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Factors affecting the bioaccessibility of fluoride from seafood products

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ABSTRACT

Fluoride is considered important for health because of its beneficial effect on the prevention of dental caries and on bone development in the child population. However, excessive intake has negative effects. The main pathway for exposure is oral, through consumption of drinking water, and some food products. Therefore its bioaccessibility (quantity of the element solubilized during the digestive process) is a parameter to be considered when estimating the risk/benefit associated with this element. The aim of the present study was to evaluate the influence of the digestion phase, gastrointestinal digestion factors (pH, pepsin and bile salt concentrations) and the presence of cations on the bioaccessibility of fluoride from seafood products.

The results show that the solubilization of fluoride takes place entirely during the gastric phase. Its bioaccessibility is strongly influenced by conditions that favor the formation of insoluble complexes of fluoride with other elements present in the matrix. The factors that are most influential in reducing its bioaccessibility are the increase in pH in the gastric phase, the presence of cations, especially in the intestinal phase, and a low concentration of bile salts.

1. Introduction

Fluorine as fluorides represents about 0.06–0.09% of the minerals present in the earth's crust (Fawell et al., 2006). Fluoride concentrations of about 1 mg/L in drinking water are considered beneficial for the prevention of dental caries and bone development (Fawell et al., 2006). However, because of its ability to bind to calcium and phosphate, excessive intake of fluoride has negative effects on the health, which appear initially as dental fluorosis and may eventually produce skeletal fluorosis (Ozsvath, 2009). Drinking water is considered the main pathway for human exposure to fluoride. The World Health Organization recommends fluoride concentrations of less than 1.5 mg/L in drinking water (WHO, 2006). It is estimated that 32% of the world's population consumes drinking water with fluoride concentrations exceeding the recommended limit. Foods can also contribute to exposure to fluoride, with the highest fluoride concentrations being found in seafood products, tea and fluoridated salt (USDA, 2004; Fawell et al., 2006).

Determination of the fluoride concentrations in foods generates the basic data to evaluate intake of this trace element. However, a more realistic approach to the estimation of the risk/benefit associated with fluoride exposure from foods should consider its bioavailability, the fraction of fluoride that finally reaches the bloodstream. In this sense, the Directorate General for Health and Consumers of European Union has indicated the need of collecting information on fluoride in food and its bioavailability (SCHER, 2011). From a study of fluoride bioaccessibility, the fraction of the element that is solubilized during gastrointestinal digestion, it is possible to make a first approach to a evaluation of its bioavailability, because bioaccessibility is indicative of the maximum quantity that could be absorbed by the intestinal epithelium.

There has been a marked increase in *in vitro* studies of the bioaccessibility of nutrients and contaminants from foods in recent years, especially contaminants, because of the little information that is available about them. Either static or dynamic methods can be used for these investigations (Venema et al., 2000; Oomen et al., 2002). Researchers generally choose static methods because of their ease of application, setting only one condition for each of the various parameters in the method (sample/digestion volume ratio, pH, concentrations of pepsin, pancreatin and bile salts). However, it has been shown that these parameters vary considerably during the digestion process (Dressman et al., 1990; Kalantzi et al., 2006). Static methods are a valid approach for a study of bioaccessibility, but it is important to know how variations in the parameters just mentioned may affect the bioaccessibility of a compound. These variations might explain differences in the absorption of a compound when it forms part of different food matrices, or differences in absorption between age groups (with

Abbreviations: TISAB, total ionic strength adjustment buffer; TIM, TNO intestinal model.

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different pHs and gastrointestinal secretions) or by groups with gastrointestinal pathologies.

The aim of the present study was to evaluate the influence of the digestion phase (gastric or intestinal), the gastrointestinal digestion factors (pH, pepsin and bile salt concentrations) and the presence of cations derived from the diet (Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}) on the bioaccessibility of fluoride from seafood products, a food group that contributes high concentrations of fluoride to the diet.

2. Materials and methods

2.1. Samples

Samples of seafood products were purchased at supermarkets in the city of Valencia (Spain). They included three species of fish: sardine (*Sardina pilchardus*), European anchovy (*Engraulis encrasicolus*) and big-scale sand smelt (*Atherina boyeri*); and two species of crustaceans: Guinea shrimp (*Parapenaeopsis atlantica*) and common prawn (*Palaemon serratus*). For preparing composites of each species, 0.45–1 kg were used. The inedible parts were removed from the samples, with the exception of common prawns and sand smelts, which are consumed whole. The samples were then cooked on a griddle, one of the customary ways of cooking for the Spanish population, without adding other ingredients. Finally they were ground and homogenized, and then kept frozen until analysis. Throughout the study, three composites of sardines (a, b, and c), two composites of anchovies and prawns (a and b) and a single composite of smelts and shrimps were employed. A detailed description of the samples of seafood products used in the study is shown in Table 1.

One portion of each sample was used to determine the fluoride concentration (Sections 2.2 and 2.9) and another portion was subjected to gastrointestinal digestion (Section 2.3).

2.2. Microwave-assisted acid digestion

The fluoride in the food samples was extracted by means of a microwave acid digestion, a method developed previously in our laboratory (unpublished data). Samples (0.5 g) were placed in a Teflon PFA vessel, treated with 4 mL of 7 mol/L HNO_3 and irradiated at 800 W (180 °C, 15 min) in a microwave accelerated reaction system (MARS) from CEM (Vertex, Spain). At the end of the digestion program the digest was placed in a plastic tube and neutralized with NaOH at pH 7.2–7.5. Deionized water was added to make up a final volume of 15 mL.

Wet digestion was applied at least in duplicate to each of the samples analyzed. An ion-selective electrode was used for the fluoride analysis (Section 2.9). Throughout the experiment the quality assurance/control of the digestion process was checked by analysing a tea certified reference material, NCS ZC73014 (National Analysis Centre for Iron and Steel, NACIS, Beijing, China; LGC Standards, Spain), with a certified value of 57 ± 15 mg/kg and 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).

2.3. Simulated gastrointestinal digestion of food samples

A static method was used, simulating the gastric and intestinal phases of the human gastrointestinal digestion process (Laparra et al., 2003). The following equipment was used in the application of the method: pH-meter (Hanna, WTW model 526, Spain), water bath with orbital shaking (Unitronic Orbital C, J.P. Selecta, Spain) and centrifuge (RC-5B Superspeed Refrigerated Centrifuge, Sorvall, Du Pont).

2.3.1. Gastric phase

The sample of cooked seafood (10 g) was weighed in an Erlenmeyer flask and 90 g of deionized water was added. The mixture was homogenized by mechanical shaking. The pH was adjusted to 2 with 6 mol/L HCl and the weight was made up to 100 g with deionized water. The mixture was then incubated at 37 °C for 2 h with shaking (120 strokes/min). Porcine pepsin was used for the gastric phase (Sigma, enzyme activity 944 U/mg of protein). A 10% solution (w/v) of pepsin was prepared in 0.1 mol/L HCl. The volume required to provide 2×10^{-3} g of pepsin/g of fresh sample, equivalent in the conditions of the method to 0.02 g pepsin/100 g of solution, was added.

2.3.2. Intestinal phase

The pH of the gastric digest was adjusted to 6.5 using 1 mol/L $NaHCO_3$ (Panreac). A solution of 0.4% (w/v) porcine pancreatin (Sigma, activity equivalent to 4 × US Pharmacopoeia specifications/mg pancreatin) and 2.5% (w/v) porcine bile extract (Sigma, glycine and taurine conjugates of hyodeoxycholic and other bile salts) was prepared in 0.1 mol/L $NaHCO_3$. The volume required to provide 5×10^{-4} g of pancreatin/g of fresh sample and 3×10^{-3} g of bile extract/g of fresh sample was added to the gastric digest. In the conditions of the method, these quantities are equivalent to 0.005 g pancreatin/100 g of solution and 0.03 g bile extract/100 g of solution. The mixture was incubated again with shaking (120 strokes/min) for 2 h at 37 °C. When the intestinal phase had finished, the pH was adjusted to 7.2 with 0.5 mol/L NaOH. The digest obtained was centrifuged (10,000 rpm/30 min/4 °C) to separate the soluble fraction (bioaccessible).

The bioaccessible fluoride concentration was calculated at least in duplicate for each of the samples. An ion-selective electrode was used for the fluoride analysis (Section 2.9). For the evaluation of fluoride bioaccessibility in each sample the following formula was used:

$$\text{Bioaccessibility} = \frac{\text{Fluoride in the bioaccessible fraction}}{\text{Fluoride in the food}} \times 100$$

2.4. Influence of the digestion phase on solubilization of fluoride

Separate portions of the same sample were weighed in Erlenmeyer flasks and 90 g of deionized water was added to each of them. The pH was adjusted to 2 with 6 mol/L HCl, and then one of the portions was subjected to the gastric phase and the other was subjected to the complete gastrointestinal phase, as described in Section 2.3. The digests obtained were centrifuged at 10,000 rpm for 30 min at 4 °C and the fluoride concentration in the soluble fraction was then determined (Section 2.9). The assays in each condition (gastric phase and gastrointestinal phase) were performed in duplicate.

2.5. Influence of pH in the gastric phase on solubilization of fluoride

Separate portions of the same sample were weighed in Erlenmeyer flasks and 90 g of deionized water was added to each of them. The pH of each portion was adjusted with 6 mol/L HCl to the values of interest (pH 2, 3, 4, 5 and 6). After making up the sample to 100 g with deionized water, the gastric phase was applied as described in Section 2.3. The gastric digests obtained were centrifuged at 10,000 rpm for 30 min at 4 °C and then the fluoride concentration in the soluble fraction was determined (Section 2.9). The assays in each pH condition were performed in duplicate.

2.6. Influence of pepsin concentration in the gastric phase on solubilization of fluoride

Separate portions of the same sample were weighed in Erlenmeyer flasks and 90 g of deionized water was added to each of them. The pH was adjusted to a value of 2 with 6 mol/L HCl, and then pepsin was added to each portion in the quantity required to reach the following proportions: 0.001, 0.002, 0.004, 0.006, 0.008 and 0.013 g pepsin/g of fresh sample, equivalent to 0.01, 0.02, 0.04, 0.06, 0.08 and 0.13 g pepsin/100 g of solution to be digested. A gastric digestion without pepsin was also assayed. Deionized water was added up to a weight of 100 g and then the gastric digestion phase was applied as described in Section 2.3. The gastric digests obtained were centrifuged at 10,000 rpm for 30 min at 4 °C and then the fluoride concentration in the soluble fraction was determined (Section 2.9). The assays at each pepsin concentration were performed in duplicate.

2.7. Influence of the bile salt concentration in the intestinal phase on solubilization of fluoride

Separate portions of the same sample were weighed in Erlenmeyer flasks, 90 g of deionized water was added to each of them and the gastric phase was simulated, as described in Section 2.3. Then the intestinal phase was applied without bile salts

Table 1
Samples of seafood products used in the assays.

Sample	Scientific name	Origin	Market form	Preparation
Sardine	*Sardina pilchardus*	Spain	Fresh; whole	Removal of intestines and heads; with bones. Grilled
European anchovy	*Engraulis encrasicolus*	Italy	Fresh; whole	Removal of intestines and heads; with bones. Grilled
Big-scale sand smelt	*Atherina boyeri*	Turkey	Frozen; whole	Grilled
Guinea shrimp	*Parapenaeopsis atlantica*	China	Frozen; cooked peeled tails	Grilled
Common prawn	*Palaemon serratus*	Spain	Fresh; cooked whole individuals	Grilled