The Influence of Cookware on the Concentration of Trace Metals and Lipid Peroxidation in Pork Muscle

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To assess cookware's influence on the concentration of essential and non-essential trace metals and on the lipid peroxidation process in pork muscle during heat treatment, meat samples were cooked without the addition of oil, salt or spices, at a temperature of 200°C ± 2°C for 50 minutes, in pans made of aluminium, ceramic-coated aluminium, brass and stainless steel. Fe, Cu, Zn, Mn, Sn, Ni, Cr, Ti, Al, Pb, Cd and U in samples were subsequently determined by optical emission spectrometry (OES). In order to assess the intensity of lipid peroxidation, peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were determined by colorimetric methods.

Key words: meat, trace elements, peroxide value, thiobarbituric acid reactive substances

The term *trace element* is used for an element occurring at levels lower than 0.01% (< 100 μg/g) [1]. Trace metals can be classified in essential trace metals, essential for a normal life (Fe, Zn, Cr, Co, Ni, Cu, Mn, Mo, Se) and non-essential trace metals [1]. Human diet is rich in essential trace metals but, due to industrialization, may also contain non-essential trace metals with toxic effects on the human body. The presence of essential trace metals above certain concentrations in food can have negative effects on both human health and the quality of foods. The negative effects on human health may be due to the fact that inadequate intake of any essential trace metal may result in specific biochemical lesions within cells of the body and development of characteristic clinical symptoms [1]. Most kitchen utensils used for food thermal cooking are made of different metals, alone or in combination. During heat preparation, cookware metals can leach into food.

In food systems, transition metals can act as catalysts of lipid peroxidation process. Thus, iron act as promoter of lipid oxidation in the presence of hydroperoxides. Iron, like other transition metal ions, is involved in one-electron redox reaction, which leads to hydroperoxide decomposition to generate alkoxyl and peroxyl radicals, which are initiators in chain reactions of the autoxidation [2, 3, 4]. Lipid oxidation products (e.g., 4-hydroxynonenal and malondialdehyde) ingested along with food represent a risk for body health. Chronic uptake of large amounts of such substances is reported to increase tumour frequency and incidence of atherosclerosis in animals [5].

This study aimed at assessing to what extent cookware made of aluminium, ceramic-coated aluminium, brass and stainless steel influence the concentration of some trace metals and lipid peroxidation in meat cooked at 200°C. To evaluate the effect of cookware on the concentration of trace metals and lipid peroxidation, the concentrations obtained for heat-treated meat were compared with concentrations found in raw meat.

Experimental part

Materials

Fresh pig meat was purchased from a farm around the city of Bucharest, Romania. The animal was slaughtered after electrical stunning and the meat was refrigerated at 4°C. The cookware was purchased from a local store (Bucharest), excepting the brass and aluminium pans, which were purchased from a craftsman.

Preparation of meat samples

At 24 hours after slaughtering, semimembranosus muscle was excised, external fat and epimysial connective tissue were removed and muscles were divided perpendicularly to their longitudinal axis into five equal fractions, and every fraction was cut into three even portions with an average weight of 250 – 260 g. The first fraction was heat-untreated and the other 4 parts were cooked in a preheated oven at 200°C in pans made of aluminium, ceramic-coated aluminium, brass and stainless steel. Meat samples were heat treated without the addition of oil, salt or spices, at a temperature of 200°C ± 2°C for 50 min; the temperature was controlled by a Eurotherm thermoregulator. After cooking, the meat was taken out of the oven and then allowed to cool to room temperature. Both raw and cooked meat samples were homogenised in a glass blender and each group was further analysed.

Chemical analysis

Determination of trace metals in meat

Drying of samples. Meat samples were weighed with an accuracy of 0.0001 g in weighing vials brought to constant weight, at a temperature of 105 ± 2°C, and then were dried in an oven at 105 ± 2°C for 8 h.

Calcination of samples. After drying, the samples were weighed with an accuracy of 0.0001 g in porcelain crucibles brought to constant mass. The crucibles were heated in the flame of a gas burner for two hours and then were placed in a furnace at 450°C for 48 h. The resulting ash was weighed on analytical balance and then subjected to disaggregation with HCl 37% diluted with bidistilled water 1:4 (v/v). After disaggregation, the samples were brought to 25 mL in volumetric flasks and then were used to determine the trace metals.

Determination of trace metals. Fe, Cu, Zn, Mn, Sn, Cr, Ni, Ti, Al, Cd and Pb were determined by Inductively-coupled argon plasma - Optical emission spectrometry (ICP-OES),

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using a spectroflame ICP model P (SPECTRO Analytical Instruments, U.S.A.). The determinations were performed in accordance with [6]. Uranium was assessed by Direct coupled plasma - Optical emission spectrometry (DCP-OES), using a SpectraSpan V (Beckman Instruments, U.S.A.) spectrometer and a standardized method was applied [7].

Determination of lipid oxidation parameters

Determination of peroxide value (PV). PV was determined according to the method of [8], modified by [9]. Ground sample (1.00 g) was homogenized with 11 mL of chloroform/methanol (2:1, v/v) mixture. The homogenate was filtered and 2 mL of 0.5% NaCl solution were added to 7 mL of the filtrate. The mixture was vortexed at a moderate speed for 30s and then centrifuged at 3,000×g for 3 min at 4°C, using a refrigerated centrifuge to separate the sample into two phases. The lower phase (3 mL) was carefully pipetted out, and 2 mL of cold chloroform/methanol (2:1) mixture were added. Then, 25 μL of 30% (w/v) ammonium thiocyanate and 25 μL of 20 mM iron (II) chloride were added to the mixture. The reaction mixture was allowed to stand for 20 min at room temperature prior to reading the absorbance at 500 nm. The blank sample was prepared in the same manner, except that chloroform/methanol mixture was used instead of sample extract. A standard curve was prepared using cumene hydroperoxide at concentrations ranging from 0.5 to 2 mg/L. PV was expressed as milligrams of cumene hydroperoxide per kilogram wet weight (mg CHP/kg WW).

Determination of 2-thiobarbituric acid reactive substances (TBARS) value. TBARS value of uncooked and cooked meat was measured using the method described by [10]. Wet sample (0.5 g) was mixed with 2.5 mL of TBA solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N HCl. The mixture was heated in boiling water for 10 min to develop a pink colour, cooled with running tap water, sonicated for 30 min, followed by centrifugation at 5,000×g at 25°C for 10 min. The absorbance of the supernatant was measured at 532 nm. Standard curve was prepared using 1,1,3,3-tetramethoxypropane at concentrations ranging from 0 to 10 ppm, and TBARS were expressed as milligram of malondialdehyde equivalents per kilogram wet weight (mg eq MDA/kg WW).

Statistical analysis

Analysis of variance was used to evaluate experimental data, and significant differences among means were determined by one-way analysis of variance (ANOVA) and Duncan's multiple range test (p = 0.05) (SPSS 10.0 for Windows).

Results and discussion

Determination of trace metals

The concentrations of trace metals as means ± standard deviation are shown in table 1.

In all cooked pork samples, the mean concentrations of trace metals Fe, Cu Zn, Mn, Sn, Ni, Cr, Ti, Al and Pb were increased compared to average levels for control (raw meat) and in some cases they significantly ($p<0.05$) depended on the pan type used for cooking.

The highest iron mean concentrations were registered in the meat cooked in brass pans (27.72 mg/kg DW), stainless steel pans (16.67 mg/kg DW), and ceramic-coated aluminium pans (16.64 mg/kg DW). Compared to Fe mean concentrations in raw meat (control), Fe average levels in meat cooked in brass pan were 3.13 times higher, and they were increased 1.88 times in meat cooked in stainless steel and ceramic-coated aluminium pans. The iron levels found in this study showed that meat thermal treatment at 200°C in ceramic-coated aluminium, brass, stainless steel and aluminium pans respectively determined the leaching of iron into the meat, but this fact was found as significant ($p<0.05$) only for brass pans. About 70% of the iron in mammals is found in haemoglobin, and about 5% to 10% is found in myoglobin. 25% of iron in

Table 1

MEAN CONCENTRATION OF TRACE METALS IN PORK (mg/kg, DRY WEIGHT) COOKED AT 200°C FOR 50 min IN PANS MADE OF ALUMINIUM, CERAMIC-COATED ALUMINIUM, BRASS AND STAINLESS STEEL

Trace metal	Control	Aluminium pan	Ceramic-coated aluminium pan	Brass pan	Stainless steel pan
Fe	8.86±0.58	11.00±3.60	16.64±2.93	27.72±9.25 a	16.67±2.90
Cu	0.09±0.02	1.73±0.39	2.44±0.58	13.58±2.93 a	1.69±0.50
Zn	21.78±5.70	37.66±8.97	38.02±5.34	63.92±13.36 a	36.06±9.25
Mn	0.09±0.02	0.95±0.13 a	0.16±0.05	0.28±0.07 a	0.15±0.03
Sn	0.11±0.04	0.64±0.18 a	0.48±0.05 a	0.50±0.26 a	0.41±0.11 a
Ni	0.06±0.02	0.71±0.29	2.24±0.58 a	1.19±0.59 a	0.97±0.37 a
Cr	0.08±0.02	0.15±0.03	0.18±0.05 a	0.26±0.05 a	0.16±0.03 a
Ti	0.027±0.00	0.20±0.04 a	0.25±0.07 a	0.40±0.08 a	0.33±0.05 a
Al	0.99±0.10	10.15±3.64 a	4.53±0.87	21.62±2.93 a	4.94±0.64 a
Pb	0.16±0.05	0.53±0.10	0.54±0.39	0.94±0.33 a	0.37±0.10
Cd	ND	ND	0.07±0.01	ND	0.06±0.01
U	ND	ND	0.026 a ±0.001	ND	ND

a – significant differences ($p<0.05$)

ND – not determined (below the limit of detection)

Values are shown as means ± standard deviation of 5 determinations the body is stored in hemosiderin, ferritin, and transferrin in the liver, spleen, and bone marrow [11].

Also, there are some nonheme iron-containing enzymes such as peroxidases, catalases and cytochrome-*c*. Iron excess is associated to hepatic injury, fibrosis and ultimately cirrhosis. Iron can initiate lipid peroxidation process by two different iron-mechanisms. First is Fenton reaction, iron forming with hydrogen peroxide the hydroxil radical (HO•), the most reactive oxygen species, which eventually initiates lipid peroxidation. In the second mechanism, iron forms with oxygen iron-oxygen complexes such as perferryl or ferryl ions with reactivities approaching to that of HO•. Lipid peroxidation produces damages in lipid membranes of cellular organelles resulting in structural and functional alterations of cell integrity [12].

Copper mean concentration in meat cooked in brass pan was 13.58 mg/kg DW, 150.89 times higher than Cu average level in raw meat. In meat cooked in ceramic-coated aluminium pans, Cu mean concentration was 2.44 mg/kg DW (27.11 times higher compared to its average level in raw meat samples), while Cu mean concentrations in meat cooked in stainless steel and aluminium pans were 1.69 and 1.73 mg/kg DW respectively (approximately 19 times higher than the values found for raw meat). The copper levels determined in this study showed that cookware type significantly influenced ($p<0.05$) the leach of copper into the meat, but this aspect was significant ($p<0.05$) only in case of brass pans. Cu is an essential trace element present in all tissues and it is needed for cellular respiration, peptide amidation, neurotransmitter biosynthesis, pigments formation and connective tissue strength [13]. Copper is a functional component of several essential enzymes, known as copper enzymes, and plays an important role in central nervous system development [13, 14]. Nevertheless, the other side of the coin is the prooxidant activity of copper in lipid oxidation. Like iron and other transition metals, Cu ions facilitate the transfer of electrons leading to increased rates of free radicals formation [15] and are effective in absorbing oxygen when added to meat [16].

Mean concentrations of zinc in cooked meat were significantly ($p<0.05$) dependent on the pan type used for cooking. In all cooked meat samples, Zn mean concentrations were increased compared to those registered in raw meat samples, and the values were significantly ($p<0.05$) higher only in case of meat samples cooked in brass pan. Zn is an essential trace element in the body and it is essential as a catalytic, structural and regulatory ion. It is involved in homeostasis, in immune responses, in oxidative stress, in apoptosis and in ageing [17]. Zn is involved in the activity of about 100 enzymes, e.g. RNA polymerase, carbonic anhydrase, Cu–Zn superoxide dismutase, angiotensin I converting enzyme. Also, it is present in Zn-fingers associated with DNA [18]. Zn toxicity has been highlighted in both acute and chronic forms; intakes of 150–450 mg of Zn per day have been associated with low Cu status, altered Fe function, reduced immune function, and reduced levels of HDL [19].

All cooked meat samples showed increased mean values of manganese compared to those found for the same element in raw meat samples, but the Mn mean concentrations were significantly ($p<0.05$) higher only in meat samples cooked in brass and aluminium pans. Mn is associated with bone development, and with amino acid, lipid, and carbohydrate metabolism [18] Manganese is component of a number of enzymes, as manganese containing superoxide dismutase Mn-SOD [20], and activates other enzymes (ex., glycosyl transferases) [21]. High doses of manganese are able to produce neurotoxicity, that may develop in human Parkinsonian syndrome [22, 23], cardiac dysfunction by blocking calcium channels [24], liver toxicity [25], fertility decreasing and increased of fetal abnormalities [26-29].

All cooked meat samples registered significantly ($p<0.05$) increased mean concentrations of tin compared to its average levels in raw meat samples. Sn is believed to be an essential trace element in some organisms, potentially including humans, although its function has not been exactly determined. It has been suggested that tin may contribute to tertiary structure of proteins and may participate in redox reactions in biological systems because the $Sn^{2+} \leftrightarrow Sn^{4+} + 2e^-$ potential of 0.13 volt is within the physiological range [30]. Inorganic tin salts are poorly absorbed (5%) and rapidly excreted in the faeces. Mutagenic studies on metallic tin and its compounds have shown fewer malignant tumours in animals exposed to tin than in controls [31].

Nickel mean concentrations in cooked meat significantly ($p<0.05$) depended on the pan type used for cooking. In all cooked meat samples, Ni mean concentrations were increased compared to its average levels found in raw meat samples, but the mean values were significantly ($p<0.05$) higher for meat samples cooked in ceramic-coated aluminium, brass and stainless steel pans. Nickel is considered a nutritionally essential trace metal for at least several animal species [32], but Ni overdoses may cause toxic effects in the respiratory tract and immune system. The exposure of the general population to nickel mainly concerned oral intake, primarily through water and food, as a contaminant in drinking water or as both a constituent and contaminant of food [32, 33].

The pan type used for cooking significantly ($p<0.05$) influenced chromium mean concentrations in cooked meat. All cooked meat samples showed increased mean values of Cr compared to those registered for the same element in raw meat samples, but its average levels were significantly ($p<0.05$) higher in meat samples cooked in ceramic-coated aluminium, brass and stainless steel pans. If chromium is an essential trace element is questionable. Some studies suggested that chromium is an essential trace element associated with carbohydrate metabolism, and chromium deficiency causes an impaired glucose tolerance [34]. Anyway, it was concluded that chromium is not an essential trace element [35], because feeding with a severely low-chromium diet (0.016 ìg/g) does not impair glucose tolerance, and the amount of Cr absorbed by humans is less than 1 ìg/day, which is much lower than the level for an essential trace element. Also, chromium intake seems to be dependent on chromium contamination during food processing and cooking.

All cooked meat samples presented significantly ($p<0.05$) increased mean concentrations of titanium compared to its average levels in control samples. The highest Ti average level was found in meat samples cooked in brass pan (0.40 mg/kg DW), and its lowest average value was found in meat samples cooked in aluminium pan (0.20 mg/kg DW).

Aluminium content varied from a mean value of 4.53 mg/kg DW in meat samples cooked in ceramic-coated aluminium pan to 21.62 mg/kg DW in meat samples cooked in brass pan. All cooked meat samples showed increased Al mean concentrations compared to its average levels determined in raw meat samples, but Al average levels were significantly ($p<0.05$) higher in meat samples cooked in brass, stainless steel and aluminium pans. In meat cooked in aluminium pan, Al mean concentration was 10.15 mg/kg DW, 10 times higher compared to raw meat (0.99 mg/kg DW). It was found that the leach of Al into meat was the most intense in case of brass pan, determining the increase of Al levels by 21.83 times. Other studies showed that aluminium is implicated in several serious conditions, such as dialysis dementia and osteodystrophy, amyotrophic lateral sclerosis, and Alzheimer's disease [36]. Previous researches demonstrated that aluminium foil used in cooking provides an easy channel for the metal to enter into the human body, the increase of cooking temperature causing more leaching. The leaching is also highly dependent on the *p*H value of the food solution, as well as salt and spices added to the food [37].

Lead mean concentrations in all cooked meat samples were increased compared to its average levels found in raw meat samples, but the only Pb average level significantly ($p<0.05$) increased was registered in case of meat samples cooked in brass pan (0.94 mg/kg DW, 5.88 times higher than control). Also, Pb mean concentrations for meat samples cooked in aluminium and ceramic-coated aluminium pans were 3.3 times higher compared control. Pb, as Cd, is a toxic metal with numerous negative effects on health such as neurotoxicity, carcinogenicity, reproductive toxicity and renal dysfunction [38, 39].

Cadmium mean concentrations in cooked meat (when detected) were higher than in raw meat samples (not detected), but they were not significantly ($p>0.05$) dependent on the pan type used for cooking. In case of meat samples cooked in ceramic-coated aluminium and stainless steel pans, Cd was detected in mean concentrations of 0.07 mg/kg DW and 0.06 mg/kg DW respectively. Cd is one of the most toxic elements to which humans can be exposed at work or in the environment [40]. Once absorbed, Cd is efficiently retained in the human body, in which it accumulates throughout life [41]. Cd is toxic for kidney, is responsible for bone damage and itai-itai disease, alteration of reproductive biology and cancer [41, 42].

The only sample in which uranium was detected and its mean value was significantly ($p<0.05$) increased was the meat cooked in ceramic-coated aluminium pan (0.026 mg/kg DW). In raw meat samples and in the other cooked meat samples, U was below the method detection limits. The leach of U into food from glass and ceramics was reported by [43]. From a uranium-glazed plate, vinegar could leach up to 31,800μg/L of uranium, and nitric acid leached 304,000 μg/L of uranium [43]. Uranium compounds were used in the colouring of ceramics and glass until the middle of the 20th century. Twenty percent of 15 uranium-glazed dinnerware samples tested contained easily removable surface compounds of natural uranium [44].

Evaluation of lipid oxidation process

Peroxide value

Lipid hydroperoxides are primary oxidation products that may break down to a variety of volatile and non-volatile secondary products. PV is an indicator of the initial stages of lipid oxidation. PV for the analysed samples as means ± standard deviation is shown in table 2.

The mean PV for raw meat was 1.271 mg CHP/kg WW, which indicates the presence of lipid peroxidation process during meat handling and refrigeration. The largest amount of lipid peroxides was found in meat cooked in ceramic-coated aluminium pan (1.974 mg CHP/kg WW). In the case meat heat-treated in other cookware, PV was lower than in uncooked meat, the lowest value being found in meat cooked brass pan (0.378 mg CHP/kg WW). Compared to uncooked meat (control), the concentration of lipid peroxides was significantly ($p<0.05$) higher in meat heat-treated in ceramic-coated aluminium pan and significantly ($p<0.05$) lower in meat heat-treated in the other cookware. For heat-treated meat, between PV and the concentration of Al a significant negative correlation was found ($r = -0.555$). Statistical correlations were not significant ($p>0.05$) between the concentration of the other metals and PV.

The lower PV found in meat heat-treated in the brass pan could be due to the higher thermal conductivity of brass, which causes a faster achievement of a temperature in meat which favours thermal decomposition of lipid peroxides. According to The Engineering Tool Box, thermal conductivity for brass is 64, for aluminium bronze is 44 and for stainless steel is 7 – 26 Btu/(hr^{0}F ft). The difference between PV for ceramic-coated aluminium pan and aluminium pan can be explained also by the difference in thermal conductivity, the ceramic-coated aluminium pan having a thickness of about 3 times higher, and the ceramic layer decreases thermal conductivity as well.

TBARS value

Thiobarbituric acid reactive substances (TBARS) are secondary oxidation products formed via the decomposition of primary lipid peroxidation products. TBARS value is an indicator of the second stages of lipid oxidation. Changes in TBARS value of meat cooked in aluminium, ceramic-coated aluminium, brass and

Table 2

PEROXIDE VALUE (PV) AND THIOBARBITURIC ACID REACTIVE SUBSTANCES VALUE (TBARS) IN MEAT COOKED AT 200°C FOR 50 MIN IN PANS MADE OF ALUMINIUM, CERAMIC-COATED ALUMINIUM, BRASS AND STAINLESS STEEL

Parameter	Control	Aluminium pan	Ceramic-coated aluminium pan	Brass pan	Stainless steel pan
PV (mg CHP/kg, wet weight)	1.271±0.369	0.609±0.072 a	1.974±0.439 a	0.378±0.120 a	0.679±0.269 a
TBARS (mg eq MDA/kg, wet weight)	0.210±0.065	0.468±0.610 a	0.406±0.612 a	0.764±0.314 a	0.555±0.036 a

a – significant differences ($p<0.05$)

Values are shown as means ± standard deviation of 5 determinations stainless steel pans are shown in Table 2. In all samples of cooked meat, TBARS values were higher than the one of uncooked meat. The lowest TBARS value was found in meat cooked in ceramic-coated aluminium pan (0.406 mg eq MDA/kg WW), while the highest TBARS value was found in meat cooked in brass pan (0.764 mg eq MDA/kg WW). Compared with heat-untreated meat, TBARS values were significantly ($p<0.05$) higher in cooked meat, regardless of the type of cookware used. TBARS value determined for meat cooked in brass pan was also significantly ($p<0.05$) higher than TBARS values determined for aluminium, ceramic-coated aluminium and stainless steel pans. Between TBARS values of cooked meat and Fe, Cu, Zn, Cr, Ti, Al and Pb concentrations were found significant correlations ($p<0.05$). TBARS values were significantly ($p<0.05$) and strongly positively correlated with concentrations of Fe ($r = 0.809$), Cu ($r = 0.814$), Zn ($r = 0.874$), Cr ($r = 0.842$), Ti ($r = 0.920$), Al ($r = 0.867$) and Pb ($r = 0.777$).

The results demonstrate that metal ions leaked from cookware favour lipid oxidation process. The meat cooked in brass pan showed the highest concentrations of Fe, Cu, Zn, Ti, Al and Pb, but also the highest concentration of secondary products of lipid peroxidation. Some researchers argued that lipid oxidation is enhanced by metals such as iron, cobalt and cooper which facilitate the transfer of electrons leading to increased rates of free radical formation [15]. It was suggested that lipid peroxidation in minced turkey muscle is primarily affected by *free* metal ions [45]. It was stated that free ionic iron plays an important role in the catalysis of lipid peroxidation and the status of ionic iron is more important than the total amount of iron [46].

Conclusions

The concentration of trace metals leaked from cookware into meat significantly depends on the type of cookware used. The highest leakage of metals was found in meat cooked in brass pan. Brass, stainless steel and aluminium pans caused significant ($p<0.05$) leakage of Al into the meat. The ceramic-coated aluminium pan caused significant ($p<0.05$) leakage of U into the meat.

Metal ions leaked from cookware favour lipid peroxidation process in meat. Between TBARS mean values found in heat-treated meat and the concentrations of Fe, Cu, Zn, Cr, Ti, Al and Pb, significant correlations were found.

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