Impact of corona discharge plasma treatment on microbial load and physicochemical and sensory characteristics of semi-dried squid (*Todarodes pacificus*)

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Abstract The use of corona discharge plasma jet (CDPJ) for the improvement of the sanitary quality of semi-dried squid was investigated. Microbial contaminants, namely aerobic, coliforms, and molds and yeasts, were detected in the samples. The CDPJ generated using 20 kV DC voltage at 58 kHz frequency was used for the decontamination treatment (1–10 min). The bacterial contaminants and molds and yeasts were inactivated in the range of 1.5–2.1 and at 1.9 log CFU/g, respectively. The inactivation pattern was well fitted to the first-order kinetics model. The CDPJ treatment for 10 min did not exert significant changes ($p > 0.05$) in VBN and the trimethylamine content of semi-dried squid as compared to untreated controls. On the contrary, significant changes ($p < 0.05$) in moisture content, water activity, pH, color, and TBARS levels were observed upon plasma exposure. However, the CDPJ-treated samples displayed slightly better sensory properties than the controls in terms of appearance, visual color, and flavor.

Keywords Corona discharge plasma jet · Decontamination · Microbial contaminant · Semi-dried squid · Physicochemical property

Introduction

In Korea and other Asian countries, semi-dried squid (*Todarodes pacificus*) is a popular seafood because of its unique flavor and taste as well as recognition as a rich

source of protein [1]. Freshly caught squid typically contains moisture levels above 80%; therefore, they are highly perishable via microbial spoilage and enzymatic degradation. Hence, squid, particularly dried squid, is usually processed into various products with extended shelf life. Although fully dehydrated fishery products can be stored for much longer periods than semi-dried ones, there is an increasing preference for the latter products owing to their better flavor and tenderness [2, 3]. Fully dried and semi-dried squid have been highly prized in the Far East for their unique flavor and often eaten directly as a snack food, side dishes, or refreshments [4].

However, as semi-dried food products contain relatively more moisture than fully dried ones, increased microbial activity is a major concern, and thus, they need better control over microbial growth. Drying of squid is generally carried out under natural (sun-dried) conditions until its moisture content drops to approximately 35–40% during winter (0 ± 3 °C) [5]; therefore, there is a greater risk of cross contamination of the products with foodborne pathogens apart from natural bio-contaminants [6, 7]. Semi-dried squid is usually preserved under frozen and refrigerated conditions; however, the shelf life of refrigerated semi-dried squid is relatively short and predominantly depends on the degree of microbial contamination. Moreover, these methods of preservation may not effectively control microbial contamination [2].

Microbial contamination during dried and seasoned squid processing has been demonstrated in a study [8], which recommended the development of sanitation standard operating procedures (SSOPs) for control of microbial contamination. In addition, dried sliced squid distributed in supermarkets and traditional markets in Korea have tested positive for coliforms, *Staphylococcus aureus*, and *Bacillus cereus* [4, 9]. Therefore, various strategies to reduce

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microbial contamination have gained momentum. Over the past few decades, non-thermal methods for microbial decontamination of foods have received much attention due to their effective antimicrobial action without affecting sensory qualities of food. Gamma radiation was used to extend the shelf life of semi-dried squid [5]. The study results showed that the total viable cells were reduced with the increase in irradiation dose, and a dose level of 7 kGy was found to be optimal for the preservation of semi-dried squid. The microbial safety and qualities of sliced dried squid were improved by electron beam irradiation at dosages of 2, 4, 8, 12, and 16 kGy [10]. A UV-C dosage of 18 kJ/m² was found to reduce the *E. coli*, *S. aureus*, and *B. cereus* contamination in sliced squid by 1.35, 0.54, and 1.05 log CFU/g, respectively [4].

Recently, non-thermal plasmas for microbial decontamination of foods have also received great attention because of their excellent microbicidal action against a wide range of microorganisms [7, 11–13]. Non-thermal plasma (NTP) is an ionized gas that consists of charged particles, electric fields, UV photons, and reactive species [13, 14]. As pulsed DC discharge plasma, low-temperature corona discharge is usually generated under atmospheric pressure and is one of the several approaches for NTP generation. Corona discharge in air generates a complex chemical composition [15]. Corona discharges have been shown to produce strongly oxidizing chemical species, namely oxygen ions, electrons, and excited neutrals [16]. Corona discharges in atmospheric air exhibit a strong bacterial-killing effect. Several attempts have been made in the past to evaluate the inactivation potential of corona plasma discharges on different microorganisms. Most of them yielded promising results [12, 17–20].

A recent study from our lab has shown the usefulness of corona discharge plasma jet (CDPJ) for microbial decontamination of dried squid shreds [7]. The study results indicated that natural contaminants of dried squid can be inactivated up to a maximum of 2 log units by CDPJ without affecting their sensory characteristics. However, the plasma effectiveness for microbial decontamination of semi-dried squid is lacking. Therefore, the present study aims to determine CDPJ efficacy for microbial load reduction in semi-dried squid and evaluates possible changes in physicochemical and sensory properties due to the plasma treatment.

Materials and methods

Semi-dried squid

Semi-dried whole flattened squid packaged in polythene bags were purchased from a local fish and seafood market

in Seongnam-city, Korea. After procurement, the samples were placed in a refrigerator at 4 °C until use. The squid mantle was cut into small pieces measuring about 3.0 × 3.0 × 0.2 cm³ (L × W × H). These pieces were used as samples in the present study.

Identification and quantification of microbial contaminants

General and selective growth media were used for microbial contaminant detection in semi-dried squid samples, and the standard plate count method was used to quantify the viable count [21]. Squid pieces were placed in separate sterile sample bags (5 g each) (3 M Korea, Seoul, Korea) and 45 ml of sterile saline solution was added to each bag. Then, using a paddle mixture (Masticator, IUL Instruments, Barcelona, Spain), the samples were homogenized for 3 min. Under aseptic conditions, aliquots (1 ml each) were taken from filtrates of the stomacher bags, serially diluted with 0.85% sterile saline, and then aliquots of each dilution were pipetted into Petri plates (pour plate method) containing melted agar (~15 ml each), before being incubated at 37 °C for 24 h.

General-purpose media used include plate count agar for aerobic bacteria and potato dextrose agar for molds and yeasts. Selective enrichment media (Difco, Becton, Dickinson and Co., Sparks, MD, USA) including eosin-methylene blue agar and thiosulfate-citrate-bile salts-sucrose agar were used for the detection of coliform bacteria and *Vibrio* spp., respectively. Also, for *L. monocytogenes* detection, Oxford Listeria Selective Agar (Merck, Darmstadt, Germany) containing Oxford Listeria Selective Supplement was used.

CDPJ generation and treatment

CDPJ was generated as discussed in our earlier publications [7, 12]. The semi-dried squid pieces were treated with the CDPJ generated at a current of 1.50 A for 0–10 min. A distance of 25 mm was maintained between the plasma-emitting electrode and the sample during treatment. Under each treatment condition, single pieces of squid taken in glass Petri plates were treated by CDPJ for a pre-arranged period. Soon after the plasma treatment, the samples were subjected to determination of microbial survival counts, quantitative color measurement, and sensory evaluation. Other analyses pertaining to physicochemical properties of semi-dried squid were conducted within 24 h post-treatment.

Modeling of inactivation

Microbial inactivation is known to follow first-order kinetics as follows (Eq. 1):

$$\log \frac{N_0}{N} = \frac{k}{2.303} \cdot t \quad (1)$$

where

N_0 is the initial microbial population, in CFU

N is the microbial population at time t , in CFU

t is exposure time (min) and k is the inactivation rate constant.

Therefore, the CDPJ inactivation of microbial contaminants in semi-dried squid was modeled using first-order kinetics.

Physicochemical analyses

The moisture content was determined using AOAC method 934.01 [22]. A humidity sensor (TR-77Ui, T & D Corporation, Nagano, Japan) was used to measure the water activity (a_w) in the squid samples.

For pH determination, each sample weighing 3 g was homogenized in 30 mL of distilled deionized water for 1 min. Whatman No. 2 filter paper was used to filter the resultant homogenates, and then, pHs of the filtrates were measured using a Mettler Toledo 320 pH meter.

A handheld colorimeter (CR-200, Konica-Minolta Inc., Tokyo, Japan) was used to determine color characteristics of semi-dried squid before and after treatment using the CDPJ. The color was measured through an 8 mm aperture of the colorimeter and using a D_{65} illuminant. Values are expressed in terms of three coordinate values, namely L^* (brightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness), in accordance with CIELAB color spaces. In addition, the color difference value (ΔE) was calculated.

A modified Conway micro-diffusion technique, as described by Miwa and Iida [23], was used to determine the volatile basic nitrogen (VBN) content of semi-dried squid, and the values are expressed as mg%.

Thiobarbituric acid reactive substance (TBARS) levels, which indicate lipid peroxidation in squid flesh, were measured using a spectrophotometric method as described by Buege and Aust [24]. Values are expressed as mg malondialdehyde (MDA)/kg of the sample.

Trimethylamine (TMA) contents of the semi-dried squid were determined using the Conway micro-diffusion method following the method of Conway and Byrne [25].

Sensory evaluation

Appearance, color, and flavor characteristics of semi-dried squid mantle meat were subjectively evaluated using the 5-point hedonic scale: 1 = dislike extremely; 5 = like extremely. A panel of 12 untrained participants (male 6, female 6, aged between 20 and 30 years) from the Dept. of

Food Science and Biotechnology, Gachon University, was involved in the evaluation of sensory qualities of the untreated and CDPJ-treated semi-dried squid samples. The samples treated at specified time intervals were subjected to sensory evaluation within 10 min after the treatment. Two samples were provided in each white plate, and the samples were coded with random three-digit numbers.

Statistical analyses

Results are presented as the mean values $\pm$ standard deviation (SD) of three replicates. All statistical analyses were conducted using the SAS software package (version 9.2; SAS Institute Inc., Cary, NC, USA). Microbial counts are expressed as log colony-forming units (CFUs) per gram of sample. The statistical significance ($p < 0.05$) of the physicochemical and sensory evaluation data was analyzed using a one-way ANOVA test (Duncan's multiple range test).

Results and discussion

Natural microflora of semi-dried squid

Semi-dried squid samples were found to contain aerobic bacteria and molds and yeasts as predominant contaminants, with mean counts of 5.42 and 4.5 log CFU/g, respectively. Other than these, coliform bacteria were detected in significant numbers at a level of 4.10 log CFU/g (Table 1). However, the common foodborne bacterial pathogens, namely *Vibrio* spp. and *L. monocytogenes*, were not detected.

The contamination of dried squid products by foodborne pathogens has been reported earlier. Dried squid samples from supermarkets in Korea were found to contain coliforms, *S. aureus*, and *B. cereus* bacteria at levels 0.40, 1.00, and 1.00 log CFU/g, respectively; these bacteria levels in samples from traditional squid market were 0.90,

Table 1 Microbial contaminants of semi-dried squid and their D -values by the CDPJ treatment

Microorganisms	Counts (log CFU/g)	D -value (min)
Aerobic bacteria	5.42 $\pm$ 0.17	4.15
Molds and yeasts	4.54 $\pm$ 0.38	5.12
Coliforms	4.10 $\pm$ 0.08	7.58
<i>L. monocytogenes</i>	ND	–
<i>Vibrio</i> spp.	ND	–

ND not detected

1.00, and 1.48 log CFU/g, respectively [4, 9]. Total aerobic bacteria in seasoned dried squid were found to be within 3.00–6.45 log CFU/g [26].

CDPJ inactivation of microorganisms and its modeling

All of the detected contaminant microorganisms of semi-dried squid were inactivated by the CDPJ treatment in a time-dependent manner. Over 0–10 min treatment, aerobic bacteria were inactivated by nearly 2.1 log CFU/g. At the same treatment duration, molds and yeasts and coliforms were reduced by 1.9 and 1.5 log CFU units per gram sample, respectively (Fig. 1). Previous studies have shown that CDPJ treatment for 3 min, under the same plasma generation and treatment conditions, was effective in inactivating microbial contaminants, namely aerobic bacteria, marine bacteria, and *S. aureus*, of dried Alaska pollock shreds and dried squid shreds by 2.5, 1.5, and >1.0 log units [27] and 2.0, 1.6, and 0.9 log units [7], respectively. The results indicate that the extent of inactivation by the CDPJ depends predominantly on the type of microorganism and the nature of the substratum. In addition, sample or substrate moisture content can affect the plasma treatment efficiency either positively or negatively. Not only does the

optimum in substrate moisture content depend on the type of substrate and microbial contamination, but the type of plasma discharge and its operating conditions are also supposed to play a role [28].

For quantitative microbial risk assessment, modeling the inactivation kinetics provides very useful information. It also offers tools to compare the significance of different process technologies on the reduction of microbial populations. Microbial inactivation generally follows first-order kinetics. Hence, it can be characterized by a single rate constant “ k ” or its reciprocal, the D -value, which provides a quantitative measure of resistance to an applied lethal agent. A linear relationship between the variables of log inactivation and plasma treatment time is shown in the scatter plots. The coefficients of determination closer to 1 imply that inactivation patterns are better explained using first-order kinetics (Fig. 1).

D -values

D -value refers to decimal reduction time. Aerobic bacteria of semi-dried squid were inactivated relatively quickly compared to molds and yeasts and/or coliform by the CDPJ treatment (Table 1). Coliforms seem less susceptible to inactivation exerted by the CDPJ as their D -value is the highest among the inactivated microbes.

Fig. 1 Time-dependent log reduction and inactivation kinetics (based on first-order inactivation kinetics) of microbial contaminants by the CDPJ treatment

Table 2 Changes in moisture content and water activity of semi-dried squid samples by the CDPJ treatment

Treatment time (min)	Moisture content	a_w
0	54.80 $\pm$ 0.75 ^a	0.98 $\pm$ 0.05 ^a
3	54.79 $\pm$ 1.05 ^a	0.97 $\pm$ 0.09 ^{ab}
5	52.94 $\pm$ 1.71 ^a	0.96 $\pm$ 0.14 ^{ab}
7	50.25 $\pm$ 2.02 ^b	0.96 $\pm$ 0.11 ^b
10	49.75 $\pm$ 2.18 ^b	0.95 $\pm$ 0.12 ^b

Values are given as mean $\pm$ SD ($n = 10$). Distinct letters within the same column indicate significant differences ($p < 0.05$)

Moisture content and water activity (a_w)

For treatment periods of up to 5 min, there was no statistically significant ($p > 0.05$) difference in the moisture content and water activity (a_w) between the untreated and CDPJ-treated semi-dried squid samples (Table 2). However, upon extending the treatment until 7 or 10 min, both the moisture content and a_w of the squid samples were significantly ($p < 0.05$) reduced in comparison to the untreated controls. The mean moisture content of the samples was within 49.7–54.8%, and a_w was within 0.95–0.98.

Instrumental color analysis

The color characteristics (L^* , a^* , and b^*) of semi-dried squid were altered significantly ($p < 0.05$) due to the CDPJ treatment for 10 min. Average values of brightness (L^*) were in the range of 96.82–99.81, and values of yellowness (b^*) were in the range of 1.73–7.60 (Table 3). No significant differences ($p > 0.05$) were observed for the redness (a^*) color value between the untreated and CDPJ-treated semi-dried squid. The magnitude of color change due to the plasma treatment is indicated by ΔE . A proportional increase was noted in ΔE values relative to the plasma treatment time. Differences in perceivable color can be analytically classified as very distinct ($\Delta E > 3$), distinct

($1.5 < \Delta E < 3$), and small differences ($\Delta E < 1.5$) [29]. ΔE values obtained under the present experimental conditions indicate “small differences” for 3 min and “very distinct” for 5, 7, and 10 min plasma treatment times. Color differences are obvious to the human eye only when $\Delta E > 3$, according to Francis and Clydesdale [30]. Therefore, color differences are visually noticeable when the plasma treatment exceeds 3 min in the semi-dried squid samples.

pH and VBN

Compared to untreated samples, pH of the CDPJ-treated (3 and 5 min) semi-dried squid samples was slightly but significantly altered ($p < 0.05$) (Table 4). A mild acidic pH (about 6.2) was observed in both types of samples. In dried fish products, pH is an important indicator of the level of lipid and protein hydrolysis [31]. The pH values indicated that proteins and lipids of semi-dried squid were not hydrolyzed substantially due to the CDPJ exposure.

The levels of VBN in semi-dried squid were not significantly ($p > 0.05$) altered due to the CDPJ treatment, as compared to the untreated control samples (Table 4). VBN, a measure of protein degradation, includes basic amines, trimethylamine, and basic nitrogenous compounds such as ammonia. The average levels of VBN were in the range 4.06–4.28 mg%. In our previous study, the average VBN levels of 51.67–53.41 mg% were noted for both untreated and CDPJ-treated fully dried squid samples [7]. The maximum VBN level in salted and dried fish products is set at 150 mg N/100 g by the Chilean Official Organization–Sernapesca [32]. The levels measured in this study were well within the prescribed limits.

Thiobarbituric acid reactive substances (TBARS)

TBARS are formed as a byproduct of lipid peroxidation. TBARS values, which represent the content of the secondary lipid oxidation products, are regarded as an important non-microbial factor responsible for meat deterioration. Compared to the untreated control, TBARS

Table 3 Changes in CIE color values of semi-dried squid by CDPJ treatment

Treatment time (min)	Color coordinates			
	L^*	a^*	b^*	ΔE
0	96.82 $\pm$ 0.35 ^b	−0.32 $\pm$ 0.06 ^a	1.73 $\pm$ 0.32 ^d	0 e
3	96.87 $\pm$ 0.83 ^b	−0.31 $\pm$ 0.04 ^a	2.73 $\pm$ 0.55 ^d	1.37 $\pm$ 0.45 ^d
5	99.15 $\pm$ 0.92 ^a	−0.42 $\pm$ 0.02 ^a	4.42 $\pm$ 0.66 ^c	3.62 $\pm$ 1.09 ^c
7	99.30 $\pm$ 0.98 ^a	−0.53 $\pm$ 0.05 ^a	6.38 $\pm$ 0.64 ^b	5.39 $\pm$ 0.12 ^b
10	99.81 $\pm$ 0.58 ^a	−0.22 $\pm$ 0.64 ^a	7.60 $\pm$ 0.74 ^a	6.61 $\pm$ 0.48 ^a

Values are given as mean $\pm$ SD ($n = 10$). Means in the same column followed by the same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test

Table 4 Changes in pH, volatile basic nitrogen (VBN) content, 2-thiobarbituric acid reactive substances (TBARS), and trimethyl amine (TMA) of semi-dried squid by CDPJ treatment

Treatment time (min)	Physicochemical properties			
	pH	VBN (mg%)	TBARS (mg MDA/kg)	TMA (mg%)
0	6.20 ± 0.05 ^{ab}	4.26 ± 0.21 ^a	0.20 ± 0.02 ^c	4.76 ± 0.84 ^a
3	6.10 ± 0.10 ^b	4.20 ± 0.22 ^a	0.21 ± 0.02 ^{bc}	4.57 ± 0.70 ^a
5	6.25 ± 0.06 ^{ab}	4.28 ± 0.19 ^a	0.24 ± 0.04 ^{ab}	4.48 ± 0.56 ^a
7	6.28 ± 0.11 ^a	4.06 ± 0.31 ^a	0.26 ± 0.01 ^a	5.04 ± 0.28 ^a
10	6.27 ± 0.08 ^a	4.09 ± 0.30 ^a	0.25 ± 0.02 ^{ab}	4.95 ± 1.26 ^a

Values are given as mean ± SD (n = 10). Distinct letters within the same column indicate significant differences ($p < 0.05$). MDA, Malondialdehyde

Table 5 Sensory properties of CDPJ-treated semi-dried squid

Sensory properties	Treatment time (min)				
	0	3	5	7	10
Appearance	3.00 ± 0.67 ^a	2.70 ± 0.67 ^a	3.10 ± 0.88 ^a	3.00 ± 1.05 ^a	3.20 ± 0.79 ^a
Color	3.10 ± 0.57 ^a	3.00 ± 0.94 ^a	2.80 ± 0.79 ^a	2.70 ± 0.67 ^a	3.30 ± 0.67 ^a
flavor	2.60 ± 0.70 ^b	3.20 ± 0.63 ^b	3.70 ± 0.67 ^{ab}	3.10 ± 0.74 ^{ab}	3.30 ± 0.82 ^a
Overall acceptance	3.10 ± 0.74 ^a	3.30 ± 0.67 ^a	3.20 ± 0.79 ^a	3.30 ± 0.67 ^a	3.30 ± 0.82 ^a

Values are given as mean ± SD (n = 10). Means in the same row followed by the same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test

levels of the CDPJ-treated semi-dried squid were increased proportionally ($p < 0.05$) to the treatment time. The average TBARS levels were in the range 0.20–0.26 mg MDA/kg of the sample (Table 4). The average TBARS levels in fully dried shreds were in the range 1.75–2.07 mg MDA/kg of the dried sample [7]. The increase in TBARS value during the CDPJ treatment may be attributed to the further oxidation of primary lipid oxidation products by reactive species generated by the plasma.

Low TBA levels are generally observed in squid even after drying. However, a significant rise of TBA levels was noted during further storage. In dried-seasoned squid, it has been shown that TBA values were progressively increased from the initial 0.886 mg MDA/kg to the final 1.95 mg MDA/kg during storage at 25 °C for 120 days [33]. As squid contain large amounts of unsaturated fatty acids, rapid oxidation of these fatty acids in the presence of oxygen and natural light can be anticipated. Apparently, a low level of lipid oxidation had occurred during cold drying. In some previous reports, a low level of lipid oxidation has been reported in fishes, especially in Alaska Pollock, during low-temperature drying [6, 31].

The rate of lipid oxidation highly depends on temperature and increases with temperature; it can double for every 10 °C increment. In this study, upon the CDPJ treatment, high plasma temperature and reactive plasma species may have increased the lipid peroxidation, especially the formation of TBARS through the decomposition of primary lipid oxidation products, in semi-dried squid samples. An

air-tight or vacuum packaging can prevent oxidative rancidity in semi-dried squid after the plasma treatment.

Trimethylamine (TMA)

Trimethylamine oxide (TMAO) and TMA are regarded as taste-active components in squid and contribute to sweetness and agreeable characteristic squid flavor, respectively [34, 35]. The levels of TMA in semi-dried squid were not significantly ($p > 0.05$) altered by the CDPJ treatment, as compared to the untreated control samples (Table 4). The mean levels of TMA in both untreated and CDPJ-treated semi-dried squid were in the range of 4.48–5.04 mg%.

Sensory characteristics

Sensory properties were evaluated in terms of appearance, color, and flavor of semi-dried squid. Appearance and visual color qualities of the CDPJ-treated samples were not significantly ($p > 0.05$) altered in comparison with untreated controls (Table 5). However, the flavor of semi-dried squid was significantly ($p < 0.05$) improved due to the CDPJ treatment, a more pronounced characteristic squid flavor was observed with CDPJ-treated samples than untreated ones. Therefore, the overall scores for acceptance of the plasma-treated semi-dried squid were slightly improved, but not significantly, when compared to untreated controls.

In conclusion, the elimination or decrease of pathogens of significance in food remains a top priority as microbial contaminants can render the food unsafe for consumption. The presence of coliforms indicates insanitation or possible contamination, though they are not necessarily pathogens. The detection of presumptive coliforms from the samples is a cause for concern with regard to food safety and necessitates the need for improvement of the sanitary quality of semi-dried squid. The CDPJ used has enabled the microbial reduction in the samples by 99%. First-order kinetics can be readily used to explain the inactivation pattern of the contaminants. Except for color, moisture, and TBARS levels, the rest of the evaluated physicochemical properties, as well as sensory qualities of semi-dried squid, were virtually unaltered by the CDPJ treatment.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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