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ORIGINAL ARTICLE

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Chemical composition and microbial loads of chicken table eggs from retail markets in urban settings of Eastern Ethiopia

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ABSTRACT

For the determination of microbial load, chemical quality, pH and cholesterol level, a total of 400 table eggs were randomly collected from 10 retailers situated at major urban settings in Eastern Ethiopia and Haramaya University Poultry Farm, Ethiopia. The data were analyzed using SAS. High moisture and cholesterol levels and low pH and huge microbial load were detected in the eggs. Exposure of eggs to sunlight in the market and transportation of eggs from farm to the retail market might influence in lowering moisture and increasing pH. Huge microbial contamination of eggs from retailers at urban settings could be due to unhygienic handling of the eggs, lack of standard storage and transportation facilities. Storing of eggs in refrigeration condition and protection of direct sun light could be helpful in maintaining quality eggs. Introduction of refrigeration systems and construction of shades in markets are suggested to maintain and improve quality of eggs sold in markets.

Keywords

Chemical Quality, Egg, Microbial Load, Retailer, Urban setting

ARTICLE HISTORY

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INTRODUCTION

The quality of egg depends on physical make-up and chemical composition of its constituent namely egg shell, albumen and yolk. Chemical compositions are important in egg product industry as the demand of liquid eggs, frozen eggs, egg powder and yolk oil increases (Song et al., 2002). However; significant percent loss occurs in albumen and yolk chemical composition and pH by long storage periods (Bufano, 2000). Environmental factors such as temperature, humidity, and the presence of CO_2 are also of prime importance to maintain of egg quality. However, eggs remained fairly acceptable sensorial upto 10 days of storage at ambient condition (Jones, 2007) and naturally occuring microorganisms on the egg shell surface and in egg contents got markedly increased during storage. Freshly laid eggs are generally devoid of organisms. However, following exposure to environmental conditions (for example, soil, dust and dirty nesting materials), eggs become contaminated with different types of microorganisms (Ellen et al., 2000).

Microbial contamination of egg has important outcome to the poultry industry and illness from contaminated egg is a serious public health problem around the world. This may induce cases of food-borne infection or intoxication to consumers, which constitute public health hazards (Osei-Somuah et al., 2003). Microbial contamination of table eggs in the process of production, handling and marketing has been therefore, of a major public health concern. Until recently, little is known regarding microbial quality of table eggs and most studies are concerned with the quality of hatching eggs (Knape et al., 2002).

Studies made elsewhere indicated that chicken eggs are important sources of microbial infection (USDA, 2005). In developed countries like United States, Canada and Japan, internal and external quality, and microbial load of table eggs is routinely evaluated before retail selling. However, such practices are not followed in Ethiopia and particularly microbial load of chicken table eggs has not been well studied. Because of the continuous

consumer demands worldwide for eggs, periodical assessment is required to offer safe and good quality eggs for consumption. Therefore, the objective of this study was to evaluate chemical composition and microbial loads of chicken table eggs at retailers' from major urban settings of eastern Ethiopia.

MATERIALS AND METHODS

Description of the Study Area: The study was carried out in major urban settings of Eastern Ethiopia namely Dire Dawa, Haramaya and Harar open markets, located 515, 508 and 527 km from Addis Ababa, respectively. The elevation above sea level of the cities varies from 950-1250, 1600-2100, and 1600-1900 m, respectively. The respective average temperature is about 18.2 -31.4 for Dire Dawa, 10-15 for Haramaya and 18-20^{o}C for Harar. The aggregate average annual rainfall that Dire Dawa, Haramaya and Harar gets are 604, 804.7 and 900 mm, respectively (Alemu, 2008). Haramaya University is located in Haramaya district at latitude 9^{o}26'N and longitude 42^{o}3'E. It elevated at 1980 meters above sea levels and receives 780 mm mean annual rainfall. The mean annual minimum and maximum temperatures are 8.5 and 24.4^{o}C, respectively (Alemu, 2008).

Chemical Analyses

pH determination: One hundred twenty (4x30) egg yolk and albumen pH of individual eggs were measured using a pH meter (Scott and Silversides, 2001). On the same day of collection, samples were tested for pH after a 3-point calibration was performed on the pH meter using 4.0, 7.0, and 10.0 buffers in chemistry laboratory of HU. Once calibrated, a small amount of albumin and yolk sample was placed into a 50 mL beaker and the probe of the meter placed into the sample.

Quantifying nutritional ingredients: Egg yolks and whites from 30 eggs were pooled into separate glass beakers and homogenized to make composite samples from each location. Moisture, ash, protein, lipid and carbohydrate contents in albumen and yolk were carried out according to AOAC (1990). All the measurements were done in duplicates in Animal Nutrition laboratory of HU. Moisture determined by drying samples in conventional oven at 105^{o}C for 24 h. Ash was determined by ashing samples using muffle furnace oven at 550^{o}C for 6 h. Crude fat/lipid was analyzed by soxhlet extraction. Protein was determined by the method of semi-microkjeldal determination of N% and the values obtained multiplied with 6.25 to calculate protein percent. Carbohydrate was calculated by subtracting moisture, ash, protein, and lipid percentages from 100.

Total egg yolk cholesterol analysis: The total egg cholesterol analysis was conducted at Addis Ababa University, Faculty of Veterinary Medicine, in Animal Physiology Laboratory. A total of 40 eggs were randomly taken and evaluated individually. Solubilization of the egg yolks from each group was performed by dispersing 1 g of liquid yolk in 27 mL of 0.85% (w/v) NaCl solutions. Samples were then gently shaken at room temperature for approximately 2hr in a tightly capped to prevent evaporation. One (1) mL solubilized yolk sample was further diluted ten-fold with the same concentration of NaCl solution and used as a working sample. Deionized water was used in yolk solubilization as control. A commercial test kit (Linear chemicals S.L. Barcelona, Spain) for cholesterol analysis was used. The sample contains 0.1 mL of the chosen concentration of NaCl (0.85%), 1 mL enzyme reagent and 0.01mL of the cholesterol sample was used as standard. A blank was prepared by substituting 0.01 mL of deionized water for cholesterol sample. The final volume of each reaction mixture was 1.11 mL. Absorbances were read at 500 nm using photometer 5010. All samples were run in duplicate and their mean was taken as a final result.

Microbial Analysis

Preparation of sample: Three (3) eggs were randomly sampled from individual retailers of the city and pooled together to form a total of thirty (30) eggs from each site. The surface of the egg was sterilized by immersing in 70% alcohol for 2 min; air dried in a sterile chamber for 10 min and then cracked with a sterile knife. Each pooled egg contents was mixed thoroughly and 25 mL of the mixed egg content inoculated into 225 mL of buffered peptone water (BPW) and homogenized for 2 min with shaker. One (1) mL of homogenized egg (yolk and white together) content was used for both TABC and TCC in a separate test tube. All the media was prepared following the manufacturer's instruction and sterilized by autoclaving at 121^{0}C for 20 min. Standard plate counts were done on plate count agar (PCA) using the pour plate technique (De Reu et al., 2005).

Total aerobic bacterial and coliform count: Counts of egg contents were determined by pooling the contents of thirty eggs in a sterile sample bag, stomaching for one (1) minute in a laboratory stomacher. Total viable count of all the egg content samples were determined

by standard plate count method as described by APHA (Alemu, 2008). Serial dilutions of the samples were done with buffered peptone water (BPW),1 mL from each dilution (10^{-1} to 10^{-5}) was pour plated on standard plate count agar (Himedia, M091A, India) and violet red bile agar (VRBA) 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 (De Reu et al., 2005). Colonies were measured as log colony-forming units (CFU) per mL using number of bacteria/mL = Number of colonies on the plate * reciprocal of the dilution of the sample.

Statistical Analysis of Data: Data collected from laboratory work were subjected to ANOVA using the General linear model (GLM) equation of Statistical Analysis System (SAS, 2008) software. When the analyses of variance indicate the existence of significant effect, then Tukey method was used to locate the means that are significantly different from each other. The model used to analyze the quantitative data was:

$Y_{ij} = \mu + A_i + e_{ij}$, *Where:* Y_{ij}*= Individual observation of egg quality,* μ *= Overall mean,* A_i *= the effect of* i^{th} *location on egg quality (i= 1-4, Dire Dawa, Haramaya, Harar and HU),* e_{ij}*= Error term*

RESULT AND DISCUSSIONS

Chemical Composition and Microbial Loads of Chicken Eggs

Albumen and yolk pH: A number of chemical changes take place in shelled eggs during storage, the first of which is the increase of albumen pH, caused by a loss of carbon dioxide through the pores in the eggshell (Rhim et al., 2004). The pH of eggs, especially the albumen; may give some indication of unbiased quality (Scott and Silversides, 2001). The mean albumen and yolk pH of table eggs collected from the farm and retailers in selected urban settings showed significant (P<0.0001) differences (**Table 1**). Li-Chan et al. (1995) reported the pH of egg yolk to be 6.0 for fresh eggs whereas the pH changes between 6.4 -6.9 during storage which similar to pH of egg yolk evaluated from retailers of open markets. The higher pH values of table eggs from retailers might be due to longer retention periods during marketing of eggs through transit which cause a loss of carbon dioxide through the pores in the eggshell. This agreed with Waimaleongora-Ek et al. (2009) who reported 7.6 to 8.7 for freshly laid eggs that increase with the increase of storage up to 9.6 to 9.7.

Albumen and yolk chemical composition: The statistical analysis for both albumen and yolk revealed no significant differences among the study sites in ash, protein and lipid percentages except moisture (**Table 1**). The low moisture in eggs evaluated from retailers indicates movement of moisture and some amino acids from albumen to yolk through transit until it reaches consumers and exposure to environmental conditions (high temperature/sunlight that lead to evaporation).

Yolk cholesterol content: For consumers of market eggs, milligrams of cholesterol per gram of egg or milligrams of cholesterol per gram of edible egg are probably the most relevant expressions of egg cholesterol. Reports on chicken egg cholesterol values show considerable variation (Hatice and Ergul, 2005). The results of the egg yolk cholesterol content evaluated from retailers of open markets was lower (**Table 1**) than the average cholesterol value of 17.41 and 17.08 mg/g for white and brown birds, respectively, as reported by Han and Lee (1992). In this study, there was significant (P<0.0001) deference in mean cholesterol contents in eggs collected from retailers and the farm. Nevertheless, the overall mean (159.55 mg/egg yolk) was lower than the recommended value of 220 mg of cholesterol per egg yolk reported by Simopoulos (2000).This might be due to the age of the hen, genotype, rearing system, diet, environmental condition and nutrition since those factors cause cholesterol variation of chickens (Shafey et al., 1992). In the study sites the majority of table eggs supplied to retailers were from free range chicken production system that results in lower cholesterol contents of the yolk because free range chickens have free access to scavenging dietary forage legumes that significantly reduce yolk cholesterol concentration along with genetic differences. This agreed with Long and Alterman (2007) also reported lower saturated fat and cholesterol from free range chicken eggs relative to intensively reared chickens.

Microbial Loads of Egg Contents: The result obtained showed microbial growth on all samples of pooled eggs when the egg contents were cultured from all the retail markets including the farm. This agrees with the report of USDA (2006) that, 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.. Likewise; Bruce and Drysdale (1994) reported that microorganisms inside an un-cracked or whole egg may be due to the presence of pathogens within the

Table1. Albumen and yolk chemical composition of table eggs at retail levels, in percent/ 100 gm.

Parameters	Destination sites					
	HUPF	Haramaya	Harar	Dire Dawa	SEM	SL
Albumen						
Moisture (%)	89.46[a]	82.34[b]	81.60[b]	84.97[ab]	1.17024	*
Ash (%)	0.47	0.68	0.56	0.51	0.03817	NS
Protein (%)	9.69	10.98	11.38	11.02	0.28189	NS
Lipid (%)	0.030	0.037	0.034	0.028	0.00213	NS
pH	7.77[b]	9.50[a]	9.48[a]	9.43[a]	0.1470	***
Yolk						
Moisture (%)	48.40[b]	50.29[a]	50.20[a]	49.51[ab]	0.31	*
Ash (%)	1.13	0.92	0.94	1.01	0.038	NS
Protein (%)	16.19	16.38	16.52	16.05	0.084	NS
Lipid (%)	33.40	32.07	32.11	32.64	0.240	NS
Carbohydrate (%)	0.88[a]	0.34[b]	0.23[b]	0.79[a]	0.107	**
Cholesterol(mg/g)	17.78[a]	15.82[b]	14.95[b]	15.27[b]	0.235	***
pH	6.05[b]	6.62[a]	6.61[a]	6.62[a]	0.0663	***

[a,b,c] Means with in a row with different superscripts are significantly different,* =Significant at (P<0.05), ***=Significant at (P<0.0001), NS=Non-significant (P>0.05), SL = significant level, SEM = standard error of mean, HUPF=Haramaya university poultry farm.

Table 2: Microbial load of table eggs (log_{10} CFU/mL) at retail levels.

Indicator Organisms	Destination sites					
	HUPF	Haramaya	Harar	Dire Dawa	SEM	SL
TABC	1.226[b]	5.378[a]	5.596[a]	5.597[a]	0.648	**
TCC	1.234[b]	5.296[a]	5.511[a]	5.487[a]	0.637	**

[a,b] Means with in a row with different superscripts are significantly different,** =Significant at (P<0.01), SEM = standard error of mean, HUPF=Haramaya university poultry farm, TABC-Total aerobic bacterial counts, TCC-Total coliform counts.

hen's ovary or oviduct before the shell forms around the yolk and albumin.

There were significant (P<0.01) difference in total aerobic and coliform counts between retailers and farm egg contents (**Table 2**). The greater incidence of total aerobic bacterial and coliform counts from retailers of open markets of urban settings could be attributed to unhygienic conditions in the markets, absence of standard storage and transporting facilities, thus the eggs are exposed to weather conditions, resulting in their contamination in the markets from relatively high humidity that contributed to the high microbial growth. It was also observed that eggs were displayed on unclean materials or floor and improperly stored for long time without any standard storage facilities which can be a source of infection. Similarly, Etches (1992) reported that, as eggs stay longer, their resistance is reduced enabling these organisms to penetrate into the egg content. Also, Arthur and Osei-Somnah (2001), reported as warm and moist litters, poor condition in the farmhouses and retail outlets were the sources of microbial growth.

The microbial load of egg contents depends on temperature and length of storage, as reported by Humphrey (1994). The microbial analysis result showed the lower microbial loads than reported by other researchers (**Table 2**). Ansah et al. (2009) reported the higher mean log viable count of table egg contents 7.26, 6.54, 7.18 and 6.9 for Kukuo, Lamashegu, Aboabo and Tamale city, respectively. Also, Obi and Igbokwe (2007) reported higher mean total viable microbial counts of greater than 7.0 log_{10} CFU/mL in retail egg contents. Conversely, Abdul et al. (2012) reported lower CFU/mL (3.02 log_{10}) of average total aerobic bacterial counts from commercially available table egg contents in Taif city (Saudi Arabia). However; in this study the average total viable and coliform count for egg content were less than the accepted 6.0 log_{10}CFU/mL, recommended by the International Commission on the Microbiological Specification for Food (ICMSF, 1998) but the result suggest that eggs from the retailers must be consumed with caution.

CONCLUSION

This study evaluates chemical and microbial quality of table eggs at retail levels from major urban settings in Eastern Ethiopia. Low moisture content and high pH value in eggs found at retail levels speculates that the eggs are stored for long duration in the hands of retailers. The overall mean of cholesterol level was 159.55 mg/egg yolk. The presence of indicator organisms in eggs of retail levels can be attributed to unhygienic conditions in the markets and poor handlings. Therefore, it is recommended to establish quality control and inspection regulations of table eggs

to offer safe and good quality eggs for consumption. Also, retailers can store their eggs under good sanitary conditions in refrigeration condition, and where facilities are not available, eggs can be protected from direct sun light by establishing shading facilities.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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