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Quantification of Fluoride in Food by Microwave Acid Digestion and Fluoride Ion-Selective Electrode

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ABSTRACT: To quantify fluoride in food it is necessary to extract the fluoride from the matrix. Dry ashing (alkali fusion) and facilitated diffusion are the methods most commonly used, but their application requires lengthy treatments. The present study proposes the use of a microwave oven and 7 mol/L nitric acid for simple, rapid digestion of foods for fluoride analysis. The analyte is subsequently quantified by fluoride ion-selective electrode. The various steps of the method were optimized and an in-house validation was performed. The limit of quantification (0.130 mg/kg), trueness (92%), recovery (84−101%), and precision (1−8%) were determined. These analytical characteristics are satisfactory and show the suitability of the method for analysis of fluoride in foods of various kinds. The method's ease of application and the use of equipment normally found in food analysis laboratories may help to further increase research on fluoride concentrations in foods consumed by the population.

KEYWORDS: *fluoride, food, microwave digestion, fluoride ion-selective electrode*

INTRODUCTION

Fluoride is a ubiquitous element that, although not essential for human development and growth, is considered beneficial because of its ability to inhibit the initiation and progression of dental caries.[1] However, excessive intake may cause dental and skeletal fluorosis and even a reduction in cognitive abilities of the child population.[1] Water and food are the main sources of human exposure to this element,[2,3] although in the child population, intake from toothpaste must also be considered.[4]

Fluoride concentrations in drinking water are regularly evaluated by administrations because most countries limit the maximum concentration. However, very little research has been done in food. The United States and Canada[4,5] have carried out diet surveys and have databases for fluoride in food. In other countries, studies are more scarce and have concentrated on specific populations or particular food groups.[3,6−12] The analytical difficulties associated with quantifying this element in food may be the reason why fluoride is not an element that is routinely analyzed by the organizations responsible for food safety and health.

The isolation of fluoride from the organic matrix is a key step in its determination. In the literature there are descriptions of methods that use mineralization by dry ashing[6,7,9,13−15] or facilitated diffusion processes.[8,11,16−21] Both methods require considerable time, more than 12 h, and are highly operator-dependent. The working conditions for dry mineralization are different from those applied for other trace elements because the digestion is performed in basic conditions, preferably NaOH, and the use of specific material such as nickel or platinum crucibles is required.[14] The alternative of using wet digestion to mineralize the organic matter has scarcely been applied for the analysis of fluoride in food. In the literature there are descriptions of the use of nitric acid for digestion in open systems with heating to 150 °C[12] and in closed vessels with heating by microwave oven.[10,22]

There are many methods for quantifying fluoride after solubilization,[23] although direct potentiometry with fluoride selective electrode is the method of choice for the determination of fluoride in foods.[9−11,14] It has advantages because of its low cost, satisfactory sensitivity and selectivity. It is easy to apply, because it only requires adjustment of the ionic strength and pH of the sample by the addition of a total ionic strength adjustment buffer (TISAB).

The aim of this study was to develop and optimize a rapid method for the analysis of fluoride in foods, based on microwave-assisted acid digestion and subsequent detection by potentiometry, in order to obtain a simple alternative to alkali fusion and facilitated diffusion.

MATERIALS AND METHODS

Apparatus. An accelerated microwave reaction system with a maximum power of 1200 W (model MARS, CEM) was used for digestion of the samples. The fluoride concentration was quantified using a fluoride ion-selective electrode (ISE DC219-F, Mettler Toledo). Other equipment used included: magnetic shaker (IKA), pH meter (model 526, Hanna WTW) and lyophilizer (model Genesis SQ 35 EL, Virtis).

Reagents. A NaF standard with a concentration of 1000 mg/L as fluoride (Panreac) was used. TISAB II was prepared in the laboratory from 58 mg/mL of NaCl (Panreac), 10 mg/mL of *trans*-1,2 diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid monohydrate (Fluka) and 57 μL/mL of glacial acetic acid (Panreac). The TISAB II pH was adjusted between 4.8 and 5.2 with 7% NaOH (w/v) (Panreac). Other reagents used were TISAB III (Scharlau), HNO_3 (Merck) and H_2O_2 (Prolabo).

Deionized water (18.2 MΩ cm) obtained with a Milli-Q water system (Millipore Inc.) was used for preparation of the solutions. The glassware and polyethylene material used were treated with 10% HNO_3 (v/v) (Merck) for one week before they were first used. After they were used in the analytical method they were treated with 10% HNO_3 (v/v) for 24 h and rinsed with deionized water.

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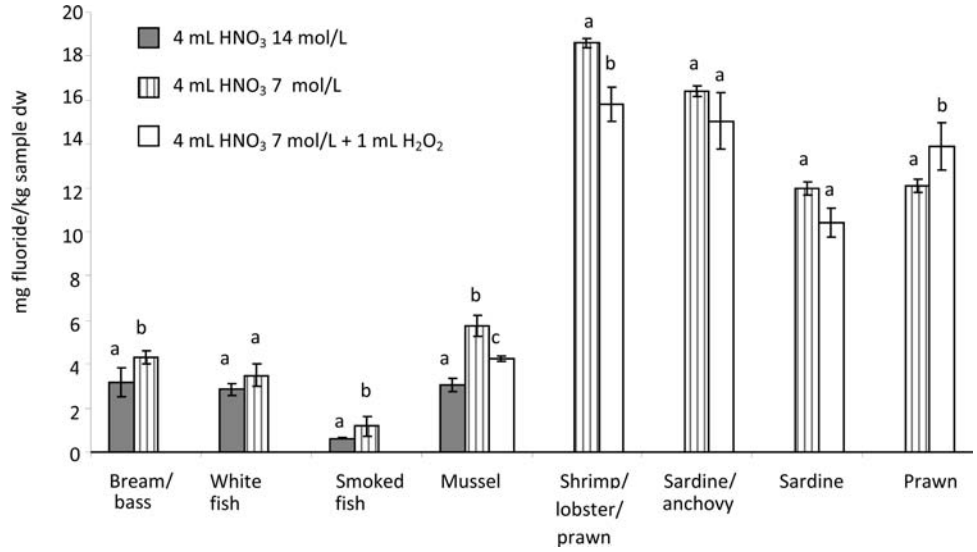

Figure 1. Effect of the oxidizing reagent used in the microwave digestion on the quantification of fluoride by ISE. Fluoride concentration expressed as mg/kg dry weight (dw) (mean ± standard deviation, $n = 3$). Different letters on bars indicate statistically significant differences ($p < 0.05$) between the treatments applied to each sample.

Samples. The samples used were seafood products, cereals, vegetables, fruits, legumes, and tubers included in a total diet study conducted by the General Public Health Administration of the Generalitat Valenciana (Valencian Community, Spain). The samples consisted of individual foods (e.g., potato, mussel) or food groups (e.g., carrot/pumpkin and sardine/anchovy). The inedible parts of the fresh foods were removed and they were then lyophilized and stored at 4 °C until analysis. A sample of tea leaves with a certified fluoride concentration of 57 ± 15 mg/kg (GBW10016, National Analysis Center for Iron and Steel, NACIS, Beijing, China) purchased from LGC Standards was used as a reference sample. We also analyzed an in-house reference material (cod flour; assigned value: 25.9 ± 3.2 mg/kg) kindly donated by Dr. Kare Julshamn (National Institute of Nutrition and Seafood Research, NIFES, Bergen; Norway).

Sample Digestion Procedure. The lyophilized samples (0.50−1.0 g) were weighed in a Teflon reactor and were then subjected to a microwave-assisted wet digestion process, using 7 mol/L HNO_3 (4−8 mL). The power applied was 800 W and the program used was: step 1: ramp from room temperature to 180 °C for 15 min; step 2: hold at 180 °C for 15 min; step 3: cool to room temperature. When the Teflon reactor had cooled, the digest was transferred to a plastic tube and the contents were adjusted to a pH close to 7 on the day of the digestion. Two solutions of NaOH were used for this purpose. Initially 8 mol/L NaOH was added until a pH close to 2 was reached and then 1.8 mol/L NaOH was added to adjust the pH to 7. Deionized water was then added to make a final volume of 15 mL. The digestion was applied at least in duplicate to each of the samples analyzed.

Fluoride Analysis. The fluoride concentration was quantified by fluoride ion-selective electrode (ISE). TISAB II was used to adjust the pH and ionic strength of the standards and the digested samples. The percentage of TISAB II in the solution to be quantified was 20% (v/v). The concentration was quantified against a fluoride calibration curve in a range of 0.010−10 mg/L prepared with a reagent blank. A MedisafeR Metalle U urine sample acquired from LGC Standards with a certified fluoride concentration (assigned value: 10 mg/L; confidence interval: 7.6−12.4 mg/L) was used for quality control of the quantification method.

RESULTS AND DISCUSSION

Microwave-assisted digestion in acid medium is considered an effective alternative in the preparation of food samples for subsequent analysis of inorganic compounds by electrochemical techniques.[24] The microwave ovens that are currently available permit simultaneous treatment of a large number of samples. By applying short treatment times and high temperatures it is possible to mineralize the organic matter without losses of analyte through volatilization. These characteristics are great advantages when it comes to analyzing fluoride in foods. Digestion by alkali fusion, the method most commonly described for this element, is tedious to apply and also requires considerable sample handling. Consideration of the time needed for the digestion, less than 30 min for microwave oven compared with 24 h for alkali fusion, is, in itself, a reason for undertaking the development and optimization of microwave-assisted wet digestion for the analysis of fluoride in food.

The first assays of acid digestion with nitric acid in closed vessels (Teflon, 120 °C, 6 h) provided higher fluoride recoveries in plant samples than those found with acid or alkali digestion in open systems.[25] Those authors attributed the improvement to the complete solubilization of the fluoride in the sample and the elimination of losses through volatilization. The use of closed systems in microwaves for the analysis of fluoride was not described until 1998, by Grobler and Louw, who used Parr bombs to obtain excellent recoveries in various samples, including food.[22] This use may be considered a late arrival in comparison with the extensive application of microwave digestion since the 1980s for the quantification of other trace elements and macronutrients.[26] At present, laboratories are still optimizing methods employing dry ashing or isolation by facilitated diffusion for the determination of fluoride in food.[14,27,28] Only Usydus et al.[10] have recently described the use of microwave for digestion of food prior to quantification of fluoride, although they do not give details of the working conditions or demonstrate the suitability of the method.

The results obtained in the optimization of the various steps involved in the proposed method of microwave acid digestion and ion-selective electrode for the determination of fluoride in food are set out in the following sections.

Optimization of Microwave Digestion Conditions and Neutralization. The most important parameters to consider in microwave oven digestion are sample weight, volume, and concentration of oxidizing reagents, temperature, microwave

power and digestion time. In this study we used the microwave conditions (800 W, 180 °C, 15 min) and sample weight (0.5 g, lyophilized) that our laboratory has found valid for complete mineralization of organic matter and analysis of inorganic contaminants in food.[29] Taking these conditions as a starting point, we assayed the suitability of various oxidizers commonly used in microwave digestion (concentrated HNO_3, diluted HNO_3 and a mixture of HNO_3 and H_2O_2) for the analysis of fluoride.

The results show that the fluoride concentration was higher in the samples digested with 4 mL of 7 mol/L HNO_3 than in the samples treated with the same volume of 14 mol/L HNO_3 (Figure 1). This difference was significant ($p < 0.05$) in most of the products analyzed. It is worth noting that the use of dilute acid can prevent the formation of insoluble salts and improve quantification of some minerals.[24] The incorporation of H_2O_2 (1 mL, 30% v/v) in the digestion process had no effect on the quantification of fluoride, and therefore its use was rejected. Consequently, 7 mol/L HNO_3 was selected as the most suitable oxidizing reagent for microwave digestion.

The digest is extremely acidic and has to be neutralized to a pH close to 7 before proceeding with the addition of TISAB and quantification by ISE. Grobler and Louw[22] used 8.5 mol/L NaOH to neutralize the microwave digest. In the present study, 8 mol/L NaOH was added until a pH close to 2 was reached and then 1.8 mol/L was added to reach pH 7. The use of two different concentrations of NaOH reduces the time needed for neutralization and for the achievement of a stable pH.

After this neutralization process the sample volume, which can vary from sample to sample, reached a maximum of 13 mL. In order to equalize the final volume of all the samples, deionized water was added to make the sample up to 15 mL. This neutralization process entails dilution of the analyte, which has a negative effect on the method's limit of quantification and makes analysis of foods with a low fluoride concentration difficult. Therefore we assayed microwave digestion of a larger sample quantity (1 g, lyophilized), increasing the oxidizer proportionally (8 mL of 7 mol/L HNO_3). Under these conditions (1 g sample/8 mL acid) sample digestion was complete, and as we kept to a volume of 15 mL after neutralization the increased sample weight and oxidizer volume enabled us to concentrate the analyte. Table 1 shows the results obtained after applying the two sample/oxidizer conditions to various seafood products. The fluoride concentrations detected did not differ significantly, and this means that the sample weight can be increased when foods with low concentrations of this element are analyzed.

Table 1. Effect of Sample Weight and Oxidizing Reagent Volume Used in the Microwave Digestion on Quantification of Fluoride by ISE[a]

sample	microwave conditions (g sample/mL 7 mol/L HNO_3)	fluoride (mg/kg)
smoked fish	0.5 g/4 mL	2.38 ± 0.12
	1 g/8 mL	2.27 ± 0.04
sardine/ anchovy	0.5 g/4 mL	12.5 ± 0.7
	1 g/8 mL	12.2 ± 0.3

[a]Fluoride concentration expressed as mg/kg dry weight (mean ± standard deviation, $n = 3$).

Selection of TISAB. In quantification by ISE it is necessary to adjust the ionic strength of the sample. This step, which always involves additional dilution, is carried out by adding TISAB, a buffer with a pH close to 5, which allows the fluoride ion to be the predominant form. It also contains chelating agents which prevent the interference that cations produce in fluoride quantification.[30]

The manufacturers of fluoride electrodes recommend the TISAB to be used. In the present study we assayed the forms most commonly employed, TISAB II 50% (v/v) and TISAB III 10% (v/v). Figure 2 shows the fluoride standard calibration curves (0.010 to 10 mg/L) obtained under both conditions. With TISAB II 50% (v/v) a linear relationship was observed over the whole analysis range, with a correlation coefficient greater than 0.999 and a slope of −58.16 mV. In the curves obtained with TISAB III (10% v/v), however, the response was not linear below 0.025 mg/L, an effect previously observed by Kjellevold Malde et al.[14] On the basis of the results obtained we decided to use TISAB II because it gave a greater linear range.

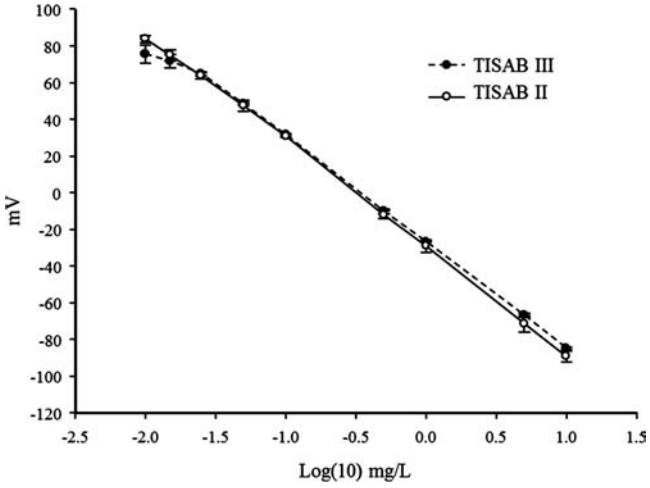

Figure 2. Fluoride calibration curve obtained with standards prepared in TISAB II 50% (v/v) and TISAB III 10% (v/v). Values expressed as mean ± standard deviation ($n = 3$).

Furthermore, in order to minimize consumption of TISAB II we evaluated the possibility of reducing its concentration to 20% (v/v). Under these conditions the slope and the ordinate at the origin of the calibration curve did not differ significantly from those found with TISAB II 50% (v/v) (data not shown). We also studied whether TISAB II 20% (v/v) is sufficient to complex the interfering cations that may be present in samples of digested food. To do this we analyzed the fluoride in the certified sample of tea leaves (GBW10016) digested and diluted with TISAB II 20% (v/v). The value found (53 ± 2 mg/kg) was within the range certified (57 ± 15 mg/kg), and therefore we considered that TISAB II 20% (v/v) was suitable for the sample analysis.

Calibration Curve. In their development of a method involving alkali fusion and ISE quantification for food analysis, Kjellevold Malde et al.[14] showed that the preparation of the calibration curve under conditions different from those used for sample preparation reduces the recovery of fluoride. In view of this, we evaluated whether this effect was also relevant in ISE quantification of the microwave digest. To do this we compared the calibration curves obtained with standard solutions prepared in deionized water and in blank digestion solution.

The linearity was satisfactory in both cases (0.9995 and 0.9993 respectively), but there were significant differences ($p < 0.05$) in the slope (−57.87 and −55.06 mV respectively) and in the ordinate at the origin (−29.04 and −22.59 respectively) which affected the recovery of fluoride standards digested in microwave. Standards of 1−100 mg/L digested and quantified against an aqueous calibration curve showed recoveries of 70−75%, less than the values considered acceptable (80−110%).[31] The recoveries increased to satisfactory values against a calibration curve prepared with reagent blanks (84−108%). Consequently, in this method the fluoride standards must be diluted with digestion blanks.

Stability of the Analyte in Solution. Quantification by ISE does not consume sample, so that the analysis of the digest can be performed as many times as desired. The limit of this way of working is determined by the stability of the fluoride in the digested samples, the concentration of which might be altered by precipitation or by processes involving release of fluoride from the material used for storage or adsorption of fluoride to it. Therefore we evaluated the effect of storage on the fluoride concentration, studying the stability of the calibration of the digest and of the digested samples diluted with TISAB II.

In the case of the standards, we compared the calibration curve obtained with standards prepared daily in TISAB II 20% (v/v) with the curve generated from standards stored at 4 °C. The results obtained showed that during storage of up to 30 days there were no significant variations ($p < 0.05$) in the slope or in the ordinate at the origin with respect to the curve prepared daily (data not shown).

To study the stability of the samples, the tea leaves certified material was used. The digested sample was stored in refrigeration and an aliquot was prepared and quantified every five days. The mean value of the fluoride found during 30 days of storage of the digest was within the certified range and had low variability (standard deviation: 2 mg/kg) and a random distribution in the results (data not shown). With regard to the stability of the sample digested and diluted with TISAB II, the mean value found during 30 days of storage was close to the value of the reference sample (found 62 mg/kg; reference 57 ± 15 mg/kg), with low variability in the measurements that were taken (standard deviation: 3 mg/kg) and without showing a tendency to produce increasing or decreasing values. Therefore the storage of standards and samples does not produce variations in the fluoride concentration, so that the steps of digestion, dilution with TISAB and quantification can be adapted to laboratory requirements.

Effect of Sample Volume on Quantification. The concentration of fluoride in food is very variable and therefore it may be necessary to use various dilutions of the microwave digest for quantification by ISE in order to increase the sensitivity of the method. In the present study we considered the effect of dilution by using samples with different fluoride concentrations: low (salmon/trout: 4 mg/kg dry weight, dw), medium (shrimp/lobster/prawn: 19 mg/kg dw) and high (tea; 57 mg/kg). Three dilutions of the digest—1/5, 2/5, and 3/5—were assayed in all of them, always keeping to TISAB II 20% (v/v) in the solution to be quantified. The results obtained did not show a matrix effect, since the dilution did not significantly affect the fluoride concentration ($p < 0.05$) in any of the samples. Therefore the dilution does not affect the ruggedness of the method. The possibility of using different dilutions broadens the range of concentrations that can be analyzed with the method developed.

Limit of Detection and Limit of Quantification. The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated as, respectively, 3 and 10 times the standard deviation of the fluoride concentration in 20 blanks. The values found were 0.0018 mg/L for the LOD and 0.0052 mg/L for the LOQ. These values are within the interval of LOD reported in the literature for fluoride analysis methods (0.002−1.4 mg/L).[32] If the differences in sample mass employed (0.5 or 1 g) and the possible dilutions for quantification by ISE (1/5 to 3/5) are taken into account, different LOQ values expressed as mg/kg are obtained. The highest LOQ, obtained with 0.5 g of sample and a 1/5 dilution factor, was 0.78 mg/kg. However, by applying the method under the most favorable conditions for sensitivity (1 g of sample and 3/5 dilution factor), the LOQ decreased to 0.130 mg/kg. This value is of the order of the value reported by Kjellevold Malde et al.[14] for alkali fusion−ISE (0.203 mg/kg). Those authors indicated that the LOQ for analysis by ISE is strongly influenced by the type of electrode used and that the effectiveness of the electrode varies with time. These aspects must be taken into account when comparing the sensitivity of the methods described in the literature.

Trueness. At present we are aware of only one food product that is marketed with a certified fluoride concentration (tea leaves, GBW10016), which is the sample that was used to evaluate trueness. Six independent samples of this certified reference material were analyzed under conditions of within-laboratory reproducibility. The results obtained (mean ± standard deviation = 52 ± 3 mg/kg) indicate a trueness of 92%, in accordance with the acceptability criteria set by the European Commission (80−110%).[31] The in-house reference material provided by the Norwegian laboratory was also analyzed. The concentration found in three independent samples of cod flour (mean ± standard deviation = 28.0 ± 0.5 mg/kg) is within the range assigned by that laboratory (mean ± standard deviation = 25.9 ± 3.2 mg/kg).

Recovery. The proposed method has been developed for the analysis of fluoride in all kinds of food. Most of these matrices are very different from tea and have lower fluoride concentrations, for which no certified reference material is available. In order to consider these matrices in the in-house validation of the method, we performed recovery assays under conditions of repeatability. Six samples were spiked with fluoride prior to digestion in the microwave: two seafood products with very different fluoride concentrations (bream/bass and shrimp/lobster/prawn) and three samples of fruit and vegetable products (banana, apple/pear, carrot/pumpkin). In each sample we assayed three levels of spiking and in each case three replicates of the analysis were performed. The results obtained (Table 2) indicated that the recoveries were satisfactory at all the spiking levels (84−101%), irrespective of the initial concentration in the sample and the kind of food. The results obtained in the recovery assays show that the method developed is not affected by losses or interference in the various steps that it comprises (digestion, neutralization and quantification by ISE).

Precision. The precision of the method was evaluated under conditions of repeatability, using samples with different fluoride concentrations (0.867−18.7 mg/kg). The precision obtained (Table 3), expressed as relative standard deviation of the results of six replicates, ranges between 1 and 8%, values below the

Table 2. Recovery of Fluoride in Seafood Products and Fruit and Vegetable Products after Addition of Various Concentrations of Fluoride[a]

product	fluoride in sample (mg/kg)	addition (mg/kg)	recovery (%)
bream/bass	4.52 ± 0.46	5	86 ± 7
		10	87 ± 4
		25	85 ± 5
shrimp/lobster/prawn	18.7 ± 0.1	20	86 ± 4
		40	85 ± 6
		60	96 ± 10
banana	1.06 ± 0.03	1	84 ± 1
		5	84 ± 1
		25	85 ± 1
apple/pear	1.27 ± 0.08	1	94 ± 1
		5	86 ± 1
		25	92 ± 1
carrot/pumpkin	1.15 ± 0.03	1	101 ± 1
		5	91 ± 1
		25	91 ± 1

[a]Fluoride concentration expressed as mg/kg dry weight (mean ± standard deviation, $n = 3$).

Table 3. Precision of the Method[a]

sample	fluoride (mg/kg)	RSD (%)
salmon/trout	1.15 ± 0.05	2
salted fish	7.78 ± 0.07	1
sardine/anchovy	17.2 ± 0.7	4
shrimp/lobster/prawn	18.7 ± 0.1	2
carrot/pumpkin	1.15 ± 0.03	2
green bean	1.12 ± 0.09	8
banana	1.06 ± 0.03	3
apple/pear	1.27 ± 0.08	6
potato	0.867 ± 0.07	3

[a]Fluoride concentration expressed as mg/kg dry weight (mean ± standard deviation, $n = 6$) and relative standard deviation expressed as percentage.

limit of 10% set by the European Commission for analyte concentrations ≥1 mg/kg.[31] The Horrat value for repeatability (observed RSDr divided by the RSDr value estimated from the modified Horwitz equation using the assumption $r = 0.66$ R)[33] is below 2 in the concentration range studied. Consequently, the method developed is acceptable, since it complies with the performance criterion for precision admitted for analysis methods.

Application to Determination of Fluoride in Food Samples. The microwave digestion−ISE method was applied to samples of various foods included in the Valencian Community Total Diet Study (Spain). The results obtained, expressed in dw and wet weight (ww), are shown in Table 4, and in all cases the coefficients of variation are less than 10%.

The fluoride concentrations in these foods range between 0.74 and 11.1 mg/kg dw, values found in samples of carrot/pumpkin and shrimp/lobster/prawn, respectively. In terms of wet weight, the concentrations range between 0.08 and 5.01 mg/kg. These values are similar to those reported in the literature.[2−4] The samples of sardine/anchovy and shrimp/lobster/prawn had high concentrations (3.05 and 2.40 mg/kg ww, respectively), which might be a result of the presence of remains of skeleton (bones or exoskeleton) in the samples analyzed. The concentration was also high in salted fish (5.01 mg/kg ww), perhaps because it was processed with salt containing high fluoride concentrations or as a result of concentration of the analyte owing to water loss during the salting process.

Table 4. Fluoride Concentrations in Food Samples[a]

sample	fluoride (mg/kg) dry weight	fluoride (mg/kg) wet weight
sardine/anchovy	11.1	3.05
bream/bass	2.58	0.88
swordfish/emperor	2.40	0.70
salmon/trout	4.17	1.34
white fish	2.86	0.63
smoked fish	2.10	0.88
salted fish	9.25	5.01
mussel	3.86	1.56
squid/cuttlefish	4.04	0.73
shrimp/lobster/prawn	9.98	2.40
rice	2.20	1.89
artichoke/leek/thistle/celery	2.83	0.31
cauliflower/broccoli/cabbage	4.76	0.60
lettuce/chicory (Belgian endive)/curly endive	1.68	0.15
aubergine/courgette/cucumber	1.89	0.20
carrot/pumpkin	0.74	0.08
bell pepper	1.34	0.14
mushrooms	3.8	0.39
banana	2.03	0.69

[a]Results expressed in mg/kg dry weight and wet weight (mean value; $n = 2$).

As discussed earlier, in this study a method for the analysis of fluoride in food by microwave acid digestion and fluoride ion-selective electrode was optimized and validated in-house. The use of microwave digestion has clear advantages over traditional methods based on alkali fusion or facilitated diffusion, particularly because of its quickness and the possibility of using the same microwave oven digest for the analysis of other minerals and trace elements. Its satisfactory analytical characteristics together with its ease of application and the use of equipment normally found in food analysis laboratories could help to increase research on fluoride concentrations in foods consumed by the population. This would allow better evaluation of exposure to this element, concerning which many countries, including Spain, have little information.

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Notes

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