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ESSENTIAL ELEMENTS IN DIFFERENT TYPES OF EGGS BY NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Eggs are excellent sources of protein and provide essential nutrients to human nutrition. Neither the color of the shell nor that of the yolk affects the egg nutritive value, even though this value can vary according to the eggs from different poultries, and as well as according to the feed given to them. The egg consumption of the Brazilian southeastern population is 9.9 g/day, which represents 0.61% of the daily food consumption per capita. The objective of this study was to determine the concentrations of the elements Br, Ca, Cr, Cs, Fe, Na, Rb, Se, Sc and Zn in three types of eggs (hen, free-range and quail eggs). The edible parts of the eggs (egg white and egg yolks) were analyzed Hard-boiled and Raw. The Neutron Activation Analysis (INAA) was applied to determine the element concentrations. The samples were irradiated for approximately eight hours in flux of 4.5-5.5 10^{12} n cm^{-2} $s^{-}1$, at nuclear research reactor IEA-R1 of the Nuclear and Energy Research Institute, IPEN/CNEN-SP, São Paulo, Brazil. There was variation in the elements concentrations among the types of eggs and between Hard-boiled and Raw eggs.

1. INTRODUCTION

Eggs have well-known nutritional properties. They are considered a complete food source with essential components to human nutrition. Egg protein is the standard by which other protein sources are measured. Besides, they contain almost every essential vitamin and mineral needed by humans as well as several other beneficial food components. Neither the color of the shell nor that of the yolk affects the egg nutritive value, even though this value can vary according to the eggs from different poultries, and as well as according to the feed given to them. They are good source of iron and phosphorus and also supply calcium, copper, iodine, magnesium, manganese, potassium, sodium, zinc, chloride and sulphur, as well as white and yolk egg have been associated with antiadhesive, immunomodulatory, anticancer, and antioxidant properties [1-3].

The egg consumption for Brazilian southeastern population was evaluated by the Second Brazilian Total Diet Study (TDS). The food consumption data were obtained from the 2008-2009 Household Budget Survey by the Brazilian Institute of Geography and Statistics (IBGE) [4]. TDS is an approach to estimate the daily dietary intakes of the essential and toxic elements for a large-scale population over a specific period of time. TDS is based on the evaluation of food samples representing the typical diet for a population [5- 6]. In the 19 food

groups of the 2nd TDS, the eggs group presents the average consumption of 9.9 g/day, which represents 0.61% of the total weight of the foods consumed by this population [4].

In spite of low egg consumption by Brazilian population, information about their trace mineral composition is required [7], since chicken eggs are increasingly recognized as an important source of nutrients, including trace elements [8-9].

In this study, three types of eggs, hen eggs, free-range eggs and quail eggs, were analyzed in order to evaluate their element composition. The eggs were analyzed both in hard boiled and raw. The hard- boiled egg means the eggs are either boiled long enough for the egg white and then the egg yolk to solidify.

2. MATERIALS AND METHODS

2.1. Collection and preparation of samples

The egg samples were collected in supermarkets of São Paulo city. A dozen hen eggs, free-range eggs and quail eggs were acquired. The samples were divided in two groups, the half of them was analyzed raw, and another half was analyzed Hard-boiled. The whole egg was analyzed (yolk and egg white).

The Raw eggs were cleaned with deionized water and divided per type. For homogenization, polyethylene tools and bottles were utilized. The utensils were previously demineralized (were placed in Extran ® 10% for at least 12 hours, rinsed with deionized water and placed in a solution of acid nitric 10% for at least 12 hours, rinsed with deionized water and dried by air). The yolk and egg white were mixed and put in small portions in polyethylene bottles, and left in freezer for 24 hours

The Hard-boiled eggs were prepared per type, cooked in pyrex pan for ten minutes with boiling MilliQ water with no spices, oils and salt, in order to not alter the elements concentrations. After cooked, the shell was removed and the Hard-boiled egg was cut in small pieces, placed in polyethylene bottles previously demineralized and stored in a freezer for approximately 24 hours.

After that, the Raw and Hard-boiled eggs were freeze-dried at -50 ºC for about 16 hours and 49 μbar pressure, in a Modelo ModulyD freeze-dryer (Thermo Electron Corporation, Millford, USA).

After freeze-drying, the samples were ground in an agata mortar, then homogenized and sieved. The samples were stored in polyethylene bottles in a freezer until analysis.

2.2. Instrumental neutron activation analysis (INAA)

2.2.1. Standard preparation

Standards of Br, Ca, Cr, Cs, Fe, Na, Rb, Se, Sc and Zn were prepared from appropriate solutions certified from SPEX; the solutions were pipetted onto Whatman 40 filter paper

using Eppendorf pipettes (Eppendorf AG, Hamburg, Germany) and dried under an infrared lamp. After drying, the standards were transferred to polyethylene bags previously cleaned. The final mass of the standards were 1.24 µg of Br, 992.9 µg of Ca, 2.48 µg of Co, 2.49 µg of Cr, 0.123 µg of Cs, 493.8 µg of Fe, 125.5 µg of Na, 9.99 µg of Rb, 0.246 µg of Sc and 2.49 µg of Se.

2.2.2. Reference material

For quality control of the INAA method, reference material with similar characteristics of the samples was analyzed. The reference material used was SRM 8415 Whole Egg from NIST (National Institute of Standards & Technology). About 150-200 mg was weight in polyethylene bags previously cleaned.

2.2.3. Irradiation

About 150-200 mg of samples and reference material were weighted in polyethylene bags previously cleaned. Samples, reference material and standards were simultaneously submitted to a neutron flux of 4.5-5.5. 10^{12} n cm^{-2} s^{-1}, for 8 h at nuclear research reactor IEA-R1 of the Nuclear and Energy Research Institute, IPEN/CNEN-SP, São Paulo, Brazil.

2.2.4. Counting System

After appropriate decay periods, γ-ray spectra of eggs samples, reference material and element standards were measured using a High-purity Ge (HPGe) detector Model POP-TOP (EG&G Ortec, Oak Ridge, TN, USA). The detector was coupled to an EG&G Ortec and associated electronics. Spectrum analysis was carried out using VISPECT software, in TURBOBASIC
language

3. RESULTS AND DISCUSSION

3.1. Quality Assurance

In each irradiation one reference material was analyzed simultaneously for quality control. The certificate reference material SRM 8415 Whole Egg from NIST (National Institute of Standards & Technology) was analyzed. The results obtained were corrected considering the residual moisture. The results are shown in Table 1.

Table 1: Results for SRM 8415 Whole Egg reference material

Element	X ± SD[a]	RSD	|RE|	CV
Ca µg/g	2442 ± 62	2.6	1.5	2480 ± 190
Cr ng/g	389 ± 19	5.0	5.2	370 ± 18
Fe µg/g	110.21 ± 0.98	0.88	1.6	112 ± 16
Na µg/g	3548 ± 100	2.8	6.0	3770 ± 340
Se ng/g	1383.5 ± 6.2	0.45	0.47	1390 ± 170
Zn µg/g	64.3 ± 3.1	4.8	4.8	67.5 ± 7.6

[a]**:** Mean and standard deviation of three determinations **RSD:** Relative standard deviation
|**RE**|**:** Relative error **CV:** certificated values

3.2. Element Concentration

This study analyzed three types of eggs in both Hard-boiled and Raw. The mean and standard deviation results of Br, Ca, Cr, Cs, Fe, Na, Rb, Se, Sc and Zn were obtained in three determinations for each type of egg. The results of eggs in dried weight are shown in Table 2. The water loss during the freeze-drying process were obtained: 75.9% for Raw hen eggs, 75.7% for Hard-boiled hen eggs, 73.3% for Raw quail eggs, 72.8% Hard-boiled quail eggs, 74.5% for Raw free-range eggs and 75.8% Hard-boiled free-range eggs.

Table 2: Concentrations of elements in each type of egg in dried weight

Sample	Raw Quail Eggs	Hard-boiled Quail Eggs	Raw hen eggs	Hard-boiled hen eggs	Raw Free-ranged eggs	Hard-boiled Free-ranged eggs
Br mg/kg	5.82±0.44	4.34±0.34	4.54±0.36	4.06±0.47	5.31±0.17	4.96±0.61
Ca mg/kg	2000±90	2148±212	1384±62	1734±173	1784±82	1473±26
Cr µg/kg	<207	<153	<203	<185	<215	<164
Cs µg/kg	19.8±1.2	20.8±2.3	22.6±4.6	16.8±1.9	19.0±5.6	7.31±0.96
Fe mg/kg	102.8±8.3	95.4±7.0	66.2±4.0	66.1±3.5	94.1±20.9	71.8±4.9
Na- mg/kg	4336±76	3476±180	4949±334	4156±270	4759±215	4796±307
Rb mg/kg	11.99±0.48	10.93±0.13	8.76±0.25	5.46±0.34	10.05±0.71	8.76±0.35
Sc µg/kg	22.67±0.32	6.8±1.0	24.5±5.6	18.5±2.0	12.9±3.4	7.06±0.91
Se µg/kg	752±20	711±84	770±6	654±66	741±59	738±79
Zn mg/kg	53.4±2.3	55.0±4.8	42.8±5.0	48.6±3.4	53.6±8.4	45.4±3.8

The element concentrations *in natura* eggs and in 100g of the eggs are shown in Tables 3 and 4, respectively. The detection limits were calculated according to Currie criterion [9]. In all types of eggs the elements Cr was lower than the detection limit. Tukey test [10] was applied to verify if there are differences among the element concentrations determined in each type of analyzed eggs. Tukey test showed that the concentrations of the most elements determined were statistically different between the egg species and between hard-boiled and raw eggs ($p<0.05$). The Se and Zn concentrations were no statistically difference.

Table 3: Concentrations of elements in egg *in natura*

Sample	Raw Quail Eggs	Hard-boiled Quail Eggs	Raw hen-white eggs	Hard-boiled hen-white eggs	Raw Free-ranged eggs	Hard-boiled Free-ranged eggs
Br mg/kg	1.55 ± 0.12^{a}	1.18 ± 0.09^{b}	1.21 ± 0.10^{b}	0.99 ± 0.12^{b}	1.35 ± 0.04^{ab}	1.20 ± 0.15^{ab}
Ca mg/kg	534 ± 24^{ab}	584 ± 58^{a}	369 ± 17^{d}	421 ± 42^{bc}	455 ± 21^{bc}	355.5 ± 6.4^{cd}
Cr µg/kg	<55.3	<41.6	<54.2	<44.9	<54.8	<39.7
Cs µg/kg	5.31 ± 0.33^{a}	5.67 ± 0.65^{a}	6.05 ± 1.25^{a}	4.1 ± 0.47^{a}	4.85 ± 1.44^{a}	1.77 ± 0.23^{b}
Fe mg/kg	27.4 ± 2.2^{a}	26.0 ± 1.9^{ab}	17.7 ± 1.1^{d}	16.08 ± 0.85^{cd}	24.0 ± 5.4^{abc}	17.4 ± 1.2^{bcd}
Na mg/kg	1158 ± 20^{ab}	946 ± 49^{c}	1321 ± 89^{a}	1010 ± 66^{b}	1213 ± 55^{ab}	1161 ± 74^{ab}
Rb mg/kg	3.2 ± 0.13^{a}	2.97 ± 0.03^{ab}	2.34 ± 0.07^{c}	1.33 ± 0.08^{d}	2.56 ± 0.18^{bc}	2.12 ± 0.09^{c}
Sc µg/kg	6.05 ± 0.09^{a}	1.87 ± 0.27^{c}	6.56 ± 1.52^{a}	4.5 ± 0.5^{ab}	3.31 ± 0.88^{bc}	1.71 ± 0.22^{c}
Se µg/kg	200.8 ± 5.5^{a}	194 ± 23^{a}	206 ± 16^{a}	159 ± 16^{a}	189 ± 15^{a}	179 ± 19^{a}
Zn mg/kg	14.26 ± 0.62^{a}	15.0 ± 1.3^{a}	11.4 ± 1.3^{a}	11.81 ± 0.82^{a}	13.7 ± 2.1^{a}	10.98 ± 0.91^{a}

Mean values followed by same letter in line indicate no difference by Tukey test ($p<0.05$)

Table 4: Concentrations of elements in 100 grams of eggs

Concentration in mg/100g						
Sample	**Raw Quail Eggs**	**Hard-boiled Quail Eggs**	**Raw hen-white eggs**	**Hard-boiled hen-white eggs**	**Raw Free-ranged eggs**	**Hard-boiled Free-ranged eggs**
Br	0.155±0.012	0.118±0.009	0.121±0.010	0.099±0.012	0.135±0.004	0.120±0.015
Ca	53.4±2.4	58.4±5.8	36.9±1.6	42.1±4.2	45.5±2.1	35.65±0.64
Cr	<5.53	<4.16	<5.42	<4.49	<5.48	<3.97
Cs	0.53±0.03	0.57±0.07	0.61±0.12	0.41±0.05	0.49±0.14	0.18±0.02
Na	115.8±2.0	94.6±4.9	132.1±8.9	101.0±6.6	121.3±5.5	116.1±7.4
Rb	0.32±0.01	0.297±0.003	0.234±0.007	0.133±0.008	0.256±0.018	0.212±0.009
Sc	0.605±0.009	0.187±0.027	0.66±0.15	0.45±0.05	0.331±0.088	0.171±0.022
Se	20.08±0.55	19.3±2.3	20.6±1.6	15.9±1.6	18.9±1.5	17.9±1.9
Zn	1.43±0.06	1.50±0.13	1.14±0.13	1.18±0.08	1.37±0.21	1.10±0.09

Table 5 shows results obtained in this study were compared to the Brazilian Food Composition Table (TACO) [11]. Table presents result only for Ca, Fe, Na and Zn for hen eggs (raw and hard boiled) and raw quail. The results obtained in this study are in agreement with TACO, except for Ca in raw quail and Na for hen eggs, which values were lower than TACO values.

Table 5: Concentrations of Ca, Fe, Na and Zn for raw quail, raw hen and hard-boiled hen eggs in this study and TACO

Element	**Values in mg/100g of eggs**					
	Raw Quail Eggs		**Raw hen eggs**		**Hard-boiled hen eggs**	
	This study	**TACO**	**This study**	**TACO**	**This study**	**TACO**
Ca	53	79	37	42	42	49
Fe	2.7	3.3	1.8	1.6	1.6	1.5
Na	116	129	132	168	101	146
Zn	1.4	2.1	1.1	1.1	1.2	1.2

CONCLUSIONS

The Neutron Activation Analysis applied to determine the element content in three kinds of egg samples showed good precision and trueness. In this study were observed a little variation of the essential trace element content between raw and hard boiled eggs.

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