Anti-oxidant activity and total phenolic content of some Asian vegetables

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Summary The anti-oxidant activity of extracts from 36 vegetables was evaluated by using a model system consisting of β-carotene and linoleic acid. The total phenolics of the extracts was determined spectrophotometrically according to the Folin–Ciocalteau procedure and ranged from 34 to 400 mg $(100\ g)^{-1}$ on a fresh weight basis. Mint, aonla, black carrots, chenopodium, fenugreek, kachnar and ginger had high phenolic contents. The anti-oxidant activity expressed as per percent inhibition of oxidation ranged from a high of 92% in turmeric extracts to a low of 12.8% in long melon. Other vegetables found to have high anti-oxidant activity (>70%) were kachnar, aonla, ginger, fenugreek, mint, beetroot, black carrots, Brussels sprouts, broccoli, lotus stem, yam, coriander and tomato. Anti-oxidant activity correlated significantly and positively with total phenolics ($r^2 = 0.6578$, $P < 0.05$). The results indicate that vegetables containing high phenolics may provide a source of dietary anti-oxidants.

Keywords Anti-oxidant activity, β-carotene, phenolics, phenols, vegetables.

Introduction

Fruits and vegetables have had conferred on them the status of functional foods (Hasler, 1998), They seem to be capable of delivering health benefits besides fulfilling physiological needs. Routine or habitual consumption of fruits and vegetables confers significant benefits to human health (Steinmetz & Potter, 1996). Epidemiological data as well as *in vitro* studies strongly suggest that foods containing phytochemicals with anti-oxidation potential have strong protective effects against major disease risks including cancer and cardiovascular diseases (Steinberg, 1991; Block *et al*., 1992; Ames *et al*., 1993; Hertog *et al*., 1993; Byers & Guerrero, 1995; Knekt *et al*., 1997; Elliot, 1999; Kaur & Kapoor, 2001).

The protective action of fruits and vegetables has been attributed to the presence of anti-oxidants, especially anti-oxidant vitamins including ascorbic acid, α-tocopherol and β-carotene (Gey *et al*., 1991; Willet, 1994; Kalt & Kushad, 2000; Prior & Cao, 2000).

However numerous studies have conclusively shown that the majority of the anti-oxidant activity may be from compounds such as flavonoids, isoflavone, flavones, anthocyanin, catechin and isocatechin rather than from Vitamin C, E and β-carotene (Wang *et al*., 1996; Kahkonen *et al*., 1999). Epidemiological studies have shown that consumption of food and beverages rich in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis by acting as anti-oxidants towards low-density lipoprotein (LDL) (Kinsella *et al*., 1993; Frankel *et al*., 1995; Landbo & Meyer, 2001). Therefore, mostly, the current focus is on the anti-oxidant action of phenolics. The anti-oxidant activity of phenolics is mainly because of their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evans *et al*., 1997). Elimination of

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synthetic anti-oxidants in food applications has given more impetus to exploring natural sources of anti-oxidants. In this context a large number of plant sources including many vegetables and fruits have been explored for their anti-oxidant potential (Willet, 1994; Al-Saikhon *et al*., 1995; Cao *et al*., 1996). Mushroom, white cabbage and cauliflower (Gazzani *et al*., 1998), garlic, broccoli, kidney and pinto beans (Vinson *et al*., 1998), beans, beet and corn (Kahkonen *et al*., 1999) have been reported to have high anti-oxidant activity. Other vegetables such as kale, spinach, brussels sprouts, alfalfa sprouts, broccoli, beets, red bell-pepper, onion, corn, eggplant, and cucumber are also rich source of anti-oxidants (Gazzani *et al*., 1998; Velioglu *et al*., 1998; Vinson *et al*., 1998).

Garlic extracts are also used as potential cardiovascular and anticancer agents (Oomah & Mazza, 2000). Mushrooms, white cabbage, cauliflower and garlic have also been shown to have strong protective activity against a number of diseases (Gazzani *et al*., 1998). Extracts of many different vegetables have antimutagenic effects. Spinach is regarded, as the 'Brain Food' needed to avoid memory loss and Alzheimer disease (Clarke, 1999). Broccoli is a potential source of glucosinolates having anticancerous activity (Fahey & Stephenson, 1999).

India is bestowed with diverse climatic conditions, conducive for the growth of different vegetables known for their nutritional values. So far, there has not been a study on anti-oxidant activity of vegetables grown and consumed here. The main objective of this study was to screen a large number of vegetables consumed in the Indian diet with respect to their total phenolic content and anti-oxidant activity.

In the present study, the anti-oxidant activity of different vegetables was evaluated by using a widely accepted model system containing β-carotene and linoleic acid (Lee & Ahn, 1985; Tsushida *et al*., 1994; Al-Saikhon *et al*., 1995; Nicoli *et al*., 1997; Gazzani *et al*., 1998).

Materials and methods

Sample preparation

Thirty-four vegetables were purchased fresh on two separate occasions from different markets. These were cleaned, washed and chopped into small pieces. Onions, potatoes, ginger, turmeric, garlic and other vegetables having dead and dry skins were processed after removal of their skins. Only edible portions of vegetables were weighed and homogenized using a Waring blender for 3 min at high speed. In addition two waste materials, pea pods and carrot leaves, were also used to assess their anti-oxidant activity and phenolic contents. Aonla (*Emblica officinalis*), although is a fruit, has been included in the study as it is consumed after cooking.

Determination of total phenolic content

Total phenolics were determined using the Folin–Ciocalteau reagent (Singleton & Rossi, 1965). Samples (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged in cold at 10 000 ***g*** for 15 min and the supernatant was saved. The residue was re-extracted twice with 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residue was dissolved in 5 mL of distilled water. One-hundred microlitres of this extract was diluted to 3 mL with water and 0.5 mL of Folin–Ciocalteau reagent was added. After 3 min, 2 mL of 20% of sodium carbonate was added and the contents were mixed thoroughly. The colour was developed and absorbance measured at 650 nm in a Bausch and Lomb spectronic-21 UVD spectrometer after 60 min using catechol as a standard. The results were expressed as mg catechol/100 g of fresh weight material.

Determination of anti-oxidant activity

For the determination of anti-oxidant activity, both aqueous and alcoholic (80%) vegetable extracts were prepared. Vegetables (1 g) were homogenized in the respective media and centrifuged at 10 000 ***g*** for 15 min. Supernatants were stored in capped tubes until further use. Anti-oxidant activity was determined according to the β-carotene bleaching method as described by Miller *et al*. (1993) with modifications (Wanasundara *et al*., 1994). β-Carotene (2 mg) was dissolved in 20 mL of chloroform. A 4 mL aliquot of the solution was added to a conical flask with

40 mg linoleic acid and 400 mg Tween-40. Chloroform was removed with a rotary evaporator at 50 °C. Oxygenated distilled water (100 mL) was added to the β-carotene emulsion mixed well and aliquots (3 mL) of the oxygenated β-carotene emulsion and 0.2 mL of water/alcoholic extracts were placed in capped culture tubes and mixed well. The tubes were immediately placed in a water-bath and incubated at 50 °C. Oxidation of the β-carotene emulsion was monitored spectrophotometerically taking absorbance at 10-min interval at 470 nm for 100 min. A control consisted of 0.2 mL distilled water instead of vegetable extract. Anti-oxidant activity was expressed as per cent inhibition relative to control using the equation

$$AA(\%) = [(\text{Degradation rate of control} - \text{degradation rate of sample})]/\text{Degradation rate of control}$$

where degradation rate $= \ln(a/b) \times 1/t$, where ln = natural log, a = initial absorbance (470 nm), b = absorbance (470 nm) at 100 min interval and t = time (min).

Results and discussion

Total phenolics

The results of phenol analysis of 33 commonly consumed vegetables in India is given in Tables 1–3. Total phenolic content of the vegetables varied from 400 mg catechol/100 g fresh weight in mint to 34 mg catechol/100 g fresh weight in round melon. Considering a large variation in the total phenolics, the vegetables were divided into three groups namely high (> 200 mg catechol/100 g), medium (100–200 mg catechol/100 g) and low (< 100 mg catechol/100 g). Interestingly mint, aonla, black carrots, chenopodium, fenugreek, beetroot, kachnar and ginger had the highest fresh weight concentration of total phenols followed by turmeric, palak, green chillies, potato and garlic. The low group was represented by lotus stem, yam, French beans, cabbage, broad beans, cauliflower, round melon and peas. The wet weight phenol content of vegetables was measured because they are purchased and eaten fresh. Vinson *et al*. (1998) observed that the phenol anti-oxidant index, a combined measure of the quality and quantity of anti-oxidants present in vegetables, was maximum in kidney bean followed by pinto bean, and garlic on a fresh weight basis while on a dry weight basis asparagus had the highest index followed by yellow onions, red onions and garlic.

Anti-oxidant activity

The presence of different anti-oxidant components in plant tissues especially fruits and vegetables make it relatively difficult to measure each anti-oxidant component separately. Therefore several methods (Al-Saikhon *et al*., 1995; Cao *et al*., 1995;

Table 1 Anti-oxidant activity and total phenolic content of vegetables (high anti-oxidant activity group)

Vegetables	Anti-oxidant activity (%)		Total phenolics [mg (100 g)$^{-1}$]
	Ethanol extract	Water extract	
Turmeric fresh (*Curcuma domestica*)	92.45	81.3	175.5 ± 7.2
Kachnar (*Bauhnia variegata*)	91.5	90.3	275.0 ± 10.1
Aonla (*Emblica officinalis*)	86.8	84.3	348.8 ± 13.4
Broccoli (*Brassica oleracea* var. *italica*)	78.4	72.5	87.5 ± 8.1
Mint (*Mentha spicata*)	77.8	73.8	399.8 ± 3.2
Brussel sprouts (*Brassica oleracea* var. *gemmifera*)	73.8	68.5	68.8 ± 1.3
Beet root (*Beta vulgaris*)	73.3	55.0	323.0 ± 11.7
Black carrots (*Daucus carota*)	73.0	61.8	350.5 ± 12.9
Fenugreek (*Trigonella foenum – graceum*)	72.8	62.8	217.5 ± 8.9
Ginger (*Zingiber officinale*)	71.8	65.0	221.3 ± 9.4
Lotus stem (*Nelumbium nelumbo*)	71.8	69.5	85.7 + 1.2
Coriander (*Coriandrum sativum*)	71.8	65.0	82.5 ± 1.9
Yam (*Dioscorea alata*)	71.0	62.8	92.0 ± 2.7
Tomato (*Lycopersicon esculentum*)	70.8	56.3	68.0 ± 1.6

Data expressed as mean ± s.e.m. of three samples analysed separately.

Table 2 Anti-oxidant activity and total phenolic content of vegetables (medium anti-oxidant activity group)

Vegetables	Anti-oxidant activity (%)		Total phenolics [mg (100 g)$^{-1}$]
	Ethanol extract	Water extract	
Palak (*Beta vulgaris*)	69.5	66.8	196.3 ± 8.1
Cabbage (*Brassica oleracea* var. *capitata*)	69.3	47.8	92.5 ± 2.4
Bell pepper *(Capsicum annuum)*	68.5	29.3	67.3 ± 1.2
Carrots (*Daucus carota*)	67.0	37.5	55.0 ± 0.9
Carrot leaves (*Daucus carota*)	66.5	63.5	115.8 ± 2.8
Mustard (*Brassica juncea*)	65.0	61.3	62.0 ± 1.1
Bathua (*Chenopodium album*)	64.5	60.5	253.5 ± 9.8
Pea pods (*Pisum sativum*)	64.0	60.5	85.5 ± 2.1
Green onion (*Allium cepa*)	63.0	46.5	63.3 ± 1.2
Potato (*Solanum tuberosum*)	62.3	62.5	149.8 ± 6.3
Garlic (*Allium sativum*)	62.1	61.8	145.0 ± 5.9

Data expressed as mean ± s.e.m. of three samples analysed separately.

Table 3 Anti-oxidant activity and total phenolic content of vegetables (low anti-oxidant activity group)

Vegetables	Anti-oxidant activity (%)		Total phenolics [mg (100 g)$^{-1}$]
	Ethanol extract	Water extract	
Onion (*Allium cepa*)	57.5	31.0	56.8 ± 1.1
Peas (*Pisum sativum*)	57.0	35.8	39.8 ± 0.6
Green chillies (*Capsicum anunum*)	54.8	46.5	115.0 ± 1.2
French beans *(Phaseolus vulgarlis)*	49.8	40.8	97.0 ± 1.1
Turnip (*Brassica rapa*)	47.8	45.8	127.0 ± 1.8
Sem (*Dolichus lablab*)	43.3	34.7	93.8 ± 1.4
Cucumber (*Cucumis sativus*)	34.3	28.0	48.0 ± 0.9
Roundmelon (*Praecitrullus vulgaris* var. *fistulosius*)	26.5	25.5	34.5 ± 1.1
Cauliflower (*Brassica oleracea* var. *botrytis*)	19.5	13.5	96.0 ± 0.9
Radish (*Raphanus sativus*)	13.0	11.9	54.5 ± 0.8
Longmelon (*Cucumis utilissimus*)	12.8	12.0	45.8 ± 0.6

Data are expressed as mean ± s.e.m. of three samples analysed separately.

Furuta *et al*., 1997; Gazzani *et al*., 1998; Velioglu *et al*., 1998; Vinson *et al*., 1998; Kahkonen *et al*., 1999; Chu *et al*., 2000; Tang *et al*., 2001) have been developed in recent years to calculate the total anti-oxidant activity of biological samples. These workers have also tried different extraction mediums to ensure the maximum extraction of the available anti-oxidants from the samples (Kahkonen *et al*., 1999). In the present study, two different solvent extraction viz. water and ethanol were used. Extraction in ethanol was found to be more efficient than water in extracting the anti-oxidants present in the vegetables, especially the carotenoids. The anti-oxidant activities of aqueous and ethanolic extracts of the 33 vegetables are given in Tables 1–3, wherein the vegetables have been placed in three different groups of high, medium and low anti-oxidant activities, respectively. The decrease in absorbance of β-carotene in the presence of different extracts with the oxidation of β-carotene and linoleic acid is shown in Figs 1–3. A large variation in the anti-oxidant activities, ranging from as high as 92% in turmeric and kachnar to as low as 13% in radish and long melon, was observed. Similar wide ranges in anti-oxidant activities has been reported earlier (Cao *et al*., 1996; Wang *et al*., 1996; Gazzani *et al*., 1998). Out of 34 vegetable extracts evaluated for anti-oxidant activity 14 vegetables were found in the group of high anti-oxidant activity ($>70\%$) (Table 1). These included turmeric, kachnar, aonla, broccoli, mint, Brussels sprouts, beetroot, black carrots, fenugreek, ginger, lotus stem, coriander, yam and tomato. The group

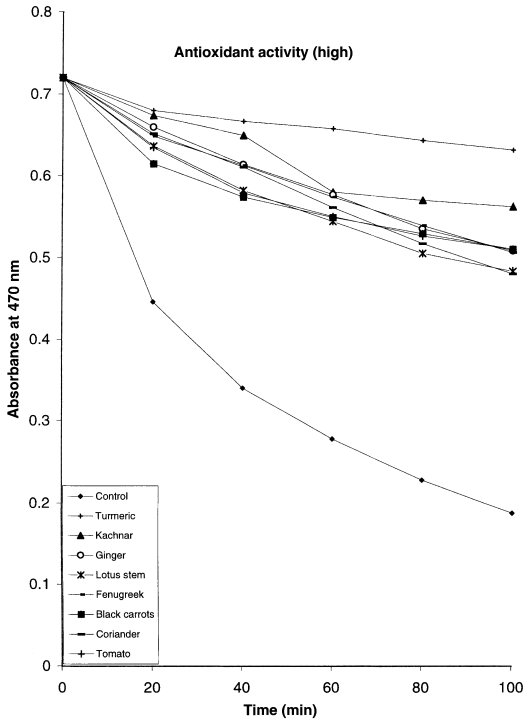

Figure 1 Anti-oxidant activity measured by bleaching of linoleic acid–carotene emulsion (low anti-oxidant activity group).

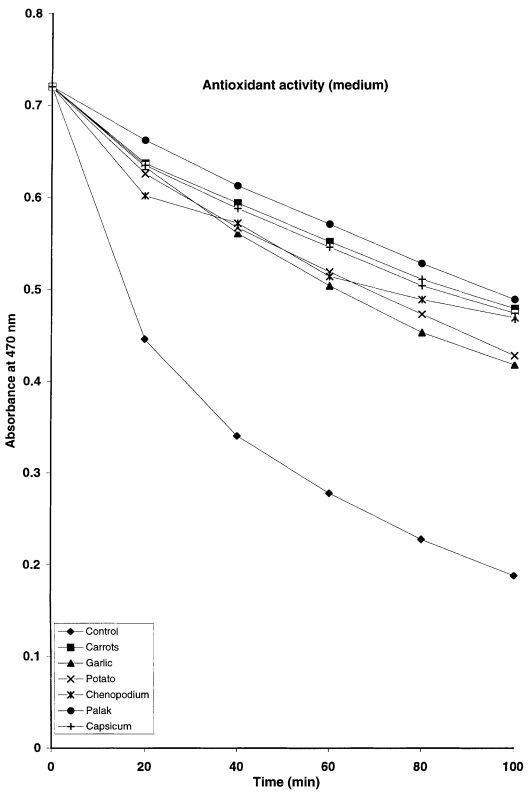

Figure 2 Anti-oxidant activity measured by bleaching of linoleic acid–carotene emulsion (medium anti-oxidant activity group).

with moderate activity (Table 2) (60–70%) was represented by palak, cabbage, bell-pepper, carrots, carrot leaves, mustard, bathua, pea pods, potatoes and garlic. Pea, onion, green chillies, French beans, sem cucumber, round melon, cauliflower, radish and long melon represented the group with low anti-oxidant activity (< 60%). The results are in close agreement with those obtained by Cao *et al.* (1996), who have identified vegetables like Brussels sprouts, beets, bell-pepper, onion, potato with strong anti-oxidant activity using oxygen radical absorbance capacity (ORAC) assay.

The anthocyanin rich vegetables like black carrot and beetroots showed high phenolic contents and correspondingly high anti-oxidant activity. The results thus confirm that anthocyanin rich vegetables possess strong anti-oxidant activity (Wang *et al.*, 1997; Velioglu *et al.*, 1998).

Interestingly all vegetables, except chenopodium (253.5 mg catechol/100 g), which were grouped in a high phenolic content group also had a high anti-oxidant activity and vegetables like radish, cauliflower, French beans, round melon and long melon, which had low phenolic contents, also had low anti-oxidant activities. On the other hand there were some vegetables like tomato, broccoli, Brussels sprouts, turmeric, lotus stem and coriander, which were characterized by moderate or low phenolic contents, but which had high anti-oxidant activities. Their high anti-oxidant activities can be explained on the basis of high anti-oxidant activity of some individual phenolic units, which may act as efficient anti-oxidants rather than contributing to high total phenolics. The scavenging action of various phenolic compounds is closely connected with their spatial conformation. The epicatechin unit of phenol has been found to be more efficient than catechin (Gaulejac *et al.*, 1999). Similar results have been reported by Chu *et al.* (2000) in vegetables like white cabbage and crown daisy, which despite having low phenolic contents had moderate anti-oxidant activity. They attribute this to the presence of some other phytochemicals such as phenolic acid, ascorbic

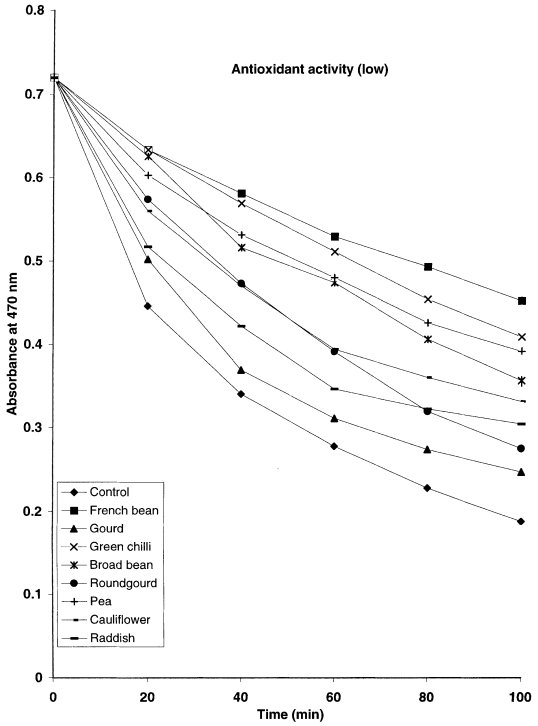

Figure 3 Anti-oxidant activity measured by bleaching of linoleic acid–carotene emulsion (high anti-oxidant activity group).

acid, tocopherol and pigments, which also contribute to total anti-oxidant activity. Based on this they recommended that anti-oxidant activity of vegetables should be evaluated by different methods rather than depending on the results of a single method. Also, Cao *et al.* (1996) stressed the measurement of anti-oxidant activity against different radicals, as fruits and vegetables appear to have an optimal mixture of various anti-oxidants. A very high anti-oxidant activity, observed in the case of fresh turmeric is attributed to Curcumin (diferuloylmethane), a phenolic compound present in *Curcuma domestica*. It is a yellow coloured polyphenolic phytochemical, which has been in use for a very long time for the treatment of swellings and wounds (Sindhu *et al.*, 1998). It has high anti-inflammatory activity (Vanketesan & Chandrakasan, 1995), antitumourogenesis (Chan *et al.*, 1998; Nakamura *et al.*, 1998), anti arthritic (Joe *et al.*, 1995) and is being developed as a nutraceutical in both dietary supplements and foods. Consumption of fresh turmeric may thus serve as an excellent source of anti-oxidant in its natural form. Dried turmeric powder is an indispensable spice used in Indian curries. The present studies highlight the importance of exploring the changes in anti-oxidant activity during drying/curing of turmeric for optimizing processing technologies. High anti-oxidant activity in bell-pepper has been attributed to the radical scavenging effect of capsanthin, which has been found to last longer than β-carotene (Matasufuji *et al.*, 1998). Similarly high anti-oxidant activity in ginger has been attributed to the active principal gingerol (Lee & Ahn, 1985; Kikuzaki & Nakatani, 1993). It is worthwhile to note that the presence of moderate anti-oxidant activities in wastes like pea pods and carrot leaves, which are usually discarded as waste can also be alternate sources of anti-oxidants.

Relationship between phenolic content and anti-oxidant activity

The relationship between total phenolic content and anti-oxidant activity of vegetables is shown in Fig. 4. The statistical analysis showed a positive and highly significant ($r^2 = 0.6578$, $P < 0.005$) relationship between total phenolics and anti-oxidant activity. Several studies have been made concerning the relationship between the phenolic structure and anti-oxidant activity (Faure *et al.*, 1990; Cuvelier *et al.*, 1992), but no relationship has been elucidated because of the many different evaluation systems used to test for anti-oxidant activity. Recently Velioglu *et al.* (1998) and Deighton *et al.* (2000) have demonstrated a linear relationship between anti-oxidant capacity and total phenolics in *Rubus* sp. However, Gazzani *et al.* (1998), Heinonen *et al.* (1998) and Kahkonen *et al.* (1999) could not find any correlation between total phenolic content and anti-oxidant activity of the plant extracts. According to them different phenolic compounds have different responses in the Folin–Ciocalteau method. Similarly the molecular anti-oxidant response of phenolics in methyl linoleate varies remarkably depending on their chemical structure (Statue-Gracia *et al.*, 1997). Thus the anti-oxidant activity of an extract could not be explained just on the basis of their phenolic content but also required their proper characterization (Heinonen *et al.*, 1998). Another Japanese study used the Folin

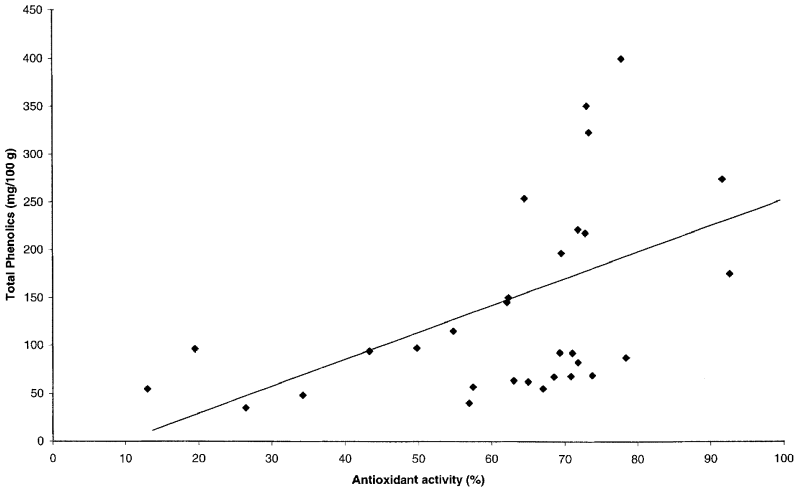

Figure 4 Relationship between total phenolic content and anti-oxidant activity of different vegetables ($P < 0.05$, $r^2 = 0.6578$).

assay for fresh vegetable extracts and measured their activity using β-carotene bleaching coupled with the oxidation of linoleic acid (Tsushida *et al.*, 1994). They found a positive correlation of anti-oxidant activity with phenol content. This correlation suggests that although the vegetables may contain other anti-oxidants such as proteins, ascorbate and the carotenoids, these do not contribute significantly to the anti-oxidant activity. Vinson & Hontz (1995), corroborated this assumption.

Conclusion

The study clearly indicates that it is important to measure the anti-oxidant activity using various radicals and oxidation systems and to take both phenolic content and anti-oxidant activity into account while evaluating the anti-oxidant potential of plant extracts. However, the model system consisting of β-carotene and linoleic acid can be used to screen large number of sources for their anti-oxidant capacity. Furthermore, in order to realize the health benefits from potential plant sources, additional information on their dietary intake and enhancing bioavailability after various processing operations is required. Currently work is underway in our laboratory to confirm the anti-oxidant activity of various fruit and other sources by using other oxidation systems and lipid models. In addition more work on optimizing processing and storage conditions for their maximum stabilization is also under progress.

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