

Effect of egg washing on the cuticle of table eggs

W. Messens¹, S. Leleu¹, K. De Reu¹, S. De Preter², L. Herman¹, J. De Baerdemaeker³, M. Bain⁴

¹*Institute for Agricultural and Fisheries Research (ILVO), Technology and Food Science Unit, Brusselssesteenweg 370, 9090 Melle, Belgium*

²*Eggology, Acaciadreef 12, 3140 Keerbergen, Belgium*

³*Department of Biosystems, Katholieke Universiteit Leuven, Kasteelpark Arenberg 30, 3001 Heverlee, Belgium*

⁴*University of Glasgow, Faculty of Veterinary Medicine, 464 Bearsden Road, Glasgow G61 1QH, Scotland, UK*

* Corresponding author: winy.messens@ilvo.vlaanderen.be

Abbreviated title: Cuticle of washed table eggs

Summary

Within the EU, egg washing is currently only permitted in Sweden and in one plant in the Netherlands mainly due to the concern that damage can occur to the cuticle during or after the washing process. In this study we compared the cuticle coverage of 400 eggs derived from either a brown and white egg laying flock which were either unwashed or washed using a standard Swedish egg washing process. The cuticle of the washed and unwashed eggs was quantified using a colorimeter by measuring the colour difference before and after staining the eggs with Tartrazine and Green S (or Edicol Pea Green) stain. The cuticle of an additional 30 eggs from each group was then visually assessed by scanning electron microscopy (SEM). The staining characteristics of the cuticle differed greatly within each group of eggs irrespective of their origin and washing did not lead to damage of the cuticle (one sided t-test with $\alpha=5\%$ comparing washed versus unwashed brown eggs and washed versus unwashed white eggs). SEM confirmed that there was no irreversible damage to the cuticle when present in the washed egg groups. In conclusion, we could find no evidence to suggest that the washing procedure used in this investigation irreversibly changed the quality of the cuticle.

Introduction

Within the EU egg washing is currently only permitted in Sweden and in one plant in the Netherlands due to wide spread concerns that potential damage to the cuticle occurs during or after washing, as a result of sub-optimal operation of apparatus. Such damage may favor trans-shell contamination with bacteria and moisture loss and thereby increase the risk to consumers particularly if subsequent drying and storage conditions are also sub-optimal (EFSA, 2005). Whilst any shell damage should be seen as a disadvantage of washing, it should be balanced against the fact that washed eggs normally have considerably lower microbiological populations on the shell. Nonetheless, the aim must be to avoid, or at least minimize any damage to the eggs natural defenses.

In a number of countries, such as the United States, Australia and Japan, egg washing has become a routine and established practice, is regarded as safe, and is perceived by consumers as an essential part of the hygienic production of eggs (Hutchison et al., 2004). In Sweden, washing has been shown to lower microbial counts on the shell surface of washed versus unwashed eggs and no movement of microbes from the shell to the egg contents has been reported. The aim of the current study was to compare the cuticle coverage by means of color measurement using a dye and the ultrastructural properties of the cuticle of washed versus unwashed eggs derived from a flock of brown and a flock of white egg layers.

Materials and methods

Eggs

Eggs were derived from either a brown and white egg laying flock which were either unwashed or washed using a standard Swedish egg washing process. The brown eggs were from a laying flock in Belgium, while the white eggs were from a laying flock in Sweden. To avoid bias, all eggs were assigned a randomized code before being assessed.

Cuticle staining

The cuticle coverage was compared of 100 eggs of each group. The cuticle of the washed and unwashed eggs was quantified using a colorimeter by measuring the colour difference before and after staining the eggs by immersion in an aqueous solution containing per liter 7.2 g Tartrazine and 2.8 g Green S (Barentz N.V., Zaventem, Belgium) (also referred to as Edicol Pea Green) for a period of 1 min. The shell was then rinsed in water to remove excess dye prior to

drying. This method was developed by Board and Halls (1973). The cuticle deposition is then measured using a colorimeter (spectrophotometer CM-2600d; Konica Minolta). It uses the L*a*b* colour space. The system measures the degree of lightness L* and the chromaticity coordinates are a* and b*. The a* and b* indicate colour directions: +a* is the red directions, -a* is the green direction, +b* is the yellow direction and -b* is the blue direction. The saturation of the colour increases when the a* and b* values increase and the point moves out from the centre. In the L*a*b* colour space, colour difference can be expressed as a single numerical value ΔE_{ab}^* defined by:

$$\Delta E_{ab}^* = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

The L*a*b* value of each egg was determined at four points around the equator before and after staining. Then the average L*a*b* before and after staining was calculated and used to calculate ΔE_{ab}^* . The higher ΔE_{ab}^* , the higher the cuticle deposition.

A two-sample one-sided t-test was performed to assess whether ΔE_{ab}^* was reduced by washing of eggs. This test was done for both brown and white eggs. Because the variable ΔE_{ab}^* within each group of eggs was not normally distributed, a box-cox transformation was performed with an outcome of $\lambda = 2$. The t-test was thus performed on the square transformed variable. Equality of variances was assumed for brown eggs as the F-ratio test gave a p-value of 0.34. For white eggs, equality of variances was rejected by the F-ratio test ($p < 0.001$). The null hypothesis is thus

$$H_0: \mu_{\text{square } \Delta E_{ab}^* \text{ washed eggs}} \geq \mu_{\text{square } \Delta E_{ab}^* \text{ unwashed eggs}}$$

The alternative hypothesis is that

$$H_a: \mu_{\text{square } \Delta E_{ab}^* \text{ washed eggs}} < \mu_{\text{square } \Delta E_{ab}^* \text{ unwashed eggs}}$$

The significance level α was set at 0.05. All analyses were done in R version 2.7.2 (2008-08-25).

Ultrastructural assessment

Ultrastructural assessment was done for 30 eggs of each group. Sections of eggshell were removed from the equator of each egg using a diamond tipped circular saw. These sections were then mounted on an aluminium stub, coated with gold palladium then viewed with an Hitachi 501B scanning electron microscope at a standard working distance of 12 mm and an accelerating voltage of 15kV. The gold palladium coating masked the colour of the eggshell which in addition to the coding system further insured a non-biased approach.

The sections of eggshell were analysed randomly using a standard magnification of $\times 500$ and then assigned a score for each of the following criteria:

- Cuticle coverage (even coverage = 0; occasionally patchy = 2; very patchy = 5; no cuticle = 8)
- Mechanical damage (none = 0; occasionally present = 2; frequently observed = 6)
- Presence of surface debris (absent = 0; isolated = 2; moderate = 5; heavy = 8)
- Exposed Pores (none visible = 0; occasional pore visible = 2; frequently observed = 5)

The scores for each category were weighted in terms of their perceived effect on the functionality of the cuticle such that the higher the score the more negative the effect. The average score for each category was then calculated for all groups of eggs.

A representative photo was also taken of each section of the eggshell (brown *versus* white; washed *versus* unwashed) at a magnification of $\times 500$ and another experienced assessor was then asked to look at these images and comment on whether they thought the image represented a washed (Y) or an unwashed (N) eggshell. The 60 brown eggs (washed and unwashed) were assessed independently of the 60 white eggs (washed and unwashed) in this exercise. The assigned category (Y or N) for each coded egg was then compared to its true source (W or UNW) to determine how many times the assessor got it right. The assessor was not told how many eggs there should be in each category in advance.

Results and discussion

In Table 1 the descriptive statistics are given and Fig. 2 depicts the ΔE_{ab}^* values of both the brown and white eggs that were either unwashed or washed. The staining characteristics of the cuticle differed greatly within each group of eggs irrespective of their origin, reflecting the natural variability in cuticle deposition. Washing did not lead to damage of the cuticle (one sided t-test with $\alpha=5\%$ comparing washed versus unwashed brown eggs and washed versus unwashed white eggs).

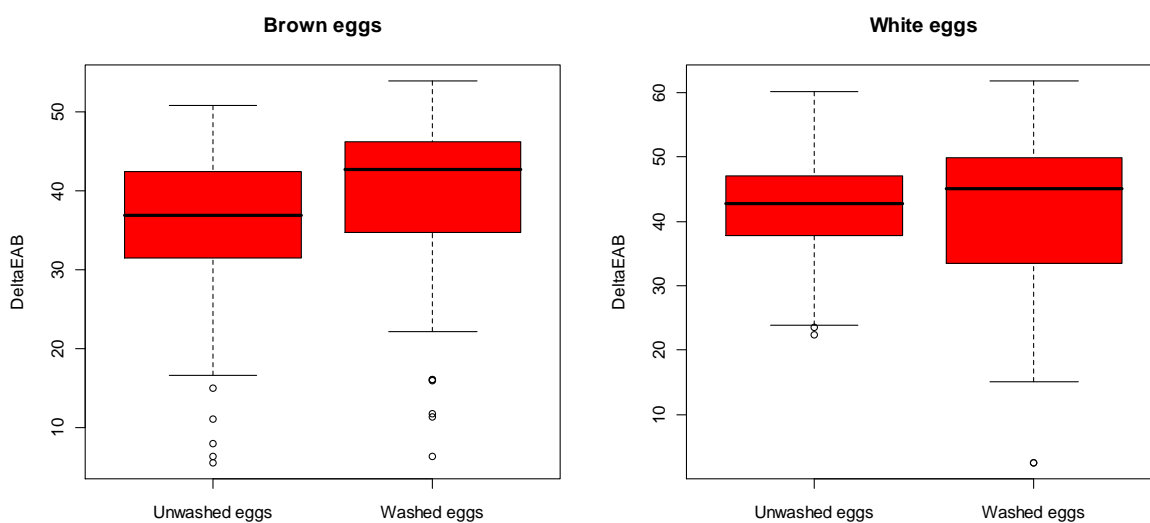
Table 1. ΔE_{ab}^* values of both brown and white eggs that were either unwashed or washed

Group	N	Mean \pm SD	Median (IQR*)	Min	Max	p-value†
Brown eggs						p = 0.9997
Unwashed	100	35.50 \pm 9.58	36.90 (31.58 – 42.34)	5.53	50.81	
Washed	100	39.61 \pm 9.65	42.77 (34.88 – 46.21)	6.43	54.04	
White eggs						p = 0.34
Unwashed	100	42.75 \pm 8.49	42.82 (37.82 – 47.04)	22.32	60.08	
Washed	100	41.29 \pm 12.14	45.01 (33.48 – 49.88)	2.44	61.87	

*IQR interquartile range (i.e. 25th to 75th percentile)

†The p-value is obtained using a two-sample one-sided t-test on the square transformed variable ΔE_{ab}^*

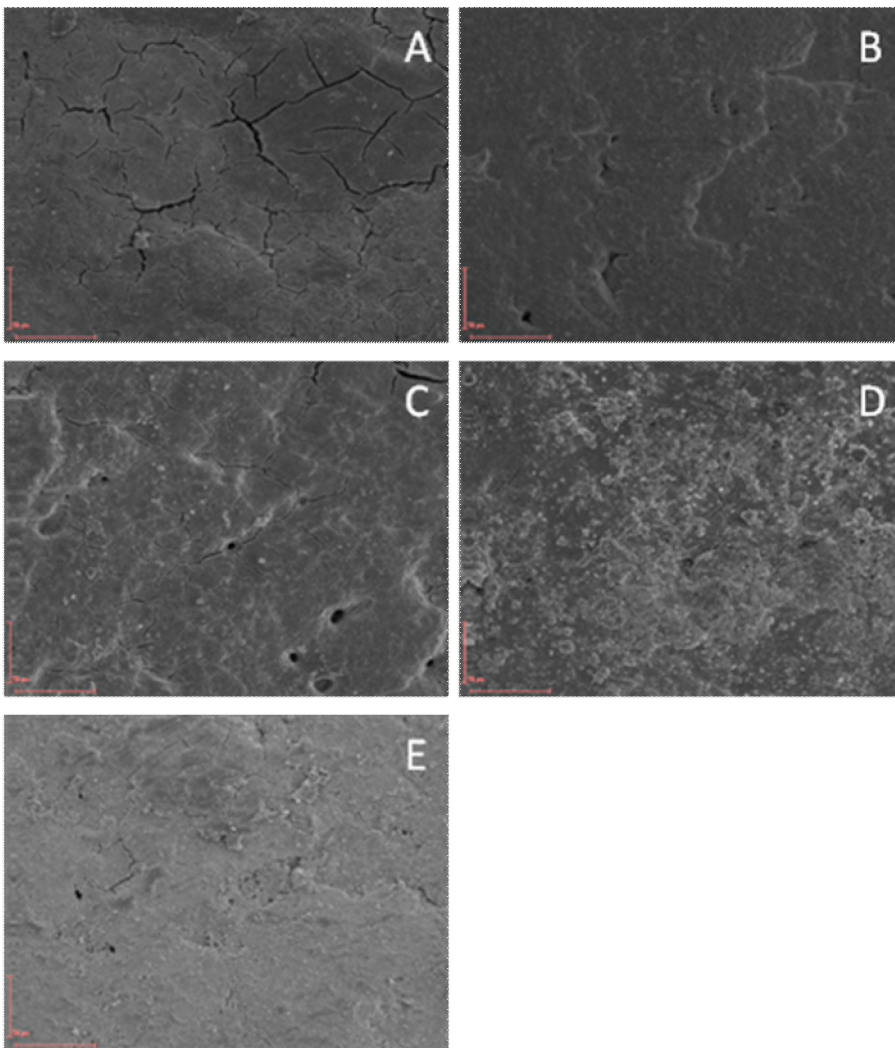
Figure 2. Boxplot presenting ΔE_{ab}^* for brown and white eggs that were unwashed and washed. The line within the box marks the median. The boundaries of the box represent the 25th and 75th percentile. Whiskers above and below the box indicate the values within 1.5 times the inter quartile range.



In Fig. 3, images are shown to give examples of the types of observation that formed the basis of the cuticle assessment carried out by SEM. The mean scores for each of the four different criteria used to assess the cuticle in each group of eggs are presented in Fig. 4. Few of the eggs assessed had a good even cuticle coverage (as depicted in Fig. 3A and awarded a score of 0). The cuticle coverage was in fact considered poor in all four groups irrespective of

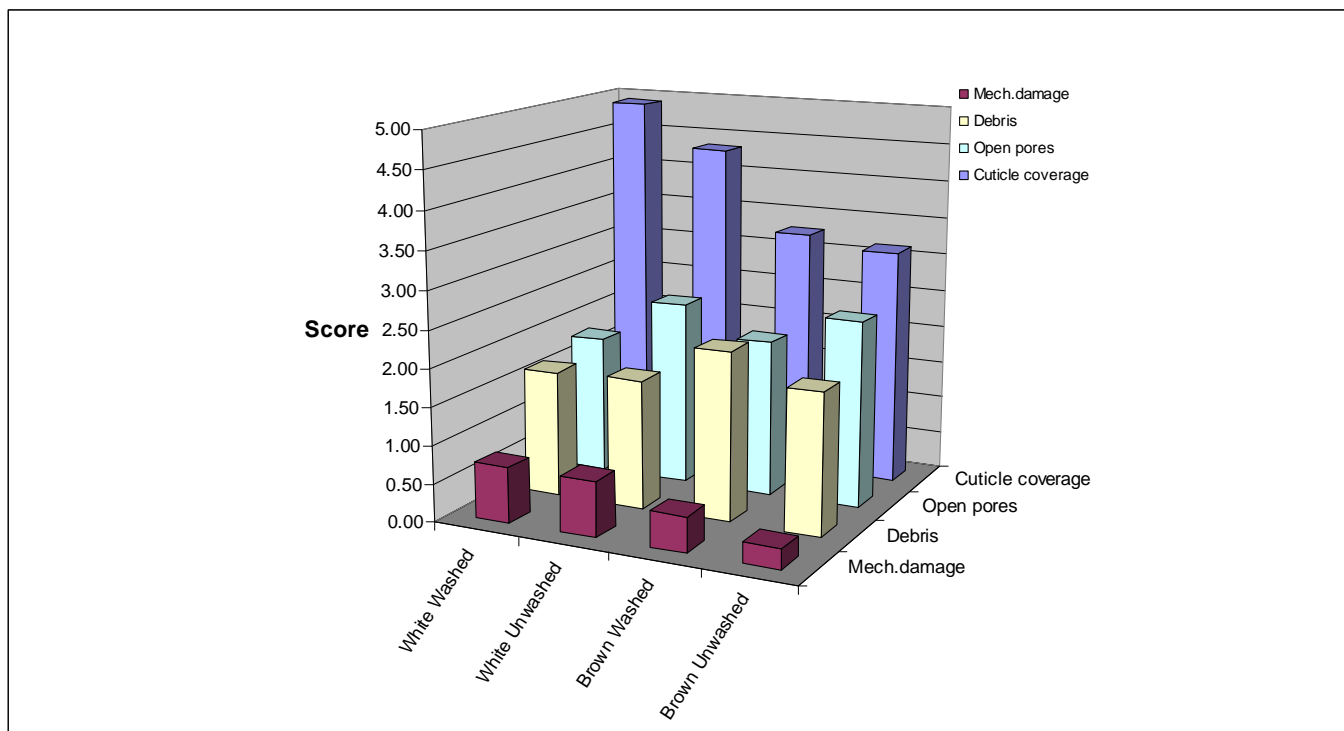
treatment or source (washed *versus* unwashed, brown *versus* white). The average scores for the white eggs were 4.9 ± 2.16 (washed) and 4.33 ± 2.11 (unwashed) whilst that for the brown eggs was 3.27 ± 2.42 (washed) and 3.13 ± 2.89 (unwashed). The moderately higher score for the white washed eggs compared to the white unwashed eggs was considered not to be related to the treatment. It has previously been suggested that washing will result in the removal of the cuticle thereby exposing the external openings of the gaseous exchange pores.

Figure 3. Examples of the types of observation that formed the basis of the cuticle assessment carried out by SEM ($\times 500$). (A) Typical SEM appearance of an egg with good cuticle coverage. The cuticle has a typical 'cracked mud' appearance which is induced as the cuticle dries just after oviposition, (B) SEM image of an egg with no cuticle. The calcite columns of the palisade layer are completely exposed, (C) SEM appearance of an egg with patchy cuticle coverage and exposed gaseous exchange pores, (D) SEM appearance of an egg in which the cuticle is covered with surface debris, (E) SEM appearance of an egg evidence of mechanical abrasion*. There is also very little by way of a cuticle on this egg.



The incidence of open pores in this study however does not appear to have been affected by the washing procedure. The mean values of the unwashed eggs (white: 2.43 ± 1.89 ; brown: 2.47 ± 2.00) was higher than the mean values for the washed eggs (white: 1.83 ± 1.70 and brown: 2.07 ± 2.01) again suggesting treatment was not the causative factor influencing this feature. Surface deposits consistent with the use of sanitizers have previously been reported on the surface of washed eggs. In the current study the washed brown eggs displayed the highest incidence of surface debris on the surface of the cuticle (2.23 ± 2.45) but this was statistically not different to that when compared to the unwashed brown eggs (1.87 ± 1.72). The scores for the white washed *versus* white unwashed eggs were comparable (1.67 ± 1.63 ; 1.70 ± 1.76). It was not possible to perform a chemical analysis on the surface debris observed but it resembled dust rather than the mesh like phosphorous rich deposits previously observed with the inappropriate usage of sanitizers. SEM allows the critical appraisal of mechanical damage to the shell resulting from the washing process. The type of mechanical damage observed is usually reflective of the type of wash action employed viz. brush versus jet washers. In this study we were not provided with any information as to the type of wash action used and from our assessment we are pleased to report that there was a very low incidence of mechanical damage with no obvious difference being observed between the washed and unwashed egg groups (white washed eggs: 4.9 ± 2.16 ; white unwashed eggs: 4.33 ± 2.11 ; brown unwashed eggs: 3.27 ± 2.42 ; brown washed eggs: 3.13 ± 2.89).

Figure 4. Comparison of mean scores for the four different criteria relating to the quality of the cuticle in white eggs (washed and unwashed) and brown eggs (washed and unwashed). N=30 samples in each group. A score of 0 is optimum in terms of quality in each category.



An alternative way of looking at our data is presented in Table 2. Here the percentage of eggs in each group which were assigned a particular score in each category is presented. More than 50% of the white eggs were scored as having a very patchy cuticle irrespective of whether they had been washed or unwashed. The washing procedure did not appear to affect the overall quality of the cuticle in either group. The brown eggs in general were of better quality in terms of their cuticle scores than the white eggs. Again, however, there is little evidence to suggest that the washing procedure per se had affected the cuticle quality in this group. The incidence of mechanical damage observed in washed versus non-washed eggs was also similar in both groups of eggs and there was no direct evidence that washed eggs had more or less surface debris than the unwashed eggs. There is also no obvious pattern in the data relating to the presence or absence of exposed pore sites.

Table 2. Percentage of eggs attaining any particular score in each category of cuticle assessment

		White-washed	White-unwashed	Brown-washed	Brown-unwashed
Cuticle coverage	0	0	3	17	23
	2	27	30	40	43
	5	50	53	33	14
	8	23	14	10	20
Mechanical damage	0	63	70	77	87
	2	37	27	23	13
	6	0	3	0	0
Presence of surface debris	0	37	40	33	27
	2	50	43	47	63
	5	13	17	10	67
	8	0	0	10	3
Exposed pores	0	33	23	37	27
	2	50	47	37	40
	5	17	30	27	33

Table 3 shows the results of the second assessors ability to correctly categorize the SEM images taken of the 60 white eggs and then the 60 brown eggs as being washed or unwashed. Only 56% of the white egg images were correctly identified as being washed, and only 50% of the washed brown eggs were likewise correctly identified. The overall conclusion was that cuticle quality was in general poor and that the washing procedure per se did not significantly alter the appearance of the cuticle to the extent that the washed eggs were instantly recognizable.

Table 3. Results of a second assessment of the cuticle carried out by an independent assessor. The number of images correctly assigned as being representative of eggs which had been 'washed' are expressed as a percentage of the total images shown (N=60).

	White eggs (N = 60 images)	Brown eggs (N=60 images)
% of images categorized correctly as being washed	56%	50%

Conclusion

From the data obtained, we could find no evidence to suggest that the washing procedure used in this investigation irreversibly changed the quality of the cuticle.

References

BOARD, R.G. and HALLS, N.A. (1973) The cuticle: a barrier to liquid and particle penetration of the shell of hen's eggs. British Poultry Science 14: 69-97.

EFSA (2005) Opinion of the scientific panel on biological hazards on the request from the commission related to the microbiological risks on washing of table eggs. The EFSA Journal 269: 1-39.

HUTCHISON, M.L., GITTINS, J., SPARKS, A.W., HUMPHREY, T.J., BURTON, C. and MOORE, A. (2004) An assessment of the microbiological risks involved with egg washing under commercial conditions. Journal of Food Protection 67: 4-11.