

Full Length Research Paper

Microbial quality and content aflatoxins of commercially available eggs in Taif, Saudi Arabia

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Contamination of eggs and egg products with microorganisms that possibly affect eggs quality and pathogen transmission induced food-borne infection or intoxication to consumers, which cause public health hazards. A total of 135 table eggs (75 processed and 60 unwashed) were collected randomly from different markets and groceries in Taif city (Saudi Arabia). Every three eggs were represented as one egg pooled sample ($n = 45$ pools). Each egg shell and content was examined for their microbiological contents in terms of aerobic, Enterobacteriaceae, fungal counts; presence of *Salmonella*, *Escherichia coli* O157:H7, *Listeria*, *Campylobacter* and the presence of total aflatoxins (AFs) levels by enzyme linked immuno-sorbent assay (ELISA). The results showed microbial growth on 37.7% (45 shell and content pools) of the examined samples and of all, total aflatoxin contamination was determined to trace amounts in three egg samples (6.6%) (ranging from <1 to 1.19 ppb). Other samples tested were found to be free from any detectable level of aflatoxins. Among unwashed eggs, one shell sample was *Campylobacter* positive, two shell samples were *Listeria* positive, no *Salmonella* and *E. coli* O157:H7 could be detected. The average overall detected aerobic, Enterobacteriaceae and mould counts was 3.02, 0.9, 1.9 log₁₀ CFU/ml, respectively. It is concluded that eggs sold in Taif city were of good quality, although occurrence of some pathogenic microorganisms. Therefore, it is recommended that table eggs should not be consumed raw.

Key words: Microbiological quality, table eggs, food-borne pathogens, aflatoxins, enzyme linked immuno-sorbent assay (ELISA), Saudi Arabia.

INTRODUCTION

Today, eggs remain a staple food within the human diet, consumed by people throughout the world. They are consumed worldwide in various dishes and considered very nutritious and a cheap source of protein (Blumenthal, 1990; Papadopoulou et al., 1997; MAFF, 2000). Though eggs are considered as complete food for growth and sustenance, studies indicated that microorganisms often contaminate eggs (Osei-Somuah et al., 2003).

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; Smith et al., 2000). Furthermore, these microorganisms may contaminate the egg contents either by penetration or withdrawal through pores of the shells (Harry, 1963; Schoeni et al., 1995), and also through the transovarian route (Bruce and Drysdale, 1994). Some other factors such as environmental temperature and humidity influence the bacterial penetration and thus, enhance the infection and spoilage (Frazier and Westhoff, 1987; Theron et al., 2003).

Food-borne diseases caused by microorganisms are a large and growing public health problem. Contamination of eggs and egg products with microorganisms can affect egg quality, which may lead to spoilage and pathogen

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transmission. This may induce cases of food-borne infection or intoxication to consumers, which constitute public health hazards. Several pathogenic microorganisms have been isolated from the surface of chicken egg shells and contents. Among them, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Escherichia coli* O157:H7, *Salmonella* and *Campylobacter* were detected (Chiesa et al., 1989; Leator and Foegeding, 1989; Ruser and Marth, 1989; Farber et al., 1992; Moore and Madden, 1993; Schoeni and Doyle, 1994; Hope et al., 2002; Adesiyun et al., 2005).

Aflatoxins contaminate a vast array of foods and agriculture commodities, and produced by certain species of fungi. Such mycotoxins pose profound challenges to food safety widespread in many countries, especially in tropical and subtropical regions where temperature and humidity conditions are optimum for growth of moulds and production of toxins. The possible transmission of such toxic residues to edible eggs results in potential hazards to human health (Martin et al., 1998). Aflatoxins are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans (IARC, 1993; Yaling et al., 2008).

Because of the continuous consumer demands worldwide for eggs, periodical assessment is required to offer safe and good quality eggs for consumption. The present investigation was, therefore, planned to assess shell and interior quality of consumed eggs at retail levels in Taif city, Saudi Arabia. Microbiological quality, presence of food pathogens and total aflatoxin residues were investigated.

MATERIALS AND METHODS

Sampling

One hundred and thirty-five (135) fresh table eggs were collected randomly from different groceries and markets in Taif city, Saudi Arabia. The eggs collected were processed and unwashed (balady) eggs. Every three eggs from each market were represented as one egg pooled sample. All 45 egg pooled samples were examined for microbial quality, total aflatoxins (AFs) as well as presence of *Listeria*, *E. coli* O157:H7, *Campylobacter* and *Salmonella* in their shells and contents. Two sampling methods were utilized: shell crush (shell and membrane emulsion) (Musgrove et al., 2005) and egg contents (Jones et al., 2004).

Microbiological analysis

Serial dilutions were done from each egg shell recovery buffer and content. Then, duplicate plating of 1 mL aliquots used for enumeration of each analyzed microbial item. Each sample was divided into two parts, one for extraction of aflatoxins and the other for microbial enumeration, detection, isolation and identification. Standard plate counts were done on plate count agar (PCA, Scharlau, Barcelona, Spain) using the spread plate technique. The samples were then incubated at 30°C for 72 h. Enterobacteriaceae counts were determined on violet red bile agar (VRBA, Oxoid), and then incubated at 37°C for 24 h. Total mould count/g of egg samples was estimated on dichloran rose bengal chloramphenicol

agar (Difco- DRBC) and the plates were then incubated at 25°C for 7 to 10 days.

For *Salmonella* isolation, egg samples were enriched in Rappaport-Vassilidis broth (RV, Oxoid, UK), followed by recovery on xylose lysine dextrose agar (XLD - Scharlau, Barcelona, Spain). For *Listeria* isolation, two stage enrichment procedures were done using *Listeria* enrichment broth (LEB - Oxoid) followed by isolation on palcam agar plates (Oxoid). For *E. coli* O157:H7, tryptone soya broth (TSB, Difco, Detroit, MI, USA) supplemented with 20 mg.L⁻¹ novobiocin (Sigma, Germany) were used. Isolation was done on MacConkey sorbitol agar plates. Thermophilic *Campylobacter* were isolated directly or after enrichment on Karmali media at 42°C (BK + BS, Biokar Diagnostic, Beauvais Cedex – France).

The methods used were of the Association of Official Analytical Chemists (AOAC, 1980) and in the compendium of methods for the microbiological examination of foods (Downes and Ito, 2001). Identification of Enterobacteriaceae and other species was made by commercially available biochemical test (API tests, BioMérieux, Lyon, France), while taxonomic identification of the different genera and species was made according to microscopic criteria in accordance with appropriate keys (Raper and Fennel, 1965; Pitt and Hocking, 1997; Klich, 2002).

Detection of aflatoxins

MaxSignal® Aflatoxin Total - enzyme linked immuno-sorbent assay (ELISA) Test Kit (Bioo Scientific Corp., Austin, TX, USA) is a competitive enzyme immunoassay and was used for the quantitative detection of AFs in the samples following manufacturer instructions. The microwells were measured at 450 nm by ELISA reader (ELx800, BioTek inc., Highland Park, Winooski, VT, USA). The optical densities of the samples were determined and compared with that of the kit standard. Egg samples were exposed to some pretreatments: the egg pooled samples were diluted with 70% methanol (1:9), mixed in a blend jar and blended for one min. Next, the homogenate was filtered through filter paper, and 50 µl of the filtrates was used per well for the assay. The test kit detection limit is 0.05 ng/g or ppb.

Data analysis

The data were subjected to two way analysis of variance and simple correlation after converting the microbial counts to a logarithmic scale (Snedecor and Cochran, 1989).

RESULTS

Table 1 showed that the average total aerobic counts associated with shells and contents of the eggs ranged from 1.1 to 5.9 log₁₀ CFU/ml. Contents from unwashed (balady) and processed eggs had aerobic counts ranged from 1.1 to 2.0 log₁₀ CFU/ml, respectively, as compared with the shells from both types (3.1 to 5.9 log₁₀ CFU/ml, respectively). The overall average of all pools was 3.02 log₁₀ CFU/ml.

The detected levels of Enterobacteriaceae ranged from < 1 to 1.5 log₁₀ CFU/ml (Table 1). The average detection level was 0.9 log₁₀ CFU/ml. The prevalence of Enterobacteriaceae was 13.3% in all of the egg pools. Bacteriological identification of randomly selected Enterobacteriaceae colonies revealed isolation of six genera, namely, *E. coli*, *Enterobacter* spp., *Citrobacter*

Table 1. Microbial contamination profiles of retail table eggs in Taif city, Saudi Arabia.

Analyzed items	Log ₁₀ CFU/ml or + (present) and – (absent)			
	Egg shell		Egg content	
	Processed	Unwashed	Processed	Unwashed
Total plate count	3.1	5.9	1.1	2.0
Enterobacteriaceae	0.9	1.5	0.0	0.6
Mould count	1.3	3.4	0.0	1.1
<i>E. coli</i> O157:H7	-	-	-	-
<i>Salmonella</i>	-	-	-	-
<i>Campylobacter</i>	-	(1/20)*	-	-
<i>Listeria</i>	-	(2/20)	-	-

*, Number in parenthesis is the number of positive isolates per number of samples.

Table 2. Identification of isolates randomly selected from violet red bile glucose agar Inoculated with 45 pooled shell and content of egg samples.

Bacterial species	Egg shells		Egg contents	
	Processed (n= 25)	Unwashed (n= 20)	Processed (n= 25)	Unwashed (n= 20)
<i>E. coli</i>	1	3	0	0
<i>Enterobacter</i> spp.	1	5	0	1
<i>Providencia</i> spp.	1	3	0	0
<i>Citrobacter</i> spp.	0	2	0	0
<i>Serratia</i> spp.	0	1	0	0
<i>Kluyvera</i> spp.	0	1	0	0

Table 3. Prevalence of isolated fungi from the examined table egg shells and contents.

Fungal species	Egg shells		Egg contents	
	Processed (n= 25)	Unwashed (n= 20)	Processed (n= 25)	Unwashed (n= 20)
<i>Aspergillus flavus</i>	0	3	0	2
<i>Aspergillus niger</i>	0	1	0	1
<i>Aspergillus fumigatus</i>	1	5	0	1
<i>Penicillium</i> spp.	2	1	3	0
<i>Cladosporium</i> spp.	4	4	5	0
<i>Mucor</i> spp.	1	2	6	0

spp., *Providencia* spp., *Serratia* spp. and *Kluyvera* spp. (Table 2).

The microbiological analysis of 45 pooled egg samples revealed two *Listeria* spp., single *Campylobacter* isolate found in the shell sample of unwashed eggs, whereas no *Salmonella* and *E. coli* O157:H7 were detected (Table 1).

For the total mould count, it was ranged from 1.1 to 3.4 log₁₀ CFU/ml, with an overall average value 1.9 log₁₀ CFU/ml for total eggs (Table 1). Mycological examination of eggs revealed presence of four fungal genera, namely, *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp. and *Mucor* spp. (Table 3).

Results of competitive ELISA revealed that AFs residue were detected in trace amounts in 3 (6.6%) out of the 45

egg pool samples examined, with a value ranged from 0.61 to 1.19 ng/g or ppb (Table 4).

DISCUSSION

Chicken eggs are a common food and one of the most versatile ingredients used in cooking, preparation of many types of dishes, both sweet and savory. 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 of studies are concerned with the quality of hatching eggs

Table 4. Results of AFs content residues in the examined 45 pooled egg samples.

Examined sample	Total No. of samples	No. of positive	AFs concentration (ug/g or ppb of egg content)
Processed eggs	25	0	ND
Unwashed eggs	20	3	0.61, 0.81, 1.19

ND, Non-detected level.

(Quarles et al., 1970; Board and Tranter, 1995; Favier et al., 2000; Knape et al., 2002). Our study indicated that 37.7% of the 45 pooled composite egg samples were contaminated with aerobic bacteria, having overall average 3.02 log₁₀ CFU/ml. The average total viable count for the shell and content were less than the accepted 6.0 log₁₀ CFU/ml, recommended by the International Commission on the Microbiological Specification for Food (ICMSF, 1998). A higher mean total viable bacterial counts > 7.0 log₁₀ CFU/ml was reported in retail eggs (Obi and Igbokwe, 2007; Ansah et al., 2009) with the shell having the highest and content the lowest load.

Microbiological analysis showed that the unwashed (balady) had more microbial load than the processed one and contamination of egg shells was greater than egg contents. Our results are in agreement with previous reports that indicated higher prevalence and counts of bacteria on unwashed egg shells as well as on egg shell relative to the contents (Labaque et al., 2003; Jones et al., 2004). Humphrey (1994) reported that the final microbial load of egg contents depends on temperature and length of storage.

Contamination with Enterobacteriaceae was used to evaluate the sanitary or hygienic quality of raw foods and also during food processing (Mercuri and Cox, 1979). In the current study, the prevalence of Enterobacteriaceae was 13.3% in all of the egg pools and thus has log average Enterobacteriaceae (0.9 CFU/ml). Similarly, other studies reported low detection level of Enterobacteriaceae (the highest concentration detected was 0.6 log₁₀ CFU/egg shell (Jones et al., 2004). Rodenburg et al. (2006) and De Reu et al. (2007) found that the log average Enterobacteriaceae egg shell contamination of table eggs were 1.5 log₁₀ CFU/eggshell. However, Musgrove et al. (2004) showed that no contamination of washed eggshells with Enterobacteriaceae within the advertised shelf life for retail eggs. A total of six Enterobacteriaceae spp. were isolated from pooled egg samples in this study agree with other published reports where *Enterobacter* spp., *Escherichia*, *Citrobacter* spp., *Providencia* spp. and *Serratia* spp. have been detected from eggs or their wash water (Moats, 1980; Papadopoulou et al., 1997; Jones et al., 2004; Musgrove et al., 2004; Obi and Igbokwe, 2007; Ansah et al., 2009).

A health issue associated with eggs is their contamination by pathogenic bacteria. In this study, 2 (1.4%) of 135 eggs (*n* = 45 pooled samples) had *Listeria*

spp. in their shells but not in the egg content. Similar prevalence was found by Nitcheva et al. (1990) who isolated *Listeria monocytogenes* from the eggshell (1 of 71 samples) but not from egg contents. In contrast, *L. monocytogenes* was isolated with high frequency from samples of eggs collected at processing plants (Leasor and Foegeding, 1989) as well as from wash water and the outer surface of the eggshell (Faber et al., 1992). Sayed et al. (2009) found that egg shells were contaminated with 7% of *Listeria* spp. while no contamination was found in egg contents. Moore and Madden (1993) reported that 72% of raw blended egg samples were positive for *Listeria* spp. of which 37.8% were identified as *L. monocytogenes*.

In the current study, a single *campylobacter* (0.7%) of 135 eggs (*n* = 45 pooled samples) isolate was detected in a shell samples with no contamination of the egg contents. *Campylobacter jejuni* is commonly associated with poultry, which might result in egg shells and egg contents contamination (Humphrey, 1994). Earlier studies showed that, even if at a low level, *C. jejuni* could be isolated from both outer (Doyle, 1984) and inner (Shanker et al., 1986) shell surfaces of eggs laid by naturally infected commercial layers or broiler breeders. Shane et al. (1986) isolated the organism from both interior surfaces of egg shell and egg contents after swabbing feces containing *C. jejuni* onto the egg surface.

E. coli O157:H7 was not isolated from any of egg samples investigated in this study. However, Schoeni and Doyle (1994) reported long colonization and shedding of *E. coli* O157:H7 in chicken ceca and *E. coli* were isolated from the shell of 14 out of 101 (13.9%) eggs but not from the albumen or yolks.

Failure to isolate *Salmonella* spp. in table eggs in the current study may owe to strict control measures applied against these bacteria. Similarly, *Salmonella* was absent in all samples analyzed (Favier et al., 2000; Anon, 2004). Other studies reported variable and very low incidence of *Salmonella* in eggs. Begum et al. (2010) only isolated three *Salmonella* strains out of 1100 domestic eggs. Musgrove et al. (2005) identified one out of 105 Enterobacteriaceae isolates, isolated from 84 shell surfaces, as *Salmonella*. Poppe et al. (1998) reported that 0.07 to 0.4% table eggs (*n* = 1/512) (egg shell and egg content) were *Salmonella*-positive. De Reu et al. (2006) found 0.18% (1/554 eggs) table eggs *Salmonella*-positive. Fourteen (14) eggs out of a total of 46200 eggs (0.03%) sampled were *Salmonella*-positive (de Boer and

Wit, 2000). This variability in *Salmonella* occurrence may be due to sample size, timing of sampling, sites of the egg that were tested, techniques used, investigations of eggs laid by artificially or naturally infected hens (Humphrey, 1994).

The fungal load found averaged $1.9 \log_{10}$ CFU/ml. however, higher fungal count was reported in table eggs (Ahmed et al., 2002; Suba et al., 2005; Salem et al., 2009) which was reported to be $>5 \log_{10}$ CFU/g. Other studies indicated lower count of $1 \log_{10}$ CFU/g in egg samples (Ahmed et al., 1987; El-Essawy et al., 1989). Jones et al. (2004) found an average fungal concentration of $1.5 \log$ CFU/egg shell in the day of egg collections while averaged $0.1 \log$ CFU/ml in the content of unwashed shell eggs. Mycological examination carried out in the current work revealed four genera, which agrees with published reports where *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp. and *Mucor* spp. have been recovered from eggs or their wash water (El-Essawy et al., 1989; Obi and Igbokwe, 2007; Salem et al., 2009).

The AFs contamination was detected to trace amounts only in three (6.7%) of egg samples ranged between <1 to 1.19 ppb. The positive samples did not exceed the maximum residual limits of aflatoxin recommended by the WHO (2005) which is 5 ppb. Similar findings were reported by Ahmed et al. (2002), Aly and Anwer (2009), Herzallah (2009), Salem et al. (2009) where aflatoxin contamination of eggs was minimal comparing with the limit recommended by WHO (2005).

In conclusion, the results showed that eggs of Taif city markets are generally in a good quality. However, because of the presence of minimal pathogenic micro-organisms in some samples, we recommend that table eggs should not be consumed raw.

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