Comparative study of chemical composition and microbial loads of chicken eggs from Kurdish local chicken and retail markets in Iraqi Kurdistan Region

Dyar Hassan Hama Kawani[(1)], Othman Kareem Qadir[(2)], Tazhan Mohamed Ameen[(3)], Jaza Ali Mohammed[(4)]

[1, 2, 3]College of Agricultural Engineering Sciences, University of Sulaimani

[4]Sulaymaniyah Agricultural Research Center

dyar.hama@univsul.edu.iq ,othman.qadir@univsul.edu.iq ,tazhan.amin@univul.edu.iq ,jaza1977.ali@gmail.com

Abstract:

The aim of the study was to compare the chemical composition particularly protein content, minerals and some heavy metals and the level of microbial contaminations between Kurdish local chicken eggs and commercially available in retail markets in Kurdistan. Total protein in white egg, minerals (Fe, Mg, Cu, and Ca), heavy metals (Pb, Cd, and Co), and microbiological quality were assessed. The results show that depending on egg type it is possible to use such nutrient claims as "high protein". The examined eggs samples comply the permissible limits for trace elements in table eggs. The findings indicate that the concentrations level of heavy metals in eggs are set within the standard limits and considered as safe for human consumption. In addion, microbial loads off egg samples were tested for aerobic plate count which shows that 46.6% of examined entire contents of egg samples (Barn, Erbil, Domestic, Iran and Turkey) fulfilled with the standards and a total bacterial count within the permissible count (2.5×10^4/g) except one sample from barn of the coliform count was confirmed *E. coli*, and one sample from barn was positive for salmonella.

Introduction:

People in Kurdistan consume caged hens which imported from different neighbour countries such as Turkey and Iran and local eggs which produced in Erbil city in Kurdiatan. Some people are consider the domestic Kurdish local chicken eggs to be superior to eggs from caged hens**.**

Eggs are an outstanding source of micro and macro nutrients, including all essential amino acids, fats, water-soluble vitamins, minerals, lecithin and unsaturated fats [1,2]. In terms of daily diet, eggs (fresh eggs) are the most important and nutritious food item, and it's moreover included in many food products serving different functions [3].

Hen eggs quality (in terms of nutrition) are effected by many factors, some of the hen age and breed, the nutrient density and composition of hen feed along with rearing system[4]. Since eggs represent an essential portion of humans' diet (children in particular) and due to the elevated pollution in the global environment with trace elements, led to an increased investigation in terms of metal contamination of eggs included food-stuffs [5]. Iron (Fe) and Copper (Cu) are some of the indispensable required trace elements for humans, however, at high concentrations all metal elements are toxic [6 and 7]. Arsenic (As), Lead (Pb) and Cadmium (Cd) are heavy metals that accumulate over time and toxic when laid, eggs are mostly found to be sterile (90%), but it's possible for the eggs to be contaminated on the outer shell surface and internally [8].

Eggs are a good source of nutrients needed for growth of pathogenic and spoilage microorganisms. Egg spoilage depends on

 ISSN 2072-3875

temperature, handling, storage, and availability of nutrients in eggs [9]. Bacteria and fungi are micro-organisms that can contaminate eggs and elevate the hazards of causing spoilage and food borne diseases since the egg defense mechanism can be evaded by these micro-organisms and then penetrating into the egg. Food poisoning caused by the Salmonella serotypes (most frequently Salmonella Enteritidis, Salmonella, and Salmonella Typhimurium) which are the mostly prevalent pathogen of eggs that are found inside the eggs shell [10]. Another source of egg contamination with pathogen is by the ovarian infection either prior to laying or afterward due to the microorganism entry into the egg (in which elevated temperature and humidity are regarded as auxiliary conditions for the infection) causing spoilage and economical damage or create hazards in terms of public health]11[.

In Kurdistan, it's vital to perform an analysis for the eggs in terms of microbial, nutritional composition due to elevated eggs consumes in the region. Eggs quality is associated by the users in terms of yolk color and freshness. Additionally, the consumers in Kurdistan regard local chicken eggs as superior when compared to eggs from caged hens. Therefore, this study was aimed to identify and address the residual concentration levels of iron, cobalt, copper and lead in eggs of domestic local hen and commercially imported eggs, and to compare the nutritional composition particularly protein content and microbial contaminations.

Materials And Methods:

Sample collection:

The commercial and domestic kurdish local egg samples were collected randomly from various retail markets in Kurdistan. The samples were analyzed at the laboratories of College of Agricultural Engineering Sciences, University of Sulaimani.

Table (1): Source of egg samples:

Sample code	Farming method	Type of eggs in the market
1	Barn eggs (control)	Farmer – Barn eggs
2	Caged chicken eggs from (Erbil)	Eggs in boxes
3	Kurdish local chicken eggs (Domesctic)	Free range
4	Caged chicken eggs from (Iran)	Eggs in boxes
5	Caged chicken eggs from (Turkey)	Eggs in boxes

Protein Determination in Eggs according to the Kjeldahl Method:

The protein content in eggs is an important and essential parameter to determine in order to ensure the quality and safety of food. The nitrogen content in eggs sample was estimated by Kjeldahl's method [12]. Approx. 1.2 g of the homogenized sample were weighed in directly into a sample tube. A portion of 20 ml of sulfuric acid and 2 Kjeldahl tablets were added and the digestion was performed. After digestion the ammonia of the sample was distilled into a boric acid solution by steam distillation and titrated with sulfuric acid.

Preparation of egg samples for determine mineral and hevy metals :

Egg samples were washed with de-ionized water, the egg yolk and white were mixed in a 200ml beaker. A portion (5-10gm) of mixed sample was accurately weighed into 100ml beaker and 5-10ml 65% concentrated HNO3 was added and covered with watch glass. After 30 min the beaker was placed on hot

plate up to 140°C until the complete decomposition of sample was achieved and the total volume was reduced to nearly 3-5ml. the digested sample was cooled and filtered into a 50ml calibrated flask, the solution was injected to OES-ICP Perkin Elmer 2100 for determine [13] .

Microbial examination of eggs:

Ten-fold serial Decimal dilutions were aseptically prepared from the rinse solutions, as well as from the homogenous egg contents using 0.1% sterile peptone water.

The egg was prepared for evacuation of its content according to the method described in A.P.H.A. egg was washed with warm water, the egg was drained and immersed in70% Alcohol for 10 min, then flamed after it has been removed from alcohol. A hole was made in the blunt end of the egg by using sterile scalpel. The contents of each group (sample) were removed aseptically and received into a sterile mixer until the sample becomes homogenous [14].

Twenty-five ml of the egg was placed in a sterile stomacher bag to which 225ml of buffered peptone water was added. The sample was homogenized in the stomacher for one minute to obtain a homogeneous primary sample. Decimal serial dilutions of the primary sample for egg content were set up using test tubes containing 9ml of the diluent of 0.1% peptone water. Serial dilutions of the rinsate were pour plated on plate count agar All the media was prepared following the manufacturer's instruction and sterilized by autoclaving at 121°C for 20 min [15].

Total aerobic bacterial and coliform count:

Total viable count of all the egg content samples were determined by standard plate count method using nutrient agar by the pour plate technique and macconkey agar medium in duplicates for total aerobic bacterial counts (TABC) and total coliform count (TCC), respectively. The plates were then incubated at 37°C for (24-48) h and plates with colonies from 30 to 300 were used for determining TCC and TABC [16]. Colonies were measured as colony-forming units (CFU) per mL using number of bacteria/mL = Number of colonies on the plate*reciprocal of the dilution of the sample(17) .

Examination for Salmonella:

Aseptically weigh 25g of egg content into a sterile 500 ml screw cap jar, add 225 ml pepton water , and mix well by swirling, loosen cap 1/2 turn and incubate for 24 hrs at 37°C. After incubation, transferred 1 ml of the culture to 10 ml of tetrathionate broth , and incubated at 37°C for 24 hrs. A loopful of tetrathionate broth culture were streaked on to three selective agar media of Salmonella-Shigella agar (SS agar) salmonella on ss agar appear colorless colonies, production of H 2 S turn the center of colony to black , and then incubated at 37°C for 24 hrs, after incubation , the plate were examined for typical colony, and suspected Salmonella colonies were sub culture to a selective MacConkey agar plate and non-selective Nutrient agar plate and incubated at 37°C for 24 hrs for more confirmation conducted to microscopic examination [18] .

Statistical analysis:

The data were statistical analysis according to the method of analysis of variance as a general test. Factorial experiment with three replications was used by (XLSAT) program ver. 7.5.2 and conducted using Complex Randomized Design (CRD). All possible comparisons among the means were carried out by using (Dunkin) test at the significant level of 0.05 after they show their significant in the general test.

Results and discussion:

Protein contents:

Table 2 shows the results of protein percentage of eggs from different egg samples shows that protein content is vary among samples of eggs between 10.30 - 13.02%. There was a significant differences in caged chicken eggs from Tureky compared to other

 ISSN 2072-3875

egg samples. Maximum value of protein was recorded in eggs from caged hens in Erbil 12.867% and minimum value was recorded in eggs from caged hens in Tureky 10.3% which was less than of the protein in barn eggs 13.023%, Kurdish local chicken eggs 12.237%, Erbil 12.867% and Iran 12%. This difference might be refer to hens feed and balanced dietary protein levels [19].

Table (2): The percentage of protein content in different type of eggs

Egg samples	**Protein %**
Barn eggs (control)	13.023[a]
Caged chicken eggs from (Erbil)	12.867[a]
Kurdish local chicken eggs (Domesctic)	12.237[a]
Caged chicken eggs from (Iran)	12.000[a]
Caged chicken eggs from (Turkey)	10.300[b]

N=3, ($p<0.05$)

Minerals:

The results showed that disparity in metals concentrations in eggs among the samples. The concentration of heavy metals in eggs are presented in (Table 3). In general, Iron (Fe) appear relatively high concentration in egg content which ranged between 1.56 to 3.84 ppm, the highest iron content was observed in Kurdish local chicken eggs which was 3.840ppm and the lowest was in caged chicken eggs from Tureky 1.560ppm. Magnesium (Mg) ranged between 12.53 in Kurdish local chicken eggs to 18.48 ppm in caged chicken eggs from Erbil. There was no significant difference in Copper (Cu) and Calcium (Ca) among the egg samples, which Cu ranged between 0.069 to 0.092 ppm, and Ca ranged between 15.12 to 20.75 ppm. These results didn't differ significantly from levels of minerals were determined in eggs by another investigators [20]. In the absence of dietary informations about the hens, the variation in egg minerlas may refers to the dietary sytem, hen age, hen strain and farming methods. Therfore, we cannot identify reasons that contributedto these variation precisely [21].

In table (4), Cobalt (Co) have been the lowest concentration ranged 0.005 to 0.006 ppm. The level of Cadmium (Cd) concentration ranged from 0.006 to 0.034 ppm. The concentration of Pb level ranged between 0.053 to 0.112 ppm. It has been reported that free-range system hens produced eggs richest in micronutrients (K, Na, Ca, Mg, Zn, Se, Mn and Fe), as it allows hens to supplement their dietary ration [22]. It is in partial agreement with our results. Content of lead and cadmium in all egg samples were below the detection level, therefore, it can be concluded that all egg samples were free of heavy metals. However, a study in Egypt has established equally noticeable contamination with Pb and Cd in both free range eggs and caged hens' eggs [23].

 ISSN 2072-3875

Table (3): Minerals in different type of eggs (ppm)

Egg samples	Means			
	Fe	**Mg**	**Cu**	**Ca**
Barn eggs (control)	1.950[b]	13.880[b]	0.087[a]	19.810[a]
Caged chicken eggs from (Erbil)	1.930[b]	18.480[a]	0.085[a]	20.750[a]
Kurdish local chicken eggs (Domesctic)	3.840[a]	12.530[b]	0.081[a]	19.250[a]
Caged chicken eggs from (Iran)	1.870[b]	14.020[b]	0.069[a]	19.200[a]
Caged chicken eggs from (Turkey)	1.560[b]	14.300[b]	0.092[a]	15.120[a]

N=3, ($p<0.05$)

Table (4): The concentraction of heavy metals in different type of eggs (ppm)

Egg samples	Means		
	Co	**Cd**	**Pb**
Barn eggs (control)	0.005[a]	0.008[b]	0.104[b]
Caged chicken eggs from (Erbil)	0.005[a]	0.034[a]	0.053[e]
Kurdish local chicken eggs (Domesctic)	0.005[a]	0.007[b,c]	0.112[a]
Caged chicken eggs from (Iran)	0.006[a]	0.008[b]	0.074[c]
Caged chicken eggs from (Turkey)	0.005[a]	0.006[c]	0.059[d]

N=3, ($p<0.05$)

Microbial contamination in eggs:

According to the results reported in Table (5), the highest values of total bacteria counts was in Barn 3×10^4, the lowest value was in Turkey, the high results may be due to bad handling of eggs during storage with high temperature specially in summer months and under humid conditions. In addition, the results for total coliform counts were positive in three treatment (Barn, Domestic, Iran) wih mean value 3×10^2, 8×10^2, 2×10^2 (cfu/ml) (Table 6), only one sample from barn of the coliform count was confirmed *E. coli*, and only one sample from barn was positive for salmonella test. The high counts of coliforms may be due to bad sanitary conditions and/or delay in eggs collection from nests which were contaminated with fecal matters.

Tested samples for aerobic plate count showed that 46.6 % of the total examined samples of entire egg contents of Barn, Turkey, Iran, local, and Erbil eggs content fulfilled with Egyptian standards with a total bacterial count within the permissible count (2.5× 104/g) stated by the (Egyptian Organization for Standardization and Quality Control, [24] of egg. The mean total viable count for the egg content lower than the accepted $10x10^5$ gm/cfu as recommended by the International Commission on the Microbiological Specification for Food ICMSF [25]. A microbial load of less than 2 log cfu packaged egg is considered an excellent commercial standard [26] .

The absence of standard structures and drainage system in the market and the relatively high humidity could have contributed to the high microbial growth. It was also found out that most retailers do not store eggs in refrigerators, thus the eggs are exposed to weather conditions, resulting in their contamination. The isolated microbes could cause severe health problems like, diarrhea, nausea and abdominal pain, since they are pathogenic. Microorganisms can be

found inside egg content; may be due to the fact that the egg emerges from the hens body through the same passageway feces is excreted and fecal contamination through the pores on the shell after they are laid.

After deposition, eggs may also come into contact with environmental bacteria which may due to temperature, soil, length of storage, dirty nesting materials, cloths, hands of poultry workers, dust, the environment, weather conditions, transporting and marketing [27]. This resulting in eventual penetration of the shell. Entrance of microorganisms into egg content may be either by penetration or with drawal through pores of the shells [28].

Table (5): The total bacterial count in different type of eggs (cfu/ml)

Egg samples	Mean of total bacteria counts (cfu/ml)
Barn eggs (control)	3×10^4
Caged chicken eggs from (Erbil)	3×10^2
Kurdish local chicken eggs (Domesctic)	3.5×10^2
Caged chicken eggs from (Iran)	4×10^2
Caged chicken eggs from (Turkey)	2×10^2

Table (6): Coliform bacteria count in different type of eggs (cfu/ml)

Egg samples	Mean of total coliform counts (cfu/ml)
Barn eggs (control)	3×10^2,
Caged chicken eggs from (Erbil)	1.0×10
Kurdish local chicken eggs (Domesctic)	8×10^2
Caged chicken eggs from (Iran)	2×10^2
Caged chicken eggs from (Turkey)	1.0×10

Conclusion:

This study evaluates chemical composition and microbial loads of table eggs at retail markets from major and Kurdish local chicken in Iraqi Kurdistan. Toxic heavy metals can have serious adverse impacts on human health. For this reason, the present investigation is mainly focused on the evaluation of Pb, Cd, Co, and Cu in egg samples, collected from the city of Kurdistan. According to the results, the concentration of essential trace metals were not found higher within the permissible limit. The results of microbial contents in eggs of retails can be attributed to unhygienic conditions in the markets and poor handlings. Therefore, it is recommended to establish and enforce quality control and inspection regulations of table eggs to provide safe and good quality eggs for consumption. Also, retailers can store their eggs under good hygienic conditions in refrigerators.

References:

1- Miranda J.M, Anton X., Redondo-Valbuena C., RocaSaavedra P., Rodriguez J.A., Lamas A., Franco C.M., Cepeda A. (2015) Egg and Egg-Derived Foods: Effects on Human Health and Use as Functional Foods. *Nutrients,Vol. 7(1), p. 706–729.*

2- Kralik G., Kralik Z. (2017) Poultry products enriched with nutricines have beneficial effects on human health. *Medicinski Glasnik, Vol. 14 (1), p. 1–7.*

3- Sharkawy, A.A. and Ahmed, Eman, Kh. (2002). Determination of lead, cadmium, mercury and copper concentrations in hen's eggs at Assiut Governorate. *Assiut Vet. Med. J., 48 (95): 45-59.*

4- Küçükyılmaz K., Bozkurt, M., Herken, E.N., Çınar M.,Çatl A.U., Bintaş E., Çöven F. (2012) Effects of rearing systems on performance, egg characteristics and immuneresponse in two layer hen genotype. *Asian-Australasian Journal of Animal Sciences, Vol. 25(4), p. 559–568.*

5- International Dairy Federation, IDF (1991). Monograph on residue and contaminants in milk and milk products. *Brussels (Belgium), chapter 6, Carl, M.(ed),112-119.*

6- Elzbieta Rolka and Mirosław Wyszkowski, (2020). Availability of Trace Elements in Soil with Simulated Cadmium, Lead and Zinc Pollution. *Minerals*, 11, 879.

7- Lane, T.W. and Morel, F.M. (2009). A biological function for cadmium in marine diatoms. *Proceeding of National Academy of Science. p. 462–31.*

8- Egg Safety Center, (2010). Pathogens. www.eggsafety.org /consumers /pathogens European Food Safety Authority, Opinion of the Scientific Panel on Biological Hazards on the Request from the Commission Related to the Microbiological Risks on Washing of Table Eggs. The UFSA Journal, 269: 1-39.

9- Al-Bahry SN, Mahmoud IY, Al-Musharafi SK and AlAli MA, (2012). Penetration of Spoilage and Food Poisoning Bacteria into Fresh Chicken Egg: A Public Health Concern. Global Journal of Bio-Science and Biotechnology, 1(1): 33-39.

10- Gaungoo Y and Jeewon R, (2013). Effectiveness of Training among Food Handlers: A Review on the Mauritian Framework. Current Researchin Nutritionand Food Science, 1(1):1-9. http://www.foodandnutritionjournal.org/?p=329.

11- Board, R.G. and Fuller, R.(1994): Microbiology of avian egg. 1st ed. Chapman and Hall p. 94 –112–128.

12. AOAC. 2000. *Association of Official Analytical Chemistry. 17th edition.* Washington DC:: Official method of Analysis".

13- Siddiqui Ishratullah, Nazami,. S. S, Khan,. F A, Bhutto,. S, Tahir,. M, Munshi,.A. B,. Syed N.(2011). Determination of some heavy metals in hen eggs using ICP-AES technique. Journal of Pak. J. Biochem. Mol. Biol , 44(4): 133-136 .

14- A.P.H.A.(American Public Health Association)(1992):Standard Method for Examination of Dairy Products.16th Ed.American Public Health Association. Washington, DC, USA.

15- De Reu K, Grijspeerdt K, Heyndrickx M, Uyttendaele M, Debevere J and Herman L, (2006). Bacterial Shell Contamination in the Egg Collection Chains of Different Housing Systems for Laying Hens. British Poultry Science, 47: 163-172.

16- De Reu K, Grijspeerdt K, Messens W, Heyndrickx M, Uyttendaele M, Debevere J, Herman L (2005). Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including Salmonella enteritidis. International Journal of Food Microbiology 112: 253-260.

17. Quinn, P.J.; Carter, M.E.; Markery, B.K. and Carter, G.R. (1994): Clinical Veterinary Microbiology. Wolfe pub. Europ limited 209–236.

18. A.O.A.C. (Association of Official Analytical Chemists) (1980): Official methods of analysis. 15th Ed. Benjamin Franklin Station. Washington.

19- M.Y. Shim, E. Song, L. Billard, S.E. Aggrey, G.M. Pesti, P. Sodsee, (2013) Effects of balanced dietary protein levels on egg production and egg quality parameters of

 ISSN 2072-3875

individual commercial layers, *Poultry Science*, Volume 92, Issue 10, Pages 2687-2696.

20- Azza M. K. Sobeih and Hanaa, M.R. Hegazy. (2011). Determination of Some Heavy Metals in Table Hen's Eggs. *Journal of American Science*;7(9). Pages 224 -229

21- Laura E. Heflin, Ramon Malheiros, Kenneth E. Anderson, LuAnn K. Johnson, Susan K. Raatz, (2018). Mineral content of eggs differs with hen strain, age, and rearing environment, *Poultry Science*, Volume 97, Issue 5, Pages 1605-1613

22- Kiczorowska B., Smolinsky W., Kwiecień M., Winiarska-Mieczan A., Rusinek – Prystupa E., Ridha A., Al-Yasiry M. (2015) Nutritional value and the content of minerals in eggs produced in large-scale, courtyard and organic systems. *Journal of Elementology, Vol. 20(4), p. 887–895*

23- Ahmed Abdel-Hameid A., Enas El-Prince M., Doha Y., Al-Shimaa M.F. (2017) Lead, cadmium and copper levels in table eggs. *Journal of Advanced Veterinary Research, Vol. 7(3), p. 66–70.*

24- Osei-Somuah A, Otsyina HR, Arthur CT, Nortey PWK, Hammond V (2003). Microbial Quality of table eggs sold on selected markets in Accra. Ghana Veterinary Medical Association Bi-Annual Newsletter, 6: 314-318.

25- International Commission on Microbiological Specifications for Foods (ICMSF) (1998). Microorganisms in Foods 5. Microbiological Specifications of food pathogens. Blackei Academic and professional. New York, NY; pp 68.

26- Bell DD, (2002). Introduction to the U.S Table-Egg Industry.In:Commercial Chicken Meat and Egg Production (Bell DD and Weaver WD Jr.eds.).5thed. pp. 931-945. Kluwer Academic.Norwall, MA.

27. Egg Safety Center 2010. Pathogens. www.eggsafety.org/consumers/pathogens. Egyptian Organization for Standardization and Quality Control (E.O.S.Q.C.)

28- Neamatallah – AA. (2009) Biosafety Against Fungal Contamination of Hen's Eggs and Mycotoxins Producing Species . *Meteorology, Environment and Arid Land Agriculture Sciences Journal*, 2: 1319-1039.

مقارنة بعض المكونات الغذائية للبيض والمحتويات الميكروبية بين الدجاج المحلي الكردي والدجاج التجاري المتوفر في إقليم كردستان العراق

ديار حسن حمة كاواني[1] ،عثمان كريم قادر[2]، تازان محمد امين[3] ، جزا علي محمد[4]

[2, 31] قسم علوم الاغذية والسيطرة النوعية ،كلية علوم الهندسة الزراعية، جامعة السليمانية

[4]مديرية البحوث الزراعية السليمانية ، وزارة الزراعة والري

المستخلص :

كان الهدف من الدراسة هي مقارنة القيمة الغذائية و خاصة محتوى البروتين والمعادن ، ثم بعض المعادن الثقيلة و التقييم المايكروبي بين بيض الدجاج المحلي الكردي والأقفاص المتوفر تجارياً في كردستان. تم تحليل متوسط عينة مكونة من خمسين بيضة من كل نوع في مختبر كلية علوم الهندسة الزراعية قسم علوم الاغذية والسيطرة النوعية بجامعة السليمانية. تظهر النتائج أنه اعتمادًا على نوع البيض كانت جميع عينات البيض آمنة من الناحية الميكروبيولوجية ولا تحتوي على معادن ثقيلة. تتوافق عينات البيض المفحوصة مع الحدود المسموح بها للعناصر النزرة في بيض المائدة ، وتشير النتائج إلى أن مستوى تركيزات المعادن في البيض في أسواق مدينة كوردستان تم وضعه ضمن الحدود المعيارية وتعتبر آمنة للاستهلاك البشري. أظهر عدد الصفائح الهوائية أن 46.6٪ من المحتويات الكاملة التي تم فحصها من محتوى البيض في قفص ، ديك رومي ، إيران ، محلي ، وبيض هولر تم استيفائها مع المعايير مع العدد الإجمالي للبكتيريا ضمن العدد المسموح به.