SHORT COMMUNICATION

Assessment of the efficacy of stable liquid chlorine dioxide (CLO_2) in disinfection of stored table eggs

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ABSTRACT

Microbiological contamination of table egg shells poses an important public health and technological challenge, particularly in the European Union, where washing and disinfection of Class A eggs are prohibited due to adverse effects associated with previously used sanitising agents. This study evaluated the efficacy of stable liquid chlorine dioxide (ClO_2) in reducing microbial load on eggshells during storage under commercial conditions. A total of 200 eggs were divided into two groups: a control group (untreated) and an experimental group treated with a fine aerosol of 50 ppm ClO_2. Microbiological cleanliness was assessed using total aerobic mesophilic bacterial count (UBAMB) and luminometry at predetermined intervals.Immediately after treatment (day 0), the experimental group showed a pronounced microbiological reduction, with median UBAMB values decreasing from 35,000 CFU in the control group to 70 CFU in the treated group ($p = 0.029$). Significant differences in luminometry values between the groups were observed on days 0 and 14 ($p = 0.028$; $p = 0.025$), indicating sustained antimicrobial activity during storage. Within the experimental group, luminometry values showed significant temporal variation ($p = 0.015$), characterised by an increase by day 7, followed by stabilisation by day 14, when values returned to levels comparable to those observed immediately after disinfection ($p = 0.980$). No significant temporal changes were detected in the control group ($p = 0.276$). Stable liquid chlorine dioxide proved to be an effective and technologically acceptable disinfectant, achieving significant microbial reduction without compromising egg integrity. These findings highlight the potential applicability of ClO_2 as a modern, non-destructive sanitising option that addresses microbiological safety concerns while meeting the hygienic and regulatory requirements related to table egg production. Further research is recommended to assess the internal quality of eggs and to explore broader microbiological indicators under varying storage and environmental conditions.

Keywords: Disinfection, eggshell, egg quality, EU legislation, stable liquid chlorine dioxide

INTRODUCTION

Eggs intended for human consumption are typically not washed or disinfected. To ensure the quality and safety of eggs, which are a highly nutritious food source, it is crucial to strictly adhere to proper technological procedures during animal management and egg collection on farms (Hutchison, 2003). Despite these measures, eggs can pose significant health risks to humans due to the presence of dangerous pathogens, such as Salmonella, Pseudomonas, and Escherichia. While table eggs are generally left unwashed, there is a growing need for a simple yet effective method to enhance the hygienic treatment of their shells (Abdulwahid, 2020). After an egg is laid, its external temperature is lower than that of the hen's body, causing the internal structures of the egg to contract. This contraction can draw surface particles through the shell's pores into the egg itself, allowing microorganisms, including various pathogens and fungi, to penetrate (Gagić, 1999). Public health considerations emphasize controlling microorganisms during the intensive production and distribution of both hatching and table eggs. Proper hygienic management of these egg categories is essential to prevent economic losses in poultry production. Foodborne illness outbreaks linked to contaminated table eggs pose a global public health concern. In contrast to table eggs, breeding eggs are often subjected to collection, washing, and routine disinfection. Controlling pathogenic and conditionally pathogenic microorganisms is fundamental to effective disinfection practices (Bermúdez-Aguirre, 2025). Research conducted in the past decade has demonstrated the successful application of disinfectants from the DioxyActiv Supra line, which utilizes stable liquid chlorine dioxide for various disinfection needs (Gagić et al., 2013a; Gagić et al., 2013b; Ališah et al., 2023; Hansung et al., 2018., Ališah, 2020), Compared to other disinfectants, especially in their gaseous forms (Kustura et al., 2012., TurtoriandBorda, 2014; Morouj et al., 2016; Rrahimi, 2021), stable liquid chlorine dioxide offers a broad spectrum of action, straightforward application, and minimal toxicity to living tissue. Additionally, it functions effectively at lower concentrations than chlorine and its compounds, which can negatively impact disinfecting efficacy and lead to harmful by-products. Unlike other recommended disinfectants, chlorine dioxide is neither toxic nor environmentally hazardous, unlike chlorine, while offering equal efficacy and greater stability compared to ozone. Furthermore, it does not leave undesirable residues that could compromise the hygienic status of treated surfaces or materials (Gagić et al., 2013a).The European Union's regulatory framework regarding the treatment of class A table eggs is based on Regulation (EC) No. 589/2008 and Delegated Regulation (EU) 2023/2465, which state that eggs for direct consumption must not be washed or disinfected before being placed on the market. This prohibition stemmed from the assumption that washing and chemical treatments could damage the shell's cuticle, disrupt the microstructure of the pores, and, therefore, increase the risk of microorganisms penetrating the interior of the egg (EFSA 2005; FSAI 2019). Historically, the most commonly used agents for disinfection included chlorine, formaldehyde, and hydrogen peroxide, whose aggressive properties could adversely affect the organoleptic properties and stability of the egg. Consequently, the European Union established a ban that remains in force today (Reg. 853/2004, Annex III, Sect. X).Recent research suggests that the prohibition on washing and disinfection no longer reflects current technological possibilities. Stable liquid chlorine dioxide (ClO_2) has emerged as one of the most promising disinfectants due to its wide spectrum of action, high stability, and minimal toxicity. Unlike traditional chlorine compounds, ClO_2 operates effectively at low concentrations and does not produce toxic by-products (Gagić et al., 2013b). Stable liquid chlorine dioxide demonstrates selective action by effectively removing pathogenic microflora while preserving beneficial saprophytic bacteria without negatively impacting product quality (Ališah et al. 2025).

There are no published studies that have addressed the issue of disinfecting stored table eggs using stable liquid chlorine dioxide. Accordingly, the aim of our research was to evaluate the effectiveness of stable liquid chlorine dioxide (ClO_2) in reducing the microbial load on eggshells during storage.

MATERIAL AND METHODS

A total of 200 table eggs (52 – 64 grams) were taken in the sorting plant of a large farm for the production of table eggs. The eggs are divided into two groups, each containing 100 pieces. The control group of eggs was not disinfected. A few hours after grading, the experimental group of eggs was returned to a sorting conveyor belt consisting of rotating roast beads and passed through a fine aerosol, prepared from 100 ml of 50 per mille solution of disinfectant based on stable liquid chlorine

dioxide (ClO_2). After treatment of the experimental eggs, both groups were stored in a cold chamber at the fridge temperature and a relative humidity (RH) of 55% to 65% for the remainder of the experiment.The number and reduction of the total number of aerobic mesophilic bacteria (UBAMB), and the intensity of luminance in phentomols (fm), were performed in the programmatically provided control terms, namely UBAMB and zero-day luminance one (1) hour after storing both groups of eggs in the refrigerator.Then, we determined only the luminance on the seventh (7) and 14th day. During the controls, we prepared six (6) aggregate samples from each egg group. One aggregate sample consisted of five (5) eggpieces.

Microbiological Method

Each swab was immersed in 10 cc of peptone water in a test tube, and the first dilution was prepared after 30 minutes at room temperature. Later, 0.1 mL of the diluted content was applied to the UBAMB medium in appropriate Petri plates and incubated at 37°C for 24 hours, followed by a reading of the results.

Luminometry Method

Luminometry only shows the degree of microbiological contamination of the controlled surface or medium by the use of ATP luminators and appropriate swabs. The criteria for determining an acceptable sanitary-hygienic cleanliness zone in accordance with USA standards are for surfaces, up to 25000 RLU, and for drinking water zero (0) RLU (Relative Light Units)

Statistical Analysis

Descriptive statistics were calculated as median, mean and interquartile range for continuous variables. Comparisons between experimental and control groups were performed using the Mann–Whitney U test on log10 (value + 1) - transformed data due to non-normal distributions. Changes over time within each group for LUM data were tested using the Kruskal – Wallis test. When significant differences were detected, pairwise post-hoc comparisons were performed using Dunn's test with Bonferroni correction for multiple testing. All analyses were conducted in R, version 4.5.1 (R Foundation for Statistical Computing, Vienna, Austria). A value of $p<0.05$ was considered statistically significant.

RESULTS

Descriptive statistics are presented in Table 1. At baseline (day 0), the control group showed higher laboratory (LAB) values (median 35000) compared with the experimental group (median 70), whereas luminometry (LUM) values were more comparable between groups (Table 1). On pairwise comparison between experimental and control groups using the Mann–Whitney U test on log10(value + 1)-transformed data, LAB values at day 0 ($W = 0$, $p = 0.029$) and LUM values at day 0 and 14 ($W = 0$, $p = 0.028$, $W=0$, $p=0.025$) were statistically significant (Table 2).

Over time, within-group for LUM data comparisons using the Kruskal–Wallis test indicated a significant change in the experimental group ($\chi^2 = 8.35$, $p = 0.015$), while in the control group remained stable ($\chi^2 = 2.58$, $p = 0.276$).

Post-hoc pairwise comparisons using Dunn's test, corrected for multiple testing, showed that in the experimental group, LUM values decreased significantly from day 0 to day 7 ($p = 0.013$), while no significant differences were observed between day 0 and day 14 ($p = 0.980$) or between day 7 and day 14 ($p = 0.187$). LUM measurements in the control group did not show significant differences between any time points.

Table 1 Descriptive statistics for control and experimental groups over a 14-days period. Values represent the mean, standard deviation (SD), median, interquartile range (IQR), and range (min-max) for each group and method at days 0, 7, and 14

Group	Method	Day	Mean	SD	Median	IQR	Min	Max
Control	Lab	0	40000	31622.8	35000	400000	10000	80000
	Lum	0	21474	14899.8	16458	13563	10059	42921
	Lum	7	66817.8	60462.4	50383.5	58505.21	14834	151670
	Lum	14	39377.8	27516.4	29480	15923.8	18622	79929

Group	Method	Day	Mean	SD	Median	IQR	Min	Max
Experimental	Lab	0	65	55.1	70	75	0	120
	Lum	0	6268	778.9	6596.5	635.5	5120	6759
	Lum	7	16655	4794.3	17794	4483.5	9987	21045
	Lum	14	8114.5	2022.4	8889	1299.5	5122	9558

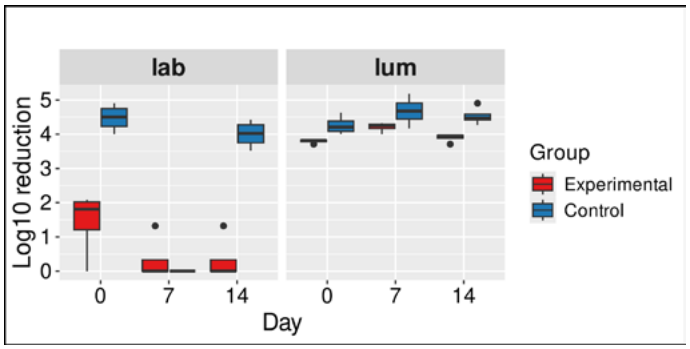

Figure 1 Boxplot distribution of log 10 (value+1) -transformed measurement values at days 0, 7, and 14 for the experimental and control groups. Data are presented separately for each method (lab and lum). For data set, whiskers present the full range of variations (minimum-maximum), box present 25th - 75th percentiles and horizontal lines in box present median values

Table 2 Results of the Mann–Whitney U test for comparing changes between the two study groups($p<0.05$)

	Day	W	p-value
Lab	Zero day	0	**0.029**
Lum	Zero day	0	**0.028**
	7^{th} day	3	0.211
	14^{th} day	0	**0.025**

DISCUSSION AND CONCLUSION

According to the available literature, we did not find any published studies that directly examined the impact of stable liquid chlorine dioxide on the hygienic quality of the eggshell. This limits the possibility of a detailed comparison with similar research. However, previous studies investigating chlorine dioxide–based disinfectants-primarily in gaseous or liquid forms-have reported notable antimicrobial efficacy (Gagić et al., 2013b; Hansung et al., 2018; Ališah et al., 2025), supporting the outcomes observed in our study. Also, Turtoi and Borda (2014) demonstrated the effectiveness of UV-based shell decontamination, while Morouj et al. (2016) and Rrahimi (2021) reported reductions in Salmonella contamination using hydrogen peroxide and sodium carbonate. These findings collectively suggest that ClO_2-based disinfection offers a technologically feasible and microbiologically effective approach to improving shell hygiene.

Results of this study clearly demonstrate that the application of stable liquid chlorine dioxide (Dioxy Activ Supra) at 50 ppm significantly improves the microbiological hygiene of table egg shells. Immediately after treatment (day 0), the experimental group exhibited a substantial reduction in microbiological load, as confirmed by significantly lower LAB values compared with the control group ($p = 0.029$). This rapid antimicrobial effect is consistent with the known oxidative modes of action of chlorine dioxide, which target essential bacterial cellular components and lead to swift inactivation of microorganisms.

The LUM data provide additional insight into the temporal dynamics of microbial contamination during storage. Significant differences between the experimental and control groups were detected on days 0 and 14, confirming that the antimicrobial advantage of chlorine dioxide persists beyond the immediate post-treatment period. Within-group comparisons further revealed a significant temporal change in the experimental group ($p = 0.015$), characterised by an

initial increase in LUM values between day 0 and day 7 ($p = 0.013$), followed by stabilisation between days 7 and 14. Importantly, no significant difference was observed between day 0 and day 14 ($p = 0.980$), which suggests that despite natural recolonisation occurring during storage, the microbial load returns to levels comparable to those measured immediately after disinfection.

By contrast, the control group exhibited no significant changes over time ($p = 0.276$), and consistently maintained higher levels of microbial contamination. These findings underscore the value of chlorine dioxide treatment in reducing initial contamination and maintaining favourable hygienic conditions throughout storage.

Overall, chlorine dioxide treatment proved to be a simple, cost-effective, and efficient method to reduce microbial contamination of table egg shells. The ability of stable liquid chlorine dioxide to maintain reduced microbial levels up to 14 days highlights its potential for use in commercial egg production systems, particularly those with extended storage periods.

This study has several limitations. Although the results confirm the reduction of bacterial load on the external eggshell surface, the internal structure of the egg (albumen, yolk, and membranes) was not examined. Therefore, it cannot be concluded with certainty that stable liquid chlorine dioxide has no toxicological or microbiological impact on internal egg components. Previous literature (e.g., Gagić et al., 2013a) provides supporting evidence, but does not offer comprehensive toxicological evidence to validate this assumption for stored table eggs fully. This gap highlights the need for future studies focusing on the internal microbiological and toxicological safety profile of eggs treated with stable liquid chlorine dioxide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTION

Conception: AA, AG; Design: AA, AG; Supervision: EŠ, AG; Materials: AA, AG; Data Collection and/or Processing: AA, AG; Analysis and/or Interpretation of the Data: AA, AG; Literature Review: AA, EŠ; Writing: AA; Critical Review: AG, EŠ

REFERENCES

Abdulwahid A, Ali H, Saeed S. 2020. Disinfection of table eggs using lemon juice as a natural biocide. Basrah J Vet Res. 19, 177-89. doi: 10.23975/bjvetr.2020.174109.

Ališah A, Gagić A, Pivić A, Varatanović M, Softić A, Lalović M, et al. 2023. Production indicators of broiler chickens fattening in the circumstances of a new approach to the organization and implementation of preventive measures on farms in Montenegro. J Hyg Engin Design. 45, 57-64.

Ališah A, Kapo-Dolan N, Pivić A, Bejdić P, Trifković J, Gagić A. 2025. Efficiency of stable liquide chlorine dioxide (clo_2) in sanitation of water distributionsystem in poultry production-synthesis oftheoretical and experimental findings. Veterinaria. 74, Suppl 1. 39-46.doi.org/10.51607/22331360.2025.74.S1.39

Ališah A. 2020. Higijenski, zdravstveno-tehnološki, etološki i ekonomski aspekti novog koncepta opšte profilakse tova brojlerskih pilića Doctoral Thesis. University of Sarajevo-Veterinary Faculty.

Bermúdez-Aguirre D, Carter J, Niemira BA. 2025. An Investigation About the Historic Global Foodborne Outbreaks of Salmonella spp. in Eggs: From Hatcheries to Tables. Compr Rev Food Sci Food Saf.24(3), e70202.doi: 10.1111/1541-4337.70202.

EUR-Lex. Commission Delegated Regulation (EU) 2023/2465 supplementing Regulation (EU) 1308/2013. [Updated 2025 November 10]. Available from: [https://eur-lex.europa.eu/eli/reg_del/2023/2465/oj/eng]

EUR-Lex. Commission Regulation (EC) No 589/2008. (EU):Laying down marketing standards for eggs. [Updated 2025 November 11]. Available from:[https://eur-lex.europa.eu/eli/reg/2008/589/oj/eng]

European Food Safety Authority (EFSA) 2005. Opinion of the Scientific Panel on Biological Hazards on risk assessment of washing of table eggs. EFSA J, 269, 1-39.

FSAI 2019. Guidance Note on Egg Washing and Grading, Food Safety Authority of Ireland, Dublin.[Updated 2025 November 09]. Available from:[https://www.fsai.ie/enforcement-and-legislation/legislation/food-legislation/eggs/egg-washing]

Gagić A, Hadžiabdić S, Vrabelj D, Ratiznojnik M, Goletić T, Kustura A, Ališah A, Rešidbegović E. 2013. Upotreba stabiliziranog tečnog hlordioksida (ClO_2) radi sanitacije sistema za napajanje u prisustvu nesilica konzumnih jaja oboljelih od koliseptikemije, Veterinaria 62a (3-4), 165-73.

Gagić A, Selimović S, Jukić S, Ališah A, KusturaA. 2013. Dileme savremene dezinfekcije: hlor ili stabilizirani tečni

hlordioksid, Veterinaria 62 b(3-4), 229-40.

Gagić A. 1999. Reprodukcija u peradarstvu, textbook, Sarajevo, Studentska štamparija Univerziteta, COBISS-ID: 6938630

Hansung C, Hyobi, Donghoon M, Seongjoon K, Nong-Hoon C. 2018. Effect of Chlorine Dioxide Gas Application to Egg Surface: Microbial Reduction Effect, Quality of Eggs, and Hatchability, Korean J Food Sci Anim Res, 38(3), 487-97.doi: 10.5851/kosfa.2018.38.3.487.

Hutchison M, Gittins J, Walker A, Moore A, Burton C, Sparks N. 2003. Washing table eggs: A review of the scientific and engineering issues. Poult Sci J, 59(2), 233-48. doi:10.1079/WPS20030015.

Kustura A, Goletić T, Hadžiabdić S, Gagić Aj, Rešidbegović E, Gagić A, et al. 2012. Higijenski aspekti inkubacije nakon tri različita sanitarna tretmana rasplodnih jaja peradi, Split-DDD i ZUPP.

EUR-Lex. Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin.[Updated 2025 November 08]. Available from: [https://eur-lex.europa.eu/eli/reg/2004/853/oj/eng].

Rrahimi N. 2021. Treatment of Egg Shells with Hydrogen Peroxide and Sodium Carbonate as a Disinfectant with Bactericidal Effect to Eliminate Salmonella Enteritidis. Eurasia Proceedings Sci, Technol, EnginMathem, 12, 1-6.

Turtoi M, Borda D. 2014. Decontamination of egg shells using ultraviolet light treatment. World's Poul Sci J, 70(2), 265-78. doi: 10.1017/S0043933914000282

Procjena efikasnosti stabilnog tečnog hlor-dioksida (CLO_2) u dezinfekciji skladištenih konzumnih jaja

SAŽETAK

Mikrobiološka kontaminacija ljuski konzumnih jaja predstavlja značajan javnozdravstveni i tehnološki izazov, osobito u Europskoj Uniji, u kojoj su pranje i dezinfekcija jaja A klase zabranjeni zbog nuspojava povezanih sa ranije korištenim sredstvima za dezinfekciju. Ovo istraživanje evaluira efikasnost stabilnog tečnog hlor dioksida (ClO2) u smanjenju mikrobiološkog optrećenja ljuski jaja za vrijeme skladištenja u komercijalnim uvjetima. Ukupno 200 jaja je podijeljeno u dvije grupe, kontrolnu (netretiranu) i eksperimentalnu koja je tretirana sa finim aerosolom od 50 ppm ClO2. Mikrobiološka čistoća je procijenjena ukupnim brojem aerobnih mezofilnih bakterija (UBAMB) i luminometrijom rađenom u prethodno određenim intervalima. Neposredno nakon tretmana (Dan 0), eksperimentalna grupa je pokazala izraženu mikrobnu redukciju sa srednjim UBAMB vrijednostima koje su se smanjile sa 35,000 CFU u kontrolnoj grupi na 70 CFU u tretiranoj grupi ($p = 0.029$). Signifikantna razlika u luminometrijskim vrijednostima između grupa je uočena u Danima 0 i 14 ($p = 0.028$; $p = 0.025$), što ukazuje na perzistirajuću antimikrobnu aktivnost za vrijeme skladištenja. Luminometrijske vrijednosti su u eksperimentalnoj grupi pokazale signifikantnu temporalnu varijaciju ($p = 0.015$) karakteriziranu rastom da dana 7, nakon čega je do Dana 14 nastupila stabilizacija, pri čemu su se vrijednosti vratile na vrijednosti zabilježene neposredno nakon dezinfekcije ($p = 0.980$). U kontrolnoj grupi nisu otkrivene nikakve temporalne promjene ($p = 0.276$). Stabilni tečni hlor dioksid se dokazao kao učinkovit i tehnološki prihvatljiv dezinficijens kojim je postignuta značajna redukcija broja mikroba, bez uticaja na integritet jaja. Ovakvi razultati naglašavaju mogućnost primjene ClO2 kao suvremene, neškodljive opcije dezinfekcije koja uzima u obzir mikrobiološke sigurnosne mjere dok zadovoljava higijenske i zakonske zahtjeve povezane sa proizvodnjom konzumnih jaja. Preporučuju se daljnja istraživanja sa ciljem procjene unutrašnje kvalitete jaja, kao i šire ispitivanje mikrobioloških indikatora u različitim uvjetima skladištenja i okoliša.

Ključne riječi: Dezinfekcija, EU zakonodavstvo, kvalitet jaja, ljuska jaja, stabilni tečni hlor dioksid