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DETERMINATION OF Br, Ca, Na, K, Fe, Rb, Se AND Zn IN MILK FORMULAS BY INAA

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ABSTRACT

In this study Br, Ca, Na, K, Fe, Se, Rb and Zn levels were determined in seven different milk formula samples. The samples were acquired in the markets of São Paulo city. After 8-hour irradiations in the IEA-R1 nuclear research reactor under a thermal neutron flux of 10^{12} n cm^{-2} s^{-1}, the essential elements were analyzed by Instrumental Neutron Activation Analysis. For validation of the methodology, NIST RM 8435 Whole Milk Powder and SRM 1549 Non Fat Milk Powder reference materials were analyzed. Almost all the milk formula samples showed the essential element concentrations (Ca, Fe, Na, K, Se and Zn) equivalent to those levels printed on the labels. Only for one sample the Fe and Zn elements were below the stated values and two samples that Zn higher compared with printed of the labels claims.

1. INTRODUCTION

Human milk is considered a rich source in nutrients for children. Due to its content and the availability of carbohydrates, proteins, fats, vitamins and minerals and also to additional biological factors human milk contributes to the child's health and well-being. These factors protect against infection and ensure optimal development of the intestinal flora [1]. Brätter and Schramelin [2] have emphasized the need of trace elements during infancy and their adequate availability in human milk. Deficiency of minor and trace elements can lead to various disorders in infants. Milk is an adequate food and it is the only source of mineral nutrients for infants [3].

The World Health Organization (WHO) recommends that infants up to 6 months be exclusively breastfed. Breastfeeding should continue along with complementary feeding until 2 years of age. However, this is not always possible due to the fact that not all mothers are able to produce sufficient amounts of milk for their infants [4].

According to *Codex Allimentarius*, the best substitute choices for maternal milk are infant milk formulas, and when prepared under proper hygienic conditions can be used to feed infants. Numerous infant products have been produced and formulated to meet the nutritional needs of healthy full-term infants. Due to variation in nutrient contents from the food sources used to the formula preparations, specifications of nutrient levels, including mineral elements, have been set to simulate levels of these nutrients similar to human milk [5,6].

In 1915 the first infant milk formula, known as "Synthetic Milk Adapted-SMA", was produced in the USA, and later a soy-based milk was produced commercially [7].

In recent years, there has been great interest to analyze infant formulas. The objective has been to compare of the essential nutrient content in human milk and to the manufactures label claims. Kashlan *et al.* [6] reported that the levels of mineral elements in different infant formulas marketed in Kuwait were above the *Codex Alimentarius* recommendation, except for a few products that were low in Fe, Zn and Cu. Variation from label claim was highest for Mn. Abbé *et al.* [8] showed that several infant formulas sold in Canada are very low in selenium. These products may not provide adequate selenium to meet the infants' requirements, particularly during the first 6 months of life, as milk provides the primary source of selenium for these infants. Plessi *et al.* [9] analyzed seven different powdered infant formulas and the results showed that the aluminum content of soy-based formulas was much higher than those presented in milk-based formulas, while that present in protein-hydrolysate formula was even higher than other milks. It is difficult to identify correctly the causes of different aluminum levels. The high aluminum levels in soy-based formulas could result from aluminum naturally present in soy-bean. Torres *et al.* [10] analyzed infant formulas which provide significantly less selenium than 10 μg/g corresponding to the recommended daily allowance for infants from 0 to 6 months.

There are no published data on element content in infant milk formulas marketed in São Paulo city. The objective of this study was to determine element (Br, Ca, Na, K, Fe, Rb, Se and Zn) content by Neutron Activation Analysis in the infant milk formulas consumed in São Paulo city and to compare with the printed labels.

Instrumental neutron activation analysis (INAA) was employed for the precise and accurate element determination, since it is a powerful method for the direct analysis of solid food samples without dissolution, thus eliminating the possibility of contamination. The technique also provides low detection limits for many inorganic constituents. Moreover, the quality control and quality assurance of the technique is also maintained through the use of certified reference materials (CRMs) [11].

2. EXPERIMENTAL

2.1 Sample Preparation

A survey was elaborated to verify the more frequently consumed infant milk formula by the local population. In this study seven milk formulas were analyzed, four being based-milk samples, two soy-based sample and one infant food.

Table 1 describes the milk formula samples analyzed with their codes used in this study. For each milk formula sample there is an adequate age range and these are also presented. Six samples are used as human milk substitute and one is an infant food.

All samples collected were in the powder form. The milk formula samples were coded and removed from their containers to the polyethylene vials, previously cleaned with deionized H_2O and 10% HNO_3 solution. The samples were stored then in a refrigerator at -4 $^{\circ}$C to maintain the product in adequate conditions.

2.2 Moisture Content Determination

For moisture content determination about 1 g of each sample was heated at 85 ^{0}C until its constant weight, using a Moisture analyzer MB45 – OHAUS Corporation, USA. The percentages of weight losses obtained varied from 1.17 to 2.93%.

Table 1. Description of the infant milk-formula samples

Sample Brands (codes)	Description	Adequate age
IF 1 -	Milk-based enriched with Fe	0 - 6 months
IF 2 -	Milk-based fortified with Fe	From 6 months on
IF 3 -	Soy-based with Fe	Infants
IF 4 -	Milk with Prebio enriched vitamin and Fe	From 1 year on
IF 5 -	Infant food enriched with vitamins and minerals	From 4 years on
IF 6 -	Soy-based with Fe	0 – 5 months
IF 7 -	Milk-based fortified with Fe	From 6 months on

2.3 Preparation of Br, Ca, Fe, Se, Na and Zn Standards

Standards of Br, Ca, Fe, Se, Na and Zn were prepared from appropriate dilutions of their Spex Certiprep stocks solutions. Aliquots (50 – 100 μL) taken from such solutions were pippeted on small sheets of Whatman 40 filter paper and dried under infrared lamp. After drying, these filter papers were placed into to clean polyethylene bags.

2.4 Neutron Irradiations

About 0.2 g of infant milk formula wheighed in polyethylene bags were irradiated together with element standards for 8 hours under a thermal neutron flux of 4.5 x 10^{12} n cm^{-2} s^{-1} at the IEA-R1 nuclear research reactor of IPEN/CNEN-SP.

After different decay periods, γ-ray activities of the irradiated infant milk formula and element standards were measured with a POP TOP model H Ge detector from EG&G ORTEC with 20% efficiency and 1.9 keV resolution at 1332.49 keV peak of ^{60}Co. The detector is coupled an electronic system composed of multi-channel analyzer, source of high tension, amplifier and a compatible microcomputer. The gamma-ray spectrum was analyzed using the VISPECT2 software. Table 2 shows the nuclear data of the radioisotopes used in this study.

Table 2. Nuclear data for the radioisotopes determined by INAA

Radionuclides used	Half-life	Gamma-ray used keV	Decay time	Counting time
^{82}Br	35.3 h	554.35	5 d	7200 s
^{47}Ca	4.54 d	1297.09	10 d	54000 s
^{59}Fe	44.5 d	1099.25	10 d	54000 s
^{24}Na	14.96 h	1368.60	5 d	7200 s
^{42}K	12.36 h	1524.58	5 d	7200 s
^{86}Rb	18.66 d	1076.60	10 -15 d	54000 s
^{75}Se	119.77 d	264.66	10 -15 d	54000 s
^{65}Zn	243.9 d	1115.55	10 - 15 d	54000 s

2. RESULTS AND DISCUSSION

Instrumental Neutron Activation Analysis (INAA) was applied to determine five essential elements (Ca, Fe, Na, K, Se and Zn) and two non essential (Rb and Br) trace elements in seven infant milk formula samples. To assess the accuracy and precision methodology two certified reference materials (CRMs) NIST-SRM-1549 Non Fat Milk Powder and NIST-RM-8435 Whole Milk Powder were also analyzed. Table 3 presents the experimental results with certified values for these elements. The values obtained for z score < 2 indicate that the results are considered satisfactory at 95% of confidence level [12]. Most of the results agree with the certified values, resulting in relatively good accuracy, except for selenium whose values were higher than that presented in the certificate

Table 3. Concentration of elements in NIST certified reference materials

Element	Non Fat Milk Powder (NIST-SRM 1549)			Whole Milk Powder (NIST-RM-8435)		
	This study (Mean ±SD) [a]	Values of Certified	z score	This study (Mean ±SD) [b]	Values of Certified	z score
Br mg/g	10 ± 1	(12)[c]	-	20 ± 2	20 ± 10	1.8
Ca mg/g	1222 ± 686	13000 ± 500	-1.6	9312 ± 911	9220 ± 490	0.19
Fe µg/g	1.8 ± 0.5	1.78 ± 0.1	0.20	2 ± 1	1.8 ± 1.1	0.18
Na mg/g	4943 ± 510	4970 ± 100	-0.27	2878 ± 268	3560 ± 400	-1.7
K mg/g	16699 ± 1653	16900 ± 300	-0.67	12692 ± 803	13630 ± 470	-2.0
Rb µg/g	10 ± 2	(11)	-	13 ± 1	(16)	-
Se ng/g	143 ± 2	110 ± 10	3.3	144 ± 122	131 ± 14	0.93
Zn µg/g	42 ± 6	46.1 ± 2.2	-1.9	26 ± 1	28 ± 3.1	-0.65

[a] Mean and Standard Deviation of eight determinations
[b] Mean and Standard Deviation of six determinations
[c] Values in parenthesis indicate informative values

The results of major and trace elements in infant milk formula samples consumed in São Paulo city are shown in Table 4 and Table 5, respectively. For a better insight the results are also presented in the Figure 1. Most formula samples analyzed in this study showed similar results for K and Se compared to the values calculated from label values. Some of them

presented high concentrations for Ca and Zn and low concentrations for Na. The most disagreement results were found for Fe in IF5, IF6 and IF7 samples and for Zn in IF7 samples. The label claims do not present values for Rb and Br elements

Table 4. Concentrations of major elements and label values in mg g^{-1} dried weight

Sample	Na		K		Ca	
	This study	Label values	This study	Label values	This study	Label values
IF 1	1.1 ± 0.1	1.2	4.4 ± 0.1	4.6	3.5 ± 0.2	3.2
IF 2	1.9 ± 0.2	2.0	5.9 ± 0.5	6.0	6.1 ± 0.6	5.8
IF 3	1.6 ± 0.1	1.8	5.3 ± 0.1	5.8	6.4 ± 0.2	5.3
IF 4	2.4 ± 0.1	2.6	8.6 ± 0.1	8.6	9.7 ± 0.5	8.3
IF 5	1.0 ± 0.2	1.3	2.8 ± 0.4	3.3	5.2 ± 0.9	4.1
IF 6	2.3 ± 0.1	2.1	5.5 ± 0.5	4.7	5.4 ± 0.4	3.9
IF 7	3.3 ± 0.2	3.0	7.8 ± 1.0	6.4	6.0 ± 0.6	5.0

Table 5. Concentrations of trace elements and label values in µg g^{-1} dried weight

Sample	Fe		Zn		Se		Br^b	Rb^b
	This study	Label values	This study	Label values	This study	Label values	This study	This study
IF 1	61 ± 3	62	44 ± 1	39	105 ± 1	100	3.5 ± 0.1	5.4 ± 0.1
IF 2	80 ± 5	82	58 ± 4	58	nd[a]	-	5.3 ± 0.3	14 ± 1
IF 3	69 ± 2	61	73 ± 1	61	134 ± 63	-	2.7 ± 0.1	0.4 ± 0.1
IF 4	65 ± 4	70	58 ± 1	59	112 ± 11	120	6.0 ± 0.3	22 ± 1
IF 5	266 ± 6	320	28 ± 1	31	252 ± 91	240	12 ± 1	1.0 ± 0.1
IF 6	102 ± 10	60	35 ± 1	35	46 ± 11	-	43 ± 1	2.9 ± 0.4
IF 7	94 ± 9	80	152 ± 5	70	nd	-	59 ± 1	2.7 ± 1.0

[a] Not detected [b] There are no data for Rb and Br in the labels

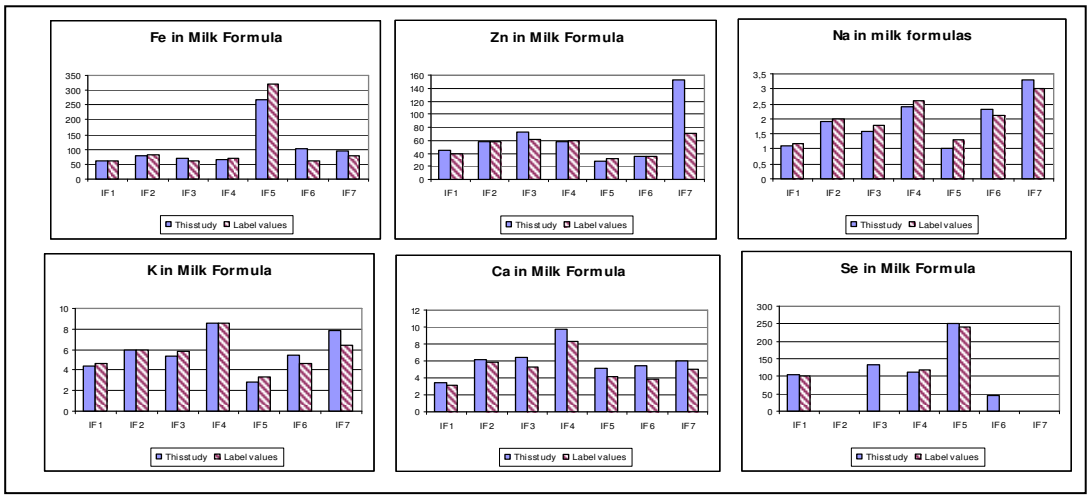

Figure 1: Histograms comparing the essential element results by INAA with label values

3. CONCLUSIONS

The results of this study showed that the most essential element levels in different infant formula marketed in São Paulo city were agreement with printed values. The difference between element levels found in this study and those presented in the labels was highest for the Fe. In conclusion the results showed that most of elements present in the formulas are very close or within the values stated in the labels. These preliminary data encourage further study for determine other essential elements and in a variety of milk formulas available on the market.

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