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FULL PAPER

PLASMA PROCESSES AND POLYMERS

Effect of cold atmospheric pressure plasma treatment of eggshells on the total bacterial count inoculated *Salmonella* Enteritidis and selected quality parameters

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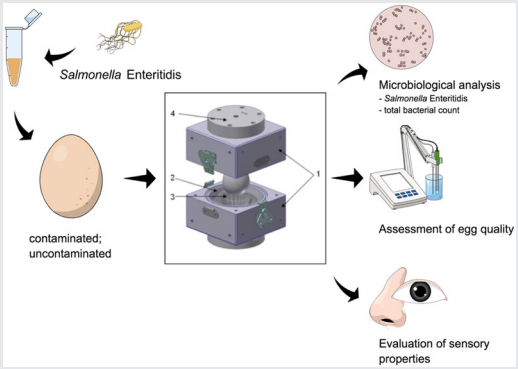
Abstract

In the European Union, foodborne outbreaks caused by *Salmonella* Enteritidis, related to eggs and egg products, have even been reported in 2018. Atmospheric pressure plasma is becoming increasingly important as a decontamination method. A semidirect cold atmospheric pressure plasma, the flexible electrode plasma source, was developed for treating whole hen's eggs. An average reduction of 1.16 and 0.95 log colony-forming units (CFU)/egg was achieved for the total bacterial count of clean and dirty eggs, respectively. An inactivation of 4.1 log CFU/egg *Salmonella* Enteritidis was achieved with artificially inoculated eggshells. Selected quality parameters and sensory properties were analysed. Overall, the present study yielded promising results for a realistic implementation of an industrial prototype plasma source.



KEYWORDS

egg quality measurements, egg surface contamination, nonthermal plasmas, *Salmonella* Enteritidis, sterilisation

1 | INTRODUCTION

In today's society, where healthy nutrition is becoming increasingly important, eggs have a particular significance; first, as a natural source of high-quality protein, essential vitamins (for example, vitamins A, D, E and K) and minerals, and second, as a versatile foodstuff that can be processed. This importance is also reflected in the development of the egg market. From 2000 to 2018, the global egg production has increased approximately by 39%.[1] In 2018, about 77

million metric tons of eggs were produced worldwide.[1] Experts from the European Commission predicted that the total egg production in the European Union (EU) and the United Kingdom (UK) would amount to 7,144 in 1,000 tons by 2020, and this upward trend is expected to continue in the coming years.[2] For example, in Germany, there was an increase in the annual egg consumption between 2006 and 2019 by about 13%, 236 eggs per person.[3] A general awareness of food safety matters is also on the rise. However, within the EU, there are currently no legal decontamination methods available for table eggs, and the washing of grade A eggs is generally prohibited.[4] Salmonellosis in humans is still the second most commonly reported bacterial disease. The only type of bacterial disease to be reported more frequently in the EU in 2018 was campylobacteriosis.[5] Although a downward trend has been established over the past decades, 91,857 cases of salmonellosis in humans were recorded in the EU in 2018.[5] In this regard, the most commonly reported serovar in 2018 was *Salmonella* (*S.*) Enteritidis.[5] On the basis of strong evidence, it has been observed that egg and egg products are still the most frequently reported vehicles (47.2%) for *S.* Enteritidis foodborne outbreaks.[5] The use of atmospheric pressure plasma is a new, alternative technology designed to achieve the gentle, yet efficient, inactivation of microorganisms.[6] Therefore, it is suitable for applications in the field of food safety. In recent years, some studies relating to the use of nonthermal plasma on eggshells have been implemented. Whereas Davies and Breslin[7] were not able to demonstrate any kind of antimicrobial effect of eggs inoculated with *S.* Enteritidis with indirect plasma treatment, Ragni et al.[8] inactivated up to 4.5 log colony-forming units (CFU)/eggshell of *S.* Enteritidis and 3.5 log CFU/eggshell of *S.* Typhimurium within 90 min, using indirect plasma treatment. Chen[9] and Wan et al.[10] even achieved a reduction of approximately 5.5 log CFU/eggshell of *S.* Enteritidis after 15 min with a direct high-voltage atmospheric cold plasma (HVACP) treatment using a modified atmospheric gas. Georgescu et al.[11] obtained a reduction below the detection limit of *S.* Typhimurium after 10 min of direct and 25 min of indirect cold atmospheric plasma treatment. Dasan et al.[12] also achieved a reduction below the detection limit of *S.* Enteritidis on the eggshell after 120 s of atmospheric pressure plasma jet (APPJ) treatment, with air being used as the feed gas. In previous investigations, we were able to demonstrate a reduction of up to 2.27 log CFU of *S.* Enteritidis/eggshell after 300 s of treatment with a direct APPJ (kINPen 09®; neoplas tools GmbH).[13] Except for the study of Ragni et al.,[8] in which the entire eggshell was inoculated, most of the studies only investigated small inoculated parts.

The principal aim of the present study was, therefore, to investigate the effects of semidirect cold atmospheric plasma treatment on whole hen's eggs with an eggshell-adapted plasma source. The most efficient reduction of bacteria should be achieved in the shortest possible treatment time with simultaneously unaffected quality parameters. The effects on the total bacterial count were established, in addition to the inactivation of *S.* Enteritidis on artificially inoculated eggshells. Furthermore, selected quality parameters and sensory properties of the eggs were analysed.

2 | EXPERIMENTAL SECTION

2.1 | Plasma source

In this study, a semidirect atmospheric pressure plasma source, namely, the flexible electrode plasma source (FEPS; INP Greifswald, Germany), was used. According to Schlüter et al.,[14] semidirect means a much larger distance between plasma and substrate than the mean free particle path. There are no interactions with charged particles and the antimicrobial effect is caused due to irradiation, long-lived radicals and metastable and inhibitory substances. This was developed by INP Greifswald and was specially adapted to the geometry of hen's eggs. It comprises two parts of a rigid case, each of which contains a flexible elastomer inlay (Figure 1). The inlay has a shape similar to the design of a citrus juicer. The electrodes are located in its grooves (with a maximum distance of 9 mm between the electrodes at the equatorial

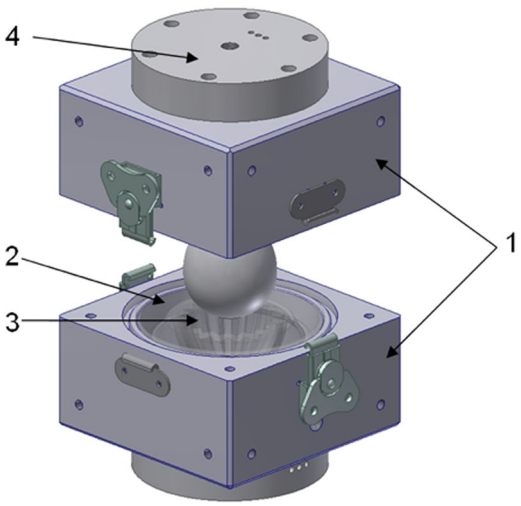


FIGURE 1 The schematic diagram of a semidirect atmospheric pressure plasma source, flexible electrode plasma source, for egg treatment. (1) Rigid case, (2) flexible elastomer inlay, (3) electrodes, and (4) connection flange with high-voltage connection and gas supply. Reproduced with permission (© 2015, INP Greifswald, Germany)

FIGURE 2 Discharge of flexible electrode plasma source. Reproduced with permission (© 2015, INP Greifswald, Germany)

plane). The applied output voltage input of the FEPS ranged between 4.8 and 5.0 kVpp at a frequency of 3.7 kHz when a feed gas flow of 100 sccm of compressed air was used. Figure 2 shows the discharge geometry of the FEPS.

2.2 | Eggs

The eggs were purchased from a local *Salmonella*-free farm and transilluminated to ensure the shells were intact. The eggs were stored for a maximum of 10 days at 8°C and stored for at least 1 hr at room temperature before atmospheric pressure plasma treatment.

2.3 | The effect of atmospheric pressure plasma treatment on the total bacterial count on the eggshell

White eggs, both clean and dirty (naturally covered with faeces, dust, feathers, etc.), were used to investigate the effect on the total bacterial count on the eggshell. On three consecutive days, five eggs were tested per day for each group (clean, dirty and control = untreated eggs). The eggs were treated with an atmospheric pressure plasma for 300 s. This was immediately followed by the determination of the total bacterial count. The eggs were transferred into sterile plastic bags (one egg per bag; Stomacher bags, Carl Roth, Karlsruhe, Germany) and rinsed with 25-ml sterile NaCl–peptone water (Roth). After 1 min of gentle movement, 0.5 ml of the rinse solution was used to estimate the viable microorganisms by plating the serially diluted rinse solution on plate count agar (SIFIN, Berlin, Germany). Furthermore, 1.0 ml of the undiluted rinse solution was plated on three agar plates (detection limit: 25 CFU/egg). The plates were incubated at 30°C for 72 hr before the counting of viable colonies.

2.4 | The effect of atmospheric pressure plasma treatment on *S.* Enteritidis inoculated on eggshells

One *S.* Enteritidis strain (phage type 4/6) isolated from an eggshell-related outbreak in humans (Robert Koch-Institute, Berlin, Germany) was chosen to inoculate the eggshells. The strain was stored in cryogenic cultures (Mast Group, Bootle, UK), transferred into nutrient broth (SIFIN) and incubated for 24 hr at 37°C. This suspension was first plated on brilliant green phenol red (BG) agar (SIFIN) and xylose lysine deoxycholate (XLD) agar (SIFIN). For the experiments, one colony of *S.* Enteritidis was transferred into nutrient broth (SIFIN) and incubated for 24 hr at 37°C. The inoculums were serially diluted to the desired *S.* Enteritidis concentration (10^4 CFU/egg). Clean eggs were inoculated with the *S.* Enteritidis suspension in a sterile plastic bag for 5 min. To determine the *S.* Enteritidis concentration in the sterile plastic bag, the serially diluted inoculation suspension was plated on XLD agar (SIFIN). A total of five eggs were inoculated per plastic bag. After inoculation, the eggs were dried in a safety cabinet for 10 min at room temperature. On two consecutive days, five untreated control eggs and five plasma-treated eggs were tested per day. The recovery method was the same, as set out above, with the exception that only XLD agar plates (SIFIN) were used and the plates were incubated at 37°C for 24 hr.

2.5 | The effect of atmospheric pressure plasma treatment on selected quality parameters

The pH value of the albumen, height of the air cell, Haugh units and eggshell staining were measured directly after the treatment to evaluate the effect of the atmospheric pressure plasma treatment on the egg quality or cuticle damage. On three consecutive days, five eggs were tested per day for each parameter. The principles of the measurement of the height of the air cell and the Haugh units are described in the Egg-Grading

Manual of the U.S. Department of Agriculture.[15] The pH value of the albumen was measured, and a comparison with untreated control eggs was carried out three times per egg, with a commercial surface pH electrode (08300700, WTW pH 330; Xylem Inc., Weilheim, Germany). The height of the air cell was measured before and after treatment using an air cell gauge. The Haugh unit was determined and a comparison was carried out with untreated control eggs using the FUTURA measurement system (Futura 2IA, Mini-DTS 2.0; Futura–Werner Fürste, Lohne, Germany).

To assess any possible negative effects of the gas plasma on the cuticle, eggshell staining was carried out. According to Mamadou, the cuticle was measured by dipping treated and untreated eggs into a 1:10 diluted carbolfuchsin solution (Roth) for 5 min.[16] Subsequently, the eggs were rinsed thoroughly with tap water. After air drying, an optical assessment of the shell colour was carried out.

2.6 | The effect of atmospheric pressure plasma treatment on sensory properties of the egg

To determine the sensory differences between treated and untreated eggs (blinded trial), a group of four sensory assessors evaluated different parameters, which are summarised in Table 1. Half of the eggs were evaluated raw and the other half were assessed hard-boiled (cooked for 10 min) directly after treatment. On three consecutive days, five raw untreated control eggs, five hard-boiled untreated control eggs and five raw and five hard-boiled plasma-treated eggs were tested per day (15 eggs/group).

2.7 | Statistical analysis

The antimicrobial efficacy of the different treatments was calculated using the following formula: Reduction factor (RF) = $\log_{10}N^{0}_{\text{mean}}$ - $\log_{10}N^{t}_{\text{single}}$.

In this formula, $\log_{10}N^{0}_{\text{mean}}$ is the mean *S.* Enteritidis or total bacterial count per egg without treatment ($n \geq 5$) and $\log_{10}N^{t}_{\text{single}}$ is the germ count on single eggs after treatment. To assess if the data set featured a normal distribution, the Kolmogorov–Smirnov test was used.[17] To compare the treatment conditions, *t* test or one-way analysis of variance with Bonferroni's post-test was carried out. For the statistical data analysis, the GraphPad software, version 4.00 for Windows (GraphPad Software, San Diego, CA) was used.

Differences were considered to be significant if *p* was less than .05.

TABLE 1 Parameters of the sensory assessment

Parameter	Raw eggs (n = 15)	Hard-boiled eggs (n = 15)
Visual appearance		
Shell	X	X
Yolk	X	X
Albumen	X	X
Inner shell membrane	X	X
Odour		
Shell	X	X
Yolk/Albumen	X	X
Consistence of the albumen (thick-to-thin albumen ratio)	X	N/A
Curvature of the yolk	X	N/A
Texture		
Yolk	N/A	X
Albumen	N/A	X

Abbreviation: N/A, cannot be analysed.

3 | RESULTS AND DISCUSSION

3.1 | The effect of atmospheric pressure plasma treatment on the total bacterial count on the eggshell

Figure 3 shows the CFU per egg of clean and dirty eggs treated with atmospheric pressure plasma and an untreated control. Total viable cell counts of clean control eggs and dirty control eggs were 7.9×10^4 and 3.3×10^5 CFU/egg, respectively. The average reduction that was achieved for clean eggs and dirty eggs was 1.16 ± 0.51 and 0.95 ± 0.63 log CFU, respectively. No significant statistical difference was established between the different test trials or between the reduction of dirty and clean eggs. However, so far, there are no other studies about the effect of atmospheric pressure plasma treatment on the total bacterial count on the eggshell. Therefore, only different methods for decontamination can be compared. Al-Bachir et al.[18] reached reductions of total microorganisms up to 2.3 log CFU/eggshell with γ irradiation. Favier et al.[19] achieved reductions up to 4.55 log CFU/egg of aerobic bacteria with UVC radiation, whereas de Reu et al.[20] reached only reductions up to 0.9 log CFU/egg of the natural bacterial load for UVC-treated clean eggs. The latter observed no significant reduction for very dirty eggs. Our results were most likely compatible with the data of de Reu et al.[20] for clean eggs.

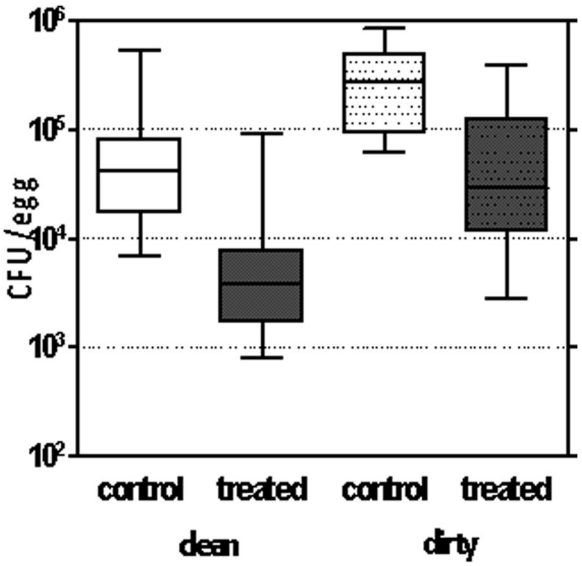


FIGURE 3 Colony-forming units per egg of clean and dirty eggs after atmospheric pressure plasma treatment and control ($n = 15$)

3.2 | The effect of atmospheric pressure plasma treatment on *S.* Enteritidis inoculated on the eggshell

The eggshells were inoculated with an average of 1.38×10^4 ($\pm 1.1 \times 10^4$) *S.* Enteritidis before treatment. Already after 300 s of treatment with atmospheric pressure plasma, no more traces of *S.* Enteritidis were detectable. Therefore, an average reduction factor of 4.1 (±0.05) log CFU was established (on XLD). However, according to Wan et al.,[10] injured cells are able to reproduce and form colonies on nonselective media (such as TSA) but not on selective media (such as XLD). They used a high-voltage atmospheric pressure plasma in a modified atmospheric gas (MA; 65% O_2, 30% CO_2, 5% N_2) for 15 min and achieved a maximum *S.* Enteritidis inactivation of 5.53 log CFU/egg on TSA and 6.37 log CFU/egg on XLD (initial contamination 10^7 CFU/egg).[10] In relation to these findings, the reduction factor of 4.1 (±0.05) log CFU that was achieved may be attenuated. Contrary to these results, Georgescu et al.[11] tested TSA and XLD medium to determine the bacterial concentration of *S.* Typhimurium and established that the reproducibility of the results was better with XLD Agar.

Davies and Breslin[7] used a Gas Plasma air ioniser for the indirect treatment of eggs inoculated with *S.* Enteritidis inside a safety cabinet with no antimicrobial effect. In contrast, Ragni et al.[8] achieved a reduction up to 4.5 log CFU/eggshell of *S.* Enteritidis and 3.5 log CFU/eggshell of *S.* Typhimurium (initial contamination 5.5–6.5 log CFU/eggshell), but only after a 90-min period (5 min in the present study) of indirect treatment with a resistive barrier discharge (RBD). They demonstrated that a higher relative humidity (RH) enhanced the effectiveness of the air plasma treatment. In contrast, pretests carried out before the present study showed that a higher RH resulted in a lower reduction factor with FEPS than dry air.

Chen[9] and Wan et al.[10] assessed two modes of HVACP treatment: a direct one, where the eggs were placed between two electrodes inside the plasma field, and an indirect one, where the eggs were located outside the plasma field. In addition, two different feed gases were used: dry air and MA gas (see above), as well as exposure times of 5–15 min. They achieved the highest reduction of approximately 5.5 log CFU/eggshell of *S.* Enteritidis after 15 min of direct treatment with MA gas. In the case of indirect treatment under the same conditions, a reduction of only 1.69 log CFU/egg was reached. Similarly, Georgescu et al.[11] investigated the inactivation of *S.* Typhimurium using dielectric barrier discharge (DBD) plasma with a direct and an indirect application. The initial contamination of *S.* Typhimurium was about 10^8 CFU/egg. For direct treatment, the He/O_2 mixture was used as the feed gas and for the indirect one, the air was used as the feed gas. They obtained the highest levels of inactivation, below the detection limit (10^2 cells/egg), at an RH of 80% after 10 min of direct and 25 min of indirect application. Dasan et al.[12] used an atmospheric pressure plasma jet for the inactivation of *S.* Enteritidis (initial concentration of 10^7 CFU/egg) on the eggshell and also achieved a reduction below the detection limit (10^2 CFU/egg) after 120 s, with air being used as the feed gas. However, taking the reduction that was achieved and the exposure times into consideration, the results of the present study were almost comparable with the findings of Dasan et al.[12] and the plasma source used in this study appears to be more effective than the plasma sources tested by Ragni et al., Chen, Wan et al., and Georgescu et al.[8–11]

To understand the differing results of the above-mentioned studies, it is important to realise that, even at an atmospheric pressure and with approximately comparable system designs, the experimental setups still differ from one study to another. Therefore, a direct comparison of the data is problematic and the differences between the studies have to be taken into account.[9,21,22] First, an indirect mode of treatment was reported to be less effective, compared with a direct one.[9–11,23–25] Misra et al.[26] explained the discrepancies associated with the recombination of the charged particles, UV radiation and short half-life species (such as O_2^-, OH^+, N_2^+, N_2O^+, etc.) before reaching the sample for indirect treatment. Only reactive species (such as O_3, O_2, NO_2 and CO) with a long

half-life were able to reach the sample outside the plasma field.

In addition, the varying durations of the plasma treatment influence the decontamination. In general, a longer exposure time leads to a decrease in the surviving bacterial population.[9,10,27–30] According to Chen,[9] a larger number of reactive gas species were generated with longer exposure times: the contact time between microorganisms and the reactive gas species increased, thus enhancing the bactericidal effect. Simultaneously, the voltage that is used must also be taken into consideration as an influencing factor. Nevertheless, a comparison between plasma sources with various designs, that is, in terms of an electrode structure or their power supply, turns out to be complicated. For instance, the electric field strength, which is an important factor for plasma ignition, strongly varies for different electrode geometries driven with the same voltage. However, more as an ordering element than as an underlying physical characteristic, various plasma sources have been discussed below, based on their maximum operating voltage. Chen[9] stated that Ragni et al.[8] used a moderate voltage of 15 kV, which may explain the relatively long exposure times in their study. In comparison, Georgescu et al.[11] used a voltage of 25–30 kV and Chen[9] and Wan et al.[10] used a voltage of 85 kV, both significantly higher than the voltage used in this study. Deng et al.[31] stated that a higher DBD voltage results in a greater number of reactive gas species and therefore greater microbial inactivation efficiency. However, in the present study, a voltage of 6 kV was used and satisfactory results were achieved. Further, the differences in the plasma sources that are used and experimental setups must be taken into account. The above-mentioned studies predominantly used DBD plasma sources in direct or indirect treatment with much greater electrode gaps or an RBD plasma source with only indirect treatment.[8–11] The newly developed plasma source of the present study adapted to eggs, featuring small electrode gaps (1.2 mm) in its construction. Therefore, it is possible to generate plasma with a relatively low-voltage output.

Furthermore, the gas composition that is used varies between the above-mentioned studies. Depending on the plasma system, one single gas or blends of two or more gases can be applied. According to Chen,[9] the gas composition determines the active species that are generated during atmospheric cold plasma treatment. For example, higher oxygen concentrations in the feeding gas led to higher amounts of O radicals, complicating a direct comparison between the studies.[29]

Finally, it should be noted that most of the studies that were carried out only investigated small inoculated parts of the eggshell. Chen[9] and Wan et al.[10] spot inoculated 0.1-ml *S.* Enteritidis inoculum on the sideway of the egg within a 3 × 3-cm area. Georgescu et al.[11] and Dasan et al.[12] spread 20 and 40 μl, respectively, of bacterial suspension on the eggshell on a 1 × 1-cm area. Only Ragni et al.[8] inoculated the entire eggshell and achieved reductions equal to our data with indirect treatment. However, a treatment time of 90 min was necessary, contrary to an only 5-min direct treatment with the FEPS presented here.

3.3 | The effect of atmospheric pressure plasma treatment on selected quality parameters

The pH value of the albumen, the height of the air cell and Haugh units demonstrated no significant statistical differences between treated and untreated eggs. The mean pH value ranged between 8.61 and 9.29, thus being in the expected range. Freshly laid eggs exhibit a pH value of 7.6. During storage, CO_2 escapes through the pores in the eggshell, which means that the pH value rises and, after a few days, reaches a stable value of 9.5.[32]

The height of the air cell (as a sign of freshness) was ≤2.5 mm before and after treatment.

The average Haugh unit measured as another indicator of freshness was 75.57 (±6.16), which is comparable with grade AA according to the definition provided by the U.S. Department of Agriculture.[15] No significant difference was found between the individual tests or between treated and untreated eggs.

Other research groups already investigated the effect of atmospheric pressure plasma on selected quality parameters of eggs. For instance, Ragni et al.[8] analysed the yolk index (another indicator of freshness), the weight loss and the albumen pH value after 90-min treatments at an RH of either 35% or 65% and 25°C. Chen[9] compared the egg weight, Haugh units, yolk colour, pH value of the albumen and yolk, vitelline membrane strength and the shell strength of eggs treated directly with HVACP in MA gas (see above) for 15 min with untreated controls. Wan et al.[10] described the same quality parameters, except the shell strength for the same plasma source. Georgescu et al.[11] assessed the albumen pH, Haugh units and yolk index, and Dasan et al.[12] evaluated the albumen and yolk pH value, yolk colour, Haugh units, and yolk index. All the above-mentioned data, including our data, showed that the different plasma treatments did not have a significant statistical influence on the quality parameters.

With regard to the intactness of the cuticle, the untreated control eggs showed a pink colour overall in our

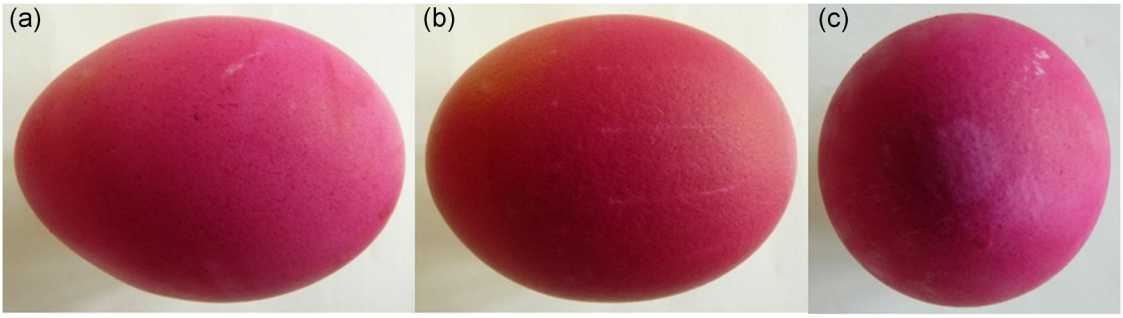


FIGURE 4 Eggshell staining of an untreated egg (a) and after plasma treatment (b and c)

study with natural variances after eggshell staining (Figure 4a). After 300 s of atmospheric pressure plasma treatment, the eggshell staining showed the pattern of the electrode arrangement of the plasma device (Figure 4b) to a minor extent. Beneath the electrodes, the eggshell was lightened or unstained, as shown in Figure 4c, which may be the result of an interaction between the cuticle and reactive species of the plasma. Otherwise, the contact of the eggshell with the elastomer inlay of the FEPS may inhibit the dyeing process. For clarification purposes, scanning electron microscopy (SEM) examinations of the cuticle should be carried out. Also, Ragni et al.[8] investigated the cuticle intactness, using also a green solution containing Tartrazine and Green S, and qualified the dye using a reflectance colorimeter. After treatment, the eggshell colour at an RH of 35% indicated that the cuticle of these eggs was able to bind more green solution, but the colour difference was very low; therefore, the authors attributed this phenomenon to the heterogeneity of the substrate of the eggs. They also analysed the cuticle using the SEM technique, as did Georgescu et al.[11] and Dasan et al.[12] In all three studies, the images showed that the atmospheric pressure plasma treatment did not damage the egg cuticle and the integrity was preserved.

3.4 | The effect of atmospheric pressure plasma treatment on sensory properties of the egg

Slight sensory deviations were detected for a total of 26 out of 30 eggs (86.6%), which were mostly caused by plasma instabilities of the prototype device. Table 2 shows the sensory deviations of the eggs in detail.

The chemical sour odour was only connected to the shell, whereas the albumen and the yolk of affected eggs still had a neutral odour.

The scorch marks on the shell and inner shell membrane were often found at the equatorial plane of the eggshell (Figure 5a,b). The odour of burned protein was connected with scorch marks on the shell in all cases and only noticeable on the inner shell membrane, the albumen and the yolk, whereas the eggshell still had a neutral odour.

In total, 23% of all eggs showed localised scorch marks. The protein coagula (Figure 5d) occurred alongside scorch marks of the shell (with one exception), which is a consequence of a high temperature developing on the eggshell, presumably by a locally too high energy input or punctiform (filamentary) discharges. Simultaneously, two eggs showed cracks on the shell (intactness

TABLE 2 Sensory deviations of atmospheric pressure plasma-treated eggs

Localisation	Deviation	Number of raw eggs ($n = 15$)	Number of cooked eggs ($n = 15$)
Shell	Chemical, sour odour	12	1
Shell	Streaky, shiny surface	5	2
Shell	Yellow strokes	1	7
Shell/inner shell membrane	Scorch mark	7	0
Yolk/albumen	Odour of burned protein	7	0
Albumen	Coagulum	6	N/A
Albumen/inner shell membrane	Yellow discolouration	0	3

Abbreviation: N/A, cannot be analysed.

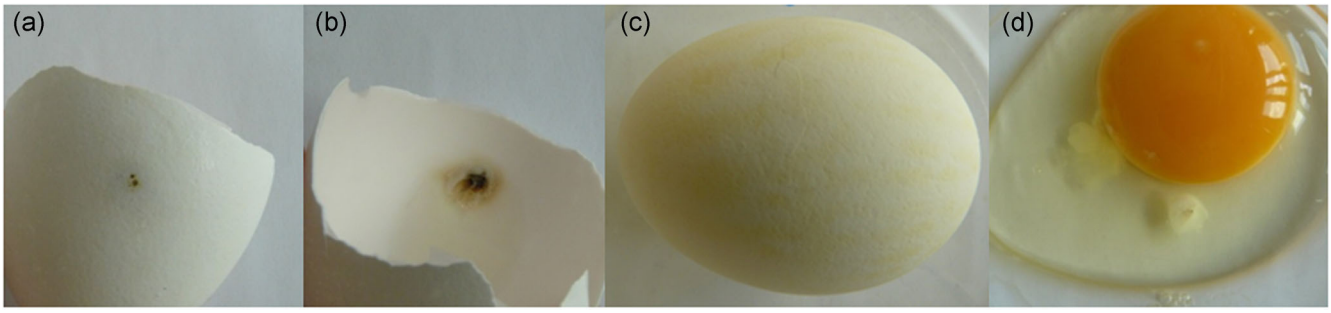


FIGURE 5 Some sensory deviations that were detected: scorch marks (a and b), yellow strokes (c), protein coagulum (d)

was verified before the treatment), which were also established to be connected to scorch marks. The streaky shell surface and the yellow strokes on the shell (Figure 5c) appear to correspond with the results of the eggshell staining.

The negative effects of the atmospheric pressure plasma treatment are caused by plasma instabilities of the prototype device. This can be minimised or prevented by using more sophisticated parameter control techniques. Natural variations in the size, perimeter and shape of eggs present a major challenge for the development of a plasma source. There is also a significant connection between the scorch marks on the eggshells and the structure of the FEPS. These were only observed at the equatorial plane of the eggshell where the two halves of the FEPS join together. Georgescu et al.[11] stated that in the case of direct DBD plasma treatment of eggs in air, hot spots on the filamentary discharge developed, which would be destructive for the eggshell in a localised manner. This may be a possible explanation for the scorch marks that were observed, the protein coagula and the odour of burned protein. However, Chen[9] and Wan et al.[10] used dry air with DBD HVACP, without reporting any negative effects on the eggshell. To clarify the cause of the negative effects that were observed in the present study, further diagnostic research on plasma stability, temperature development and reactive species that are generated is necessary to optimise the FEPS equipment.

4 | CONCLUSION

In the present study, a statistically significant reduction of *S.* Enteritidis (4.1 log CFU) was achieved with the FEPS plasma source adapted to eggs (after a 300-s treatment time and a feed gas flow of 100 sccm of compressed air). Furthermore, no significant differences in the pH value of the albumen, the height of the air cell and Haugh units were observed after atmospheric pressure plasma treatment. However, slight changes of the eggshell staining in odour and local scorch marks could be determined. Presumably, the first ones are likely to be negligible. To eliminate the local scorch marks, in particular, the plasma source must be optimised. In comparison to other plasma treatment systems, the use of compressed air, the short exposure time of 300 s (exposure time and achieved a reduction of *S.* Enteritidis were comparable or better than in other studies), the low-voltage output and the modular construction of the FEPS constitute advantages of this plasma treatment system. Furthermore, the whole egg was inoculated, and for the first time, sensory properties were analysed. Ultimately, practicability, technical feasibility for industrial applications and cost–benefit considerations determine the current plasma system. The modular design and the use of compressed air allow a realistic industrial application of the newly developed plasma source. Any arrangement of the plasma source in form and number to large modules (pallets) is possible. An adaptation and integration into a continuously running production chain can be implemented. By exchanging the individual modules, simple repair and assembly are possible. However, for a final recommendation, a field test has to be carried out, which was beyond the objective of this study.

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AUTHOR CONTRIBUTIONS

M. M. carried out the experiments, collected data, analysed and interpreted the results and drafted the paper. C. W. designed the study, interpreted the results and drafted the paper. P. G. B., J. E. and K.-D. W. designed and supervised the project, drafted and revised the paper. T. W. performed the plasma experiments together with M. M.

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