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Study of the influence of pulsed electric field pre-treatment on quality parameters of sea bass during brine salting

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Innovative Food Science and Emerging Technologies Study of the influence of pulsed electric field pre-treatment on quality parameters of sea bass during brine salting --Manuscript Draft--

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Abstract:	Pulsed electric field (PEF), as an emerging technique, has recently gained increased popularity in food processing and preservation. However, applications in the seafood industry are still scarce. In the present study, sea bass samples were subjected to PEF pre-treatment prior to brine salting to verify the possible acceleration of the brining rate, increasing the salt uptake and ensuring the homogeneous salt distribution in the muscle. The applied intensity of the current was set at 10 and 20 A (corresponding to a field strength of 0.3 and 0.6 kV/cm) prior to sea bass salting in brine with 5 and 10% salt concentration, respectively. The results have shown that PEF pretreatment could effectively shorten the brine salting time compared to control samples (from 5 to 2 days), or increase the salt uptake up to 77%, ensuring at the same time its homogenous distribution in the muscle. However, myofibrillar protein solubility was significantly reduced in PEF pretreated samples. At the same time, no significant differences in water holding capacity and water activity between PEF pre-treated and untreated samples were found during the whole salting period. Freezable water was influenced by PEF application, but the effect was significant only at the lowest salt concentration during the first period of the salting process. Industrial relevance: PEF-assisted brining appears a promising technology in the firsh processing industry due to its efficacy in reducing the salt brining time, increasing the mass transfer and enhancing the diffusion of brine into the muscle to ensure the homogeneous distribution of salt in it. The increased salt uptake of the PEF-treated samples compared to control samples shows future potentiality of using PEF prior to salting in the fish processing industry.
Response to Reviewers:	

Highlights

- PEF pre-treatment allowed to shorten brining times in sea bass fillets
- NaCl uptake was increased in seabass fillets compared to untreated samples
- Water state and distribution was only slightly affected by PEF treatment
- Reduction of myofibrillar protein solubility during brining was observed

1	Study of the influence of pulsed electric field pre-treatment on quality
2	parameters of sea bass during brine salting
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29 Abstract

Pulsed electric field (PEF), as an emerging technique, has recently gained increased popularity in 30 food processing and preservation. However, applications in the seafood industry are still scarce. 31 In the present study, sea bass samples were subjected to PEF pre-treatment prior to brine salting 32 33 to verify the possible acceleration of the brining rate, increasing the salt uptake and ensuring the homogeneous salt distribution in the muscle. The applied intensity of the current was set at 10 and 34 20 A (corresponding to a field strength of 0.3 and 0.6 kV/cm) prior to sea bass salting in brine with 35 5 and 10% salt concentration, respectively. The results have shown that PEF pretreatment could 36 37 effectively shorten the brine salting time compared to control samples (from 5 to 2 days), or 38 increase the salt uptake up to 77%, ensuring at the same time its homogenous distribution in the muscle. However, myofibrillar protein solubility was significantly reduced in PEF pretreated 39 40 samples. At the same time, no significant differences in water holding capacity and water activity between PEF pre-treated and untreated samples were found during the whole salting period. 41 42 Freezable water was influenced by PEF application, but the effect was significant only at the lowest salt concentration during the first period of the salting process. 43

44

45 Industrial relevance:

PEF-assisted brining appears a promising technology in the fish processing industry due to its efficacy in reducing the salt brining time, increasing the mass transfer and enhancing the diffusion of brine into the muscle to ensure the homogeneous distribution of salt in it. The increased salt uptake of the PEF-treated samples compared to control samples shows future potentiality of using PEF prior to salting in the fish processing industry.

51

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53 Keywords: pulsed electric field, brine salting, sea bass, water distribution, LF-NMR

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57 **1.** Introduction

Fish is a highly perishable raw material where deterioration caused by biochemical phenomena
and microorganisms begin soon after slaughtering. Proper handling and preservation practices are
therefore needed to prolong the shelf life of the product (Nagarajarao, 2016).

61 Salting is one of the oldest preservation methods used for long time storage of fish. Salted pelagic fish was well known to the old civilizations including the ancient Greeks and the Romans, the 62 63 Vikings and other populations that lived on the shores of the Mediterranean Sea and the Atlantic 64 Ocean. Today, a variety of salted pelagic fish products including sardines, anchovies, sea bass, 65 bacalao, herring i.e., as well as Scandinavian dried and salted cod called *klippfisk*, literally "clifffish", are produced under the common name of "salted fish products" and marketed in many 66 countries of the Mediterranean and the North Sea regions. Due to a fairly good market price and 67 high palatability, these product commodities have become popular and highly appreciated in 68 Europe and the USA. Along with the changes of lifestyle and growing consumer demands towards 69 ready-to-eat, healthy and tasty foods, lightly salted fish products are currently gaining more and 70 more popularity (Fan, Luo, Yin, Bao, & Feng, 2014). 71

Salting is one of the simplest methods of preserving large quantities of fish from spoilage. Salt is 72 73 usually used at concentrations high enough to preserve the fish. Salting can be also used as a preliminary operation in smoking, drying and cooking processes helping to improve sensory 74 75 parameters and increase the shelf-life of the final product (Bras & Costa, 2010). Salt can interact with proteins to increase hydration and water holding capacity of fish muscle thus improving its 76 77 textural parameters. Increasing the water holding capacity of fish muscle helps to decrease cooking 78 loss, thereby enhancing the tenderness and juiciness of the final product. Sodium chloride (NaCl), 79 the common salt, is the main ingredient used in fish salting. It acts as a preservative by dehydration 80 and osmotic pressure inhibiting bacterial growth and deactivating enzymes. Even at low concentrations, NaCl possesses some preservative action (Lupín, Boeri, & Moscidar, 1981). Other 81 82 substances such as herbs, spices, sugar or antioxidants can also be used in the fish salting process 83 to improve sensory attributes of the product, modify flavor and reduce shrinkage after salting. The 84 conventional fish salting methods include dry-salting and wet-salting. During dry salting, the salt (traditionally sodium chloride) and other ingredients from the curing mixture (sugars and spices) 85

are applied to the fish surface. Wet salting is performed by immersing the product into brine or 86 injecting the brine directly into the fish muscle (Birkeland, Skåra, Bjerkeng, & Rørå, 2003; Hall, 87 2011). The concentration of salt in the brine affects the weight gain, water holding capacity and 88 commercial quality of the end product (Nguyen, Thorarinsdottir, Gudmundsdottir, Thorkelsson, 89 & Arason, 2010). Weight gain of salted fish products depends on the ability of the myofibril 90 proteins to retain water inside the muscle affected by the salting procedures applied 91 (Thorarinsdottir, Arason, Sigurgisladottir, Valsdottir, & Tornberg, 2011). The brining time usually 92 varies from 2 to 10 days depending on the desired level of salt in the muscle. During immersion 93 brining, fish is covered with brine for a period of time and held at a temperature between 0 to 4°C. 94 In injection salting, the brine is injected into the fish fillet using a set of needles making this a 95 faster method than immersion brining. 96

97 Myofibrillar proteins are of great importance for the functional properties of light-salted fish products, such as water holding capacity (WHC). It is well known that salting of fish alters protein 98 extractability and thermal denaturation and aggregation of many muscle proteins (Nguyen, 99 100 Thorarinsdottir, Gudmundsdottir, Thorkelsson, & Arason, 2010), which in turn affects the WHC. 101 Salting also affects the proteolytic activity responsible for degradation of myofibrils and connective tissue proteins, as well as extra-cellular matrix (Thorarinsdottir, Arason, 102 103 Sigurgisladottir, Valsdottir, & Tornberg, 2011). Thus, the influence of salting on the distribution of water within the muscle may be related to direct effects of salt on changes in structural 104 105 components of the muscle (Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergsson, 2002; Larsen & Elvevoll, 2008). It is also assumed that the main components of fish muscle (proteins, 106 107 lipids and salts) influence the arrangement of water molecules in a product matrix, thereby having an effect on the product quality and shelf-life (Pacetti et al., 2015). Therefore, it is important to 108 109 study how the salt content and water distribution within the muscle may affect water holding capacity of the product. Low-field nuclear magnetic resonance (LF-NMR) has been employed in 110 the food industry to study water mobility and distribution within the fish muscle (Løje, Green-111 Petersen, Nielsen, Jørgensen, & Jensen, 2007; Aursand, Gallart-Jornet, Erikson, Axelson, & 112 Rustad, 2008). This technique has been suggested a tool for rapid and non-destructive analysis of 113 114 water mobility and identification of intra-myofibrillar or extra-myofibrillar water components (Andersen and Jørgensen, 2004; Jensen, Jørgensen, Nielsen, & Nielsen, 2005; Løje, Green-115 116 Petersen, Nielsen, Jørgensen, & Jensen, 2007) in the muscle.

117 The migration of salt from brine to fish matrix is generally quite slow. Different brining methods have previously been tested to accelerate salt transport through the product, for instance high 118 intensity ultrasound brining and marinating (Chemat, Zill-e-Huma, & Khan, 2011; Turhan, 119 120 Saricaoglu, & Oz, 2013), pulsed vacuum brining (Andres, Rodrigues-Barona, Barat, & Fito, 2002), and vacuum tumbling (Mathias, Jittinandanana, Kenney, & Kiser, 2003; Esaiassen et al., 2004). 121 Pulsed electric field (PEF), as an emerging technology, has great potential to contribute to 122 123 improved salting of fish products through enhanced diffusion of salt into the fish muscle (Hafsteinsson Gudmundsson Arnarson Jonsson, & Siguroardottir, 2000). However, to our 124 knowledge, no studies have so far been published on PEF applications for salting of fish. Even 125 though the concept of PEF was introduced to the food industry about 50 years ago, this technique 126 can be still considered an emerging technology due to the recent developments related to microbial 127 inactivation applications and improvement of mass transfer through cell disruption (Gómez et al., 128 2019). In general, PEF technique applies high voltage pulses of short duration to food placed 129 between two electrodes, resulting in specific structural modifications of the tissue including the 130 disruption of cell membrane (Barba et al., 2015). Under the application of the high electric field 131 132 pulses, the membrane permeability is increasing due to either enlargement of existing pores or generation of new ones (Gómez et al., 2019). This concept was previously applied in the seafood 133 134 industry with the aim of enhancing water holding capacity of fish and tenderization of shellfish products (Klonowski, Heinz, Toepfl, Gunnarsson, & Porkelsson, 2006). PEF has also been 135 136 suggested as a promising technique for accelerating mass transfer which could potentially be used as a pre-treatment in the fish drying process (Gómez et al., 2019). 137

138 Therefore, the main aim of the present study was to investigate whether the PEF pre-treatment can be applied to accelerate the brining process and ensure a uniform distribution of salt within the 139 140 muscle of fish, evaluating mass transfer kinetic and, in parallel, water state and distribution. The study aims also at investigating the effect of PEF pre-treatment on quality parameters of sea bass 141 during salting. It is well known that PEF may affect the extractability and aggregation of proteins, 142 since electroporation within the muscle tissue can result in chemical modifications by the 143 formation of free radicals which can further alter the structure of proteins and the intermolecular 144 145 forces (Gudmundsson & Hafsteinsson, 2001; Zhao, Sun & Tiwari, 2019). Therefore, this research also investigated the effect of different PEF pre-treatments on protein functionality by evaluating 146 147 water holding capacity and protein solubility.

148

- 149 2. Materials and Methods
- 150

151 **2.1. Materials**

Sea bass (*Dicentrarchus labrax*) were supplied by Tagliapietra e Figli s.r.l. (Venice, Italy) in May 2019. The day after catch, the fish were delivered to Economia del Mare (Cesenatico, Italy) where they were gutted, filleted and de-skinned. The sea bass fillets were placed on ice in Styrofoam boxes and transported to the CIRI-Agrifood laboratory in Cesena (Italy), where the experiment was carried out in the same day. Commercial salt 'Sale alimentare di Sicilia' from Italkali s.r.l. (NaCl ~98%) was used for brines preparation.

158

159 **2.2. PEF pre-treatment and brine salting**

160 Sea bass fillets were cut into small pieces $(8.3 \pm 0.2 \text{ g each})$ with the dimensions of length $2.3 \pm 0.2 \text{ cm}$, width $3.1 \pm 0.4 \text{ cm}$ and height $1.3 \pm 0.5 \text{ cm}$.

162 Prior to salting, the obtained sea bass pieces were subjected to PEF pre-treatment, performed using

- a lab scale PEF unit Mod. S-P7500 delivering a maximum output current and voltage of 60A and
 8kV, respectively (Alintel, Bologna, Italy). The generator provides monopolar rectangular-shape
- pulses and adjustable pulse duration (5-20 μ s), pulse frequency (50-500 Hz) and total treatment
- time (1-600 s). The treatment chamber (50 mm length x 50 mm width x 50 mm height) consisted
- 167 of two parallel stainless-steel electrodes (3 mm thick) with a 47 mm fixed gap. Output voltage and
- 168 current were monitored using a PC-oscilloscope (Picoscope 2204a, Pico Technology, UK). Sea
- bass pieces were treated at room temperature in tap water delivering n = 1000 pulses at fixed pulse
- width (10 \pm 1 µs), frequency (100 Hz), repetition time (10 \pm 1 ms) and selecting two different
- 171 current intensities, 10A and 20A, corresponding to values of electric field strengths of 0.3 and 0.6
- 172 kV/cm and specific energy input of 0.25 ± 0.01 and 1.01 ± 0.03 kJ/kg, respectively. The process
- 173 parameters were chosen on the basis of preliminary experimental trials.
- The sea bass pieces were randomly distributed into the three experimental groups (two PEF-treated and one control samples) and salted by immersion into a brine with two different salt (NaCl) concentrations in tap water (5% and 10% (w/w)) and in closed plastic containers (500ml) each
- 177 containing a ratio of 4 to 1 w/w brine/fish. Five independent replicates were considered for each

sample type and for each sampling time. The salting process was carried out in a cold room at 0-4°C for 2, 5 and 8 days according to the experimental plan displayed in **Table 1**.

180 At each sampling day, sea bass samples were randomly collected and analyzed. Changes in weight yield, water-holding capacity, water activity, freezable water by differential scanning calorimetry 181 182 and water behavior and distribution inside the muscle by LF-NMR as affected by different PEF pre-treatment and salting parameters, were studied directly after each sampling day at the 183 184 laboratories of the University of Bologna (Cesena, Italy). The remaining experimental samples from each treatment were frozen at -80°C and transported to Norwegian University of Science and 185 Technology (Trondheim, Norway) for determination of water and salt content, pH and protein 186 solubility. 187

Analyses were performed in 3-6 replicates for each sample as described in detail in the followingsection.

190

191 **2.3. Physico-chemical analyses**

192 2.3.1 Mass transfer parameters

193 Weight yield

The fish samples were weighed raw and after each sampling day. The weight yield was determined
with respect to the weight of the raw fillets as described by Thorarinsdottir, Arason, Bogason, &
Kristbergsson (2004).

197

198 Water content

Water content was determined by drying a sample of 2 g at 105 °C for 24 h to a constant weight,
according to the official method (AOAC 2005). Finely chopped fish obtained from 5 individual
pieces was mixed and analysed in triplicate.

202

203 Salt (NaCl) content

Salt content in all sea bass samples was determined by titration according to AOAC 976.18 (1995).
Briefly, the fish obtained by 5 different pieces was minced with a kitchen blender (Bosch 600W,
Gerlingen, Germany), and 2 g of the resulting mince was weighed in a 150 ml glass beaker, filled
with 80 ml warm distilled water (60°C) and mixed for 5 min until a homogeneous mixture was
obtained. Then, 1 ml of 1M HNO₃ was added to the mixture, the electrode type AgCl 32 and

burette tip was placed in the solution, and the titration was performed with an automatic titrator
(mod. TitroLine 7800, Xylem Analytics, Mainz, Germany). The analysis was performed in three

replicates and the results were expressed in % salt as a mean value \pm SD.

212

The total water and NaCl weight changes $(\Delta M^0_t, \Delta M^w_t \text{ and } \Delta M^{NaCl}_t, \text{ respectively})$ of salted samples were determined with Eqs (1), (2) and (3) as follow:

215
$$\Delta M^{\circ}{}_{t} = \frac{(M^{\circ}{}_{t} - M^{\circ}{}_{0})}{M^{\circ}{}_{0}}$$
(1)

216
$$\Delta M_{t}^{w} = \frac{(M^{\circ}_{t} \cdot x_{t}^{w} - M_{0}^{o} \cdot x_{0}^{w})}{M^{\circ}_{0}}$$
(2)
217
$$\Delta M_{t}^{NaCl} = \frac{(M^{\circ}_{t} \cdot x_{t}^{NaCl} - M^{\circ}_{0} \cdot x_{0}^{NaCl})}{M^{\circ}_{0}}$$
(3)

217 $\Delta M_t^{NaCl} = \frac{(M^* t^* x_t^{-M^* - M^* 0^* x_0^{-M^* 0^*})}{M^\circ_0}$ (3) 218 where M°_t and M°₀ are the sea bass weights, x^w_t and x^w₀ are the water weight fractions, and x^{NaCl}_t 219 and x^{NaCl}₀ are the NaCl weight fractions, at sampling time *t* and before the salting process *0*, 220 respectively.

221

222 2.3.2 Water state and mobility

223 Water activity

Water activity was measured with a Water Activity Meter mod. AQUALAB, (Decagon Devices, US). Briefly, the fish samples were cut into small pieces $(0.2 \times 0.2 \text{ cm})$ and introduced into sample holders prior to the analysis. Between measurements, the samples were covered with lids and protected with parafilm. For each of the experimental groups, four measurements were performed and the mean value \pm SD was calculated.

229

230 Differential scanning calorimetry (DSC)

A differential scanning calorimeter (DSC) mod. Q20 (TA Instrument, Germany), equipped with a 231 low- temperature cooling unit (TA-Refrigetated Cooling System90).) was used to assess freezable 232 water content (FW, g/g of water) and to evaluate the effect of processing on protein denaturation. 233 Temperature and melting enthalpy calibrations were performed with ion exchanged distilled water 234 (mp 0.0°C) and indium (mp 156.60°C), while heat flow was calibrated using the heat of fusion of 235 indium ($\Delta H = 28.71$ J/g). For the calibration, the same heating rate and dry nitrogen gas flux of 50 236 ml/min used for the analysis were applied. Each sample was weighed (about 15 mg) into a $50-\mu$ L 237 aluminum pan, sealed hermetically and frozen at -40°C. Frozen samples were then loaded into the 238

DSC instrument. The heating rate of DSC scans was 5°C/min over a range of -40 to 90°C. Empty
aluminum pans were used as reference and for baseline corrections. Eight replications for each
sample were performed and results were elaborated through PeakFit Software (SeaSolve Software
Inc. Framingham, MA, USA).

243 The FW was determined as follows:

244

245
$$FW = \frac{\Delta H_m}{\Delta H_W}$$
 (4)

246

where ΔH_w (325 J/g) is the latent heat of melting per gram of pure water at 0°C, and ΔH_m (J/g) is the measured latent heat of melting of water per gram of sample obtained by the integration of the melting endothermic peak. FW was further related to the water content and expressed as grams per gram of water content (FW^w).

PeakFit Software (SeaSolve Software Inc. Framingham, MA, USA) was used to analyse thermal data and
obtain deconvoluted peaks and calculate relative melting enthalpy.

253

254 *LF-NMR*

A 10 mm deep slice was cut from each sample, then cylinders (6 mm diameter) of about 400 mg 255 256 were obtained with a cork borer. Signals weighted by T2 were registered with the CPMG pulse 257 sequence (Meiboom & Gill, 1958), using a Bruker mod. Minispec PC/20 spectrometer operating 258 at 20 MHz. Each measurement consisted in 30K points, spaced 0.080 ms. Subsequent scans were separated by a recycle delay of 3.5 s. The specified interpulse spacing avoided sample overheat 259 but allowed the observation of the protons with T2 higher than a few milliseconds. UPEN software 260 (Borgia, Brown, & Fantazzini, 1998) allowed to obtain an overview of the protons T2 distributions 261 (the relaxograms) by inverting the T2-weighted signals towards a semi-continuous distribution of 262 exponential curves, according to Eq. (5): 263

264
$$I(2\tau n) = \sum_{i=1}^{M} I_0(T_{2,i}) \exp(-2\tau n/T_{2,i})$$
 (5)

where 2τ is the CPMG interpulse spacing, n is the index of each CPMG point while I0 is the intensities of each T2 component extrapolated at t = 0, sampled logarithmically. As some components resulted as partially overlapped in the relaxograms from several samples, we observed them separately by fitting the T2-weighted signals to the sum of an increasing number of exponential curves. An F-test showed that the optimum ratio between fitting ability and complexity
of the model was reached for most samples with three exponentials. Six measurements were
performed for each of the experimental sets.

272

273 2.3.3 Protein functionality

274 *pH*

pH was measured at room temperature by inserting electrode directly into the sea bass mince (mod.
MP-220 pH-meter, Mettler-Toledo, Hong Kong) according to Thorarinsdottir, Arason, Bogason,
& Kristbergsson (2004). Prior to pH measurements, the pH meter was calibrated with standard
buffer solutions. The measurements were performed at least in triplicate, and the mean value ± SD
was calculated.

280

281 **Protein solubility**

Water and salt soluble proteins were determined in white muscle extracts according to a modification of the methods of Licciardello et al (1982), as previously described by Hultmann & Rustad (2002). The amount of proteins in the extracts was determined with BioRad protein assay after centrifugation at 8000 g and 4°C for 20 min, using gamma globulin as a standard. The analyses were run in triplicate and the mean value \pm SD was calculated.

287

288 Water Holding Capacity (WHC)

WHC of sea bass samples was measured according to the method described by Thorarinsdottir, Arason, Bogason, & Kristbergsson (2004), as follows. The minced samples were placed in centrifuge tubes and centrifuged at 200 g for 10 min (0–4 $^{\circ}$ C). The weight (g) of the fish pieces before and after the centrifugation was determined. WHC was expressed as the amount of released water divided by the original weight (g) of the sample before centrifugation. Four replicates were performed for each treatment group.

295

296 **2.4. Statistical analysis**

The data sets from the experiment were analyzed by Statistica 8.0 software (StatSoft, Tulsa, USA)
The effect of the parameters of PEF treatment (PEF), NaCl concentration (Salt) and brining time

299 (Time) and their interaction on dependent variables was evaluated through the factorial Analysis

- 300 of Variance (ANOVA). Statistical significance of the experimental data was verified using Tukey
- 301 as post-hoc (p<0.05). To establish a relationship between certain parameters, Pearson correlations 302 were calculated. Differences were considered significant at p<0.05.
- 303
- **304 3. Results and discussion**
- 305

306 3.1 Mass transfer parameters

Fig. 1 reports the total weight change (A), water (B) and salt uptake (C) mass fraction of control
and PEF (0.3 and 0.6 kV/cm) treated sea bass samples during the brining process at 5% and 10%
salt concentrations.

In control samples, weight increased between 24 and 26 % during the first 5 days of brining. 310 However, on the last day of brine salting, the weight yield of control samples was reduced up to -311 0.13% and 2.56% for 5% and 10% salt concentration in the brine, respectively. The lowest weight 312 yield in the control group on day eight may possibly be explained by an inhomogeneous salt 313 distribution within the inner and outer parts of the fish muscle at the beginning of brining, leading 314 315 to disintegration of the fish muscle pieces in the last part of the experiment, as previously showed by Thorarinsdottir, Arason, Bogason, & Kristbergsson (2004). Differently PEF treated samples 316 317 showed a constant increase of weight during the entire brining period. While no significant differences were observed compared to the control until the 5th day of salting, on the 8th day all 318 319 PEF treated samples (0.3 and 0.6 kV/cm) reached a weight gain of 28-32%.

The total water content in the sea bass samples varied from 73.9 to 88.7 % (w/w) during brine 320 salting. In all samples, water uptake (Fig. 1B) was observed until the 5th day, when samples 321 immersed in the 5% salt brine showed significantly higher values compared to samples in the 10% 322 323 one. However, no differences were observed among the control and the PEF treated samples in each of the 2 groups (0.3 and 0.6 kV/cm). At the 8th day, the water uptake showed a drastic drop 324 325 for both the control samples, as already observed with the total weight change. PEF treated samples 326 in the 5% brine, did not show a further water uptake, while samples in the 10% brine showed a 327 further increase. All PEF treated samples showed similar water fraction values at the end of the 328 brining period.

Initial salt content of sea bass fillets was 0.01 g/100g. Salt weight fraction changes are reported in **Fig. 1C**. In control samples, an increase of salt content was observed until the 5th day, reaching values of 0.03 and 0.07 that corresponded to 2.7 and 5.9 % of net salt content for the 5 and 10%
brining respectively. Hence, as expected, the salt uptake was driven by concentration gradients
between the muscle and brine, similarly to previous studies (Nguyen, Thorarinsdottir,
Gudmundsdottir, Thorkelsson, & Arason, 2010). However, as observed for the weight and water
uptake, on the last day of brining, the salt fraction decreased to values corresponding to 0.46 and
2.05% for the 5 and 10% brining respectively.

337 Following PEF pre-treatment, there was a general increase of the salt uptake in all samples at the end of the salting process. After two days, both 10 and 20A PEF (0.3 and 0.6 kV/cm) treated 338 samples were significantly higher compared to their respective controls, while after 5 days, only 339 the 10A sample and the 20A sample in the 5% brine. Salt concentration in PEF treated sea bass 340 fillets increased slightly between the 5th and the 8th day, but, although samples treated at 10A (0.3 341 kV/cm) showed and increasing trend, differences were not statistically significant. The higher salt 342 weight fractions reached corresponded to a salt content in the samples of 4.47 and 6.84 g/100g for 343 the 5 and 10% brining respectively, showing an increase of 77 and 35% compared to the highest 344 salt content obtained in control samples at day five. 345

Applying PEF pretreatment allowed to reach a similar salt uptake after 2 days of brining, instead of 5 days in the control samples, thus reducing the time necessary for the process.

348 PEF has previously been shown to increase mass transfer in other animal and vegetable foods, such as ham, cured and salted meat, potato crisps, dried fruits etc. (Gómez et al., 2019). 349 350 Electroporation is one of the several complex mechanisms attributed to this phenomenon. It was previously assumed that a greater number of pores in the muscle emerges with increasing the 351 352 electric field intensity, which is why generally a mass transfer increases is obtained (Gómez et al., 353 2019). Electroporation has been shown to cause increased inter-myofibrillar spacing in fish and 354 meat products (Gómez et al., 2019) which could aid mass transfer, thus increasing the salt uptake 355 by the muscle. Therefore, we suggest that in the present study electroporation facilitated the salt uptake by the fish through increasing the extra-cellular spaces in the muscle serving as additional 356 channels for diffusion of brine. Moreover, Klonowski, Heinz, Toepfl, Gunnarsson & Porkelsson 357 (2006) found a more porous structure in cod fillets pre-treated by PEF, that might have aided the 358 359 diffusion of salt. Even though this effect was observed with the application of a higher electric field strength (2kV/cm) compared to the ones applied in this present research (0.3-0.6kV/cm), it is 360 361 possible that a change on the flesh structure might have happened.

362 The increase of salt concentration in the tissue results, especially at the level of myofibrils, in 363 greater water absorption and swelling under certain conditions (Krasnow, Loss, Ahrens, & Fiore 364 III, 2013). This phenomenon is linked to the action of Cl⁻ chloride anions, which tend to associate with the positively charged groups of proteins. Positive charges are neutralized and therefore the 365 repulsive force of negative charges increases. The intra-myofibrillary space expands due to the 366 repulsive forces and a greater water retention capacity is determined. However, brines with a saline 367 concentration above 10-15% can lead to an opposite effect, worsening the water retention capacity. 368 In this case the salting-out phenomenon may occur: the ions in excess of Cl-, not being able to 369 interact with the positive charges of the proteins already occupied by the other ions, interfere with 370 371 them for the interaction with the water molecules, sequestering the solvation water and causing the loss of solubility and the precipitation of proteins (Aberoumand e Nejad, 2015; Kalra, Tugcu, 372 373 Cramer, & Garde, 2001; Offer e Trinick, 1983). This phenomenon, however, was not observed in PEF treated samples by Klonowski, Heinz, Toepfl, Gunnarsson & Porkelsson (2006), although the 374 375 final salt concentration was higher.

We hypothesize that, contrarily to control samples, PEF treatment in the range of 0.3 and 0.6 kV/cm promoted a more homogeneous distribution of NaCl within inner and outer parts on the fish muscle due to formation of small pores in the muscle, facilitating the mass transfer and leading to enhanced diffusion of salt from the brine to the muscle.

380

381 3.2 Water state and distribution

The water activity (a_w) of untreated sea bass samples was 0.990 ± 0.002 . As shown in Fig. 2, fish 382 383 tissue brining resulted in a significant decrease of water activity, explained by the bonding of 384 residual fluid from the fish muscle by salt through ionic interactions. These interactions reduce the 385 amount of free water contained in the fish muscle, thus lowering water activity of the product (Lupín, Boeri, & Moscidar, 1981). Statistical analysis showed that only the NaCl concentration in 386 387 the brine had a significant (p < 0.05) influence on water activity of sea bass samples during salting, leading to values in the range of 0.966 to 0.972 and 0.941 to 0.949, during the salting period for 388 389 the 5 and 10% concentration respectively. Neither PEF intensity (0.3 and 0.6 kV/cm) nor duration 390 of brine salting did affect water activity of the fish samples.

According to different authors (da Silva Carneiro et al., 2016; Mudalal, Petracci, Tappi, Rocculi,

392 & Cavani, 2014), there are three different water populations in muscle tissues, the first one (below

393 5%) exists as true hydration water that is strictly bound to proteins by macromolecular of 394 multimolecular adsorption, the second is water located inside organized protein structures (intra-395 myofibrillar), and the third one, which is the major one (>70%), is the extra-myofibrillar water, easily mobilizable. The first one is not free; it has an ice-like structure (liquid crystal), it is 396 unfreezable, unaffected by charges on the muscle protein (pH), and it is unavailable to participate 397 in reactions. From a calorimetric point of view, freezable water (FW) is usually associated to the 398 399 second two fractions, representing the water affected during processing. FW assessment by DSC has been used to determine the gross phase changes of water in polymeric networks (Capitani et 400 al., 2003) and in food systems, such as meat (Venturi et al., 2007; Petracci et al., 2012; Mudalal, 401 402 Petracci, Tappi, Rocculi, & Cavani, 2014).

Fig. 3A reports, as an example, the obtained thermograms of sample C10 at different brining times 403 (zero to height days). As it is possible to observe, the FW peak was actually composed by two 404 superimposed peaks, melting at slightly different temperatures. While in the fresh sample, this 405 difference was small, with the first melting at around -3°C and the second melting at around 0°C 406 being almost indistinguishable, as the brining time increased, the first peak appeared at lower 407 408 temperatures, until reaching -6°C after 8 days. In order to better understand the phenomena, the total melting enthalpy of FW were calculated and the relative amount of the two peaks were 409 410 plotted, as shown in Fig. 3B (example of raw thermogram) and 3C (example of deconvoluted thermogram) respectively. 411

412 Fig. 4 shows the total FW^w content, (4A), the fraction of peak 1 (4B) and the melting temperature of the first peak (4C). In the fresh sample, total FW^w content was 0.69 g/g water. In control samples 413 414 immersed in the 5% NaCl brine, this value increased slightly after two days. However, the increase of salt concentration led to a decrease of the FW^w to the initial values. The first raise was probably 415 416 due to a fast water uptake that increased the general mobility of the water. However, the simultaneous increase of salt concentration probably counterbalanced this effect. However, 417 differences were not significant. In PEF treated samples, no differences were observed compared 418 to initial value at all brining days. 419

For samples in the 10% NaCl brine, the total FW^w water content showed a slight decrease that was
maintained during all brining time, but without significant differences among the samples. The

422 water uptake, as shown in Fig. 1A was similar for the two salt concentrations (Fig. 1B). However,

samples in the 10% solution showed, as expected, a higher salt diffusion during brining (Fig. 1C),
this is the reason for the lowering of FW^w.

Hence, it is possible to observe that the total FW^w was fairly constant in all samples; however, if 425 we take into account the two different peaks, it is possible to observed that, while initially the 426 427 majority of the water was melting at 0°C (about 80%), as brining proceeded, the fraction (peak 1) melting at lower temperature increased progressively. In samples in the 10% solution, the increase 428 429 occurred after the first two days and then values remained similar (between 0.88 and 0.95), while for the 5% samples, the transition was more progressive. The decrease in FW^w and melting 430 temperature depends on the balance between the water uptake and the salt concentration in the 431 432 tissue. Although at the end of the eighth day values were similar for all samples, control samples (C5) showed higher values for peak 1 after two and five days, showing a slower decrease of the 433 melting temperature transition. As shown by Fig. 1C, in PEF treated samples, salt concentration 434 increased more compared to the control, corroborating the hypothesis of the observed differences. 435 436 Moreover, in Fig. 4C the melting temperature related to peak 1 was evaluated for all samples during brining. In the 5% samples the temperature did not change, while for the 10% samples a 437 438 significant decrease was observed already after two days. Hence, DSC data were able to discriminate samples according to the concentration of salt in the brine showing a proportional 439 440 reduction of freezable water and a decrease of the melting temperature due to the increasing salt content. However, few significant differences were observed among samples. This was not 441 442 expected since a higher amount of salt found in PEF treated samples compared to control at different brining times for both 5% and 10% samples. Moreover, the effect of 'salting out' 443 444 observed in the control samples, was not reflected in the FW measurements. This might be due to 445 a different distribution of salt in the tissue as hypothesized earlier. Indeed, sampling procedure is 446 pivotal for DSC analysis, since the small sample size (about 15 mg). Hence, although we took 447 extra care in collecting representative samples, this could be one of the reasons for the observed unexpected behavior. However, considering that, to our knowledge, there are no reports of FW^w 448 measure by DSC in fish samples during brining, so it is not possible to compare results giving a 449 450 more exhaustive explanation of the obtained results.

Low-resolution NMR has been successfully used in many previous studies to investigate water mobility and distribution in fish and meat samples subjected to salting (da Silva Carneiro et al., 2016; Gudjónsdóttir, Arason, & Rustad, 2011; Aursand, Gallart-Jornet, Erikson, Axelson, & 454 Rustad, 2008; Wu et al., 2006). As in previous studies, in the present research it was possible to reveal the presence of 3 water populations (displayed in **Fig. 5**), characterized by short, medium 455 456 and long proton relaxation times. W_B (T₂=1-3 ms) relates to water bound by secondary bonds to the proteins, W₁ (T₂=40-80 ms) describes capillary water found in the myofibrillar network, while 457 W_2 (T₂=100-190 ms) is mechanically immobilized water or extra-myofibrillar which can be further 458 released as drip loss. Table 2 reports the relative intensities expressed as arbitrary units (AU) and 459 460 the T_2 of the three water populations for all the analyzed seabass samples. According to Aursand, Gallart-Jornet, Erikson, Axelson, & Rustad (2008) populations W1 and W2 represent more than 461 90% of the total water in the muscle. 462

In the present study, an evident migration of water from pools W_B and W₁ towards pool W₂, with 463 longer relaxation times was observed from the untreated raw sample to all brined samples. This 464 465 indicates a migration of water from the myofibrillar network towards extra-myofibrillar pools. Indeed, NaCl not only has a preservation effect, but it also acts as a structures-breaker, allowing 466 the muscle fibers to expand and entrap water. This occurs due to electrostatic repulsion within the 467 myofibrils, exposing protein sidechains to water binding (Strasburg, Xiong & Chiang, 017). 468 469 Similar results were found in the study of Aursand, Gallart-Jornet, Erikson, Axelson, & Rustad (2008) investigating water distribution and behavior in brine salted cod and salmon by low-field 470 471 NMR technique. However, in the present research, apart from a few exceptions, no significant differences were observed among samples, neither according to NaCl concentration, nor according 472 473 to the treatment. The only variable that showed consistently a significant effect on water distribution parameters was brining time (p<0.001). 474

475 With regard to relaxation times (Table 2), Wu et al (2006) found a decrease for the bound water (T_{2B}) and an increase related to T_{21} and T_{22} populations during salting of pork meat. In the present 476 477 research T_{2B} showed a decrease but the difference was not significant. Instead, salting in 5% and 10% NaCl brine, led to a shift toward longer relaxation times for the other two water populations. 478 T_{21} (intra-myofibrillar water) shifted from about 45 ms to 65-85 ms, while T_{22} (extra-myofibrillar 479 water) from about 106 ms to 130-190 ms, directly reflecting the increased amount of water, which 480 481 was also observed in other studies conducted on brine salting of fish (Aursand, Gallart-Jornet, 482 Erikson, Axelson, & Rustad, 2008). However, also for this parameter, few significant differences were observed. Specifically, while in T₂₂ a significant increase was found during brining time, no 483

484 differences were observed among samples according to the PEF treatment (0.3 and 0.6 kV/cm). A 485 significant effect was found only for brining time and for NaCl concentration for T_{21} and T_{22} .

486

487 3.3. Protein functionality

The pH values of sea bass samples after PEF-treatment and salting performed for 2, 5 and 8 days 488 are shown in Table 3. Untreated sample showed an initial value of 6.7 that decreased progressively 489 490 during brining, but the only significant differences was observed for C10 after 8 days (pH= 6.18). The results of PEF treated samples (0.3 and 0.6 kV/cm) have shown significantly lower pH values 491 compared to control samples on day 2 and 5 of brining. This could be due to a release of ions from 492 493 PEF-disrupted cells or structural changes of proteins allowing release of acidic groups (Zhao, Sun, & Tiwari, 2019). Values, however, did not change during brining but apart from the initial 494 decrease, remained stable. Nevertheless, result of multifactorial ANOVA showed that this 495 parameters is influences significantly by all considered variables and their interaction. 496

- WHC of sea bass samples (**Table 3**) showed very small variations remaining in the range of 97.7 to 98.99%. In some samples, a slight but not always significant increase of WHC appeared. This may have been due to the increased salt concentration as observed by Thorarinsdottir, Arason, Bogason, & Kristbergsson (2004) and Aursand et al (2008). However, no significant effect of PEF pre-treatment (0.3 and 0.6 kV/cm) or of salt concentration on WHC during salting period was observed in the present study. The only variable affecting WHC was indeed brining time and its interaction with other variables.
- The solubility of sarcoplasmic and myofibrillar proteins in sea bass samples during brine salting is reported in **Fig. 6 A** and **B**.
- 506 Solubility of water soluble (sarcoplasmic) protein was strongly and significantly reduced during 507 brining in all samples. In seabass brined in the 10% NaCl solution, PEF treated samples showed 508 always significantly lower values compared to the control, but with no differences according to 509 the intensity of the electric field applied, 0.3 or 0.6 kV/cm. For samples in the 5% brine solution,
- 510 differences were not always significant.
- 511 Solubility of salt-soluble (myofibrillar) proteins showed a very different behavior. In control
- samples, it did not change compared to the initial untreated sample for all brining times. Instead,
- 513 PEF treated samples reported a remarkable decrease already after 2 days for both 0.3 and 0.6

kV/cm treated samples. However, there were no differences in the values found between salt
concentration and during brining.

516

517 *3.4 Correlation results*

In order to get a better understanding on the observed phenomena and of their relation, correlations among the parameters of mass transfer, water mobility and distribution, and protein functionality measured in the sea bass samples were evaluated through the Pearson's correlation. Results are shown in **Table 4**.

522 ΔM^{o}_{t} is positively correlated to both ΔM^{w}_{t} and ΔM^{NaCl}_{t} , as they showed similar behavior during 523 brining, but it was also negatively correlated to W_B and to the solubility of both water- and salt-524 soluble proteins. No significant correlation was observed with any of the other parameters, that, as 525 observed before, did not reflect the effect of salting out.

Water activity and total FW were positively correlated (0.64), however, the evolution of peak 1 of
FW (water fraction freezing at a lower temperature) was actually correlated to all the other water
state and mobility parameters, measured by LF-NMR and solubility of water-soluble proteins.

- 529 Specifically, the solubility of myofibrillar proteins positively correlated with W_B-water pool expressing water bound by secondary bonds to the proteins in PEF-treated samples, while the 530 531 solubility of sarcoplasmic proteins negatively correlated with W_2 -water pool representing mechanically immobilized water. This suggests that the water pool W_B diffused to the extra-532 533 myofibrillar spaces of the fish muscle (W₂-water pool) as a result of the PEF-induced increased solvation. Supported by previous investigations (Nguyen, Thorarinsdottir, Gudmundsdottir, 534 535 Thorkelsson, & Arason, 2010), this could be caused by the reduced hydration due to the increased solvation capacity of salt ions that reduced the hydrodynamic radius of proteins, increasing 536 537 substantially protein-protein interactions compared to protein-water interactions. The weaker 538 associations between the water molecules bound to proteins resulted in their increased mobility and penetration into extra-myofibrillar spaces of the muscle. At the same time, polar and 539 hydrophobic interactions between proteins became stronger, contributing to their increased 540 hydrophobicity and aggregation (Stefansson & Hultin, 1994; Lin & Park, 1998). 541
- 542

543 **4.** Conclusions

The results of this study have shown that PEF treatment at 0.3-0.6 kV/cm allowed to significantly increase the salt uptake during sea bass brining, that may be due to a more homogeneous distribution of salt in the fish muscle. The study of water state and distribution however did not show many differences among samples that were generally discriminated according to the concentration of salt in the brining solution but not to the PEF treatment applied. On the other side, a remarkable reduction of myofibrillar protein solubility was observed, as a consequence of the application of the electric field.

To sum up, the obtained results suggest that PEF pre-treatment allowed to obtain a significant reduction of the duration of salt brining (more than 50%) or an increase of salt uptake (up to 77%) compared to conventional brining process. However, aspects related to the effect on protein structure and functionality should be further clarified, and different parameters of this innovative processing deeply investigated.

556

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704 **Figure captions**

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Figure 1. Total weight change (ΔM^{0}_{t}) (A), water uptake (ΔM^{w}_{t}) (B) and NaCl uptake (ΔM^{NaCl}_{t}) (C) of control and PEF treated sea bass samples during the brining process at 5% and 10% salt concentrations. Results are expressed as means ± standard deviations (error bars) of n=5. Values with different letters in the auxiliary tables differ significantly (p<0.05).

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Figure 2. Water activity of control and PEF treated sea bass samples during the brining process at 5% and 10% salt concentrations. Results are expressed as means \pm standard deviations (error bars) of n=4. Values with different letters in the auxiliary table differ significantly (p<0.05).

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Figure 3. Example of (A) the obtained thermograms for sample C10 at different brining times (0
to 8 days), (B) of a raw thermogram and (C) of a deconvoluted thermogram related to freezable
water (FW^w).

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Figure 4. DSC data of (A) freezable water (FW^w) content, (B) fraction of the first peak composing FW and (C) melting temperature of water of control and PEF treated sea bass samples during the brining process at 5% and 10% salt concentrations. Results are expressed as means \pm standard deviations (error bars) of n=8. Values with different letters in the auxiliary tables differ significantly (p<0.05).

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Figure 5. Three typical transverse relaxation time relaxograms (T_2) obtained on a control sample at day 0 (dashed black line) and at day 8 (solid black line) and on sample salted in 10% brine and treated at 10 A (solid gray line). To allow for a direct comparison among them, the intensities are scaled so that the total area equals one arbitrary unit.

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Figure 6. Content of (A) water- and (B) salt-soluble proteins (% net weight) of control and PEF treated sea bass samples during the brining process at 5% and 10% salt concentrations. Results are expressed as means \pm standard deviations (error bars) of n=3. Values with different letters in the auxiliary tables differ significantly (p<0.05).













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Conflict of interest

The authors declare no conflict of interest.

CRediT Author statement

Janna Cropotova: Conceptualization, Formal analysis, Investigation, Writing - Original Draft; Silvia Tappi: Formal analysis, Investigation, Writing - Original Draft, Visualization; Jessica Genovese: Formal analysis, Investigation, Writing - Review & Editing; Pietro Rocculi: Supervision, Funding acquisition, Writing - Review & Editing; Luca Laghi: Formal analysis, Writing - Review & Editing: Marco Dalla Rosa: Supervision Turid Rustad: Project administration, Writing - Review & Editing.