Free Radical Research, August 2005; 39(8): 893–904

Taylor & Francis
Taylor & Francis Group

Effect of vegetables on human phenolsulfotransferases in relation to their antioxidant activity and total phenolics

CHI-TAI YEH, & GOW-CHIN YEN

Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan

Accepted by Professor J. Yodoi

(Received 23 September 2004; in revised form 17 March 2005)

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

Abstract
Epidemiology studies have shown that consumption of fruits and vegetables is associated with the prevention of chronic diseases such as cancer and cardiovascular disease. Induction of cellular phase II detoxifying enzymes is associated with cancer preventive potential. Phenolsulfotransferases (PSTs) are traditionally known as phase II drug-metabolizing or detoxifying enzymes that facilitate the removal of drugs and other xenobiotic compounds. Phenolic acids are known to increase the activities of PSTs. In the present study, human $HepG_2$ cells were used as model to investigate the influence of twenty vegetables on human PST activity and to evaluate the relationships to their antioxidant activity and total phenolics content. The result showed that PST-P activity was significantly ($p < 0.01$) induced by asparagus, broccoli, cauliflower, celery and eggplant, whereas PST-M activity was induced by asparagus, broccoli, carrot, eggplant and potato at a concentration of 100 μg/ml. The vegetable extracts that induced both forms of PSTs activities were found to have higher antioxidant capacities and total phenolic content in the oxygen radical absorbance capacity (ORAC) and Folin-Ciocalteu assay. The major polyphenols in broccoli, the most potential inducer in both forms of PSTs activities, was antioxidant phenolic acids. HPLC retention times and standard spiked indicated the presence of gallic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, gentisic acid and ferulic acid in broccoli. The overall effect of vegetables tested on the activity of PST-P was well correlated to their ORAC value and total phenolics content ($r = 0.82, p < 0.05$ and $r = 0.78, p < 0.05$). These results imply that vegetables have a capability of inducing PST activity, and the PST induction may be possibly ascribed to antioxidant phenolic acids in vegetable extracts.

Keywords: *Sulfate conjugation, phenolsulfotransferase, vegetables, antioxidant activity, total phenolics*

Abbreviations: *AAPH, 2,2′-Azobis (2-amidinopropane)-hydrochloride; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GST, glutathione S-transfersase; GAE, gallic acid equivalent; HPLC, high performance liquid chromatography; $ORAC_{ROO\cdot}$, oxygen radical absorbance capacity; PST-P, P-form phenolsulfotransferases; PST-M, M-form phenolsulfotransferase; β-PE, β-phycoerythnin; QR, quinone reductase; RT-PCR, reverse transcription polymerase chain rection; TEAC, trolox equivalent antioxidant capacity; UGTs, UDP-glucoronosyl transferase*

Introduction

Epidemiology studies have shown that dietary patterns were significantly associated with the prevention of chronic diseases such as heart disease, cancer, diabetes and Alzheimer's disease [1]. Consumption of fruits and vegetables has been highly associated with the reduced risk of cancer [2]. Fruits and vegetables furnish an abundance of nutrients, especially vitamins and minerals, to the diet of individuals. They also contain non-nutritive constituents, such as fiber and phenolic compounds, the latter which have been implicated in conferring biological and beneficial health effects in test animals and human [3]. Recent

Correspondence: G.-C. Yen, Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan. Fax: 886 4 2285 4378. E-mail: gcyen@dragon.nchu.edu.tw

ISSN 1071-5762 print/ISSN 1029-2470 online © 2005 Taylor & Francis Group Ltd
DOI: 10.1080/10715760500150424

RIGHTSLINK

investigations show that there is a profound link between the dietary habits and the incidence of cancer and heart diseases in humans, and the non-nutritive constituents appear to play a role in preventing the development of these diseases [4]. Many of the beneficial health effects of non-nutritive constituents of vegetables have been known to originate from or be closely associated with their antioxidant properties [5]. It is widely accepted that induction of phase II detoxification enzymes is a major strategy for protecting cells against a variety of endogenous and exogenous toxic components, such as reactive oxygen species and chemical carcinogens [6]. Various synthetic organic compounds, such as *tert*-butylhydroquinone (t-BHQ), butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA), have been reported to be potent chemopreventive agents because they can induce phase II enzymes in cultured murine hepatoma cells [7]. Many of the non-nutritive food components, such as phenolics and sulfur-containing compounds, including glucosidenolates and their metabolites, have also been shown to possess cancer chemopreventive properties [8].

Phenolsulfotransferase (PST) catalyzes the sulfation of various endogenous compounds, drugs and xenobiotics as well as in steroid biosynthesis, catecholamine metabolism and thyroid hormone homeostasis [9]. At least two major forms of human PST enzymes have been characterized biochemically from liver, blood platelets and other tissues, the phenol-preferring PST (PST-P) and the monoamine neurotransmitter-preferring PST (PST-M). Our previous studies revealed that *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid, and coumaric acid all could increase the activities of both PST-P and PST-M. These phenolic acids also possessed antioxidant capacity in the oxygen radical absorbance capacity (ORAC) and TEAC assays [10]. Furthermore, in both two-compound and three-compound combinations with each of other phenolic acids, gallic acid and gentisic acid exhibit the potential synergistic effects in the promotion of PSTs activities [11]. The overall effects of phenolic acids on the activities of PST-P and PST-M are highly correlated to their ORAC values, suggesting that antioxidant phenolic acids might alter sulfate conjugation. The antioxidant activity of several plant materials has recently been reported [12]. Nevertheless, in the literature data regarding the effect of vegetables extracts on phenosulfotransferase activities is limited.

Hepatoma cell line ($HepG_2$) which not only resembles morphologically normal hepatocytes [13] but has also been shown to retain many of the enzymes involved in xenobiotic metabolism, including a functional Ah receptor [14], an inducible sulphotransferase [15] and an inducible NAD(P)H quinone oxidoreductase [16]. In this cell line, phenolsulfotransfease is also inducible, and the predominant isoforms present in control cell were phenol (P) and monoamine (M) form of the human phenolsulphotransferase [17]. On this basis we have used a highly differentiated human hepatoma cell line, $HepG_2$ as a model to assess the influence of vegetables extracts on the activity of human phenolsulfotransferase (PST-P or PST-M) in this study. In addition, the antioxidant capacity, total polyphenol content and composition of vegetable extracts were investigated by ORAC and Folin-Ciocalteu assay [18]. The effect of vegetables on the activities of both forms of phenolsulfotransferase in relation to their antioxidant capacities was also determined.

Materials and methods

Materials

Fresh vegetables (asparagus, broccoli, cabbage, carrot, cauliflower, celery, Chinese chive, cucumber, eggplant, garlic, leek, lettuce, onion, potato, shiitake, snap bean, soybean sprouts, spinach, sweet potato and tomato) were purchased form a local supermarket in Taichung, Taiwan (Table I). These twenty vegetables were selected on the basis of consumption per capita data in the Taiwan. [^{35}S]-labeled 3′-phosphoadenosine-5′-phosphor sulfate ($PAPS^{35}$) (1.0–1.5 Ci/mmol) was purchased from DuPont-New England Nuclear (Boston, MA). β-phycoerythnin (β-PE) from *Porphydium cruentum* was purchased from Sigma Co. (St. Louis, MO). 2,2′-Azobis (2-amidinopropane)-hydrochloride (AAPH) was purchased from Wako Chemicals (Osaka, Japan). 6-Hydrooxy-2,5,7,8-tetramethyl- chroman-2- carboxylic acid

Table I. Vegetables used in this study.

Vegetable	Scientific name	Vegetable	Scientific name
Asparagus	*Asparagus officinalis L.*	Leek	*Allium ampeloprasum L.*
Broccoli	*Brassica oleracea L.*	Onion	*Allium cepa L.*
Cabbage	*Brassica oleracea L.*	Potato	*Solanum tuberosum L.*
Carrot	*Daucus carota L.*	Shiitake	*Lentinus edodes Sing.*
Cauliflower	*Brassica oleracea L.*	Snap bean	*Phaseolus sativum L.*
Celery	*Apium graveolens L.*	Soybean sprouts	*Glycine max Merr.*
Chinese chive	*Allium tuberosum Rottler*	Spinach	*Spinacia oleracea L.*
Cucumber	*Cucumis sativus L.*	Sweet potato	*Ipomoea batatas Poir.*
Eggplant	*Solanum melongena L.*	Tomato	*Lycopersicon esculentum Mill.*
Garlic	*Allium sativum L.*	Lettuce	*Lactuca sativa L.*

Edible parts from the twenty different vegetables were extracted with methanol.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

RIGHTSLINK

(Trolox) was obtained from Aldrich (Milwaukee, WI). $ABTS^{2-}$(2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) as sulfonic acid was obtained from Sigma Co. (St. Louis, MO).

Preparation of the vegetable extracts

An edible part of the vegetables that is normally eaten was weighted and rapidly frozen in liquid nitrogen, followed by freeze-drying and grinding to produce a fine powder. Subsequently, freeze-dried vegetables were accurately weight into 1.0-g aliquots and 10 ml of methanol extraction solvent was added. The mixture was shaken at 600 rpm at room temperature on an orbital shaker for an hour. Methanol was completely removed by rotary evaporation, and residue was dissolved in 75 mM potassium phosphate buffer solution (pH 7.4). Before addition to the cell culture, all were filtered through a 0.22 μm filter. The concentrations shown are expressed as fresh weight (FW) of vegetable per milliliter of culture medium.

Cell culture

Human hepatoma cells ($HepG_2$ cells were obtained form the Bioresource Collection and Research Center (BCRC, Food Industry Research and Development Institute, Hsin Chu, Taiwan). Cells were grown in Dulbecco's modified Eagles's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (GIBCO BRL, Grand Island, NY), 100 U/ml penicillin, 100 μg/mL streptomycin, 0.37% (w/v) $NaHCO_3$, 0.1 mM NEAA, 1 mM sodium pyruvate, and 0.03% L-glutamine at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Assay of phenolsulfotransferase activity

The PST induction activity of different vegetables was determined by mean of PST assay. The cells were grown in 12-well plates (Costar 3524, Corning Inc., Corning, NY) for 24 h and then exposed to different vegetables (100 μg/ml) for 24 h. Growth medium and gallic acid were used as negative and positive control, respectively [10]. Treated cells were scraped off, washed and suspended in ice-cold 5 mM potassium phosphate buffer, pH 7.4, before homogenization to produce a cell homogenate. Aliquots of the cell homogenate were collected and immediately tested for PST activity. The incubation mixture contained 100 μl of 0.1 M potassium phosphate buffer (pH 7.0), 20 μl of the $HepG_2$ cell homogenates, 20 μl of the substrate, and 20 μl [^{35}S]-labeled PAPS (final concentration 6.7 μM) was added at successive intervals to tubes at 37°C in a water bath, and the reaction was terminated after 20 min by addition of 0.1 M barium acetate (200 μl). Any unreacted PAPS, free sulfate or protein was precipitated by two additions of 0.1 M barium hydroxide (200 μl) followed by 0.1 M zinc sulphate (200 μl). After centrifugation (11,500*g* for 3 min), 500 μl of the supernatant was thoroughly mixed with 4 ml scintillant, and radioactivity was measured by liquid scintillation spectrometry, and all assay were performed in triplicate. The protein content of cell homogenates was determined using a Bio-Rad protein assay kit.

RNA extraction and RT-PCR

RT-PCR was performed to determine the levels of P-form and M-form phenolsulfotransferase gene expression. $HepG_2$ cells (1×10^6 in 10 ml medium) were plated in 100-mm tissue culture dishes. After pre-incubation for 24 h, $HepG_2$ cells were exposed to three major vegetables (100 μg/ml), sulforaphane (20 μM), and polyphenolic compounds (20 μM) for 24 h. Cellular RNA was extracted with a TRIzol RNA isolation kit (Life Technologies, Rockville, MD) as described in the manufacturer's manual. The 987 bp target in PST-P cDNA (GenBank accession no. L10819) was amplified, using the sense primer (5′-ATGGAGCTGATCCAGGACAC-3′) at positions 39–58 and anti-sense primer (5′-TGACCTACCGTC-CCAGGCCC-3′) at positions 1006–1025. The 987 bp target in PST-M (GeneBank accession no. L19956) was amplified, using the sense primer (5′-ATGGAGC-TGAT-CCAGGACAC-3′) at positions 139–158 and anti-sense primer (5′-TGAGCCACTGTGCCTG-ACTC-3′) at positions 1106–1125. As a housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was amplified, using the sense primer (5′ -GACCCCTTCATTGACCTCAAC-3′) at positions 143 162 and anti-sense primer (5′-CATACCAG-GAAATGAGCTTG-3′) at positions 965–984. Briefly, from each sample, 250 ng of RNA was reverse-transcribed, using 200 units of Superscript II reverse transcriptase, 20 units of RNase inhibitor, 0.6 mM of dNTP and 0.5 μg/μl of oligo (dT) 12–18. Then, PCR analyses were performed on the aliquots of the cDNA preparations to detect PST-P, PST-M and GAPDH (as an internal standard) gene expression, using the FailSafe PCR system (Epicenter Technologies, Madison, WI, USA). The reactions took place in a volume of 50 μl, containing (final concentration) 50 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MnCl_2$, 0.2 mM dNTP, 2 units of Taq DNA polymerase and 50 pmol of 5′ and 3′ primers. After initial denaturation for 2 min at 95°C, 30 cycles of amplification (at 95°C for 1 min, 60°C for 1 min, and 72°C for 1.5 min) were performed, followed by a 7-min extension at 72°C.

Analysis of PCR products

A 10 μl aliquot from each PCR reaction was electrophoresed in a 1.8% agarose gel, containing 0.2 μg/ml ethidium bromide. The gel was then

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

RIGHTSLINK

photographed under ultraviolet transillumination. For quantification, the PCR bands on the photograph of the gel were scanned using a densitometer, linked to a computer analysis system. We normalized the PST-P and PST-M signal, relative to the corresponding GAPDH signal, from the same sample, expressing the data as the PSTs/GAPDH ratio.

Oxygen-radical absorbance capacity assay

The automated ORAC assay was carried out on a Fluostar Galaxy plate reader (BMG LabTechologies, GmbH, Offenburg, Germany) with fluorescent filter (ex. 540 nm; em. 565 nm). The procedure was based on a previous report of Cao et al. [19], with a slight modification. Briefly, in the final assay mixture, β-PE (16.7 nM) was used as a target of free radical (or oxidant) attack with AAPH (40 mM) as a peroxyl radical generator. Trolox (1–4 μM) was used as a standard and prepared fresh daily. The analyzer was programmed to record the fluorensence of β-PE in every 5 min after AAPH was added. All fluorescent measurements were expressed in relative to the initial reading. Final results were calculated using the differences of area under the β-PE decay curves between the blank and a sample and expressed as μmol of trolox equivalents (TE) per gram of FW vegetable samples.

Total phenolic content and characterization of phenolic compounds

The content of total phenolic was analyzed spectrophotometrically using the Folin-Ciocalteu colorimetric method and calculated using gallic acid as a standard [20]. All vegetable extracts were diluted with distilled water to obtain reading within the standard curve ranges of 0–500 μg of gallic acid/ml. Briefly, 100 μl of standard gallic acid solution or diluted vegetable extracts was mixed with 0.5 ml of distilled water in a test tube followed by the addition of 100 μl of Folin-Ciocalteu reagent. The samples were mixed well and then allowed to stand for 5 min before 1.25 ml of a 2% sodium carbonate aqueous solution was added. Samples were allowed to stand for 30 min at room temperature before the absorbance was measured at 750 nm versus the blank using a Hitachi spectrophotometer (model U-3000). Results were expressed as milligrams of gallic acid equivalent (GAE) per gram of FW vegetable samples. The asparagus, broccoli and eggplant extracts were hydrolyzed with 2.5 N HCl at 100°C for 2 h to break glycosidic bonds, and the released aglycons were extracted into ethyl acetate until they became colorless. The extracts were combined, evaporated to dryness on a rotary evaporator, and then dissolved in 5 ml of methanol. The crude extracts were filtered through a 0.45 μm filter before HPLC analysis. The filtrates were analyzed by HPLC (Hitachi, Japan), using the LiChrosphere RP-18 column ($150 \times 4\,mm^2$, 5 μm) and photodiode array detector (measured at 280 nm). Elution was carried out at room temperature and utilized 2% (v/v) acetic acid in water as solvent A and 0.5% acetic acid in water and acetonitrile (50:50, v/v) as solvent B. The elution gradient program was as follows: 10% B to 55% B (50 min), 55% B to 100% B (10 min), 100% B to 10% B (5 min) at a flow rate of 1 ml/min. The injection volume for standard sample extracts was 10 μl. Five phenolic acids (gallic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, gentisic acid, and ferulic acid) were quantified using the external standard method. Quantification was based on peak area. Calibration curves of the standards were made by diluting stock standards in methanol to yield 0–300 μg/ml (phenolic acids). Linear regression was fitted to the data to obtain regression coefficients >0.99 for phenolic acids standard curves. BHA was used as the internal standard to calculate the loss of phenolic acids. Spectra were recorded from 200 to 600 nm.

Statistical analysis

Correlation and regression analyses and principal component analysis were performed using SigmaPlot scientific graph system. The component loadings included ORAC, total phenolic content, and activities of P-form and M-form phenolsulfotransferase. Analysis of variance was performed using ANOVA procedures. Significant differences ($p < 0.05$) between means were determined using Duncan's multiple ranged tests.

Results

Effect of vegetables on the phenosulfotransferase activity and mRNA in $HepG_2$ cells

In the present study, the effects of twenty vegetables extracts on the sulfation of *p*-nitrophenol and dopamine in $HepG_2$ cells were determined. The vegetables used in this study are shown in Table I. The results shown in Table II demonstrated that PST-P and PST-M activity of control (without vegetables extracts) were 22 ± 3 and 68 ± 4 pmol/min/mg protein, respectively. Moreover, the addition of vegetable extracts could cause the effect of both forms of PST activities. The induction folds of vegetable extracts on human $HepG_2$ cells are also presented in Table II. It was found that asparagus, broccoli and eggplant showed the highest inducing effects on both form of PSTs activities at a concentration of 100 μg/ml ($p < 0.01$). Although cauliflower, celery and snap bean showed the

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

RIGHTSLINK

Table II. Effect of vegetable extracts on phenolsulfotrnasferase activity in $HepG_2$ cells.

Vegetables (Scientific name)	PST activity (pmol/min/mg protein)	
	p-nitrophenol	Dopamine
Asparagus (*Asparagus officinalis L.*)	36 ± 1 (1.6)**	109 ± 1 (1.6)**
Broccoli (*Brassica oleracea L.*)	41 ± 2 (1.8)**	116 ± 4 (1.7)**
Cabbage (*Brassica oleracea L.*)	25 ± 1 (1.1)	68 ± 2 (1.0)
Carrot (*Daucus carota L.*)	23 ± 1 (1.0)	102 ± 1 (1.5)**
Cauliflower (*Brassica oleracea L.*)	41 ± 2 (1.8)**	54 ± 3 (0.8)
Celery (*Apium graveolens L.*)	39 ± 3 (1.7)**	75 ± 6 (1.1)
Chinese chive (*Allium tuberosum Rottler*)	23 ± 2 (1.0)	96 ± 4 (1.4)*
Cucumber (*Cucumis sativus L.*)	27 ± 3 (1.2)	82 ± 3 (1.2)
Eggplant (*Solanum melongena L.*)	42 ± 1 (1.8)**	116 ± 5 (1.7)**
Garlic (*Allium sativum L.*)	25 ± 4 (1.1)	75 ± 2 (1.1)
Leek (*Allium ampeloprasum L.*)	27 ± 1 (1.2)	68 ± 7 (1.0)
Lettuce (*Lactuca sativa L.*)	20 ± 1 (0.9)	55 ± 1 (0.8)
Onion (*Allium cepa L.*)	18 ± 3 (0.8)	75 ± 2 (1.1)
Potato (*Solanum tuberosum L.*)	25 ± 2 (1.1)	102 ± 6 (1.5)**
Shiitake (*Lentinus edodes Sing.*)	25 ± 1 (1.1)	68 ± 2 (1.0)
Snap bean (*Phaseolus sativum L.*)	32 ± 2 (1.4)*	75 ± 1 (1.1)
Soybean sprouts (*Glycine max Merr.*)	23 ± 1 (1.0)	68 ± 1 (1.0)
Spinach (*Spinacia oleracea L.*)	18 ± 2 (0.8)	55 ± 1 (0.8)
Sweet potato (*Ipomoea batatas Poir.*)	25 ± 3 (1.1)	68 ± 2 (1.0)
Tomato (*Lycopersicon esculentum Mill.*)	25 ± 2 (1.0)	68 ± 1 (1.0)

$HepG_2$ cells were treated with each vegetable extracts for 24 h, the both forms of PSTs activities were then being measured. PST-P and PST-M activity of control (without vegetable extracts) were 22 ± 3 and 68 ± 4 pmol/min/mg protein, respectively. The vegetables extracts concentration used in phenolsulfotransferase assay was 100 μg/ml. Values in parentheses represent the induction folds of vegetables extracts on human $HepG_2$ sulfotransferase. Data are expressed as mean specific activity ± SD from three experiments. Significantly different form the control cells: $^*p < 0.05$, $^{**}p < 0.01$.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

significant induction on PST-P, they had weaker induction on PST-M activity ($p < 0.05$). Carrot, Chinese chive and potato could markedly induce the PST-M activity by 1.4–1.5-fold ($p < 0.01$). Cabbage, cucumber, garlic, leek, potato, shiitake and sweet potato showed lower inducing effects on PST-P activity, whereas celery, cucumber, garlic, onion and snap bean had slight induction on PST-M activity by 1.1–1.2-fold ($p > 0.05$). Moreover, other vegetables (lettuce, shiitake, soybean sprouts, spinach, sweet potato and tomato) showed no influence on both PST-P and PST-M activities. To further evaluate the PST-P and PST-M mRNA expression induced by three major vegetables, we first evaluated PST-P and PST-M mRNA expression in response to 100 μg/ml extracts of broccoli, eggplant and asparagus in $HepG_2$ cells. RT-PCR analyses were performed to examine the steady-state levels of PST-P and PST-M mRNA in $HepG_2$ cells after treatment with the extracts of three major vegetables for 24 h. As shown in Figure 1, PST-P gene expression was elevated by all three vegetables and broccoli elicited the strongest inductive effect among them. Similarly, PST-M gene expression was also increased by treatments with broccoli, eggplant and asparagus. The housekeeping gene GADPH showed no change in the experiments. Therefore, we believe that increased both forms of PSTs activities by three major vegetables in $HepG_2$ cells were regulated through transcriptional activation.

Effect of broccoli-derived chemicals on P-form phenolsulfotransferase activity and mRNA in $HepG_2$ cells

To further compare the effects of broccoli, sulforaphane and phenolic acids on PST-P activity

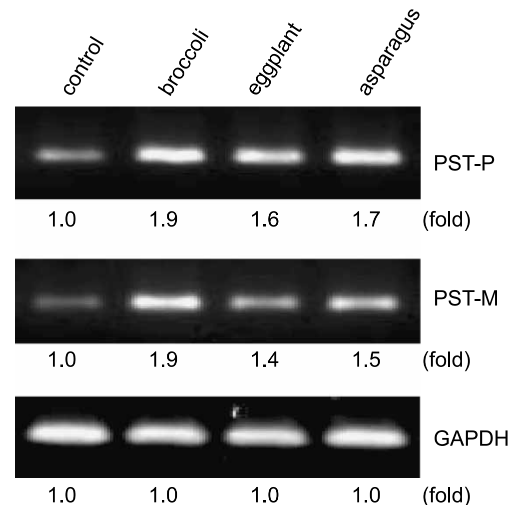

Figure 1. Effects of broccoli, eggplant and asparagus extracts on PSTs mRNA expression. $HepG_2$ cells were cultured at approximately 60–70% confluence and then treated with 100 μg/ml broccoli, eggplant and asparagus extracts. Expression of PSTs mRNA was analyzed by RT-PCR. GAPDH, the housekeeping gene, was used as an internal control. Induction folds of PST-P and PST-M were calculated as the intensity of the treated samples relative to the control by densitometry. The images shown are examples of two separate experiments.

RIGHTSLINK

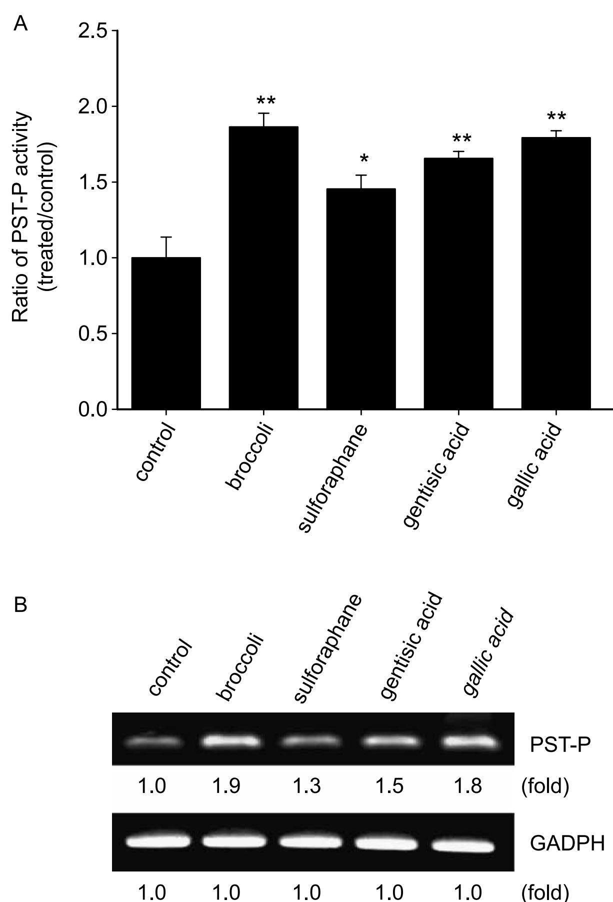

Figure 2. Induction of P-form PSTs activity and mRNA expression in $HepG_2$ cells by broccoli, sulforaphane, and phenolic acids. $HepG_2$ cells were treated with broccoli extract (100 μg/ml), sulforaphane (20 μM), gentisic acid (20 μM), and gallic acid (20 μM) for 24 h. PST-P activity (A) and PST-P mRNA expression (B) were measured as described in the "Materials and methods" section. The results are represented as the ratio of PST-P activity in the treated cells to control. Each value of the ratio of PST-P activity represents the mean ± SD of three independent experiments. $*p < 0.05$ and $**p < 0.01$ vs. control. The mean uninduced activity in the control cells was 22 ± 3 pmol/min/mg protein.

in the $HepG_2$ cells, the PST-P inducing activity by these compounds in $HepG_2$ cells was then evaluated. The basal specific activity of PST-P in $HepG_2$ cells was 22 ± 3 pmol/min/mg protein. As shown in Figure 2A, the broccoli extracts, sulforaphane, gentisic acid, and gallic acid were found to significantly increase PST-P activity. The greatest proportionate induction of PST-P activity was observed in treatment with broccoli extracts by inducing PST-P activity with a maximum of 1.8-fold. Treatment with sulforaphane at 20 μM induced PST-P activity about 1.4-fold in $HepG_2$ cells. At concentration of 20 μM, both gentisic acid and gallic acid showed a significant increase of PST-P activity, with a 1.6- and 1.7-fold induction, respectively. By contrast, PST-M activity was also increased to the same extent by these compounds (data not shown). The effects of broccoli, sulforaphane and phenolic compounds on PST-P mRNA expression were also investigated by using RT-PCR assay. As shown in Figure 2B, the levels of PST-P mRNA in $HepG_2$ cells grown in normal cell culture medium without the addition of phenolic compounds were barely detectable; however, PST-P mRNA expression was significantly induced after the $HepG_2$ cells were incubated with all these compounds for 24 h. Treatment of $HepG_2$ cells with the broccoli extracts containing the mixtures of isothiocyanates and polyphenolic compounds substantially increased PST-P mRNA expression. This increase was comparable to the induction of PST-P mRNA affected by sulforaphane and phenolic acids at 20 μM. Broccoli extracts showed the most potent inducing effect, elevating PST-P mRNA level to 1.9-fold. The inductions of PST-P mRNA expression by sulforaphane, gentisic acid and gallic acid at 20 μM were 1.3-, 1.5-, and 1.8-fold, respectively. The housekeeping gene GADPH showed no change under all these compounds treatments.

Phenolic content and antioxidant activity in vegetables

The total phenolics content and total antioxidant capacity (expressed as an $ORAC_{ROO\cdot}$ value) of 20 vegetables are shown in Table III. Phenolic contents were expressed as milligrams of gallic acid equivalents per g of FW vegetables. Among all the vegetables analyzed broccoli had the highest amount of total phenolics content (7.0 ± 0.3 mg/g of vegetable), followed by asparagus (5.5 ± 0.2 mg/g of vegetable), eggplant and snap bean (4.8 ± 0.1 and 4.5 ± 0.1 mg/g of vegetable, respectively), celery (3.7 ± 0.2 mg/g of vegetable), cauliflower (3.5 ± 0.2 mg/g of vegetable) and garlic (3.2 ± 0.2 mg/g of vegetable). Sweet potato had the lowest total phenolics content (1.6 ± 0.3 mg/g of vegetable) of the 20 vegetables, which was just slightly lower than spinach (2.0 ± 0.1 mg/g vegetable). It is interesting to note that asparagus, broccoli, and eggplant had relatively higher amounts of total phenolics content compared to the other vegetables in this study ($p < 0.05$). Broccoli and sweet potato had the highest and lowest overall total phenolics content with 7.0 ± 0.3 and 1.6 ± 0.3 mg/g vegetable, respectively.

The antioxidant activities against peroxyl radicals ($ORAC_{ROO\cdot}$ activity) of 20 vegetables are shown in Table III. The data indicated that broccoli had the highest $ORAC_{ROO\cdot}$ value (45 ± 1 μmol of trolox equiv./g of FW vegetable, $p < 0.05$) among the 20 vegetables tested. Asparagus, cauliflower, celery, snap bean, cabbage, eggplant and garlic had $ORAC_{ROO\cdot}$ values within the range of 34–41 μmol of trolox equiv./g. Soybean sprouts, onion, leek, Chinese chive and spinach were in the lower $ORAC_{ROO\cdot}$ values (13–24 μmol of trolox equiv./g of FW vegetable). Of the tested vegetables including broccoli, celery, asparagus, cauliflower, snap bean, eggplant, leek and

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

RIGHTSLINK

Table III. Total phenolic content and antioxidant activity ($ORAC_{ROO\cdot}$) in vegetables.

Vegetables (Scientific name)	(mg/g)	$ORAC_{ROO\cdot}$ (μmol of TE/g)
Asparagus (*Asparagus officinalis L.*)	5.5 ± 0.2	39 ± 4
Broccoli (*Brassica oleracea L.*)	7.0 ± 0.3	45 ± 1
Cabbage (*Brassica oleracea L.*)	2.7 ± 0.3	33 ± 1
Carrot (*Daucus carota L.*)	2.3 ± 0.1	28 ± 1
Cauliflower (*Brassica oleracea L.*)	3.5 ± 0.2	39 ± 2
Celery (*Apium graveolens L.*)	3.7 ± 0.2	41 ± 2
Chinese chive (*Allium tuberosum Rottler*)	2.1 ± 0.1	20 ± 1
Cucumber (*Cucumis sativus L.*)	2.3 ± 0.3	26 ± 3
Eggplant (*Solanum melongena L.*)	4.8 ± 0.1	35 ± 2
Garlic (*Allium sativum L.*)	3.2 ± 0.1	34 ± 2
Leek (*Allium ampeloprasum L.*)	3.3 ± 0.2	21 ± 2
Lettuce (*Lactuca sativa L.*)	3.1 ± 0.1	28 ± 3
Onion (*Allium cepa L.*)	2.1 ± 0.2	23 ± 2
Potato (*Solanum tuberosum L.*)	3.3 ± 0.2	30 ± 1
Shiitake (*Lentinus edodes Sing.*)	2.3 ± 0.1	28 ± 1
Snap bean (*Phaseolus sativum L.*)	4.5 ± 0.3	38 ± 3
Soybean sprouts (*Glycine max Merr.*)	3.5 ± 0.2	24 ± 1
Spinach (*Spinacia oleracea L.*)	2.0 ± 0.1	13 ± 4
Sweet potato (*Ipomoea batatas Poir.*)	1.6 ± 0.3	28 ± 1
Tomato (*Lycopersicon esculentum Mill.*)	2.7 ± 0.1	30 ± 2

Data expressed as mean ± SD from three experiments. Data expressed as milligrams of gallic acid equivalents per gram of fresh weight (FW) of vegetables. Data expressed as μmol of trolox equivalents per gram of FW vegetables.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

cabbage all acted as antioxidant against peroxyl radical (ROO·) in a dose-dependent manner (data not shown). In addition, the results obtained from $ORAC_{ROO\cdot}$ assay were similar to those of the total polyphenolic assay. In general, vegetable extracts that induced both forms of PSTs activities were found to have higher antioxidant capacities.

HPCL analysis of phenolic acids in vegetables

Our previous studies revealed that *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid, and *p*-couamric acid all could increase the activities of both PST-P and PST-M. These phenolic acids also possessed antioxidant capacity in the ORAC and TEAC assay [10]. From this background, phenolic acids are presumed to be a potential inducer on both forms of PSTs activities in vegetables. Among the 20 vegetable samples, the extracts of asparagus, broccoli and eggplant were the most potential inducer in both forms of PSTs activities. To determine the main antioxidant compound in polyphenol, the phenolic composition in vegetables was assayed. The phenolic chromatograms from asparagus, broccoli and eggplant after hydrolysis with acid are shown in Figure 3 and Figure 3A shows the chromatograms of phenolic acids standard. Figure 3B–D represents the chromatograms of extracts of asparagus, broccoli and eggplant, respectively. As the results in Table IV show, asparagus, broccoli and eggplant contained large amount of polyphenols, especially gallic acid, *p*-hydroxybenzoic acid, *p*-couamric acid, gentisic acid, and ferulic acid. The amount of gallic acid ranged from trace in asparagus and eggplant to 200 ± 40 μg/g of extracts in broccoli. The amount of *p*-hydroxybenzoic acid ranged from trace in eggplant to 477 ± 38 μg/g of extract in asparagus. *p*-Coumaric acid in both broccoli and eggplant were 1306 ± 42 and 1733 ± 40 μg/g of extract, respectively, which was the highest compared to other vegetables. Gentisic acid and ferulic acid were also found to be highest in broccoli, with a content of 561 ± 19 and 1059 ± 64 μg/g of extract, respectively. From this result, it can be concluded that phenolic acid in polyphenol of vegetables extract is the main responsible antioxidant and that the phenolic acid in vegetable has a positive inducing ability on phenolsulfotransferase activity.

Relationship between antioxidant activity, total phenolic content, and phenolsulfotransferase activity

There was a direct relationship between total phenolic content and total antioxidant activity in extracts of different vegetables ($r = 0.75$, $p < 0.05$; data not shown). The higher total phenolics content in vegetables resulted in higher total antioxidant activity. The influence of vegetables on PST-P activity in relation to their antioxidant activity is presented in Figure 4. It was found that there is a significant linear correlation between the influences of vegetables on PST-P activity. A correlation coefficient ($r = 0.82$, $p < 0.05$) was observed between the influence of vegetables on PST-P activity and their ORAC values (Figure 4A). In addition, the influence of vegetables on PST-P activity was linearly related to the total

RIGHTSLINK

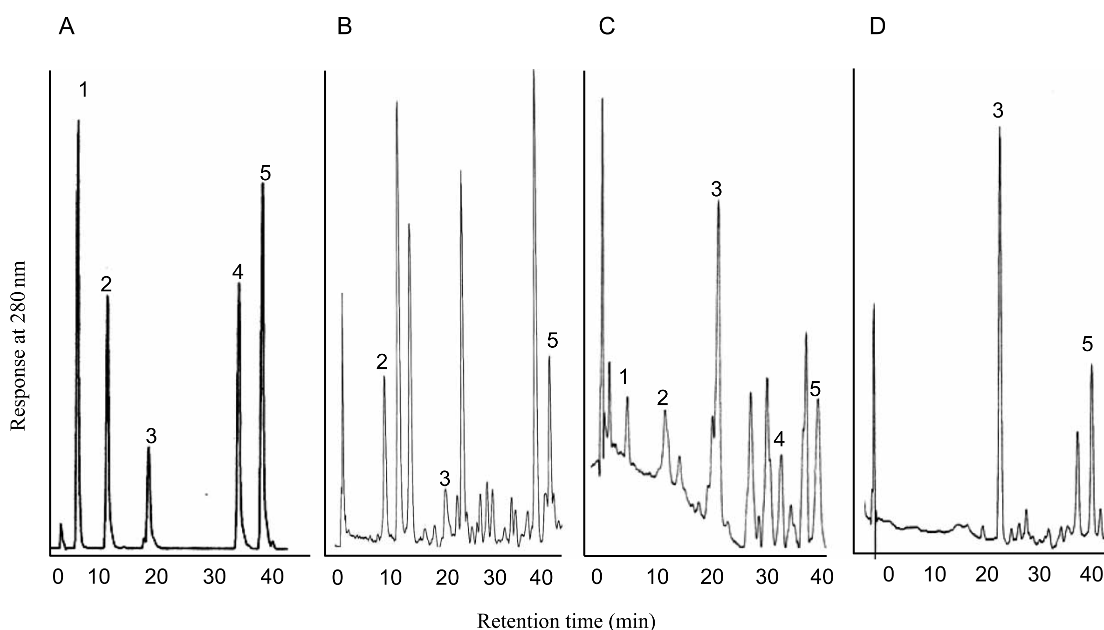

Figure 3. HPLC chromatogram of phenolic acids standards and water extract from vegetables: (A) phenolic acids standards; (peak identification: 1, gallic acid; 2, *p*-hydroxybenzoic acid; 3, gentisic acid; 4,ferulic acid; 5, *p*-coumaric acid); (B) asparagus; (C) broccoli; (D) eggplant.

phenolics measurement ($r = 0.78$, $p < 0.05$) (Figure 4B). A similar result can be seen in Figure 5, where there is a significant linear correlation between the influence of vegetables on PST-M activity and their ORAC values ($r = 0.76$, $p < 0.05$) (Figure 5A) or total phenolics ($r = 0.70$, $p < 0.05$) (Figure 5B). These results suggest that vegetables tested to the activity of PST-P and PST-M are well correlated to their antioxidant activity and total phenolics.

Discussion

This paper describes the influence of twenty vegetables (Table I) on the activity of both forms of PSTs in relation to their antioxidant activity and total phenolics. The results demonstrated that PST-P activity was significantly ($p < 0.01$) induced by asparagus, broccoli, cauliflower, celery and eggplant, whereas PST-M activity was marked induced by asparagus, broccoli, carrot, eggplant and potato at a concentration of 100 μg/ml. Among vegetables extracts tested, asparagus, broccoli and eggplant were the most effective inducer on the activity of both PST-P and PST-M (Table II). Thus, RT-PCR analysis was performed to obtain further evidence that broccoli, eggplant and asparagus are potent inducer of PST-P. The result (Figure 1) showed that all of these three vegetables had markedly increased PST-P gene expression in the $HepG_2$ cells, and broccoli elicited the strongest inductive effect among them. Broccoli and eggplant was reported to significantly induce quinone reductase (QR) activity at the concentration of 100 μg/ml [21]. Broccoli sprouts is an exceptionally rich source of inducer of phase II enzymes that protect against chemical carcinogens [22]. Preclinical and clinical evaluation of broccoli supplements as inducers of glutathione S-transferase activity, suggesting that the GST activity of blood lymphocytes may be used as a biomarker of the responsiveness of colon tissue to chemopreventive regimens [23]. Cauliflower was found to be a potent inducer of both glutathione S-transferase and quinone reductase [24]. Our results agreed with those reports and also found that some vegetables were effect of both forms of PST activities. In contrast, the results presented in the present study comprise the first report to show the asparagus, broccoli and eggplant could enhance the activity of both PST-P and PST-M.

Table IV. Contents of total phenolic acid in vegetables extract.

	μg/g of fresh weight vegetables				
	Gallic acid	*p*-Hydroxybenzoic acid	*p*-Coumaric acid	Gentisic acid	Ferulic acid
Asparagus	trace	447 ± 38	183 ± 16	151 ± 20	461 ± 42
Broccoli	200 ± 40	233 ± 11	1306 ± 42	561 ± 19	1059 ± 64
Eggplant	trace	trace	1733 ± 40	trace	936 ± 36

Data expressed as mean ± SD from three experiments.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

RIGHTSLINK

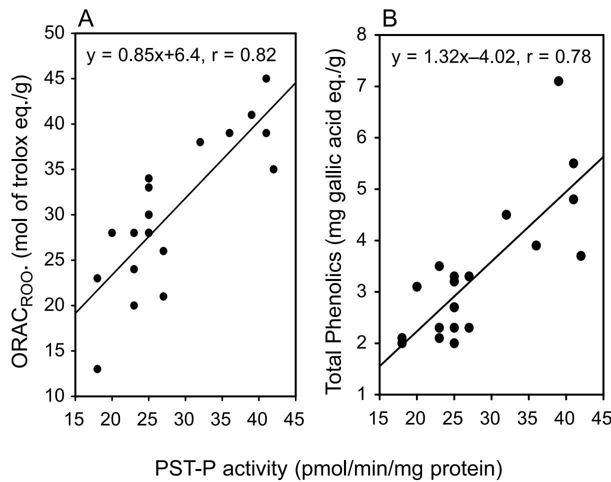

Figure 4. Effects of vegetables on P-form phenolsulfotransferase activity$_{(x)}$ (pmol/min/ mg protein) in relation to their (A) ORAC$_{ROO(Y)}$ (μmol of trolox eq./g). and (B) Total phenolics $_{(y)}$ (mg gallic acid eg./g). Each value is the mean ± SD of three experiments.

There is much evidence which suggests that high intake of cruciferous vegetables is associated with a lower incidence of cancer. The important groups of compounds that have this property are organosulfur compounds, such as the isothiocyanates [25]. Sulforaphane is one member of the isothiocyanate class of cancer chemopreventive compounds that has been shown to be effective in blocking initiation and progression of carcinogenesis [26]. Several studies have demonstrated that sulforaphane can potently induce phase II detoxifying enzymes, which contributes to its chemopreventive functions [27]. In this study, we compared the effect of broccoli, sulforaphane and phenolic acids on PST-P activity and mRNA expression in the $HepG_2$ cells. Our data revealed that broccoli is a potent inducer on PST-P activity and mRNA expression in $HepG_2$ cells. In contrast, the results presented in the present study comprise the first report to show that sulforaphane, gallic acid and gentisic acid could enhance the activity and mRNA expression of PST-P (Figure 2A and 2B). Comparing isothiocyanate with polyphenolic compounds indicated that the phenolic acids, mainly gentisic acid and gallic acid caused strong inducing activity on PST-P activity and mRNA expression. The sulforaphane, an active constituent isolated from broccoli, had lower inducing activities than polyphenolic compounds. This is in a good agreement with the previous work of Zhang et al. [25] that sulforaphane is a potent inducer of phase II detoxification enzyme such as quinine reductase (QR), UDP-glucoronosyl transferase (UGTs), and glutathione transferase (GSTs). Yeh and Yen [10,11] also reported that gallic acid and gentisic acid were effective inducer of the phenosulfotransferase (PSTs). Our results indicated that polyphenolic compounds showed in general higher inducing activity than sulforaphane in $HepG_2$ cells. Therefore, it can be inferred that the increase in PST-P mRNA by sulforaphane and phenolic acids might be regulated mainly at the transcription level.

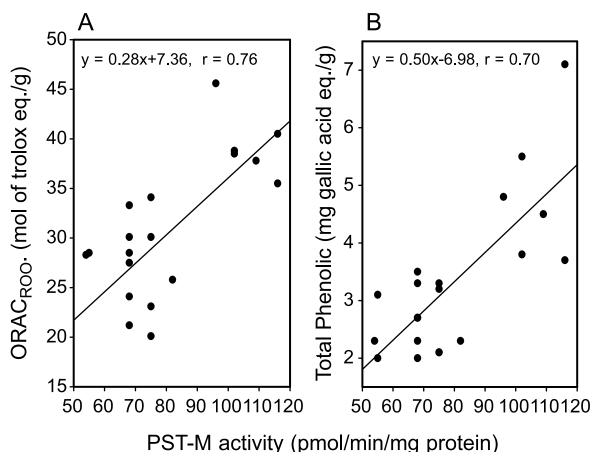

Figure 5. Effects of vegetables on M-form phenolsulfotransferase activity$_{(x)}$ (pmol/min/mg protein) in relation to their (A) ORAC$_{ROO(Y)}$ (μmol of trolox eq./g). and (B) Total phenolics $_{(y)}$ (mg gallic acid eg./g). Each value is the mean ± SD of three experiments.

The most common antioxidants present in vegetables were vitamin C and E, carotenoids, polyphenolics, and thiol (SH) compounds, etc. The chemical diversity of antioxidant makes it difficult to separate and quantify individual antioxidant from the vegetable matrix. A wide range of methods has been described in the literature for assessing antioxidant activity of vegetables [28]. The ORAC assay is one of the methods used to evaluate the antioxidant capacity of various biological substrates, ranging from pure compounds such as phenolic acids [10] and flavonoids [29] to complex matrices such as vegetables [12] and animal tissues [30]. In this study, the ORAC assay was used to evaluate the antioxidant activity of vegetables that increased the activity of PST. Our results clearly demonstrated that all vegetables tested in this study had antioxidant activities against peroxyl radical (Table II). The rank order based on the absolutely ORAC mean values is broccoli > celery > asparagus ≒ cauliflower > snap bean > eggplant > garlic > cabbage > potato ≒ tomato > carrot ≒ shiitake ≒ lettuce > cucumber > soybean sprouts > leek > onion > Chinese chive > spinach. In general, vegetable extracts that induced both forms of PSTs activities were found to have higher antioxidant capacities. There was a significant correlation between the influence of vegetables on both forms of PST activities and their ORAC values ($r = 0.82$, $p < 0.05$; $r = 0.76$, $p < 0.05$) (Figure 4A and 5A).

The USDA recommends daily consumption of five servings of vegetables partly on the basis of accumulated evidence that the phytochemicals in these horticultural products are beneficial to human health. The combined antioxidant activity of chemical

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

RIGHTSLINK

constituents in fruit and vegetables tissues is thought to be one key factor [31]. Fruit and vegetables containing high levels of antioxidants (mainly plant phenolic acids) can prevent the oxidative stress caused by free radical. It has been proposed that phenolic acids are the major contributor to the antioxidant capacity of vegetables [32]. Thus, increased consumption of vegetables containing high levels of polyphenolic has been recommended to reduce cellular oxidative damage in the human body [33]. Our previous studies revealed that *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid, and coumaric acid all could increase the activities of both PST-P and PST-M. These phenolic acids also possessed antioxidant capacity in the ORAC and TEAC assays [10]. Furthermore, in both two-compound and three-compound combinations with each of other phenolic acids, gallic acid and gentisic acid exhibit the potential synergistic effects in the promotion of PSTs activities [11]. Consequently, understanding the polyphenolic distribution profile in vegetables is of primary importance. In the present study, asparagus, broccoli and eggplant had relatively higher amounts of total phenolic contents compared to the other vegetables ($p < 0.05$) (Table III), whereas the extracts of asparagus, broccoli and eggplant were the most potential inducer in both forms of PSTs activities ($p < 0.01$) (Table II). There was a significant correlation between the influence of vegetables on PST activity and their total phenolic content ($r = 0.78$, $p < 0.05$; $r = 0.70$, $p < 0.05$) (Figures 4B and 5B). In order to establish whether phenolic acids make a contribution in the induction of both forms of PST activities, an additional study was conducted in vegetables by HPLC with diode-array detection. The HPLC system successfully separated the five phenolic acids in the vegetable extracts (Figure 3). Some active phenolic acids, such as gallic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, gentisic acid and ferulic acid could be resolved in this system (Table IV). Phenolic acids, especially hydroxycinnamic and hydroxybenzoic acids are secondary plant products and commonly found in plant-derived food-stuffs. Li et al. [34] using high performance liquid chromatographic assay, have reported that the simple polyphenols such as cinnamic and benzoic acid derivatives are highly widespread in vegetables. The hydroxycinnamic acid esters in broccoli are highly effective in trolox equivalent antioxidant capacity (TEAC) and inhibition of iron/ascorbate-induced lipid damage [35]. Whitaker and Stommel [28] determined that the hydroxycinnamic acid conjugates are the major class of polyphenols in various commercial eggplant cultivars. The cell walls of asparagus contains a range of phenolic esters, most notable are the *trans*-isomers of *p*-coumaric (PC) and ferulic acid (FA) [36]. Our results agreed with those reports and also found the active components in the extract of asparagus, broccoli and eggplant that may induce PST activity are phenolic acids.

There is much evidence that elevated tissue levels of phase II detoxification enzymes are associated with decreased susceptibility to chemical carcinogenesis [37]. The phenolsulfotransferase (PSTs) are the main phase II sulfate conjugation enzymes for catecholamines, thyroid hormones, and drugs. Our results suggest that extracts from asparagus, broccoli and eggplant are effective inducer on both forms of PST in human $HepG_2$ cells. The present study further demonstrates that simple phenolic acids such as gallic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, gentisic acid, and ferulic acid are at relatively high level in vegetables. It is generally assumed that the dietary compounds responsible for these protective effects are antioxidant nutrients. Such compounds as flavonoids, phenolic acids, glucosinolates, sulphur compounds and indoles have been shown to alter the levels of phase I and phase II drug metabolizing enzymes [38]. Ferulic acid and *p*-coumaric acid have been reported to act as scavengers of thiol free radical. In addition, ferulic acid has a strong antioxidant activity by preventing oxidative DNA damage induced by Fenton reaction [39]. Gentisic acid was reported to have an inhibitory action in myeloperoxidase system and was able to impair the tyrosyl radical catalyzed low-density lipoprotein peroxidation [40]. Some fruit and vegetables contain a group of natural phenolic acids that have not only a high antioxidant activity but also a good antioxidant quality [41]. Because of their ubiquitous presence in vegetables, humans consume phenolic acids on a daily basis. The estimated range of consumption is 25 mg–1g a day depending on diet (fruit, vegetables, grain, teas and coffees) [42]. Therefore, the supplementation of natural phenolic acids through a balanced diet containing enough fruits and vegetables could be most effective in inducing of phase II chemoprotective enzymes.

In conclusion, our results demonstrate that asparagus, broccoli, cauliflower, celery, eggplant and snap bean were found to increase the PST-P activity. However, asparagus, broccoli, carrot, Chinese chive, eggplant and potato could increase the activity of PST-M. In addition, asparagus, broccoli, cauliflower, celery, eggplant and snap bean had antioxidant capacity in the ORAC assay system. There was a significant correlation between the activity of both forms of PST and the antioxidant capacity of ORAC value by vegetables ($r = 0.82$, $p < 0.05$ and $r = 0.72$, $p < 0.05$). Furthermore, asparagus, broccoli and eggplant had relatively higher amounts of total phenolic contents compared to the other vegetables tested in this study ($p < 0.05$). There is also a direct induction of PSTs mRNA by these three vegetables in $HepG_2$ cells. The asparagus, broccoli and eggplant contained large amount of polyphenols, especially gallic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid,

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

RIGHTSLINK

gentisic acid and ferulic acid. Therefore, we have compared three broccoli-derived chemicals in the induction of PST-P in $HepG_2$ cells. Sulforaphane, gentisic acid and gallic acid are all potent inducers of PST-P activity and mRNA. In contrast, polyphenolic compounds mainly gentisic acid, and gallic acid caused strong inducing activity than sulforaphane, an active constituent isolated from broccoli. The results of this study suggest that phenolic acids in vegetables may play an important role in both forms of PSTs activities. Since PST is a key enzyme to catalyze the xenobiotics metabolism, the increased activity of PST will therefore promote the efficiency of detoxification. Our results have provided more understanding on the effect of vegetables on human PST activities as well as information on the antioxidant phenolic acids may play an important role in the effect of vegetable foods. The biological implications of these finding could be important for understanding the vegetables rich in antioxidant phenolic acids that may have a great potential in inducing of phase II chemoprotective enzymes.

Acknowledgements

This research work was partially supported by the National Science council, the Republic of China, under grant NSC92-2313-B005-067.

References

[1] Willett WC. Balancing life-style and genomics research for disease prevention. Science 2002;296:695–698.
[2] Gariballa SE, Silnclair AJ. Nutrition, aging and ill health. Br J Nutr 1998;80:7–23.
[3] Bresnick E, Birt DF, Wolterman K, Wheeler M, Markin RS. Reduction in mammary tumorigenesis in the rat by cabbage and cabbage residue. Carcinogenesis 1990;11:1159–1163.
[4] Willett WC. Micronutrients and cancer risk. Am J Clin Nutr 1994;59:162–165.
[5] Hanasaki Y, Ogawa S, Fukui S. The correlation between active oxygen scavenging and antioxidative effects of flavonoids. Free Radic Biol Med 1994;16:845–850.
[6] Kensler TM. Chemoprevention by inducers of carcinogen detoxification enzymes. Environ Health Perspect 1997;105: 965–970.
[7] Wilkinson J, Clapper ML. Detoxification enzymes and chemoprevention. Proc Soc Exp Biol Med 1997;216:192–200.
[8] Tawfiq N, Heaney RK, Plumb JA, Fenwick CR, Musk SRR, Williamson G. Dietary glucosinolates as blocking agents against carcinogenesis: Glucosinolate breakdown products assessed by induction of quinone reductase activity in murine hepa lclc7 cells. Carcinogenesis 1995;16:1191–1194.
[9] Brix LA, Nicoll R, Zhu X, McManus ME. Structural and functional characterization of human sulfotransferase. Chem Biol Interact 1998;109:123–127.
[10] Yeh CT, Yen GC. Effects of phenolic acids on human phenolsulfotransferases in relation to their antioxidant activity. J Agric Food Chem 2003;51:1474–1479.
[11] Yeh CT, Shih PH, Yen GC. Synergistic effect of antioxidant phenolic acids on human phenolsulfotransferase activity. J Agric Food Chem 2004;52:4139–4142.
[12] Cao G, Sofic E, Prior RL. Antioxidant capacity of tea and common vegetables. J Agric Food Chem 1996;44:3426–3431.
[13] Bouma ME, Rogier E, Verthier N, Labarre C, Feldmann G. Further cellular investigation of the human hepatoblastoma-derived cell line $HepG_2$: Morphology and immunocytochemical studies of hepatic secreted proteins. *In Vitro* Cell Dev Biol 1989;25:267–275.
[14] Roberts EA, Johnson KC, Harper PA, Okey AB. Characterization of the Ah receptor mediating aryl hydrocarbon hdyrolase induction in the human cell line $HepG_2$. Arch Biochem Biophys 1990;276:442–450.
[15] Dawson JR, Adams DJ, Wolf CR. Induction of drug metabolizing enzymes in human liver cell line $HepG_2$. FEBS Lett 1985;183:219–222.
[16] Backman L, Appelkvist EL, Sundberg A, Teclebrhan H, Brunk U. Modulation of metabolism in $HepG_2$ cell upon treatment with cyclosporin A and Nva2-eyclosporin. Exp Mol Pathol 1991;54:242–254.
[17] Shwed JA, Walle UK, Walle T. $HepG_2$ cell line as a human model for sulphate conjugation of drugs. Xenobiotica 1992;22:973–982.
[18] Gao G, Alessio HM, Culter RG. Oxygen-radical absorbance capacity assay for antioxidants. Free Radic Biol Med 1995;14:303–311.
[19] Cao G, Sofic E, Prior RL. Antioxidant and pro-oxidant behavior of flavonoids: Structure–activity relationship". Free Radic Biol Med 1993;22:749.
[20] Taga MS, Miller EE, Pratt DE. Chia seeds as a source of natural lipid antioxidants. J Am Oil Chem Soc 1984;61:928–931.
[21] Hashimato K, Kawamata S, Usui N, Tanaka A, Uda Y. *In vitro* induction of the anticarcinogenic marker enzyme, quinone reductase, in human hepatoma cells by food extracts. Cancer Lett 2002;180:1–5.
[22] Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc Natl Acad Sci 1997;94:10367–10372.
[23] Clapper ML, Szarka CE, Pfeiffer GR, Graham TA, Balshem AM, Litwin S, Goosenberg EB, Frucht H, Engstrom PF. Preclinical and clinical evaluation of broccoli supplements as inducers of glutathione S-transferase activity. Clin Cancer Res 1997;3:25–30.
[24] Williamson G, DuPont MS, Wanigatunga S, Heaney RK, Musk SRR, Fenwick GR, Rhodes MJC. Induction of glutathione S-transferase activity in $HepG_2$ cell by extracts from fruits and vegetables. Food Chem 1997;60:157–160.
[25] Zhang Y, Kensler TW, Cho CG, Posner GH, Talalay P. Anticarcinogenic activities of sulforaphane and structureally related synthetic norbornyl isothiocyanates. Proc Natl Acad Sci USA 1994;91:3147–3150.
[26] Thimmulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M, Biswal S. Identification of Nrf2-regulated genes induced by the chempreventive agent sulforaphane by oligonucleotide microarray. Cancer Res 2002;62:5196–5203.
[27] Parnaud G, Li P, Cassar G, Rouimi P, Tulliez J, Combaret L, Gamet-Payrastre L. Mechanism of sulforaphane-induced cell cycle arrest and apoptosis in human colon cancer cells. Nutr Cancer 2004;48:198–206.
[28] Proteggente AR, Pannala AS, Paganga G, van Buren L, Wagner E, Wiseman S, van de Put F, Dacombe C, Rice-Evans CA. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radic Res 2002;36:217–233.
[29] Cao G, Verdon CP, Wu AHB, Wang H, Prior RL. Automated oxygen radical absorbance capacity assay using the COBAS FARA II. Clin Chem 1997;41:1738–1744.
[30] Kohen R, Beit-Yannai E, Berry EM, Tirosh O. Overall low molecular weight antioxidant activity of biological fluids and

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

RIGHTSLINK

tissues by cyclic voltammetry. Methods Enzymol 1999; 300:285–296.
[31] Whitaker BD, Stommel JR. Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. J Agric Food Chem 2003; 51:3448–3454.
[32] Tanaka T, Kojima T, Kawamori T, Wang A, Suzui M, Okamoto K, Mori H. Chemoprevention of diethynitro-samine- induced hepatocarcinogenesis by a simple phenolic acid protocatechuic acid in rats. Cancer Res 1993;53:2775–2779.
[33] Prior RL. Fruits and vegetables in the prevention of cellular oxidative damage. Am J Clin Nutr 2003;78:570–578.
[34] Li P, Wang XQ, Wang HZ, Wu YN. High performance liquid chromatographic determination of phenolic acids in fruits and vegetables. Biomed Environ Sci 1993;6:389–398.
[35] Plumb GW, Price KR, Rhodes MJ, Williamson G. Antioxidant properties of the major polyphenolic compounds in broccoli. Free Radic Res 1997;27:429–435.
[36] Rodriguez-Arcos RC, Smith AC, Waldron KW. Effect of storage on wall-bound phenolics in green asparagus. J Agric Food Chem 2002;50:3197–3203.
[37] Talalay P. Chemoprotection against cancer by induction of Phase 2 enzymes. BioFactors 2000;12:5–11.
[38] Valerio Jr., LG, Kepa JK, Pickwell GV, Quattrochi LC. Induction of human NAD(P)H:quinone oxidoreductase (NQO1) gene expression by the flavonol quercetin. Toxicol Lett 2001;119:49–57.
[39] Aruoma OI. Antioxidant action of plant foods: Use of oxidative DNA damage as a tool for studying antioxidant efficacy. Free Radic Res 1999;30:419–427.
[40] Hermann M, Kapiotis S, Hofbauer R, Seelos Ch, Held L, Gmeiner B. Salicylate promotes myeloperoxidase-initiated LDL oxidation: Antagonization by its metabolite gentisic acid. Free Radic Biol Med 1999;26:1253–1260.
[41] Vinson JA, Hao Y, Su X, Zubik L. Phenolic antioxidant quantitiy and quality in foods: Vegetables. J Agric Food Chem 1998;46:3630–3634.
[42] Clifford MN. Chlorogenic acids and other cinnamates -nature, occurrence, and dietary burden. J Sci Food Agric 1999;79: 362–372.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/29/11
For personal use only.

RIGHTSLINK