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Monitoring antimicrobial residues in table eggs in Aswan governorate markets and their impact on egg quality and public health

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Abstract

Background: Organic egg is among the most common organic foods offered for sale in Egyptian markets in recent years, and consumers buy them at a higher price because they believe organic eggs are safer and have superior nutritional value than conventional eggs.

Aim: The present work aimed to monitor antimicrobial residues in brown table eggs, whether conventional or organic type, in Aswan governorate markets and assessed their physical and chemical quality and their public health hazards.

Methods: Brown table egg samples (n = 400 total) were randomly selected in the present study, in which they represented two equal groups (n = 200 each) including conventional eggs and organic eggs. Eggs were collected from different retail stores in the Aswan governorate, Egypt. Egg samples were subjected to thorough physical and chemical quality evaluation as well as an assessment of antimicrobial residues.

Results: The results reported that organic eggs were cleaner and had a better odor, less blood, and meat spots, but smaller with more shell cracks than conventional eggs. Chemical analysis of some nutrient contents in the egg yolk revealed significantly higher nutritive values of organic eggs than that of conventional ones as the organic eggs contain significantly higher levels of vitamin A and vitamin D/D3 and significantly lower values of cholesterol, calcium, magnesium, and zinc than those in conventional eggs. Disc diffusion assay has been used for monitoring antimicrobial residues in egg samples. The results have shown that all examined organic eggs were free from antimicrobial residues, while 12% and 8% of conventional egg yolk and white were positive for antimicrobial residues, respectively.

Conclusion: The study concludes the higher nutritive value of organic eggs compared with the conventional type because of their significantly higher contents of vitamins A and D and their significantly lower contents of cholesterol. Moreover, organic eggs were free from antimicrobial residues which maximizes their public health benefits..

Keywords: Antimicrobial residues, Chemical and physical quality, Conventional eggs, Nutritive egg values, Organic eggs.

Introduction

Eggs are considered a highly valuable and economical source of animal protein (Żakowska-Biemans and Tekień, 2017). Egg protein is second to breast milk in its benefits and has the closest nature to breast milk protein. Moreover, the human body can successfully absorb approximately 98% of the protein in eggs (Xia *et al.*, 2022).

In Egypt, chicken eggs are considered an important component of human daily nutrition intake, one of the most popular foods, and one of the least expensive when compared to other nutrients from animal sources (Karmi *et al.*, 2019). Eggs often provide people of all ages with well-balanced nutrients; they contribute 20% to 30% of the vitamins E, A, and B-12 and 10% to 20% of the dietary folate for egg consumers. Furthermore, eggs are suitable for many adult therapeutic diets because of their ease of digestion and low-calorie content (Ebied *et al.*, 2021).

In recent years, medical and nutritional scientists discover new functions and nutritional values of eggs in health care such as the prevention and treatment of atherosclerosis, improvement of intelligence,

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nourishment of the brain, delaying of aging, prevention of cancer, and protection of liver (Xia *et al.*, 2022).

Housing systems may exert a significant impact on poultry products and their quality. Whenever it concerns the performance, health, and welfare of the birds, every housing system has benefits and drawbacks. For layer chickens, the ideal housing design should be considered to maximize egg production and egg quality properties (Batkowska *et al.*, 2014), Regarding the layers of housing systems in Egypt, egg production, egg weight, and egg mass were better obtained in the cage system rather than floor system (Abo Ghanima *et al.*, 2020). Furthermore, customers are becoming more and more interested in poultry products from alternative housing systems because they are natural, organic, and contain fewer harmful substances to human health (Da Silva Pires *et al.*, 2021a, 2021b). The housing system effect on the egg quality produced by layers is conflicting, some research reporting no difference in egg quality (Ahammed *et al.*, 2014). Meanwhile, a very recent study emphasizes that more optimum production results could be obtained by alternative housing systems (free-range system) (Alig *et al.*, 2023a).

Currently, because of the presence of toxic substance residues such as antimicrobials, heavy metals, mycotoxins in conventionally produced food, the demand for organic food has increased (Śmiechowska and Dmowski, 2005). Antimicrobials are typically used by poultry farmers as drugs in drinking water or feed to prevent or treat illnesses as well as a growth stimulator (Hafez *et al.*, 2013). Tetracyclines, quinolones, aminoglycosides, macrolides, beta-lactams, and sulfonamides are just a few of the antibiotic families used to treat egg-laying chickens that are almost identical to those used in human medicine. As a result, their residues are prohibited in human food (Stolker and Brinkman, 2005).

Additionally, the use of antimicrobials in laying hens without proper control may result in the accumulation of residues in eggs, causing a risk to the health of consumers. When the utilization of antibiotics is required for illness treatment or prevention, a withdrawal period may be observed until the residues vanish or become insignificant (Nisha, 2008). Antimicrobial residues in eggs may lead to toxicity, skin rashes, allergic reactions, antibiotic resistance, and the emergence of cross-resistance across other antibiotics with comparable structural features or the same mode of action (Roberts, 2004; Shehata *et al.*, 2014).

In organic chicken farms, the use of antibiotics is prohibited; instead, the birds may get vaccines as a kind of preventative medicine and nutritional supplements containing extra minerals and vitamins. Hens consume feed that is certified organic and free of animal byproducts. Access to the outdoors, direct sunlight, shade, exercise areas, and fresh air are requirements for successful poultry farming (Henry, 2002; USDA, 2009; Ebied *et al.*, 2021).

Most consumers prefer organic food because they believe it is safe, devoid of chemical residues, palatable, and nutritious (Fesseha and Aliye, 2019). Because of the higher cost of their cultivation, acquisition, processing, and distribution as well as additional costs related to product certification and segregation; organic eggs are more expensive to purchase than comparable conventional eggs. The production of organic eggs and organic foods, in general, is currently constrained by the high costs and the scarcity of organic grains (Hafez *et al.*, 2013). However, the advantages of organic products inspire consumers to pay more for a nutritious and safe product, which encourages retailers to expand the markets to meet consumer demand and fuels major economic growth (Ruiz *et al.*, 2018).

For maintaining a safe industry and encouraging people to buy healthy products, the government must tighten the control measures over the markets and collect samples regularly to ensure that it is free from harmful residues and meets international standards.

Accordingly, the current work was conducted to monitor antimicrobial residues in brown table eggs of both the conventional and organic types in Aswan governorate markets. We assessed the differences in physical and chemical properties and their impact on egg quality and public health to assure that organic eggs meet consumer needs and expectations.

Materials and Methods

Samples collection

Brown table egg samples (n = 400) were purchased from supermarkets in Aswan governorate, Egypt, where they are divided into two equal groups (n = 200 for each) including conventional eggs and organic eggs. Samples were transported to the laboratory in a cooler as quickly as possible and kept in a refrigerator until examination for physical and chemical quality. All the samples of organic eggs were in their first third of shelf-life and were stored refrigerated, while the conventional eggs were stored at room temperature in markets and their shelf-life was roughly considered by a physical examination of yolk position by candling.

Physical examination of exterior eggs quality traits

A digital balance was used to measure eggs' weight. Then, the form, cleanliness, color, and soundness of the shells were assessed visually. Additionally, the odor and shell texture were checked by smell and palpation, respectively. According to the United States Department of Agriculture's Food Safety and Inspection Service, an exterior physical evaluation was conducted (USDA, 2000).

Physical examination of interior eggs quality traits

Quality standard procedures were the suitable technique adopted for measuring the quality of internal egg parts (Monira *et al.*, 2003; Fayeye *et al.*, 2005). The general appearance of the internal egg components, smell and any other defects or abnormalities were observed. For estimation of the yolk and albumen and heights, an

electronic tripod micrometer was used. pH values were detected by the pH meter.

Determination of cholesterol levels in eggs yolk

The titrimetric method, according to AOAC 941.09 protocol (AOAC, 2005) was employed to estimate the cholesterol contents of eggs' yolk.

Determination of vitamin A and D3 levels in eggs yolk

Eggs' yolk contents of vitamins D3 and A were measured by an official method using a spectrophotometer (AOAC, 1995).

Determination of minerals contents in eggs yolk

An atomic absorption spectrometer (Unicam 929) was used according to Szymanek *et al.* (2019) to measure the mineral contents in egg yolk, including calcium (Ca), magnesium (Mg), and zinc (Zn).

Detection of antimicrobial residues, i.e. disc assay screening method

According to the method described by Karmi *et al.* (2019), a qualitative disc assay method was used for measuring antimicrobial residues in egg yolk and white. *Bacillus stearothermophilus* was used as an indicator organism, whereas discs were immersed into the egg yolk and white separately. Thereafter, the discs were fixed on the surface of agar plates with two control discs of ciprofloxacin (positive control) and distilled water (negative control). The plates were then incubated at 37°C for 24 hours. The inhibition zone around the discs was measured and recorded. The disc would be considered as positive for antimicrobial residue presence when 1 mm or more of diameter represented by an inhibition zone around the disc had been described.

Statistical analysis

Obtained data were analyzed using IBM Statistical Package for the Social Sciences Statistics for Windows, Version 20.0. (IBM Corp., Armonk, NY). Data were entered as categorical or numerical, as appropriate. Quantitative data were presented as mean ± SD. The significance between two parametrically distributed quantitative variables was measured by an independent sample *t*-test. The result is considered statistically significant when $p < 0.05$.

Ethics approval

All animal procedures performed in this study were conducted in accordance with and approved by the ethical committee of the Faculty of Veterinary Medicine, Assiut University, Egypt, and in accordance with the Egyptian bylaws and OIE animal welfare standards for animal care and use in research and education.

Results

In the current study, the external and internal qualities of 200 conventional and 200 organic eggs sold in retail markets in the Aswan Governorate were compared. The results as shown in Table 1, have shown that the percentage of organic eggs with a dirty shell, rough shell, tainted odor, small blood spots, and meat spots were less than those in the conventional eggs, but organic eggs contained more shell cracks than conventional eggs, while neither conventional nor organic eggs having embryo, discolored white, bloody yolk or white, stuck yolk and mold growth. With reference to these results, it was noted that organic eggs have better physical properties than conventional eggs, but organic eggs have a thinner and weaker shell with more cracks than conventional eggs. The shell of the normal egg should be smooth, clean, and free from cracks to avoid penetration by microorganisms.

The obtained data in the present study shows that eggs from the organic system are remarkably smaller and lighter than conventional eggs whereas the mean value of egg weight, albumen weight, albumen height, yolk weight, yolk height, and Haugh unit are significantly ($p < 0.05$ or $p < 0.01$) higher in conventional eggs

Table 1. Physical examination of exterior and interior raw egg quality traits in conventional ($n = 200$) and organic ($n = 200$) eggs.

	Conventional eggs		Organic eggs	
	No.	%	No.	%
Dirty shell	12	6	7	3.5
Cracked shell	8	4	14	7
Rough shell	10	5	8	4
Tainted egg	3	1.5	0	0
Embryonated egg	0	0	0	0
Discolored white	0	0	0	0
Small blood spots	13	6.5	5	2.5
Bloody eggs	0	0	0	0
Stuck yolk	0	0	0	0
Meat spots	5	2.5	3	1.5
Mold growth	0	0	0	0

(No.): Number of positive.

than those in organic eggs. Moreover, no significant alterations in values of albumen pH or yolk pH are demonstrated between organic eggs and conventional eggs through the current study (Table 2).

The present work reports that cholesterol content in organic egg yolk is significantly ($p < 0.01$) lower than those in conventional egg yolk. The data reported in Table 3 have pointed out that the mean levels of vitamins A and D are significantly ($p < 0.01$) higher in organic egg yolk compared with conventional egg yolk, which indicates a significant increase in the level of vitamins A and D in organic eggs. Currently, the reported results as in Table 3 indicate that the mineral contents of egg yolk in conventional table eggs are remarkably greater than those in organic ones whereas levels of Ca, Mg, and Zn, in the yolk of organic eggs are significantly lower than those in conventional type.

The microbial inhibition test used in the present study is a qualitative assay used for quick and easy screening of many samples to detect many antibiotics. Table 4 shows that all examined organic eggs were free from antimicrobial residues, while 12% and 8% of conventional egg yolk and conventional egg white were positive for antimicrobial residues, respectively.

Discussion

The production of organic food has been based on special standards, so it is considered safe food and environmentally friendly, and free from chemical residues (Kouba, 2003; Newerli-Guz and Śmiechowska, 2004). Chicken eggs are one of the most specific foods that are organically produced worldwide. Organic eggs, also called firewood eggs or native eggs, are laid by free-range or native hens, whereas those hens have been raised on natural organic food and grown in healthy natural environments (Dangour *et al.*, 2010).

Consumers form their first impressions of products based on their external appearance of eggshells, but the physical features of the internal egg components are as crucial for ensuring the safety, soundness, and wholesomeness of the product. Table eggs are like a sealed box; if they do not meet expectations, consumer confidence begins to decline (Ansah *et al.*, 2009). In the current study, the external and internal qualities of 200 conventional and 200 organic eggs sold in retail markets in the Aswan Governorate were compared. The results have shown that the percentage of organic eggs with a dirty shell, and other quality parameters is lower than that of the conventional eggs. The shell of the normal egg should be smooth, clean, and free from cracks to avoid penetration by microorganisms according to the guidelines established by the USDA (USDA, 2000). Eggs get contaminated during the laying process, transportation, and storage, or from fecal matters, litter materials, dust, clothes, and hands of the workers (De Reu *et al.*, 2006). Eggs with a dirty shell are of lower quality, and in certain countries, they cannot be sold or used for human consumption (Jacob *et al.*, 2011).

Rough shell defect may be inherited, or because of excessive use of antibiotics, excess Ca supplementation, copper deficiency, and some diseases such as infectious laryngotracheitis, Newcastle disease, and infectious bronchitis (Beyer, 2005). While shell cracks may be because of bad handling or thinning of eggshell. Different feeding regimen that does not provide the organic hens with the sufficient amount of micronutrients needed to increase their production can have negative effects on eggshell breaking strength (Minelli *et al.*, 2007). Similar results were reported previously (Hafez *et al.*, 2013; Ebied *et al.*, 2021).

Small blood spots are caused by the rupture of small blood vessels and ovarian hemorrhage during egg formation. This defect may be inherited, or because of ovarian diseases, vitamin K deficiency, high ambient temperature, old age hens, and using sulfa drugs and antibiotics (Jacob *et al.*, 2011). Meat spot is defined as any foreign body inside the egg except the heat spot. They may be fatty, fleshy. or liver-like materials floating freely in the white or embedded in chalazae or attached to the yolk. Egg with meat spot and tainted eggs are considered unfit for human consumption (Karmi *et al.*, 2019).

Consumers usually prefer larger eggs (USDA, 2000), variations in egg weight among different sources can affect different egg parts, this difference may be attributed to the age and strain of the hens (Alsaffar *et al.*, 2013), nutritional contents of the hens' diet (i.e., protein, fat, minerals, vitamins and total energy), housing system and density, environmental stress, health status, and feed intake (Attia *et al.*, 2014; Bovera *et al.*, 2014).

The obtained data in the present study (Table 2) indicates that eggs from the organic system are remarkably smaller and lighter than conventional eggs whereas the mean value of egg weight, albumen weight, albumen height, yolk weight, yolk height, and Haugh unit are significantly higher in conventional eggs than those in organic eggs. Moreover, no significant alterations in values of albumen pH or yolk pH are demonstrated between organic eggs and conventional eggs through the current study. The results were consistent with the previous articles reported by Śmiechowska and Dmowski (2005); Minelli *et al.* (2007); Hafez *et al.* (2013).

The alteration in size, weight, pH values, albumin, and yolk highest may be due to the way the eggs were stored at the point of sale. Refrigeration of egg at the point of sale is not required in European Union (EU) and Latin America to avoid excessive rising in cost (Da Silva Pires *et al.*, 2020). In Egypt, too that is not required.

Da Silva Pires *et al.* (2021a, 2021b) reported that egg coating is also a potent factor to reduce weight loss by covering the shell pores. The egg quality may

Table 2. Mean values of raw eggs characteristics traits in conventional (n = 200) and organic (n = 200) eggs.

	Conventional eggs	Organic eggs	p value
Egg weight (g)	62.18 ± 4.37	52.31 ± 3.21**	0.0001
Albumen weight (g)	33.27 ± 1.87	27.26 ± 2.43**	0.0001
Albumen height (mm)	6.12 ± 0.52	5.46 ± 0.52**	0.0002
Yolk weight (g)	18.84 ± 1.93	16.17 ± 1.23**	0.0001
Yolk height (cm)	1.43 ± 0.12	1.31 ± 0.06**	0.0003
Haugh unit	73.20 ± 1.94	71.92 ± 1.95*	0.0412
Albumen pH	9.18 ± 0.36	9.41 ± 0.41	0.0686
Yolk pH	6.11 ± 0.25	6.32 ± 0.23	0.079

Significant when the values of organic eggs compared with those of conventional eggs ($p < 0.05$; **$p < 0.01$).

Table 3. Mean values of some nutrient contents levels in conventional (n = 200) and organic (n = 200) eggs.

	Conventional eggs	Organic eggs	p value
Cholesterol (mg/g yolk)	15.8 ± 0.71	13.6 ± 0.83**	0.0001
Vitamin A (µg/100g)	135 ± 4.83	154 ± 6.72**	0.0001
Vitamin D/D3 (µg/100g)	2.1 ± 26	2.8 ± 0.35**	0.0001
Calcium (mg/100 g)	143.45 ± 3.63	131.52 ± 2.86**	0.0001
Magnesium (mg/100 g)	25.64 ± 3:34	18.71 ± 1.92**	0.0001
Zink (mg/100 g)	4.37 ± 0.65	3.21 ± 0.48**	0.0001

Significant when the values of organic eggs compared with those of conventional eggs ($p < 0.01$).

Table 4. Detection of antimicrobial residues in the yolk and white of examined eggs in conventional (n = 200) and organic (n = 200) eggs.

	Conventional eggs		Organic eggs	
	No.	%	No.	%
Eggs yolk	12	12	0	0
Eggs white	8	8	0	0

(No.): Number of positive.

be affected also due to the farming systems, birds' genotype, nutrition strategy, and cages used can have a direct influence on egg quality (DalleZotte *et al.*, 2021; Alig *et al.*, 2023b).

In human nutrition, cholesterol levels in different foods are of concern. Cholesterol is a very important biological molecule that has a significant role in membrane structure and is a precursor for steroid hormone synthesis, vitamin D, and bile acids. However, the increased cholesterol level in the blood than normal is very dangerous, as serum cholesterol is a major risk factor for human cardiovascular diseases (CVD) such as coronary heart disease and stroke (Tabas, 2002) may occur. The present work reports that the cholesterol content in organic egg yolk is significantly lower than those in conventional egg yolk. These results are like the previous report by Durguti *et al*. (2019). Moreover, cholesterol deposition on egg yolk may be affected by feed, inclusion of specific foods in the chicken ration. Unsaturated fatty acid-rich vegetable oils are used to alter the lipid profile of the egg and reduce the cholesterol content (Faitarone *et al.*, 2013). In 1968, the American Heart Association recommended that dietary cholesterol consumption be only 300 mg daily and emphasized that only three yolks should be consumed per week. However, recent epidemiological studies usually indicate a lack of association between dietary cholesterol and/or egg intake and the risk of developing CVD in the general population (Blesso and Fernandez, 2018).

The crisis of malnutrition and hunger is currently of unprecedented proportions, not only because of a shortage of food but also limited nutrient contents. The variation in nutrient composition between conventional and organic eggs has gradually aroused the attention of consumers and researchers as well (Xia *et al.*, 2022). Vitamins and minerals are among the most important nutrients (Szymanek *et al.*, 2019). The data reported

in the current study have pointed out that the mean levels of vitamins A and D are significantly higher in organic egg yolk compared with conventional egg yolk, which indicates a significant increase in the level of vitamin A and D in organic eggs. The levels of vitamins and minerals in the egg yolk depend on the rearing system, which may reflect the effect of feed on the concentration of these elements (Brodacki *et al.*, 2018). For human health and nutrition, vitamins A and D are very important elements. Vitamin D is responsible for the maintenance of normal teeth and bones, absorption of phosphorus and Ca, normal muscle function, and to the maintenance of normal immune system function, whereas vitamin A is responsible for the maintenance of normal immune system function, healthy skin and contributes to normal vision (Réhault-Godbert *et al.*, 2019). Egg yolk is a vitamin-rich food that contains all vitamins (A, D, E, K, B1, B2, B5, B6, B9, and B12) except vitamin C (ascorbic acid). Generally, eating one egg per day covers 5% to 15% of the daily humans' requirements from vitamins (Réhault-Godbert *et al.*, 2019).

Besides being smaller, organic eggs also have less micronutrient, i.e. Calcium (Ca) and Magnesium (Mg), than conventional eggs as reported by Mesias *et al.* (2011); Kucukyilmaz *et al.* (2012); Saad Eldin and Raslan (2018); Philippe *et al.* (2020); Selim and Hussein (2020). Currently, the reported results indicate that the mineral contents of egg yolk in conventional table eggs are remarkably greater than those in organic ones, whereas levels of Ca, Mg, and Zn, in yolk of organic eggs are significantly lower than those in conventional type. In contrary, Szymanek *et al.* (2019) reported the opposite result. On the other side, Ca is very important for building and maintaining bones and teeth. It also has a significant role in nervous system function, as it stimulates the release of neurotransmitters or by transferring nerve impulses. Studies show that Ca deficiencies remain a serious nutritional problem (Kumssa *et al.*, 2015). Moreover, Zinc (Zn) helps in wound healing, growth, maintenance of tissues, and blood formation. More than 200 proteins contain Zn and, besides some hormones, need Zn as testosterone, insulin, and adrenal corticosteroids (O'Dell, 1992). Magnesium is a very important mineral, it has many functions in the body including maintenance of mitochondrial function or oxidative phosphorylation processes, regulating blood pressure, entering over 300 enzyme reactions in the body, helping nerve and muscle function, and supporting the immune system (Coudray *et al.*, 2005; Bologa *et al.*, 2013).

The microbial inhibition test used in the present study is a qualitative assay used for quick and easy screening of many samples to detect many antibiotics (Karmi *et al.*, 2019). Growth inhibition of the test bacteria method is applied in the European Community since January 2003 (European Commission Decision 96/23/EC). Antibacterial residues in eggs may be produced by the administration of antibacterial medications to laying hens via food or drinking water by veterinarians for therapy, prophylaxis, growth promotion and other management practices in laying hens that lead to accumulation of antibiotics in the body and other hen's products such as eggs and meat (Ezenduka *et al.*, 2011; Shehata *et al.*, 2014; Ehsani and Hashemi, 2015). The presence of antibiotic residues in eggs represents a public health threat and a matter of significant concern to public health agencies as it causes hypersensitivity reaction, development of resistant strains of bacteria to these antibiotics, destruction of gastrointestinal flora, etc. (Ehsani and Hashemi, 2015). The present study shows that all examined organic eggs were free from antimicrobial residues, while 12% and 8% of conventional egg yolk and conventional egg white were positive for antimicrobial residues, respectively. Similar results were reported by Hafez *et al.* (2013); Shehata *et al.* (2014); Karmi *et al.* (2019); Ebied *et al.* (2021).

Conclusion

Our study concludes higher nutritive values of organic eggs in comparison with conventional types because of their significantly higher contents of vitamins A and D and their significantly lower contents of cholesterol than those of conventional eggs. Organic eggs are considered a safe and healthy food, as they are free from antimicrobial residues and contaminants. However, organic eggs are smaller, have less Ca, Mg, Zn, weak shell, and are more expensive than conventional eggs. Drug residues in eggs have serious dangerous effects on human health, hence periodical examination of eggs in local markets for the presence of antibacterial residues should be done, poultry farms must be kept under veterinary supervision and application of good hygienic practices during egg production. Drug withdrawal times must be strictly followed post administration according to the manufacturer's recommendations.

Authors' contributions

All authors prepared conception and design of study. AMZ, MAA, MIK, and EE conducted the field study and samples collection. AMZ, MIK, HHM, MAA, and EE conducted laboratory analyzes. AA, AK, AMZ, and HHM statistically analyzed the data. EE, MIK and MAA performed analysis, data curation and interpretation of data. AMZ, MIK, AK, and AA drafted the manuscript. MAA, HHM, EE, and AK and carried out final writing, critical review and revision. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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