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Received for review July 23, 1985. Accepted December 6, 1985. Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. No. 1448-E, 1985 series. This work was supported by the USA–Israel Binational Agricultural Research and Development Fund (BARD).

Determination of Fluoride in Foods

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With the development of modern analytical methods and techniques for fluoride analysis the acid-diffusible (ionic plus acid-labile) and total fluorine content of a food can be reliably determined. The difference between these values represents nonionic form(s) not released under conditions of acid diffusion. Higher amounts of fluorine were generally found with the fluoride ion specific electrode when ashed foods were analyzed employing the open-ashing–heat-facilitated diffusion method or the oxygen bomb–reverse extraction technique for isolation of the fluoride than when acid-labile fluoride was isolated from an unashed sample by heat- or silicone-facilitated diffusion. Similar results were obtained by both diffusion techniques. The simplest method for isolation of acid labile fluoride is by silicone-facilitated diffusion. Recovery of added fluoride by all methods and techniques was, in most instances, excellent.

The development of modern reliable analytical methods and techniques for fluoride analyses has made the determination of the acid-diffusible (ionic plus acid-labile) fluoride and total fluorine in foods possible. Nonionic fluorine is defined as the difference between the total fluorine and acid-diffusible fluoride. The reliability of some results previously obtained with routine fluoride analytical methods for foods has been questioned (Dabeka et al., 1979; Singer and Ophaug, 1979; Singer et al., 1980; Taves, 1979). There is evidence that methods based on the separation of fluoride from an unashed sample by acidic diffusion and determination of the isolated fluoride with some colorimetric methods may yield erroneous results. The use of a colorimetric reagent (American Conference of Governmental Industrial Hygienists, 1969) for the analysis of fluoride in diffusates from unashed commercially prepared infant foods by Dabeka et al. (1979) suggested that the fluoride concentration found was significantly lower when the same diffusates were analyzed with the fluoride ion specific electrode. Much higher results have been obtained for the analyses of fluoride in some foods with the eriochrome cyanine R colorimetric method (Megregian, 1954) than when the fluoride was determined with the fluoride ion specific electrode (Singer and Ophaug, 1979; Singer et al., 1980; Taves, 1979). The difference was not attributable to fluoride in any form.

Recognizing the problems encountered in determination of fluoride in foods, Dabeka et al. (1979) attempted to develop a simple, accurate, routine method for fluoride determination in unashed infant foods based on the isolation of the fluoride from the sample by microdiffusion from perchloric acid (47 °C for 16 h) and the subsequent measurement of the ion in the diffusate with the fluoride ion specific electrode. Their relative standard deviations varied from 4 to 20% from day to day. A collaborative study of 12 laboratories using the method described by Dabeka et al. (1979) with an added freeze-drying step demonstrated an interlaboratory mean coefficient of variation of 21.6% (Dabeka and McKenzie, 1981). They suggested the elimination of this added step to overcome some of the variations. Attempts to apply a routine physical method based on the $^{18}F(P,P'\partial)^{19}F$ reaction have not been promising (Shroy et al., 1982).

There obviously is need for documentation of the reliability of analytical procedures for the determination of fluoride and total fluorine in foods. The present investigation was undertaken to determine whether simple rapid methods not requiring specialized equipment could be recommended for the accurate determination of various forms of fluoride in foods and for determining how much of the total fluorine is nonionic.

PROCEDURES

The U.S. Food and Drug Administration (FDA) has maintained a program of Market Basket food collections of young males 15–19 years of age in order to estimate the dietary intake of certain metals and to monitor pesticide residues in the food chain. Between 1975 and 1982 these collections were comprised of 117 food items placed into composite food groups (Tables II and III) by the FDA based on the relative quantities of each item found in the average diet of the young male living in the United States (U.S. Food and Drug Administration Compliance Program Guidance Manual, 1977). Appropriate samples of 11 composites from six Market Baskets collected prior to 1982 were analyzed by four fluoride techniques. These techniques are contrasted in Table I. Foods may be ashed prior to analysis for fluoride to convert some or all nonionic fluorine to the ionic form, which is detected with the fluoride ion specific electrode. Some fluorocarbons may be lost during open ashing because of their volatility (Venkateswarlu, 1975A), and total fluorine in the sample, for this reason, may be underestimated with these procedures. There is some evidence that such fluorocarbons are

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0021-8561/86/1434-0510$01.50/0

Table I. Methods Employed for Fluoride Isolation from Foods Prior to the Fluoride Determination with the Fluoride Ion Specific Electrode

	unashed samples		ashed samples	
factors	heat-fac diff	silicone-fac diff	open-ashing–heat-fac diff	oxygen bomb–rev extrctn
temp, °C				
ashing			500	
diff	60	room temp	60	
ashing vessel			platinum dishes	stainless steel
diff dish	polypropylene Conway dishes	polystyrene Petri dish	polystyrene Petri dish	none
blank (~μg of F^-)	0.06	0.04	0.17	0.15
rel ease	2	1 (easiest)	3	4 (diff)
sample size, g	1–2	1–8	1–10	*b*
no. anal.[a]	30/2 days	30/2 days	20/3 days	4/day
fluorine det	acid diffusible	acid diffusible	total	total

[a] Time required to completely analyze a number of samples with all MV determinations read at equilibrium. [b] Pellet dry weight should not exceed 1 g.

Table II. Mean Fluoride Content[a] of Food Groups from Four Market Basket Collections Obtained by Three Methods

	techniques		
food groups	unashed samples: heat-fac diff	open-ashing–heat-fac diff	oxygen bomb–rev extrctn
dairy products	0.06 ● 0.013	0.15 ± 0.040	0.15 ± 0.031
meat, fish, poultry	0.30 ± 0.006	0.35 ± 0.069	0.46 ± 0.088
grain and cereal products	0.30 ± 0.017	0.34 ± 0.040	0.44 ± 0.042
potatoes	0.12 ± 0.017	0.13 ± 0.007	0.23 ± 0.033
green leafy veg	0.11 ± 0.036	0.14 ± 0.019	0.15 ± 0.031
legumes	0.31 ± 0.056	0.34 ± 0.060	0.34 ± 0.055
root vegetables	0.08 ± 0.023	0.10 ± 0.014	0.10 ± 0.013
misc veg and veg products	0.19 ± 0.035	0.23 ± 0.024	0.29 ± 0.026
fruits	0.11 ± 0.017	0.10 ± 0.019	0.11 ± 0.036
sugar and adjuncts	0.28 ± 0.076	0.28 ± 0.050	0.21 ± 0.032
beverages, including water	0.72 ± 0.246	0.78 ± 0.120	0.72 ± 0.121

[a] Mean (ppm) ± SEM.

present in the average diet. It is not known, however, how much is ingested, or whether this is a significant contribution to the total fluoride intake. The fluoride in most foods is diffusible from acidic solution (Taves, 1983), but some foods, because of the possible presence of nonionic fluorine, should be ashed prior to determination of their total fluorine content.

Ashing Procedures. When the open-ashing technique was employed to measure total fluorine in food, 1–10 g was placed in uncovered platinum dishes, fixed with low-fluoride calcium phosphate (10 mg) or magnesium oxide (50 mg), and ashed at 500 °C in a muffle furnace. The fluoride in the ash was isolated by overnight diffusion from perchloric acid at 60 °C (Singer and Armstrong, 1965). The blank determination carried through the procedure without sample was determined routinely and averaged 0.17 μg of fluoride. In the oxygen bomb–reverse extraction technique, because of the heat produced, no more than an equivalent of 0.7 g of dry sample was placed between two layers of filter pulp (0.3 g), lyophilized, and fired in a Paar oxygen bomb. Thus, the maximum pellet weight fired wa 1 g or less. The total fluorine in the incinerated sample was isolated by extraction in diphenylsilanediol and reverse extraction into NaOH (Venkateswarlu, 1975B).

Nonashing Procedures. Acid-diffusible fluoride (ionic plus acid labile) was also isolated from unashed samples by two acidic diffusion techniques.

(1) A *heat-facilitated diffusion* technique was used to isolate fluoride by overnight diffusion from perchloric acid at 60 °C (Singer and Armstrong, 1965). The unashed sample (1–2 g) was placed directly into polypropylene Conway dishes into which 25% perchloric acid (4 mL) was added. The diffused fluoride was trapped in alkali (500 μL of 0.5 N NaOH) placed in the center well.

Table III. Recovery of Fluoride Added to Foods (%)[a]

	range of fluoride added/ sample, μg	unashed samples diff	ashed samples	
food groups			open-ashed diff	oxygen bomb–extrctn
dairy products	0.10–0.50	100 ± 2.0 (3)	97 ± 4.0 (5)	97 ± 3.6 (9)
meat, fish, poultry	0.10–0.50	91 (91, 91)	99 ± 1.5 (11)	101 ± 5.2 (7)
grain and cereal products	0.10–0.50	99 ± 1.7 (4)	94 ± 2.0 (7)	92 ± 2.3 (7)
potatoes	0.20–0.50	92 ± 1.0 (3)	101 ± 2.0 (9)	89 ± 1.8 (8)
green leafy veg	0.20–0.50	100 ± 0.3 (3)	106 ± 2.0 (3)	77 ± 6.5 (7)
legumes	0.40–0.50	100 ± 0.3 (3)	98 ± 1.7 (14)	100 ± 3.8 (7)
root veg	0.10–0.50	102 (102, 102)	100 ± 1.9 (6)	87 ± 4.0 (8)
misc veg and veg products	0.10–0.50	104 ± 3.5 (4)	104 ± 2.5 (5)	59 ± 7.9 (6)
fruits	0.10–0.40	99 ± 0.7 (3)	100 ± 2.6 (6)	89 ± 10.6 (6)
sugar and adjuncts	0.25–0.50	100 (95, 100)	104 ± 4.4 (3)	91 ± 2.1 (4)
beverages, including water	0.50	101 (101, 101)	101 ± 1.3 (7)	96 ± 3.1 (5)

[a] Mean (%) ± SEM (number of determinations).

(2) In the *silicone-facilitated diffusion*, a modification of the procedure described by Taves (1968) for fluoride in plasma, 1–8 g of food was placed in the bottom half of a polystyrene Petri dish (100 × 15 mm) and mixed with 10–12 mL of redistilled water before adding 8 mL of concentrated perchloric acid (70%). A small polystyrene weighing boat ($1^3/_4$ in. by $1^3/_4$ in.) containing 0.5 mL of 0.5 N NaOH was floated on the acid containing the dispersed sample to collect the diffused fluoride. The rim of the lower half of the Petri dish was coated with silicone grease and sealed when the cover was placed on top of the lower dish. A 50-μL portion of 4% hexamethyldisiloxane (HMDS) in 95% ethyl alcohol was rapidly added through a small hole in the cover that was then sealed with a dab of silicone grease. The HMDS catalyzed diffusion of fluoride from the acidic solution at ambient temperature. For convenience the fluoride in the sample was isolated after an overnight diffusion.

The fluoride isolated by each method was determined with the fluoride ion specific electrode in a solution adjusted to pH 5.0 with TISAB buffer (Orion Research,

Cambridge, MA 02139) by comparison to standards of known fluoride content.

RESULTS

A general description of each food class analyzed and the mean fluoride content obtained for the composites of four Market Baskets with three methods are given in Table II. The recovery of added inorganic fluoride, not exceeding 50% of the native fluoride found in the sample, was determined after being carried through an entire procedure (Table III).

The average mean blanks (±SEM (number of determinations)) found with the techniques employed to isolate fluoride by heat-facilitated diffusion from ashed samples and silicone-facilitated and heat-facilitated diffusion from unashed samples were 0.17 ± 0.007 (51), 0.04 ± 0.004 (12), and 0.06 ± 0.005 (27) μg, respectively. The blank obtained with the oxygen bomb–reverse extraction technique was 0.15 ± 0.12 (12) μg. The appropriate blank was subtracted from the fluoride found in each analysis, and the value in Table II is the fluoride concentration in the food group.

DISCUSSION

Taves (1983) compared the fluoride content of ashed and unashed specimens of 93 different foods, from which fluoride was isolated by silicone-facilitated diffusion and determined with the fluoride ion specific electrode. The results for seven of these comparisons showed a discrepancy of at least 25%. The greatest percent differences (ashed/unashed results) were found for Rice Krispies (633), Shredded Wheat Miniatures (154), pepper (158), roast beef (58), and grapefruit juice (44). The greatest percentage differences we observed (Table III) for the determination of fluoride in the same sample with oxygen bomb–reverse extraction technique and heat-facilitated acidic diffusion from the unashed sample were for dairy products (275), potatoes (202), root vegetables (165), miscellaneous vegetables and products (157), and grains and cereal products (148). The mean ratio (ashed/unashed results) for all 11 composites is 1.31. This suggests that only a part of the fluorine present in some unashed samples is determined. Venkateswarlu (1975A) suggested, quite correctly, that the two basic problems that could be encountered in the open ashing of samples containing submicrograms of fluorine are loss of fluorine and extraneous fluoride contamination. In most foods, an exception being some dairy products, the inorganic fluoride levels are sufficiently high so that contamination did not appear to be a serious problem. We have demonstrated with perfluorooctanoic acid, a model nonionic fluoride compound, that theoretical amounts of fluoride bonded to carbon can be obtained after incineration in a Paar oxygen bomb where the potential problems of volatility and incomplete incineration that may be encountered in open ashing are eliminated (Ophaug and Singer, 1980). Approximately 20% of the theoretical amount of fluoride from this compound was found when the sample was analyzed after being ashed in an open dish (Guy et al., 1976; Ophaug and Singer, 1980).

Generally the oxygen bomb–reverse extraction technique gave results that were similar to or greater than those found when the sample was ashed in the open platinum dish prior to isolation of the "total" fluorine by heat-facilitated diffusion (Table II). The results obtained with the oxygen bomb–reverse extraction technique were, in most cases, higher than those obtained when the acid-labile fluoride was isolated by diffusion from an unashed sample. The mean ratios of results for four Market Basket collections obtained with the oxygen bomb–reverse extraction technique and that found after the fluoride was isolated (1) by heat-facilitated diffusion from an unashed sample and (2) by heat-facilitated diffusion from a sample ashed in an open platinum dish were 1.47 ± 0.159 and 1.16 ± 0.082, respectively. The difference between the concentrations of fluoride reported in an unashed and an ashed sample (Table II) reflects the amount of nonionic fluorine in the food that is converted to the ionic form, retained, and measured in the ashed sample. Of all composites analyzed the highest mean percent of total fluorine in the nonionic form was found in dairy products (52 ± 17.7%). The concentration of fluoride in this composite (Table II) is extremely low. Excluding the water and beverage composite, the percent of the fluorine that was nonionic in all of the other composites from the four Market Baskets ranged from 0 to 82.

The recovery with each method of an increment of ionic fluoride added to representative samples is shown in Table III. In general the recovery of an inorganic fluoride addition (0.10–0.50 μg), not exceeding half of the native fluoride content of a food, with the methods employed in this investigation were good, although in some instances somewhat lesser recovery was obtained with the oxygen bomb technique. The concentration of fluoride determined with the oxygen bomb–reverse extraction technique was adjusted for the recovery of added fluoride found for the food group.

The mean acid-diffusible fluoride concentration of the food classes analyzed in this study varied from 0.06 to 0.72 ppm. The total fluorine in the foods varied between 0.10 to 0.78 ppm. Most of the food classes had a mean fluoride or fluorine concentration of 0.30 ppm or less (Table II). It is obvious that when very low levels of fluoride are found in a food using two methods, even though the results are only slightly different, a relatively large ratio may be calculated. Although the fluoride concentration (ppm) may sometimes be low, the weight of sample that can be taken for analysis allows one to accurately determine the fluoride concentration in the sample. The blank for the procedure was, therefore, not a problem.

If the objective of an investigation is to determine the acid-diffusible fluoride content of foods, there is little difference between the results obtained when the fluoride is isolated from an unashed sample by silicone-facilitated or heat-facilitated acidic diffusion. The simplicity of the silicone-facilitated diffusion method (unashed sample) and its low and consistent blanks as well as ease in which large numbers of food samples can be analyzed recommends this method over heat-facilitated diffusion for determining the acid-labile fluoride. If a determination of the total fluorine (ionic and nonionic) content of the food is desired, one must employ methods in which the sample is ashed prior to isolating the fluoride. In this investigation the ashing of most samples in an open platinum dish provided similar estimates of total fluorine content as obtained with oxygen bomb–reverse extraction technique. Fats and lipids cannot be analyzed accurately with the open-ashing technique since physical loss of sample usually occurs. In this study this composite was not analyzed with this technique. If a slightly more accurate determination of the total fluorine content of a food that may contain volatile fluorocarbons is desired, the oxygen bomb–reverse extraction method is recommended despite being time consuming, complex, and limited in the number of samples that can be analyzed daily.

Registry No. Sugar, 57-50-1; F_2, 7782-41-4; F^-, 16984-48-8.

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Received for review September 12, 1985. Accepted February 3, 1986. Supported by Grant DE01850 from the National Institutes of Health, Bethesda, MD.

Hydrolysis of 4-Nitrophenyl Organophosphinates by Arylester Hydrolase from Rabbit Serum

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The substrate specificity of arylester hydrolase partially purified from rabbit serum was studied. It was found that 10 of 13 4-nitrophenyl organophosphinate compounds tested were substrates for the enzyme. Michaelis constants were determined and ranged from 0.021 mM for 4-nitrophenyl methyl(2-furyl)phosphinate to 0.49 mM for 4-nitrophenyl bis(chloromethyl)phosphinate as compared to 0.61 mM for ethyl paraoxon. Specific activities for a number of other substrates were also determined for this enzyme.

Arylester hydrolase (arylesterase EC 3.1.1.2) is an enzyme that can detoxify the oxon metabolites of various organophosphorothioate insecticides such as paraoxon. The enzyme was first studied by Mazur (1946) and Aldridge (1953). Mazur found that rabbit serum was capable of hydrolyzing paraoxon and DFP. Aldridge demonstrated that the paraoxon hydrolyzing activity was enzymatic and that it was heat labile, pH dependent, and substrate concentration dependent. This enzyme has been referred to as paraoxonase, since paraoxon hydrolysis has been used to measure its activity, and also as phosphotriesterase because it does not hydrolyze monoesters of orthophosphoric acid (Aldridge and Reiner, 1972). Paraoxonase has been purified from sheep serum by Main (1960), but much of the work on paraoxonase specificity has been done with crude serum or tissue (Lenz et al., 1973; Zech and Zurcher, 1974; Chemnitius et al., 1983). Since there have been few purifications of arylesterase, its substrate specificity is unclear. A few organophosphonates were not hydrolyzed by rabbit serum (Lenz et al., 1973).

We report hydrolysis of certain 4-nitrophenyl organophosphinates by arylesterase partially purified from rabbit serum. The study of the metabolism of phosphinates is very important because they are a class of organophosphorus compounds that do not undergo the "aging" reaction and as such should not be capable of producing organophosphate-induced delayed neuropathy. These compounds provide transient protection against organophosphorus compounds that do cause delayed neuropathy (Johnson, 1982). Phosphinates could also provide prophylaxis against acute poisoning by phosphates and phosphonates.

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MATERIALS AND METHODS

Chemicals. Organophosphinates I–XIII were provided by the U.S. Army Medical Research Institute of Chemcal Defense (Aberdeen Proving Ground, MD) following synthesis by Ash-Stevens, Inc. (Detroit, MI) (Lieske et al., 1982, 1984). Paraoxon, MOPS [3-(3-morpholino)-propanesulfonic acid], 1- and 2-naphthyl acetate, 4-nitrophenyl acetate, and 4-nitrophenyl butyrate were from Sigma Chemical Co. (St. Louis, MO), methyl paraoxon was from the U.S. Environmental Protection Agency, and parathion and methyl parathion were from Monsanto Agricultural Products Co. Phenyl thioacetate, phenyl thiopropionate, and phenyl thiobutyrate were generously provided by Dr. A. R. Main, North Carolina State University. Ethanethiol and butyryl chloride were purchased from Aldrich Chemical Co. (Milwaukee, WI). Ethyl thioacetate and ethyl thiopropionate were from Fairfield Chemical Co. (Blythewood, SC) and were distilled before use. Bio-Rad protein assay reagent was purchased from Bio-Rad Laboratories (Richmond, CA).

Handling of Organophosphinates. The organophosphinates have mammalian toxicities comparable to paraoxon and were handled accordingly. The LD_{50} values for some of the phosphinates in rats are published (Lieske et al., 1984).

Synthesis of Ethyl Thiobutyrate. Ethyl thiobutyrate was synthesized by an adaption of procedures described by Booth and Metcalf (1970). Butyryl chloride and ethanethiol were added in a 1.5:1 molar ratio and refluxed for 2 h. The reaction mixture was cleaned up by distillation.

0021-8561/86/1434-0513$01.50/0