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NEUTRON ACTIVATION ANALYSIS APPLIED TO NUTRITIONAL AND FOODSTUFF STUDIES

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ABSTRACT

Neutron Activation Analysis, NAA, has been successfully used on a regularly basis in several areas of nutrition and foodstuffs. NAA has become an important and useful research tool due to the methodology's advantages. These include high accuracy, small quantities of samples and no chemical treatment. This technique allows the determination of important elements directly related to human health. NAA also provides data concerning essential and toxic concentrations in foodstuffs and specific diets. In this paper some studies in the area of nutrition which have been carried out at the Neutron Activation Laboratory of IPEN/CNEN-SP will be presented: a Brazilian Total Diet Study: Nutritional Element Dietary Intakes of São Paulo State Population; a study of trace element in maternal milk and the determination of essential trace elements in some edible mushrooms.

1. INTRODUCTION

Information about the nutritional status in individuals and populations is essential in order to initiate any necessary intervention. This information comes from evaluation measurements of nutrient requirements and studies of the uptake and bioavailability of mineral and vitamins [1-2]. For this reason, determination and quantification of nutrients in foodstuffs and diets are fundamental in order to achieve adequate dietetic planning.

Besides that, the necessity of healthy and good quality foodstuffs and diets requires the ability to detect the presence of possible contaminants, as well as, nutritional composition of the foodstuffs. In terms of health and nutritional safety, to know the levels of nutrients and/or toxic elements consumed by the population through foodstuffs has become of great importance.

Many analytical methods have been used in food and diet analysis in order to establish a great number of nutritional elements, with high sensitivity and accuracy. Neutron Activation Analysis, NAA, has been successfully used on a regularly basis in several areas of nutrition

and foodstuffs. NAA has become an important and useful research tool due to the methodology's advantages. These include high accuracy, small quantities of samples and no chemical treatment. This technique allows the determination of important elements directly related to human health. NAA also provides data concerning essential and toxic concentrations in foodstuffs and specific diets [3-5].

In this paper, some studies in the area of nutrition that have been carried out in the last 4 years at the Neutron Activation Laboratory of IPEN/CNEN-SP will be presented. A Brazilian Total Diet Study: Nutritional Element Dietary Intakes of São Paulo State Population; a study of trace element in colostrum milk and the determination of toxic and essential trace elements in some edible mushrooms. In these studies, INAA was used to determine the concentration of essential minor ant trace elements in foodstuffs.

In the Total Diet Study (TDS), trace element concentrations were determined in 24 of the most consumed food groups of a Market Basket of São Paulo State - Brazil. This Market Basket included 57 foods consumed more than 2g/day/person according to the National Household Food Budget Survey from July 2002 to June 2003, conducted by the Brazilian Institute for Geography and Statistics (IBGE). The foods were grouped in 24 food groups previously defined and the element contents were determined by INAA [6-7].

Deficiency of minor and trace elements can lead to various disorders in the early stages of child development. During early childhood trace element requirements, are more critical due to faster growth rates [8-9]. In this study, human colostrum samples from mothers of pre-term and term newborns were studied.

Mushrooms are excellent nutritional sources since they provide proteins, fibers and mineral, such as K, P, Fe. They have also been the focus of medical research [10-11]. In Brazil mushrooms are not consumed in large quantities by the general population since people know little about the nutritional and medicinal benefits that mushrooms offer. Hence, the study of mushrooms intends to contribute to a better understanding of the essential element content in edible mushrooms, which are currently commercialized in São Paulo state.

2- EXPERIMENTAL PART

2.1- Collection of samples:

2.1.1- Total diet samples:

The foods were collected in the restaurants of the University of São Paulo and kitchen preparation of foods was carried out in the COSEAS restaurant. The inedible portions (like bone, fruit peels) were discarded and the foods were prepared ready-to-consume. Foods of the same group were mixed in proportions based on available consumption data from the IBGE survey.

2.1.2.- Colostrum Milk samples:

Colustrum milk samples were collected from two mother groups; pre-term (15) and full term (15) babies after first and fourth day pos-partum.

Human colostrum samples (circa of 5 mL) were collected by manual expression from individual women directly into acid-rinsed 25-mL polyethylene bottles. The bottles were weight and frozen at -20^{0}C.

The colostrum milk study was approved by the Ethical Commission from the Clinical Hospital from the University of São Paulo, Brazil.

2.1.3 – Mushroom samples

Edible mushrooms were acquired in retail stores and directly from producers of various cities of São Paulo state. Eight fresh species were collected: Shitake (*Lentinus edodes*), Shimeji escuro (*Pleurotus ostreatus*), Shimeji branco (*Pleurotus flórida,)* Paris Champignon (*Agaricus bisporus*), Hiratake (*Pleurotus ssp*), Salmão Rosa (*Pleurotus salmoneostramineus),* Porto Bello *(Agaricus sp*) e Eringue (*Pleurotus Eryngü*). Only one sample (*A. bisporus*) was in conserve. About 400g were collected for each species. The mushroom samples were washed with Milli Q H_2O and cut in small pieces with plastic knife and put in Petri plates or plastic recipients.

2.2 – Sample Preparations:

To determine the minor and trace element concentrations in the food samples by Instrumental Neutron Activation Analysis, the samples should be preferentially in the dry powder form. In this way, the samples were then freeze-dried for 10 to 15 hours at 51 ^{o}C in the Thermo Electron Corporation (Modulyyo Model) freeze-dryer. After the freeze-drying process, the samples were ground and homogenized in a domestic blender with Ti blades. These samples were then stored in pre-cleaned polyethylene vials until analysis.

2.3.- Instrumental Neutron Activation Analysis

2.3.1- Preparation of element standards

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

2.3.2. Sample and Standard Irradiations

About 0.15 to 0.20 g of freeze-dried samples were irradiated with standards of the elements and food reference materials for 30 seconds and for 8 hours under thermal neutron flux of 5 x 10^{12} n cm^{-2} s^{-1} at the IEA-R1 nuclear research reactor of IPEN/CNEN-SP.

2.3.3. Gamma Spectrometry

After appropriate decay periods, γ-ray spectra of the samples, element standards and the reference materials were measured using two counting systems: Ortec EG&G and Canberra high resolution solid state Ge detectors with 20% efficiency and 1.9 keV resolution for

1332.49 keV peak of ^{60}Co. These detectors were coupled to an EG&G Ortec and Canberra card and associated electronics. Spectrum analysis was carried out using VISPECT 2 software.

3- RESULTS AND DISCUSSION

The validation of the analytical method applied was carried out by analysis of various food certified reference materials Mussel Tissue SRM 2976, Wheat Flour SRM 1567[a], Whole Milk Powder RM 8435 from the National Institute of Standards and Technology (NIST-USA) and Mixed Polish Herb (MPH-2) from the Institute of Nuclear Chemistry and Technology- (INCT- Poland). The results showed good agreement with the certified values as can be verified in Table 1.

Table 1: Element concentrations in the food reference materials by INAA

Elements	Mixed Polish Herb		Mussel Tissue		Wheat Flour		Whole Milk Powder	
	This study[a]	Certified Value	This study[a]	Certified Value	This study[a]	Certified Value	This study[a]	Certified Value
Br mg kg^{-1}	8.08±0.36	7.71±0.61	-	-	-	-	20±2	20±10
Ca %	1.10±0.10	1.08±0.07	-	-	nd	0.0191±0.0004	0.923±0.091	0.922±0.049
Cr µg kg^{-1}	1777±159	1690±130	524±41	500±160	-	-	nd	500[b]
Fe mg kg^{-1}	503±19	460[b]	172± 7	171.0±4.9	13±4	14.1±0.5	2±1	1.8±1.1
Na %[1]	0.0351±0.003	0.0350[b]	-	-	-	0.0061	0.288±0.27	0.35±0.40
K %	1.92 ±0.18	1.91 ±0.12	-	-	0.138±0.010	0.133±0.003	1.27±0.08	1.36±0.47
Se mg kg^{-1}	nd	-	2.12±0.12	1.80±0.15	1.13±0.26	1.1±0.2	nd	131±14
Zn mg kg^{-1}	32±2	33.5±2.1	143±7	137±13	12±2	11.6±0.4	25±2	28±3

[a] mean and standard deviation of 4 individual determinations
[b] informative value.
nd: not determined

In the Total diet study the Cr, Fe, Se and Zn concentrations were determined by INAA in the 24 food groups of the Market Basket. In Table 2 these results are presented.

As the data shows, sweets and breads, which are industrialized foods, showed the highest Cr concentrations. The food groups composed of vegetables showed the lowest concentration of this element. Iron concentrations in the food groups ranged from 0.05 mg kg^{-1} (alcoholic beverages) to 45 mg kg^{-1} (biscuits). The breads, flours and biscuits showed the highest Fe concentrations. It probably occurred due to iron-fortified wheat flour. Selenium was determined in 15 of the 24 analyzed food groups. Thus, in 9 food groups, the Se level was lower than the detection limit. Selenium concentrations ranged from 2.93 µg kg^{-1} (coffee) to

129 µg kg^{-1} (poultry and ready-made dishes). Food groups containing meats had the highest concentrations of Se, mainly the food groups based on poultry (poultry and ready-made dishes). Zinc concentrations ranged from 0.02 mg kg^{-1}(alcoholic beverages) to 88.6 mg $kg^{-1.}$ (standard grade beef). The food groups composed of meats had the highest Zn concentrations.

Table 2: Trace element concentrations in the food groups

Food Groups	Cr µg kg^{-1}	Fe mg kg^{-1}	Se µg kg^{-1}	Zn mg kg^{-1}
Cereals	8.41	0.84	nd	5.08
Leguminous	35.8	20.7	nd	10.1
Leafy vegetables	39.2	2.24	nd	1.62
Fruity vegetables	4.18	2.44	nd	1.32
Tuberous vegetables	6.47	3.85	nd	3.08
Tropical fruits	20.7	2.05	nd	1.35
Other fruits	13.6	1.21	nd	0.62
Flours	46.1	39.5	62.1	9.97
Pasta	18.1	19.9	12.8	2.17
Breads	203	44.3	20.6	10.4
Biscuits	85.1	45	25.4	9.5
Prime grade beef	55.4	25.1	97.9	50.5
Standard grade beef	99.4	31	74.4	88.6
Pork meat	106	17.7	99.2	10.9
Other meats	80.6	12.9	84.3	26.7
Poultry	53.9	7.42	129	18.7
Milk/cream	nd	0.30	11	3.53
Other dairy products	21.1	0.35	11.2	2.83
Sugars	43.8	1.39	14.9	0.25
Sweets	735	35.7	54.9	4.17
Sauces	57.1	7.30	nd	1.42
Alcoholic beverages	10.6	0.05	nd	0.02
Coffee	2.21	0.61	2.93	0.10
Ready-made dishes	26.4	7.51	129	16.1

nd: not determined

As it can be observed from Table 3, the mean concentrations of Fe, Mg, Na, Se and Zn were higher in the colostrum milk of mothers of pre-term babies. However, higher concentrations of Ca and K were found in the colostrum of full term mothers as compared of pre-term mothers.

Cluster Analysis was applied to verify the degree of similarity between the groups of colostrum samples analyzed and its resulted the formation of two different groups. In the first group G1, most of samples presented higher concentrations of Cl, Fe, Se and Zn, except for K that presented low concentration in this group. In the second group G2, showed the samples that presented lower concentration in Cl, Mg and Na. However, this group presented wide

range in the concentrations of all elements determined. In both groups, there are samples belonging from the pre-term and full term colostrum samples.

Table 3: Element concentrations in colostrum samples (mg mL^{-1})

Elements	Pré-term colostrum milk		Full-term colostrum milk	
	mean ±sd	range	mean ±sd	range
Ca	224 ± 77	130 - 345	244 ± 81	156 - 489
Cl	972 ± 260	638 - 1388	861 ± 266	552 - 1409
Fe	0.71 ± 0.51	0.15 - 1.81	0.52 ± 0.36	0.14 - 1.72
K	718 ± 258	289 - 983	651 ± 161	385 - 938
Mg	23 ± 14	4.2 - 53	17 ± 8	5.8 - 38
Mn	0.036 ± 0.047	<0.01 - 0.09	0.030 ± 0.020	<0.01 - 0.066
Na	799 ± 476	278 - 1748	632 ± 424	237 - 1726
Se	39 ± 25	14 - 85	18.9 ± 8.4	8.0 - 40
Zn	12 ± 9	4.4 - 33	8.0 ± 2.7	4.8 - 12.6

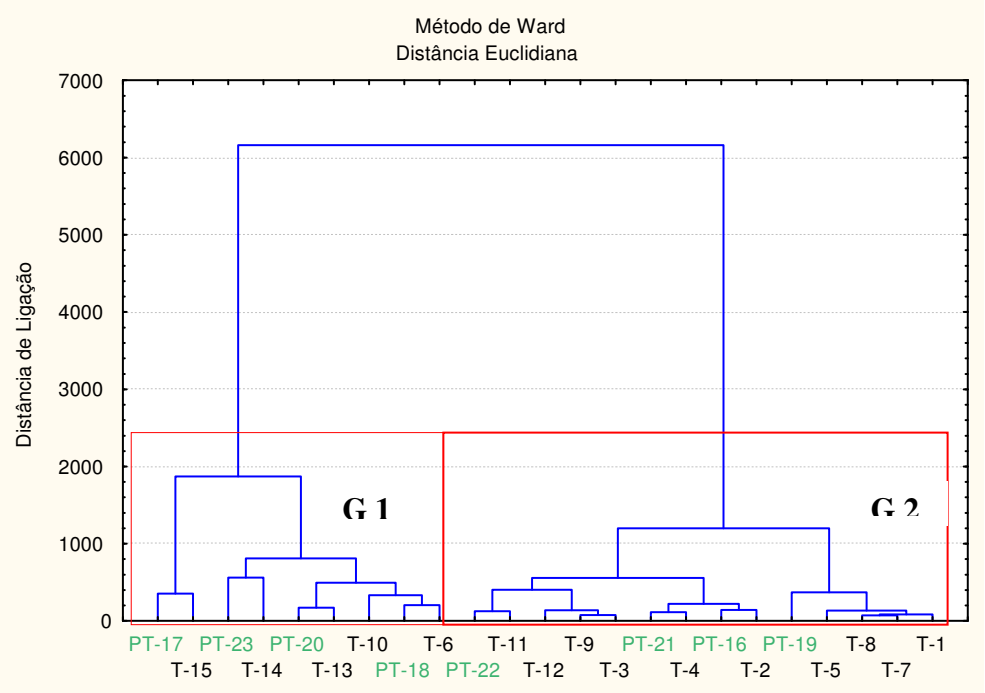

Figure.1 – Cluster analysis to the colostrum samples

Table 4 shows mean results for the elements Br, K, Na, Fe and Zn obtained of triplicate determinations in edible mushrooms. All results presented are related to the dry mass of the samples.

Large variability can be observed among mushroom species in relation to their Br, K, Na, Fe and Zn content. However, the specimens of the same species (*L.edodes*) collected in different

regions showed similar levels of the essential element. The difference is not greater than twofold for K and Na. For Br and Fe the levels are almost equal. The higher difference was obtained for Zn.

Table 4 Element concentrations in different samples of edible mushrooms (mg kg^{-1} dry weight.)

Mushrooms	City	Br	K	Na	Fe	Zn
Lentinus edodes	São Paulo	0.18 ± 0.02	17634 ± 336	74 ± 5	18 ± 2	85 ± 2
Lentinus edodes	Suzano	0.13 ± 0.01	14090 ± 3589	57 ± 4	21 ± 1	33 ± 2
Lentinus edodes	Mirandópolis	0.17 ± 0.01	14215 ± 1212	69 ± 10	22 ± 0.5	64 ± 2
Lentinus edodes	Juquitiba	0.18 ± 0.01	20674 ± 994	72 ± 8	22 ± 2	44 ± 4
Pleurotus almoneostramineus	São Paulo	1.80 ± 0.08	24054 ± 2261	17 ± 1	101 ± 2	119 ± 3
Agaricus bisporus	São Paulo	1.90 ± 0.06	21960 ± 377	2437 ± 35	101 ± 7	61 ± 4
Agaricus bisporus in conserve	São Paulo	0.92 ± 0.02	4631 ± 1032	5556 ± 520	130 ± 5	21 ± 2
Pleurotus flórida	São Paulo	0.31 ± 0.03	12343 ± 705	38 ± 1	93 ± 3	79 ± 5
Pleurotus ostreatus	São Paulo	0.50 ± 0.01	15117 ± 737	32 ± 2	76 ± 6	97 ± 8
Agaricus sp	São Paulo	2.7 ± 0.16	33857 ± 1499	311 ± 60	220 ± 7	63 ± 2
Pleurotus Eryngü	São Paulo	0.30 ± 0.02	20887 ± 321	132 ± 4	32 ± 0.3	78 ± 2
Pleurotus ssp	São Paulo	0.37 ± 0.05	17535 ± 1107	27 ± 1	122 ± 3	95 ± 5
Average		0.79	18083	735	80	70
Minimum		0.13	4631	17	18	21
Maximum value		2.7	33857	5556	220	119

In general, large variability can be observed among mushroom species in relation to their Br, K, Na, Fe and Zn content. The edible mushrooms analyzed presented high K and Zn content, confirming that mushrooms can be considered a good source for these essential elements. The low Na level is a good nutritional benefit for the consumer.

4. CONCLUSIONS

This paper presents the results obtained in three studies carried out at the LAN laboratory where Instrumental Neutron Activation Analysis was applied in the nutrition area. In this studies the methodology showed good precision and high accuracy and allowed to determine the essential elements at different levels in many kinds of foods, as showed in the Total diet study, where cereals, meats and oils samples were analyzed.

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