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Original research article

Naturally occurring dietary salicylates: A closer look at common Australian foods

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ABSTRACT

Dietary salicylates may have similar benefits and/or adverse symptoms as documented for Aspirin. To develop dietary strategies, data on salicylate content of food is essential, but the available literature is limited and controversial. Hence the aims of this study are to apply and validate a reliable methodology to determine the salicylate content of common foods, and compare with recently published data. Gas chromatography-mass spectrometry (GC–MS) was used with SA-d6 (deuterated salicylic acid) as an internal standard to analyse 112 common Australian food items pooled from ten different sources. Technical sextuplicates show a coefficient of variation of 3.03%. SA content ranged from 1.28–26.93 (vegetables), 2.13–36.90 (fruits), 2.80–604.97 (herbs/spices) and 2.04–51.48 (beverages) mg/kg. SA was undetected in oils, sugars and cereals analysed. The results reveal inconsistencies within the extant literature and a pressing need for further research extending the analysis to a broader range of food items.

1. Introduction

Human health is greatly influenced by dietary choices (Abnet et al., 2015; McCarroll et al., 2015). Many plant derived secondary metabolites are capable of effecting biological activities that may in turn affect human health. (Acamovic and Brooker, 2005). Salicylic acid (SA) in plants is one such compound. Salicylic acid (2-hydroxybenzoic acid) is a phytohormone that is generated from the phenyl propanoid pathway (Ververidis et al., 2007). In plants, SA occurs in three major forms – free salicylic acid, its carboxylated esters and phenolic glycosides (Chadha and Brown, 1974). SA is primarily involved in plant immunity (Yalpani et al., 1993). Buchner in 1828 first isolated natural SA as salicin from wilow bark (Mahdi et al., 2006). Chemical synthesis of SA was done by Kolbe in 1860. Eventually, Hoffman (1987) chemically acetylated SA to produce acetylsalicylic acid or aspirin (Mahdi et al., 2006).

Detection of SA in serum and in urine of subjects not taking aspirin suggest a dietary source of this chemical in humans (Shaukat et al., 2011). Significantly higher levels of SA were found in serum of vegetarians not taking aspirin with reported levels comparable to patients taking 75 mg of aspirin (Lawrence et al., 2003). The salicylate content of blood and urine has also been shown to increase following consumption of a meal, indicating that the dietary source of SA is bio-available (Paterson et al., 2006).

Several benefits of aspirin include lowered risk of death from colorectal cancer, protection against cardiovascular disease and possible prevention of pre-eclampsia (Chan et al., 2008; Kim et al., 2014; Oyola and Kirley 2015; Voelker, 2014). However, conditions related to aspirin hypersensitivity including ailments of skin (urticaria, angioedema) and respiratory tract (rhinitis, asthma) have also been reported (Kowalski et al., 2013). Gastrointestinal symptoms have also been implicated in SA hypersensitivity (Raithel et al., 2005). However, whether or not the levels of SA

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obtained from dietary sources have the same benefits/ailments as aspirin is not yet clearly known.

As SA is a plant hormone, the levels may fluctuate with differences in variety, storage, processing or growing conditions. The concentrations of SA in spices can differ; for example, cumin from two different sources contained 1629 mg/100 g and 980 mg/100 g (Paterson et al., 2006). However, the differences in some cases may be attributed to the analytical method used for SA detection; for example, total concentrations of SA levels in blueberries range from 27.6 mg/kg to 0.57 mg/kg when HPLC (High performance liquid chromatography) was used. (Swain et al., 1985; Wood et al., 2011). This difference may be attributed to decreased sensitivity due to use of UV detector in the former (Scotter et al., 2007). Thus although there are a few studies looking at SA levels in food, the information is limited and somewhat controversial.

The aims of this study were first, to develop methodology using gas chromatography–mass spectroscopy (GC–MS) to accurately determine the content of total SA (both free and bound forms) of an array of common Australian fruits, vegetables, beverages, herbs, spices, cereals and oils, and secondly, to compare the results with currently published data.

2. Materials and methods

2.1. Reagents

All chemical reagents were sourced from Sigma-Aldrich (Castle Hill, NSW, Australia): salicylic acid, >99% (2-hydroxybenzoic acid), N-methyl-N-(trimethylsilyl) trifluroacetamide (MSTFA) > 98.5%; Salicylic acid-d6, Sodium hydroxide (NaOH),> = 97.0%, pellets., Hydrochloric acid (HCl), Ethyl acetate (EthOH), Ethyl alcohol (CH_3CH_2OH). Ethyl acetate and ethyl alcohol were LC–MS (Liquid chromatography–mass spectrometry) and HPLC grade respectively.

Sample collection and determination of serving size. 112 food samples were collected in total. The food sampling procedure followed the protocol of the Food Standards Australia New Zealand (FSANZ, Canberra, Australia). Each sample of fruits and vegetables was purchased from one supermarket and one green grocer in five different areas of Victoria. Thus ten samples of each type of fruit and vegetable were pooled. 500 g of each of the fruit and vegetable, and three bunches of each of the herbs were purchased from the different outlets. As for the three commercially-available brands of spices, beverages, oils and cereals were purchased from one supermarket. If three different brands were not available, three different batches of the same brand were purchased. The edible portions of each of the pooled food samples were blended together; 100 g were aliquoted, freeze-dried and stored at −20 °C for GC–MS analysis. The weight of serving size of each food item was determined using FoodWorks 7 nutrition software for diet and recipe analysis and also by weighing food in-house.

2.2. Determination of total SA and free SA content in foods

2.2.1. Determination of free SA and total SA content in fruits, vegetables, herbs, spices, cereals

In plants, salicylates occur in three major forms – free SA and bound SA (phenolic glycosides and carboxylic esters) (Chadha and Brown, 1974). The protocol for extraction of SA in foods was adopted from Paterson et al. (2006)(Paterson et al., 2006) with some modifications as follows. For total SA extraction 50 mg of finely powdered aliquots of freeze-dried samples or 1 g of liquid samples were suspended in 2 mL of 2.5 mol/L NaOH. This mixture was left to hydrolyse for 24 h at room temperature followed by addition of 10 μg of SA-d6 as an internal standard. 1 mL of 5.3 mol/L HCl was added to the alkaline mixtures to yield a final concentration of 0.1 mol/L of HCl. The acidified mixtures were extracted five times with 3 mL of ethyl acetate. The combined extracts were evaporated to dryness with a GeneVac EZ-2 centrifugal evaporator at 55 °C. The dried samples were stored under Argon pending derivatization. The extracted SA was then derivatized by adding 600 μL of MSTFA to the dried extracts, vortexed for 30 s and was allowed to react at 60 °C for 30 min, as previously described (Pfleger et al., 1992). The stock standard solution (138 μg/mL) was prepared by adding 10 μg of SA-d6 to 600 $\in^1/_4$L aqueous solution of SA (1 mM) followed by 200 μL of HCl (1 M). Five different dilutions of this standard were prepared in ethyl acetate ranging from 138 μg/mL to 13.8 μg/mL. For determination of free SA, 50 mg of freeze dried samples were acidified with 1 mol/L HCl to a final concentration of 0.1 mol/L. The alkaline hydrolysis step with NaOH was excluded. The standards and samples were extracted and derivatized as detailed above. The derivatized samples were stored at −20 °C until thawed for analysis by GC–MS.

2.2.2. Determination of total SA content in oils

The protocol for extraction of total SA in oil was performed as previously described (Owen et al., 2000) with some modifications as follows. 10 g of olive or sunflower oil at room temperature was weighed out and 10 μg of SA-d6 was added to the samples and the standards. The mixture was vortexed for 2 min at 3000 rpm on Biocote vortex in continuous mode. The samples were extracted 5 times by adding 4 mL of Methanol followed by vortexing (3000 rpm, 2 min) and centrifugation (2000 rpm, 30 min). The top phenolic fraction was pooled in a separate tube and the combined extracts were evaporated to dryness with a GeneVac EZ-2 centrifugal evaporator at 55 °C. The dried samples were stored under Argon pending derivatization. The extracted SA was then derivatized by adding 600 μL of MSTFA to the dried extracts, vortexed for 30 s and was allowed to react at 60 °C for 30 min. The stock standard solution (138 μg/mL) was prepared by adding 10 μg of SA-d6 to 600 μL solution of SA in methanol (1 mM) followed by 200 μL of HCl (1 M), extracted, evaporated and derivatized as above. Five different concentrations of standards were prepared in ethyl acetate ranging from 138 μg/mL to 13.8 μg/mL. The derivatized samples were stored at −20 °C until thawed for analysis by GC–MS.

2.3. GC–MS conditions

The derivatized sample extracts were transferred into sample vials for GC–MS analysis by Chemical Analysis, Croydon, Melbourne. The analysis was done on Agilent 6890 interfaced with

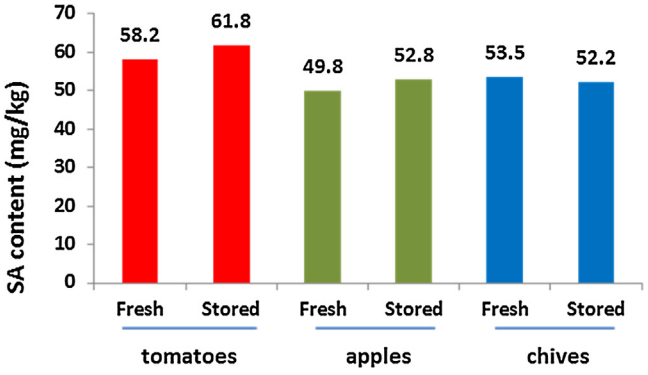

Fig. 1. Similar levels of total SA detected in fresh and stored food samples. Ten samples of each food item were pooled together. The stored samples were homogenized, freeze dried and stored in dark at −20 °C.

HP5973 mass selective detector. Separation via gas chromatography was performed using a HP–5 ms Ultra inert capillary column (5% phenyl methyl siloxane) with dimensions of 30 m × 250 mm × 0.25 μm. One μl was injected using split injection and a helium flow rate of 1 mL/min. The GC column temperature program used was 50 °C for 2 min, increased at a rate of 1 °C per min–300 °C. The mass spectrometer was set at electron energy of 70 eV in full scan mode. MS scan range was 40–400 amu. The derivatized SA peak and SA-d6 peak were identified by extracting ions 267 *m*/*z* and 271 *m*/*z* respectively. Quantitation was carried out using extracted ion chromatographic areas.

2.4. Quality assurance procedures

A calibration curve was created by diluting the standards in ethyl acetate resulting in a five-point concentration range. All samples were quantified against the curve. The calibration curve was created by plotting the ratio of the peak area of the standard and the peak area of the internal standard against concentration. Linearity (R^2) was determined to be >0.99.

Free SA was also measured in three of the tested food samples and compared with their total SA content. This was done to confirm that the methodology for extracting total SA (free plus bound SA)

Table 1a
Total salicylic acid content of vegetables reported in wet weight and unit converted to mg/kg. All analyses are with fresh vegetables unless otherwise stated.

Vegetable	Variety	Salicylic acid content (mg/kg)				
		Current study	Wood et al. (2011)	Venema et al. (1996)	Swain et al. (1985)	Robertson and Karmode (1981)
Asparagus	–	8.21	1.29	–	14	–
Bean sprouts	–	1.28	–	–	0.6	–
Beetroot	–	26.93	–	–	1.8	–
Broccoli	–	11.01	0	–	6.5	–
Brussel sprouts	–	8.60	–	–	0.7	–
Bok choy	–	1.84	–	–	–	–
Capsicum	red	8.85	0.09	–	–	0.04
Cauliflower	–	5.86	0.01	–	–	0.07
Corn on cob fresh	–	16.48	–	–	1.3	0.73
Cabbage	common	2.55	0	–	0	0.01
Cucumber	common peeled	5.81	0.02	–	–	–
	long peeled	2.93	0.002	–	–	–
	other[a]		0.02	0.08	7.8	–
Celery	–	2.79	0.04	–	0	–
Chilli	red	6.57	–	–	12	–
Choy sum	–	6.89	–	–	–	–
Eggplant	–	7.86	0	–	8.8	–
Endive	baby	3.85	–	–	19	–
Fennel	top	3.67	–	–	–	–
	bulb	1.29	–	–	–	–
Green beans	–	13.88	0.07	–	1.1	–
Leek	–	4.10	–	–	0.8	–
Lettuce	cos	15.82	–	–	–	–
	iceberg	2.69	0.05	–	–	–
	butter	8.79	–	–	–	–
	other[a]	–	–	–	0	–
Mushroom	button	8.16	0.13	–	–	–
Onion	white	5.11	0.08	–	–	–
	spanish	12.85	–	–	1.6	–
	other[a]	–	–	–	1.6	–
Parsnip	–	3.90	–	–	4.5	–
Peas	green	25.52	–	–	0.4	0.02
	sugar snap	4.85	–	–	–	–
Potato	sweet yellow	21.15	–	–	4.8	–
	white	4.64	0.02	–	1.2	–
	other[a]	–	–	–	–	0.04
Pumpkin	butternut	8.69	–	–	–	–
	jap	11.19	–	–	–	–
Rocket leaves	–	15.62	–	–	–	–
Radish	–	16.93	–	–	12.4	–
Red kidney beans	–	6.02	–	–	–	–
Spinach	–	2.29	–	–	5.8	–
Swede	–	9.40	0.07	–	0	–
Tomato	canned diced	6.42[b]	0.13	–	5.3[c]	–
	paste	10.81[d]	–	0.75	4.3[e], 5.7[f], 14.4[g]	–
	cherry	7.05	–	–	–	–
	sun-dried	18.63	–	–	–	–
	table/common	3.18	0.13	0.36	–	0.05
	roma	5.02	0.13	–	–	–
	other[a]	–	–	–	1.3	–
Zucchini	–	6.13	–	–	10.4	–

[a] variety not reported.
[b] Ardmona, Homebrand, Valverde pooled.
[c] Letona.
[d] Homebrand, Leggos, Coles pooled.
[e] Tom Piper.
[f] Campbell.
[g] Leggos.

Table 1b
Total salicylic acid content of fruits reported in wet weight and unit converted to mg/kg. All analyses are with fresh fruit unless otherwise stated.

Fruit	Variety	Salicylic acid content (mg/kg)				
		Current study	Wood et al. (2011)	Venema et al. (1996)	Swain et al. (1985)	Robertson and Karmode (1981)
Apple	granny smith[c]	9.70	0.55	–	5.9[b]	–
	pink lady[c]	9.02	–	–	–	–
	pink lady[b]	2.93	–	–	–	–
	golden del[c]	3.18	–	–	0.8[b]	–
	golden del[b]	3.20	–	–	–	–
	other[a]	–	–	<0.02	–	–
Apricots	–	8.26	–	0.01	25.8	0.03
Avocado	haas	29.72	–	–	–	–
	other*a*	–	–	–	6	–
Banana	common	5.39	0.34	–	0	0.05
Blueberry	–	9.12	0.57	–	–	–
Cantaloupe	–	5.02	0.11	–	15	–
Coconut	dried	22.26	–	–	2.6	–
Dates dry	–	36.90	–	–	45	–
Grapes	thompson[d]	8.31	0.02	0.03	–	–
	ralli[d]	7.65	0.02	0.03	–	–
	red malaita	–	–	–	9.4	–
	sultana	–	–	–	18.8	–
	other[a]	–	–	0.03	–	–
Kiwi fruit	–	16.61	0.31	–	3.2	0.02
Lemons	–	6.74	–	–	1.8	–
Mandarin	imperial	2.70	–	–	5.6	–
Mango	–	7.09	0.03	–	1.1	–
Nashi pear	–	3.23	–	–	–	–
Nectarine	–	13.28	3.29	0.87	4.9	–
Olives	black[e]	26.75	–	–	–	–
	other[a]	–	–	–	3.4	–
Orange	naval	2.13	–	<0.02	–	–
	other[a]	–	0.11	–	23.9	–
Passionfruit	–	12.40	–	–	1.4	–
Pawpaw	–	4.79	–	–	0.8	–
Peach	white	3.30	0.12	–	5.8	–
Pear	packham[c]	2.95	–	–	0	–
	packham[b]	12.90	–	–	–	–
	other[a]	–	0.23	–	–	–
Persimmon	–	5.91	–	–	1.8	–
Pineapple	–	7.29	-	–	21	–
Plum	tegan blue	6.35	–	–	–	–
	blood	–	–	–	2.1	–
	kelsey	–	–	–	0.9	–
	wilson	–	–	–	1.1	–
	other[a]	–	0.01	–	–	0.03
Pomegranate	–	15.17	–	–	0.7	–
Raspberry	–	10.52	0.09	–	31.4	–
Strawberry	–	6.98	0.61	0.65	13.6	–
Watermelon	–	4.80	–	–	4.8	0.07

[a] Variety not reported.
[b] Unpeeled.
[c] Peeled.
[d] Seedless.
[e] Coles smartbuy, Coles pitted, deli pitted pooled.

was efficient. Total SA was also measured in stored vs freshly prepared samples to ensure that there was no significant loss of SA upon storage. Six replicates of sample preparation for fresh cherry tomatoes were run to ensure that the intra sample variability was minimum.

3. Results

3.1. Total versus free salicylic acid detection

In order to determine the relative amount of SA that was in the soluble component of foods, one item from each of the food groups – cherry tomatoes (vegetable), peeled granny smith apples (fruit) and chives (herbs) – was subjected to different extraction processes that detected the free and total (free and bound SA) SA content. The total SA content of cherry tomatoes was 7 mg/kg compared to a free SA content of 0.3 mg/kg. In contrast, free SA was not detected in granny smith apples and chives in which total SA content was 0.9 mg/kg and 0.8 mg/kg respectively.

3.2. Effect of storage on salicylic acid levels

Concentrations of SA were determined in samples of cherry tomatoes, granny smith apples and chives that were collected in 2006/2007, freeze-dried and stored at −20 °C in the dark, and in freshly prepared samples. As shown in Fig. 1, the concentrations of SA were similar in the stored and freshly prepared samples.

3.3. Control for intra-sample variability

Intra-sample variability was investigated by repeating the experiments six times with the same sample (fresh cherry tomatoes). The average concentration of SA was 53.37 mg/kg, CV

Table 1c
Total salicylic acid content of herbs, spices, nuts, seeds analysed.

Herbs, Spices, Nuts and seeds	Variety	Current study	Salicylic acid content (mg/kg)					
			Wood et al. (2011)	Herrmann and Nagel (2009)	Paterson et al. (2006)	Venema et al. (1996)	Variyar and Bandhopadhay (1995)	Swain et al. (1985)
Spices								
Black pepper	powder	45.72[a]	–	3.0	90	3.05	–	62
Cinnamon	powder	57.24[a]	0.78	10	642, 120[c]	23.8	122	152
Coriander	powder	207.18[a]	–	1.0	27	–	–	–
Cumin	powder	604.97[a]	29.76	–	16294,980[c]	–	–	450
Turmeric	powder	38.83[a]	20.88	17	3505, 2889[c]	–	–	764
Herbs								
Basil leaves	fresh	3.24	–	–	–	–	–	
	powder	–	–	–	–	–	–	34
Chives	fresh	20.16	–	–	–	–	–	0.3
Garlic	fresh	17.05	–	–	56	–	–	1
Ginger	fresh	31.57	–	–	35	–	–	45
Lemon grass	fresh	3.59	–	–	–	–	–	–
Parsley leaves	fresh	2.80	–	–	–	–	–	0.8
Rhubarb	fresh	6.91	–	–	–	–	–	1.3
Vanilla bean	extract	130.26[d]	–	–	–	–	–	–
	essence	–	–	–	–	–	–	14.4
Nuts & seeds								
Almond	fresh	47.09[b]	–		–	–		30
Cashew nut	fresh	41.10[b]	–		–	–		0.7
Pumpkin seeds	fresh	17.58[b]	–	–	–	–	–	–

[a] Homebrand, Hoyts, Woolworths select pooled.
[b] Coles, Freshlife, Lucky pooled.
[c] two separate sources.
[d] 3 batches of Queens pooled.

Table 1d
Total SA content of beverages, sugars, oils, cereals analysed.

Beverages	Variety	Brands pooled	Salicylic acid content (mg/L)				
			Current study	Wood et al. (2011)	Paterson et al. (2006)	Venema et al. (1996)	Swain et al. (1985)
Drinking chocolate[a]	powder	Victoria original, Nestle Alpenblend, Abundant Earth organic	51.48	–	–	–	–
Tea	Chamomile	Lipton, Tasty, Twinnings	4.72	–	–	–	0.6
	English Breakfast	Twinnings, Dilma, Madura	2.40	–	–	–	–
		Twinnings	–	–	–	–	30
	Peppermint	Twinnings, Tasty, Nerada	2.19	–	–	–	11
	other[b]	–	–	–	–	0.42	–
Coffee	Instant decaff	Nescafe Blend, Moccona Classic Decaf, Republica 100% pure decaf	2.05	–	–	–	–
		Nescafe, Moccono	–	–	–	–	0
	Instant regular	Moccona Classic Medium Roast, Nescafe Blend 43, International Roast	2.04	–	–	–	–
		Nescafe	–	–	–	–	5.9
		Moccono	–	–	–	–	6.4
	other[b]	–	–	–	–	0.37	–
Sugar	Caster sugar	CSR, Homebrand, Billington	nd	–	–	–	0
	Maple syrup	Woolworths select, S&Wpremium, Queen	nd	–	–	–	–
		Campbell	–	–	–	–	0
	Soft brown sugar	CSR, Homebrand	nd	–	–	–	–
	White granulated	–	–	–	–	–	0
Oil	Olive	Balducci extra virgin, Moro, Bertolli	nd	–	–	–	–
	Sunflower	Homebrand, Golden fields, Crisco	nd	–	–	–	–
Cereals/Flour	White rice flour	Mckenzie's[c]	nd	–	–	–	–
	White rice grains	–	–	–	–	–	0
	White quinoa flour	Mckenzies[c]	nd	–	–	–	–
	White quinoa flake	Macro organic[c]	nd	–	–	–	–

[a] Expressed in mg/kg.
[b] Brands not reported.
[c] Three different batches of the same brands were pooled together. *nd* not detected.Tea prepared by soaking one tea bag in 250 mL water for 2 min. Coffee prepared by adding 1tsp of coffee powder to 250 mL water.

3.03%, SD 1.62 and CI range 6.37% indicating minimal intra-sample variability.

3.4. Salicylic acid content in foods commonly available in Australia

The foods examined for total salicylates are listed in Tables 1a–1d . For comparative purposes, SA content reported in previous literature is also included. Most of the results are distinctly different from those previously published. From the range of foods tested, eggplants, bean sprouts, parsnips, dates, watermelon and ginger show similar SA content as reported previously. SA was not detected in oils, sugars and cereals. The total SA content ranged from 1.29 mg/kg (fennel bulb) to 86.18 mg/kg (butternut pumpkin) in vegetables; 2.13 mg/kg (navel oranges) to 36.9 mg/kg (dry dates) in fruits; 3.24 mg/kg (basil leaves) to 604.97 mg/kg (ground cumin) in herbs and spices and from 2.04 mg/kg (instant coffee) to 51.48 mg/kg (drinking chocolate) in beverages analysed. Representative chromatograms of standards and a sample are depicted in Figs. 2 and 3respectively.

For clinical dietetic practice, it is important to formulate diets based on serving size. Tables 2a–2c summarizes SA content of foods based on serving size. SA content in vegetables range from 0.05 mg/serve (celery) to 6 mg/serve (butternut pumpkin); fruits range from 0.04 mg/serve (lemon) to 3.54 mg/serve (dry dates); herbs and spices range from 0.01 mg/serve (ginger) to 1.21 mg/serve (cumin); and beverages range from 0.51 mg/serve(coffee) to 1.18 mg/serve (chamomile tea).

4. Discussion

Given the importance of plant metabolites in human health (Kennedy, 2014), having accurate information about food content of SA is important for those who have potential health issues because of salicylate sensitivity or for the potential use of dietary SA to prevent illness. The current study addresses the controversy over the actual food content by the application of state-of-the-art technologies to measure SA concentrations and by following FSANZ guidelines in the collection and processing of food samples.

The variance of food content of SA in the literature has at least in part been attributed to the analytical methodologies applied. For example, criticisms from authors have included – the use of structurally related phenolic acids in the assay, poor extraction efficiency (Duthie and Wood, 2011), use of non-specific UV detector leading to interference and overestimation of SA content and isocratic elution used in HPLC leading to co-elution of compounds leading to interference in the fluorescence intensity (Paterson et al., 2006). For this reason, we have applied in the current study the use of GC–MS technology, have used an internal standard (deuterated SA) to optimise the accuracy of detection and quantification, and have utilised N-Methyl-N-(trimethylsilyl)

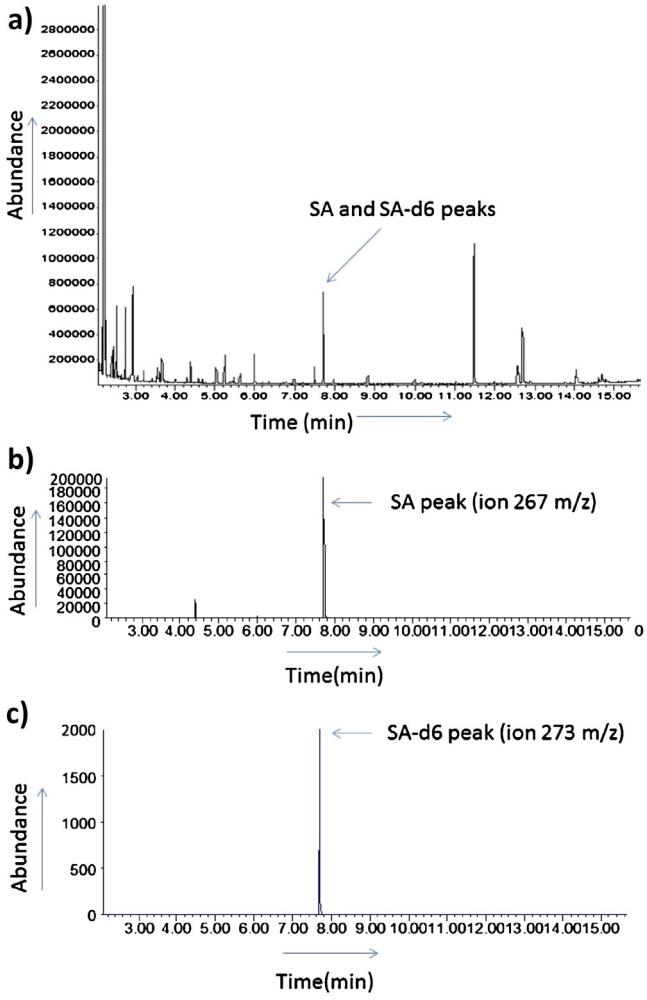

Fig. 2. Representative Chromatogram of a standard. a) Total ion chromatogram of SA and SA-d6 peaks in a standard. b) Extracted ion chromatogram of SA peak and c) SA-d6 peak. Ion 267*m*/*z* and ion 273*m*/*z* represent SA and SA-d6 respectively.The SA and D6-SA have the same retention time of ~7.9 min.

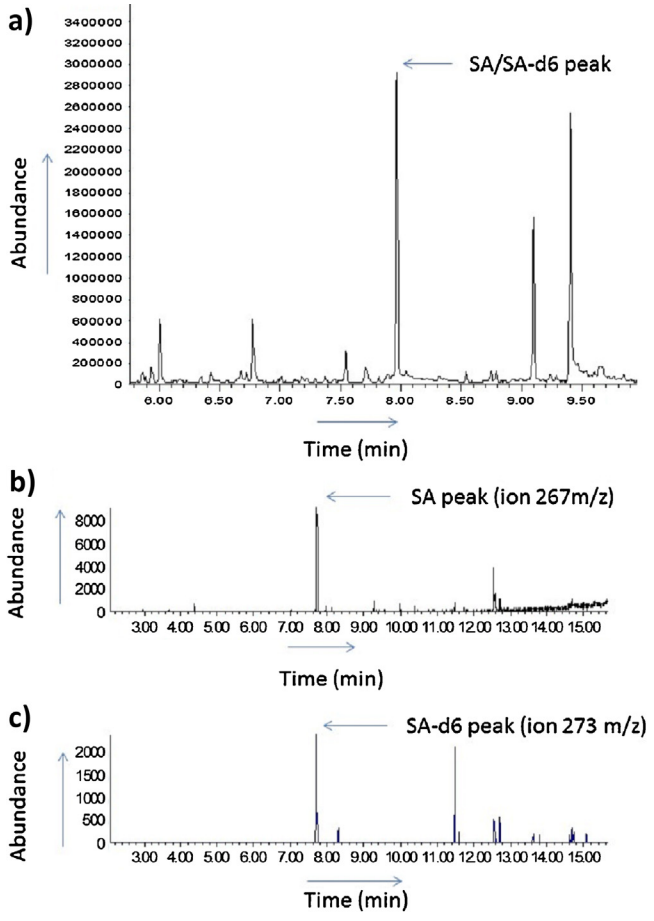

Fig. 3. Representative Chromatogram of a sample. a) Total ion chromatogram of SA and SA-d6 peaks in a sample (zoomed) b) Extracted ion chromatogram of SA peak and c) SA-d6 peak. Ion 267*m*/*z* and ion 273*m*/*z* represent SA and SA-d6 respectively. The SA and D6-SA have the same retention time of ~7.9 min.

Table 2a
Salicylic acid (SA) content of vegetables expressed in serving size (mg/serve).

Vegetables	Variety	Serving size (g)	SA (mg/serve)
Asparagus	–	30	0.25
Bean	sprouts	102	0.13
Beetroot	–	41	1.10
Broccoli	–	47	0.52
Brussels sprouts	–	38	0.33
Bok choy	–	85	0.16
Capsicum	red	52	0.46
Cauliflower	–	66	0.39
Corn on cob	fresh	85	1.4
Cabbage	common	94	0.24
Cucumber	common	64	0.37
	long	64	0.19
Chilli	red	28	0.18
Choy sum	–	60	0.01
Celery	–	19	0.05
Eggplant	–	41	0.32
Endive	baby	40	0.15
Fennel	top	12	0.34
	bulb	49	0.06
Green beans	–	86	1.19
Leek	–	83	0.34
Lettuce	butter	35	0.31
	cos	35	0.55
	iceberg	35	0.09
Mushroom	button	74	0.60
Onion	white	36	0.18
	spanish	45	0.58
Parsnip	–	62	0.24
Peas	green	72	1.84
	sugar snap	60	0.29
Pumpkin	butternut	60	0.52
	jap	60	0.67
Potato	sweet yellow	140	2.96
	white	122	0.57
Rocket leaves	–	35	0.55
Radish	–	40	0.68
Red kidney beans	canned cooked	95	0.57
Spinach	–	38	0.09
Swede	–	70	0.66
Tomato	canned diced	92	0.59
	paste	17	0.18
	cherry	68	0.48
	sun-dried	16	0.30
	table/common	119	0.38
	roma	46	0.23
Zucchini	–	66	0.40

Table 2b
Salicylic acid (SA) content of fruits expressed in serving size (mg/serve).

Fruits	Variety	Serving size (g)	Â SA (mg/serve)
Apple	golden delicious[a]	165	0.52
	golden delicious[b]	165	0.9
	granny smith[a]	165	1.6
	pink lady[a]	165	0.48
	pink lady[b]	165	1.49
Apricot	–	112	0.92
Avocado	haas	80	2.38
Banana	common	100	0.54
Blueberry	–	28	0.26
Cantaloupe	–	100	0.50
Coconut	dry	37	0.82
Dates	dry	96	3.54
Grapes	ralli[c]	150	1.15
	thompson[c]	150	1.25
Kiwifruit	–	85	1.14
Lemons	–	6	0.04
Mandarin	imperial	86	0.23
Mango	–	20	0.14
Nectarine	–	151	2
Olive	black	6	0.16
Orange	naval	130	0.28
Passionfruit	–	23	0.28
Pawpaw	–	74	0.35
Peach	white	145	0.48
Pear	packham [a]	166	0.49
	packham [b]	166	2.13
	nashi	187	0.6
Persimmon	–	170	1.0
Pineapple	–	82	0.60
Pomegranate	–	73	1.10
Berry	raspberry	45	0.47
	strawberry	96	0.67
Watermelon	–	286	1.37

[a] Peeled.
[b] Unpeeled.
[c] Seedless.

trifluroacetamide (MSTFA) as the derivatizing agent as silylation is the most prevalent method for producing narrow and symmetrical peaks (Duncan et al., 1984; Kataoka, 2005). A similar methodology has been used previously but their aim was to determine free SA levels in food samples (Scotter et al., 2007). Since both free and bound SA in foods are bioavailable, (Paterson et al., 2006) we attempted to quantify total SA content in foods using a similar methodology. This is what has primarily motivated our research.

Differences in the levels of SA content in the same food item using a consistent methodology has also been reported (Paterson et al., 2006). This variation may be due to differences in the variety or growing conditions of the foods analysed. For example, apples of different varieties have different polyphenol composition (Vrhovsek et al., 2004) and organic foods have been shown to contain higher levels of SA than its non-organic counterpart (Baxter et al., 2001). In order to minimise such variance, two strategies were undertaken. First, in order to give a more accurate estimation of the population average of SA content of that food item, we have pooled ten samples of each of the fruit and vegetable analysed compared with previous reports in which six was the maximum number of samples collected per food item, (Wood et al., 2011) a single sample was analysed, (Variyar and Bandyopadhyay, 1995) or no information on sample collection was reported (Robertson and Kermode, 1981). Secondly, care has also been taken in pooling samples of the same variety. Some previous studies have not provided varietal information.

The results for SA content in the current study show several variations from that published in previous literature except eggplants, parsnips and watermelon. In many cases, comparison with previous literature is not possible due to lack of varietal information (e.g. avocados, pears in Table 1b), different varieties analysed (e.g. grapes, plum in Table 1b) or due to a different forms of the foods analysed (e.g. peeled vis-à-vis unpeeled apples and fresh vis-à-vis powdered basil leaves). Where comparison is possible, the SA levels obtained were, in most cases, higher than that previously reported. This difference may partly be attributed to the increased precision of quantitation due to use of SA-d6 as an internal standard, more extensive extraction, more efficient derivatization and the extensive pooling of food samples. Difference in origin may also play a role in the SA content. Previous literature has reported a 649 mg difference in cumin obtained from two different sources (Paterson et al., 2006).

Two commonly used oils in Australia – sunflower and olive oils – had no detectable SA content. This is supported by the fact that seed oils, like sunflower oil, are devoid of phenolics, and olive oil, although containing simple phenolics, secoiridoids and lignans, are devoid of SA (Owen et al., 2000). SA was also not detected in any of the sugars analysed. This is in agreement with previous literature, where 23 phenolics were isolated in Canadian maple syrup but not SA (Li and Seeram, 2010). Also granulated white sugar was shown to be devoid of SA (Swain et al., 1985). Quinoa and rice flour showed no detectable SA content, even though rice

Table 2c
Salicylic acid (SA) content of herbs, spices, nuts, seeds & beverages expressed in serving size (mg/serve).

Food			Serving size (g)	SA (mg/serve)
Herbs	Basil leaves[a]		16	0.05
	Chives[a]		4	0.08
	Garlic root[a]		3	0.05
	Ginger root [a]		0.01	0.11
	Lemongrass[a]		4	0.02
	Parsley leaves[a]		16	0.04
	Rubarb[a]		52	0.36
	Vanilla bean extract		4	0.52
Spices	Black pepper[b]		2	0.09
	Cinnamon bark[b]		2	0.11
	Coriander seed[b]		2	0.41
	Cumin seed[b]		2	1.21
	Turmeric root[b]		2	0.08
Nuts & seeds	Almond		24	1.13
	Cashew nut		24	0.98
	Â Pumpkin seeds		30	0.53
Beverages	Coffee[c]	Instant decaf	2	0.51
		Instant regular	2	0.51
	Drinking chocolate		14	0.72
	Tea[d]	Camomile	2	1.18
		English breakfast	2	0.6
		Peppermint	2	0.55

[a] Fresh.
[b] Dry powdered.
[c] Coffee prepared by adding 1teaspoon of coffee in 250 mL boiling water.
[d] Tea prepared by soaking 1tea bag (2 g) in 250 mL boiling water for 2 min.

seedlings are reported to contain SA (0.01–37.19 μg/g fresh weight) (Silverman et al., 1995). Absence of SA in adult plants may be due to its concentration in the leaves (Yang et al., 2004).

SA levels in raw foods can be affected by the way the food is consumed. In the current study, SA content in unpeeled pink lady apple is 9.03 mg/kg as opposed to 2.93 mg/kg in the peeled variety. This may indicate maximum concentration of SA in the skin. There is evidence suggesting that polyphenol content of skin of fruits can be significantly higher than the pulp (Fattouch et al., 2008). On the other hand, SA content in beverages like tea may be affected by the method of preparation. In our study, SA content of English Breakfast tea was reported to be 2.40 mg/L which is significantly different from that reported previously – 30 mg/L (Swain et al., 1985). This difference may partly be due to the difference in soaking time. In a previous study, it was reported that a longer soaking time of tea at 100 °C was associated with a higher percentage of polyphenols in the water extract of tea (Zhou et al., 2000). This may be applicable for SA content as well.

New information on SA food content is also reported in the current study. First, the content of bok choy, butter and cos lettuce, rocket leaves, fennel, canned kidney beans, pink lady apple, nashi pear, quinoa, brown sugar and drinking chocolate have been documented. Secondly, we have listed SA levels of foods based on the serving size. This information is required while formulating diets in clinical dietetic practice. A food which seems to be high in SA may be well tolerated by a patient with SA hypersensitivity if the serving size is small as the total SA entering the body may be quiet negligible. For example spices like cumin with SA content of 604.97 mg/kg only delivers 1.21 mg of SA per serve as a single serve of cumin is 2 g. Hence, categorisation of foods into the level of SA content based on clinical trials is necessary.

In conclusion, methodologies applied to the measurement of SA content of food have been optimised – via attention to analytical methodological issues, by sampling techniques used, and by paying due attention to variety, seasonal variation, origin, mode of consumption and preparation – to provide the most accurate estimation in a field steeped in variability and controversy. The results provide a valuable addition to the existing body of literature although the analyses need to be extended to a greater number of food items.

Conflicts of interest

The authors have no conflict of interest to declare.

Author contributions

Sreepurna Malakar and Jacqueline Barrett designed the experiments. Sreepurna Malakar performed the experiments, collected and analyzed research data and drafted the manuscript. Peter Gibson and Jane Muir critically reviewed and edited the manuscript and supervised the entire study. All authors read and approved the final manuscript.

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