Shell quality effect on *Salmonella* Enteritidis (SE) and *Escherichia coli* behavior on chicken eggs surface submitted to an oscillating temperature regime and the risk of bacteria penetration in the content

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The objectives of this work were to demonstrate the behaviour of *Salmonella* Enteritidis and *Escherichia coli* on eggshell surface when they are submitted to temperature oscillation and to investigate the risk of bacteria penetration in chicken eggs submitted to this temperature condition. Eggs were separated in two blocks (no shell defects and little shell defects) by ovoscopy. The eggshell defects don't declassify eggs to consumer use. The shell surfaces had been contaminated with chicken faeces previously inoculated with SE and *E. coli*, on their meridional side (about 1/8 of their total area). They had been submitted to an oscillation temperature regime (8°C – zero up to 48 hours; 30°C – 49 up to 72 hours and 8°C – 73 up to 336 hours). Bacterial counts were performed at defined times. The shells were submitted to a surface washing with buffered peptone water, albumen and yolk were analyzed by decimal dilution. Five samples were analyzed per time and per shell and plated in agar MacConkey. The Exact Fischer’s Test was used to show statistical differences between shell conditions, survival of bacteria on shell surface and their penetration in the egg contents. SE and *E. coli* had remained viable and maintained their counts on shell surface until the end of experiment (336 hours) in all samples, no shell defects and shell defects. The penetration in the content occurred only in eggs with shell defects during the 48 hours storage at 30°C. In these conditions, SE and *E. coli* can survive in shell surface and may allow the egg content contamination by the consumer use. When egg shell presented defects, the albumen and yolk contamination probability rised although no statistical significance was observed.

Key-words: chicken eggs; bacteria penetration; food safety

Introduction

Egg shell, even when intact, allows microorganisms to penetrate into its contents. There are several factors that decide this penetration, including: variations in temperature, the integrity of the shell and its quality. These factors, especially combined, may increase the frequency of penetration. The storage temperature of the eggs is a limiting factor in the multiplication of the micro-organisms, even low temperatures allow the presence of micro-organisms on the egg surface (Simon, Ayres and Kraft, 1970; Gentry and Quarles, 1972; Radkowski, 2002; and Aydin *et al*, 2004). Despite this, dirty eggs when stored at a high temperature, experience a significant decrease in bacterial contamination on the surface of the shell (Gentry and Quarles, 1972). Oliveira and Silva (2000) observed a reduction in the quantity of viable SE cells on shells contaminated with pieces, even after the first 24 hours of storage.
at room temperature (25°C). According to Radkowski (2002), the process of conservation can allow the condensation of humidity on the surface of chicken eggs if there is temperature variation. Ernst et al (1998) relate the accumulation of humidity through condensation to a high frequency of internal contamination by salmonella, mainly when dealing with eggs with defective shells. Stadelmann (1995) relates that condensation occurs when refrigerated eggs are exposed to higher temperatures. The regime of temperature variation simulates what happens in practise when eggs are subject to breakage on the cold chain or when inadequately stored domestically (in the refrigerator door). When refrigerated eggs are subject to an elevated temperature the phenomenon of “sweating” occurs, or rather the condensation of water on their surface (Ernst et al op. cit). Eggs with intact shells can have their contents contaminated by micro-organisms including Salmonella (Todd, 1996). The degree of invasion is dependent on several factors such as the quantity of water, humidity and the temperature difference between the egg and the liquid (Berang et al, 1999). Before laying, the egg is at the internal temperature of the bird; 42°C. On contact with the environment the egg cools and the contents contract to form a negative pressure in the interior. The possibility of contamination of the contents of chicken eggs increases if there is a positive difference of temperature (the temperature of the egg is higher than that of the environment). Even in eggs of low quality, bacterial contamination depends on temperature and humidity, associated with the quality of the shell, there is also a relationship with the number of pores. (Nascimento and Solomon, 1991). Santer and Peterson (1974) observed that there that eggs with an excellent shell quality had a lower index of contamination (21%) when compared to eggs considered to be of medium or bad quality; which, respectively, had 27% and 54% of eggs contaminated. This was after 24 hours of contamination of the shells by immersion in a culture broth containing salmonella and using a temperature gradient. Studies have proven that eggs with cracked shells are a source of salmonelllas (Todd, 1996). According to D’Aoust, Stoltland and Randall (1980), between 8 and 13% of cracked eggs showed salmonella in their contents or shells, while, for eggs with intact shells, only 2% showed the presence of micro-organisms. Also, Perales and Audicana (1989), found salmonellas in 5% of eggs with cracked shells and in 0.6% of intact eggs submitted to microbiological analysis. The presence of organic material on the eggs favours the invasion of the contents by micro-organisms, even after disinfection (Padron 1990). The environment of the laying hens, if inadequately hygienic, is also an important factor that predisposes bacterial penetration (Bruce and Drysdale 1994). Eggs laid in nests or cages contaminated with faeces have a greater chance of having their contents invaded by micro-organisms. Board and Tranter (1994) relate that the main sources of contamination of the egg surface at laying, apart from the cloaca, are dust, soil and faeces. Oliveira and Silva (2000) verified the presence of SE in the interior of table eggs that had been contaminated on the surface by contaminated pieces and stored under different temperature conditions. The objective of the study was to demonstrate the behaviour of Salmonella Enteritis and Escherichia Coli on the surfaces of dirty eggs of varying shell quality submitted to variation in temperature, and investigate the risks of bacterial penetration in chicken eggs submitted to this condition of storage.

**Materials and Methods**

**Samples:** Chicken eggs 60g+3g by weight and a maximum of 10 hours from laying. These were classified by the quality of egg shell, by ovoscopy, as with no defects or with small defects in the shell (not unclassified commercially in natura).

**Contamination of the eggs:** The surface of with previously sterilised chicken faeces inoculated with Salmonella Enteritis and Escherichia Coli on their meridinal side (totalling ⅛ of their area). The inoculation of the faeces was conducted in such a way as to give 10⁷/sample unit of each of the micro-organisms.

**Temperature regime:** the contaminated eggs were submitted to the following regime: 8°C from 0 to 48 hrs; 30°C from 49 to 72 hrs; 8°C from 73 to 336 hrs.

**Bacterial count:** the counting was conducted at predetermined times (0, 24, 72, 168 and 336 of storage). The eggs were washed in buffered peptone water to recover the micro-organisms present on the shell. They were drained and immersed in alcohol 70⁰GL for 30 minutes and then dried and scorched. After aseptic breaking the yolk and albumen were separated and diluted in buffered peptone
water (decimal dilution). Five samples were analysed each time. The samples were plated on MacConkey agar and incubated for 24-48 hours at 35°C.

Statistical analysis using the Exact Fischer Test was conducted to identify the statistical difference between, the conditions of the shell (with and without defects), bacterial survival on the shell and penetration to the contents.

Results and discussion

SE and *E. coli* remained viable and stable on the surface of the eggs throughout the study, in all samples independent of the condition of the shell (table 1). There was no significant difference in the behaviour of micro-organisms between the intact eggs and those with defects (α of 0.05). In fact, the micro-organisms remained viable throughout the time period. The behaviour, on the shell, of both micro-organisms under study, needs also to be considered an important factor in the contamination of eggs, since they remain viable and a stable contaminant throughout the study, in the conditions studied (in the presence of organic material, dirty eggs).

Table 1: Contaminated media of *Salmonella* Enteritis and *Escherichia coli* on the surface of eggs, with intact and defective shells, contaminated via faeces and stored in a regime of varying temperatures and relative humidity.

<table>
<thead>
<tr>
<th>t/T*</th>
<th>SE Contamination (logUFC/egg)</th>
<th>E. coli Contamination (logUFC/egg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eggs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intact Shell</td>
<td>Defective Shell</td>
</tr>
<tr>
<td>0/8°C</td>
<td>3.093422</td>
<td>3.093422</td>
</tr>
<tr>
<td>24/8°C</td>
<td>3.823474</td>
<td>3.071882</td>
</tr>
<tr>
<td>48/30°C</td>
<td>4.056905</td>
<td>4.622214</td>
</tr>
<tr>
<td>72/30°C</td>
<td>4.037426</td>
<td>4.594393</td>
</tr>
<tr>
<td>168/8°C</td>
<td>4.369216</td>
<td>4.565848</td>
</tr>
<tr>
<td>336/8°C</td>
<td>5.193125</td>
<td>5.033424</td>
</tr>
</tbody>
</table>

*time/temperature

The presence of humidity on the surface of the eggs due to variation in temperature, allows the permanence of micro-organisms on the surface, this was allied with the keeping of the product at 8°C for most of the experiment. Simons, Ayres and Kraft (1970), Gentry and Quarles (1972), Oliveira and Silva (2000), Radkowski (2002), and Aydin *et al.* (2004), have proved the presence of viable bacterial cells on the shells of intact eggs for longer when they are subjected to refrigeration temperatures (10°C or less).

The behaviour, on the shell, of both micro-organisms under study, needs also to be considered an important risk factor in the contamination of eggs. Notice that there is a tendency for the micro-organisms to multiply under the study conditions (in the presence of organic material, dirty eggs), when the eggs are subjected to the study conditions.

Table 2: Presence of *Salmonella* Enteritis and *Escherichia coli* in the albumen and yolk of chicken eggs (both intact and with defects), contaminated via faeces and subjected to a regime of varying temperature and relative humidity.

<table>
<thead>
<tr>
<th>t/T***</th>
<th>SE Contamination (logUFC/egg)</th>
<th>E. coli Contamination (logUFC/egg)</th>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Intact Shell</td>
<td>Defective Shell</td>
</tr>
<tr>
<td>0/8°C</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
<tr>
<td>24/8°C</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>48/30°C</td>
<td>1/5</td>
<td>2/5**</td>
</tr>
<tr>
<td>72/30°C</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>168/8°C</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>336/8°C</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

n.r. = not realized  
** count obtained  
* presence after incubation for 24 hours at 35°C  
*** time/temperature
Table 2 shows the results for the presence of the micro-organisms under study. It can be seen that eggs with defective shells show the invasion of the internal contents after 24 hours under the regime of high temperature (48th hour of storage). However, for the same storage time, only one sample unit of those with intact shells showed the presence of SE in the albumen. Although there was no significant difference under the Fischer Test ($\alpha$ of 0.05), it could be noticed that there was a greater tendency for bacterial penetration in defective eggs subjected to the condensation of humidity. A bacterial count could only be conducted for eggs with defective shells, indicating that a larger number of bacterial cells penetrated into the contents of the eggs. This compared to intact eggs, where only the presence of SE could be identified. The greater the level of the initial contamination the greater was the possibility that bacteria could reach sufficient quantities to provoke damage to the health of the consumer. Even if only one egg was contaminated with a large number of bacterial cells, mixing it with other eggs or other ingredients in the preparation of a meal raised the risk of disease. There is already a relationship with the manifestation of disease and the quantity of agent ingested by the host. Ernst et al (1998) concluded, in their study simulating the condensation of humidity on the surface of the eggs that the quality of the shell seems to be a more important impediment to the penetration of SE into the contents than the presence of humidity alone. The methodology used in their study was to immerse the samples in a culture broth containing the micro-organism, which could have promoted a greater contamination of the albumen and yolk. The tendency of a greater number of samples with defects to be invaded by micro-organisms, in this experiment shows that even when contamination is confined to the surface, there could be a greater risk of penetration of SE into the eggs’ contents if they are subjected to the condensation of humidity.

As regards E. Coli, it can be verified that a greater number of intact eggs have their contents invaded (when compared to eggs with defective shells). However this difference is not significant under the Fischer Test ($\alpha$ of 0.05). This occurrence proves the description by Tood (1996) that even intact eggs can have their contents contaminated with micro-organisms, mainly if they were subjected to certain conditions, such as the presence of humidity or temperature difference. In eggs with defective shells, however, a smaller number of sample units had been contaminated, it was noticed that the bacterial load was larger, and it was already possible to conduct a bacterial count, a different situation to that with intact eggs. It can be verified, as well, that there was a major occurrence of contaminated eggs when those with defective shells were examined. However, there wasn’t, in this case, any significant difference under the Fischer Test ($\alpha$ of 0.05). This trend was also observed in the behaviour of SE under the experimental conditions. Ernst et al (1998) relate the accumulation of humidity by condensation to the greater frequency of internal contamination of the eggs by Salmonellas, mainly when dealing with eggs with defective shells. The authors, working with various qualities of shell, concluded that the integrity of the shell is more important in the prevention of invasion than condensation itself. On the other hand, it could be noticed that even for intact chicken eggs, this condensation coupled to inappropriate temperatures could promote the entry of bacterial pathogens into the contents. This fact could represent a risk to public health.

After this study it can be affirmed that if the demands of hygiene and adequate storage are not met then the risk of the survival of E Coli and SE on the shells of chicken eggs, as well as their entry into the contents, represents a serious risk to human health. This risk occurs when the eggs are eaten raw or are inadequately heated, or even by cross contamination, since the responsible agents can remain viable on the surface of the shells.

References


