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NEUTRON ACTIVATION ANALYSIS CHARACTERIZATION PROCEDURES FOR FISH CONSUMED AT SÃO PAULO CITY

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ABSTRACT

The characterization of edible tissues of fishes consumed by humans is very important for determination of several toxic and potentially toxic elements, ensuring the food safety. The Instrumental Neutron Activation Analysis (INAA) comparative method allows the determination of several of these elements, as well as others, for example of nutritional character. This study is part of the International Atomic Energy Agency (IAEA) technical cooperation project of Latin America and Caribbean countries to ensure the quality of food and biomonitoring of contaminants in molluscs and fishes. Ten specimens of 5 of the most consumed fish in São Paulo city: whitemouth croaker (*Micropogonias Furnieri*), smooth weakfish (*Cynoscion learchus*), common snook (*Centropomus undecimalis*), Brazilian sardine (*Sardinella brasiliensis*) and bluefish (*Pomatomus Saltatrix*) were analyzed. Complete procedures for analysis, which includes purchase in the largest warehouse in Latin America, transport to the laboratory, storage, freeze-drying, milling, weighting and others preparations of the subsamples, and the short irradiation parameters for the determination of Br, Cl, K, Mn and Na are reported. Results obtained for macro and microelements are presented and are in agreement with analysis of oyster tissue and mussel tissue certified reference materials under the same irradiation conditions, with z-score values ranging from -3.0 to 2.2.

1. INTRODUCTION

Fish consumption is important in human feeding, because it contains vitamins, minerals [1] and amino acids [2]. The benefits of fish consumption are mostly associated with their high quality protein content and of two kinds of omega 3 poly unsaturated fatty acids: eicosapentaenoic acid (EPA) and docosahexanoic acid (HPA), which contributes to lower cholesterol levels [1, 3, 4, 5].

Despite the benefits of fish consumption and Brazil having an extensive coastline, the Brazilian population only reached the consumption recommended by World Health Organization (OMS) of 12 kg/year person, according the Ministry of Fisheries and Agriculture in 2013 [6].

The fishes that are commonly consumed by humans are relatively situated at the top of the aquatic food chain, and may accumulate inorganic contaminants from water, sediments and food [7, 8, 9]. Most chemical reactions that explain the toxicity of these contaminants at cellular level involve electron transfer, free radical formation, their influence in the DNA chain and competitions with the essential elements [10].

Considering the risks and benefits, several researchers in the area of food safety assurance [11, 12, 13] have produced studies in this area of fish evaluation.

This paper describes procedures for analysis of micro, macro and toxic elements in fish by the comparative INAA technique, in the framework of Technical Cooperation Project of the International Atomic Energy Agency (IAEA) for Latin American and Caribbean countries in assurance of food quality and biomonitoring of contaminants in shellfish and fish (Project Number RLA/5/504, ARCAL CIII) [14].

2. EXPERIMENTAL

This study reports from the purchase of fishes to the subsample preparation for irradiation, and the parameters for the determined elements by INAA.

2.1. Fish Sampling

Ten individuals of the species most commercialized at São Paulo city: whitemouth croaker (*Micropogonias Furnieri*), smooth weakfish (*Cynoscion learchus*), common snook (*Centropomus undecimalis*), Brazilian sardine (*Sardinella brasiliensis*) and bluefish (*Pomatomus saltatrix*), were purchase in CEAGESP, the largest warehouse of Latin America, being transported between layers of ice to the Neutron Activation Analysis Laboratory (IPEN - CNEN/SP). The fish remained in refrigerators until the time of their preparation.

2.1.1. Sample preparation

After the samples were purchased, they were weighed, washed with purified water (Milli-Q® 18.2 Ω), the tissues commonly edible by Brazilians were freeze-dried (Thermo Savant Modulyo D, Thermo Electron Co.), milled in blender adapted with titanium blades, homogenized (Homogenizer MA 201/30) and stored in decontaminated flasks. The residual humidity content was measured before the analysis by the oven drying method until constant mass was obtained.

2.2. Instrumental Neutron Activation Analysis (INAA)

Approximately 200 mg of the powdered samples were weighted in analytical balance (Shimadzu AEM-5200) in previously decontaminated 2 x 1.5 cm polyethylene bag (24 h in 10% v/v Merck HNO_3) and sealed (Selapack). A similar procedure was performed for two certified reference materials (CRM) *Perna perna* mussel [15] and NIST SRM 1566b, Oyster Tissue.

Elemental standard solutions (Spex Certiprep) were pipetted into filter papers strips (Whatman 40) using Eppendorf pipettes with previously checked nominal volumes. For some elements, it was necessary to dilute the standard solutions before pipetting them. The papers strips were dried at room temperature in a laminar flow hood then were folded and placed in polyethylene bags of the same sample size.

Each irradiation batch consisted of a sample or CRM and elemental standards. They were irradiated in the Nuclear Reactor IEA-R1 (IPEN - CNEN/SP) by the pneumatic station for 20 s under neutron flux of 1.9×10^{12} cm^{-2} s^{-1}.

After irradiation, it was possible to determine the elements Br, Cl, K, Mg, Mn and Na by gamma spectrometry of radionuclides of these elements, performed in a Canberra HPGe detector (model GC2018) coupled to Canberra DSA 1000 digital spectral analyzer. Further information about the radionuclides used for the element determinations is show in Table 1 [16].

Table 1: Parameters of radionuclides used in comparative INAA.

Element	Radionuclide	Energy (keV)	Half-Life
Bromine	^{80}Br	616.3	17.68 min
Chlorine	^{38}Cl	1642.7	37.24 min
Potassium	^{42}K	1524.6	12.360 h
Magnesium	^{27}Mg	843.3	9.458 min
Manganese	^{56}Mn	846.8	2.5785 h
Sodium	^{24}Na	1368.6	14.9590 h

Regarding the measuring conditions, standards of the radionuclide ^{27}Mg was measured for a period of 300 s, followed by ^{80}Br, ^{38}Cl, ^{42}K, ^{56}Mn and ^{24}Na for 1,800 s. Subsamples were measured twice, just after leaving the reactor for a period of 300 s, and after 2,700 s decay for 3,600 s. The calculation of mass fraction was performed using Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1. Quality Assurance

Results obtained experimentally by the comparative method of INAA for the *Perna perna* mussel and Oyster Tissue 1556b CRMs, their certified values and calculation of z-scores are presented in Table 2. The z-score was obtained using the modified Horvitz equation [17]. The Oyster Tissue CRM has no certified and reference value for Br. Values of z-score varied between ± 3, indicating that the comparative INAA method is adequate for determining the mass fraction of Br, Cl, K, Mg, Mn and Na in the fish samples.

Table 2: Mass fractions obtained by comparative INAA (mean values and expanded uncertainties, k = 2, dry weight, n = 5) and the certified values of reference materials.

Element	CRM			
	Mussel *Perna Perna*		Oyster Tissue 1556b	
	Obtained value (certified value)	z-score	Obtained value (certified value)	z-score
Br (mg kg^{-1})	245 ± 28 (250 ± 0.42)	-0.27	-	-
Cl (%)	3.82 ± 0.08 (3.62 ± 0.43)	1.5	0.523 ± 0.013 (0.514 ± 0.010)	0.35
K (%)	0.866 ± 0.088 (0.81 ± 0.11)	1.4	0.585 ± 0.032 (0.652 ± 0.009)	-2.4
Mg (%)	0.405 ± 0.031 (0.360 ± 0.043)	2.2	0.1083 ± 0.0092 (0.1085 ± 0.0023)	-0.03
Mn (mg kg^{-1})	23 ± 1 (23.4 ± 3.1)	-0.16	16.3 ± 0.4 (18.5 ± 0.2)	-1.2
Na (%)	2.243 ± 0.029 (2.27 ± 0.36)	-0.31	0.2840 ± 0.0040 (0.3297 ± 0.0053)	-3.0

3.2. Mass Fraction Determination in Fish Sample

Table 3 shows the mean mass fraction of Br, Cl, K, Mg, Mn and Na of ten specimens of each species.

Table 3: Element mass fraction in wet weight in fish samples (mean ± SD and range in parenthesis).

Element	Fish species				
	Cynoscion leiarchus	*Centropomus undecimalis*	*Micropogonias furnieri*	*Sardinella brasiliensis*	*Pomatomus saltatrix*
Br, mg kg^{-1}	7.0 ± 2.0 (4.1 – 11.0)	4.8 ± 1.3 (2.9 - 6.9)	4.18 ± 0.62 (3.10 - 5.11)	3.95 ± 0.59 (3.02 - 4.71)	5.86 ± 0.73 (4.74 - 6.75)
Cl, g kg^{-1}	1.59 ± 0.38 (0.938 - 2.29)	0.79 ± 0.17 (0.58 - 1.0)	0.946 ± 0.070 (0.834 - 1.05)	0.911 ± 0.090 (0.794 - 1.08)	1.06 ± 0.15 (0.782 - 1.24)
K, g kg^{-1}	3.20 ± 0.58 (2.65 - 4.63)	3.58 ± 0.35 (2.79 - 4.02)	3.54 ± 0.46 (2.91 - 4.81)	5.0 ± 1.5 (3.8 - 8.5)	6.70 ± 0.40 (5.99 - 7.16)
Mg, g kg^{-1}	0.253 ± 0.035 (0.198 - 0.300)	0.274 ± 0.041 (0.172 - 0.308)	0.225 ± 0.027 (0.182 - 0.272)	0.381 ± 0.054 (0.282 - 0.449)	0.426 ± 0.041 (0.366 - 0.479)
Mn, mg kg^{-1}	0.094 ± 0.025 (0.057 - 0.142)	0.064 ± 0.016 (0.037 - 0.083)	0.100 ± 0.038 (0.042 - 0.170)	0.64 ± 0.42 (0.34 - 1.7)	0.118 ± 0.035 (0.064 - 0.193)
Na, g kg^{-1}	1.08 ± 0.31 (0.805 - 1.57)	0.613 ± 0.067 (0.50 - 0.69)	0.688 ± 0.091 (0.596 - 0.835)	0.624 ± 0.071 (0.532 - 0.753)	0.85 ± 0.14 (0.67 - 1.1)

Similar results were obtained for the Br, K and Na elements in *Cynoscion leiarchus*, *Centropomus undecimalis*, *Micropogonias furnieri* and *Sardinella brasiliensis* analyzed by INAA in other study [18]. In both cases, the K mass fractions were the highest for the analyzed elements.

This paper is part of a study that is based on the analysis of toxic elements, and therefore on food safety. Results for micro and macro elements are presented because the technique used has multi-elemental character. Long irradiations will also be carried out in the already prepared samples and will allow the analysis of the toxic elements As and Zn, which are present in national and international legislations [19, 20, 21].

In order to analyze others toxic elements, Atomic Absorption Spectrometry (AAS) will be used, being As, Cd and Pb by Electrothermal Atomization (ET AAS) and Hg by Cold Vapor (CV AAS).

4. CONCLUSIONS

The procedure for the preparation of edible fish tissue for analysis by INAA was adequate, with z-score results of the used CRMs under the same irradiation condition with values between -3.0 and 2.2. Although food safety has been discussed in this paper, conclusions in this direction require further analyses, such as the obtained with long irradiation INAA, AAS and arsenic speciation, which will be carried out in the near future.

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REFERENCES

1. M. Al-Busaidi, P. Yesudhanon, S. Al-Mughairi, W. A. K. Al-Rahbi, K. S. Al-Harthy, N. A Al-Mazrooei, S. H. Al-Habsi, "Toxic metals in commercial marine fish in Oman with reference of national and international standards", *Chemosphere*, **85**, pp.67–73 (2011).
2. M. O Aremu, O. E. Ekunode, "Nutritional evaluation and functional properties of *Clarias lazera* (African Catfish) from river Tammah in Nasarawa State, Nigeria", *Am. J. Food Technol.,* **3**, pp.264–274 (2008).
3. C. Gonçález, M. Isabel, "Ácidos Grasos Omega 3: Beneficios y Fuentes, *Interciencia*, **27,** pp.128-136. (2002).
4. T. W. Clarkson, "The three modern faces of mercury"*, Environ. Health Perspect.,* **110,** pp.10–23 (2002).
5. J. L. Dominogo, A. Bocio, G. Flaco, J. M. Llobet, "Benefits and risks of fish consumption. Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants", *Toxicology,* **230**, pp.219–226 (2007).

6. “Consumo de pescado no Brasil aumenta 23,7% em dois anos”, http://www.brasil.gov.br/economia-e-emprego/2013/10/consumo-de-pescado-no-brasil-aumenta-23-7-em-dois-anos (2013).
7. F. Yilmaz, N. Ozdemir, A. Demirak, A. L. Tuna, “Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus*”, *Food Chem.*, **100**, pp.830–835 (2007).
8. S. Zhao, C. Feng, W. Quan, X. Chen, J. Niu, Z. Shen, “Role of living environments in the accumulation characteristics of heavy metals in fishes and crabs in the Yangtze River Estuary, China”, *Mar. Pollut. Bull.*, **64**, pp.1163–1171 (2012).
9. Kh. M. El-Moselhy, A. I. Othman, H. Abd El-Azem, M. E. A. El-Metwally, “Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt”, *Egypt. J. Basic Appl. Sci.*, **1**, pp.97–105 (2014).
10. B. Halliwell, J. M. C. Gutteridge, *Free Radicals in biology medicine*: Oxford, Oxford & UK, 1996.
11. O. Emily, A. L. Choi, M. R. Karagas, K. Mariën, C. M. Rheinberger, R. Schoeny, E. Sunderland, S. Korrick, “Which fish should I eat ? Perspectives influencing fish consumption choices, *Environ. Health Perpect.*, **120**, pp.790–798 (2012).
12. M. R. Neff, S. P. Bhavsar, F. J. Ni, D. O. Carpenter, K. Drouillard, A. T. Fisk, M. T. Arts, “Risk-benefit of consuming Lake Erie fish”, *Environ. Res.*, **134**, pp.57–65 (2014).
13. J. Hoekstra, A. Hart, H. Owen, M. Zeilmaker, B. Bokkers, B. Thorgilsson, H. Gunnlaugsdottir, “Fish, contaminants and human health: Quantifying and weighing benefits and riks, *Food Chem. Toxicol.*, **54**, pp.18–29 (2013).
14. “Technical Cooperation Report for 2012”, https://www.iaea.org/About/Policy/GC/GC57/GC57InfDocuments/English/gc57inf-4_en.pdf (2013).
15. “The Lund/LBLN Nuclear Data Search: Nuclide search”, http://nucleardata.nuclear.lu.se/toi/ (1998).
16. “Preparo e caracterização de um material de referência de mexilhão *Perna perna*”, http://www.teses.usp.br/teses/disponiveis/85/85131/tde-21062011-093245/pt-br.php (2010).
17. M. Thompson, “Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing”, *Analyst*, **125**, pp.385–386 (2000).
18. K. C. Fabiano, A. P. Lima, M. B. A. Vasconcellos, E. G. Moreira, “Contribution to food safety assurance of fish consumed at São Paulo city by means of trace element determination”, *J. Radioanal. Nucl. Chem.*, **309**, pp.383–388 (2016).
19. “Agência Nacional de Vigilância Sanitária (ANVISA). Resolução - RDC No. 42. de 29 de agosto de 2013. Dispõe sobre o Regulamento Técnico Mercosul sobre Limites Máximos de Contaminantes inorgânicos em alimentos”, http://portal.anvisa.gov.br/documents/33916/393845/RDC%2Bn%C2%BA%2B42_2013_final.pdf/eec629cf-8d17-422b-a362-366b275c1a00?version=1.0 (2013).
20. “Ministério da Saúde Fixa limites máximos de tolerância de contaminantes químicos em alimentos”, https://www.univates.br/unianalises/media/imagens/Anexo_XI_61948_11.pdf (1998).
21. “Maximum levels of contaminants and natural toxicants”, http://www.foodstandards.gov.au/code/Documents/Sched%2019%20Contaminant%20MLs%20v157.pdf (1991).