; lane 4, Fenton's reagent + DNA + 2 U super oxide dismutase, lanes 5, 6, 7, 8, 9, 10, 11 and 12, Fenton's reagent + DNA + (40 and 20 μg/1.0 ml) extracts of outer layers of violet, red, green and white varieties of *Allium cepa* bulbs, respectively.

Acknowledgements

The authors are thankful to Dr. Rakesh Tuli, Director, National Botanical Research Institute, Lucknow, for his keen interest, guidance and for providing research facilities and to Miss Jyoti Barthwal, for MS/MS studies.

References

Apati, P., Szentmihalyi, K., Kristo, Sz. T., Papp, I., Vinkler, P., Szoke, E., et al. (2003). Herbal remedies of Solidago, correlation of phytochemical characteristics and antioxidative properties. *Journal of Pharmaceutical and Biomedical Analysis, 32*, 1045–1053.

Bajpai, M., Pande, A., Tewari, S. K., & Prakash, D. (2005). Phenolic contents and antioxidant activity of some food and medicinal plants. *International Journal of Food Sciences and Nutrition, 56*, 287–291.

Bilyk, A., Cooper, P., & Sapers, G. (1984). Varietal difference in distribution of quercetin and kaempferol in onion tissue. *Journal of Agricultural and Food Chemistry, 32*, 274–276.

Bingham, M., Gibson, G., Gottstein, N., Pascual-Teresa, S. D., Minihane, A. M., & Rimbach, G. (2003). Gut metabolism and cardio protective effects of dietary isoflavones. *Current Topics in Nutraceutical Research, 1*, 31–48.

Challier, B., Pernau, J., & Viel, J. (1998). Garlic, onion and cereal fibre as protective for breast cancer: a French case study. *European Journal of Epidemiology, 14*, 739–747.

Crozier, A., Lean, M. E. J., McDonald, M. S., & Black, C. (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onion, lettuce and celery. *Journal of Agricultural and Food Chemistry, 45*, 590–595.

Decker, E. A., & Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and Food Chemistry, 38*, 674–678.

de Groot, H., & Rauen, U. (1998). Tissue injury by reactive oxygen species and protective effects of flavonoids. *Fundamental and Clinical Pharmacology, 12*, 249–255.

Dorant, E., Van Den Breandt, P., & Goldbohm, A. (1995). Allium vegetable consumption, garlic supplement intake and female breast carcinoma incidence. *Breast Cancer Research and Treatment, 33*, 163–170.

Emmons, C. L., & Peterson, D. M. (1999). Antioxidant activity and phenolic contents of Oats, Groats and Hulls. *Cereal Chemistry, 76*, 902–906.

Fossen, T., Pedersen, A. T., & Andersen, O. M. (1997). Flavonoids from red onion (*Allium cepa*). *Phytochemistry, 47*, 281–285.

Fukushima, S., Takada, N., Hori, T., & Wanibuchi, H. (1997). Cancer prevention by organosulfur compounds from garlic and onion. *Journal of Cellular Biochemistry Supplement, 27*, 100–105.

Gazzani, G., Papetti, A., Massolini, G., & Daglia, M. (1998). Anti and prooxidant activity of water-soluble components of some common diet vegetables and the effect of thermal treatment. *Journal of Agricultural and Food Chemistry, 46*, 4118–4122.

Halliwell, B. (1995). Oxygen radical, nitric oxide and human inflammatory joints disease. *Annals of the Rheumatic Diseases, 54*, 505–510.

Halliwell, B., & Gutteridge, J. M. C. (1999). *Free radicals in biology and medicine*. Oxford: Oxford University Press.

Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry, 165*, 215–219.

Kroyer, G. T. (2004). Red clover extract as antioxidant active and functional food ingredient. *Innovative Food Science and Emerging Technologies, 5*, 101–105.

Lean, M., Norrozi, M., Kelly, L., Burrns, J., Talwar, D., Satter, N., et al. (1999). Dietary Flavanoids protect diabetic human lymphocytes against oxidant damage to DNA. *Diabetes, 48*, 176–181.

Lee, J. C., Kim, H. R., Kim, J., & Jang, Y. S. (2002). Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. Saboten. *Journal of Agricultural and Food Chemistry, 50*, 6490–6496.

Lin-Chin, Y. R., Huang, C. Y., & Wen, K. C. (2000). Evaluation of quantitative analysis of flavonoid aglycones in *Gingko biloba* extract and its products. *Journal of Food and Drug analysis, 8*, 289–296.

Nilsson, J., Stegmark, R., & Akesson, B. (2004). Total antioxidant capacity in different pea (*Pisum sativum*) varieties after blanching and freezing. *Food Chemistry, 86*, 501–507.

Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochemical and Biophysical Research Communication, 46*, 849–864.

National Onion Association (NOA). Onions – phytochemical and health properties. Available from www.onions.usa.org.

Nuutila, A. M., Puupponen-Pimiä, R., Aarni, M., & Oksman-Caldentey, K. M. (2003). Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chemistry, 81*, 485–493.

Patil, B., Pike, L., & Yoo, K. (1995). Variation in the quercetin content in different coloured onions. *Journal of American Society for Horticultural Science, 120*, 909–913.

Ragazzi, E., & Veronese, G. (1973). Quantitative analysis of phenolic compounds after thin layer chromatographic separation. *Journal of Chromatography, 77*, 369–375.

Rhodes, M., & Price, K. (1996). Analytical problems in the study of flavanoid compounds in onions. *Food Chemistry, 57*, 113–117.

Sanchez-Rabaneda, F., Jauregui, O., Casals, I., Andres-Lacueva, C., Izquierdo –Pulido, M., & Lamuela-Raventos, M. R. (2003). Liquid chromatography/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). *Journal of Mass Spectrometry, 38*, 35–42.

Sestili, P., Guidarelli, A., Dacha, M., & Cantoni, O. (1998). Quercitin prevents DNA single strand breakage and cytotoxicity caused by tert-butylhydroperoxide: Free radical scavenging versus iron chelating mechanism. *Free Radical Biology and Medicine, 25*, 196–200.

Shutenko, Z., Henry, Y., Pinard, E., Seylaz, J., Potier, P., Berthet, F., et al. (1999). Influence of the antioxidant quercitin *in vivo* on the level of nitric oxide determined by electron paramagnetic resonance in rat brain during global ischemia and reperfusion. *Biochemical Pharmacology, 57*, 199–208.

Stajner, D., & Varga, I. S. (2003). An evaluation of the antioxidant abilities of *Allium* species. *Acta Biologica Szegediensis., 47*, 103–106.

Suzuki, Y., Masahi, I., Segami, T., & Ito, M. (1998). Anti-ulcer effects of antioxidants, Quercitin, α-tocopherol, nefedipine & tetracycline in rats. *Japanese Journal of Pharmacology, 78*, 435–441.

Vinson, J. A. (1998). Flavonoids in foods as in vitro and in vivo antioxidants. In B. Ma (Ed.), *Flavonoids in the living system* (pp. 151–164). New York: Plenum Press.

Yen, G. C., & Duh, P. D. (1994). Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen. *Journal of Agricultural and Food Chemistry, 42*, 629–632.

Yin, M., & Cheng, W. (1998). Antioxidant activity of several *Allium* members. *Journal of Agricultural and Food Chemistry, 46*, 4097–4101.

Zhao, C., Dodin, G., Yuan, C., Chen, H., Zheng, R., & Fan, Z. J. B. (2005). "In vitro" protection of DNA from Fenton reaction by plant polyphenols verbascoside. *Biochimica Biophysica Acta, 1723*, 114–123.