



Influence of Cooking by Boiling on Lead and Cadmium in Meat and Liver of Chickens

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A B S T R A C T

Heavy metals are widely known as environmental pollutants due to their toxicity, heat stability, long staying in the atmosphere, and their capability to accumulate in the body. The current experiment investigates the concentration of lead (Pb) and cadmium (Cd), which are considered heavy metals in the liver and meat of fresh and imported frozen chicken, by studying the effect of cooking process using the boiling method. A total of 80 samples were randomly collected and examined: 40 frozen (10 from each of the liver and meat) before and after cooking) and 40 fresh (10 from each the liver and meat) before and after cooking) from different regions in AL-Karkh Province, Baghdad. Atomic Absorption Spectrometry device was used to evaluate the concentration of Pb and Cd. The results revealed that, in the concentration of Pb in the frozen cooked liver was significantly higher 321.8 ppm in comparison to fresh uncooked liver was 84.20 ppm. In contrast, meat samples indicated that neither cooking nor storage significantly affected the concentrations of Pb. The results also indicated that Cd concentration in meat tissue was significantly higher in fresh cooked (133.7 ppm) compared to frozen cooked (24.30 ppm). For correlation, a non-significant correlation between Pb and Cd concentration at the 0.05 level in liver and meat before and after cooking was recorded. The present study concluded that cooking by boiling method which is a traditional method that is used in Iraqi kitchens, can cause an increase in Pb, and Cd concentration. High concentrations of Pb or Cd in meat and liver may come from the environment, food consumed by chickens, cooking procedures, pans used, and sources of water may affect metal concentrations.

Keywords: chicken, meat, liver, cadmium, lead, cooking

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INTRODUCTION

Poultry products, like eggs, liver, gizzard, heart and meat, are all primary sources of energy, protein, minerals and vitamins worldwide. Poultry meat have lower fat and higher protein compare with red meat, so it helps the consumers to have a normal physiological function of the body organs. The prices of these products are affordable; also, they provide the daily recommended allowances of energy, trace minerals and proteins (1, 2). Heavy metals contamination is a worldwide food safety concern; the contamination can take place in a number of food products such as vegetables, fruits, and meat (3, 4).

However, poultry products may be exposed to contamination of heavy metals from the food chain or the environment via the inhalation of contaminated air or drinking contaminated water; as well as, contamination could also occur as a result of human activities like agricultural and industrial processes (2, 5). Furthermore, to formulate poultry feed, minerals with high concentrations are added to the poultry diet to achieve the bird's requirements and sometimes it becomes above the birds' needs. Also, the poultry diet, in lots of cases, contains fish or bone meals added to the diet because they have a higher nutritional value such as essential amino acid, vitamins, and minerals. Those meals could be a source of contamination

with heavy metals due to mining activity, water contamination, and effluent treatment activities in animal raising regions (6, 7). In nature, 35 metals are present such as iron and copper, about 23 of them have a high specific density that has an atomic weight of more than 40.04 amu, which is called heavy metals (8, 9). Lead (Pb), nickel (Ni), zinc (Zn), cobalt (Co), cadmium (Cd), copper (Cu), and others are heavy metals that are present in the biota. They are also called trace elements that have an essential role in the different metabolic processes of animals, microorganisms, and plants. These elements are important for different biochemical and physiological functions in the body; if they are not available in adequate amounts, they may cause various deficiency diseases. Meanwhile, if it exists in big doses, it may lead to toxicities (10, 11).

Cd is an uncommon metal that is rarely found in soil and water. In recent years, the production of Cd has risen because it is a major component of nickel-cadmium batteries and pigments. Soil and water could be contaminated with Cd from atmospheric pollution, sewage, fossil fuels burning, and mining (12, 13). Osteoporosis could lead to skeletal damage, is one of the significant effects of exposure to Cd (14, 15). In addition, pulmonary edema may progress to death with severe exposure to Cd (16).

Pb concentration varies in different food products. It mainly originates from the deposition of the atmosphere and adherence of Pb particles in soil to plants. According to Nasreddine et al. (17) and Zeinali et al. (18), about half of Pb consumption by humans occurs from food, while the other half originates from plants. High Pb concentration in humans can cause damage to internal organs like the central nervous system; it also can reduce the ability to create new blood cells. Kidneys are the most sensitive to chronic exposure to Pb in adults. Humans can be exposed to contamination with Pb through water, food, air, and dust, but food is considered as the main source of contamination (19, 20).

Recently, many studies investigated the content of heavy metals in eggs, meat, meat products, the liver, gizzard and heart of chicken. Research conducted by Korish and Attia (21) concluded that Cd and Pb concentrations were not recorded in table eggs or fresh and frozen chicken meat. In contrast, the study exhibited a high concentration of heavy metals in the liver where the Cd and Pb intake for adults was above the daily recommended allowance. Another study was done by Sadeghi et al. (6) to evaluate the levels of Cd and Pb in the liver, heart, and muscle of chicken samples; higher levels of heavy metals were recorded in the liver and heart compared with muscle. Otherwise, a few studies focused on the cooking effect of different methods on the concentration of heavy metals. Research was conducted by Abou-Raya et al. (22) to study the concentration of Cd and Pb in Bolti fish (*Tilapia* sp) that were collected from farms, lakes and markets in Egypt. Both frying and grilling samples were used to estimate the Cd and Pb concentrations. The results indicated that Cd and Pb concentrations were reduced by frying and got higher by grilling.

Therefore, the objective of the current research was to study the concentrations of Cd and Pb in fresh and frozen meat and the liver of chickens in Al-Karkh region, Baghdad province, and evaluate the effect of cooking by boiling on level of Cd and Pb.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was granted according to the local committee of care and use of animals in research at the College of Veterinary Medicine, University of Baghdad (Approval number P.G/399 dated February 2, 2024).

Sample Collection

A total of 80 samples were randomly collected and examined from different regions in Al-Karkh region, Baghdad province, 40 samples from imported frozen meat and liver and 40 from fresh meat and liver of chickens. The samples were examined for Cd and Pb concentration in liver and meat before and after cooking by boiling method using metal pans. Different brands of the frozen liver and meat chickens that were available in local markets were collected randomly for examination. While for the fresh meat and liver, chicks were slaughtered for the collection of liver and meat from the same chick.

Sample Preparation

Polyethylene bags were used to collect the meat and liver samples, and then all the samples were preserved at low temperatures (4°C) until the heavy metal determination. For meat samples, they were taken from the thigh and breast areas of chickens and then pooled as one sample for the metal detection. All samples were analyzed by using atomic absorption spectrometry (Model: AA-7000, Shimadzu, Japan). A cathode lamp was operated and used based on the manufacturer's instructions for Cd (228.8 nm, 8 mA and slit 0.5 nm) and Pb analysis (283.0 nm, 10 mA and slit 0.5 nm). Samples were washed with distilled water to eliminate contamination. The original standard solution (1000mg/mL in 2% HNO₃) for Cd and Pb elements was prepared. Then, 4 standard solutions were prepared by dilution of the original one in order to use in the calibration curve.

Cooking Processes

The meat and liver of fresh and frozen chickens were cooked by boiling method using metal pans and a kitchen gas stove. Tap water was used to wash and/or cook the meat. The water was put in the cooking pan, and when the water reached the boiling point, about 25 g from each sample were put in the pan and cooked for 25-30 min.

Analytical Procedure

Determinations of metal concentrations were based on the wet weight of samples. One g sample from liver or meat tissue was weighed and placed in 100 mL conical flask, then 5 ml of concentrated nitric acid (HNO₃ 70%) was added, followed by 1 mL perchloric acid (HClO₄); the sample was

left at room temperature for an hour. Then the sample was placed on a hot plate (100°C) until violet vapors appeared. The temperature then rose to (150-200°C) until white fumes appeared. The digestion process was completed when the final solution became pale yellow, and filter paper (Whatman 0.45) was used then for sample filtration. Finally, the volume of the solution was completed with distilled water and acidified with (1% HNO₃) up to 25 mL to read the sample using the graphite furnace technique of atomic absorption spectrometer. The analysis was done according to methods described by (23).

Statistical Analysis

Data on Cd and Pb concentration in chicken meat and liver tissue samples were independently exposed to two-way analysis of variance (ANOVA) by using the Standard Least Squares procedure of JMP Pro 16.0.0 software (SAS, Institute Inc., Cary, NC, USA) (24). The model for analysis included the fixed factors of cooking (before vs. after), storage (fresh vs. frozen), and their interaction (cooking × storage). Using the Least Significant Difference (LSD) post-hoc test, significant main effects with interaction were identified. Person correlation analysis for fresh chicken meat and liver samples, both pre- and post-cooking, was performed to evaluate any potential association between Pb and Cd. Results with $P \leq 0.05$ were considered statistically significant.

RESULTS

The effect of cooking and storage conditions on Pb and Cd concentrations in the liver and meat of the chicken are presented in Table 1. There were significant main effects of cooking ($P=0.017$) and storage ($P=0.009$) for Pb. A significant interaction effect for cooking × storage was observed ($P=0.013$). Liver Pb was significantly higher (321.8 ± 76.73 ppm) in the frozen cooked liver compared to the fresh uncooked liver (84.20 ± 19.41 ppm). However, the results of meat tissue indicated that neither cooking ($P=0.834$) nor storage ($P=0.379$) significantly affected Pb concentration. Furthermore, there was no significant interaction among cooking × storage ($P=0.694$) on Pb concentration.

There were no significant main effects of cooking ($P=0.665$) and storage ($P=0.075$) on the concentration of Cd in liver tissue. Additionally, there was no significant interaction between cooking and storage ($P=0.977$). Meanwhile, the main effect of cooking on Cd concentration in meat tissue was not significantly different ($P=0.731$). However, the Cd concentration of storage for meat tissue showed a significant effect ($P=0.005$). So, Cd concentration in meat tissue was significantly higher in fresh/cooked compared to frozen/cooked. For interaction, there was no significant interaction among cooking × storage ($P=0.596$).

Table 1. Effect of cooking and storage conditions on lead (Pb) and cadmium (Cd) concentrations (ppm) in chicken liver and meat

Cooking	Storage	Pb (ppm)		Cd (ppm)	
		Liver	Meat	Liver	Meat
Before	Fresh	84.20±19.41 ^b	215.8±162.5	144.3±42.69	127.8±43.35 ^a
	Frozen	89.69±37.56 ^b	421.6±173.7	72.11±30.97	51.90±25.37 ^{ab}
After	Fresh	79.50±22.85 ^b	245.4±159.9	162.9±47.44	133.7±36.51 ^a
	Frozen	321.8±76.73 ^a	324.4±142.1	88.40±36.69	24.30±7.440 ^b
Main Effect					
Cooking					
Before		86.9±20.60 ^B	319±118	108.2±26.96	89.85±25.94
After		200.7±47.9 ^A	285±105	125.7±30.41	79.00±22.05
Storage					
Fresh		81.85±14.6 ^Z	230.6±111	153.6±31.13	130.8±27.59 ^X
Frozen		205.7±49.4 ^X	373.0±110	80.26±31.19	38.10±10.36 ^Z
P-values					
Cooking		0.017	0.834	0.665	0.731
Storage		0.009	0.379	0.075	0.005
Cooking × Storage		0.013	0.694	0.977	0.596

Values are means ± SEM, n = 10 per treatment group. ^{A-B} Means followed by different uppercase letters in the same column are different from each other in cooking factor. ^{Z-X} Means followed by different uppercase letters in the same column are different from each other in storage factor. ^{a-b} Means followed by different lowercase letters in the same column or row are different from each other in interaction effect cooking × storage

Additionally, Person correlation coefficients were calculated to evaluate the linear relationship between Pb and Cd concentrations in fresh chicken meat and liver tissue before and after cooking. The coefficients were calculated to detect the degree to which Pb and Cd concentrations in these tissues are linearly related, giving insights into the potential interactions of these metals within biological matrices.

According to Table 2, there was a weak negative correlation between Pb and Cd concentrations in the liver before cooking ($r=-0.1413$, $P=0.697$), and the correlation

was not significant at the 0.05 level. The correlation between Pb and Cd after cooking in the liver was also strongly negative ($r=-0.5515$, $P=0.098$). This means as Pb concentration increases, Cd concentration leans towards decrease, and vice versa. This correlation was not statistically significant at the 0.05 level.

For the liver after cooking, a strong negative correlation between Pb and Cd concentrations ($r=-0.5515$, $P=0.098$). This means that as Pb concentration increased, Cd concentration tended to decrease, and vice versa. However, this correlation was not statistically significant at 0.05 level.

In the case of meat before cooking, a moderated inverse correlation was detected between Pb and Cd levels ($r=-0.2989$, $P=0.401$). This means a moderated inverse pattern in the concentrations of these elements, but the relationship was neither strong nor consistent. Like the previous finding, this correlation did not demonstrate statistical significance at the 0.05 level.

Finally, in meat samples after cooking, a weak correlation was noted between Pb and Cd concentration ($r=-0.0961$, $P=0.792$), which reflects a minimal inverse trend in the concentrations of both elements. However, the correlation was neither marked nor reliable. This finding also did not achieve statistical significance at the 0.05 level.

Table 2. Person correlation coefficient (r) between Pb and Cd concentrations in fresh chicken liver and meat tissues

Tissue	Cooking	r	P -value
Liver	Before	-0.1413	0.697
Liver	After	-0.5515	0.098
Meat	Before	-0.2989	0.401
Meat	After	-0.0961	0.792

DISCUSSION

Chicken meat and liver are widely consumed in Iraq. Consuming contaminated feed and environmental pollution, such as contaminated water and industry effluents, could expose chickens to heavy metals (25).

Heavy metal's harmful effects can include a physiological and deleterious effect on the metabolism of the cell. Moreover, it has an oxidative effect on biological macromolecules that influence nuclear protein and DNA (26-29). Some heavy metals are extremely important to keep the different biochemical and physiological functions in indispensable organisms in insignificant amounts; conversely, these metals become unsafe when they exceed exact standards. It is approved that those metals can cause toxicity and malfunctions (30-32). The contamination with heavy metals could be different from one element to another (33-35), mostly influenced by the type of environment, soil, geographical area and animal species (36, 37).

As it is also known, cooking under specific circumstances alters the amount of chemical pollutants in food (38, 39). In the current study, cooking with boiling method cause a significant higher Pb concentration in frozen compared with fresh liver tissue. However, the Cd concentration in meat was significantly higher in fresh cooked compared to frozen cooked meat. The increasing in those concentrations may be due to cooking conditions such as time temperature, water used in cooking, and type of diet chicken consumed or from the environment.

It has been stated that cooking may reduce the content of those metals in some foods while it may also absorb metals if the water used for cooking is contaminated (40). Research was done by (41) to evaluate the concentration of some heavy metals with and without heat in some organs of chicken (meat, gizzard and liver). The metals included Pb and Cd, and samples were collected from five different regions of Damietta governorate in Egypt. This study found

that $Pb > Cd$ in chicken livers, the concentration of Pb was above the safe limit while Cd concentration was below the allowable limits. Through the results, this study stated that cooking with boiling had reduced some heavy metal concentrations except Pb concentration. This study agreed with our finding that Cd concentration in fresh meat increased after cooking.

Another study was carried out by Joyce et al. (42) to investigate different cooking methods (frying, boiling and grilling) on some heavy metal concentrations, including Pb and Cd, in smoked and fresh game meat; cooking with boiling and frying methods had a significant increase in Pb concentration which agreed with our results, cooking with boiling increase the Pb concentration in frozen liver samples. However, same study showed that grilling method reduced the concentration of Pb. In relation to cooking effects on Pb and Cd concentration levels in foods, a study was done by Perello et al. (39) by collecting random samples of different foods such as fish (hake, tuna and sardine), meat (thigh and breast of chickens, veal rib and steak of lamb). Samples were cooked by using (grilled, fried, roasted and boiled); the results showed that the highest concentrations of Pb in raw and cooked samples were determined in fish with the ability to rise after cooking. Nevertheless, the levels of Cd samples were near their detection limits.

Several researchers were also contacted to study the contamination with different heavy metals on chickens' edibles and muscles. An experimental was carried out on different tissues of market chickens (liver, heart and muscle), which determined the contents of Pb and Cd in Mashhad, Iran; this study indicated higher heavy metals in heart and liver samples in comparison to muscle samples (6). Also, a study was done by Ali et al. (43) on twenty samples of chicken liver (frozen and alive) from the markets of Erbil, Iraq in order to investigate the concentration of ten heavy metals, including Cd and Pb. The results indicated that Cd concentration in liver samples was significantly lesser than the allowed limit specified by World Health Organization (WHO), while the Pb concentration significantly exceeded the permissible limits. Additionally, Cd and Pb concentration was evaluated in the kidney, heart and meat of sheep, cows and chickens from the Baquba and Howaydir markets in Baquba, Diyala province, Iraq. This research concluded that meat has the highest significant metal levels while the kidney and heart have the lowest levels; the concentration of Pb and Cd exceed the allowed limits set by WHO (44).

Experiments were also done on different types of fish; a study conducted by Ouda et al. (45) on catfish body parts showed no accumulation of toxic heavy metals at high rates. In contrast, another experiment done by Ahmed (46) and Kareem et al. (47) on carp fish (*Cyprinus carpio* L.) revealed that Pb and Cd concentrations were higher than other heavy metals that were detected. Additionally, Jaber et al. (48) and Mustafa et al. (49) stated that toxic heavy metals can accumulate in the muscles of fish, which is the main source for consumption by humans and affect the public health of humans.

Thus, the toxicity of heavy metals causes them to accumulate in biological tissue, a procedure known as bioaccumulation and biomagnifications. Metals bioaccumulate in living organisms due to exposure to metals in the environment and food, including animal foods like fish, poultry, and humans (50).

To sum up, heavy metals are present in the meat and liver of poultry from the environment and the food consumed when they are alive. However, meat and liver processing and storage may influence the accumulation of those metals by increasing their concentrations inside the tissue. Additionally, cooking methods such as boiling may extract heavy metals from cooking vessels into the food and cause an increase in metal concentrations. In the current study, the rise of Pb concentration in frozen liver and the Cd concentration in fresh meat after cooking may occur due to the effect of cooking with boiling or the source of water used and also be caused by the type of cooking pan that was used. Thus, in order to ensure the safety and quality of meat and liver of chickens' consumed by humans, further investigations should be done with larger sample sizes to reveal a clear picture of heavy metal contamination.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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تأثير الطبخ بالغليان على تراكيز الرصاص والكاديوم في لحوم وأكباد الدجاج

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الخلاصة

نُفذت المعادن الثقيلة من الملوثات المنتشرة بشكل واسع في البيئة وقد تؤدي الى حدوث التسمم وتمتاز ببقائها لمدة طويلة في الغلاف الجوي فضلاً عن قابليتها على التراكم في جسم الإنسان. هدفت هذه الدراسة الى معرفة تراكيز عناصر الكاديوم والرصاص في لحوم وأكباد الدجاج المجمد المستورد والطازج ودراسة تأثير الطهي بالسلق (الغليان) على تركيز هذه المعادن. تم جمع وفحص ٨٠ عينة عشوائية وبواقع ٤٠ عينة مجمدة (١٠ من كل من الأكباد ولحوم الدجاج) قبل وبعد الطبخ و ٤٠ عينة طازجة (١٠ من كل من الأكباد ولحوم الدجاج) قبل وبعد الطبخ من عدة مناطق من جانب الكرخ- بغداد. ولمعرفة تركيز الكاديوم والرصاص قبل وبعد الطبخ تم فحص العينات بجهاز مطياف الامتصاص الذري. أظهرت نتائج الدراسة أن تركيز الرصاص كان أعلى معنوياً في أكباد الدجاج المطبوخ المجمد (٣٢١,٨) جزءاً في المليون مقارنة بالكبد الطازج غير المطبوخ (٨٤,٢٠) جزءاً في المليون. بينما في عينات اللحوم لم يؤثر الطهي أو الخزن بشكل معنوي على تركيز الرصاص. زيادة على ذلك أظهرت النتائج أيضاً أن تركيز الكاديوم في لحوم الدجاج الطازج المطبوخ كان أعلى معنوياً (١٣٣,٧) جزءاً في المليون مقارنة بالمجمد المطبوخ (٢٤,٣٠) جزءاً في المليون. ولاتوجد علاقة على مستوى معنوية (٠,٠٥) بين تركيز كل من الرصاص والكاديوم في أكباد ولحوم الدواجن قبل وبعد الطهي. نستنتج من الدراسة الحالية أن الطبخ بالغليان وهي إحدى الطرق التقليدية المستخدمة في المطابخ العراقية قد تؤدي الى ارتفاع تركيز كل من الكاديوم والرصاص. أن هذه الزيادة في تركيز الرصاص والكاديوم في الأكباد ولحوم الدجاج قد تكون بسبب التلوث البيئي. العلف المستهلك من قبل الدجاج. طرق الطبخ المستخدمة. أو اني الطهي المستخدمة علاوة على نوعية المياه المستخدمة في الطهي.

الكلمات المفاحية: الدجاج، اللحم، الكبد الكاديوم، الرصاص، الطبخ