



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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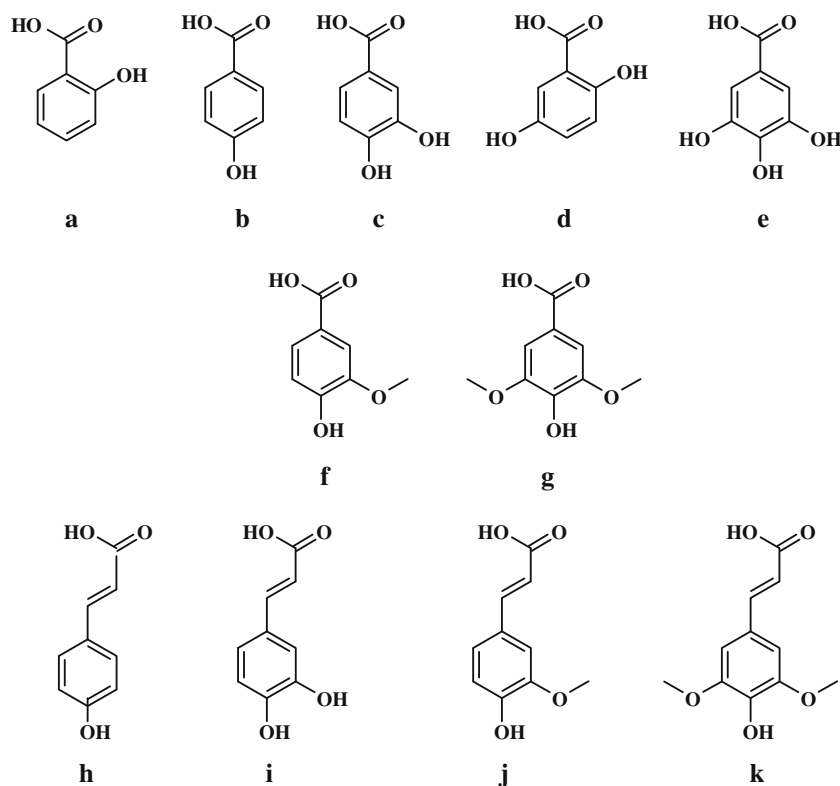


Fig. 1. Structures of the predominant benzoic (C6C1; a–g) and cinnamic acids (C6C3; h–l) found in commonly consumed and locally produced Scottish fruits. Salicylic acid (a) *p*-hydroxybenzoic acid (b) protocatechuic acid (c) gentisic acid (d) gallic acid (e) vanillic acid (f) syringic acid (g) *p*-coumaric 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°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 cm^3), in which the pH was reduced to pH 2 with HCl (6 mol dm^{-3}), extracted into ethyl acetate (EtOAc; 50 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°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 mol dm^{-3}). Sodium hydroxide (4 mol dm^{-3}) was added to give a final concentration of 1 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 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 mol dm^{-3}). HCl (10 mol dm^{-3}) was added to give a final concentration of 2 mol dm^{-3} and the sample incubated with stirring at 95°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

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