Impact of ozone and UV irradiation sanitation treatments on the survival of *Salmonella* and the physical–chemical characteristics of hen eggs

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Primary Audience: Egg Quality Assurance Personnel, Researchers

SUMMARY

Salmonella is the second main cause of foodborne illness in poultry production. It is one of the most problematic zoonoses in terms of public health worldwide because of the difficulty in controlling it and its significant morbidity and mortality rates. Recent surveys have shown that small flocks of laying hens have the same or higher prevalence of salmonellosis than larger flocks, mainly due to a lack of control actions, that is, the control of mice and wild animals, employees, and poor management practices. In this regard, different physical and chemical procedures have proven efficacious for reducing external and/or internal *Salmonella* contamination. This research evaluated the effect of ozone and UV-C rays on *Salmonella* growth and hen egg quality. Microbiological evaluation was performed on 120 eggs: negative control (C−), eggs not contaminated with *Salmonella*; positive control (C+), eggs contaminated; contaminated ozonate-treated (O, 600 mg/h for 2 h); and contaminated UV-C–irradiated (ƛ, 254 nm for 15 s) eggs. Moreover, 30 eggs were used (10/group) for the quality assessments of the C, O, and UV-C groups. A 2 $\log_{10}$ CFU/g reduction in *Salmonella* on contaminated eggs was found in the UV-C group compared with the C+ and O groups. Compared with UV-C treatment, ozonization reduced the amount of yolk tocols and carotenoids (by 2 times). The lipid oxidative status decreased (−1.5 times), similar to the cholesterol level (−28.5%), whereas the amount of cholesterol and its oxidized products increased (+82.1%) in the O group compared with the C group. UV-C irradiation is an effective strategy to reduce *Salmonella* contamination in eggs without negatively affecting the quality. Therefore, UV treatments remain among the more promising procedures.

Key words: eggs, ozone, UV, antioxidants, cholesterol, oxidation, *Salmonella*

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DESCRIPTION OF PROBLEM

Eggs are one of the most commonly consumed foods; approximately 1,140 billion eggs are produced per year, and 700 billion/yr are consumed worldwide [1, 2]. They are a less-expensive food protein source (0.30 dollar/g protein, [2]), and from a nutritional viewpoint, they are considered a functional food because of their high nutritional content [2, 3]. However, eggs are one of the main food vehicles of

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pathogens; for example, *Salmonella* is a potential food safety risk in eggs. Generally, there are 2 possible transmission routes: 1) horizontal, by penetration through the eggshell from the colonized gut or from contaminated feces during or after oviposition and 2) vertical, by the direct contamination of the yolk, albumen, eggshell membranes, or eggshells before oviposition [2]. *Salmonella* is the second main cause of foodborne illness in poultry production, after *Campylobacter* [4], with a mortality rate of less than 1%. It is one of the most problematic public health zoonoses worldwide, owing to its high endemicity, but mainly because of the difficulty in controlling it [5]. An estimated 2 million illnesses/yr occur because of *Salmonella*-contaminated eggs in the United States, whereas in Europe, the range is 7,400 cases/yr [6].

Recent surveys have shown that small flocks of laying hens have the same or higher prevalence of salmonellosis than do larger flocks (from 26 to 69% depending on the countries) [7]. A lack of control actions (such as the control of mice and wild animals, employees, and poor management practices) is the major risk factor for these small flocks [7].

Different physical and chemical procedures have proven efficacious for reducing external and/or internal *Salmonella* contamination [8]. Egg decontamination can be performed using several nonthermal methods, such as irradiation, acidic electrolyzed water, and high-pressure carbon dioxide [9–11]. In addition, pasteurization methods, including freeze-drying, hot air, hot water, infrared, atmospheric steam, microwave heating, and radiofrequency heating, can be applied [12–14]. However, these processes can also influence the qualitative characteristics of eggs, rendering these methods far from ideal. In particular, heat treatment and gamma irradiation have been demonstrated to greatly reduce *Salmonella enteritidis* contamination, but they compromise the quality of the eggs [9] and are often not permitted in some countries (i.e., European Union; [15]). Moreover, sometimes the equipment used is expensive and requires additional costs that cannot be supported by small-scale farms.

Ideally, a valid process should not leave any residues in the food, should be inexpensive, and should not reduce the nutritional or chemical characteristics of the product, thus combining safety and nutritional quality. Therefore, ozone and UV treatments remain among the more promising procedures.

UV irradiation is already used for packaging sterilization, and it has been demonstrated that no adverse effects (e.g., cytotoxicity [16, 17]) are caused by its use. Furthermore, the Food and Drug Administration has approved the use of short UV-C wavelengths for the treatment of many liquid foods (i.e., water and juices) to inactivate pathogenic microbes [18]. Similarly, ozone has been approved for use as a disinfectant or sanitizer in foods and food processing in the United States [19], for example, disinfection of poultry carcasses and chilled water in the poultry industry [20].

In summary, the main benefits of these treatments for food processing are as follows: 1) they are nonthermal ways to control pathogens and microbes, 2) they use nonchemical agents to treat food (no residues are left in the food or water), and 3) they are compatible with organic production systems [15].

However, there is little information available on the effect of these processes on the qualitative properties of eggs [8].

In particular, there are no scientific studies available on the effect of ozone and UV irradiation on cholesterol and its derived products. This represents an important area of investigation considering that cholesterol constitutes 2% of the egg lipid fraction [21].

In light of this, the objective of this research was to analyze the ability of simple and inexpensive decontamination processes (ozone and UV-C) to reduce *Salmonella* contamination on hen eggs and to preserve their quality.

MATERIALS AND METHODS

Egg Sampling and Treatments

During the experiment, 150 homogenous hen eggs were used: 120 for the microbiological evaluation and 30 for the qualitative analysis (10 eggs per group). The trial was repeated 3 times; a total of 450 eggs were used. The eggs were purchased from a commercial farm [22], which uses brown Hy-Line hens reared on the floor and fed

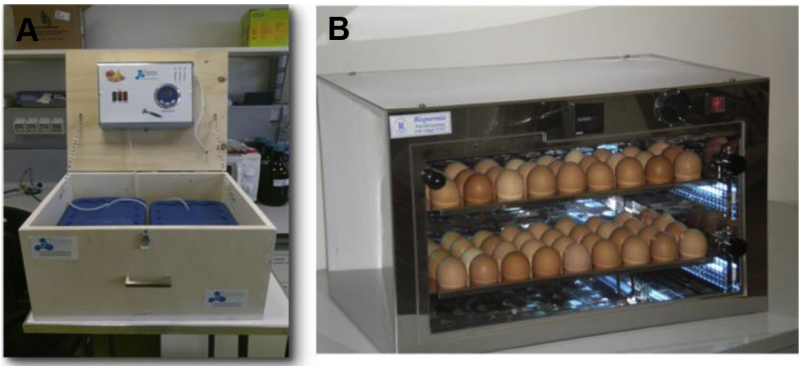

Figure 1. Sterilization equipment used for the experiment: A) ozone was provided by Ozono Disinfezioni [23]; the total dimensions were 90 cm L × 80 cm H × 20 cm W, and the cost was in the range of $400 to $450; B) UV-C irradiation was provided by [24]. The total dimensions were 70 cm L × 50 cm H × 55 cm W, and the cost was in the range of $300 to $400.

the same commercial diet (main nutrients: 16% crude proteins, 16% ash, 4% fat, and 3.7% fiber; main minerals: 5.1% calcium, 0.5% phosphorus, and 0.2% sodium; main amino acids: 0.65% lysine and 0.30% methionine).

For the microbiological evaluation, 120 eggs were divided into 4 groups, replicated 3 times: 1) negative control (C−), eggs not experimentally contaminated with *Salmonella*; 2) positive control (C+), eggs experimentally contaminated with a cell suspension of 3 *Salmonella* serotypes (*Salmonella typhimurium*, *S. enteritidis,* and *Salmonella senftenberg*); 3) eggs experimentally contaminated as described previously and then treated with ozone (group O; model AISI 304, [23]; at a flow rate of 600 mg/h for 2 h; Figure 1A); 4) eggs experimentally contaminated and then UV irradiated (group UV-C; UV light model STP-120 [24], pumped through a Teflon tubing bundle arranged around 5 central low-pressure mercury UV-C lamps emitting at a 254 nm wavelength for 15 s, as per the manufacturer's protocols; Figure 1B).

Quality assessment was performed on control eggs (C, no treatments), ozonate eggs (O, Figure 1A), and UV-C irradiated eggs (Figure 1B).

Microbiological Evaluation

Bacterial Strains and Inoculation of Eggs. A multistrain mix of *S. enterica* ser. Typhimurium WDCM 00031, *S. enterica* ser. *Enteritidis*, and *S. enterica* ser. *Senftenberg*, isolated from eggs (collection IZSUM 17/13 and IZSUM 72/14, respectively), was used. The strains were grown aerobically at 37°C for 24 h in brain heart infusion (cod. 4012302; [25]). Counts were confirmed by serial 10-fold dilution and inoculation on chromogenic salmonella medium (cod. 4053402 and cod. 4240013; [25]) plates incubated at 37°C for 24 h. Each of the 3 strains was combined in an equal quantity at a concentration of approximately 8 log CFU/mL. Eggs were spotted with 500 µL of serial 10-fold dilutions of the mixed cultures made in physiological saline (0.9 g/100 mL NaCl) to obtain a concentration of the *Salmonella* strains of approximately 10^5 CFU/egg.

Detection and Enumeration of Salmonella in Eggs. The positive control (C+) and treated groups (O and UV) were tested for the presence and enumeration of *Salmonella* spp. after surface contamination (T0), after treatment (T1), and after 6 and 24 h of treatment (T6 and T24, respectively). The eggs were broken, the inner contents were discarded, and the eggshells were diluted in 90 mL of buffered peptone water (cod. 4012782; [25]). Viable bacteria counts were obtained by seeding 10-fold dilution series made in physiological saline onto chromogenic salmonella medium. If the bacteria were less than detectable levels (1.95 log CFU/mL) of the method, a value of −0.5 log CFU/mL was assigned [26].

The detection of *Salmonella* spp. was performed using the AFNOR BIO method and UNI EN ISO to

confirm positive results. Uncontaminated shell eggs (C−) were tested for the presence of *Salmonella* spp. to check that they were *Salmonella*-free. Serological typing was also performed on *Salmonella* isolates.

Qualitative Evaluations

Egg Characteristics and Physical–Chemical Composition of the Yolk. The percentage of the albumen, yolk, and shell was calculated based on the whole egg weight. The pH was measured with a Knick digital pH meter [27] after homogenization of the yolk. Albumen consistency was tested by calculating the Haugh unit (HU) value of each egg:

$$HU = 100 \log[H - g(30W * 0.37 - 100) / 100 + 1.9]$$

where, H is the maximum height of thick albumen in millimeters, W is the weight of the egg in grams, and g is a constant (32.2) related to the constant of gravitation.

Yolk color intensity was established using the Roche color fan scale (1–15) and the CIE-Lab colorimetric method. Color parameters (L*, a*, b*) were evaluated using a tristimulus analyzer (Minolta Chroma Meter CR-200, [28]). The L*a*b* color system consists of a luminance or lightness component (L*) and 2 chromatic components: the a* that ranges from green (−a*) to red (+a*), and the b* that ranges from blue (−b*) to yellow (+b*). The colorimeter was calibrated using a standard white plate. The chemical composition of the egg yolk was determined as per the method described by the AOAC for freeze-drying egg samples [29].

Antioxidant Compounds in Egg Yolk. The different isoforms of vitamin E (tocols α and β+γ, δ, tocopherol T, and tocotrienol T_3) in the yolk were quantified using HPLC as reported by Mattioli et al. [30]. Then, 0.1 g of freeze-dried egg yolk was saponified in 60 g/100 mL KOH in ethanol in a thermostat bath at 50°C for 1 h. Then, the content was sonicated and extracted twice with 10 mL of *n*-hexane. The upper phase was collected, dried with nitrogen and then reconstituted in 200 μL of acetonitrile, and 50 μL was injected into the HPLC system (model AS 950-10, [31]) on a Synergi Hydro-RP column (4 μm, 4.6 × 100 mm, [32]). The different isoforms were identified using an FD detector (FP-1525 model, [33]) at the excitation and emission wavelengths of 295 and 328 nm, respectively, and quantified using external calibration curves prepared with increasing amounts of pure tocols in ethanol.

The main carotenoids in the yolk and their derivative (retinol) were determined by the same HPLC system previously described. The solvent system consisted of solution A (methanol/water/acetonitrile, 10:20:70, v/v/v) and solution B (methanol/ethyl acetate, 70:30, v/v), following the elution program reported in the study conducted by Mattioli et al. [30]. The detector was a UV–visible spectrophotometer (UV2075 Plus, [33]) set at a wavelength of 450 nm for lutein, zeaxanthin, and β-carotene and at 325 nm for retinol. The different carotenoids were identified and quantified by comparing the sample to pure commercial standards [34].

Fatty Acid Profiles of the Egg Yolk. The fatty acid profiles of the egg yolk were determined by gas chromatography after lipid extraction as per the method described by Folch et al. [35]. One microliter of lipid extract was evaporated under a stream of nitrogen, and the residue was derived by adding 0.5 mL of KOH in methanol (11.2 g/100 mL). After incubation at 80°C for 1 h, methyl esters were extracted using *n*-hexane and 1 μL was injected into a gas chromatograph (CP-3800, [36]) equipped with a flame ionization detector. The fatty acid methyl esters (FAMEs) were separated using a capillary column (30 m × 0.25 mm I.D., [37]) coated with a DB-wax stationary phase (film thickness of 0.25 mm). The operating conditions used during the column injection were as follows: the temperatures of the injector and detector were set at 270°C and 280°C, respectively, and the detector gas flow rates were 50 mL/min for H and 100 mL/min for air. The oven temperature was programmed to provide a good peak separation as follows: the initial oven temperature was set at 130°C; this temperature increased at a rate of 4.0°C/min to 180°C and was held for 5 min; the temperature was then increased at a rate of 5.0°C/min to 230°C, and the final temperature was held for 5 min. Helium was used as a carrier gas at a constant flow rate of 3 mL/min. Individual FAMEs were identified by referring to the retention time of FAME authentic

standards. For the quantitative analysis, C21:0 methyl ester was added before extraction as an internal standard.

Cholesterol and Its Oxidized Products. Cholesterol and its oxidized products (COPs) were extracted from yolk samples using *n*-hexane. Briefly, 0.1 g of freeze-dried and ground yolk was subjected to saponification in a 2 g/100 mL KOH–ethanol solution for 1 h at 50°C. At the end of the saponification, 2 further extractions using n-hexane and sonication (30% for 10 s; model U50, [38]) were carried out. The mixture was vortexed and centrifuged at 900 × *g* for 5 min. The hexane extract was collected and dried under a stream of N_2. The residue was suspended in 3 mL of the mobile phase and filtered through a syringe with regenerated cellulose (RC Phenex 4 mm, 0.26 μm size, [32]) before being injected into the HPLC system.

The quantification of cholesterol and COPs was performed using an HPLC/UV-vis system (model pump PU1850, equipped with an autosampler, model AS 950-10, [33]) with an analytical C18 reversed-phase column (particle size ODS-2.5 M, 4.6 mm internal diameter, [39]). The mobile phase was composed of a mixture of acetonitrile/isopropanol (70:30, v/v) and was released at a flow rate of 1.5 mL/min. The injected volume was 10 μL.

The identification of sterols was performed using a UV detector (Model 2075 Plus, [33]) set at λ 210 nm for cholesterol, 206 nm for 7-hydroxycholesterol (7-OH), and 233 nm for 5-cholesten-3β-ol-7-one (7-Keto) using a programmed step run of 20 min and quantified using a calibration curve with increasing amounts of pure standard solutions in isopropanol.

Lipid Oxidation Status. The thiobarbituric acid reactive substance (TBARS) values were assessed according to Cherian et al. [40]. Briefly, 2 g of egg yolk samples were weighed in 50 mL test tubes, and 18 mL of 3.86 g/100 mL perchloric acid was added. The samples were homogenised for 15 s, 50 μL of butylated hydroxytoluene (4.5 g/100 mL ethanol) was added to each sample during homogenization to control lipid oxidation, and the samples were centrifuged at 6000 × *g* for 10 min. The homogenate was filtered through a Whatman no. 1 filter paper. Two milliliters of filtrate was mixed with 2 mL of 0.3 g/100 mL thiobarbituric acid in distilled water and incubated in the dark at room temperature for 15 to 17 h. Absorbance was determined at 531 nm. The results were expressed as μg of malondialdehyde (MDA) per gram of fresh yolk.

Statistical Evaluation

The effect of treatments (ozone and UV-C) on the quality traits of eggs (physical–chemical properties, levels of fatty acids, antioxidants, and cholesterol derivatives and oxidative status) were analyzed by one-way ANOVA, and significant differences were determined using the post hoc Bonferroni test at the level of $P < 0.05$ [41]. Survival curves of *Salmonella* pooled strains ($\log_{10}$ CFU/mL) under different conditions (C+, O, and UV-C) were constructed using DMFit, version 2018, [42] by fitting the data to the model of Baranyi and Roberts [43].

RESULTS AND DISCUSSION

The objective of the present study was to evaluate the efficacy of ozone and UV-C irradiation on *Salmonella* contamination and on the egg quality of laying hens. *Salmonella* contamination is considered a relevant hygiene issue, especially in small-scale farms that are not controlled by an authorized agency or when a Hazard Analysis Critical Control Point (HACCP, [44]) plan is not available.

The use of nonthermal decontamination methods, including ozonization and UV-C, has been extensively considered over the last few years [45]. In fact, the adverse effects of high temperature on some components of food (fatty acids, vitamins, cholesterol) are widely known [46].

The antimicrobial effect of the ozone and UV-C treatments was examined on eggs experimentally contaminated by a multistrain mix of *S. enterica* (Figure 2). After UV-C irradiation of the experimentally *Salmonella*-contaminated eggs, the *Salmonella* count was significantly decreased ($P < 0.05$) compared with that of the positive control and ozone groups (T1, 2.32 vs. 5.57 and 4.79 log CFU/egg, respectively). Six hours after treatment (T6), the *Salmonella* count decreased in all groups, mainly due to the loss of growth substrates on the egg surfaces (lack of

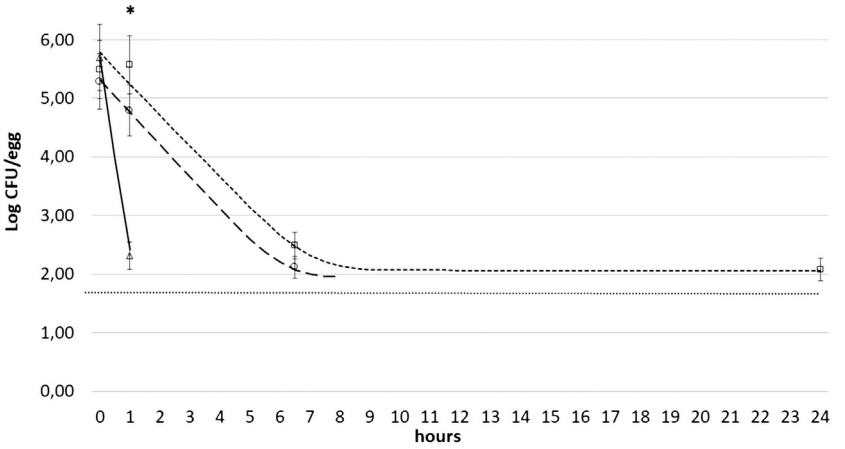

Figure 2. Mean values (square, control; circle, ozone; triangle, UV-C), upper and lower 95% CI limits and survival curves of *Salmonella serovars* in shell eggs. The curves were estimated using the Baranyi and Roberts model [43]. Points represent the averages of microbial counts performed in triplicate with 3 eggs per experimental condition. * $P < 0.05$.

feces), but the level was below the limit of quantification (LOQ = 1.95 log CFU/mL) in the UV-C group. After 24 h of treatment, the *Salmonella* count was also below the limit of quantification in the O group. However, using the AFNOR BIO 12/16–09/05 method, it was possible to reveal the presence of *Salmonella* spp. after 24 h in all the samples from the C+ and O groups, whereas some eggs in the UV-C group (33%, data not shown, $P = 0.20$) were negative for *Salmonella*.

UV-C irradiation is a promising preservation technology for food that may be a substitute for thermal processing. It was demonstrated that the short wavelength UV-C spectrum acts against a wide range of microorganisms without negatively affecting the nutritional quality of food. Pasquali et al. [47] found that the total colony count of UV-treated eggs was approximately 1 $\log_{10}$ CFU/g lower than that of untreated eggs (2.27 vs. 3.29 $\log^{10}$ CFU/g, respectively). Furthermore, Gopisetty et al. [17] observed a non-negative effect of the UV-C irradiation (for 403 s) of cranberry-favored water on some antioxidant compound (anthocyanin and ascorbic acid) content. In this context, UV-C light seems to penetrate cells and thereby prevent the replication of microorganisms because of its absorption by nucleic acids (forming pyrimidine dimers) [48].

In the present study, we detected *Salmonella* after 24 h in both the ozone and control groups, suggesting that ozone had no effect on bacterial depletion. Conversely, in many other studies (reviewed by Pandiselvam et al. [49]), ozone

Table 1. Effect of Ozone and UV-C on the Physical Characteristics of Hen Eggs (n = 30).

Physical characteristics	Control	Ozone	UV-C	Pooled SE
Egg weightg	60.76	61.02	60.55	2.21
Shell%	12.13	12.05	12.00	0.26
Albumen%	60.16	60.20	60.30	1.24
Yolk%	27.55	27.52	27.60	1.05
pH	5.98	5.95	5.97	0.04
Haugh Index, Haugh unit	118.70	119.50	119.80	1.27
Roche Color, Roche unit	8.50	8.40	8.50	1.00
CieLab Color				
L*	60.30	60.00	60.30	2.67
a*	−7.62	−8.05	−7.35	0.92
b*	42.60	39.90	40.80	4.31

Abbreviation: SE, standard error.

Table 2. Effect of Ozone and UV-C on the Chemical Composition of Egg Yolk (n = 30).

Chemical composition	Control	Ozone	UV-C	Pooled SE
Moisture, % d.m.	46.05	45.10	45.10	1.09
Lipid, % d.m.	32.22	33.02	32.98	1.46
Protein, % d.m.	18.31	18.16	18.34	0.89
Ash, % d.m.	3.42	3.72	3.58	0.03

Abbreviation: SE, standard error.

alone or in combination with other treatments was capable of reducing *Salmonella* colonization in eggs. It is likely that the temperature of the egg, the flow rate, and the duration of exposure to ozone largely affect its decontamination power. However, exposing hen eggs to ozone endangered the nutritive characteristics of the eggs.

No significant differences in the egg characteristics, physical–chemical traits (Tables 1 and 2), or yolk fatty acid profiles (Table 3) were found between the treated egg groups (both ozone and UV-C groups) and the control group. A small change in the amount of oleic acid ($P = 0.06$) was found in UV-C eggs (81.77 vs. 93.72 and 100.81 mg/g yolk in C and O groups, respectively), whereas the saturated fatty acids (mainly palmitic acid) tended to be lower in the ozonated eggs (84.06 vs. 90.41 and 90.24 mg/g yolk in C and UV-C, respectively).

Conversely, many differences were found in the levels of antioxidants in the egg yolks (Table 4). Compared with the control condition and UV-C treatment, egg ozonization reduced the amount of α- and β+γ-tocotrienols (by 2.8 and 1.7 times, respectively), α- and β+γ-tocopherols (by 3.3 and 3.2 times, respectively), and carotenoids (by 2 times less). In particular, the total amount of tocopherols per egg in the ozonized samples was 99.12 vs. 320.00 and 311.20 μg for the control and UV-C groups, respectively. Ozone also reduced the carotenoids in the egg yolk compared with the trend for tocopherols (120.11 vs. 247.23 and 238.00 μg/egg for the control and UV-C groups, respectively).

In addition, the lipid oxidative status declined in the ozone group (Table 5; TBARS value: 0.15 vs. 0.10 and 0.09 μg MDA/g yolk for the control and UV-C groups, respectively).

The ozonized eggs had reduced antioxidants (tocopherols: −70%; carotenoids: −50%; Figure 3) and cholesterol, whereas the oxidized derivatives of cholesterol and TBARS largely increased. The COP concentration of these eggs was double than that of the UV-C irradiated eggs, suggesting a worse oxidative status, which was also confirmed by the TBARS value. Ozone easily penetrates eggshells and dissolves in the albumen and yolk [50]. In particular, antioxidants, fatty acids, and cholesterol (approximately 200 mg/egg) are the main target molecules of oxidation in the egg [51].

The total cholesterol content was also lower in the ozone group (7.94 mg/g egg) than in the C and UV-C groups. In line with the reduction in cholesterol and the increase in lipid oxidation, the total COPs (mainly 7-OH) were higher in the O group than in the other 2 groups. Fuhrmann et al. [50] demonstrated that the

Table 3. Effect of Ozone and UV-C on the Fatty Acid Profile of Egg Yolk (mg/g Yolk, n = 30).

	Experimental groups			
Fatty acids	Control	Ozone	UV-C	Pooled SE
C14	1.04	1.00	1.08	0.90
C16	57.54	54.31	59.13	20.11
C16:1cis9	11.80	11.44	11.24	2.56
C18	31.83	28.75	30.04	7.45
C18:1cis9 n-9	93.72	100.81	81.77	46.22
C18:2cis n-6, LA	31.00	29.35	31.04	8.87
C18:3 n-3, ALA	2.04	2.40	2.68	0.79
C20:4 n-6, AA	4.24	5.54	4.33	0.99
C20:5 n-3, EPA	0.55	0.47	0.63	0.11
C22:5 n-3, DPA	0.08	0.08	0.08	0.03
C22:6 n-3, DHA	0.75	0.67	0.70	0.23
SFA	90.41	84.06	90.24	22.76
MUFA	105.52	102.25	93.01	35.76
PUFA	39.21	38.99	40.12	3.22
PUFA n-6	35.79	35.36	35.99	11.8
PUFA n-3	3.42	3.63	4.13	1.23
n-6/n-3	10.48	9.76	8.72	1.34

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linolenic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; PUFA n-3, polyunsaturated fatty acids of n-3 series; PUFA n-6, polyunsaturated fatty acids of n-6 series; SE, standard error; SFA, saturated fatty acid.

Table 4. Main Antioxidant Compounds of the Egg Yolk (n = 30) in the Different Experimental Groups.

Antioxidant compounds	Control	Ozone	UV-C	Pooled SE
α-Tocotrienol, μg/g yolk	0.71[b]	0.04[a]	0.31[b]	0.33
(β+γ)-Tocotrienol, μg/g yolk	0.40[b]	0.35[a]	0.28[a]	0.03
δ-Tocopherol, μg/g yolk	1.42[b]	0.84[a]	1.40[b]	0.26
α-Tocopherol, μg/g yolk	15.71[b]	4.28[a]	15.15[b]	2.58
(β+γ)-Tocopherol, μg/g yolk	0.41[b]	0.14[a]	0.40[b]	0.04
Σ Tocopherols, μg/egg	320.00[b]	99.12[a]	311.20[b]	15.76
Retinol, μg/g yolk	3.10[b]	1.56[a]	2.87[b]	2.28
Lutein, μg/g yolk	3.21[b]	2.26[a]	3.00[b]	1.56
Zeaxanthin, μg/g yolk	10.24[b]	4.35[a]	10.00[b]	4.17
β-Carotene, μg/g yolk	0.43[b]	0.21[a]	0.29[a]	0.02
Σ Carotenoids, μg/egg	247.23[b]	120.11[a]	238.00[a]	12.00

[a,b] on the same line indicates $P < 0.05$.

Abbreviation: SE, standard error.

alterations in the whole egg caused by ozone were dose dependent; low ozone doses (10–20 mL/L of ozone flow for 120 min) mainly affected the egg surface, whereas a higher dose (50 mL/L of ozone flow for 60 min) negatively affected the main components of the eggs (vitamin E, oleic acid, SH groups, arachidonic acid, and DNA). However, no studies on COPs have been reported.

Comparing the trend for the antioxidant levels and oxidation status of eggs subjected to the different treatments revealed that the values of the UV-C group were similar to those of the control group ($P > 0.05$), whereas compared with the control conditions, ozone treatment reduced antioxidants (tocopherols: −69.2% and carotenoids: −51.6%) and cholesterol (−28.5%) while simultaneously increasing COPs (+82.1%). UV-C irradiation resulted in similar amounts of antioxidants (−4.2 and −3.9% for tocopherols and carotenoids, respectively), cholesterol, and COPs as the control (Figure 3).

Many studies have been carried out to define the COP level in eggs due to their copious use in human diets. The most abundant COPs in eggs and egg products are 7α-hydroxycholesterol, 7ß-hydroxycholesterol, and their dehydrogenation products 7α-ketocholesterol, 5,6α-epoxycholesterol, 5,6ß-epoxycholesterol, and cholestantriol, as well as 20ß-hydroxycholesterol and 25-hydroxycholesterol [46, 52].

Mazalli and Bragagnolo [46] found small amounts of COPs in fresh eggs enriched with n-3 fatty acids and showed that 7α and 7β-OH increased in fried eggs and were subsequently converted into 7-Keto. In agreement, Obara et al. [52] found that 7α-OH was produced in egg powder during storage, and Zardetto and Dalla Rosa [53] reported an increase in cholesterol oxidation in egg pasta due to heat treatments (100°C for 120 min). In the present study, the main COPs detected were 7-OH and 7-Keto; these oxidation products are biologically active and have negative effects on human health, such as promoting atherogenesis, carcinogenesis, and neurodegenerative diseases [52].

A strong effect of UV-C and ozone treatments is also reported on the fatty acid composition of food; however, studies on egg fatty acids are lacking. For instance, ozone has different affinities for individual fatty acids [52]:

Table 5. Cholesterol and Its Oxidation Products in the Egg Yolk (n = 30) of the Different Experimental Groups.

Oxidative products	Control	Ozone	UV-C	Pooled SE
Cholesterol, mg/g egg	11.10[b]	7.94[a]	10.40[b]	3.01
7-OH, μg/g egg	1.85[a]	3.78[b]	1.72[a]	1.05
7-Keto, μg/g egg	0.60[a]	0.71[b]	0.57[a]	0.10
Σ COPS, μg/g egg	2.45[a]	4.49[b]	2.29[a]	0.23
TBARS, μg MDA/g yolk	0.10[a]	0.15[b]	0.09[a]	0.01

[a,b] on the same row indicates $P < 0.05$.

Abbreviations: 7-Keto, 5-cholesten-3β-ol-7-one; 7-OH, 7-hydroxycholesterol; COPs, oxidized cholesterol products; SE, standard error; TBARS: thiobarbituric acid reactive substances.

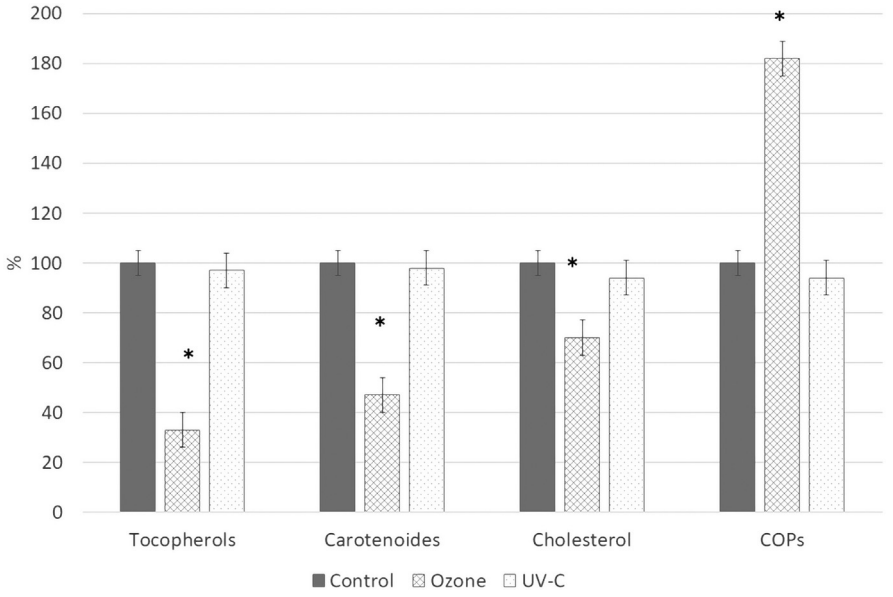

Figure 3. Effect of treatments on tocopherols, carotenoids, cholesterol, and oxidized cholesterol products (COPs) in eggs (% change with respect to the control, 95% upper and lower limits). The gray bar represents the control group, the hatched bar represents the ozone group, and the dotted bar represents the UV-C group. * $P < 0.05$.

as a monounsaturated fatty acid, oleic acid ranks higher than linoleic acid in its reactivity to ozone. In addition, it has been observed that ozone not only alters fatty acids but also forms reactive secondary products such as aldehydes that can attack other molecules, for example, the exposure of unsaturated fatty acids to ozone leads to the formation of acrolein [48].

According to the results presented here, the ozonized eggs showed only minor differences (oleic acid content, $P = 0.06$) in fatty acid composition. Evidently, the ozonization dose used caused limited damage to fatty acids; thus, no significant effects were observed. The TBARS value, which is an indirect method to evaluate the content of aldehydes, could be considered a less effective way to estimate the lipid oxidative status because ozone mainly causes the generation of their intermediate product [48], which is not detectable with this method. In contrast, the UV-C treatment better preserved the nutritional quality of the eggs, showing COPs, TBARS, and antioxidant values similar to those of the control eggs.

CONCLUSIONS AND APPLICATIONS

1. Currently, owing to the increasing attention of the consumer about the origin of food, the purchase of niche products from small producers (small farms) is common; one of these products is hen eggs. For this reason, the investigation of simple, rapid, and inexpensive decontamination methods that are compatible with organic production systems and can be used directly by farmers has become of great importance for providing high-quality and healthy food.
2. UV-C irradiation could be considered an effective strategy to reduce *Salmonella* contamination in eggs. This method 1) did not negatively affect the quality of the eggs, in contrast to the ozone treatment (which largely increased the COP amount and lipid oxidation), 2) is easy to use (light the lamp and expose the eggs for a few seconds), and 3) the equipment has a low cost (less than $400).

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