Hindawi
Journal of Food Processing and Preservation
Volume 2023, Article ID 5542051, 8 pages
https://doi.org/10.1155/2023/5542051

Research Article

Heavy Metal Residue (As, Cd, Hg, and Pb) in Hen Eggs after Applying Different Cooking Methods

Hedayat Hoseini,[1] **Fatemeh Mohammadi-Nasrabadi**,[2] **Abdolsamad Abedi**,[2] **Negar Rostami**,[2] **Sara Bazzaz**,[2] **and Fatemeh Esfarjani**[2]

[1]*Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[2]*Food and Nutrition Policy and Planning Research Department, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Correspondence should be addressed to Fatemeh Esfarjani; fesfarjani@hotmail.com

Received 26 June 2023; Revised 4 October 2023; Accepted 21 October 2023; Published 16 November 2023

Academic Editor: Ali Akbar

As a nutritious food, eggs are capable of accumulating heavy metals and are a good indicator of environmental contamination. This study is aimed at determining the levels of heavy metal residues (As, Cd, Hg, and Pb) in hen eggs after applying different cooking methods. In an experimentally designed study, forty-four hen egg samples were selected from 22 best-selling brands of supermarkets in Tehran and categorized into one of four groups (raw, dry-frying, and boiling with and without eggshells). The levels of heavy metal residues were analyzed by using inductively coupled plasma mass spectrometry (ICP-MS). The average levels of heavy metals (As, Cd, Hg, and Pb) in raw samples were 0.307, 1.654, 0.121, and 6.5 (μg.kg^{-1}), respectively. After applying cooking methods, the residue of two heavy metals in boiling without eggshells (As: 0.228 ± 0.197; Cd: 1.985 ± 0.037) was lower than boiling with eggshells (As: 0.457 ± 0.918; Cd: 2.11 ± 0.223 μg.kg^{-1}), while the dry-frying method had the lowest level of heavy metal residue (As: 0.222 ± 0.109; Cd: 1.54 ± 0.223) ($p < 0.05$) and could be identified as efficient in reducing the amount of these heavy metals. In addition, different cooking methods did not make a significant difference in other heavy metal residues (Hg and Pb). To explain this reduction, two hypotheses can be proposed to explain the reduction of heavy metals after applying different cooking methods. First, the eggshell is permeable; the second is the association of heavy metals bonding with sulfur groups and weight loss due to evaporation. All heavy metal residues were found to be within acceptable permissible thresholds. Therefore, policymakers should protect the health of the population by continuously monitoring heavy metals in foods and prioritizing education and research on how to reduce them in the food chain.

1. Introduction

Food chain pathways and dietary consumption are the primary causes of toxic heavy metal exposure in humans [1]. Food pollution with heavy metals is the main threat to human health, as they cause many diseases and complications. High concentrations of toxic heavy metals (As, Cd, Pb, Cr, Zn, Cu, Co, Fe, and Ni) in the environment, contaminate the water, soil, fauna, and flora [2, 3]. These metals mostly accumulate in agricultural soils, later showing up in food and ultimately causing health problems [4, 5]. Heavy metals are present in the environment due to both natural processes and pollutants from human activity [6].

Hen eggs are a valuable supply of high-quality protein and vital nutrients [7–10]. Compared to other sources of animal proteins, hen eggs are a necessary part of the food pattern and are more affordable worldwide [11, 12]. In addition, hen eggs contain a staggering number of biologically active components to provide nutrients suitable for practically any age [13–15]. Worldwide, the average annual per capita consumption of hen eggs was 250. The annual number per capita egg consumption in the USA, Japan, Turkey, and Iran was 280, 335, 135, and 192, respectively [12, 16].

Eggs may become contaminated with heavy metals through drinking water and chicken feed, both of which are highly affected by the environment [17]. Eggs are capable of accumulating heavy metals and are a good indicator of environmental contamination, too [18–20].

According to the Environmental Protection Agency (EPA), a group of four heavy metals that includes arsenic (As), mercury (Hg), cadmium (Cd), and lead (Pb) is the most prevalent in the environment [21]. These metals gradually spread in water and subsurface soil because they are not degradable and have a long persistence, allowing them to accumulate and amplify along the food chain [22]. They can cause both carcinogenic and noncarcinogenic consequences, as well as a variety of detrimental health issues with long-lasting impacts [23]. Negative effects can occur through skin contact, inhalation, and ingestion via water, air, and soil [21, 24]. When heavy metals enter the human food chain after consumption, they react with proteins and enzymes and can become stable oxidants in the acidic environment of the stomach. This causes the body to accumulate heavy metals [25].

Heat (boiling, baking, roasting, frying, and grilling) is applied to food in different ways to improve its hygienic quality by inactivating pathogenic microorganisms to enhance flavor and taste, increase shelf life [17], increase food safety, enhance nutrient quality, and digest proteins better; so, it is recommended to heat hen eggs before consumption [26, 27].

Many studies have been conducted to investigate the negative and positive effects of processing in reducing hazardous components in food. Some studies suggest that heating food greatly reduces the amount of heavy metals. A wide variety of food products have been assessed, including fish and seafood, seaweed, fruits, vegetables, rice, milk, and bread. Based on their findings, they have developed protocols and proposed chemical agents to minimize the amount of toxic elements in final food products [2, 28].

The different methods of cooking have been reported to significantly affect the amounts of contaminants such as heavy metals present in the final product [29]. Cooking methods such as boiling and dry-frying can alter the quantity of hazardous elements through the loss of water and volatile ingredients, the solubilization of the metal, and to some extent, metal binding to macronutrients, including proteins, lipids, and carbohydrates. Harmful substances are eliminated from food during dry-frying, boiling, or canning operations and can be transferred to the frying pan, boiling water, or cooking stocks because they are not evaporated or converted into safer compounds. However, some cooking techniques, including herbal marinating, can lower the bioaccessibility of hazardous substances by combining them with other substances to produce inaccessible complexes [28].

This study was conducted in the framework of a heavy metal monitoring plan and risk assessment in the food chain. Although numerous studies have been conducted on the amount of heavy metals in hen eggs, to the best of our knowledge, this is the first study in which two methods of hen egg cooking, boiling (with and without shell), and dry-frying are compared on the residues of heavy metals (As, Cd, Hg, and Pb).

2. Material and Methods

2.1. Samples. In the experimental design, forty-four hen egg samples (from each pack of 12, 2 eggs) were randomly chosen from 22 best-selling brands of supermarkets in Tehran, Iran, in February (2023). All samples were initially checked with candles, and only undamaged and fresh eggs (eggs without pinholes, clearly clean eggs, and no cracks) were chosen. All the hen eggs were cleaned with deionized water and categorized into one of four groups (raw, dry-frying, boiling with and without eggshells). Before usage, deionized water was utilized to wash all the glassware (even ceramic nonstick pots and pans) (Figure 1).

Boiling group with eggshells: the deionized water was used to boil hen eggs with shells in ceramic nonstick pots for 10 minutes, and then the shells were removed ($n = 11$).

Boiling group without eggshells: the deionized water was used to boil. After that, unshelled hen eggs were put in ceramic nonstick pots and boiled for 8 minutes, and then filter papers were used to remove water from hen eggs (poached) ($n = 11$).

Dry-fried group (without using any oil or butter): whole edible hen eggs (yolk and white) mixed with a glass stirring rod in a beaker) to avoid metal contamination(and then dry-fried for 5 minutes in ceramic nonstick pans which is similar to grilling ($n = 11$).

Raw group: raw hen eggs, whole (yolk and white), mixed with a glass rod in a glass beaker (without any cooking) ($n = 11$).

2.2. Samples Preparing for Laboratory. All samples were first cooled at room temperature in Pyrex plates before being put in zipped-lock bags and tagged with a code. Each sample was thoroughly mixed and homogenized before being transported to the laboratory for analysis of heavy metal residues (As, Cd, Pb, and Hg). The samples were stored in a refrigerator at 4°C immediately before the preparation steps. To prevent chemical contamination, sterile and chemical-resistant flask containers were used. Initial analytical work began immediately after cleaning all equipment with dilute nitric acid (HNO_3 10%) and then rinsing with deionized water.

The whole egg ingredients (yolk and white) were put in a petri dish to dry in the 70°C oven for 24 hours until it turns into a fine powder [30]. For the digestion of .05 grams of dried egg samples, 10 ml of 65% nitric acid and 37% hydrogen peroxide (v/v) were purchased (Merk KGaA, Darmstadt, Germany).

The solution was left at room temperature for one night until it was clear. The digestion was stopped at 150°C after 4 hours after bringing the solution to room temperature (22–23°C). The solution was then diluted to 50 ml with deionized water, filtered with 0.451 of acid-resistant filter paper, and stored for later examination [31]. Using the heat-block-assisted acid digestion method and the inductively coupled plasma mass spectrometry (ICP-MS) methodology following the FDA (Food and Drug Administration) Elemental Analysis

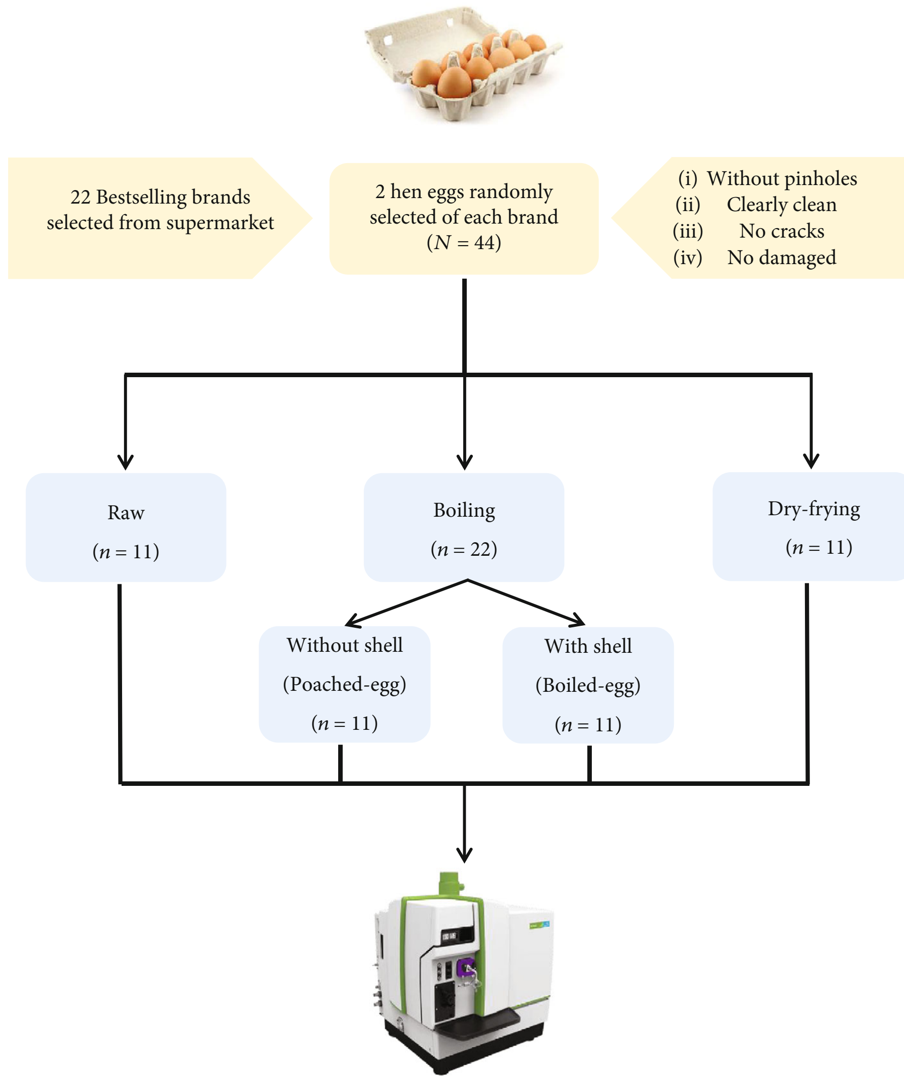

Figure 1: Flow chart of the experimental design.

Manual [32], the total amount of As, Pb, Cd, and Hg was analyzed. The blank solution was made similarly but without the use of hen egg. The criteria for detection in hen eggs using ICP-MS are listed in Table 1. The method employed in ICP-MS included ionizing the sample with inductively coupled plasma. ICP-MS is well-known for its application in detecting exceedingly low quantities of different nonmetals and metals in liquid samples. For determining the level of heavy metals in the food industry, this method is thought to be the fastest and most reliable. Estimation was done using ICP-MS (ULTIMA2, 6100 DRC-e Perkin Elmer Elan) [31].

The calculation of the limit of detection (LOD $\mu g\,kg^{-1}$) based on the measurement results obtained with blank filters in the present study was 0.0003. The results were given in micrograms per kilogram of the sample's moist weight. The limit of quantification (LOQ) was estimated to be 0.001 for both metals [33].

2.3. Statistical Analysis. The statistical analysis was performed with the SPSS software version 20. The means with standard errors of values were estimated by one-way analysis of variance (ANOVA). Differences between means were compared by Tukey's method, and $p < 0.05$ was considered significant.

3. Results and Discussion

3.1. Heavy Metal Residue in Raw Hen Eggs. According to the results of heavy metals in raw samples in the present study, the average levels of As, Cd, Hg, and Pb were 0.307, 1.654, 0.121, and 6.5 ($\mu g.kg^{-1}$), respectively. Compared to the provisional tolerated weekly intake (PTWI) of As, Cd, Hg, and Pb of 15, 7, 4, and 25 ($\mu g\,kg^{-1}$ BW/week), all heavy metal residues (As: 0.75, Cd: 4.12, Hg: 0.30, and Pb: 16.25) were determined to be below acceptable permitted values ([34–40]. On another hand, comparing the results of this study with Iranian assessments [30, 41–43] indicated that during almost a decade, food authorities have paid more attention to this issue.

Table 1: Conditions of ICP-MS apparatus for determining As, Pb, Cd, and Hg in hen eggs.

Parameter	Value
Radiofrequency	1200 W (40 Mhz)
Plasma gas (argon) flow	16 l/min
Nebulizer gas (argon) flow	1 l/min
Read delay and analysis speeding	30 s
Wash	60 s
Wash speeding	30 rpm
Dwell time	50 ms
Resulting/amu 10% peak	0.7
Integration time	3.5
Linear working (total element) (ppb)	0.053
Precision %RSD $n = 10$	1.3
Addition/recovery	93-103
Repetition	3
Limit of detection (LOD) ($\mu g\,kg^{-1}$)	0.00033
Limit of quantification (LOQ) ($\mu g\,kg^{-1}$)	0.001

3.2. Effect of Cooking Methods Boiling (with and without the Shell) and Dry-Frying on Heavy Metal Residue in Hen Eggs. Table 2 displays the ICP-MS results on the effect of different cooking methods in terms of heavy metal residues ($\mu g.kg^{-1}$). The remaining arsenic in boiling with eggshell (0.457 ± 0.918) was more than boiled without eggshell (0.228 ± 0.197) and dry fried eggs (0.222 ± 0.109). This difference was significant by using the ANOVA test ($p < 0.05$). Also, residual cadmium in hen eggs was shown between boiling (with shell 2.118 ± 0.223; without shell 1.985 ± 0.037) and dry-frying (1.548 ± 0.223). Dry-frying also lowered arsenic and cadmium concentrations to a significant degree compared to raw eggs ($p < 0.05$). Probably, the eggshell prevents the transfer of As or Cd to the boiling water. The extent of this reduction varies depending on the severity of the element. Heavy metals may be bonded to soluble amino acids and uncoagulated proteins. The mechanism is believed to be the leaching of heavy metals into boiling water due to protein denaturation and solubilization from cooking temperatures. Metals bound more loosely to egg components are released [44].

In general, the results of the present study showed a higher residual of heavy metals including arsenic and cadmium in boiled egg with shell in comparison to raw samples; despite the lack of statistical significance, this increase can be related to the increased permeability of the eggshell during heating [45]. According to the results of Hincke et al. [46], an eggshell has many pores in its structure [46]. So, it is permeable, and these little alterations might be put on by the surroundings of the chicken, human contamination from handling eggs, the container used to keep the eggs, recycled materials, or anything else that contaminates the eggshell's surface that plays a slight role in these changes, boiled with eggshell compared to the results of raw hen eggs. Therefore, the eggshell was suitable as an indicator of heavy metal pollution [47].

In one study, Canadian researchers investigated the effect of cooking on residual arsenic levels. The results showed that the decrease in arsenic in cooked fish was due to weight reduction in fish which led to an increase in the concentration of arsenic [48]. In another study [49], it was claimed that reductions in arsenic levels during cooking were due to the dissolving or evaporating of the metalloid. In contrast, the increase in total arsenic levels was caused by the concentration of arsenic resulting from the loss of water and other soluble compounds during cooking. Arsenic species can be classified as either lipid-soluble or water-soluble arsenic based on their chemical characteristics. Water-soluble As includes both inorganic and organic chemicals, and they can dissolve in water during cooking. However, lipid-soluble arsenic can be dissolved in oil or other fats during cooking. Therefore, understanding the characteristics of different arsenic species can help reduce exposure to this toxic metalloid [50].

Although the level of Pb residue decreased (with eggshell $6.285 \pm 1.672 >$ without eggshell $5.714 \pm 1.889 >$ dry − frying 5.457 ± 1.672), it was not significant. The amount of mercury residue level in hen eggs after boiling decreased lightly (with shell 0.100 ± 0.166 and without shell 0.120 ± 0.005), despite a slight increase in the dry-frying method (0.122 ± 0.012), and, it was also not significant. Mercury and lead showed little change or inconsistent effects from cooking methods. They may bind more strongly within eggs and resist removal through cooking. The mineralization of the shell is characterized by the precipitation of calcium carbonate on the egg membrane [51]. As the eggshell is mainly inorganic, Hg is rarely deposited in the eggshell [52]. It seems that mercury has a preference for forming bonds with sulfur (-SH) groups in addition to the water loss due to heating, which can explain the slight increase in concentration during grilling [25].

When hen eggs are dry-fried, water molecules inside the hen egg are heated and gain enough energy to evaporate, and the reduction of some heavy metal concentrations, such as As, Cd, and Pb, might be due to the loss of volatile substances during evaporation. The extent of evaporation depends on several factors, such as the cooking temperature, duration, and the amount of egg surface exposed to the air [28]. The fact indicates that the concentration of crude protein (amino acids containing sulfur) in food is inversely correlated with the quantity of heavy metals (As, Cd, Hg, Pb), indicating that heavy metals (As, Cd, Hg, Pb) are binding to the SH groups of proteins [49].

Several investigations claimed that the amount of heavy metals in food had significantly decreased after cooking. Cooking parameters, such as duration, temperature, and cooking medium, were factors in studies that showed a reduction of hazardous components [28, 53]. Boiling and grilling are both widely used cooking methods that can alter the amount of harmful elements present in food. These changes are brought about by the loss of water and volatile compounds during cooking, as well as the solubilization of metals and, to a lesser extent, metal binding to macronutrients like proteins, lipids, and carbohydrates. Harmful elements that are removed from food during frying, boiling, or canning are not converted to safer compounds or evaporated during the cooking process. However, these materials can migrate to the frying oil, the boiling water, or the cooking stocks [28].

Table 2: Comparing the mean ± SE of the heavy metals residues of raw and two methods (boiling and dry-frying) on hen eggs.

Heavy metals (μg.kg^{-1})	Raw ($n = 11$)	Boiling		Dry-frying ($n = 11$)
		With shell ($n = 11$)	Without shell* ($n = 11$)	
As	$0.307 \pm 0.109^{a\dagger}$	0.457 ± 0.918^{a}	0.228 ± 0.197^{b}	0.222 ± 0.109^{b}
Hg	0.121 ± 0.015	0.100 ± 0.166	0.120 ± 0.005	0.122 ± 0.012
Cd	$1.654 \pm 0.261^{a\dagger}$	2.118 ± 0.223^{a}	1.985 ± 0.037^{a}	1.548 ± 0.223^{b}
Pb	6.5 ± 2.470	6.285 ± 1.672	5.714 ± 1.889	5.457 ± 1.672

*Poached. †Significant differences between ^{a} and ^{b} in terms of As and Cd concentrations between boiling with shell, without shell, and dry-frying ($p < 0.05$).

Particular attention was placed on the chemical binding of both the organic and inorganic forms of each element in diverse diets when discussing the reduction of hazardous elements. Additionally, the types of these reactions as well as the chemical groups and ligands by which food items bond with metals are discussed [28]. Since heating methods in different organs of the body have not shown the same results such as in cows' meat, the grilling method reduced Pb concentrations, while in the chicken liver, the level of Pb was above the maximum allowable level after boiling and grilling [54]. By selecting an appropriate preparation procedure, it is possible to reduce the amount of harmful ingredients in a food product.

3.2.1. Based on the Findings, Two Hypotheses Were Proposed

(1) *Eggshell Has Many Pores in Its Structure* [46]. So, the eggshell is permeable; therefore, these little alterations might be put on by the surroundings of the chicken, human contamination from handling eggs, the container used to keep the eggs, recycled materials, or anything else that contaminates the eggshell's surface

(2) *Water Loss and Evaporation.* It was suggested that a heating process could reduce the levels of heavy metals by causing the loss of water and volatile materials, causing the metals to dissolve, and causing partial metal binding to macronutrients such as proteins, lipids, and carbohydrates, as well as the interaction of heavy metals with the sulfur group (-SH). Mijin et al. were found to bind heavy metal ions to different sites in egg ovalbumin before thermal treatment. Such binding could not cause a change in the heavy metal level of the hen egg after heating [44].

Tehran is one of the world's most polluted cities [55]. This pollution can affect human health directly or indirectly through contamination of the food chain. Chickens play an essential role in the food chain, and their contamination can have irreparable consequences. Hen eggs may become tainted with heavy metals through chicken feed and drinking water, both of which are primarily influenced by the environment. Consistently consuming heavy metals in food at hazardous levels may have negative impacts on humans by impairing a variety of biological and metabolic systems [11].

3.2.2. Limitation. The loss of metals by evaporation, particularly for mercury, cannot be ruled out since we were unable to measure the temperature, which was identified during the cooking operations. Another limitation was that we did not have access to drinking water or chicken feed from the chicken farms from which we obtained the hen eggs for examination. Also, the detection of heavy metals from eggshells and boiled water is suggested for future research.

4. Conclusions

Hen eggs are a valuable supply of high-quality protein and vital nutrients and are more affordable worldwide. Also, hen eggs are capable of accumulating heavy metals and are a good indicator of environmental contamination. The result of different cooking methods showed that the residue of dry-fried samples had the lowest level of the two heavy metals (As and Cd). All heavy metal residues were found to be within acceptable permissible thresholds. Therefore, policymakers should protect the health of the community and prioritize continuous national monitoring of food as well as education and research in the field of reducing heavy metals in the food chain. It can also design programs on how to reduce these heavy metals and improve guidelines to protect consumer health and increase food safety.

Data Availability

The data will be available upon request from the corresponding author.

Ethical Approval

The Ethics Committee of the National Nutrition and Food Technology Research Institute, Faculty of Nutrition Science and Food Technology, Shahid Beheshti University of Medical Sciences, approved the project by ethical code: IR.SBMU.nnftri.Rec.1400.074. This article did not contain any studies involving human participants performed by any of the authors. Also, it did not contain any studies involving animals performed by any of the authors. Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been completely observed by the authors. All authors have read and confirmed the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest to disclose.

Authors' Contributions

HH and FE were involved in all parts of the study including designing, supervision, data collecting, and experimental measuring. AA did the statistical analysis and drafted the manuscript. FMN contributed to the conceptualization, methodology, and data interpretation and reviewed the manuscript. NR and SB contributed to the data gathering, experimental measuring, and drafting of the manuscript.

Acknowledgments

This study was supported fully by the National Nutrition and Food Technology Research Institute (NNFTRI), Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Grant No.: 30109). The authors would like to thank the Research Council of National Nutrition and Food Technology Research Institute, Faculty of Nutrition Science and Food Technology, Shahid Beheshti University of Medical Sciences, for their support, and the Saba analyze (HMS) laboratory, validated by Iran Food and Drug (IFDA), for the assistance in conducting measurement tests.

References

[1] M. S. Islam, M. K. Ahmed, M. Raknuzzaman, M. Habibullah-Al-Mamun, and M. K. Islam, "Heavy metal pollution in surface water and sediment: a preliminary assessment of an urban river in a developing country," *Ecological Indicators*, vol. 48, pp. 282–291, 2015.

[2] S. Ghasemi, M. Hashemi, M. Gholian Aval et al., "Effect of baking methods types on residues of heavy metals in the different breads produced with wheat flour in Iran: a case study of Mashhad," *Journal of Chemical Health Risks*, vol. 12, no. 1, pp. 105–113, 2022.

[3] B. Xiong, T. Xu, R. Li et al., "Heavy metal accumulation and health risk assessment of crayfish collected from cultivated and uncultivated ponds in the middle reach of Yangtze River," *Science of the Total Environment*, vol. 739, article 139963, 2020.

[4] A. Jose and J. G. Ray, "Toxic heavy metals in human blood in relation to certain food and environmental samples in Kerala, South India," *Environmental Science and Pollution Research*, vol. 25, no. 8, pp. 7946–7953, 2018.

[5] R. Singh, N. Gautam, A. Mishra, and R. Gupta, "Heavy metals and living systems: an overview," *Indian Journal of Pharmacology*, vol. 43, no. 3, pp. 246–253, 2011.

[6] V. Masindi and K. L. Muedi, "Environmental contamination by heavy metals," *Heavy Metals*, vol. 10, pp. 115–132, 2018.

[7] S. S. Morris, K. Beesabathuni, and D. Headey, "An egg for everyone: pathways to universal access to one of nature's most nutritious foods," *Maternal & Child Nutrition*, vol. 14, no. S3, article e12679, 2018.

[8] C. Nimalaratne and J. Wu, "Hen egg as an antioxidant food commodity: a review," *Nutrients*, vol. 7, no. 10, pp. 8274–8293, 2015.

[9] S. Réhault-Godbert, N. Guyot, and Y. Nys, "The golden egg: nutritional value, bioactivities, and emerging benefits for human health," *Nutrients*, vol. 11, no. 3, p. 684, 2019.

[10] I. Seuss-Baum, "Nutritional evaluation of egg compounds," in *Bioactive Egg Compounds*, pp. 117–144, Springer, 2007.

[11] Y. Fakhri, A. Mohseni-Bandpei, G. O. Conti et al., "Systematic review and health risk assessment of arsenic and lead in the fished shrimps from the Persian gulf," *Food and Chemical Toxicology*, vol. 113, pp. 278–286, 2018.

[12] S. F. Talakesh, A. Bahonar, S. Mashayekhi, and V. Rahmanian, "Estimation of per capita consumption of chicken egg and attitude of Tehrani families towards factors influencing egg consumption in 2018," *Journal of Nutrition and Food Security*, vol. 5, no. 3, pp. 209–217, 2020.

[13] E. Abeyrathne and D. Ahn, "Isolation of value-added components from egg white and their potential uses in food, nutraceutical and pharmaceutical industries," in *Handbook of Eggs in Human Function*, R. R. Watson and F. DeMeester, Eds., pp. 35–52, Wageningen Academic Publishers, 2015.

[14] M. Anton, F. Nau, and C. Guérin-Dubiard, "Bioactive fractions of eggs for human and animal health," in *Improving the Safety and Quality of Eggs and Egg Products*, pp. 321–345, Elsevier, 2011.

[15] Y. Mine, "Egg proteins and peptides in human health-chemistry, bioactivity and production," *Current Pharmaceutical Design*, vol. 13, no. 9, pp. 875–884, 2007.

[16] H. Demirulus, "The heavy metal content in chicken eggs consumed in Van Lake territory," *Ekoloji*, vol. 22, no. 86, pp. 19–25, 2013.

[17] J. Pokorny, *Changes of Nutrients at Frying Temperatures. Frying of food. Oxidation, Nutrient and Non-Nutrient Antioxidants, Biologically Active Compounds and High Temperatures*, Frying of Food Technomic Publishing Co. Lancaster, 1999.

[18] H. Aliu, S. Dizman, A. Sinani, and G. Hodolli, "Comparative study of heavy metal concentration in eggs originating from industrial poultry farms and free-range hens in Kosovo," *Journal of Food Quality*, vol. 2021, Article ID 6615289, 7 pages, 2021.

[19] G. V. Goran, E. Badea, M. L. Buga, and V. Crivineanu, "Cadmium, lead and zinc levels in organic and conventional eggs," *Agrolife Scientific Journal*, vol. 8, no. 1, pp. 107–112, 2019.

[20] W. F. Saad Eldin and A. A. Raslan, "Residues of some toxic heavy metals and trace elements in chicken eggs," *Zagazig Veterinary Journal*, vol. 46, no. 1, pp. 8–16, 2018.

[21] M. Kaur, A. Kumar, R. Mehra, and I. Kaur, "Quantitative assessment of exposure of heavy metals in groundwater and soil on human health in Reasi district, Jammu and Kashmir," *Environmental Geochemistry and Health*, vol. 42, no. 1, pp. 77–94, 2020.

[22] D. Shikha and P. K. Singh, "In situ phytoremediation of heavy metal–contaminated soil and groundwater: a green inventive approach," *Environmental Science and Pollution Research*, vol. 28, no. 4, pp. 4104–4124, 2021.

[23] M. Sieghardt, E. Mursch-Radlgruber, E. Paoletti et al., "The abiotic urban environment: impact of urban growing conditions on urban vegetation," in *Urban Forests and Trees*, pp. 281–323, Springer, 2005.

[24] S. Borowska and M. M. Brzóska, "Metals in cosmetics: implications for human health," *Journal of Applied Toxicology*, vol. 35, no. 6, pp. 551–572, 2015.

[25] J. O. Duruibe, M. Ogwuegbu, and J. Egwurugwu, "Heavy metal pollution and human biotoxic effects," *International Journal of Physical Sciences*, vol. 2, no. 5, pp. 112–118, 2007.

[26] P. Evenepoel, B. Geypens, A. Luypaerts, M. Hiele, Y. Ghoos, and P. Rutgeerts, "Digestibility of cooked and raw egg protein in humans as assessed by stable isotope techniques[123]," *The Journal of Nutrition*, vol. 128, no. 10, pp. 1716–1722, 1998.

[27] K. Zaheer, "An updated review on chicken eggs: production, consumption, management aspects and nutritional benefits to human health," *Food and Nutrition Sciences*, vol. 6, no. 13, pp. 1208–1220, 2015.

[28] P. Hajeb, J. J. Sloth, S. Shakibazadeh, N. Mahyudin, and L. Afsah-Hejri, "Toxic elements in food: occurrence, binding, and reduction approaches," *Comprehensive Reviews in Food Science and Food Safety*, vol. 13, no. 4, pp. 457–472, 2014.

[29] K. Joyce, B. Emikpe, D. Asare et al., "Effects of different cooking methods on heavy metals level in fresh and smoked game meat," *Journal of Food Processing & Technology*, vol. 7, no. 9, pp. 9–11, 2016.

[30] H. Hoseini, A. S. Abedi, F. Mohammadi-Nasrabadi, Y. Salmani, and F. Esfarjani, "Risk assessment of lead and cadmium concentrations in hen's eggs using Monte Carlo simulations," *Food Science & Nutrition*, vol. 11, no. 6, pp. 2883–2894, 2023.

[31] B. Meermann and V. Nischwitz, "ICP-MS for the analysis at the nanoscale–a tutorial review," *Journal of Analytical Atomic Spectrometry*, vol. 33, no. 9, pp. 1432–1468, 2018.

[32] P. J. Gray and W. Cunningham, "Inductively coupled plasma collision cell quadrupole mass spectrometric determination of extractible arsenic, cadmium, chromium, lead, mercury, and other elements in food using microwave-assisted digestion: results from an FDA interlaboratory study," *Journal of AOAC International*, vol. 102, no. 2, pp. 590–604, 2019.

[33] M. Esposito, S. Cavallo, E. Chiaravalle et al., "Trace elements in free-range hen eggs in the Campania region (Italy) analyzed by inductively coupled plasma mass spectrometry (ICP-MS)," *Environmental Monitoring and Assessment*, vol. 188, no. 6, pp. 1–9, 2016.

[34] EFSA Panel on Contaminants in the Food Chain (CONTAM), "Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food," *EFSA Journal*, vol. 10, no. 12, p. 2985, 2012.

[35] FAO/WHO, *Food and Agriculture Orgnization/World Health Organization, Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1956-2003), (First through Sixty First Meetings)*, ILSI Press International Life Sciences Institute, 2004.

[36] FAO/WHO, "Food and Agriculture Orgnization/World Health Organization, Joint FAO/WHO expert committee on food additives, summary report of the seventy-third meeting of JECFA," WHO Technical Report Series, Geneva, Switzerland, 2010.

[37] ISIRI, *Institute of Standards and Industrial Research of Iran, Food & Feed-Maximum Limit of Heavy Metals*, p. 210, ISIRI press, Tehran, Iran, 1st edition, 2010.

[38] Food Joint, "73rd meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Geneva, Switzerland, 8-17 June 2010," *FAO JECFA Monographs*, vol. 10, pp. 1–150, 2010.

[39] Joint FAO/WHO Expert Committee on Food Additives (JECFA) and World Health Organization, *Safety evaluation of certain contaminants in food: prepared by the seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*, World Health Organization, 2011.

[40] Joint FAO/WHO Expert Committee on Food Additives (JECFA) and World Health Organization, "Evaluation of certain contaminants in food: eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives," World Health Organization, 2017.

[41] S. Abbasi Kia, G. Jahed Khaniki, N. Shariatifar, S. Nazmara, and A. Akbar Zadeh, "Contamination of chicken eggs supplied in Tehran by heavy metals and calculation of their daily intake," *Journal of Health in the Field*, vol. 2, no. 4, pp. 44–51, 2017.

[42] S. Farahani, N. Eshghi, A. Abbasi, F. Karimi, E. Shiri Malekabad, and M. Rezaei, "Determination of heavy metals in albumen of hen eggs from the Markazi Province (Iran) using ICP-OES technique," *Toxin Reviews*, vol. 34, no. 2, pp. 96–100, 2015.

[43] J. Salar-Amoli and T. Ali-Esfahani, *Determination of Hazardous Substances in Food Basket Eggs in Tehran*, A preliminary study. Paper presented at the Veterinary Research Forum, Iran, 2015.

[44] N. Mijin, J. Milošević, S. Stevanović et al., "Amyloid-like aggregation influenced by lead(II) and cadmium(II) ions in hen egg white ovalbumin," *Food Hydrocolloids*, vol. 136, p. 108292, 2023.

[45] D. Fromm, "Permeability of the egg shell as influenced by washing, ambient temperature changes, and environmental temperature and humidity," *Poultry Science*, vol. 39, no. 6, pp. 1490–1495, 1960.

[46] M. T. Hincke, Y. Nys, J. Gautron, K. Mann, A. B. Rodriguez-Navarro, and M. D. McKee, "The eggshell: structure, composition and mineralization," *Frontiers in Bioscience-Landmark*, vol. 17, no. 1, pp. 1266–1280, 2012.

[47] T. Dauwe, L. Bervoets, R. Blust, R. Pinxten, and M. Eens, "Are eggshells and egg contents of great and blue tits suitable as indicators of heavy metal pollution?," *Belgian Journal of Zoology*, vol. 129, pp. 439–447, 1999.

[48] R. W. Dabeka, A. D. McKenzie, G. M. Lacroix et al., "Survey of arsenic in total diet food composites and estimation of the dietary intake of arsenic by Canadian adults and children," *Journal of AOAC International*, vol. 76, no. 1, pp. 14–25, 1993.

[49] R. Wauchope, "Uptake, translocation and phyrotoxicity of arsenic in plants," in *Paper Presented at the Arsenic: Industrial, Biomedical, Environmental Perspectives. Arsenic Symposium, Van Nostrand Reinhold Company*, pp. 348–374, Gaithersburg, MD. New York, 1983.

[50] C. S. Cobbett, "Phytochelatins and their roles in heavy metal detoxification," *Plant Physiology*, vol. 123, no. 3, pp. 825–832, 2000.

[51] M. Hincke, J. Gautron, A. Rodriguez-Navarro, and M. McKee, "The eggshell: structure and protective function," in *Improving the Safety and Quality of Eggs and Egg Products*, pp. 151–182, Woodhead Publishing Series in Food Science, Technology and Nutrition, 2011.

[52] Z. Wang, J. Liao, X. Guo, X. Li, and S. Y. Kwon, "Total mercury in different egg tissues provides insights to mercury metabolisms in bird bodies," *Ecotoxicology and Environmental Safety*, vol. 249, article 114336, 2023.

[53] M. Menon, W. Dong, X. Chen, J. Hufton, and E. J. Rhodes, "Improved rice cooking approach to maximise arsenic removal while preserving nutrient elements," *Science of the Total Environment*, vol. 755, Part 2, p. 143341, 2021.

[54] A. F. Hasballah, "Evaluation of some heavy metals in cows meat and some chicken organs in Damietta governorate markets and effect of the heat on their levels," *Mansoura University*, vol. 48, no. 3, pp. 131–143, 2019.

[55] H. Goharipour and S. S. Firoozabadi, "Assessing the impacts of changes in the currency exchange rate on air pollution in Tehran: a sectoral review," *European Journal of Business and Management Research*, vol. 7, no. 3, pp. 12–19, 2022.