ORIGINAL CONTRIBUTION

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Salicylic acid in soups prepared from organically and non-organically grown vegetables

Summary Background Salicylic acid is a chemical signal in plants infected by pathogens and it is responsible for the anti-inflammatory action of aspirin. Patients who take aspirin have a reduced risk of

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developing atherosclerosis and colorectal cancer, both of these pathologies having an inflammatory component. Dietary salicylic acid may help to prevent these conditions. We wondered if foods made from organically-reared plants might have a higher content of salicylic acid than those made from non-organic plants, since the latter are more likely to be protected from infection by the application of pesticides. Objective To determine if organic vegetable soups have a higher salicylic acid content than non-organic vegetable soups. Methods The contents of salicylic acid in organic and non-organic vegetable soups purchased from supermarkets were determined. Salicylic acid was identified by varying the chromatographic conditions and comparing the retention times of the unknown substance in the extracts with salicylic acid; by treating extracts of the soups with salicylate hydroxylase; and by using GCMS. Salicylic acid was determined by using HPLC

with electrochemical detection. Results Salicylic acid was present in all of the organic and most of the non-organic vegetable soups. The median contents of salicylic acid in the organic and non-organic vegetable soups were 117 (range, 8–1040) ng \cdot g⁻¹ and 20 (range, 0–248) ng \cdot g⁻¹ respectively. The organic soups had a significantly higher content of salicylic acid (p=0.0032 Mann Whitney U test), with a median difference of 59 ng \cdot g⁻¹ (95% confidence interval, 18–117ng \cdot g⁻¹). Conclusions Organic vegetable soups contained more salicylic acid than non-organic ones, suggesting that the vegetables and plants used to prepare them contained greater amounts of the phenolic acid than the corresponding non-organic ingredients. Consumption of organic foods may result in a greater intake of salicylic acid.

Key words salicylic acid – organic food – vegetable soups

Introduction

Despite the scarcity of scientific comparisons of their chemical constituents, it is widely perceived that organic foods are safer and more nutritious than non-organic foods. Salicylic acid is present in many foods derived from plants [1], where it functions as a chemical signal

whose concentration is increased when plants become infected by pathogens [2]. Foodstuffs produced from plants reared organically, which are not protected by the application of pesticides, may therefore contain more salicylic acid.

In a recent study we showed that salicylic acid is a normal constituent of serum in individuals not consuming salicylate drugs [3]. We also reported higher $\frac{34}{5}$ serum concentrations of salicylic acid in vegetarians than in non-vegetarians, and found that there was overlap in the serum salicylic acid concentrations of the vegetarians and patients taking low dose aspirin [4]. These results indicate that dietary fruits and vegetables are a source of salicylic acid, an anti-inflammatory compound that may be responsible for some of the health benefits of a diet rich in fruits and vegetables [5]. By measuring the salicylic acid content of various organic and non-organic soups made from plants or plant products, we now test the hypothesis that food prepared from organically grown vegetables contains more salicylic acid.

Methods

We examined 2 non-organic home-made soups, and 22 different non-organic and 11 organic vegetable soups (Table 1) obtained from supermarkets and shops in Dumfries, Scotland. The soups were homogenised (Ul-tra-turrax) for 5 min and portions (1g) were acidified and extracted; the substances extracted were separated by HPLC and salicylic acid was quantified electrochemically, according to our method for determining the acid in serum [4].

The identity of the substance present in the extracts of the soups which eluted at the retention time (Rt) of salicylic acid was investigated by 1) altering the chromatographic conditions, by using isocratic elution with methanol (50% vol/vol) in citrate buffer, 30 mmol/l, pH 4.0 (mobile phase A from the above method), 2) treating extracts of three soups with salicylate hydroxylase, an enzyme which catalyses the conversion of salicylic acid into catechol, as described previously (3), 3) collecting the fraction that was eluted from the HPLC column between 1 min prior to and 1 min after the Rt of salicylic acid, with the detector switched off. The material eluted was treated with 2 ml of acetyl chloride in methanol (300 µl of acetyl chloride added to 10 ml of methanol containing 0.1% water) and the mixture was heated at 60 °C for 2h and then evaporated at 60 °C with a stream of nitrogen. The residue was dissolved in ethyl acetate (2 ml) and portions $(1 \mu \text{l})$ applied to a GC column; DB-5 capillary column (30 m \times 0.25 mm) fitted to a Fisons 8000 series gas chromatograph, interfaced with an MD 800 mass spectrometer (Thermoquest, Manchester, UK). The temperature of the column was increased from 54 °C at 11 °C/min until it reached 300 °C. The injection port temperature was 200 °C. The mass spectrometer electron energy was + 70 eV set in full scan mode.

The efficiency of extraction was investigated by adding 15 ng of salicylic acid to 1 g portions of five of the soups prior to extraction. The intra-assay coefficient of variation (CV) for the determination of salicylic acid was calculated from duplicate determinations of the **Table 1** The soups investigated and their salicylic acid content

Organic soups (n = 11)		Salicylic acid content (ng · g ^{−1})
Baxters	Tomato and Vegetable	134
Go-organic	Tomato and Basil	8
Organic soups	Cream of Tomato	22
Baxters	Carrot and Parsnip with Nutmeg	120
Organic soups	Vegetable	63
Organic soups	Lentil	117
Simply	Mediterranean Tomato	54
Simply	Carrot and Coriander	1040
Suma	Carrot and Coriander	138
Suma	Spicy Lentil	301
Tesco	Tomato	34
Non-organic soups (n = 24)		
Asda	Country Vegetable	85
Baxters	Lentil and Vegetable	nd
Baxters	Traditional Tomato	nd
Asda	Tomato	5
Campbell	Tomato	22
Asda	Vegetable	60
Heinz	Vegetable	32
Baxters	Mediterranean Tomato	nd
Campbells	Mediterranean Tomato	8
Heinz	Mediterranean Tomato	248
St Michaels	Mediterranean Tomato	19
Baxters	Carrot and Coriander	nd
St Michaels	Carrot and Coriander	21
Heinz	Carrot and Coriander	16
Campbells	Carrot and Coriander	34
Campbells	Asparagus	16
Campbells	Tomato and Red Pepper	22
Tesco	Tomato and Red Pepper	8
Со-ор	Tomato and Lentil	23
Heinz	Lentil	73
Home-made	Lentil	36
Heinz	Cream of Tomato	9
St. Michaels	Tomato and Basil	13
Home-made	Scotch Broth	160

nd not detected

content in 22 soups. The inter-assay CVs for the determination of salicylic acid in 4 different soups were also determined. (The contents of 2 soups were determined on 4 separate occasions and the contents of the other 2 were determined 5 times.) Salicylic acid was determined in two different batches of eight soups to assess the variation of content of different batches.

The salicylic acid contents of the soups were not normally distributed and median values and the range observed are reported. Comparison of the medians was carried out using the Mann Whitney U test (two-tailed).

Results

An unknown substance present in the extracts of most of the soups had Rt similar to that of salicylic acid (see Fig. 1a). The substance and salicylic acid had Rt of 7.35 and 7.36 min respectively when isocratic elution and mobile phase A were used. When the extracts of soup were treated with salicylate hydroxylase the unknown substance disappeared (Fig. 1a). The unknown substance isolated from soup had, after esterification, a mass spectrum which most closely fitted that of methyl salicylate (first hit, 89.7 % match, Pfleger, Mauer and Weber spectral library). All of the organic and 20 out of 24 of the non-organic vegetable soups contained salicylic acid.

The median contents of salicylic acid in the organic and non-organic soups were 117 (range, 8–1040) ng \cdot g⁻¹ and 20 (range, 0–248) ng \cdot g⁻¹ respectively, the organic soups having a significantly higher content (p=0.0032, Mann-Whitney U test), with a median difference of 59 ng \cdot g⁻¹ (95% confidence interval, 18–117 ng \cdot g⁻¹). Examination of several soups produced by one manufacturer revealed that all four of the non-organic soups investigated were devoid of salicylic acid (see Fig. 1b for example), whereas salicylic acid was present in both of the other organic soups. The intra-assay CV was 1.2% and inter-assay CVs of 2.4% (mean value of 5 determinations, 8 ng \cdot g⁻¹), 6.8% (mean value of 4 determinations, 15 ng \cdot g⁻¹), 0.7% (mean value of 5 determinations, 41 ng \cdot g⁻¹) and 3.6% (mean value of 4 determinations, 83 ng \cdot g⁻¹) were observed for 4 different soups. The efficiency of extraction of exogenous salicylic acid was found to be 78%, 72%, 83%, 77% and 84% respectively, when salicylic acid (15 ng) was added to 5 different soups (1 g portions) prior to extraction. Eight commercial soups were investigated for variation of salicylic acid content between batches. In 4 out of the 8 the variation was less than 10%.

Discussion

Our results show that salicylic acid is present in all of the organic, and most of the non-organic vegetable soups we examined. Moreover, they show that organic vegetable soups have a higher content of salicylic acid than non-organic ones, suggesting that the vegetables and plants used to prepare them contained greater amounts

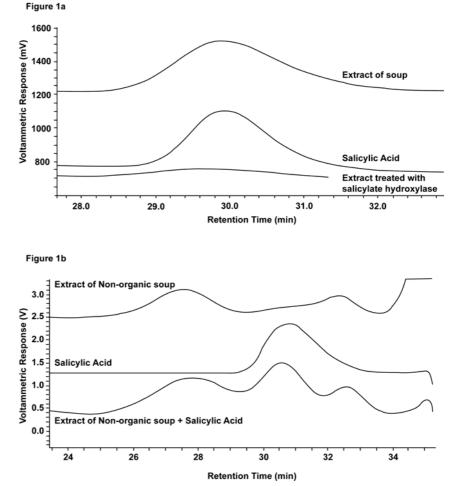


Fig. 1 Chromatography of salicylic acid and extracts of soup: **a** extract of a soup before and after treatment with salicylate hydroxylase, **b** non-organic soup extracted before and after addition of salicylic acid $(15 \text{ ng} \cdot \text{g}^{-1})$.

of the phenolic acid than the corresponding non-organic ingredients.

Other factors, however, might have influenced our results. It is likely that the different types of soup we investigated were prepared by using different procedures, employing different varieties of vegetables and plants which had been grown, stored and handled under different climatic and environmental conditions. All of these factors are believed to affect the content of nutrients in foodstuffs derived from plants [6]. It is interesting to note, however, that the organic soups prepared by a certain manufacturer contained salicylic acid, whereas the non-organic soups prepared by them were devoid of the phenolic acid. The minor ingredients used in the manufacture of the soups are important also; certain herbs and spices have high contents of salicylic acid [1], and the proportions of these in the soups investigated might explain the difference recorded. Lastly, a combination of all of these factors may be responsible for our finding. Other work has identified differences between organic and non-organic foods. However, it is worthy of note that a recent comparison of organic and non-organic apples has indicated that the former might have a higher content of nutrients [7].

Aspirin is consumed on a massive scale. It is prescribed mainly for its anti-platelet effect in cardiovascular disease [8], but it is thought also to decrease the risk of developing colorectal cancer [9]. Aspirin is metabolised rapidly to generate salicylic acid [10], the major metabolite, which is probably responsible for its anti-inflammatory effect [11]. Inflammatory processes are important in various chronic pathologies, including atherosclerosis [12] and cancer [13], the risks of which are reduced by consumption of aspirin. Whether or not salicylic acid derived from dietary sources is beneficial to health has not been established. Its effects have not been investigated, but it has been suggested that because our daily intake might be less than 10 mg, and is not in the form of aspirin, its effects are unlikely to be significant [14]. However, concentrations of salicylic acid in the serum of vegetarians who do not take aspirin overlap with those in the serum of patients who have taken low doses of aspirin over a long period of time [4]. An increased dietary intake of salicylic acid might be most effectively achieved by consuming fruits, vegetables, herbs and spices, and this report highlights the difference in the content of salicylic acid in organic and nonorganic vegetable soups. Further studies are required to establish whether this difference occurs in other foodstuffs, and whether the consumption of organic foods results in a significantly increased dietary intake of salicylic acid.

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References

- 1. Venema DP, Hollman PCH, Janssen KPLTM, Katan MB (1996) Determination of acetylsalicylic acid and salicylic acid in foods, using HPLC with fluorescence detection. J Agric Food Chem 44:1762–1767
- Dempsey D'MA, Shah J, Klessig DF (1999) Salicylic acid and disease resistance in plants. Crit Rev Plant Sci 18:547-575
- Paterson JR, Blacklock CJ, Campbell G, Wiles D, Lawrence JR (1998) The identification of salicylates as normal constituents of serum: a link between diet and health? J Clin Pathol 51:502–505
- 4. Blacklock CJ, Lawrence JR, Wiles D, et al. (2001) Salicylic acid in the serum of subjects not taking aspirin. Comparison of salicylic acid concentrations in the serum of vegetarians, non-vegetarians, and patients taking low dose aspirin. J Clin Pathol 54:553–555

- 5. Paterson JR, Lawrence JR (2001) Salicylic acid: a link between aspirin, diet and the prevention of colorectal cancer. Q J Med 94:445–448
- Goldman IL, Kader AA, Heintz C (1999) Influence of production, handling, and storage on phytonutrient content of foods. Nutrition Reviews 57:S46–52
- Reganold JP, Glover JD, Andrews PK, Hinman HR (2001) Sustainability of three apple production systems. Nature 410:926–930
- Antiplatelet Trialists Collaboration (1994) Collaborative overview of randomised trials of antiplatelet treatment. I. Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. B M J 308:81–106
- Baron JA, Sandler RS (2000) Nonsteroidal anti-inflammatory drugs and cancer prevention. Annu Rev Med 51: 511–523

- Needs CJ, Brooks PM (1985) Clinical pharmacokinetics of the salicylates. Clinical Pharmacokinetics 10:164–177
- 11. Higgs GA, Salmon JA, Henderson B, Vane JR (1987) Pharmacokinetics of aspirin and salicylate in relation to inhibition of arachidonate cyclooxygenase and antiinflammatory activity. Proc. Natl. Acad. Sci USA 84:1417-1420
- Ross R (1999) Atherosclerosis an inflammatory disease. N Engl J Med 340: 115–126
- Balkwill F, Mantovani A (2001) Inflammation and cancer: back to Virchow. Lancet 357:539–545
- 14. Janssen PLTMK, Hollman PCH, Reichman E, Venema DP, van Staveren WA, Katan MB (1996) Urinary salicylate excretion in subjects eating a variety of diets shows that amounts of bioavailable salicylates in foods are low. Am J Clin Nutr 64:743–747