Cold atmospheric pressure air plasma jet disinfection of table eggs

1 **Cold atmospheric pressure air plasma jet disinfection of table eggs:**
2 **Inactivation of *Salmonella enterica*, cuticle integrity and egg quality**
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Abstract

17 Eggshell cuticles are first lines of defense against egg-associated pathogens, such as *Salmonella*
18 *enterica* serovar Enteritidis (SE). Infections from eggs contaminated with this strain remain a
19 significant risk. In addition, changes in the cuticle are closely related to changes in egg safety. The
20 upcoming non-thermal atmospheric pressure plasma technology enables high rate of microbial
21 inactivation at near-ambient temperatures, making it ideal for food safety applications. This study
22 examines the effects of a cold atmospheric pressure air plasma jet (CAAP-J) on eggshell cuticle
23 and egg quality whilst inactivating SE. Samples of shell eggs inoculated with SE (7 $\log_{10}$ cfu/egg)
24 were used to test the decontamination performance of the device with an industrial CAAP-J with
25 different power levels (600-800 W), exposure times (60-120 s), at a certain distance from the
26 plasma jet (20 mm) and air flow rate (3600 L/h). It was found that the best results were obtained
27 by reducing the number of SE colonies on the eggs' surface to below the detection limit (10
28 cfu/egg) after 120 s at maximum plasma power. After CAAP-J treatment, the temperature remains
29 below 50.5°C there by minimizing the risk of altering egg quality. All specific measurements (egg
30 white pH, yolk pH, yolk color, HU, and eggshell breaking strength) have shown that CAAP-J
31 treatment has no negative effect on egg quality. No changes in eggshell cuticle quality after CAAP-
32 J treatment was confirmed through scanning electron microscope (SEM).

33 **Keywords:** *Salmonella,* Cold plasma, Eggshell decontamination, ATR-FTIR, Egg quality,
34 chemical composition
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1. Introduction

Salmonella contamination of eggs is one of the leading reasons for food-borne human outbreaks global (Kouam et al., 2018; Wan et al., 2017; Lin et al., 2021). *Salmonella enterica* serotype Enteritidis (SE) is the most common *Salmonella* serotype found in eggs and is a Gram-negative bacterium that causes foodborne salmonellosis (Shah et al., 2017). Egg contamination with *Salmonella* generally occurs in two ways: contamination of the inner eggs and contamination of the outer shell surface.The outer surface of the eggs must have a contaminated environment to cause contamination of the shells, which could eventually result in internal contamination by bacteria penetrating through the shell. In *Salmonella* control programs, it is important to properly handle eggshells (Lin et al., 2021a, 2021b).

The washing of eggs is one of the most common methods of decontamination in poultry farms in Asian countries, the United States (USA), Canada and Australia. However, in European Union countries, washing or cleaning eggs in shell is not allowed, because these procedures can damage the outer layer of the egg (cuticle) and increase the risk of eggs becoming infected with microorganisms including *Salmonella* getting into the eggs. In addition, the decontamination of eggs by gamma rays or electron beams has been banned in Europe (Muñoz et al., 2015; Afari et al., 2016; Dasan et al., 2018). Therefore, the poultry industry need to have effective and economical disinfection systems in place to ensure consumers have safe eggs that are easily accessible . Technologies are currently being developed to improve both the quality and intrinsic functional properties of eggs or their components. A variety of technologies, including pulsed electric fields.

A variety of technologies including pulsed electric fields (Liu et al., 2019), high-pressure processing (Naderi et al., 2017), ultrasonics (Yüceer & Caner, 2020), microwaves (Li et al., 2018), radio frequency (Yang et al., 2019), and ultraviolet light (Holck et al., 2018) have been used for egg products. Using novel technologies over thermal treatments have many advantages, especially when it comes to protect the heat-sensitive nature of eggs; however, eggs may lose some of their sensory and nutritional properties with these technologies (Afraz et al., 2020). It is possible to inactivate microbes by cold plasma (CP) technology, while perserving the quality of fresh products (Wang et al., 2022).

65 CP is not only chemical-free and environment friendly, but also has a high potential to reduce the
66 microbial load of eggs while maintaining the quality characteristics of the product (Lin et al.,
67 2021a). This treatment also inactivates a variety of pathogens such as viruses, fungi and spores.
68 However, different parameters and mechanisms influence the extent of damage that occurs under
69 such disinfection technique (Barroug et al., 2021). Some of the major reactive agents responsible
70 for inactivating microbial targets are reactive oxygen species (ROS) (superoxide anion, singlet
71 oxygen, and ozone), reactive nitrogen species (RNS) (atomic nitrogen, nitric oxide, and excited
72 nitrogen), neutral particles, charged particles and ultraviolet (UV) light (Patange et al., 2019; Ng
73 et al., 2021). Etching of bacterial cell surfaces, erosion of their morphology, damage to nucleic
74 acids, oxidation of proteins and loss of viability are the mechanisms attributed to CP disinfection
75 (Ulbin-Figlewicz et al., 2015).

76 The severity of damage varies depending on the different parameters and mechanisms of CP. There
77 are both process factors (intensity of energy source, duration of treatment, type of exposure (direct
78 or indirect), gas mixture, product factors (type of treated samples (liquid, solid, or semi-solid),
79 surface topology and characteristics of target cells)) that influence the antimicrobial effect of CP
80 (Lu et al., 2014; Patange et al., 2019; Barroug et al., 2021). Bourke et al. (2017; 2018) reviewed
81 various types of food samples, such as fresh fruits and vegetables, meat and milk products, and
82 fruit juices, that were nonthermally decontaminated with atmospheric plasma treatments. Several
83 studies have demonstrated the lethal effect of CP against bacteria on food contact surfaces (Dasan
84 & Boyaci, 2018; Ansari et al., 2022; Sruthi et al., 2022).

85 It is interesting to note that the standard measurements of eggshell quality do not take into account
86 the quality of the cuticle, which is an important selection tool for increasing productivity in the
87 poultry industry. Nevertheless, there are several methods for determining cuticle quality. It is
88 possible to analyze a large number of samples using these methods, but do not provide information
89 about the cuticle's chemical composition. Cuticles function differently depending on their chemical
90 composition. For example, protein content affects cuticle resistance to bacterial invasion. Fourier
91 transform infrared spectroscopy with attenuated total internal reflection (ATR-FTIR) is a
92 characterization method that measures cuticle thickness, amount and chemical composition
93 (Rodríguez-Navarro et al., 2013; Bain et al., 2019; Réhault-Godbert et al., 2021; Kulshreshtha et
94 al., 2022).

The aim of this study is to investigate the effect of industrial cold atmospheric pressure air plasma jet (CAAP-J) treatment of commercial table eggs (refrigerated chicken eggs) to control SE on the shell surface. A major advantage of this type of plasma system is its ability to operate at atmospheric pressure in air. In addition, the CAAP-J used in the current study provides larger plasma sizes and faster processing speeds than similar systems. SE on eggshells was evaluated using air as the process gas for different treatment times and plasma power levels. After CAAP-J treatment, egg quality was also evaluated by measuring cuticle, Haugh unit (HU), yolk color, albumen and yolk pH. In the present study, the ATR-FTIR technique was used to characterize the quality of the cuticle, including its chemical composition.

2. Materials and methods

2.1. Bacterial strain and cell suspension preparation

SE culture was obtained from the Poultry Science Department, Tarbiat Modares University (Tehran, Iran). The stock culture was maintained at -80°C with 50% glycerol. To prepare a fresh working culture, 200 μL of the frozen culture was inoculated in 10 mL. For eggshell inoculation, the cell suspension was incubated at 37°C for 24 h in Tryptic Soy Broth (TSB) (DifcoTM, MD, USA). To determine the bacterial concentration, the strain was inoculated onto Xylose Lysine Deoxycholate Agar (XLD) (DifcoTM, MD, USA). The cell concentration in the initial inoculum was 8 $\log_{10}$ CFU/mL (Wan et al., 2017).

2.2. SE inoculation on shell eggs

Grade A eggs were purchased from a local bulk supplier (Telavang Company, Tehran, Iran). Eggs were used only if they did not contain surface residues. At first, wipes sprayed with 70% ethanol were carefully used to decontaminate the surfaces of the eggs. A laminar flow hood was used to dry the pre-decontaminated eggs for 10 min before inoculation. To reach 10^8 CFU/mL, the SE inoculum was centrifuged at 7000 x g for 5 min at 4°C and resuspended in new TSB. An area of 36 spots was spot inoculated with 0.1 mL SE inoculum on the lateral surface of the eggs. To dry the eggs, they were placed in a laminar flow cabinet after inoculation for 30 min. After this procedure, a constant population of 10^7 cfu/egg SE cells was transferred to the inoculated eggs (Wan et al., 2017).

2.3. Cold plasma treatment

The CAAP-J from Kavosh Yaran Fann-e Pouya Company (model: ACPJ-17A, Iran) was used for eggshell disinfection. Air compressor, power supply, and plasma jet nozzle had the three main subsystems. A power generator provides the energy required to generate ionization in the plasma nozzle between two electrodes. A peak voltage of 10 kV and a frequency of 20 kHz are generated by this power source, which generates an average power of approximately 700 W. The compressor blows dry and clean air into the nozzle at a constant flow rate of 60 L/min. Fig. 1 shows the schematic diagram of the plasma nozzle, in which the high-frequency arc discharge is combined with the airflow to produce a uniform plasma discharge (Maroofi et al., 2020).

2.4. Evaluation of bacteria survival on the egg surface

Positive controls (inoculated, untreated), negative controls (uninoculated, untreated) and treated eggs (inoculated) were analyzed for microbial population recovery. Serially diluted suspensions of the resulting solution were prepared with water containing 0.1% peptone and plated onto XLD agar (specific for *Salmonella*) according to Miles et al., 1938. After 24 h of incubation at 37°C, counts were performed. For each experiment, at least three replicates were performed (control and CAAP-J treatment) (Wan et al., 2017).

2.5. Egg quality assessment

A comparison of untreated and plasma-treated eggs was performed using egg white pH, yolk pH, yolk color, HU, and eggshell breaking strength. An Egg Multi-Tester (EMT- 5200, Robotmation Co, Japan) was used to measure HU in eggs. Egg white and yolk pH were measured using a pH meter (model 220, Denver Instrument, USA) and a pH probe (IQ150, Spectrum Technologies, Israel). Two points 7.0 and 10.0, were used to calibrate the pH meter prior to testing and pH measurements were taken in triplicate after measuring egg quality. Eggshell strength was determined using an eggshell force gauge (model 0502, Tacknox Headquarters Factory, South Korea).

Several photographs of the samples were used for processing and analysis to determine the color of the yolk. The digital camera (model 6.3, Nokia Inc, India) was held under a certain lighting condition at a vertical distance of 20 cm from each yolk sample. Only the highest resolution and quality images were selected for image analysis. The images were captured at resolution of 1080

154 × 2340 pixels, then they were transferred to a PC and processed using MATLAB R2020b. Data

155 processing was employed to extract the colorimetric data. The variables measured are L^*, a^*, and

156 b^*, where L^* is the light index, a^* and $-a^*$ are the redness and greenness, b^* and $-b^*$ are the

157 yellowness and blueness. Color changes (ΔE_{ab}^*), chroma (C^*) and hue (H^*) are calculated as

158 follows:

159 $\Delta E_{ab}^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$ (1)

160 $C^* = \sqrt{a^{*2} + b^{*2}}$ (2)

161 $H^* = \tan^{-1}(\frac{b^*}{a^*})$ (3)

162 In this case, ΔL^{*2}, Δa^{*2} and Δb^{*2} were all calculated based on the untreated samples. Color changes

163 after CAAP-J treatment are measured by ΔE_{ab}^*(Dasan et al., 2018; Lin et al., 2021 b).

2.6. Scanning electron microscope observation

165 To assess the extent of cuticle damage caused by CAAP-J treatment, scanning electron microscope

166 (SEM) (FEI Quanta, Thermo Fisher Scientific Inc, USA) was used. Eggshell pieces of 1×1 cm^2

167 were coated with a thin layer (5 nm) of gold-palladium for SEM analysis. SEM in high vacuum

168 mode was used to analyze the samples after coating with an accelerating voltage of 20 kV (Dasan

169 & Boyaci, 2018).

2.7. Optical emission spectroscopy

171 A computer-controlled optical spectrometer recorded the emission spectra of shell eggs treated

172 with CAAP-J. The optical emission spectroscopy (OES) (Emerald C0R10, Teksan Co, Iran) data

173 was collected over a wavelength range from 200 to 1100 nm with a resolution of 0.5 nm to identify

174 CP species and detect their excited states. The atomic spectra database was used to analyze the

175 corresponding spectra and identify the species (Maroofi et al., 2020).

2.8. Infrared spectroscopy

177 An infrared spectrometry study was performed using the methods described by Rodríguez-Navarro

178 et al. (2013) and Kulshreshtha et al. (2022). Infrared spectra were recorded at a resolution of 2 cm$^-$

179 ^{1} over 100 scans using an FTIR spectrometer using eggshells that were approximately 0.5×0.5

180 cm^2 were pressed against the ATR diamond crystal window (Spectrum 400, PerkinElmer Co,

181 USA). Water, proteins, and polysaccharides are determined using the peak area of the absorption

182 peaks associated with a particular molecular group (i.e., O-H: water, amide: proteins, C-O-C:

183 polysaccharides). Integrated areas of main bands were measured and normalized to the total

184 spectrum area to resolve overlapping peaks (e.g., carbonate in 900-1800 cm^{-1}; O-H in 900-4000

185 cm^{-1}) (Rodríguez-Navarro et al., 2013; Muñoz et al., 2015; Kulshreshtha et al., 2018, 2022).

186 **2.9. Temperature monitoring**

187 The surface temperature of the eggs was measured using an infrared-based thermographic camera

188 (Fluke Ti32, Evrett, USA). In order to effectively study the effect of plasma gas on artificially

189 contaminated eggs, the infrared camera was placed horizontally in close contact with a plasma gas

190 generator during each treatment to ensure that the temperature of the egg did not rise above a

191 certain threshold (50.5°C) and did not affect the quality of the egg. The plasma-treated samples

192 and the control samples were examined in three replicates.

193 **2.10. Statistical analysis**

194 Statistical significance of differences between treatments was determined by factor analysis of

195 variance (ANOVA) and the LSD test ($p < 0.01$). The SAS 9.0 statistical package (SAS Inc,

196 Chicago, IL) was used for data analysis.

197

198 **3. Results and discussion**

199 **3.1. *Salmonella* inactivation**

200 Table 1 shows the recovery results for inoculated chicken eggs from selective (XLD agar) medium

201 after CAAP-J treatment. Direct exposure to CAAP-J resulted in a significant ($p < 0.01$) reduction

202 in the population of SE. The decrease was proportional to the increase in time and power. No

203 viable cells were detected after the plasma process at 800 W in 120 s. Thus, after 120 s of CAAP-

204 J treatment, SE was inactivated by more than 7 $\log_{10}$. Treatment of the eggshell with plasma for

205 120 s eliminates the microorganisms on the surface. Due to the higher plasma power used for

206 bacterial inactivation, a shorter treatment time was required to detect the surviving cells. In this

207 study, the artificial contamination of eggshells was in the order of 7 $\log_{10}$ (cfu/egg), while in

208 another study, aerobic microorganisms were found in eggshells in concentrations of 10^3-10^4

209 cfu/egg (Musgrove et al., 2005; Dasan & Boyaci, 2018).

210 A higher initial microbial load can result in stacked cells on the sample surface as they are shielded

211 from plasma species generated during CAAP-J, there by reducing the effectiveness of inactivation

212 (Fernández & Thompson, 2012). Treatment of the eggshell with plasma for 120 s eliminates the

213 microorganisms on the surface. Pasteurization of shell eggs was predicted to have a positive
214 impact, which reduced the level of SE by three logs, which caused to reduce diseases by this
215 organism till 70% (USDA/FSIS, 2013). It is therefore possible to reduce the risk of salmonellosis
216 by treating eggshells with CAAP-J within a reasonable time using this alternative method.

217 The minimum plasma inactivation time of SE cells was 60 s at a distance of 20 mm from the
218 electrode structure and when the distance increased, the number of surviving microorganisms was
219 significantly high. SE reduction appears to be influenced by the distance between the plasma
220 nozzle and eggshell. Inactivation effects of CAAP-J were significantly reduced when sample
221 distance was increased from plasma. Plasma temperature and energy as well as the reactive species
222 composition are affected by the distance between the plasma source and the treated sample. There
223 is evidence that sterilisation efficacy decreases with increasing distance (Moritz et al., 2021). As
224 the distance between the sample and the reactive species increased, fewer reactive species reached
225 the sample and the microorganisms were less inactivated (Fricke et al., 2012).

226 The use of plasma or another processing method could either cause the cell to die or damage it,
227 rendering the cell unusable for culture or not completely inactivated. In addition, previous studies
228 have shown that the damage to the microorganisms caused by plasma processes was not temporary,
229 so that the damage to the cell structure could not be repaired during storage. After plasma
230 treatment, the damaged cells remained viable but could not grow due to the damage sustained
231 during storage. For this reason, the number of surviving cells would not increase after plasma
232 treatment but, on the contrary, is expected to decrease during storage after plasma treatment. Major
233 disadvantages could be the longer treatment times required for *Salmonella* reduction and the use
234 of relatively expensive process gases (Dasan et al., 2016b; Dasan et al., 2017; Saremnezhad et al.,
235 2021b).

236 Both the generation of the plasma (CAAP-J) and the gas used as the process gas (air) could play a
237 role in achieving this high level of inactivation in a relatively short time. A greater number of
238 chemically active species were found in the air than in other gases. Compared to other gases, air
239 contained a larger number of chemically active species. A number of radicals are involved in the
240 inactivation of air plasma, including O_3, OH and NO radicals, NO_2^- and NO_3^-; in addition, H_2O_2 is
241 formed by OH radical reactions and also plays an important role. As well as facilitating the
242 movement of chemically active species, frequent air circulation serves as a means of refreshing

243 the air in the plasma zone. As a result, inactivation with plasma generated in still air is significantly

244 less effective as the active species move extremely slowly to the surface of the egg (Georgescu et

245 al., 2017; Moritz et al., 2017; Ott et al., 2021).

246

247 **Table 1** Numbers of SE cells ($\log_{10}$ cfu/egg) surviving exposure to CAAP-J for different treatment times

Time (s)	Power (W)		
	600 W	700 W	800 W
0	7.22 ± 0.20^a	7.22 ± 0.20^a	7.22 ± 0.20^a
60	5.70 ± 1.03^b	5.40 ± 1.40^b	5.55 ± 0.58^b
90	5.24 ± 0.55^b	5.09 ± 0.17^b	4.67 ± 0.13^b
120	5.10 ± 0.45^b	5.00 ± 0.75^b	$< 1^c$

248 1^c represents the detection limit
249 Mean ± standard error is used to express the results
250 The different letters indicates a significant difference (p < 0.01)

251

252 **3.2. Egg quality analysis**

253 Table 2 compares untreated (control), and CAAP-J treated eggs with respect to quality parameters.

254 There was no statistically significant difference in any of the parameters analyzed. For comparison,

255 the HU of control eggs was 67.8, while after treatment, the HU ranged from 56.77 to 62.20. Plasma

256 was found not to change eggshell strength; the pH of albumen and yolk, significantly after CAAP-

257 J treatment.

258

259 **Table 2** Egg quality parameters after CAAP-J treatment
260

Time (s)	Treatments	HU	Force strength eggshell (N)	Albumen pH	Yolk pH
0	Control	67.80 ± 7.73^a	37.37 ± 1.18^a	9.25 ± 0.17^a	7.21 ± 0.18^a
60	600 W	56.77 ± 8.90^a	39.24 ± 5.30^a	9.25 ± 0.08^a	6.98 ± 0.13^a
	700 W	67.00 ± 11.51^a	37.96 ± 6.08^a	9.28 ± 0.25^a	6.91 ± 0.37^a
	800 W	62.43 ± 11.01^a	29.33 ± 9.81^a	9.26 ± 0.14^a	6.92 ± 0.56^a
90	600 W	56.63 ± 18.77^a	32.96 ± 3.43^a	9.60 ± 0.18^a	7.09 ± 0.19^a
	700 W	60.33 ± 7.61^a	39.34 ± 8.34^a	9.44 ± 0.15^a	6.91 ± 0.18^a
	800 W	67.17 ± 14.04^a	36.98 ± 4.32^a	9.43 ± 0.04^a	6.92 ± 0.15^a
120	600 W	63.67 ± 4.84^a	34.24 ± 2.75^a	9.55 ± 0.24^a	7.24 ± 0.19^a
	700 W	57.10 ± 12^a	36.59 ± 1.47^a	9.47 ± 0.15^a	6.97 ± 0.03^a
	800 W	62.20 ± 4.70^a	32.25 ± 10.79^a	9.37 ± 0.21^a	6.84 ± 0.34^a

261 Mean ± standard error is used to express the results
262 The difference letters indicates a significant difference (p < 0.01)

263

264 The color parameters of each experiment are shown in Table 3. There was no visual difference in

265 color between the treated egg yolk samples and the control samples. It was not significant to detect

266 any differences in color parameters after CAAP-J treatment. Natural pigments called carotenoids

9

267　give egg yolks their yellow to dark bright orange color. Processed shell eggs are therefore at risk

268　of color fading due to oxidation of the carotenoids. No significant difference in yolk color and

269　overall change in color (ΔE_{ab}^{*}) was observed after CAAP-J treatment.

270

271　**Table 3** Egg yolk color parameters after CAAP-J treatment

Time (s)	Treatments	L^{*}	a^{*}	b^{*}	C^{*}	H^{*}	ΔE_{ab}^{*}
0	Control	77.33 ± 0.58^{abc}	0.39 ± 0.54^{abc}	40.00 ± 1.01^{abc}	40.00 ± 0.01^{abc}	89.44 ± 0.24^{ab}	-
60	600 W	80.60 ± 0.04^{a}	1.04 ± 0.61^{a}	37.99 ± 0.80^{c}	38.00 ± 0.04^{c}	88.43 ± 0.04^{b}	3.89 ± 0.28^{ab}
	700 W	78.25 ± 0.38^{abc}	0.34 ± 0.04^{abc}	41.11 ± 0.74^{a}	41.11 ± 0.22^{a}	89.53 ± 0.21^{a}	1.44 ± 0.28^{a}
	800 W	72.85 ± 0.49^{c}	0.39 ± 0.54^{abc}	42.12 ± 0.88^{a}	42.13 ± 0.45^{a}	89.47 ± 0.45^{a}	4.95 ± 0.54^{ab}
90	600 W	82.62 ± 0.67^{a}	1.06 ± 0.59^{a}	39.89 ± 0.97^{bc}	39.90 ± 0.34^{bc}	88.44 ± 0.34^{b}	5.33 ± 0.59^{abc}
	700 W	82.93 ± 1.41^{a}	1.05 ± 0.04^{a}	38.42 ± 1.35^{bc}	38.43 ± 0.17^{bc}	89.44 ± 0.17^{b}	5.85 ± 0.04^{abc}
	800 W	76.04 ± 0.06^{bc}	0.86 ± 0.86^{abc}	40.64 ± 0.64^{ba}	40.65 ± 0.52^{ba}	88.79 ± 0.43^{b}	1.51 ± 0.86^{a}
120	600 W	80.83 ± 1.75^{ab}	0.97 ± 0.30^{ba}	37.99 ± 0.85^{c}	38.00 ± 0.24^{c}	88.54 ± 0.24^{a}	4.08 ± 0.03^{ab}
	700 W	77.37 ± 0.32^{abc}	0.16 ± 0.03^{bc}	42.16 ± 1.71^{a}	42.16 ± 0.32^{a}	89.78 ± 0.16^{a}	2.17 ± 0.03^{ac}
	800 W	78.03 ± 0.08^{abc}	0.08 ± 0.08^{c}	38.33 ± 0.26^{c}	38.33 ± 0.08^{c}	89.88 ± 0.08^{a}	1.84 ± 0.08^{a}

272　Mean ± standard error is used to express the results
273　The difference letters indicates a significant difference (p < 0.01)
274

275　### 3.3. Temperature measurement

276　To maintain the quality of the sample during CP treatment, the CAAP-J system parameters such

277　as input power, treatment time, the temperature during treatment and gas type should be optimized

278　(Gavahian et al., 2018). In the experiments, it was found that the maximum temperature of the

279　eggshell surface depended on the exposure time. As the exposure time increased, the range of

280　maximum temperature increased. Around this area of longer axis (length), the temperature dropped

281　rapidly. At a distance of 20 mm, a 120 s treatment at maximum power (800 W) with an air gas

282　flow rate of 3600 L/h resulted in a maximum temperature of 50.5°C. By increasing the distance to

283　30 mm, the temperature dropped to 40.6 °C. An exposure time of 60 s was carried out with 600 W

284　and an air admixture of 20 mm, the lowest measured temperature was 35°C. During all

285　experiments, the temperature on the eggshell did not rise above 50.5°C. Eggshell temperature was

286　found to be affected by several factors, including the distance between the eggshell and the plasma

287　jet nozzle, the amount of gas supplied to the nozzle, the rate at which the gas flowed, the input

288　power, and the exposure time. Similar results have been shown by other researchers (Moritz et al.,

289　2017; Hernández-Torres et al., 2022).

3.4. Scanning electron microscope observations

Eggshell damaged cuticle can lead to a lower freshness index as well as bacterial invasion. For better understanding the destructive changes in the surface layers of the cuticle after CAAP-J treatment, SEM analysis of the egg surface was performed. A comparison of SEM images of the egg surface before and after plasma treatment is shown in Fig. 2. This was the best treatment condition (800 W, 120 s) for inactivation of SE. As shown in the images, these eggs have a well-covered cuticle with the typical appearance of cracked mud. The protective egg cuticle was preserved after CAAP-J treatment with no visible microscopic damage. According to a study by Chen et al. (2019) and Dominguez-Gasca et al. (2017), surface cuticle and egg freshness quality are closely related together. In these studies, bacteria were less likely to penetrate through the cuticle of the eggs, as less damage to the cuticle resulted in higher freshness indices. Consequently, CAAP-J treatment did not cause cuticle damage compared to the control, making it a suitable technology for pathogen inactivation.

3.5. Optical emission spectroscopy of CAAP-J discharge

Fig. 3 shows the emission spectra of the CAAP-J treatment. A large part of the emission is in the range from 212 to 400 nm in the near UV spectrum of air. The singlet oxygen atom emission line was observed at 777 nm. There is a high intensity of nitrogen and oxygen emission lines in this spectrum, as would be expected from atmospheric air plasma. The different states of nitrogen, oxygen and hydrogen are reflected in the emission lines in the Fig. 3. It is known that a plasma discharge produces a large number of excited atomic species (such as H, O and N) and molecular bands (such as N_2^+, N_2, NO, O_2, OH). These molecules are able to generate electron impact excitations and dissociations, which generally act as antimicrobial agents.

According to these results, CAAP-J induces reactive nitrogen species (RNS) as well as reactive oxygen species (ROS). The formation of OH radiators increases the killing rate of bacteria and endospores. The low intensity of the OH peak is indicative of the nonthermal nature of the plasma source employed in this study. It is well known that NO is biologically significant and has strong antibacterial properties (Lin et al., 2016; Maroofi et al., 2020; Salgado et al., 2021; Ansari et al., 2022). The use of different gases in plasma generates a variety of radicals and species, which means that the chemical effects of plasma vary depending on the gas. The results of this study are

320 similar to those of other studies on atmospheric pressure plasma (Misra et al., 2014; Sarangapani

321 et al., 2016; Tolouie et al., 2021).

322

323 **3.6. Infrared spectroscopy**

324 According to Fig. 4, the main chemical components of the eggshell cuticle show a change in ATR-

325 FTIR peak intensity. Detailed information on the chemical composition and quality of the cuticle

326 was obtained from ATR-FTIR analysis of eggshell surfaces. According to the FTIR spectra of the

327 cuticle, the following features emerge: O-H and amide A groups are associated with water and

328 proteins in the band from 3700 to 2500 cm^{-1}; the peak of the protein amide I group is at 1630 cm^{-1}

329 . It also shows peaks from about 1419 to 1424 cm^{-1}, which are related to carbonate groups in the

330 calcite crystals formed from the eggshells and carboxylate groups in amino acid residues.

331 Polysaccharides are associated with a broad band at 1100 to 990 cm^{-1}. Smaller bands associated

332 with lipid C-H groups appear in the spectra between 2876 and 2923 cm^{-1}.

333 In their IR spectra, thin cuticle eggshells showed strong carbonate peaks but weak amide or

334 polysaccharide bands. With decreasing shell coverage or thickness, the eggshell mineral became

335 more exposed at the surface and therefore enhanced its carbonate peak. As a result, the spectrum

336 of an eggshell without a cuticle would consist of pure calcite (the mineral that makes up the

337 eggshell). The main transmission bands in the spectrum of calcite were identified at 713, 875,

338 1419-1424, 1031-1164, and 1799-1800 cm^{-1}, which are associated with carbonate groups

339 (Dominguez-Gasca et al., 2017; Réhault-Godbert et al., 2021; Kulshreshtha et al., 2022).

340 Sulfates, polysaccharides, and proteins (amides) appear strongly and positively correlated peaks

341 in Fig. 5. All these molecular components make up form glycoproteins, which are the main organic

342 components of the cuticle. The intensity of the amide peaks and the main carbonate peaks showed

343 a significant negative correlation. Proteins covering the surface of eggshell may decrease when the

344 cuticle coverage decreases. A decrease in the amount of protein may also be caused by an increase

345 in the amount of mineral carbonate substrate exposed to the eggshell surface, which then produces

346 more IR spectrum emission. Therefore, the intensity ratio between the main carbonate peak and

347 the amide I peak can be used as an indicator of cuticle quality. Comparing the intensity ratio

348 between polysaccharide and amide peaks can be useful for determining the degree of glycosylation

349 of cuticle proteins (Rodríguez-Navarro et al., 2013; Poyatos Pertiñez et al., 2020; Réhault-Godbert

350 et al., 2021).

351   To explain the increase in the cuticle signal, it is important to note that as the thickness of the
352   cuticle increases, cuticle components (namely the proteins) become more prominent, and the
353   underlying signal of the shell carbonate decreases. Plasma-treated samples exhibit an increase in
354   hydrophilic materials, resulting from the increase in absorbance between 3700 and 2500 cm^{-1}.
355   Plasma treatment significantly increasing the hydrophilicity and adhesion of eggshell surfaces to
356   air humidity (Holc et al., 2021). On the other hand, the heat of the plasma treatment on the
357   eggshells causes the proteins to gain energy and literally break the bonds between the parts of the
358   amino acid strands, causing the proteins to denature.

359   The quality of the egg's interior can be affected by lower humidity. If this factor is not controlled,
360   the eggs may lose moisture. The water can drain through the porous shell, resulting in weight loss.
361   It is common for eggs to lose two to three percent of their weight, which is hardly noticed by the
362   consumer. When losses exceed this threshold, egg contents become smaller and air cells are
363   enlarged (Criteria, 2016). Due to the hydrophilic nature of plasma and the egg's ability to absorb
364   moisture from the environment, moisture loss in the egg can be prevented, but the heat generated
365   by the plasma must be optimized.

366

4. Conclusion

368   CAAP-J treatment for 120 s resulted in a maximum SE inactivation of > 7 $\log_{10}$ cfu/egg when
369   tested on XLD agar. CAAP-J process parameters such as power levels and treatment time were
370   found to have strong interactions with microbial inactivation. Given the natural contamination of
371   eggshells with SE of 3 to 5 log (cfu/egg), the inactivation values obtained can be considered
372   promising from a safety point of view. The results of this study suggest that CAAP-J could be an
373   effective method for eggshell decontamination, significantly reducing pathogenic SE and natural
374   bacteria without affecting egg quality. Although no undesirable changes in standard quality
375   characteristics have been observed, the chemical composition of eggs has not yet been studied in
376   relation to the effects of plasma on it. In this study, a novel analytical method was used to examine
377   egg proteins, lipids and other components in interaction with reactive plasma species. It is possible
378   to improve the quality of eggs and safety of the eggs using the analytical method that consists of
379   infrared spectroscopy (ATR-FTIR). Additionally, the SEM images of the eggs show that CAAP-J
380   treatment did not damage the protective egg cuticle and that the integrity of the egg was not
381   compromised.

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Author's Contribution

Baphareh Abdoli: conceptualization, designed and performed the experiments, investigation, analyzed the data and wrote the main manuscript text; *Mohammad Hadi Khoshtaghaza*: project supervision, validation, review and editing; *Hamid Ghomi*: overall direction and planning related to conducting plasma tests, validation, review and editing; *Mohammad Amir Karimi Torshizi*: project advice, developed the conceptualization, review's formal analysis, methodology, investigation, validation, review and editing; *Saman Abdanan Mehdizadeh*: evaluation of machine vision techniques, validation, review and editing; *Gholamreza Pishkar*: consultant in the industry; *Ian C. Dunn*: project advice, validation, review and editing.

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Data availability

The authors confirm that the data of the result of this paper will be available to the journal by requesting. .

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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585
586   **Figure captions**

587   Fig. 1. The schematic representation of CAAP-J for eggshell disinfection treatment.

588   Fig. 2. Surface analysis of eggs by SEM (A) control (egg without CAAP-J treatment), (B) CAAP-J-treated

589   eggs.

590   Fig. 3. OES spectrum of the CAAP-J treatment sample.

591   Fig. 4. ATR-FTIR spectra of eggshell cuticle chemical components.

592   Fig. 5. Identifies the chemical components of the eggshell cuticle by measuring the intensity of the ATR-
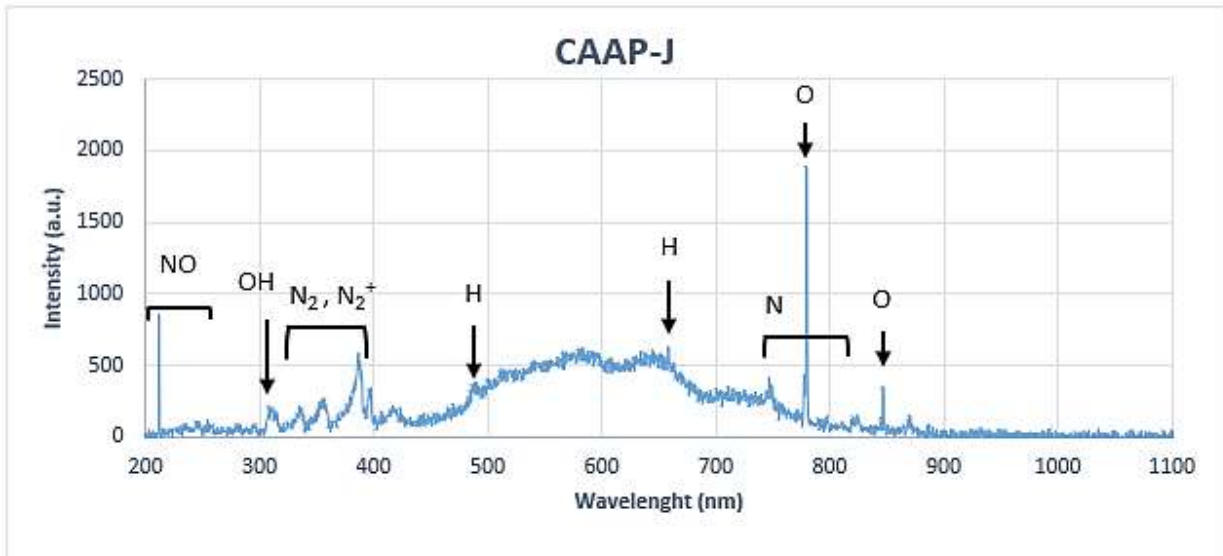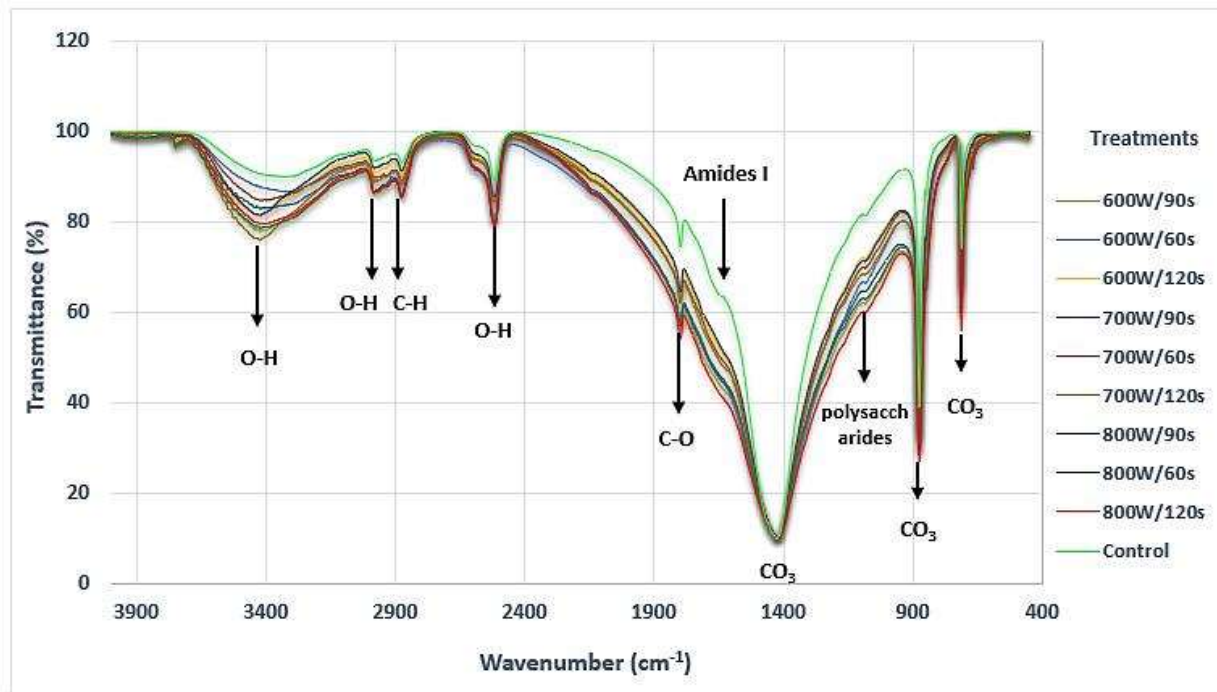
593   FTIR peaks.

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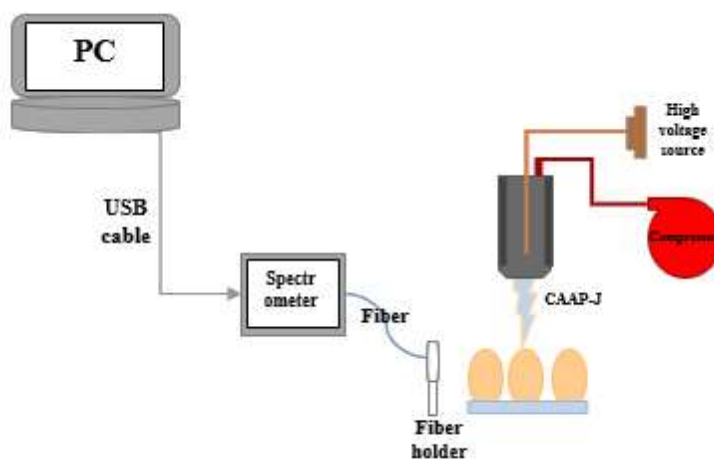
595
596   **Fig. 1.** The schematic representation of CAAP-J for eggshell disinfection treatment.
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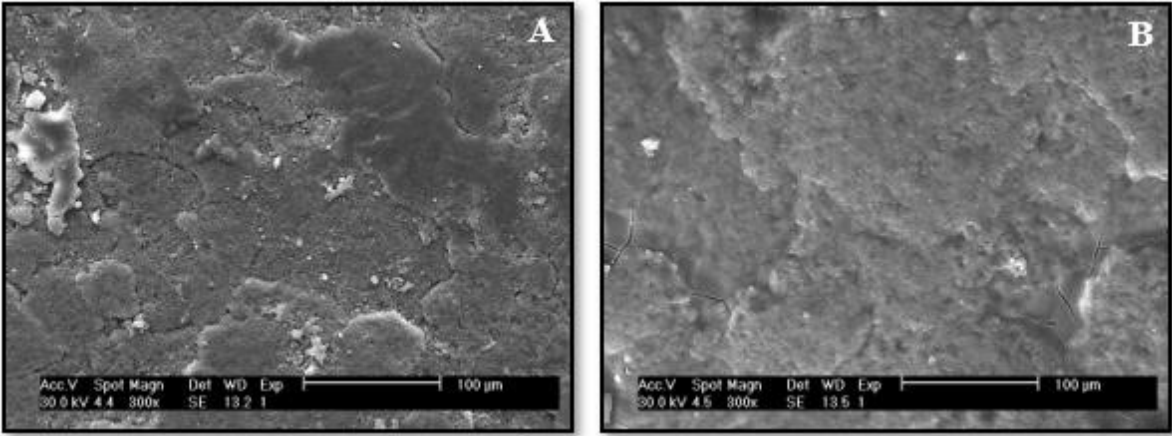
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599 **Fig. 2.** Surface analysis of eggs by SEM (**A**) control (egg without CAAP-J treatment), (**B**) CAAP-J-treated eggs.
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603 **Fig. 3.** OES spectrum of the CAAP-J treatment sample.
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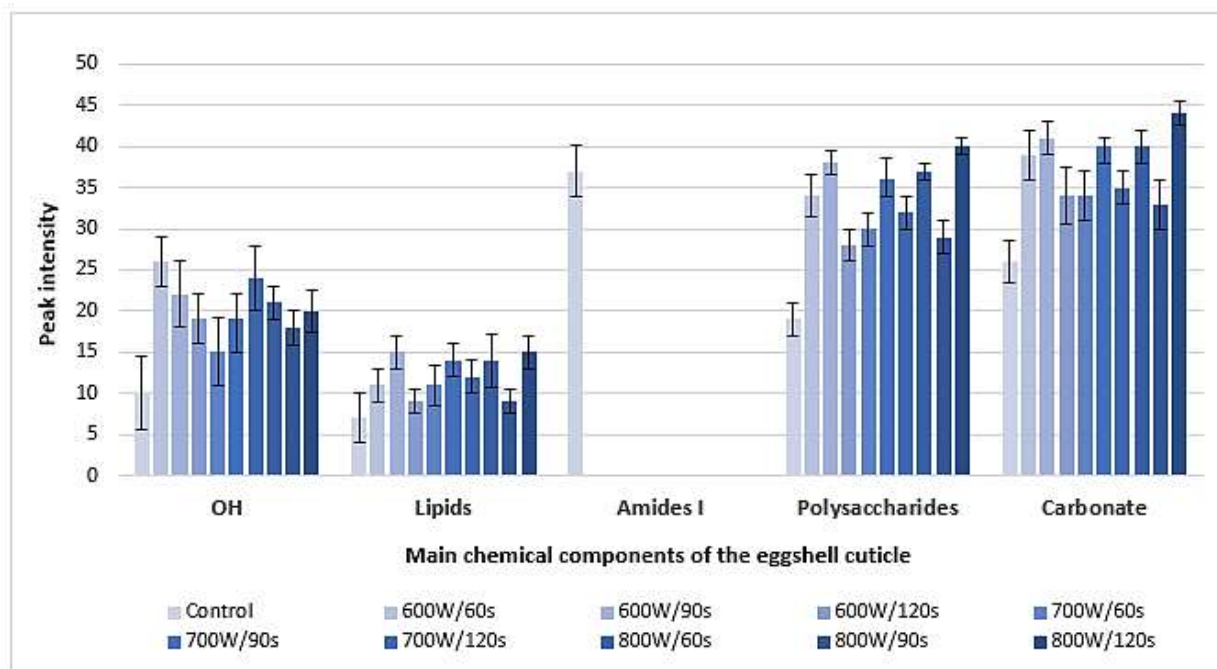606 **Fig. 4.** ATR-FTIR spectra of eggshell cuticle chemical components.
607
608

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610 **Fig. 5.** Identifies the chemical components of the eggshell cuticle by measuring the intensity of the ATR-FTIR peaks
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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: