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Antioxidant activity of asparagus

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A report prepared for the
New Zealand Asparagus Council

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1 Executive summary

This report describes the results of an initial project undertaken for the New Zealand Asparagus Council to investigate the health benefits of asparagus (*Asparagus* spp.). The first part of the report is a review of the scientific literature on the health benefits of asparagus. Different parts of asparagus (shoots, storage roots and seeds) have been identified as having antioxidant, antitumour, antifungal, antimutagenic, diuretic, cytotoxic, antiviral and molluscicide properties. Chemical constituents that are present in asparagus with potential health benefits include: flavonoids, oligosaccharides (in particular fructans), amino acid derivatives, sulfur-containing amino acids and saponins. Of these, flavonoids, fructans and saponins are discussed in more detail in this report.

Asparagus has very strong antioxidant activity compared to most other common vegetables.

The second part of this report details the results of a series of experiments to investigate the levels and activity of antioxidants present in New Zealand-grown asparagus (*Asparagus officinalis*). Six cultivars or breeding lines were examined, and comparisons were also made between fresh and canned asparagus. The average contents of antioxidant components for asparagus were:

- **phenolics** (determined by HPLC): 103 mg/100 g FW for fresh asparagus and 47 mg/100 g FW for canned asparagus,
- **carotenoids**: 0.98 mg/100 g FW for fresh asparagus and 0.54 mg GAE/100 g FW for canned asparagus,
- **antioxidant vitamins**: these were not measured as part of this project but typical values, from the New Zealand Food Composition Tables, are 11 mg/100 g FW for vitamin C and 0.16 mg/100 g FW for vitamin E.

Although the antioxidant activity of asparagus is not as high as berry fruit, a single serving of asparagus can provide more antioxidant activity than many supplements on the market that are promoted for their antioxidant activity.

Quantifying "total phenolics" by Folin-Ciocalteu reagent gave an approximate values for total phenolics plus vitamin C. These figures were, on average, 117 mg GAE/100 g FW for fresh asparagus and 60 mg GAE/100 g FW for canned asparagus. The average **antioxidant activity** of fresh asparagus was 664 µmol TEAC/100 g FW and 303 µmol TEAC/100 g for canned asparagus. There were some variations in antioxidant components and activity between the cultivars examined. Compared to other vegetables, asparagus has very strong antioxidant activity; of the vegetables we have examined to date only watercress and red leaf lettuce have had higher activity on an equal weight basis. Asparagus has greater antioxidant activity than other vegetables such as kumara, broccoli and onions, which are known for their antioxidant properties.

Further research is required to substantiate the health benefits of asparagus. This will firstly include seeing how activity varies from season to season and screening further cultivars to see if there are others that have higher antioxidant activity.

These results indicate that there is potential for asparagus to make a significant contribution to the antioxidant activity of the diet. Further research is required to substantiate the health benefits of asparagus and to examine the feasibility of extracting a nutraceutical from waste streams.

2 Background

2.1 Antioxidants

Antioxidants have received a lot of press for their possible role in the prevention of many degenerative diseases. They are a group of compounds that provide protection against the harmful effects of free radicals and other reactive oxidants. Free radicals have been implicated in numerous diseases, including the universal degenerative diseases such as cancer, heart disease and cataracts, and diseases with more specific causes such as Parkinson's disease and pancreatitis. These free radicals have one or more unpaired electrons and can react with a range of biological molecules, which can result in cell damage. They are generated in the body all the time as a by-product of breathing oxygen, exercising and breaking down food for energy. Many environmental factors such as UV exposure from sunlight, smoking, pollution and exposure to some chemicals also produce free radicals.

Consumption of antioxidants may help prevent these diseases because they include free radical scavengers, enzymes that remove reactive oxidants and systems that repair oxidative injury.

Several classes of antioxidants are commonly found in many fruits, vegetables, grains and beverages. These compounds have varying antioxidant activity and are consumed in the diet in quite different quantities.

Antioxidants also have an important role in helping to prevent undesirable changes in foods, such as fats and oils becoming rancid.

Details on the following antioxidants are presented below:

- carotenoids,
- phenolics,
- micronutrients, and
- antioxidant vitamins.

2.1.1 Carotenoids

Carotenoids are the major yellow, orange and red pigments found widely in nature. Common carotenoids include ß-carotene, α-carotene, lycopene (the red compounds present in tomatoes) and lutein. These carotenoids vary in their antioxidant activity, for example lycopene has much stronger activity than ß-carotene. Some carotenoids, such as ß-carotene, also have provitamin A activity (that is they are converted to vitamin A in the human body), but this is not directly related to antioxidant activity. Good sources of carotenoids are dark green vegetables such as broccoli and spinach and yellow, orange and red fruit and vegetables such as apricots, carrots, kumara (sweet potato), peppers and tomatoes.

2.1.2 *Phenolics*

Flavonoids and other phenolics are a diverse group of compounds of which various classes are important antioxidants. These compounds include anthocyanins, flavan-3-ols, flavanones, flavones, flavonols and proanthocyanins. As with the carotenoids, there is considerable variation in the antioxidant activity of the different flavonoid compounds. Various classes of flavonoids are present in most fruit and vegetables but are especially high in apples, blackcurrants, grapes and onions. The anthocyanins are responsible for the red, blue and purple colours of some fruits and vegetables such as apples, red cabbage and berry fruits. They are often present in very large quantities in the skin compared to the flesh, so peeling a fruit can have a large bearing on dietary intake. Beverages such as tea and red wine are also important dietary sources of flavonoids. Flavonoids make a very important contribution to the antioxidant potential of the diet and on a milligram per day basis the intake of flavonoids exceeds that of carotenoids, vitamin E and probably vitamin C, but there is a lack of good data. Some flavonoids may have other modes of action, independent of antioxidant activity, such as anticancer, antimutagenic, immune-stimulating, anti-allergic and antiviral effects and the isoflavonoids have oestrogenic activities. A number of other phenolic compounds, such as phenolic acids, have been identified as having antioxidant activity. These compounds include caffeic, chlorogenic, coumaric and ellagic acids. Phenolic acids are present in virtually all edible parts of fruit and vegetables, and compounds with strong antioxidant activity are especially high in berry fruit and grapes.

2.1.3 *Micronutrients*

Dietary intake of some micronutrients (e.g. selenium, zinc and sulfur-containing compounds) can increase levels of enzymes involved in free radical scavenging and the repair of oxidative injury. Good sources of these micronutrients are wholegrain cereals, onions, garlic and mushrooms. The levels of micronutrients in crops can be related to soil content, for example New Zealand soils are low in selenium and hence produce grown here is also lower in selenium. The sulfur compounds present in the Alliums onions and garlic have been reported to have various health benefits, which may be independent of antioxidant activity.

2.1.4 *Antioxidant vitamins*

Ascorbic acid (vitamin C) is an essential water soluble vitamin, with antioxidant and immune stimulating properties, that is not manufactured in the human body. Cigarette smoking and high alcohol intake can deplete levels of vitamin C in the body. Vitamin C is high in blackcurrants, citrus, kiwifruit, leafy greens and broccoli. The other major antioxidant vitamin is vitamin E, which usually refers to a mixture of biologically active tocopherols. These are lipid soluble and potent inhibitors of lipid peroxidation. Major sources of vitamin E in the diet are cold-pressed vegetable oils, nuts, seeds and wholegrain cereals. Although some antioxidant supplements contain vitamin A it is not an antioxidant (although some carotenoids have provitamin A activity).

2.2 Antioxidant components in asparagus

In terms of antioxidant components, asparagus contains phenolics, carotenoids and vitamin C as well as some micronutrients (although these are not discussed here).

Phenolics: There are a number of reports on the phenolic composition of asparagus although very few quantitative data are available. Compounds present include phenolic acids (Smith & Stanley, 1989), flavonols (Kartnig et al., 1985) and anthocyanins, which are responsible for the purple colour (Mazza & Miniati, 1993). Vinson et al. (1998) reported the total polyphenol content of asparagus as 80.9 mg/100 g FW.

Carotenoids: There are limited data on the quantities and composition of carotenoids in asparagus spears. Carotenoids are important antioxidants but are generally present at much lower concentrations than the phenolics. Asparagus contains approximately 400 µg of ß-carotene equivalents per 100 g FW (which equates to 70 µg/100 g of total vitamin A equivalents). Overseas data give ß-carotene contents in the range 0.43 to 0.7 mg/100 g FW (Gross, 1991).

Antioxidant vitamins: Asparagus is reported as a good source of vitamin C (Visser et al., 1990), although there appears to be considerable variation between cultivars. Values have been reported for New Zealand grown cultivars from 5 to 18 mg /100 g FW (Burlingame et al., 1997; Visser et al., 1990). Vitamin E is not present in many fruit and vegetables and asparagus only contains very low levels (0.16 mg/100 g FW) (Visser et al., 1990).

There have been a few overseas studies on the antioxidant activity of asparagus (see Section 3.1), but no studies in New Zealand. These studies have not been particularly comprehensive in determining all the antioxidant components in asparagus and their relationship to antioxidant activity. This report provides a more detailed investigation of the antioxidant activity of New Zealand grown asparagus.

3 Literature review - the health benefits of asparagus

A review of the scientific literature on health benefits of eating asparagus was conducted. Health benefits have been attributed to shoots, which are the edible parts, the storage roots and seeds. The seeds have been used as coffee substitutes, in diuretic preparations, laxatives, remedies for neuritis and rheumatism, to relieve toothache, to stimulate hair growth and as cancer treatments in Chinese and Indian systems of medicine (Shao et al., 1997). Different parts of asparagus have been identified as having antioxidant (Tsushida et al., 1994; Vinson et al., 1998), antitumour (Ogino et al., 1997), antifungal, antimutagenic, diuretic, cytotoxic, antiviral and molluscicide properties (Shao et al., 1997). Chemical constituents of asparagus with health benefits include: flavonoids, oligosaccharides (in particular fructans), amino acid derivatives, sulfur-containing amino acids and saponins. Some of these are discussed in more detail below, but there is little information on some of the other components present in asparagus.

3.1 Flavonoids and antioxidant activity

In recent years flavonoids, and other phenolics, have received considerable attention due to their strong antioxidant activity, as well as other health benefits. There is strong evidence that a diet rich in fruit and vegetables (and moderate consumption of red wine) offers protection against degenerative diseases of ageing such as cancer and heart disease. The protection that these dietary components provide had been attributed to the various antioxidants contained in them (for background information on antioxidants see Section 2). Although there has been considerable interest in the health benefits of fruit and vegetables, the antioxidants present in asparagus have received comparatively little study. One study from the USA included asparagus (although probably only a single cultivar) and this showed it to rank very highly in terms of quality and quantity of antioxidants. Using the phenol antioxidant index, asparagus ranked fifth out of 23 vegetables on a fresh weight basis (Vinson et al., 1998). The polyphenol content of asparagus was reported as 80.9 mg/100 g. In the only other study found to include asparagus the antioxidant activity of 43 vegetable extracts was measured, using the rate of discoloration of beta-carotene and the oxidation of emulsified linoleic acid as indicators (Tsushida et al., 1994). Thirteen vegetable extracts showed high antioxidant activity and this group included asparagus. A strong correlation (R^2=0.77) was noted between polyphenol content of the extracts and their antioxidant activity. The main antioxidant in asparagus, determined by MS, was rutin, a flavonol glycoside (Tsushida et al., 1994). The only other report of antioxidant activity was from *Asparagus racemosus*, which had potent antioxidant properties *in vitro* in mitochondrial membranes of rat liver (Kamat et al., 2000).

3.2 Saponins

Saponins are a large and widely distributed group of plant substances named from their ability to form strongly foaming, soap-like solutions with water. Many kinds of biological activities of saponins have been reported (Price et al., 1987), a number of which are beneficial, including: cholesterol lowering effects (Lasztity et al., 1998, Price et al., 1987), a role in cancer protection (Steinmetz & Potter, 1996), antiviral activity against HIV *in vitro* (Lasztity et al., 1998) and antimicrobial activity (Grover & Rao, 1988). However, saponins are also classed as antinutrients; they may reduce the availability of some nutrients and cause growth inhibition (Thompson, 1993).

The genus *Asparagus* is cited as medicinally important, due largely to its richness in saponins. A review of phytochemical studies on *Asparagus* species (published up to 1994) has been conducted by Ahmad et al. (1996). Although there are a number of reports on the composition of saponins in asparagus (Ahmad et al., 1996) there are only a few reports on the biological activities of these saponins. Reports include spermicidal activity (Pant et al., 1988) and inhibition of growth of some fungi (Shimoyamada et al., 1990). The crude saponins from the shoots and seeds of *Asparagus officinalis* have been shown to have antitumour activity; they inhibited the growth of human leukaemia cells in culture in a dose and time-dependent manner (Shao et al., 1996, 1997). The 'bottom cut' of white asparagus has been investigated as a potential source of antifungal saponins (Shimoyamada et al., 1990). However, saponins are responsible for a bitter taste, especially in the butt of the spear (Shimoyamada et al., 1996).

3.3 Fructose-containing oligosaccharides (fructans)

Fructans are high molecular weight polysaccharides of fructose units that are linked glycosidically. They have been shown to provide a variety of health benefits for humans and animals and have attracted considerable attention in recent years. About 15% of higher plants and numerous crops store fructans. Fructans are mostly obtained from chicory, but also Jerusalem artichoke, transgenic potato and sugar beet lines. Vegetable crops like onion, leek, asparagus, and sprouts from cereals contain rather high amounts of fructans and may become more important in the near future as there are increased demands for 'functional foods'. They may help to reduce the risk of disorders such as constipation and infectious diarrhoea as well as diseases such as cancer, osteoporosis, cardiovascular disease, obesity, and non-insulin dependent diabetes (Roberfroid & Delzenne, 1998). Fructans may stimulate the growth of specific micro-organisms in the human colon (Ernst & Feldheim, 2000) that have both beneficial and pathogenic potentials (Gibson, 1998). There is now much interest in manipulating the microbiota composition in order to improve health. The prebiotic approach dictates that non-viable food components are specifically fermented in the colon by indigenous bacteria thought to be of positive value, e.g. bifidobacteria, lactobacilli. Any food ingredient that enters the large intestine is a candidate prebiotic. However, to be effective, selectivity of the fermentation is essential. Most current attention and success has been derived using non-digestible oligosaccharides, such as fructans. The fructans present in asparagus meet these prebiotic criteria, but there is limited research on their specific effects.

4 Experimental

4.1 Collection of samples

Four asparagus (*Asparagus officinalis* L.) cultivars were examined (a white cultivar, a purple cultivar, UC 157 and Jersey Giant) along with two Crop & Food Research lines that have shown promise in taste panel assessments (for the purposes of this report named C&F A and C&F B). All samples were taken from Crop & Food Research trials at Lincoln. Asparagus was harvested at six different dates during the growing season with at least six spears sampled per date. Spears of fairly uniform size were selected.

Canned asparagus was purchased from a local supermarket. Two brands were analysed for the purposes of this report. They are referred to as Brands X and Y.

4.2 Preparation of samples

Asparagus was weighed (to obtain fresh weight), freeze-dried and reweighed (to obtain dry weight). Dry weights were fairly consistent between cultivars (varying between 8.6 and 9.7%). For each cultivar/breeding line the samples collected at different dates were pooled and ground to obtain a homogeneous powder.

For each sample approximately 2 g equivalent of fresh weight was weighed out and extracted with two 10 ml portions of 80% acetone. These aliquots were pooled, centrifuged and the supernatant used for subsequent analysis. Triplicate extractions were performed on each sample.

4.3 Quantification of antioxidant components

4.3.1 Total phenolics

Total phenolics were measured in the acetone extracts using Folin-Ciocalteu's reagent (Spanos & Wrolstad, 1990). Some of the acetone extracts had to be concentrated before analysis to bring them into an acceptable absorbance range. Gallic acid was used to prepare a standard curve and results were expressed in milligrams of gallic acid equivalents per gram fresh weight (mg GAE/g FW). Phenolic assays were carried out in duplicate on each sample.

4.3.2 Profile of phenolic compounds

Phenolics were analysed using an HPLC method developed for apples (Lister et al., 1994). A rutin (quercetin-3-rutinoside) standard was used for quantification of flavonols, gallic acid for phenolic acids, catechin for flavan-3-ols and cyanidin-3-rutinoside for the anthocyanins. Total phenolics were also calculated by this method from the sum of all the individual components.

4.3.3 Carotenoids

Carotenoid levels were determined spectrophotometrically in the acetone extracts after partitioning into petroleum ether and saponification, using the method of Knee (1972). For the basis of calculation the extinction coefficient used was that of β-carotene ($E^{1\%}_{1cm} = 2500$).

4.4 Measurement of antioxidant activity

Antioxidant activity was measured using a modified ABTS assay (Miller & Rice-Evans, 1996, 1997). Triplicate assays were performed on each extract and at three different dilutions. The assay system is based on generating a free radical (which is coloured) and the ability of an extract to quench the radical and return it to a non-coloured "harmless" form. This method compares antioxidant activity of the extracts to Trolox, a water-soluble vitamin E analogue. Results are expressed as the amount of Trolox equivalent antioxidant capacity per 100 grams (•mol TEAC/100 g FW), which represents the amount of Trolox (vitamin E) that gives the same response as one gram of the extract.

5 Results and discussion

5.1 Quantification of antioxidant components

5.1.1 *Total phenolics*

The levels of total phenolics present in asparagus are given in Table 1. On average, fresh asparagus had a total phenolic content of 117.4 mg/100 g FW. There was some variation between cultivars with Jersey Giant having the highest level followed closely by the purple cultivar and C&F B. The white cultivar and C&F A had the lowest levels with UC 157 falling in the middle. Ideally, measurements should be taken in another season to determine if these are consistent values for each cultivar or just due to the particular season and growing environment. These factors are known to have influences on phenolic levels. The phenolic contents reported here are higher than those reported by Vinson et al. (1998) of 80.9 mg/100 g FW. However, no details were given of the samples used in this publication apart from the fact that they were being purchased from local supermarkets (Pennsylvania, USA). It is possible that phenolic levels are higher in New Zealand grown produce as we have higher light levels, which are known to induce flavonoid (phenolic) levels.

The canned asparagus had much lower phenolic levels (on average 60 mg/100 g FW) than the fresh asparagus (approximately 50%). Despite the two brands actually coming off the same production line there was considerable variation in the levels between the two. However, only one can was analysed of each brand. The difference may reflect variation in the batch of asparagus used (possibly different cultivars) or the time from canning to analysis. From these limited results it cannot be determined which is more critical. Phenolics are water-soluble compounds so they may leach into the brine. Analysis of the canning liquid may provide an indication if this is the case, but was not carried out as part of this project. Over time, rutin (a major flavonoid present in asparagus – see Section 5.1.2) can precipitate giving a yellowish powder, particularly in reaction with tin ions (Mazza & Miniati, 1993).

One problem with using Folin-Ciocalteu reagent for estimating total phenolics is that it is susceptible to interference from reducing substances such as ascorbic acid. Thus, the "total phenolic" measurements are actually phenolics plus vitamin C. For this reason total phenolics were also quantified by HPLC, using the sum of individual compounds.

Table 1: Levels of phenolics, carotenoids and antioxidant activity in a range of asparagus samples.

Sample description	"Total phenolics"[a] (mg GAE[b]/100 g FW)	Total phenolics[c] (mg/100 g FW)	Carotenoids (mg/100 g FW)	Antioxidant activity (μmol TEAC[d]/100 g FW)
Fresh				
White	109.24	103.62	0.55	606.79
Purple	123.90	100.15	0.62	753.50
UC 157	115.85	112.08	0.93	678.88
Jersey Giant	126.54	100.26	0.93	701.17
C&F A	108.34	94.81	1.45	566.11
C&F B	120.50	104.76	1.39	674.84
Average	117.40	102.61	0.98	663.55
Canned				
Brand X	56.62	42.99	0.37	289.03
Brand Y	63.34	50.11	0.70	316.98
Average	59.98	46.55	0.54	303.01

[a] quantified using Folin-Ciocalteu reagent (so this figure is actually an estimation of phenolics plus vitamin C, see Section 5.1.1).
[b] GAE = gallic acid equivalents (see Section 4.3.1).
[c] quantified by HPLC (see Section 5.1.2).
[d] TEAC = Trolox equivalent antioxidant capacity (see Section 4.4).

5.1.2 Profile and quantification of individual phenolic compounds

HPLC analysis of the phenolic compounds present in asparagus revealed the presence of flavonols, anthocyanins, phenolic acids and flavan-3-ols (see Appendix). The major compound was a flavonol, quercetin-3-rutinoside. There were three flavonols present in much smaller amounts—two other quercetin glycosides and a kaempferol glycoside. From the literature these could be quercetin-3-galactoside, quercetin-3-rhamnoside and kaempferol-3-rutinoside, but these were not confirmed with standards. There has been a report of quercetin-3-glucoside (Kartnig et al., 1985), but we did not detect it. In addition, there were between two and eleven flavonols and flavones present in trace amounts.

In the purple cultivar there were two cyanidin glycosides present. The retention times matched cyanidin-3-glucoside and cyanidin-3-rutinoside (although the spectra were fractionally different). Traces of anthocyanin were also present in all samples apart from the white and canned. Robinson & Robinson (1934) reported that the red pigment of asparagus was a cyanidin diglucoside, but Wann & Thompson (1965) were the first to isolate and characterise the two main pigments as cyanidin-3-glucoside and cyanidin-3,5-diglucoside, possibly acylated with carboxylic acids. Francis (1967) identified cyanidin-3-glucosylrutinoside as the main pigment, followed by cyanidin-3-rutinoside in the reddish bracts of Mary Washington asparagus. Peonidin-3-glucosylrutinoside and peonidin-3-rutinoside were also reported to be present.

In addition to anthocyanins and flavonols, there were significant amounts of flavan-3-ols (catechins) and phenolic acids (up to 11 compounds in total). None of these were identified. There is little information in the literature regarding these compounds in asparagus. HPLC analysis of blanched asparagus gave tentative identification of seven compounds: 4-hydroxybenzoic acid, caffeic acid, vanillic acid, syringic acid, p-coumaric acid, syringaldehyde and ferulic acid (Smith & Stanley, 1989).

The levels of total phenolics as quantified by HPLC are shown in Table 1. The figures are slightly lower than for "total phenolics" as quantified by Folin-Ciocalteu reagent. However, as discussed above, the Folin method is due to interference from vitamin C, and thus the HPLC quantification provides a more accurate estimation of total phenolics.

5.1.3 *Carotenoids*

The levels of carotenoids in asparagus were comparatively low compared to the phenolics (Table 1). There were significant differences between the cultivars; carotenoid levels were generally lower in canned (average of 0.54 mg/100 g FW) than fresh asparagus (average of 0.98 mg/100 g FW). Carotenoids are relatively labile and thus some may have been destroyed during the processing. Unlike the phenolics, there is less chance of carotenoids leaching into the brine as they are not particularly water-soluble.

There is very little information on the complete analysis of carotenoids present in asparagus compared to many other vegetables. In green asparagus the ß-carotene content has been reported to range between 0.43 and 0.7 mg/100 g fresh weight (Gross, 1991). However, the data on which these figure are based are very old and their accuracy may be questionable. Our total carotenoid levels are higher than these values as they account for all carotenoids not just ß-carotene. A total carotene content of 0.85 mg/100 g FW has been reported, with 84% of the carotene fraction cited as being ß-carotene (Beadle & Zscheile, 1942), but non-carotene carotenoids may also be present. ß-carotene has been reported as highest in green asparagus (0.48 mg/100 g fresh wt) with violet and white asparagus containing 0.14 and 0.1 mg/100 g respectively (Dobreanu et al., 1983). Our results show similar trends with the white asparagus having the lowest total carotenoids and the purple also being lower than the green cultivars.

5.1.4 *Vitamin C*

Due to the financial limitations of this project vitamin C analyses were not conducted as part of this project. However, since the initial measure of total phenolics actually includes vitamin C (Section 5.1.1) the difference between this figure and the phenolics quantified by HPLC (Table 1) probably provides some indication of the vitamin C content. The accuracy of this is questionable although it is clear that there are probably significant differences between cultivars. Some data on the vitamin C content of asparagus are available from The New Zealand Food Composition Tables (Burlingame et al., 1997) and Composition of New Zealand Foods - 2. Export fruits and vegetables (Visser et al., 1990). Steamed and drained asparagus (mixed cultivars) contained 5 mg vitamin C/100 g FW while canned and drained (var. altilis) contained 11 mg/100 g FW (Burlingame et al., 1997). It is surprising that the canned asparagus had a higher vitamin C content since a significant proportion of vitamin C is generally destroyed during processing. However, some variation in the vitamin C content between the different cultivars is likely, and could explain differences. This is backed by data giving the vitamin C content of cv. Mary Washington as 18 mg/100 g FW (Visser et al., 1990). However, it is difficult to make a direct comparison as this was an uncooked sample. Cooking generally destroys some vitamin C, but it would not usually be as high as 75% following light steaming.

5.1.5 Comparisons with other vegetables

Table 2 shows some comparisons in antioxidant components between different vegetables. Asparagus has very high phenolic levels, with only red lettuce and kumara being higher. Carotenoid levels are average, although not as high as carotenoid-rich vegetables such as carrots, squash and tomatoes. Asparagus is at the lower end in terms of vitamin C content.

Table 2: Comparisons of antioxidant components in selected fresh vegetables (values for each vegetable represent the average of several cultivars).

Vegetable	"Total phenolics"[a] (mg GAE/100 g FW)	Total carotenoids (mg/100 g FW)	Vitamin C[b] (mg/100 g FW)
Asparagus	117.4	0.98	11
Broccoli	74.1	1.47	110
Carrot	35.6	8.83	7
Cauliflower	35.0	0.24	60
Kumara	123.1	0.54	35
Lettuce – green	24.4	0.83	12
Lettuce – red	182.0	1.90	19
Onion	55.4	0.12	7
Potato	35.0	Trace	9
Squash	35.0	7.13	25
Tomato	25.7	4.34	24

[a] quantified by Folin-Ciocalteu reagent.
[b] most vitamin C data from NZ Food Composition Tables, rest from personal data.

5.2 Measurement of antioxidant activity

Asparagus had very strong antioxidant activity; the extracts had to be diluted considerably to bring them into the assay range. On average, fresh asparagus had antioxidant activity of 664 µmole TEAC/100 g FW. There was some variation between different cultivars (Table 1 and Figure 1). These differences probably relate to differences in levels of antioxidant components (see Section 5.1). It is difficult to make direct comparisons between our results and those reported in the literature (Tsushida et al. 1994; Vinson et al., 1998) as quite different methods of analysis were used. However, these reports come to similar conclusions—that asparagus ranks highly compared to many other vegetables.

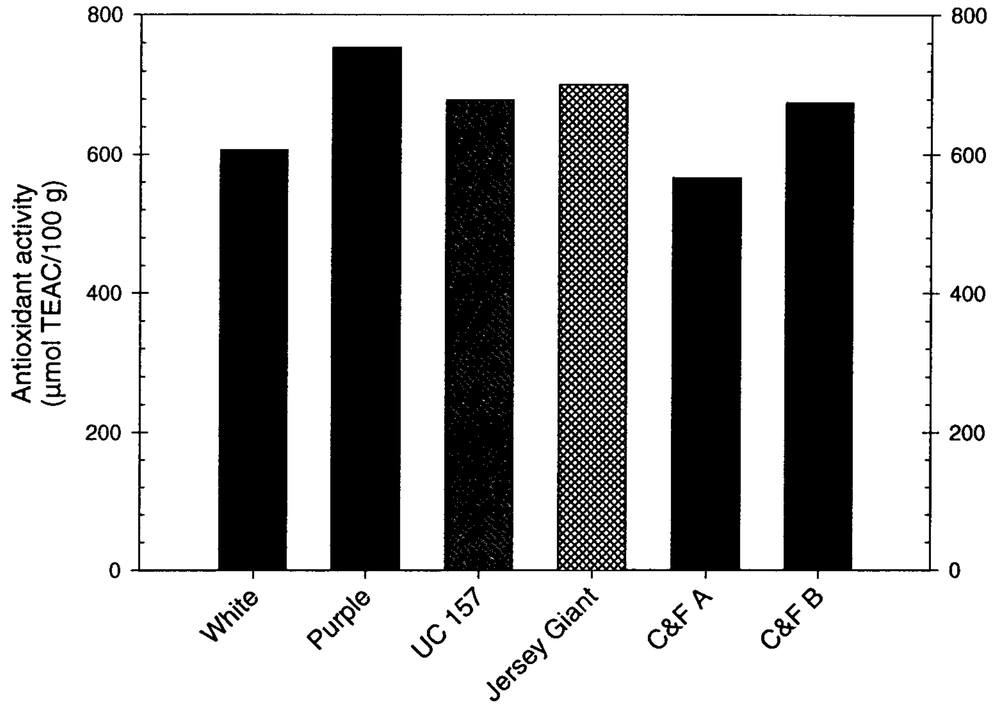

Figure 1: Antioxidant activity of different asparagus cultivars/breeding lines.

5.2.1 *Comparison between fresh and canned asparagus*

There was a small difference in antioxidant activity between the two brands of canned asparagus with Brand X having activity of 289 µmole TEAC/100 g FW and Brand Y having 317 µmole TEAC/100 g FW (Table 1). These differences probably related to the differences in phenolic and carotenoid content for the reasons discussed above. As expected, canned asparagus had much lower antioxidant activity than fresh asparagus (Figure 2). This lower activity is probably due to the lower phenolic and carotenoid contents of canned asparagus compared to fresh (Section 5.1.1, Table 1).

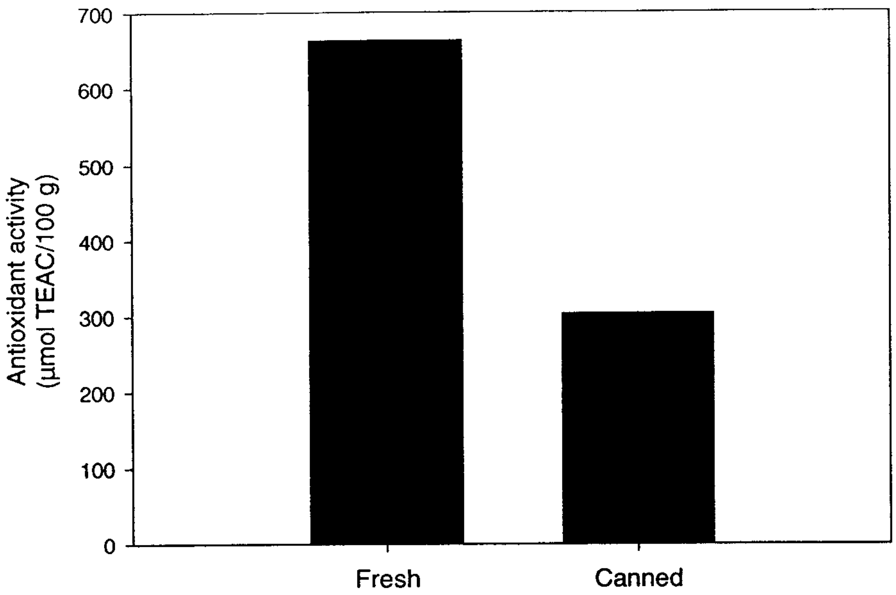

Figure 2: Comparison of antioxidant activity between fresh and canned asparagus. Values represent the average of the samples analysed.

5.2.2 *Contribution of carotenoids and vitamin C to antioxidant activity*

Based on the antioxidant activity of a pure compound (e.g. vitamin C, or •-carotene, one of the most common carotenoids) and knowing the concentration of that compound in the sample the theoretical contribution it makes to antioxidant activity can be calculated. If these calculations are made for the asparagus samples we can determine the relative contribution of different compounds to antioxidant activity. The contribution of carotenoids to the antioxidant activity is extremely small, approximately 4 µmol/100 g for fresh asparagus and 2 µmol/100 g for canned asparagus (which is less than 1% of the total activity in both cases). Using the vitamin C figures from the New Zealand Food Composition Tables the contribution of vitamin C to antioxidant activity is 28 µmol/100 g for fresh (or 100 µmol/100 g using the figure from Visser et al., 1990) and 62 µmol/100 g for canned, that is approximately 4% (or 15% using the figure of Visser) and 20% respectively. However, it is likely that some vitamin C is lost during our sample preparation so these figures may not be exact but they provide some indication of the contribution. It is clear from these data that neither vitamin C nor carotenoids are the major contributors to antioxidant activity of asparagus, as measured by the ABTS assay.

5.2.3 *Relationship between phenolic content and antioxidant activity*

In all samples of asparagus (fresh and canned) there was a useful correlation between phenolic content, as measured by HPLC, and antioxidant activity (adjusted R^2=0.88) (Figure 3a). For this correlation the intercept on the y axis indicates that compounds other than phenolics make a small contribution to antioxidant activity. The relationship is even stronger when phenolic content, as measured by Folin-Cioaclteu reagent, is used (adjusted R^2=0.96) (Figure 3b), since this also accounts for vitamin C. The intercept on the x axis (phenolic content) indicates that not all "phenolics" contribute to antioxidant activity. From these figures it appears that phenolics are the greatest contributor to antioxidant activity in asparagus, but phenolics plus vitamin C are a very good indicator of antioxidant activity.

For the phenolics it is very difficult to calculate a theoretical contribution to antioxidant activity because a diversity of compounds is present, each of which varies considerably in its antioxidant activity. One way to look at the relationship is to calculate the ratio of antioxidant activity to phenolic content. Overall, for asparagus this ratio is 5.5:1, with slightly lower values for the canned asparagus (closer to 5:1) and higher for the purple (6:1). These values are higher than for most other vegetables (average of 3.7:1), although not as high as berryfruit (12.7:1) and cereal grains (11.5:1). There are a number of reasons for the high ratio. Firstly, it may be that the particular compounds present in the asparagus have very high antioxidant activity or that there are more low molecular weight compounds. This is backed up by the HPLC data, which showed rutin as the major phenolic. This compound has very high antioxidant activity compared to most other phenolics. Another explanation is that this ratio may also indicate that there are other compounds present that contribute to antioxidant activity in the asparagus, although these are clearly not the carotenoids or vitamin E (Section 5.2.2). There may be other compounds present in asparagus with antioxidant activity that we have not accounted for. A further explanation is that there are synergies between the particular compounds in asparagus, resulting in a higher antioxidant activity than expected from amounts of individual compounds. Such synergies have recently been shown for compounds present in grape skins and seeds.

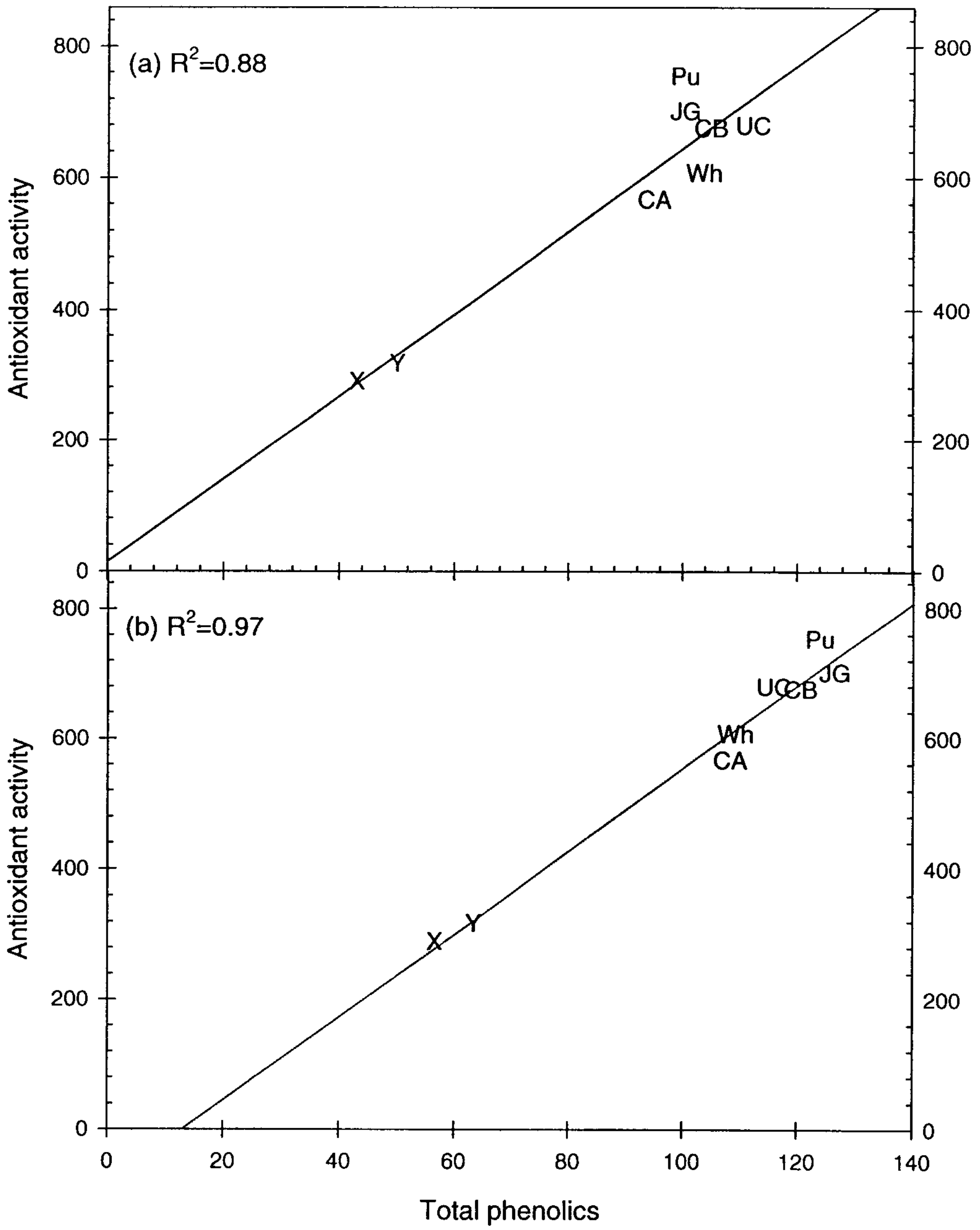

Figure 3: Relationship between phenolic content and antioxidant activity in asparagus: (a) phenolics quantified by HPLC and (b) phenolics as quantified by Folin-Ciocalteu reagent (thus phenolics plus vitamin C). Each letter symbol represents a sample (Wh=white; Pu=purple; UC=UC157; JG=Jersey Giant; CA=Crop & Food selection A; CB=Crop & Food selection B; X=canned, Brand X; Y=canned, Brand Y). The line on each graph is the regression line.

5.2.4 *Comparisons with other vegetables*

Figure 4 shows how asparagus compares with other vegetables grown in New Zealand vegetables in terms of antioxidant activity. Comparisons are given both for an equal weight basis and on serving size (for example in a meal you would not eat the same weight of lettuce as you would potato or kumara). For all the vegetables there was a strong correlation between antioxidant activity and phenolic content. However, there are some slight variations, for example asparagus is similar to onion in that they both have slightly higher antioxidant activity than would be predicted from their total phenolic content. This probably relates to similarities in their phenolic composition—they both have high levels of quercetin glycosides. Kumara has a higher level of total phenolics than asparagus but asparagus has higher antioxidant activity. This is probably due to the fact that kumara mainly contains phenolic acids, which have relatively low antioxidant activity compared to quercetin glycosides.

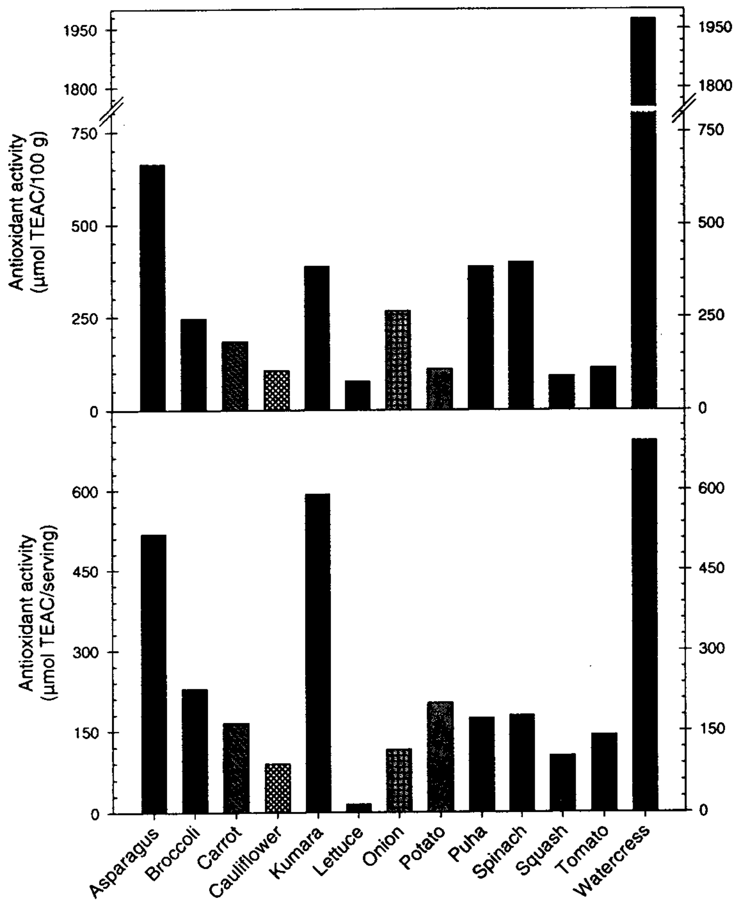

Figure 4: Antioxidant activity of selected fresh vegetables. Top graph shows comparisons on an equal weight basis while the bottom graph shows comparisons on a serving basis.

5.2.5 *Potential of asparagus to deliver health benefits*

There is considerable potential for asparagus to make a contribution to the antioxidant intake in the diet. Table 3 shows the antioxidant activity of various plant-based foods and antioxidant supplements. Although the antioxidant activity of asparagus is not as high as berry fruit, a single serving of asparagus can provide more antioxidant activity than many supplements on the market that are promoted for their antioxidant activity. Figures from the recent National Nutrition Survey show 16% of New Zealanders consume asparagus at least once per week (Russell et al., 1999). Although a considerable proportion of the volume consumed would be canned, even canned asparagus had higher antioxidant activity than many fresh vegetables. There is considerable merit in encouraging asparagus consumption.

Table 3: Typical antioxidant activity of a range of plant-based foods and supplements.

Dietary source			Antioxidant activity (µmol TEAC/100 g[a])
Berry fruit	Blackcurrants		19300
	Blueberries		7750
	Boysenberries		9270
Cereals	Barley		2653
	Maize		694
	Oats		275
	Rye		826
	Wheat		442
Supplements	Selenium ACE		900
	ACE		855
	Antioxidant mix 1		1183
	Antioxidant mix 2		27
	Pine bark		189
	Concentrated fruit extract		436
	Concentrated vegetable extract		75
Vegetables	**Asparagus**	**fresh**	**664**
		canned	**303**
	Broccoli		246
	Carrot		185
	Cauliflower		106
	Kumara		385
	Lettuce – green, heart		77
	Lettuce – red, leaf		986
	Onion		267
	Potato		109
	Puha		384
	Spinach		396
	Squash		90
	Tomato		112
	Watercress		1975

6 Conclusions and future research

Asparagus has very strong antioxidant activity compared to most other common vegetables. This activity is largely due to phenolics, such as rutin, with a smaller contribution made by vitamin C. These results indicate that there is potential for asparagus to make a significant contribution to the antioxidant activity of the diet. Further research is required to substantiate the health benefits of asparagus. This will firstly include seeing how activity varies from season to season and screening further cultivars to see if there are others that have higher antioxidant activity. This may then lead to trials to determine whether asparagus can increase the antioxidant activity of plasma using a small animal model. There may also be potential to extract a nutraceutical from waste streams (e.g. bottom cut discarded from canning process). However, in this project we only looked at the activity in the trimmed spear. We need to measure the activity in the butt end of the spear as it is possible that activity is concentrated in the growing tip.

7 Acknowledgements

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Appendix HPLC traces of phenolic compounds in asparagus

Typical HPLC traces of phenolic compounds in asparagus (example shown is for the purple cultivar):

(a) Detection at 280 nm – shows virtually all phenolics but the only wavelength where flavan-3-ols & proanthocyanins are detected

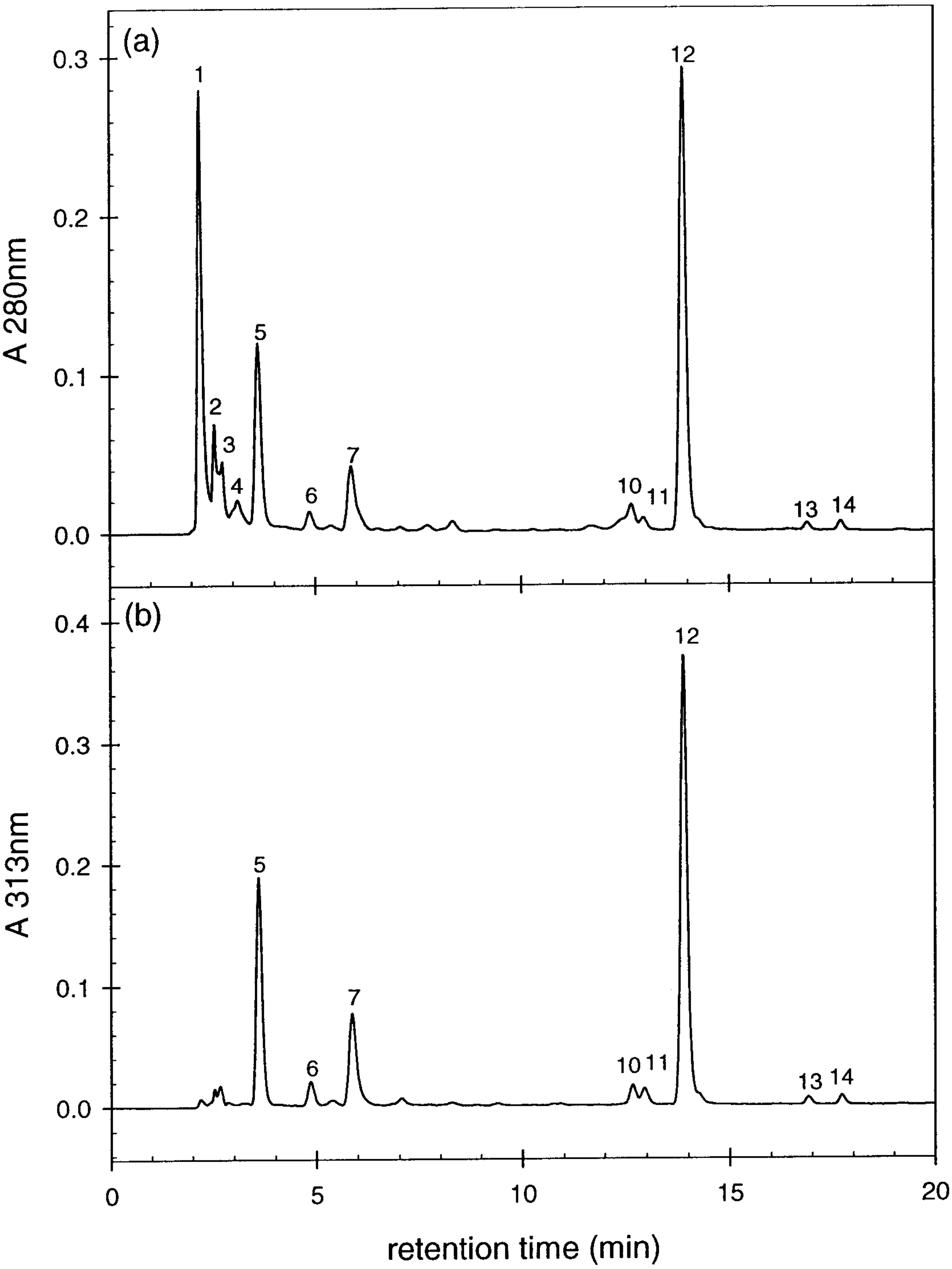
(b) Detection at 313 nm – phenolic acids

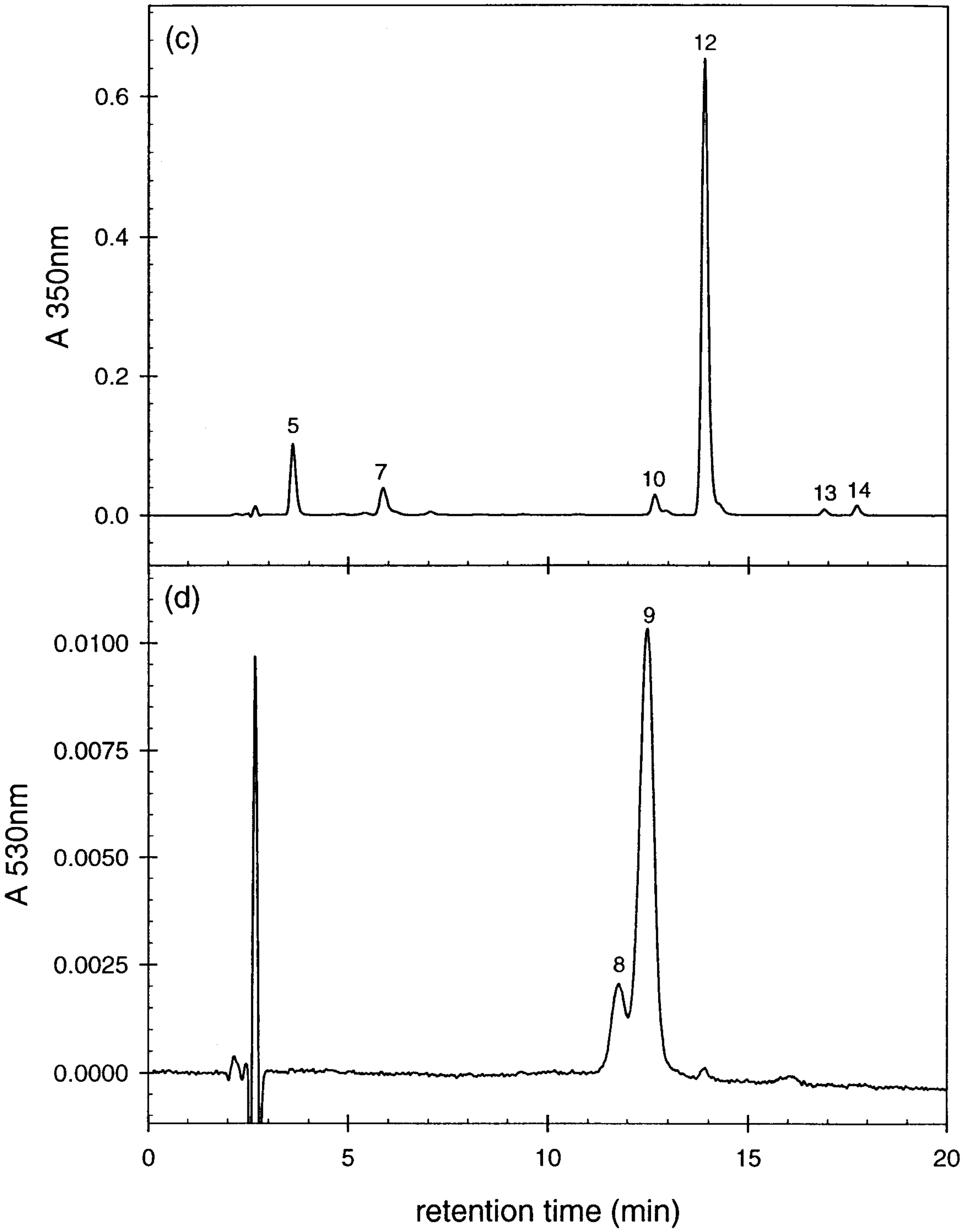
(c) Detection at 350 nm - flavonols,

(d) Detection at 530 nm – anthocyanins

Compounds present:

1-4	Flavan-3-ols & proanthocyanins
5-7	Phenolic acids
8 & 9	Cyanidin glycosides
10	Quercetin glycoside
11	Phenolic acid
12	Rutin (quercetin-3-rutinoside)
13	Kaempferol glycoside
14	Quercetin glycoside


(a)
A 280nm
0.3
0.2
0.1
0.0
1
2
3
4
5
6
7
10
11
12
13
14
(b)
A 313nm
0.4
0.3
0.2
0.1
0.0
5
6
7
10
11
12
13
14
0
5
10
15
20
retention time (min)



(c)
A 350nm
0.6
0.4
0.2
0.0
5
7
10
12
13
14
(d)
A 530nm
0.0100
0.0075
0.0050
0.0025
0.0000
8
9
0
5
10
15
20
retention time (min)