

Fluoride concentration in Peruvian salts and the standardization of analysis with specific electrode by the direct method

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According to the Peruvian legislation, salt for human consumption should contain 200-250 mg F/kg, but there is limited data showing whether this requirement is being accomplished. **Aim:** In this pilot study, we evaluated the fluoride concentration in samples of salt marketed in Lima, Peru, using a standardized protocol with ion-specific electrode by direct method (ISE). **Methods:** Seven 1 kg salt packages of four brands were purchased in two supermarkets of Lima. Six aliquots of each package were weighed and dissolved in the proportion of 0.025 g/mL of water. Duplicates of 1.0 mL of these solutions were mixed with 1.0 mL of TISAB II and fluoride concentration was determined with ISE calibrated with standards fluoride solutions ranging from 0.25 to 16.0 µg F/mL. The ionic strength of the standards was adjusted with p.a NaCl (25 mg/mL). In addition, triplicates of 15 g of each salt package were fractionated in a set of sieves (0.590 to 0.177 mm) to determine the homogeneity of fluoride concentration in salt. **Results:** In four packages the mean fluoride (mean±SD,n=6) concentration (mg F/kg) was in agreement to the Peruvian regulation (214.5±10.4; 221.8±14.3; 226.9±19.1 and 237.2±52.0 mg F/kg), but in 3 packages it was lower (145.2±7.9; 145.7±23.3 and 158.4±20.6 mg F/kg). Variability in fluoride concentration was observed within the same brand and among brands. Also, the fluoride concentration was not homogeneous in none of the salt samples, ranging from 72.0 to 1449.7 mg F/kg. **Conclusion:** The findings suggest that the manufacturing and sanitary surveillance of fluoridated salt in Peru should be improved.

Keywords: Fluoridation. Dental caries. Ion-Selective Electrodes

INTRODUCTION

Oral health approaches based on the safe use of fluorides have contributed worldwide to the reduction of dental caries¹. Among the ways of use of fluoride, the community based is considered the one with greater coverage, allowing the benefit of all social classes. Salt fluoridation is a community based strategy to prevent tooth decay and it has been recommended as an effective alternative to water fluoridation²⁻⁵. In addition, salt fluoridation has been implemented in several Latin American countries, reaching nearly 200 million people^{6,7}.

On the other hand, an adequate fluoride surveillance system is required to guarantee that any program of fluoride use reaches the maximum anticaries benefits with the minimal risk to develop undesirable side effects, such as dental fluorosis⁸. One of the advantages of salt fluoridation compared to water would be the facility to monitor the fluoride concentration, because in any country the number of salt producers to be monitored is very smaller than the number of water treatment plants. Despite this advantage, fluoride concentrations below or above those established by local legislations have been found in salt brands sold in El Salvador, México, Colombia and Peru⁹⁻¹⁵.

In Peru, the fluoridated salt program was set in 1984¹⁶ and the fluoride concentration in salt for human consumption should be from 200 to 250 mg F/kg¹⁷. Official data about the fluoride concentration in Peruvian salts have not been reported and searching the literature it was found only one evaluation carried out 12 years ago¹⁵. Ten brands of salt were purchased in 34 markets from the province of Trujillo, north coast of Peru. However, only one brand was fluoridated and the concentration found was below the legislation (152 ppm F).

Considering the scarce and limited data found, the aim of this study was to report updated findings about the fluoride concentration in salts brands sold in Peru.

MATERIAL AND METHODS

This exploratory in vitro study, blind regarding the analyst, determined the fluoride concentration in four fluoridated salt brands of human consumption found in Lima, Peru. Seven 1 kg packages were purchased in 2013, in two supermarkets of Lima city. It was recorded the producer, batch, validity, type of granulation, ingredients and fluoride concentration declared on the package label (Table 1).

Standardization of the sample salt weight taken for analysis

The fluoride concentration in salt could be influenced by the amount of salt sample taken for analysis if the mixture of NaCl and the fluoride salt used is not homogeneous. Therefore, to obtain more accurate results, it was investigated which would be the weight of salt aliquots representative of the mean fluoride concentration in the salt package. For analysis, salt aliquots from 0.025 to 2.5 g (± 0.01) were dissolved in purified water at a ratio of 1:40 (w/v). Fluoride concentration was determined using ion-specific electrode (ISE) by the direct method and the concentration of NaCl in fluoride standard solutions was adjusted according to the sample¹⁸. The variation coef-

Table 1. Producer, code for analysis, salt brands, batch and validity, granulation type, fluoride concentration and ingredients declared on the label

Producer	Code	Salt brands	Batch and validity	Type of granulation	Fluoride concentration declared (mg F/kg)	Ingredients
QUIMPAC S.A.	1	Pura Sal yodada cocina	LT.LN 10910131 FV 091015_17:19_3	fine	200-250	KF, KIO ₃ , SiO ₂ (max1%)
Idem	2	Pura Sal yodada cocina	LT.LN 11 210 131 FV 121015_01:24_2	fine	Idem	Idem
Idem	3	Em Sal yodada cocina	LT. LN11109131 FV 1109154_06:15_1	fine	Idem	Idem
Idem	4	Em Sal yodada cocina	LT. LN 12710131 FV. 271015_02:38_1	fine	Idem	Idem
Idem	5	Sal Extra Refinada-Marina	LT.LN11310131 FV 131015_04:06_1	refined	Idem	Idem
Idem	6	Sal Extra Refinada-Marina	LT.LN127101311 FV 271015_04:18_3	refined	Idem	Idem
Idem	7	Em Sal yodada mesa	P 16.09.13 V 16.09.15 13:47 B2	fine	Idem	Idem

ficient of the fluoride concentration in the salt samples weighing less than 2.5 g was high (up to 30 %), while in samples weighing around 2.5 g it was lower (10 %). Thus, salt aliquots with weight of around 2.5 g were taken for this study.

Standardization of fluoride determination with ion selective electrode (ISE) by the direct method

The analysis of the fluoride concentration with ISE by direct method is subjected to interferers^{19,20}. Calcium-ferrocyanide, calcium carbonate, calcium triphosphate, magnesium oxide, magnesium hydroxide and magnesium carbonate are usually added to salt as anticaking agents²¹. Ca⁺⁺ and Mg⁺⁺ ions form complexes with fluoride that could interfere with the determination of fluoride in salt using ISE by the direct method²². Another problem is the ionic strength of the salt solutions prepared for analysis because it decreases the activity of the fluoride ion in solution. The interference of Ca⁺⁺ and Mg⁺⁺ could be controlled by the presence of chelating agents added in the buffer solution used for the analysis, such as CDTA presents in TISAB II (Total ionic strength adjustment buffer). The effect of the ionic strength could be controlled by preparing a very diluted salt solution for analysis. However, as described in the previous item, it is necessary to weigh a representative amount

of salt to obtain a more accurate fluoride concentration in salt. Thus, the concentration of salt in the solution prepared may surpass the concentration of NaCl (1.0 M) contained in the TISAB II buffer. This problem has been solved adding pure NaCl in the TISAB II buffer to prepare the fluoride standard solutions used in the calibration curve¹⁸, as is described further.

To test the effect of the ionic strength on the accuracy of the fluoride concentration determination, salt sample solutions from 0.5% to 20% (w/v) were prepared. Fluoride concentration was determined using calibration curves made with standard fluoride solutions adjusted or not (raw data) with pure NaCl (Sigma Aldrich, Lot # 70M027330V) according to the percentage of salt in the solution prepared for analysis.

Figure 1 shows fluoride concentration (mg F/kg) found in salt according to the amount of salt (g) dissolved in 100 mL of purified water, determined from calibration curves made with standard fluoride solutions not adjusted (raw data) or adjusted (data normalized) according to the percentage of NaCl in the samples. If the salt concentration in the standard solutions is not adjusted (below curve), the fluoride concentration is underestimated from 4% to 15%. Also, the data shows that a more accurate fluoride concentration is obtained when salt is prepared at proportion of around 2 g of salt in 100 mL of water and the standard solutions are adjusted with pure NaCl. Below and above this weight, the concentration of fluoride is underestimated. Thus, a final proportion around 2.5 g of salt dissolved in 100 mL of purified water was used for the analysis of salt samples.

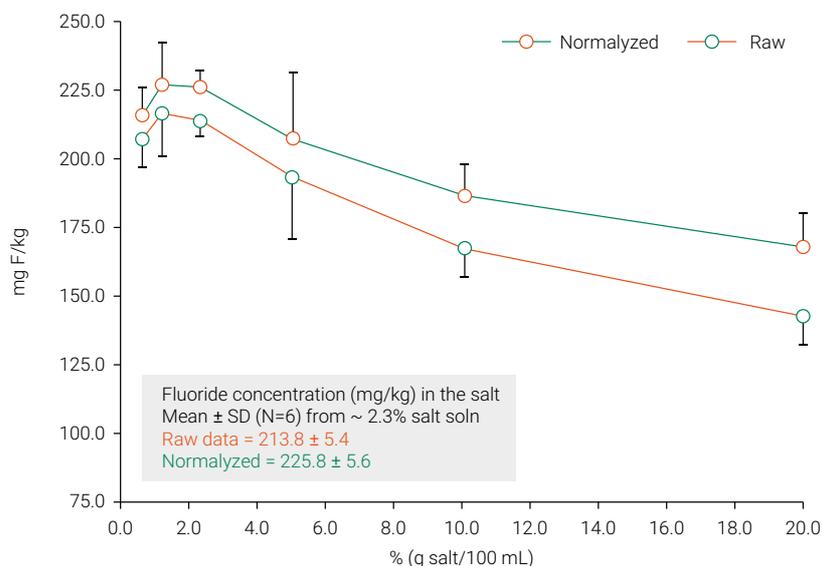


Figure 1. Concentration of fluoride found in salt (mg F/kg) according to the amount (g) of salt dissolved in 100 mL of purified water, determined from calibration curves made with standards fluoride solutions adjusted or not with p.a NaCl according to the % of salt in the samples.

Salt sample harvest and preparation for analysis

The salt packages were manually agitated for 2 min for homogenization. Six aliquots weighting 2.5 g (± 0.01) were harvested from the top of each package and dissolved in the proportion of 0.025 g/mL of purified water. To each duplicate of 1 mL of the prepared solutions, 1 mL of TISAB II was added (1:1; v/v). Fluoride analysis was carried out as is described further.

Assessment of the fluoride concentration homogeneity in the salt package

To determine the homogeneity of fluoride in the salt packages, triplicates of 15 g of each package were fractionated using a set of sieves (Telatest) with tyler of 28 (0.59 mm), 35 (0.42 mm), 60 (0.250 mm) and 80 (0.177 mm). After 1 min of manual agitation, the fractions of salt that passed through the 28-60 tyler as well as the retained in the tyler 80 (particles from < 0.177 to > 0.590) were collected and dissolved in the proportion of 0.025 g/mL of purified water. Solutions were prepared as described before for fluoride determination.

Fluoride analysis

The fluoride concentration in the salt solutions was determined using ion-specific electrode (Orion 96-09, Thermo Scientific, Cambridge, MA, EUA) coupled to an ion analyzer (Orion Star A214; Thermo Scientific, EUA). This equipment was previously calibrated with standard fluoride solutions ranging from 0.25 to 16.0 $\mu\text{g F/mL}$, mixed in ratio (1:1; v/v) with TISAB II added of 0.025 g NaCl (p.a) /mL¹⁸.

Microsoft Excel software was used to estimate by linear regression the relationship between the logarithm of the fluoride concentration in the standards and the mV. The linear correlation coefficient was of 0.999. The regression was used to determine the fluoride concentration in the samples and the average coefficient of variation for the duplicate analysis was of 1.3 %.

For the data analysis, it was calculated the mean ($\pm\text{SD}$; n=6) fluoride concentration in every package and the mean ($\pm\text{SD}$; n=3) and range of fluoride concentration in the fractionated samples of salt. The results were expressed in mg F/kg and compared with the range of fluoride concentration demanded by the Peruvian legislation.

RESULTS

Only 4 brands of fluoridated salt were found in two supermarkets of Lima, all from the same manufacturer, QUIMPAC, that is responsible for 60% of salt marketed in Peru. From 3 brands were purchased 2 packages, one in each supermarket, but one brand was found in only one supermarket. Seven salt packages from different batches were purchased and analyzed (Table 1).

Five out of the seven (71%) salt samples analyzed were fine granulated and the remaining two (29%) refined granulated. All the samples were iodized and had silicon dioxide (max 1%) as the anticaking agent. The fluoride agent declared by the manufacturer in all the samples was KF and the final fluoride concentration declared was in the range of 200 to 250 mg F/kg.

Results showed that the mean (\pm SD; n=6) of fluoride concentration (mg F/kg) of the salt samples ranged from 145.2 to 237.2 mg F/kg (Table 2). In four packages, the fluoride concentration was according to the Peruvian legislation (salts code 2,4,6 and 7), but, in three of them (salts code 1,3 and 5) it was lower.

Table 2. Code, salt brands and mean (\pm SD; n= 6) of fluoride concentration (mg F/kg) found in the samples of salt

Code	Salt brands	*mg F/kg
1	PuraSal yodada cocina	145.7 \pm 23.3
2	PuraSal yodada cocina	214.5 \pm 10.4
3	emsal yodada cocina	145.2 \pm 7.9
4	emsal yodada cocina	221.8 \pm 14.3
5	Sal extra refinada-Marina	158.4 \pm 20.6
6	Sal extra refinada-Marina	226.9 \pm 19.1
7	emsal yodada mesa	237.2 \pm 52.0

* Expected: 200-250 mg F/kg, according to Peruvian regulation

The fluoride concentration (\pm SD; n=3) found (mg F/kg) in the fractionated samples of salt is shown in Table 3. The concentration in the fractions of the salt code 2 was the most heterogeneous (from 116.2 to 1449.7), while in salts 3 and 5 it was the most homogeneous (from 164.6 to 221.3 and 164.6 to 221.3, respectively).

Table 3. Mean (\pm SD; n=3) of fluoride concentration (mg F/kg) found in the fractions of the salt samples and the range found

Code	Salt Brands	Particle size (mm)					Range
		< 0.177	0.1770-250	0.250-0.420	0.420-0.590	> 0.590	
1	PuraSal yodada cocina	332.4 \pm 228.6	181.6 \pm 32.2	137.0 \pm 11.8	98.6 \pm 27.9	126.6 \pm 23.2	98.6-332.4
2	PuraSal yodada cocina	125.6 \pm 11.0	116.2 \pm 3.1	202.5 \pm 65.3	1449.7 \pm 1496.1	271.0 \pm 12.4	116.2-1449.7
3	emsal yodada cocina	115.1 \pm 19.0	155.8 \pm 39.1	127.1 \pm 46.3	159.4 \pm 49.5	167.5 \pm 14.3	115.1-167.5
4	emsal yodada cocina	141.6 \pm 7.1	72.0 \pm 16.5	152.3 \pm 39.7	420.9 \pm 25.9	234.8 \pm 9.8	72.0-420.9
5	Sal extra refinada-Marina	218.7 \pm 35.8	221.3 \pm 65.4	178.7 \pm 24.3	164.6 \pm 14.4	167.5 \pm 53.3	164.6-221.3
6	Sal extra refinada-Marina	382.0 \pm 62.7	297.3 \pm 91.7	159.8 \pm 11.0	120.9 \pm 12.6	275.3 \pm 112.7	120.9-382.0
7	emsal yodada mesa	375.2 \pm 28.6	290.7 \pm 40.4	211.4 \pm 10.7	121.9 \pm 12.1	93.7 \pm 13.6	93.7-375.2

DISCUSSION

The monitoring of the fluoride concentration in salt is necessary to guarantee the maximum anticaries benefits with the minimal risk to develop unacceptable dental

fluorosis in the population. To determine an accurate and precise fluoride concentration in salt is essential to have a valid methodology of analysis. Additionally, the analysis should be feasible to be conducted in any country where salt fluoridation programs were implemented.

Our findings showed that accurate fluoride concentration in salt was found when salt sample solutions were prepared at concentration of around 2.5 % (w/v) and the ionic strength of the fluoride standards were adjusted with pure NaCl (Fig 1). These results were obtained using ion specific electrode (ISE) by direct analysis but a publication has suggested that the determination of fluoride in salt using ISE should be made after microdiffusion and not by the direct analysis¹⁹. However, according to this publication suggesting that microdiffusion should be used¹⁹, fluoride concentration determined in the salt by the direct analysis was made from a 10% salt solution and the ionic strength of the samples was not adjusted. As Fig 1 shows, if a salt sample solution is prepared at 10% and the electrode is calibrated without adjustment of the ionic strength, the fluoride concentration would be underestimated in 27%, compared with the concentration found from analysis of 2.5% salt solution. It should be emphasized that even with the adjustment of the ionic strength, the fluoride concentration in the salt from analysis of a 10% solution would still be underestimated in 18% (Fig.1). In conclusion, to obtain accurate results is necessary to weigh an amount of salt that is representative of the fluoride concentration in the salt (at around 2.5 g) which may be dissolved in the proportion of 0.025 g/mL of water. Also, the ionic strength of the fluoride standards must be properly adjusted with NaCl chemically pure.

Regarding the results found and the surveillance system of the salt fluoridation program in Peru, the local legislation establishes that the adequate range of fluoride in salt for human consumption should be from 200 to 250 mg F/kg¹⁷. Thus, fluoride concentration in salt below or above this range would not have a maximum anticaries benefit or would provoke unacceptable fluorosis, respectively. According to the findings (Tab 2), 57% of the samples analyzed showed fluoride concentration according to the legislation and the remaining salt samples (43%) showed concentration lower than the minimum recommended.

Additionally, it was observed variability of the mean fluoride concentration within the same brand (Tab 2). As described in the results item, all the seven packages of salt analyzed were produced by the same manufacturer but were products of 4 brands (Table 1). Moreover, three of the brands analyzed (PuraSal yodada cocina, Emsal yodada cocina and Sal extra refinada-Marina) were purchased in duplicate but from different batches (Tab 1). Table 2 shows that one sample from each brand presented adequate fluoride concentration, but its duplicate did not. Considering that all salts were produced by the same manufacturer and probably by the same process of fluoride addition, the findings suggest that the program of quality control of fluoride concentration during salt production is deficient.

Moreover, when the data of mean fluoride concentration in the salts (Table 2) are confronted with the data of fluoride concentration in the fractions of salts sieved (Table 3), other problems are noticed. Thus, although the mean fluoride concentration found in salts code 2, 4 and 6 (table 2) are according the Peruvian legislation, they were not the most homogeneous (Table 3) compared with salts batches 1, 3 and 5. Fluoride concentration in the fractions of salts 2, 4 and 6 ranged respectively from 116.2 to

1449.7 (12-fold), 72.0 to 420.9 (6-fold) and 120.9 to 380.2 (3-fold), while in salts 1, 3 and 5 ranged from 98.6 to 332.4 (3-fold), 115.1 to 167.5 (1.4-fold) and 164.6 to 221.3 (1.3-fold). Therefore, for example, when people use a small amount of salts code 2, 4 or 6 in a salad than a larger amount to cook foods, they were subjected to greater variability of fluoride ingestion.

Although the sampling of salts analyzed in our study was limited to only 7 packages of salt, we compared our findings (Table 2) with reports from Peru and other countries, where the program of salt fluoridation was implemented¹⁰⁻¹⁵. Sunohara¹⁵ analyzed in 2005 ten brands of Peruvian salts. Fluoride was found in only one brand (EMSAL Yodada de Mesa) but fluoride concentration was below the legislation (152 ppm F). In this same brand (Table 2, code 7), we found 237.2 ppm F. This difference found in the same brand shows deficiency of a program of quality control of fluoride concentration during the production of salt.

The results that we found in Peruvian salts (Table 2) are similar to findings for 75 Mexican salts analyzed in 2002 and 2003 because 50% of the samples were in agreement with the local legislation (200-250 mg F/kg)¹⁰. However, other analyses conducted in Mexican salts have found worst results. Analyses made in 1995¹¹ and in 2008¹² showed respectively that only 1 and 7% of the salt samples presented fluoride concentration in accordance with the local legislation.

Regarding labeling, all the Peruvian salt brands selected are in agreement with the Peruvian norm of salt for human consumption¹⁷ in terms of the declaration of the fluoride concentration in mg/kg of salt, method used for fluoride addition, sanitary registration number, batch, date of production and expiration. The discrepancy between the mean fluoride concentration found (Table 2) and that declared by the manufacturer (Table 1) on the label of samples code 1, 3 and 5 is due to the deficiency of quality control of fluoride concentration during salt production, as described before.

Although the present pilot study was limited to samples of salts found in Lima, the impact of fluoride concentration found (Table 2) for the Peruvian population, considering the anticaries benefits and the risks of dental fluorosis of the salt fluoridation program implemented in Peru may be discussed. People who use salt brands 2, 4, 6 and 7 would have the maximum anticaries effect but who use salts 1, 3 and 5 would be less protected. However, dental caries is a chronic disease and the effect of fluoride on caries is local, not systemic²³. Therefore, the time of maintenance of an effective fluoride concentration in the mouth to interfere with the caries process should be considered than "optimal" concentration for short time. Also, it is not known whether salts with concentration of around 150 mg F/kg (Tab 2) would be less effective in terms of caries prevention regarding salts with concentration of 200-250 mg F/kg.

Concerning dental fluorosis, the risk is not increased because none of the salt samples presented mean fluoride concentration above the established range (Table 2). However, when the salt was fractioned it was found in the sample code 2 a fraction with 1449.7 mg F/kg (Tab 3). Although this concentration is around 6-fold the upper limit, there is a few concern because it was found in very small salt particle (0.420-0.59 mm). A small particle could be used by chance for example to add salt to a tomato slice but not to cook, when larger amount of salt is used. Therefore, assuming that a 20 kg child added

0.1 g of salt with concentration of 1449.7 mg F/kg to a tomato slice, he would intake 0.15 mg of fluoride. Thus, the child would be subjected to the dose of 0.0075 mg F/kg of body weight, which is 10 times lower the upper limit dose for unacceptable risks of fluorosis²⁴. Indeed, Peruvian children are not exposed to dose of risks of fluorosis from foods cooked with fluoridated salt²⁵. In addition, dental fluorosis is a chronic disease and the time of duration of an "optimun" dose is more relevant than sporadic higher dose. However, this discussion about fluorosis risks of the Peruvian fluoridated salt program is not considering the effect of fluoridated salt for people living in areas containing natural fluoride. In fact, the Peruvian regulation set that "the population who is exposed to water containing natural fluoride at concentration of 0.7 ppm or greater does not need consume fluoridated salt". However, it does not establish any mechanism to avoid that fluoridated salt is market in these areas.

The overall findings of this pilot study about fluoride concentration in salts marketed in Lima, Peru, and the publication cited¹⁵, alert about the need to improve the monitoring of the fluoride concentration in salt that is responsibility of manufacturers and governmental entities. It is reasonable to think that the balance between the anticaries potential and low risk of fluorosis requires an adequate concentration of fluoride in salt.

Based on this pilot finding, we concluded that the manufacturing and surveillance system of fluoridated salt in Peru should be reinforced.

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