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Health benefits of New Zealand vegetables

A report prepared for the

New Zealand Vegetable and Potato Growers' Federation Inc.

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CROP & FOOD RESEARCH
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1 EXECUTIVE SUMMARY

The general aim of our work is to increase the consumption of fruit and vegetables by providing knowledge of beneficial compounds present in New Zealand-grown produce. The consumption of fruit and vegetables in the diet is associated with a lower disease incidence, particularly degenerative diseases associated with aging (e.g. cancer and heart disease), and improved well-being. There is a vast array of components present in fruit and vegetables with anticancer, antimutagenic, immune-stimulating, anti-allergic and antiviral activities; these compounds are often termed **nutraceuticals**. One of the mechanisms of their protective action is thought to be their antioxidant activity. **Antioxidants** are compounds that provide protection against the harmful effects of free radicals and other reactive oxidants. Vitamin C, vitamin E and ß-carotene (provitamin A) have been regarded as the major nutritional antioxidants for many years. More recently other constituents of fruits and vegetables, for example carotenoids such as lycopene (the red pigment in tomatoes) and phenolics including some flavonoids (e.g. quercetin present in many fruit and vegetables), have been shown to have significant antioxidant activity.

In this project we measured the major nutraceuticals and antioxidant activity in a range of New Zealand-grown vegetables (which are important horticultural crops and/or we have identified as having significant potential for study). The vegetables examined in this study were: broccoli, carrots, cauliflower, garlic, kumara, lettuce, onions, potatoes, squash and tomatoes. Red varieties of lettuce, kumara and potatoes were also examined along with standard cultivars. Antioxidant components quantified were: total carotenoids, total phenolics and vitamin C. Composition of the individual carotenoids and phenolics, including phenolic acids and flavonoid subclasses (anthocyanins, flavones, flavonols, catechins and proanthocyanins), were also examined. Antioxidant activity was measured by two different assay systems: an ABTS assay and the coupled oxidation of ß-carotene and linoleic acid. Vegetables with **very strong** activity in both assays were kumara and red lettuce while broccoli, carrot and onion gave **good** activity in both assays. Heart lettuce, buttercup squash and tomatoes gave good activity in one assay only and the remaining vegetables performed poorly in both assays. The levels of the various classes of antioxidant components were correlated with antioxidant activity, and a strong correlation was found between antioxidant activity and total phenolics. This indicates that phenolics may be one of the most important classes of nutraceuticals in terms of their contribution to antioxidant activity. However, further studies of their metabolism in humans and *in vivo* reaction mechanisms are required before definite conclusions can be drawn.

2 INTRODUCTION

The general aim of our work is to increase the consumption of fruit and vegetables by providing knowledge of beneficial compounds present in New Zealand-grown produce. There is strong evidence that a diet rich in fruit and vegetables (and moderate consumption of red wine) offers protection against degenerative diseases associated with aging, such as cancer and heart disease. The protection that these dietary components provide had been attributed to the various antioxidants they contain. Although there is a huge amount of data on the various classes of antioxidants present in plants there are still many significant gaps in our knowledge. Many values for compounds, such as the flavonoids, in plants are based on food analysis techniques, which are now considered inappropriate. Consequently, these estimations are incorrect. Also, estimations have often been based on the whole food rather than edible or consumed parts. Most work on measuring antioxidant activity has focused on individual compounds (often from the point of view of supplementation) and has not necessarily measured the concentrations of beneficial compounds typically found in fruit and vegetables.

The aim of our current research is to collect data on the levels and composition of antioxidants in New Zealand-grown vegetables and compare our data with measures of the antioxidant activity of these vegetables. Later studies will involve more detailed research to obtain more conclusive medical evidence for the health benefits of particular compounds. From this information we will be able to make better recommendations on the types and amounts of vegetables that should be eaten, which will provide a useful marketing tool for New Zealand's vegetable industry. This type of research also has many benefits for New Zealanders. The high costs of heart disease in New Zealand mean that even a small reduction in the incidence of heart attacks will produce substantial savings to society, let alone the individual.

3 METHODS

3.1 Measurements of nutraceutical components

Vegetables were purchased locally and the most commonly available cultivars were used, although these were not always clearly specified in retail outlets. To eliminate variation between individual vegetables within a cultivar samples were freeze-dried and ground to a fine powder to obtain a homogeneous material. This powder was used for all assays and allowed direct comparisons to be made between results. The exception was lettuce, which did not form a powder after freeze drying but produced tough, fibrous sheets. Compounds of interest were not fully extracted from this material and so fresh samples of lettuce were used. The following vegetable parts were used for analysis: broccoli—florets and stalks minus woody ends; carrots—whole root; cauliflower—florets and stalks; garlic—peeled cloves; kumara—whole with skin on; lettuce—leaves, minus stalk core; onion—peeled bulb; potato—whole with skin on; squash—flesh, skin and seeds removed; tomato—whole. Acetone extracts of each vegetable were prepared for all assays, except measurement of vitamin C, as this solvent proved to be the most efficient for extracting compounds. Methanol, while effective in extracting the phenolics, did not give total extraction of the carotenoids in some vegetables.

3.1.1 Carotenoids

Total carotenoid contents of the vegetables were determined spectrophotometrically after partitioning the acetone extracts into petroleum ether and saponification. HPLC was used to provide an indication of the key carotenoids (standards were available for ß-carotene, lycopene, α-carotene, ß-cryptoxanthin, lutein and zeaxanthin).

3.1.2 Phenolics/flavonoids

Total phenolics were determined in the vegetable extracts using Folin-Ciocalteu's reagent (Spanos & Wrolstad 1990). TLC and HPLC were used to give an indication of the numbers and types of different phenolic compounds present in the vegetables. The diverse spectral properties meant it was difficult to make quantitative calculations without running many standards and identifying individual compounds (which was beyond the scope of this project).

3.1.3 Vitamin C

Vitamin C (L-ascorbic acid) was measured in the vegetables by standard methods.

3.2 Measurement of antioxidant activity

Two different assay systems were used to measure the antioxidant activity of the vegetable extracts. These assay systems have different reaction mechanisms and thus give different measures of antioxidant activity; compounds may behave differently in the two assays. Acetone extracts were also used for these assays as this solvent extracted both carotenoids and phenolics, enabling these compounds to be assayed together to measure total antioxidant activity rather than just certain classes of compound.

3.2.1 ABTS assay

The first measure of antioxidant activity was obtained using a modified ABTS assay (Miller & Rice-Evans 1996). This method compared antioxidant activity of the vegetable extracts with Trolox—a water-soluble vitamin E analogue.

3.2.2 Coupled oxidation of ß-carotene and linoleic acid

Antioxidant activity was also assessed using the coupled oxidation of ß-carotene and linoleic acid (Taga et al. 1984) in the presence of vegetable extracts.

3.2.3 Correlation of nutraceuticals with antioxidant activity

Correlations were made between antioxidant components and antioxidant activity measured by the two assay systems.

4 RESULTS AND DISCUSSION

4.1 Measurements of nutraceutical components

4.1.1 Carotenoids

Carotenoid-rich vegetables were carrot, squash and tomato (Table 1), but they had different compositions of individual carotenoids (Table 2). ß-carotene was the predominant carotenoid in carrots while smaller amounts of α-carotene and lutein were present. In buttercup squash lutein was the major carotenoid, but this vegetable also contained significant quantities of ß-carotene and smaller amounts of α-carotene and violaxanthin. Tomatoes were the only vegetable to contain lycopene. It was the major carotenoid in this vegetable and smaller amounts of ß-carotene and lutein were present. Several other carotenoids were detected in the vegetables, but were present in smaller amounts. Broccoli, kumara and lettuce had moderate levels of carotenoids and would make a significant contribution to the intake of nutraceuticals in the diet. Cauliflower, garlic, onion and potato had very low levels of carotenoids and would not make a contribution to dietary intake. Red-skinned kumara had much lower levels than gold kumara, a result that was predictable because of the much lighter yellow colour of its flesh. Even the gold kumara used in this study had fairly pale flesh and some of the more orange cultivars would undoubtedly have higher carotenoid levels.

Table 1: Concentrations (mg/100 g FW) of antioxidant components in common vegetables.

Vegetable	Total carotenoids	Total phenolics	Vitamin C
Broccoli	1.41	83.1	111.5
Carrot	8.83	40.2	1.0
Cauliflower	0.24	35.0	54.4
Garlic	0.10	9.6	5.5
Kumara - red skin	0.48	154.4	31.3
Kumara - gold	1.64	78.5	45.2
Lettuce - green heart	0.83	24.4	5.3
Lettuce - red leaf	1.90	182.0	9.5
Onion	0.14	66.8	7.1
Potato - Rua	t[1]	38.3	13.4
Potato - Red Desiree	nd[2]	41.8	5.5
Buttercup Squash	7.36	35.0	25.2
Tomato	4.10	28.8	29.6

[1] t trace.

[2] nd not detected.

Table 2: Carotenoid composition.

Vegetable	ß-Carotene	α-Carotene	Lutein	Lycopene
Broccoli	✓		✓✓	
Carrot	✓✓✓✓	✓✓	✓	
Cauliflower	✓		✓	
Garlic	✓			
Kumara - red	✓			
Kumara - gold	✓✓	✓		
Lettuce - green heart	✓		✓	
Lettuce - red leaf	✓✓		✓✓	
Onion	✓			
Potato - Rua	✓			
Squash	✓✓✓	✓	✓✓✓✓	
Tomato	✓✓		✓	✓✓✓✓

✓	< 0.5 mg/100 g FW
✓✓	0.5-1 mg/100 g FW
✓✓✓	1-2 mg/100 g FW
✓✓✓✓	> 2 mg/100 g FW

4.1.2 Phenolics/flavonoids

All vegetables contained phenolics. In many of the vegetables studied phenolics were the predominant compounds, and were often present in very large amounts (Table 1). Red leaf lettuce and red-skinned kumara had very high levels and broccoli, gold kumara and onion also had fairly high levels. The remaining vegetables, except for garlic, had significant levels of phenolics and would make a major contribution to dietary intake. For kumara and lettuce there was a big difference in the levels of total phenolics between the red and standard cultivars; the red cultivars were much higher in phenolics in both cases. However, the two potato cultivars had very similar concentrations of phenolics.

In the vegetables studied phenolics were comprised of phenolic acids and various flavonoid subclasses: anthocyanins, flavones, flavonols, catechins and proanthocyanins (Table 3). Red leaf lettuce contained the greatest diversity of components, with at least 15 compounds present in significant amounts. Kumara had very high levels of phenolic acids and their composition appears to be similar to that of phenolic acids in lettuce and potato. Of the flavonoid subclasses, flavonols were present in many of the vegetables although in relatively smaller amounts than the phenolic acids. Flavonol levels were very high in red leaf lettuce and reasonably high in broccoli, onion and

tomato. Anthocyanins are the compounds usually responsible for the red, blue and purple colours of fruits and vegetables and hence were only present in the three red vegetable cultivars (not tomatoes where the red colour is due to the carotenoid lycopene). Levels were very high in red lettuce but fairly low in red kumara and potato due to the fact that in these latter two vegetables they were only present in the skin, which is only a small proportion of the vegetable weight. The closely related catechins and proanthocyanins were present in red cultivars of kumara, lettuce and potato, but also at reasonable levels in tomato with traces in Rua potato. The flavones were only present in significant amounts in lettuce and were higher in the red leaf lettuce than the green heart lettuce.

Table 3: Presence of phenolic subclasses.

Vegetable	Anthocyanins	Flavones	Flavonols	Catechins and Proanthocyanins	Phenolic acids
Broccoli			✓		✓
Carrot					✓
Cauliflower					✓
Garlic					✓
Kumara - red	✓		✓	✓	✓
Kumara - gold			✓		✓
Lettuce - green heart		✓	✓		✓
Lettuce - red leaf	✓	✓	✓	✓	✓
Onion			✓		✓
Potato - Rua			✓	✓	✓
Potato - Red Desiree	✓		✓	✓	✓
Squash					✓
Tomato			✓	✓	✓

4.1.3 Vitamin C

There was considerable variation between the vegetables in terms of the levels of Vitamin C (Table 1). Broccoli had much higher levels of vitamin C than the other vegetables while cauliflower, kumara, buttercup squash and tomatoes had reasonable levels. The remaining vegetables were relatively low in vitamin C. Once again, there were significant differences in the levels of vitamin C between the red and standard types of kumara, lettuce and potato.

4.2 Measurement of antioxidant activity

4.2.1 ABTS assay

Using this assay the levels of antioxidant activity were in the following order: lettuce (red leaf) > kumara (gold) > kumara (red skin) > onion > broccoli > carrot > potato (red skin) > garlic > potato (Rua) > cauliflower > buttercup squash > tomato > lettuce (green heart). The activity of red lettuce was exceptionally high while kumara (both gold and red) had fairly high activity and broccoli, carrot and onion had moderate activity (Table 4). The other vegetables had fairly low antioxidant activity. In some cases activity of the vegetables was expected to be higher based on vitamin C content. This may indicate that more complex reactions are taking place, e.g. because oxidation reactions are taking place in the plant extracts some of the antioxidant capacity is involved in negating these reactions.

4.2.2 Coupled oxidation of ß-carotene and linoleic acid

This assay measured the bleaching of ß-carotene that results from oxidation reactions involving the degradation products of linoleic acid and the ability of antioxidants to reduce this oxidation reaction. Without antioxidants in the assay system complete decolourisation of ß-carotene occurred after 20-30 minutes. When vegetable extracts containing antioxidants were added decolourisation was inhibited to varying degrees (Fig. 1). From absorbance readings taken at 15 and 30 minutes an average percentage of inhibition was obtained for each vegetable (Table 4); the higher the percentage inhibition the greater the antioxidant activity. Antioxidant activity of the vegetables was in the following order kumara (red skin) > kumara (gold) > lettuce (red leaf) > lettuce (green heart) > broccoli > carrot > onion > tomato > buttercup squash > potato (red skin) > potato (Rua) > garlic > cauliflower. The order of the antioxidant activity of the vegetables is different when measured using this assay than the ABTS assay. These differences probably reflect the different reaction mechanisms and the variation in the composition of nutraceuticals in the vegetables. The shapes of the decolourisation curves varied slightly for some of the vegetables indicating that different reaction mechanisms may be taking place. Vitamin C gave no activity with this assay, even at the maximum concentration used (which was limited by its solubility).

Table 4: Antioxidant activity of vegetables.

Vegetable	ABTS assay (mM/g FW TEAC[1])	ß-Carotene assay (% inhibition)[2]
Broccoli	2.26	33.6
Carrot	1.80	26.8
Cauliflower	0.96	3.3
Garlic	1.04	6.2
Kumara - red	3.75	56.0
Kumara - gold	4.08	50.6
Lettuce - green heart	0.70	36.9
Lettuce - red leaf	8.97	47.3
Onion	2.48	26.2
Potato - Rua	0.99	8.2
Potato - Red Desiree	1.18	16.4
Squash	0.82	23.0
Tomato	0.76	23.7

[1] Trolox-equivalent antioxidant activity.

[2] Compared to control (no extract) which was 0% (complete decolourisation after 30 minutes).

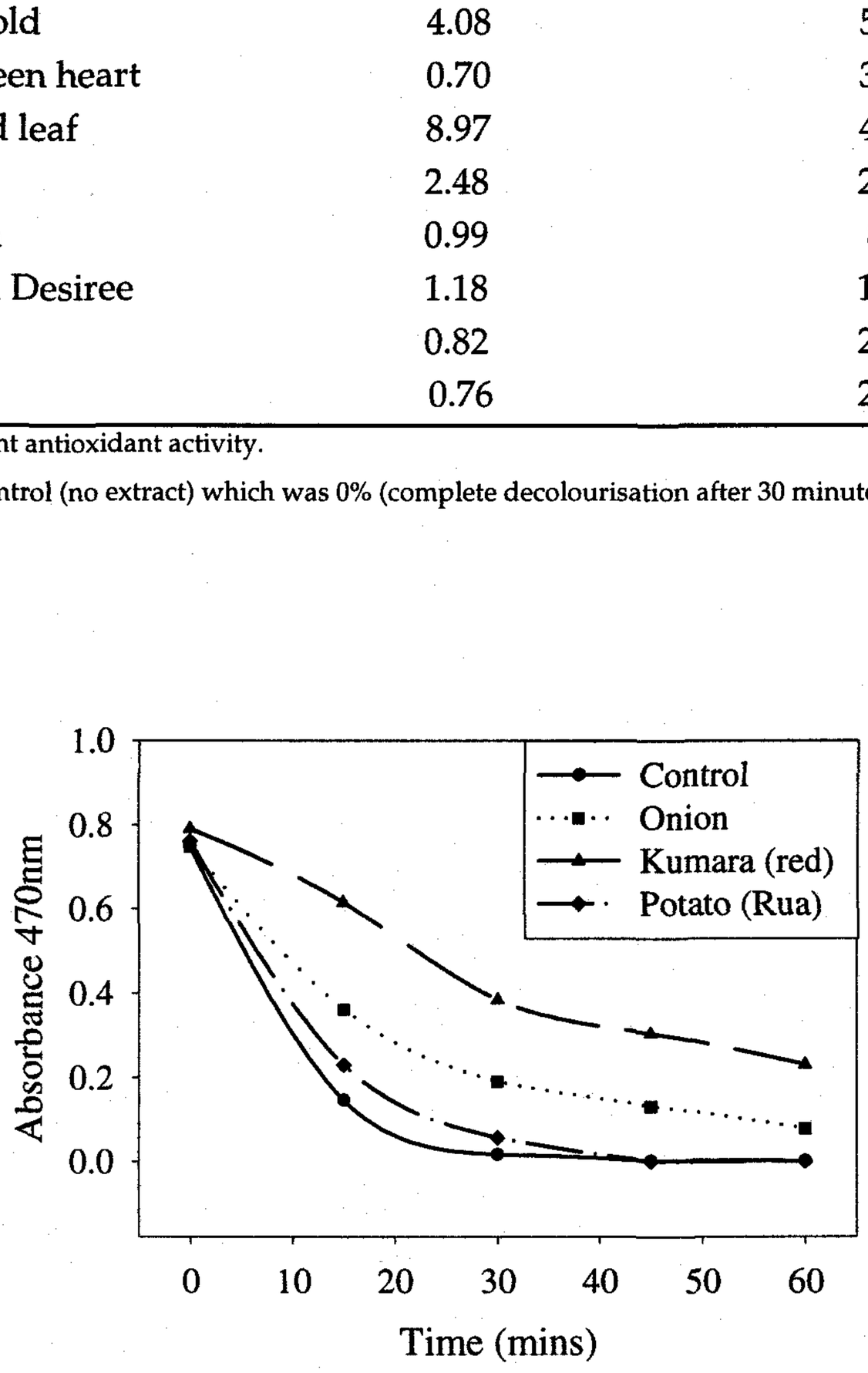

Figure 1: **Effect of vegetable extracts on oxidation of β-carotene by linoleic acid degradation products. The control contained no antioxidant.**

4.2.3 *Correlation of nutraceuticals with antioxidant activity*

Correlations were calculated between antioxidant components and antioxidant activity, as measured by the two assays. There was a very strong correlation between total phenolics and the ABTS assay (r^2 = 0.854) although the correlation between phenolics and the linoleic acid assay was not as strong (r^2 = 0.509). The correlation between other nutraceutical components and antioxidant activity was poor. This means that of the classes of nutraceuticals the phenolics are possibly the most important group of antioxidants.

4.3 FfRST bid

A bid to the Foundation for Research, Science and Technology was prepared to extend our work in the area of antioxidants. This bid was successful in obtaining partial funding of $200 000 p a for the next two years. The work proposed will include more detailed identification and quantification of antioxidant components in a range of fruit and vegetables (with a particular focus on Maori and Pacific Island foods). It will develop further improved antioxidant assay systems to mimic probable reactions that occur in the body, identify synergies between compounds, and measure the absorption and metabolism of flavonoids ingested in the diet.

5 FUTURE PLANS

In addition to the studies on antioxidants that will be carried out as part of our FfRST bid various other aspects are worth investigating over the next few years. They will require additional funding.

- **Distribution of antioxidants:** We know that in many cases phenolics are distributed in different parts of the vegetable. Comparisons need to be made between parts of the vegetable, e.g. skin versus flesh and the effect of, for example, peeling on antioxidant activity. There may also be opportunities for using vegetable wastes, such as peels, to provide a natural source of antioxidants.

- **Cultivar variations:** From previous studies on apples and potatoes we know that there are big differences in the levels of phenolics between different cultivars. Improved understanding of these variations and how phenolics levels are inherited may be usefully incorporated into breeding programmes to develop vegetables with increased levels of antioxidants, especially phenolics.

- **Environment:** Many environmental conditions and cultural practices can have an effect on the levels of antioxidants. Light is known to increase the levels of flavonoids and, because New Zealand has high UV light levels, it may be that New Zealand-grown produce has an added 'health advantage'. Study of these conditions and practices could provide useful marketing information.

- **Processing:** There has been little study of the effect of processing on antioxidant activity and how antioxidant activity in the raw vegetable relates to a processed product. Many factors may influence the composition of antioxidants in a product. For example, pH can be an important factor because under certain conditions ascorbic acid (vitamin C) is converted to dehydroascorbic acid and can act as an oxidant rather than an antioxidant. Knowledge in this area may be used to minimise losses in activity during processing.

6 REFERENCES

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