Emirates Journal of Food and Agriculture. 2016. 28(5): 348-352
doi: 10.9755/ejfa.2015-07-478
http://www.ejfa.me/

REGULAR ARTICLE

Development of chlorine dioxide gas generation chamber to prevent spoilage of eggs

Gopiesh Khanna[1], Anil Kumar Chauhan[2], Sun Chul Kang[2]*

Department of Biotechnology, Daegu University, Kyoungsan, Kyoungbook 712-714, Republic of Korea
1st and 2nd author equally contributed to this work.

ABSTRACT

In the present study, we evaluated the efficacy of gaseous chlorine dioxide in a plastic chamber for preserving unwashed eggs. A time period of 30 days at 3-day intervals was used to evaluate freshness of eggs, including total microbial counts, *E. coli*, *Coliform*, and *Salmonella* spp count. We also determined the yolk index and pH level of eggs at intervals of 1 week and 5 days, respectively. The results for total microbial counts revealed that chlorine dioxide at a concentration of 100 parts per million by volume (ppmv) could potentially kill all microbes within 3 days of the evaluation period. Freshness of eggs improved in the presence of chlorine dioxide (50 and 100 ppmv) compared to that of eggs without chlorine dioxide treatment. At a higher concentration of chlorine dioxide (100 ppmv), reduction of the yolk index was less compared to control eggs without treatment. Further, the pH level of control unwashed eggs were lower than that of eggs treated with chlorine dioxide. The results of the present study suggest that treatment of unwashed eggs with chlorine dioxide can be effective for long-term storage with maintenance of quality.

Keywords: Chlorine dioxide gas; Yolk index; Egg quality control; Albumin pH

INTRODUCTION

Chlorine dioxide (ClO_2) has recently been acknowledged as one of the most prominent and effective chemicals for deodorizing and sterilizing food products. The chemical has been proven as having outstanding germicidal power, as it has 2.5-fold stronger oxidative potential than ordinary chlorine disinfectants (Han et al, 2003; Volk et al, 2002). Chlorine dioxide gas has been widely used to sterilize water due to its efficacy in breaking down phenolic compounds as well as its potential to remove odors (Dychdala et al., 1968). There have been several reports suggesting its strong toxic effect towards foodborne pathogens (Park and Kang, 2015), especially *Salmonella typhimurium* and *Listeria monocytogenes* (Shin et al., 2004), which make it a potential candidate in food industries.

In addition to its efficacy for food and water disinfection, chlorine dioxide has been shown to play a role in medical science (Noszticzius et al., 2013). The potential of chlorine dioxide for killing oral bacteria has been widely studied, as clinical and laboratory investigations have demonstrated that stabilized chlorine dioxide oral rinse reduces bacteria numbers in the mouth, essentially eliminating oral malodor, as well as signs of gum disease (Drake and Villhauer, 2011). A study carried out by Wilson et al. suggested that chlorine dioxide treatment can inhibit growth of fungi and molds which are associated with many human diseases including sick building syndrome (SBS) (Wilson et al., 2005).

Eggs have always been accepted as one of the most important sources of nutrition for humans. As such, eggs of high quality under hygienic conditions are in demand worldwide and play a great role in the economy (Park et al, 2003). However, maintaining quality is a critical and difficult parameter for most egg-producing farms. Besides the beneficial aspects of eggs, a number of foodborne illnesses and diseases are associated with egg consumption by human. Therefore, the inner quality of eggs during storage, including integrity, aesthetics, and microbiological contamination of the albumin and yolk, has become a major concern (Koelkebeck, 2003). Deterioration of egg internal quality begins soon after the egg is laid due

***Corresponding author:**
Sun Chul Kang, Department of Biotechnology, College of Engineering, Daegu University, Kyoungsan, Kyoungbook 712-714, Republic of Korea. Phone: +82-53-850-6553, Fax: +82-53-850-6569, E-mail: sckang@daegu.ac.kr

Received: 01 July 2015; **Revised:** 06 March 2016; **Accepted:** 08 March 2016; **Published Online:** 18 March 2016

to various factors such as high temperature and aging (Olobatoke and Mulugeta, 2012).

In the present study, we developed a specialized chlorine dioxide gas generator based on the membrane electrode assembly and a small-sized light-weight compact system. To validate the instrument, we further evaluated the efficacy of chlorine dioxide produced by the instrument for preventing egg spoilage.

MATERIALS AND METHODS

Materials

Freshly laid unwashed eggs were purchased from the Department of Agriculture, Daegu University.

Treatment of eggs

A total of 150 eggs were randomly divided in three groups: control unwashed eggs, eggs treated with 50 parts per million volume (ppmv) of chlorine dioxide, and eggs treated with 100 ppmv of chlorine dioxide. Treatment of eggs with chlorine dioxide was carried out in a mechanical chamber specifically designed to produce chlorine dioxide. Unwashed control eggs without treatment were kept at room temperature.

Development of chlorine dioxide generation chamber

The chlorine dioxide gas was generated by a chlorine dioxide generator (PurgoFarm Inc., Korea) following the electrochemical method. Briefly, aqueous $NaClO_2$ was passed through a patented multi-porous membrane electrode assembly (MEA) producing highly pure chlorine dioxide gas (>98% Chlorine dioxide). After a series of electrochemical reactions, chlorine dioxide gas was blown out through a chlorine dioxide vent into a collecting chamber. During treatment, the chlorine dioxide concentration in the chamber was monitored using a PortaSens II gas leak detector (Analytic Technology, Inc., USA).

Determination of total bacterial counts on eggshells

Determination of total bacterial numbers on the eggshell surface was carried out by following a previously reported method (Mallmann et al. 1953). A total of 10 eggs from each group at intervals of 3 days were placed into Whirl-Pak sampling bags (Sigma-Aldrich, USA) containing 0.1% peptone water and mixed by shaking the bag (10 min). The solution was diluted, plated on Luria-Bertani agar, and incubated at 37 ºC for 2 days. After the incubation period, bacterial colonies were counted.

Determination of *E. coli* and *Coliform* on eggshells

Determination of *E. coli* and *Coliform* numbers was carried out as described above. The diluted solution was plated on chromogenic medium (CM0956, Oxoid, UK) to differentiate *Coliform* and *E. coli*. After an incubation period of 24 hrs at 37 ºC, colonies were counted and presented as CFU/ml.

Determination of *Salmonella* species on eggshells

Determination of *Salmonella* species was carried out by diluting the shell solution every 3 days, followed by plating on *Salmonella*-specific chromogenic agar medium (CM1092 Oxoid, UK) for 18 hrs at 37 ºC. After completion of the incubation period, the colonies were counted and expressed as CFU/ml.

Determination of yolk index

Yolk index refers to the morphology of egg yolk, which includes the height and diameter of the yolk [yolk index (YI) = Yolk Height (mm)/Yolk diameter (mm) x 100]. Yolk height was measured by a tripod micrometer, and yolk width was measured with digital calipers.

Determination of albumin pH

Albumin was separated from the yolk and mixed thoroughly, after which measurement of pH was done with a digital pH meter (Orion Research., Inc., USA).

Statistical analysis

A total of 50 eggs per group were used to analyze the data, and the experimental results are depicted as the mean ± standard error of the mean (SEM).

RESULT AND DISCUSSION

Development of chlorine dioxide generation chamber

There are three different types of gas generation methods based on electrolysis. The first and the second types are characterized by the presence of a diaphragm seal in the device, whereas the last type is known as membrane electrode assembly (MEA). A gas generator with no diaphragm seal is unable to prevent unintended reactions between NaOH and newly produced chlorine dioxide, resulting in low gas productivity (Fig. 1). On the other hand, a gas generator with a diaphragm seal is unable to achieve high gas productivity due to increased resistance caused by conduction by the diaphragm itself. Salt can be added to increase gas productivity, but salt can have corrosive effects and does nothing to prevent formation of chlorine gas and its disinfectant by-products (DBP). On the other hand, MEA allows efficient production of high-purity chlorine dioxide gas without follow-up procedures to counter the negative effects of conduction resistance. Conduction resistance is lower in MEA compared to other gas-generating devices due to the absence of physical gaps between positive and negative electrodes. This highly efficient production of chlorine dioxide can be even more

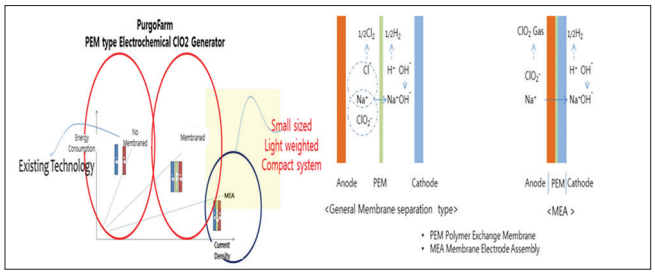

Fig 1. Graphical representation of modified chlorine dioxide gas generation system and principle of gas generation.

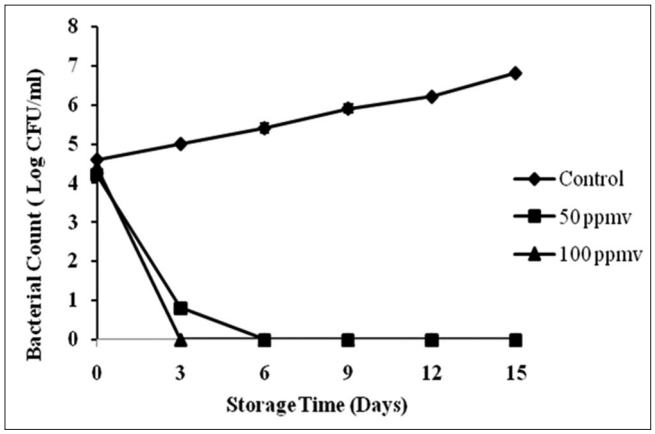

Fig 2. Effect of chlorine dioxide (50 and 100 ppmv) on total microbial counts on eggshell surface every 3 days. Results are depicted as the mean of triplicate biological determinations ± standard error of the mean (SEM).

improved without having to increase the size of the MEA device.

Determination of total bacterial counts

The bacterial population inside the eggs is mostly responsible for spoilage during storage (Gentry and Quarles, 1972). In the present study, we evaluated the total numbers of bacteria in both treated and untreated eggs daily. We observed that total numbers of bacteria decreased with increasing concentrations of chlorine dioxide. At concentrations of 50 and 100 ppmv of chlorine dioxide, all bacteria were killed within 3 days of storage (Fig. 2). On the other hand, elevation of bacterial counts was observed in control untreated eggs. We also evaluated total bacterial numbers on eggshells, which are more prone to infection and can transport bacteria inside to the albumin and yolk. The results suggest that gaseous chlorine dioxide even at a low concentration of 50 ppmv can efficiently kill bacteria and can protect eggs from bacterial infection.

Determination of *E. coli* and *Coliform* counts

E. coli and *Coliform* bacteria are important in deteriorating internal quality of eggs. We evaluated the potential of chlorine dioxide gas for removing *E. coli* and *Coliform* bacteria. At 50 and 100 ppmv of chlorine dioxide, the

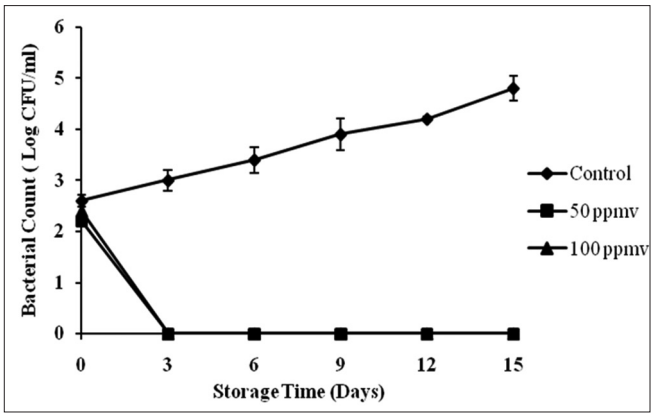

Fig 3. Effect of chlorine dioxide (50 and 100 ppmv) on *Coliform* counts on eggshell surface every 3 days. Results are depicted as the mean of triplicate biological determinations ± standard error of the mean (SEM).

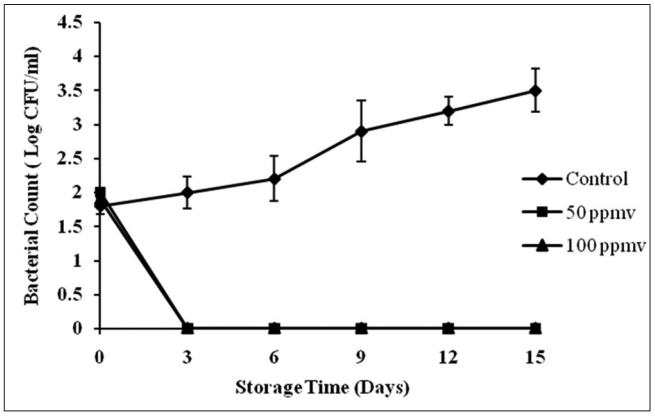

Fig 4. Effect of chlorine dioxide (50 and 100 ppmv) on *E. coli* counts on eggshell surface every 3 days. Results are depicted as the mean of triplicate biological determinations ± standard error of the mean (SEM).

number of colony forming units (CFU) of both bacteria was 0 after 3 days of incubation. On the other hand, control untreated cells showed an increase in the number of CFU at different time periods. After 15 days of storage, the number of *Coliform* was 4.8 log CFU/ml (Fig. 3) and 3.5 log CFU/ml in the case of *E. coli* (Fig. 4), for control eggs.

These results suggest that both concentrations of chlorine dioxide (50 and 100 ppmv) could kill bacteria on eggshells, thus protecting eggs from spoilage. Both bacteria have been found to be involved in dairy and food product spoilage during long-term storage. Although a variety of sanitizers have already been introduced to reduce contamination of food products by these bacteria, chlorine dioxide has more beneficial effects. The antibacterial effect of chlorine dioxide might be due to its intense toxic nature towards the microorganism which protected the eggshell in our study.

Determination of *Salmonella* species

Salmonella is another important bacterium involved in the spoilage of eggs during storage, and serious illness

can result if eggs contaminated with *Salmonella* are consumed. There are many methods to reduce *Salmonella* contamination of eggs, including washing eggs with hot water. Here, we examined the efficacy of chlorine dioxide to reduce *Salmonella* contamination of eggs during storage and found that all concentrations of chlorine dioxide (50 and 100 ppmv) showed potential to kill *Salmonella* spp. on the surface of eggshells. After 3 days of storage, log CFU was found to be 0 for eggs treated with chlorine dioxide at 50 and 100 ppmv, whereas control untreated eggs showed an increased number of *Salmonella* with each day. After 15 days of storage, the number of *Salmonella* species was 2.6 log CFU/ml (Fig. 5).

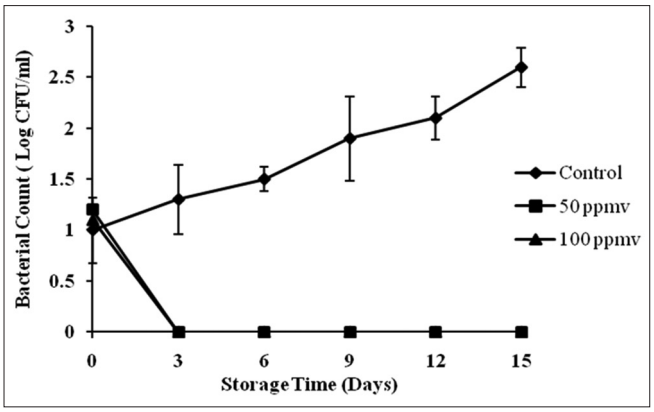

Fig 5. Effect of chlorine dioxide (50 and 100 ppmv) on *Salmonella* species counts on eggshell surface every 3 days. Results are depicted as the mean of triplicate biological determinations ± standard error of the mean (SEM).

Determination of yolk index

Inner quality of eggs is defined by the yolk index, which continuously decreases during storage of eggs due to diffusion of water from the albumin to yolk. Diffusion of water from the albumin to yolk dilutes and alters the normal structure of the yolk, resulting in a reduced yolk index (Scott and Silversides, 2000; Silversides and Scott, 2001). In the present study, we have evaluated the effects of different concentrations of chlorine dioxide on the yolk index, which represents the inner quality of eggs during 1 month of storage. We found that the yolk index of eggs treated with chlorine dioxide at concentrations of 50 and 100 ppmv decreased at a slower rate, even after 30 days (30.74 and 32.4%, respectively), compared to that of control untreated eggs, which decreased to 28.9% after 30 days (Fig. 6). This property of chlorine dioxide suggests its utility in preservation of eggs at room temperature.

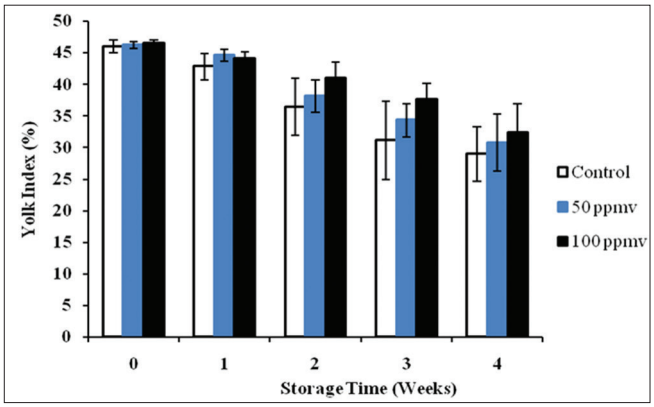

Fig 6. Effect of chlorine dioxide (50 and 100 ppmv) on yolk index of eggs determined at 1-week intervals. Results are depicted as the mean of triplicate biological determinations ± standard error of the mean (SEM).

Detection of albumin pH

The most important criterion for assessing egg quality is the pH level of albumin, which increases due to loss of CO_2. Carbon dioxide is continuously released from eggs during storage through pores in the eggshell (Powrie, 1973; French and Tullet, 1991). In our experiment, we measured a slight increase in the pH level of eggs treated with both concentrations of chlorine dioxide (50 and 100 ppmv). However, the pH level increased at a slower rate upon 100 ppmv of chlorine dioxide compared to 50 ppmv after 30 days (pH levels of 8.5 and 8.21 at 50 and 100 ppmv, respectively). On the other hand, the pH level of albumin from control untreated eggs continuously increased in accordance with time after 30 days of storage (pH level of 8.9) (Fig. 7). These results suggest that chlorine dioxide can potentially protect against elevation of pH in eggs during storage, ultimately preventing spoilage of eggs.

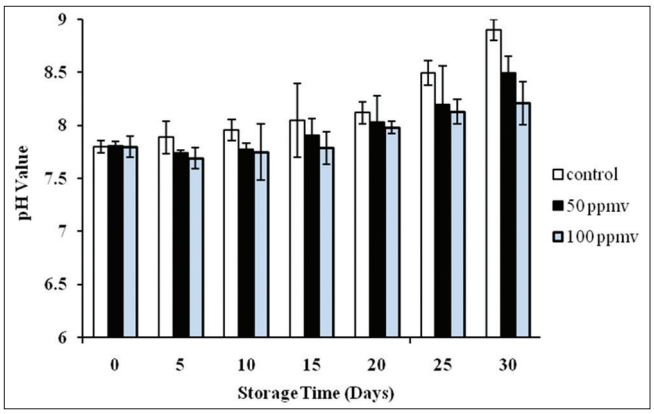

Fig 7. Effect of chlorine dioxide (50 and 100 ppmv) on albumin pH determined at 5-day intervals for 1 month. Results are depicted as the mean of triplicate biological determinations ± standard error of the mean (SEM).

CONCLUSION

Egg spoilage is responsible for great economic loss worldwide, as eggs are a main nutritional source. As such, maintenance of egg freshness has long been a main concern. There have been numerous reports attempting to develop procedures or potent compounds that can be used for long-term of storage of eggs while retaining their

internal quality. The results of the present study suggest that our specialized chamber producing chlorine dioxide can be used to preserve eggs at room temperature by inhibiting bacterial penetration into the albumin and yolk. Further, the potential of chlorine dioxide to inhibit pH elevation in the albumin as well as maintain the yolk index suggests its efficacy in preventing egg spoilage. Collectively, these results indicate that chlorine dioxide can be used for the preservation of eggs during long-term storage by retaining internal quality.

Funding

This research was supported by the Daegue University research grant.

Author contributions

SCK, GK and AKC designed the work, GK and AKC carried out the experiments and wrote the manuscript. SCK reviewed the manuscript and made the corrections.

REFERENCES

Drake, D. and A. L. Villhauer. 2011. An *in vitro* comparative study determining bactericidal activity of stabilized chlorine dioxide and other oral rinses. J. Clin. Dent. 22: 1-5.

Dychdala, G. R. 1968. Chlorine and chlorine compounds. In: Lawrence, C. A. and S. S. Block, (Eds.), Disinfection, Sterilization, and Preservation, Lea & Febiger, Philadelphia, PA, p. 294.

French, N. A. and S. G. Tullet. 1991. Variation in the eggs of poultry species. Avian Incubation 22nd Poultry Science Symposium, Butterworth-Heinemann Ltd., Pp. 59-77.

Gentry, R. F. and C. L. Quarles. 1972. The measurement of bacterial contamination on eggshell. Poult. Sci. 51: 930-933.

Han, Y., R. H. Applegate, R. H. Linton and P. E. Nelson. 2003. Decontamination of *Bacillus thuringiensis* spores on selected surfaces by chlorine dioxide gas. J. Environ. Health. 66: 16-21.

Koelkebeck, W. K. 2003. What is egg quality and conserving it. University of Illinois Extension Publications, Urbana-Champaign, IL.

Mallmann, W. L., L. E. Dawsan, B. M. Sultzer and H. L. Wright. 1953. Studies in microbiological methods for predicting shelf life of dressed poultry. Food Technol. 7: 122-125.

Noszticzius, Z., M. Wittmann, K. Kály-Kullai, Z. Beregvári, I. Kiss, L. Rosivall and J. Szegedi. 2013. Chlorine dioxide is a size-selective antimicrobial agent. PLoS One. 8: e79157.

Olobatoke, R. Y. and S. D. Mulugeta. 2012. The effect of dietary garlic powder and a low temperature on the physical quality of stored eggs. S. Afr. J. Anim. Sci. 42: 540-544.

Park, Y. S., I. J. Yoo, K. H. Jeon, H. K. Kim, E. J. Change and H. I. Oh. 2003. Effects of various eggshell treatments on the egg quality during storage. Asian Aust. J. Anim. Sci. 16: 1224-1229.

Park, S. H. and D. H. Kang. 2015. Antimicrobial effect of chlorine dioxide gas against foodborne pathogens under differing conditions of relative humidity. LWT-Food Sci. Technol. 60: 186-191.

Powrie, W. D. 1973. In: Stadelman, W. J. and O. J. Cotterill, (Eds.), Egg Science and Technology, The AVI Publishing Company Inc., Westport, Connecticut.

Scott, T. and F. G. Silversides. 2000. The effect of storage and strain of hen on egg quality. Poult. Sci. 79: 1725-1729.

Shin, J. H., S. Chang and D. H. Kang. 2004. Application of antimicrobial ice for reduction of foodborne pathogens (*Escherichia coli* O157:H7, *Salmonella Typhimurium, Listeria monocytogenes*) on the surface of fish. J. Appl. Microbiol. 97: 916-922.

Silversides, F. G. and T. A. Scott. 2001. Effect of storage and layer age on quality of eggs from two different lines of hens. Poult. Sci. 80: 1240-1245.

Volk, C. J., R. Hofmann, C. Chauret, G. A. Gagnon, G. Ranger and R. C. Andrews. 2002. Implementation of chlorine dioxide disinfection: effects of the treatment change on drinking water quality in a full-scale distribution system. J. Environ. Eng. Sci. 1: 323-330.

Wilson, S. C., C. Wu, L. A. Andriychuk, J. M. Martin, T. L. Brasel, C. A. Jumper and D. C. Straus. 2005. Effect of chlorine dioxide gas on fungi and mycotoxins associated with sick building syndrome. Appl. Environ. Microbiol. 71: 5399-5403.