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Antioxidant activity of potatoes

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Contents

1 Executive summary

This report describes the results of project undertaken for the Potato Industry Research & Development Grants Committee of Vegfed to investigate the antioxidant activity of New Zealand-grown potatoes. Whole potato samples (unpeeled, i.e. skin and flesh) of ten cultivars or breeding lines exhibited considerable variation in skin and flesh colour. For four of these cultivars, comparisons were also made between skin and flesh. The average contents of antioxidant components in potatoes were:

- **phenolics**:
  - whole potato - 16.8 mg/100 g (fresh weight) FW for 'non-coloured' flesh (white, cream and pale yellow cultivars) and 176.2 mg/100 g FW for coloured flesh (purple, Urenika).
  - 'flesh only' - 12.4 mg/100 g FW for 'non-coloured' flesh and 140.0 mg/100 g FW for coloured flesh (Urenika).
  - 'skin only' - 110.5 mg/100 g FW for non-coloured skin (Ilam Hardy), 170.3 mg/100 g FW for cultures with coloured skin but white or cream flesh (Red Rascal and 2880-2), and 398.4 mg/100 g FW for the coloured skin/coloured flesh cultivar (Urenika).
- **anthocyanins**: These compounds were only present in coloured skins and flesh.
  - whole potato - 0.40 mg/100 g FW for coloured skin but 'non-coloured' flesh (Red Rascal, 1021/5 & 2880-2) and 54.8 mg/100 g FW for coloured flesh (Urenika).
  - 'flesh only' - Urenika 43.7 mg/100 g FW
  - 'skin only' - 5.14 mg/100 g FW for cultivars with coloured skin but 'non-coloured' flesh (Red Rascal & 2880-2) and 87.6 mg/100 g FW for coloured flesh (Urenika).
- **carotenoids**:
  - whole potato - 0.03 mg/100 g FW for 'white' flesh, 0.06 mg/100 g FW for 'cream' flesh and 0.13 mg/100 g FW for 'yellow' flesh.
  - 'flesh only' - 0.04 mg/100 g FW ('white' flesh only).
  - 'skin only' - 0.03 mg/100 g FW ('white' flesh only).
- **antioxidant vitamins**: these were not specifically measured as part of this project but values, from the New Zealand Food Composition Tables, are approximately 10 mg/100 g FW for vitamin C and 0.07 mg/100 g FW for vitamin E.

- **antioxidant activity**:
  - whole potato (i.e. unpeeled) - 86.0 µmol TEAC/100 g FW for 'non-coloured' flesh (i.e. white, cream and pale yellow cultivars) and 755.5 µmol TEAC/100 g FW for coloured flesh (purple, Urenika).
  - 'flesh only' - 72.1 µmol TEAC/100 g FW for 'non-coloured' flesh and 698.0 µmol TEAC/100 g FW for coloured flesh (Urenika).
  - 'skin only' - 495.1 µmol TEAC/100 g FW for non-coloured skin (Ilam Hardy) and 1116.56 µmol TEAC/100 g FW for coloured skin (Red Rascal, Urenika and 2880-2).

There were some large variations in antioxidant components and activity between the cultivars examined, mainly due to colour variations. The coloured skin potatoes generally had slightly higher antioxidant activity and Urenika, which had purple skin and flesh, had several fold higher phenolic content and antioxidant activity. When all samples of potatoes were included there was a very strong correlation between phenolic content and antioxidant activity (R^2=0.99).

Compared to other vegetables potatoes have fairly weak antioxidant activity and relatively low levels of most antioxidant components. Despite this potatoes probably do make a significant contribution to the antioxidant activity of the diet since they are eaten in larger amounts and more regularly than many other vegetables. Potato skins alone are relatively high in activity and thus may have potential for development of 'healthier' snack products or for preparation of extracts for inclusion in supplements. Cultivars with coloured skins have higher activity and thus greater potential than standard 'non-coloured' varieties and Urenika, with strongly coloured flesh, has even greater potential. The use of these coloured varieties could also add novelty colour value. Further research is required to determine the potential of waste streams for extraction of a nutraceutical and to substantiate the health benefits of potato phenolics.

2 Background

An earlier report by the author has described the results of a literature review undertaken for the potato sector of Vegfed to describe the nutritional status and health benefits of potatoes (Lister & Monro 2000). It highlighted the current knowledge and identified some of the future prospects and the possible next generation of key attributes that may be used for product development and marketing strategies. One of these was antioxidant activity.

2.1 Antioxidants

Antioxidants have received a lot of press for their possible role in the prevention of many degenerative diseases of ageing (e.g. cancer and heart disease). Antioxidants are a group of compounds that provide protection against the harmful effects of free radicals and other reactive oxidants. They include free radical scavengers, enzymes that remove reactive oxidants and

systems that repair oxidative injury. Free radicals are chemicals that have one or more unpaired electrons and can react with a range of biological molecules, which can result in cell damage. Free radicals are generated in our 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.

Antioxidants have an important role in helping to prevent undesirable changes in foods, such as fats and oils becoming rancid. However, they can also play a protective role in our body. Free radicals have been implicated in numerous diseases including the universal degenerative diseases of aging such as cancer, heart disease, cataracts, Parkinson's disease and pancreatitis. Thus consumption of antioxidants may help prevent these diseases. There are several classes of antioxidants commonly found in fruits, vegetables, grains and beverages. These compounds have varying antioxidant activity and are consumed in the diet in quite different quantities.

2.1.1 Carotenoids

Carotenoids are the major yellow, orange and red pigments found widely in nature. Common carotenoids include ß-carotene, ∀-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 (eg. selenium, zinc and sulphur-containing compounds) can increase levels of enzymes involved in free-radical scavenging and repairing 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 NZ soils are low in selenium and hence produce grown here is also lower in selenium. The sulphur 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, not manufactured in the human body, with antioxidant and immune stimulating properties. 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 Potato antioxidants

2.2.1 Antioxidant components

In terms of antioxidant components potatoes contain phenolics and vitamin C, but generally no or very low levels of carotenoids and only traces of vitamin E.

***Phenolics*:** Potato tubers contain a number of phenolic compounds but their percentage is rather low (Lister & Monro 2000). There are a number of reports on the phenolic composition although there is limited quantitative data. There has been a comprehensive study of the composition of NZ-grown potatoes, a PhD thesis completed by Lewis (1996). Chlorogenic acid may constitute up to 90% of the total polyphenolic content of potatoes, while other phenolic acids include protocatechuic, sinapic, coumaric and vanillic. Other polyphenolic compounds present in potatoes include flavanones (naringenin and eriodictyol), flavan-3-ols (catechin and epicatechin) and flavonols (kaempferol and sometimes quercetin glycosides) (Lewis 1996). In some cultivars anthocyanins are present, which are responsible for the red/purple colour (Mazza & Miniati 1993). Many of these compounds are present in fairly low concentrations. Vinson et al. (1998) reported the total polyphenol content of potatoes as 28 mg/100 g FW. There is about ten times as much phenolic compounds in the peel as in the flesh of the potato

(Lisi|ska & Leszcy|ski 1989). The chemistry, biochemistry and dietary role of potato polyphenols has been reviewed by Friedman (1997).

***Carotenoids*:** Carotenoids are important antioxidants but are generally present at much lower concentrations than the phenolics. The flesh of potato varieties is often tinged with yellow to a greater or lesser extent and this is mainly due to the presence of carotenoids. The main carotenoid constituents of potato tubers are the xanthophylls: violaxanthin, lutein and lutein-5,6-epoxide, with small amounts of neoxanthin and neoxanthin-A. β-carotene, a common carotenoid in many other plants, and also present in the aerial parts of the potato plant, is absent or present in only trace amounts in the tubers (Burton 1989). There is a direct correlation between yellow flesh colour and total carotenoid content, which is a heritable characteristic. Typical 'white' flesh potatoes contain 0.01-0.05 mg of carotenoids per 100 g FW while varieties with 'yellow' flesh contain 0.11-0.34 mg/100 g FW (Gross 1991).

***Antioxidant vitamins*:** Of all the vitamins, potatoes contain vitamin C in the highest quantity (Lister & Monro 2000). The vitamin C (present as ascorbic acid and dehydroascorbic acid) level in potato tubers ranges from 1 to 54 mg per 100 g FW, although most frequently it is between 10 and 25 mg/100 g. The highest concentration of vitamin C is found in the vicinity of the vascular system and is lowest in the pith and skin. There has been some breeding of potatoes to increase the levels of vitamin C. Vitamin E is not present in many fruit and vegetables, and potatoes only contain trace levels (typically around 0.07 mg/100 g FW) (Visser et al. 1990).

2.2.2 Antioxidant activity

There have been a few overseas studies on the antioxidant activity of potatoes and only a brief study in New Zealand. Polyphenolic compounds in potatoes show antioxidative activity in several systems. In a US study total phenol content of potato (peeled) was 28 mg per 100 g FW and it was ranked 20th out of 23 commonly consumed vegetables. However, it was ranked 9th in terms of antioxidant activity (Vinson et al. 1998). This study gave potatoes as the 4th greatest contributor to phenolics consumed per day from vegetables. Chlorogenic acid from potato has been found to be an effective inhibitor of lipid oxidation (Al-Saikhan et al. 1995). Extracts from potato peels have been shown to possess strong antioxidant activity, attributed mostly to their chlorogenic, protocatechuic and caffeic acid contents (Onyeneho & Hettiarachchy 1993). Extracts prepared from red peels have been shown to have stronger activity than those from brown peels and is probably due to strong antioxidant activity of the anthocyanins and higher total phenolic content.

New Zealand studies (Lister & Podivinsky 1998; Lister 1999) have measured the antioxidant activity of New Zealand-grown potatoes. Red Desiree ranked 9th out of 15 vegetables and Rua was 11th. Antioxidant activity was correlated with phenolic content of the vegetables and the two potato cultivars ranked 8th and 10th for phenolic content respectively. Peeling the potato considerably reduced both the phenolic content and the antioxidant activity. Other potato cultivars have higher phenolic contents (Lewis 1996) and thus may have higher antioxidant activity. This report provides a more detailed investigation of the antioxidant activity of a variety of NZ-grown potatoes.

3 Methods

3.1 Analysis of potato tubers

3.1.1 Collection of samples

Ten cultivars/breeding lines were examined in this study; details of these are given in Table 1 and photographs are shown in Figure 1. All samples were taken from the same Crop & Food Research trial at Lincoln.

Table 1: Descriptions of potato cultivars/breeding lines used in this study.

Cultivar/Breeding Line	Use	Skin colour	Flesh colour
Agria	fresh/crisp	yellow	yellow
Ilam Hardy	fresh	white	white
Nadine	fresh	white	white
Red Rascal	fresh	red	white
Rua	fresh	white	white
Russet Burbank	french fry	brown	white
Urenika (Congo)	fresh	purple	purple
1021/5	crisp	red	white
2852-5	crisp	white	cream
2880-2	fresh	purple	cream

3.1.2 Preparation of samples

For each cultivar or breeding line six tubers of reasonably uniform size where possible (weights of individual tubers are given in Appendix I) were selected. Each tuber selected was cut in half, then half was used for 'whole' treatment. For four cultivars only (Ilam Hardy, Red Rascal, Urenika and 2880-2) the other half of each tuber was peeled carefully with a potato peeler and used for a 'skin only' sample. The remaining flesh, minus skin was used for a 'flesh only' sample.

Composite potato samples were weighed (to obtain fresh weight), freeze-dried, reweighed (to obtain dry weight) and ground to a homogeneous powder. Dry weights were generally fairly consistent between cultivars (varying between 18 and 25%) with the exception of Urenika and 1021/5 which were 31 and 29% respectively (Appendix II).

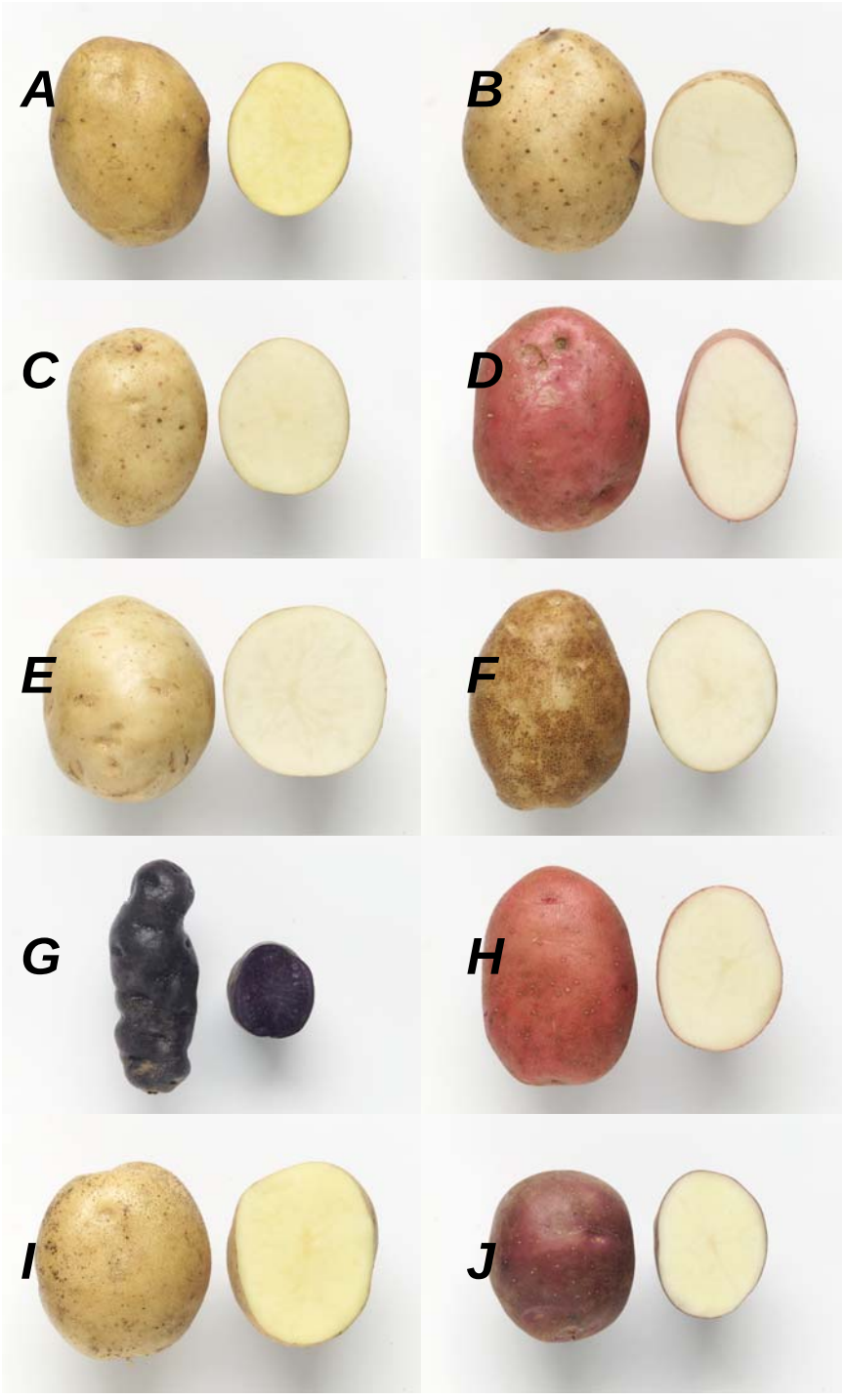

Figure 1: Potato cultivars/breeding lines used in this study: A - Agria, B – Ilam Hardy, C – Nadine, D – Red Rascal, E – Rua, F – Russet Burbank, G – Urenika, H – 1021/5, I – 2852-5, J - 2880-2.

For each sample approximately 2 g equivalent of fresh weight was weighed out and extracted with 15 ml of 80% acetone for four hours. These samples were then centrifuged and the supernatant used for subsequent analysis. Triplicate extractions were performed on each sample.

3.1.3 Quantification of antioxidant components

Total phenolics (Folin Method)

Total phenolics were measured in the acetone extracts using Folin-Ciocalteu's reagent (adapted from the method of Spanos & Wrolstad (1990)). Some 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 100 gram fresh weight (mg GAE/100 g FW). Phenolic assays were carried out in duplicate on each sample.

One problem with this particular assay is that it is susceptible to interference by reducing substances such as ascorbic acid. Thus, the total phenolic measurements by this method are actually phenolics plus vitamin C. For this reason total phenolics were also quantified by HPLC, using the sum of individual compounds.

HPLC of phenolic compounds

Phenolics were analysed using an HPLC method based on that developed for apples (Lister et al. 1994) with slight modifications to improve separation of potato compounds (Lewis 1996). Where identifications could be made samples were quantified on the basis of a pure standard of that compound (if available). Where standards were not available or specific identification could not be made, a chlorogenic acid standard was used for quantification of phenolic acids, catechin for flavan-3-ols, rutin (quercetin-3-rutinoside) for flavonols, naringenin for flavanones and cyanidin-3-rutinoside for the anthocyanins. Total anthocyanins and total phenolics were calculated by this method from the sum of the individual components.

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 for a mixture of carotenoids ($E^{1\%}_{1cm}$=2500).

3.1.4 Measurement of antioxidant activity

ABTS assay

The main assay used for measuring antioxidant activity was a modified ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assay (Miller & Rice-Evans 1996 & 1997). 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. Triplicate assays were performed on each extract and at three different dilutions. Results are expressed as the amount of Trolox equivalent antioxidant capacity per 100 grams fresh weight (µmol TEAC/100 g FW), which represents the amount of Trolox (vitamin E) that gives the same response as one hundred grams of potato.

Other assays

Six samples were selected for preliminary screening using two other assays for measuring antioxidant activity. Samples were: Agria, Ilam Hardy, Red Rascal, Urenika, 1021/5 and 2880-2 (all whole potato samples, i.e. skin and flesh). Both assays were carried out on a microtitre plate with nine wells per sample and at least two separate runs were performed on each extract.

A TRAP (total peroxyl radical-trapping potential) assay (based on the method of Valkonen & Kuusi (1997)) was used to examine the radical scavenging ability of potato extracts in an aqueous system. Peroxyl radicals were continuously generated by thermal decomposition of AAPH (2,2-diazobis(2-amidinopropane) dihydrochloride) and scavenging by antioxidants was determined as the length of the lag phase and was expressed relative to Trolox.

The modified FOX assay (based on the method of Hermes-Lima et al. (1995)) measures the ability of antioxidants to inhibit light-induced peroxidation of lipid. For ease of interpretation IC_{50} values shown in this report represent the volume of intralipid for which peroxidation is inhibited 50% by one hundred grams of potato.

3.2 Analysis of transgenic potato plants

3.2.1 Samples

Potatoes genetically modified for resistance to aphids were analysed. The genetic engineering involved the insertion of a gene responsible for the manufacture of the enzyme glutathione reductase. Overexpression of this enzyme in potatoes is expected to maintain cellular ascorbic acid in the reduced state and thus could possibly have an effect on cellular antioxidant activity.

3.2.2 Sample preparation

Samples were tissue culture plantlets and only shoots (leaves and stem) were used for analysis. Tissue (0.5 to 1 g) was ground in liquid nitrogen and extracted with 10 ml of 80% acetone for four hours. These samples were then centrifuged and the supernatant used for subsequent analysis.

3.2.3 Analyses

Total phenolics were quantified as per section 3.1.3 and antioxidant activity was measured by the ABTS assay (section 3.1.4).

4 Results and discussion

4.1 Potato tubers

4.1.1 Quantification of antioxidant components

Total phenolics (Folin-Ciocalteu reagent)

One problem with this particular assay is that it is susceptible to interference by reducing substances such as ascorbic acid. Thus, the total phenolic measurements are actually phenolics plus vitamin C. The levels of total phenolics (plus vitamin C) present in potatoes are given in Table 2. On average fresh whole potatoes (excluding the highly coloured flesh Urenika) had a total phenolic content of 28.6 mg GAE/100 g FW (range 22.7-35.7). It can be seen that there is some variation between cultivars with the purple Urenika having significantly higher phenolic content at 128.2 mg GAE/100 g FW. Two of the other cultivars/breeding lines with red or purple coloured skins had higher phenolic content than non-coloured cultivars (Red Rascal 35.7 mg GAE/100 g FW and 2880-2 35.2 mg GAE/100 g FW), but the red-skinned line 1021/5 had the lowest level (22.7 mg GAE/100 g FW). Ideally measurements should be taken in another season to determine if these are consistent values for each cultivar or just due to factors such as the particular season and growing environment, which are known to have influences on phenolic levels. The average phenolic content reported here is similar to that reported by Vinson et al. (1998) of 28 mg/100 g FW with the Folin-Ciocalteu reagent. However, the value obtained for Rua in this study (31.3 mg GAE/100 g FW) was slightly lower than that recorded in 1998 (38.3 mg GAE/100 g FW) (Lister & Podivinsky 1998). This difference may due to a number of factors including environmental conditions, as mentioned above.

Phenolic levels (quantified by HPLC) in ‘flesh only’ samples (Table 2) were lower than for the respective whole potato samples. This is due to the concentration of phenolics being higher in the skin than the flesh; phenolics were 2 to 8 times higher in the ‘skin only’ than for the respective ‘flesh only’ samples. Phenolics were much higher in the coloured skin samples tested (average of 107.3 mg GAE/100 g FW for Red Rascal and 2880-2, and 398.4 for Urenika) than the non-coloured Ilam Hardy skin (110.5 mg GAE/100 g FW).

Table 2: Levels of phenolics and carotenoids in a range of potato samples.

Sample description	Tissue type	Total phenolics + vitamin C[a] (mg GAE[b]/100 g FW)	Total phenolics[c] (mg/100 g FW)	Anthocyanins[c] (mg/100 g FW)	Carotenoids (mg/100 g FW)
Agria	skin & flesh	23.91	11.85	0.00	0.134
Ilam Hardy	skin & flesh	26.49	14.20	0.00	0.026
	flesh only	20.02	11.03	0.00	0.028
	skin only	90.89	110.48	0.00	0.025
Nadine	skin & flesh	27.00	14.37	0.00	0.030
Red Rascal	skin & flesh	35.72	24.76	0.44	0.028
	flesh only	24.67	12.91	0.00	0.023
	skin only	154.87	187.57	5.68	0.040
Rua	skin & flesh	31.27	19.39	0.00	0.041
Russet Burbank	skin & flesh	29.40	21.42	0.00	0.022
Urenika	skin & flesh	128.21	176.16	54.77	0.044
	flesh only	116.70	139.98	43.72	0.050
	skin only	249.57	398.36	87.58	0.032
1021/5	skin & flesh	22.72	9.60	0.22	0.038
2852-5	skin & flesh	25.44	12.63	0.00	0.054
2880-2	skin & flesh	35.17	22.95	0.54	0.075
	flesh only	26.53	13.10	0.00	0.073
	skin only	141.22	153.01	4.60	0.035

[a] quantified using Folin-Ciocalteu reagent (see section 3.1.3)
[b] GAE = gallic acid equivalents (see section 3.1.3)
[c] quantified by HPLC (see section 3.1.3)

Profile & quantification of individual phenolic compounds

The levels of total phenolics as quantified by HPLC are shown in Table 2. It can be seen that the figures are lower than for total phenolics as quantified by Folin-Ciocalteu reagent, since this later method also accounts for vitamin C. The Folin-Ciocalteu method would appear to underestimate phenolic content when some specific compounds are present (e.g. those higher in potato skins and also Urenika). Thus, the HPLC quantification provides a more accurate estimation of total phenolics. However, even our HPLC quantification is only an estimate since standards were not available for all compounds and thus responses were based on comparative compounds, which may give slightly different responses. Comparisons between potato samples are valid.

HPLC analysis of the individual phenolic compounds present in potatoes revealed the presence of phenolic acids, flavonoids and anthocyanins. There were significant differences in composition of the phenolics between the different cultivars/breeding lines and there were also differences between

skin and flesh. The major compound in virtually all samples was chlorogenic acid, with other phenolic acids, such as protocatechuic, sinapic and vanillic, also being present in relatively high proportions. The flavonoids in the tubers were flavones (probably naringenin and eriodictyol glycosides), flavonols (catechin and epicatechin) and flavonols (kaempferol and quercetin glycosides). These flavonoids were present in very low concentrations and were higher in skin than flesh.

Levels of total anthocyanins (the red/purple pigments) are also given in Table 2. The anthocyanin composition varied significantly between the different coloured skin samples. Urenika contained two main anthocyanins, which were the same in both skin and flesh. These were probably petunidin-3-(*p*-coumaroyl-rutinoside)-5-glucoside and malvidin-3-(*p*-coumaroyl-rutinoside)-5-glucoside. Red Rascal differed in that the skin contained primarily pelargonidin-3-(*p*-coumaroyl-rutinoside)-5-glucoside and a smaller amount of a peonidin glycoside. The purple-skinned line 2880-2 contained predominantly one anthocyanin, petunidin-3-(*p*-coumaroyl-rutinoside)-5-glucoside, present in much lower concentration than in the purple Urenika.

Carotenoids

The levels of carotenoids in potatoes were very low compared to the phenolics (Table 2) and also much lower than the vitamin C content. Whole potatoes (skin and flesh) had an average carotenoid content of 0.05 mg/100 g FW. However, there was considerable variation between the cultivars based on appearance of the flesh. The 'white' flesh cultivars had an average content of 0.03 mg/100 g FW and for 'cream' flesh the average was 0.06 mg/100 g FW. Agria, the yellow-fleshed cultivar, had the highest carotenoid content at 0.134 mg/100 g FW. Carotenoids were generally slightly higher in the flesh than in the skin, with the exception of Red Rascal. The values obtained in this study are comparable with literature values for typical 'white' flesh potatoes of 0.01-0.05 mg/100 g FW and 'yellow' flesh of 0.11- 0.34 mg/100 g FW (Gross 1991). Thus, it would be expected that 'cream' fleshed potatoes would have carotenoid levels in between these two ranges, as was the case in this study.

Vitamin C

Due to the financial limitations of this project, individual vitamin C analyses were not conducted as part of this project. However, since the initial measure of total phenolics actually includes vitamin C (section 4.1.1), the difference between this figure and the phenolics quantified by HPLC (Table 2) probably provides some indication of the vitamin C content. Values for whole potatoes would appear to be between 8 and 13 mg/100 g FW. The accuracy of this is questionable since for some samples the Folin method underestimates certain phenolics. However, it is clear that there are probably some differences between cultivars, although not as great as for phenolics and carotenoids. Some data on the vitamin C content of potatoes is 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). The value for uncooked potatoes (Ilam Hardy) was 12

mg/100 g FW (Visser et al. 1990), which is in line with values estimated in this study.

4.1.2 *Comparisons with other vegetables*

Table 3 shows some comparisons in antioxidant components between different vegetables. It can be seen that non-coloured flesh potatoes have low phenolic levels, but the purple-fleshed Urenika ranked a lot higher than many other vegetables. Carotenoid levels in potatoes are very low compared to all the other vegetables examined. Potatoes have a lower vitamin C content, although because they are consumed so frequently they do make significant contribution to the daily intake of this vitamin (Lister & Monro 2000).

Table 3: Comparisons of antioxidant components in selected fresh vegetables (values for each vegetable usually 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			
Non-coloured flesh	**28.6**	**0.05**	**11.8**
Purple flesh (Urenika)	**128.2**	**0.04**	**ND[c]**
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
[c] ND = not determined

4.1.3 *Measurement of antioxidant activity*

ABTS assay

Potatoes showed fairly weak antioxidant activity with the exception of Urenika and 'skin only' samples (Table 4). On average whole non-coloured flesh potatoes had antioxidant activity of 86 µmole TEAC/100 g FW. There was some variation between different cultivars (Table 4 and Figures 2 & 3).

These differences probably relate to differences in levels of antioxidant components (Table 2).

It is difficult to make direct comparisons between our results and those reported already in the literature (Al-Saikhan et al. 1995; Onyeneho & Hettiarachchy 1993; Vinson et al. 1998) as quite different methods of analysis were used. However, they all come to similar conclusions in that potatoes rank fairly low compared to many other vegetables on an equal weight basis but skins are a good source of antioxidants. The antioxidant activity of Rua in this study (80.4 µmol TEAC/100 g FW) was lower than for our previous study in 1998 (109 µmol TEAC/100 g FW) and probably relates to differences in phenolic content noted above.

Table 4: Antioxidant activity in a range of potato samples.

Sample description	Tissue type	ABTS assay (µmol TEAC[a]/100 g FW)	TRAP assay (µmol TRAP[b]/100 g FW)	Modified FOX assay IC_{50} (mg FW equiv/ml Intralipid)
Agria	skin & flesh	84.68	330.4	255.4
Ilam Hardy	skin & flesh	75.86	262.0	167.9
	flesh only	60.17	ND	ND
	skin only	495.07	ND	ND
Nadine	skin & flesh	86.94	ND	ND
Red Rascal	skin & flesh	95.32	370.4	221.7
	flesh only	74.60	ND	ND
	skin only	833.09	ND	ND
Rua	skin & flesh	80.41	ND	ND
Russet Burbank	skin & flesh	87.40	ND	ND
Urenika	skin & flesh	755.54	1075.2	1286.6
	flesh only	698.01	ND	ND
	skin only	1729.49	ND	ND
1021/5	skin & flesh	80.62	332.9	87.1
2852-5	skin & flesh	78.06	ND	ND
2880-2	skin & flesh	104.96	340.3	274.8
	flesh only	81.51	ND	ND
	skin only	787.10	ND	ND

ND = not determined
[a] TEAC = Trolox equivalent antioxidant capacity (see section 3.1.4)
[b] TRAP = total peroxyl radical-trapping potential (see section 3.1.4)

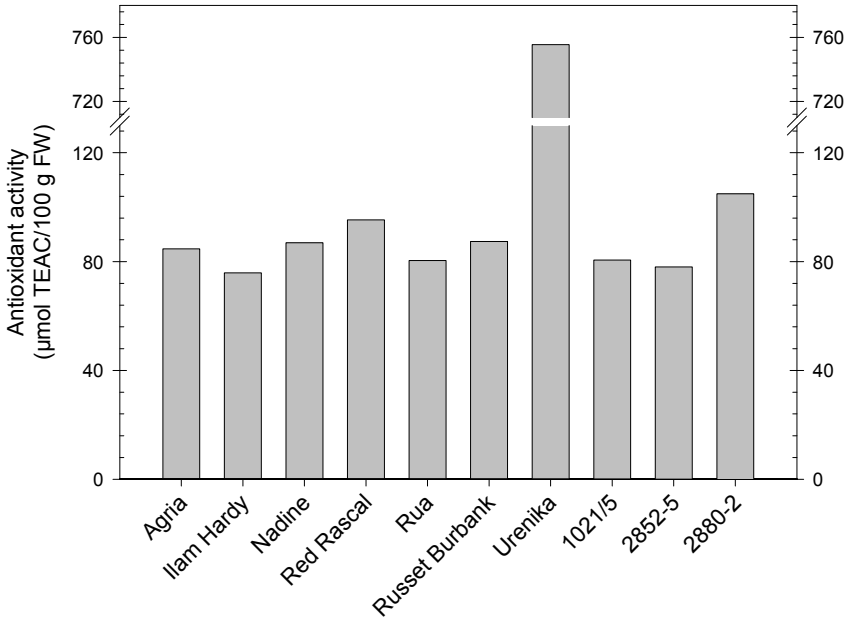

Figure 2: Antioxidant activity (as measured by the ABTS assay) of whole potatoes (skin plus flesh).

Other assays

Figure 4 shows the antioxidant activity of whole potatoes as analysed by the three different assay systems used (data also given in Table 4). It can be seen that Urenika gave very high activity in all three assay systems. The other cultivars/breeding lines varied slightly in their response in the different assays. For example, 1021/5 did not perform very well in the modified FOX assay. Agria may have been expected to perform slightly better in this assay which measures the ability to inhibit lipid peroxidation. This cultivar contained the highest level of carotenoids, which are lipid-soluble compared to phenolics and vitamin C, which are water-soluble.

Relationship between phenolic content and antioxidant activity

When samples of potatoes were included there was a very strong correlation between phenolic content, as measured by HPLC, and antioxidant activity, as measured by the ABTS assay (R^2=0.99). Urenika was very influential in determining this correlation, but it did not appear to have a different relationship from other cultivars. A very similar relationship was observed between phenolic content, as measured by Folin-Ciocalteu reagent (i.e. phenolics plus vitamin C), and antioxidant activity. From these figures it appears that phenolics are the greatest contributor to antioxidant activity in potatoes. Vitamin C is probably also important but is less variable in its content and therefore does not have as great an influence on variation in activity between cultivars. Carotenoids showed no relationship with antioxidant activity and make a minimal contribution to the total activity (R^2=0.02).

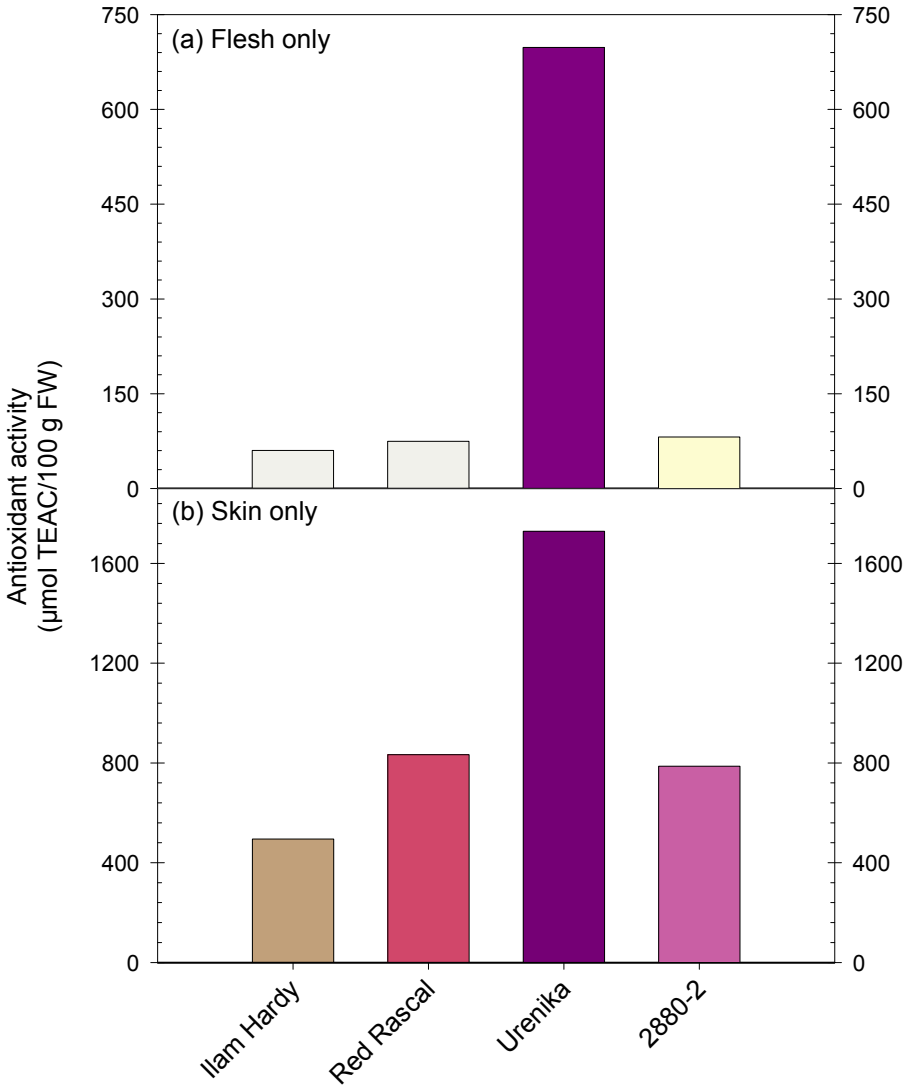

Figure 3: Antioxidant activity (as measured by the ABTS assay) of different potato cultivars in two tissues.

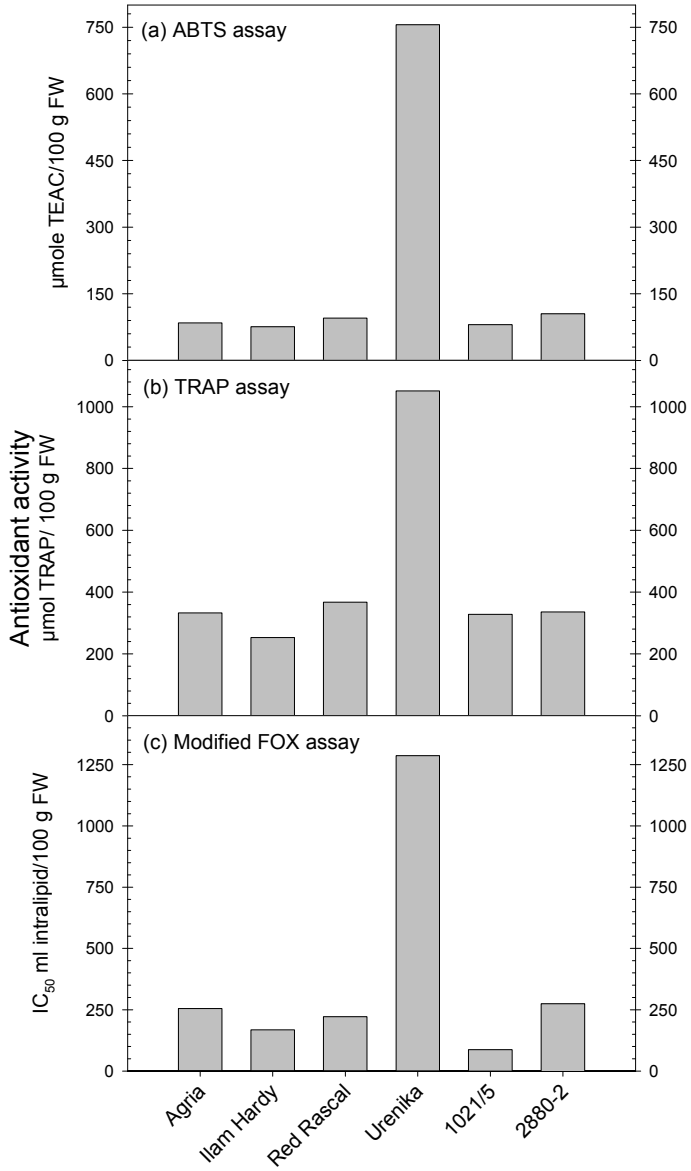

Figure 4: Antioxidant activity of whole potato samples as measured by three different assay systems.

Comparisons with other vegetables

Figure 5 shows how potatoes compare with other NZ-grown 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). It can be seen that standard potato cultivars with 'white', 'cream' or 'yellow' flesh showed relatively low activity. Despite this potatoes probably do make a significant contribution to the antioxidant activity of the diet, since they are eaten in larger amounts and more regularly than many other vegetables (Russell et al. 1999). On the other hand the purple-fleshed Urenika has very high antioxidant activity, particularly on a per serving basis. In fact a single serving of Urenika would provide more antioxidant activity than any of the so-called antioxidant supplements on the market that we have tested.

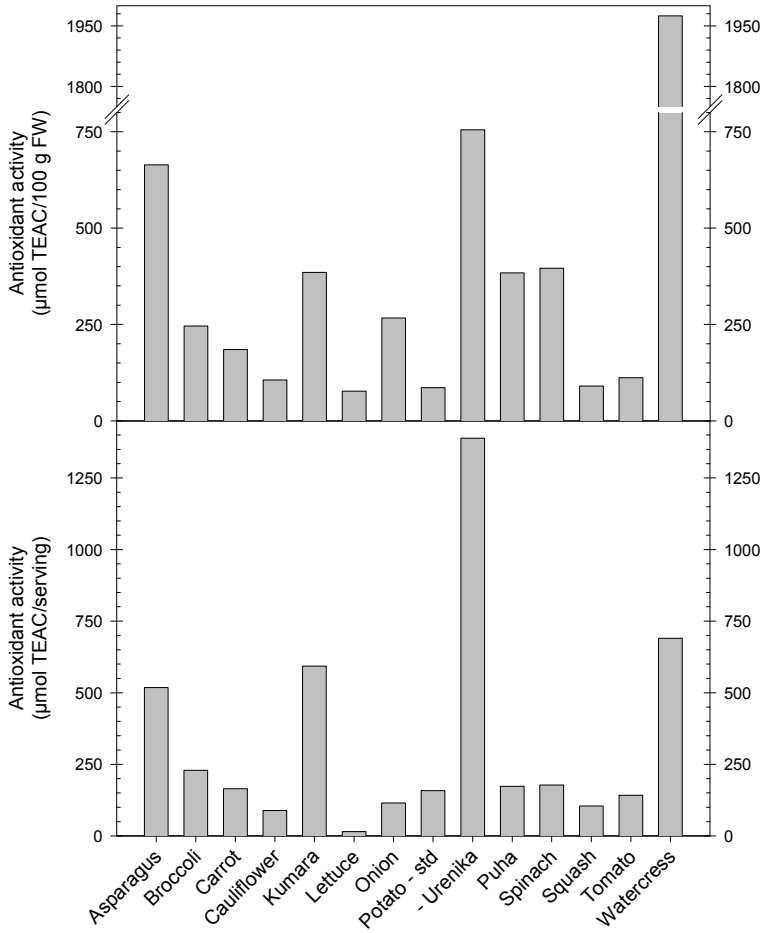

Figure 5: Comparisons of antioxidant activity (measured by ABTS assay) of selected fresh vegetables. Top graph shows comparisons on an equal weight basis while the bottom graph shows comparisons on a serving basis.

4.2 *Analysis of transgenic potato plants*

Table 6 shows the composition of the transgenic potato plants analysed. There was considerable variation between the different lines. Except for Line ERG-04, all lines were higher in antioxidant activity than the control, which had not been transformed, and in a couple of cases the activity was more than twice the control. In some cases the level of activity did appear to correlate with activity of the enzyme product of the introduced gene. The genetic engineering involved the insertion of a gene responsible for the manufacture of the enzyme glutathione reductase (GR). Overexpression of this enzyme in potatoes is expected to maintain cellular ascorbic acid in the reduced state. It would appear that, as postulated, this may have had an effect on cellular antioxidant activity.

These preliminary results are very interesting and indicate there may be significant gains in antioxidant activity from manipulation of enzyme antioxidant systems. To be meaningful these experiments really need to be repeated as there was considerable variations between the plantlets, such as in leaf size, which could affect phenolic levels. These plants will be put into the glasshouse in the coming year to produce tubers for analysis.

Table 6: Characteristics, phenolic content and antioxidant content of transgenic potato plants.

Potato line	Glutathione content[a]	GR activity[a]	Southern GR[a,b]	Chlorophyll content	Total phenolics (mg/100 g FW)	Antioxidant activity (µmol TEAC/100 g FW)
Control	100	100	-	3.36	66.08	319.65
ERG-04	70	147	3	4.47	68.61	308.85
ERG-05	77	74	0	4.22	77.53	338.19
ERG-11	81	64	3	5.05	119.54	466.88
ERG-12	85	66	3	6.50	122.05	490.29
ERG-13	94	72	2	6.86	121.10	479.86
ERG-14	133	261	?	8.09	71.10	354.45
ERG-15	156	81	2	3.82	115.35	449.89
ERG-16	129	159	4	6.10	150.03	584.68
ERG-17	99	87	1	7.15	145.71	508.12
ERG-18	96	71	2	5.89	134.25	539.69
ERG-19	101	82	1	4.09	115.94	529.94
ERG-20	141	81	8	4.68	109.14	602.64
ERG-21	171	200	7	5.17	105.92	447.69
ERG-22	126	143	1	8.04	194.53	798.98
ERG-24	122	186	2	4.07	110.70	424.90
ERG-25	150	230	1	4.63	133.08	537.43
ERG-26	122	174	1	5.72	206.28	719.75

[a] Data supplied by Dr Tony Conner, Crop & Food Research
[b] No. of copies of GR gene by Southern analysis

5 Conclusions and future research

Compared to other vegetables potatoes have fairly weak antioxidant activity and relatively low levels of most antioxidant components. Despite this, potatoes probably do make a significant contribution to the antioxidant activity of the diet, since they are eaten in larger amounts and more regularly than many other vegetables. The purple-fleshed cultivar Urenika had very high antioxidant activity and this could possibly be used as a marketing strategy. Potato skins alone were also relatively high in activity and thus have potential for development of 'healthier' snack products that could include them. Another possibility is that extracts prepared from potato skins could be included in supplements or some food products (i.e. as a nutraceutical). The use of coloured skins or coloured flesh could also add novelty colour value. Further research is required to determine the potential of waste streams for extraction of a nutraceutical and to substantiate the potential health benefits of potato phenolics.

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Appendices

Appendix I Individual tuber weights

Cultivar/Breeding line	Individual tuber weights (g)						Average weight (g)
	1	2	3	4	5	6	
Agria	177	175	203	182	298	142	196
Ilam Hardy	210	229	175	227	242	206	215
Nadine	175	237	250	176	213	233	214
Red Rascal	147	262	360	211	205	190	229
Rua	205	224	227	253	188	253	225
Russet Burbank	264	208	352	263	228	207	254
Urenika (Congo)	100	127	124	102	168	98	120
1021/5	178	308	187	214	266	265	236
2852-5	325	305	178	171	209	187	229
2880-2	149	103	117	90	91	103	109

Appendix II Percentage dry matters for potato cultivars/breeding lines used in this study

Cultivar/Breeding line	Whole (skin plus flesh)	Skin only	Flesh only
Agria	23.8		
Ilam Hardy	22.1	14.8	23.0
Nadine	18.8		
Red Rascal	24.1	20.6	22.3
Rua	23.4		
Russet Burbank	24.6		
Urenika (Congo)	31.0	33.7	31.7
1021/5	28.7		
2852-5	23.2		
2880-2	25.2	23.2	24.9