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Antioxidant and radical scavenging activities of polyphenols from apple pomace

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Abstract

The antioxidant properties of apple polyphenols were evaluated using the β-carotene/linoleic acid system, DPPH radical and superoxide scavenging activities. The polyphenols examined were epicatechin, its dimer (procyanidin B2), trimer, tetramer and oligomer, quercetin glycosides, chlorogenic acid, phloridzin and 3-hydroxy-phloridzin. All the compounds showed strong antioxidant activities, and their DPPH-scavenging activities were 2–3 times and superoxide anion radical-scavenging activities were 10–30 times better than those of the antioxidant vitamins C and E.

1. Introduction

Apple pomace is a major global waste product generated primarily during apple juice processing and in New Zealand the amount was $2–4\times10^6$ tonnes in 1997 alone (Kennedy et al., 1999). A great deal of effort has been expended to utilize this waste in a variety of ways such as in biogas and energy production, as food ingredients, and speciality chemicals, including apple seed oils (Carson, Collins & Penfield, 1994; Hang, 1987; Kennedy, 1994; Kennedy et al., 1999). Currently, apple pomace is still grossly under-utilized and is used mostly as stock feed in New Zealand. In this context it is desirable to seek higher value products from apple pomace which could be economically worthwhile for commercial exploitation.

Our current interest in apple pomace was instigated by the perceived health-protecting properties of apple, exemplified by the old adage "an apple a day keeps the doctor away". This belief is now being supported by recent data which have suggested that the risk of heart attack was 49% lower for men who ate 110 g or more apple a day compared to those who ate less (18 g) (Hertog, Feskens, Hollman, Katan & Kromhout, 1993). There is now increasing evidence to suggest that many age-related human diseases such as heart disease, cancer, inflammation, arthritis, immune system decline, brain dysfunction and cataracts are the result of cellular damage by free radicals and antioxidants in our diet could play an important role in such disease prevention (Aruoma, 1998; Block & Langseth, 1994; Gordon, 1996; Halliwell & Gutteridge, 1989). This has fueled much public interest in natural antioxidants and has led to an extensive search for effective antioxidants in nature (Frankel, Huang, Aeschbach & Prior, 1996; Madsen & Bertelsen, 1995; Shahidi & Wanasundara, 1992), especially those that are present naturally in human diets (Vinson, Hao & Zubik, 1998; Wang, Cao & Prior, 1996). Our interest is focused on the apple polyphenols which are present in the skin and hence the pomace from juice pressing. The aim of this research is to investigate apple pomace as a potential source of natural polyphenols for use as dietary or food antioxidants. We have previously reported the isolation and characterization of polyphenols in apple pomace (Foo & Lu, 1999; Lu & Foo, 1997) and, in continuation of that work, this report deals with the antioxidant properties of the apple polyphenols derived from the pomace.

2. Materials and methods

The apple polyphenols used in this work were isolated from gala apple pomace obtained from Frucor (formerly ENZA) processors in Hastings, New Zealand, and characterised as previously described (Foo & Lu,

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1999; Lu & Foo, 1997). Their purity was determined as being greater than 90% using HPLC analysis. Other chemicals were purchased as follows: α-tocopherol (97%), β-carotene (95%) and linoleic acid (99%) from Aldrich Chemical Company; ascorbic acid from BDH Chemicals Ltd; 1, 1-diphenyl-2-picrylhydrazyl (DPPH), xanthine (99%), xanthine oxidase (25 units) and superoxide dismutase (75000 units) from Sigma, nitro blue tetrazolium chloride (NBT), sodium dodecylsulfate (SDS) and Tween 20 from Serva Feinbiochemica GmbH and Co. Solvents were distilled before use.

2.1. Antioxidant activity of apple polyphenols using the β-carotene/linoleic acid system (Taga, Miller & Pratt, 1984)

One millilitre of a solution of β-carotene in chloroform (3.34 mg/ml) was pipetted into a flask containing 40 mg linoleic acid and 400 mg Tween 20. The chloroform was removed by rotary evaporation at 40°C for 5 min and, to the residue, 100 ml of distilled water was added slowly with vigorous agitation, to form an emulsion. A 5 ml aliquot of the emulsion was added to a tube containing 0.2 ml of the antioxidant solution at 200 mg/1 and the absorbance was measured at 470 nm, immediately, against a blank, consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 40°C and the absorbance measurements were made again at 15 min intervals.

2.2. Free radical scavenging activity of apple polyphenols using DPPH

The free radical scavenging activity of apple polyphenols was measured using the method of Brand-Williams, Cuvelier and Berset (1995) with some modification. A 0.1 mM solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) in methanol was prepared and to 2 ml of this solution was added 0.1 ml of an antioxidant solution in methanol at different concentrations. The decrease in absorbance at 517 nm was measured at 0, 5 and then every 15 min until the reaction reached a plateau. The percentage of DPPH remaining at the steady-state was calculated as a function of the molar ratio of antioxidant to DPPH. The EC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated from the results.

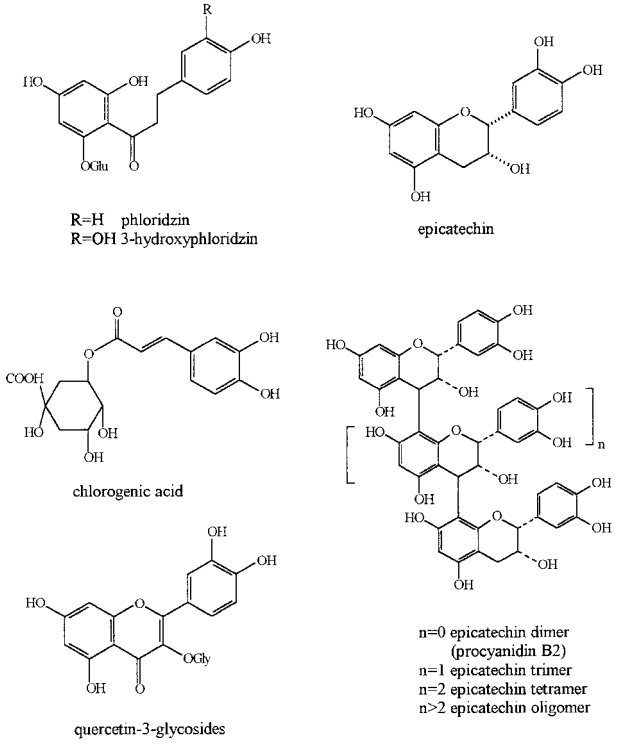
2.3. Superoxide anion radical-scavenging activity of apple polyphenols

The superoxide radical-scavenging activity was performed according to the procedure of Okamura, Mimura, Yakou, Niwano and Takahara (1993). 0.1 ml standard solution (5, 10, 25, 50, 100, 200 units/ml) of aqueous superoxide dismutase (SOD) or a polyphenol solution in DMSO (0.2 mg/ml) was added to a 1 ml mixture of 0.4 mM xanthine and 0.24 mM nitro blue tetrazolium chloride (NBT) in 0.1 M phosphate buffer (pH 8). A 1 ml solution of xanthine oxidase (0.049 units/ml), diluted in 0.1 M phosphate buffer (pH 8) was added and the resulting mixture incubated in a water bath at 37°C for 20 min. The reaction was terminated by adding 2 ml of an aqueous solution of 69 mM sodium dodecylsulfate (SDS) and the coloration of NBT was measured at 560 nm. The SOD equivalent activity of polyphenols was calculated as equivalent SOD unit/mg from the SOD standard curve.

3. Results and discussion

3.1. Antioxidant activity of apple polyphenols using β-carotene/linoleic acid method

In the β-carotene/linoleic acid assay the procyanidins and quercetin glycosides (see Scheme 1 for their chemical structures) showed the highest activity while phloridzin had only moderate activity (Figs. 1 and 2). Among procyanidins, the oligomeric procyanidins were most potent followed by the higher molecular weight procyanidin polymers while the monomeric epicatechin was the least active. In the flavonoids, quercetin, the aglycone had a higher antioxidant activity than its glycosides on a weight basis but this difference was more



Scheme 1. Chemical structures of polyphenols from apple pomace.

attributed to its lower molecular weight rather than structural changes. The lower antioxidant activity of phloridzin was consistent with other studies (Dziedzic & Hudson, 1983; Dziedzic, Hudson & Barnes, 1985) which confirmed that a catechol B-ring was essential for good antioxidant activity. This was further corroborated by the higher activity observed for 3-hydroxyphloridzin, which has an additional hydroxyl in the B-ring.

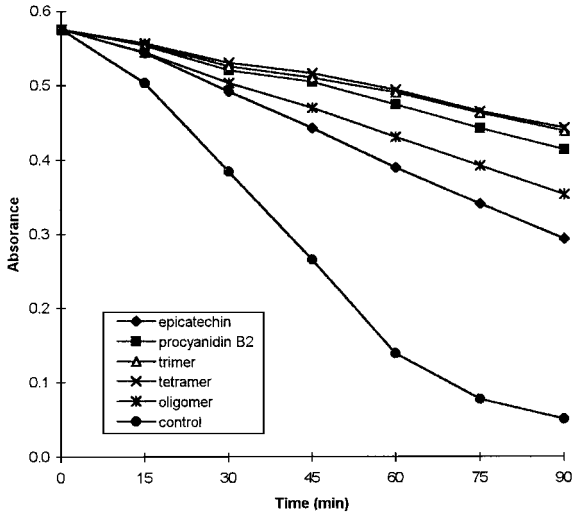


Fig. 1. Antioxidant activity of procyanidins, at 200 mg/l in the β-carotene/linoleic acid system.

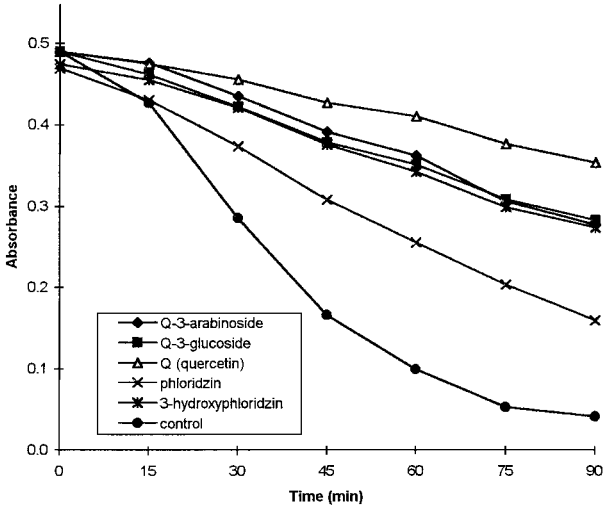


Fig. 2. Antioxidant activity of flavonoid glycosides at 200 mg/l in the β-carotene/linoleic acid system.

3.2. DPPH-free radical-scavenging activity of apple polyphenols

All the apple polyphenols examined were found to possess good DPPH-scavenging activity. The EC_{50} values (see Table 1) showed that the DPPH-scavenging activity decreased in the following order: quercetin glycosides $\geqslant$ procyanidins $>>$ chlorogenic acid $\approx$ 3-hydroxyphloridzin $>>$ phloridzin. With the exception of phloridzin, all polyphenols were better than vitamins C and E as DPPH free radical scavengers.

All the quercetin glycosides had EC_{50} values of 0.10–0.11 while the procyanidins had comparable or slightly higher EC_{50} values, indicating that these two classes of compounds were the most potent DPPH scavengers among the apple polyphenols. Their radical-scavenging activity was significantly better than chlorogenic acid, which had an EC_{50} of 0.24 and the dihydrochalcones, with EC_{50} 0.60 for phloridzin. These findings were consistent with the results reported by Bors, Heller, Michel and Saran (1990).

Using pulse radiolysis, they had demonstrated that the *o*-dihydroxy (catechol) structure in the B-ring, the 2,3-double bond in conjunction with a 4-oxo function and the additional presence of both 3- and 5-OH groups were three structural groups important for maximal radical-scavenging potential. The lower radical-scavenging activity of phloridzin (EC_{50} value of 0.60), compared with its analogue, 3-hydroxyphloridzin (EC_{50} value of 0.24), confirmed the importance of the o-dihydroxy B-ring for enhanced activity.

Procyanidin B2 (epicatechin dimer) on a molar basis had an EC_{50} value of 0.06 but, when calculated on a per epicatechin unit, the EC_{50} value was doubled to 0.12, showing it to be a little weaker than the quercetin glycosides (EC_{50} of 0.1–0.11). In a similar manner, both the epicatechin trimer and tetramer had EC_{50} of 0.115 on a per epicatechin unit basis and were slightly more effective

Table 1
Free radical-scavenging activity (EC_{50}) of polyphenols of apple pomace

Compound	EC_{50}	Compound	EC_{50}
Phloridzin	0.60	Epicatechin	0.135
3-Hydroxyphloridzin	0.24	Epicatechin dimer (procyanidin B2)	0.06 (0.12[a])
Chlorogenic acid	0.24	Epicatechin trimer	0.115[a]
Quercetin-3-glucoside	0.10	Epicatechin tetramer	0.115[a]
Quercetin-3-arabinoside	0.11	Epicatechin oligomer	0.15[a]
Vitamin C	0.35	Vitamin E	0.30

[a] Molar concentrations based on epicatechin unit.

scavengers than the dimer procyanidin B2, which in turn was superior to its monomer epicatechin (EC_{50} of 0.135). This trend did not extend to higher molecular weight procyanidins where the higher epicatechin oligomers (EC_{50} value of 0.15) showed the least activity in the epicatechin series, possibly due to increasing crowding and less availability of those affected hydroxyls to readily donate hydrogen. This observation was consistent with the report that both the procyanidin dimers, B1 and B3 were more effective in scavenging free radicals than monomer catechin (Ariga, Koshiyama & Fukushima, 1988).

3.3. Superoxide anion radical-scavenging activity of apple polyphenols

The superoxide-scavenging activity of apple polyphenols was determined using the cellular xanthine/xanthine oxidase system as a superoxide source. It should be noted, however, that these polyphenols could possess more than one mode of action in this assay system. They may participate as xanthine oxidase inhibitors (Aucamp, Gaspar, Hara & Apostolides, 1997; Chan, Wen & Chiang, 1995; Cotelle, Bernier, Catteau, Pommery, Wallet & Gaydou, 1996) as superoxide scavengers (Cos et al., 1998) or more as likely both. The activity is expressed here as equivalent SOD units for simplicity rather than to denote a mode of action. The results displayed in Fig. 3 showed that the oligomeric procyanidins (epicatechin trimer and tetramer) were particularly effective superoxide scavengers (with values around 300 SOD units/mg), followed by the dimer procyanidin B2 (242 SOD units/mg), monomer epicatechin (189 SOD units/mg) and the higher oligomers (142 SOD units/mg). The procyanidin series were all much more effective than quercetin glycosides which had 119-132 SOD units/mg. Chlorogenic acid and 3-hydroxyphloridzin both had SOD activity comparable to that of quercetin glycosides. Phloridzin had the lowest SOD activity which may be attributed to the limited contribution of a monophenol B-ring structure. Quercetin had 209 SOD units/mg of activity which was higher than its glycosides. The higher scavenging activity might be explained by the fact that sugar moieties did not contribute to the scavenging activity. Quercetin is reported to possess SOD activity as high as 2000 SOD units/mg (Okamura et al, 1993). This anomalously high value could be due to the non-linearity of the SOD standard curve used in the estimation of activity. When higher concentrations of compounds were tested, their SOD activity increased dramatically; thus caution needs to be taken when using this method. For best results,

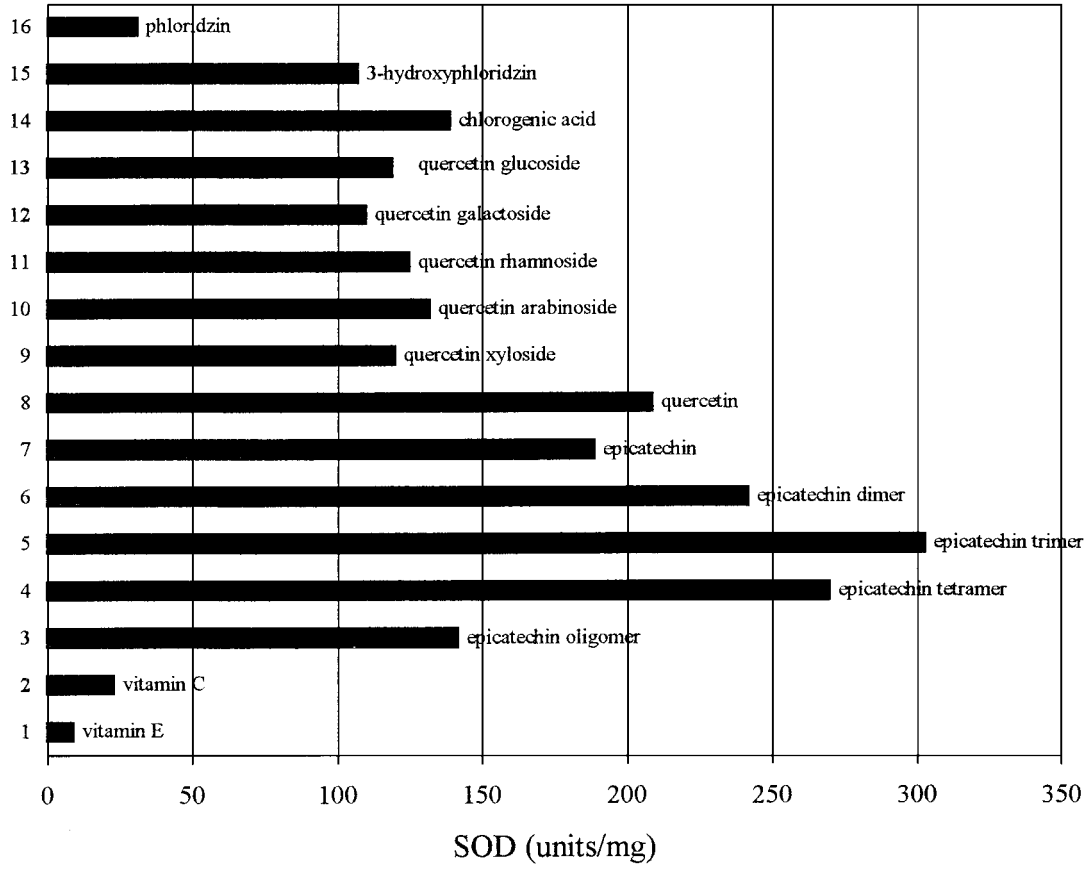


Fig. 3. Superoxide-scavenging activity of apple polyphenols.

the antioxidant concentrations should not exceed 0.2 mg/ml. Our results for epicatechin (189 SOD units/mg) and quercetin (209 SOD units/mg) were comparable with the IC_{50} value (μM) of 1.59 for epicatechin (Mw 290) and 1.63 for quercetin (Mw 302) reported by Cos et al. (1998).

All the apple polyphenols, including phloridzin, were superior to vitamins C and E which had activities of 23 and 9 SOD units/mg, respectively, as superoxide anion radical-scavengers. The procyanidins, in particular, were found to have very potent SOD activity, being 10–30 times more active than the vitamins. The foregoing results showed that the epicatechin, procyanidins, flavonoid glycosides and chlorogenic acid were the most active antioxidant constituents in apple pomace. These are the compounds which should form the target of commercial utilization of the pomace.

4. Conclusion

Polyphenols responsible for the antioxidant activity in apple are still present in the pomace, which could be commercially exploited. A range of polyphenolic compounds isolated from apple pomace, comprising phloridzin, 3-hydroxyphloridzin, chlorogenic acid, epicatechin, epicatechin dimer (procyanidin B2), trimer, tetramer and oligomer, and quercetin glycosides, were evaluated for their antioxidant, DPPH free radical- and superoxide anion radical-scavenging properties. All the apple polyphenols exhibited good antioxidant properties, having 2–3 times the DPPH-scavenging and 10–30 times superoxide-scavenging activities of vitamins C or E. The lower molecular weight procyanidins and the quercetin glycosides, in particular, showed excellent activity in the assays. The apple pomace could provide a cheap and readily available source of dietary antioxidants.

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