



Effect of corona discharge plasma on microbial decontamination of dried squid shreds including physico-chemical and sensory evaluation



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ABSTRACT

Non-thermal techniques for microbial decontamination in foods are becoming more promising. This work aims to evaluate the suitability and effectiveness of corona discharge plasma jet (CDPJ) for the inactivation of microbial contaminants of dried squid shreds. CDPJ was generated using 20 kV pulsed DC voltage and at a 58 kHz frequency. Upon the CDPJ treatment (0–3 min) of dried shreds, contaminants namely aerobic bacteria, marine bacteria and *Staphylococcus aureus* were inactivated by 2.0, 1.6, and 0.9 log units, respectively. Also, a 0.9 log reduction of yeasts and molds contaminants was observed. The inactivation pattern fitted well to the pseudo-first-order model rather than first-order kinetic model. The CDPJ treatment did not exert statistically significant ($P > 0.05$) changes in color characteristics and volatile basic nitrogen content of dried squid shreds as compared with untreated controls. In contrast, the moisture and thiobarbituric acid reactive substances levels of shreds were significantly ($P < 0.05$) altered by the plasma exposure. However, the treatment exerted no significant ($P > 0.05$) impact on the sensory characteristics of dried squid shreds. The CDPJ was found to be effective for microbial decontamination of real-world samples of dried squid. This technology can readily be applied to commercial dried squid processing.

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1. Introduction

In countries with long coastlines, seafood is a major source of proteins and unsaturated lipids for human nutrition. The popular seafood squid (*Todarodes pacificus*) contains a significant (13.0–19.2%) amount of protein with all the essential amino acids in a good balance required by the human body, and thus squid meat may be considered nutritionally a very good source of protein (Bano, Shakir, Begum, & Qadri, 1992; Deng et al., 2012). As the fresh squid contains a moisture level of more than 80%, they are often preserved in the dry form for food use. Dried squid, made simply by drying after removal of the internal organs, has been highly prized in the Far East (Japan, Korea, China, Philippines and Vietnam, etc.) for its characteristic flavor and often eaten directly as a snack food, side dishes or refreshments (Lee, Park, & Ha, 2015).

Traditionally, fresh squid are processed by hand and sun-dried (Sheehy & Vik, 1980), therefore, there is a greater risk of cross-contamination of product with foodborne pathogens apart from

natural bio-contaminants. In a study, the microbial contamination during seasoned and dried squid processing, through the apparatus, machines, and employee's gloves at each step in processing, was demonstrated (Choi, Park, & Shin, 2012). The results obtained indicate that sanitation standard operating procedures (SSOP) must be developed for control of microbial contamination in seasoned and dried squid processing. It has also been reported that dried sliced squid distributed in supermarkets and traditional markets in Korea have tested positive for coliforms, *S. aureus* and *Bacillus cereus* (KMFDS 2012; Lee et al., 2015). Furthermore, a relatively high level of bacterial contamination in dried and seasoned seafood products compared with other food products was reported in a study (Kim, Kim, Kang, Hwang, & Rhee, 2013). Such high contamination levels are believed to originate from the raw materials (such as squid, octopus and filefish) and/or the manufacturing process. Therefore, various strategies to reduce microbial populations have received greater importance to render seafood products safe for human consumption.

In recent years, much research has been focused on non-thermal methods of bio-decontamination of foods, such as use of high hydrostatic pressure, pulsed electric fields, ionizing radiation, high intensity ultrasound, and non-thermal plasma. Earlier, some non-

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thermal decontamination techniques have been applied to improve the microbiological quality of dried squid. Electron beam irradiation at dosages of 2, 4, 8, 12 and 16 kGy was successfully applied to improve the microbial safety and qualities of sliced dried squid. In that study, the decimal reduction dose (D_{10} value) of total bacteria count, yeast and mold, coliforms in sliced dried squid were 8.57, 4.60, and 8.10 kGy, respectively (Ko, Ma, & Song, 2005). Lee et al. (2015) investigated the decontaminating effects of different doses of UV-C light at 253.7 nm (0–18 kJ/m²) on *E. coli*, *S. aureus* and *B. cereus* in contaminated sliced squid surfaces. The counts of all the three bacteria were significantly ($P < 0.05$) reduced by the increase of UV-C dosage. Upon using the UV dosage of 18 kJ/m², the *E. coli*, *S. aureus* and *B. cereus* bacteria were reduced by 1.35, 0.54 and 1.05 log CFU/g, respectively. Recently, non-thermal plasmas (NTPs) for bio-decontamination of foods have also received substantial attention owing to their excellent antimicrobial activity against a wide range of microorganisms (Kim, Puligundla, & Mok, 2015; Ma et al., 2015; Misra, Tiwari, Raghavarao, & Cullen, 2011; Puligundla, Kim, & Mok, 2015).

Nonthermal plasma is an ionized gas that consists of charged particles, electric fields, UV photons and reactive species (Deng, Shi, & Kong, 2006; Ma et al., 2015). Corona discharge (a pulsed DC discharge plasma) is one of several approaches for NTPs generation under atmospheric pressure conditions. These discharges in air have a complex chemical composition and are not been fully understood (Timoshkin et al., 2012). Corona discharges are known to produce chemically active species, namely oxygen ions and other charged species, which act as very strong oxidizers (Deng et al., 2007). Corona discharges generated in atmospheric air have a strong bactericidal effect. Several attempts have been made in the past to study the inactivation effect of different corona discharges on microbes (Fletcher et al., 2007, pp9; Julak, Kriha, & Scholtz, 2006; Kim et al., 2015; Korachi, Turan, Senturk, Sahin, & Aslan, 2009; Machala, Chladekova, & Pelach, 2010, pp7). Recently, we have shown the effectiveness of corona discharge plasma jet (CDPJ) for inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on both fresh and frozen pork (Choi, Puligundla, & Mok, 2016). In the present study, CDPJ was used to decontaminate dried squid shreds, and possible changes in physicochemical and sensory properties of the shreds due to the plasma treatment were evaluated.

2. Materials & methods

2.1. Dried squid samples

Unpackaged dried squid shreds were purchased at Moran market (Seongnam-si, Gyeonggi-do, Korea). The samples were then sealed in plastic bags to protect the product from moisture gain and stored at refrigeration temperature (4 °C) until use. Shreds were measured about 7–10 cm long, 0.3–0.5 cm in width and 0.1–0.2 cm thick.

2.2. Identification & enumeration of microbial contaminants

Microbial contaminants of dried squid shreds were detected using general and selective growth media, and viable counts were enumerated using standard plate count method (KFDA, 2011). Shreds (10 g) were taken in a sterile sample bag (3M Korea, Seoul, Korea), and sterile saline solution (90 ml) was added to it. Then, the sample was homogenized in a paddle blender for 3 min (Masticator, IUL Instruments, Barcelona, Spain). Under sterile conditions, aliquots (1 ml each) from filtrate were removed from the stomacher bag, serially diluted with 0.85% sterile saline, and aliquots of each dilution were pipetted into petri plates (pour plate method) with

melted agar (~15 ml), and incubated at 37 °C for 24 h. General purpose media namely plate count agar (PCA) and potato dextrose agar (PDA) were used for mesophilic aerobic bacteria, and yeasts and molds detection and enumeration, respectively. Selective enrichment media (Difco, Becton Dickinson and Co., Sparks, USA) used include marine agar (MA) for marine bacteria, eosin-methylene blue agar for *E. coli*, Baird-Parker agar for *S. aureus*, xylose-lysine-deoxycholate (XLD) agar for *Salmonella* spp., thiosulfate-citrate-bile salts-sucrose (TCBS) agar for *Vibrio* spp., and Oxford Listeria-selective agar supplemented with Oxford Listeria-selective supplement (Merck, Darmstadt, Germany) for *Listeria monocytogenes*.

2.3. CDPJ generation and squid shreds treatment

CDPJ was generated as discussed in our earlier publications (Choi et al., 2016; Kim et al., 2015). The plasma generation system consisted of power supply, electrode assembly, air blower and sample treatment plate. The CDPJ used for experimentation was generated using 220 V AC power with an output voltage of 20 kV DC, at a current of 1.50 A, and a frequency of 58 kHz. A centrifugal air blower operating at a constant rotational speed of 3312 rpm was used for plasma jet/plume creation. Air velocity at the electrodes tip (with 5 mm inter-electrode gap) was 2.5 m/s. The size of emission slit in electrode housing unit was 6 × 35 mm. The shreds were treated with the CDPJ for 0, 1, 2 and 3 min. A span length of 25 mm was maintained between the plasma electrode and sample. The sample size for each treatment condition was 10 ($n = 10$). For each treatment cycle, shreds (10 g) were aseptically taken in a glass petri dish and exposed to the CDPJ for a predetermined amount of time. Immediately after the plasma treatment, samples were tested for surviving microbes, and subjected to instrumental color measurement and sensory evaluation. The physicochemical analyses were conducted within 24 h post-treatment. All samples were stored in airtight dark bottles and kept at 25 °C until analysis.

2.4. Modeling of inactivation

Microbial inactivation models were developed based on pseudo-first-order kinetics or Singh-Heldman (2009) model, as described in our earlier publication (Kim et al., 2015).

2.5. Physicochemical analyses

2.5.1. Moisture content and water activity

Moisture content was measured using the 105 °C drying method (AOAC, 2000). The water activity of shreds was analyzed using a humidity sensor (TR-77Ui, T&D Corporation, Nagano, Japan).

2.5.2. Instrumental color measurement

Color characteristics of untreated and CDPJ-treated squid shreds were determined using a colorimeter (CR-200, Konica-Minolta Inc., Tokyo, Japan) with an 8.0 mm aperture and D_{65} illuminant; and values are expressed in terms of the L^* (brightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness). Also, the total color difference (ΔE) was calculated.

2.5.3. pH measurement

For pH measurement, sample weighing 3 g was homogenized with 30 ml of distilled deionized water for 1 min. The homogenate was filtered through Whatman No. 2 filter paper, and then the pH of filtrate was measured using a Mettler Toledo 320 pH-meter.

2.5.4. Volatile basic nitrogen (VBN)

VBN content of dried squid shreds was determined using the

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