Protective Activity of Water Soluble Components of Some Common Diet Vegetables on Rat Liver Microsome and the Effect of Thermal Treatment

Gabriella Gazzani,*,† Adele Papetti,† Maria Daglia,† Francantonio Bertè,‡ and Cesarina Gregotti‡

Department of Pharmaceutical Chemistry, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy, and Institute of Pharmacology, University of Pavia, Piazza Botta 10, 27100 Pavia, Italy

The water soluble antioxidant properties of carrot, cauliflower, celery, eggplant, mushroom, garlic, onion, white cabbage, white potato, tomato, yellow bell pepper, and zucchini were investigated. Vegetable juices were obtained by centrifugation, and each antioxidant property was determined in terms of the protective activity (PA%) against rat liver microsome lipid peroxidation induced by CCl_4 and measured by malondialdehyde release. All juices were found to be active. PA was very high (90−100%) and constant (relative standard deviation (RSD) = 4−7) for mushroom, garlic, cauliflower, and potato. For white cabbage, zucchini, and eggplant the PA reached similar values (80%) but was less constant (RSD = 12−15). Onion and yellow bell pepper showed high PA (75%) which was more variable (RSD = 20−24), and the PA for tomato and celery was less high (50%) and very variable (RSD = 25), especially in the case of carrot juice (6%) (RSD = 50). The juices were also analyzed after different technological treatments (boiling, freezing, and freeze-drying). In general, boiling and freezing juices resulted in a slightly decreased PA while freeze-drying slightly increased their PA values. Cluster analysis was carried out considering the PA values of the variously treated juices and their relative RSD values and permitted us to subdivide the vegetables according to their behavior. Juice components were separated according to their polarity on a Baker C_{18} cartridge. Bound and unbound fractions obtained from each vegetable juice were shown to be protective against lipid peroxidation with the exception of the bound yellow bell pepper fraction. The results pointed out different interactions between the vegetable juice components.

Keywords: *Edible vegetables; antioxidants; liver microsomas; lipid peroxidation*

INTRODUCTION

Epidemiological studies have pointed out clear relations between diet and cancer (Doll and Peto, 1981; Byers et al., 1990) and coronary heart desease (Hemeda and Klein,1990; Renaud and de Lorgeril, 1992). The protective effects against various forms of neoplastic and heart diseases (Ames, 1983, 1989; Caragay, 1992; Kinsella et al., 1993) follow the consumption of vegetables and fruits. The principal agents responsible for the protective effects are considered to be vitamins A, C, and β-carotene because of their antioxidant and antiradical properties. In recent years it became apparent that other dietary components, in particular phenols which are ubiquitous in plants, can strongly contribute to the positive effects of vegetable foods (Jones et al., 1995; Nakagami et al., 1995).

Polyunsaturated fatty acids or fatty acyl side chains in biological membranes can be peroxidized in the presence of enzymes or in their absence by exposure to reactive oxygen species and to transition metal ions in a free radical chain reaction. This results in lipid peroxidation which can be deleterious for membrane permeability and can produce toxic compounds for humans. Phenols, because of their molecular structures which include an aromatic ring with hydroxyl groups containing mobile hydrogens, are very efficient scavengers of peroxyl radicals (Halliwell, 1990; Aruoma, 1994). These compounds, especially flavonoids, were also found to act as powerful inhibitors of both 5-lipoxygenase and cyclo-oxygenase, probably by scavenging chain-propagating peroxyl free radicals that are formed within the active site of the enzymes (Laughton et al., 1991). Moreover, the action of phenolic compounds can be related to their capacity to reduce and chelate ferric iron which catalyzes lipid peroxidation.

Recently, a number of studies were carried out to assess antioxidant and antiradical activities in vitro (Bilyk et al., 1984; Hertog et al., 1992; Ishii et al., 1996) and ex vivo of isolated plant constituents such as flavonoids (Laughton et al., 1991), organosulfur compounds (Horie et al., 1992; Wang et al., 1996), and vegetable extracts (Horie et al., 1989; Gebhardt, 1997) or natural compounds isolated from them (Horie et al., 1989) against oxidative degradation of lipids.

In this study we investigated the water soluble proantioxidant activity of 12 vegetables commonly used in the mediterranean diet, the influence of technological treatment on such activity, and the possible analogies between the behaviors of each vegetable analyzed.

Such activity was studied ex vivo as protective activity against lipid peroxidation of microsome membrane hepatocyties induced by CCl_4 (Brent and Rumack, 1993). This xenobiotic induces rapid extensive pathological changes in liver tissue well defined at the

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

† Department of Pharmaceutical Chemistry.

‡ Institute of Pharmacology.

10.1021/jf980301g CCC: $15.00
Published on Web 09/19/1998

biochemical and ultrastructural levels (Baskin and Salem, 1997).

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.), 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. All vegetable juices were prepared as described previously (Gazzani et al., 1998). Each juice was subdivided into four batches: the first was immediately analyzed, the second batch was boiled for 30 min at 102 °C before analysis, the third was freeze-dried and the residue was dissolved in distilled water, and the last batch was frozen for either 3 or 6 months.

Biological Lipid Peroxidation Assay (Protective Activity, PA%). Liver microsomes were prepared from male Wistar rats weighing 200−250 g following the method of Horie et al. (1988). The microsomal pellets obtained were suspended either in 0.1 M sodium phosphate buffer, pH 7.4 (control sample), or in vegetable juice (sample) to make a total volume of 6 mL. An aliquot (0.1 mL) of the obtained suspension was immediately removed and used for determination of microsomal proteins (Lowry et al., 1951).

The remaining preparation was added to NaCl (1 mL, 140 μM), EDTA (1 mL, 50 μM), and sodium phosphate buffer (1 mL, 0.1 M, pH 7.4) and then subdivided into two aliquots of 4 mL, respectively. All samples were stoppered, and N_2 was bubbled through the solution at 37 °C for 15 min to obtain anaerobic conditions for the following induction of the lipid peroxidation.

To one group of samples were then added NADP (0.5 mL, 500 μM), G6P (0.5 mL, 250 μM), and CCl_4/EtOH (20 μL, 50% v/v). An equivalent amount of buffer was instead added to the second group.

Both samples were placed in a shaking water bath at 37 °C for 30 min, and then the equal volumes of 30% trichloroacetic acid (TCA) at 0 °C and 0.75% thiobarbituric acid (TBA) (Plaa and Hewitt, 1982) were added.

The reaction mixtures were heated in boiled water for 15 min, kept in ice for 5 min, and then centrifuged for 10 min at 3000 rpm to separate corpuscolate particles.

The absorbance of supernatant was read in a spectrophotometer (λ = 545 nm) using the second series of samples to bring the spectrophotometer to zero to correct for interference due to color and thiobarbituric acid-reactive substances (TBA-RS) naturally occurring in vegetable juices.

The protective activity was expressed as the percentage decrease of TBA-RS relative to the control using the equation

$$PA\% = (a - b)/a \times 100 \quad (1)$$

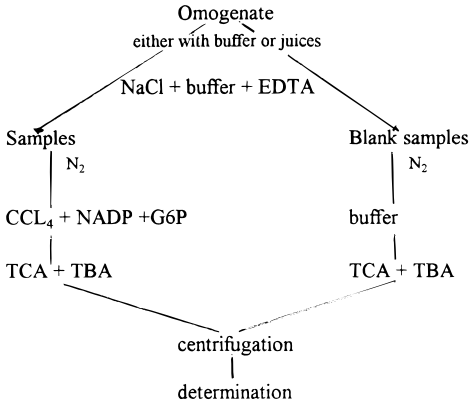
where a is the TBA-RS in control sample and b represents the TBA-RS in sample.

The schematic sequence of the operations described is reported in Scheme 1.

Solid-Phase Extraction of the Vegetable Juices. Each vegetable juice was separated into two fractions using a Bakerbond C_{18} solid-phase extraction (SPE) cartridge preconditioned with 5 mL of methanol−Millipore grade distilled water (30:70, v/v). After sample loading (6 mL), the cartridge was washed with 12 mL of water and bound compounds were eluted with 18 mL methanol−water (50:50, v/v). After evaporation of the solvent, each fraction was dissolved in a 6 mL aliquot of distilled water and tested for biological lipid peroxidation assay.

Statistical Analysis. Values represent means of 10 replications. Data were analyzed with the statistical analysis package Statgraphics by Statistical Graphic Corp., STSC, Inc., MD (version 5, 1991). The Multivariate data set was processed using the cluster analysis procedure, and to visually compare the different clusters, the star symbol plot procedure was used.

Scheme 1



RESULTS

Vegetable juices were prepared working either at 2 or at 25 °C. There were no significantly different PA values so only the values given by the juices obtained at 25 °C are reported. The same juices were also analyzed after boiling, freezing, and freeze-drying. The results obtained were reported in Table 1 with the juice volumes given by each vegetable and the relative standard deviation (RSD).

Cauliflower, garlic, mushroom, and potato juices always possessed a very strong and constant protective activity. In fact, in their presence TBA-RS formation in microsomes during lipid peroxidation was almost completely suppressed (>90%). All the other vegetable juices (with the exception of carrot) inhibited lipid peroxidation more than 50%, but the activity of each juice was not constant (RSD > 10), especially for yellow bell pepper and tomato. Carrot juice showed the highest variability, and the mean of PA values were very low.

On comparing the RSD value relative to juice volume to that relative to the PA value of each vegetable, it was found that the PA variability of a vegetable juice could not always be attributed to variability in water content of the vegetable.

Technological treatments either positively or negatively influenced the protective activity of the vegetable juices depending on the type of treatment and on the vegetable considered.

Boiling of juices for 30 min, a procedure commonly used in home cooking of most vegetables, generally caused a slight decreases in PA values, but in the case of celery and tomato, decreases of more than 50% were registered. Small increases in PA were found for mushroom and white cabbage. Only in the case of carrot, boiling gave rise to a very high increase in PA values. With regard to PA variability generally, RSD values decreased following heat treatment. This is remarkable, especially in the case of carrot. PA variability increased only for cauliflower, eggplant, onion, and white potato.

With regard to freezing, a slight decrease in the PA of vegetable juices was found in most cases. Analyses were carried out on juices both after 3 and 6 months of freezing. The results of both sequences of experiments did not show significant differences (p > 0.005). For this reason the reported data are relative to 3 months.

Table 1. Protective Activity of Tested Vegetables[a]

vegetable	mL/g	RSD	technological treatment	PA%	RSD
carrot	0.51	0.089	untreated	6	52.15
			boiling	32	19.37
			freezing	28	30.05
			freeze-drying	10	35.43
cauliflower	0.33	0.044	untreated	93	6.28
			boiling	79	9.92
			freezing	91	10.49
			freeze-drying	91	6.30
celery	0.55	0.097	untreated	58	16.06
			boiling	27	7.21
			freezing	55	19.54
			freeze-drying	49	24.61
eggplant	0.46	0.078	untreated	60	12.48
			boiling	56	16.54
			freezing	72	18.54
			freeze-drying	70	10.16
garlic	0.39	0.073	untreated	94	7.53
			boiling	90	1.08
			freezing	99	0.58
			freeze-drying	100	0.10
mushroom	0.37	0.053	untreated	97	4.66
			boiling	100	1.00
			freezing	95	0.10
			freeze-drying	100	0.10
onion	0.56	0.064	untreated	81	14.05
			boiling	76	22.07
			freezing	78	17.69
			freeze-drying	68	17.52
tomato	0.55	0.078	untreated	63	21.69
			boiling	27	16.35
			freezing	52	20.10
			freeze-drying	58	26.83
white cabbage	0.46	0.063	untreated	80	18.92
			boiling	86	14.85
			freezing	77	14.43
			freeze-drying	84	10.57
white potato	0.39	0.061	untreated	93	5.32
			boiling	83	6.15
			freezing	91	11.38
			freeze-drying	86	7.80
yellow bell pepper	0.68	0.091	untreated	75	24.14
			boiling	68	20.07
			freezing	72	22.59
			freeze-drying	82	15.63
zucchini	0.52	0.053	untreated	72	15.47
			boiling	51	9.96
			freezing	67	13.06
			freeze-drying	89	10.42

[a] Test carried out with BHT 0.05, 0.1, and 0.2% solutions gave PA values of 57, 57, and 63%, respectively.

Freezing seemed to give rise to small decreases in PA (<5%) for cauliflower, mushroom, onion, white potato, and white cabbage. Decreases were less than 10% for yellow bell pepper and zucchini and 17% for tomato. There was a slight increase for celery and garlic (5%), a notable increase for eggplant (20%), and very high increase for carrot (400%). For carrot, garlic, mushroom, white cabbage, yellow bell pepper, and zucchini, PA values were found to be stabilized by freezing (see RSD values); while for cauliflower, celery, eggplant, potato, and especially for onion, PA values were more variable. Tomato and yellow bell pepper PA variability did not seem to be influenced by the freezing process.

Conversely, in most cases freeze-drying determined an increase in the PA values of vegetable juices. Increases were less than 10% for garlic, mushroom, white cabbage, and yellow bell pepper but were higher for eggplant, zucchini, and especially for carrot (77%). Decreases were less than 10% for cauliflower, tomato, and white potato but higher for celery and onion (16%).

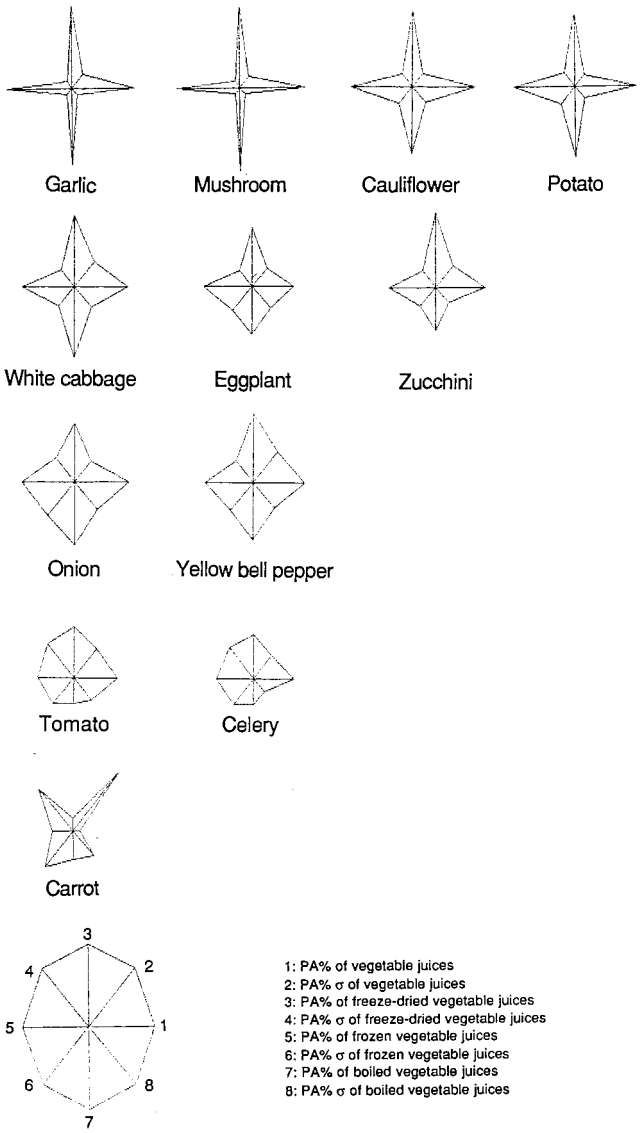


Figure 1. Star symbol plot of tested vegetables.

After freeze-drying, the PA values were less variable for eggplant, garlic, mushroom, white cabbage, yellow bell pepper, zucchini, and especially carrot. The greater variation in data was found for celery, onion, tomato, and white potato. Freeze-drying did not seem to influence variability in PA values of cauliflower juices only.

Cluster analysis allowed us to compare the behavior of each vegetable with all others. Analogies were determined by the PA values of untreated, boiled, frozen, and freeze-dried juices and by their relative RSD. Following cluster analysis vegetables could be grouped into five clusters. The first one contains mushroom, garlic, cauliflower, and potato and is always characterized by having a very strong and constant PA. Another group consisted of cabbage, eggplant, and zucchini whose activity was high but rather variable. High and variable activity was found in the case of onion and yellow bell pepper. Celery and tomato showed lower but more variable activity. Carrot showed a poor PA and a very high variability. The analogies were found to be evident in the graphic representation carried out using the star symbol plot procedure. It uses the shortest ray to plot the smallest value in each variable and uses the longest ray to plot the largest value (Figure 1).

Water soluble vegetable juice components were sepa-

rated according to their polarity on a Bakerbond C_{18} SPE cartridge. Both fractions (bound and unbound) of each vegetable juice were tested. All fractions obtained were shown to be protective against lipid peroxidation with the exception of the bound yellow bell pepper fraction which was only slightly prooxidant. The two fractions obtained from celery and tomato each showed about half the value of the unfractioned juices. The two fractions from garlic and onion both showed activity values similar to that of the relative unfractioned juices. In the case of eggplant, mushroom, white cabbage, white potato, yellow bell pepper, and zucchini, the unbound fractions gave much higher PA values than those of relative bound fractions. In the case of cauliflower, the bound fraction was more active than the unbound one. Both carrot fractions gave PA values higher than that of the unfractioned juice. The sum of the PA values of the bound and unbound fractions were close to that of the relative unfractioned juices for celery, tomato, white cabbage, yellow bell pepper, and zucchini. For cauliflower, eggplant, garlic, and onion, the sum of the PA values of the bound and unbound fractions was about twice the PA value of the unfractioned juices and was 10 times that for carrot.

Table 2. Protective Activity (PA%) of SPE C_{18} Cartridge Bound and Unbound Fractions

	PA%	
vegetable	unbound fraction	bound fraction
carrot	36	24
cauliflower	64	100
celery	27	29
eggplant	85	24
garlic	93	100
mushroom	100	60
onion	80	85
tomato	20	24
white cabbage	57	29
white potato	96	34
yellow bell pepper	64	−6
zucchini	65	6

DISCUSSION

The results show that all vegetable juices can influence microsomial lipid peroxidation in varying degrees. The protective activity is very high and constant for several vegetables while for some others it was found to be less high and very variable. In the first case we can hypothesize the vegetable as being a biological system where the redox balance is such that it is not significatively influenced by the climatic growth conditions, growth or ripening stage, and temperature and duration of storage.

Technological treatments, which are useful for long-term vegetable storage, do not have much influence on antioxidant properties. A significant increase in activity was registered only in the case of vegetables that showed low and variable PA values. This seems to indicate that the oxidants, or those interacting with antioxidant compounds, are susceptible to technological treatments. Thermal treatment generally causes a decrease in protective activity (with the exception of carrot) in contrast to what was registered in another paper when the antioxidant activity was tested in vitro by chemical assay (Gazzani et al., 1998). This seems to indicate that the thermal inactivation of oxidant substances, probably enzymes, apparent in vitro, is not revealed by biological assay where the antioxidant defense mechanisms of living beings may come into play. Conversely, this test can probably reveal the slight negative influence of thermal treatment on the antioxidant components.

The results obtained and the cluster analysis did not show any correlation between the protective activity of the vegetables, their behavior, or their botanic class or family. For example, onion and garlic are in two different clusters as are white cabbage and cauliflower or carrot and celery. Furthermore, all four examined Solanaceae are in four different clusters.

The comparison between the AA, reported in another paper (Gazzani et al., 1998), and PA values obtained from a chemical and a biological assay, respectively, seems to indicate that the antioxidant properties of vegetable juices can be better explained ex vivo than in vitro (Table 2), and this is also reminiscent of the efficiency of antioxidant defense mechanisms of living beings. The greater in vitro antioxidant activity of carrot is probably due to the presence of soluble compounds in it which cannot act as antioxidants in the biological system used.

The PA of the bound and unbound juice fractions, obtained according to their polarity, point out the different interactions between the juice components of the vegetables. For yellow bell pepper, tomato, and zucchini, interactions gave rise to a slight increase of the juice PA, while in the case of cauliflower, eggplant, garlic, onion, and particularly carrot the results indicate a strong negative synergism.

The comparison between the results given by the bound and unbound fractions in the chemical assays (where many fractions were prooxidant, especially in the first period of the reaction) and in the biological assays (where only yellow bell pepper bound fraction showed a very low prooxidant activity) also seems to confirm the importance of the protection systems in aerobic living beings. After inactivation of prooxidant substances, antioxidant activity can then be expressed.

An investigation concerning antioxidant and protective properties of other dietary vegetables is in progress. The results of this study, apart from supplying us with interesting information about other common components of our diet, will also allow us to test the feasability of an in vitro assay for extrapolating the probable activity of juices ex vivo and in vivo.

Moreover, we are studying the properties of the water insoluble components in all vegetables already taken into consideration in the present study.

ACKNOWLEDGMENT

The authors thank Fabrizio Rossi and Gaetano Viani for their technical assistance.

LITERATURE CITED

Ames, B. N. Dietary carcinogens and anti-carcinogens. *Science* **1983**, *221*, 1256−1260.

Ames, B. N. Endogenous oxidative DNA damage, aging, and cancer. *Free Radical Res. Commun.* **1989**, *7*, 121−127.

Aruoma, O. I. Nutrition and health aspects of free radicals and antioxidants. *Food Chem. Toxicol.* **1994**, *32* (7), 671−683.

Baskin, S. I.; Salem, H. *Oxidants, antioxidants, and free radicals*; Taylor & Francis publisher: Washington, 1997.

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

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.

Brent, J. A.; Rumack, B. H. Role of free radicals in toxic hepatic iniury. Are free radicals the cause of toxin-induced liver injury? *Clin. Toxicol.* **1993**, *31* (1), 173−196.

Caragay, A. B. Cancer preventive foods and ingredients. *Food Technol.* **1992**, *46* (4), 65−68.

Doll, R.; Peto, R. The causes on cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* **1981**, *66*, 1192−1308.

Gazzani, G.; Papetti, A.; Daglia, M. Anti- and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *J. Agric. Food Chem.* **1998**, *46*, 4118−4122.

Gebhardt, R. Antioxidative and protective properties of extracts frim leaves of the artichoke (*Cynara scolymus L.*) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. *Toxicol. Appl. Pharmacol.* **1997**, *144*, 279−286.

Halliwell, B. How to characterize a biological antioxidant. *Res. Communs.* **1990**, *9* (1), 1−32.

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.

Horie, T.; Murayama T.; Mishima T.; Itoh, F.; Minamide Y., Fuwa T.; Awazu S. Protection of liver microsomal membranes from lipid peroxidation by garlic extracts. *Planta Med.* **1989**, *55*, 506−508.

Horie, T.; Awazu, S.; Itakura, Y.; Fuwa, T. Identified diallyl polysulfides from an aged garlic extract which protects the membranes from lipid peroxidation. *Planta Med.* **1992**, *58* (5), 468−469.

Ishii, G.; Mori, M.; Umemura, Y. Antioxidative activity and food chemical properties of anthocyanins from the colored tuber flesh of potatoes. *Nippon Shokuhin Kagaku Kogaku Kaishi* **1996**, *43* (8), 962−966.

Jones, D. P.; Kagan, V. E.; Aust, S. D.; Reed, J. D.; Omaye, S. T. Impact of nutrients on cellular lipid peroxidation and antioxidant defense system. *Fundam. Appl. Toxicol.* **1995**, *26*, 1−7.

Kinsella, J. E.; Frankel, E.; German, B.; Kanner, J. Possible mechanismsfor the protective role of antioxidants in wine and plant foods. *Food Technol.* **1993**, *47*, 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.

Lowry, O. H.; Rosenbrough, N. J.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

Nakagami, T.; Nanaumi-Tamura, N.; Toyomura, K.; Nakamura, T.; Shigehisa, T. Dietary flavonoids as potencial natural biological response modifiers affecting the autoimmune system. *J. Food Sci.* **1995**, *4*, 653−656.

Plaa, G. L.; Hewitt, W. R. Toxicology of the liver. *Target Organ Toxicol. Ser.* **1982**, 217−225.

Renaud S.; de Lorgeril M. Wine, alcohol, platelets and the French paradox for coronary heart disesase. *Lancet* **1992**, *339*, 1523−1526.

Wang, E. J.; Li, Y.; Lin, M.; Chen, L.; Stein, A. P.; Reuhl K. R.; Yang, C. S. Protective effects of garlic and related organosulfur compounds on acetaminophen-induced hepatotoxicity in mice. *Toxicol. Appl. Pharmacol.* **1996**, *136*, 146−154.

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.

JF980301G