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Effects of dielectric barrier discharge (DBD) generated plasma on microbial reduction and quality parameters of fresh mackerel (*Scomber scombrus*) fillets

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ABSTRACT

The effect of atmospheric cold plasma generated by a novel in-package dielectric barrier discharge (DBD) on microbial and quality parameters of mackerel fillets was investigated. DBD voltage (70 kV and 80 kV) and treatment time (1, 3 and 5 min) were studied. Within 24 h of DBD treatment, spoilage bacteria (total aerobic psychrotrophic, *Pseudomonas* and lactic acid bacteria) were significantly reduced. However, significant effects on lipid oxidation parameters (PV, Dienes) were observed for the treated samples. Both studied treatment factors, treatment voltage and time, significantly affected anti-microbial efficacy and lipid oxidation. Nevertheless, no changes in pH or colour (except for L*) were observed. These results suggest atmospheric cold plasma generated by DBD could be implemented as technology for fish processing, retaining product quality over its shelf life. However, further investigations are needed in order to implement this technology and to control and mitigate its limitations, mainly associated to increased oxidation.

Industrial relevance: Cold atmospheric plasma (CAP) has gained attention as an emerging and non termal technology for decontamination of food. This technology has been used on fruits and vegetables successfully for the inactivation of food-borne pathogens. However, this technology has not been investigated in fish, being a highly persibale product.

The use of dielectric barrier discharge (DBD) to produce cold plasma showed a potential industrial application at low cost and convenience. Cold plasma was found to be effective for reducing the main problem of oily fish quality such as the spoilage bacteria. However, this technology seems to accelerate oxidative pathways; for this reason, further studies to investigate the use of antioxidants in combination with cold plasma as "hurdle technology" to minimise this negative effect are suggested.

1. Introduction

Several research works have identified the potential of atmospheric cold plasma for decontamination of foods. The term "plasma" refers to a partially or wholly ionised gas. Nonthermal or cold plasmas are considered to be in a state of nonthermal equilibrium (Schlüter et al., 2013; Surowsky, Schlüter, & Knorr, 2015). Although they contain high temperature electrons, the neutrals, ions, and radicals remain close to room temperature and as such they are considered cold plasmas with limited macro heating of material to which they interface with (Misra et al., 2015; Mishra, Bathia, Pal, Visen, & Trivedi, 2016; Min et al., 2017). Cold plasma may be generated by a diversity of electrical discharges such as DC glow discharge, radio frequency (RF) discharge, dielectric barrier discharge (DBD), atmospheric pressure plasma jet (APPJ), microwave and pulsed power discharge.

Industrially cold atmospheric plasma equipment has not implemented in food industry for direct food contact but is employed for packaging and label modification. The main limitation is the necessity of working under vacuum and thus incompatible with food processing (Misra, Keener, Bourke, Mosnier, & Cullen, 2014). Experimentally plasma jet and dielectric barrier discharges (DBD) have been studied. Plasma jet typically operate with noble gases (Misra, Tiwari, Raghavarao, & Cullen, 2011), which increase the cost of treatment. Ideally, ambient air would be use in the cold plasma technology in

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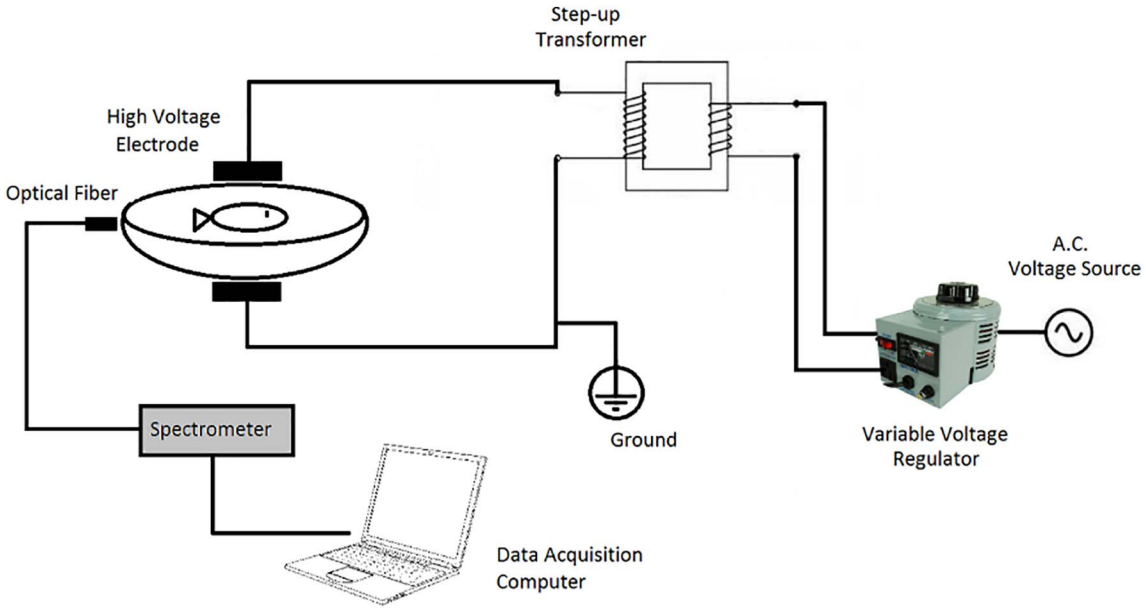

Fig. 1. Schematic of the experimental set-up for DBD of Atlantic mackerel fillets.

order for food industry to implement this technology. In this sense, previous studies have demonstrated the application of dielectric barrier discharges to generate atmospheric cold plasma inside sealed packages filled with air through the application of sufficiently high voltages (Misra, Keener, et al., 2014; Misra, Patil, et al., 2014; Misra, Ziuzina, Cullen, & Keener, 2013; Pankaj, Misra, & Cullen, 2013). Dielectric barrier discharges are generated when high voltage is applied across the electrodes. These discharges generate energetic electrons that dissociate oxygen molecules by direct impact. This single O atom combines with oxygen molecules (O_2) to form ozone gas (Misra, Keener, et al., 2014; Misra, Patil, et al., 2014). Another advantage, in comparison with other cold plasma's generators, is the treatment takes place inside sealed packages, which eliminates the risk of post-process contamination and facilitates rapid treatment times as the resultant reactive species are contained within the package and continue to act post treatment (Misra, Keener, et al., 2014; Misra, Patil, et al., 2014).

Dielectric barrier discharges have successfully applied on food retaining food quality in dry products such as wheat flour (Misra et al., 2015), legumes such as peas (Bußler et al., 2015), dried laver (Kim, Puligundla, & Mok, 2015) or vegetables such as tomatoes (Pankaj et al., 2013; Misra, Keener, et al., 2014; Misra, Patil, et al., 2014), spinach (Klockow & Keener, 2009) and fruits such as strawberries (Misra, Keener, et al., 2014; Misra, Patil, et al., 2014), grapes (Moon, Noh, Moon, & You, 2016), fresh-cut melon (Tappi et al., 2015) and orange juice (Almeida et al., 2015).

The activity of microorganism is the main factor limiting the shelf life in fresh fish (Ólafsdóttir et al., 1997). However, there are no available studies concerning the effects of cold plasma on the spoilage microbiology and quality of fish. Plasma sources for decontamination of foodstuff such as meat, have been reported (Kim, Yong, Park, Choe, & Jo, 2013; Kim et al., 2011; Noriega, Shama, Laca, Díaz, & Kong, 2011; Rød, Hansen, Leipold, & Knøchel, 2012). However, the suitability of ACP for high lipid containing food is doubted. These products are susceptible to detrimental effects of oxidation due to the formation of hydroxyl acids, keto acids, short-chain acids and aldehydes (Misra et al., 2011). The effect of ACP on food quality parameters in meat products has not been studied extensively. Further studies should be conducted to clarify these results. Kim et al. (2011) did not find any significant changes due to plasma jet treatment (pH, TBARS, microscopic observation) except for colour, mainly L* values of the meat surface was increased. Rød et al. (2012) demonstrated that TBARS values of plasma treated samples increased with power and storage time but that the plasma did not induce measurable colour differences. Kim et al. (2013) found lower TBARS values in plasma treated bacon than the control upon treatment, but increased subsequently as a function of storage. Also, significant reductions in the sensory quality parameters were observed in plasma treated samples. There are few reports of DBD treatment on meat products. Jayasena et al. (2015) observed minor deterioration of fresh pork and beef quality. Only high exposure time (10 min) caused lipid oxidation. Furthermore colorimetric measures showed that L* was not affected by DBD, whereas a* values were lowered significantly after 5 and 7.5 min of DBD exposure. Wang, Zhuang, and Zhang (2016) demonstrated that DBD was effective in inhibiting spoilage bacteria for inoculated chicken carcasses.

The objectives of this study were to investigate the effects of different conditions (voltages and times) for DBD on the treatment of fish spoilage bacteria, and how this affects lipid oxidation and the physicochemical characteristics of Atlantic mackerel fillets.

2. Material and methods

2.1. Chemicals

All the chemicals were analytical grade obtained from Sigma-Aldrich (Wicklow, Ireland). All the solvents were HPLC grade and also purchased from Sigma-Aldrich (Wicklow, Ireland). Culture media were supplied by Oxoid (Basingstoke, UK).

2.2. Product characteristics

Six kilos of Atlantic mackerel (*Scomber scombrus*) caught in early February 2015 were purchased in Stevie Connolly Seafood (Dublin, Ireland). The average weight for each fillet was 100 g.

2.3. In-package plasma treatment

Two fillets were packaged in commercial 270 μm-thick polyethylene terephthalate trays (150 mm × 70 mm × 35 mm) and sealed with a high barrier-50 μm film. A plasma discharge was generated inside the trays using dielectric set-up, as shown in Fig. 1. The package was placed between two circular aluminium plate electrodes (outer diameter = 158 mm) with a contact surface area of 249.64 cm^2. A 2 mm thick

polypropylene sheet was used to stabilize the discharge. The electrode separation was adjusted to the tray height of 35 mm. The applied voltage to the electrode was controlled using a set-up transformer (Phoenix Technologies, Inc., USA) at a fixed frequency of 50 Hz, the input to which is regulated using a variable transformer. The samples were treated in triplicate at two discrete voltages of 70 and 80 kV for different treatment times (1, 3 and 5 min). The experiment was performed in duplicate. The atmospheric air conditions at the time of treatment were 15 °C and 50% relative humidity, measured using a humidity-temperature probe connected to a data logger (Testo 176T2, Testo Ltd., UK). Control and treated packages were stored at 4 °C. In order to maximize antimicrobial efficacy of the treatment, samples were stored for 24-h, allowing interaction of the metastable reactive species with the product and subsequent reaction or reversion of the reactive species to the original gas composition (Ziuzina, Patil, Cullen, Keener, & Bourke, 2013; Misra et al., 2013).

2.4. Effect of cold plasma on microbiological growth

Fish samples (10 g) were aseptically transferred into bags (Seward 80 bags, United Kingdom) with 90 mL of sterile maximum recovery diluent (MRD) and homogenised with a Stomacher blender for 5 min (Seward, London, UK). For each sample, appropriate serial decimal dilutions were prepared in MRD for the following microorganism counts:

(i) Total aerobic mesophilic bacteria were determined using Tryptic Glucose Yeast Agar (PCA) with 1% NaCL after incubation at 30 °C for 72 h.

(ii) Total aerobic psychrotrophic bacteria on 1% NaCl PCA spread plates, incubated at 15 °C for 72 h.

(iii) Lactic acid bacteria (LAB) on double-layer Man Rogosa Sharpe medium incubated at 30 °C for 72 h.

(iv) *Pseudomonas* on spread plates of Pseudomonas Agar Base with added CFC (Cetrimide, Fucidine, Cephalosporine) supplement for *Pseudomonas* spp. incubated at 25 °C for 48 h.

2.5. Effect of cold plasma on physicochemical parameters

2.5.1. pH

The pH of fillets was measured at room temperature using a portable pH meter (Orion Research Inc., Boston, MA 02129, USA).

2.5.2. Proximate composition

Moisture content was gravimetrically determined according to AOAC (1995). Total lipids were extracted from 10-g samples with methanol/chloroform (1:1, v:v) according to the Bligh and Dyer (1959).

2.6. Effect of cold plasma on lipid oxidation

2.6.1. Fatty acid composition (FA)

The fatty acid profile of the samples was determined in triplicate from the Bligh & Dyer extracts. The lipid-containing chloroform phase was separated and evaporated to dryness under nitrogen. The remaining residue was dissolved in 1 mL of hexane and a methylation procedure carried out by adding 100 μL of 0.5 M methanolic KOH and leaving the reaction for 10 min at room temperature (RT). The upper layer was transferred to a 2-mL vial. Analysis of fatty acid methyl esters (FAME) were carried out on a gas chromatograph Agilent 7890A (Agilent Technologies, PA, California, USA) equipped with a DB-23 column 60 m × 0.32 mm, (0.25 μm film thickness) (Agilent Technologies, Palo Alto, CA, USA) and a flame ionisation detector. Helium was used as the carrier gas. The oven temperature was programmed to 50 °C for the first 7 min and increased to 200 °C at a rate of 25 °C/min; then, the temperature was increased to 230 °C at a rate of 3 °C/min and held for 26 min. Injector and detector temperatures were 250 °C and 280 °C, respectively. One μL of the hexane extract was injected in split mode (ratio 25:1), and FAMEs were identified by comparison of retention times with those of 37 FAME's standard mix (Supelco, Sigma Aldrich, CO). Polyene ratio was calculated on the basis of fatty acid composition, being ([20:5] + [22:6]) 100 / [16:0]. The ratio ω6/ω3 was also estimated.

2.6.2. Peroxide value (PV)

PV was measured directly on the Bligh & Dyer extract according to the method described by the International IDF Standards (1991). Results were expressed in milliequivalents of O_2 per kilogram of oil.

2.6.3. Conjugated hydroperoxides (Dienes)

Conjugated hydroperoxides were measured on the Bligh and Dyer extract dissolved in hexane, as described by Undeland, Stading, and Lingnert (1998). The absorbance was measured at 268 nm and results were calculated as mmoles of hydroperoxides per kilogram of oil.

2.6.4. Thiobarbituric acid reactives substances (TBARS)

Samples were analysed using the methodology described by Vyncke (1975) on a 5% trichloracetic acid extract of the restructured fish muscle. Results were expressed as mg of malondialdehyde (MDA) per kilogram of fish.

2.7. Effect of cold plasma on colour

The colour parameters lightness (L*), redness (a*) and yellowness (b*) were measured using a colourimeter (Colour Quest XE Hunter Lab, Northants, UK). The illuminant was D65 (colour temperature of 6504 K) and the standard observer was 10°. The colourimeter was standardised using a light trap and a white calibration plate. Measurements were taken on the samples packaged in transparent plastic bags at three different points.

2.8. Effect of cold plasma on protein structure and water distribution

2.8.1. Nuclear magnetic resonance (NMR) measurement

Ten grams of mackerel were placed in sealed NMR tubes and held at 25 °C in a water bath for 1 h. NMR data were generated using a Mara Ultra Instrument (Oxford Instruments, Abington, UK) with a resonance frequency of 23.2 MHz. Transverse measurements (T_2) were conducted using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with the resultant relaxation decays analysed by tri-exponential unsupervised fitting in the RI Win-DXP software (version 1.2.3; Oxford Instruments).

2.9. Statistical analysis

The data were subjected to One-way ANOVA. Fisher LSD (Least Significant Difference) test was applied for determining group differences at 95% confidence level. Statgraphics Centurion XVI was used for carrying out the statistical analysis.

3. Results & discussion

3.1. Effect of cold plasma on microbiological growth

Fig. 2 shows the changes in the microbial flora of Atlantic mackerel subjected to ACP. The initial total aerobic mesophilic bacteria (control) were 4.1 ± 0.07 log CFU g^{-1}, which is comparable to values reported in the literature of between 3.0 and 5.0 log CFU g^{-1} on filleted fish (Chytiri, Chouliara, Savvaidis, & Kontominas, 2004; Dalgaard, Gram, & Huss, 1993). There was no significant ($P > 0.05$) reduction in the total aerobic mesophilic count. However, psychrotrophic bacteria, LAB and *Pseudomonas* counts were significantly ($P < 0.05$) reduced due to DBD.

Total aerobic psychrotropic bacteria incubated at 15 °C were higher in Atlantic mackerel compared to total aerobic mesophilic bacteria.

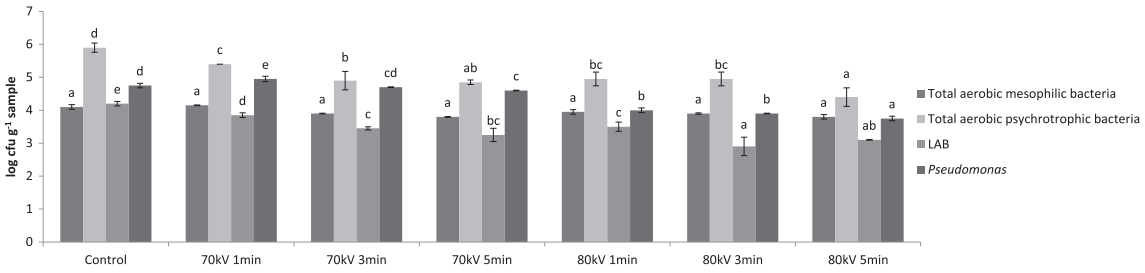

Fig. 2. Total aerobic mesophilic bacteria, psychrotrophic bacteria, LAB and *Pseudomonas* (log CFU g^{-1}samples) counts in Atlantic mackerel subjected to different DBD treatments. Different letters have mean values that are significantly different ($P < 0.05$) due to the treatment among the same microorganism.

Similarly, Núñez-Flores, Castro, López-Caballero, Montero, and Gómez-Guillén (2013) showed differences in the total microorganism counts depending upon the incubation temperature related to the nature of psychrotrophic microbiota predominant in fish. The reduction of total aerobic psychrotrophic bacteria was more dependent on plasma exposure time than on voltage applied. In any case, the most effective reduction was achieved using the highest voltage and time (80 kV, 5 min).

LAB counts were lower than *Pseudomonas* counts. LAB were also found to be more dominant storage. This finding can be explained due to the inhibition of other bacteria by the formation of lactic acid and bacteriocins (Gram & Dalgaard, 2002). Besides, LAB can grow under both anaerobic and aerobic conditions. Other authors found *Pseudomonas* to be the dominant microflora for filleted chilled fish (Chytiri et al., 2004). Both microorganisms (LAB and *Pseudomonas*) were found to display greater sensitivity to applied voltage than treatment time. Kim et al. (2011) studied the effect of atmospheric pressure plasma on inactivation of pathogens (*L. monocytogenes*, *E. coli* and *S. typhimurium*). Concerning LAB and *Pseudomonas*, the previous work along with the present study, demonstrated that plasma treatment at high voltage was effective for reducing fish spoilage microorganism over short time periods. Wang et al. (2016) demonstrated the effectiveness of DBD at 55 kV for 3 min for inactivation of *Pseudomonas fluorescens* isolated from chicken carcasses and suspended in liquid media.

Ozone is one key metastable generated in large quantities by DBDs and which can be measured relatively easily inside of the package Wang et al. (2016) claimed that ozone is a relatively long half-life compared to other reactive species formed during discharge. Other reactive oxygen species (ROS) such as atomic oxygen (O) and hydroxyl radicals (OH) are also generated and they can react with almost all bacteria cell resulting in damage to DNA proteins, lipids and membranes (Kim et al., 2011; Kim et al., 2013). The prototype in-package system used in this study has previously been characterised using electrical and optical diagnostics (Moiseev et al., 2014). The post-discharge gas composition within the sealed packages was quantified using UV–Vis absorption spectroscopy. The concentration of ozone and nitrogen oxides (O_3, NO_2, NO_3, N_2O_4) was found to increases with treatment time however a strong decrease in O_3 levels was observed with increases in relative humidity. The decrease in O_3 and an abundance of nitrogen oxides is ascribed to high specific power densities in the closed container and to increasing RH levels. Humid air large gap DBD plasmas in closed containers generate along with O_3, high levels of nitrogen oxides and HNOx (x = 1, 4) acids which can be linked to bactericidal effects.

3.2. Effect of cold plasma on physicochemical parameters

3.2.1. pH

The pH values were similar to values reported in the literature for Atlantic mackerel (Senturk & Alpas, 2013). Atlantic mackerel did not show any clear trend in pH behaviour after plasma exposure (Fig. 3).

There were no differences on the pH levels of Atlantic mackerel after DBD treatments with the exception of 80 kV at 5 min. These results were consistent with Kim et al. (2011) and Ulbin-Figlewicz, Brychcy, and Jarmoluk (2013), where cold plasma did not induce pH changes.

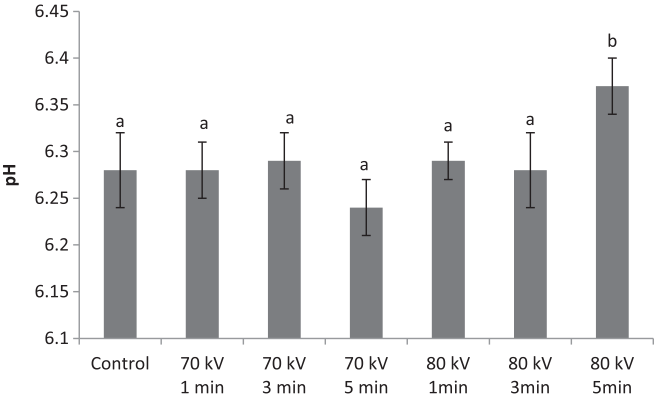

Fig. 3. pH of Atlantic mackerel fillets submitted different DBD treatments. Values (mean ± standard deviation. n = 3) followed by different lowercase letter in same column are significantly different ($P < 0.05$).

The pH values of the samples submitted to 80 kV at 5 min were clearly the highest. Furthermore, there was not an apparent relationship between pH and bacterial growth. pH has been reported to be a poor indicator of microbial growth and fish freshness (Alfaro, Hernández, Baliño-Zuazo, & Barranco, 2013). Nevertheless, Senturk and Alpas (2013) specified that fresh Atlantic mackerel with a pH higher than 7.00 was considered to be spoiled. All treatments tested maintained values under this critical limit.

3.2.2. Proximate composition

Moisture and fat content of Atlantic mackerel were found to be in the range of 69.91–68.11 and 14.14–14.9 g 100 g^{-1}, respectively. Both results agreed with previous studies on Atlantic mackerel (Aubourg, Rodríguez, & Gallardo, 2005; Torres, Vázquez, Saraiva, Gallardo, & Aubourg, 2013). Fat and moisture content did not change for the treatment conditions employed.

3.3. Effect of cold plasma on lipid oxidation

3.3.1. Fatty acid composition (FA)

Analysis of fatty acid profiles detected that docosahexaenoic acid (DHA, C22:6 n-3) was the most abundant polyunsaturated fatty acid, and palmitic acid (C16:0) and oleic acid (C18:1, n-9) the main saturated and monounsaturated fatty acids, respectively. This is in agreement with previously reported data (Maestre, Pazos, & Medina, 2011).

Fatty acids composition of the treated samples was modified by ACP, as shown in Table 1. The levels of oleic acid (C18:1, n-9) and eicosapentaenoic acid (EPA, C20:5 n-3) were lower for the control samples compared to plasma treated samples. These reductions can be attributed to the reactive oxygen and nitrogen species generated and retained within the package.

Polyene ratio also reflected the loss of ω3 PUFAs (C: 20:5 and C: 22:5) for the control samples. Another study showed that there was no

Table 1
Main fatty acid composition of Atlantic mackerel submitted different DBD treatments.

	16:00	18:1, n-9	20:5 n-3	22:6 n-3	Polyene ratio
Control	1.00 ± 0.00d	0.12 ± 0.00a	0.30 ± 0.05ab	0.91 ± 0.01b	1.20 ± 0.04
70 kV 1 min	0.66 ± 0.01abc	0.26 ± 0.01b	0.28 ± 0.03a	1.00 ± 0.00c	1.95 ± 0.06
70 kV 3 min	0.74 ± 0.16bc	0.29 ± 0.07bc	0.62 ± 0.12c	1.00 ± 0.00c	2.27 ± 0.69
70 kV 5 min	0.83 ± 0.01cd	0.40 ± 0.00d	0.59 ± 0.08c	1.00 ± 0.00c	1.91 ± 0.13
80 kV 1 min	0.54 ± 0.02a	0.34 ± 0.01cd	0.46 ± 0.03bc	1.00 ± 0.00c	2.71 ± 0.08
80 kV 5 min	0.62 ± 0.04ab	0.34 ± 0.00cd	1.00 ± 0.00d	0.42 ± 0.02a	2.27 ± 0.09

Values (mean ± standard deviation. n = 3) followed by different lowercase letter in same column are significantly different ($P < 0.05$).

Table 2
Lipid oxidation markers (Peroxide Value, Dienes) of Atlantic mackerel submitted to different DBD treatments.

	PV (meq. active oxygen/kg lipids)	Dienes (mmol of hydroperoxides/kg lipid)
Control	6.89 ± 0.00a	1.42 ± 0.19a
70 kV 1 min	8.97 ± 0.74a	2.17 ± 0.28ab
70 kV 3 min	21.87 ± 0.59b	2.37 ± 0.12b
70 kV 5 min	35.44 ± 6.16c	2.78 ± 0.55b
80 kV 1 min	17.59 ± 3.23b	1.46 ± 0.36a
80 kV 3 min	35.75 ± 0.09c	2.25 ± 0.30b
80 kV 5 min	37.57 ± 2.49c	5.56 ± 1.33c

Values (mean ± standard deviation. n = 3) followed by different lowercase letter in same column are significantly different ($P < 0.05$).

significant correlation between total amount of PUFAs and mackerel shelf life in term of oxidation (Maestre et al., 2011).

3.3.2. Lipid oxidation parameters (PV, Dienes, TBARS)

Primary oxidation was followed by PV and Dienes assessment (Table 2). Control samples had 6.89 milliequivalents of O_2 per kilogram of oil. Ozogul and Balikci (2013) reported similar initial PV. A significant ($P < 0.05$) primary oxidation (PV and Dienes) development was observed for DBD treatment. A comparison of different voltages (70 kV and 80 kV) and treatment time (1, 3 and 5 min) showed both variables increased the rate of oxidation. Similarly, Joshi et al. (2011) suggested that lipid oxidation is proportional to the amount of plasma energy applied. Van Durme, Nikiforov, Vandamme, Leys, and De Winne (2014) also revealed that cold plasma caused the formation of several volatiles related to lipid oxidation. ACP can generate reactive species that have strong oxidation capacities. During DBD treatment, free radicals are generated that trigger lipid oxidation (Kim et al., 2013).

TBARS values ranged from 0.74 ± 0.01 to 0.75 ± 0.00 mg of malondialdehyde (MDA) per kilogram of fish. There were no significant differences ($P > 0.05$) between control and samples submitted to ACP treatment. The effect of ACP on lipid oxidation measured through TBARS values is not clear. Whereas, Rød et al. (2012) and Kim et al. (2013) reported higher TBARS values for plasma treated samples on bresaloa and pork loin, Kim et al. (2011) found lower TBARS values of plasma treated bacon, however this trend reverted over storage. The TBARS values of DBD plasma treated pork and beef samples were unmodified up to a treatment time of 7.5 min (Jayasena et al., 2015).

3.4. Effect of cold plasma on colour

Colour has a direct influence on the acceptance of fish and influences consumers' decision to purchase (Rodriguez-Turienzo et al., 2011). The impact of ACP on the colour of Atlantic mackerel is shown in Table 3.

No clear trend was found between any plasma treated conditions, similar to those reported by Rød et al. (2012). However, a significant decrease in L* (lightness) was evident for the plasma treated samples compared to control samples. Kim et al. (2011, 2013) also observed that

Table 3
Colour (L*, a* and b*) of Atlantic mackerel submitted to different DBD treatments.

	L*	a*	b*
Control	57.42 ± 2.15d	3.67 ± 0.63ab	14.12 ± 2.75ab
70 kV 1 min	53.72 ± 2.98bc	4.48 ± 0.33c	13.26 ± 1.56ab
70 kV 3 min	51.70 ± 0.50ab	3.96 ± 0.06bc	13.21 ± 0.55ab
70 kV 5 min	53.87 ± 1.26bc	4.19 ± 0.29bc	15.13 ± 0.12b
80 kV 1 min	55.37 ± 0.86cd	3.19 ± 0.35a	15.15 ± 0.42b
80 kV 3 min	50.65 ± 0.93a	4.4 ± 0.49c	13.44 ± 0.15ab
80 kV 5 min	55.91 ± 0.47cd	3.97 ± 0.28bc	11.52 ± 2.32a

Values (mean ± standard deviation. n = 3) followed by different lowercase letter in same column are significantly different ($P < 0.05$).

the L* value decreased for bacon and pork loin treated with ACP. There were not differences between ACP treated samples and controls for a* and b* values. These results indicated that the ACP did not influence on the colour markedly.

3.5. Effect of cold plasma on protein structure and water distribution

NMR provides useful information about the interactions between water and myofibrillar meat proteins (Table 4). Three peaks are thought to be directly related to three water components in muscle tissue. The first component is water closely associated ("bound") with macromolecules (T_{2b}). T_{21} is immobilised water, which is located in the protein-dense myofibrillar network. The final component is extramyofibrillar water or free water (T_{22}). Table 4 shows that water tightly bound to macromolecules (T_{2b}) is not influenced by DBD treatment. Similarly, Bertram et al. (2001) demonstrated the T_{2b} did not reflected any mechanical stress and micro or macro-structural changes in the meat matrix. In all treatments, the majority of water is trapped by the dense myofibrillar network (T_{21}). A significant decrease in T_{21} was however observed for plasma treated samples. This is probably due to the fish structure alteration, which will weaken the matrix that is trapping the T_{21} water. Simultaneously, extramyofibrillar water (T_{22}) increased as a consequence of the release of trapped water (T_{21}).

Table 4
NMR parameters (T_{2b}, T_{21}, T_{22}) area of Atlantic mackerel submitted to different DBD treatments.

	T_{2b}	T_{21}	T_{22}
Control	8814 ± 3879a	331,221 ± 1466d	10,327 ± 1573a
70 kV 1 min	22,221 ± 1959b	300,835 ± 3826c	10,867 ± 1562ab
70 kV 3 min	14,873 ± 4195a	295,586 ± 1015b	14,576 ± 2572c
70 kV 5 min	11,306 ± 1213a	290,037 ± 8038b	22,705 ± 3159d
80 kV 1 min	12,774 ± 1033a	307,940 ± 8025c	15,586 ± 2653c
80 kV 3 min	14,029 ± 1033a	300,017 ± 1840c	13,511 ± 3111bc
80 kV 5 min	0 ± 0	273,197 ± 3769a	22,685 ± 870d

Values (mean ± standard deviation. n = 3) followed by different lowercase letter in same column are significantly different ($P < 0.05$).

4. Conclusions

DBD is shown to be a potential treatment for reducing the spoilage bacteria (total aerobic psychrotrophic bacteria, *Pseudomonas* and lactic acid bacteria). Treatment voltage and time were both found to have significant effects on microbial inactivation. Results indicated that ACP caused changes in immobilised (T_{21}) and extramyofibrillar (T_{22}) water, but no differences were found in water closely associated to molecules (T_{2b}). Processing conditions of DBD treatment (voltage and time) rendered the mackerel more susceptible to lipid oxidation. However, ACP does not affect adversely physicochemical parameters such as pH and colour. Overall, ACP showed promising results in reducing microbiological load of mackerel fish, although lipid oxidation should be further controlled.

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