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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 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.
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

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

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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 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.

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

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).

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 Vikings and other populations that lived on the shores of the Mediterranean Sea and the Atlantic Ocean. Today, a variety of salted pelagic fish products including sardines, anchovies, sea bass, *bacalao*, herring i.e., as well as Scandinavian dried and salted cod called *klippfisk*, literally "cliff-fish", are produced under the common name of "salted fish products" and marketed in many countries of the Mediterranean and the North Sea regions. Due to a fairly good market price and high palatability, these product commodities have become popular and highly appreciated in Europe and the USA. Along with the changes of lifestyle and growing consumer demands towards ready-to-eat, healthy and tasty foods, lightly salted fish products are currently gaining more and more popularity (Fan, Luo, Yin, Bao, & Feng, 2014).

Salting is one of the simplest methods of preserving large quantities of fish from spoilage. Salt is 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 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 textural parameters. Increasing the water holding capacity of fish muscle helps to decrease cooking loss, thereby enhancing the tenderness and juiciness of the final product. Sodium chloride (NaCl), the common salt, is the main ingredient used in fish salting. It acts as a preservative by dehydration and osmotic pressure inhibiting bacterial growth and deactivating enzymes. Even at low concentrations, NaCl possesses some preservative action (Lupín, Boeri, & Moscidar, 1981). Other substances such as herbs, spices, sugar or antioxidants can also be used in the fish salting process to improve sensory attributes of the product, modify flavor and reduce shrinkage after salting. The 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) are applied to the fish surface. Wet salting is performed by immersing the product into brine or injecting the brine directly into the fish muscle (Birkeland, Skåra, Bjerkeng, & Rørå, 2003; Hall, 2011). The concentration of salt in the brine affects the weight gain, water holding capacity and commercial quality of the end product (Nguyen, Thorarinsdottir, Gudmundsdottir, Thorkelsson, & Arason, 2010). Weight gain of salted fish products depends on the ability of the myofibril proteins to retain water inside the muscle affected by the salting procedures applied (Thorarinsdottir, Arason, Sigurgisladottir, Valsdottir, & Tornberg, 2011). The brining time usually varies from 2 to 10 days depending on the desired level of salt in the muscle. During immersion brining, fish is covered with brine for a period of time and held at a temperature between 0 to 4°C. In injection salting, the brine is injected into the fish fillet using a set of needles making this a faster method than immersion brining.

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 extractability and thermal denaturation and aggregation of many muscle proteins (Nguyen, Thorarinsdottir, Gudmundsdottir, Thorkelsson, & Arason, 2010), which in turn affects the WHC. Salting also affects the proteolytic activity responsible for degradation of myofibrils and connective tissue proteins, as well as extra-cellular matrix (Thorarinsdottir, Arason, 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 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, 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 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 the food industry to study water mobility and distribution within the fish muscle (Løje, Green-Petersen, Nielsen, Jørgensen, & Jensen, 2007; Aursand, Gallart-Jornet, Erikson, Axelson, & Rustad, 2008). This technique has been suggested a tool for rapid and non-destructive analysis of water mobility and identification of intra-myofibrillar or extra-myofibrillar water components (Andersen and Jørgensen, 2004; Jensen, Jørgensen, Nielsen, & Nielsen, 2005; Løje, Green-Petersen, Nielsen, Jørgensen, & Jensen, 2007) in the muscle.

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 intensity ultrasound brining and marinating (Chemat, Zill-e-Huma, & Khan, 2011; Turhan, Saricaoglu, & Oz, 2013), pulsed vacuum brining (Andres, Rodrigues-Barona, Barat, & Fito, 2002), and vacuum tumbling (Mathias, Jittinandanana, Kenney, & Kiser, 2003; Esaiassen et al., 2004). Pulsed electric field (PEF), as an emerging technology, has great potential to contribute to improved salting of fish products through enhanced diffusion of salt into the fish muscle (Hafsteinsson Gudmundsson Arnarson Jonsson, & Siguroardottir, 2000). However, to our knowledge, no studies have so far been published on PEF applications for salting of fish. Even though the concept of PEF was introduced to the food industry about 50 years ago, this technique can be still considered an emerging technology due to the recent developments related to microbial inactivation applications and improvement of mass transfer through cell disruption (Gómez et al., 2019). In general, PEF technique applies high voltage pulses of short duration to food placed between two electrodes, resulting in specific structural modifications of the tissue including the disruption of cell membrane (Barba et al., 2015). Under the application of the high electric field 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 industry with the aim of enhancing water holding capacity of fish and tenderization of shellfish products (Klonowski, Heinz, Toepfl, Gunnarsson, & Þorkelsson, 2006). PEF has also been 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).

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 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 during salting. It is well known that PEF may affect the extractability and aggregation of proteins, since electroporation within the muscle tissue can result in chemical modifications by the formation of free radicals which can further alter the structure of proteins and the intermolecular 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 water holding capacity and protein solubility.

2. Materials and Methods

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.

2.2. PEF pre-treatment and brine salting

Sea bass fillets were cut into small pieces (8.3 ± 0.2 g each) with the dimensions of length 2.3 ± 0.2 cm, width 3.1 ± 0.4 cm and height 1.3±0.5 cm.

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 μs), 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 of two parallel stainless-steel electrodes (3 mm thick) with a 47 mm fixed gap. Output voltage and 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 ± 1 μs), frequency (100 Hz), repetition time (10 ± 1 ms) and selecting two different current intensities, 10A and 20A, corresponding to values of electric field strengths of 0.3 and 0.6 kV/cm and specific energy input of 0.25 ± 0.01 and 1.01 ± 0.03 kJ/kg, respectively. The process 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 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**.

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 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 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 Technology (Trondheim, Norway) for determination of water and salt content, pH and protein solubility.

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

2.3. Physico-chemical analyses

2.3.1 Mass transfer parameters

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).

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.

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 HNO_3 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 ± SD.

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

$$\Delta M^\circ{}_t = \frac{(M^\circ{}_t - M^\circ{}_0)}{M^\circ{}_0} \quad (1)$$

$$\Delta M_t^w = \frac{(M^\circ{}_t \cdot x_t^w - M_0^o \cdot x_0^w)}{M^\circ{}_0} \quad (2)$$

$$\Delta M_t^{NaCl} = \frac{(M^\circ{}_t \cdot x_t^{NaCl} - M^\circ{}_0 \cdot x_0^{NaCl})}{M^\circ{}_0} \quad (3)$$

where $M^\circ{}_t$ and $M^\circ{}_0$ are the sea bass weights, $x^w{}_t$ and $x^w{}_0$ are the water weight fractions, and $x^{NaCl}{}_t$ and $x^{NaCl}{}_0$ are the NaCl weight fractions, at sampling time *t* and before the salting process *0*, respectively.

2.3.2 Water state and mobility

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 x 0.2 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 ± SD was calculated.

Differential scanning calorimetry (DSC)

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

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).

The FW was determined as follows:

$$FW = \frac{\Delta H_m}{\Delta H_w} \quad (4)$$

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.

LF-NMR

A 10 mm deep slice was cut from each sample, then cylinders (6 mm diameter) of about 400 mg were obtained with a cork borer. Signals weighted by T2 were registered with the CPMG pulse sequence (Meiboom & Gill, 1958), using a Bruker mod. Minispec PC/20 spectrometer operating 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 but allowed the observation of the protons with T2 higher than a few milliseconds. UPEN software (Borgia, Brown, & Fantazzini, 1998) allowed to obtain an overview of the protons T2 distributions (the relaxograms) by inverting the T2-weighted signals towards a semi-continuous distribution of exponential curves, according to Eq. (5):

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

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.

2.3.3 Protein functionality

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.

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 ± SD was calculated.

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 °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.

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 (Time) and their interaction on dependent variables was evaluated through the factorial Analysis of Variance (ANOVA). Statistical significance of the experimental data was verified using Tukey as post-hoc ($p<0.05$). To establish a relationship between certain parameters, Pearson correlations were calculated. Differences were considered significant at $p<0.05$.

3. Results and discussion

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. However, on the last day of brine salting, the weight yield of control samples was reduced up to -0.13% and 2.56% for 5% and 10% salt concentration in the brine, respectively. The lowest weight yield in the control group on day eight may possibly be explained by an inhomogeneous salt distribution within the inner and outer parts of the fish muscle at the beginning of brining, leading 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 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 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 salting. In all samples, water uptake (**Fig. 1B**) was observed until the 5th day, when samples immersed in the 5% salt brine showed significantly higher values compared to samples in the 10% 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 for both the control samples, as already observed with the total weight change. PEF treated samples in the 5% brine, did not show a further water uptake, while samples in the 10% brine showed a further increase. All PEF treated samples showed similar water fraction values at the end of the 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.

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 samples were significantly higher compared to their respective controls, while after 5 days, only the 10A sample and the 20A sample in the 5% brine. Salt concentration in PEF treated sea bass fillets increased slightly between the 5th and the 8th day, but, although samples treated at 10A (0.3 kV/cm) showed and increasing trend, differences were not statistically significant. The higher salt weight fractions reached corresponded to a salt content in the samples of 4.47 and 6.84 g/100g for the 5 and 10% brining respectively, showing an increase of 77 and 35% compared to the highest salt content obtained in control samples at day five.

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.

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). 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 electric field intensity, which is why generally a mass transfer increases is obtained (Gómez et al., 2019). Electroporation has been shown to cause increased inter-myofibrillar spacing in fish and meat products (Gómez et al., 2019) which could aid mass transfer, thus increasing the salt uptake 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 channels for diffusion of brine. Moreover, Klonowski, Heinz, Toepfl, Gunnarsson & Þorkelsson (2006) found a more porous structure in cod fillets pre-treated by PEF, that might have aided the 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 possible that a change on the flesh structure might have happened.

The increase of salt concentration in the tissue results, especially at the level of myofibrils, in greater water absorption and swelling under certain conditions (Krasnow, Loss, Ahrens, & Fiore 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 repulsive force of negative charges increases. The intra-myofibrillary space expands due to the repulsive forces and a greater water retention capacity is determined. However, brines with a saline concentration above 10-15% can lead to an opposite effect, worsening the water retention capacity. In this case the salting-out phenomenon may occur: the ions in excess of Cl-, not being able to interact with the positive charges of the proteins already occupied by the other ions, interfere with 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, Cramer, & Garde, 2001; Offer e Trinick, 1983). This phenomenon, however, was not observed in PEF treated samples by Klonowski, Heinz, Toepfl, Gunnarsson & Þorkelsson (2006), although the 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.

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 tissue brining resulted in a significant decrease of water activity, explained by the bonding of residual fluid from the fish muscle by salt through ionic interactions. These interactions reduce the 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 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 the 5 and 10% concentration respectively. Neither PEF intensity (0.3 and 0.6 kV/cm) nor duration 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, & Cavani, 2014), there are three different water populations in muscle tissues, the first one (below

5%) exists as true hydration water that is strictly bound to proteins by macromolecular of multimolecular adsorption, the second is water located inside organized protein structures (intra-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 unfreezable, unaffected by charges on the muscle protein (pH), and it is unavailable to participate in reactions. From a calorimetric point of view, freezable water (FW) is usually associated to the 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 al., 2003) and in food systems, such as meat (Venturi et al., 2007; Petracci et al., 2012; Mudalal, Petracci, Tappi, Rocculi, & Cavani, 2014).

Fig. 3A reports, as an example, the obtained thermograms of sample C10 at different brining times (zero to height days). As it is possible to observe, the FW peak was actually composed by two superimposed peaks, melting at slightly different temperatures. While in the fresh sample, this difference was small, with the first melting at around -3°C and the second melting at around 0°C being almost indistinguishable, as the brining time increased, the first peak appeared at lower 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 plotted, as shown in **Fig. 3B** (example of raw thermogram) and **3C** (example of deconvoluted thermogram) respectively.

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 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 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, differences were not significant. In PEF treated samples, no differences were observed compared to initial value at all brining days.

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 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 we take into account the two different peaks, it is possible to observed that, while initially the 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 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 temperature depends on the balance between the water uptake and the salt concentration in the 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 melting temperature transition. As shown by **Fig. 1C**, in PEF treated samples, salt concentration increased more compared to the control, corroborating the hypothesis of the observed differences. 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 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 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 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' observed in the control samples, was not reflected in the FW measurements. This might be due to a different distribution of salt in the tissue as hypothesized earlier. Indeed, sampling procedure is pivotal for DSC analysis, since the small sample size (about 15 mg). Hence, although we took 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 measure by DSC in fish samples during brining, so it is not possible to compare results giving a 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, &

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 and long proton relaxation times. W_B (T_2=1-3 ms) relates to water bound by secondary bonds to the proteins, W_1 (T_2=40-80 ms) describes capillary water found in the myofibrillar network, while W_2 (T_2=100-190 ms) is mechanically immobilized water or extra-myofibrillar which can be further released as drip loss. Table 2 reports the relative intensities expressed as arbitrary units (AU) and the T_2 of the three water populations for all the analyzed seabass samples. According to Aursand, Gallart-Jornet, Erikson, Axelson, & Rustad (2008) populations W_1 and W_2 represent more than 90% of the total water in the muscle.

In the present study, an evident migration of water from pools W_B and W_1 towards pool W_2, with longer relaxation times was observed from the untreated raw sample to all brined samples. This 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 the muscle fibers to expand and entrap water. This occurs due to electrostatic repulsion within the myofibrils, exposing protein sidechains to water binding (Strasburg, Xiong &Chiang, 017). 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 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 to the treatment. The only variable that showed consistently a significant effect on water distribution parameters was brining time ($p<0.001$).

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 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. T_{21} (intra-myofibrillar water) shifted from about 45 ms to 65-85 ms, while T_{22} (extra-myofibrillar water) from about 106 ms to 130-190 ms, directly reflecting the increased amount of water, which was also observed in other studies conducted on brine salting of fish (Aursand, Gallart-Jornet, Erikson, Axelson, & Rustad, 2008). However, also for this parameter, few significant differences were observed. Specifically, while in T_{22} a significant increase was found during brining time, no differences were observed among samples according to the PEF treatment (0.3 and 0.6 kV/cm). A significant effect was found only for brining time and for NaCl concentration for T_{21} and T_{22}.

3.3. Protein functionality

The pH values of sea bass samples after PEF-treatment and salting performed for 2, 5 and 8 days are shown in **Table 3.** Untreated sample showed an initial value of 6.7 that decreased progressively 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 compared to control samples on day 2 and 5 of brining. This could be due to a release of ions from 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 decrease, remained stable. Nevertheless, result of multifactorial ANOVA showed that this parameters is influences significantly by all considered variables and their interaction.

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**.

Solubility of water soluble (sarcoplasmic) protein was strongly and significantly reduced during brining in all samples. In seabass brined in the 10% NaCl solution, PEF treated samples showed always significantly lower values compared to the control, but with no differences according to the intensity of the electric field applied, 0.3 or 0.6 kV/cm. For samples in the 5% brine solution, differences were not always significant.

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, 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.

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**.

ΔM^{o}_{t} is positively correlated to both ΔM^{w}_{t} and ΔM^{NaCl}_{t}, as they showed similar behavior during brining, but it was also negatively correlated to W_B and to the solubility of both water- and salt-soluble proteins. No significant correlation was observed with any of the other parameters, that, as 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.

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 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-myofibrillar spaces of the fish muscle (W_2-water pool) as a result of the PEF-induced increased solvation. Supported by previous investigations (Nguyen, Thorarinsdottir, Gudmundsdottir, 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 substantially protein-protein interactions compared to protein-water interactions. The weaker 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 hydrophobic interactions between proteins became stronger, contributing to their increased hydrophobicity and aggregation (Stefansson & Hultin, 1994; Lin & Park, 1998).

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.

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Figure captions

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).

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 ± standard deviations (error bars) of n=4. Values with different letters in the auxiliary table differ significantly (p<0.05).

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).

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 ± standard deviations (error bars) of n=8. Values with different letters in the auxiliary tables differ significantly (p<0.05).

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.

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 ± standard deviations (error bars) of n=3. Values with different letters in the auxiliary tables differ significantly (p<0.05).

Fig. 1

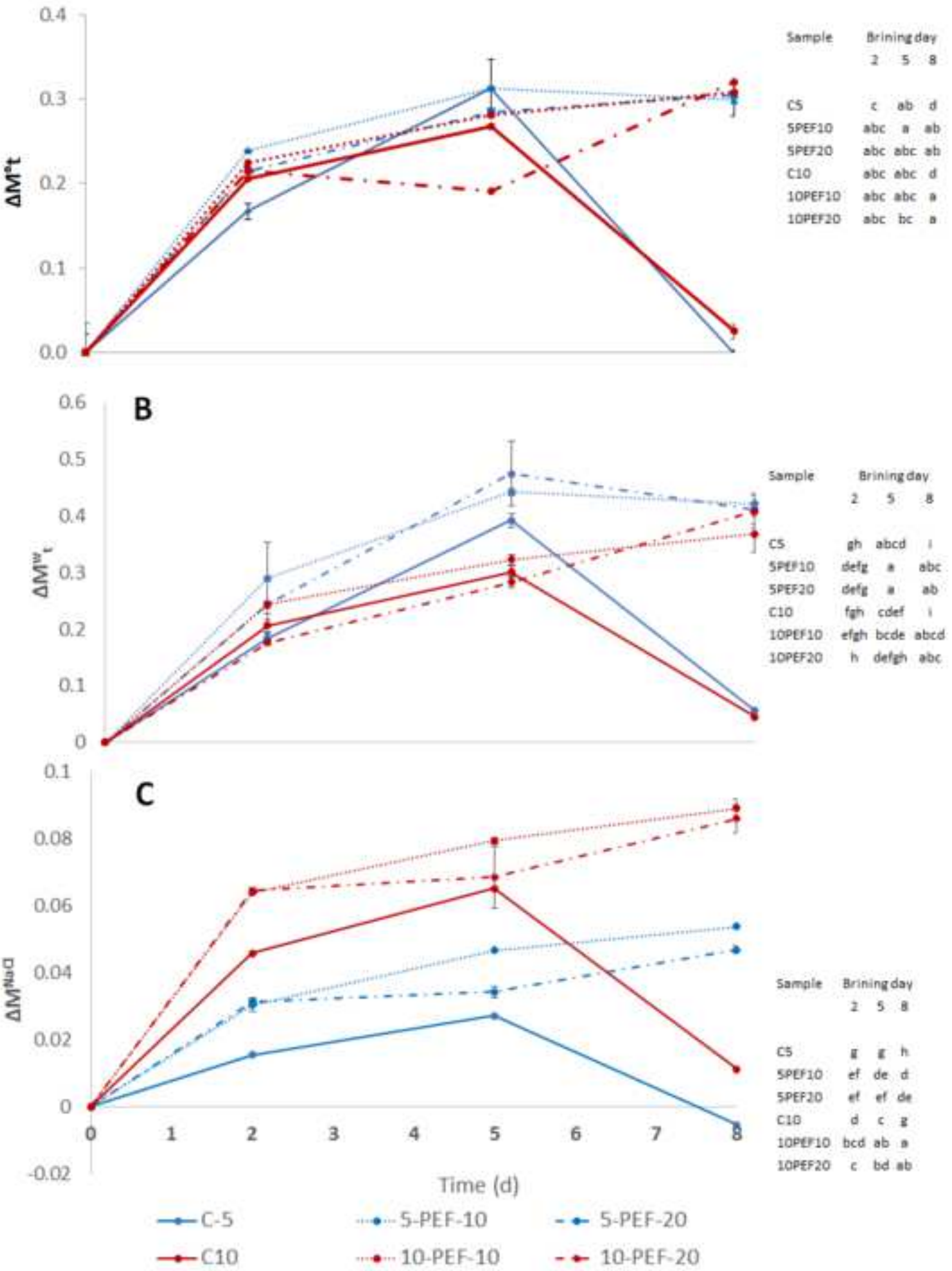

ΔM°t
0.4
0.3
0.2
0.1
0.0
Sample Brining day
2 5 8
C5 c ab d
5PEF10 abc a ab
5PEF20 abc abc ab
C10 abc abc d
10PEF10 abc abc a
10PEF20 abc bc a
B
ΔM^w_t
0.6
0.5
0.4
0.3
0.2
0.1
0
Sample Brining day
2 5 8
C5 gh abcd i
5PEF10 defg a abc
5PEF20 defg a ab
C10 fgh cdef i
10PEF10 efgh bcde abcd
10PEF20 h defgh abc
C
ΔM^NaCl
0.1
0.08
0.06
0.04
0.02
0
-0.02
0 1 2 3 4 5 6 7 8
Time (d)
Sample Brining day
2 5 8
C5 g g h
5PEF10 ef de d
5PEF20 ef ef de
C10 d c g
10PEF10 bcd ab a
10PEF20 c bd ab
C-5
5-PEF-10
5-PEF-20
C10
10-PEF-10
10-PEF-20

Fig. 2 Click here to access/download;Figure;Fig. 2x.tif

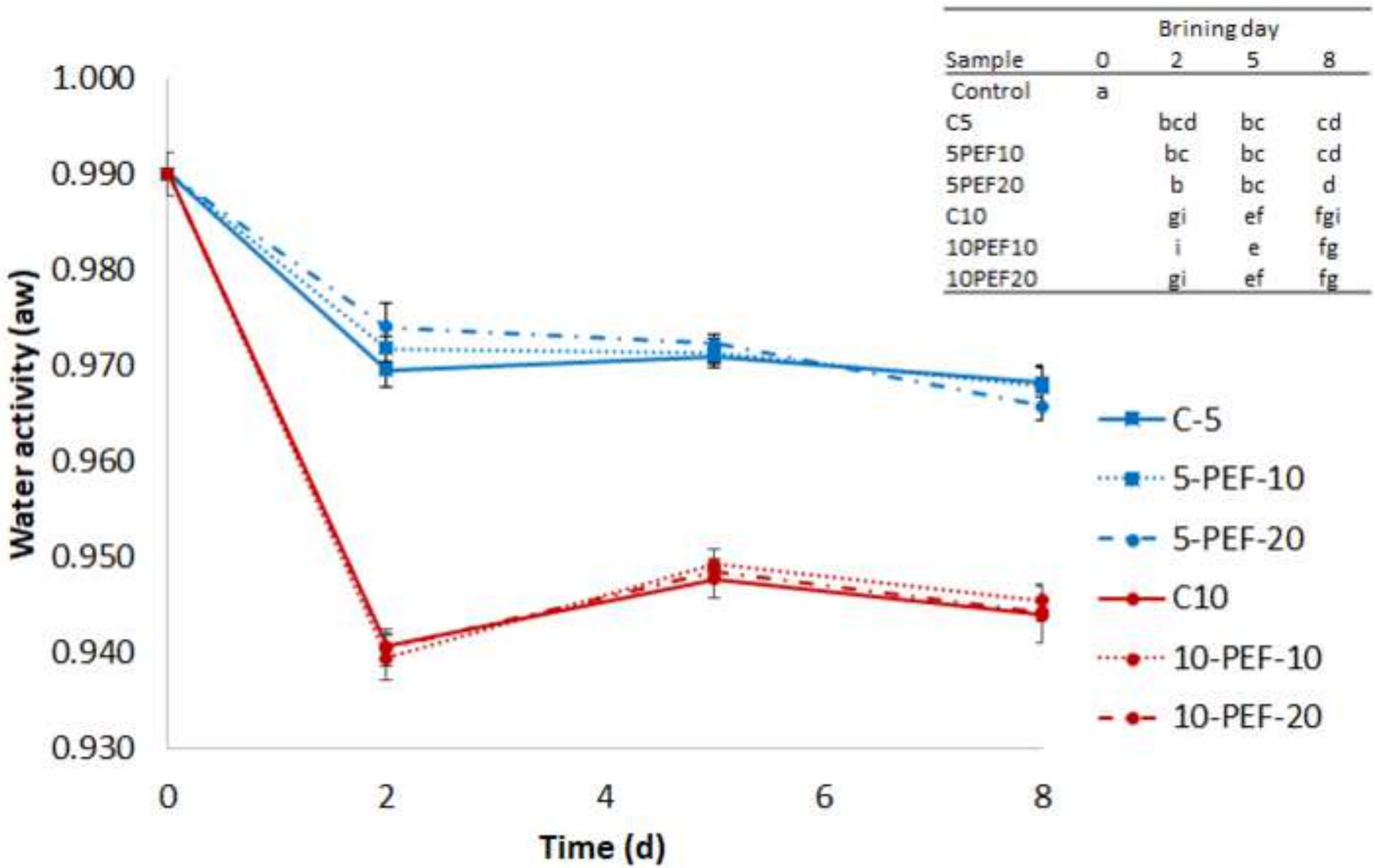


Fig. 3

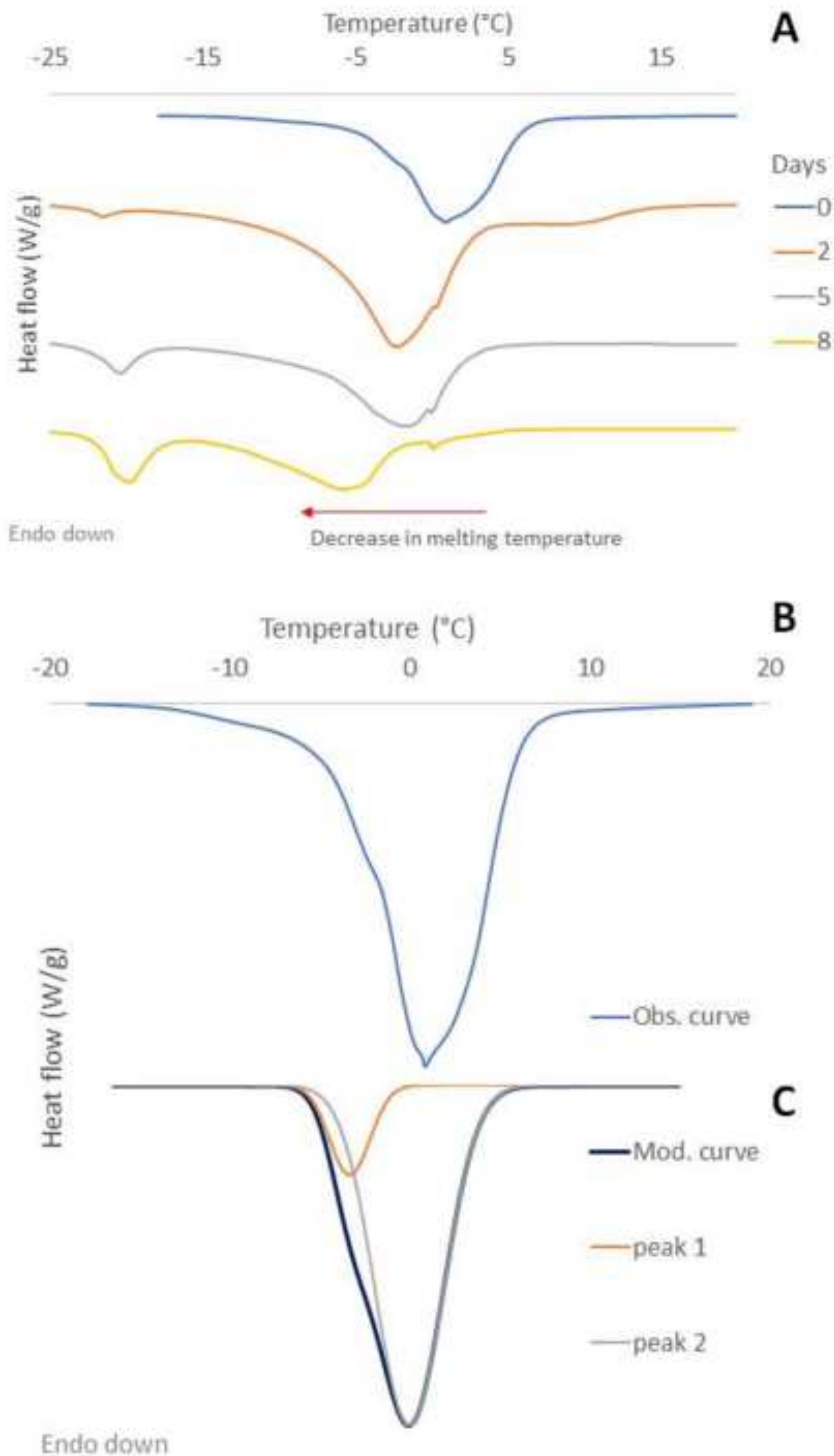

A
Temperature (°C)
-25
-15
-5
5
15
Heat flow (W/g)
Days
0
2
5
8
Endo down
Decrease in melting temperature
B
Temperature (°C)
-20
-10
0
10
20
Heat flow (W/g)
Obs. curve
C
Mod. curve
peak 1
peak 2
Endo down

Fig. 4

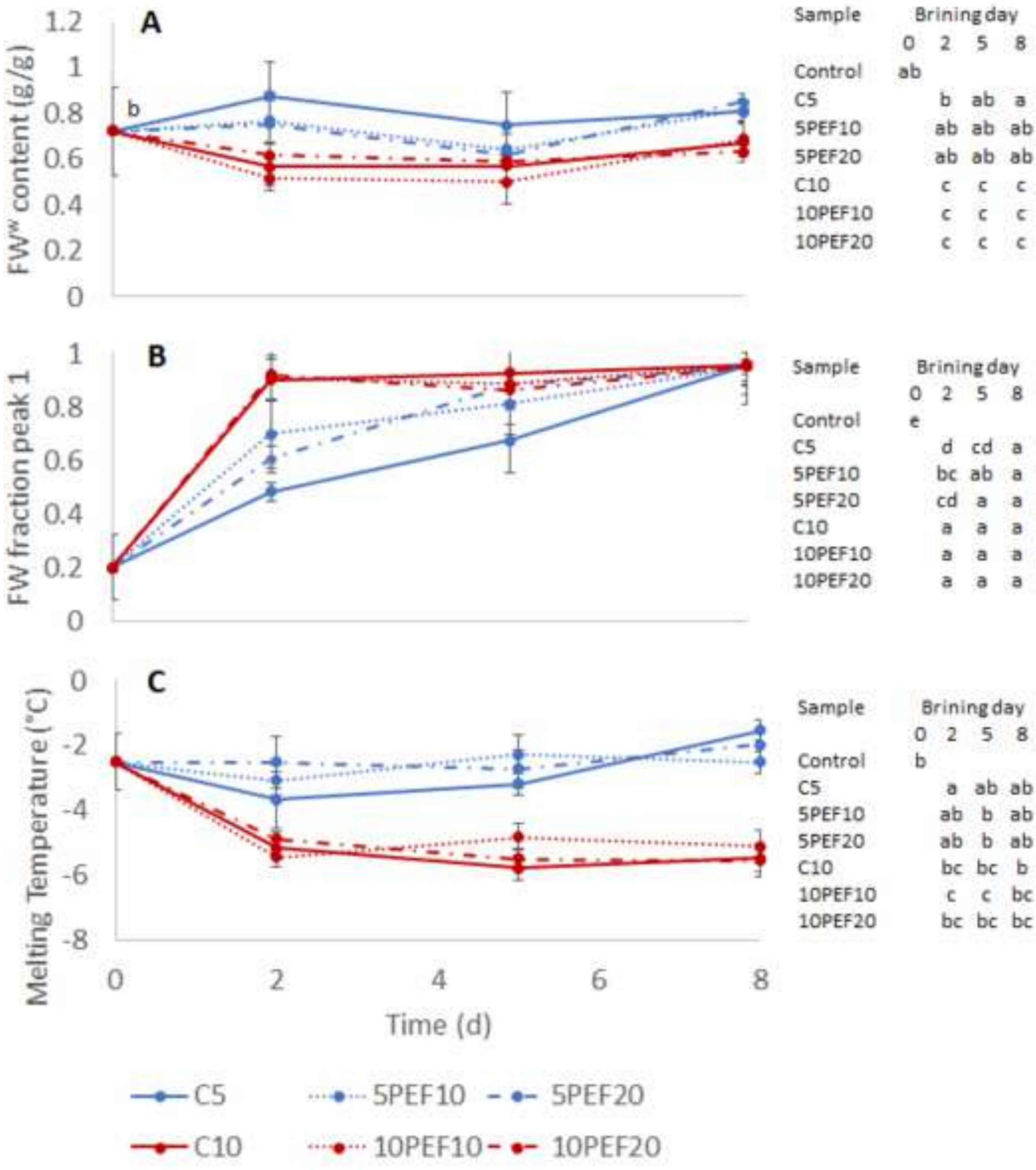
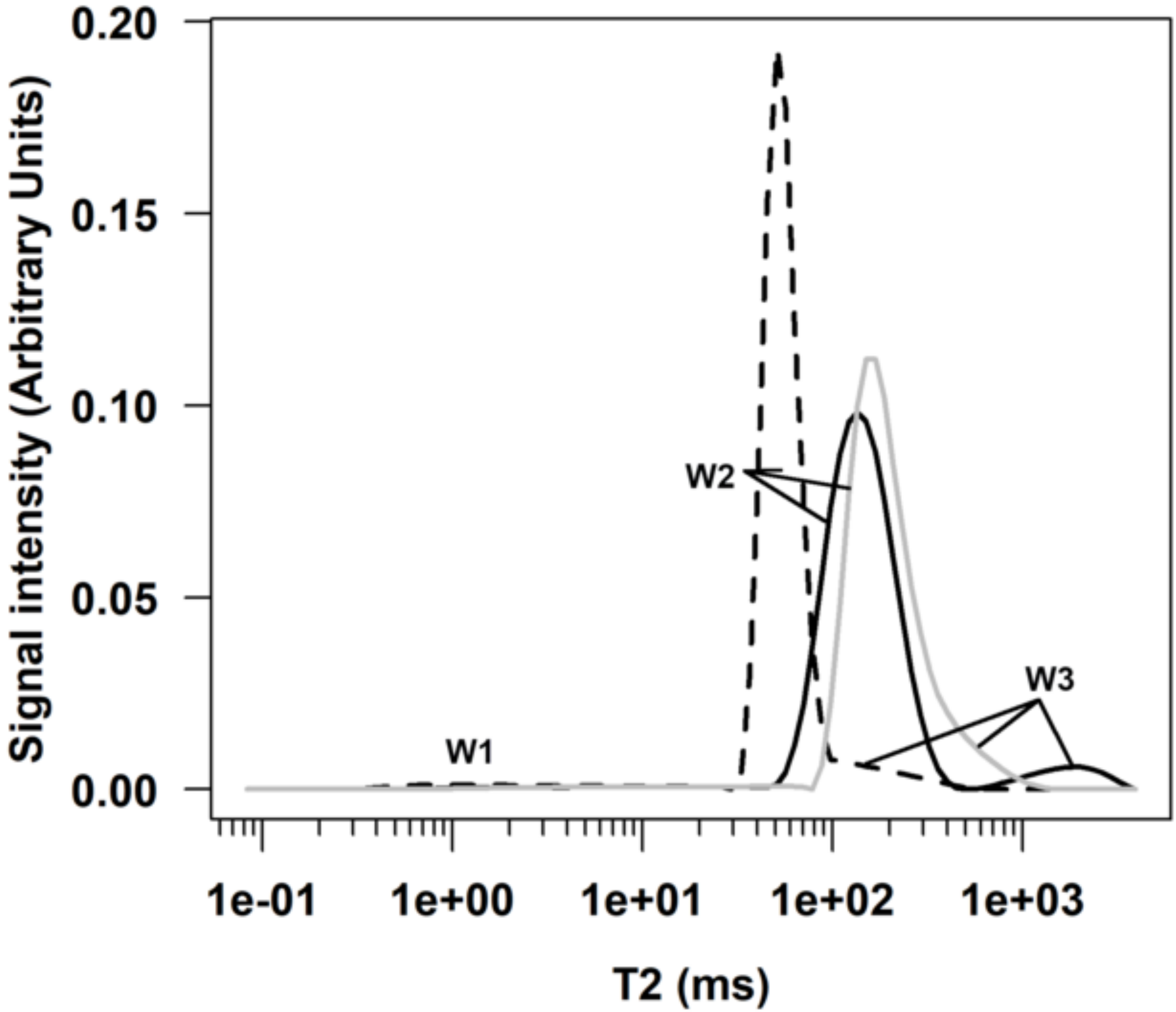
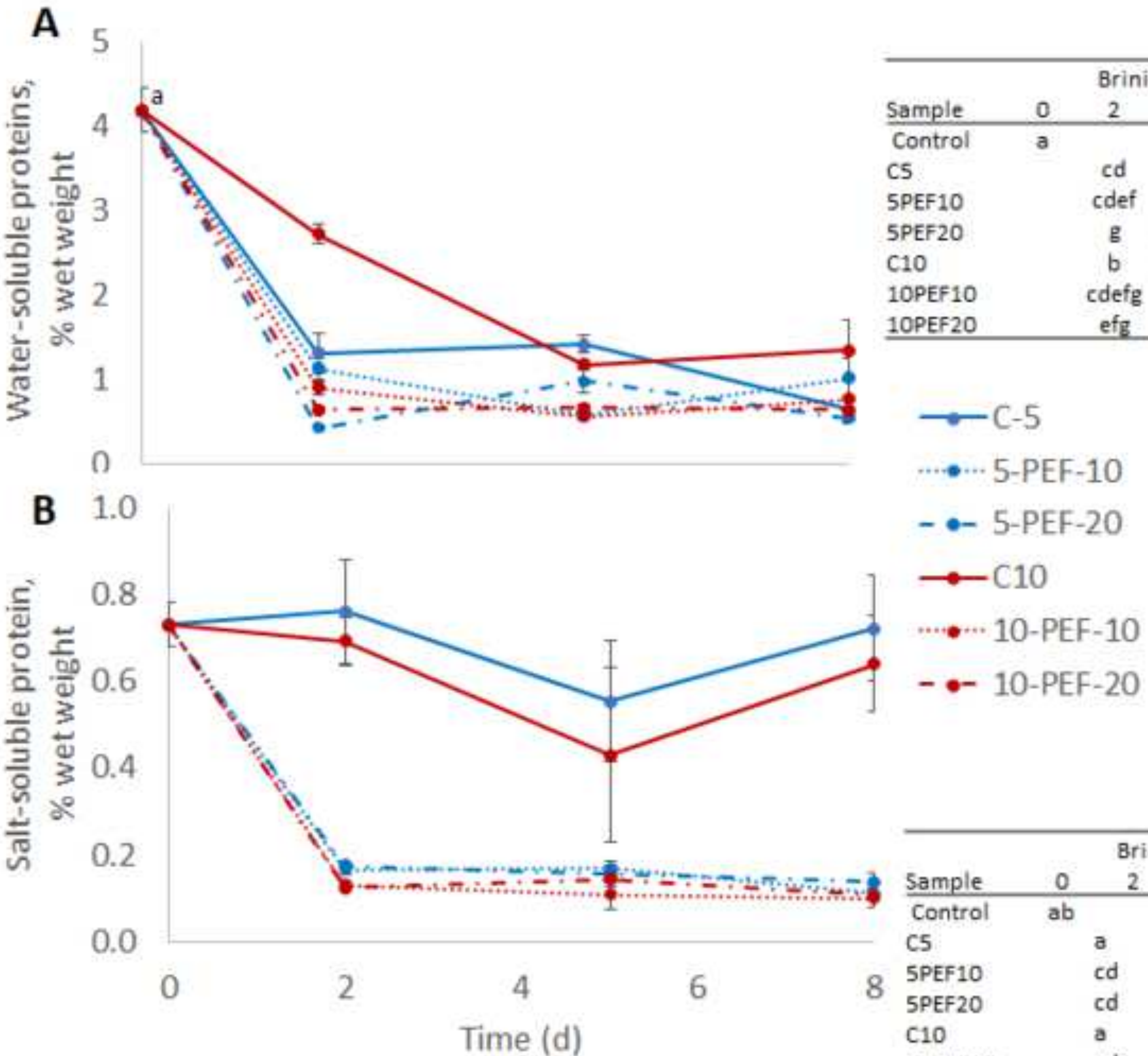

A
FW^w content (g/g)
1.2
1
0.8
0.6
0.4
0.2
0
b
Sample Brining day
0 2 5 8
Control ab
C5 b ab a
5PEF10 ab ab ab
5PEF20 ab ab ab
C10 c c c
10PEF10 c c c
10PEF20 c c c
B
FW fraction peak 1
1
0.8
0.6
0.4
0.2
0
Sample Brining day
0 2 5 8
Control e
C5 d cd a
5PEF10 bc ab a
5PEF20 cd a a
C10 a a a
10PEF10 a a a
10PEF20 a a a
C
Melting Temperature (°C)
0
-2
-4
-6
-8
Sample Brining day
0 2 5 8
Control b
C5 a ab ab
5PEF10 ab b ab
5PEF20 ab b ab
C10 bc bc b
10PEF10 c c bc
10PEF20 bc bc bc
0 2 4 6 8
Time (d)
C5
5PEF10
5PEF20
C10
10PEF10
10PEF20

Fig. 5



Signal intensity (Arbitrary Units)
0.20
0.15
0.10
0.05
0.00
W1
W2
W3
1e-01
1e+00
1e+01
1e+02
1e+03
T2 (ms)

Fig. 6



Sample	0	2	5	8
	Brining day			
Control	a			
C5		cd	c	efg
5PEF10		cdef	efg	cdefg
5PEF20		g	cdefg	fg
C10		b	cdefg	cd
10PEF10		cdefg	fg	efg
10PEF20		efg	efg	efg

Sample	0	2	5	8
	Brining day			
Control	ab			
C5		a	ab	ab
5PEF10		cd	cd	d
5PEF20		cd	cd	cd
C10		a	abc	ab
10PEF10		cd	d	d
10PEF20		cd	Cd	d

Table 1

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Table
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Table 4

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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.