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The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables

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Abstract

Regular consumption of dietary antioxidants may reduce the risk of several serious diseases. As vegetables are a major source of antioxidants it is desirable to assess their antioxidant activity and compare different processing and preparation methods. The total antioxidant activity was determined in water- and lipid-soluble extracts from fresh, stored and frozen vegetables. The contribution of individual compounds to total antioxidant activity was estimated. In stored vegetables at ambient or chill temperatures antioxidant activity declined. Blanching and freezing of peas and spinach reduced water-soluble antioxidant activity by 30 and 50%, respectively, thereafter levels remained constant on storage at −20 °C. Samples of frozen peas and spinach purchased from retail outlets had substantially higher antioxidant activity than canned or jarred samples. In a comparison of cooking methods, microwave and boiling for short periods had a negligible effect on total antioxidant activity, but substantial losses occurred after prolonged boiling in water.
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Keywords: Antioxidants; Vegetables; Storage; Cooking; Ascorbic acid; Chlorophyll; Flavanoids

Industrial relevance: With the increasing interest in functional foods it is surprising that only a limited amount of information exists regarding the total antioxidative properties of vegetables and the impact of postharvesting technologies. A simple method has been developed capable of being applicable over a wide concentration range and for water and lipid soluble components. The paper offers clear suggestions about the optimum method of heat processing and thus contributes to an overall assessment of optimum means for gentle processing for maximum retention of the nutritional quality of plant foods.

1. Introduction

Epidemiological studies have shown a strong and consistent protective effect of vegetable consumption against the risk of several age-related diseases such as cancer, cardiovascular disease, cataract and macular degeneration (Block, Patterson & Subar, 1992; Hertog, Feskens, Hollmann, Katan & Kromhout, 1993; Jacques, Hartz, Chylack, McGandy & Sadowski, 1988; Steinmetz & Potter, 1996; Law & Morris, 1998; Sarma, Brunner, Evans & Wormald, 1994; Kritchevsky et al., 1998; Varma, Devamanoharan & Morris, 1995). A growing body of evidence suggests that it is compounds with antioxidant activity that play a major role in explaining the benefits of vegetable consumption.

Vegetables and other natural products contain many hundreds of compounds with potential antioxidant activity and it is impractical to quantify all of these individually. Therefore, techniques have been developed that attempt to determine the total antioxidant activity of a food. Although it is not clear whether analytical estimates of total antioxidant activity predict beneficial effects after consumption, these methods do allow a comparison to be made between different vegetables and between different methods of storage, processing and cooking.

Most studies on antioxidants in food have concentrated on the measurement of specific antioxidants, e.g. vitamin C, flavanoids, etc. However, the total antioxidant activity of vegetables has been demonstrated in a few publications (Cao, Sofic & Prior, 1996; Rice-Evans & Miller, 1996; Szeto, Thomlinson & Benzie, 2002), and the results of these studies show that compared with

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other foods, vegetables are a rich source of compounds with antioxidant activity. In a comparison of 22 different vegetable types (Cao et al., 1996) showed that antioxidant activity ranged from 0.5 to 19.4 μm Trolox equivalents·gFW^{-1}. In a further comparison of 17 different vegetable types (Szeto et al., 2002) showed that antioxidant activity ranged from 0.29 to 5.22 μm ascorbate equivalents·gFW^{-1}.

After harvest and before consumption, vegetables may be stored for varying periods of time and may be processed and prepared under a wide variety of conditions. Previous studies of vegetable antioxidant activity have mostly been carried out on extracts of unprocessed and uncooked samples (Cao et al., 1996). Many vegetables are however consumed after prolonged periods of storage and after a variable degree of processing and cooking. It has been observed that the conditions of storage, processing and preparation have very significant effects on the level of ascorbate in vegetables (Favell, 1998).

The main objective of the current study was to make a preliminary assessment on the effects of storage, processing and cooking on vegetable antioxidant activity. In the present study, both water- and lipid-soluble antioxidant activities were determined in extracts of peas, spinach, green beans and carrots. The specific contributions of two potentially important components to total antioxidant activity (ascorbate and non-protein sulfydryl, NPSH) were assessed and the effects of various cooking regimes on the antioxidant activity of peas and spinach were also determined.

A method of assaying for water-soluble antioxidants based on the reduction of the 2,2′-azino*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation (Rice-Evans & Miller, 1994) has been used previously in this laboratory to estimate total antioxidant activity. However, this method was found to be not strictly time- and concentration-dependent for some antioxidants (Schofield & Braganza, 1996) and an alternative method, the FRAP assay (Ferric Reducing Ability of Plasma, Benzie & Strain, 1996) based on the reduction of ferric iron has therefore been developed for use with vegetables extracts. This method was chosen as it is simple to use, gives a linear response over a large concentration range and can be made applicable to both water- and lipid-soluble components. Additionally, it uses equipment commonly available in most laboratories, and can process samples relatively quickly.

2. Materials and methods

2.1. Vegetables

Commercially available samples of fresh, frozen, canned and jarred vegetable products were purchased from supermarkets. In addition antioxidant activities were measured in peas and spinach (strains of the *Avola* and *Ballet* cultivars, respectively) grown at Unilever R&D Colworth (Sharnbrook, Bedfordshire, UK) and sampled at harvest maturity. After harvest, samples of peas and spinach were stored in plastic bags at 20 °C or at 4 °C or were blanched and frozen using an approximation of commercial conditions (peas: 85 s at 97±1 °C, then cooled with cold water for 90 s, frozen at −30 °C for 40 min, spinach: 90 s at 97±1 °C, then cooled with cold water for 90 s, frozen at −30 °C for 40 min). Frozen samples were then stored in plastic bags at −20 °C.

2.2. Cooking regimes

Microwave—225 g of peas and 15 ml water were placed in a Pyrex bowl. The dish was covered with film with two slits and cooked in a commercially-available 750 W microwave oven for 2 min. The peas were stirred and microwaved for a further 2 min then drained and cooled on ice. Frozen spinach (240 g) was cooked for 2.5 min in a covered Pyrex bowl in a commercially-available 750 W microwave oven without additional water. The spinach was stirred and microwaved for a further 2 min then drained and cooled on ice. These cooking methods are the on-pack recommended cooking instructions.

Boiling—225 g of peas were added to 125 ml of boiling water. The water was brought back to the boil and the pan covered. After simmering for 3 min, the peas were drained and cooled on ice. Frozen spinach (220 g) was added to 65 ml of boiling water. The water was brought back to the boil and the pan covered. After simmering for 4.5 min, the spinach was drained and cooled on ice. These cooking methods are the on-pack recommended cooking instructions.

'Overcooked'—225 g of peas were added to 300 ml of boiling water. The water was brought back to the boil and the pan covered. After simmering for 8 min, the peas were then drained and cooled on ice. Frozen spinach (225 g) was added to 225 ml of boiling water. The water was brought back to the boil and the pan covered. After simmering for 15 min, the spinach was drained and cooled on ice.

2.3. Extract preparation for total antioxidant activity

Trichloracetic acid (5%) was added to samples, they were ground in a pestle and mortar at 4 °C and left on ice for 30 min to precipitate protein, then centrifuged (400×*g*, 20 min, room temperature) to remove debris. The supernatant was analysed for water-soluble antioxidant activity. To prepare a lipid-soluble extract, a method based on the extraction of carotenoids from food matrices was used (C. Dacombe, personal communication); 200 mg of solid magnesium carbonate and

10 ml extraction solvent (tetrahydrofuran without butylated hydroxytoluene: methanol, 1:1 v/v) was added to the pellet obtained from the water-soluble extract preparation. The resulting slurry was mixed, centrifuged and the supernatant decanted. The extraction was repeated twice and the supernatants combined. 10 ml of petroleum ether (40–60 °C) and 10 ml 10% (w/v) sodium chloride solution was added and the mixture shaken gently. The top ether phase was removed and the ether extraction repeated twice. The combined ether fractions were dried under nitrogen.

2.4. Water-soluble antioxidant assay

This was based on the method of Benzie & Strain (1996). Briefly, 2.25 ml FRAP reagent (16.67 mM ferric chloride and 8.33 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) in 250 mM acetate buffer, pH 3.6) was added to a test tube at 37 °C, 225 μl water and 75 μl of the supernatant was added and the contents mixed. After incubation for 30 min, the absorbance was measured at 593 nm. This was compared with an 5% trichloracetic acid blank and ascorbic acid standards prepared in 5% trichloracetic acid at concentrations up to 0.25 mM. The absorbance produced from both pea and spinach extraction was dose-dependent up to an absorbance value of 0.9, equal to an ascorbate equivalence value of 0.5 mM (data not shown).

2.5. Lipid-soluble antioxidant assay

The organic residue was redissolved in propanol: acetone (2:1 v/v) containing 1% (v/v) Triton X-100, with the aid of a sonic bath (Ultrasonics Ltd., USA). This extract was then used in the FRAP assay, which was carried out as for the water-soluble antioxidants, except that 1% Triton X-100 is included in the FRAP reagent. The assay was calibrated using ascorbate (prepared in 40 mM hydrochloric acid, but with the addition of the propanol/acetone/Triton X-100 solvent in the assay).

2.6. Ascorbate assays

These were based on the methods of Foyer et al. (1995) and Wimalasiri & Wills (1983). To the sample for assay trichloracetic acid (5%, w/v) was added and the mixture ground in a pestle and mortar at 4 °C, left on ice for 30 min to precipitate protein and centrifuged (21 000 × *g*, 30 min, 4 °C).

2.7. High performance liquid chromatography assay

20 μl of the supernatant (diluted to 50% (v/v) with 2.0 M sodium dihydrogen phosphate) was injected onto a Phenomenex ODS (octadecylsilane) column, (300 × 4.6 mm) and eluted with an isocratic mobile phase of 0.2 M sodium dihydrogen phosphate at a flow rate of 1 ml·min^{-1}. Ascorbate was detected in the eluate using a Gilson UV spectrophotometer set at 254 nm. Ascorbate concentration was determined by comparison with an ascorbate standard prepared in trichloracetic acid at a concentration of 50 mg·ml^{-1} and diluted as for the samples. To determine whether interfering compounds were co-eluting with the ascorbate peak, 130 μl of sodium acetate buffer (4.5 M, pH 6.2) was added to 1 ml of the diluted vegetable lysate, mixed and a Boehringer ascorbate oxidase spatula added. No interfering compounds were detected in the eluate.

2.8. Ascorbate oxidase assay

Supernatant was added to 1.0 M sodium phosphate buffer (pH 6.0) and the absorbance at 265 nm measured, 400 U·ml^{-1} ascorbate oxidase added and mixed. The absorbance at 265 nm was measured again and the decrease calculated. The assay was calibrated using ascorbate standards (0–1 mM) in trichloracetic acid.

2.9. Non-protein sulfydryls

5,5′dithio-*bis*-nitrobenzoic acid (2 mM) in phosphate buffer (1.0 M at pH 7.4) and aliquots of the sample supernatant were mixed, the mixture was left at room temperature for 15 min and the absorbance at 412 nm determined. The absorbance was compared to a range of glutathione standards and the non-protein sulfydryl content expressed as glutathione equivalents. This was then multiplied by 0.079 to give an antioxidant value in ascorbate equivalent.

2.10. Chlorophyll determination

This is based on a published method (Arnon, 1949), 2 g of vegetables were ground in a pestle and mortar in 7 ml of distilled water at 4 °C. Eight millilitres of acetone was then added to 2 ml of the suspension. After mixing and centrifugation, the absorbance of the extracted chlorophyll was measured at 663 and 645 nm.

2.11. Extraction of flavanoid-O-glycosides (FOG)

This is based on a published method (Hempel & Böhm, 1996). Vegetable samples were freeze-dried and ground, 80 ml methanol was added to 1–3 g of sample and the slurry mixed for 15 min at 50 °C. This was then centrifuged (900 × *g*, 10 min, 15 °C). The pellet was re-extracted twice with 30 ml 70% aqueous methanol at 50 °C and the supernatants combined. This was then dried by rotary evaporation, with residual methanol being removed under a stream of nitrogen. The extract was dissolved in water and passed through a 10 cm

polyamide column (pre-swollen in 15% aqueous methanol and washed in 100% methanol, then water). The column was washed with 100 ml water and the flavanoids eluted with 250 ml methanol. The eluate was dried by rotary evaporation to remove the methanol. The residue was redissolved in water, freeze-dried and weighed. The antioxidant activity was determined using a solution of the residue dissolved in methanol and diluted 10-fold in 40 mM aqueous hydrochloric acid.

2.12. Reagents

Ascorbate oxidase spatulas were obtained from Boehringer-Mannheim (Lewes, East Sussex, UK). TPTZ was obtained from the Fluka chemical Company, (Poole, Dorset, UK). All other chemicals were obtained from the Sigma-Aldrich Company Ltd. (Poole, Dorset, UK) and Fisher Scientific (Loughborough, Leicestershire, UK).

2.13. Statistical analyses

Antioxidant activities have been expressed as nanomoles per gram wet weight of tissue to allow for direct comparison between the different molecules. Values in tables are the arithmetical means of determinations ± the S.E. Values in the figures are the arithmetical means of determinations ± the range. Differences were assessed using the Student's '*t*-test', with significance set at $P \leq 0.05$.

3. Results and discussion

3.1. Total antioxidant activity of individual components

The ascorbate equivalents of selected compounds found in plant foods are shown in Table 1.

Table 1
Ascorbate equivalents of antioxidants

Antioxidant	Assay used	Ascorbate equivalents
Gallic acid	Water-soluble	2.92
Chlorophyll *a*	Lipid-soluble	2.11
(+)-Catechin	Water-soluble	1.78
(−)-Epicatechin	Lipid-soluble	1.76
Chlorophyll *b*	Lipid-soluble	1.69
Caffeic acid	Lipid-soluble	1.68
Quercetin	Lipid-soluble	1.54
Ferulic acid	Lipid-soluble	1.24
α-Tocopherol	Lipid-soluble	1.02
L-Ascorbic acid	Water-soluble	1.00
Rutin	Lipid-soluble	0.89
β-Carotene	Lipid-soluble	0.54
L-Cysteine	Water-soluble	0.18
p-Coumaric acid	Lipid-soluble	0.16
Linolenic acid	Lipid-soluble	0.12
Glutathione	Water-soluble	0.079
L-Tyrosine	Water-soluble	0.046
Naringin	Water-soluble	0.033
L-Tryptophan	Water-soluble	0.015
Putrescine	Water-soluble	0.005
Spermine	Water-soluble	0.005
Spermidine	Water-soluble	0.004
D-Fructose	Water-soluble	0.000
D-Glucose	Water-soluble	0.000
Sucrose	Water-soluble	0.000

Compounds were dissolved in 40 mM hydrochloric acid or in propanol:acetone (2:1 v/v) containing 1% (v/v) Triton X-100. Antioxidant activity was assayed was described at a pH of 3.6.

3.2. The effects of blanching, freezing and storage on antioxidant activity and ascorbate levels

In spinach the activity of both ambient and chilled samples declined with time of storage. A loss of approximately 50% was caused by blanching and thereafter the activity in frozen samples remained constant (Fig. 1). In peas, the activity of samples stored at 4 and at 20 °C remained approximately constant until 7 days after harvest. A loss of approximately 20% was caused by blanching and thereafter the activity in frozen samples remained constant (Fig. 1). The lipid-soluble antioxidant activity in both peas and spinach (ambient, chilled and frozen) remained stable up to 21 days of storage at either 4 or 20 °C and there was no effect of blanching and freezing (data not shown). In both peas and spinach, the ascorbate content declined on chilled and ambient storage (Fig. 1). In spinach, the ascorbate content was not detectable in ambient samples after 3 days and in chilled samples after 21 days. Although some ascorbate remained in peas stored at ambient and chilled conditions, levels declined to lower than found in frozen peas.

From the present study it is clear that a substantial proportion of the total antioxidant activity of a vegetable may be lost during storage after harvest and that storage at typical chill temperature (4 °C) may reduce the rate of loss. A further large proportion of antioxidant activity (particularly water-soluble activity) may also be lost when vegetables are commercially processed by canning or jarring. In both processes vegetables are usually heated to high temperatures in water (typically 95 to 121 °C for several min), potentially causing thermal decomposition of antioxidants and leaching of water-soluble antioxidants into the liquor. In comparison, the losses caused by blanching and freezing were relatively small, particularly in peas. Similarly, losses of ascorbate have been shown to be significantly higher in canned and jarred vegetables compared with frozen vegetables (Favell, 1998).

3.3. Comparison of frozen and canned vegetables

The total antioxidant activity of a range of different

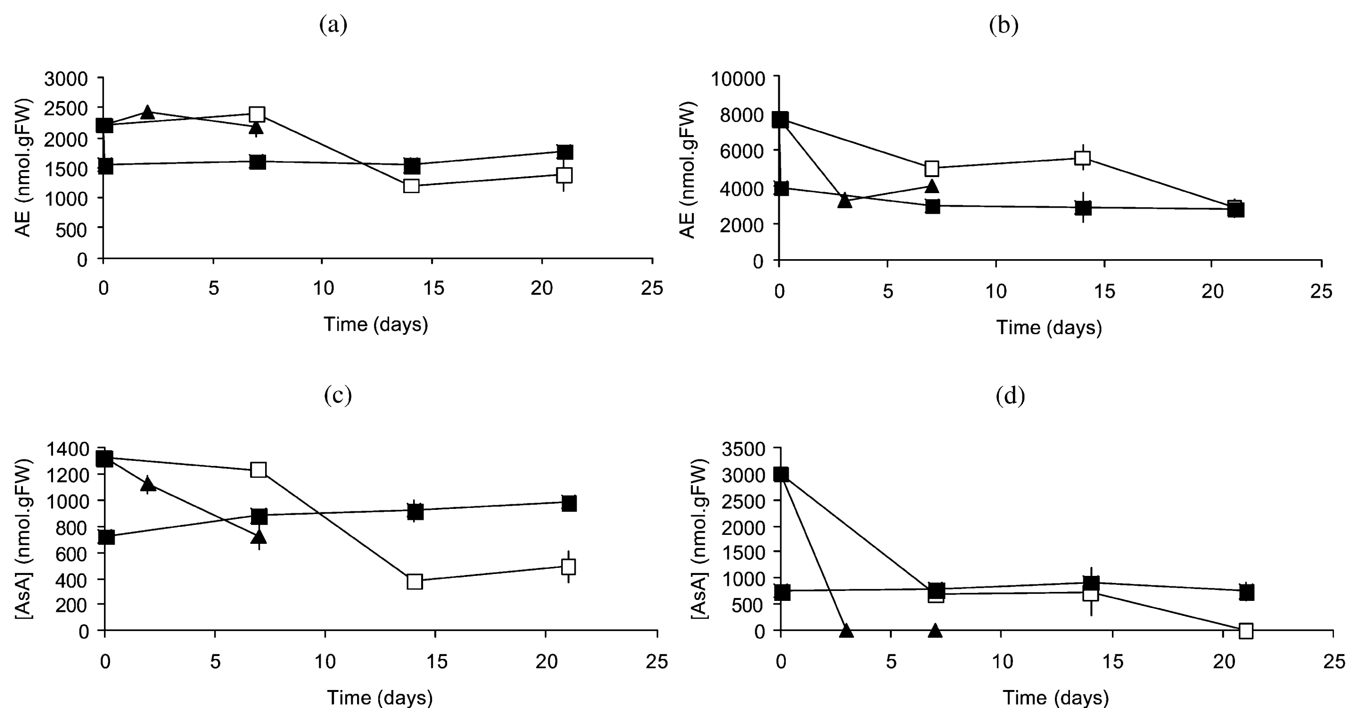

Fig. 1. The effect of storage on the total antioxidant activity and ascorbic acid content of peas and spinach. (a) Antioxidant activity of stored peas (b), antioxidant activity of stored spinach (c), ascorbic acid content of stored peas and (d) ascorbic acid content of stored spinach. Antioxidant activity in (a) and (b) is the sum of the water- and lipid-soluble activities. In all figures, triangles = ambient storage, open squares = chilled storage and closed squares = frozen storage. Also, zero is the time at which the vegetables were harvested and/or blanched. The decrease immediately following time zero in the frozen samples is the reduction in activity and content due to blanching and freezing. Data were not collected for vegetables stored at ambient temperature after 7 days due to autolysis in the tissue making the vegetable completely inedible. Values are the means of two samples, error bars are equivalent to the range. AE = Ascorbate Equivalents, AsA = ascorbic acid.

vegetables (purchased from supermarkets) is shown in Table 2. Leaf spinach showed the highest total antioxidant activity, followed by chopped spinach. Peas were also high in antioxidant activity, with less activity observed in green beans and carrots. Carrots and canned spinach had a relatively high lipid-soluble antioxidant activity, compared with their total activities. Approximately half of the activity in the individual vegetables was accounted for by ascorbate. Spinach contained the highest level of unknown water-soluble antioxidants.

Table 2
The total antioxidant activity and composition of vegetables

Vegetable	Total water-soluble antioxidant activity (ascorbate equivalents) ($nmol \cdot g^{-1}$)	Ascorbic acid ($nmol \cdot g^{-1}$)	Non-protein sulfydryl antioxidant activity (ascorbate equivalents) ($nmol \cdot g^{-1}$)	Unknown water-soluble antioxidant activity (ascorbate equivalents) ($nmol \cdot g^{-1}$)	Total lipid-soluble antioxidant activity (ascorbate equivalents) ($nmol \cdot g^{-1}$)
Canned peas	910 ± 30 (13)	268 ± 28 (13)	11 ± 2 (13)	631 ± 26 (13)	120 ± 18 (3)
Jarred peas	926 ± 11 (3)	405 ± 29 (3)	20 ± 2 (3)	501 ± 39 (3)	138 ± 7 (3)
Frozen peas	1749 ± 131 (7)	1102 ± 77 (7)	42 ± 3 (7)	605 ± 147 (7)	129 ± 7 (3)
Fresh peas	2225 ± 132 (12)	1041 ± 69 (12)	63 ± 4 (12)	1121 ± 132 (12)	152 ± 14 (3)
Canned spinach	1832 ± 107 (13)	162 ± 39 (13)	18 ± 2 (13)	1652 ± 124 (13)	1035 ± 141 (3)
Frozen chopped spinach	3673 ± 61 (3)	1214 ± 43 (3)	45 ± 6 (3)	2414 ± 90 (3)	641 ± 68 (3)
Frozen leaf spinach	5367 ± 877 (3)	1990 ± 320 (3)	62 ± 12 (3)	3315 ± 578 (3)	790 ± 138 (3)
Fresh spinach	7020 ± 745 (15)	1642 ± 367 (15)	73 ± 6 (15)	5306 ± 480 (15)	457 ± 52 (5)
Frozen green beans	1233 ± 82 (4)	718 ± 124 (4)	21 ± 1 (4)	495 ± 164 (4)	143 ± 19 (2)
Frozen carrots	585 ± 84 (3)	291 ± 47 (3)	27 ± 7 (3)	267 ± 30 (3)	297 ± 14 (3)

Samples were processed as detailed in 'Section 2', and the results expressed in terms of ascorbate equivalents. Values are expressed as the mean in $nmol \cdot g^{-1}$ fresh (or frozen) weight ± the standard error. Numbers in parentheses = number of samples assayed.

When antioxidant activities were ranked for different processing methods for peas, they appeared in the order of fresh > frozen > canned ≡ jarred, the differences being statistically significant. For spinach the ranking of total antioxidant activity was fresh ≥ frozen leaf > frozen chopped > canned, the differences (apart from fresh and frozen leaf) were statistically significant.

The ascorbate concentrations of peas were ranked in the order of frozen ≥ fresh > jarred > canned, the differences being statistically significant. In spinach, the rankings were frozen leaf ≥ fresh > frozen chopped > canned, again with the differences being statistically significant. From the total antioxidant assay and measurement of ascorbate content, it is evident that this vitamin forms a major component of the water-soluble antioxidant activity (approx. 63% in frozen peas and approx. 37% in frozen leaf spinach). It is noteworthy that a large proportion of total antioxidant is made up of unknown compounds, particularly in spinach.

Within a plant tissue there are likely to be many thousands of compounds with antioxidant activity that may have potential health benefits for consumers of vegetables. Partial characterisation of this activity was attempted for peas and spinach. The contribution of ascorbate to the total water-soluble antioxidant activity of peas was relatively large, whereas its contribution in spinach was smaller (47 and 23% in fresh peas and spinach, respectively). In both peas and spinach a large proportion of the water-soluble antioxidant activity could not be attributed to ascorbate or non-protein sulfydryl compounds (50 and 76% in fresh peas and spinach, respectively).

3.4. Chlorophyll content

The chlorophyll content of supermarket frozen peas was 261 ± 11 nmol·g^{-1} (chlorophyll *a*) and 50 ± 2.2 nmol·g^{-1} (chlorophyll *b*). In frozen spinach, there was 2124 ± 249 nmol·g^{-1} and 218 ± 41 nmol·g^{-1} of chlorophyll *a* and *b*, respectively. These figures can be expressed as ascorbate equivalents as the AE of pure chlorophyll *a* and *b* are 2.11 and 1.69, respectively. However, when pure chlorophylls were dissolved in 5% TCA, the value for chlorophyll *a* decreased to 1.00, whereas the value for chlorophyll *b* remained unchanged. As chlorophyll is extracted in TCA for the antioxidant assay, the ascorbate equivalents were assessed using the latter figures, producing values of 124 ± 5.4 AE nmol·g^{-1} (chlorophyll *a*) and 84.2 ± 3.7 AE nmol·g^{-1} (chlorophyll *b*) for peas and 1005 ± 118 AE nmol·g^{-1} (chlorophyll *a*) and 368 ± 69 AE nmol·g^{-1} (chlorophyll *b*) in spinach.

Although chlorophyll is not considered to be a human dietary antioxidant, it was expected that the chlorophyll from peas and spinach would be a contributor to the in vitro antioxidant activity. It was determined in the study that the antioxidant activity of chlorophyll *a* was reduced in the presence of acid (TCA), although that of chlorophyll *b* was unaffected. If this is taken into account, the total contribution of chlorophyll in the assay would be 208 AE nmol·g^{-1} in frozen peas (34% of the unknown water-soluble activity) and 1373 AE nmol·g^{-1} in frozen spinach (41% of the unknown water-soluble activity). Although these figure account for a significant proportion of the antioxidant activity of these vegetables in vitro, their contribution in vivo would be expected to be small, as the antioxidant activity is completely destroyed by 1.0 M hydrochloric acid, simulating the conditions in the human stomach (data not shown).

3.5. Flavanoids

Measurements of total polyphenols were only made in spinach, as flavanoids have not been detected in significant quantities in peas. 6.07 mg of total polyphenols were extracted from 1 g of frozen spinach and gave an antioxidant activity of 0.783 mmol·g^{-1} of solid, equivalent to 4753 nmol·g^{-1} of spinach tissue.

It is known that 1 g of spinach contains approximately 0.150 mg of flavanoid-*O*-glycosides and 0.45 mg of hydroxycinnamate esters (Amitori, Komori & Kawasaki, 1986; Herrmann, 1989) and these compounds would form at least 10% of the 6 mg of polyphenols extracted from frozen spinach. Given that the unknown water-soluble antioxidant value for frozen spinach is 3315 ± 578 nmol·g^{-1} and the total polyphenol value is 4753 nmol·g^{-1}, this would suggest these compounds could make up all of the unknown activity, within experimental error. However, as the form (aglycone or glycoside) in which flavanoids are absorbed by the human body is still debated, their contribution to the antioxidant activity in vivo cannot be determined. However, spinach flavanoid-*O*-glycosides have been characterised and shown to have antioxidant activity in vitro (Bergman, Varshavsky, Gottlieb & Grossman, 2001). There is now growing evidence for the correlation between dietary flavanoids and the reduction of serious diseases, such as coronary heart disease (Hertog et al., 1993, Hirvonen et al., 2001), cardiovascular disease (Yochum, Kushi, Meyer & Folsom, 1999) and lung cancer (le Marchand, Murphy, Hankin, Wilkens & Kolonel, 2000; Knekt et al., 1997). Therefore, a diet rich in flavanoids could be advantageous to health.

3.6. Other antioxidants

The polyamines are also relatively abundant (1000 nmol·g^{-1}) in spinach (Kotzabasis, Fotinou, Roubelakis-Angelakis & Ghanotakis, 1993) and are known to act as antioxidants in some in vitro systems (Løvaas, 1991). However, as coumaric acid and the polyamines have AE values of 0.156 and 0.005, respectively, these com-

Table 3
The effect of cooking on the total antioxidant activity and composition of frozen vegetables (frozen peas and frozen chopped spinach)

	Total water-soluble antioxidant activity (ascorbate equivalents) ($nmol \cdot g^{-1}$)	Ascorbic acid ($nmol \cdot g^{-1}$)	Non-protein sulfydryl antioxidant activity (ascorbate equivalents) ($nmol \cdot g^{-1}$)	Unknown water-soluble antioxidant activity (ascorbate equivalents) ($nmol \cdot g^{-1}$)	Total lipid-soluble antioxidant activity (ascorbate equivalents) ($nmol \cdot g^{-1}$)
Uncooked peas	1712	1062	603	47	115
Microwaved peas	1758	927	788	43	109
Boiled peas	1462	645	771	46	126
Overcooked peas	1129	410	678	41	123
Uncooked spinach	3411	600	2756	55	553
Microwaved spinach	3565	633	2871	61	691
Boiled spinach	3400	451	2877	72	706
Overcooked spinach	2039	159	1843	37	832

Samples were processed as detailed in 'Section 2', and the results expressed in terms of ascorbate equivalents. See 'Section 2' for details of cooking protocols. Values are expressed as the mean in $nmol \cdot g^{-1}$ fresh (or frozen) weight.

pounds would not be expected to contribute significantly to the water-soluble antioxidant activity. Phenolic compounds other than flavanoids will also contribute to antioxidant activity, albeit to a lesser degree. Some 4-hydroxyphenylacetic acid was detected in spinach (Guo, Cao, Sofic & Prior, 1997) and the amino acid tyrosine contains a phenolic moiety and will act as an antioxidant. Most of the tyrosine will be sequestered in proteins and will not contribute to the assay, and this together with its low ascorbate equivalent value means that it will not make a large contribution to the total antioxidant activity. The same arguments also apply to the indole-containing amino acid tryptophan. Several vitamins other than A, C and E can act as antioxidants. Riboflavin can be thought of as a reducing agent, but its low concentration in plants and its low ascorbate equivalent activity means that it would not significantly affect the total antioxidant activity. The same arguments apply to ubiquinone and vitamin K. The sugars common in plants (sucrose, glucose and fructose) do not act as antioxidants. Unsaturated fatty acids such as linoleic and linolenic acids will act as antioxidants because of their double bonds, and they are found in relatively high concentration in plants (8700 and 1800 $nmol \cdot g^{-1}$, in peas, respectively, and 990 and 4800 $nmol \cdot g^{-1}$, in spinach, respectively, Souci, Fachman & Kraut, 1989). These acids could make a significant contribution to the total activity if (a) they are active as glycerides and (b) can form sufficient micelles to act in water-soluble assay. However, as negligible antioxidant activity was detected in chloroform extracts of the TCA extracts of the vegetables, these explanations are unlikely.

3.7. The effects of cooking on antioxidant activity

Cooking peas by microwaving caused no significant losses of water- or lipid-soluble antioxidant activities or ascorbate (Table 3). Cooking by boiling produced only a small loss of water- and lipid-soluble antioxidant activities, but a 39% loss of ascorbate. Overcooking caused losses in water-soluble antioxidant activity and ascorbate of 34 and 61%, respectively. NPSH and unknown antioxidant levels were unaffected by cooking. Cooking spinach by microwaving resulted in no loss of water- or lipid-soluble antioxidant activities and no loss of ascorbate (Table 3). Although a slight increase in all of the known water-soluble antioxidant activities was observed (4–6%) this is unlikely to be statistically significant. A larger increase was observed in the small lipid-soluble antioxidant activity (25%); this could be due to the additional disruption of the cellular structure releasing more carotenoid compounds (Castenmiller, West, Linssen, van het Hof & Voragen, 1999). The boiling method did not significantly reduce the water- or lipid-soluble antioxidant activities, but caused a 25% loss of ascorbate. Overcooking caused losses of 40, 33 and 74% in water-soluble antioxidant activities, unknown water-soluble antioxidant activity and ascorbate, respectively. NPSH were unaffected by any method of cooking.

4. Conclusions

Compared with most foods, vegetables contain a very high total antioxidant activity. This is probably highly significant in explaining the beneficial effects of vegetable consumption that has been observed in many epidemiological studies. In measuring the total antioxidant activity in vegetables processed under different conditions, we have demonstrated that frozen vegetables have similar antioxidant activities to the equivalent vegetables purchased fresh from supermarkets, and much higher levels compared with canned and jarred vegetables. As expected from previous publications, antioxi-

dant activity is lost on storage of fresh vegetables after harvest, however, appropriate cooking methods retain total antioxidant activity, although overcooking may result in substantial losses.

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