



## Phenolic acid content of fruits commonly consumed and locally produced in Scotland

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### ABSTRACT

Despite fruit, vegetables and many processed products counting towards achieving the recommended five-a-day strategy, it is inevitable that produce choice will affect the benefits delivered. Fruits locally produced and commonly consumed in Scotland were compared for their phenolic acid content and form. The phenolic acid composition was highly variable, but the locally produced fruits were significantly ( $p < 0.001$ ) higher in total concentration (1.61–4.89 g/kg compared to 0.06–0.22 g/kg). The majority of the phenolic acids were conjugated to other plant components, suggesting that any health benefits derived from these compounds are likely to be after they are released/metabolised by the colonic microbiota. Although the potential protective effects of the individual compounds will not be ascertained until the exact role of these compounds in disease prevention has been clarified, it is clear that the total amount of phenolic acids in the diet will vary enormously depending on the types of fruits consumed.

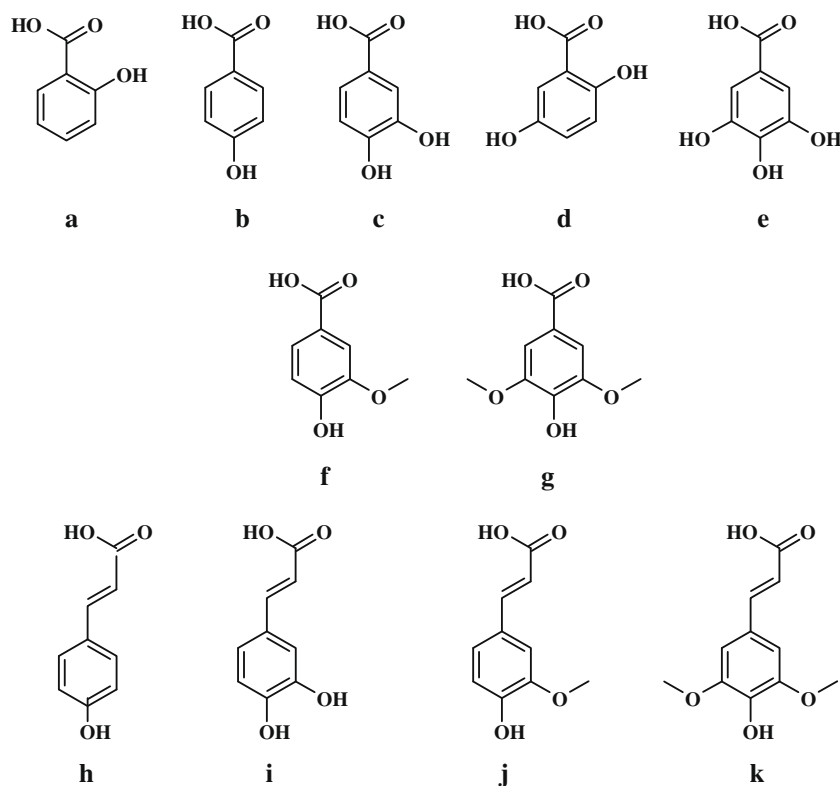
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### 1. Introduction

To address Scotland's poor dietary and nutritional status, one strategic government policy is to encourage the consumption of fruit and vegetables. Decades of research have associated increased fruit and vegetable consumption with disease prevention and in particular with diseases of the gastrointestinal tract (Riboli & Norat, 2003; Shannon, White, Shattuck, & Potter, 1996). Although, it is suggested that fruit, vegetables and many of their processed products count towards achieving the recommended five-a-day strategy, it is without doubt that the choice of produce will affect the potential benefits delivered. Of the many phytochemicals in plant-based foods considered beneficial for human health, phenolic compounds have received much attention (Surh, 1999). The phenylpropanoids and their derivatives are of particular interest, as these secondary metabolites are produced in plants in response to stress (Nicholson & Hammerschmidt, 1992). It is likely, that the properties required by the plant (e.g. anti-oxidant and radical scavenging ability) also impart some of the potential protective properties of these compounds in the diet (Fig. 1). The presence of these phenolic metabolites in plants is dependent on

the environmental conditions under which it is grown, the content will vary not only with species, but within species and higher concentrations of certain phenolic compounds are more likely in plants grown in more hostile environments. There is now much evidence to suggest that soft-flesh fruits, commonly referred to as 'berries', may have beneficial effects against several human cancers and that lower molecular weight phenolic compounds may be the active constituents (Boivin, Blanchette, Barrette, Moghrabi, & Beliveau, 2007; Kresty et al., 2006; Seeram, 2008). Fruits such as strawberries and raspberries are traditionally a part of the Scottish diet. Although still locally produced, diet surveys and supermarket statistics suggest that fruits mostly produced outside Scotland such as bananas, apples, oranges, pears and grapes are more commonly consumed (Duthie et al., 1991). However, relatively little information is available regarding the phenolic composition of fruits both grown and consumed in Scotland. Also, often overlooked are the phenolic acids, and in particular those attached to other plant components. These esterified phenolic compounds represent a major fraction, which after consumption and metabolism, are likely to be bio-available and possibly bio-active in the gastrointestinal tract (Glinghammar, Holmberg, & Rafter, 1999; Haza, Glinghammar, Grandien, & Rafter, 2000; Jenner, Rafter, & Halliwell, 2004; Karlsson et al., 2005). In this study we have measured the total amount of potentially bio-available phenolic acids

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**Fig. 1.** Structures of the predominant benzoic (C6C1; a–g) and cinnamic acids (C6C3; h–l) found in commonly consumed and locally produced Scottish fruits. Salicylic acid (a) *p*-hydroxybenzoic acid (b) protocatechuic acid (c) gentisic acid (d) gallic acid (e) vanillic acid (f) syringic acid (g) *p*-coumaric acid (h) caffeic acid (i) ferulic acid (j) and sinapic acid (k).

present in selected Scottish fruits produced locally and therefore, not subject to long-term storage. These have been compared with the phenolic acid content of five commonly consumed fruits, which are representative of a typical five-a-day fruit selection in Scotland.

## 2. Materials and methods

### 2.1. General reagents

Standard phenolic acids and general laboratory reagents were purchased from Sigma/Aldrich (Gillingham, UK).

### 2.2. Fruits

Raspberries (*Rubus idaeus* L.; Autumn Bliss; Scotland), gooseberries (*Ribes uva-crispa* L.; Whinhams Industry; Scotland), blackcurrants (*Ribes nigrum* L.; Ben Sarek; Scotland) and strawberries (*Fragaria ananassa* L.; Elsanta; Scotland) were all purchased directly from a fruit farm in Tayside, Scotland. Banana (*Musa acuminata* L.; Cavendish; Dominican Republic), Apple (*Malus domestica* L.; Braeburn; England), Pear (*Pyrus communis* L.; Conference; Holland), White Grapes (*Vitis vinifera* L.; Thompson; South Africa) and Oranges (*Citrus sinensis* L.; Navel-late; Spain) were purchased from a local supermarket. All fruits were considered to be ripe and were prepared in accordance with the predominant method of consumption. This involved removing any remaining leaves and stalks from the raspberries, gooseberries, blackcurrants, strawberries and grapes, removing a small cored segment containing the seeds from the apples and pears, removing the outer skin from the bananas and both the outer peel and seeds from the oranges.

### 2.3. Extraction and analysis of phenolics acids from fruits

All fruits were washed, sectioned where necessary, weighed and stored at  $-80^{\circ}\text{C}$ . They were then lyophilised (Heto Lab Equipment; Allerød; Denmark) and the moisture loss recorded. They were freeze-milled (Spex 6700; Edison; USA) and the powder stored in a desiccator prior to extraction. Samples (2 g dry weight;  $n = 3$ ) were suspended in water ( $100\text{ cm}^3$ ), in which the pH was reduced to pH 2 with HCl ( $6\text{ mol dm}^{-3}$ ), extracted into ethyl acetate (EtOAc;  $50\text{ cm}^3$ ) and the layers separated by centrifugation (15 min;  $3800g$ ). The extraction was repeated three times and the EtOAc extracts combined. The organic layer was left to stand over sodium sulphate (anhydrous) for one hour and filtered through number one filter paper (Whatman; England) washing the sodium sulphate with EtOAc (anhydrous). The combined organic layers were then evaporated to dryness under reduced pressure at temperature not exceeding  $40^{\circ}\text{C}$  and stored in a desiccator prior to analysis by HPLC. The pH of the aqueous fraction was increased to pH 7 with sodium hydroxide ( $4\text{ mol dm}^{-3}$ ). Sodium hydroxide ( $4\text{ mol dm}^{-3}$ ) was added to give a final concentration of  $1\text{ mol dm}^{-3}$  and the sample stirred at room-temperature for 4 h under nitrogen. The pH was reduced to pH 2 with HCl ( $6\text{ mol dm}^{-3}$ ) and the samples extracted into EtOAc ( $50\text{ cm}^3 \times 3$ ) and processed as described above. The pH of the aqueous fraction was then increased to pH 7 with NaOH ( $4\text{ mol dm}^{-3}$ ). HCl ( $10\text{ mol dm}^{-3}$ ) was added to give a final concentration of  $2\text{ mol dm}^{-3}$  and the sample incubated with stirring at  $95^{\circ}\text{C}$  for 30 min, cooled and extracted with EtOAc ( $50\text{ cm}^3 \times 3$ ) and processed again as described above. The extracts were then re-dissolved in methanol and filtered through a  $0.2\text{ }\mu\text{m}$  polyvinylidene fluoride membrane. Separation of the phenolic compounds was by HPLC (Spectra SYSTEM; Thermo Fisher Scientific; UK) using a Polar-RP column ( $250 \times 4.6\text{ mm}$ ;  $4\text{ }\mu\text{m}$ ) (Phenomenex; UK) with

AcCN and trifluoroacetic acid (0.05% v/v; pH 2.3) and employing gradient elution: 11–14% AcCN (35 min), 14–50% AcCN (5 min), 50% AcCN (10 min) and 50–11% AcCN (5 min). Detection was at 215 and 280 nm and the metabolites were quantified by post-extraction internal standardisation (4-hydroxyacetovanillone) with reference to their relative retention times ( $t_R$ ) and use of response factors calculated from pure compounds (Table 1, Figs. 2 and 3). The limits of detection for quantification were lower than  $100 \text{ ng dm}^{-3}$ . The identity of the phenolic acids was also confirmed by LC-MS (Finnigan MAT 900; Bremen, Germany) ( $t_R$  and Molecular Ion).

### 3. Results

The locally produced Scottish fruits had significantly ( $p < 0.01$ ) higher concentrations of phenolic acids ( $1.61\text{--}4.89 \text{ g kg}^{-1}$  dry weight) than the commonly consumed fruits ( $0.07\text{--}0.22 \text{ g kg}^{-1}$  dry weight). The fruits were highly variable in their composition with strawberries, raspberries and blackcurrants having the most similar profiles and all having gallic acid as the principle component (29%, 48% and 31%, respectively). In these fruits, gallic acid was mostly found conjugated to other plant components. With the exception of apples (which were particularly rich in the free form

of *p*-hydroxybenzoic acid), the conjugated phenolic acids were the major fraction for all fruits. Cinnamic acids, and in particular ferulic acid is considered to have a structural role in cross-linking wall polymers and this component was particularly high in bananas (69%), oranges (28%) and strawberries (14%). This is likely to be due to the esterification of ferulic acid to cross-link the fibrous strands in bananas (Oliveira et al., 2006), the presence of achenes in strawberries (Aaby, Wrolstad, Ekeberg, & Skrede, 2007) and the remaining albedo in the edible portion of oranges (Kanes, Tisserat, Berhow, & Vandercook, 1993). Gooseberries and blackcurrants both belong to the Grossulariaceae family, and although similar in terms of phenolic composition, blackcurrants were particularly rich in gallic acid, whereas conjugated *p*-coumaric acid was the principle phenolic acid in gooseberries (31%). Hydroxylated phenolic acids were the major phenolic acid fraction in gooseberries (93%), blackcurrants (92%) and grapes (96%), with the bulk of these acids (>82%) also being esterified to other components. Apples were the only fruit found to be rich in free hydroxylated phenolic acids (42%) and this was due to *p*-hydroxybenzoic acid being the major component of this fraction (34%). Pears were exceptional in that they were the only fruit that were particularly rich in methylated phenolic acids, with 70% of the phenolic acids being dimethylated (syringic and sinapic acid) compared to less than 23% for all of the other fruits analysed.

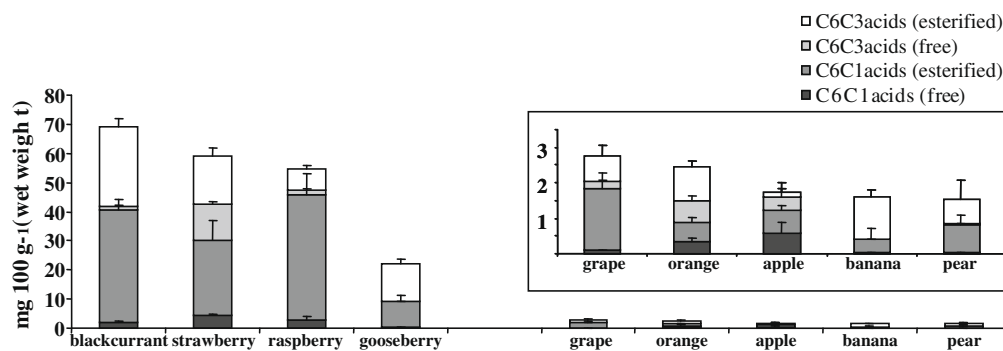


Fig. 2. Comparison of cinnamic (C6C3) and benzoic (C6C1) acids in commonly consumed and locally produced Scottish fruits. Values are specified on a wet weight basis in  $\text{mg } 100 \text{ g}^{-1}$ , which corresponds to approximately one fruit portion and are given as mean  $\pm$  standard deviations ( $n = 3$ ).

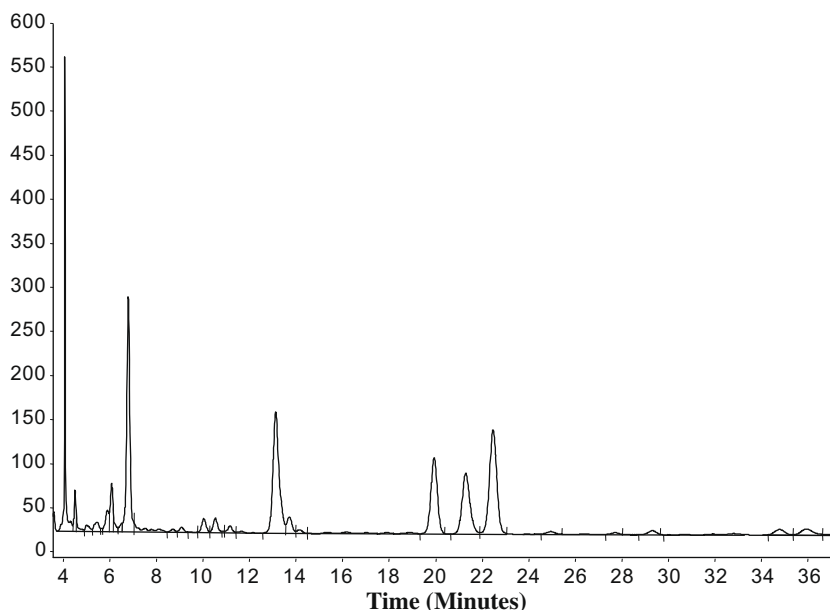


Fig. 3. Typical chromatogram in this case showing the acid-labile phenolic acids liberated from Gooseberries.

#### 4. Discussion

There were significant ( $p < 0.01$ ) differences between the commonly consumed and locally produced Scottish fruits in terms of total phenolic acid concentrations, whether this is expressed as dry (Table 2) or wet weight (Fig. 2). Although this current study does not focus on direct comparisons between countries of origin or storage conditions, the disparity was consistent for all fruits studied and of significant magnitude to be of interest in terms of potential health benefits. Whether the phenolic acids were found to be in the free form or conjugated to other plant components is likely to have an effect on bio-availability. Conjugated compounds, particularly those attached to cell wall components such as polysaccharides and lignin are more likely to be bio-available for microbial metabolism and uptake in the colon (Poquet, Clifford, & Williamson, 2008). Free phenolic acids may be directly absorbed in the small intestine, either for rapid metabolism and/or excretion.

**Table 1**  
Phenolic acid IUPAC names, synonyms, HPLC-UV retention times ( $t_R$ ) and molecular ions ( $M^+$ ).

Phenolic acid (IUPAC name)	Synonym	$t_R$ (min)	$M^+$ (amu)
3,4,5-Trihydroxybenzoic acid	Gallic acid	4.46	169.00
3,4-Dihydroxybenzoic acid	Protocatechuic acid	6.72	153.10
4-Hydroxybenzoic acid	<i>p</i> -Hydroxybenzoic acid	10.40	137.00
2,5-Dihydroxybenzoic acid	Gentisic acid	11.58	153.10
3-(3,4-Dihydroxyphenyl)acrylic acid	Caffeic acid	12.85	179.00
4-Hydroxy-3-methoxybenzoic acid	Vanillic acid	13.45	167.00
4-Hydroxy-3,5-dimethoxybenzoic acid	Syringic acid	15.37	197.00
3-(4-Hydroxyphenyl)acrylic acid	<i>p</i> -Coumaric acid	22.21	163.10
3-(4-Hydroxy-3-methoxyphenyl)acrylic acid	Ferulic acid	29.23	193.10
3-(4-Hydroxy-3,5-dimethoxyphenyl)acrylic acid	Sinapic acid	31.71	223.10
2-Hydroxybenzoic acid	Salicylic acid	33.71	137.00

**Table 2**  
Phenolic acids content of fruits.

Moisture (%)	Raspberry	Gooseberry	Blackcurrant	Strawberry	Banana	Apple	Pear	Grapes	Orange
	84.4	86.4	79.0	87.9	73.9	85.2	84.5	69.4	88.8
<i>Phenolic acid (free)</i>									
Gallic	5.73 ± 0.53	1.27 ± 0.50	5.63 ± 0.64	1.67 ± 0.29	0.02 ± 0.03	0.11 ± 0.04	n.d.	0.17 ± 0.03	n.d.
Protocatechuic	3.83 ± 0.83	27.22 ± 2.13	10.45 ± 1.70	39.09 ± 2.07	0.16 ± 0.02	3.37 ± 0.84	0.45 ± 0.24	1.67 ± 0.68	2.53 ± 1.19
<i>p</i> -Hydroxybenzoic	33.31 ± 6.54	n.d.	4.33 ± 0.97	193.91 ± 19.88	n.d.	34.09 ± 18.62	0.48 ± 0.15	1.20 ± 0.60	17.41 ± 6.32
Gentisic	n.d.	2.01 ± 0.40	n.d.	30.85 ± 2.10	n.d.	n.d.	n.d.	n.d.	0.20 ± 0.12
Caffeic	2.33 ± 0.62	13.23 ± 1.45	3.73 ± 0.38	n.d.	n.d.	n.d.	n.d.	7.03 ± 1.04	1.00 ± 0.82
Vanillic	24.58 ± 6.32	n.d.	15.01 ± 2.54	98.46 ± 3.73	0.30 ± 0.01	n.d.	n.d.	n.d.	4.86 ± 1.35
Syringic	107.51 ± 47.28	n.d.	n.d.	n.d.	0.21 ± 0.10	1.10 ± 0.64	n.d.	n.d.	2.53 ± 0.76
<i>p</i> -Coumaric	n.d.	n.d.	19.83 ± 11.79	n.d.	n.d.	11.65 ± 10.39	n.d.	n.d.	6.65 ± 2.74
Ferulic	74.33 ± 17.20	n.d.	n.d.	567.89 ± 28.82	n.d.	n.d.	n.d.	n.d.	30.06 ± 3.99
Sinapic	36.89 ± 10.01	n.d.	n.d.	450.30 ± 27.54	n.d.	13.42 ± 18.38	0.96 ± 0.63	n.d.	17.28 ± 2.96
Salicylic	7.64 ± 4.77	n.d.	62.09 ± 5.44	n.d.	n.d.	n.d.	0.37 ± 0.20	n.d.	2.37 ± 0.61
<i>Phenolic acid (conjugated)</i>									
Gallic	1669.25 ± 256.24	43.59 ± 15.63	999.18 ± 82.06	1416.04 ± 443.09	3.22 ± 1.42	0.44 ± 0.15	0.45 ± 0.23	1.29 ± 0.26	n.d.
Protocatechuic	273.26 ± 68.81	389.80 ± 100.52	355.91 ± 53.78	76.15 ± 17.59	n.d.	28.47 ± 5.51	2.65 ± 0.59	21.17 ± 2.32	1.79 ± 0.98
<i>p</i> -Hydroxybenzoic	676.31 ± 76.59	120.75 ± 19.75	259.16 ± 16.41	429.52 ± 58.12	1.62 ± 0.69	n.d.	n.d.	6.94 ± 1.35	15.12 ± 7.71
Gentisic	n.d.	41.28 ± 6.30	77.37 ± 6.87	89.79 ± 17.80	0.64 ± 0.54	2.29 ± 0.99	5.81 ± 3.81	2.99 ± 0.31	0.92 ± 0.29
Caffeic	63.95 ± 8.05	354.51 ± 60.82	537.23 ± 58.43	130.97 ± 61.85	n.d.	1.77 ± 0.40	2.81 ± 0.48	0.40 ± 0.20	3.63 ± 0.46
Vanillic	94.34 ± 26.65	48.21 ± 6.07	83.40 ± 6.22	47.38 ± 10.36	1.04 ± 1.12	5.21 ± 1.14	7.98 ± 1.03	0.22 ± 0.21	13.74 ± 1.39
Syringic	5.91 ± 3.07	n.d.	10.27 ± 1.25	n.d.	3.54 ± 6.92	n.d.	33.03 ± 11.16	n.d.	0.59 ± 0.41
<i>p</i> -Coumaric	224.64 ± 37.62	504.81 ± 62.83	591.31 ± 58.18	1107.61 ± 142.65	3.47 ± 3.33	2.00 ± 0.55	5.84 ± 3.28	16.31 ± 5.97	20.89 ± 10.76
Ferulic	127.74 ± 12.85	67.27 ± 5.37	120.96 ± 13.99	121.79 ± 29.90	40.07 ± 7.98	0.56 ± 0.17	0.10 ± 0.05	n.d.	30.01 ± 2.79
Sinapic	42.89 ± 9.33	n.d.	37.34 ± 4.69	n.d.	0.27 ± 0.53	6.34 ± 4.93	34.87 ± 32.92	2.93 ± 1.66	28.86 ± 2.94
Salicylic	25.13 ± 5.57	n.d.	55.76 ± 9.41	93.17 ± 9.57	3.93 ± 0.68	6.73 ± 1.53	1.93 ± 0.59	9.50 ± 1.36	16.97 ± 1.95

Conjugated phenolic acids are a summation of both the alkali- and acid-labile fractions. Values are specified on a dry weight basis in mg kg<sup>-1</sup> and are given as mean ± standard deviations ( $n = 3$ ). Not detected = n.d.

With regard to the potential health effects of the individual phenolic acids, the form in which the compounds are available will be important. Conjugated compounds available in the colon will be extensively metabolised by the gut microflora (Blaut, Schoefer, & Braune, 2003; Clavell, Borrmann, Braune, Dore, & Blaut, 2006). It is likely that these metabolites, which are in contact with the gut wall, could impart a protective effect on human health (Glinghammar et al., 1999; Haza et al., 2000; Karlsson et al., 2005). In previous studies, we have shown that metabolism of ferulic acid by a faecal inoculum resulted in the formation of various metabolites which had significantly different inflammatory effects in colon cells in culture (Russell, Drew, Scobbie, & Duthie, 2005). However, free phenolic acids that are absorbed prior to metabolism could have a direct cellular effect (Kountouri, Mylona, Kaliora, & Andrikopoulos, 2007; Garcia-Alonso, Ros, Vidal-Guevara, & Periago, 2007). Conversely, absorption and excretion of these low molecular weight compounds could be a mechanism for removing potentially toxic compounds from the diet. Absorption of protocatechuic acid has been shown to modulate the activity of xenobiotic detoxifying enzymes (Krajka-Kuzniak, Szaefer, & Baer-Dubowska, 2008), suggesting a potential role of these compounds in the removal of carcinogens.

For all fruits studied, with the exception of apple, conjugated phenolic acids were the predominant fraction. Therefore, the bulk of the phenolic compounds are likely to be available for metabolism by the gut microbiota. For diseases of the gastrointestinal tract, potential protective effects derived from fruit phenolics are likely to be due to the metabolites obtained from this fraction. However, only once these metabolites are identified and the role of these compounds in disease prevention ascertained would the potential health benefits become clear. What is clear, is that fruit selection will determine the types of phenolic acids delivered and that the total amount of phenolic acids in the diet will vary enormously depending on the types of fruits consumed. It is likely that locally produced Scottish soft-fruits will deliver a higher concentration of phenolic acids compared to the current most commonly consumed fruits.

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