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Research Article

Microbiological Quality of Table Eggs Sold at Different Sales Location

Fatma YENİLMEZ^{1*}, Ayşen BULANCAK²

ABSTRACT

In this study, we will compare the microbiological quality of table eggs and aim to shed light on consumer preferences in terms of egg safety. Totally 150 eggs randomly selected and grouped into three groups from 225 eggs which have been purchased 5 different market brands (in cooler cabinet), grocery brand (open) and bazaar (village egg). A total number of Mesophilic Aerobic Bacteria (TMAB), total mold-yeast, *Enterobacteriaceae* loads and the presence of *Salmonella* spp. were determined by using commercial kits. According to the research results, market eggs shell had the lowest load in terms of TMAB and mold-yeast ($P \leq 0.05$), but higher *Enterobacteriaceae* load ($P > 0.05$). *Salmonella* spp. determined in one sample from the village and 2 samples from grocery eggs, but none from market eggs. TMAB and total mold-yeast amount were found to be highest in village egg albumens. Market egg albumens and yolk samples were detected free from microorganisms. The results of the analysis show that it would be more safety to prefer market eggs from the cooler cabinets with cold chains. It is necessary to be more careful about village egg consumption because of their microorganism load which can penetrate into eggs by outdoor sale conditions like heat and moisture.

Keywords: Consumer preference, egg, microbiological load, sales location

Farklı Satış Noktalarındaki Sofralık Yumurtalarda Mikrobiyolojik Kalite

ÖZ

Bu araştırmada, market (dolap kullanan), bakkal (açıkta) ve pazarlarda (köy yumurtası) satışa sunulan sofralık yumurtaların mikrobiyolojik kalitesi karşılaştırılarak yumurta güvenliği açısından tüketici tercihlerine ışık tutmak amaçlanmıştır. Üç farklı grubun her biri için 5 farklı noktadan satın alınan toplam 225 yumurta içerisinde rastgele seçilen 150 adet yumurtada; Toplam Mezofilik Aerobik Bakteri (TMAB), toplam küf-maya, *Enterobacteriaceae* yükleri ve *Salmonella* spp. varlığı araştırılmıştır. Yapılan analizler sonucunda, market yumurtası kabuklarında TMAB ve küf-maya yükünün en düşük ($P \leq 0.05$), *Enterobacteriaceae* yükünün ise en yüksek düzeyde olduğu tespit edilmiştir ($P > 0.05$). *Salmonella* spp. varlığı köy yumurtalarında bir, bakkal yumurtalarında 2 numunede belirlenmiş, market yumurtalarında ise rastlanmamıştır. TMAB ve toplam küf-maya yükü köy yumurtası akında en yüksek düzeyde belirlenirken, market yumurtası ak ve sarı örneklerinde mikroorganizmaya rastlanmamıştır. Analizlerden elde edilen sonuçlar soğuk zincir ve dolap kullanan market yumurtalarını tercih etmenin daha güvenli olacağını göstermektedir. Açıkta satılan yumurtalarda sıcaklık, nem ve olumsuz çevre koşullarından dolayı yumurta içerisine nüfuz edebilecek mikroorganizmalar nedeniyle köy yumurtası tüketiminde daha dikkatli olunması gerekmektedir.

Anahtar Kelimeler: Tüketici tercihi, yumurta, mikrobiyolojik yük, satış yeri

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Introduction

Animal origin foods such as meat and egg products of poultry are concerned as the general reason for a food-borne infection induced by micro-organisms (Sabarinath *et al.*, 2009; Casey *et al.*, 2012). Egg quality can be affected by the contamination of eggs and products with microbes. This can lead to pathogen transmission and consequently spoilage and this causes foodborne infection or consumer poisoning.

Microbial contaminations of eggs commonly take place within few seconds after oviposition, transaction and till consumption (Indhu *et al.*, 2014). Eggs may be infected vertically by; microorganisms from the blood of digestive tract, pass through egg yolk by blood (Gordon and Tucker, 1965) and horizontally depending on the environmental conditions after oviposition by various organisms (*Streptococcus* and *coli-acrogens* at artificial insemination) (Harry, 1963), cloacal contact with nest and litter material during oviposition. Other factors may also affect bacterial contamination such as dust in barns and storerooms, shell hygiene or structure (cracks, the existence of cuticle and membrane quality), season and storage conditions (Mallet *et al.*, 2010).

The contamination of the eggshells with microorganisms is mostly through feces. Whenever eggs are laid, they can be contaminated with fecal material and microorganisms may pass through the shell and membranes by vacuum effect which occurred by heat loss of egg after lay. Microorganisms may reach the egg content by unsuitable long storage and transfer conditions (Keller *et al.*, 1995). Contaminated eggs and products may lead to serious health risks when consumed raw or uncooked. The shelf life and food safety of eggs may be adversely affected by high levels of contamination. Nowadays good egg perception of consumers has changed from eggshell hygiene and physical properties into microbial unity by increasing awareness of food safety issues.

Many kinds of bacteria, such as *Escherichia*, *Micrococcus*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Aeromonas*, *Enterobacter*, *Proteus*, *Pseudomonas* have been determined on the shells of table eggs. Similarly, mold and yeast were also determined (Mayes and Takeballi, 1983; Ricke *et al.*, 2001; Musgrove *et al.*, 2004). On the other hand, the gram-negative *Enterobacteriaceae* group isolated as a major contaminant of commercial chicken eggs (Arathy *et al.*, 2009; Sabarinath *et al.*, 2009). *Salmonella* is a gram-negative, selective anaerobic bacteria of the family *Enterobacteriaceae*. And eggs are one of the most common foods that cause *Salmonella* infections (Akbaş, 2014).

Storing eggs by cooling is a good method to prevent the growth of pathogens such as *Salmonella* spp. (EFSA, 2005). Some regulations regarding eggs in different regulations are stated as follows; transport and storage of eggs should be carried out at a constant temperature, providing the best hygiene conditions (EC, 2004-853), eggs must be purchased within 21 days after laying (EC, 2004-853) and the expiry date of eggs must be determined 28 days after laying (EC, 2008-589). Turkey is also a candidate country for future membership of the European Union. Turkish government prepared regulations for egg and egg products as Turkish Food Codex Microbiological Criteria (Regulation on Turkish Food Codex Microbiological Criteria, 2011) and Egg Notification (Regulation on Turkish Food Codex Egg Notification, 2014). Turkish regulations asked for storage at 5-8°C from the 18th day onwards after the lay of eggs. *Enterobacteriaceae* load must be lower than 10^2 and free from *Salmonella* spp.

In light of the above information, this study was conducted to appraise and analyze the impact of different sale locations (market, grocery and bazaar) on microbiological load of table eggs. To investigate TMAB, Mold and Yeast, *Enterobacteriaceae* and *Salmonella* spp. loads of table eggs from the shell, albumen and yolk contents.

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Material and Methods

Sampling

In this study 225 eggs (7 days old, weighing between 63-72 g Large) have been purchased from 5 different market brands (in cooler cabinet), grocery brand (open) and bazaar (village egg). The egg samples were carried to lab immediately and prepared for microbiological analysis. A total of 150 eggs randomly selected and grouped into three (50 eggs for each group): Group1 from the market brand (in cooler cabinet + 4 °C), Group2 from grocery brand (open + 24°C room temperature) and Group3 from bazaar (village egg, environmental temperature). Eggshells, albumen and yolk were analyzed with commercial kits for TMAB, mold and yeast, *Enterobacteriaceae* and *Salmonella* load (log CFU/egg).

Preliminary Preparations for Analysis

Preparations of Egg Shells: Every 5 eggs were put in a sterile plastic bag, and bags considered as one composite sample. Buffered peptone water (BPW) was (100 ml) poured into the egg samples in sterile bags and stirred, washed and scrubbed with fingers (ISO 6579:1993) for five minutes. Then 225 ml Tryptone Soya Broth (TSB) and 25 ml rinse of BPW mixed well and incubated 18-24 hours at 37 °C.

Preparations of Egg Albumen and Yolk Content: After sterilization of eggshells with 70% alcohol; they were broken and separated into contents of yolk and albumen. Each sample from yolk and albumen were pooled in sterile beaker glass to form one sample. Then 225 ml TSB and 25 ml of egg contents mixed for 30 seconds and incubated 18-24 hours at 37°C.

Total Mesophilic Aerobic Bacteria (TMAB) Count

Tenfold dilution was obtained from sterile peptone water up to 10^{-9} from homogenized egg contents or eggshells. 1 ml sample with 9 ml TPS inoculated onto PCA (Plate Count Agar) and incubated 24-48 hours at 37 °C. After incubation colonies counted by Most Probable Number (MPN) Method and calculated by logarithmic Colony Forming Unit (log CFU) per egg.

Total Mold-Yeast Count

For mold-yeast counts, homogenized egg contents or eggshells dilutions used up to 10^{-9} with 0.5 ml sterile pipets and spread plate technique on Potato Dextrose Agar (PDA) plates with drigalski spatula. Plates incubated five days at 25°C and colonies counted (by MPN Method) and calculated by log CFU per egg.

Enterobacteriaceae Count

Tenfold dilution was obtained from sterile peptone water up to 10^{-4} from homogenized egg contents or eggshells. *Enterobacteriaceae* were enumerated according to Roberts and Greenwood, (1995) with a 1 ml sample onto Violet Red Bile Glucose agar by double plating method (VRBG) (ISO 4832, 2006). Samples were incubated at 30-32°C (24 hours), colonies counted and calculated by log CFU per egg.

Salmonella spp. Detection

To start *Salmonella* spp. detection; the homogenized egg contents and eggshell samples were pre-enriched with peptone water. Then 0.1 ml samples were enriched with Rappaport Vassiliadis and incubated 24 ± 3 hours at $41.5 \pm 1^\circ\text{C}$. Parallel aliquots (1 ml) from enriched solution added on 10 ml Selenite Cystine Broth Base (SC) and incubated 24 ± 3 hours at $37 \pm 1^\circ\text{C}$. Each enriched sample plated on Brilliant Green agar (BPLS) and Xylose Lysine Deoxycholate Agar (XLD) at the same time and incubated 24 ± 3 hours at $37 \pm 1^\circ\text{C}$. Unconfirmed positive colonies were stabbed on both Lysine Iron agar and Triple Sugar Iron agar (TSI) and incubated 24 hours at 37 °C. Positive black tubes with colonies verified with enzyme tests after incubation.

Statistical Analysis

All data obtained from the experiment were analyzed using the IBM SPSS 19.0 (2010) statistical software package program. The normal distribution of data was analyzed as a completely randomized variance design (ANOVA) and the Tukey test was used for the comparison of means. Statistical significance level was defined as $P \leq 0.05$.

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Results and Discussion

The highest rate of bacterial load detection of table eggs occurred on the eggshell (140/150), followed by albumen (100/150) and none at yolk contents (0/150) (Table I). There were significant differences ($P \leq 0.05$) between shell and albumen detections at the market, grocery and bazaar sale conditions.

Results from the microbial analysis of the Total number of Mesophilic Aerobic Bacteria (TMAB) showed that table eggs collected from grocery and bazaar are more contaminated than eggs from markets. TMAB, mold and yeast,

Enterobacteriaceae were detected in each sale condition of the table eggshells. Bacterial load detection for albumen and yolk contents tested during the study was negative at market sale condition.

The level of TMAB of the surface of the eggshell ranges from $10^{3.8}$ to $10^{7.0}$ CFU/egg, with an average level around $10^{4.5}$ CFU/egg in previous studies (Jones *et al.*, 2004; Musgrove *et al.*, 2005; De Reu *et al.*, 2008; 2009; Nordenskjöld, 2010; Englmaierova *et al.*, 2014; Bulancak *et al.*, 2016; İncili *et al.*, 2019).

Table I. Microbiological load of table eggs from sale conditions (log CFU/egg)

		Market (n=50)	Bazaar (n=50)	Grocery (n=50)	P Values
TMAB	Shell	8,91 ^a	10,51 ^b	11,66 ^b	0.00
	Albumen	0,00 ^a	5,13 ^b	4,20 ^b	0.00
	Yolk	0,00	0,00	0,00	-
Mold- Yeast	Shell	6,84 ^a	9,36 ^b	8,52 ^b	0.00
	Albumen	0,00 ^a	3,19 ^c	1,72 ^b	0.01
	Yolk	0,00	0,00	0,00	-
<i>Enterobacteriaceae</i>	Shell	4,15	3,43	3,03	0.80
	Albumen	0,00	0,00	0,00	-
	Yolk	0,00	0,00	0,00	-

^{a-c} means followed by different letters in the same row are significantly different.

Significant sample type × sale condition interaction ($P \leq 0.05$)

CFU = colony-forming units

But in this study TMAB load on shell and albumen detected highest as 11.66 and 4.20 log CFU/egg at the grocery; and 10.51 and 5.13 log CFU/egg at bazaar conditions respectively (Table I, $P \leq 0.05$). Our result for the eggshell load of market condition (8.91 log CFU/egg) in agreement with results of Ansah *et al.*, 2009 (7.56 log CFU/egg) and Chaemsanit *et al.*, 2015 (7.2 to 8.00 log CFU/egg). De Reu *et al.* (2008) stated the limit of 5 log CFU/egg, which can refer to eggshells of acceptable hygienic quality. International Commission on the Microbiological Specification for Food (ICMSF) was identified as acceptable limits of the mean for the eggshell total viable count and mean log as $10 + 10^5$ and 6.00. And in our study, this count was higher than the specified.

The results show that the current situation is unacceptable according to FAO / WHO egg production standards for all sales conditions.

Mold and yeast load from the shell of bazaar table eggs found the highest amount as 9.36 log CFU/egg. This can be explained by changeable temperature and humidity levels of store conditions of table eggs. The temperature seems to have affected a total load of microorganisms. Mold and yeast load at shell increase by nearly 3 log CFU/egg at the grocery (open + 24 °C room temperature) and bazaar higher environmental temperatures instead of market (in cooler cabinet + 4°C) condition. Mold and yeast detected at albumen content of eggs as 3.19 log CFU/egg at the bazaar and 1.72 log

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CFU/egg at grocery sale condition ($P \leq 0.05$). Mold and yeast are not detected at market albumens. Karadal *et al.*, (2018) investigate the microbiological quality of the market and village eggs sold at retail in Niğde and Kayseri (Central Anatolian Region of Turkey). Results in this study for eggshell load and albumen content are in agreement with our results (6.80 and 6.97 log CFU/egg for market and village eggs respectively, albumen negative at the market condition). Likewise, Ahmed *et al.*, (2002) found mold and yeast load on eggshells ≥ 5 log CFU/egg. Jones *et al.* (2004) found the lowest yeast and mold levels at 2 weeks of storage (1.3 log CFU/ml) at unwashed eggshells. They stored eggs one week more than ours. Microbial populations reached the highest concentration of 2.9 and 2.6 log CFU/ml at 8 and 10 weeks, respectively, as storage time increased. Bahobail *et al.*, (2012) study mold and yeast contamination at one week stored eggs found 1.1-3.4 log CFU/egg. Salem *et al.* (2009) pointed out that feeds, unhygienic barn and storage conditions may cause the highest level of mold and yeast contamination on shell of table eggs. Likewise, Tomczyk *et al.* (2019) reported that higher humidity (95%) and temperature (20 °C) during the egg storage period may cause the development of fungi in the albumen. In our study mold and yeast load considered to be significantly high because of high temperature and humidity factors.

Enterobacteriaceae loads obtained as 4.15, 3.43 and 3.03 log CFU/eggshell respectively for the market, bazaar and grocery sale conditions (Table I). The difference between the sales conditions was not significant ($P > 0.05$). Albumen and yolk contents are free from *Enterobacteriaceae* load. İncili *et al.*, (2019) found *Enterobacteriaceae* load as 1.23 and 1.30 log CFU/eggshell and 0.71 and 0.70 log CFU/egg for the content of conventional and village eggs, respectively. Their results are in the agreement with our study; that there was no difference between the conventional (market and grocery) and village (bazaar) eggshells in terms of the number of *Enterobacteriaceae* ($P > 0.05$). Wall *et al.* (2008) found a significantly

higher proportion of *Enterobacteriaceae* load in furnished (1-2.3%) than in conventional cages (5.80%). Gole *et al.* (2013) and De Reu *et al.* (2009) reported 1.46 and 1.51 log CFU/eggshell from furnished cages respectively. Musgrove *et al.* (2005) also reported 2.29 log CFU/eggshell from commercial egg processors. In Roberts *et al.* (2014) study *Enterobacteriaceae* load was relatively low (1.63 log CFU/egg) in furnished cage eggs and significantly higher (2.10 log CFU/egg) in conventional cage eggs. The change in *Enterobacteriaceae* loads might be depending on the sampling method. Such as eggs from the cage front directly or from a commercial facility as Musgrove's study (2005). Al-Ashmawy, (2013) reported less contamination for *Enterobacteriaceae* at white and brown eggshells (4.9×10 , 6.3×10 log₁₀ CFU/g) than home-produced (1.2×10^2 log₁₀ CFU/g) table eggs from his study. Jones and Musgrove (2007) noticed a higher *Enterobacteriaceae* load (3.40 log CFU/eggshell) in agreement with our study result.

Enterobacteriaceae levels may be an important criterion in terms of food quality and hygiene of processing conditions (Carter and Cole, 1990). This kind of eggs did not meet the quality standard allowed for this bacteria group as a maximum 10^2 log CFU/egg for the retail of European Communities (1993), EFSA (2014) and Turkish food safety criteria ISO 21528-2 (2011).

The positive shell emulsion samples for *Salmonella* spp. were detected in grocery (2/50) and bazaar (1/50) table eggs. Overall there were 2.0% (3/150) positive at eggshell samples. Albumen and yolk contents are free from *Salmonella* spp. (Table II). Similar findings were reported by Ansah *et al.* (2009), Chaemsanit *et al.* (2015) and Stepień (2010) reported a 3.2% prevalence of *Salmonella* spp. on eggshells. In this study, *Salmonella* spp. has not been found internal content of eggs. This finding is in parallel with Stepień-Pysniak (2005) who made a survey about Australian commercial eggs. Gole *et al.* (2013) found 4.51% (14/310) *Salmonella* spp. positive on

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eggshell and negative at internal content of the egg. Incili *et al.* (2019) found *Salmonella* spp. load as 0.69-1.39% at eggshell and 0-0.69% for the content of conventional and village eggs, respectively. Their results are in the agreement

with our study; that there was no difference between the conventional (market and grocery) and village (bazaar) eggshells in terms of the number of *Salmonella* spp. ($P > 0.05$).

Table II. *Salmonella* spp. prevalence of table eggs from sale conditions

		Market (n=50)	Bazaar (n=50)	Grocery (n=50)	%	P Values
<i>Salmonella</i> spp.	Shell	0/50	1/50	2/50	2.00	0.05
	Albumen	0/50	0/50	0/50	-	-
	Yolk	0/50	0/50	0/50	-	-

Results were considered significant when $P \leq 0.05$.

Adesiyun *et al.* (2005) recovered *Salmonella* spp. at layer farms of Trinidad as 3.8% from eggshell samples and 1.2% in egg contents (one-day-old egg). They reported that the risk of table egg-induced gastroenteritis is important in Trinidad. They have associated this with, particularly salmonellosis, which occurs as a result of consumption of raw or improperly cooked eggs or egg products. *Salmonella* spp. was not isolated from farm hen egg contents in Awny *et al.* (2018) study. While they were isolated from Balady hens' egg content and ducks' eggs content with the incidences of 4 and 8%, respectively. Fikiin *et al.* (2020) did not detect *Salmonella* spp. in poultry farms. And they reported that it may be the result of strict spraying and good care practices in poultry farms.

Eggshell surface contamination level and type; may be affected by sanitary conditions of the breeding environment, practices, housing system, geographical area and season. Also, it can occur from one egg to another during egg storage, transport and packaging processes (Techer *et al.*, 2013; Englmaierova *et al.*, 2014). It has been reported that 44-68% of salmonellosis disorders in the EU are caused by practices during the processing and consumption of eggs and egg products (Hilbert *et al.*, 2014; EFSA, 2015; Fikiin *et al.*, 2020). Eggs should be stored below 7 °C to control or decrease the microbial load of the eggshell surface (Aygün, 2017). This could also answer

the question of why supermarkets had lower amounts of *Salmonella* spp. than the minimarkets which store the eggs at room temperature. Studies have proven that the rate of changes at unfavorable quality parameters slows down significantly ($P < 0.05$) by cold storage (8 °C) and lower air humidity conditions (Messens *et al.*, 2005; Nordenskjöld, 2010; Eke *et al.*, 2013; Jones *et al.*, 2018; Tomczyk *et al.*, 2019). Martelli and Davis (2012) were reported that refrigeration decrease SE growth and metabolic activity on the eggshell. Zeidler (2002) was recommended keeping raw eggs at 4-8 °C to decrease heat resistance and growth of the *Salmonella* spp. Pasquali *et al.* (2016) were reported that a storage temperature of 4 °C has the strongest inhibiting effect on *Salmonella* spp., compared with 8 and 20 °C (Fikiin *et al.*, 2020).

Conclusions

The results of the analysis show that it would be more safety to prefer market eggs from the cooler cabinet which comes with cold chains. In Turkey egg, consumer groups preferred village eggs between the rates of 83.25-92.8% as healthy (Mızrak *et al.*, 2012; İskender *et al.*, 2014). The eggs from grocery and village should be therefore taken with caution because of their microorganism load. The public should be warned and educated about the risks of consumption of raw and undercooked egg and egg products. Although the eggs were highly contaminated with total bacteria the absence of

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Salmonella spp. at the market, eggs are an encouraging factor for consumers. The existing egg handling norms should be revised by introducing transparent and concrete concerns at storage and transport conditions. Food safety and quality characteristics based on temperature and humidity should be determined and applied to a much greater extent.

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