Original

Determination of Total and Ionic Fluoride in Formula Milk Produced in Japan

乳児用調整粉乳中の総フッ素およびイオン型フッ素の測定

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概要: 母乳および乳児用調整粉乳中のフッ素の測定は、測定法の進歩にもかかわらず、多有機質中の微量フッ素測定の困難さから、イオン型フッ素と総フッ素の割合およびその存在様態なども明らかではない。

本研究ではわが国における市販乳児用調整粉乳20種について、4種の測定法を行ない、それぞれの値を比較検討した。

試料は市販乳児用調整粉乳（5社、20種類、そのうち6種は大豆ベース2種を含む特殊調整粉乳）を製造会社の指定する標準濃度に蒸留水で希釈した（0.13~0.17g/ml）。また微量拡散の操作条件を検討するため市販牛乳2種を用いた。

フッ素の測定はフッ素イオン電極による直接測定（IE法）以外はガスクロマトグラフィーにより行なった。前処理法として①微量拡散法（Dif法）②パイロハイドロリシス法（PyH法）③低温灰化法（LTA法）を用いたが、それらの装置と条件、フッ素の添加回収率等についてはすでに報告している。Dif法については、拡散時間（1~24時間）と過塩素酸の濃度（2.7Mと9.2M）をかせ、乳児用調整粉乳と牛乳について検討した。

その結果、イオン型フッ素を測定するIE法とDif法によるフッ素測定値は殆どの場合一致せず、Dif法からの値の方が高かった。我々がすでに検討した牛乳中のイオン型フッ素は濃い酸を用い、12時間以上拡散を行なってもIE法の値を上回ることにはなかったが、それと反対の現象をみとめた。Dif法の再検討を行なった成績も同様にDif法の値がIE法の値より高く、これらの結果は乳児用調整粉乳中のイオン型フッ素には、フリー（free）と酸不安定（acid labile）の2つの型が存在することを示唆している。

総フッ素については、LTA法で測定した値がPyH法で測定した値よりわずかに高い傾向がみられた（Wilcoxon's signed ranks test）。しかし、PyH法の値とDif法の値は数種の例外を除いて近似していた。このことから、乳児用調整粉乳中のフッ素測定において、Dif法を簡易法として用いることができるであろう。

今回測定した乳児用調整粉乳のフリーのイオン型フッ素は0.010~0.178 μ g/ml; 総フッ素は0.033~0.210 μ g/ml（PyH法）を示し、それらの平均値は0.048と0.070 μ g/mlとなった。また、大豆ベースを含む特殊調

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整粉乳中のフッ素の平均値については試料数が少ないので別個に求めているが、必ずしも高いとはいえない。

Key words: Ionic fluoride, Total fluoride, Infant formula milk, Measurement

索引用語: イオン型フッ素, 総フッ素, 乳児用調整粉乳, 測定法

Introduction

Determining the fluoride (F) level in infant formula milk and human milk is important to estimate the amount of F intake in early infancy. In recent years, reports¹⁻³⁾ of an increased prevalence of dental fluorosis in both fluoridated and nonfluoridated areas have led to a reassessment of the amount of F being ingested by infants. To achieve such a reassessment, it is essential to have accurate determination methods for ionic and total (ionic plus bound) F in infant foods.

Although in the early 1970s the determination of F in milk was done for both ionic and total F⁴⁻⁷⁾, Singer *et al.*⁸⁾ and Taves⁹⁾ reported that there was no discrepancy between the values of total F obtained by the ashing method and those of ionic F obtained by the unashing method in most of the food groups studied. A recent collaborative study by Dabeka *et al.*¹⁰⁾ has recommended a method based on acid diffusion from unashed samples for determining F in infant foods. Given these circumstances, current studies¹¹⁻¹⁷⁾ which have evaluated the F content of infant foods have measured only ionic F by various diffusion methods.

However, according to a report by van Staden *et al.*¹⁸⁾, they measured ionic and ionizable F in milk using various reported diffusion methods and found that the recovery of ionic F was insufficient in almost all the methods examined. Rao¹⁹⁾ stated in his review that no convenient and broadly accepted method had yet been found to determine the amount of F in foods and beverages. The determination of total F in milk is difficult because milk contains high amounts of organic matter while its F content is low. Therefore, different analytical procedures have produced a wide range of reported F levels in milk, and the ratio of ionic and total F levels in milk is not yet clear.

In this context, we previously reported total and ionic F levels in cow' milk measured by three analytical procedures²⁰⁾. This time, we determined and compared the results of total and ionic F content in formula milk samples obtained in Japan using four analytical procedures.

Materials and methods

Formula milk: Twenty kinds of powdered formula milk (5 manufacturers), regular type milk-based and specialized formula including soy-based products, were purchased locally and used for analysis. We determined the F content in formula milk diluted with distilled water according to the manufacturer's directions. The concentrations of milk powder were from 13 to 17 g per 100 ml of distilled water.

Cows' milk: Two kinds of cows' milk were purchased and used for examining the microdiffusion operating conditions compared with those of formula milk.

F analysis: Ionic F was determined by the F ion selective electrode (IE) method and the microdiffusion (Dif) method, while pyrohydrolysis (PyH) and low temperature plasma ashing (LTA) were used for pretreatment of the total F determination. The F separated from the milk component was measured by gas chromatography (GC). Although we have already reported the procedures for the above-mentioned methods²⁰⁻²⁴⁾, in this study we used strontium as a fixative for the LTA method.

Reexamination of the Dif method: The effects of diffusion time (1-24 hrs) and acid concentrations (2.7 M and 9.2 M perchloric acid) on the F values in formula milk and cows' milk were examined again.

Table 1 Total and ionic F levels in formula milk diluted with distilled water

Manu- facturer	No	Milk conc. (g/ml)	F conc.※ (μg/ml)				Note
			Free ionic	Ionizable	Total		
			IE	Dif	PyH	LTA	
A	1	0.14	0.041	0.052	0.054	0.067	(2)
	2	0.136	0.053	0.080	0.085	0.101	
	3	0.14	0.035	0.069	0.075	0.074	
	4	0.15	0.063	0.075	0.081	0.093	(3)
	5	0.15	0.057	0.073	0.077	0.083	
	6	0.15	0.091	0.109	0.133	0.113	
B	7	0.13	0.038	0.051	0.061	0.073	(4)
	8	0.13	0.020	0.039	0.041	0.047	
	9	0.14	0.040	0.082	0.100	0.100	
	10	0.145	0.178	0.203	0.210	0.239	(1)
	11	0.135	0.010	0.026	0.033	0.031	
C	12	0.13	0.037	0.038	0.037	0.038	Soy-based
	13	0.13	0.019	0.022	0.036	0.038	
	14	0.17	0.079	0.084	0.083	0.093	
	15	0.15	0.029	0.047	0.058	0.063	
D	16	0.13	0.031	0.061	0.061	0.058	
	17	0.13	0.028	0.029	0.038	0.044	
	18	0.14	0.052	0.062	0.062	0.072	
E	19	0.147	0.026	0.025	0.038	0.035	
	20	0.14	0.043	0.043	0.053	0.060	
Wilcoxon's test							
			* *		* *		*
Mean			0.048	0.063	0.070	0.076	

Statistically significant *: $p < 0.05$ **: $p < 0.01$

Note (1) : Low sodium formula

(2) : Lactose free formula

(3),(4) : Protein hydrolysate formula

※ : Average of 3 tests for each sample.

Results

F levels in formula milk: The F levels in formula milk determined by four methods are shown in Table 1 and the relationships of each value are shown in Fig. 1. The ionic F values determined by the IE and the Dif methods did not agree with each other in almost all the samples: the values from the Dif method were higher than the values from the IE method. These findings revealed a large difference from the results in our previous experiments on cows' milk²⁰. The total F values obtained by the LTA method were generally slightly higher than those of the PyH method.

Fig. 1 Relationships of F values in formula milk obtained by the four methods

the same as previous experimental data²⁰; the F values given by the Dif method did not exceed the values which were measured by the IE method even after a diffusion time of 24 hours. However, in the case of formula milk, the F values given by the Dif method were higher than those of the IE method even after 1 hour of diffusion with 2.7 M perchloric acid.

Discussion

Ionic F in formula milk: It is now generally agreed that although the F content in drinking water is in ionic or free form, foods and beverages contain both ionic and nonionic, or bound forms of F. Moreover, the analysis of ionic F in drinking water is relatively straightforward; on the other hand, no convenient and adequate method is generally accepted for the analysis of total F in foods and beverages at this time¹⁹.

Even though the use of the diffusion technique for the determination of ionic F in milk is generally

Using Wilcoxon's signed ranks test, statistically significant differences were observed between the F values obtained with the IE method and those of the Dif method ($p < 0.01$), and differences were also observed between the F values from the PyH method and those from the LTA method ($p < 0.05$).

The ionizable F values from the Dif method showed only a little difference from the total F values given by the PyH method although there were some exceptions, and the PyH method always gave slightly higher values than the Dif method.

The F values of arithmetic mean in the formula milk diluted with distilled water obtained by the four methods were 0.048 for the IE, 0.063 for the Dif, 0.070 for the PyH, and 0.076 µg/ml for the LTA.

The ratios of F values given by the Dif, PyH, and LTA methods to the free ionic F given by the IE method are shown in Table 2. The ratios of total F to free ionic F had a wide range, from 1.0 to 3.3 (in the PyH method). The ratios of acid labile F (subtracting the F value obtained by the IE method from the F value obtained by the Dif method) to free ionic F showed a range from -0.04 to 1.6.

Reexamination of the Dif method: The effects of diffusion time and perchloric acid concentrations on the F values determined in the formula milk and the cows' milk are indicated in Fig. 2. The results of the cows' milk were

Table 2 Ratios of F values to free ionic F obtained by the IE method

Milk No	Ratios of F values to free ionic F			
	Acid labile	Ionizable	Total	
	(Dif-IE)/IE	Dif/IE	PyH/IE	LTA/IE
1	0.27	1.27	1.32	1.63
2	0.51	1.51	1.60	1.91
3	0.97	1.97	2.14	2.11
4	0.19	1.19	1.29	1.48
5	0.28	1.28	1.35	1.46
6	0.20	1.20	1.46	1.24
7	0.34	1.34	1.61	1.92
8	0.95	1.95	2.05	2.35
9	1.05	2.05	2.50	2.50
10	0.14	1.14	1.18	1.34
11	1.60	2.60	3.30	3.10
12	0.03	1.03	1.00	1.03
13	0.16	1.16	1.89	2.00
14	0.63	1.06	1.05	1.18
15	0.62	1.62	2.00	2.17
16	0.97	1.97	1.97	1.87
17	0.04	1.04	1.36	1.57
18	0.19	1.19	1.19	1.38
19	-0.04	0.96	1.46	1.35
20	0.00	1.00	1.23	1.40
Mean	0.43	1.43	1.65	1.75
±SD	0.45	0.45	0.56	0.52

considered very accurate, the values reported from different laboratories continue to span a wide range and many different opinions still exist¹⁸⁾. According to some reports^{4,7)} some part of the F which is added to the milk remains in a free ionic state while the remainder becomes bound to the milk constituents.

We have reported²⁰⁾ that the direct measurement of ionic F in cows' milk by an ion-selective electrode made it possible to measure the F concentration down to a third decimal place with good reproducibility by correcting the calibration curve with a suitable standard solution between each measurement. This time, measurements of ionic F in formula milk by the IE method were performed in the same manner as in cows' milk and good reproducibility was also observed.

In reexamination of the Dif method, which showed higher F values from the Dif method than those from the IE method only for the formula milk, we discovered that there were two forms of ionic F in formula milk, one free and the other acid labile. In blood serum, however, Paez *et al.*²⁵⁾ reported that a labile fraction can be detected in a combined or organic fraction depending on the intensity of the treatment applied in order to obtain the separation of total F. In the formula milk we found the acid labile form, which could not be found in blood serum and cows' milk in our previous studies^{20,22)}.

Total F in formula milk: The values for total F in the LTA method generally were slightly higher than in the PyH method. We believe these differences were related to the specific character of the

Fig. 2 Effect of diffusion time and acid concentrations on F values

F pretreatment methods; that is, with the PyH method, the F isolated from samples was determined; however, with the LTA method, the F which remained in the ashed samples was determined. As we have experienced in the ashing of cows' milk²⁰⁾, the F values from the LTA method were affected by ashing time; ashing time beyond 8 hours resulted in a gradual increase in F values.

Although a significant difference was recognized statistically in the F values between the Dif method and the PyH method, both values showed considerable agreement in each sample. Our results are in close agreement with Taves's report⁹⁾ that no discrepancy between the F values from ashed (total F) and unashed (inorganic F) samples was found in most of the food groups treated. Therefore, when highly precise data and special equipment are not required for the experiments, we recommend that the Dif method on unashed samples be used in place of the determination of total F on ashed samples in formula milk.

Comparison with other published data: Recently, Watanabe *et al.*²⁰⁾ reported that the total F values in formula milk purchased locally in Japan and diluted with distilled water were from 0.03 to 0.07 $\mu\text{g/ml}$. McKnight-Hanes *et al.*¹⁵⁾ reported that soy-based formulas contained higher levels of F than their milk-based counterparts. We purchased and used twenty kinds of powdered formula milk, regular type milk-based formula, and specialized formula including soy-based products. The specialized formula milk is produced for infants who require special treatment for metabolic disorders; these formulas are low sodium, lactose free and protein hydrolysate. Because the number of samples used in our experiment was limited, we did not separately calculate the average F concentrations in the specialized formulas or in the regular formulas. However, the F concentrations in the specialized formulas had levels similar to those of the regular formulas, although there were a few exceptions.

Our results for total F ranged from 0.033 to 0.210 $\mu\text{g}/\text{ml}$ (obtained by the PyH method), equivalent to 0.004 to 0.031 mg F/100 kcal; the average was 0.070 μg F/ml, equivalent to 0.01 mg F/100 kcal. These figures do not exceed the upper limit of 0.06 mg F/100 kcal for powdered formulas recommended by Ekstrand²⁷⁾.

Ekstrand stated in his paper²⁷⁾ that the F concentrations ranged from 0.1 to 0.8 mg/l for ready-to-feed milk-based formulas and this variation depended mainly on the F concentration of water used in preparing the products. In Japan, because the amount of F in most water supplies is low (0.1–0.2 mg/l), the F concentrations in formula milk diluted with tap water would not reach the intake of 0.1 mg F/kg body weight/day commonly believed to be associated with increased risk of developing dental fluorosis²⁸⁾.

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