Anti- and Prooxidant Activity of Water Soluble Components of Some Common Diet Vegetables and the Effect of Thermal Treatment

Gabriella Gazzani,* Adele Papetti, Gabriella Massolini, and Maria Daglia

Department of Pharmaceutical Chemistry, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy

The pro-antioxidant activity of carrot, cauliflower, celery, eggplant, garlic, mushroom, onion, white cabbage, white potato, tomato, yellow bell pepper, and zucchini was investigated. Juices obtained by centrifugation of vegetables were treated at different temperatures (2, 25, 102 °C) and assessed for antioxidant activity (AA) using a model system β-carotene−linoleic acid. Antioxidant activity of all vegetable juices showed a linear correlation with time. The equations of all straight lines obtained showed positive slope values indicating either an increase in antioxidant activity or a decrease in prooxidant activity during the reaction. Negative intercept values were found when the juices showed prooxidant activity at least during the first phase of the reaction. Mushroom and white cabbage always showed more than 80% AA, while cauliflower, celery, and eggplant showed such high AA only after boiling. Tomato and yellow bell pepper were always prooxidant. Cluster analysis allowed the vegetables to be divided into five groups according to their anti- and prooxidant behavior as a function of thermal treatment and reaction time. Vegetable juice components were separated on a Bakerbond C_{18} solid-phase extraction cartridge according to their polarity, and the AA of the bound and unbound fractions of each vegetable was also tested.

Keywords: *Vegetables; lipid peroxidation; antioxidants; prooxidant*

INTRODUCTION

Lipid peroxidation is a major cause of the decrease in the flavor and nutritive value of fat and oil products. This reaction generates very reactive oxygen compounds which in humans are responsible for causing or accelerating chronic disease states such as cardiovascular, neoplastic, inflammatory, and amyloidosis pathologies and aging (Havesteen, 1983; Caragay, 1992; McCord, 1994).

Since lipid peroxidation is a chemical reaction with low activation energy, the rate of this reaction is not significantly diminished by lowering the storage temperature of bulk diet lipids. As a consequence synthetic antioxidants are often incorporated to prevent peroxidation. In recent years the use of these compounds has been restricted because of the possibility of their toxic and carcinogenic effects (Imida et al., 1983; Maeura et al., 1984; Haigh, 1986; Van der Heijden et al., 1986; Van Esch, 1986). On the other hand, during the past two decades interest in lipid peroxidation has surpassed food technology. It has been realized that compounds deriving from lipid peroxidation and their degradation products have harmful effects on biological systems. Furthermore, a number of plant constituents have been recognized to have positive effects against the oxygen reactive compounds in biological systems (Hemeda and Klein, 1984). It is thus possible that a more in-depth knowledge about plant food properties can help to discover natural components that can act as antioxidants in vitro and in vivo.

A number of reports studied the antioxidant properties of either isolated plant constituents such as flavonoids (Hertog et al., 1992a,b; Lee et al., 1995), flavonols (Bilyk et al., 1984), essential oils (Farag et al., 1989), and tannins (Laughton et al., 1991) or plant extracts obtained from vegetables, fruits, spices, and wine (Onyeneho and Hettiarachchy, 1992; Kinsella et al., 1993). The effects of processing on food antioxidant properties (Gazzani, 1994; Al-Saikan et al., 1995; Nicoli et al., 1997) were also extensively studied.

The aim of this paper was to assess the antioxidant activity (AA) of 12 common edible vegetables widely consumed in the mediterranean diet. Such activity is determined on vegetable juices obtained by simple centrifugation and filtration at 2 °C, to minimize the effects of handling. It is well known that many factors (i.e., the antioxidant concentrations, temperature and pH of the medium, the occurrence of chemicals with either positive or negative synergism) can strongly influence antioxidant activity. To evaluate the effects of storage and thermal treatment to which vegetables are commonly subjected, juices were also analyzed after storage at room temperature (25 °C) and boiling (102 °C). The antioxidant activity was determined using a model system containing β-carotene−linoleic acid which is widely used to assess antioxidant activity of vegetable extracts (Tsushida et al., 1994; Lee et al., 1995; Al-Saikhan et al., 1995; Nicoli et al., 1997). Furthermore, total phenolic content, reducing substances, and peroxidase activity were determined to verify any relation with antioxidant activity.

MATERIALS AND METHODS

Vegetable Samples. The vegetables purchased in September from a local supermarket were carrot (*Daucus carota* L.), cauliflower (*Brassica oleracea* L. var. botrytis), celery (*Apium graveolens* L.), eggplant (*Solanum melongena* L.),

* Corresponding author [telephone +39 0382 507373; fax +39 0382 422975; e-mail gazzani@chifar.unipv.it].

10.1021/jf980300o CCC: $15.00
Published on Web 09/19/1998

Table 1. Description of Tested Vegetables

common name	Latin name	class, order, family	mL/g	RSD	pH	RSD
carrot	*D. carota*	Apiaceae	0.51	0.089	6.83	0.27
cauliflower	*B. oleracea convar. botrylis* var. botyrlis	Brassicaceae	0.33	0.044	6.96	0.37
celery	*A. graveolens* var. dulce	Apiaceae	0.55	0.097	6.32	0.09
eggplant	*S. melongena*	Solanaceae	0.46	0.078	6.14	0.59
garlic	*A. sativum L.*	Liliaceae	0.39	0.073	6.13	0.23
mushroom	*P. campestris*		0.37	0.053	6.93	0.40
onion	*A. cepa*	Liliaceae	0.56	0.064	5.94	0.18
tomato	*L. esculentum*	Solanaceae	0.55	0.078	4.31	0.11
white cabbage	*B. oleracea convar. capitata* var. alba	Brassicaceae	0.46	0.063	6.37	0.34
white (Irish) potato	*S. tuberosum*	Solanaceae	0.39	0.061	6.27	0.12
yellow bell pepper	*C. annuum*	Solanaceae	0.68	0.091	4.96	0.08
zucchini	*C. pepo convar. giromontina*	Cucurbitaceae	0.52	0.053	6.78	0.23

garlic (*Allium sativum* L.), mushroom (*Psalliota campestris*), onion (*Allium cepa* L.), tomato (*Lycopersicon esculentum* Mill. L.), white cabbage (*Brassica oleracea* L. var. *alba* L.), white (Irish) potato (*Solanum tuberosum* L.), yellow bell pepper (*Capsicum annuum* L.), and zucchini (*Cucurbita pepo* L. *convar. giromontiina Greb.*).

Sample Preparation. Vegetables were washed (carrot, eggplant, garlic, onion and white potato were also peeled), weighed, cut into small pieces, homogenized, and then centrifuged for 4 min to completely separate the juice from each vegetable. The volume and pH value (Table 1) of each vegetable juice were measured, and then the juice was subdivided into three batches. The juice was filtered on Ruudfilter Schleicher Schuell 1573 (no. 314709, diameter 190 mm) and then on Millipore membranes of cellulose acetate/cellulose nitrate mixed esters (0.45 μm). Two lots of each juice were filtered in an ice bath (2 °C), and a third batch was filtered at room temperature (25 °C). Filtration resulted in loss of most of the juice coloration. The batch filtered at 2 °C was immediately analyzed, while the second batch was boiled for 30 min, the time commonly used in home cooking of most vegetables. The temperature during boiling was experimentally measured and resulted to be 102 ± 0.5 °C. The batch filtered at 25 °C was maintained at room temperature for 3 h before analysis.

In Vitro Antioxidant Assay. The antioxidant activity of the vegetable juices, based on coupled oxidation of β-carotene and linoleic acid, were evaluated following the method of Taga et al. (1984) with some modifications. β-carotene (5 mg) (Merck) was dissolved in 50 mL of chloroform solution. A 3 mL aliquot of β-carotene chloroform solution was added to a conical flask along with 40 mg of linoleic acid (Merck) and 400 mg of Tween 20 (Merck). Chloroform was evaporated to dryness under reduced pressure at low temperature (less than 30 °C). Distilled water (100 mL) was added to the dried mixture, and the mixture was shaken. Four aliquots (400 μL) of vegetable juices were added to 5 mL of β-carotene emulsion in test tubes, and the mixture was mixed well (samples). In preliminary tests the adding of juices showed no significant change in sample pH. For one sample the absorbance at 470 nm was immediately measured using the spectophotometer, and for the other samples absorbance was measured after 10, 20, and 30 min of incubation in a water bath at 50 °C. Each sample was read against an emulsion prepared as described but without β-carotene (blank). To correct for the influence of the juice color in calculating β-carotene degradation rate (dr), four aliquots (400 μL) of each juice were added to 5 mL of blank (blank samples). These mixtures for each time point were spectrophotometrically read, and the absorbance measured was subtracted from that of the corresponding sample. The dr of β-carotene was calculated by first-order kinetics:

$$(\ln(A_0/A_t))/t = \text{dr of sample} \quad (1)$$

where A_0 = absorbance of the sample − absorbance of blank sample at time 0 (absorbance was read immediately after the addition of juice), A_t = absorbance of the sample − absorbance of blank sample at time t, and t = 10 or 20 or 30 min of incubation at 50 °C.

$$(\ln(a_0/a_t))/t = \text{dr of control sample} \quad (2)$$

where 400 μL of distilled water was added to 5 mL of β-carotene emulsion and treated as the corresponding sample, a_0 = absorbance of the control sample at time 0, and a_t = absorbance of the control sample at time t.

Antioxidant activity was expressed as the percent of inhibition relative to the control using the equation:

$$\text{AA \%} = \frac{\text{dr control sample} - \text{dr sample}}{\text{dr control sample}} \times 100 \quad (3)$$

An aliquot of 200 mg of butylhydroxytoluol (BHT, Fluka) was added to 2000 mg of Tween 20 and 100 mL of distilled water in a conical flask. The emulsion was mixed well, and an aliquot of 100 μL was diluted with 300 μL of distilled water and assayed for AA.

Solid-Phase Extraction (SPE) of the Vegetable Juices. Each vegetable juice filtered at 2 °C was separated into two fractions using a Bakerbond C_{18} SPE cartridge. The C_{18} cartridge was preconditioned with 5 mL of methanol−Millipore grade distilled water (30:70, v/v). After the sample was loaded (3 mL), the C_{18} cartridge was washed with 6 mL of water and C_{18} bound compounds were eluted with 9 mL of methanol−water (50:50, v/v). After evaporation of the solvents, each fraction (bound and unbound) was dissolved in a 3 mL aliquot of bidistilled water and tested for antioxidant assay.

Statistical Analysis. Values represent means of 10 replications. Data were analyzed with the statistical analysis package Statgraphics by Statistical Graphic Corp. (version 5,1991).

Slope and intercept of the fitted lines (percentage of antioxidant activity versus time for each vegetable juice following the different heat treatment) were calculated by simple regression procedure (linear model). The multivariate data set was processed using cluster analysis procedure, and to visually compare the different clusters the star symbol plot procedure was used.

RESULTS

Table 1 reports some chemical, botanical characteristics and the volume of raw juice obtained from 1 g of the tested vegetables.

The data concerning the percentage of antioxidant activity calculated after 10, 20, and 30 min of reaction at 50 °C for each vegetable juice at the different temperatures are reported in Table 2.

When prepared at 2 °C, most vegetable juices showed initial (after the first 10 min of reaction) prooxidant activity. This activity was very high for eggplant, tomato, and yellow bell pepper and decreased with time

Table 2. Antioxidant Activity Percentage of Tested Vegetables and Regression Analysis Results Produced by the Simple Regression Procedure

		AA%						
vegetable	thermal treatment (°C)	10 min	20 min	30 min	RSD	slope	intercept	*r*
carrot	2	−57	11	64	33.56	6.05	−115	0.997
	25	24	37	75	15.50	2.55	−6	0.996
	102	37	50	77	9.15	2.00	15	0.980
cauliflower	2	−40	−1	38	3.21	3.90	−79	1.000
	25	−14	8	32	12.02	2.30	−37	0.999
	102	68	70	86	4.43	0.90	57	0.912
celery	2	−68	2	47	39.40	6.25	−121	0.992
	25	−100	−16	25	21.92	5.75	−121	0.981
	102	71	83	86	18.91	0.75	65	0.945
eggplant	2	−307	−85	0.02	61.58	15.35	−438	0.968
	25	−24	22	42	13.01	3.30	53	0.975
	102	81	86	90	9.84	0.45	77	0.998
garlic	2	−90	−6	42	16.92	6.60	−150	0.988
	25	−89	11	56	6.37	7.25	−152	0.977
	102	26	54	76	6.34	2.50	2	0.998
mushroom	2	87	92	96	2.12	0.45	83	0.997
	25	78	92	94	2.12	0.80	72	0.918
	102	93	94	95	0.70	0.10	92	1.000
onion	2	−32	28	52	9.29	4.20	−68	0.971
	25	−3	15	46	2.83	2.45	−30	0.988
	102	−20	44	63	19.89	4.15	−54	0.954
tomato	2	−621	−341	−175	93.41	22.30	−825	0.989
	25	−648	−319	−106	22.63	27.10	900	0.992
	102	−261	−101	−53	63.52	10.40	−346	0.973
white cabbage	2	66	78	82	8.66	0.80	59	0.961
	25	50	72	80	11.01	1.50	37	0.966
	102	52	66	87	21.38	1.75	33	0.993
white potato	2	*a*	*a*	*a*	*a*	*a*	*a*	*a*
	25	*a*	*a*	*a*	*a*	*a*	*a*	*a*
	102	63	73	75	23.56	0.60	58	0.933
yellow bell pepper	2	−432	−288	−143	236.37	14.45	−577	1.000
	25	−843	−473	−172	350.70	33.55	−1157	0.988
	102	−667	−381	−261	346.50	20.30	−842	0.973
zucchini	2	−137	−36	26	47.96	8.15	−212	0.991
	25	38	69	80	68.59	2.10	20	0.964
	102	34	51	60	11.53	1.30	22	0.985
BHT (0.5 mg/mL)		81	86	90	0.50	0.45	77	0.998

[a] Not determined because of turbidity of the β-carotene−linoleic acid system after the addition of the juice.

becoming antioxidant at the end of the monitoring period (30 min) in the case of all vegetable juices except those of tomato and yellow bell pepper.

When prepared and stored at room temperature (25 °C), the juice of celery, tomato and yellow bell pepper revealed an initial prooxidant activity higher than that of the same juices prepared at 2 °C, while in the case of cauliflower and eggplant this activity was lower; in contrast, carrot and zucchini were antioxidant. The initial AA of white cabbage juice prepared at 2 °C slightly increased when the juice was treated at 25 °C. For the same treatment the final AA increased in the cases of carrot, eggplant, garlic, and zucchini; decreased for cauliflower, celery, and onion; and did not significantly change for the other vegetables. The prooxidant activity of tomato juice decreased while that of yellow bell pepper increased. After adding white potato juice obtained at 2 and 25 °C in the β-carotene−linoleic acid system, the solution was turbid, and so the assay could not be carried out.

With regard to boiled juices, they were found to be antioxidant even after 10 min of reaction, with the exception of onion juice that initially only showed weak prooxidant activity and of tomato and yellow bell pepper juices which were always found to be prooxidant. Results showed that in general the AA of the juices increased appreciably after heat treatment.

The juice activities were either constant or variable as shown by the relative standard deviation (RSD) values relative to AA depending on type of vegetable and on thermal treatment. Mushroom juice activity was almost constant at all temperatures applied, while activity was variable for all other vegetables especially for eggplant, tomato, and yellow bell pepper. In the cases of carrot, celery, garlic, mushroom, zucchini, tomato, and particularly eggplant juice, AA was stabilized by boiling (lower RSD values); conversely a marked increase in variability was found in the case of onion, white cabbage, and yellow bell pepper (higher RSD values).

The percentage of antioxidant activity (AA%) of all vegetable juices increased steadily as a function of reaction time, and the estimated fitted lines presented a correlation coefficient (*r*) that was always higher than 0.90 (Table 2). All the estimated slopes presented positive values that increased correspondingly with increasing activity during the reaction. For each type of vegetable, different slope values, at the different temperatures, showed that anti- or prooxidant activity was influenced by thermal treatment.

With regard to the intercepts, a positive value indicated that the juice always possessed antioxidant properties, while a negative value showed that it possessed, at least initially, prooxidant activity.

Cluster analysis, a procedure which allows one to group observations from a multivariate data set into clusters of similar points, was carried out by considering the slope and intercept values of the fitted lines esti-

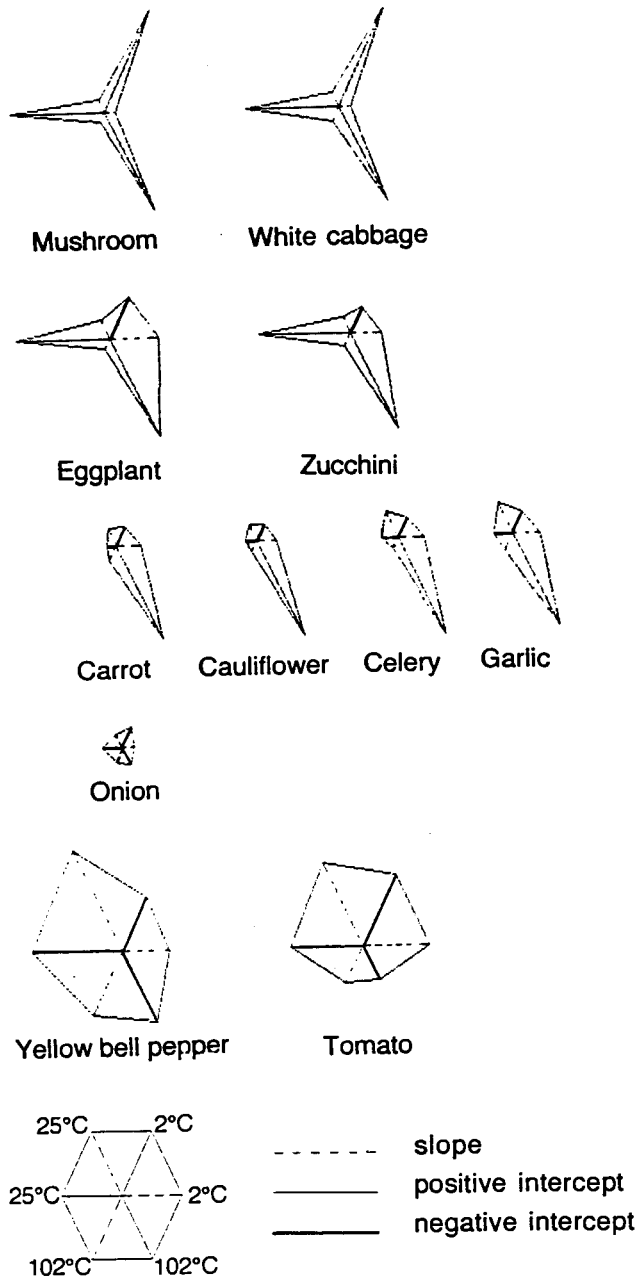

Figure 1. Star symbol plot of tested vegetables.

mated at 2, 25, and 102 °C (Table 2) for each vegetable (with the exception of white potato), and so one can discriminate vegetables showing similar behavior. Analogies between tested vegetables were clearly evident in the graphic representation of the estimated slopes and intercepts carried out using the star symbol plot procedure that allows one to visually compare different observations (Figure 1). Mushroom and white cabbage represented the best cluster that was found to have an initial high antioxidant activity which scarcely increased during the monitoring and was hardly influenced by thermal treatment. In the case of eggplant and zucchini a similar cluster was found at 25 and 102 °C, even if AA % values registered after 30 min were lower. Their behavior was different at 2 °C because at this temperature eggplant and zucchini juices were found to be prooxidant. Carrot, celery, cauliflower, and garlic formed a cluster whose activity increased appreciably as a function of reaction time and especially following thermal treatment at 102 °C. Onion differed from all the other vegetables because boiled juice presented, but only initially, light prooxidant activity. Tomato and yellow bell pepper formed the last cluster that always showed high prooxidant activity that persisted regardless of reaction time and thermal treatment.

Table 3. Antioxidant Activity (AA%) of SPE C_{18} Cartridge Unbound and Bound Fractions

	AA%			
	unbound fraction		bound fraction	
vegetable	10 min	30 min	10 min	30 min
carrot	−21	91	0	85
cauliflower	−88	2	44	78
celery	−275	−64	1	33
eggplant	−650	−85	6	37
garlic	−68	81	29	81
mushroom	48	78	74	84
onion	25	77	−7	49
tomato	−342	−124	6	53
white cabbage	22	74	39	62
yellow bell pepper	−910	−419	−430	−369
zucchini	−620	−164	−175	−92

To achieve preliminary information about the substances with prooxidant or antioxidant activity present in the tested vegetables and point out eventual synergism, each juice was fractionated by solid-phase extraction giving a least polar (bound) and a most polar (unbound) fraction (Table 3). After 10 min of reaction the unbound fraction of all vegetables was found to be prooxidant with the exception of that of mushroom, onion, and white cabbage that were antioxidant. At the end of the monitoring period, carrot, cauliflower, and garlic became antioxidant while the other remained prooxidant.

With regard to bound fractions, onion, yellow bell pepper, and zucchini were initially prooxidant, but at the end of the reaction period only yellow bell pepper and zucchini remained prooxidant. All the other bound fractions showed antioxidant activity.

The reducing substances, the content of total polyphenols, and the peroxidase activity were determined on all vegetable juices. These results are not reported because multivariate analysis of the data sets did not show any correlation between the factors or between the factors and AA%.

DISCUSSION

The results show that, in the experimental conditions described, all vegetable juices influence lipid peroxidation. Their behavior differed according to the type of vegetable, time and temperature of reaction, thermal treatment to which the vegetable juices were subjected, and volume of juice added to the system.

Each variable considered had different effects on AA depending on the type of vegetable, apart from the reaction time that always positively influenced activity. In fact, in the case of all vegetable juices, either increased antioxidant activity or decreased prooxidant activity was found with prolonged reaction time. This is probably because the oxidant substances that contribute to the redox equilibrium in the biological systems considered have high reactivity and trasformation or denaturation rates so that the activity of antioxidant compounds that often appears to be efficient and persistent can be revealed. Mushroom and white cabbage appear to be biological systems in which antioxidant compounds are very powerful and stable so that

they efficiently and persistently protect lipids against peroxidation. The high variability of AA values found in most cases can be determined by the influence that factors such as climatic growth conditions, growth and ripening stage, and temperature and duration of storage can have on the redox balance of a vegetable system. However, comparing the RSD value relative to the juice volume of each vegetable to that relative to its AA value determined that the AA variability of a vegetable could be attributed to its variation in water content.

In most cases oxidants were found to be thermolabile as clearly shown by the faster and higher antioxidant activity registered in boiled juices, especially during the first reaction period. In these cases it is reasonable to suggest that prooxidant activity is due to peroxidases which are inactived at high temperature, while tomato and yellow bell pepper prooxidant activity, unaffected by heat treatment, can be attributed to the presence of thermostable chemicals.

The separation of juice components according to their polarity revealed the presence of compounds with different properties which sometimes interfered with each other. An oxidant fraction (unbound) could be isolated from tomato juice. This fraction seems to be able to counteract the action of a reducing fraction (bound). In yellow bell pepper more components with oxidant properties that seemed to negatively interact, producing a lower oxidant effect than the juice "in toto", were found. Carrot and garlic both gave two fractions (bound and unbound) that showed higher AA values than the relative unfractioned juices, indicating a particularly strong negative synergism. In contrast, a positive synergism was found to exist between bound and unbound fractions obtained from zucchini. In fact, each separate zucchini fraction was prooxidant while the unfractioned juice showed antioxidant activity. Mushroom and white cabbage seem to lack oxidant compounds or, if present, they appare to be inactive.

These findings further confirm that anti-prooxidant properties of a compound are strongly influenced by a number of factors and by the reaction conditions, so it is very difficult to extrapolate the effects of tested vegetables on human health and safety. This leads us to extend our investigations on biological systems where interactions between the various components of vegetables and the matrix can be subjected to strong modifications and where new factors, such as protective mechanisms with which living organisms are equipped to render inactive xenobiotic and nocive substances, may take part. Furthermore, investigations are currently under way to study anti-prooxidant properties of water insoluble vegetable components.

LITERATURE CITED

Al-Saikhan, M. S.; Howard, L. R.; Miller, J. C. Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum*, L.). *J. Food Sci.* **1995**, *60*, 341−343.

Belitz, H. D.; Grosch, W. Vegetables and their products. In *Food Chemistry*; Springer-Verlag: Berlin, 1987; pp 549−572.

Bilyk, A.; Cooper, P. L.; Sapers, G. M. Varietal differences in distribution of quercetin and kaempferol in onion (*Allim cepa* L.) tissue. *J. Agric. Food Chem.* **1984**, *32*, 274−276.

Byers, T.; LaChance, P.; Pierson, H. New directions: the diet-cancer link. *Patient Care* **1990**, *24*, 34−48.

Caragay, A. B. Cancer-preventive foods and ingredients. *Food Technol.* **1992**, *4*, 65−68.

Farag, R. S.; Badel, A. Z. M. A.; Hewedi, F. M.; El-Baroty, G. S. A. Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. *J. Am. Oil Chem. Soc.* **1989**, *66*, 792−799.

Gazzani, G. Anti and prooxidant activity of some dietary vegetables. *Riv. Sci. Aliment.* **1994**, *23*, 413−420.

Haigh, R. Safety and necessity of antioxidants: EEC approach. *Food Chem. Toxicol.* **1986**, *24*, 1031−1036.

Havesteen, B. Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol.* **1983**, *32* (7), 1141−1148.

Hemeda, H. M.; Klein, B. P. Effects of naturally occurring antioxidants on peroxidase activity of vegetable exstracts. *J. Food Sci.* **1990**, *55*, 184−185.

Hertog, M. G. L.; Hollman, P. C. H.; Venema, D. P. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J. Agric. Food Chem.* **1992a**, *40*, 1591−1598.

Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. Content of potentially anticarcinogenic flavonoids in 28 vegetables and 9 fruits commonly comsumed in The Netherlands. *J. Agric. Food Chem.* **1992b**, *40*, 2379−2383.

Imida, K.; Fukushima, S.; Shivai, T.; Ohtani, M.; Nakanishi, K.; Ito, N. Promoting activities of butylated hydroxyanide and butylated hydroxytoluene on 2-stages urinary bladder carcinogenesis and inhibition of γ-glutamyl transpeptidase-positive foci development in the liver of rats. *Carcinogenesis* **1983**, *4*, 895−899.

Kinsella, J. E.; Frankel, E.; German, B.; Kanner, J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol.* **1993**, *4*, 85−89.

Laughton, M. J.; Evans, P. J.; Moroney, M. A.; Hoult J. R. S.; Halliwell B. Inhibition of mammalian 5-lipoxygenase and cyclo-oxigenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. *Biochem. Pharmacol.* **1991**, *42* (9), 1673−1681.

Lee, Y.; Howard, L. R.; Villalòn, B. Flavonoids and antioxidant activity of fresh pepper. *J. Food Sci.* **1995**, *60*, 473−476.

Maeura, Y.; Weisburger, J. H.; Williams, G. Dose-dependent reduction of N-2-fluorenylacetamide-induced liver cancer and enhancement of bladder cancer in rats by buthylated hydroxytoluene. *Cancer Res.* **1984**, *44*, 1604−1608.

McCord, J. M. Free radicals and prooxidants in health and nutrition. *Food Technol.* **1994**, *5*, 106−120.

Nicoli, M. C.; Anese, M.; Parpinel, M. T.; Franceschi, S.; Lerici, C. R. Loss and or formation of antioxidants during food processing and storage. *Cancer Lett.* **1997**, *114*, 71−74.

Onyeneho, S. N.; Hettiarachchy, N. S. The effect of nany bean hull extract on the oxidative stability of soy and sunflower oils. *J. Agric. Food Chem.* **1991**, *39*, 1701−1704.

Taga, M. S.; Miller, E. E.; Pratt, D. E. Chia seeds as a source of natural lipid oxidant. *J. Am. Oil Chem. Soc.* **1984**, *61* (5), 928−931.

Tsushida, T.; Suzuki, M.; Kurogi, M. Evaluation of antioxidant activity of vegetable extracts and determination of some active compounds. *Nippon Kogyo Gakkaishi* **1994**, *41* (9), 611−618.

Van der Heijden, C. A.; Janssen, P. J. C. M.; Strik, J. J. T. W. A. Toxicology of gallates: a review and evalaution. *Food Chem. Toxicol.* **1986**, *24*, 1067−1070.

Van Esch, G. J. Toxicology of *tert*-butyl-hydroquinone (TBHQ). *Food Chem. Toxicol.* **1986**, *24*, 1063−1066.

Received for review March 23, 1998. Revised manuscript received July 27, 1998. Accepted July 28, 1998. This work was supported by a grant from M.U.R.S.T. (FAR).

JF980300O