

*Original Research***Sanitization of Table Eggs with Treatment of Hydrogen Peroxide and Ultraviolet Radiation****A. S. Rathod¹, R. N. Waghmare^{2*}, V. V. Deshmukh³, M. S. Vaidya⁴, S. V. Londhe⁵ and S. S. Gaikwad⁶**

College of Veterinary and Animal Sciences, (MAFSU) Parbhani, Maharashtra, INDIA

^{1, 2 & 3}Department of Veterinary Public Health⁴Department of Animal Genetics and Breeding⁵Department of Livestock Products Technology⁶Department of Animal Biotechnology***Corresponding author:** rupeshwaghmare@gmail.com

Rec. Date:	Oct 17, 2019 06:33
Accept Date:	Feb 15, 2020 10:07
DOI	10.5455/ijlr.20191017063328

Abstract

Use of ultraviolet radiation and hydrogen peroxide for improvement of microbial quality of table eggs and assuring safety is under studied in India. Considering this fact in the present study, a total of 432 Table eggs were procured from local retail markets of Parbhani city and treated with UV radiation at different exposure time of 1 (UV-1), 2 (UV-2) and 4 (UV-4) minutes alone and with 1.5 % H₂O₂ (HP) and analyzed for microbial quality of eggshell surface. Efficacy of 'HP+UV-2' & 'HP+UV-4' treatment was found to be significant ($p < 0.05$) in reducing total viable count of eggshell surfaces whereas *Staphylococcus* spp. contamination was significantly ($p < 0.05$) reduced up to 1.5 log₁₀ cfu by use of 'HP+UV-4' treatment. Comparative effect of various treatments on yeast and mould counts indicated non-significant difference except for HP+UV-4. The study concluded that the combination of 'HP+UV-4' found to be superior than other treatment groups in enhancing microbial shell surface quality of Table eggs to safeguard the public health.

Key words: Hydrogen Peroxide, Microbial Quality, Sanitization, Table Eggs, UV Radiation**How to cite:** Rathod, A., Waghmare, R., Deshmukh, V., Vaidya, M., Londhe, S., & Gaikwad, S. (2020). Sanitization of Table Eggs with Treatment of Hydrogen Peroxide and Ultraviolet Radiation. International Journal of Livestock Research, 10(3), 99-107. doi: 10.5455/ijlr.20191017063328**Introduction**

The poultry industry of India has changed dramatically over the last 50 years. Due to the organized efforts from integrators and farmers the poultry industry has grown and specialized in meat production and egg production. The Indian poultry sector has been one of the fastest growing sectors, with annual growth of about 8.5 % in eggs and 7.8 % in meat production (GOI, 2019). Egg is considered as proteinous food, which

contain many vitamins and minerals except vitamin C and fully mixed egg contains about 65% water, 12% proteins and 11% fat (Jay *et al.*, 2005). Many nutrient substances present in eggs create an excellent environment for the development of bacterial microflora, including pathogenic bacteria (Stepien, 2010). Egg spoilage is usually associated with freshly and poorly stored eggs owing to poor storage conditions, like relatively high humidity, which contributes to the high microbial growth. Microbes of egg many cause severe health problems like diarrhea, nausea and abdominal pain, since they are pathogenic (Ansah *et al.*, 2009).

In order to achieve the objective of food safety in modern egg production and processing systems, sanitization of eggs with physical and chemical methods has become a common practice. Combination of physical and chemical methods should be explored for sanitization of eggshell surfaces mitigating the negative effects of heat on the functional properties of egg (Favier *et al.*, 2001 and O'bryan *et al.*, 2017). In order to preserve the microbial quality and reduce the potential for contamination of eggs with pathogenic microorganism sanitization of the eggshell surfaces during processing at egg production facilities is an important step. UV light may be lethal to most microorganisms found on eggshell surfaces (Sastri *et al.*, 2000 and Wells *et al.*, 2010). Similarly, research shows that lower concentrations of H₂O₂ can effectively reduce microbial levels from eggshell surfaces (Bayliss and Waites, 1982; Padron, 1995 and Wells *et al.*, 2010). Morouj *et al.* (2016) reported that hydrogen peroxide (H₂O₂) and UV treatment resulted in the greatest reductions in eggshell aerobic plate counts compared to other treatments throughout egg storage. Therefore, keeping in view the relative advantages of ultraviolet radiation and hydrogen peroxide in consideration to safeguard the public health from various pathogens, assuring safety and quality of shell eggs, the present study was designed with the objective to study effect of hydrogen peroxide along with and without ultra violet radiation treatment on microbial quality of table eggs.

Materials and Methods

Sample Collection

A total of 432 table eggs comprising of 144 samples (each sample contains 3 eggs) were randomly collected from different markets of Parbhani city and soon after collection the samples were transported to the laboratories within one hour under aseptic condition in sterile polybags.

Calculation of Surface Area of Eggs

Weighing of table egg was done for calculation of surface area for estimating Colony Forming Units of microbial load. A method of Bonnet *et al.* (1965) modified by Wall *et al.* (2008) was used for estimating the colony forming units (cfu) as cfu/cm².

$$S = 4.68 P^{(2/3)}$$

Where,

S = surface in cm^2 , P = egg weight in grams.

Table 1: Design of experiment

S. No.	Group Code	Treatment	Number of Samples
1.	C	Control without treatment	18
2.	HP	Hydrogen peroxide(H_2O_2) 1.5%	18
3.	UV-1	UV radiation – 1 min exposure	18
4.	UV-2	UV radiation – 2 min exposure	18
5.	UV-4	UV radiation – 4 min exposure	18
6.	HP+UV-1	UV radiation 1 min exposure + Hydrogen peroxide(H_2O_2) 1.5%	18
7.	HP+UV-2	UV radiation 2 min exposure + Hydrogen peroxide (H_2O_2) 1.5%	18
8.	HP+UV-4	UV radiation 4 min exposure + Hydrogen peroxide (H_2O_2) 1.5%	18
Total No. of samples*			144
Total number of eggs collected (144 x 3)			432

Sanitization of eggs with Hydrogen Peroxide (HP) and Ultraviolet (UV) radiation

The table eggs were treated with 1.5 percent Hydrogen peroxide by using Hand sprayer purchased from local market whereas, for UV treatment kept in cabinet provides 250 nm UV rays where eggs were given exposure time of 1, 2 and 4 minutes. In UV cabinet eggs were placed in between two UV tubes so that all surfaces of eggs exposed with UV light. Details of control and treatment group is depicted in Table 1.

Microbial Analysis

Microbial quality of eggshell surfaces of Table eggs with and without treatment with HP and UV radiation at different exposure time were assessed and evaluated. A method of Musgrove *et al.* (2005) was used for collection of eggshell rinsate sample with some modification. Three eggs were aseptically transferred to sterile polyethylene bag containing 300 ml peptone water. The surface of each egg was gently rubbed manually through the bag for 1 min. Egg was then removed and transferred to sterile bag separately. Rinsate was immediately used for estimation of TVC (BAM, 1998) and Differential Count in relation to *Staphylococcus* spp. (ISO-6888-2: 1999) and Yeast and Mould (ISO-21527-1). Isolation and identification of *Salmonella* spp. was done as per the ISO 6579: 1993, Reaffirmed 2005 method.

Results and Discussion

Average Eggshell Surface Area of Collected Samples

The average shell surface area in cm² of collected egg samples was calculated and results are presented in Table 2. The average surface area of various treatment groups varied from 45.63±0.40 to 46.10±0.44 cm² with mean average of 46.070±0.39 cm². Data pertaining to the surface area of samples was further subjected to statistical analysis. Average eggshell surface area amongst the different groups of treatment did not differ significantly. The non-significant difference indicated that eggs from various treatment group and control were of similar size and did not have effect on microbial load.

Table 2: Details of average shell surface area of Table eggs samples collected under various groups.

S. No.	Group code	Average ± SE (n=3)	
		Weight (grams)	Surface area (cm ²)
1.	C	32.03352±0.47	46.10695±0.44 ^N
2.	HP	31.90±0.42	45.987±0.39 ^N
3.	UV-1	31.813±0.41	45.90114±0.39 ^N
4.	UV-2	31.586±0.37	45.6874±0.35 ^N
5.	UV-4	31.580±0.49	45.67269±0.47 ^N
6.	HP+UV-1	31.81296±0.42	45.63115±0.40 ^N
7.	HP+UV-2	31.58593±0.27	45.77817±0.26 ^N
8.	HP+UV-4	31.58019±0.45	45.8492±0.42 ^N
	Average	31.99 ±0.41	46.07±0.34

Mean average surface areas do not differ significantly *p<0.05

Effect of Sanitation on Microbial Quality of Eggshell Surfaces

The mean TVC, *Staphylococcus* spp. and *Yeast and Mould* counts of eggshell surfaces of Control ('C') and Treatment groups ('HP', 'UV-1', 'UV-2', 'UV-4', 'HP+UV-1', 'HP+UV-2' and 'HP+UV-4') are given in Table 3.

Table 3: Comparative analysis of effect of H₂O₂, UV radiation and H₂O₂ with UV radiation on microbial quality of eggshell surface.

Microbial count (log ₁₀ cfu/cm ²)	Group code							
	C	HP	UV-1	UV-2	UV-4	HP+UV-1	HP+UV-2	HP+UV-4
TVC	6.331±0.04 ^a	5.883±0.10 ^b	5.629±0.15 ^{bc}	5.607±0.10 ^{bc}	5.368±0.14 ^{bc}	5.596±0.12 ^{cd}	5.248±0.14 ^{de}	4.994±0.16 ^e
<i>Staphylococcus</i> spp.	5.303±0.04 ^a	4.574±0.16 ^{bc}	4.334±0.16 ^{cd}	4.293±0.15 ^{cd}	4.111±0.16 ^{de}	4.67±0.07 ^b	4.209±0.09 ^d	3.801±0.12 ^e
Yeast and Mould	3.715±0.04 ^a	3.384±0.08 ^{bc}	3.427±0.09 ^{bc}	3.505±0.09 ^{ab}	3.191±0.07 ^c	3.274±0.05 ^c	3.189±0.05 ^c	2.899±0.17 ^d

a, b, c, d, e means with different superscript in a row differ significantly * p < 0.05

Total Viable Count

Total viable counts (TVC) of eggshell surfaces given sanitary status at which the eggs are being handled in farm to retail process. The higher TVC counts indicated unhygienic handling and storage (Gole *et al.*, 2013). A significantly ($p < 0.05$) higher TVC count was observed in control group as compared to treatment groups. However, comparisons within UV treatment at various exposure time group indicate non-significant difference ($p < 0.05$) but lower TVC count in 'UV-4' was observed. Significant reduction in TVC count of eggshell surfaces by UV radiation at 1 to 5 minutes exposure were reported by several research workers (Kuo *et al.*, 1997; Chavez *et al.*, 2002; Coufal *et al.*, 2003; Patil, 2013 and Melo *et al.*, 2019). UV radiation in the range of 250 to 260 nm was found to be lethal to various microorganisms including bacteria and Yeast and Mould (Bintsis *et al.*, 2000; Patil, 2013). The observations in present study indicated that TVC of eggshell surfaces can be effectively reduced by exposing eggs to UV radiation for 4 minutes. Wells *et al.* (2010) also reported 1.5 \log_{10} cfu reductions in TVC of eggshell surfaces after UV radiation (250 nm) for 4 minutes.

Significantly ($p < 0.05$) lower TVC count was observed in hydrogen peroxide (HP) alone group in comparison with Control ('C') group. Effectiveness of hydrogen peroxide in eliminating eggshell surface microflora was reported by Sheldon and Brake (1991). Similarly, Wells *et al.* (2010) reported effectiveness of 1.5 percent hydrogen peroxide in reduction of bacterial count from eggshell surfaces. In present study effects were on similar lines in relation with control of shell surface microflora. The comparison of mean TVC count of groups ('HP+UV-1', 'HP+UV-2' and 'HP+UV-4') indicates significantly ($p < 0.05$) lower count in 'HP+UV-4' group. The results are in agreement with earlier work carried out by Morouj *et al.* (2016). The present findings are also in accordance with Woodring (2011) and Wells *et al.* (2010) where reduction in total viable counts of eggshell surfaces were recorded 2 to 3 \log_{10} cfu/egg after treating samples with hydrogen peroxide and UV radiation. In comparison among all groups of experiment ('HP', 'UV-1', 'UV-2', 'UV-4', 'HP+UV-1', 'HP+UV-2' and 'HP+UV-4') the efficacy of 'HP+UV-2' & 'HP+UV-4' treatment was found to be significant ($P < 0.05$) than other treatments in controlling shell surface microflora. It was also observed that combination of Hydrogen peroxide and UV radiation with exposure time of 2 and 4 minutes were equally effective in reducing total viable count of eggshell surfaces.

Staphylococcus spp.

Staphylococcus spp. is one of the predominant Gram-positive pathogens found on eggshell surface (Anshah *et al.*, 2009). Significantly ($p < 0.05$) lower count of *Staphylococcus spp.* was seen on UV treatment group compared to control group; however non-significant difference was observed within UV treatment groups. Observations indicated that *Staphylococcus spp.* contamination can be reduced by exposing the eggshell surfaces to UV radiation for 1 to 4 minutes. The results of present study are not in agreement with study

carried out by Kuo *et al.* (1997). They did not observe significant reduction in *Staphylococcus* spp. count after exposing eggs to UV radiation for 15 minutes. The results are corroboration with earlier studies carried out by Reu *et al.* (2006) and Patil (2013). The comparison of 'C' and 'HP' indicated that eggshell surface count of *Staphylococcus* spp. was significantly ($p < 0.05$) lower in 'HP' group. Comparatively higher reduction of *Staphylococcus* spp. was reported by Maktabi *et al.* (2018) by using 0.5 percent Hydrogen peroxide. French *et al.* (2004) also reported bactericidal effect of hydrogen peroxide on *Staphylococcus aureus* isolated from poultry environment. The comparison of mean *Staphylococcus* spp. count of all groups ('C', 'HP+UV-1', 'HP+UV-2' and 'HP+4') revealed significantly ($p < 0.05$) lower count in 'HP+UV-4' group. Current results are in agreement with earlier studies carried out using Hydrogen peroxide or UV radiation individually or in combination (Reu *et al.*, 2006; Wells *et al.*, 2010; Gottselig, 2011 and Patil, 2013).

In comparison among all groups of experiment ('HP', 'UV-1', 'UV-2', 'UV-4', 'HP+UV-1', 'HP+UV-2' and 'HP+UV-4') the efficacy of 'HP+UV-4' treatment was found to be significant ($P < 0.05$) than other treatments which indicated that *Staphylococcus* spp. contamination could be significantly ($p < 0.05$) reduced up to 1.5 log₁₀ cfu. It was also observed that 'UV-4' and 'HP+UV-2' treatment was at par with each other in control of *Staphylococcus* spp. contamination.

Yeast and Mould

Yeast and Mould are ubiquitous in nature. Eggs were contaminated because of improper environmental and storage conditions (Arthur and Somuah, 2001). The yeast and mould count was significantly ($p < 0.05$) higher in 'C' group as compared to other groups of experiment ('HP', 'UV-1', 'UV-2', 'UV-4', 'HP+UV-1', 'HP+UV-2' and 'HP+UV-4'). Within the UV treatment groups 'UV-4' group had significantly ($p < 0.05$) lower count than 'UV-1' and 'UV-2' group. Results are suggestive of high efficacy of 'UV-4' radiation method in reduction of yeast and mould contamination. Findings of the present study are on similar lines with earlier work carried out by Kuo *et al.* (1997) and Patil (2013). Several other workers also used UV radiation to control yeast and mould growth on food products (Coufal *et al.*, 2003 and Melo *et al.*, 2019). Eggshell surface count of yeast and mould was significantly ($p < 0.05$) lower in 'HP' group than 'C' group. Sheldon and Brake (1991) reported 97.1 percent reduction in yeast and mould count of eggshell surfaces treated with 5 percent Hydrogen peroxide. Maktabi *et al.* (2018) reported effect of Hydrogen peroxide on yeast and mould counts of eggshell surfaces.

The comparison of mean *Yeast* and *Mould* count of combination treatment groups ('C', 'HP+UV-1', 'HP+UV-2' and 'HP+4') reported significantly ($p < 0.05$) lower count in 'HP+UV-4' group. Rotem *et al.* (1985) reported that UV light was effective in killing fungal spores. Comparison of effect of various

treatments on yeast and mould counts indicates non-significant difference except for HP+UV-4 this shows superiority of 'HP+UV-4' treatment over other treatment groups

Salmonella spp.

In current study none of the eggshell surface sample from Control ('C') as well as from all Treatment groups of experiment were found positive for *Salmonella* spp. Earlier Berrang *et al.* (1995); Gao *et al.* (1997); Coufal *et al.* (2003) and Keklik *et al.* (2010) reported that ultraviolet radiation was safe decontamination agent for reduction of *Salmonella* spp. from egg surface. Various researchers reported that Hydrogen peroxide is effective in reduction of *Salmonella* spp. from eggshell surfaces (Padron, 1995; Cox *et al.*, 2002 and Buhr *et al.*, 2013). Researchers observed that effectively used Hydrogen peroxide and UV radiation to reduce *Salmonella* spp. contamination from eggshell surface (Gottselig, 2011 and Morouj *et al.*, 2016). Chemical and physical methods are used since long for egg sanitation (Shane and Faust, 1996, Chavez *et al.*, 2002; Coufal *et al.*, 2003, Reu *et al.*, 2006; Wells *et al.*, 2010; Patil, 2013 and Maktabi *et al.*, 2018).

Conclusion

The findings of this study imply that combination of hydrogen peroxide (1.5% v/v) with UV radiation (4 minutes) is an effective method to reduce shell surface microflora of table eggs. Moreover, combination of various physical and chemical methods needs to be evaluated for their effectiveness in reducing shell surface microflora.

Acknowledgement

The authors are grateful to Associate Dean, College of Veterinary and Animal Sciences, MAFSU, Parbhani for providing necessary facilities and guidance at various stages of the experiment.

References

1. Ansah T., Dzoagbe G.S.K., Teye G.A., Addey S. and Danquah J.K. (2009). Microbial quality of table eggs sold on selected markets in the Tamale municipality in the Northern region of Ghana. *Livestock Research for Rural Development*. 21(8): 1-6.
2. Arthur C. T. and Somuah Osei. A. (2001). Sources of microbial contamination in smoked anchovies. *Science Technology* 45:29.
3. Bacteriological Analytical Manual (1998). 8th edition publication by FDA, U.S. Bayliss C.E., and Waites W.M. (1982). Effect of simultaneous high intensity ultraviolet radiation and hydrogen peroxide on bacterial spores. *Journal Food Science and Technology*. 17: 467-470.
4. Berrang M. E., Cox N. A., and Bailey J. S. (1995). Efficacy of ultraviolet light for elimination of *Salmonella* on broiler hatching eggs. *Journal of Applied Poultry Research*, 4: 422-429.
5. Bintsis T., Litopoulou T. E. and Robinson R. K. (2000). Existing and potential applications of ultraviolet light in the food industry – a critical review. *Journal of the Science of Food and Agriculture*. 80: 637–645.
6. Bonnet Y. and Mongin P. (1965). Mesure de la surface de. *Ann Zootech*. 14: 311–317.

7. Buhr R. J., Spickler J. L., Ritter A. R., Bourassa D. V., Cox N. A., Richardson L. J., and Wilson J. L. (2013). Efficacy of combination chemicals as sanitizers of Salmonella-inoculated broiler hatching eggshells. *Journal of Applied Poultry Research*, 22:27–35.
8. Chavez C., Knape K. D., Coufal C. D. and Carey J. B. (2002). Reduction of Eggshell aerobic plate counts by ultraviolet radiation. *Poultry Science*, 81:1132–1135.
9. Coufal C. D., Chavez C., Knape K. D. and Carey J. B. (2003). Evaluation of a method of ultraviolet light sanitation of Broiler hatching eggs. *Journal of Poultry Science*. 82: 754-759.
10. Cox N. A., Mauldin J. M., Kumararaj R., and Musgrove M. T. (2002). Ability of hydrogen peroxide and timsen to eliminate artificially inoculated Salmonella from fertile Broiler eggs. *Journal of Applied Poultry Research*, 11: 266–269.
11. Favier G.I., Escudero M.E., Velazquez L., and Guzman A.M.S. (2001): Reduction of *Yersinia enterocolitica* and mesophilic aerobic bacteria in egg- shell by washing with surfactants and their effect on the shell microstructure. *Food Microbiology*, 17: 73-81.
12. French G. L., Otter J. A., Shannon K. P., Adams N. M. T., Watling D. and Parks M. J. (2004). Tackling contamination of the hospital environment by methicillin resistant *Staphylococcus aureus* (MRSA): a comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination. *Journal of Hospital Infection*, 57: 3137.
13. Gao F., Stewart L. E., Joseph S. W. and Can L. E. (1997). Effectiveness of ultraviolet irradiation in reducing the numbers of Salmonella on eggs and egg belt conveyor materials. *Applied Engineering in Agriculture*, 13(3): 355-359.
14. Gole V.C., Chousalkar K.K. and Roberts J.R. (2013). Survey of *Enterobacteriaceae* contamination of table eggs collected from layer flocks in Australia. *International Journal of Food Microbiology*, 164:161–165.
15. Gottselig S. M. (2011). Microbial reduction on eggshell surfaces by the use of hydrogen peroxide and ultraviolet light Unpublished M.V.Sc. thesis submitted to the office of post graduate studies of Texas A&M University, Texas.
16. Government of India, (2019). Basic Animal husbandry Statistics, Department of Animal husbandry and Dairying, Ministry of Fisheries, Animal Husbandry & Dairying, Krishi Bavan, New Delhi.
17. International Standards Office, (1993). ISO 6579: (Reaffirmed at 2005), Methods for detection of Bacteria responsible for food poisoning part-3 general guidance on methods for the detection of *Salmonella* (Second revision).
18. International Standards Office, (1999). ISO 6888-2: (Reaffirmed at 2012) Indian standard methods for detection of Bacteria responsible for food poisoning part- 8 horizontal method for enumeration of Coagulase-positive *Staphylococcus* (*Staphylococcus Aureus* and other species) section- 2, Technique using rabbit plasma fibrinogen agar medium.
19. International Standards Office, (2008). ISO 21527-1, First edition, Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of *Yeasts* and *Moulds*.
20. Jay J.M., Golden D.A. and Loessner M.J. (2005): Modern food microbiology, (7thed.). *Springer Verlag* Kuo F.L., Ricke S. C. and Carey J. B (1997). Shell egg Sanitation: UV radiation and egg rotation to effectively reduce populations of aerobes, *Yeasts* and *Moulds*. *Journal of Food Protection*, 60(6):694-697.
21. Keklik N. M., Demirci A., Patterson P. H., and Puri V. M. (2010). Pulsed UV light inactivation of *Salmonella enteritidis* on eggshells and its effects on egg quality. *Journal of Food Protection*, 73(8): 1408–1415.
22. Maktabi S., Zarei M. and Rashnavady R. (2018) Effect of sequential treatments with sodium dodecyl sulfate and citric acid or hydrogen peroxide on the reduction of some foodborne pathogens on eggshell. *Iranian Journal of Veterinary Research*, 19(2)-(63):113-117.
23. Melo E. F., Climaco W. L. S., Triginelli M. V., Vaz D. P., de Souza M. R., Baiao N. C., Pompeu M. A., and Lara L. J. C. (2019). An evaluation of alternative methods for sanitizing hatching eggs. *Poultry Science*, vol 98: 1–8.

24. Morouj N., Al-Ajeeli T., Taylor T. M., Alvarado C. Z. and Coufal C. D. (2016). Comparison of eggshell surface sanitization technologies and impacts on consumer acceptability. *Poultry Science Association*, 95:1191–1197.
25. Musgrove M. T., Jones D. R., Northcutt J. K., Cox N. A. and Harrison M. A. (2005). Shell rinse and shell crush methods for the recovery of aerobic microorganism and *Enterobacteriaceae* from shell eggs. *Journal of Food Protection*, 68: 2144-2148.
26. O'Bryan C. A., Ricke S. C. and Crandall P. G. (2017). Chemical and physical sanitation and pasteurization methods for intact shell eggs. Producing safe eggs: *Microbial Ecology of Salmonella*, 373-390.
27. Padron N. M. (1995). Egg dipping in hydrogen peroxide solution to eliminate *Salmonella typhimurium* from eggshell membranes. *Avian Disease*, 39: 627–630.
28. Patil G. V. (2013) Studies on efficacy of sanitizers on table eggs. Unpublished M.V.Sc. thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur.
29. Reu De. K., Grijspeerdt K., Mssens W., Heyndrickx M., Uyttendaele M., Debevere J. and Herman L. (2006). Egg shell factors influencing eggshell penetration and whole egg contamination by different bacteria including *Salmonella enteritidis*. *International Journal of Food Microbiology*, 122: 253-260.
30. Rotem J., Wooding B., and Aylor D. E. (1985). The role of solar radiation, especially ultraviolet, in the mortality of fungal spores. *Phytopathology* 75:510-514.
31. Sastry S.K., Datta K. and Worobo R. W. (2000). Ultraviolet light. *Journal of Food Science*, 65: 90–92.
32. Shane S. M. and Faust A. (1996). Evaluation of sanitizers for hatching eggs. *Journal of Applied Poultry Research*, 5:134-138.
33. Sheldon B. W. and Brake J. (1991). Hydrogen peroxide as an alternative hatching Egg disinfectant. *Poultry Science* 70:1092-1098.
34. Stepien D. (2010) Occurrence of Gram-negative bacteria in hens' eggs depending on their source and storage conditions. *Polish Journal of Veterinary Sciences*, 13: 507-513
35. Wall H., Tauson R. and Sorgjerd S. (2008). Bacterial contamination of Eggshells in furnished and conventional cages. *Journal of Applied. Poultry Research*, 17:11-16.
36. Wells J. B., Coufal C. D., Parker H. M. and McDaniel C. D. (2010). Disinfection of eggshells using ultraviolet light and hydrogen peroxide independently and in combination. *Poultry Science*, 89: 2499–2505.
37. Woodring K. S. (2011). Quality and sensory attributes of shell eggs sanitized with combination of hydrogen peroxide and ultraviolet light unpublished M.V.Sc. thesis submitted to the office of graduate studies of Texas A&M University, Texas.