Urinary salicylate excretion in subjects eating a variety of diets shows that amounts of bioavailable salicylates in foods are low[1–3]

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ABSTRACT Intake of acetylsalicylic acid reduces the risk of cardiovascular disease and is associated with a decreased risk for colorectal cancer. Amounts of salicylates in foods are thus of interest, but data are scarce and controversial. We gave 58 μmol (10.5 mg) pure acetylsalicylic acid or 66 μmol (9.1 mg) salicylic acid to six volunteers and recovered 77–80% in 24-h urine samples. Thus, urinary excretion is a valid indicator for intake of free forms of (acetyl)salicylic acid. To estimate the bioavailable salicylate contents of diets, we subsequently studied salicylate excretion in 17 volunteers from 14 countries and four continents who ate a wide variety of self-selected diets. Median 24-h urinary salicylate excretion was 10 μmol (range: 6–12 μmol). Values increased with the fiber content of the diet ($r = 0.73$), suggesting that vegetable foods are the main sources of salicylates. However, amounts of salicylates in a variety of diets are evidently low and probably insufficient to affect disease risk. *Am J Clin Nutr* 1996;64: 743–7.

KEY WORDS Acetylsalicylate, aspirin, excretion, diet, human, salicylate, urine

INTRODUCTION

Acetylsalicylic acid, aspirin, has a variety of biological effects. It is effective in the prevention of cardiovascular disease in doses as low as 30 mg/d (1–3). Intake has also been associated with a decreased risk for colorectal cancer (4, 5). Feingold (6) suggested that the intake of dietary salicylates causes hyperactivity in children, but this could not be substantiated in properly controlled trials (7, 8).

Plants contain natural salicylates, but data on the salicylate contents of foods are scarce and controversial (9–15; AR Swain, RH Lobley, AS Truswell, unpublished observations, 1985). Swain et al (13, 14) suggested that a normal mixed diet provides 72–1448 μmol/d (10–200 mg/d) total salicylates, and significant but unknown amounts of acetylsalicylate. In contrast, we (15, 16) found total salicylate contents of only 0–7 nmol/g (0–1 μg/g) in vegetables and fruits, and 20–200 nmol/g (3–28 μg/g) in herbs and spices. We could not confirm the presence of acetylsalicylate in any of the 30 Dutch products studied; the limit of detection was 0.1 nmol/g (0.02 μg/g) for fresh, and 1.4 nmol/g (0.2 μg/g) for dry products. Uncertainties about concentrations of salicylates in the diet may arise because of a limited selection of foods or differences in analytical techniques; for instance, liberation of salicylates from a plant matrix is notoriously difficult. Acetylsalicylate is excreted in urine mainly as various salicylates (17, 18). Swain et al (13; AR Swain, RH Lobley, AS Truswell, unpublished observations, 1985) showed that this is also the case with other salicylates in foods.

We therefore assessed the validity of using excretion of salicylates in urine as a marker for salicylate intake. We then investigated the urinary excretion of salicylates in subjects with a wide range of dietary habits to estimate the bioavailable salicylate contents of human diets.

SUBJECTS AND METHODS

Subjects

Six Dutch women working at our university participated in a preliminary study to check the validity of urinary salicylates as a marker of intake. These control subjects were aged 27 ± 4 y and ate a normal mixed Western diet.

We next recruited volunteers from the town and surroundings of Wageningen. We sought nonresidents from foreign countries and Dutch subjects eating nontraditional diets to maximize the chances of encountering a wide range of salicylate intakes. All applicants had to speak English or Dutch. Twenty-nine volunteers responded to posters in university buildings, apartments, shops, ethnic restaurants, and community centers for foreigners. We excluded one applicant because she was pregnant, five because their dietary habits deviated too much from their native habits, one because she used medication, and another five at random. Six men and 11 women aged 29 ± 7 y ($\bar{x}$ ± SD) with a mean body mass index (in kg/m^2) of

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21 ± 3 participated in the study. They habitually ate a wide variety of diets (**Table 1**). Thirteen subjects came from abroad and most of them were temporarily living in the Netherlands; four were Dutch.

All subjects were healthy on the basis of a medical questionnaire and negative checks for urinary protein and glucose. They did not smoke and had not used any regular or homoeopathic medication or dietary supplements for at least 1 mo before the study, except for seven women who took oral contraceptives. We instructed subjects to use no dietary supplements or medication during the study; only the use of oral contraceptives was permitted. We supplied them with Naproxen (naproxenum; Pharmachemie BV, Haarlem, Netherlands), which is a salicylate-free pain medication.

The protocol was approved by the Medical Ethics Committee of the Wageningen Agricultural University Department of Human Nutrition. We explained the protocol fully to the subjects who gave their written informed consent.

Study design

The six control subjects of the validation study participated in a randomized crossover study for 3 d separated by washout periods of 14 d. They swallowed an indistinguishable capsule containing either pure salicylic acid, or acetylsalicylic acid, or nothing as a placebo together with a *para*-aminobenzoic acid capsule. On analysis, capsules contained 58 ± 1 μmol (10.5 ± 0.2 mg; $n = 3$) acetylsalicylic acid, 66 ± 2 μmol (9.1 ± 0.2 mg; $n = 3$) salicylic acid, or 78.2 ± 0.6 mg *para*-aminobenzoic acid ($n = 3$). The 17 subjects with unusual diets participated in the study for 1 d.

We visited the six subjects participating in the validation study and the 17 subjects with the different diets at their homes and explained the protocol. We provided them with bottles to sample urine and the herbs and spices that they used in cooking. We also gave them a box containing dry ice, a household scale, a diary, and three capsules containing *para*-aminobenzoic acid, which served to check the completeness of the 24-h urine samples (19).

The day after this visit participants swallowed the first capsule of *para*-aminobenzoic acid just before breakfast, the second before lunch, and the third before dinner. They collected urine for 24 h from the time they swallowed the first capsule. The volunteers collected each urine sample in a separate bottle containing 0.225 g thimerosal (T5125; Sigma, Axel, Netherlands) as a preservative. They wrote the times of urine collections on each bottle, and put filled bottles on dry ice immediately. The 6 subjects of the validation study and the 17 subjects eating their specific diets maintained their usual eating and drinking habits during the study day.

All subjects weighed and recorded in a diary all foods and drinks they consumed during the days of urine collection. They saved samples of all herbs and spices in amounts equal to those that they had used that day. They put dried herbs and spices in plastic bottles and fresh ones on dry ice. Subjects recorded in the diary any signs of illness, medications used, and times at which they had swallowed the capsules.

A trained dietitian (ER) checked the food records at the subjects' homes on day 2. She asked about possible deviations from dietary habits, adverse effects, illness, medications, and visits to a dentist or doctor, and collected the urine samples and herbs and spices. We assigned random codes to the urine samples and stored them at −20 °C. We weighed the samples of herbs and spices.

Chemical analyses

We weighed the thawed 24-h urine samples and pooled them by subject. We took 10-mL aliquots of the 24-h pools and stored the samples at −20 °C until analyzed.

We determined concentrations of total salicylates in the 24-h pools after hydrolysis with 5 mol HCl/L for 2 h at 120 °C according to Swain (13). The residue of the ether extract was taken up in 1.5 mL acetonitrile:water:acetic acid (25:75:5, by

TABLE 1
Origin and diet of 17 subjects eating a variety of self-selected diets who participated in the study of urinary salicylate excretion

Subject	Country of origin	Diet		
		Type of diet	Typical foods consumed	Foods excluded
1	United States	Macrobiotic	Whole grains, vegetables, fruit, sea weeds, soy products	Meat, dairy products, eggs, coffee, black tea
2	China	Chinese	Meat, plant products, spices	Alcoholic beverages
3	Czechoslovakia	East European	Meat, potatoes, vegetables, tea, bread	
4	Ethiopia	African	Bread, fish, chicken, spices	Other sea foods, pork, turkey, alcoholic beverages
5	Finland	Scandinavian	Rye bread, knäckebröd, dairy products	Coffee, meat, alcoholic beverages
6	India	Asian	Rice, vegetables, pulses, spices	Meat, fish, alcoholic beverages
7	Indonesia	Asian	Rice, meat, soy products	Pork
8	Italy	Mediterranean	Pasta, fruit, olives and olive oil, herbs	Alcoholic beverages
9	Lithuania	East European	Meat, eggs, potatoes, vegetables	
10	Malaysia	Asian	Rice, meat, fish, vegetables, fruit, herbs, spices	Pork (products), alcoholic beverages
11	Mexico	South American	Tortilla, fruit, vegetables, meat, cheese, hot sauce	Sea foods, milk
12	Netherlands	Lactoovovegetarian	Plant products	Animal products, dairy, eggs
13	Netherlands	Vegetarian	Plant products, dairy products	Meat, fish
14	Netherlands	"Prehistoric"	Uncooked and unprocessed products, fruit, vegetables	Cooked foods
15	Netherlands	Western	Bread, dairy products, meat, potatoes, vegetables	
16	Suriname	Surinamese	Rice, vegetables, grains, pulses, soy products, spices	Beef
17	Turkey	Middle Eastern	Rice, vegetables, meat, tea, pulses	Pork

vol) and filtered through a 0.45-μm filter for organic solvents (Acrodisc CR PTFE; Gelman Sciences, Ann Arbor, MI). We injected 10 μL onto a Lichrospher 100 RP18 (Merck, Darmstadt, Germany) column (4.6 × 250 mm, 5-μm particle size) by using 85% methanol:water:phosphoric acid (40:60:0.2, by vol) as the mobile phase at a flow rate of 0.9 mL/min. The eluent was mixed with 0.15 mL 1 mol NaOH/L per minute in a postcolumn stainless steel reaction coil (0.5 mm × 5 m) placed in a waterbath at 60 °C. Fluorescence was measured at 400 nm with a Merck Hitachi F-1050 (Tokyo) fluorescence detector with the excitation wavelength set at 300 nm. A urine control sample was included in each series of analyses; the relative SD of the between-run variation was 9%, and of the within-run variation 2%. The limit of detection was 0.1 nmol/g (0.02 μg/g) urine. We measured *para*-aminobenzoic acid photometrically using fluorescamine (F9015; Sigma, Axel, Netherlands) after hydrolysis of urine samples with 0.7 mol HCl/L for 40 min at 100 °C (20).

Data analysis

Recoveries of salicylates in the validation study with salicylic and acetylsalicylic acid supplementation were corrected for salicylate excretion with placebo. We calculated the intake of energy and nutrients of the 17 subjects eating unusual diets using the Netherlands Nutrient Data Bank NEVO (21), and calculated the daily intake of herbs and spices using the weights of the duplicate portions. We did not calculate the food intake of the six participants in the validation study.

To estimate the range of salicylate intake we calculated the intake of dietary salicylate of two subjects having the highest (nos. 6 and 14; Table 1) and two having the lowest (nos. 10 and 16; Table 1) excretion of salicylates using their food records and salicylate concentrations of foods as determined by Swain et al (13, 14), Robertson and Kermode (11), Herrmann (10), Janssen et al (15), and Venema et al (16).

RESULTS

Two subjects (nos. 1 and 7; Table 1) were unwilling to take *para*-aminobenzoic acid; the other 15 and the 6 subjects of the validation study stated that they had swallowed all capsules of *para*-aminobenzoic acid. Mean recovery of *para*-aminobenzoic acid in the 24-h urine pools was 80 ± 14% ($\bar{x}$ ± SD) in the 15 diet study subjects and 86 ± 10% in the 6 validation study subjects.

Mean recovery of administered acetylsalicylic or salicylic acid in 24-h urine of the six validation study subjects was 80 ± 18% for salicylic acid and 77 ± 10% for acetylsalicylic acid (**Figure 1**). Median salicylate excretion was 10 μmol/24 h, with a range of 6–12 μmol/24 h (1.4 mg/24 h, range: 0.8–1.6 mg/24 h) for the six subjects (Figure 1) on the day they took no salicylic or acetylsalicylic acid.

In the 17 subjects with nontraditional diets, energy intake was 9.0 ± 3.1 MJ (range: 3.8–16.9 MJ), of which 15 ± 4% (range: 10–24%) was provided by protein, 51 ± 12% (range: 31–71%) by carbohydrates, and 33 ± 10% (range: 13–47%) by fat; only 2 of the 17 subjects consumed alcohol (nos. 3 and 13; Table 1). Median intake of vegetable protein was 7% of total energy intake (range: 4–12%), and intake of dietary fiber was 1.7 g/MJ (range: 0.6–5.7 g/MJ) (**Figure 2**).

Median excretion of salicylates in urine for the 17 subjects eating unusual diets was 10 μmol/24 h (1.4 mg/24 h), with a range of 4–34 μmol/24 h (0.5–4.7 mg/24 h; Figure 2). Salicylate excretion correlated positively with intake of dietary fiber ($r = 0.73$, $P < 0.01$) and with intake of protein from vegetable sources ($r = 0.42$, $P = 0.10$).

Calculations of the intake of dietary salicylate of four subjects (nos. 6, 10, 14, and 16; Table 1) by using salicylate contents of foods as determined by Swain et al (13, 14) produced intake values that were 2 to 18-fold higher than the amounts excreted in urine (**Table 2**). Calculating the intake of dietary salicylate using salicylate contents of foods as deter-

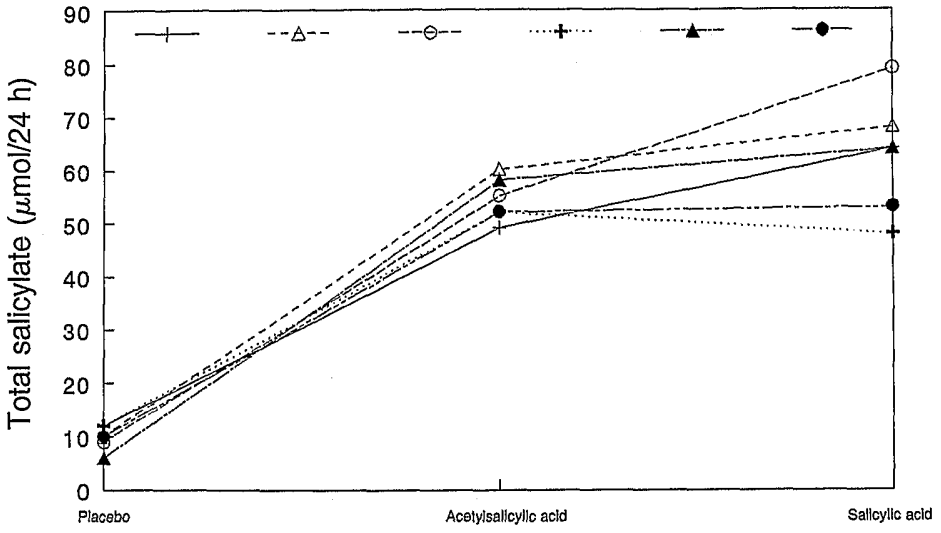

FIGURE 1. Urinary excretion of salicylates in six healthy volunteers participating in a randomized validation study lasting 3 d, separated by washout periods of 14 d. On each urine collection day subjects swallowed a capsule containing 66 ± 2 μmol (9.1 ± 0.2 mg) pure salicylic or 58 ± 1 μmol (10.5 ± 0.2 mg) acetylsalicylic acid, or nothing as placebo. Each symbol represents a different subject. To convert salicylate values to mg/24 h, multiply by 0.13812.

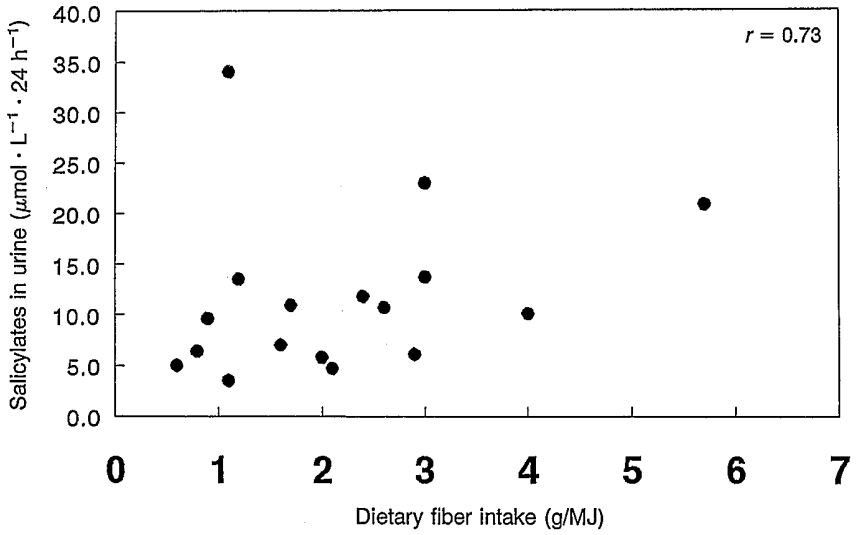

FIGURE 2. Urinary excretion of salicylates versus intake of dietary fiber in 17 healthy volunteers eating a variety of self-selected diets. To convert salicylate values to mg/24 h, multiply by 0.13812.

mined by us (15, 16) or others (10, 11), revealed lower values (Table 2).

DISCUSSION

We found that subjects eating self-selected diets high in a variety of plant foods excreted only a few milligrams of salicylates per day (Figures 1 and 2). When we gave subjects small amounts of pure acetylsalicylic or salicylic acid we recovered ≈80% in urine (Figure 1).

Our assay only measures salicylate and its conjugates. However, a small fraction of the salicylates are metabolized to gentisic acid, which would escape detection (18). Thus, urinary excretion is a valid marker for intake, even at low amounts. Our recoveries of *para*-aminobenzoic acid indicate that the 24-h urine samples were nearly complete.

Our results are at variance with those of Swain et al (13; AR Swain, RH Lobley, AS Truswell, unpublished observations, 1985). They reported urinary salicylate excretions of 825 ± 43 μmol/24 h (114 ± 6 mg/24 h; $\bar{x}$ ± SEM, n = 25) after consumption of a diet providing ostensibly 637 μmol salicylate/d (88 mg/d) for 2 d; salicylate excretions were 268 ± 29 μmol/24 h (37 ± 4 mg/24 h, n = 28) after a diet providing 0 mg salicylates/d for 2 d (13). Thus, Swain et al (AR Swain, RH Lobley, AS Truswell, unpublished observations, 1985) recovered 86% (n = 24) of dietary salicylate in urine.

Swain et al (13, 14) also reported 10–100-fold higher concentrations of total salicylates in foods than we (15, 16) and others (9–12) did. A plausible interpretation of these discrepancies is that the assays used by Swain et al lacked specificity, and that substances other than salicylates were included in the salicylate figures. Estimated dietary salicylate intake data for the four subjects in Table 2, using the data of Herrmann (10), Robertson and Kermode (11), and ourselves (15, 16) may be somewhat underestimated due to the limited number of foods analyzed for salicylate compared with the number of foods Swain et al analyzed (13, 14). The correlation between salicylate excretion and dietary intake of fiber or vegetable protein that we found confirmed that plant foods are the major source of dietary salicylates. Therefore, we think the possible underestimation cannot be large because salicylate concentrations of the most important salicylate sources—plant foods—have been

TABLE 2
Excretion of salicylates in urine of four healthy volunteers eating an Indian (no. 6), Malaysian (no. 10), "prehistoric" (no. 14), or Surinamese (no. 16) diet, respectively[1]

Subject	Urinary salicylates	Estimated intake of dietary salicylates[2]			
		Swain et al (13, 14)	Robertson and Kermode (11)	Herrmann (10)	Janssen et al (15, 16)
	μmol/24h		*μmol/24h*		
6	34	62	0	1	2
10	4	54	0	1	2
14	23	211	1	5	4
16	5	83	1	3	3

[1] Subjects weighed and recorded all foods and drinks consumed during the urine-collection day in a diary and sampled equal amounts of the herbs and spices they used that day.

[2] We calculated the intake of dietary salicylates using the food records, the weights of the herbs and spices consumed, and salicylate contents of foods as determined by Swain et al (13, 14), Robertson and Kermode (11), Herrmann (10), and Janssen et al (15, 16). To convert salicylate values to mg/24 h, multiply by 0.13812.

given by Robertson and Kermode (11), Herrmann (10), and ourselves (15, 16). If we correct our salicylate excretion data using a salicylate recovery of 100%, we estimate that even pure vegetable diets provided < 43 μmol (6 mg) salicylates/d. Even if most of this would be in the form of acetylsalicylate, which is highly unlikely (15, 16), these intakes are still too low to affect risk for coronary heart disease or colon cancer.

At the same time, our data suggest that worries about adverse effects of dietary salicylates in children are unfounded. The data on salicylates in the Dutch Food Intolerance Databank (22) overestimate dietary salicylate intake because these data are derived almost entirely from Swain et al (13, 14). Even if dietary salicylates affect behavior, which is doubtful (7, 8), our results suggest that amounts of salicylate in foods are so low that diet may be ignored as a source.

We found that salicylate excretion in urine is a valid indicator for the intake of salicylates and that daily excretion of salicylates in urine is very low in subjects eating a variety of self-selected diets. We conclude that the amount of dietary (acetyl)salicylate is probably too low to affect disease risk.

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REFERENCES

1. Fuster V, Dyken ML, Vokonas PS, Hennekens C. Aspirin as a therapeutic agent in cardiovascular disease. Circulation 1993;87:659–75.
2. Badimon JJ, Fuster V, Chesebro JH, Badimon L. Coronary atherosclerosis; a multifactorial disease. Circulation 1993;87(suppl 2):3–16.
3. The Dutch TIA Trial Study 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;325:1261–6.
4. Giovannucci E, Rimm EB, Stampfer MJ, et al. Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. Ann Intern Med 1994;121:241–6.
5. Giovannucci E, Egan KM, Hunter DJ, et al. Aspirin and the risk of colorectal cancer in women. N Engl J Med 1995;333:609–14.
6. Feingold BF. Hyperkinesis and learning disabilities linked to the ingestion of artificial food colours and flavours. J Learn Disabil 1976;9:19–27.
7. Conners CK, Goyette CH, Southwick DA, et al. Food additives and hyperkinesis: a controlled double-blind experiment. Pediatrics 1976;58:154–66.
8. Harley JP, Ray RS, Tomasi L, et al. Hyperkinesis and food additives: testing the Feingold hypothesis. Pediatrics 1978;61:818–28.
9. Herrmann K. Occurrence and content of hydroxycinnamic and hydroxybenzoic compounds in foods. CRC Crit Rev Food Sci Nutr 1989;28:4315–47.
10. Herrmann K. Salicylsäure und andere verbreitete Hydroxybenzoesäuren und deren natürlich vorkommende Verbindungen in Lebensmitteln. (Salicylic acid and other hydroxybenzoic acids and their naturally occurring compounds in foods.) Ernährungswiss Umschau 1990;37: 108–29(in German).
11. Robertson GL, Kermode WJ. Salicylic acid in fresh and canned fruit and vegetables. J Sci Food Agric 1981;32:833–6.
12. Robertson GL. Salicylic acid in grapes. Am J Enol Vitic 1983; 34:42–3.
13. Swain AR. The role of natural salicylates in food intolerance. PhD thesis. Sydney, Australia: University of Sydney, 1988.
14. Swain AR, Dutton SP, Truswell AS. Salicylates in foods. J Am Diet Assoc 1985;85:950–60.
15. Janssen PLTMK, Katan MB, Hollman PCH, Venema DP. No aspirin in red wine. Lancet 1994;344:762.
16. Venema DP, Hollman PCH, Janssen PLTMK, Katan MB. Determination of acetylsalicylic and salicylic acid in foods, using HPLC with fluorescence detection. J Agric Food Chem 1996;44:1762–7.
17. Ellenhorn MJ, Barcelouse DG, eds. Medical toxicology: diagnosis and treatment of human poisoning. Salicylates. New York: Elsevier, 1988:563–71.
18. Shen J, Wanwimolrule S, Roberts MS. Novel direct high-performance liquid chromatographic method for determination of salicylate glucuronide conjugates in human urine. J Chromatogr 1991;565:309–20.
19. Bingham S, Cummings JH. The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 h urine collections in man. Clin Sci 1983;64:629–35.
20. Eisenwiener Von HG, Morger F, Lergier W, Gillessen D. Die Bestimmung der *p*-Aminobenzoesäure mit Fluram im Urin nach Durchführung des Pankreasfunktionstests mit Bentiromid. (Determination of *p*-aminobenzoic acid in urine using fluram after assaying pancreas functionality with bentiromid.) J Clin Chem Clin Biochem 1982;20: 557–65(in German).
21. Stichting Nederlands Voedingsstoffenbestand. NEVO tabel. (NEVO table.) Den Haag: Voorlichtingsbureau voor de Voeding, 1993(in Dutch).
22. ALBA Databank. Salicylaten in voedingsmiddelen. (Salicylates in foods.) Zeist: TNO Voeding, 1993(in Dutch).