Determination of Acetylsalicylic Acid and Salicylic Acid in Foods, Using HPLC with Fluorescence Detection

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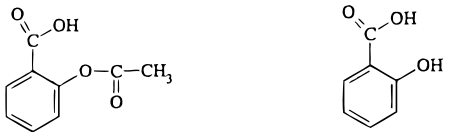
We developed a specific and sensitive HPLC method with fluorescence detection for the determination of free acetylsalicylic acid, free salicylic acid, and free salicylic acid plus salicylic acid after alkaline hydrolysis (free-plus-bound) in foods. Acetylsalicylic acid was detected after postcolumn hydrolysis to salicylic acid. With the method for free acetylsalicylic acid and salicylic acid, recovery was 95−98% for acetylsalicylic acid added to foods and 92−102% for salicylic acid. Recovery of added salicylic acid was 79−94% for the free-plus-bound salicylic acid method. The limit of detection was 0.02 mg/kg for fresh and 0.2 mg/kg for dried foods for all substances. We did not find acetylsalicylic acid in any of 30 foods previously thought to be high in salicylates. The contents of free-plus-bound salicylic acid and of free salicylic acid ranged from 0 to 1 mg/kg in vegetables and fruits and from 3 to 28 mg/kg in herbs and spices. Thus the tested foods did not contain acetylsalicylic acid and only small amounts of salicylic acid. Our data suggest that the average daily intake of acetylsalicylic acid from foods is nil and that of salicylic acid is 0−5 mg/day.

Keywords: *Acetylsalicylic acid; aspirin; salicylic acid; phenolic acids; foods; HPLC; determination; fluorescence detection*

INTRODUCTION

Acetylsalicylic acid or 2-acetoxybenzoic acid (Figure 1) are the formal names of a drug commonly known as aspirin. Aspirin is effective as a profylactic against coronary heart disease in doses as low as 30 mg/day (Roth et al., 1994; the Dutch TIA Trial Group, 1991; Hennekens et al., 1989; Antiplatelet Trialists' Collaboration, 1994). Acetylsalicylic acid may also prevent colon cancer (Giovannucci et al., 1994) and pregnancy induced pre-eclampsy (Hauth et al., 1993). If acetylsalicylic acid would be present in foods, a diet rich in acetylsalicylic acid should have an antithrombotic effect. Not much is known about the presence of acetylsalicylic acid in food. Swain (1984) suggested that acetylsalicylic acid was present in 37 out of 56 foods studied. However, only qualitative data were given, obtained with thin layer chromatography.

Feingold (1976) suggested that the Kaiser−Permanente diet, which eliminates all artificial food colors and flavors, as well as foods containing natural salicylates, improves behavioral disturbance in children. Although this could not be substantiated in properly controlled trials, belief in the efficacy of dietary treatment with salicylate-free diets is firmly held (Wender, 1986). Hypersensitivity is one of the reported adverse reactions of medication containing salicylates, but improvement by a salicylate-free diet is highly unlikely (Häberle, 1987). In spite of this, there is still much interest in salicylate levels of foods (Databank ALBA, 1993).



Acetylsalicylic acid Salicylic acid

Figure 1. Structure of acetylsalicylic acid and salicylic acid.

The number of publications regarding the content of salicylates in foodstuffs is limited and contradictive (Swain et al., 1985; Robertson and Kermode, 1981; Herrmann, 1989). Previous food analyses were performed using HPLC with UV detection at 235−245 nm, which is prone to interferences. We therefore adapted an HPLC method with fluorescence detection (Siebert and Bochner, 1987) for food analysis and measured free acetylsalicylic acid, free salicylic acid, and free-plus-bound salicylic acid, i.e. free salicylic acid plus salicylic acid after alkaline hydrolysis, in 30 common foods previously reported to contain salicylates.

MATERIALS AND METHODS

Foods and Sample Preparation. We selected foods that were earlier reported to have a high content of salicylic acid and/or are known to be consumed in large amounts.

We purchased three samples of 500 g of each type of fresh fruit or vegetable at different local supermarkets in the summer of 1994. Fresh foods were processed within 24 h, nonedible parts were removed, and the three samples were combined per product to a composite sample by mixing equal amounts. One packing of each of three different brands of processed foods was purchased, again in local supermarkets in the summer of 1994, and combined to a composite sample.

Fresh and canned foods were chopped under liquid nitrogen immediately after cleaning. The frozen samples were ground using a food processor, and extracted immediately. None of

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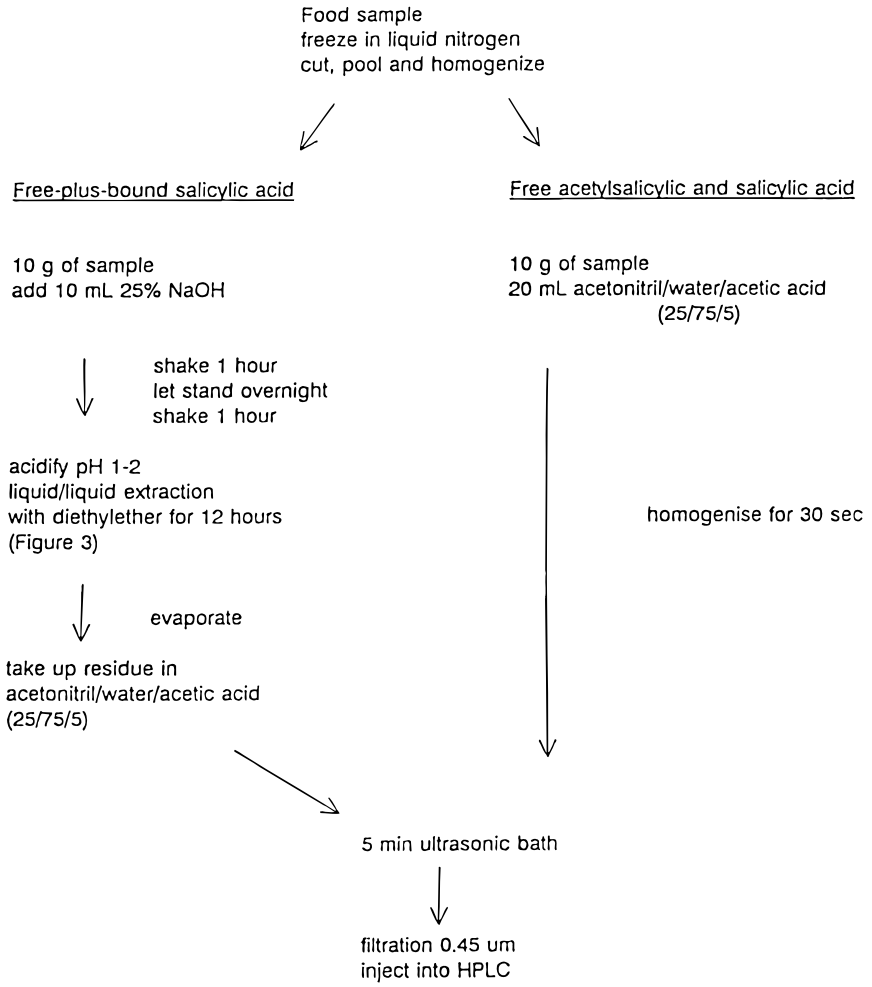

Figure 2. Methods for extraction of free-plus-bound salicylic acid and of free acetylsalicylic and salicylic acid.

the samples were freeze-dried because of the risk of sublimation of the salicylic acid. Dried foods were ground without pretreatment.

We prepared tea from 1500 mL of boiling water and three tea bags of different brands each containing 4 g of tea. Tea bags were removed after 5 min, and the liquid was allowed to cool. We prepared coffee by pouring 1 L of boiling water on 50 g of a mixture of three brands of ground coffee contained in a paper filter. The liquid was allowed to cool.

Extraction and Hydrolysis. Samples were protected from direct daylight during the entire extraction procedure (Figure 2). We weighed out 10.00 g of the freshly ground fresh foods or 1.00 g of the dried foods and added 9 mL of water to the dried foods.

Determination of Free Acetylsalicylic Acid and Salicylic Acid. We homogenized samples with 20 mL of acetonitrile/water/acetic acid (25/75/5) for 30 s in a Waring blender at high speed and sonicated for 5 min.

Free-plus-Bound Salicylic Acid. We essentially followed the method of Swain (1984): food samples were mixed with 10 mL of NaOH (250 g/L) for 1 h on a rotary shaker (New Brunswick Sc.) at 250 rpm. Extracts were left to stand overnight at room temperature, shaken for 1 h, acidified to pH 1−2 with 10 M HCl, and transferred quantitatively with water into a liquid/liquid extractor (Figure 3). The extractor was placed onto a heated flask, and diethyl ether was added in the extractor until about 50 mL flowed over into the heated flask. We extracted the samples on two successive days for 6 h each day. Diethyl ether was evaporated carefully until almost dry, and the last bit of ether was evaporated at room temperature, to avoid sublimation of salicylic acid. The residue was taken up in 25 mL of acetonitrile/water/acetic acid (25/75/5) and sonicated for 5 min.

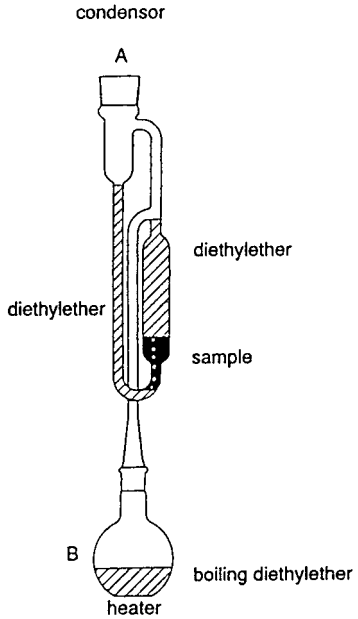

Figure 3. Liquid/liquid extraction apparatus for the continuous water/diethyl ether extraction of salicylic acid from hydrolyzed food samples. Acidified liquid sample after alkaline hydrolysis is introduced into A. Diethyl ether is added in A until flask B contains 50 mL. Heating is started after connecting the extractor with the condensor.

We filtered approximately 2 mL of each sample extract through a 0.45 μm filter for organic solvents (Acrodisc CR) prior to injection into the HPLC system.

Standards. Acetylsalicylic acid (Sigma A-5376) and salicylic acid (Sigma S-0875), were dissolved in acetonitrile/acetic acid (99/1) to a concentration of 1 g/L. Acetylsalicylic acid standards were made daily and salicylic acid standards once a week. Further dilutions were made daily in acetonitrile/water/acetic acid (25/75/5) which is similar to the eluent, thus preventing injection problems. Calibration curves were constructed in the range of 2−20 μg/L for acetylsalicylic acid and 10−300 μg/L for salicylic acid. We identified the peaks by comparing retention times of samples with those of standards. Occasionally, peaks were confirmed by addition of standards to the sample extracts.

Chromatography. The HPLC system consisted of a Perkin-Elmer ISS-100 automatic injector, a Gynkotek 480 pump, and a Lichrospher RP-18 (Merck) column (4 × 250 mm, 5 μm) protected by a Perisorb RP-18 (3.9 × 40 mm, 30−40 μm) precolumn both placed in a column oven set at 30 °C, a fluorescence detector (Merck Hitachi F-1050) with excitation wavelength at 300 nm, and emission wavelength at 400 nm, and a postcolumn stainless steel reaction coil (0.5 × 5000 mm) immersed in a glycerol-filled bath set at 60 °C. Acetylsalicylic acid and salicylic acid were separated with an eluent consisting of methanol/water/phosphoric acid 85% (40/60/0.2), at a flow rate of 0.9 mL/min. Acetylsalicylic acid was hydrolyzed to salicylic acid in the postcolumn reactor by addition of 0.15 mL/min of NaOH (1 M) to the eluent using a Gilson minipuls-3 pump.

RESULTS

The salicylates in foods are partly bound as esters or glycosides (Herrmann, 1990). We used alkaline hydrolysis plus ether extraction to liberate matrix-bound salicylates and to convert salicylate esters and glycosides to their parent salicylic acid. Values thus obtained were named "free-plus-bound salicylic acid". Acetylsalicylic acid is unstable in alkali. We therefore used a gentle extraction to detect acetylsalicylic acid; this technique also detects the extractable or "free" salicylic acid.

Contents were measured with an optimized HPLC method with fluorescence detection.

Optimization of Column and Eluent. We tested a Novapak RP-18, 150 × 3.9 mm, 4 μm column (Waters Associates, Milford MA); a Lichrospher RP-18, 250 × 4 mm, 5 μm column (Merck); and a Inertsil ODS 2, 150 × 4.6 mm, 5 μm column (GL Sciences Inc.) with UV detection at 245 nm. Dissociation of salicylic acid (pK_a = 3.0) is an important variable in its chromatographic behavior. We therefore tested acetic acid and phosphoric acid as eluent acidifiers, taking care that the pH of the eluent remained between pH 2.2 and 2.4. Methanol/water and acetonitrile/water both with acetic acid or phosphoric acid were used.

The Inertsil column showed low plate numbers for both acetylsalicylic acid (N = 3200) and salicylic acid (N = 5700) with acetonitrile/water/phosphoric acid. Therefore it was not tested with any of the other eluents. Acetylsalicylic acid showed symmetric peaks on the Novapak and the Lichrospher column with all eluents, but salicylic acid showed variable degrees of peak tailing. The Lichrospher column was superior for both acetylsalicylic acid (N = 11 200) and salicylic acid (N = 11 500) with all mobile phases. Phosphoric acid was more compatible with the postcolumn reaction than acetic acid, because a lower amount of sodium hydroxide was required to neutralize it. We therefore chose methanol/water/phosphoric acid as a mobile phase and the Lichrospher as a column (Figure 4a).

Postcolumn Hydrolysis. Acetylsalicylic acid can be made fluorescent by conversion to salicylic acid. Postcolumn hydrolysis conditions were essentially as described by Siebert and Bochner (1984), but the use of 0.2% instead of 0.1% of phosphoric acid in the eluent and 1 M instead of 0.5 M NaOH resulted in somewhat better plate numbers.

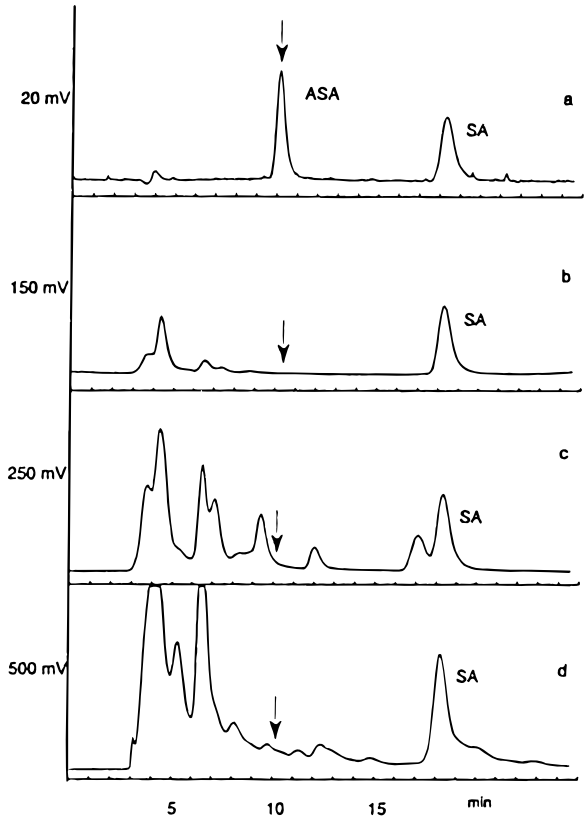

Figure 4. Typical chromatograms of acetylsalicylic acid and salicylic acid in foods. Peaks: ASA, acetylsalicylic acid; SA, salicylic acid. Arrows indicate retention time of acetylsalicylic acid [(a) standards, (b) tea extract, (c) wine, (d) thyme]. Detection: Fluorescence after postcolumn hydrolysis of acetylsalicylic acid; excitation, 300 nm; emission, 400 nm; flow rate, eluent 0.9 mL/min, sodium hydroxide (1 M) 0.15 mL/min.

Acetylsalicylic acid hydrolysis was complete at 60 °C using a coil of 0.5 mm × 5 m and addition of 0.15 mL/min of 1 M NaOH. Salicylic acid proved to be stable under these conditions. Excitation was at 300 nm and emission at 400 nm. The fluorescence of salicylic acid was about 8 times higher at alkaline than at acid pH. Phenolic compounds that possibly interfere in UV detection, such as cinnamic and hydroxybenzoic acids, showed little fluorescence under these conditions. The sensitivy of the fluorescence detection of salicylic acid was about a factor 1000 higher than that by ultraviolet light absorption at 235−245 nm.

Stability of Acetylsalicylic Acid and Salicylic Acid. We tested the stability of acetylsalicylic acid in various extraction solvents by comparing UV spectra of the solutions. The wavelength of maximum UV absorbance in eluent of salicylic acid is 303 nm and that of acetylsalicylic acid is 275 nm. Acetylsalicylic acid hydrolyzed rapidly to salicylic acid when dissolved in methanol. The stability was better in the presence of acetic acid plus methanol or acetonitrile (Table 1).

Salicylic acid was stable for more than 1 week in all solvents if kept in the dark, as judged by its unchanged UV spectrum.

We never observed any degradation of acetylsalicylic acid or salicylic acid in the HPLC elution pattern.

Table 1. Stability of Acetylsalicylic Acid in Various Solvents[a]

solvent	time	loss (%)
methanol	4 h	7
methanol	1 day	22
methanol/1% acetic acid	5 days	2
acetonitrile/1% acetic acid	5 days	3
acetonitrile/water/acetic acid (25/75/5)	5 days	2
acetonitrile/water/H_3PO_4 (25/75/0.2)	5 days	3

[a] All determinations are based on single sample analysis; concentrations during storage were 1 mg/mL, and during measurement, 20 μg/mL, except for acetylsalicylic acid in water where we used a saturated solution.

Table 2. Recovery of Acetylsalicylic Acid When Added to Various Foods[a]

	acetylsalicylic acid		
product	amount in food (mg/kg)	amount added (mg/kg)	recovery (%)
grape	<0.02	0.075	98
tomato	<0.02	0.077	98
thyme	<0.02	0.15	95

[a] Means of duplicate analyses, except for thyme which was analyzed in quadruplicate.

Sample Extraction and Hydrolysis. *Free Acetylsalicylic and Salicylic Acids.* Acetylsalicylic acid added as a solution in acetonitrile to samples showed a recovery ranging from 95 to 98% (Table 2) and for added free salicylic acid from 92 to 102% (Table 3). Recoveries did not change after extracts had been stored for 24 h.

Free-plus-Bound Salicylic Acid. We extended the ether extraction as used by Swain (1985) to 12 h, because extraction was not complete after 5 h (Table 4). This was probably due to a less efficient construction of our extraction apparatus (Figure 3). After the first 12 h of extraction, a new flask with 50 mL of ether was placed on the extraction apparatus and the ether was refluxed for an additional 3 h. This extension of the extraction period added little to the salicylic acid yield: the gain was 0% for wine and 5% for thyme.

In the free-plus-bound method acetylsalicylic acid was completely hydrolyzed to salicylic acid. Methylsalicylate, a flavorant, was also hydrolyzed completely.

The mean free-plus-bound salicylic acid content of a sample of freeze-dried endives analyzed on 6 different days was 0.132 mg/kg; the coefficient of variation between days was 8%. Recovery of salicylic acid added as a solution in acetonitrile to samples in the free-plus-bound method ranged from 79 to 94% (Table 2).

Determination in Foods. No acetylsalicylic acid was detected in any of the food samples (Table 5). Salicylic acid was frequently detected in vegetables and fruits though in concentrations of only 0−1 mg/kg. Some herbs and spices like thyme, rosemary, and cinnamon contained amounts up to 28 mg/kg. The poor duplicates of the tomato sample (Table 4) may be due to the fact that the sample was not homogeneous; pits and peel were very difficult to grind.

Figure 4 shows some typical chromatograms of the separation of salicylic acid and acetylsalicylic acid in various foods.

DISCUSSION

We found that foods previously reported to be important sources of salicylic acid and potential sources of acetylsalicylic acid (Swain, 1984, 1985) contained low amounts of salicylic acid and no acetylsalicylic acid. We used a sensitive and specific HPLC method with post-column hydrolysis of acetylsalicylic acid and fluorescence detection optimized for the determination in foodstuffs. For the acetylsalicylic acid content in foods, we used a fast extraction into a solvent in which acetylsalicylic acid was stable. The solubility of acetylsalicylic acid in the solvent was sufficient, so that any free acetylsalicylic acid present in the food sample would be extracted.

None of the 30 products tested by us contained measurable amounts of acetylsalicylic acid. Swain (1984) tested 56 foods with a qualitative thin layer chromatographic method and found acetylsalicylic acid in 14 and both acetylsalicylic acid and salicylate in 23. The identity of the acetylsalicylate spot on the thin layer plate was confirmed by coelution of a standard and by the slow hydrolysis to salicylic acid that occurred on the thin layer plate. Swain extracted acetylsalicylic acid by alkaline extraction for 2 h (Fazio, AOAC, 1990). In our hands all acetylsalicylic acid was hydrolyzed completely to salicylic acid under those conditions.

Swain et al. (1985) found salicylic acid contents ranging from 0 to 60 mg/kg in vegetables and fruits and from 0 to 2180 mg/kg in condiments. Other investigators found much lower contents: Robertson and Kermode (1981) found salicylic acid in the range 0.01−0.82 mg/kg in vegetables and fruits, and Herrmann (1990), using a less sensitive method, found only traces of salicylic acid in vegetables and fruits. Herrmann (1990) also determined the presence of salicylates in herbs and spices at levels of 0−81 mg/kg (Table 5) and questioned the high values found by Swain. Differences might arise by differences in origin, processing, or storage. Therefore, we measured salicylates in composite samples of three different origins or brands. Swain did find a high natural variation amounting to 10-fold differences. However, all our results are much lower than the lowest value given by Swain. This excludes natural variation as a possible explanation for these discrepancies. Discrepancies in salicylates contents between extraction methods may arise through differences in the extent of liberation of matrix-bound salicylates. However, though we used essentially the same extraction method as

Table 3. Recovery of Salicylic Acid When Added to Various Foods[a]

	amount in food free-plus-bound salicylic acid	amount added salicylic acid	recovery	
product	(mg/kg)	(mg/kg)	free salicylic acid method (%)	free-plus-bound salicylic acid method (%)
brewed tea	0.500	0.400	99	88
grape	0.032	0.075	102	90
tomato	0.225	0.200	102	79
thyme	16.1	10.0	92	94

[a] Means of duplicate analyses, except for thyme which was analyzed in quadripulate; for method, see text.

Table 4. Influence of Extraction Time on the Amounts of Salicylic Acid Extracted from Different Foods by the Free and Free-plus-Bound Methods[a]

product	free salicylic acid (mg/kg)	free-plus-bound salicylic acid 5 h (mg/kg)	free-plus-bound salicylic acid 12 h[b] (mg/kg)
grape	<0.02	0.020	0.028
tomato	0.05	0.236	0.276[c]
thyme	no determination	12.05	15.85

[a] Means of duplicate determinations. [b] After 5 h new solvent in heated flask; cf. Materials and Methods. [c] Poor duplicate, sample possibly not homogeneous.

Swain we found much lower contents. Possibly the HPLC separation and UV detection of Swain was not specific enough and some other component coeluted at the same time as salicylic acid.

Muller and Fugelsang (1994) published salicylic acid contents in red wine of 11−21.5 mg/L. Again we believe that these values are too high (Janssen, 1994). In four wine samples from different countries we found 0.26−0.71 mg/kg. This agrees with data of Robertson (1983) of 0.04 mg/kg in grapes and 0.08 mg/kg in fermented juice.

Little free salicylic acid is present in fresh products because salicylate is bound as ester or as glycoside (Herrmann, 1989). In most of the processed products salicylic acid apparently was liberated by food processing.

The difference in the amounts of free salicylic acid and free-plus-bound salicylic acid for peppermint (Table 5) is probably due to methylsalicylate used as a flavorant.

Some herbs and spices contain rather high amounts of salicylic acid, but their consumption is low. Using the food intake data of the Dutch Food Consumption Survey (1993) and our data of free-plus-bound salicylic acid, we estimate the intake of salicylates in The Netherlands 0−5 mg/day.

Our data suggest that the acetylsalicylic acid and the salicylic acid content in a normal mixed daily diet is too low to produce measurable physiological effects *in vivo*.

Table 5. Content of Free Acetylsalicylic Acid and Salicylic Acid and of Free-plus-Bound Salicylic Acid in Various Foodstuffs[a]

	content in edible part of food					
	this study			published values for salicylic acid		
product	free acetylsalicylic acid (mg/kg)	free salicylic acid (mg/kg)	free-plus-bound salicylic acid (mg/kg)	Swain (1985) (mg/kg)	Herrmann (1990) (mg/kg)	Robertson (1981) (mg/kg)
Fresh or Canned Vegetables and Fruits						
apples with peel	<0.02[b]	<0.02[b]	<0.02[b]	0.8−5.9 av 4	<1	0.04
blueberry jam	<0.02	0.55	0.67			
canned apricot	<0.02	0.13	0.1	14.2	<1	0.03
canned cherries	<0.02	0.27	0.36	3−27.8	<1−2	
cucumber with peel	<0.02	0.02	0.077	7.8[c]	<1−3	
grape	<0.02	<0.02	0.03	0.94−1.88		0.04
nectarine	<0.02	0.03	0.87	4.9		0.04
orange	<0.02	<0.02	<0.02	23.9	<1	0.07
strawberry	<0.02	0.03	0.65	13.6	0−2	0.04
tomato with peel	<0.02	0.11	0.36	1.3	<1−1	0.05
Herbs and Spices						
cinnamon	<0.2	6.6	23.8	152	10	
mild curry powder	<0.2	3.69	5.55	2180		
French mustard	<0.2	0.2	0.48	260[d]	19−39	
oregano	<0.2	11.8	19.9	660		
hot paprika powder	<0.2	1.73	2.98	2030		
black pepper	<0.2	1.18	3.05	62	3	
rosemary	<0.2	6.76	28.4	680	21	
thyme	<0.2	11.95	12.8[e]	1830	11	
Beverages						
pilsner beer	<0.02	<0.02	<0.02	3[f]		
brewed coffee	<0.02	0.24	0.37	0−9[d]		
brewed tea	<0.02	0.33	0.42	30[g]		
red French Bordeaux wine	<0.02	0.65	0.71	9		
red Spanish Rioja wine	<0.02	0.68	0.70			
red Italian Chianti wine	<0.02	0.32	0.32			
red Californian wine	<0.02	0.26	0.28			
Miscellaneous						
grape currant	<0.2	0.32	0.41	58		
honey	<0.2	0.59	0.66	25−112 av 63		
licorice	<0.2	1.25	1.62[e]	79.6−97.8		
peppermint	<0.2	<0.2	0.07	7.7−75.8 av 27.8		
grape raisins	<0.2	0.60	0.98	66.2−78		
tomato paste	<0.02	0.48	0.75	4.3−14.4 av 8.1		0.07

[a] All analyses were an single analysis, except when the content was >1.0 mg/kg for high-consumption foods or 10 mg/kg for low-consumption foods; samples were then reanalyzed in duplicate. [b] Detection limit. [c] Without peel. [d] Powder. [e] Mean of three analyses. [f] Not pilsner. [g] Bag.

LITERATURE CITED

Antiplatelet Trialists' Collaboration: Collaborative overview of randomized trials of antiplatelet treatment. Part 1: Prevention of death, myocardial infarction and stroke by prolonged antiplatelet therapy in various categories of patients. *Br. Med. J.* **1994**, 91.

Databank ALBA. *Salicylaten in voedingsmiddelen (Salicylates in foodstuffs)*; TNO-voeding: Zeist, The Netherlands, 1993.

Dutch Food Comsumption Survey. *Zo eet Nederland, 1992 (That's the way Holland is eating, 1992);* Voorlichtingsbureau van de voeding: Den Haag, 1993.

The Dutch TIA Trial Group. A comparison of two doses of aspirin (30 mg vs. 283 mg a day) in patients after a transient ischemic attack or minor ischemic stroke. *N. Engl. J. Med.* **1991**, *325*, 1261−66.

Fazio, T. Food additives: Direct. In *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed.; Helrich, K., Ed.; Association of Official Analytical Chemists, Inc.: Arlington, VA, 1990; pp 1155−1156.

Feingold, B. F. Hyperkinesis and learning disabilities linked to ingestion of artificial food colors and flavors. *J. Learn. Disabil.* **1976,** *9*, 19−27.

Giovannucci, E.; Rimm, E. B.; Stampfer, M. J.; Colditz, G. A.; Ascherio A.; Willett, W. C. Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. *Ann. Intern. Med.* **1994**, *121*, 241−246.

Häberle, M. Salicylate und biogene Amine-natürliche Inhaltsstoffe von Nahrungsmitteln als Auslöser von Pseudoallergien (Salicylates and biogenic amines−natural compounds of foodstuffs as inducers of pseudo-allergies). *Ernährungs-Umschau* **1987**, *34*, 287−296.

Hauth, J. C.; Goldenber, R. L.; Parker, R.; Philpis, J. B.; Copper, R. L.; Dubard, M. B.; Cutter, G. R. Low-dose aspirin therapy to prevent eclampsia. *Am. J. Abstet. Gynecol.*, **1993**, *168*, 1083−1093.

Hennekens, C. H.; Buring, J. E.; Sandercock, P.; Collins, R.; Peto, R. Aspirin and other anti-platelet agents in the secondary and primary prevention of cardiovascular disease. *Circulation* **1989**, *80*, 749−56.

Herrmann, K. Occurence and content of hydroxycinnamic and hydroxybenzoic compounds in foods. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 4315−4347.

Herrmann, K. Salicylsaüre und andere verbreitete Hydroxybenzoësaüren und deren natürlich vorkommende Verbindungen in Lebensmitteln (Salicylic acid and other widespread hydroxybenzoic acids and their naturally occurring derivatives in foodstuffs). *Ernahrungs-Umschau*, **1990**, *37*, 108−112.

Janssen, P. L. T. M. K.; Katan M. B.; Hollman P. C. H.; Venema D. P. No aspirin in red wine. *Lancet*, **1994**, *344*, 762 (letter).

Muller, C. J.; Fugelsang, K. C. Take two glasses of wine and see me in the morning. *Lancet* **1994**, *343*, 1428−1429 (letter).

Robertson, G. L. Salicylic acid in grapes. *Am. J. Enol. Vitic.* **1983**, *34*, 42−43.

Robertson, G. L.; Kermode, W. J. Salicylic acid in fresh and canned fruit and vegetables. *J. Sci. Food Agric.* **1981**, *32*, 833−836.

Roth, G. J.; Calverley D. C. Aspirin, platelets, and thrombosis: theory and practice. *Blood* **1994**, *83*, 885−898.

Siebert, D. M.; Bochner, F. Determination of plasma aspirin and salicylic acid concentrations after low aspirin doses by HPLC with post-column hydrolysis and fluorescence detection. *J. Chromatogr.* **1987**, *420*, 425−431.

Swain, A. R. Ph.D. Thesis, University of Sydney, Australia, 1984.

Swain, A. R.; Dutton, S. P.; Truswell, A. S. Salicylates in foods. *J. Am. Dietetic Assoc.* **1985**, *85*, 950−960.

Wender, E. H. The food additive-free diet in the treatment of behavior disorders: a review. *J. D. Behav. Pediatr.* **1986**, *7*, 35−42.

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