Copper content differences in meat products, fish and shellfish

N. Bilandžić[1], M. Đokić[2], M. Sedak[1], D. Sokolić-Mihalak[3], M. Jurković[2], A. Gross Bošković[2]

scientific paper

Summary

Copper concentrations were measured in a 65 samples marketed and collected in Croatia in summer 2012: meat samples (bovine, pig, sheep), meat products (sausages, pâté), fish and fish products and shellfish (mussels, oysters). Mean Cu levels were (mg/kg): all meat 0.77, sausages 0.69, pâté 2.24, fish 0.23, fish products 1.20, mussels 0.81, oysters 30.0. Significant differences were found between the foods groups. The estimated mean daily intake (EDI) of Cu in selected food contributing to the recommended dietary allowance (RDA) were (%): meat 1.54; sausages 1.38, pâté 4.48, fish, fish products and mussels < 1, oysters 10.0. The average daily intake of Cu represented % of the provisional maximum tolerable daily intakes (PMTDI) value were less than 0.9% in meat and meat products, less than 0.085% in fish, fish products and mussels and 2% for oysters. The highest Cu contents measured in oysters, suggesting that this species may be included in a diet as a good source of Cu. In conclusion, the analytical data obtained indicate there are no health risks from the consumption of the tested food items. In order to estimate sea contamination in Croatia, Cu levels in oyster samples should be investigated for different oyster farm locations.

Keywords: *Copper; Meat; Meat products; Fish and products; Shellfish; ICP-OES; Croatia*

Introduction

Essential trace elements such as Fe, Co, Cu, Mn, Ni and Zn need to be consumed in adequate amounts for normal physiological functioning of the human body (Nasreddine et al., 2010). Essential elements deficiencies in humans are widespread throughout the world and may play a negative role in children's development, pregnancy and elderly health (Grantham-McGregor and Ani, 2001; Black, 2003). Copper has multiple functions and a biochemical role in the promotion of health and optimising production and reproduction. These functions include iron utilisation, oxidation-reduction reactions, and a role as a cofactor for enzymes involved in glucose metabolism and synthesis of haemoglobin, connective tissue and phospholipids, and the maintenance of nervous system structure and function (Salgueiro et al., 2000; Nardi et al., 2009).

Copper deficiency may cause impaired energy production, abnormal glucose and cholesterol metabolism, increased oxidative damage, increased tissue iron content, altered structure and function of circulating blood and immune cells, abnormal neuropeptide synthesis and processing, and may increase the risk of developing coronary heart disease (Saari, 2000; Harris, 2003; Uriu-Adams and Keen, 2005). Similar to Cu deficiency, Cu toxicity can result in significant oxidative stress and subsequent tissue damage. Acute Cu toxicity can cause a number of pathologies resulting in abdominal pain, nausea, vomiting, headache, lethargy, diarrhoea, tachycardia, respiratory difficulties, haemolytic anaemia, gastrointestinal bleeding, liver and kidney failure and death. Chronic Cu toxicity can result in liver disease and severe neurological defects (Uriu-Adams and Keen, 2005).

Copper is found in almost all types of foods and food products of animal and plant origin. Factors affecting Cu levels in meat and meat products may be environmental conditions, type of pasture and genetic characteristics of the animals, and technological treatments used in the production of meat products (Demirezen and Uruc, 2006). Reports show that red meat is one of the major sources of minerals and provides the high bioavailable essential minerals in human diet (Santaella et al., 1997; Leblanc et al., 2005; Cabrera et al., 2010; Noël et al., 2012). Therefore a low meat intake, especially red meat, is recommended to avoid the risk of cancer, obesity and metabolic syndrome (Biesalski, 2005). Meat and meat products are widely consumed in the world due to their high protein content. Meat products are often consumed worldwide due to their convenience and affordability for most working families. Fermented meat, such as sausages and pâté as minced and sterilized meat, are also very popular products in Croatia. Fish is known for its low levels of saturated fat and high content of omega fatty acids which are known to support good health (Ikem and Egiebor, 2006). Although fish and seafood can contribute to achieving the recommended daily intake of essential elements, also may contribute to excessive exposure of environmental pollutants and toxic elements (Guérin et al., 2011).

---

[1] **Nina Bilandžić, PhD**, Scientific Advisor, **Maja Đokić, BSc**, **Marija Sedak, BSc,** Laboratory for residue determination, Department for Veterinary Public Health, Croatian Veterinary Institute, Zagreb

[2] **Darja Sokolić-Mihalak**, **Marina Jurković**, **Andrea Gross Bošković**, Croatian Food Agency, I. Gundulića 36b, Osijek, Croatia

Table 1 **Operating conditions for Optima 8000 ICP-OES.**

Parameter	Value
Plasma viewing mode	Axial and radial
Read time	5 s
Measurement replicates	3
Generator of radio frequency	40 MHz
RF incident power	1500 W
Plasma argon flow rate	8 L/min
Nebulizer argon flow rate	0.55 L/min
Auxiliary argon flow rate	0.4 L/min
Sample uptake rate	1 mL/min
Inner diameter of the torch injector	2.0 mm
Nebulizer type	concentric glass (Meinhard)
Spray chamber type	Glass cyclonic spray chamber

The aim of this study was to determine and compare Cu content in different foods of animal origin commonly consumed on the Croatian market, i.e. in meat and meat products and in fish and shellfish. Results were also compared with data from other countries.

Materials and methods

Sample collection

A total of 65 food samples of animal origin were collected in Croatian market places during summer 2012. Meat product samples included 12 pâté and 10 sausage samples. Meat samples (muscle *Longissimus dorsi)* were collected from: 6 beef, 5 pigs and 4 sheep. Fish samples (6 mackerel (*Scomber japonicus)* and 6 picarel (*Spicara smaris))* were obtained from fish markets in Croatian coastal cities. An addition three fish products were also obtained in marketplaces in the Croatian capital. Shellfish samples of four mussels (*Mytilus galloprovincialis)* were collected from a fish market on the Croatian coast. Oyster, 9 samples of European flat oyster (*Ostrea edulis)* were obtained from shellfish production farms in Mali Ston Bay on the south-eastern Adriatic Sea.

Following collection, samples were stored in polyethylene bags, labelled and frozen at -18°C prior to analysis.

Sample preparation

Reagents HNO_3 and HCl were analytical grade reagent (Kemika, Croatia). All solutions were prepared and dilluted 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 double deionised water and drying prior to use. Calibrations were prepared with Cu standard solutions of 1 g/l (Perkin Elmer, USA). The stock solution was diluted in HNO_3 (0.2%).

Meat and meat products, fish and shellfish 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 high-pressure laboratory microwave oven Multiwave 3000 (Anton Paar, Ostfildern, Germany) was employed to perform 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 minutes. The second step at a potency of 1000 W (ramp 5 min) was held for 5 minutes. The third step at a potency of 1400 W (ramp 5 min) was held for 10 minutes. A blank digest was carried out in the same way.

Digested samples were diluted to a final volume of 50 ml with double deionised water. Concentrations of Cu were determined on a wet weight basis as mg/kg. All samples were run in batches that included blanks, a standard calibration curve, two spiked specimens. Detection limits were determined as the concentration corresponding to three times the standard deviation of ten blanks. Data quality was checked by analysis of the recovery rate using certified reference materials: mussel tissue (ERM-CE278, IRMM, Belgium) and dogfish muscle (DORM-3, National Research Council, Canada)

Quantification of Cu

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 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 instrumental operating conditions used are shown in Table 1.

Determination of daily intake

The estimated daily intake (EDI) was calculated by the equation (Juan et al., 2010):

EDI = [(Mean of mg per kg of food) multiply by (Daily Intake of food)] divided by [Adult body weights (60 kg)].

Statistical analysis

Statistical analysis was performed using the Statistica 6.1 software (StatSoft^{Ò} Inc., Tulsa, USA). Concentrations were expressed as mean ± standard deviation, median, minimum and maximum values. One-way analysis of variance was used to test for differences in food Cu levels. In addition, differences between the Cu concentrations in different food items were analysed using the *t*-test. Results were considered significant at $p < 0.05$.

Results and Discussion

The results obtained in quality control checks of Cu concentrations were in good agreement with certified values. A reference sample of muscle tissue (ERM-CE278, IRMM, Belgium) was analyzed ($n = 5$) and the recovery was 99.3 ± 4.6%. A reference sample of dogfish muscle (DORM-3, National Research Council, Canada) was analyzed ($n = 5$) and the recovery was 101.9 ± 4.2%. Detection limits (mg/kg) of Cu in different food samples were: muscle tissue 0.0015, meat products 0.002, fish 0.0021.

Table 2 **Copper concentrations in meat and meat products, fish, fish products and shellfish.**

Food	N	Mean ± SD (mg/kg)	Median (mg/kg)	Minimum (mg/kg)	Maximum (mg/kg)
Meat					
Beef	6	0.78 ± 0.43 ^{f}	0.68	0.39	1.62
Pork	5	0.77 ± 0.22 ^{f}	0.74	0.52	1.09
Sheep	4	0.76 ± 0.12 ^{f}	0.75	0.63	0.91
All	15	0.77 ± 0.29 ^{abdf}	0.74	0.39	1.62
Meat products					
Sausages	10	0.69 ± 0.35 ^{af}	0.56	0.24	1.22
Pate	12	2.24 ± 2.48 ^{aaf}	1.43	0.41	7.58
All	22	1.54 ± 1.96 ^{f}	0.75	0.24	7.58
Fish and products					
Fish	12	0.23 ± 0.18 ^{bcdf}	0.18	0.01	0.59
Fish products	3	1.20 ± 1.14 ^{bcf}	1.19	0.064	2.35
Shellfish					
Mussel	4	0.81 ± 0.035 ^{f}	0.8105	0.774	0.868
Oyster	9	30.01 ± 9.31 ^{f}	26.9	19.6	42.9

Vertically, letters show statistically significant differences between samples: meat and meat products

^{a} ($P < 0.05$); fish and fish products, all meat and meat products ^{b} ($p < 0.001$), ^{c} ($p < 0.01$), ^{d} ($p < 0.05$);

oyster and all meat, meat products, fish, fish products, mussel ^{f} ($p < 0.001$)

Table 3 **Estimation of the daily intakes (EDIs) of an adult Croatian population to Cu and contribution to food recommended and toxicological nutritional reference values.**

Food	EDI^{a} (mg/kg/BW/day)	Contribution of mean to RDA^{b} (%)	Contribution of mean to PMTDI^{c} (%)
Meat	1.54	0.17	0.31
Meat products			
Sausages	1.38	0.15	0.28
Pate	4.48	0.50	0.89
Fish and products			
Sea fish	0.077	0.009	0.015
Fish products	0.40	0.044	0.080
Shellfish			
Mussel	0.27	0.03	0.054
Oyster	10.0	1.11	2

^{a} EDI was calculated by the equation: [(Mean of mg per kg of food) per (Daily Intake of food)] divided by [Adult bodyweights (60 kg)] (Juan et al., 2010)

^{b} RDA for Cu = 900 μg/day/person

^{c} PMTDI for Cu = 0.5 mg/kg/BW/day (FAO/WHO, 2007).

Mean Cu concentrations and ranges measured in meat and meat products, fish and fish products and shellfish are presented in Table 2. The concentrations obtained were in the range 0.01 mg/kg in fish to a maximal value of 42.9 mg/kg measured in oyster. Analysis of variance of Cu concentrations showed statistically significant differences between the tested food groups ($p < 0.01$).

The recommended dietary allowance (RDA) of Cu is 900 μg/day and the tolerable upper intake level (UL) is 10 μg/day for adults (Institute of Medicine, 2001). The World Health Organization (WHO) also determined as a risk assessment a provisional maximum tolerable daily intake (PMTDI) of 0.5 mg/kg BW/day (FAO/WHO, 2007). The upper tolerable intake level of Cu for children (1–3 years old) and males/females (19–70 years old) is 1 and 10 mg/kg, respectively (Institute of Medicine, 2001). In some countries, maximal permissible levels (MPL) for Cu in food have been determined: 20 mg/kg in fish in Turkey, 30 mg/kg in shellfish molluscs in Australia and 30 mg/kg (food category not specified) in Brazil and Malaysia (Amiard et al., 2008). However, in Croatia there is no national legislation regarding Cu levels in food and products of animal or plant origin.

Table 4 **Copper content (mg/kg) in different food items in other countries**

Food/ Country	Meat	Bovine meat	Pork meat	Sausage	Fish	Shellfish
Brasil	1.4 ^{a2}	0.8-0.9 ^{a1}	0.5-0.9 ^{a1}	0.8-1.0 ^{a1}	0.27 ^{a1} 0.32 ^{a2} 1.2-2.9 ^{a3}	
France	0.78 ^{b1} 0.804 ^{b2}				0.41 ^{b1} 0.686 ^{b2} 0.381 ^{b3}	7.05 ^{b1} 6.13 ^{b2} mussel 1.01 ^{b3} oyster 12.9 ^{b3} oyster 6.4, 78 ^{b4} oyster 13, 58 ^{b4}
Nigeria	1.6-3.8 ^{c}	0.89 ^{c}	2.87 ^{c}		1.0-3.33 ^{c}	
Spain		0.63-1.68 ^{d1} 1.66 ^{d2} 4.4 ^{d3}				
Turkey	0.94 ^{e1}			0.883 ^{e1}	1.1-2.5 ^{e2} 0.84-1.83 ^{e3} 0.32-6.48 ^{e4} 0.65-2.78 ^{e5} 0.34-7.05 ^{e6} 0.56-3.6 ^{e7}	
Lebanon	1.644 ^{f}				0.254 ^{f}	

^{a1} Ferreira et al. (2005); ^{a2} Nardi et al. (2009); ^{a3} Medeiros et al. (2012)

^{b1} Leblanc et al. (2005); ^{b2} Noël et al. (2012); ^{b3} Guérin et al. (2011); ^{b4} Amiard et al. (2008)

^{c} Onianwa et al. (2001)

^{d1} López Alonso et al. (2000); ^{d2} López Alonso et al. (2004); ^{d3} Sedki et al. (2003)

^{e1} Demirezen and Uruc (2006); ^{e2} Tuzen and Soylak (2007); ^{e3} Uluozlu et al. (2007); ^{e4} Türkmen et al. (2008) ;

^{e5} Tuzen (2009); ^{e6} Türkmen et al. (2009); ^{e7} Mendil et al. (2010)

^{f} Nasreddine et al. (2010)

In this study, the selected food items represented only a portion of food items used in the average daily intake of the average individual. In a previous study of dietary exposure to Cu, it was concluded that food groups that contributed the most to the Cu intake were breads and cereals (44.1%), followed by meat and poultry (13.3%) and vegetables (10.8%) (Nasreddine et al., 2010). Table 3 shows the estimated daily intake (EDI) of Cu based on the concentrations found in this study calculated by taking into account the recent published data for average consumption of food in Croatia (g/day per adult): 120 for meat and meat products, 50 for poultry meat; 20 for fish and fish (Antonić et al., 2007).

The estimated mean daily intakes of Cu in meat of species tested were 1.54 mg/day in meat, 1.38 mg/day in sausages and 4.48 mg/day in pâté, thus contributing only 0.17%, 0.15% and 0.5% of the RDA values, respectively (900 mg/day). For fish, fish products and mussel EDI values were 0.077 mg/day, 0.4 mg/day and 0.27 mg/day, respectively, contributing less than 0.045% of the RDA values. However, EDI values calculated for oysters were the highest, at 10 mg/day and contributing 1.11% of the RDA value. The average daily intake of Cu represented % of the PMTDI value (500 mg/kg BW/day) were less than 0.9% in meat and meat products, less than 0.085% for fish, fish products and mussels and 2% for oysters. The estimated daily intakes calculated suggested a low contribution of the tested food items for the dietary intake of this element. Therefore, there is no risk in accordance to the permissible tolerable limits.

Copper contents measured in beef, pork and sheep meats were similar and within the range 0.39 to 1.62 mg/kg. This is in agreement with literature values reported in meat of different species in Turkey and France (Leblanc et al., 2005; Demirezen and Uruc, 2006; Noël et al., 2012), but lower than results obtained in Brazil, Nigeria and Spain (Onianwa et al., 2001; Sedki et al., 2003; López Alonso et al., 2004; Nardi et al., 2009). Copper concentrations in different types of food presented in studies from other countries are presented in Table 4. Results obtained in this study were lower than those previously measured in Croatia (Bilandžić et al., 2012).

In previous studies conducted in different countries, Cu levels in beef meat ranged from 0.10 to 2.60 mg/kg in Venezuela (Huerta-Leidenz et al., 2003), from 0.4 to 0.9 mg/kg in Italy (Lombardi-Boccia et al., 2005), 0.77 mg/kg in Switzerland and 0.50 mg/kg in the USA (Gerber et al., 2009). The Cu levels in pork obtained in this study were 3.7-times lower than those found in Nigeria (Onianwa et al., 2001). Differences in Cu concentrations between different muscles has been presented in recent studies in cattle (Lombardi-Boccia et al., 2005; Cabrera et al., 2010; García-Vaquero et al., 2011). It was shown that the most active muscles with less fat content, i.e. diaphragm and cardiac muscles, have the highest essential and the lowest non-essential trace element accumulation in comparison with other muscles.

In the present study, the mean Cu levels measured in pâté were 3-times higher than the mean measured for all meat samples. Significantly higher Cu concentrations were found in meat products such as pâté than in meat ($p < 0.05$). However, significantly higher Cu levels were determined in meat and meat products than in fish ($p < 0.001$; $p < 0.05$).

In the scarce data regarding element levels in meat products such as sausage from Brazil and Turkey, similar Cu concentrations to those measured in the present study were determined (Ferreira, Gomes and Chaves, 2005; Demirezen and Uruc, 2006).

In this study, Cu contents in fish samples ranged from 0.01–0.59 mg/kg. These results are similar to results found in studies from Brazil and France (Leblanc et al., 2005; Nardi et al., 2009; Guerin et al., 2011; Noël et al., 2012), though higher levels have been reported in different studies from Turkey (Uluozlu et al., 2007; Türkmen et al., 2008; Tuzen, 2009; Mendil et al., 2010). This is explained by the fact that the seas around Turkey are highly industrialized (Demirezen and Uruc, 2006). The mean concentration of Cu in fish products in this study is about 5-times higher than in fish. These results were similar to canned fish products from Turkey, with Cu levels ranging from 1.1–2.5 mg/kg and 0.125–2.653 mg/kg (Tuzen and Soylak, 2007; Mol, 2011), but much lower than those in another study from Turkey with Cu ranges from 7.1–45.7 mg/kg measured in frozen anchovies (Celik and Oehlenschlager, 2007).

In the present study, Cu concentrations determined ranged from 0.774–0.868 mg/kg in mussels and 19.6–42.9 mg/kg in oysters. Significantly higher Cu levels were measured in oysters than in all meat, meat products, fish, fish products and mussels ($p < 0.001$, all). These findings indicate that oysters are a good source of dietary Cu and it is advisable to include it in diet as an alternative to meat. Results obtained for Cu concentrations in mussels were similar to those measured in France (Guerin et al., 2011). However, the Cu content measured in oysters was more than 2-times higher than values recently reported from France. Also, Cu levels measured in oysters were 130-times higher than those found in fish. Previous studies determined that the essential element content in molluscs and crustaceans is dependent on the contamination of the water at sampling sites, the coastal region or open sea (Schertz & Kirchhoff, 2006; Amiard et al., 2008). Variations in Cu concentrations with regard to sampling site contamination was found for the oyster species *Ostrea edulis* (coasts of Brittany and France) and *Crassostrea gigas* (French Atlantic coast) from non-contaminated and contaminated sampling sites. Differences in Cu concentrations in tissues at non-contaminated and contaminated locations were determined (mg/kg): *Ostrea edulis* 6.4 and 78; *Crassostrea gigas* 13 and 58 (Amiard et al., 2008). In the same study, the maximal concentrations of Cu were measured in the range 134–148 mg/kg in the oyster species *Saccostrea cucullata* from the rocky shores off Hong Kong (Amiard et al., 2008). The highest Cu contents measured in oysters, suggesting that this species may be included in a diet as a good source of Cu. In order to estimate sea contamination in Croatia, Cu levels in oyster samples should be investigated for different oyster farm locations.

In conclusion, the analytical data obtained indicate there are no health risks from the consumption of the tested food items.

Acknowledgements

We sincerely thank all those who contributed to the study. The authors are grateful to Marijana Fluka and Mirjana Hren for sample preparation and support.

References

Amiard, J.-C., C. Amiard-Triquet, L. Charbonnier, A. Mesnil, P. S. Rainbow, W.-X. Wang (2008): Bioaccessibility of essential and non-essential metals in commercial shellfish from Western Europe and Asia. Food Chem. Toxicol. 46, 2010–2022.

Antonić Degač, K., Z. Laido, A. Kaić-Rak (2007): Dietary and nutritional status characteristics of Croatian's population. Croat. J. Pub. Health. 3, 9.

Biesalski, H.-K. (2005): Meat as a component of a healthy diet – are there any risks or benefits if meat is avoided in the diet? Meat Sci. 70, 509–524.

Bilandžić, N., M. Đokić, M. Sedak, I. Varenina, B. Solomun Kolanović, D. Oraić, S. Zrnčić (2012): Determination of copper in food of animal origin and fish in Croatia. Food Control, 2012; 27: 284-288.

Black, M. (2003): Micronutrient deficiencies and cognitive functioning. J. Nutr. 133, 3927S–3931S.

Cabrera, M. C., A. Ramos, A. Saadoun, G. Brito (2010): Selenium, copper, zinc, iron and manganese content of seven meat cuts from Hereford and Braford steers fed pasture in Uruguay. Meat Sci. 84, 518–528.

Celik, U., J. Oehlenschlager (2007): High concentrations of cadmium, lead, zinc and copper in popular fishery products sold in Turkish supermarkets. Food Chem. 18, 258–261.

Demirezen, D., K. Uruc (2006): Comparative study of trace elements in certain fish, meat and meat products. Meat Sci. 74, 255–260.

FAO/WHO (2007): Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1956–2007) (first through 68th meetings). Food and Agriculture Organization of the United Nations and the World Health Organization, ILSI Press International Life Sciences Institute, Washington, DC. <http://jecfa.ilsi.org/> (accessed July 2012).

Ferreira, K. S., J. C. Gomes, J. B. P. Chaves (2005): Copper content of commonly consumed food in Brazil. Food Chem. 92, 29–32.

García-Vaquero, M., M. Miranda, J. L. Benedito, I. Blanco-Penedo, M. López-Alonso (2011): Effect of type of muscle and Cu supplementation on trace element concentrations in cattle meat. Food Chem. Toxicol. 49, 1443–1449.

Gerber, N., R. Brogioli, B. Hattendorf, M. R. Scheeder, C. Wenk, D. Günther (2009): Variability of selected trace elements of different meat cuts determined by ICP-MS and DRC-ICPMS. Animal 3, 166–172.

Grantham-McGregor, S. M., C. C. Ani (2001): The role of micronutrients in psychomotor and cognitive development. Brit. Med. Bull. 55, 511–527.

Guérin, T., R. Chekri, C. Vastel, V. Sirot, A., J.-L. Volatier, J.-C. Leblanc, L. Noël (2011): Determination of 20 trace elements in fish and other seafood from the French market. Food Chem. 127, 934–942.

Huerta-Leidenz, N., L. Arenas de Moreno, O. Moron-Fuenmayor, S. Uzcátegui-Bracho (2003): Composición mineral del músculo longissimus crudo derivado de canales bovinas producidas y clasificadas en Venezuela. Arch. Latinoam. Nutric. 53, 96–101.

Ikem, A., **N. O. Egiebor** (2006): Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama (United States of America). J. Food Compos. Anal. 18, 771–787.

Institute of Medicine (2001): Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. A Report of the Panel of Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Uses of Dietary Reference Intakes and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. National Academy Press, Washington, DC. 1–773.

Juan, C., J. C. Moltó, J. Mañes, G. Font (2010): Determination of macrolide and lincosamide antibiotics by pressurised liquid extraction and liquid chromatography-tandem mass spectrometry in meat and milk. Food Contr. 21, 1703–1709.

Leblanc, J.-C., T. Guérin, L. Noël, G. Calamassi-Tran, J.-L. Volatier, P. Verger (2005): Dietary exposure estimates of 18 elements from the 1st French Total Diet Study. Food Addit. Contam. 22, 624–641.

Lombardi-Boccia, G., S. Lanzi, A. Aguzzi (2005): Aspects of meat quality: trace elements and B vitamins in raw and cooked meats. J. Food Comp. Anal. 18, 39-46.

López Alonso, M., J. L. Benedito, M. Miranda, C. Castillo, J. Hernández, R. F. Shore (2000): Arsenic, lead, copper and zinc in cattle from Galicia, NW Spain. Sci. Total. Environ. 246, 237–248.

López Alonso, M., F. Prieto Montaña, M. Miranda, C. Castillo, J. Hernández, J. L. Benedito (2004): Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. BioMetals, 17, 389–397.

Medeiros, R. J., L. M. G. dos Santos, A. S. Freire, R. E. Santelli, A. M. C. B. Braga, T. M. Krauss, S. C. Jacob (2012) : Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil. Food Contr. 23, 535–541.

Mendil, D., Z. Demirci, M. Tuzen, M. Soylak (2010): Seasonal investigation of trace element contents in commercially valuable fish species

Unterschiede in Bezug auf Kupferkonzentration in Fleischerzeugnissen, Fischen und Muscheln

Zusammenfassung

Es wurden Kupferkonzentrationen (Cu) in 65 Mustern in Musterproben bzw. gekauft in Kroatien im Sommer 2012, bestimmt: Fleisch (Rinder, Schweine, Schafe), Fleischerzeugnisse (Würste, Pasteten), Fische und Fischerzeugnisse und Muscheln (Miesmuscheln, Austern). Mittlere Konzentrationen Cu (mg/kg) sind: alle Fleischmuster 0,77, Würste 0,69, Pastete 2,24, Fisch 0,23, Fischerzeugnisse 1,20, Muscheln 0,81, Austern 30,0. Es wurden statistisch bedeutende Unterschiede unter Nahrungsmittelgruppen festgestellt. Bewertete mittlere Tagesmengen der Einnahme (EDI) von Cu in der geprüften Nahrung, die der empfohlenen Einnahmemenge durch die Nahrung (RDA) beitragen, sind (%): 1,54 Fleisch, 1,38 Würste, 4,48 Pasteten, sowie < 1 Fisch, Fischerzeugnisse und Muscheln, 10,0 Austern. Durchschnittliche Tageseinnahmen von Cu ausgedrückt als % von vorläufig maximaler genehmigter Tageseinnahme (PMTDI) sind < 0,9 % für Fleisch und Fleischerzeugnisse, < 0,085 % für Fisch, Fischerzeugnisse und Miesmuscheln und 2 % für Austern. Die höchste Konzentration von Cu wurde in Austern bestimmt, was darauf hinweist, dass diese Sorte in die Nahrung als gute Quelle von Cu eingeschlossen werden kann. In der Schlussfolgerung zeigen die analytisch erzielten Resultate, dass keine wissenschaftlichen Risiken bestehen, die geprüften Nahrungsmittel zu konsumieren. Um Verunreinigungen des Meeres in Kroatien zu bewerten, soll der Inhalt von Cu in Austern aus verschiedenen Lokalitäten der Austerfarmen geprüft werden.

Schlüsselwörter: *Kupfer, Fleisch, Fleischerzeugnisse, Fische und Erzeugnisse, Muscheln, ICP-OES, Kroatien*

Differenze nelle concentrazioni di rame nei prodotti di carne, nel pesce e nei molluschi bivalvi

Sommario

Le concentrazioni di rame (Cu) sono state rilevate in 65 campioni campionati, o meglio acquistati, in Croazia nell'estate 2012: carne (bovina, suina, ovina), prodotti a base di carne trasformata (salsicce, pâté), pesce e prodotti a base di pesce trasformato e molluschi bivalvi (cozze, ostriche). Ecco le concentrazioni medie di Cu (mg/kg) rilevate: in tutti i campioni di carne 0,77, nelle salsicce 0,69, nel pâté di carne 2,24, nel pesce 0,23, nei prodotti a base di pesce trasformato 1,20, nei molluschi bivalvi (cozze) 0,81, nelle ostriche 30,0. Sono state accertate differenze statisticamente significative tra gruppi di alimenti. L'assunzione giornaliera stimata (EDI) di Cu negli alimenti esaminati che contribuiscono alla dose giornaliera consigliata (RDA) sono: 1,54 di carne; 1,38 di salsicce, 4,48 di pâté di carne e <1 per il pesce, i prodotti a base di pesce trasformato e i molluschi bivalvi (cozze), 10,0 di ostriche. I valori di assunzione media giornaliera di Cu, espressi come % della dose giornaliera massima tollerabile provvisoria (PMTDI), sono < 0,9% per la carne e i prodotti a base di carne trasformata, < 0,085 % per il pesce, i prodotti a base di pesce trasformato e le cozze, e il 2% per le ostriche. Nelle ostriche è stata riscontrata la maggior concentrazione di Cu, il che significa che quest'alimento potrebbe essere incluso nella dieta equilibrata come una buona fonte di Cu. In conclusione, i risultati analitici ottenuti mostrano che non sussistono rischi per la salute a seguito del consumo degli alimenti campionati e analizzati. Per esaminare il tasso d'inquinamento del mare in Croazia, è necessario, quindi, esaminare il contenuto di Cu nelle ostriche provenienti da impianti di acquacoltura ubicati in aree differenti.

Parole chiave: *rame; carne; prodotti a base di carne trasformata; pesce e prodotti a base di pesce; molluschi bivalvi; ICP-OES; Croazia*

from the Black Sea, Turkey. Food Chem. Toxicol. 48, 865–870.

Mol, S. (2001): Determination of trace metals in canned anchovies and canned rainbow trouts fish marketed in Turkey. Food Chem. Toxicol. 49, 348–351.

Nardi, E. P., F. S. Evangelista, L. Tormen, T. D. Saint´Pierre, A. J. Curtius, S. S. de Souza, F. Barbosa (2009): The use of inductively coupled plasma mass spectrometry (ICP-MS) for the determination of toxic and essential elements in different types of food samples. Food Chem. 112, 727–732.

Nasreddine, L., O. Nashalian, F. Naja, L. Itani, D. Parent-Massin, M. Nabhani-Zeidan, N. Healla (2010): Dietary exposure to essential and toxic trace elements from a total diet study in an adult Lebanese urban population. Food Chem. Toxicol. 48, 1262–1269.

Noël, L., R. Chekri, S. Millour, C. Vastel, A., Kadar, V. Sirot, J.-C. Leblanc, T. Guérin (2012): Li, Cr, Mn, Co, Ni, Cu, Zn, Se and Mo levels in foodstuffs from the 2nd French TDS. Food Chem. 132, 1502–1513.

Onianwa, P. C., A. O. Adeyemo, O. E. Idowu, E. E. Ogabiela (2001): Copper and zinc contents of Nigerian foods and estimates of the adult dietary intakes. Food Chem. 72, 89–95.

Saari, J. T. (2000): Copper deficiency and cardiovascular disease: role of peroxidation, glycation, and nitration. Can. J. Physiol. Pharm. 78, 848–855.

Salgueiro, M. J., M. Zubillaga, A. Lysionek, M. I. Sarabia, R. Caro, T. De Paoli (2000): Zinc as an essential micronutrient: A review. Nutr. Res. 20, 737–755.

Santaella, M., I. Martínez, G. Ros, M. J. Periago (1997): Assessment of the role of meat cut on the Fe, Zn, Cu, Ca and Mg content and their in vitro availability in homogenised weaning foods. Meat Sci. 45, 473–483.

Scherz, H., E. Kirchhoff (2006): Trace elements: in foods: Zinc contents of raw foods—A comparison of data originating from different geographical regions of the world. J. Food Comp. Anal. 19, 420–433.

Sedki, A., N. Lekouch, S. Gamon, A. Pineau (2003): Toxic and essential trace metals in muscle, liver and kidney of bovines from a polluted area of Morocco. Sci. Total. Environ. 317, 201–205.

Tuzen, M., M. Soylak (2007): Determination of trace metals in canned fish marketed in Turkey. Food Chem. 101, 1378–1382.

Tuzen, M. (2009): Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey. Food Chem. Toxicol. 47, 1785–1790.

Türkmen, M., A. Türkmen, Y. Tepe, A. Ates, K. Gokkus (2008): Determination of metal contamination in sea food from Marmara, Aegean and Mediterranean Sea: Twelve fish species. Food Chem. 108, 794–800.

Türkmen, M., A. Türkmen, Y. Tepe, Y. Töre, A. Ates (2009): Determination of metals in fish species from Aegean and Mediterranean Seas. Food Chem. 113, 233–237.

Uluozlu, O. D., M. Tuzen, D. Mendil, M. Soylak (2007): Trace metal content in nine species of fish from the Black and Aegean Seas, Turkey. Food Chem, 104, 835–840.

Uriu-Adams, J. Y., R. B. Rucker, J. F. Commisso, C. L. Keen (2005): Diabetes and dietary copper alter (67)Cu metabolism and oxidant defense in the rat. J. Nutrit. Biochem. 16, 312–320.,

Received: 26 July 2013
Accepted: 2 Sep 2013

Chemical evaluation of the quality of meat of broilers fed with the supplement from button mushroom, *Agaricus bisporus*

Mršić[1], G., M. J. Petek[1], S. Srečec[2], Ž. Cvrtila Fleck[3], M. Živković[4], K. Špiranec[5], D. Špoljarić[6],
D. Mihelić[5], L. Kozačinski[3], M. Popović[6]

short communication

Summary

The effect of added supplement from button mushroom, Agaricus bisporus of the quality of broilers meet was researched in this paper. Nutritional content of an animal feed is influenced not only by nutrient content but also by many other aspects such as, feed presentation, hygiene, digestibility, and effect on health. Usage of antibiotic growth promotors is abandoned in poultry production as well, it is neccesery to find alternative strategies for control and prevention of infections. Broilers buttlock and white meat from seven broilers from each experimental group were taken in order to examine the effect of addition of the supplement of white button mushroom to the controlled food intended for broilers on chemical quality of broilers meat using standard chemical methodes. Based on the percentages of water, protein, fat and ash in meat of broilers fed with food additive made of Agaricus bisporus we conclude that it is characterized by a low energy value, and as such can be considered as favorable dietary product, so called "light meat" intended for human consumption.

Key words: *broiler, Agaricus bisporus, light meat, antibiotic growth promotors*

Introduction

Danger of use of antibiotic growth promotors in food for animals and/or misuse of antibiotic growth promotors, led to ban of their use in European Union (Regulation EC No. 1831/2003). Consequences of the prohibition of the antibiotic growth promotors are lower usability of food, reduction of production feature and higher mortality and morbidity rate of animals. Therefore it is necessery to find alternative and sustainable methods of control of stress factors on animal health with apropriate feeding systems. So, today, when the usage of antibiotic growth promotors is abandoned in poultry production as well, it is neccesery to find alternative strategies for control and prevention of infections. However, alternative strategies could have a big impact on well being of animals raised for human consumption and therefore increase the risk of ailment of animals and people from various diseases. Connecting food with possible cause of diseases, developed so called "functional food" concept. Food is considered functional if its composition contains substances which in a positive way affect normal functioning of organism. Such substances are called food supplements, medicinal food or food for special medical purposes. Food for animals must ensure sufficient quantities of digestible nutrients without pathogens, protect animals from oxidative stress, minimize diseases outbreaks and maintain an effective immune system. Having that in mind the wider scientific community researched numerous types of mushrooms and proved their beneficial effects (Aida et al., 2009). Torus, mycelium and spores of mushrooms accumulate a series of bioactive metabolites with immunomodulatory, antiinflammatory, anticancer, antioxidant and antimicrobial effect (Hu et al., 2004, Špoljarić et al., 2011). In accordance with article 17. of regulation (EU) No. 1831/2003 on animal nutrition supplements, Commission founded Register of animal nutrition supplements, whereby recommending natural animal nutrition supple-

---

[1] **doc. dr. sc. Gordan Mršić**; **dr. sc. Maja Jelena Petek** Forensic Science Centre "Ivan Vučetić", Ministry of Interior, Ilica 335, Zagreb, Croatia
[2] **dr. sc. Siniša Srečec**, prof., Visoko gospodarsko učilište u Križevcima, Milislava Demerca 1, Križevci, Croatia
[3] **prof. dr. sc. Željka Cvrtila Fleck**; **prof. dr. sc. Lidija Kozačinski**, Department of Hygiene,Technology and Food safety, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, Croatia
[4] **Mario Živković, dr. med.**, **Farm Živković**, Kvarte 125, Perušić, Croatia
[5] **Katarina Špiranec, dr. med. vet.**; **prof. dr. sc. Damir Mihelić**; Department of Anatomy, Histology and Embriology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, Croatia
[6] **dr. sc. Daniel Špoljarić**; **prof. dr. sc. Maja Popović**; Department of Biology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, Croatia