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Characterization of food antioxidants, illustrated using commercial garlic and ginger preparations

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Methods for the characterization of antioxidants are presented and illustrated by their application to commercial garlic and ginger preparations, since it has been widely speculated that garlic and ginger might be beneficial to human health because they exert 'antioxidant activity'. The sample of commercial ginger powder, tested at concentrations up to 5 mg/ml, inhibited the peroxidation of phospholipid liposomes, but a sample of one commercial garlic preparation was less effective. Both preparations could scavenge peroxyl radicals, but the garlic preparation was again less effective.

The ginger and garlic preparations were powerful scavengers of hydroxyl radicals ($OH^{\cdot}$) and were able to react with hypochlorous acid (HOCl) at a rate sufficient to protect catalase and alpha-1-antiproteinase against inactivation. However, they could also interact with iron chelates to facilitate $OH^{\cdot}$ generation from H_2O_2 ('pro-oxidant' activity). Ginger (but not the garlic preparation) also exerted pro-oxidant action in the bleomycin assay, accelerating damage to DNA in the presence of a bleomycin–ferric iron complex.

Our results illustrate the use of antioxidant characterization methods. © 1997 Elsevier Science Ltd

INTRODUCTION

Generation of reactive oxygen species (ROS) beyond the antioxidant capacity of a biological system gives rise to *oxidative stress*. There are suggestions that oxidative stress plays a role in heart diseases, malaria, neurodegenerative diseases, AIDS, cancer and in the aging process (Riemersma *et al.*, 1991; Emerit & Chance, 1992; Yoshikawa, 1993; Halliwell & Gutteridge, 1989; Sies, 1991; Aruoma, 1993*a*; Slater, 1989; Jenner, 1991). In addition, antioxidants are used during food manufacture to minimize free radical damage to lipids in foods (St. Angelo, 1992; Hudson, 1990; Löliger, 1991).

Plant-derived 'antioxidants' such as vitamin E, vitamin C and flavonoids are becoming increasingly suggested as important dietary antioxidant factors that may help to protect against some of the above diseases. In addition, there is a growing interest in the use of 'natural' antioxidants for food preservation (Hertog *et al.*, 1993; Löliger, 1991; Brownridge, 1993; Block *et al.*, 1992; Shahidi & Wanasundara, 1992; Ames *et al.*, 1993; Aruoma, 1994*a*; Bonorden & Pariza, 1994; Laranjinha *et al.*, 1994; Stavric *et al.*, 1992). Thus garlic products have been suggested to be beneficial to human health (Dorant *et al.*, 1993; Lau, 1989, 1990; Fenwick *et al.*, 1985), perhaps by exerting antioxidant activity (Prasad *et al.*, 1995; Masuda *et al.*, 1995). For example, consumption of garlic tablets (Kwai®) has been reported to decrease the susceptibility of human low density lipoproteins to oxidation (Phelps *et al.*, 1993), which could give garlic an anti-atherosclerotic effect (Steinberg *et al.*, 1989). Garlic oil has also been found to have potent antioxidant activities in various oils (Shahidi & Wanasundara, 1992). Garlic has been shown to reduce radicals generated by the Fenton reaction and those present in cigarette smoke (Török *et al.*, 1994). Aged garlic extract has recently been shown to protect vascular endothelial cells from H_2O_2-induced oxidant injury (Yamasaki *et al.*, 1994).

Unfortunately, the word 'antioxidant' means different things to different people. Often (e.g. by food scientists) the term is implicitly restricted to chain-breaking inhibitors of lipid peroxidation, such as α-tocopherol. However, free radicals generated *in vivo* damage many other targets, including proteins, DNA and small molecules (Halliwell *et al.*, 1989). Hence a broader definition of an antioxidant is 'any substance that, when present at low concentrations compared to those of an oxidizable

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substrate, significantly delays or prevents oxidation of that substrate' (Halliwell, 1990). The term 'oxidizable substrate' includes every type of molecule found *in vivo*. This definition emphasizes the importance of the damage target studied and the source of ROS used when antioxidant action is characterized. It is perfectly possible for an antioxidant to protect, for example, lipids against oxidative damage whilst accelerating damage to other biological molecules (Aruoma *et al.*, 1990, 1992, 1993; Laughton *et al.*, 1989).

In evaluating the likelihood that a dietary constituent or other putative antioxidant actually exerts such an effect *in vivo*, it is important to ask certain questions (Table 1). Simple experiments can answer some of these questions, and the results often allow one to dismiss the proposed antioxidant ability: a compound that is a poor antioxidant *in vitro* is unlikely to be any better *in vivo*. The present article illustrates a few of the many approaches to characterization of direct antioxidant activity that we have developed (Halliwell, 1990; Aruoma *et al.*, 1990). Two obvious (but often forgotten) additional points are:

- A compound should be tested at concentrations achievable *in vivo* or in the food matrix.
- In assaying putative antioxidants one should use ROS that are relevant to the food matrix and, if the antioxidant is for use *in vivo* or can be absorbed from food, ROS that are created *in vivo* (Table 2).

In the present paper, we have applied some of our assays to characterize the direct antioxidant/pro-oxidant actions of commercial samples of Kwai®, a garlic preparation. We have also compared it with a sample of commercial ginger powder, since several of the constituents of ginger have been shown to exert antioxidant activities *in vitro* (Aeschbach *et al.*, 1994; Rajakumar *et al.*, 1994; Masuda & Jitoe, 1994; Masuda *et al.*, 1995; Prasad *et al.*, 1995).

MATERIALS AND METHODS

A sample of Kwai® (prepared by lyophilizing fresh Chinese garlic cloves as described by Lichtwer Pharma, GmBH, Germany) was purchased from the local supermarket. Dried and ground ginger was provided by Seven Seas Ltd, Marfleet, Hull, UK.

Table 1. Questions to ask when evaluating 'antioxidants' *in vivo*

1. What biomolecule is the antioxidant supposed to protect? Does enough antioxidant reach that target *in vivo* or in the food matrix?
2. How does it protect—by scavenging ROS, preventing their formation or repairing damage?
3. If the antioxidant acts by scavenging, can the resulting antioxidant-derived radicals themselves cause damage?
4. Can the antioxidant cause damage in other biological systems? For example, can it be pro-oxidant to other targets?

Other chemicals and reagents were of the highest quality available and were purchased either from Sigma Chemical Company (Poole, Dorset, UK) or BDH Chemical Company (Gillingham, Dorset, UK).

Assays

Peroxidation of phospholipid liposomes and reaction with trichloromethylperoxyl radical

The ability of the samples to inhibit lipid peroxidation at pH 7.4 was tested using ox-brain phospholipid liposomes prepared as described in Quinlan *et al.* (1988). Peroxidation was started by adding $FeCl_3$/ascorbate (100 μM final concentration of each) to the reaction mixture. Incubations were at 37 °C for 60 min.

At the end of this incubation period, 0.1 ml of 2% (w/v) butylated hydroxytoluene (BHT) was added to each mixture followed by addition of 1 ml each of 1% (w/v) thiobarbituric acid (TBA) and 2.8% (w/v) trichloroacetic acid. The solutions were heated in a water bath at 80 °C for 20 min to develop the adduct. Addition of BHT to the reaction mixtures minimises erroneous increases in colour due to iron ion-dependent hydroperoxide decomposition during the acid heating stage. The $(TBA)_2$-MDA chromogen was extracted into 2 ml butan-1-ol and the extent of peroxidation measured in the organic layer as absorbance at 532 nm.

Reaction with trichloromethylperoxyl radical was conducted using the Linear Accelerator Facility at the Paterson Institute, Christie Hospital, Manchester, UK. Reaction mixtures contained 1% (v/v) CCl_4 and 50% (v/v) propan-2-ol in 10 mM KH_2PO_4–KOH buffer, pH 7.4 and 0.5% (w/v) of the samples of garlic and ginger.

Deoxyribose assay to assess reaction with hydroxyl radical and pro-oxidant action

The deoxyribose assay allows determination of the rate constant for reaction between antioxidants and hydroxyl radicals. This assay has also been adapted to assess

Table 2. Relevant ROS for examining potential antioxidants

In vivo	Food matrix
$O_2^{\cdot-}$ (superoxide radical)	$O_2^{\cdot-}$
singlet O_2	singlet O_2
H_2O_2 (hydrogen peroxide)	H_2O_2
lipid peroxides	lipid peroxides
HOCl (hypochlorous acid)*	—
$RO^{\cdot}$ (alkoxyl radicals)†	$RO^{\cdot}$
$RO_2^{\cdot}$ (peroxyl radicals)†	$RO_2^{\cdot}$
$NO^{\cdot}$ (nitric oxide), $NO_2^{\cdot}$ (nitrogen dioxide), $ONOO^-$ (peroxynitrite)	$NO^{\cdot}$, $NO_2^{\cdot}$, $ONOO^-$ (nitrite preservation)
$OH^{\cdot}$ (hydroxyl radical)	$OH^{\cdot}$

*HOCl is produced by activated phagocytes in the human body. Many bleaches and disinfectants contain its sodium salt (NaOCl, sodium hypochlorite), so it is possible that food constituents might sometimes come into contact with this molecule.

†Radicals formed by the breakdown of lipid hydroperoxides, either thermally (as in heated oils/fats) or catalyzed by transition metal ions (both in foods and *in vivo*).

pro-oxidant actions and was conducted essentially as described in Halliwell *et al.* (1987).

Iron dependent DNA damage to assess pro-oxidant actions

The bleomycin–ferrous ion complex can damage deoxyribose in DNA to form base propenals which can be detected by the thiobarbituric acid test (Gutteridge *et al.*, 1981; Petering *et al.*, 1990). If the added antioxidant can reduce a bleomycin–ferric iron complex, it stimulates DNA damage. The experiment was conducted essentially as described in Aruoma, 1993*b*. Control experiments showed that the garlic and ginger preparations tested did not interfere with measurement of base propenals.

Reactions with hypochlorous acid

Reaction with hypochlorous acid (HOCl) was studied using the elastase assay (Wasil *et al.*, 1987), with some modifications (Aruoma *et al.*, 1992). For the assay, 75 μM HOCl (produced immediately before use by adjusting NaOCl to pH 6.2 with dilute H_2SO_4) and the compounds to be tested were incubated in a final volume of 1.0 ml in phosphate buffered saline (140 mM NaCl, 2.7 mM KCl, 16 mM Na_2HPO_4 and 2.9 mM KH_2PO_4). To the reaction mixture, α_1-antiproteinase (Sigma type A9024) (4 mg/ml) was added. This allowed any HOCl remaining to inactivate α_1AP. After 20 min further incubation, 0.05 ml of 5 mg/ml elastase (Sigma type E0258) was added. Any HOCl remaining was diluted out to the point at which it cannot affect elastase, by addition of 2 ml of phosphate buffered saline (PBS). This mixture was allowed to stand for 30 min to allow any α_1AP still active to inhibit elastase. The elastase activity remaining was measured by adding 0.1 ml of a 5 mg/ml solution of elastase substrate (*N*-succinyl-trialanyl-*p*-nitroanilide), and monitoring increases in absorbance at 410 nm. Control experiments, carried out to test for any direct effects of ginger and garlic upon elastase or α_1-antiproteinase, showed that neither of them interfered with the assay system.

Reaction of HOCl with catalase was studied essentially as described in Aruoma *et al.* (1987). A solution of 83 μM catalase in phosphate-buffered saline (PBS) was incubated (where applicable) with 1.1 mM HOCl for 15 min at 37 °C and/or in the presence of ginger and garlic. Ginger was prepared to a 50 mg/ml concentration in H_2O which was mixed and then sonicated for 15 min. This was then spun at 3200 rpm for 30 min at room temperature to remove major cloudiness. Because some cloudiness remained, each experimental spectrum was run against a control baseline, which contained the volume of the ginger preparation used in the experiment and PBS. Garlic was treated in the same way; there was no need to centrifuge the mixture, however.

Table 3. Inhibition of phospholipid liposome peroxidation

Preparations at respective concentrations tested	Extent of peroxidation as A_{532}	% Inhibition of peroxidation
0	1.540	—
Ginger sample (mg/ml)		
0.10	0.980	36
0.50	0.953	38
1.0	0.810	47
2.0	0.577	63
4.0	0.467	70
5.0	0.404	74
Garlic sample (mg/ml)		
0.5	1.419	8
1.0	1.407	9
2.0	1.265	18
4.0	1.011	34
5.0	0.875	43

Experiments were conducted essentially as described in the Materials and Methods section. Values are the means from duplicates which varied by no more than 10%. Garlic and ginger samples were dissolved in ethanol and used at the final concentration shown.

RESULTS

The object of this paper was to illustrate methods of *in vitro* antioxidant characterization, using commercial preparations of ginger and garlic, such as might be bought by a consumer interested in their health-promoting/antioxidant effects. We also tested for pro-oxidant activity. The data are purely illustrative: it is possible that other commercial garlic/ginger preparations, or even other batches of the same product, might exert different actions.

Inhibition of phospholipid liposome peroxidation and scavenging of trichloromethyl peroxyl radical

Ox-brain phospholipid liposomes undergo rapid non-enzymic peroxidation when incubated in the presence of $FeCl_3$ and ascorbic acid. The effects of ginger and garlic preparations on liposomal peroxidation are shown in Table 3. The ginger sample, tested at a concentration of up to 5 mg/ml, inhibited phospholipid peroxidation. Higher concentrations could not be tested because of interference with the assay used to measure peroxidation. The garlic sample had much less inhibitory effect. None of the compounds stimulated peroxidation under any reaction conditions tested.

Inhibition of lipid peroxidation can occur by several mechanisms (Halliwell, 1990), including chelation of metal ions and scavenging of peroxyl radicals, important intermediates in lipid peroxidation. The latter property was investigated directly using trichloromethyl peroxyl radical ($CCl_3O_2^{\cdot}$), a reactive organic radical frequently used in studies to assess the ability of a compound to react with peroxyl radicals (Aruoma *et al.*, 1992; Alfassi *et al.*, 1993; Lal *et al.*, 1988). $CCl_3O_2^{\cdot}$ was generated by radiolysis of an aqueous mixture of propan-2-ol and CCl_4:

$$e^-aq + CCl_4 \rightarrow {}^{\cdot}CCl_3 + Cl^- \qquad (1)$$

$$OH^{\cdot} + CH_3CHOHCH_3 \rightarrow H_2O + CH_3\dot{C}OHCH_3 \quad (2)$$

$$CH_3\dot{C}OHCH_3 + CCl_4 \rightarrow CH_3COCH_3 + H^+ + \dot{C}Cl_3 + Cl^- \quad (3)$$

$$\dot{C}Cl_3 + O_2 \rightarrow CCl_3O_2^{\cdot} \quad (4)$$

No absorbance changes at any of the wavelengths examined were observed when a 0.5% w/v solution of the garlic sample was included in the reaction mixture, suggesting a slow reaction (if any) with $CCl_3O_2^{\cdot}$. By contrast, a 0.5% w/v solution of the ginger sample did produce small changes in absorbance at 390, 380 and 320 nm, consistent with a reaction taking place. These data are consistent with Table 3, perhaps suggesting that the ginger sample was a better inhibitor of lipid peroxidation because of its greater ability to react with peroxyl radicals.

Reactions with hydroxyl radicals in the deoxyribose assay

Hydroxyl radicals ($OH^{\cdot}$) were generated in a reaction mixture containing ascorbate, H_2O_2 and Fe^{3+}–EDTA at pH 7.4 [eqns (5) and (6)]. The ascorbic acid greatly increases the rate of $OH^{\cdot}$ generation by reducing iron and maintaining a supply of Fe^{2+}–EDTA [eqn (5)]:

$$Fe^{3+}\text{–EDTA} + \text{ascorbate} \rightarrow Fe^{2+} - \text{EDTA} + \text{oxidised ascorbate} \quad (5)$$

$$Fe^{2+}\text{–EDTA} + H_2O_2 \rightarrow Fe^{3+} - \text{EDTA} + OH^{\cdot} + OH^- \quad (6)$$

The generation of $OH^{\cdot}$ in the system is measured by its ability to degrade the sugar deoxyribose into fragments that react on heating with thiobarbituric acid at low pH to form a pink colour, measured at 532 nm.

The ginger and garlic preparations were found to inhibit deoxyribose degradation competitively (Fig. 1), showing that they are scavengers of $OH^{\cdot}$ radicals. Control experiments showed that the compounds did not scavenge H_2O_2 or interfere with the deoxyribose assay at the concentrations shown in Fig. 1 (although they did at higher concentrations). The chemical rate constants for the reaction of ginger and garlic with $OH^{\cdot}$ cannot be calculated because the constituents of these complex preparations active in $OH^{\cdot}$ scavenging are unknown.

The deoxyribose assay also makes it possible to obtain information on the likelihood that molecules could chelate iron ions and stop them from *catalyzing* $OH^{\cdot}$ formation. When iron is added to the assay mixture as $FeCl_3$ instead of as ferric–EDTA, some of the Fe^{3+} ions bind to deoxyribose, and damage to the sugar becomes 'site-specific', i.e. the $OH^{\cdot}$ formed by bound iron ions immediately attacks the deoxyribose (Gutteridge, 1984; Aruoma *et al.*, 1987; Halliwell *et al.*, 1989). The ability of a substance to inhibit deoxyribose degradation under these reaction conditions is a measure of its ability to chelate iron ions and interfere with $OH^{\cdot}$ generation (Gutteridge, 1984, 1987; Aruoma *et al.*, 1988; Aruoma & Halliwell, 1988). The garlic and ginger preparations were effective inhibitors in this system (Fig. 1).

When ascorbate is omitted from the deoxyribose reaction mixture, the ability of added compounds to reduce the Fe^{3+}–EDTA complex [eqn (5)], acting as pro-oxidants, can be tested (Halliwell, 1990; Aruoma *et al.*, 1992; Aruoma, 1994*b*). Both ginger and garlic preparations had weak pro-oxidant action at low concentration, in the presence of EDTA (Table 4), but not in the absence of both EDTA and ascorbate. The pro-oxidant effect was not seen at higher concentrations, perhaps because it is outweighed by the $OH^{\cdot}$-scavenging activity of the extracts.

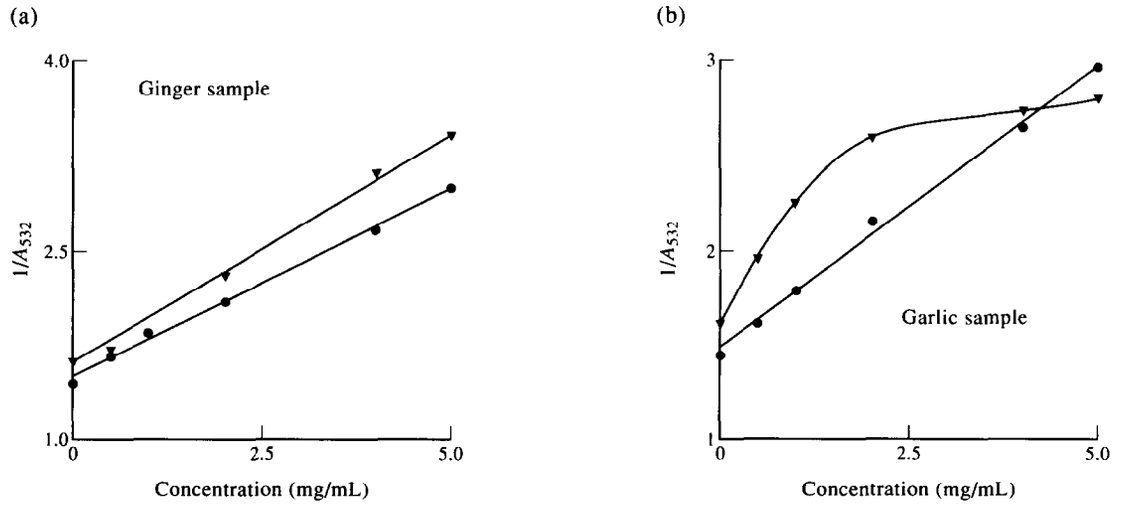

Fig. 1. Scavenging of hydroxyl radical by ginger and garlic preparations. Experiments were conducted essentially as described in Halliwell *et al.* (1987) and in Aruoma (1994*a*). ●: EDTA present; ▼: EDTA absent.

Table 4. Pro-oxidant effects of garlic and ginger on iron-dependent hydroxyl radical generation as measured by the deoxyribose assay

	EDTA present Ascorbate absent	
Additions to reaction mixture mg/ml	A_{532} nm	% stimulation
None	0.169	—
Ginger		
0.5	0.220	30
1.0	0.230	36
2.0	0.257	52
4.0	0.171	1
5.0	0.165	0
Garlic		
0.5	0.252	49
1.0	0.295	74
2.0	0.256	51
4.0	0.154	0
5.0	0.145	0

Experiments were conducted essentially as described by Halliwell *et al.* (1987), except that ascorbate was omitted. All other concentrations quoted were final concentrations in the respective assay mixtures. Values are the means of absorbances from duplicates which varied by less than 10%. The garlic and ginger preparations were used as suspensions in aqueous medium.

Effect of the ginger and garlic samples upon bleomycin–iron mediated DNA damage

Mixtures of bleomycin and ferric ions, in the presence of reducing agents, mediate damage to DNA which can be detected using a TBA assay. It may be seen from Table 5 that the ginger sample promoted DNA damage in the bleomycin–ferric iron system in a concentration dependent manner, but the garlic sample had no effect.

Reaction with hypochlorous acid and effects on the activity of α_1-antiproteinase

Reaction with hypochlorous acid (HOCl) was studied using the elastase assay. Since α_1-antiproteinase is an important inhibitor of serine proteinase enzymes in human body fluids, a good test of the ability of a substance to scavenge HOCl is to examine its action in protecting the elastase-inhibitory capacity of α_1-antiproteinase against inactivation by HOCl (Wasil *et al.*, 1987). The ginger and garlic preparations were found to be powerful scavengers of HOCl (Table 6). This was further demonstrated by the ability of the substances to protect catalase against HOCl attack (Aruoma *et al.*, 1987). Both the ginger and garlic preparations provided protection to the catalase against HOCl, as shown by spectral changes (Fig. 2). The samples did not themselves affect the spectrum of catalase nor did they exhibit catalase-like activity.

DISCUSSION

The premise that oxidative stress plays a role in the pathology of human diseases has provoked the evaluation of 'natural' and 'synthetic' antioxidant compounds for the treatment of the diseases. Dietary 'antioxidants' can exert a number of effects *in vivo*, e.g. promoting increased synthesis of endogenous antioxidant defences or themselves acting directly as antioxidants. Clearly, for any proposed antioxidant to have a physiologically meaningful effect, it must become absorbed and presented to the site of intended action at a concentration that actually exerts an 'antioxidant' effect (Aruoma, 1994*a*; Halliwell, 1990). However, the feasibility of a compound's exerting direct antioxidant effects can be evaluated by *in vitro* tests that investigate how the putative antioxidant can (or cannot) react with biologically-relevant oxygen derived species. In the present study, we have applied established *in vitro* assays to screen the antioxidant/pro-oxidant actions of samples of commercially available ginger powder and garlic (Kwai®). We

Table 5. DNA damage by ferric bleomycin

Compounds added	Concentrations	DNA damage A_{532} nm	
		A	B
None, control		0.002	0.001
Ascorbate*	0.24 (mM)	2.22	2.19
Ginger	0.50 mg/ml	0.278	0.061
	1.0	0.478	0.05
	2.0	0.778	0.129
	4.0	0.916	0.220
	5.0	0.851	0.190
Garlic	0.50 mg/ml	0.021	0.00
	1.0	0.044	0.00
	2.0	0.009	0.00
	4.0	0.005	0.00
	5.0	0.00	0.00

* Ascorbate was used as a positive control.
Experiments were performed as described in the Materials and Methods section. The table shows the extent of DNA damage measured by absorbance changes at 532 nm. The mean values quoted, varied by no more than 10% in three separate experiments. A = compounds used as suspensions in aqueous medium; B = compounds dissolved in absolute ethanol

Table 6. Reactions of garlic and ginger samples with hypochlorous acid and protection of alpha-1-antiproteinase

Addition to reaction mixture	Elastase activity A_{410} nm min^{-1}	Activity of α_1AP in inhibiting elastase (%)
Elastase alone	0.702	—
Elastase + α_1AP (0.4 mg/ml)	0.044	94
Elastase + HOCl (0.12 mM) + α_1AP (0.4 mg/ml)	0.707	0
Elastase + HOCl, + ginger (5.0 mg/ml) + α_1AP	0.025	96
Elastase + HOCl, + garlic (5.0mg/ml) + α_1AP	0.103	85

The experiments were conducted essentially as described in the Materials and Methods section. Values of the A_{410} measurement quoted are the means from duplicate independent experiments with variations of $\leq 5\%$

emphasize that only one batch of each was tested, and our data must not be regarded as valid for other batches or for other commercial preparations. We used these batches only as illustrations of methodology.

The ginger and garlic preparations were good scavengers of $OH^{\cdot}$ and HOCl. However, they could also exert 'pro-oxidant' effects, stimulating $OH^{\cdot}$ generation. Ginger, but not garlic, promoted DNA damage in the

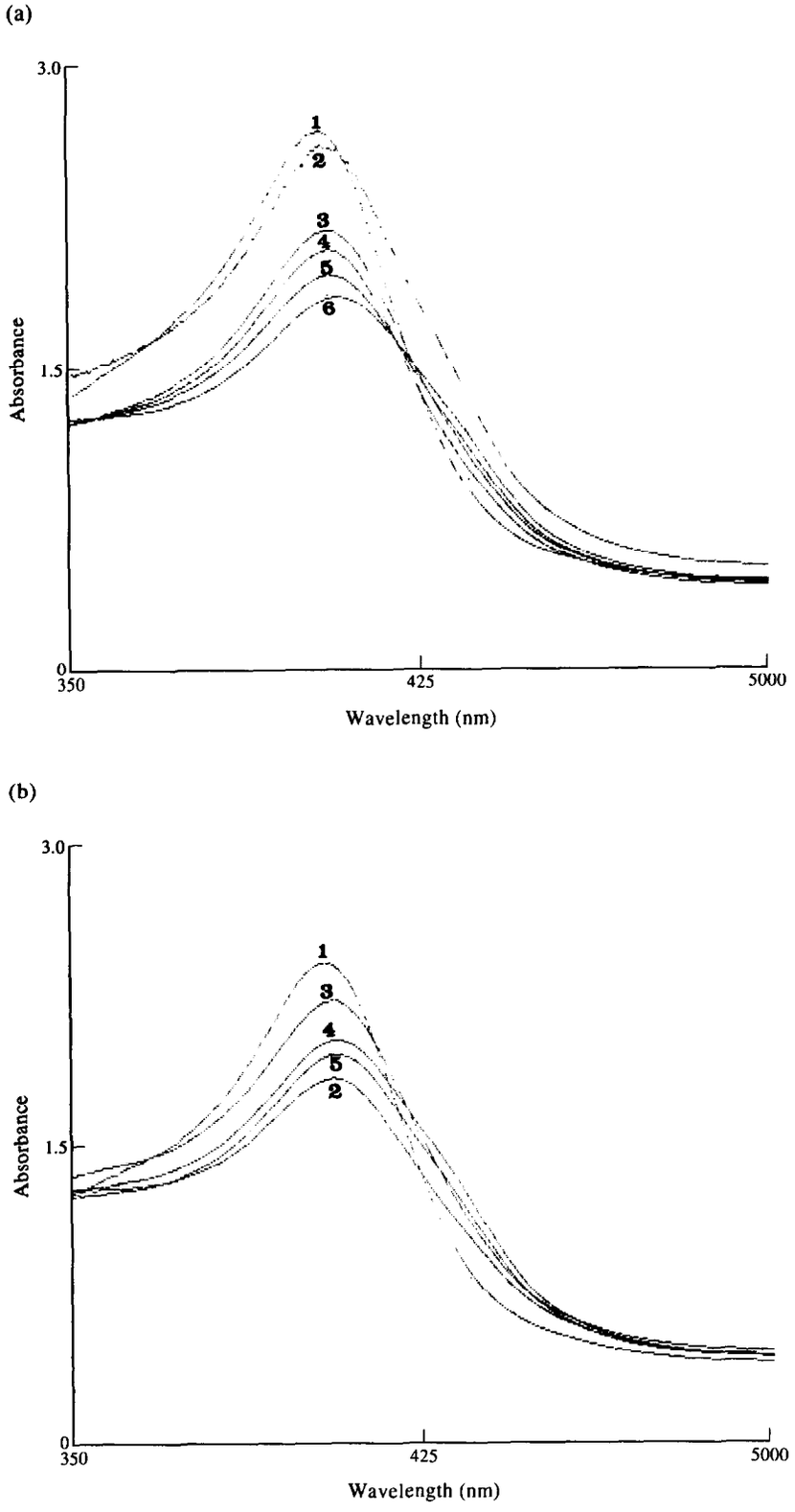

Fig. 2. Protection of catalase by ginger and garlic samples against the action of HOCl. Plot A; Ginger preparation: line 1 18.7 μM catalase; line 2 catalase + 1.6 mM HOCl + 1.67 mg/ml ginger; line 3 Catalase + 1.6 mM HOCl + 0.83 mg/ml ginger; line 4 Catalase + 1.6 mM HOCl + 0.33 mg/ml ginger; line 5 Catalase + 1.6 mM HOCl + 0.167 mg/ml ginger; line 6 Catalase + 1.6 mM HOCl. Plot B: Garlic preparation: line 1 18.7 μM catalase; line 2 Catalase + 2.5 mM HOCl; line 3 Catalase + 2.5 mM HOCl + 5 mg/ml garlic; line 4 Catalase + 2.5 mM HOCl + 3.33 mg/ml garlic; line 5 Catalase + 2.5 mM HOCl + 1.67 mg/ml garlic.

bleomycin–iron system. These 'pro-oxidant' effects presumably involve interaction with iron ions, and their relevance *in vivo* or in the food matrix would depend on the availability of such ions.

The ginger preparation, but not the garlic, reacted with trichloromethylperoxyl radicals. This might explain the ability of the ginger sample to offer greater protection against the peroxidation of phospholipid liposomes accelerated by iron(III)/ascorbate. 6-Gingerol and zingerone are among the active components of ginger powder and Aeschbach *et al.* (1994) showed that the former (but not zingerone) is a powerful inhibitor of phospholipid peroxidation.

Our data show that one sample of one commercially-available garlic and ginger does exert antioxidant effects in some *in vitro* systems, but can be pro-oxidant in others. The activity of the single garlic sample tested needed high concentrations, which might be far in excess of those that could be achieved *in vivo* in consumers of it, perhaps suggesting that this preparation would be unlikely to exert *direct* antioxidant effects *in vivo*. There are of course many factors that would influence the bioavailability of the active constituents (Southgate *et al.*, 1989) and it is possible that other batches/preparations would be more effective or that the compounds could act in other beneficial ways.

What is the biological relevance of 'pro-oxidant' effects? In general, iron ions 'catalytic' for free radical reactions are safely sequestered in the human body. However, they can become available at sites of tissue injury, e.g. in advanced atherosclerotic lesions and so the widespread use of 'natural' antioxidants needs to be approached with caution. The limited pro-oxidant activity of samples tested suggests that this is unlikely to be a significant problem.

As far as the ability of nutrients and drugs to act as antioxidants *in vivo* is concerned, specific assays are being developed to measure rates of oxidative damage to proteins, DNA and lipids (reviewed in Aruoma, 1994*a*, 1996; Halliwell, 1994, 1996). Steady-state and total body oxidative damage to these molecular targets can now be approximated, providing a tool to examine the effects of 'antioxidants' *in vivo*.

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