Research Article

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Ozone treatment of shell eggs to preserve functional quality and enhance shelf life during storage

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Abstract

BACKROUND: Eggs have long been recognised as a source of high-quality proteins. Many methods exist to extend shelf life of food and one of them is ozone treatment, which is an emerging technology for disinfecting surfaces in the food industry. This study aimed to extend the shelf life of fresh eggs using gaseous ozone treatments at concentrations of 2, 4 and 6 ppm with exposure times of 2 and 5 min during storage for 6 weeks at 24 °C. The effect of the treatments on interior quality and functional properties of eggs is also reported.

RESULTS: Ozone concentration and exposure time significantly affected the Haugh unit (HU), yolk index, albumen pH, relative whipping capacity (RWC), and albumen viscosity of eggs during the storage. Control eggs had the highest albumen pH and lowest albumen viscosity. Attributes such as albumen pH and RWC of eggs exposed to ozone treatments were better than the control samples. The measurement results showed that ozone concentration at 6 ppm and exposure time of 5 min can be applied to fresh eggs and extend shelf life up to 6 weeks at 24 °C storage period.

CONCLUSION: Ozone treatments helped to maintain egg quality for a longer time. Ozone concentrations at 2 and 4 ppm showed promising results in maintaining internal quality and functional properties of fresh eggs during storage. Ozone at high concentration (6 ppm) caused a detrimental effect on eggshell quality. As a result, this study demonstrated that ozone treatments of 2, and especially 4 and 6 ppm concentration maintained eggshell quality during the storage.

Keywords: gaseous ozone; shell eggs; egg quality; functionality; physicochemical properties; albumen and egg yolk

INTRODUCTION

Eggs have been valuable food source due to their high nutritional value. Eggs are also used widely by the food industry due to their unique multifunctional properties. Even though the eggshell is a remarkable protective package, eggs have a short shelf life. Immediately after eggs are laid, their chemical, physical, microbiological and functional properties change and deterioration begin.[1,2] These natural degradations could result in significant economic losses for the egg industry. A small percentage improvement in the overall quality and shelf life would result in significant savings to the egg industry considering sheer production capacity of the fresh eggs. There has been increasing interest in using alternative emerging technologies as food preservation methods. Nowadays, several non-thermal methods, including high pressure, ultrasound and ozonation, have been studied as food preservation technologies. Among these methods, ozone treatments have gained interest for extending the shelf life of perishable foods. Ozone (O_3), a highly reactive and an effective antimicrobial agent, generates no residual chemicals.[3,4] The US Department of Agriculture (USDA) and the US Food and Drug Administration (FDA) approved gaseous and aqueous ozone as an antimicrobial agent for direct use in food applications.[4–6] Ozone is also approved in the US on meat and poultry products in accordance with industrial standards of good manufacturing practice (21 CFR 173.368; FDA 2003). As a result, there is a growing research interest in use of ozone applications for extending shelf life of food products.[3,7–10]

In the last decade the efficacy of ozonation on the surface of fresh eggs have been studied.[4,11–16] Rodriguez-Romo *et al.*[15] reported that gaseous ozone can penetrate through eggshell pores. Fuhrmann *et al.*[11] showed that even at low ozone concentrations, cuticula proteins of the egg can be destroyed by oxidation of amino acids and three-dimensional structures. Goo-Hee and Kyung-Haeng[12] studied the effects of gaseous ozone (38.8 ppm) for 10 to 30 min treatment times on the egg's physical and chemical characteristics including Haugh unit (HU), yolk colour, pH of egg albumen and yolk, foaming ability, foam stability and lipid oxidation development. The results of that study showed that ozone-treated eggs were no different than controls when stored

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Table 1. Effect of the ozone treatment on egg weight loss (%) during 5 weeks of storage

Ozone concentration (ppm)	Weight loss (%)					
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks
0 (control)	0.00 ± 0.00^{Aa}	2.22 ± 0.16^{Ba}	3.50 ± 0.40^{Ca}	5.02 ± 0.40^{Da}	6.84 ± 0.28^{Ea}	7.99 ± 0.26^{Fa}
2	0.00 ± 0.00^{Aa}	2.10 ± 0.14^{Ba}	2.66 ± 0.47^{Bb}	3.85 ± 0.91^{Cb}	5.14 ± 0.88^{Db}	7.32 ± 0.91^{Eab}
4	0.00 ± 0.00^{Aa}	1.95 ± 0.19^{Ba}	2.92 ± 0.24^{Cb}	4.35 ± 0.47^{Dbc}	5.16 ± 0.79^{Eb}	6.94 ± 0.57^{Fb}
6	0.00 ± 0.00^{Aa}	2.03 ± 0.19^{Ba}	3.13 ± 0.30^{Ca}	4.73 ± 0.50^{Dc}	5.62 ± 0.43^{Eb}	7.16 ± 0.33^{Fb}

Data are expressed as mean ± standard deviation.
$^{a-c}$Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
$^{A-F}$Means in the same row with different superscript capital letters are significantly different ($P < 0.05$).

at 4 °C for 14 days. The effects of gaseous ozone on extending the shell of eggs and internal quality, in particular the functional properties of eggs, have not been extensively studied. Therefore, it is important to investigate the effects of gaseous ozone treatments on shell egg interior quality for long term storage at room temperature.

The first objective of this study was to evaluate internal qualities (pH, Haugh unit, and yolk index) of fresh shell eggs under various ozone concentrations and treatment times. The second objective was to measure functional properties (viscosity, relative whipping capacity) of the shell eggs during storage at ambient conditions; and the third objective was to measure eggshell strength during storage at 24 °C.

MATERIALS AND METHODS

Eggs

Clean, white-shell (Lohmann White laying hen breed), grade AA, unwashed, 1-day-old fresh shell eggs weighing 55–60 g were obtained from A.B. Foods (Balikesir, Turkey).

Ozone generation equipment and treatments

Gaseous ozone was produced using a plate type ozone generator (model TKZ-6G, H series; Teknozone, Izmir, Turkey) in a custom-made ozone glass vessel with a diffuser as a gaseous ozone injector.[17] Concentration was monitored continuously using an ozone detector (Teknozone). Treatments consisted of control (untreated) eggs, and eggs treated with gaseous ozone at concentrations of 2, 4 and 6 ppm, and exposure times of 2 and 5 min. Treatments were performed in a custom-made chamber which had a capacity of five eggs at 24 °C. After treatments, eggs were placed in plastic eggs trays and stored under ambient conditions at 24 °C for 6 weeks.

Weight loss

Weight loss (%) of fresh eggs was calculated by subtracting the final weight of the egg from the initial weight and dividing by the initial weight and multiplying by 100 as described in Caner and Cansýz.[18]

Ten separate eggs (3 × 10) were marked and placed into vials per treatment. Weights of ozone-treated and untreated shell eggs were recorded by using a sensitive laboratory electronic balance throughout the storage period.

Haugh unit and yolk index

Ten eggs (3 × 10) were used per treatment each week to measure Haugh unit and yolk index. Haugh unit (HU) was calculated by using the formula[19] Haugh unit $= 100 \times \log(h - 1.7\,W^{0.37} + 7.6)$, where h is the height of the thick albumen (in mm) and W is the weight of the egg (in g). The parameter h was recorded by averaging three measurements carried out in different points of thick albumen at a distance of the 10 mm from the yolk using a digital calliper (CD-15CP; Mitutoya Ltd, Hampshire, UK).[19]

Yolk index was calculated as yolk height divided by yolk width. Yolk height and width were measured with digital callipers (CD-15CP; Mitutoyo Ltd), without removing albumen.[20]

pH measurements

Egg albumen (3 × 10 eggs) for each treatment was homogenised for 20 s using a Waring Blender Model 32 BL 80 (Waring, Torrington, CT, USA) and then the pH of egg yolk was measured using a pH 210 meter (Hanna Instruments, Woonsocket, RI, USA).[19]

Albumen viscosity

The eggs were broken, chalaza separated, and the albumen was collected in a vessel for measuring viscosities. Ten eggs (3 × 10) were used for each treatment to measure albumen viscosity in each week. Albumen viscosity [millipascal second (mPa s)] was measured at 20 °C using the Brookfield viscometer (Model DV II + Pro D 220, TC-502 Rheocalc software; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). The spindle (UL Adaptor at 30 rpm) was selected based on the torque measurement between 10 and 100%. Results were recorded after a 20 s rotation of the spindle.[19]

Foaming properties

Relative whipping capacity (RWC) and foam stability of the egg albumen and whole egg samples (20 °C) were measured with minor modifications as described by Li-Chan and Nakai.[21] Ten eggs from each treatment were used to determine the foaming properties. Foam was obtained at room temperature by whipping 75 mL of egg albumen/whole egg in a Hobart Mixer N50CE (Hobart Foster Scandinavia A/S, Aalborg, Denmark) at speed 2 for 90 s, then on speed 3 for 90 s. Foam density was calculated from the mass of a given volume of foam, and foam stability as the percentage of liquid held-drained was measured with a graduated cylinder after the foam was allowed to rest for 1 h, as described by Nicorescu *et al.*[22] The stability of foam was measured in the same vessel as the volume of released fluid at the bottom, 1 h after whipping. Egg RWC was calculated as follows: volume (%) = [(volume of prepared foam × volume of liquid drainage)/original volume of liquid] × 100.[19]

Eggshell breaking strength (puncture strength)

Measurement was performed on each egg using a Texture analyser (TA.XT-Plus; Texture Technologies, Scarsdale, NY, USA). Twenty separate eggs per treatment were taken for the top and the bottom sides. Each egg was mounted on a platform and eggshells were punctured at top and bottom using 3 mm probe at 5 mm s^{-1} constant speed with a 30-kg load cell in a compression mode. The force required puncturing eggshell as kilograms force (kg_f) was recorded and expressed as shell strength of eggshell.[19]

Data analysis

Analysis of variance was carried out on all the measured parameters among the control and ozone concentrations and exposure times on eggs to determine significant difference during storage (6 weeks at 24 °C). The experiments were repeated three times. Statistical procedures were done using least square means (LSM-PROG GLM) of the statistical analysis software program (SAS Institute Inc., Cary, NC, USA). Statistical significance was defined as *P*-values of <0.05.

RESULTS AND DISCUSSION

Weight loss

The weight loss of control (untreated) and ozone-treated eggs (2, 4 and 6 ppm concentrations with exposure times of 2 and 5 min) is shown in Table 1. Weights of the eggs decreased significantly during storage. The weight loss was higher for the control group (7.99%) as compared with the 2 ppm (7.32%), 4 ppm (6.94%), and 6 ppm (7.16%) treated eggs (Table 1). All gaseous ozone treatments slowed down the weight loss of eggs ($P < 0.05$) during storage period. Differences in weight loss between 4 ppm and 6 ppm ozone-treated eggs were not significant after 2 weeks of storage.

Ozone treatments at 4 and 6 ppm prevented weight loss ($P < 0.05$) compared with control eggs. The present results are in agreement with Qing[23] who showed that ozone treatments slowed down the water loss of eggs during storage.

Haugh unit and egg grade

The Haugh unit (HU) is a measurement of egg protein quality. Higher HU values mean higher protein quality.[18,19] Table 2 shows the changes in HU values of untreated and ozone-treated eggs during storage for 6 weeks at 24 °C. Statistical analysis showed that the interactions of three factors (Week*Concentration*Time) were significant. In our study, HU decreased with increasing storage time, which is in agreement with previous publications.[24,25] A decrease in HU was induced by albumen thinning due to age-related changes occurring in ovomucin. Thinning was due to the destruction of the lysozyme–ovomucin complex when loss of CO_2 occurred.[19,26] As eggs age, water migrates from the albumen to the yolk and the strength of the vitellin membrane decreases, leading to lower HU values.[21,27] In addition, the decrease in HU values is associated with low pH of ozone-treated eggs and is the result of radical chain reactions on some component of albumen. It is possibly due to the oxidation of hydroxyl amino acids,[28] with the loss of CO_2 from egg as the pH becomes more basic and structural changes take place in the albumen. Also, reports by others showed that ozone can penetrate through egg shells.[11,15] These results were in agreement with Fuhrmann *et al.*[11] Rodriguez-Romo and Yousef[29] and Qing.[23] The result of the mechanism is a thinning of the albumen and affects HU. The present study showed that the shelf life of eggs was prolonged (grade A) by at least 1 week by ozone treatments, especially with 4 ppm.

Table 2. Effect of the ozone treatment (for 2 and 5 min) on Haugh unit (HU) and egg grade during 6 weeks of storage

Ozone concentration (ppm)	Haugh unit values and egg grades															
	0 week		1 week		2 weeks		3 weeks		4 weeks		5 weeks		6 weeks			
	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min		
0 (control)	83.06 (AA)* ± 0.38^{Aal}	82.09 (AA) ± 0.34^{Aal}	79.30 (AA) ± 0.39^{Bal}	80.78 (AA) ± 0.48^{Aal}	75.77 (AA) ± 0.38^{Cal}	76.50 (AA) ± 0.61^{Bal}	68.22 (A) ± 0.65^{Dal}	69.49 (A) ± 0.46^{Cal}	67.38 (A) ± 0.83^{Dal}	67.20 (A) ± 0.40^{Cal}	59.40 (B) ± 0.29^{Eal}	59.32 (B) ± 0.32^{Dal}	45.18 (B) ± 0.58^{Fal}	45.05 (B) ± 0.48^{Dal}		
2	83.74 (AA) ± 0.39^{Aal}	84.29 (AA) ± 0.30$^{Aal.II}$	79.63 (AA) ± 0.36^{Bal}	79.28 (AA) ± 0.80$^{Bal.II}$	77.14 (AA) ± 0.63^{Cal}	78.28 (AA) ± 0.80$^{Bal.II}$	71.40 (A) ± 0.98^{Dall}	73.57 (AA) ± 0.88^{CbII}	69.45 (A) ± 0.93^{Eall}	70.20 (A) ± 0.69^{Dall}	64.80 (A) ± 0.82^{Fall}	66.28 (A) ± 0.55^{Eall}	53.47 (B) ± 0.9^{Gall}	52.87 (B) ± 0.7^{Fall}		
4	84.43 (AA) ± 0.22^{Aal}	84.69 (AA) ± 0.24$^{Aal.II}$	79.73 (AA) ± 0.70^{Bal}	78.32 (AA) ± 0.58^{Bal}	77.26 (AA) ± 0.88^{Cal}	77.98 (AA) ± 0.94$^{Cal.II}$	74.37 (AA) ± 0.77^{Dall}	76.94 (AA) ± 0.70^{CbII}	70.33 (A) ± 0.70^{Eall}	72.61 (AA) ± 0.96^{DbII}	65.63 (A) ± 0.35^{Fall}	67.85 (A) ± 0.77$^{FbII.III}$	52.07 (B) ± 0.7^{Gall}	55.54 (B) ± 0.9^{FbII}		
6	84.00 (AA) ± 0.24^{Aal}	85.49 (AA) ± 0.25^{Aall}	79.56 (AA) ± 0.75^{Bal}	79.97 (AA) ± 0.59^{Bal}	77.69 (AA) ± 0.71^{Cal}	79.39 (AA) ± 0.60^{CbII}	76.82 (AA) ± 0.79^{CaIV}	78.08 (AA) ± 0.88^{CaIII}	72.75 (AA) ± 0.76^{DaIII}	72.64 (AA) ± 0.64^{DaIII}	69.00 (A) ± 0.76^{EaIII}	69.34 (A) ± 0.65^{EaIII}	54.38 (B) ± 0.51^{FaIII}	55.64 (B) ± 0.59^{FaIII}		

Data are expressed as mean ± standard deviation.
A–^{G}Different superscript uppercase letters donate significant differences between storage times in same treatment and time ($P < 0.05$).
a–^{b}Different superscript lowercase letters donate significant differences between treatment times in same treatment and storage time ($P < 0.05$).
I–^{IV}Different roman numbers donate significant differences between treatments in same treatment time and storage time ($P < 0.05$).
* Uppercase letters in parentheses are egg grades, where AA refers to HU > 72; A, HU = 71–60; B. HU = 59–31; C. HU < 30.

Table 3. Effect of the ozone concentration and egg storage time on yolk index during 6 weeks of storage

	Yolk index						
Ozone concentration (ppm)	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
0 (control)	0.47 ± 0.01^{Aa}	0.43 ± 0.01^{Ba}	0.40 ± 0.01^{Ca}	0.35 ± 0.01^{Da}	0.31 ± 0.01^{Ea}	0.29 ± 0.01^{Fa}	0.26 ± 0.01^{Ga}
2	0.48 ± 0.01^{Ab}	0.44 ± 0.01^{Ba}	0.42 ± 0.01^{Cb}	0.38 ± 0.01^{Db}	0.35 ± 0.01^{Eb}	0.32 ± 0.01^{Fb}	0.30 ± 0.01^{Gb}
4	0.50 ± 0.01^{Ac}	0.44 ± 0.01^{Ba}	0.43 ± 0.01^{Bb}	0.41 ± 0.79^{Cc}	0.37 ± 0.01^{Dc}	0.32 ± 0.01^{Ec}	0.32 ± 0.01^{Gc}
6	0.51 ± 0.01^{Ad}	0.45 ± 0.01^{Ba}	0.44 ± 0.01^{Bc}	0.42 ± 0.43^{Cc}	0.39 ± 0.01^{Dd}	0.36 ± 0.01^{Ed}	0.34 ± 0.01^{Gd}

Data are expressed as mean ± standard deviation.
$^{a-d}$Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
$^{A-G}$Means in the same row with different superscript uppercase letters are significantly different ($P < 0.05$).

Table 4. Effect of ozone concentration and treatment time (2 and 5 min) on yolk index

	Yolk index	
Ozone concentration (ppm)	2 min	5 min
0 (control)	0.36 ± 0.07^{a}	0.36 ± 0.07^{a}
2	0.38 ± 0.06^{b}	0.39 ± 0.06^{b}
4	0.40 ± 0.05^{c}	0.42 ± 0.05^{c}
6	0.41 ± 0.05^{d}	0.43 ± 0.05^{d}

Data are expressed as mean ± standard deviation.
$^{a-d}$Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).

Table 6. Effect of ozone concentration and egg treatment time (2 and 5 min) on albumen pH

	Albumen pH	
Ozone concentration (ppm)	2 min	5 min
0 (control)	8.97 ± 0.23^{Aa}	9.01 ± 0.22^{Aa}
2	8.43 ± 0.14^{Ab}	8.35 ± 0.13^{Bb}
4	8.30 ± 0.14^{Ac}	8.22 ± 0.15^{Bc}
6	8.13 ± 0.13^{Ad}	8.04 ± 0.19^{Bd}

Data are expressed as mean ± standard deviation.
$^{a-d}$Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
^{A,B}Means in the same row with different superscript uppercase letters are significantly different ($P < 0.05$).

Yolk index

Weakening of the vitellin membranes, reduction of the total solid, and liquefaction of the yolk was caused mainly by osmotic diffusion of water from the albumen during storage.[19,27,30] Table 3 and Table 4 show yolk index values of untreated and ozone-treated eggs during 6 weeks of storage. The yolk index (YI) of the control eggs was significantly lower than that of the ozone-treated eggs after 2 weeks of storage. Storage time had a significant effect on YI. After 6 weeks of storage, the YI values of control eggs were 0.26, while 2, 4 and 6 ppm ozone-treated eggs had the values of 0.30, 0.32 and 0.34, respectively. YI values of ozone-treated eggs at 6 weeks were different to the YI of control eggs at 4 weeks (Table 3). The YI of eggs exposed to 6 ppm ozone concentration was significantly higher than 2 and 4 ppm after 3 weeks (Table 3). The YI of eggs increased along with 2, 4 and 6 ppm ozone concentrations for both 2 and 5 min exposure times, respectively (Table 4). Furthermore, the YI of eggs exposed to the 2 min ozone concentrations (2, 4 and 6 ppm) was significantly higher than 5 min (Table 4). Application of ozone preserved the yolk quality at least 2 weeks longer than control. Goo-Hee and Kyung-Haeng[12] showed that 15 and 30 min ozone-treated eggs had higher YI values than control and 5 min treated eggs. According to Yuceer and Caner[19] Caner[24] and Bhale *et al.*,[31] inhibiting albumen liquefaction and water uptake by the yolk led to a reduced rate of water and CO_2 loss from the albumen and resulted in minimised yolk quality loss.

pH measurement

Freshly laid eggs have an albumen pH that lies between 7.6 and 8.5, and contain 1.44–2.05 mg CO_2 g^{-1} of albumen.[19,32] During storage of fresh eggs with the loss of CO_2, the egg's pH becomes more basic; and structural changes take place in the albumen due to the deterioration of albumen quality. After eggs have been laid, the pH of the albumen increases from near neutral (pH 7.0) to as high as 9.5 owing to the release of CO_2 from the breakdown of

Table 5. Effect of the ozone concentration and egg storage time on albumen pH during 6 weeks of storage

	Albumen pH						
Ozone concentration (ppm)	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
0 (control)	8.64 ± 0.02^{Aa}	8.76 ± 0.05^{ABa}	8.84 ± 0.03^{Ba}	9.03 ± 0.03^{Ca}	9.13 ± 0.02^{CDa}	9.20 ± 0.05^{DEa}	9.27 ± 0.04^{Ea}
2	8.59 ± 0.03^{Aa}	8.29 ± 0.02^{Bb}	8.31 ± 0.02^{Bb}	8.30 ± 0.04^{Bb}	8.30 ± 0.05^{Bb}	8.38 ± 0.02^{Bb}	8.59 ± 0.05^{Cb}
4	8.55 ± 0.03^{Aa}	8.16 ± 0.06^{Bc}	8.17 ± 0.02^{Bc}	8.17 ± 0.04^{Bc}	8.19 ± 0.06^{Bc}	8.21 ± 0.04^{Bc}	8.36 ± 0.09^{Cc}
6	8.49 ± 0.03^{Ab}	8.00 ± 0.06^{Ad}	7.98 ± 0.07^{Ad}	7.97 ± 0.05^{Ad}	8.01 ± 0.06^{ABd}	8.01 ± 0.09^{ABd}	8.09 ± 0.09^{Bd}

Data are expressed as mean ± standard deviation.
$^{a-d}$Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
$^{A-E}$Means in the same row with different superscript uppercase letters are significantly different ($P < 0.05$).

Table 7. Effect of egg storage time after ozone treatment (2 and 5 min) on albumen pH

	Albumen pH	
Storage time (weeks)	2 min	5 min
0	8.57 ± 0.05^{Aa}	8.53 ± 0.06^{Aa}
1	8.28 ± 0.23^{Ab}	8.22 ± 0.27^{Ab}
2	8.29 ± 0.27^{Ab}	8.21 ± 0.29^{Abc}
3	8.27 ± 0.30^{Abc}	8.24 ± 0.35^{Abc}
4	8.36 ± 0.35^{Ac}	8.28 ± 0.40^{Acd}
5	8.37 ± 0.39^{Acd}	8.27 ± 0.39^{Ad}
6	8.56 ± 0.39^{Aa}	8.41 ± 0.41^{Be}

Data are expressed as mean ± standard deviation.
[a–e] Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
[A,B] Means in the same row with different superscript uppercase letters are significantly different ($P < 0.05$).

carbonic acid in albumen, resulting in changes in the bicarbonate buffer system.[19,32,33] The escape of CO_2 through the pores of the shell leads to an increase in pH, especially in the albumen.

In our study, albumen pH increased, in control and ozone-treated eggs (Table 5, Table 6 and Table 7), but the application of ozone seemed to prevent drastic changes in albumen pH (Table 5, Table 6 and Table 7). The albumen pH values in control eggs were higher than in ozone-treated eggs during storage (Table 5 and Table 6). The albumen pHs for control eggs ranged from 8.64 to 9.27 at the end of storage (Table 5). For treated eggs, albumen pH values were stable and reached 8.49–8.09 (6 ppm), 8.55–8.36 (4 ppm), and 8.59 (2 ppm) during storage. Also, the pH of eggs exposed to ozone for 2 and 5 min was significantly different, with 6 ppm ozone being the lowest, respectively (Table 6). These findings showed that 6 ppm gaseous ozone treatments were most effective in maintaining albumen values during 6 weeks of storage. Since CO_2 is expected to act as a radical scavenger, and ozone decomposition gives rise to radical species, ozone treatment would be expected to decrease levels of CO_2 in the albumen. The sustained lower pH is probably the result of radical chain reactions on some components of albumen, possibly due to the oxidation of hydroxyl amino acids.[34]

The significantly lower albumen pH found in ozone-treated eggs during storage suggested that the ozone concentration and exposure times were effective in decreasing the rate of albumen liquefaction, thus helping to maintain albumen quality by controlling albumen pH. Although yolk pH increased during storage, the increase was lower than initial pH up to 4 weeks for all ozone concentrations.

Table 9. Effect of the ozone concentration and egg treatment time (2 and 5 min) on egg yolk pH during 6 weeks of storage

	Yolk pH	
Ozone concentration (ppm)	2 min	5 min
0 (control)	6.40 ± 0.11^{Aa}	6.41 ± 0.10^{Aa}
2	6.25 ± 0.09^{Ab}	6.20 ± 0.10^{Bb}
4	6.15 ± 0.10^{Ac}	6.12 ± 0.09^{Ac}
6	6.10 ± 0.08^{Ad}	6.07 ± 0.07^{Ad}

Data are expressed as mean ± standard deviation.
[a–d] Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
[A,B] Means in the same row with different superscript uppercase letters are significantly different ($P < 0.05$).

Yolk pH was also increased by storage time. The results agree with those of Yuceer and Caner,[19] Caner,[24] Walsh *et al.*,[35] Ahn *et al.*[36] and Scott and Silversides.[32] The pH of yolk in freshly laid eggs is generally about 6.0 and gradually increases to 6.4–6.5 during storage.[36] Table 8 and Table 9 shows yolk pH values of untreated and ozone-treated eggs during storage for 6 weeks at 24 °C. For ozone-treated eggs, pH of yolk values reached to 6.14 (6 ppm), 6.27 (4 ppm), and 6.38 (2 ppm) after the storage period. Eggs treated with 6 ppm ozone had significantly lower yolk pH values than 4 ppm and 2 ppm treated eggs after 6 weeks (Table 8). Ozone-treated eggs had significantly lower yolk pH values than control eggs. These findings showed that 6 ppm ozone treatment was effective in maintaining pH of fresh eggs during storage. Ozone-treated eggs with exposure times of 2 and 5 min were significantly different (Table 9).

Foaming properties

Foam stability is determined by measuring the loss of liquid resulting from destabilisation, i.e. leakage, measuring volume decrease or density increase with time. Foam stability reflects the water-holding capacity of the foam.[37] The stability or drainage volume of foam is influenced by the thickness of the interface, foam size distribution, interface permeability, and surface tension. This interferes with the formation of a cohesive film at the air/water interface, causing a decrease in foam stability.[38,39] The pH in the aqueous phase determines the magnitude and nature of protein charges and therefore affects the foaming properties.[37–41]

Table 10 and Table 11 show relative whipping capacity values of albumen at different time–concentrations of ozone treatments

Table 8. Effect of ozone concentration and egg storage time on egg yolk pH during 6 weeks of storage

	Yolk pH						
Ozone concentration (ppm)	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
0 (control)	6.24 ± 0.01^{Aa}	6.32 ± 0.02^{ABa}	6.37 ± 0.02^{BCa}	6.42 ± 0.02^{BCDa}	6.45 ± 0.01^{CDa}	6.48 ± 0.03^{DEa}	6.57 ± 0.08^{Ea}
2	6.23 ± 0.01^{Aa}	6.12 ± 0.05^{Bb}	6.17 ± 0.06^{Bb}	6.18 ± 0.06^{Bb}	6.21 ± 0.06^{Bb}	6.27 ± 0.08^{Bb}	6.38 ± 0.07^{Cb}
4	6.23 ± 0.02^{Aa}	6.02 ± 0.03^{Bc}	6.05 ± 0.03^{BCc}	6.08 ± 0.02^{BCc}	6.11 ± 0.02^{Cc}	6.17 ± 0.07^{Cc}	6.27 ± 0.08^{Dc}
6	6.23 ± 0.02^{Aa}	5.99 ± 0.02^{Ad}	6.02 ± 0.01^{BCc}	6.05 ± 0.01^{BCc}	6.06 ± 0.01^{BCc}	6.09 ± 0.06^{CDc}	6.14 ± 0.04^{Dd}

Data are expressed as mean ± standard deviation.
[a–d] Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
[A–D] Means in the same row with different superscript uppercase letters are significantly different ($P < 0.05$).

Table 10. Effect of ozone concentration and egg storage time on albumen RWC (relative foaming capacity) during 6 weeks of storage

Ozone concentration (ppm)	Albumen relative foaming capacity						
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
0 (control)	1352.0 ± 27.39^{Aa}	1108.3 ± 37.64^{Ba}	908.3 ± 37.64^{Ca}	800.0 ± 31.69^{Da}	670.0 ± 27.39^{Ea}	508.3 ± 37.64^{Fa}	333.3 ± 40.81^{Ga}
2	1345.0 ± 33.43^{Aa}	1175.0 ± 39.89^{Bb}	1022.2 ± 26.35^{Cb}	900.0 ± 33.33^{Db}	758.3 ± 46.87^{Eb}	633.3 ± 38.92^{Fb}	512.5 ± 48.27^{Gb}
4	1366.0 ± 38.92^{Aa}	1268.2 ± 40.45^{Bc}	1127.3 ± 60.68^{Cc}	1006.0 ± 35.03^{Dc}	850.0 ± 36.93^{Ec}	720.0 ± 48.3^{Fc}	641.7 ± 46.87^{Gc}
6	1383.3 ± 24.62^{Aa}	1340.9 ± 37.54^{Bd}	1230.0 ± 48.30^{Cd}	1118.0 ± 33.71^{Dd}	955.0 ± 28.38^{Ed}	840.9 ± 37.56^{Fd}	725.0 ± 26.1^{Gd}

Data are expressed as mean ± standard deviation.
$^{a-d}$Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
$^{A-G}$Means in the same row with different superscript uppercase letters are significantly different ($P < 0.05$).

Table 11. Effect of ozone concentration and egg treatment time (2 and 5 min) on albumen RWC (relative foaming capacity) during 6 weeks of storage

Ozone concentration (ppm)	Albumen relative foaming capacity	
	2 min	5 min
0 (control)	809.5 ± 319.6^{Aa}	812.5 ± 339.5^{Aa}
2	882.5 ± 296.5^{Ab}	923.1 ± 279.8^{Bb}
4	964.5 ± 267.6^{Ac}	1030.5 ± 254.2^{Bc}
6	1067.1 ± 240.9^{Ad}	1100.0 ± 246.3^{Bd}

Data are expressed as mean ± standard deviation.
$^{a-d}$Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
^{A,B}Means in the same row with different superscript uppercase letters are significantly different ($P < 0.05$).

Table 13. Effect of ozone concentration and egg treatment time (2 and 5 min) on whole eggs relative whipping capacity (RWC) during 6 weeks of storage

Ozone concentration (ppm)	Whole eggs relative whipping capacity	
	2 min	5 min
0 (control)	483.3 ± 186.6^{Aa}	475.0 ± 195.7^{Aa}
2	558.5 ± 176.4^{Ab}	603.8 ± 162.0^{Bb}
4	642.3 ± 158.3^{Ac}	660.0 ± 150.7^{Bc}
6	680.0 ± 144.5^{Ad}	696.3 ± 139.3^{Ad}

Data are expressed as mean ± standard deviation.
$^{a-d}$Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
^{A,B}Means in the same row with different superscript uppercase letters are significantly different ($P < 0.05$).

and changes during storage for 6 weeks at 24 °C. A significant interaction between the ozone concentration and storage time was observed for albumen foaming properties ($P < 0.05$). A decrease in RWC was observed during storage, confirming earlier results obtained by Yuceer and Caner.[19] Albumen RWC values significantly decreased from 1352 to 333.3 (control), 512.5 (2 ppm), 641.7 (4 ppm), and 725.0 (6 ppm) at the end of storage (Table 10). There were significant differences between the RWC of albumen control and ozone treatments at 2, 4, 6 ppm concentrations. The ozone treatments could improve foaming properties of eggs (Table 10). The RWC of eggs exposed to gaseous ozone for 2 and 5 min was significantly different (Table 11). Higher foam expansion indicates that more air was trapped in the foam and albumen reduces the surface tension and interfacial tension to a level sufficiently low to form the interfacial film that exceeds the critical thickness. As time passed, the films became progressively thinner and ruptured.[19,42,43] Throughout the storage period, fluid is lost by lamellar water drainage, resulting in foam collapse.[43] After 6 weeks of storage, the reductions in RWC were 72.86% (control), 56.14% (2 ppm), 43.91% (4 ppm) and 42.79% (6 ppm).

There were also significant differences in the RWC of whole eggs for control, and 2 ppm, 4 ppm and 6 ppm treatments and also in exposure times (2 and 5 min) (Table 12, Table 13 and Table 14). The ozone treatments caused maintained the stability of foam. These results clearly demonstrated that ozone treatments maintained both RWC of albumen and whole eggs (foaming properties: whipping capacity) by preventing changes in the pH during storage (Table 12, Table 13 and Table 14).

Table 12. Effect of ozone concentration and egg storage time on whole eggs relative whipping capacity (RWC) during 6 week of storage

Ozone concentration (ppm)	Whole eggs relative whipping capacity						
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
0 (control)	758.3 ± 37.6^{Aa}	675.0 ± 27.3^{Ba}	531.3 ± 25.8^{Ca}	491.7 ± 37.6^{Ca}	360.0 ± 22.3^{Da}	300.0 ± 31.6^{Da}	216.7 ± 25.8^{Ea}
2	762.5 ± 31.0^{Aa}	759.1 ± 20.2^{Ab}	695.5 ± 56.8^{Bb}	595.8 ± 39.6^{Cb}	554.5 ± 52.2^{Cb}	386.4 ± 50.4^{Db}	316.7 ± 32.5^{Eb}
4	775.0 ± 26.1^{Aa}	804.2 ± 33.4^{Ac}	760.0 ± 31.6^{ABc}	725.0 ± 26.3^{Bc}	637.5 ± 37.6^{Cc}	486.4 ± 32.3^{Dc}	387.5 ± 31.0^{Ec}
6	800.0 ± 36.9^{Aa}	831.8 ± 25.2^{Ad}	804.5 ± 26.9^{Ac}	736.4 ± 23.3^{Bc}	681.8 ± 25.3^{Cc}	541.7 ± 28.8^{Dd}	445.08 ± 33.4^{Ed}

Data are expressed as mean ± standard deviation.
$^{a-d}$Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
$^{A-E}$Means in the same row with different superscript uppercase letters are significantly different ($P < 0.05$).

Table 14. Effect of egg storage time, after ozone treatment for 2 and 5 min, on whole eggs relative whipping capacity (RWC) during 6 weeks of storage

	Whole eggs relative whipping capacity	
Storage time (weeks)	2 min	5 min
0	771.4 ± 37.3^{Aa}	780.0 ± 33.4^{Aa}
1	778.6 ± 53.7^{Aa}	782.6 ± 65.8^{Aa}
2	697.4 ± 94.9^{Ab}	739.5 ± 100.8^{Ab}
3	647.5 ± 95.2^{Ac}	657.9 ± 101.7^{Ab}
4	563.2 ± 110.3^{Ad}	617.5 ± 102.9^{Ad}
5	425.5 ± 89.5^{Ae}	467.5 ± 96.3^{Ae}
6	347.6 ± 79.8^{Af}	371.4 ± 85.9^{Af}

Data are expressed as mean ± standard deviation.
$^{a-f}$Means in the same column with different superscript lowercase letters are significantly different ($P < 0.05$).
^{A,B}Means in the same row with different superscript uppercase letters are significantly different ($P < 0.05$).

Albumen viscosity

Viscosity of the albumen affects the functional properties of eggs during whipping and emulsifying.[44–46] Thick albumen progressively liquefies and thins with time, transforming itself into thin albumen due to the changes in the complex lysozyme–ovomucin. Any adverse effects on the viscosity of albumen affect these properties and would make eggs unsuitable for industrial use.[37,44,47] Albumen viscosity may be used as a tool for assessing egg quality, in that the gelatinous structure of thick albumen changes physical and chemical characteristics and gradually breaks down into a clear liquid losing its consistency during storage. Albumen viscosity is also a potential tool for the assessment of egg quality. The viscosity of albumen values reported by others varied between 10 and a few hundred mPa s.[38,48] Variation between measurements is perhaps caused by discrepancies in sample preparation and measurement protocol. Albumen, a pseudoplastic fluid, is a thixotropic material[42] and its viscosity depends on the shear force. The viscosity of the albumen decreased during storage (Table 15) confirming earlier results obtained by Kannan *et al.*[44] and Kemps *et al.*[37]

The statistical analyses showed that interactions of (Week*Concentration*Time) ($P < 0.05$) were important. The albumen viscosity for control eggs ranged from 67.04 initially to 5.08 at the end of storage (Table 15). For treated eggs, albumen viscosity values decreased to 6.78 and 6.04 (6 ppm), 6.99 and 6.43 (4 ppm), and 7.64 and 6.78 (2 ppm). The albumen viscosity depends on the ovomucin–lysozyme complex.[47] When lysozyme is present in the complex, it becomes stronger and its destabilisation changes due to pH increase during storage.[37,38,42,47,48] The liquefaction of albumen occurs because of the increasing of pH. It is influenced by the ovomucin–lysozyme complex, which results in changes in viscosity of the albumen during storage.[38,42,48] It is possible that the ozone treatments minimise changes in carbohydrate and protein moieties involved in formation of ovomucin complex, resulting in a loss of gel-like structure during storage and minimise changes in pH and maintained albumen quality.[27]

Eggshell breaking strength

Eggshell quality plays an important role for commercial handling and storage. Shell quality declines, as the hens become older. The eggshell should be as strong as possible to maximise the number of eggs reaching the consumer.[19,49] Any negative impact on shell

Table 15. Effect of the ozone treatment (for 2 and 5 min) on albumen viscosity (20 s) during 6 weeks of storage

Ozone concentration (ppm)	Albumen viscosity (cp)													
	0 week		1 week		2 weeks		3 weeks		4 weeks		5 weeks		6 weeks	
	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min
0 (control)	66.15 ± 0.63^{Aal}	67.04 ± 0.50^{Aal}	62.00 ± 0.70^{Bal}	61.43 ± 0.68^{Bal}	$52.15 \pm 0.49^{Cal,II}$	51.43 ± 0.32^{Cal}	40.75 ± 0.91^{Dal}	41.01 ± 0.76^{Dal}	28.20 ± 0.65^{Eal}	28.54 ± 0.67^{Eal}	13.02 ± 0.42^{Fal}	13.14 ± 0.43^{Fal}	5.24 ± 0.42^{Gal}	5.08 ± 0.44^{Gal}
2	68.37 ± 0.66^{Aal}	68.92 ± 0.45^{Aal}	61.58 ± 0.47^{Bal}	61.25 ± 0.66^{Bal}	50.85 ± 0.54^{Cal}	$53.40 \pm 0.60^{Ca,II}$	$42.80 \pm 0.52^{Da,II}$	$42.52 \pm 0.66^{Da,II}$	31.88 ± 0.55^{Eal}	32.20 ± 0.49^{Eal}	15.69 ± 0.42^{Fal}	14.55 ± 0.56^{Fal}	7.64 ± 0.96^{Gal}	6.78 ± 0.95^{Gal}
4	69.15 ± 0.57^{Aal}	66.81 ± 0.55^{Aal}	59.78 ± 0.43^{Bal}	60.67 ± 0.58^{Bal}	$49.96 \pm 0.98^{Ca,II}$	$49.78 \pm 0.50^{Cb,II}$	$43.59 \pm 0.49^{Da,II}$	$42.61 \pm 0.40^{Da,II}$	32.29 ± 0.73^{Eal}	31.96 ± 0.50^{Eal}	14.83 ± 0.39^{Fal}	14.68 ± 0.32^{Fal}	6.99 ± 0.66^{Gal}	6.43 ± 0.47^{Fal}
6	67.28 ± 0.53^{Aal}	68.83 ± 0.71^{Abl}	68.69 ± 0.87^{Aal}	69.74 ± 0.67^{Aal}	53.69 ± 0.40^{Bal}	$55.22 \pm 0.47^{Ba,II}$	$43.93 \pm 0.47^{Ca,II}$	$46.34 \pm 0.53^{Ca,II}$	$30.20 \pm 0.70^{Da,II}$	$33.81 \pm 0.47^{Db,II}$	15.10 ± 0.45^{Eal}	14.73 ± 0.36^{Eal}	6.78 ± 0.49^{Fal}	$6.04 \pm 0.38^{Db,II}$

Data are expressed as mean ± standard deviation.
$^{A-G}$Different superscript uppercase letters donate significant differences between storage times in same treatment and time ($P < 0.05$).
$^{a-b}$Different superscript lowercase letters donate significant differences between treatment times in same treatment and storage time ($P < 0.05$).
$^{I-IV}$Different superscript roman numbers donate significant differences between treatments in same treatment time and storage time ($P < 0.05$).

Table 16. Effect of ozone concentration, egg storage time and treatment time on eggshell breaking strength (kg_f) at both the top and bottom during 5 weeks of storage

Parameter	Eggshell breaking strength (kg_f)	
	At top	At bottom
Ozone concentration (ppm)		
0 (control)	4.13 ± 0.20^{A}	4.11 ± 0.23^{A}
2	3.72 ± 0.19^{B}	3.66 ± 0.24^{B}
4	3.35 ± 0.18^{C}	3.49 ± 0.28^{C}
6	3.00 ± 0.17^{D}	3.31 ± 0.27^{D}
Storage time (weeks)		
0	3.63 ± 0.48^{A}	3.74 ± 0.39^{A}
1	3.59 ± 0.42^{A}	3.68 ± 0.34^{AB}
2	3.49 ± 0.40^{B}	3.62 ± 0.37^{ABC}
3	3.42 ± 0.39^{BC}	3.532 ± 0.34^{BC}
4	3.40 ± 0.38^{BC}	3.47 ± 0.36^{C}
5	3.32 ± 0.39^{C}	3.46 ± 0.30^{C}
Treatment time (min)		
2	–	3.62 ± 0.35^{A}
5	–	3.53 ± 0.37^{B}

Data are expressed as mean ± standard deviation.
$^{A-D}$Means in the same column, for the same parameter, with different capital letters are significantly different ($P < 0.05$).

strength will result in significant eggs lost. Average breaking strength of the eggs decreased from 4.13 to 3.74 kg by 6 weeks storage. Ozone treatments decreased breaking and puncture strength (Table 16). Statistical analyses showed that interactions of factors (Week*Concentration*Time) were not significant. However, the main effects of storage time and ozone concentration were statistically significant (Table 16). The shell strength of eggs depends on storage time, ozone concentration and treatment time. The puncture strength of eggshells exposed to ozone concentrations of 2, 4, 6 ppm was significantly lower than control. Control eggs exhibited the highest puncture strength among treatments (Table 16). Proteins are the major constituents of organic matters in eggshell membranes. Lipids and carbohydrates are other organic materials with small amounts.[50] In our study, it is possible that organic materials on the shell were attacked by ozone and the eggshell's protective cover was damaged during the oxidation process,[51] because, carbon–carbon double bonds, sulfur and nitrogen atoms in the amino acid chains of egg shells are the target points for ozone.[52] In addition, ozone might react quickly with polysaccharides and lipids leading to breakage of glycosidic bonds in the shell membrane.[8]

These results clearly show the effect of ozone on eggshell properties and they agree with the those relating to ozone reduced shell strength obtained by Kampf *et al.*[39]

CONCLUSION

We can conclude from the present study that gaseous ozone concentration between 4 ppm and 6 ppm could be used to maintain the interior quality of eggs and extend the shelf life of fresh eggs. The exposure (5 min) of eggs to 6 ppm ozone concentration had a negative impact on egg quality during 6 weeks storage. The high concentration of gaseous ozone (6 ppm) and higher application times present detrimental effects on egg shell and quality during storage. There were significant increases in albumen height, HU, and yolk height of the eggs depending on the time and ozone concentration. Ozone could be a viable alternative for maintaining the functional properties (HU, YI, pH, viscosity, and RWC) and extending the shelf life during long-term storage of eggs. These parameters are also used to determine albumen quality and could be an index for egg freshness.

Gaseous ozone has a potential as an emerging technology to maintain fresh egg quality with functional properties and also extend the shelf life during long-term storage at room temperature. Further research should be conducted using different treatment times and different concentrations on various foods. Ozone applications, together with thermal and non-thermal treatments, also need to be investigated.

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