Research Note

Modified-atmosphere packaging of hen table eggs: Effects on pathogen and spoilage bacteria

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ABSTRACT As part of a more comprehensive research activity on the use of modified-atmosphere packaging for the improvement of quality and functional properties of table eggs, the effects of air, 100% CO_2, and 100% O_2 packaging were also evaluated on the survival of experimentally inoculated pathogen bacteria (*Salmonella* Enteritidis, *Escherichia coli*, and *Listeria monocytogenes*) as well as on spoilage bacteria (total aerobic mesophilic bacteria) on table eggs during 30 d of storage at 4, 25, and 37°C by colony count method. In general, temperatures played a major role, rather than gasses, in influencing the bacterial survival. In particular, the lowest microbial loads were registered at 4°C on *E. coli* and spoilage bacteria, whereas 37°C was the best storage temperature to avoid the psychrotropic microorganism *L. monocytogenes* development regardless of the gas used. One hundred percent CO_2 packaging, in association with a low storage temperature (4°C), had a significant positive effect in reducing *Salmonella* loads. On eggs inoculated with *L. monocytogenes* and stored at 4°C as well as on eggs containing only spoilage bacteria and stored at 25°C, 100% CO_2 resulted the best gas in comparison with air and O_2. One hundred percent CO_2 packaging showed no negative effect on pathogen survival compared with air. Although further improvements are required to control RH within packaging to limit bacteria growth/survival, in view of the positive effects of CO_2 packaging on quality traits of table eggs, 100% CO_2 packaging might represent a promising innovative technique for the maintenance of egg characteristics during transport, retail, and domestic storage.

Key words: table egg, modified-atmosphere packaging, pathogen bacteria, spoilage bacteria

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INTRODUCTION

In the current year 2012, Council Directive 1999/74/EC, defining minimum standards for the welfare of laying hens, is banning conventional cage systems in favor of enriched cages or alternative systems. However, keeping hens on the floor or outdoor could present an increased risk of bacterial contamination (EFSA, 2005). Differently from what happens in United States, where table eggs are washed, oiled, and stored at refrigerated temperatures, in the European Union no treatments are currently allowed on table eggs and room temperature is the only storage condition permitted over the table egg shelf-life of 28 d. In this regard, the introduction of efficient measures to reduce eggshell contamination by *Salmonella* Enteritidis or other bacterial pathogens, and thus to prevent any potential or additional food safety risk for human health, may be envisaged.

Modified-atmosphere packaging (**MAP**) is a widely used food preservation technique, which extends the shelf-life of foods by inhibiting chemical, enzymatic, and microbial spoilage. Traditionally used gases are CO_2, O_2, N_2, or different combinations of them (Rajkovic et al., 2010). Carbon dioxide is chemically reactive and presents a high solubility in water and fat. It is typically used to prevent aerobic spoilage. Nitrogen is an inert tasteless gas with low solubility in water, which is often used as a filler gas. Oxygen atmospheres are primarily used for fresh red meats to maintain the desirable color.

Active MAP can be combined with water absorbers to modify the in-package RH and to improve the positive effects of packaging gases on the quality and safety maintenance of the product (Villaescusa and Gil, 2003).

On fresh eggs, high CO_2 atmosphere packaging has a documented positive effect both on the quality maintenance of the product and on the technological properties of the egg constituents (Moran, 1937; Cotterill and Gardner, 1956; Rocculi et al., 2009, 2011). In particular, the 100% CO_2 packaging reduced the Haugh unit decrease and the pH increase during egg storage

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(Rocculi et al., 2009). These findings were associated to a statistically higher foam stability of the albumen (Rocculi et al., 2011).

From a microbiological point of view, the positive effect of MAP on the inhibition of spoilage bacteria growth has been widely documented in several food products (Genigeorgis, 1985; Hintlian and Hotchkiss, 1987; Wimpfheimer et al., 1990; Faber, 1991; Rajkovic et al., 2010). However, no data are available on table eggs.

The effect of MAP on pathogenic organisms is more complex. *Salmonella* Enteritidis survival on sterile graded carrots was substantially maintained at 4°C both under aerobic storage and low CO_2 and O_2 atmosphere (Tassou and Boziaris, 2002). In contrast, in a similar atmosphere the death rate of *Salmonella* Enteritidis inoculated on cherry tomatoes was faster than in samples stored in air both at 7 and 22°C (Daş et al., 2006). Positive gas-dependent effects on bacterial growth inhibition were observed on meat. Michaelsen et al. (2006) observed, after 35 d of storage, a 2-log reduction on both the *Salmonella* Typhimurium and *L. monocytogenes* loads on artificially inoculated pork chops stored at 10°C and packed in high CO_2 atmosphere in comparison with vacuum packed samples. A positive effect was registered also on poultry carcasses, on which a statistically significant reduction of natural contaminating psychrophiles was registered on 100% CO_2 packed poultry carcasses, whereas 100% O_2 showed a positive effect in reducing the *Campylobacter* load (Byrd et al., 2011).

This research is a part of a more comprehensive research activity on the effect of MAP on table eggs. In particular, the positive effects of MAP on quality indices of table eggs as well as on functional properties of egg constituents were documented in 2 previous published papers, which suggested the use of 100% CO_2 packaging for table eggs intended for albumen-based food production (i.e., meringue preparation; Rocculi et al., 2009, 2011). To assess possible side effects of MAP from a microbiological point of view, in the present study the effects of air, 100% CO_2, and 100% O_2 packaging were evaluated on the survival of experimentally inoculated pathogen bacteria (*Salmonella* Enteritidis, *Escherichia coli*, and *Listeria monocytogenes*) as well as on spoilage bacteria (aerobic mesophilic bacteria) on table eggs during 30 d of storage at 4, 25, and 37°C.

MATERIALS AND METHODS

Eggs and MAP Apparatus

Eggs were obtained from a flock of 32-wk-old Hy-Line brown hens reared in conventional cages. The average weight of eggs was 68.05 (±4.23) g. Eggs were packed as previously described by Rocculi et al. (2009). Briefly, 720 eggs were placed on 144 plastic supports (Celplast srl., Padova, Italy). Each support enclosing 5 eggs was packed in a high barrier multilayer pouch (Reber snc, Reggio Emilia, Italy), which was filled with gas using a quaternary mixer model KM100–4 (Witt-Gasetechnik, Witten, Germany) and a gas flushing welding machine model Multiple 315 (Orved srl, Venezia, Italy).

Due to moisture release from egg constituents through the eggshell, the RH in high barrier packages dramatically increases and the contemporaneous water condensation on eggshell surface can strongly favor the growth of both pathogen and spoilage bacteria. To reduce this side effect, moisture absorbers were included in each packaging. The absorbent amount has been chosen as the one leading to an increase of the weight loss of packed eggs associated with an aerobic mesophilic bacteria load similar to the one of nonpacked eggs. Before MAP, in each package, 5 porous paper pouches (one for each egg) containing 75 g of silica gel each have been inserted as water absorbers.

The water absorber was selected in a preliminary experiment where the weight loss of the product [g/100 g of initial fresh weight (**FW**), g/100 g of FW] and the aerobic mesophilic bacteria load were monitored on nonpacked eggs, as well as on eggs packed in air with and without silica gel, after 1, 10, 21, and 30 d of storage at 25°C. Twenty egg packages of 5 eggs each (5 packages per each sampling time) were prepared and analyzed.

Microbiological Tests

Inoculum Preparation. The inocula for surface contamination of table eggs were prepared from broth cultures of *Salmonella* Enteritidis (MB2509 strain streptomycin resistant) in Brain Heart Infusion broth (BHI, Oxoid, Milan, Italy) supplemented with 25 mg/kg of streptomycin (Sigma, Milan, Italy), *Escherichia coli* (*E. coli* mutant 10, *E. coli* ATCC 25922 induced to nalidixic acid resistance) in BHI supplemented with 20 mg/kg of nalidixic acid (Sigma) and *L. monocytogenes* ATCC 13932 in Listeria Enrichment Broth Base (Oxoid) with added Listeria Selective Enrichment Supplement (Oxoid). Two aliquots (50 mL) of each broth culture were centrifuged at 3,000 × *g* for 10 min. Cell pellets were suspended in sterile physiological saline. These working suspensions had an optical density at 600 nm of approximately 0.4 for *Salmonella* Enteritidis, 0.5 for *E. coli* and 0.9 for *L. monocytogenes*, all corresponding to approximately 10^8 cfu/mL confirmed by colony count.

Experimental Inoculation of Shell Eggs. For experimental infection, each egg was washed with distilled deionized water (22 to 25°C), and then sanitized by dipping it in ethanol (70%, vol/vol) for 30 min as described by Hammack et al. (1993). Sanitized shell eggs were aseptically dried at room temperature for approximately 40 min before inoculation. Dried, sanitized shell eggs were dipped for 10 s into the bacterial working suspension. Experimentally inoculated shell eggs were aseptically dried at room temperature for approximately 1 h before packaging.

Table 1. Weight loss [g/100 g of initial fresh weight (FW)] of nonpacked eggs (control), eggs packed in high-barrier multilayer pouches (pack), and eggs packed in high-barrier multilayer pouches with silica gel (75 g for each egg; Pack-Abs) for water regulation in the package headspace during 30 d of storage at 25°C

Time (d)	Control (g/100 g of FW)	Pack (g/100 g of FW)	Pack-Abs (g/100 g of FW)
0	0	0	0
1	$0.32 \pm 0.05^{b,A}$	$0.11 \pm 0.01^{a,A}$	$0.25 \pm 0.03^{b,A}$
10	$2.47 \pm 0.16^{c,B}$	$0.26 \pm 0.03^{a,B}$	$0.60 \pm 0.09^{b,B}$
21	$5.02 \pm 0.56^{c,C}$	$0.44 \pm 0.09^{a,C}$	$1.14 \pm 0.13^{b,C}$
30	$7.18 \pm 1.18^{c,D}$	$0.57 \pm 0.09^{a,C}$	$1.39 \pm 0.29^{b,C}$

[a–c]Mean values ± SD (n = 5) within a row with different superscripts differ significantly ($P < 0.05$).

[A–D]Mean values ± SD (n = 5) within a column with different superscripts differ significantly ($P < 0.05$).

Quantification of Eggshell Contamination. The contamination of eggshells was assessed at 1, 10, 21, and 30 d of storage at different temperatures (4°C, 25°C, 37°C) and packing gases (CO_2, O_2, and air) using 5 egg replicates for each combination of storage duration, storage temperature, gas and pathogen/aerobic mesophilic bacteria. The initial eggshell bacterial load was assessed on 5 eggs replicates at d 0 before packaging. Five sanitized not inoculated packed eggs per day, stored at 25°C, were used as negative control. Each egg was broken, the inner content discarded, and the eggshell used for the enumeration of bacteria. In particular, each weighed eggshell was diluted with 9 vol of physiological saline (0.9% NaCl). Viable cells on eggshells were enumerated by 1:10 serial dilutions in physiological saline followed by plating 100 μL of each dilution on the appropriate agar medium in duplicate: Brilliant Green Agar (BGA; Oxoid) supplemented with 25 mg/kg of streptomycin (Sigma; *Salmonella* enumeration), McConkey Agar (Oxoid) supplemented with 20 mg/kg of nalidixic acid (Sigma; *E. coli* enumeration), *Listeria* selective Agar Base (*Listeria* enumeration; Oxoid), and Plate Count Agar (PCA; Oxoid; aerobic mesophilic bacteria enumeration). Plates were incubated as follows: 37°C for 24 h, BGA and McConkey agar; 37°C for 48 h, *Listeria* selective Agar Base; 30°C for 36 h, aerobic mesophilic bacteria. In case of 0 to 4 colonies, the value of 0 cfu/g of eggshell was assigned. To allow a log-transformation, counts were given of 1 cfu/g of eggshell upon observing no colonies. The corresponding detection limit was 10^2 cfu/g of eggshell.

Statistical Analysis

The significance of the effects of the different MAP, and temperatures was evaluated by factorial ANOVA statistics followed by Fisher post hoc test for statistical comparison of log means of bacterial recovery on eggshells. The differences with $P \leq 0.05$ were considered statistically significant.

RESULTS

Weight loss results of nonpacked (control) and packed eggs with and without (pack) water absorbers in air are reported in Table 1. Egg enclosure in multilayer high barrier pouches caused a strong weight loss reduction of the product (pack eggs: 0.6 g/100 g of FW: 7.20 g/100 g of FW at d 30) as well as a higher aerobic mesophilic bacteria load (pack eggs: 5.5 $\log_{10}$ cfu/g of eggshell vs. control: 2.1 ± 0.5 $\log_{10}$ cfu/g of eggshell, unpublished data). The use of water absorber (75 g of silica gel for each egg) increased the egg weight loss of about 3 times, and it was sufficient to obtain a strong decrease of the microbial load down to 3.0 ± 1.2 $\log_{10}$ cfu/g of eggshell comparable with the load on nonpacked eggs (unpublished data).

The results on the survival of pathogen as well as spoilage bacteria on table eggs during 30 d of storage at 4, 25, and 37°C are reported in Tables 2 to 5.

During storage, the load of *Salmonella* Enteritidis significantly decreased on air-packed eggs stored at 4 and 37°C, whereas it was maintained in all the other conditions (air 25°C, 100% CO_2, and 100% O_2). At 30 d of storage, eggs packed with 100% CO_2 and stored at 4°C showed a *Salmonella* load up to 2 log lower than those stored at 25 or 37°C (3.9 $\log_{10}$ cfu/g of eggshell at 4°C vs. 5.9 $\log_{10}$ cfu/g of eggshell at 25°C vs. 5.7 $\log_{10}$ cfu/g of eggshell at 37°C; $P < 0.05$; Table 2).

The loads of *E. coli* significantly decreased on air and 100% O_2 packed eggs during storage at 4°C, whereas they were maintained or increased in all the other conditions. Again the best condition of storage was 4°C corresponding to the lowest *E. coli* load (Table 3).

The loads of *L. monocytogenes* in air and 100% O_2 packed eggs were maintained during storage at 4°C, whereas they decreased in all the other conditions. The best temperature corresponding to the lowest *L. monocytogenes* load from d 10 in all 3 different conditions was 37°C. This result is in line with the psychrotropic nature of this pathogen. From the d 21 at 4°C, 100% CO_2 packed eggs had a *L. monocytogenes* load up to 2 log lower than air-packed eggs stored at the same temperature (3.6 $\log_{10}$ cfu/g of eggshell, 100% CO_2 at 4°C vs. 5.3 $\log_{10}$ cfu/g of eggshell air at 4°C; $P < 0.05$; Table 4).

During storage, the load of spoilage bacteria increased at 25°C in all 3 types of gas packaging, whereas it was maintained at 4 and 37°C. Indeed, on air packaging at d 21, the load at 25°C was around 2 log higher than the load recorded at 4°C and 1 log higher than the one at 37°C (4.4 $\log_{10}$ cfu/g vs. 2.2 $\log_{10}$ cfu/g and 3.2 $\log_{10}$ cfu/g; $P < 0.05$). A significant lower load was registered from d 10 onward on the eggshells of 100% CO_2 packed eggs stored at 25°C. In detail, at d 30 the spoilage bacteria load on 100% CO_2 packed eggs was 2.6 $\log_{10}$ cfu/g compared with 3.7 $\log_{10}$ cfu/g of air-packed eggs and 3.4 $\log_{10}$ cfu/g of 100% O_2 packed eggs stored at the same temperature ($P < 0.05$; Table 5).

Table 2. *Salmonella* Enteritidis enumeration ($\log_{10}$ cfu/g of eggshell ± SD) on packed table eggs during 30 d of storage at 4°C, 25°C, and 37°C

Time (d)	Air			100% CO_2			100% O_2		
	4°C	25°C	37°C	4°C	25°C	37°C	4°C	25°C	37°C
0					5.2 ± 0.3				
1	5.2 ± 0.1^{abc,C}	5.8 ± 0.4^{c,AB}	5.6 ± 0.4^{bc,CD}	4.5 ± 1.3^{a,A}	5.3 ± 0.2^{abc,AB}	5.6 ± 0.5^{bc,A}	4.8 ± 0.8^{ab,A}	5.0 ± 0.7^{abc,A}	5.3 ± 0.6^{bc,AB}
10	5.2 ± 0.3^{bc,C}	6.3 ± 0.5^{d,AB}	5.5 ± 0.6^{bc,CD}	4.8 ± 0.7^{b,A}	5.4 ± 1.1^{bcd,AB}	5.6 ± 1.0^{bcd,A}	5.0 ± 0.6^{bc,A}	5.9 ± 0.9^{cd,A}	5.1 ± 1.1^{b,cAB}
21	4.9 ± 0.3^{abc,BC}	6.2 ± 0.4^{d,AB}	5.1 ± 0.7^{abcd,BC}	4.8 ± 0.4^{abc,A}	4.9 ± 0.9^{abc,A}	5.7 ± 1.5^{bcd,A}	4.5 ± 1.0^{ab,A}	6.0 ± 0.9^{cd,A}	4.3 ± 1.4^{a,A}
30	4.5 ± 0.7^{abc,AB}	5.7 ± 0.4^{c,A}	4.0 ± 0.7^{ab,B}	3.9 ± 1.1^{a,A}	5.9 ± 1.6,^{cB}	5.7 ± 1.9^{c,A}	4.4 ± 0.5^{abc,A}	5.6 ± 1.8^{bc,A}	4.5 ± 1.7^{abc,A}

$^{a–d}$Mean values ± SD (n = 5) within a row with different superscripts differ significantly ($P < 0.05$).

$^{A–D}$Mean values ± SD (n = 5) within a column with different superscripts differ significantly ($P < 0.05$).

Table 3. *Escherichia coli* enumeration ($\log_{10}$ cfu/g of eggshell ± SD) on packed table eggs during 30 d of storage at 4°C, 25°C, and 37°C

Time (d)	Air			100% CO_2			100% O_2		
	4°C	25°C	37°C	4°C	25°C	37°C	4°C	25°C	37°C
0					4.0 ± 0.7				
1	4.8 ± 0.7^{bC}	5.4 ± 0.3^{bA}	5.3 ± 0.3^{bA}	3.0 ± 1.8^{aA}	4.9 ± 0.5^{bA}	5.3 ± 0.4^{bA}	5.1 ± 0.3^{bD}	5.0 ± 1.4^{bA}	5.5 ± 0.9^{bA}
10	4.7 ± 0.2^{aC}	5.7 ± 1.4^{abcAB}	6.1 ± 1.3^{bcA}	4.7 ± 0.3^{aB}	6.5 ± 0.5^{cdB}	7.3 ± 0.1^{dB}	5.3 ± 0.1^{abD}	5.6 ± 0.6^{abcA}	5.6 ± 1.7^{abcA}
21	4.4 ± 0.2^{abBC}	5.8 ± 1.0^{bcAB}	6.1 ± 1.9^{cdeA}	3.9 ± 1.0^{aAB}	7.3 ± 0.6^{deC}	7.6 ± 0.2^{eB}	3.7 ± 0.3^{aC}	4.7 ± 1.2^{abcA}	6.1 ± 2.0^{cdA}
30	3.9 ± 0.9^{aB}	6.6 ± 0.8^{bcB}	7.9 ± 0.2^{dB}	3.7 ± 0.7^{aAB}	6.4 ± 0.4^{bB}	7.5 ± 0.1^{cdB}	3.2 ± 0.7^{aB}	6.9 ± 0.3^{bB}	6.1 ± 1.4^{bcA}

$^{a–e}$Mean values ± SD (n = 5) within a row with different superscripts differ significantly ($P < 0.05$).

$^{A–D}$Mean values ± SD (n = 5) within a column with different superscripts differ significantly ($P < 0.05$).

Table 4. *Listeria monocytogenes* enumeration ($\log_{10}$ cfu/g of eggshell ± SD) on packed table eggs during 30 d of storage at 4°C, 25°C, and 37°C

Time (d)	Air			100% CO_2			100% O_2		
	4°C	25°C	37°C	4°C	25°C	37°C	4°C	25°C	37°C
0					5.1 ± 0.3				
1	5.3 ± 0.5^{a,A}	5.6 ± 0.6^{ab,B}	5.9 ± 0.2^{ab,C}	5.4 ± 0.4^{a,C}	6.0 ± 0.4^{b,B}	5.7 ± 0.4^{ab,C}	5.7 ± 0.4^{ab,B}	5.3 ± 0.7^{a,C}	6.1 ± 0.7^{b,C}
10	5.5 ± 0.3^{c,A}	5.0 ± 0.7^{b,B}	2.7 ± 0.1^{a,B}	4.9 ± 0.4^{b,BC}	5.2 ± 0.6^{bc,AB}	2.7 ± 0.1^{a,B}	4.9 ± 0.3^{b,AB}	4.9 ± 0.6^{b,C}	2.7 ± 0.1^{a,B}
21	5.3 ± 0.8^{f,A}	3.8 ± 1.0^{cde,A}	3.1 ± 0.6^{abc,B}	3.6 ± 0.8^{bcd,A}	4.9 ± 0.4^{f,AB}	2.8 ± 0.1^{ab,B}	4.6 ± 1.1^{ef,AB}	4.5 ± 0.5^{def,BC}	2.7 ± 0.1^{a,B}
30	5.5 ± 0.1^{c,A}	3.7 ± 0.1^{b,A}	1.7 ± 0.1^{a,A}	3.8 ± 0.5^{b,A}	4.1 ± 1.6^{b,A}	1.7 ± 0.1^{a,A}	5.1 ± 0.7^{c,AB}	3.4 ± 0.7^{b,A}	1.7 ± 0.1^{a,A}

$^{a–f}$Mean values ± SD (n = 5) within a row with different superscripts differ significantly ($P < 0.05$).

$^{A–C}$Mean values ± SD (n = 5) within a column with different superscripts differ significantly ($P < 0.05$).

Table 5. Total aerobic mesophilic bacteria enumeration ($\log_{10}$ cfu/g of eggshell ± SD) on packed table eggs during 30 d of storage at 4°C, 25°C, and 37°C

Time (d)	Air			100% CO_2			100% O_2		
	4°C	25°C	37°C	4°C	25°C	37°C	4°C	25°C	37°C
0					3.6 ± 1.4				
1	2.5 ± 0.7^{b,B}	1.9 ± 0.17^{a,bA}	1.8 ± 0.17^{a,A}	2.4 ± 0.9^{ab,AB}	2.2 ± 0.7^{ab,A}	1.7 ± 0.1^{a,A}	2.2 ± 0.7^{ab,AB}	2.1 ± 0.6^{ab,A}	1.7 ± 0.1^{a,A}
10	1.7 ± 0.1^{a,A}	4.3 ± 0.3^{d,C}	2.3 ± 0.7^{ab,A}	1.8 ± 1.0^{a,A}	3.5 ± 0.7^{c,C}	1.7 ± 0.1^{a,A}	2.1 ± 0.7^{a,AB}	3.5 ± 0.7^{c,B}	2.9 ± 0.1^{bc,B}
21	2.2 ± 0.5^{a,AB}	4.4 ± 0.3^{c,C}	3.2 ± 0.4^{b,B}	1.7 ± 0.1^{a,A}	3.1 ± 1.0^{b,BC}	2.0 ± 0.4^{a,A}	2.2 ± 0.6^{a,AB}	3.9 ± 0.3^{c,B}	2.9 ± 0.3^{b,B}
30	1.9 ± 0.5^{a,AB}	3.7 ± 0.3^{c,B}	1.9 ± 0.2^{a,A}	2.2 ± 0.3^{ab,AB}	2.6 ± 0.4^{b,ABC}	1.8 ± 0.1^{a,A}	1.9 ± 0.5^{a,A}	3.4 ± 0.4^{c,B}	1.9 ± 0.5^{a,A}

$^{a–d}$Mean values ± SD (n = 5) within a row with different superscripts differ significantly ($P < 0.05$).

$^{A–C}$Mean values ± SD (n = 5) within a column with different superscripts differ significantly ($P < 0.05$).

DISCUSSION

On table eggs, positive effects of 100% CO_2 packaging on quality indices as well as on functional properties of egg constituents were documented (Rocculi et al., 2009, 2011). In the present study, the effect of MAP on table eggs was investigated from a microbiological point of view. In particular, the aim of this study was to evaluate the effect of 100% CO_2 and 100% O_2 modified atmospheres in comparison with air on survival of pathogen and spoilage bacteria on packed eggs stored till one month at 3 different temperatures (4, 25, and 37°C).

The initial level of spoilage bacteria (aerobic mesophilic bacteria) of table eggs in this study (3.6 $\log_{10}$ cfu/g of eggshell corresponding to approximately 4.5 $\log_{10}$ cfu/eggshell for an average eggshell weight of 8 g) was comparable with bacterial eggshell contaminations reported in previous studies (10^4 to 10^5 cfu/eggshell; EFSA, 2005; De Reu et al., 2005).

In the present study, a lower decrease rate was observed in air packed eggs during storage at 25°C in comparison with nonpacked and nontreated table eggs inoculated with the same pathogens and stored over 1 mo at room temperature (Manfreda et al., 2010; Pasquali et al., 2010). In this study, a *Salmonella* survival with a constant load was observed on packed eggs, whereas 1 log reduction of the *Salmonella* load was observed in nonpacked ones (Pasquali et al., 2010). The *E. coli* and *L. monocytogenes* loads on nonpacked eggs showed a fast decrease reaching the detection limit concentration after 15 and 10 d of storage, respectively (Manfreda et al., 2010), whereas in the present study *E. coli* grew with a 1 log increase and *L. monocytogenes* survived on packed eggs. Although in this study moisture absorbers have been included, these results suggest that the control of RH within each package should be further improved.

A statistically significant temperature-dependent effect was observed in packed eggs. In particular, the lowest microbial loads were registered at 4°C on *E. coli* and spoilage bacteria, whereas 37°C was the best storage temperature for the psychrotropic microorganism *L. monocytogenes* regardless of the gas used. On *Salmonella* inoculated table eggs, the positive effect of storage at 4°C was registered in association with 100% CO_2 packaging. The bacterial survival on food packed in modified atmospheres is strictly linked to the temperature of storage with chilling temperatures described as the most favorable from a microbiological point of view. Hulánková et al. (2010) found a substantial maintenance of the initial load of *Salmonella* Enteritidis PT8 experimentally inoculated on the surface of chilled chicken legs stored at 3°C and packed in both high CO_2 and O_2 atmospheres for 14 d. Similarly, decreases of approximately 1.5 and of 4 $\log_{10}$ cfu/g were observed on fresh beef meat and cherry tomatoes stored at chill temperatures and inoculated with *Salmonella* Typhimurium and *Salmonella* Enteritidis, respectively, and packed in high and low CO_2 atmosphere, respectively (Skandamis et al., 2002; Daş et al., 2006). However, due to possible water condensation on the surface of table egg, caused by temperature fluctuations during storage and transport, currently in Europe the recommended egg storage temperature, from farm to retail, is room temperature.

Statistically significant gas-temperature dependent effects were also registered in this study. In particular in comparison with air and 100% O_2, a positive effect of 100% CO_2 was observed at 4°C in eggs inoculated with the psychrotropic microorganism *L. monocytogenes* and at 25°C in eggs containing spoilage bacteria. These findings confirm previous results of positive effects of 100% CO_2 on psychrophiles and total aerobes in poultry carcasses (Byrd et al., 2011). On table eggs, further studies are required to confirm the positive effect of 100% CO_2 on both pychrotrophic and psychrophil component of spoilage bacteria as well as to confirm positive effect on the shelf-life of table eggs. It is important to underline that no negative effects of 100% CO_2 or 100% O_2 in comparison with air packaging has been observed in any of the tested temperatures.

In conclusion, 100% CO_2 gas was effective in controlling spoilage bacteria (total aerobes) and had no negative effects on pathogen growth or survival compared with air. Although further improvements are required to control RH within packaging to limit bacteria growth/survival, in view of the positive effects of CO_2 packaging on quality traits of table eggs, 100% CO_2 packaging might represent a promising innovative technique for the maintenance of egg characteristics during transport, retail, and domestic storage.

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REFERENCES

Byrd, J. A., A. R. Sams, B. M. Hargis, and D. J. Caldwell. 2011. Effect of selected modified atmosphere packaging on *Campylobacter* survival in raw poultry. Poult. Sci. 90:1324–1328.

Cotterill, O. J., and F. Gardner. 1956. Retention of interior shell egg quality with carbon dioxide. Poult. Sci. 35:1138.

Daş, E., G. C. Gürakan, and A. Bayindirli. 2006. Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of *Salmonella* Enteritidis on cherry tomatoes. Food Microbiol. 23:430–438.

De Reu, K., K. Grijspeerdt, M. Heynfrickx, J. Zoons, K. De Baere, M. Uyttendaele, J. Debevere, and L. Herman. 2005. Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. Br. Poult. Sci. 46:149–155.

EFSA (European Food Safety Authority). 2005. The welfare aspects of various systems of keeping laying hens. The EFSA Journal 197:1–23.

Faber, J. M. 1991. Microbial aspects of modified-atmosphere packaging technology—A review. J. Food Prot. 54:58–70.

Genigeorgis, C. A. 1985. Microbial and safety implications of the use of modified atmospheres to extend the storage life of fresh meat and fish. Int. J. Food Microbiol. 1:237–251.

Hammack, T. S., P. S. Sherrod, V. R. Bruce, G. A. June, F. B. Satchell, and W. H. Andrews. 1993. Research note: Growth of *Salmonella enteritidis* in Grade A eggs during prolonged storage. Poult. Sci. 72:373–377.

Hintlian, C. B., and J. H. Hotchkiss. 1987. Microbiological and sensory evaluation of cooked roast beef packaged in a modified atmosphere. J. Food Process. Pres. 11:171–179.

Hulánková, R., G. Bořilová, and I. Steinhauserová. 2010. Influence of modified atmosphere packaging on the survival of *Salmonella* Enteritidis PT 8 on the surface of chilled chicken legs. Acta Vet. (Brno.) 79:127–132.

Manfreda, G., C. Cevoli, A. Lucchi, F. Pasquali, A. Fabbri, and A. Franchini. 2010. Hot air treatment for surface decontamination of table eggs experimentally infected with *Salmonella*, *Listeria*, and *Escherichia coli*. Vet. Res. Commun. 34:S179–S182.

Michaelsen, A. R., J. G. Sebranek, and J. S. Dickson. 2006. Effects of microbial inhibitors and modified atmosphere packaging on growth of *Listeria monocytogenes* and *Salmonella enterica* Typhimurium and on quality attributes of injected pork chops and sliced cured ham. J. Food Prot. 69:2671–2680.

Moran, T. 1937. Gas storage of eggs. J. Soc. Ind. Chem. 56:96T.

Pasquali, F., A. Fabbri, C. Cevoli, G. Manfreda, and A. Franchini. 2010. Hot air treatment for surface decontamination of table eggs. Food Contr. 21:431–435.

Rajkovic, A., N. Tomic, N. Smigic, M. Uyttendaele, P. Ragaert, and F. Devlieghere. 2010. Survival of *Campylobacter jejuni* on raw chicken legs packed in high-oxygen or high carbon dioxide atmosphere after decontamination with lactic acid/sodium lactate buffer. Int. J. Food Microbiol. 140:201–206.

Rocculi, P., E. Cocci, F. Sirri, C. Cevoli, S. Romani, and M. Dalla Rosa. 2011. Modified atmosphere packaging of hen table eggs: Effects on functional properties of albumen. Poult. Sci. 90:1791–1798.

Rocculi, P., U. Tylewicz, A. Pekoslawska, S. Romani, F. Sirri, V. Siracusa, and M. Dalla Rosa. 2009. MAP storage of shell hen eggs, Part 1: Effect on physico-chemical characteristics of the fresh product. LWT-Food Sci. Technol. 42:758–762.

Skandamis, P., E. Tsigarida, and G.-J. E. Nychas. 2002. The effect of oregano essential oil on survival/death of *Salmonella typhimurium* in meat stored at 5°C under aerobic, VP/MAP conditions. Food Microbiol. 19:97–103.

Tassou, C. C., and J. S. Boziaris. 2002. Survival of *Salmonella enteritidis* and changes in pH and organic acids in grated carrots inoculated or not with *Lactobacillus* sp. and stored under different atmospheres at 4°C. J. Sci. Food Agric. 82:1122–1127.

Villaescusa, R., and M. I. Gil. 2003. Quality improvement of *Pleurotus* mushrooms by modified atmosphere packaging and moisture absorbers. Post. Biol. Technol. 28:169–179.

Wimpfheimer, L., N. S. Altman, and J. H. Hotchkiss. 1990. Growth of *Listeria monocytogenes* Scott A, serotype 4 and competitive spoilage organisms in raw chicken packaged under modified atmospheres and in air. Int. J. Food Microbiol. 11:205–214.