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Article

An Overview on Chemical Hazards and Detection Methods in Egg

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Abstract: Egg has been an important nutritional deity food for human since ancient times. It is one of the highly nutritious foods among the human food stuff cycle and can be an effective delivery system for health – regulating nutrients, vitamins, proteins, fat etc. Despite the nutritional content of eggs, some potential health risks are associated with their consumption, including exposure to environmental contaminants and in some instances individual allergies. Eggs might contain elevated levels of heavy metals originated mainly from food and water feed, which are mainly influenced by the surrounding environment. Antibiotics are used by the veterinarians and poultry industry to enhance growth rates, health of the birds and etc. Although antibiotics have considerable benefits in most cases, the illegal use of these drugs has led to the accumulation of toxic antibiotic residues in edible poultry products destined for human consumption. Aflatoxins are toxic and carcinogenic secondary metabolites produced by some strains of *Aspergillus flavus, Aspergillus parasitscus* during their growth on feed. Aflatoxin-contaminated feed may affect the growth and health of poultry and the possible transmission of such toxic residues to edible eggs resulting in potential hazards to human health. Organochlorine pesticides (OCPs) have been used in the public health sector for disease vector control and in agriculture to control crop pests for the past several decades in Jordan. They are characterized by low water solubility and high lipid solubility, leading to their bioaccumulation in fatty tissues. Therefore, they can accumulate in human body fats and the environment posing problems to human health. This study is an overview of the most important chemical hazards caused by the consumption of eggs.

Key word: Eggs, Hazards, Contamination

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1. Introduction

Eggs are nutritious and economic foods in the human diet and are included in several food products due to their various functions in the body (Tyokumbur & Daramola, 2014). Eggs store significant amounts of protein that are useful to the body. According to the European Commission (EC) Nutrition & Health Claims regulation (European Parliament and Council, 2007) a ‘source’ claim can be made for foodstuffs that meet at least 15% of the Recommended Daily Amount (RDA) per 100g, while a ‘rich in’ claim applies when nutrient levels exceed 30% RDA. To that end, the average egg is high in protein, ‘a source’ of vitamin A, folate, choline, phosphorus and selenium, and ‘rich in’ vitamin D, riboflavin, vitamin B12, biotin and iodine. The lipid matrix within the egg yolk is believed to enhance the bioavailability of nutrients, such as lutein. Eggs provide the richest mixture of essential amino acids (Layman &n Rodriguez, 2009), which is important for children, adolescents and young adults since protein is required to sustain growth and build muscle (Cook et al., 2005).

Global egg consumption has tripled in the past 40 years with consumer quality expectations increasing just as rapidly .The middle class population can afford to buy eggs for consumption across major Asian nations such as China and India. This led to constant increase in the per capita consumption of eggs. Since the population in the region is high and on the rise, the smallest rise in the per capita consumption would trigger a significant upswing in the retail egg market and demand. Overall, world countries vary largely in egg consumption levels. Annual consumption of eggs determined largely by the country’s wealth, ranges from 300 g/person in African countries to 19.1 kg in Japan. Only 9 of 43 sub-Saharan African countries have an average consumption higher than 2 kg while most Asian and American people eat at least twice that amount (Zaheer, 2015).

2. Chemical Hazards in Egg

Hazardous substances, defined as persistent, bio-accumulative and toxic substances (PBTs), are chemicals are not degrade easily in the environment. They typically accumulate in fatty tissues and are slowly metabolized often resulted in increasing their concentration through the food chain (Salar-Amoli & Ali-Esfahani, 2015). The quality of the egg depends on factors before the laying phase and after oviposition. Hen´s health, feed safety and environmental quality are intrinsic factors that define the quality of the laid egg. After oviposition, environmental conditions, grading and pack systems, processing, handling and transportation start to have influence, particularly on the shelf-life and internal quality of the eggs. As part of the assessment of eggs and egg products, we looked into the potential risks that may occur as a result of the use, or presence of various chemicals at different points in the egg supply chain. Chemical contamination may be introduced via feed, water and veterinary treatment during the primary production stages and via food additives, cleaning chemicals and chemicals that migrate from packaging materials during the manufacture and sale of egg products.

Pesticides, antibiotics, hormones, toxins, fertilizers, fungicides, heavy metals, PCB's, Color additives, inks, indirect additives and packaging materials are hazardous substances in food (Salar-Amoli & Ali-Esfahani, 2015).

2.1. Heavy Metals

Chicken egg contains some metals and minerals naturally. Minerals have important biological functions, making them necessary to be taken in adequate amounts (Demirulus, 2013). Trace metals like Cr, Co, Cu, Fe, Mn and Zn are essential metals, also called micronutrients, and are toxic when taken in excess of requirements Whereas, especially lead (Pb), Cadmium (Cd) mercury (Hg) and arsenic (As) are non-essential metals and even toxic in trace amount. Global environmental pollution with trace elements has increased the investigations concerning metal contamination of food-stuffs including eggs, which represent an important part of humans diet, especially children (Tyokumbur & Daramola, 2014).

2.1.1. Determination methods of heavy metals

There are many methods for the determination of heavy metals in samples (Baranowska & Srogi, 2000). Recently the establishment of inductively coupled plasmaoptical emission spectroscopy (ICP-OES) including a charge-transfer device with a detector ensures the possibility of development of flexibility, consumer programmability, joint multi-element capability, increasing spectral information and sample analyses. The low detection limit, high quantum yield and wide linear dynamic range made ICP-OES a viable alternative to the other techniques (Dospatliev et al., 2012).

The elements were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES); atomic absorption spectrophotometer and Flame Atomic Absorption Spectrometry (FAAS); gas chromatography-electron capture detector, hydride-generation atomic absorption spectrometry, cold-vapor atomic absorption spectrometry and conductively coupled plasma atomic optical spectrometry were used to determine the levels of OCs, As, Hg and the others, respectively.

The results of studies on heavy metal contamination in eggs reported from different country are shown in table 1 (Abdel-Wahhab et al., 2012, Al-Ashmawy, 2013, Azza,Hanaa, 2011, Demirulus, 2013, Siddiqui et al., 2011, Ul Islam et al., 2014)**.**

Table 1. Eggs and heavy metal contamination reported from different countries

	Egg samples	year	Lead			Cadmium			Arsenic			Copper		
			max	mean	Min	max	mean	min	max	mean	min	max	mean	**min**
1	**IRAN**	2015	5.363	0.749	0.225	6.368	0.248	0.025	0.125	0.078	0.075	-	-	-
2	**PAKISTAN**	2014	0.464	-	0.058	0.087	-	0.042	-	-	-	-	-	-
3	**PAKISTAN**	2006	0.63	-	0.52	0.08	-	0.07	-	-	-	0.82	-	0.74
4	**INDIA**	2013	0.9	0.4	0.1	15.4	8.0	2.3	94.4	34.3	3.5	2.1	1.4	0.6
5	**TURKEY**	2009	-	0.11	-	-	3.8	-	-	0.18	-	-	0.9	-
6	**EGYPT**	2011	2.24	0.131	ND	ND	ND	ND	ND	ND	ND	0.939	0.583	0.044
7	**PALESTIN**	2011	1.93	0.27	0.02	125	35.67	5	-	-	-	4.60	2.7	1.83
8	**NIGERIA**	2003	-	0.59	-	-	0.07	-	-	-	-	-	0.78	-
9	**U.K (London)**	2011	-	0.15	-	-	-	-	-	-	-	-	6.6	-
10	**EGYPT NORTH DELTAD**	2012	-	0.228	-	-	0.015	-	-	0.025	-	15.5	6.1	0.62
11	**EGYPT MIDDLE DELTAD**	2012	-	0.303	-	-	0.012	-	-	0.033	-	13.4	6.7	3.2
12	**EGYPT SOUTH DELTAD**	2012	-	0.178	-	-	0.006	-	-	0.023	-	9.3	5.6	3.09
13	**UPPER EGYPT**	2012	-	0.172	-	-	0.007	-	-	0.029	-	11.1	0.93	1.98
14	**PALESTIN**	2012	-	1.39	-	-	0.13	-	-	-	-	-	4.6	-
15	**MALASIA**	2011	-	0.42	-	-	0.06	-	-	0.3	-	-	-	-
16	**BANGLADESH**	2009	-	12.1	-	-	1.04	-	-	-	-	-	0.64	-
17	**BELGIUM**	2006	12.58	7.94	1.03	0.49	0.33	0.07	20.41	14.26	6005.0	622.17	511.58	329.20

-: not reported

2.2. Antibiotics

Antibiotics are naturally occurring, semi-synthetic and synthetic compounds with antimicrobial activity that can be applied parentally, orally or topically. It is estimated that 100–200 thousand tons of antibiotic substances are annually produced in the world (Mahmoudi & Norian, 2015). According to

WHO, about half of the worldwide-produced antibiotics are consumed for non-human applications (Mahmoudi & Norian, 2015). Antibiotics are used by the veterinarians and poultry industry to enhance growth rates, health of the birds, feed efficiency, egg production and for therapeutic reasons to reduce the incidence of poultry disease (Donoghue, 2003). Use of antimicrobials is strictly regulated by the Food and Drug Administration (FDA) and the USDA to warrant their safety and efficacy. The inappropriate use and handling of these antibiotics have led to occurrence of harmful residues in edible poultry tissues (meat and eggs) and other animal products like milk (JUDITH, 2014). Veterinary drug residues in poultry meat and eggs may be produced by exposing chickens to drugs or contaminants in a number of ways which include:

• Illegal or extra-label uses of drugs
• Use of feed unintentionally cross contaminated during feed mixing
• Use of mislabeled feed
• Pesticide, chemical or heavy metal contamination of feed ingredients or water

Antibiotic residues in foods of animal products can lead to losses in the food industry through international trade (Abasi et al., 2009).

Some of the commonly used antibiotics today in poultry industries in developing countries like Nigeria are Tylosine, Neomycin, Gentamycin, Tetracyclines (Chlortetracycline, Oxytetracycline), Sulfonamides (Sulfadimethoxine, Sulfamethazine, Sulfathoxazole), Penicillin (Ampicillin), Enrofloxacin, Erythromycine and vaccines (Sasanya et al., 2005).

Failure to adhere strictly to the withdrawal period (Drug withdrawal (WDTs) time is the time required for drug residue to reach a safe concentration for human or animal consumption, defined as MRL (Mahmoudi & Norian, 2015) or the Maximum Residual Limits (MRL) of antibiotics in poultry production is reported to be the primary cause of antibiotic residues in edible poultry tissues and products like eggs (Njoga, 2015). Withdrawal periods ranging from a few days to a few weeks are recommended for approved veterinary drugs (Njoga, 2015).

2.2.1. Techniques for screening of antibiotic residues

Various methods using for antibiotic residue in foods including: Microbiological methods like Agar gel diffusion test, analytical techniques that are used for the screening of veterinary drug residues include Radio Immunoassay (RIA), Enzymes Immunoassay (ELYSA) (Javadi & Mirzaie et al., 2009) and Thin Layer Chromatography (TLC) (Haughey & Baxter, 2006). Confirmatory tests applied to screening samples that require further investigation are usually based on Mass Spectrometric (MS) techniques such as Liquid Chromatography (MS/LC) (Simon & Baxter, 2006; Muhammed et al., 2009). Other techniques used for the detection and analysis of drug residues in edible tissues are High Performance Liquid Chromatography (HPLC) (Olatoye & Ehinmowo, 2009; Muhammed et al., 2009), Gas Chromatography (Muhammed et al., 2009) and Paper Chromatography (Nisha, 2008). In recent years, the demand for increased testing with regard to food safety has led to the development of new technologies for screening of drug residues in food of animal origins. The advent of a Surface Plasmon Resonance (SPR) based biosensor is one of such technologies. Solid Phase Micro- Extraction (SPME) and Micro dialysis methods are also modern analytical techniques used for drug residues analysis in edible animal tissues. Among these methods, Microbiological methods are the most common and practical method to determine antibiotic residues in food, because, these methods are compared with other methods in terms of cost and time savings are more Advantageous (Pikkemaat, 2009). Nowadays, four plate test (FPT) is commonly used for antibiotic residues screening (Kilinc & Cakli, 2008; Okerman et al., 1997).

2.2.2. Antibiotic residue reports in egg

Ehsani and Hashemi (2015) tried to determine the antibacterial drug residues in 200 commercial eggs distributed in Urmia, Iran. The results of their study revealed that twenty five samples (12.5%) of the pre-pared eggs were positive for antibacterial substances and all of them were related to macrolides group. Quite consistent with the results of current study other researchers diagnosed macrolides group as the major contaminant antibiotics in the egg yolk by FPT method (Ehsani & Hashemi, 2015).

Mehtabuddin et al (2012) analyzed 30 eggs samples for sulfonamide residues, out of which 9 (30 %) samples had detectable levels of sulfonamide residues and 10% egg samples exceeded recommended maximum residual level and were unfit for human consumption(Mehtabuddin et al., 2012). The results of sulfonamide residues in egg samples are in line with Sasanya et al. (2005) who detected sulfonamide residues in 98% egg samples. Substantiated the results of this study that sulfonamide residual problem exists in most developing countries. However, the detection level was much higher than the present study. Chowdhury et al. (2015) reported this research was undertaken to detect and determine concentration or level of antibiotic residues in milk and egg of local and commercial farms in Chittagong district of Bangladesh December 2011 to June 2012 (Chowdhury, Hassan et al., 2015). Microbial inhibition test (MIT) and thin layer chromatography (TLC**)** were used for screening and ultra-high performance liquid chromatography was used to estimate the concentrations of antibiotic residues in 200 samples.

The concentration of antibiotic residues determined by UHPLC was higher than minimum residue level. It is alarming for public health safety.

Mahmoudi and Norian (2015) detected the enrofloxacin residue in chicken eggs from Northwest regions of Iran (Tabriz, Urmia and Ardabil cities). The results of FPT revealed that up to 60.66% of the samples were contaminated with the antibiotics residue; the egg samples of Ardebil

(42.85%) and Tabriz (27.27%) had the highest and lowest percentage of contamination respectively. The ELISA assay showed that out of 91 positive samples in FPT, 78 samples (85.71%) were positive for enrofloxacin. ELISA analyses demonstrated that both maximum levels of (56.17 ppb) and the highest contamination rate (43.58%) were observed in Ardabil egg samples (Mahmoudi,Norian, 2015). Al Hazzani et al. (2014) randomly collected ninety egg samples (30 each of brown, white farm eggs and balady eggs) from different markets in Cairo and Giza cities. Collected samples were analyzed for detection of the antibacterial and aflatoxins (B1, B2, G1 and G2) residues in addition to study the effect of heat treatment (boiling and frying) on antibacterial and aflatoxins residues in positive samples (Al Hazzani et al., 2014).

The current results of FPT revealed that the incidence of antibacterial residues were 6.6%, 20% and 13.3% in balady, brown farm eggs and white farm eggs respectively. The different heat treatment completely degraded the antibacterial residues in balady eggs. However, some traces of antibacterial residues were existed after boiling and frying of farm eggs. Nearly similar results were reported by Antown and Hassan (2002) and Fath EL- Bab (2012). While lower results were obtained by Salem et al. (2009). Residues of furazolidone, chloramphenicol, sulphaquinoxaline, nitrofurazone, tetracyclines and other antimicrobial agents were detected in eggs of treated chickens (Roudaut et al., 1989; Tropilo & Stepien, 1989).

2.3. Pesticides

The OCP residues may concentrate in the adipose tissues and in the blood serum of animals leading to environmental persistence, bioconcentration and biomagnification through the food chain. Human body also gets contaminated. So far it has been reported that humans are mainly exposed to OCPs through ingestion. A study conducted in Tianjin, China, revealed that inhalation and dermal contact contributed to only 5.1% and 13.5% of the total intakes of DDTs and HCHs by adults, while ingestion through diet was responsible for 94.9% and 86.5% of the total, respectively (Goutner et al., 1997).

In the study by Aulakh et al. (2006) all the egg samples had values above the MRL for total HCH and heptachlor epoxide, while seven egg samples exceeded MRL for total DDT (Aulakh et al., 2006). Their result show contamination to α-HCH, β-HCH, γHCH , HCH- *o,p*, DDE- *p*, DDE -*o,p*, DDD -*p,p*, DDD -*o,p*, DDT- *p,p*-DDT ,∑-DDT, Endosulfan sulfate, Heptachlor epoxide in egg is, 0.04 ± 0.002, 0.16 ± 0.119, 0.06 ± 0.002, 0.26 ± 0.012, 0.16 ± 0.004, 0.08 ± 0.055, 0.13 ± 0.015, 0.05 ± 0.039, 0.02 ± 0.013, 0.07 ± 0.002, 0.51 ± 0.069, 0.17 ± 0.003, 0.11 ± 0.007, respectively. Reports also showed the presence of pesticide residues in poultry feed at the farm revealed that there was no apparent seasonal variability for the presence of residues. It appeared that sources of poultry feed for the farm remained the same and there were no appreciable differences between the contaminations of feed from different sources. The PPO-DDT was detected of eggs, chicken and meat with mean concentration of 0.142, 0.018 and 0.064 mg kg^{-1} fat, respectively. Eggs have the highest concentration compared to chicken and meat. The results indicated that 28% (38.134), of the examined eggs, were contaminated with OCP residues. The order of contamination in the analyzed samples was as meat > eggs > chicken. However, 39% (9.23) eggs and 4.6% (6.131) meat contaminated samples showed concentration above the MRLs of the FAO/WHO. The high levels of OCP residues measured in eggs in this study are surprising and raise questions about pesticides banned for more than 20 years in Jordan (Ahmad et al., 2010). But according to some reports, DDT is usually not detected anymore or only at trace level in commercial eggs, as observed in Spain (Fontcuberta, Arqués et al., 2008). in Belgium (17.30 ng/g^{-1} fat) (Van Overmeire et al., 2006). or in Sweden (6.6 ng g_1 fat) (Darnerud, Atuma et al., 2006). However, high level of DDT (457 ng/g^{-1} fat) was found in home-produced eggs in Belgium (Van Overmeire et al., 2009).

Surveys made in Iran (Hashemy-Tonkabony & Mosstofian, 1979) and in Kenya (Ayas et al., 1997) found levels of chlorinated hydrocarbons in eggs which exceeded the WHO levels in the case of Iran and some highly contaminated samples in Kenya.

2.4. Aflatoxin

The toxins produced by fungi can cause disease in animals and birds in which their direct or acute symptoms can be observed. They also can act by reducing the immune system of livestock and poultry and susceptible them to the pathogen, or could be entered in the human food chain through milk, meat and eggs, and cause danger to human health (Karami-Osboo et al., 2012). According to the FAO information, mycotoxins are worldwide problem. According to the FAO approximately 25% of world's grain supply is contaminated with mycotoxins. The greatest economic impact of mycotoxin contamination is felt by crop and poultry producers, as well as food and feed producers.

Aflatoxin is the most commonly occurring mycotoxin in Iran. Aflatoxins are a group of secondary metabolites produced by a certain species of fungus of the genus *Aspergillus* (especially *A. flavus* and *A. parasiticus*). These fungi are capable of growing and contaminating the grains and cereals at any time before and after the harvest, during storage, transportation and processing of feed ingredients and the formulated feeds after processing (Khoshpey et al., 2011).

Effects of aflatoxins on animals will vary depending on the concentration and duration of contact, breed, and diet. Natural zeolites are good additives which could be used for controlling fungal toxins (Allcroft, 1969).

2.4.1. Analytical methods

The most common analytical methods employed for aflatoxin determination are TLC, HPLC and ELISA. Among them, ELISA is often used for routine screening due to its several advantages, such as rapidity, simplicity and coste-ffectiveness (Rosi et al., 2007; Kursat et al., 2011).

2.4.2. Reports of aflatoxin contamination in egg

According to the reports of Pourelmi et al. (2013) the highest and lowest aflatoxin contaminations were 0.083 ng /ml and 0.050 ng /ml respectively. These values are lower than the usual limit of aflatoxin in feed (12 ng/ml) (Pourelmi et al., 2013).

There was no significant difference between industrial eggs from different farms of Mazandaran (P>0.05). There also was no significant difference between local eggs from different regions of Mazandaran (P>0.05). Numerically the local eggs had higher values of aflatoxin contamination in comparison to industrial eggs. Whereas in local eggs the aflatoxin concentration was not less than 0.080 (ng/ml) but in industrial eggs the highest value was 0.081 (ng/ml). Aflatoxin contamination of feedstuffs has been reported to be of a wide range from 1 to 900µg/kg in commonly used ingredients as well as mixed feed samples in developing countries (Manafi et al., 2012).

According to the study that was revealed that number of positive tested egg samples for total aflatoxins (B1, B2, G1&G2) residues in balady, brown farm eggs and white farm eggs were 30%, 16.6%and 20%; respectively. The mean values of aflatoxins residues in balady was 6.7 ± 1.7 ppb with the minimum and maximum values were 0.9 ppb and 14.3 ppb; respectively. The mean values of aflatoxins residues in brown farm eggs were 3.2 ± 1.2 ppb, with the minimum and maximum values were 0.34 ppb and 7.3 ppb; respectively and the mean values of aflatoxins residues in white farm eggs was 4.34 ± 1.1 ppb with the minimum and maximum values were 0.75 ppb and 9.1 ppb; respectively (Ozturk et al., 2014). These result were higher than those previously reported by Salem et al. (2009), but lower than those reported by Hassan (1995) who detected aflatoxin G2 residue contamination (80µg/kg) in baldy egg samples (Bahobail et al., 2012).

In a study done by Salem et al. (2008) a total of fifty random table egg samples were collected from EL-Beida city markets and Aflatoxin residues were detected only in 7 samples and the mean aflatoxin content residues was 0.542 ± 0.226 ppb (Bahobail et al., 2012).

Aflatoxin was analyzed in 80 eggs samples, collected from central areas of Punjab, Pakistan by Iqbal et al. (2014), reported that 28% samples of eggs were found positive with Aflatoxin contamination. The level of total Aflatoxine was 1.97 ± 0.94. In previous studies very limited reports have been documented for the presence of aflatoxin in eggs. Markov et al. (2013) from Croatia have documented that mycotoxins were detected in 64% of the 90 samples analyzed and found that concentration of AFB1 ranged to 3.0 mg/kg.(Iqbal et al., 2014)

Herzallah (2009) has documented the levels of Aflatoxin B1, B2, G1 and G2 in the analyzed food products, The average level of Aflatoxin B1 in total 10 samples of eggs was found 1.43 mg/kg, slightly lower than comparing to present findings of Aflatoxin B1 in 40 form eggs (2.41 mg/kg), and higher when compared to the level of 0.79 mg/kg AFB1 in 40 domestic eggs samples (Herzallah, 2009).

3. Conclusions

The chemical hazards and contaminations in production from animal origin particularly dangerous for human health, so it is essential to monitor their contamination in food production. Eggs are nutritious and economic foods in the human diet and are included in several food products due to their various functions in the body. Despite the nutritional content of eggs, some potential health risks are associated with their consumption, including exposure to environmental contaminants and in some instances individual allergies. Standardized and updateable analytical protocols need to be established to determine contamination of egg from legitimate chemical usage. A worldwide concerted effort is required to uphold the all-natural, wholesome and “clean and green” image of egg.

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