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Detection of heavy metal residues in some cooked meat served at the restaurant level.

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ABSTRACT

Lead, cadmium, and copper, extremely hazardous heavy metals, provide a risk to human health in the form of acute, chronic, fatal, and sub-lethal toxicity because they cannot be eliminated or broken down by heat treatment or environmental deterioration. The purpose of the current study was to evaluate the lead, cadmium, and copper residues in meals and their acceptance for human consumption. Ninety random samples of boiled, roasted, and grilled meat (30 of each) were collected from a restaurant in Cairo governorate. The results showed that the mean values lead, cadmium and copper were 0.21 ± 0.01, 0.13 ± 0.01 and 1.96 ± 0.14 mg/kg in boiled meat, respectively, 0.27 ± 0.01, 0.18 ± 0.01 and 2.34 ± 0.16 mg/kg in roasted meat, respectively, and 0.38 ± 0.02, 0.25 ± 0.01 and 2.51 ± 0.22 mg/kg in grilled meat, respectively. The percentages of samples that exceeded the permissible limits recommended by EOS (2010) were 36.67 %, 43.33%, and 53.34% for lead in boiled, roasted, and grilled meat, respectively, for cadmium were 23.33%, 33.33% and 46.67%, respectively. The sample of cooked meat from the three types has no deviations in copper element (0%). We concluded that heavy metal residues presence is high in grilled meat, followed by roasted meat, and finally in boiled meat. We recommend excluding sources of heavy metal residues e.g., ready-to-eat food preparation. Moreover, cooking should take place inside restaurants away from lead gasoline pollution especially in locations with high traffic and avoid grilling meat over charcoal.

1. INTRODUCTION

Due to the traditional variety, affordable costs, adaptability, satiety value, and taste, meat, and meat products are essential components of many people's daily diets. The majority of meat's nutritional value comes from its high protein content. Additionally, the body can benefit from the valuable source of energy provided by animal fats. The full range of vitamin B, as well as key minerals like iron, phosphorus, and zinc, are frequently found in meat. Some of these vitamins, like vitamin B12, are exclusively present in animal-based diets.

Throughout their lives, people are exposed to chemical pollutants like antibiotics, hormones, pesticides, and heavy metals. The potential for environmental contaminants, especially heavy metals, to contaminate food, has increased as a result of technological advancements in food production and processing. Animals that consume these pollutants deposit residues in their flesh (Sabir et al., 2003). Heavy metals are common in the environment where individuals frequently consume them through food (Harmanescu et al., 2011). There is a chance that many pesticides will be utilized in the production of agricultural crop commodities, which could expose animals indirectly through feed and leave residues in animal products. Additionally, pesticides can be consumed by cattle through their food or water and may only be partially removed throughout the slaughter process (Mac Lachlan and Bhula, 2008).

Various manifestations of heavy metal poisoning may exist depending on its chemical form, tissue affinity, route of ingestion, dose, sex, and age whether exposure is short-term or long-term (Johri et al., 2010). Heavy metals have been linked to several clinical disorders, including cancer, and are known to accumulate poisons that can interfere with vital physiological processes in living cells.

Lead is a neurotoxic and metabolic poison that binds to a number of different cellular components, including vital enzymes and renders them inactive (Cunningham and Saigo, 1997). The oral symptoms of lead poisoning, however, include stomatitis with ulcers, a lead line of blue gingiva, and grey patches within the buccal mucosa (Bryson, 1989). As an accumulative toxin, lead has a hematological effect because it prevents hemoglobin formation and shortens the lifespan of erythrocytes so, Anemia could arise (Alberti and Fidainz, 2002). It has an impact on the nervous system and makes people irritable (Mert, 1987).

Cadmium raises AST, ALP, and ALT activity in the liver, as well as levels of urea and cholesterol. On the other hand, it lowers albumin and protein levels, causing significant damage to various organs such as the kidneys, testicles, and spleen (Rhman et al., 2011). Derivatives of cadmium such as cadmium sulfate, cadmium oxide, and cadmium chloride, on the other hand, are carcinogenic. Furthermore, cadmium chloride can disrupt fertility and injure the fetus (Court et al., 2011).

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In mice, ascorbic acid and glutathione levels are considerably changed as a result of copper's hepatotoxic effects. Additionally, because copper damages cell lining, it can alter the amounts of glutathione and malonaldehyde. Due to its effects on hepatic cells with granular damage, necrosis, and dysfunction, copper sulphate can harm the liver. According to Oguz et al. (2010), copper is quite toxic and has a detrimental impact on everything alive. However, as a supplement, copper gluconate is added to newborn meals. Chromium copper arsenate (CCA), which is also employed as a protective coating for wood-building materials, has been linked to kidney injury, necrosis, and malfunction of the tubules (Rita et al., 2009).
In this research, we aimed to determine the presence of heavy metal (lead, cadmium, and copper) residues in meat meals cooked in different forms of cooking, such as boiled, roasted, and grilled, and compare the results with internationally permissible limits, and study the extent of the influence of the cooking method on the presence of such heavy metal residues.

2. MATERIAL AND METHODS

This study was approved by Institutional Animal Care and Use Committee, Faculty Of Veterinary Medicine, Benha University (Approved Number: BUFVTM 01-08-23)

2.1. Sample collection:
From a restaurant in the Cairo governorate, 90 random samples of meat meals - 30 each of boiled, roasted, and grilled meat were taken. The obtained samples were individually packaged in sterile polyethylene bags and transported without undue delay to the lab for analysis of their chemical purity.

2.2. Heavy metals determination:
The obtained samples were examined for lead, cadmium, and copper levels based on wet weight (mg/Kg).

2.3. Washing techniques (AOAC, 2006):
Cleaning the equipment is an essential first step in avoiding contamination with the tested elements. Glasses and vessels were properly cleaned with deionized water before being submerged in hot, diluted HNO_3 (10%) for 24 hours to guarantee that all the equipment was free of metals.Many times using deionized water, and dried. Following a two-hour soak in water and soap, the digestive vessels were cleaned with tap water several times. Three times: once with distilled water, once with a mixture of 10% HNO_3, 250 ml deionized water, and 80 ml hydrogen peroxide. Following a thorough cleaning with deionized water, all containers were placed in an incubator to dry naturally while being free of pollutants and dust.

2.4. Digestion step:
In a screw-capped tube, two grams of each sample were precisely macerated using a sharp scalpel before being digested in 10 ml of a digestion solution (60 ml of 65% nitric acid and 40 ml of 70% perchloric acid) (Deng et al., 2007).
The tubes were securely fastened, the contents were violently shaken, and they were allowed to stand in the ambient temperature for an entire night. Additionally, for four hours, the tubes were heated in a water bath., from 60 to 110 °C, to guarantee that the samples were completely digested. Every 30 minutes for the duration of the heating, the digesting tubes were violently shaken. To ensure that the substances were fully assimilated, after, warming to room temperature was allowed for the tubes., diluted with 1 ml deionized water (30%), and then heated in a water bath at 70 °C. At this stage, every organic matrix has been eliminated. Each tube was filled with 25 ml of deionized water before being filtered through Whitman filter paper No. 42. The Gathered filtrates were stored under ambient conditions, and their levels of cadmium, lead, and copper were identified. The test tubes were Pyrex glass and covered with polyethylene film.

2.5. Making standard and blank solutions:
The operator's manual for the atomic absorption spectrophotometer served as the foundation for the instrumentation protocols used for various studies. However, the same procedures and materials were employed in the preparation of blank and standard solutions as in wet digestion (Shibamoto and Bjeldanes, 2000). The blank solution was created using nitric acid and H_2O_2, it was filtered after being diluted with deionized water. To take into consideration any possible metal contamination of the substances, the blank's value was taken out of the final calculations. Additionally, to make the standard solutions, nitric acid in the ratio of 10 parts to 1-part H_2O_2 was utilized., which was then diluted with 25 parts deionized water to achieve varying strengths using pure certified metal standards.

2.6. Evaluation:
With the use of a flame atomic absorption spectrometer (VARIAN, Australia, model AA240 FS), After aspirating the digest, blanks, and standard solutions, the calcium concentrations were determined. The instrument's instructions state that it can operate as an auto sampler, a digital absorbance readout, and a concentration readout under the following situations:

Heavy metal state	Lead	Cadmium	Copper
Wave length of a lamp (nm)	283.3	228.8	324.8
Light flow (m/amp)	10	4	12
Rate of fuel flow	1.4	1.2	1.2
Used gas	Argon	Argon	A-AC*
Counting time (seconds)	4.0	4.0	4.0
Limit of detection (ppb)	8-40	0.2-0.8	0.5-1.0

A-AC*= Air/Acetylene

2.7. Calculation the amount of residual heavy metals:
Direct measurements of lead, cadmium, and copper absorbency were made using a digital scale., and the formula was used to calculate their concentration: C=R x (D/W). Where, C= heavy metal concentration (wet weight). R= Digital scale reading of AAS. D= The prepared sample's dilution. W= sample Weight.
N.B. Additionally, each sample that was analyzed had its amount of each heavy metal in the blank solution calculated and subtracted.

2.8. Statistical Analysis:
Analysis of Variance (ANOVA) test was applied for statistical evaluation of the obtained results for each parameter according to Feldman et al. (2003). Differences in mean of analyzed data were considered significant at $P<0.01$.

3. RESULTS

The data shown in table (1) clearly showed that the lead contents in the tested samples of boiled meat, roasted meat and grilled meat varied from 0.02 to 0.42 with a mean value of 0.21 ± 0.01, 0.04 to 0.49 with a mean value of 0.27 ± 0.01, 0.07 to 0.63 with a mean value of 0.38 ± 0.02 mg / kg, respectively. The significant differences ($P<0.01$) as indicated.

Table 1 Statistical analysis of lead (mg/kg) in cooked meat at the restaurant level (n=30).

Food item	+ve samples		Min.	Max.	Mean ± S.E*
	No	%			
Boiled meat	15	50	0.02	0.42	0.21 + 0.01 [c]
Roasted meat	18	60	0.04	0.49	0.27 + 0.01 [b]
Grilled meat	19	63.33	0.07	0.63	0.38 + 0.02 [a]

The maximum permissible limit of lead in all three types of meat is 0.1 mg / kg according to Egyptian organization of standardization "EOS" (2010). So, based on the data in table (2) the percentage of unaccepted samples of boiled, roasted and grilled meat are 36.67 %, 43.33 %, 53.33 % respectively.

Table 2 Acceptability of cooked meat samples according to lead content (n=30).

Locality	Maximum Permissible Limit (mg/kg)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Boiled meat	0.1	19	63.33	11	36.67
Roasted meat	0.1	17	56.67	13	43.33
Grilled meat	0.1	14	46.67	16	53.33
Total (90)	0.1	50	55.56	40	44.45

* Egyptian Organization of Standardization "EOS" (2010)

The data shown in table (3) clearly show that the cadmium contents in the tested samples of boiled meat, roasted meat and grilled meat varied from 0.01 to 0.27 with a mean value of 0.13 ± 0.01, 0.01 to 0.32 with a mean value of 0.18 ± 0.01, 0.03 to 0.41 with a mean value of 0.25 ± 0.01 mg / kg, respectively. The significant differences ($P<0.01$) as indicated.

Table 3 Statistical analysis of cadmium (mg/kg) in cooked meat at the restaurant level (n=30).

Food item	+ve samples		Min.	Max.	Mean ± S.E*
	No	%			
Boiled meat	12	50	0.01	0.27	0.13 ± 0.01 [c]
Roasted meat	14	60	0.01	0.32	0.18 ± 0.01 [b]
Grilled meat	17	63.33	0.03	0.41	0.25 ± 0.01 [a]

The maximum permissible limit of cadmium in all three types of meat are 0.05 mg/kg according to Egyptian organization of standardization "EOS" (2010). So, based on the data in table (4) the percentage of unaccepted samples of boiled, roasted and grilled meat are 23.33 %, 33.33 %, 46.67 % respectively.

Table 4 Acceptability of cooked meat samples according to cadmium content (n=30).

Locality	Maximum Permissible Limit (mg/kg) *	Accepted samples		Un-Accepted samples	
		No.	%	No.	%
Boiled meat	0.05	23	76.67	7	23.33
Roasted meat	0.05	20	66.67	10	33.33
Grilled meat	0.05	16	53.33	14	46.67
Total (90)	0.05	59	65.56	31	34.45

* Egyptian Organization of Standardization "EOS" (2010)

The data shown in table (5) clearly showed that the copper contents in the tested samples of boiled meat, roasted meat and grilled meat varied from 0.68 to 3.51 with a mean value of 1.96 ± 0.14, 0.92 to 3.83 with a mean value of 2.34 ± 0.16, 1.35 to 4.26 with a mean value of 2.51 ± 0.22 mg/kg, respectively. The significant differences ($P<0.01$) as indicated.

Table 5 Statistical analysis of copper (mg/kg) in cooked meat at the restaurant level (n=30).

Food item	+ve samples		Min.	Max.	Mean ± S.E*
	No	%			
Boiled meat	30	100	0.68	3.51	1.96 ± 0.14 [c]
Roasted meat	30	100	0.92	3.83	2.34 ± 0.16 [b]
Grilled meat	30	100	1.35	4.26	2.51 ± 0.22 [a]

The maximum permissible limit of copper in all three types of meat is 20 mg / kg according to food stuffs cosmetics and disinfectant act (2002). So, based on the data in Table (6). All examined meat meal samples were accepted.

Table 6 Acceptability of cooked meat samples according to copper content (n=30).

Food item	Maximum Permissible Limit (mg/kg) *	Accepted samples		Un-Accepted samples	
		No.	%	No.	%
Boiled meat	20	30	100	0	0
Roasted meat	20	30	100	0	0
Grilled meat	20	30	100		0
Total (90)	20	90	100	0	0

* Food Stuffs Cosmetics and Disinfectant Act (2002)

4. DISCUSSION

Chemical compounds as heavy metals cannot be reduced or broken down by heat treatment., and they can cause a variety of health risks to humans, including acute, long-term, deadly, and sub-lethal toxicity (Shaltout et al., 2015). The environment is poisoned as a result of the negative effects of heavy metals (bioaccumulation and biomagnification in food chains), which directly affect public health (Hussain et al., 2012). Lead has been associated to reduce intellectual with reducing development in children as well as Adults with high blood pressure and cardiovascular disease. The high lead levels could be caused by airborne traces, water contamination, and tainted exhaust and effluents from moving cars, among other things (Shaheen *et al.,* 2005). Lead is predominantly eliminated through the kidneys but can also be eliminated through the digestive tract along with bile. Lead in blood has a half-life around 30 days. and in bone, which can account for up to 94% of the body's lead load, it is 20–30 years (Patocka and Cern, 2003). Lead is an extremely toxic element that can harm fetuses since it stays in pregnant women and mothers who breastfeed their children, having the same harmful effects on the central nervous system (Dora, 2004).

Table (1) showed that the average lead levels (mg/kg) in the tested meat meal samples were 0.21 ± 0.01, 0.27 ± 0.01 and 0.38 ± 0.02 for boiled meat, roasted meat and grilled meat, respectively. Also, the different subscript letters in the same column are significant ($P<0.01$).

According to the findings in Table (2), the unacceptable percentage samples of boiled meat, roasted meat and grilled meat based on their levels of lead were 36.67%, 43.33%, and 53.33%, respectively according to the Egyptian Organization of Standardization "EOS" (2010) which mandated that lead shall not have a maximum residual limit greater than 0.1 (mg/kg).

Cadmium poisoning can be seen in several ways. It can replace zinc and other metals in certain enzymatic reactions of the organism, interfering with some pathological processes like renal dysfunctions, hypertension, arteriosclerosis, growth inhibition, harm to the neurological system, bone demineralization, and disturbance of the endocrine system (Lafuente et al., 2004).

According to the findings in Table (3), the tested meat meal samples' mean cadmium levels (mg/kg) were 0.13 ± 0.01, 0.18 ± 0.01, and 0.25 ± 0.01 for boiled meat, roasted meat, and grilled meat, respectively. Additionally, there is significance (P0.01) for the various subscript letters within the same column.

According to the findings in Table (4), the unacceptable percentage samples of boiled meat, roasted meat, and grilled meat based on their levels of cadmium were 23.33%, 33.33%, and 46.67%, respectively according to the Egyptian Organization of Standardization "EOS" (2010) which mandated that cadmium's maximum permissible residual value is not higher than 0.05 (mg/kg).

Another necessary element known as a vital role in the biochemistry and physiology of living things is copper, which serves as an enzyme co-factor. Additionally, Cu is a crucial component for cellular respiration. Overexposure to Cu, however, may cause oxidative stress to the organelles of the cell (Darwish et al., 2014).

Table (5) showed that the average copper levels (mg/kg) in the tested meat meal samples were 1.96 ± 0.14, 2.34 ± 0.16, and 2.51 ± 0.22 for boiled meat, roasted meat, and grilled meat, respectively. Also, the different subscript letters in the same column are significant ($P<0.01$).

According to the findings in Table (6), all the examined samples were acceptable based on the Food Cosmetics, and Disinfectant Act (2002) which recommended keeping copper residue at a maximum of 20 milligrams per kilogram.

5. CONCLUSIONS

This study verified the presence of lead and cadmium in the highest percentage in grilled meat, followed by roasted meat, and boiled meat containing the lowest percentage, of these two elements, while all samples of the three types of meat were devoid of the presence of copper in them. It is better to prepare and eat meat meals at home to avoid exposure to the presence of heavy elements such as car exhausts and the surrounding environment, and this would reduce the percentage of presence and exposure to such harmful elements and the chance of their accumulation in the body. As a result, it is advised that regular monitoring of these items is crucial concerning any chemical dangers that could be harmful to human health.

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