Study on the microbial quality of experimentally washed eggs

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Summary
This study was performed to investigate the effects of washing on the microbial quality of table eggs and on the cuticle structure. Data are needed to qualify the risk for the consumers and to evaluate the marketing ability of washed eggs. For that 1500 fresh laid hen’s eggs were examined. The total viable count on the egg shell was determined from washed and unwashed eggs. Washed eggs showed significantly lower counts than the unwashed control group. The air cell height and the penetration of microorganisms into the egg content of washed and unwashed eggs was examined after a storage of 18 and 28 days at 20°C combined with 70 and 85% relative humidity respectively. There was no statistical significance between the air cell height of both washed and unwashed eggs and no significant difference between percentage of bacterial penetration of washed and unwashed eggs. The effect of washing on the cuticle was detected using Evans Blue and scanning electron microscopy. Washing the eggs with warm water resulted in slight damages but using of disinfecting agents leads to complete removal of the cuticle.

Introduction
The modified regulation EEC 1907/90 will possibly allow the washing of eggs under certain conditions. If these eggs conform the class A criteria, they could be marketed labelled as “washed eggs” on the pack. One reason for a possible changing of the community legislation is that in some Member States such as Sweden the washing of eggs is common and the consumer’s preference is in favour of buying washed eggs.

Additionally the question of washing eggs gains much more interest because of the Council Directive 1999/74/EC which is laying down minimum standards for the protection of laying hens and bans the use of battery cages within the European Community from 01.01.2012. From this time only alternative keeping systems like free range systems, deep litter systems, multilevel aviaries or furnished cages will become licensed in whose consequence a higher rate of soiled eggs must be expected (Table 1).

Table 1  Rate of soiled eggs in different keeping systems (%).

<table>
<thead>
<tr>
<th>Rate of soiled eggs (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage</td>
<td>Alternative systems</td>
</tr>
<tr>
<td>2.2</td>
<td>5.0-18.6</td>
</tr>
<tr>
<td>1.5</td>
<td>11.7</td>
</tr>
<tr>
<td>9.0</td>
<td>Deep litter system</td>
</tr>
<tr>
<td>1.0</td>
<td>1.3-3.2</td>
</tr>
<tr>
<td>1.8</td>
<td>2.3-6.4</td>
</tr>
<tr>
<td>2.4</td>
<td>5.0</td>
</tr>
</tbody>
</table>

References

FAL 1982, cit. in Bessei et al. (2001)."
However, the discussion on egg washing is very controversial. There is one view that washing eggs damages the cuticle of the eggshell (Sparks and Burgess 1993) which consists of two layers with a thickness of 0.5 until 30 µm in total. The cuticle covers most of the pores of the shell and offers a natural barrier to contaminants, and also may cover up evidence of poor husbandry and hygiene standards on farms. Damages of this barrier could implement an increased health hazard because of penetrating pathogens such as Salmonella Enteritidis (S.E). However, Hutchison et al. (2004) could not isolate Salmonella from yolk or albumen of artificially contaminated eggs washed by an optimal protocol (spray jet washing). More historical long term storage-studies of Gržímek (1936) and Vadehra et al. (1970) proved that washed eggs also spoiled more quickly. More recent micro-structural studies of Kim and Slavik (1996) confirmed that some washing chemicals and sanitizers which are recommended can etch the cuticle, whereas water-washed control eggs looked similar to unwashed eggs except for minor scratches on the cuticle.

The other view, particularly in Sweden, Japan and USA, is that washing eggs reduces the presence of contaminants on the shell picked up during the egg production process and minimized in that way also the risk of horizontally-transmitted contamination of the yolk by S.E. (Mauldin and Buhr 1993, ICMSF 1998, Hutchison et al. 2003, 2004). However, the contribution of washing in improving the reduction of Salmonella risk remains unclear and is largely unproven by published data (Hutchison et al. 2003).

Current egg washing practice forms a part of the egg packaging companies, if possible within 24 h of lay, and can be divided in 4 stages (Hutchison et al. 2003): 1st) pre-washing: wetting with spray of warm water of about 40°C; 2nd) washing: either rubbing with rotating brushes in side-to side motion with 40-50°C hot water or with high pressure jet systems; 3rd) rinsing: clean hot water (up to 60°C) to remove debris and 4th) drying and further treatment (e.g. oiling). In USA it has been estimated that 50% of washed eggs are oiled, which seems not necessary when eggs distributed below 12°C and likely to be consumed quickly (Hutchison et al. 2003).

The washing procedure/practice seems to be crucial for the microbiological quality of washed eggs (Srikaeo and Hourigan 2002, Hutchison et al. 2003). Besides of high temperatures between 41-44°C (Mauldin and Buhr 1993, Australian Egg Industry Association 1998) pH-values about 11 of the washing water are demanded by Catalano and Knabel (1994). Further, the concentration of iron in the washing water should be less than 2 ppm otherwise an increased spoilage can be expected because of saturation of the iron chelating properties of ovotransferrin (Garibaldi and Bayne 1960, 1962).

However, even if eggs are washed by an optimal protocol there are a number of concerns. Potential risks for washed eggs are that all brushes and also high-pressure jet systems have a potential to damage the eggs. A condensation or so-called sweating because of an insufficient drying procedure or caused by movement of eggs in and out of cold stores could implement a faster penetration of bacteria through the shell.

Our extensive microbiological study was conducted to show if washed eggs still ensure the safety of the consumer and / or whether there are any disadvantages regarding the shelf life of the eggs. The answer to this question is essential for their marketing ability and simultaneously of great importance in the process of introducing alternative keeping systems in Europe.

Material and methods
Up to now 1500 intact hen’s eggs (Hisex White) from a local farm in Saxony (Germany) were examined.

Fresh-laid optically clean and soiled eggs (faecal material, dust, blood, egg content or feathers on the surface) from a cage husbandry were washed for one minute under flowing tap water (without any chemicals or sanitizers) with temperatures from 38 to 42°C using a brush followed by air-drying for about 2-3 minutes to simulate the commercial practice. In one trial hypochlorite-containing water (18-21 ppm) was used and the effect on the cuticle was proved.

For evaluating the effect of washing on the eggs the following investigations were performed:

**EFFECT OF WASHING ON THE CUTICLE**

a) Fresh laid washed and unwashed eggs (n=30) were coloured with Evans Blue, a diazo-dye which strongly interacts with cuticle-proteins according to the method of Mamadou (1990).

For that eggs were dipped into 250 ml 10% Evans Blue (Fluka, 46160) for five minutes, rinsed for another 10 seconds with tap water, dried and graded using an self made index covering 8 nuances from 0 (white colour; almost no cuticle) to 7 (deep blue colour, intact cuticle).
b) Further, scanning electron microscopy (SEM) was used to define the cuticle structure before and after the washing procedure. Shell samples from the sharp and blunt edges and from the equatorial regions of fresh laid washed/unwashed eggs (n=20) were broken into pieces (0.7x0.7 cm) and dehydrated for a couple of days in a vacuum container, coated with gold for a better conductivity (MED 020, Bal-Tec) and investigated by using an 1430 scanning electron microscope (SEM 1430, LEO).

**TOTAL VIABLE COUNT (TVC) ON THE SHELL BEFORE AND AFTER WASHING**

To determine the TVC on the shell the method of surface rinsing and shaking was used. Washed and unwashed eggs (n=80) were given into a sterile bag containing 25 ml of NaCl-peptone water (Sifin, TN 1137) and shaken by hand for 1 minute. Serial dilutions were made and surface-plated on plate count agar (Sifin, TN 1189). After an aerobic or anaerobic incubation of 48 hours at 30°C and 37°C respectively the TVC was enumerated.

**PENETRATION OF MICROORGANISMS INTO THE EGG CONTENT**

Both, washed eggs and the unwashed control group were stored up to 18 days and to the end of the given shelf life of 28 days considering two different defined storage conditions: 20°C combined with a relative humidity (rh) of 70 and 85% respectively (see Table 2).

**Table 2 Experimental setup.**

<table>
<thead>
<tr>
<th>Storage time</th>
<th>18 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>70% rh</td>
<td>85% rh</td>
</tr>
<tr>
<td>No. of washed eggs</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>No. of unwashed eggs</td>
<td>150</td>
<td>150</td>
</tr>
</tbody>
</table>

The fresh laid eggs were candled and eggs with any shell damages were not used in these experiments. Further the air cell was measured at the day of lay and at the 18th and 28th day of storage. In the same intervals the TVC of the egg content was enumerated (see Table 2 and additionally at the day of lay: n=170 were investigated). For that eggs each were opened at the air cell (blunt edge) after disinfection the shell surface using ethanol. After opening the inner shell membranes the egg content was mixed. 36 ml of the content were given into 100 ml buffered peptone water (Sifin, TN 1137), incubated for 48 hours at 30°C and streaked directly on plate count agar (Sifin, TN 1189). 1 ml of the mixed egg content was taken separately for determining the TVC on plate count agar (Sifin, TN 1189) after 48 hours at 30°C if a growth in the enrichment occurred. Colonies were counted and still have to be identified biochemically.

**Results and discussion**

The results so far are summarized in the following Tables and Figures.
EFFECT OF WASHING ON THE CUTICLE (SEM-ANALYSES AND EVANS BLUE)


Pictures 2a-c show in the view from above and cross-sectional a complete homogeneous contiguous cuticle on the shell. All pores are covered by the cuticle.

The ultra structural analysis of the washed eggs shows that the cuticle was slightly damaged by the washing procedure but not removed completely (Figure 2a). The cuticle became rougher and thinner and more pores became visible. An interesting finding were pores plugged by cuticle material (Figure 2b). More investigations are required to confirm this as a result of washing.
These results were supported by colouring the cuticle with Evans blue. Unwashed eggs (n=10) showed an index of 4.4 (mean value) and water washed eggs (n=10) an index of 2.0 (significant difference, t-test, p<0.05). If eggs (n=10) are washed with hypochlorite-containing water for one minute and rubbed with a brush the index decreased to 1.0 and the SEM analyses confirmed that cuticle was removed almost completely (Figure 2c, cuticle-free shell).

TOTAL VIABLE COUNT (TVC) ON THE SHELL BEFORE AND AFTER WASHING
Figure 3 demonstrates the reduction of the TVC (mean value of aerobic and anaerobic colony counts) on the shell of both soiled and optically clean eggs after washing with warm tap water.

* below detection rate

AIR CELLS AND PENETRATION OF MICROORGANISMS INTO THE EGG CONTENT
Figure 4 characterizes the development of the air cell height of washed and unwashed eggs over the storage period at the two storage conditions.
There was no statistical significance (One way Anova, p>0.05) between the air cells of both washed and unwashed eggs within in one storage condition. As expected the relative humidity influences the grade of evaporation. The air cells of the eggs held at 85% relative humidity had significant smaller air cells after the storage time of 28 days.

**Tables 3 and 4** Percentage of microbial positive egg content after the 18 and 28-day storage at the two storage conditions (aerobic TVC).

**Storage condition 1: 20°C 70% relative humidity**

<table>
<thead>
<tr>
<th></th>
<th>0 d</th>
<th>18 d storage</th>
<th>28 d storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>E</td>
<td>D</td>
</tr>
<tr>
<td>Washed</td>
<td>1.2%</td>
<td>2.9%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Unwashed</td>
<td>7.3%</td>
<td>3.3%</td>
<td>8.0%</td>
</tr>
<tr>
<td>Washed</td>
<td>5.3%</td>
<td>9.3%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Unwashed</td>
<td>6.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D: Directly plated</td>
<td>E: Enrichment</td>
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**Storage condition 2: 20°C 85% relative humidity**

<table>
<thead>
<tr>
<th></th>
<th>0 d</th>
<th>18 d storage</th>
<th>28 d storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>E</td>
<td>D</td>
</tr>
<tr>
<td>Washed</td>
<td>1.2%</td>
<td>2.9%</td>
<td>2.0%</td>
</tr>
<tr>
<td>Unwashed</td>
<td>7.3%</td>
<td>4.0%</td>
<td>7.6%</td>
</tr>
<tr>
<td>Washed</td>
<td>4.7%</td>
<td>6.0%</td>
<td>4.7%</td>
</tr>
<tr>
<td>Unwashed</td>
<td>6.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D: Directly plated</td>
<td>E: Enrichment</td>
<td></td>
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</tbody>
</table>

Tables 3 and 4 show no significant difference between washed and unwashed eggs after the 18 or 28-day storage (Chi quadrate-test, p>0.05). Even the higher relative humidity of 85% had no significant influence on the ability of bacteria to penetrate into egg yolk.

Summarizing all the results so far the following conclusions can be made. A washing practice with warm water leads to a thinner cuticle whereas an adding of disinfecting agents like hypochlorite destroys the cuticle almost completely which confirms the results of Kim and Slavik (1996). The ultra structural analyses also showed that on water washed eggs some pores were struggled with cuticle material. This might be a possible reason for the insignificant difference of the air cell heights of washed and unwashed eggs and for nearly the same low rate of penetrated organisms.

The washing procedure resulted in an about 3-log reduction of the shell germ account which is similar to the investigations of Hutchison *et al.* (2004) with a more than 5-log reduction of Salmonella of artificially contaminated eggs after spray jet washing. The reduced count on the shell could be another explanation for the low penetration rate of organisms into the egg content. A higher relative humidity of 85% resulted as expected in a lower air cell height but was not followed by a higher rate of microbial positive egg content after the storage period of 28 days.

From the results demonstrated above cannot be concluded that there is a higher potential risk for consumer's safety by egg washing but further investigations are planned to confirm our preliminary findings.

**References**


Srikaeo, K. and Hourigan, J. A. (2002) The use of statistical process control (SPC) to enhance the validation of critical control points (CCPs) in shell egg washing. *Food Control* **13**: 263-273