



## Shelf-life extension of herring (*Clupea harengus*) using in-package atmospheric plasma technology

I. Albertos<sup>a,\*</sup>, A.B. Martin-Diana<sup>a,\*</sup>, P.J. Cullen<sup>b</sup>, B.K. Tiwari<sup>c</sup>, K. Shikha Ojha<sup>c</sup>, P. Bourke<sup>b</sup>, D. Rico<sup>a</sup>

<sup>a</sup> Agrarian Technological Institute of Castille and Leon (ITACyL), Ctra. Burgos Km 119, Finca Zamadueñas, 47071 Valladolid, Spain

<sup>b</sup> School of Food Science & Environmental Health, Dublin Institute of Technology, Dublin, Ireland

<sup>c</sup> Teagasc Food Research Centre, Ashtown Dublin 15, Ireland

### ARTICLE INFO

#### Keywords:

*Clupea harengus*  
Cold plasma  
Fish spoilage  
Fish quality  
Lipid oxidation

### ABSTRACT

Atmospheric cold plasma is a green and emerging technology, highly interesting to the food industry for its application. Dielectric Barrier Discharges (DBD) can generate atmospheric cold plasma inside sealed packages filled with air through the use of high voltages. This study investigated the use of a large gap DBD design to generate a plasma discharge within the headspace of packaged herring fillets, and its effects on microbiological and quality markers of the fish stored for 11 days at 4 °C. DBD plasma treatment conditions were 70 kV or 80 kV for 5 min treatment time.

Results showed that the microbial load (total aerobic mesophilic, total aerobic psychrotrophics, *Pseudomonas*, lactic acid bacteria and *Enterobacteriaceae*) were significantly ( $p < 0.05$ ) lower in the treated samples compared to the control samples. However, samples exposed to the lowest applied voltage better retained key quality factors (lower oxidation and less colour modification). DBD-treatment caused a reduction in trapped water in the myofibrillar network (T21), identified using low-field nuclear magnetic resonance of protons (<sup>1</sup>H LF NMR).

The results indicate that in-package plasma treatment could be employed as an effective treatment for reducing spoilage bacteria in fish.

**Industrial relevance:** Dielectric Barrier Discharge (DBD) was evaluated as a treatment for highly perishable fish products. DBD's is a safety and lower costs alternative for processing industry. DBD has been scarcely tested on fish; this work showed results on quality and shelf-life of a highly perishable fish species, which might serve as reference for processing optimisation of fish products minimally processed by DBD.

### 1. Introduction

Atlantic herring (*Clupea harengus*) is one of the most important pelagic fish species in Europe, containing high levels of long chain polyunsaturated fatty acids (PUFA). Despite the health benefits of PUFA, these species are underutilised due to their susceptibility to oxidation, which is directly related to the production of off-flavours and off-odours (Harris & Tall, 1994), leading to a reduced shelf life and nutritional values. Microbial activity is the main factor limiting the shelf life of fresh fish (Ólafsdóttir et al., 1997).

Temperature-based preservation processing (cooling, super chilling and freezing) is a classic technique to increase the shelf-life in fish. However, these traditional technologies present many inconveniences. Cooling only maintains freshness over a short period of time and freezing imparts significant changes in the structural properties of the muscle (Aubourg, Rodríguez, & Gallardo, 2005; Hamre, Lie, & Sandnes, 2003; Sampels, 2015).

Non-thermal processing technologies have been effective in processing foods with higher nutritive values and fresh-like attributes. For example, high voltage electric field (HVEF) has shown promising results in frozen tuna, increasing thawing rate and reducing volatile nitrogen produced by microorganisms (Mousakhani-Ganjeh, Hamdami, & Soltanizadeh, 2015, 2016). However, these novel technologies may be limited in their use on fish due to oxidation and colour modifications, for instance, high pressure treatment (Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014) and irradiation (Sant'Ana & Mancini-Filho, 2000).

Atmospheric cold plasma is an emerging technology currently under active research. 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. DBD designs offer the feasibility of generating the discharge under atmospheric conditions within the confines of sealed packages through the application of

\* Corresponding authors.

E-mail addresses: [albmunir@itacyl.es](mailto:albmunir@itacyl.es) (I. Albertos), [mardiaan@itacyl.es](mailto:mardiaan@itacyl.es) (A.B. Martin-Diana).

<http://dx.doi.org/10.1016/j.ifset.2017.09.010>

Received 30 June 2017; Received in revised form 13 September 2017; Accepted 13 September 2017  
Available online 18 September 2017

1466-8564/ © 2017 Elsevier Ltd. All rights reserved.

sufficiently high voltages (Misra, Keener, Bourke, Mosnier, & Cullen, 2014; Misra, Ziuzina, Cullen, & Keener, 2013; Pankaj, Misra, & Cullen, 2013). These discharges generate energetic electrons that dissociate oxygen molecules by direct impact. Generated single O atom scan combine with oxygen molecules (O<sub>2</sub>) to form ozone gas (Misra et al., 2014). DBD designs offer several advantages in comparison with other atmospheric pressure plasma sources. Firstly, air is readily employed as the inducer gas, reducing the cost of treatment which is important for processing high volume low added value produce. Also, treatment can be performed inside the sealed package, 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 et al., 2014).

The effectiveness of atmospheric cold plasma has been demonstrated for meat inoculated with pathogenic bacteria (Kim et al., 2011; Kim, Yong, Park, Choe, & Jo, 2013; Noriega, Shama, Laca, Díaz, & Kong, 2011; Rød, Hansen, Leipold, & Knöchel, 2012). However, the application of atmospheric cold plasma for control of spoilage bacteria has received little attention (Wang, Zhuang, & Zhang, 2016) with sparse data reported on plasma processing of fish.

The suitability of atmospheric air plasma for high lipid products is unknown. Such products are susceptible to notable changes due to oxidation from the formation of hydroxyl acids, keto acids, short-chain acids and aldehydes (Misra, Tiwari, Raghavarao, & Cullen, 2011). The effect of atmospheric air plasma discharges on food quality of meat products has not been conclusive (Jayasena et al., 2015; Kim et al., 2011; Kim et al., 2013; Rød et al., 2012).

Albertos et al. (2017) investigated the effect of different process conditions (voltage and time) on the microbial and quality parameters of fish products after DBD treatment and post treatment storage of 24 h. The aim of the present study was to evaluate the use of optimal conditions on the microbiological and physicochemical changes and lipid oxidation process of herring during chilled storage at 4 °C after in-package atmospheric plasma treatment.

## 2. Material and methods

### 2.1. Chemicals

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

### 2.2. Produce characteristics

Atlantic herring (*Clupea harengus*) were purchased at Stevie Connolly Seafood Company (Dublin, Ireland).

### 2.3. In-package plasma treatment

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 a dielectric set-up. The packages were placed between two circular aluminium plate electrodes (outer diameter = 158 mm) with a contact surface area of 249.64 cm<sup>2</sup>. A 2 mm thick polypropylene sheet acted as the dielectric barrier and used to stabilize the discharge. The electrode separation was adjusted to the tray height of 35 mm. The applied voltage to the positive 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 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). After treatment,

the control and treated packages were stored at 4 °C and analysed on days 1, 3, 6, 9 and 11 of storage.

### 2.4. Microbiological analysis

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 were determined using Tryptic Glucose Yeast Agar (PCA) with 1% NaCl after incubation at 30 °C for 72 h.

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

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

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

(v) *Enterobacteria* were determined on double-layer Violet Red Bile Glucose (VRBG) incubated at 37 °C for 24 h.

Counts were done by using pour plate and spread techniques as described in the Official Methods of Analysis of AOAC International guidelines (AOAC, 2012).

### 2.5. pH

Two grams of fish muscle was homogenised in 10 mL of distilled water. The pH of the homogenised samples were measured directly at room temperature using a portable pH meter (Orion Research Inc., Boston, MA 02129, USA) with a glass pH probe (Glass electrode: model EC-2010-06, Refex Sensors, Ltd. Westport, Ireland).

### 2.6. Thiobarbituric acid reactive substances (TBARS)

Malondialdehyde (MDA) was measured using the methodology described by Vyncke (1975) for a 5% trichloroacetic acid extract of the fish muscle. Results were expressed as mg of malondialdehyde (MDA) per kilogram of fish. TBARS results were expressed as µM/g and calculated using the following equation:

$$\text{TBARS (mg/kg)} = (\text{Ac} \times \text{V})/\text{W}$$

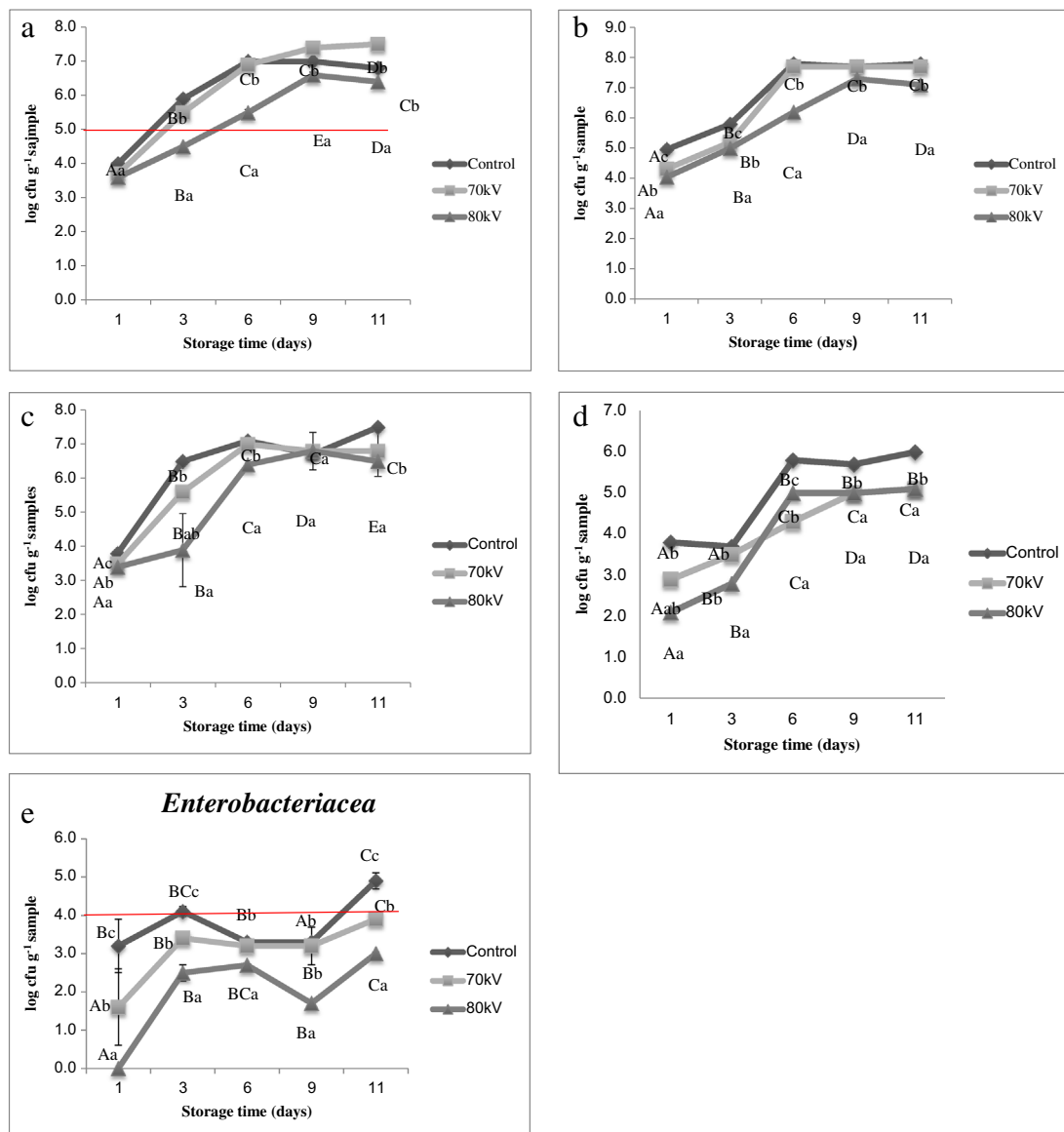
where Ac is the amount determined from the calibration curve and W is the weight of the sample taken while V is volume in mL or dilution factor of the total extract prepared.

### 2.7. Colour

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

### 2.8. Low-field nuclear magnetic resonance of protons (<sup>1</sup>H LF NMR) relaxometry

<sup>1</sup>H NMR was carried out following the method describe by Ojha, Kerry, and Tiwari (2017). Ten grams of herring 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 (T2) were conducted using the Carr-Purcell-Meiboom-



**Fig. 1.** a: Total aerobic mesophilic ( $\log \text{cfu g}^{-1}$ ) counts in Atlantic herring fillets subjected to different processing treatments and stored at 4 °C for 11 days. Values (mean  $\pm$  standard deviation,  $n = 3$ ) followed by the same lowercase letter in same day are not significantly different ( $p > 0.05$ ). Values (mean  $\pm$  standard deviation,  $n = 3$ ) followed by the same uppercase letter in same treatment are not significantly different ( $p > 0.05$ ). b: Total aerobic psychrotrophic ( $\log \text{cfu g}^{-1}$ ) counts in Atlantic herring fillets subjected to different processing treatments and stored at 4 °C for 11 days. Values (mean  $\pm$  standard deviation,  $n = 3$ ) followed by the same lowercase letter in same day are not significantly different ( $p > 0.05$ ). Values (mean  $\pm$  standard deviation,  $n = 3$ ) followed by the same uppercase letter in same treatment are not significantly different ( $p > 0.05$ ). c: *Pseudomonas* ( $\log \text{cfu g}^{-1}$ ) counts in Atlantic herring fillets subjected to different processing treatments and stored at 4 °C for 11 days. Values (mean  $\pm$  standard deviation,  $n = 3$ ) followed by the same lowercase letter in same day are not significantly different ( $p > 0.05$ ). Values (mean  $\pm$  standard deviation,  $n = 3$ ) followed by the same uppercase letter in same treatment are not significantly different ( $p > 0.05$ ). d: LAB ( $\log \text{cfu g}^{-1}$ ) counts in Atlantic herring fillets subjected to different processing treatments and stored at 4 °C for 11 days. Values (mean  $\pm$  standard deviation,  $n = 3$ ) followed by the same lowercase letter in same day are not significantly different ( $p > 0.05$ ). Values (mean  $\pm$  standard deviation,  $n = 3$ ) followed by the same uppercase letter in same treatment are not significantly different ( $p > 0.05$ ). e: *Enterobacteriaceae* ( $\log \text{cfu g}^{-1}$ ) counts in Atlantic herring fillets subjected to different processing treatments and stored at 4 °C for 11 days. Values (mean  $\pm$  standard deviation,  $n = 3$ ) followed by the same lowercase letter in same day are not significantly different ( $p > 0.05$ ). Values (mean  $\pm$  standard deviation,  $n = 3$ ) followed by the same uppercase letter in same treatment are not significantly different ( $p > 0.05$ ).

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

Data were analysed by multifactor analysis of variance (multifactor ANOVA) of each variable, using Statgraphics software (Centurion XVI; Statistical Graphics Co., Rockville, MD, USA) for different coating treatment over storage. In case of significant differences, Fisher LSD (Least Significant Differences) range test ( $p < 0.05$ ) was used.

## 3. Results and discussion

### 3.1. Microbiological analyses

Microbial populations of the treated samples were significantly reduced ( $p < 0.05$ ) as compared to control samples (Fig. 1). Total aerobic mesophilic, total aerobic psychrotrophics, *Pseudomonas*, LAB and *Enterobacteriaceae* displayed similar inactivation behaviours. The antibacterial activity of ozone in fish has been previously reported by others authors (Bono & Badalucco, 2012; Campos, Rodríguez, Losada, Aubourg, & Barros-Velázquez, 2005; Gonçalves, 2009).

Fish samples showed initial total aerobic mesophilic counts (Fig. 1a)

**Table 1**  
pH in Atlantic herring fillets subjected to different processing treatments and stored at 4 °C for 11 days.

	Day 1	Day 3	Day 6	Day 9	Day 11
Control	<sup>A</sup> 6.23 ± 0.01 <sub>c</sub>	<sup>A</sup> 6.31 ± 0.07 <sub>b</sub>	<sup>C</sup> 7.04 ± 0.01 <sub>c</sub>	<sup>C</sup> 7.02 ± 0.01 <sub>b</sub>	<sup>B</sup> 6.90 ± 0.01 <sub>a</sub>
70 kV 5 min	<sup>A</sup> 6.12 ± 0.01 <sub>a</sub>	<sup>A</sup> 6.14 ± 0.01 <sub>a</sub>	<sup>B</sup> 6.82 ± 0.01 <sub>b</sub>	<sup>C</sup> 7.02 ± 0.03 <sub>b</sub>	<sup>D</sup> 7.32 ± 0.07 <sub>c</sub>
80 kV 5 min	<sup>A</sup> 6.14 ± 0.00 <sub>b</sub>	<sup>B</sup> 6.20 ± 0.01 <sub>ab</sub>	<sup>C</sup> 6.73 ± 0.03 <sub>a</sub>	<sup>D</sup> 6.90 ± 0.01 <sub>a</sub>	<sup>E</sup> 7.16 ± 0.01 <sub>b</sub>

Values (mean ± standard deviation, n = 3) followed by different uppercase letter in same row are significantly different (p < 0.05). Values (mean ± standard deviation, n = 3) followed by different lowercase letter in same column, for each parameter, are significantly different (p < 0.05).

of 10<sup>4</sup> cfu/g, similar to values reported by other authors for herring (Lyhs, Lahtinen, & Schelvis-Smit, 2007; Özogul, Taylor, Qantick, & Özogul, 2000; Randell, Hattula, & Ahvenainen, 1997).

Total aerobic psychrotropic (Fig. 1b) showed a significantly faster growth than total aerobic mesophilic. The initial counts (4.95 ± 0.07–4.05 ± 0.06 cfu/g) values were consistent with data reported by Lyhs et al. (2007). These results suggest that psychrotropic is the predominant microflora in fish (Nuñez-Flores, Castro, López-Caballero, Montero, & Gómez-Guillén, 2013). The psychrotropic counts maintained acceptable limits by day 6. After this day, only the highest voltage (80 kV for 5 min) treatment was effective in maintaining acceptable psychrotropic counts (Commission Regulation (EC) 2073/2005).

*Pseudomonas* was also evaluated (Fig. 1c) since it is dominant in refrigerated fish (Corbo et al., 2008; Gram & Dalgaard, 2002; Karim et al., 2011) and its ability to cause spoilage odour is demonstrated (Corbo et al., 2008). Furthermore, *Pseudomonas* counts have been associated with the formation of histamine in fish (Ahmed, Mansour, Mohamed, Deabes, & Salah El din, D. A., 2012; Economidou, Gousia, Kemetzi, Sakkas, & Papadopoulou, 2017; Morii, Taylor, & Murray, 1986). Initially, both DBD treatments (70 kV and 80 kV for 5 min) showed strong effectiveness against *Pseudomonas*. This effect was significantly higher (p < 0.05) at the higher voltage which resulted in the greatest inactivation efficiency at the beginning of storage (Day 1, 3 and 6). As shown in Fig. 1c, *Pseudomonas* growth was more steady from day 6. This fact can be explained because *Pseudomonas* is a strict aerobic microorganism and is unable to survive in the absence of oxygen (Mexis, Chouliara, & Kontominas, 2009).

LAB are also part of the natural microflora of fresh Atlantic herring and associated with fish spoilage (Françoise, 2010). The initial counts were from 2.10 ± 0.01 (80 kV for 5 min) to 3.80 ± 0.71 cfu/g (control) on day 1, and reached counts from 5.10 ± 0.02 cfu/g (80 kV for 5 min) to 6.05 ± 0.07 (control) on day 11 (Fig. 1d). Although LAB are not dominant in the microflora of raw herring (Lyhs et al., 2007), they typically gain importance during storage. As a result, the growth of the Gram-negative aerobic bacteria group (predominantly *Pseudomonads*) is reduced over storage time and LAB emerge as important microorganisms for spoilage (Françoise, 2010). Furthermore, LAB inhibit the growth of other bacteria due to the formation of lactic acid and bacteriocins or by competition for nutrients, and this may contribute to their selection during spoilage seafood products (Gram & Dalgaard, 2002). These findings showed a reduction of LAB as a result of DBD treatment. At the beginning of the storage period (day 1 and 3), only the higher voltage of 80 kV was effective in the reduction of LAB. From day 6, treatment at 70 kV resulted in a reduction of LAB compared to the control. In fact, no noticeable differences in the voltage used were observed at the end of storage period.

*Enterobacteriaceae*, is a hygiene indicator, associated with polluted water, delay in chilling after catch (Chouliara, Sawaidis, Riganakos, & Kontaminas, 2004) or contamination in the filleting process (Moini et al., 2009). According to Lyhs et al. (2007), the maximum limits for acceptability of unpacked herring is 10<sup>4</sup> cfu/g counts. Samples treated at 70 kV and 80 kV fulfilled with this requirement over storage (Fig. 1e). This species of bacteria is the least dominant compared to the others studied. Similarly, Chytiri, Chouliara, Savvaidis, and

Kontaminas (2004) reported that *Enterobacteriaceae* grew less than that of other Gram-negative psychrotrophic spoilage. Their minor abundance can be attributed to a lower growth rate compared to other Gram-negative psychrotrophic bacteria, making them poor competitors (Bahmani et al., 2011).

Ozone is one key metastable compound generated in large quantities by DBDs treatments and one which can be measured relatively easily inside the package. Wang et al. (2016) reported that ozone has a relatively long half-life compared to other reactive species formed during the 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 cells, 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<sub>3</sub>, NO<sub>2</sub>, NO<sub>3</sub>, N<sub>2</sub>O<sub>4</sub>) was found to increase with treatment time; however, a strong decrease in O<sub>3</sub> levels was observed with increases in relative humidity. The decrease in O<sub>3</sub> and concentration of nitrogen oxides are associated with high specific power densities in the closed container and to increasing relative humidity levels. Relative humidity levels are found to significantly influence the levels of nitrogen oxides and HNO<sub>x</sub> (x = 1, 4) acids which can be linked to bactericidal effects.

### 3.2. pH

Changes in the pH levels of herring fillets after DBD are shown in Table 1. DBD treatment produced a significant decrease in pH. This acidification has been also reported by Kim et al. (2013) and Wang et al. (2016) in samples treated with DBD plasma systems. The formation of nitric acid (HNO<sub>3</sub>) and nitrous acid (HNO<sub>2</sub>) is attributed to H<sup>•</sup> dissociation during treatment (Oehmigen et al., 2010).

In general, pH values significantly increased over the storage period, although samples treated with plasma showed lower increases, especially for the highest voltage (80 kV). This increase in pH could be due to alkaline compounds from protein and nucleotide decomposition in the muscle during the post-mortem period (Mexis et al., 2009). Consequently, DBD exerted a significant protective effect, reducing the protein decomposition.

At the end of storage, this trend changed, which the control samples showing the significantly lowest values. The pH values were in agreement with the LAB counts. Thus, pH decreased probably due to the growth of LAB and lactic acid formation (Picouet, Cofan-Carbo, Vilaseca, Ballbè, & Castells, 2011).

### 3.3. Thiobarbituric acid reactive substances (TBARS)

Atlantic herring is found to be more susceptible to oxidation than other fish species due to its high content of PUFA (Hamre et al., 2003). Therefore, it is a good food matrix to study the effect of plasma on lipid oxidation. Kim et al. (2011) concluded that more studies must be conducted to clarify the effect of cold atmospheric plasma on the lipid oxidation of muscle foods. Possible pro-oxidant effects of ozone on fish

**Table 2**  
TBARS in Atlantic herring fillets subjected to different processing treatments and stored at 4 °C for 11 days.

	Day 1	Day 3	Day 6	Day 9	Day 11
Control	<sup>A</sup> 0.17 ± 0.01 <sub>a</sub>	<sup>B</sup> 0.36 ± 0.01 <sub>a</sub>	<sup>C</sup> 0.48 ± 0.01 <sub>a</sub>	<sup>D</sup> 0.60 ± 0.00 <sub>a</sub>	<sup>E</sup> 0.65 ± 0.00 <sub>a</sub>
70 kV 5 min	<sup>A</sup> 0.17 ± 0.01 <sub>a</sub>	<sup>B</sup> 0.36 ± 0.00 <sub>a</sub>	<sup>C</sup> 0.48 ± 0.01 <sub>a</sub>	<sup>D</sup> 0.63 ± 0.00 <sub>b</sub>	<sup>E</sup> 0.75 ± 0.00 <sub>b</sub>
80 kV 5 min	<sup>A</sup> 0.19 ± 0.00 <sub>b</sub>	<sup>B</sup> 0.42 ± 0.00 <sub>b</sub>	<sup>C</sup> 0.53 ± 0.00 <sub>b</sub>	<sup>D</sup> 0.78 ± 0.01 <sub>c</sub>	<sup>D</sup> 0.77 ± 0.00 <sub>c</sub>

Values (mean ± standard deviation, n = 3) followed by different uppercase letter in same row not significantly different (p < 0.05).

Values (mean ± standard deviation, n = 3) followed by different lowercase letter in same column, for each parameter, are significantly different (p < 0.05).

constituents has not been extensively studied to date (Gonçalves, 2009). Ozone applied as ozonated water for sanitizing of fish was described as a potent oxidizer (Crapo, Himelbloom, Vitt, & Pedersen, 2004).

TBARS is related to secondary oxidation products; they accumulated and, as expected, increased for all treatments during the storage period (Table 2). However, the differences among TBARS values for the control and 70 kV samples were not significant (p > 0.05) until the end of storage, mainly day 9. Gray, Gomaa, and Buckley (1996) established that TBARS values above 0.5 mg MDA/kg were detectable as off-flavour by an organoleptic panel. Consequently, for the 80 kV treated samples, off-flavours are likely to be detectable from day 6, whereas these values were exceeded in the other samples from day 9 of storage.

These results were consistent with other works reported by other authors. Rød et al. (2012) also demonstrated that TBARS values of jet plasma treated ready to eat meat (bresaola) were higher as a result of increasing treatment power and storage time. Similar results were reported by Kim et al. (2011, 2013) for bacon and pork loin, respectively. Cold plasma also produces precursors of lipid oxidation such as hydroperoxides (Kim et al., 2013). Jayasena et al. (2015) applied DBD treatment for pork butt and beef loin at 40 kV for 2.5, 5, 7.5 and 10 min, showing that DBD-plasma only affected lipid oxidation after a 10-min treatment.

### 3.4. Colour

Colour changes of herring fillets treated with DBD over storage are shown in Table 3. DBD treatment significantly reduced the lightness of the product (L\*). Several works (Kim et al., 2011; Kim et al., 2013) indicated that L\* values decrease with DBD plasma treatment. This reduction of L\* values was found to be proportional to the voltage applied. However, treated samples maintained stable levels of L\* values over the storage period.

A significant loss of redness (a\*) was observed for the treated samples and at the beginning of storage (Day 3) probably to oxidation of heme proteins, haemoglobin and myoglobin, which are red in their reduced form and brown in their oxidised ferric form. Colour changes in a\* are associated with lipid oxidation and have been widely reported.

**Table 3**

Colour parameters: lightness (L\*), redness (a\*), yellowness (b\*) in Atlantic herring fillets subjected to different processing treatments and stored at 4 °C for 11 days.

	Day 1	Day 3	Day 6	Day 9	Day 11
L*					
Control	<sup>BC</sup> 56.38 ± 0.26 <sub>c</sub>	<sup>C</sup> 55.19 ± 3.76 <sub>a</sub>	<sup>BC</sup> 54.92 ± 0.94 <sub>ab</sub>	<sup>A</sup> 49.58 ± 4.32 <sub>a</sub>	<sup>AB</sup> 52.41 ± 1.66 <sub>a</sub>
70 kV 5 min	<sup>A</sup> 52.08 ± 0.16 <sub>b</sub>	<sup>A</sup> 50.86 ± 0.60 <sub>a</sub>	<sup>B</sup> 59.88 ± 0.97 <sub>b</sub>	<sup>A</sup> 49.99 ± 6.05 <sub>a</sub>	<sup>A</sup> 49.73 ± 3.09 <sub>a</sub>
80 kV 5 min	<sup>A</sup> 50.21 ± 0.02 <sub>a</sub>	<sup>A</sup> 51.21 ± 2.03 <sub>a</sub>	<sup>A</sup> 50.10 ± 6.17 <sub>a</sub>	<sup>B</sup> 58.41 ± 0.94 <sub>b</sub>	<sup>A</sup> 51.74 ± 0.59 <sub>a</sub>
a*					
Control	<sup>B</sup> 4.40 ± 0.03 <sub>c</sub>	<sup>A</sup> 0.75 ± 0.14 <sub>c</sub>	<sup>AB</sup> 2.37 ± 0.64 <sub>a</sub>	<sup>AB</sup> 1.62 ± 0.62 <sub>a</sub>	<sup>B</sup> 3.85 ± 0.72 <sub>a</sub>
70 kV 5 min	<sup>BC</sup> 3.79 ± 0.03 <sub>b</sub>	<sup>A</sup> 0.24 ± 0.06 <sub>b</sub>	<sup>AB</sup> 1.49 ± 0.45 <sub>a</sub>	<sup>BC</sup> 2.41 ± 0.41 <sub>a</sub>	<sup>C</sup> 4.42 ± 0.43 <sub>a</sub>
80 kV 5 min	<sup>C</sup> 3.05 ± 0.05 <sub>a</sub>	<sup>A</sup> 0.15 ± 0.03 <sub>a</sub>	<sup>C</sup> 3.04 ± 0.37 <sub>a</sub>	<sup>B</sup> 2.03 ± 0.31 <sub>a</sub>	<sup>D</sup> 5.35 ± 1.50 <sub>a</sub>
b*					
Control	<sup>B</sup> 10.61 ± 0.04 <sub>a</sub>	<sup>C</sup> 11.47 ± 0.51 <sub>a</sub>	<sup>D</sup> 13.72 ± 1.77 <sub>b</sub>	<sup>A</sup> 7.87 ± 1.02 <sub>a</sub>	<sup>E</sup> 15.83 ± 1.37 <sub>b</sub>
70 kV 5 min	<sup>A</sup> 11.15 ± 0.06 <sub>b</sub>	<sup>AB</sup> 13.26 ± 0.78 <sub>b</sub>	<sup>AB</sup> 14.21 ± 0.66 <sub>b</sub>	<sup>AB</sup> 12.37 ± 2.65 <sub>b</sub>	<sup>B</sup> 14.74 ± 1.62 <sub>b</sub>
80 kV 5 min	<sup>A</sup> 12.02 ± 0.07 <sub>c</sub>	<sup>A</sup> 12.15 ± 0.69 <sub>ab</sub>	<sup>A</sup> 10.06 ± 1.57 <sub>a</sub>	<sup>B</sup> 16.12 ± 1.82 <sub>c</sub>	<sup>A</sup> 11.14 ± 1.27 <sub>a</sub>

Values (mean ± standard deviation, n = 6) followed by the same uppercase letter in same row are not significantly different (p > 0.05).

Values (mean ± standard deviation, n = 6) followed by the same lowercase letter in same column, for each parameter, are not significantly different (p > 0.05).

These results were in agreement with the oxidative measurement by TBARS. Similarly, other authors have observed a decrease in a\* values as a result of cold atmospheric plasma treatment (Jayasena et al., 2015; Kim et al., 2013; Rød et al., 2012). However, from day 6 no significant (p > 0.05) differences were found between any of DBD-treated and control samples.

Yellowness (b\*) increased significantly in treated samples possibly due to the oxidised heme proteins (met-heme proteins), resulting in a greenish colour. Furthermore, an increase in yellowness (b\*) in samples was reported, which may be due to hydrogen peroxide formation, which reacts with myoglobin (Jayasena et al., 2015). Control and 70 kV samples tended to become more yellow over the storage period, while samples treated at the highest voltage (80 kV) caused a major rise after treatment but less fluctuation over storage.

These data suggests that increasing the voltage might accelerate the formation of hydrogen peroxide and other radicals involved in lipid oxidative reactions.

### 3.5. Water mobility during storage by <sup>1</sup>H LF NMR relaxometry

Low-field nuclear magnetic resonance of protons (<sup>1</sup>H LF NMR) relaxometry provides useful information about the interaction between water and myofibrillar muscle proteins as it is governed by the exchange of water protons and exchangeable protons in proteins. Fig. 2 shows the distribution of relaxation times of fish muscles plasma treated at 70 kV and 80 kV for 5 min and the control which were subsequently chill stored for 11 days. The majority of the water present in the samples was T21 in time range of 46.4–79.7 ms, which represent water trapped by the dense myofibrillar network. A minor band was observed at relaxation times corresponding to T22 in time range of 338–666 ms which represents extra myofibrillar water. Fig. 2 shows a decrease in the intensity peak for the water population at the T21 band for the control compared to DBD treated samples. However, after 11 days of storage water at the T21 band was found to increase with an increase in water proportion at T22. Higher proportion of water population at T22 after DBD treatment compared to the control indicates an increase in free moisture. However, an increase in water proportion

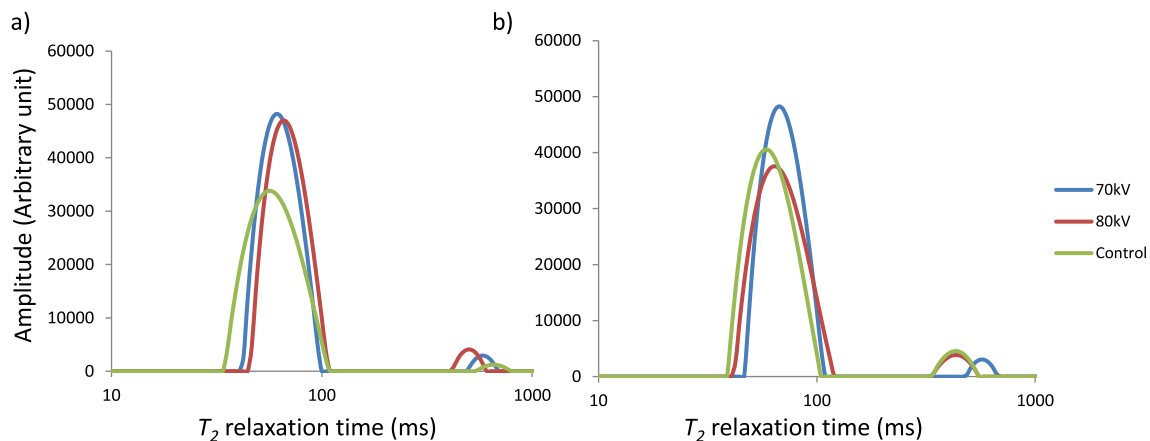


Fig. 2.  $^1\text{H}$  LF NMR distribution of  $T_2$  relaxation times of the following storage at  $4\text{ }^\circ\text{C}$  after treatment (a) and after 11 day storage (B) for Plasma treated and control samples.

at T22 after day 11 indicates an increase in free water, probably occurring due to chilled storage. The changes observed in the water population at T22 after plasma treatment and during storage can be attributed to morphological changes during treatment and/or storage (Belton, Hills, & Raimbaud, 1988; Sánchez-Valencia, Sánchez-Alonso, Martínez, & Careche, 2015) and also due to migration of intra myofibrillar water to extracellular water during storage (Sánchez-Alonso, Moreno, & Careche, 2014).

#### 4. Conclusions

In-package plasma treatment is shown to be a suitable treatment for Atlantic herring fillets, reducing microbial growth (Total aerobic mesophilic, total aerobic psychrotrophics, *Pseudomonas*, LAB and *Enterobacteriaceae*) over chilled storage. The use of higher voltage (80 kV) was more effective in inhibiting microbial counts; however, lower voltage treatments (70 kV) resulted in less negative effects on key quality parameters, such as lipid oxidation and colour modifications. Therefore, DBD plasma applied under conditions of 70 kV for 5 min seems to be a potential technology for preserving or extending the shelf-life of fresh fish fillets. The inclusion of coatings or active compounds could delay or reduce the effect of DBD without any negative effect on microbial deactivation. For this reason, further studies are required to improve and optimize the use of DBD in food matrices with highly content in lipids.

#### Acknowledgements

This work was supported by the Regional Government of Castille and Leon, Spain (Project NUGAFU-PEP 2011/769).

#### References

Ahmed, S. A., Mansour, H. A., Mohamed, L. A., Deabas, M., & Salah El din, D. A. (2012). Biogenic amines and its relation with microbial load in some fish products. *Global Veterinaria*, 8(6), 583–590.

Albertos, I., Martín-Diana, A. B., Cullen, P. J., Tiwari, B. K., Ojha, S. K., Bourke, P., ... Rico, D. (2017). Effects of dielectric barrier discharge (DBD) generated plasma on microbial reduction and quality parameters of fresh mackerel (*Scombercombrus*) fillets. *Innovative Food Science and Emerging Technologies*. <http://dx.doi.org/10.1016/j.ifset.2017.07.006>.

AOAC International (2012). *Official methods of analysis* (19th edition). Editor Dr. G.W. Latimer. Published by AOAC International.

Aubourg, S. P., Rodríguez, A., & Gallardo, J. M. (2005). Rancidity development during frozen storage of mackerel (*Scombercombrus*): Effect of catching season and commercial presentation. *European Journal of Lipid Science and Technology*, 107(5), 316–323.

Bahmani, Z. A., Rezai, M., Hosseini, S. V., Regenstein, J. M., Böhme, K., Alishahi, A., & Yadollahi, F. (2011). Chilled storage of golden gray mullet (*Liza aurata*). *LWT - Food Science and Technology*, 44, 1894–1900.

Belton, P., Hills, B., & Raimbaud, E. (1988). The effects of morphology and exchange on

proton NMR relaxation in agarose gels. *Molecular Physics*, 63, 825–842.

Bono, G., & Badalucco, C. (2012). Combining ozone and modified atmosphere packaging (MAP) to maximize shelf-life and quality of striped red mullet (*Mullus surmuletus*). *LWT - Food Science and Technology*, 47(2), 500–504.

Campos, C. A., Rodríguez, O., Losada, V., Aubourg, S. P., & Barros-Velázquez, J. (2005). Effects of storage in ozonised slurry ice on the sensory and microbial quality of sardine (*Sardina pilchardus*). *International Journal of Food Microbiology*, 103(2), 121–130.

Chouliara, I., Sawaidis, L. N., Riganakos, K., & Kontaminas, M. G. (2004). Preservation of salted, vacuum-packaged, refrigerated sea bream (*Sparus aurata*) fillets by irradiation: Microbiological, chemical and sensory attributes. *Food Microbiology*, 21, 351–359.

Chytiri, S., Chouliara, I., Savvaids, I. N., & Kontaminas, M. G. (2004). Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. *Food Microbiology*, 21(2), 157–165.

Corbo, M. R., Speranza, B., Filippone, A., Granatiero, S., Conte, A., Sinigaglia, M., & Del Nobile, M. A. (2008). Study on the synergic effect of natural compounds on the microbial quality decay of packed fish hamburger. *International Journal of Food Microbiology*, 127(3), 261–267.

Crapo, C., Himelbloom, B., Vitt, S., & Pedersen, L. (2004). Ozone efficacy as a bactericide in seafood processing. *Journal of Aquatic Food Product Technology*, 13(1), 111–123.

Economou, V., Gousia, P., Kemetzi, D., Sakkas, H., & Papadopoulou, C. (2017). Microbial quality and histamine producing microflora analysis of the ice used for fish preservation. *Journal of Food Safety*, 37(1).

Françoise, L. (2010). Occurrence and role of lactic acid bacteria in seafood products. *Food Microbiology*, 27(6), 698–709. <http://dx.doi.org/10.1016/j.fm.2010.05.016>.

Gonçalves, A. A. (2009). Ozone – an emerging technology for the seafood industry. *Brazilian Archives of Biology and Technology*, 52(6), 1527–1539.

Gram, L., & Dalgaard, P. (2002). Fish spoilage bacteria — Problems and solutions. *Current Opinion in Biotechnology*, 13(3), 262–266.

Gray, J. I., Gomaa, E. A., & Buckley, D. J. (1996). Oxidative quality and shelf life of meats. *Meat Science*, 43(Suppl. 1), 111–123.

Hamre, K., Lie, Ø., & Sandnes, K. (2003). Seasonal development of nutrient composition, lipid oxidation and colour of fillets from Norwegian spring-spawning herring (*Clupeaharengus* L.). *Food Chemistry*, 82(3), 441–446.

Harris, P., & Tall, J. (1994). Rancidity in fish. In J. Allen, & R. Hamilton (Eds.). *Rancidity in foods* (pp. 256–272). London: Chapman and Hall.

Jayasena, D. D., Kim, H. J., Yong, H. I., Park, S., Kim, K., Choe, W., & Ho, C. (2015). Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: Effects on pathogen inactivation and meat-quality attributes. *Food Microbiology*, 46, 51–57.

Karim, N. U., Kennedy, T., Linton, M., Watson, S., Gault, N., & Patterson, M. F. (2011). Effect of high pressure processing on the quality of herring (*Clupeaharengus*) and haddock (*Melanogrammus aeglefinus*) stored on ice. *Food Control*, 22(3–4), 476–484.

Kim, B., Yun, H., Jung, S., Jung, Y., Jung, H., Choe, W., & Jo, C. (2011). Effect of atmospheric pressure plasma on inactivation of pathogens inoculated onto bacon using two different gas compositions. *Food Microbiology*, 28(1), 9–13.

Kim, H., Yong, H. I., Park, S., Choe, W., & Jo, C. (2013). Effects of dielectric barrier discharge plasma on pathogen inactivation and the physicochemical and sensory characteristics of pork loin. *Current Applied Physics*, 13(7), 1420–1425.

Lyhs, U., Lahtinen, J., & Schelvis-Smit, R. (2007). Microbiological quality of maatjes herring stored in air and under modified atmosphere at 4 and 10 °C. *Food Microbiology*, 24(5), 508–516.

Medina-Meza, I. G., Barnaba, C., & Barbosa-Cánovas, G. V. (2014). Effects of high pressure processing on lipid oxidation: A review. *Innovative Food Science and Emerging Technologies*, 22, 1–10.

Mexis, S. F., Chouliara, E., & Kontaminas, M. G. (2009). Combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets stored at 4 °C. *Food Microbiology*, 26(6), 598–605.

Misra, N. N., Keener, K. M., Bourke, P., Mosnier, J., & Cullen, P. J. (2014). In-package atmospheric pressure cold plasma treatment of cherry tomatoes. *Journal of Bioscience and Bioengineering*, 118(2), 177–182.

Misra, N. N., Tiwari, B. K., Raghavarao, K. S. M. S., & Cullen, P. J. (2011). Nonthermal

- plasma inactivation of food-borne pathogens. *Food Engineering Reviews*, 3(3–4), 159–170.
- Misra, N. N., Ziuzina, D., Cullen, P. J., & Keener, K. M. (2013). Characterization of a novel atmospheric air cold plasma system for treatment of packaged biomaterials. *Transactions of the ASABE*, 56(3), 1011–1016.
- Moini, S., Tahergorabi, R., Hosseini, S. V., Rabbani, M., Tahergorabi, Z., Feàs, X., & Aflaki, F. (2009). Effect of gamma radiation on the quality and shelflife of refrigerated rainbow trout (*Oncorhynchus mykiss*) filets. *Journal of Food Protection*, 72, 1419–1426.
- Moiseev, T., Misra, N., Patil, S., Cullen, P., Bourke, P., Keener, K., & Mosnier, J. (2014). Post-discharge gas composition of a large-gap DBD in humid air by UV-Vis absorption spectroscopy. *Plasma Sources Science and Technology*, 23(6), 1–13.
- Morii, H., Taylor, L. Y., & Murray, C. K. (1986). Formation of histamine by luminous bacteria isolated from scombroid fish. *Nippon Suisan Gakkaishi*, 52(12), 2135–2141.
- Mousakhani-Ganjeh, A., Hamdami, N., & Soltanizadeh, N. (2015). Impact of high voltage electric field thawing on the quality of frozen tuna fish (*Thunnus albacares*). *Journal of Food Engineering*, 156, 39–44.
- Mousakhani-Ganjeh, A., Hamdami, N., & Soltanizadeh, N. (2016). Thawing of frozen tuna fish (*Thunnus albacares*) using still air method combined with a high voltage electrostatic field. *Journal of Food Engineering*, 169, 149–154.
- Noriega, E., Shama, G., Laca, A., Díaz, M., & Kong, M. G. (2011). Cold atmospheric gas plasma disinfection of chicken meat and chicken skin contaminated with *Listeria innocua*. *Food Microbiology*, 28(7), 1293–1300.
- Núñez-Flores, R., Castro, A. X., López-Caballero, M. E., Montero, P., & Gómez-Guillén, M. C. (2013). Functional stability of gelatin-lignosulphonate films and their feasibility to preserve sardine fillets during chilled storage in combination with high pressure treatment. *Innovative Food Science and Emerging Technologies*, 19, 95–103.
- Oehmigen, K., Hähnel, M., Brandenburg, R., Wilke, C., Weltmann, K., & Von Woedtke, T. (2010). The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. *Plasma Processes and Polymers*, 7(3–4), 250–257.
- Ojha, K. S., Kerry, J. P., & Tiwari, B. K. (2017). Investigating the influence of ultrasound pre-treatment on drying kinetics and moisture migration measurement in *Lactobacillus sakei* cultured and uncultured beef jerky. *LWT - Food Science and Technology*, 81, 42–49.
- Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., ... Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. *Trends in Food Science and Technology*, 8(8), 258–265.
- Özogul, F., Taylor, K. D. A., Quantick, P., & Özogul, Y. (2000). Chemical, microbiological and sensory evaluation of Atlantic herring (*Clupea harengus*) stored in ice, modified atmosphere and vacuum pack. *Food Chemistry*, 71(2), 267–273.
- Pankaj, S. K., Misra, N. N., & Cullen, P. J. (2013). Kinetics of tomato peroxidase inactivation by atmospheric pressure cold plasma based on dielectric barrier discharge. *Innovative Food Science and Emerging Technologies*, 19, 153.
- Picouet, P. A., Cofan-Carbo, S., Vilaseca, H., Ballbè, L. C., & Castells, P. (2011). Stability of sous-vide cooked salmon loins processed by high pressure. *Innovative Food Science and Emerging Technologies*, 12(1), 26–31.
- Randell, K., Hattula, T., & Ahvenainen, R. (1997). Effect of packaging method on the quality of rainbow trout and baltic herring filets. *LWT - Food Science and Technology*, 30(1), 56–61.
- Rød, S. K., Hansen, F., Leipold, F., & Knöchel, S. (2012). Cold atmospheric pressure plasma treatment of ready-to-eat meat: Inactivation of *Listeria innocua* and changes in product quality. *Food Microbiology*, 30(1), 233–238.
- Sampels, S. (2015). The effects of storage and preservation technologies on the quality of fish products: A review. *Journal of Food Processing and Preservation*, 39(6), 1206–1212.
- Sánchez-Alonso, I., Moreno, P., & Careche, M. (2014). Low field nuclear magnetic resonance (LF-NMR) relaxometry in hake (*Merluccius merluccius*, L.) muscle after different freezing and storage conditions. *Food Chemistry*, 153, 250–257.
- Sánchez-Valencia, J., Sánchez-Alonso, I., Martínez, I., & Careche, M. (2015). Low-field nuclear magnetic resonance of proton (1H LF NMR) relaxometry for monitoring the time and temperature history of frozen hake (*Merluccius merluccius*, L.) muscle. *Food and Bioprocess Technology*, 8(10), 2137–2145.
- Sant'Ana, L. S., & Mancini-Filho, J. (2000). Influence of the addition of antioxidants in vivo on the fatty acid composition of fish filets. *Food Chemistry*, 68(2), 175–178.
- Vyncke, W. (1975). Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in mackerel (*Scomber scombrus* L.). *Fette, Seifen, Anstrichmittel*, 77(6), 239–240.
- Wang, J., Zhuang, H., & Zhang, J. (2016). Inactivation of spoilage bacteria in package by dielectric barrier discharge atmospheric cold plasma—Treatment time effects. *Food and Bioprocess Technology*, 1–5.