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Original Research Article

Determination of zinc concentrations in foods of animal origin, fish and shellfish from Croatia and assessment of their contribution to dietary intake

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ABSTRACT

Zinc concentrations were determined in a total of 121 samples of meat and meat products, fish and fish products and shellfish collected and marketed in Croatia. The concentrations were determined in the following ranges (mg/kg): meat 4.65–64.9, meat products 4.55–51.8, fish 3.12–19.5, fish products 12.3–31.2, mussels 21.1–30.9, oysters 129–431. The highest and lowest mean zinc levels determined were 222 mg/kg in oysters and 6.15 mg/kg in fish. Average zinc recovery of the method used was 100.4% with precision expressed as RSD of 1.5%. Significant differences were found among the tested food items. The estimated mean daily intake (EDI) of zinc in different types of food contributing to the recommended dietary allowance (RDA) for women and men were (%): meat 0.13–1.29 and 0.09–0.94; oysters 0.93 and 0.67, meat products < 0.5, both; fish, fish products and mussels < 0.15, both. The ranges of the average daily intake of zinc of the provisional maximum tolerable daily intakes (PMTDI) were (%): meat and oysters 1.05–10.3; meat products < 5; fish, fish products and mussels < 1. The results found in tested foods items were similar to results from other countries used for comparison.

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1. Introduction

Essential elements required for adequate physiological functions and available through dietary intake are copper, cobalt, iron, zinc, sodium, calcium, potassium, magnesium, manganese, selenium and chromium. The major source of human exposure to different elements is through the food chain. Minerals deficiencies in humans are widespread in the world and may play a negative role in children's development, pregnancy and elderly health (Grantham-McGregor and Ani, 2001; Black, 2003). Mineral elements persistent in the environment because they do not easily biodegrade, and can be considered chemical pollutants and potential food contaminants.

Zinc is an important trace element with multiple biochemical functions in biological systems. It is present in a large number of proteins, and also as a physiological constituent of the oxidant defence system and membrane-bound zinc with structural, regulatory and antioxidant functions (Scherz and Kirchhoff, 2006). In the human genome, zinc-binding proteins account for nearly half of the transcription regulatory proteins (Tupler et al., 2001).

Inadequate zinc absorption, zinc losses and induced zinc deficiency in humans causes disorders such as diarrhoea, cerebral function disturbances, growth retardation, declining immunological defence, eye and skin lesions, malfunctioning of wound healing and other skin diseases (Roth and Kirchgaessner, 1991). Zinc toxicity is reflected in both acute and chronic forms. Intakes of 150–450 mg per day lead to altered Fe function, reduced Cu status, reduced levels of HDL and reduced immune function (Hamilton et al., 2000).

The primary dietary sources of zinc are red meat, seafood, poultry, grains, dairy, legumes and vegetables (Biesalski, 2005). Furthermore, zinc from meat has a much greater bioavailability than zinc from vegetables, and other substances present in the diet

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may increase (citric acid, histidine, cystein) or decrease (phytate, oxalate) the absorption of zinc (Groff and Grooper, 2000). Meat and fish products are important parts of the human diet and provide trace and essential elements. Meat is widely consumed in the world due to its high protein content, and fish is also known to be low in saturated fat and to contain *n*-3 fatty acids that support good health (Ikem and Egiebor, 2006). Meat products and canned fishes are often consumed worldwide due to their convenience and affordability for many working families. Meat products such as sausages as fermented meat and pâté as minced and sterilized meat are popular products in Croatia.

Zinc is found in almost all type of foods and food products of animal and plant origin. The content of zinc in meat and meat products depends on the environmental conditions, type of pasture and genetic characteristics of organisms, and also on the technological treatments used in the production of meat products (Demirezen and Uruc, 2006). Accordingly, meat and particularly offal such as liver are important and good sources of zinc in human nutrition (WHO, 1996). Seafood consumption has a positive impact regarding high levels of *n*-3 fatty acids and positive impacts on bone mineral density (Amiard et al., 2008). Consumption of seafood can contribute to achieving the recommended levels of daily intake, especially in developing countries where there is a deficiency of zinc.

The aim of this study was the assessment of zinc concentrations in selected food items, i.e. in meat and meat products, fish, fish products and shellfish consumed on the Croatian market. The results were assessed through the calculation of dietary exposures and comparisons with the recommended dietary allowances (RDA) and maximum tolerable daily intakes (PMTDIs) recommended by the World Health Organization.

2. Materials and methods

2.1. Sampling

A total of 78 food samples of animal origin representing traditional meat and meat products were collected from farmers and meat industries from Croatia in spring/summer 2012. Samples included: muscle tissue of 7 bovine, 10 pigs, 5 poultry, 3 horses and 5 sheep; 6 pig kidneys and 42 samples of meat products (canned meat, sausages and pâté). *Longissimus dorsi* muscle was collected from all animals and breast muscle from poultry. The 23 fish samples (10 mackerel, *Scomber japonicus*; 8 red mullet, *Mullus surmuletus*; 5 picarel, *Spicara smaris*) were obtained from local fishers at fish markets in Croatian coastal cities. Five fish products from fishery industries in Croatia were also tested. Shellfish samples of 6 mussels (*Mytilus galloprovincialis*) were collected from a fish market on the Croatian coast. Nine samples of European flat oysters (*Ostrea edulis*) were obtained from shellfish production farms from the Mali Ston Bay in the south-eastern Adriatic Sea. Following collection, samples were stored in polyethylene bags, labelled and frozen at −18 °C prior to analysis. Analyses were performed within two months after sample collection.

2.2. Reagents

All reagents were of analytical reagent grade; HNO_3 (65%, v/v) and H_2O_2 (30%, v/v) were purchased from Kemika (Zagreb, Croatia). All solutions were prepared and diluted with ultra-pure water (18 MΩ cm) generated by the purification system NIRO VV UV UF 20 (Nirosta d.o.o. Water Technologies, Osijek, Croatia). Plastic and glassware were cleaned by soaking in diluted HNO_3 (1/9, v/v) and by subsequent rinsing with ultra-pure water and drying prior to use.

Standard stock solution (purity 99.9999%) containing 1000 mg/L of zinc was purchased from Perkin Elmer (Waltham, Massachusetts, USA) and used to prepare calibration standards. The stock solution and working standards were diluted in 0.2% (v/v) HNO_3. Calibration curve contained four calibration points were prepared at concentration levels 0.01 mg/L, 0.5 mg/L, 1 mg/L and 2 mg/L.

Certified Reference Material of dogfish muscle (DORM-3) was purchased from National Research Council (Ottawa, Canada).

Ultra pure grade carrier argon (Ar, 99.9995% pure) was supplied by UTP d.o.o. (Pula, Croatia).

2.3. Sample preparation

Muscle and fish samples (0.5 g) were digested with 4 mL of HNO_3 (65%, v/v) and 2 mL of H_2O_2 (30%, v/v) in a microwave oven. A blank digest was carried out in the same way. A high-pressure laboratory microwave oven Multiwave 3000 (Anton Paar, Ostfildern, Germany) was employed to perform the acid digestion of samples. The digestion program began at a potency of 500 W, then ramped for 1 min, after which samples were held for 4 min. The second step at a potency of 1000 W (ramp 5 min) was held for 5 min. The third step at a potency of 1400 W (ramp 5 min) was held for 10 min. A blank digest was carried out in the same way.

Digested samples were diluted to a final volume of 50 mL with ultra-pure water. All metal concentrations were determined on a wet weight basis as mg/kg. Detection limits were determined as the concentration corresponding to three times the standard deviation of twenty blanks. All specimens were run in batches that included blanks, a standard calibration curve, two spiked specimens and one duplicate. In order to check the method for accuracy and precision, certified reference material of dogfish muscle (DORM-3) was analysed for zinc. The reference material was treated and analysed under the same conditions as the samples. According to mean measured values ($n = 4$) of 51.5 mg/kg and certified values of 51.3 ± 3.1 mg/kg, average zinc recovery was 100.4%. The precision expressed as RSD% was 1.5%. Detection limits (mg/kg) of zinc in different food samples were: 0.0015 in muscle tissue, 0.0021 in fish muscle and 0.002 in meat products.

2.4. Instrumentation

An inductively coupled plasma optical emission spectrometer (ICP OES) with axial and radial viewing plasma configuration Model Optima 8000 (Perkin Elmer, Waltham, Massachusetts, USA) operating at a 40 MHz free-running ratio-frequency and provided with an S 10 autosampler (Perkin-Elmer) was utilized. The nebulization system was equipped with a chemical-resistant concentric glass nebulizer coupled to a glass cyclonic spray chamber. A torch with an alumina-made injector was used. The polychromator, equipped with an Echelle grating, had a spectral range of 160–900 nm and a resolution of 0.009 nm at 200 nm. The UV-sensitive dual backside illuminated Charge-Coupled Device (CCD) array detector was used. The CCD-array detector collects both the analyte spectra and nearby background spectra, allowing for simultaneous background correction and providing improved precision and sample throughput. The instrumental operating conditions used are shown in Table 1.

2.5. Calculation of estimated daily intake

The estimated daily intake (EDI) was calculated by the equation (Copat et al., 2013): EDI (mg/kg BW/day) = [(Zinc concentration; mg/kg) per (Meal size or Daily Intake of food; kg)] divided by [Adult body weights (60 kg)].

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