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Surface decontamination of eggshells by using non-thermal atmospheric plasma

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ABSTRACT

In this study, the possibility of using an effective short time non-thermal plasma (NTP) treatment to inactivate *Salmonella enterica* serovar Enteritidis on eggshell surface was investigated. The eggshells were artificially contaminated with *S.* Enteritidis at an initial concentration of 10^7 cfu/egg and then treated with an atmospheric pressure plasma jet by using air as process gas under different experimental settings with various frequencies (20–25 kHz) and reference voltages (100–80%), exposure times (60–120 s), distances from plasma jet (15 or 40 mm) and gas flow rates (2000–3000 L/h). The best result was obtained at maximum plasma power of 655 W (25 kHz–100% V), where *S.* Enteritidis concentration on egg surface was reduced below the detection limit (10^2 cfu/egg) after 120 s of treatment. The temperature remained below 35 °C after all plasma treatments in order to minimize the risk of egg quality alterations. Specific measurements demonstrated that there were no negative effects on egg quality after NTP treatment. The effect of plasma process on the egg cuticle was demonstrated by using scanning electron microscopy.

1. Introduction

In most countries who publish data on foodborne diseases, *Salmonella* spp. is identified as one of the target pathogens of concern (Olaimat and Holley, 2012; Raybaudi-Massilia et al., 2009) and the cause of a prevalent foodborne illness called Salmonellosis (Galis et al., 2013; Howard et al., 2012), a serious health problem with great economic impact (Gantois et al., 2009; Messens et al., 2007). It is estimated that approximately 1.2 million illnesses and 450 deaths occur due to non-typhoidal Salmonella annually in the United States (Scallan et al., 2011). *Salmonella enterica* serovar Enteritidis, a predominant Salmonella serotype, is the leading cause of laboratory confirmed infections (CDC, 2014).

Salmonellosis is the second most common zoonosis in humans in the EU (EFSA and ECDC, 2015) which could be traced back to raw or undercooked eggs and egg products. In order to prevent this, poultry industry should have antimicrobially effective and cost efficient disinfection systems to provide consumers' easy access to safe eggs.

The conventional method used in the USA, Canada, Asia and Australia for egg decontamination is washing, which is a standard procedure applied in poultry farms (Afari et al., 2016; Bialka et al., 2004; Cao et al., 2009). But European Union countries do not allow washing or cleaning of shell eggs (EC, 2003) because these processes can affect the egg outer layer (cuticle) and rise the risk of penetration of dangerous microorganisms such as *Salmonella* to the eggshell (Munoz et al., 2015). Egg decontamination by gamma rays or electron beams irradiation (Farkas, 1998; Pinto et al., 2004) is also prohibited in Europe (EC, 1999).

Several alternative approaches for decontamination of egg shells have been proposed, including pasteurization by hot air, atmospheric steam and infrared radiation (James et al., 2002; Pasquali et al., 2010), pulsed light technology (Hierro et al., 2009; Lasagabaster et al., 2011), ultrasonic treatment (Sert et al., 2013) slightly acidic electrolyzed water (Cao et al., 2009) or ozone and UV radiation (Fuhrmann et al., 2010; Rodriguez-Romo and Yousef, 2005; Turtoi and Borda, 2014). These methods have given successful results to varying degrees, but they may induce unfavorable effects on nutritive quality and functional properties of eggs.

Cold atmospheric pressure plasma has been demonstrated as a potential alternative to conventional methods due to its non-thermal nature and proven potential to enhance microbiological safety and maintain quality characteristics of a wide range of foods within short processing times (Bourke et al., 2017). Plasma is usually generated by exposing a gas (or a gas mixture) to an electric field that accelerates the charged particles producing collisions with heavy species. In this way, gas plasma is generated as an ionized gas containing free electrons and neutral reactive species such as atoms, molecules and radicals (Moisan et al., 2001). Ions and reactive species, when they are in an excited

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state, can lose their internal energy through collisions with other particles or surfaces or by emitting photons in the UV range. These factors, separately or in synergistic combination, are the main factors responsible for germicidal effect of gas plasma (Laroussi and Leipold, 2004).

Several mechanisms can lead to inactivation effect of non-thermal plasma. Reactive oxygen and nitrogen species (RONS) such as atomic oxygen (O), ozone (O_3), hydroxyl (OH), NO, and NO_2 can cause oxidation of membranes (particularly if rich in lipids) and amino acids, and inhibition of respiration (Eto et al., 2008; Kim et al., 2009; Li et al., 2013; Lu et al., 2014; Ziuzina et al., 2014). Charged particles (electrons and ions) accumulate on the surface of cell membrane and induce its rupture due to electrostatic forces (Dobrynin et al., 2009; Fridman et al., 2007; Lu et al., 2014). Exposure to UV photons can modify DNA and cause consequent improper cell replications (Boudam et al., 2006).

Extensive research on the use of cold plasmas to inactivate microorganisms is a relatively recent phenomenon. Bourke et al. (2017) reported non-thermal atmospheric plasma treatments for decontamination of many food samples including fresh fruits and vegetables, meat and meat products, milk and dairy products, fruit juices, etc. In addition, the results of our previous studies also demonstrated the lethal effect of cold plasma on inactivation of indicator bacteria on food-contact surfaces (Dasan et al., 2017b), in fruit juices (Dasan and Boyaci, 2017) and decontamination of pathogenic fungus from hazelnut (Dasan et al., 2016b, 2017a) and maize samples (Dasan et al., 2016a). Minimized water usage, lack of chemical residue, use of atmospheric air for plasma generation, low treatment temperatures and working at atmospheric pressure are some of the advantages that broaden the scope of food processing. Discharges at atmospheric pressure and at low temperatures make decontamination process more practical, inexpensive, and suitable for continuous process and scale-up while preserving the product quality.

There are few studies using atmospheric plasma for decontamination of eggshells. Reductions up to 4.5 and 3.5 log cfu/eggshell by using air with higher moisture contents (65%) as process gas for 90 min of treatment were obtained for *S*. Enteritidis and *S*. Typhimurium, respectively (Ragni et al., 2010). In another inactivation study of *S*. Typhimurium on eggshell, a 6 log reduction was achieved after 10 and 25 min plasma treatments using dielectric barrier discharge (DBD) plasma with He/O_2 mixture, in direct and indirect mode, respectively (Georgescu et al., 2017). Additionally, Wan et al. (2017) reported a reduction of 5 log units for *S*. Enteritidis on egg surfaces directly treated under modified atmospheric gas for 15 min with a DBD plasma. In another study, reduction factors ranged between 0.22 and 2.27 log CFU were achieved in *S*. Enteritidis by atmospheric pressure plasma jet with varying process gases (Moritz et al., 2017). Although the results obtained in these studies seem to be promising, the required treatment times to observe significant reductions in *Salmonella* population are considerably high for industrial applications. Also, the process gases used are either expensive or need to be modified in terms of moisture content.

The present study aims to investigate the effect of non-thermal atmospheric plasma (NTP) treatment of refrigerated chicken eggs to control *Salmonella enterica* serovar Enteritidis on the shell surface. In order to eliminate the disadvantages stated above, an atmospheric plasma jet moving on an x–y direction system was used, which allows movement of the jet and scans the surface. This type of plasma source is suitable for continuous processing and enables to get involved in a production line. The effect of NTP treatment on the survival of *S*. Enteritidis on eggshell was evaluated by using air as process gas for varying treatment times, exposure powers, distances from plasma nozzle and gas flow rates. Egg quality was also evaluated after NTP treatment which included cuticle, Haugh unit, yolk index, yolk color, albumen and yolk pH.

2. Material and methods

2.1. Bacterial strain and cell suspension preparation

The tested strain, *Salmonella enterica* serovar Enteritidis (ATCC BAA-1045), was obtained from Food Engineering Department, Hacettepe University (Ankara, Turkey). Stock culture was stored with 50% glycerol at − 80 °C. Fresh working culture was prepared by inoculating 200 μL of frozen culture in 10 mL Tryptic Soy Broth (TSB) (Merck KGaA, Darmstadt, Germany) and after an aerobic incubation at 37 °C for 24 h, the cell suspension was used for egg shell inoculation. In preliminary experiments, the bacterial concentration was determined by inoculating the strain on Tryptone Soy Agar (TSA) (Merck KGaA, Darmstadt, Germany) medium and also on Xylose Lysine Deoxycholate Agar (XLD) (Merck KGaA, Darmstadt, Germany). The initial inoculum cell concentration was 8.98 ± 0.49 log (cfu/mL).

2.2. Preparation and inoculation of eggs

Fresh, medium A grade eggs (66.87 ± 0.55 g) were purchased from a local grocery store. The initial natural bacterial flora present on the surface of eggshells was determined to be 4.76 ± 1.01 log (cfu/egg) by plating onto TSA in triplicate for six randomly picked eggs. For decontamination of the outer shell, the egg surfaces were dipped in 70% ethanol for 1 min. The eggs were dried at room temperature in a laminar flow cabinet, for about 40 min before inoculation. After pre-decontamination with ethanol, no viable cells were counted. Then, 40 μL of bacterial suspension were spread by pipetting on the wider side of eggshell on an area of approximately 1 cm × 1 cm. After inoculation, the eggs were left to dry for another 60 min in a laminar flow cabinet at room temperature. This provides a *S*. Enteritidis concentration of about 7.16 ± 0.10 log (cfu/egg) on the eggshells.

2.3. Non-thermal atmospheric plasma generation

Non-thermal atmospheric plasma system (Plasmatreat GmbH, Steinhagen, Germany) consists of a 3 × 400 V–16 A power generator, a plasma jet, a high voltage transformer and a pressure supply control unit. The plasma was generated through a high-voltage discharge with a single electrode, forming a plasma discharge that exits the jet nozzle at high velocity onto the surface being treated. The plasma jet has a double nozzle system which rotates around the rotary axis and gives an intensive and homogeneous treatment. The rotary nozzle provides a continuous and uniform plasma treatment of surfaces with lower temperature increase. Additionally, this plasma jet was mounted on an x–y motion system which enables movement of the plasma jet through determined x and y coordinates and scan the surface material being treated (Fig. 1). The atmospheric plasma system could be operated using air as process gas, in the range of 100–70% reference voltage and 18–25 kHz frequencies with a gas flow of 1000–5000 L/h. Such plasma source produces a three-dimensional cone-shaped plasma jet that rotates around its own axis extending out of capillary tube to the length of about 30 mm with diameters of 15–30 mm.

2.4. Non-thermal plasma treatment of egg shell

The eggs were placed in a plexiglass egg tray which could hold 3 eggs for each plasma treatment operated in atmospheric air at room temperature of 20 °C (Fig. 1). Distance of plasma nozzle tip from the samples were fixed at 15 mm. NTP was directly applied using air at two different flow rates (2000–3000 L/h) at varying plasma powers (25 kHz–100% V (655 W); 20 kHz–100% V (575 W); 20 kHz–80% V (460 W)) for 60, 90 and 120 s to investigate the lethal effect of plasma parameters. Additionally, in order to investigate the effect of distance from the plasma jet, the eggs were treated at optimum parameters corresponding to the best inactivation results where the distance was