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Free salicylic acid and acetyl salicylic acid content of foods using gas chromatography–mass spectrometry

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Abstract

Dietary salicylates inhibit cycloxygenase-2 and may therefore have anti-inflammatory properties similar to those of aspirin. Individuals that are sensitive to aspirin may also be intolerant to non-acetylated salicylates and could benefit from a low salicylate diet. A total of 76 foodstuffs comprising fruit (16), fresh and prepared vegetables (13), herbs and spices (12), flavourings and sauces (9), beverages (20) and miscellaneous foods (6) were analysed using gas chromatography with mass spectrometric detection and ^{13}C carboxyl SA as internal standard. Thirty-seven of the samples contained detectable SA, the highest levels being found in dried herbs (up to 28.6 mg/kg), whereas only one sample (curry sauce) contained detectable ASA at 0.34 mg/kg. Limits of detection for both SA and ASA were matrix-dependent and ranged from 0.008 to 0.23 mg/kg. The results show many inconsistencies with previous data and highlight the need for analysis of a wider range of foods and drinks that are currently available.

Keywords: Salicylic acid; Acetyl salicylic acid; Aspirin; Cycloxygenase-2; Diet; GC–MS

1. Introduction

It is established that aspirin (ASA) irreversibly inhibits prostanoid biosynthesis from arachidonic acid via acetylation of an essential serine at the active sites of cycloxygenase-1 (COX-1) and cycloxygenase-2 (COX-2). The net result is inhibition of prostaglandin, thromboxane and prostacyclin synthesis, with increased generation of leukotrienes and hydroxy-eicosatretraenoic acids that are associated with allergic, inflammatory and immune function (Amman & Peskar, 2002). This explains the use of aspirin as an anti-inflammatory, analgesic and anti-thrombotic agent. More recently, epidemiological studies and intervention trials suggest that supression of COX-2 may protect against certain cancers, particularly colorectal cancer, by reducing the transcription of prostaglandin H(2)-synthase (Paterson, Baxter, Lawrence, & Duthie, 2006) and may reduce the risk of Alzheimer's disease (Broe et al., 2000).

Non-acetylated salicylate derivatives inhibit COX-2 gene expression (Amman & Peskar, 2002). This could explain why equimolar doses of gaultherin and aspirin produce comparable anti-inflammatory effects in mice (Zhang, He, Ding, & Du, 2006). Salicylates that occur naturally in plants (Rainsford, 1984) and those added to food, drinks and oral care products as preservatives and flavourants (Perry, Dwyer, Gelfand, Couris, & McCloskey, 1996) may therefore have clinical significance.

Willow leaf tea containing salicin (salicyl alcohol) was administered to women in labour by Hippocrates as early as 400 BC. It has recently been suggested that the low incidence of colorectal cancer in the Indian population may be related to the high salicylate content of curry spices (Baxter, Paterson, Wiles, Graham, & Shirastava, 2005; Paterson, Srivastava, Baxter, Graham, & Lawrence, 2006) and

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that salicylates in fruit and vegetables may have contributed to a decline in cardiovascular disease (Ingster & Feinleib, 1997).

Individuals that are sensitive to aspirin may suffer with urticaria, angiooedema, rhinitis, bronchial asthma and recurrent nasal polyps (Jantti-Alanko, Holopainen, & Malmberg, 1989; Samter & Beers, 1968). Similar responses have been observed after exposure to non-acetylated salicylates (Chudwin et al., 1986). Corder and Buckley (1995) suggested that doses of salicylate as low as 2.6 mg could induce bronchoconstriction in the most sensitive individuals. The chronic nature of some of these clinical presentations, without other obvious cause, may suggest an underlying aetiology related to dietary salicylate.

A low salicylate diet may be of clinical benefit to such affected individuals. This cannot be established however, until the salicylate content of different food and drinks is known. Data on the salicylate content of foods are scarce and contradictory. Our aim was to develop an accurate analytical method to measure the salicylate and ASA content of food and drinks, with a view to the development of a method for measuring serum and urine salicylate levels as a biomarker in future clinical trials.

1.1. Analytical methods

Most of published analytical methods for the determination of salicylates in foods have employed reverse-phase HPLC, though other workers have used direct spectrofluorimetry (Robertson & Kermode, 1981) or amperometric biosensors (Ehrendorfer, Sontag, & Pittner, 1996) with limited success. Swain, Dutton, and Truswell (1985) coupled isocratic RP-HPLC with UV detection to measure free plus bound SA in a wide range of foodstuffs, which required an overnight hydrolysis and 5 h solvent extraction. Venema, Hollman, Janssen, and Katan (1996) developed this approach by using post-column hydrolysis with fluorometric detection to give a 1000-fold increase in sensitivity and improved selectivity over UV detection. Overnight hydrolysis was used to liberate bound salicylate followed by a 12 h extraction. Some chromatographic peak tailing and interference of co-extractives with the ASA peak was evident. This method has been adapted for the determination of salicylates in other matrices such as urinary salicylate (Janssen et al., 1996), serum (Blacklock et al., 2001), plasma (Dadgar, Climax, Lambe, & Darragh, 1985) and soups prepared from organically and non-organically grown vegetables (Baxter, Graham, Lawrence, Wiles, & Paterson, 2001). Moreover, differences in the levels of salicylates found in foods by various authors have been attributed to differences in origin, food processing, storage or analytical methods (Janssen et al., 1996). HPLC separation with UV detection was considered to be too non-specific while fluorimetric detection was very specific and more sensitive. Paterson et al. (2006) have reported recently on the total and free SA content of spices and in blood and urine in order to assess its bioavailability, using HPLC with electrochemical detection. GC–MS was used to confirm the presence of SA in samples following acetylation.

1.2. Previous studies

Data on the salicylate contents of foods are sparse and contradictory. The total salicylate content of foodstuffs include the contribution of free SA, ASA and other salicylates, mainly methyl esters and glucosides of SA. Robertson and Kermode (1981) reported on the concentrations of SA in fresh fruit (0.02–0.10 mg/kg) and fresh vegetables (0.01–0.1 mg/kg) compared to those found in canned products (0.01–0.82 mg/kg). A total of 333 food items were analysed for total salicylates in a comprehensive study by Swain et al. (1985), where higher values were compared to previously published data. The reported ranges of salicylate content included vegetables (0–60 mg/kg), fruits including dried fruits (0.8–78 mg/kg), condiments (notably herbs and spices 0.8–2180 mg/kg) and beverages (dried coffee and tea 0–73.4 mg/kg). In a similar study, much smaller variation in results was reported (Venema et al., 1996) where both free and bound SA and ASA were determined. No ASA was found in any of 30 foods previously reported by Swain et al. (1985), whereas the contents of free plus bound SA and free SA ranged from 0 to 1 mg/kg in vegetables and fruits and from 3 to 28 mg/kg in herbs and spices.

These and other studies have been reviewed by Janssen, Hollman, Venema, van Staveren, and Katan (1996) who concluded that differences in salicylate contents found in foods may be caused by differences in origin, processing, storage or by differences in analytical methods. Moreover, the authors also stated that discrepancies in salicylate contents between extraction methods may arise through differences in the extent of liberation of matrix-bound salicylates. The reported SA levels in a range of tomato soups varied from not detectable to 0.248 mg/kg (Baxter et al., 2001), and in which organic carrot and coriander soup contained the highest amount of SA at 1.04 mg/kg. This was considered likely to relate to the herbs and spices used in the soup, and to the organic method of vegetable production. For example, polythene mulch increases the hectare yield of total salicylates by promoting biomass accumulation (Heiska et al., 2005).

Recent studies by Baxter et al. (2005) and Paterson et al. (2006) reported free and total SA levels in spices that were comparable to those reported by Swain et al. (1985) apart from cumin, for which the former reported a level of ca. 1.5 wt%.

2. Materials and methods

2.1. Reagents and materials

^{13}C Carboxyl salicylic acid (99%) internal standard was obtained from ICON Isotopes, Summit NJ, USA. Salicylic acid (99%) and acetyl salicylic acid (min 99.5%) were

obtained from Sigma–Aldrich, Gillingam, UK. *N*,*O*-Bis(trimethylsilyl)acetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was obtained from Perbio Science UK Ltd., Cramlington, UK. All other reagents were HPLC grade unless specified otherwise.

2.2. Sample preparation

Fresh fruit and vegetable samples were cryogenically blended within 24 h of purchase and analysed immediately. Other samples that were not homogeneous were blended using an Ultra Turrax T-25 homogenizer. Vegetables were cooked in boiling water for 20 min. The red pepper was fried in 10 mL of corn oil at a moderate heat for 15 min. Tea and coffee samples were prepared as infusions in boiling water (5 min) and treated as liquid samples.

2.3. Analysis

Samples were mixed thoroughly to ensure homogeneity. Depending upon sample type (dried or fresh), 1–10 g ± 0.2 g was weighed into a 100 mL Duran bottle with screw lid. Water was added to dry samples at a ratio of 1:9 (w/v). For liquid samples, 1.0 mL was pipetted into the Duran. To the sample was added internal standard solution (200 μL, 100 μg/mL in water) followed by 20 mL of extraction solvent (acetonitrile: water: acetic acid, 25:75:5 v/v/v). The samples were blended using an Ultra Turrax T25 homogenizer for 2–3 min and allowed to settle for at least 10 min. If no supernatant was visible, the mixture was centrifuged at 2500 rpm for 5 min.

A 1 mL aliquot of the supernatant was transferred to a 10 mL PTFE centrifuge tube and the solvent removed under a stream of N_2 on a thermostatically controlled dry block heater at 35 °C. Care was taken to ensure that the tubes were removed as soon as the residue was dry. The residue was redissolved in 200 μL of dichloromethane with gentle swirling to collect any residue from the sides of the tube.

The dichloromethane solution was transferred to a 2 mL glass vial and 200 μL of BSTFA/TMCS derivatising agent added. The vials were crimp sealed and heated on a water bath at 60 °C for 1 h with occasional swirling, after which they were allowed to cool to room temperature prior to analysis by GC–MS.

2.4. GC–MS conditions

The system comprised a HP 5890 series II gas chromatograph with HP 5971 series mass selective detector and HP model 7673 GC/SFC injector in splitless mode (Hewlett Packard, UK). The column was an Ultra 1 (100% dimethylpolysiloxane) 25 m × 0.2 mm × 0.11 μm film thickness (Esslab, Hadleigh, UK) and the carrier gas was helium at 1.2 mL/min.

The injector temperature was set at 250 °C and the injector volume at 1 μL. The initial oven temperature was 40 °C, which was held for 1 min then increased at a rate of 10 °C/min to 300 °C and held for10 min. The MS scan range was 50–450 amu.

Chromatograms were reconstructed from fragment ions at *m*/*z* 267 and 268 for the silylated derivatives of SA and ^{13}C-SA, respectively ($[M-CH_3]^+$), and from *m*/*z* 195 for the silylated derivative of ASA $[M-CH_3O]^+$.

Table 1
Limits of detection for SA and ASA in foods using GC–MS

Amount of sample	Free SA	Free ASA
1 mL	0.01 mg/L	0.01 mg/L
1 g	0.2 mg/kg	0.2 mg/kg
5 g	0.2 mg/kg	0.2 mg/kg
10 g	0.2 mg/kg	0.2 mg/kg

2.5. Analytical quality assurance

From 100 μg/mL stock standard solutions of SA and ASA in water, a series of five calibration standards were prepared over the concentration range 0 to ca. 2.5 μg/mL. The calibration standards were treated as for sample supernatants except that 200 μl of 5 μg/mL internal standard solution was added to 1 mL of standard. Calibration lines were constructed for SA and ASA for each analytical batch by plotting standard concentration against the ratio obtained by dividing the peak area of the standard by the peak area of the internal standard. All calibration curves obtained were linear ($r^2 = >0.99$).

Reagent blanks were extracted with each batch to show that no interferences were present. Limits of detection (LODs) were calculated from three times the standard deviation of instrument baseline noise, corrected for dilution and sample weight (Table 1).

Reagent blanks spiked at ca. 25 mg/kg were extracted with each analytical batch. For SA ($n = 6$) the recovery range was 88–103.6% (mean 95.5%, RSD 7.4%) and for ASA ($n = 6$), the recovery range was 86–93.5% (mean 89.8%, RSD 5.9%).

An in-house reference material comprising paprika powder containing SA was analysed with each batch ($n = 16$) in order to ensure that the method was under control. The mean value obtained was 5.6 mg/kg SA (ASA < 0.2 mg/kg), with an RSD of 10.2%.

3. Results

The analytical results are given in Table 2 and have been corrected for recovery using the factors obtained from spiked reagent blanks on an analytical batch basis. Of the 76 samples analysed, only one sample (curry sauce) contained detectable ASA at 0.34 mg/kg. Detectable amounts of SA were found in 7 of the 16 fruit samples ranging from 0.2 to 3.2 mg/kg, 5/13 vegetable samples (0.3–1.3 mg/kg), 2/6 miscellaneous foods (0.3–0.7 mg/kg), 8/12 herbs and spices (5.1–28.6 mg/kg), 8/9 flavourings

Table 2
Free SA and ASA contents of foods

Sample description	Typical portion or ingredient size	Free SA (mg/kg)[a]	Calculated intake of free SA per portion (mg)	Free ASA (mg/kg)[a]	Calculated intake of free ASA per portion (mg)
Fruit					
Apples (Granny Smith)	100 g	**0.7**	**0.07**	<0.2	<0.02
Apples (Golden Delicious)	100 g	<0.2	<0.02	<0.2	<0.02
Banana	100 g	**0.4**	**0.04**	<0.2	<0.02
Oranges	160 g	<0.2	<0.03	<0.2	<0.03
Clementines	60 g	<0.2	<0.01	<0.2	<0.01
Lemons	1 slice 20 g	**3.2**	**0.06**	<0.2	<0.01
Peaches	110 g	<0.2	<0.02	<0.2	<0.02
Nectarines	150 g	**0.3**	**0.05**	<0.2	<0.03
Kiwi	60 g	<0.2	<0.01	<0.2	<0.01
Pears	170 g	<0.2	<0.03	<0.2	<0.03
Plums	55 g	**0.5**	**0.03**	<0.2	<0.01
Grapes (Green)	100 g	**0.6**	**0.06**	<0.2	<0.02
Currants	25 g	<0.2	<0.01	<0.2	<0.01
Sultanas	30 g	<0.2	<0.01	<0.2	<0.01
Grapefruit	340 g	**0.2**	**0.07**	<0.2	<0.07
Mango	150 g	<0.2	<0.03	<0.2	<0.03
Vegetables					
Red Peppers (fresh)	160 g	<0.2	<0.03	<0.2	<0.03
Red Peppers (cooked)	160 g	<0.2	<0.03	<0.2	<0.03
Tomato (fresh)	85 g	**1.3**	**0.11**	<0.2	<0.02
Tomato (cooked)	25 g	<0.2	<0.01	<0.2	<0.01
Tomato Puree	85 g	**0.3**	**0.03**	<0.2	<0.02
Tomato soup	220 g	<0.2	<0.04	<0.2	<0.04
Baked beans	135 g	<0.2	<0.03	<0.2	<0.03
Carrots (fresh)	80 g	**0.5**	**0.04**	<0.2	<0.02
Carrots (cooked)	80 g	<0.2	<0.02	<0.2	<0.02
Cucumber	60 g	**0.4**	**0.02**	<0.2	<0.01
Peas (frozen)	70 g	**0.3**	**0.02**	<0.2	<0.02
Sweetcorn (frozen)	85 g	<0.2	<0.02	<0.2	<0.02
Broccoli (frozen)	85 g	<0.2	<0.02	<0.2	<0.02
Miscellaneous foods					
Honey	17 g	<0.2	<0.01	<0.2	<0.01
Raspberry jam	18 g	<0.2	<0.01	<0.2	<0.01
Strawberry yoghurt	125 g	<0.2	<0.03	<0.2	<0.03
Sweet & sour sauce	150 g	<0.2	<0.03	<0.2	<0.03
Curry sauce	150 g	**0.7**	**0.11**	**0.3**	**0.05**
Bolognaise sauce	90 g	**0.3**	**0.03**	<0.2	<0.02
Herbs and spices					
Curry powder	3 g	**15.2**	**0.05**	<0.2	<0.01
Chilli powder	3 g	<0.2	<0.01	<0.2	<0.01
Garlic (fresh)	1 g	<0.2	<0.01	<0.2	<0.01
Pepper (ground)	2 g	**5.7**	**0.01**	<0.2	<0.01
Ginger (fresh)	1 g	<0.2	<0.01	<0.2	<0.01
Cinnamon powder	3 g	**5.8**	**0.02**	<0.2	<0.01
Paprika	3 g	**5.6**	**0.02**	<0.2	<0.01
Dried mixed herbs	3 g	**22.3**	**0.07**	<0.2	<0.01
Oregano (dried)	3 g	**26.0**	**0.08**	<0.2	<0.01
Thyme (dried)	3 g	**28.6**	**0.09**	<0.2	<0.01
Parsley (dried)	3 g	<0.2	<0.01	<0.2	<0.01
Mint (dried)	3 g	**14.4**	**0.04**	<0.2	<0.01
Flavourings and sauces					
Savoury spread (yeast-based)	1 g	**3.8**	<0.01	<0.2	<0.01
Beef stock cube	7 g	**2.5**	**0.02**	<0.2	<0.01
Gravy granules	5 g	**0.5**	<0.01	<0.2	<0.01
Worcestershire sauce	5 g	<0.2	<0.01	<0.2	<0.01
Salad cream	12 g	**0.6**	<0.01	<0.2	<0.01
Tomato ketchup	12 g	**0.3**	<0.01	<0.2	<0.01
Horseradish sauce	12 g	**1.2**	**0.01**	<0.2	<0.01

(*continued on next page*)

Table 2 (*continued*)

Sample description	Typical portion or ingredient size	Free SA (mg/kg)[a]	Calculated intake of free SA per portion (mg)	Free ASA (mg/kg)[a]	Calculated intake of free ASA per portion (mg)
Malt vinegar	5 g	**0.05**	<0.01	<0.01	<0.01
White Wine vinegar	5 g	**0.15**	<0.01	<0.01	<0.01
Beverages					
Coffee, ground (10% infusion)	260 g	**6.7**	**1.74**	<0.2	<0.01
Coffee, instant (5% solution)	260 g	**6.8**	**1.77**	<0.2	<0.01
Peppermint tea (1% infusion equivalent)	260 g	**0.14**	**0.04**	<0.01	<0.01
Lemon tea (1% infusion equivalent)	260 g	**0.02**	<0.01	<0.01	<0.01
Camomile tea (1% infusion equivalent)	260 g	**0.08**	**0.02**	<0.01	<0.01
Loose tea (1% infusion equivalent)	260 g	**1.06**	**0.28**	<0.01	<0.01
Red wine	125 g	**0.67**	**0.08**	<0.01	<0.01
White wine	125 g	**0.80**	**0.10**	<0.01	<0.01
Lager	287 g	**0.06**	**0.02**	<0.01	<0.01
Bitter	287 g	**0.01**	<0.01	<0.01	<0.01
Cider	287 g	<0.01	<0.01	<0.01	<0.01
Gin	25 g	<0.01	<0.01	<0.01	<0.01
Vodka	25 g	<0.01	<0.01	<0.01	<0.01
White rum	25 g	<0.01	<0.01	<0.01	<0.01
Orange squash	50 g	<0.01	<0.01	<0.01	<0.01
Orange juice	160 g	<0.01	<0.01	<0.01	<0.01
Apple water (Sparkling)	330 mL	<0.01	<0.01	<0.01	<0.01
Peach water (Sparkling)	330 mL	**0.04**	<0.01	<0.01	<0.01
Lemonade	330 mL	<0.01	<0.01	<0.01	<0.01
Bitter lemon	330 mL	**0.09**	**0.03**	<0.01	<0.01

[a] mg/L for liquid samples.

and sauces (0.05–3.8 mg/kg) and 12/20 beverages (0.01–6.8 mg/kg).

The analytical limits of detection for both free SA and ASA ranged from 0.01 to 0.2 mg/kg depending upon sample size, which in turn was matrix-dependent. Also in Table 2 are given typical portion sizes for each sample along with associated intake estimates.

4. Discussion

4.1. Analytical method

While the method reported here was being developed, Deng, Zhang, Zhang, Qian, and Zhu (2003) reported a method for the determination of SA in plant materials using gas chromatography (GC) with mass spectrometric (MS) detection. Salicylic acid in tomato leaves was extracted with 9:1 (v/v) methanol:chloroform and derivatised with BSTFA. Quantitative analysis was carried out by GC–MS using mandelic acid as internal standard but ASA was not determined. As in our study, the derivative of SA was monitored using the m/z 267 fragment ion which was the most abundant. No other qualitative identification criteria were used other than peak retention time. Using this method, analysis was completed in 2 h. The method reported here was developed along similar lines for speed and selectivity. The incorporation of ^{13}C-SA as internal standard allowed improved accuracy and precision.

4.2. Salicylate levels

4.2.1. Fruit and vegetables

From the 29 fruit and vegetables analysed, lemons yielded the highest total SA content (3.2 mg/kg). However one slice of lemon (20 g) would only provide ca. 0.06 mg SA. Drinks that contained lemon (bitter lemon, lemon tea and lemonade) contained significantly less (0.09, 0.03 and <0.01 mg/kg, respectively).

Fresh tomatoes and carrots contained higher SA levels (1.3 mg/kg and 0.5 mg/kg, respectively) than their cooked (<0.16 mg/kg) or pureed (tomato puree 0.3 mg/kg) equivalents. This suggests that SA is lost during food processing and cooking. This may also explain why SA levels in raspberry jam were less than 0.2 mg/kg. Our results contradict previous findings of higher levels of free SA in tomato puree or soup compared with fresh tomato (Robertson & Kermode, 1981; Venema et al., 1996) and in cream-style sweet corn compared with whole kernels (Robertson & Kermode, 1981). Although plums and green grapes contained 0.5 mg/kg and 0.6 mg/kg SA, respectively, SA in currants and sultanas were less than 0.20 mg/kg. This contradicts previous findings (Venema

et al., 1996) and suggests that SA is lost during the drying process.

We found less than 0.2 mg/kg SA in the sample of tomato soup which compares well with the figure reported by Baxter et al. (2001) of 0.248 mg/kg.

Our results for apples are commensurate with the levels of free SA and ASA reported by other workers (Robertson and Kermode, 1981; Venema et al., 1996; Janssen, Hollman, Reichman, et al., 1996; Janssen, Hollman, Venema, et al., 1996), in that they are 10–100-fold lower than those reported by Swain et al. (1985) of 0.8–5.9 mg/kg. We found SA in banana (0.4 mg/kg) and frozen peas (0.3 mg/kg), which contradicts the previous findings of Swain et al. (1985) at 0 and 0.04 mg/kg, respectively.

4.2.2. Herbs and spices

SA levels were highest in thyme, oregano, dried mixed herbs, curry powder and dried mint (14.4–28.6 mg/kg). This principle is consistent with previous findings although the quantity and order of magnitude has differed (Swain et al., 1985; Venema et al., 1996). Lower amounts were found in ground pepper and paprika (5.7 and 5.1 mg/kg, respectively), with less than 0.2 mg/kg SA in chilli powder, fresh garlic, fresh ginger and dried parsley.

4.2.3. Flavourings and sauces

Of the nine items analysed, yeast-based savoury spread, beef stock cube and horseradish sauce contained the highest levels of SA (3.8–1.2 mg/kg). Tomato ketchup and tomato puree both contained 0.3 mg/kg. Swain et al. (1985) reported 643 mg/kg SA in Worcester sauce, whereas the SA level in the sample that we tested was less than 0.2 mg/kg.

Curry sauce was the only item tested that contained ASA (0.3 mg/kg) with a free SA level of 0.7 mg/kg. This is lower than the recent study by Baxter et al. (2005) who reported SA levels in cumin, paprika, turmeric of 16290, 1040 and 3500 mg/kg, respectively and a 545 g portion of vindaloo curry with 94 mg SA. Clearly the ingredients used in the curry sauce in our study and the vindaloo curry in the Baxter et al. (2005) study are likely to be totally different. However, curry sauce may have a more potent clinical effect than non-acetylated SA since it inhibits both COX-1 and COX-2 (Amman & Peskar, 2002).

4.2.4. Beverages

Our study found highest levels of SA in both the ground coffee infusion (10%) and the instant coffee solution (5%) at 6.7 and 6.8 mg/kg, respectively. The free SA result for ground coffee is higher than that reported by Venema et al. (1996) at 0.24 mg/kg for a 5% infusion. The results for the tea samples have been normalised for a 1% infusion. The highest level of free SA was found in loose tea (1.06 mg/kg), which is consistent with the result reported by Venema et al. (1996) at 0.33 mg/kg for a 0.8% infusion. The sample of white wine had a slightly higher SA content than red wine (0.80 mg/kg compared with 0.67 mg/kg, respectively). Previous studies have suggested differences related to species of grape (Venema et al., 1996). All other beverages tested contained low SA levels.

HPLC methods used for the determination of free and bound SA and ASA tend to be lengthy and prone to interference, especially when using UV detection. Our analytical method is rapid and suitable for use on a variety of different foods and drinks. The use of GC–MS is highly specific and the incorporation of ^{13}C-SA as an internal standard allowed better control over analyte losses. Hence we believe that this method also has the potential to be successfully developed for the rapid and therefore more cost-effective determination of SA and ASA biomarkers in clinical samples using an isotope dilution approach.

Because we have reported results for free SA and ASA only, comparison of results from previous studies is not straightforward. Differences in analytical methodologies (particularly extraction and chromatographic separation efficiency, detection mode and sensitivity) and sample variability (e.g. plant species and varieties) exacerbate this problem. These comments notwithstanding, our results show some agreement but also several inconsistencies with previous data and highlights the need for analysis of a wider range of foods and drinks that are currently available for both free and bound salicylates. This would benefit individuals who have salicylate intolerance.

This study provides data for the study of the relationship between important clinical conditions such as asthma and nasal polyposis and dietary salicylate. There is growing interest in the influence of diet on health and this may be an important area where such dietary manipulation can significantly affect the impact of a common and serious condition.

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References

Amman, R., & Peskar, B. A. (2002). Anti-inflammatory effects of aspirin and sodium salicylate. *European Journal of Pharmacology, 447*, 1–9.

Baxter, G. J., Graham, A. B., Lawrence, J. R., Wiles, D., & Paterson, J. R. (2001). Salicylic acid in soups prepared from organically and non-organically grown vegetables. *European Journal of Nutrition, 40*, 289–292.

Baxter, G., Paterson, J., Wiles, A., Graham, A., & Shirastava, R. (2005). Salicylic acid: the spice of life: a pilot study. *Proceeding of the Nutrition Society*(A), 64.

Blacklock, C. J., Lawrence, J. R., Wiles, D., Malcolm, E. A., Gibson, I. H., Kelly, C. J., et al. (2001). Salicylic acid in the serum of subjects not taking aspirin. Comparison of SA concentrations in the serum of vegetarians, non-vegetarians, and patients taking low dose of aspirin. *Journal of Clinical Pathology, 54*, 535–553.

Broe, G. A., Grayson, D. A., Creasey, H. M., Waite, L. M., Casey, B. J., & Bennett, H. P. (2000). Anti-inflammatory drugs protect against Alzheimer disease at low doses. *Archives of Neurology, 57*, 1586–1591.

Chudwin, D. S., Strub, M., Golden, H. E., Frey, C., Richmond, G. W., & Luskin, A. T. (1986). Sensitivity to non-acetylated salicylates in a patient with asthma, nasal polyps, and rheumatoid arthritis. *Annals of Allergy, 57*, 133–134.

Corder, E. H., & Buckley, C. E. (1995). Aspirin, salicylate, sulfite and tartrazine induced bronchoconstriction. Safe doses and case definition in epidemiological studies. *Journal of Clinical Epidemiology, 48*, 1269–1275.

Dadgar, D., Climax, J., Lambe, R., & Darragh, A. (1985). High performance liquid chromatographic determination of certain salicylates and their major metabolites in plasma following topical administration of a liniment to healthy subjects. *Journal of Chromatography, 342*, 315–321.

Deng, C., Zhang, X., Zhang, J., Qian, J., & Zhu, W. (2003). Rapid determination of SA in plant materials by gas chromatography–mass spectrometry. *Chromatographia, 58*, 225–229.

Ehrendorfer, M., Sontag, G., & Pittner, F. (1996). Determination of salicylate in beverages and cosmetics by use of an amperometric biosensor. *Fresenius Journal of Analytical Chemistry, 356*, 75–79.

Heiska, S., Rousi, M., Turtola, S., Meier, B., Tirkkonen, V., & Julkunen-Titto, R. (2005). The effect of genotype and cultivation method on the total salicylate yield of dark-leaved willows (*Salix myrsinifolia*). *Planta Medica, 71*, 1134–1139.

Ingster, L. M., & Feinleib, M. (1997). Could salicylates in food have contributed to the decline in cardiovascular disease mortality? A new hypothesis. *American Journal of Public Health, 87*, 1554–1558.

Janssen, K. P. L. T. M., Hollman, P. C. H., Reichman, E., Venema, D. P., van Staveren, W. A., & Katan, M. B. (1996). Urinary salicylate excretion in subjects eating a variety of diets shows that amounts of bioavailable salicylates in foods are low. *American Journal of Clinical Nutrition, 64*, 743–747.

Janssen, K. P. L. T. M., Hollman, P. C. H., Venema, D. P., van Staveren, W. A., & Katan, M. B. (1996). Salicylates in foods. *Nutrition Reviews, 54*, 357–359.

Jantti-Alanko, S., Holopainen, E., & Malmberg, H. (1989). Recurrence of nasal polyps after surgical treatment. *Rhinology*(Suppl. 8), 59–64.

Paterson, J. R., Srivastava, R., Baxter, G. W., Graham, A. B., & Lawrence, J. R. (2006). Salicylic acid content of spices and its implications. *Journal of Agricultural and Food Chemistry, 54*, 2891–2896.

Paterson, T. J., Baxter, G., Lawrence, J., & Duthie, G. (2006). Is there a role for dietary salicylates in health? *Proceedings of the Nutrition Society, 65*, 93–96.

Perry, C. A., Dwyer, J., Gelfand, J. A., Couris, R. R., & McCloskey, W. W. (1996). Health effects of salicylates in foods and drugs. *Nutrition Reviews, 54*, 225–240.

Rainsford, K. D. (1984). *Aspirin and the salicylates*. London: Butterworth.

Robertson, G. L., & Kermode, W. J. (1981). Salicylic acid in fresh and canned fruit and vegetables. *Journal of the Science of Food and Agriculture, 32*, 833–836.

Samter, M., & Beers, R. F. (1968). Intolerance to aspirin clinical studies and consideration of its pathogenesis. *Annals of Internal Medicine, 68*, 975–983.

Swain, A. R., Dutton, S. P., & Truswell, A. S. (1985). Salicylates in foods. *Journal of the American Dietary Association, 85*, 950–960.

Venema, D. P., Hollman, P. C. H., Janssen, K. P. L. T. M., & Katan, M. B. (1996). Determination of ASA and SA in foods using HPLC with fluorescence detection. *Journal of Agricultural and Food Chemistry, 44*, 1762–1767.

Zhang, B., He, X., Ding, Y., & Du, G.-H. (2006). Gaultherin, a natural salicylate derivative from *Gaultheria yunnanensis*: towards a better non-steroidal anti-inflammatory drug. *European Journal of Pharmacology, 530*, 166–171.