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Effect of electrical conductivity on the inactivation of *Anisakis* spp. by PEF

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ABSTRACT

Pulsed Electric Fields (PEF) have proven effective in inactivating *Anisakis* in fish fillets. One of the parameters conditioning the lethal efficacy of PEF is the electrical conductivity of the treatment medium in which the fish is immersed. However, the underlying cause of this increased efficacy remains unknown. It was hypothesized that the difference in electrical conductivity between fish cells and *Anisakis* larvae could influence the electric field applied to each cell type.

As measuring cells' electric fields is difficult, the study adopted an alternative dual approach. First, based on the experimental data, a mathematical equation was developed to predict *Anisakis* inactivation in hake belly fillets within the study's parameter ranges (1–5 kV/cm, 10–40 kJ/kg, 7–30 μs pulse width, and 0.4–10 mS/cm). Additionally, an increase in electrical conductivity under the same PEF treatment was experimentally observed to result in a greater degree of parasite inactivation. To investigate this phenomenon, the numerical simulation strategy was employed to estimate the electric field strength affecting the elements in the treatment chamber. The results showed that as the electrical conductivity of the medium increased, the electric field strength in the parasite also increased, thus explaining the greater inactivation observed.

1. Introduction

Anisakis spp. is a nematode parasite belonging to the Anisakidae family (Aibinu et al., 2019; Grabda, 1976). *Anisakis* has a complex life cycle involving multiple hosts and four larval stages. After the release of eggs by marine mammals, they develop into first-stage larvae (L1) within the egg, then molt into second-stage larvae (L2) which reach a size of approximately 4–6 mm before hatching into the water. These are ingested by crustaceans, where they develop into third-stage larvae (L3) measuring approximately 10–30 mm in length, which are the infective form for fish and squid. When these are eaten by marine mammals, L3 larvae mature into fourth-stage larvae (L4) and then into adults (EFSA, 2010; ELIKA, 2023; Smith, 1983). Humans become accidental hosts when they consume fish without previously inactivating the parasite (Aibinu et al., 2019; ELIKA, 2023). Human consumption of live *Anisakis* spp. larvae causes symptoms including nausea, vomiting, abdominal pain, and allergic reactions (EFSA, 2010; Hochberg & Hamer, 2010). Due to the popularity of sushi and sashimi in Japan, that country reports 20,000 annual cases of anisakiasis, more than any other nation (Sugiyama et al., 2022). In Spain, the consumption of raw or undercooked fish (anchovies in vinegar sauce are particularly popular) makes Spain the second-highest country in number of reported incidences of anisakiasis (Daschner et al., 2000; Herrador et al., 2019).

European legislation Regulation No. 853/2004 indicates that the whole product be frozen at a temperature of −20 °C or lower for a minimum period of 24 h or at −35 °C or lower for a minimum of 15 h if the fish is going to be consumed raw. The effectiveness of freezing has been demonstrated; however, freezing/thawing impacts fish quality. After thawing, the texture is softer, and changes in color and flavor can be observed (Chai et al., 2005; Leygonie et al., 2012).

Recent studies (Abad et al., 2023, 2024; Onitsuka et al., 2022, 2024) have demonstrated the effectiveness of Pulsed Electric Fields (PEF) technology as a possible alternative to freezing for *Anisakis* inactivation, with minimal effects on fish quality.

Pulsed Electric Fields technology consists of immersing a product in an aqueous solution (treatment medium) placed between two

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electrodes; high-intensity electric fields ranging from 0.5 to 30 kV/cm are applied by intermittent pulsing for short durations, usually to the order of microseconds. The temperature increase induced by PEF is minimal (Raso et al., 2022; Zhang et al., 1995).

Previous studies have shown that *Anisakis* inactivation increases with higher treatment intensity, electric field strength, specific energy, and pulse width in three studied species: horse mackerel (*Trachurus japonicus*), salmon (*Oncorhynchus keta*), and hake (*Merluccius merluccius*) (Abad et al., 2023, 2024; Onitsuka et al., 2022, 2024). A further factor worthy of consideration is the electrical conductivity of the treatment medium. As Onitsuka et al. (2022) demonstrated, an increase in the electrical conductivity of the treatment medium results in a greater degree of *Anisakis* inactivation. However, the mechanism behind this effect remains unclear. It could be hypothesized that the higher electrical conductivity of fish cells compared with the conductivity of *Anisakis* nematodes (Choudhury et al., 2002) could affect the electric field applied to each cell type; moreover, the electric field could also be affected by the electrical conductivity of the treatment medium. The lower the electrical conductivity of the parasite, the higher the field strength, leading to a greater degree of inactivation. This would explain the lethal effect a PEF procedure can exert on *Anisakis* larvae without affecting fish quality. However, under lab conditions it would be difficult to measure electric field strength at each location of the treatment chamber: parasite, fish meat, and treatment medium. Numerical simulation tools could be useful to predict and study this phenomenon. The study's aim was therefore twofold. Firstly, the effect of electrical conductivity in the treatment medium on *Anisakis* inactivation was investigated in two scenarios: *Anisakis* larvae in saline solution (without fish) and *Anisakis* larvae in naturally parasitized hake bellies. Secondly, to explain the observed differences in *Anisakis* inactivation, numerical simulations were used to determine the electric field strength to which the parasites, fish meat, and treatment medium would be exposed during PEF treatments.

2. Material and methods

2.1. Anisakis spp. and hake bellies

Hake (*Merluccius merluccius*) bellies naturally parasitized with *Anisakis* spp. were obtained from hakes purchased in a local supermarket. Bellies were stored in trays and covered with aluminum foil at 4 °C for up to 4 days post-purchase, as it has been demonstrated that at these storage temperatures their viability is not affected (Lanfranchi & Sardella, 2010; Pascual et al., 2010).

2.2. PEF treatment of Anisakis spp. larvae in saline solution and fish belly

PEF treatments were applied to *Anisakis* spp. and to naturally parasitized hake bellies in saline solution with several different electrical conductivities. For the treatments of *Anisakis* in saline solution, parasites were removed from the hake bellies with tweezers and stored them in saline solution (0.85 % NaCl) (Oh et al., 2014) in Petri dishes before introducing them into the PEF treatment chamber. Following extraction, the larvae were kept for 30–60 min at room temperature prior to assessing their viability using the mechanical stimulation technique described by EFSA (2010). This method involves gently stimulating the *Anisakis* larvae with tweezers to determine the presence or absence of movement; if they show movement, they are considered alive; otherwise, they are regarded as inactivated. On the other hand, to prepare the treatments of *Anisakis* in parasitized fish bellies, naturally infected fish belly meat was cut into pieces measuring 4.5 x 2.5 x 0.5 (±0.2) cm and introduced those pieces into the PEF treatment chamber. In both cases, the treatment chamber was filled with saline solutions of varying electrical conductivities, ranging from 0.4 to 10 mS/cm. The saline solutions were a mix of water and NaCl (PanReac, Barcelona, Spain) prepared in different proportions with the aim of obtaining varying electrical conductivities. The electrical conductivity of the saline solution (treatment medium) was quantified with an electrical conductivity probe (Almemo FYA641LF series, Alhborn, Germany) at 5 ± 1 °C. Following the PEF treatment, the temperature in thermal center of the fish, as well as that of the water of the treatment chamber, was measured using a K-type thermocouple temperature probe (FTA 15 P1, Almemo series, Alhborn, Germany) connected to a data logger (Almemo 2590, Alhborn, Germany). In no case did it exceed 20 °C.

The PEF equipment used in this study was the PEFPilot™ Dual system fabricated by ELEA (Quakenbrück, Germany). It applies square wave pulses of 7 μs and maximum frequencies of 400 Hz. The maximum voltage is 24 kV, and the current is 1300 A. The treatment chambers for these experiments contained two parallel square stainless-steel electrodes (laterally measuring 8 cm) with a gap of 8 cm between them.

To evaluate the effect of pulse width, a lab-scale PEF system was used: the EPULSUS-PM-10, 2 kW from Energy Pulse System (Lisbon, Portugal). It applies square wave pulses of a maximum frequency of 200 Hz and pulse widths ranging from 1 to 200 μs. The maximum applicable voltage and current are 10 kV and 180 A, respectively. The processing parameters were recorded with an oscilloscope (Tektronix, TDS 220, Wilsonville, OR, USA) connected to a voltage probe (Tektronix, P6015A, Wilsonville, OR, USA) and an amperage probe (Stangenes Industries Inc. Palo Alto, CA, USA). The treatment chamber consisted of two parallel circular stainless-steel electrodes of a 5 cm radius with an adjustable electrode gap. In this portion of the investigation, the gap used was 2 cm. Due to electric current limitations, the effect of electrical conductivity in this system could only be evaluated at 1 kV/cm.

For both systems, the specific energy applied was calculated based on Equation (1):

$$W = \frac{1}{m}\int_0^t \sigma E^2 dt \qquad \text{(Equation 1)}$$

where σ is the electrical conductivity of the treated medium or product (S/m), E is the electric field strength (V/m); t is the total time (s) during which the electric field strength is applied, and m the weight of the treated medium.

After each PEF treatment, five batches of ten *Anisakis* larvae were removed from the treatment chamber and stored them in Petri dishes with saline solution (0.85 % NaCl) at 4 °C for 3 h, given that previous studies have shown that after 3 h, there is no difference in variability (Abad et al., 2023). For the assessment of PEF treatment lethality in fish bellies, after PEF treatment, bellies were stored under refrigeration for 3 h, as (Abad et al., 2023) previously mentioned. After this period five batches of ten *Anisakis* spp. were removed from each piece of belly, followed by the same procedure as for *Anisakis* in saline solution. To determine the viability of *Anisakis* after each type of PEF treatment, the mechanical stimulation technique recommended by EFSA (2010) was applied. Each PEF treatment condition was applied at least in triplicate.

The fish samples were derived from different hakes, processed on separate days. Each piece of fish represents an independent sample, as the hake were processed in distinct batches, with individual pieces being randomly selected.

To evaluate the influence of electrical conductivity of saline solutions and the influence of PEF parameters (field strength, pulse width, and specific energy) on the viability of *Anisakis* in hake belly, a multiple regression was conducted based on the experimental results regarding *Anisakis* viability, excluding any non-significant values ($p < 0.05$). To perform this regression, Design-Expert 13 software package (Stat-Ease Inc., Minneapolis, MN, USA) was used. The chosen ranges for each parameter were: 1–5 kV/cm electric field strength, 10–40 kJ/kg specific energy, 7–30 μs pulse width, and a saline solution electrical conductivity of 0.4–10.0 mS/cm. The equation was validated with new data obtained at laboratory scale and at pilot-plant scale within the equation's range, featuring almost 20 new PEF treatment conditions, at least in triplicate,

including field strength, specific energy, pulse width, and electrical conductivity of saline solution. To determine the resulting equation's accuracy, R^2, adjusted R^2, and root mean square error (RMSE) were applied (Baranyi et al., 1999).

2.3. Numerical simulation

COMSOL Multiphysics 5.3. was the software used to carry out numerical simulations. A parallel electrode chamber was simulated with two stainless steel square electrodes of 3 cm length and 3 cm height, with a gap of 3 cm between them (Fig. 1). This configuration was selected based on previous studies (Astráin-Redín et al., 2023; Moya et al., 2022). Previous experiments conducted in a pilot-plant-scale treatment chamber yielded results that closely resembled those of a simulated chamber, with no statistically significant differences. Therefore, only the simulations under this condition are presented.

The electric field strength variable (kV/cm) can be written as a function of electrical potential, V (V). Based on charge conservation, the governing equation for electrical potential can be written as shown in Equations (2) and (3):

$$E = \nabla V \quad \text{(Equation 2)}$$

$$\nabla^* J = \nabla J^* [s(T)\nabla V] = 0 \quad \text{(Equation 3)}$$

where J (A) represents the electric current density, and σ (T) the electrical conductivity.

Two scenarios were simulated: ten *Anisakis* larvae in saline solution (0.4–10.0 mS/cm), and ten *Anisakis* larvae inside a piece of fish (representing a parasitized hake belly) immersed in saline solution (0.4–10.0 mS/cm). *Anisakis* were simulated as cylinders with 0.1 cm radius and 1 cm length, and the piece of fish as a 2-cm-side cube. In the simulation, all larvae and fish meat pieces were located in the center with respect to the treatment chamber; in the case of larvae in fish belly, the simulated parasites were uniformly distributed inside the piece of fish meat (Fig. 1).

To optimize the mesh size, a sensitivity analysis was conducted by testing various configurations, taking the electric field as a key variable to evaluate the impact of mesh refinement. The selected mesh consisted of 40,173 nodes and 234,064 quadratic tetrahedral elements.

Selected parameters were voltage and electrical conductivity of *Anisakis*, fish meat (in this case, hake), and treatment medium. The selected voltage varied from 3000 to 15,000 V, with achieved field strengths ranging from 1 to 5 kV/cm. The range of treatment medium conductivities selected extended from 0.4 to 10.0 mS/cm. The electrical conductivity of hake was 6.0 ± 0.3 mS/cm, and the electrical conductivity of *Anisakis* nematodes was 1.63 ± 0.02 mS/cm. This value corresponded to the mean value determined by two different methods, as explained below, due to the inherent difficulty in accurately measure the electrical conductivity of *Anisakis* larvae. The electrical conductivity of hake and *Anisakis* parasites was experimentally determined with an LCR

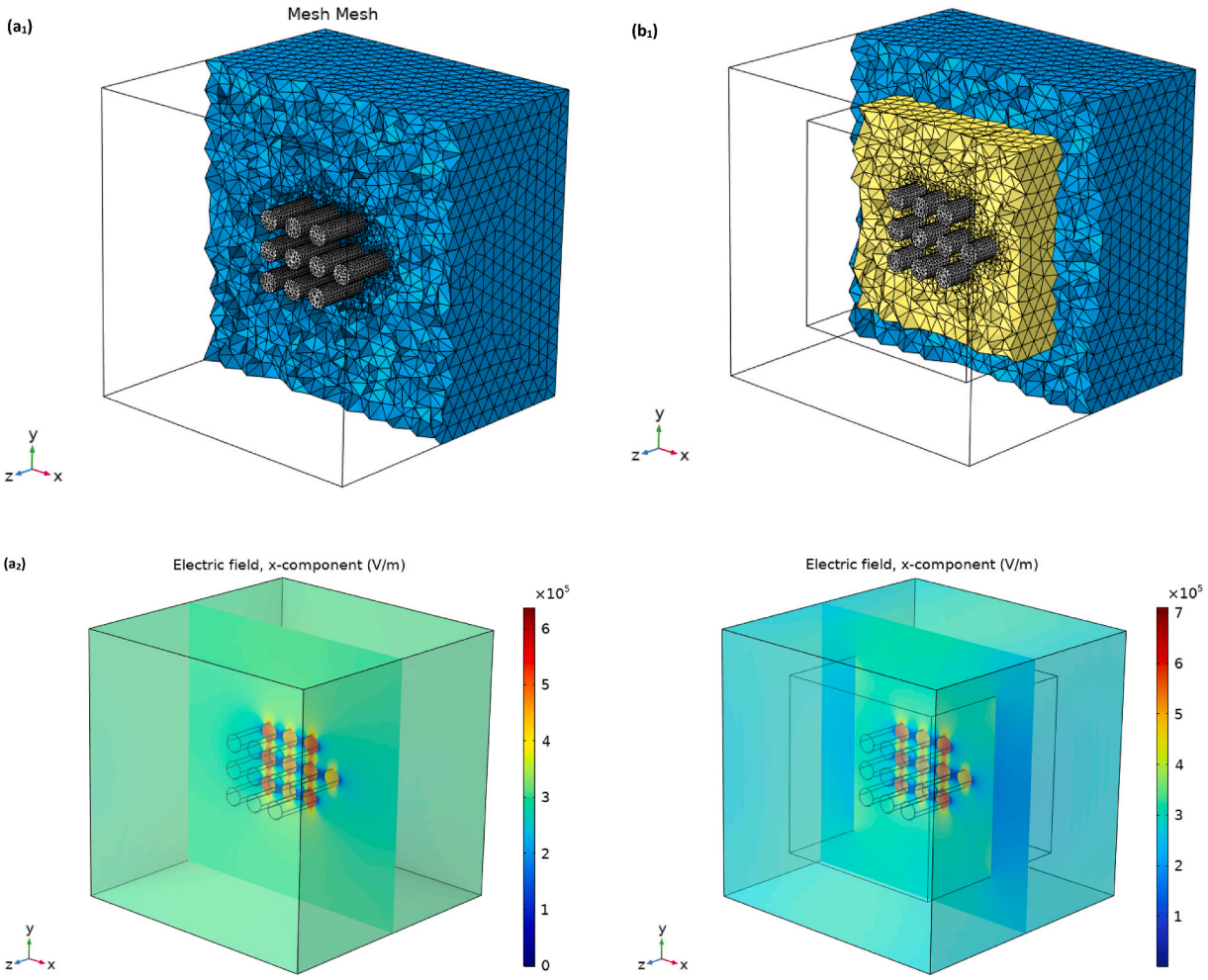

Fig. 1. Finite element mesh geometry and boundary conditions. Two different scenarios were simulated: (a) ten *Anisakis* nematodes in saline solution (a_1: mesh) (a_2; electric field), and (b) ten *Anisakis* nematodes inside a piece of fish (simulating a parasitized hake belly) immersed in saline solution (b_1: mesh) (b_2; electric field). *Anisakis* nematodes were simulated as a cylinder of 0.1 cm radius and 1 cm length, and the piece of fish as a cube of 2 cm side.

meter (REED R5001, Wilmington, United States), applying the following equation (Chen et al., 2022):

$$s = \frac{1}{R} x \frac{L}{A} \quad \text{(Equation 4)}$$

where R is the resistance of the sample (*Anisakis* or hake) (Ω), L is the gap between the two electrodes (m), in this case 0,008 m, and A is the area of the electrode (m^2); in this specific case, the electrode measured 0.4168 x 10^{-4} m^2. The frequency range at which the samples' electrical resistance was determined was: 100, 120, 1,000, 10,000, and 100,000 Hz. At each frequency, at least four samples and three replicates of each sample were taken. In the case of fish samples, it was sufficient to directly probe different tissue samples using the measurement equipment. However, due to the small size of *Anisakis*, a large number of parasites had to be extracted, thoroughly washed to remove residual fish tissue, dried with paper to eliminate water, and then crushed. Due to the complexity of measuring the electrical conductivity of *Anisakis*, the ground sample was also measured directly using a conductivity probe (Almemo FYA641LF series, Alhborn, Germany), yielding a value of 1.24 ± 0.06 mS/cm. Additionally, washed and dried *Anisakis* were placed into a circular PEF treatment chamber (1 cm radius, 0.5 cm gap) until the chamber was completely filled of parasites. These samples were subjected to several pulsed electric field treatments, measuring the delivered amperage in the treatment chamber of the first and second pulses with an amperage probe (Stangenes Industries Inc. Palo Alto, CA, USA and recorded with an oscilloscope (Tektronix, TDS 220, Wilsonville, OR, USA). Using these measurements and applying Equation (1) (in this case for a gap of 0.02 m, and an area of the electrode of 0.8 x 10^{-4} cm^2), the electrical conductivity was calculated, resulting in an average value of 2.00 ± 0.60 mS/cm.

The numerical simulations aimed to assess the electric field to which *Anisakis* larvae would be subjected when receiving a PEF treatment in saline solution and in fish meat. Using the data obtained from numerical simulations at different electrical conductivities of the treatment medium and selected field strengths in the PEF system, a multiple regression was performed in which non-significant values ($p < 0.05$) were eliminated. The regression was conducted with the Design-Expert 13 software package (Stat-Ease Inc., Minneapolis, MN, USA). To determine the final equation's accuracy, R^2, R^2-adjusted, and root mean square error (RMSE) were used (Baranyi et al., 1999).

2.4. Evaluation of fish microbiota after PEF treatments

Microbiological analyses were conducted on control and PEF-treated fish fillets subjected to the same PEF treatment, but using media with two different electrical conductivities, 0.4 mS/cm and 8 mS/cm, with the aim of evaluating the impact of the medium's electrical conductivity on microbial growth during PEF treatment. The PEF treatment applied was 3 kV/cm, 20 kJ/kg, and 7 μs of pulse width.

Hake fillets (4.5 × 2.5 cm) were introduced into the PEF treatment chamber and completely covered with saline solutions of two different electrical conductivities: 0.4 mS/cm and 8 mS/cm. PEF treatment was then applied, following the same procedure previously described for *Anisakis* inactivation. Control samples were subjected to the same procedure but without the application of PEF. After treatment, fillets were placed in trays and packaged under a modified atmosphere composed of 50 % CO_2 and 50 % N_2 (ULMA, SMART-400, Gipuzkoa, Spain). The trays were stored at 4.0 ± 0.5 °C for 8 days, with sampling performed on days 0, 2, 4, 6, and 8.

For each sampling point, 10.0 ± 0.3 g of fish muscle was removed using sterile tweezers and a scalpel, transferred into a sterile stomacher bag (VWR, Radnor, USA), and mixed with 90 mL of sterile peptone water (APT, Oxoid, Basingstoke, UK). The mixture was homogenized for 60 s at 230 rpm using a Stomacher® 400 Circulator (Seward, Worthing, UK). The specific microbial groups analyzed, culture media, incubation temperatures, durations, and detailed growth conditions are provided in Table 1. The plating method followed was similar to the one reported by Antunes-Rohling et al. (2019). All microbiological analyses were conducted in triplicate, with each replicate consisting of two individual portions of 10 g each.

3. Results and discussion

3.1. Effect of electrical conductivity on the survivability of Anisakis spp. to PEF

Fig. 2 shows the viability of *Anisakis* treated in saline solution (Fig. 2A) and in naturally parasitized hake belly (Fig. 2B) following several PEF treatment conditions (varying in terms of field strength, specific energy, and pulse width) in media of distinct electrical conductivity. Inactivation increased with the electrical conductivity of the medium in all cases when applying the same PEF treatment, and inactivation was more pronounced in saline solution than when parasites were in the fish meat. In saline solution at 1 kV/cm, survivability decreased from 75 % to 55 % when conductivity ranged from 1 to 5 mS/cm (circles in Fig. 2A), whereas in hake belly, survivability was only 93 % (empty circles in Fig. 2B). The higher PEF resistance of *Anisakis* larvae in fish has been attributed to the fact that the parasite is naturally encysted in the fish's internal tissues (Ángeles-Hernández et al., 2020). A study by Abad et al. (2024) showed that field strengths of 5 kV/cm and specific energies of 30 kJ/kg were necessary to achieve 90–99 % inactivation of *Anisakis* when the parasite was naturally present in hake and treated in 0.4 mS/cm water solution (some of these data are also included in Fig. 2B - hexagon). In this current study, it was surmised that increasing the electrical conductivity of the medium would make it possible to reduce the intensity of PEF treatments, mainly in terms of field strength. It can be observed that a PEF treatment of 3 kV/cm and an electrical conductivity of 0.4 mS/cm, the survival percentage was 44 % in saline solution (squares in Fig. 2A), 90 % in hake applying a specific energy of 20 kJ/kg (full squares in Fig. 2B) and 78 % in hake applying a specific energy of 40 kJ/kg (empty squares in Fig. 2B), always with pulses of 7 μs. However, when increasing the electrical conductivity to 6 mS/cm in saline solution, or to 8 mS/cm when treating hake, almost complete inactivation of the parasites was achieved. Even more, at 8 mS/cm and 3 kV/cm (squares in Fig. 2B), similar inactivation was obtained when treating at 0.4 mS/cm and 5 kV/cm (hexagons). *Anisakis* spp. inactivation data obtained at low electrical conductivities, specifically in solutions with an electrical conductivity similar to water, agree with the rates of *Anisakis* inactivation described by Abad et al. (2023, 2024). Likewise, the effect of the PEF parameters was similar to results in literature. Apart from field strength and specific energy, the pulse width parameter also proved to be of interest. If pulse width were enlarged to 30 μs, a lower field strength or specific energy (and thus also a shorter treatment time) would be required, as observed in Fig. 2B for treatments of 1 kV/cm, 20 kJ/kg, and 30 μs (full circles), and treatments applied at 1.2 kV/cm, 40 kJ/kg, and 7 μs (empty triangles) at different electrical conductivities.

Previous studies (Onitsuka et al., 2023; 2024) conducted on artificially parasitized salmon (*Oncorhynchus keta*) and horse mackerel (*Trachurus japonicus*) showed that the electrical conductivity of the treatment medium is a parameter worthy of consideration when estimating the parameters necessary for PEF to inactivate *Anisakis* in fish; those two studies described behaviors similar to the inactivation variability shown in Fig. 2, both in saline solution (larvae extracted from hake) and in naturally parasitized hake belly (*Merluccius merluccius*). Therefore, varying the electrical conductivity of the treatment medium could be an interesting possibility when seeking to increase the overall lethality of PEF treatments applied to *Anisakis*.

To further explore the effect of varying PEF parameters on the survivability of *Anisakis* in hake, including the effect of electrical conductivity, multiple regression analysis was conducted using the data shown

Table 1
Growth conditions applied to the microbial populations analyzed.

Microbial group	Agar	Temperature	Time	Atmosphere	Plating
Aerobic Psychrotrophes	LH Agar[a]	7 °C	10 d	Aerobic	Spread
Anaerobic Psychrotrophes	LH Agar[a]	7 °C	10–12 d	Anaerobic	Spread
Pseudomonas	GSP Agar[b]	25 °C	24–48 h	Aerobic	Spread
Shewanella	Iron Agar[c]	25 °C	3–4 d	Aerobic	Spread
Lactic Acid Bacteria	Elliker Agar[d]	25 °C	24–48 h	Aerobic	Pour
Enterobacteriaceae	VRBG Agar[e]	37 °C	48 h	Aerobic	Spread (double layer)

[a] Long and Hammer Agar (Broekaert et al., 2011).
[b] Glutamate Starch Phenol Red Agar (Sigma-Aldrich, Steinheim, Germany) + Penicillin G (Sigma-Aldrich, Steinheim, Germany).
[c] Iron Agar (Lingby) (Conda, Madrid, Spain).
[d] Elliker Broth (Sigma-Aldrich, Steinheim, Germany) + Bacteriological Agar (Oxoid, Basingstoke Hants, UK).
[e] Violet Red Bile Glucose Agar (VRBG) (Oxoid, Basingstoke Hants, UK).

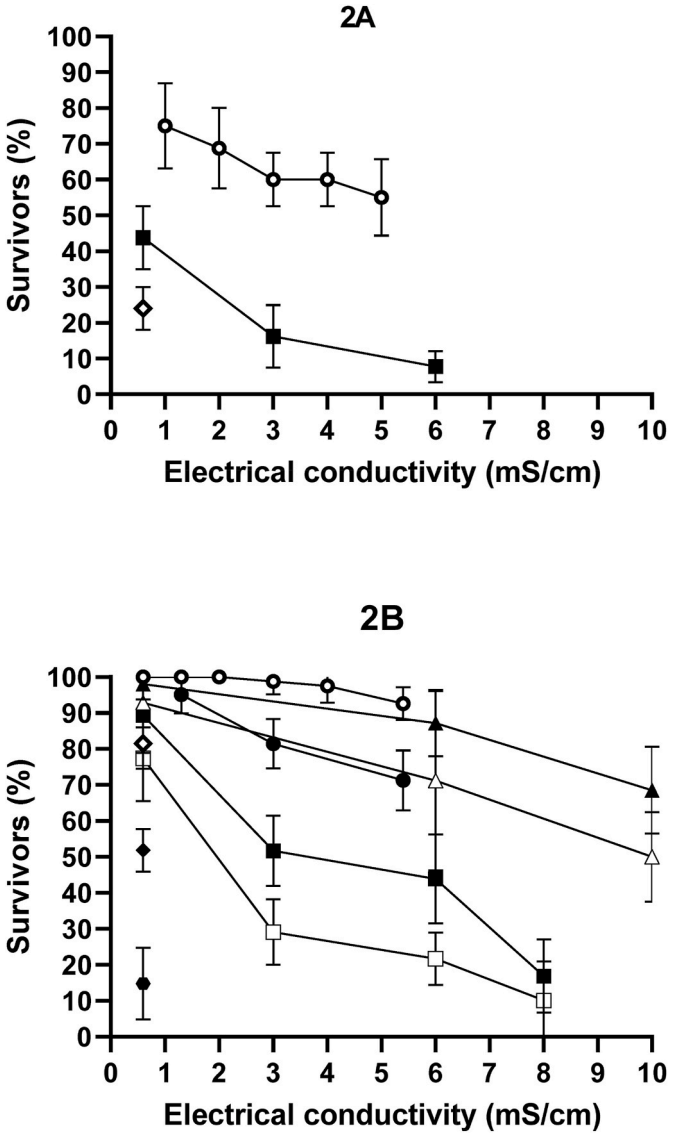

Fig. 2. Influence of electrical conductivity of the treatment medium on the percentage of *Anisakis* spp. survivors when subjected to PEF treatments in saline solution (Fig. 2A) or in hake bellies (Fig. 2B) at different pulse widths, specific energies and electric field strengths. (○) 1 kV/cm; 10 kJ/kg; 30 μs; (●) 1 kV/cm; 20 kJ/kg; 30 μs; (▲) 1.2 kV/cm; 20 kJ/kg; 7 μs; (△) 1.2 kV/cm; 40 kJ/kg; 7 μs; (■) 3 kV/cm; 20 kJ/kg; 7 μs; (□) 3 kV/cm; 40 kJ/kg; 7 μs; (⋄) 3 kV/cm; 30 kJ/kg; 20 μs; (◆) 4 kV/cm; 30 kJ/kg; 20 μs; (●) 5 kV/cm; 30 kJ/kg; 20 μs.

in Fig. 2 and further data obtained in a broader range of conditions: field strength (1–5 kV/cm); specific energy (10–40 kJ/kg), pulse width (7–30 μs), and electrical conductivities (0.4–10.0 mS/cm). Table 2 shows the obtained polynomial equation (Equation (5)) with the corresponding coefficients of each parameter and the goodness of fit. Thus, the percentage of survivors (%S) to a PEF treatment can be calculated based on the following equation:

$$\%\,S = 137.0772 - 13.9833{*}E - 0.9658{*}W - 0.3737{*}P - 2.0305{*}\sigma{*}E \quad \text{(Equation 5)}$$

where E is the field strength, W the specific energy, P the pulse width, and σ the electrical conductivity.

Equation (5) was validated with newly obtained experimental results from PEF treatments applied to *Anisakis* nematodes in saline solution and *Anisakis* nematodes in hake belly (naturally parasitized), as shown in Fig. 3. This Figure shows the relationship between the experimental survivability of *Anisakis* obtained in the laboratory and the survivability estimated based on Equation (5) presented in Table 2. As observed, both datasets' goodnesses of fit were good, with R^2 values of 0.812 and 0.930 for saline solution and hake belly, respectively. In the case of saline solution, the equation overestimated the inactivation of *Anisakis*. This was expected since Equation (5) was developed with the results obtained in hake belly. For the latter, the data fits the equivalence line, thus confirming Equation (5)'s goodness of fit. These results once more confirmed that when the parasites are located inside the meat, it is necessary to apply stronger PEF treatments than when they are in saline solution, as previously noted by Abad et al. (2024) and Onitsuka et al. (2024). Based on Equation (5), Fig. 4A and B were built. These figures present theoretical estimations derived from the mathematical model. Fig. 4A estimates the percentage of *Anisakis* survivors when treated in hake belly at different field strengths, electrical conductivities, specific energies, and pulse widths. Fig. 4B estimates the PEF treatment conditions required to achieve 99 % *Anisakis* inactivation in hake bellies. Due to limitations of the PEF systems, some of the predictions at the highest field strengths (over 3 kV/cm) and electrical conductivities (4–8 mS/cm

Table 2
Polynomial equation (Equation (5)) describing the percentage of survivability (S) of *Anisakis* larvae in hake belly after applying PEF treatments of different electric field strength (E), specific energy (W), pulse width (P), and electrical conductivity (σ). Statistical significance (p value) and the 95 % confidence limits (CL) of each parameter are included.

% S *Anisakis* = $b_0 + b_1 \times E + b_2 \times W + b_3 \times P + b_4 \times \sigma \times E$
$R^2 = 0.9347$; R^2 adjusted = 0.9256; RMSE = 7.079

	Coefficient	p value	CL (-95 %)	CL (+95 %)
b_0	137.0772	<0.0001	122.3964	151.7580
b_1	−13.9833	<0.0001	−15.8976	−12.0689
b_2	−0.9658	<0.0001	−1.3140	−0.6177
b_3	−0.3737	0.0456	−0.7395	−0.0079
b_4	−2.0305	<0.0001	−2.5352	−1.5259

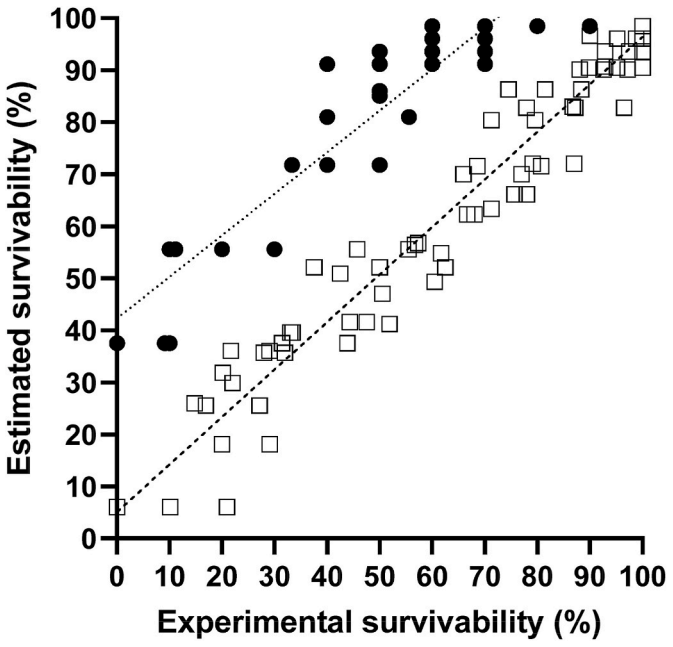

Fig. 3. Relationship between the survivability estimated with Equation (5) (shown in Table 1) and the experimentally measured survivability of *Anisakis* when located inside the fish meat (□) or immersed in saline water solution (●) after PEF treatments of different field strengths, pulse widths, energies, and electrical conductivities.

depending on the field strength) are outside the experimental conditions, but predictions have been done to show the effect of conductivity and PEF in the *Anisakis* possible lethality. Fig. 4A clearly shows the effect of electrical conductivity of the treatment medium on the viability of *Anisakis* while applying the same PEF treatment. Survivability was reduced from 51 % to almost 0 % for PEF treatments of around 4 kV/cm, 20 kJ/kg, with pulses of 7 μs, and treating in media of 1 or 8 mS/cm, respectively. This graph also shows that lethality increased with pulse width and specific energy, enabling a reduction of the field strength required to achieve a certain level of inactivation. On the other hand, Fig. 4B also allows us to assess the influence of electrical conductivity on the field strength required to reduce 99 % of the *Anisakis* population in hake belly at two levels of specific energy and pulse width. Field strength can be reduced from 6 to 3.3 kV/cm when treating hake belly at 1 or 8 mS/cm, applying 30 kJ/kg with pulses lasting 20 μs.

Based on the obtained results, the effect of the electrical conductivity of the treatment medium on the survivability of *Anisakis* is thus clear. However, the mechanisms behind this effect have not been discussed. The equations governing PEF technology (including Equations (1)–(4)) make it clear that electrical conductivity must be affecting PEF parameters. However, this occurs in different locations of the treatment chamber, where products and substances with differing electrical conductivity (fish meat, *Anisakis*, treatment medium) would result in different field strengths when applying the same voltage to the treatment chamber electrodes. As measuring this key parameter at various locations is highly difficult, the use of numerical simulation tool results essential to estimate the field strength at each location.

3.2. Determination of electric field strength by numerical simulation

Fig. 5 shows the field strength applied at the central axis based on a top view of the treatment chamber when applying 9 kV at two different electrical conductivities of the treatment medium: 0.4 (Fig. 5A) and 8.0 mS/cm (Fig. 5B). Four *Anisakis* larvae were simulated represented as cylinders, taking into account their electrical conductivity (1.63 ± 0.2 mS/cm). In both scenarios, the field strength of the treatment medium

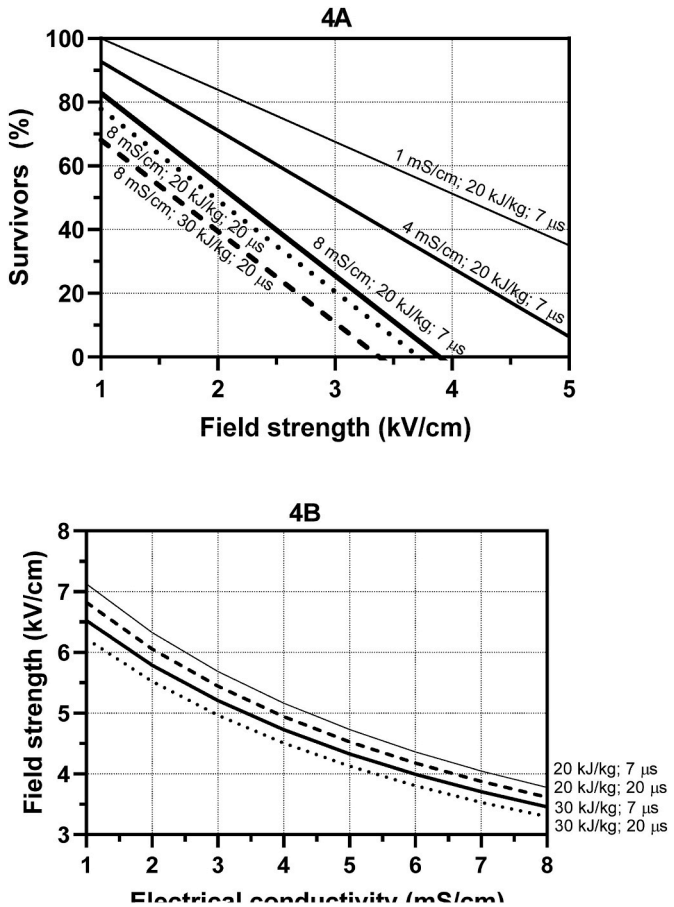

Fig. 4. Estimations of the percentage of *Anisakis* survivors when treated in hake belly at different field strengths, electrical conductivities, specific energies and pulse widths (Fig. 4A), and PEF treatment conditions required to achieve 99 % *Anisakis* inactivation in hake bellies (Fig. 4B) as predicted by Equation (5).

almost remained constant independently of its electrical conductivity (3 kV/cm). However, the electric field strength in the *Anisakis* larvae varied depending on the conductivity of the treatment medium: in media of lower conductivity (0.4 mS/cm) than the one of the parasites, after a peak in the intersection medium-parasite, the field strength decreased up to a mean field strength inside the *Anisakis* of 1.23 ± 0.06 kV/cm; when the conductivity of the medium was higher (8 mS/cm), the field strength rose up to 4.57 ± 0.37 kV/cm. In other words, field strength would increase by almost 3 kV/cm when electrical conductivity was augmented. This result, which is provided by numerical simulation, could explain the greater degree of *Anisakis* inactivation observed with increased electrical conductivity in the laboratory, as shown in Fig. 2A.

When numerical simulation was applied featuring *Anisakis* larvae inside a piece of fish, a new scenario emerged. Similarly to Figs. 5 and 6 shows the field strength at each point in the central axis of the treatment chamber for a voltage of 9 kV at 0.4 mS/cm (Fig. 6A) and 8 mS/cm (Fig. 6B) of electrical conductivity of the treatment medium in which a piece of fish (6.0 ± 0.3 mS/cm) is immersed. As observed, the electric field strength of the treatment medium decreases when electrical conductivity increases. In contrast, the field strength estimated in the nematodes and in the piece of hake was higher along with the electrical conductivity of the medium. If the electrical conductivity of the treatment medium were 0.4 mS/cm, the field strength received by *Anisakis* would be 1.01 ± 0.20 kV/cm. However, if the electrical conductivity of the medium were 8 mS/cm, the resulting field strength would be 4.64 ± 0.27 kV/cm. That is around 4.6-fold greater, which could explain the greater degree of inactivation observed in Fig. 2B and the inactivation predicted by Equation (5) as observed in Fig. 4A when treating *Anisakis*

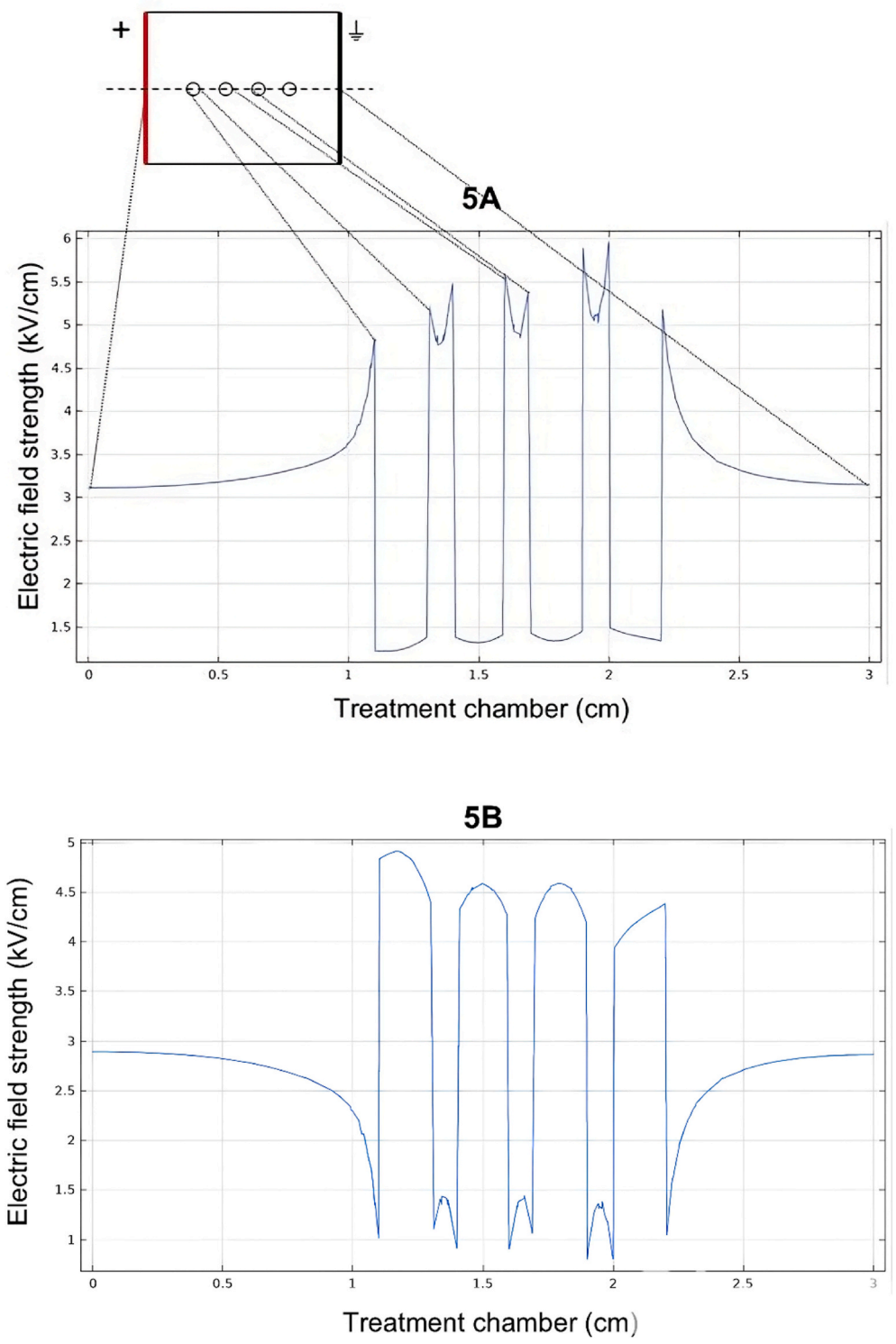

Fig. 5. Electric field strength on the central axis (dotted line in the schematic representation) of a 3-cm-gap treatment chamber and electric field strength operating on schematic *Anisakis* larvae when applying 9 kV in media of two different electrical conductivities 0.4 (Fig. 5A) and 8 mS/cm (Fig. 5B). To clarify results, a scheme of the treatment chamber has been included.

in the fish belly. In the meantime, the field strength in the piece of hake varied from 0.51 ± 0.09 kV/cm at 0.4 mS/cm to 2.8 ± 0.52 kV/cm at 8 mS/cm. Although the field strength also increased in the fish, it was around 2-fold lower than the field strength achieved in the parasite alone. This difference in field strength between the parasite and the fish meat could explain why hake quality was not affected by PEF treatments that inactivated *Anisakis*, as described by Abad et al. (2023, 2024) and Onitsuka et al. (2022, 2024). These authors did not detect differences in water holding capacity and cooking loss of fish fillets untreated and PEF treated during 7 days of shelf-life at 4 °C (Abad et al., 2023, 2024). Additionally, an evaluation of the possible effect of electrical conductivity when applying PEF on the microbiota of hake fillets has been done in this work as it is discussed later.

It has to be pointed out that all the simulations and estimations that

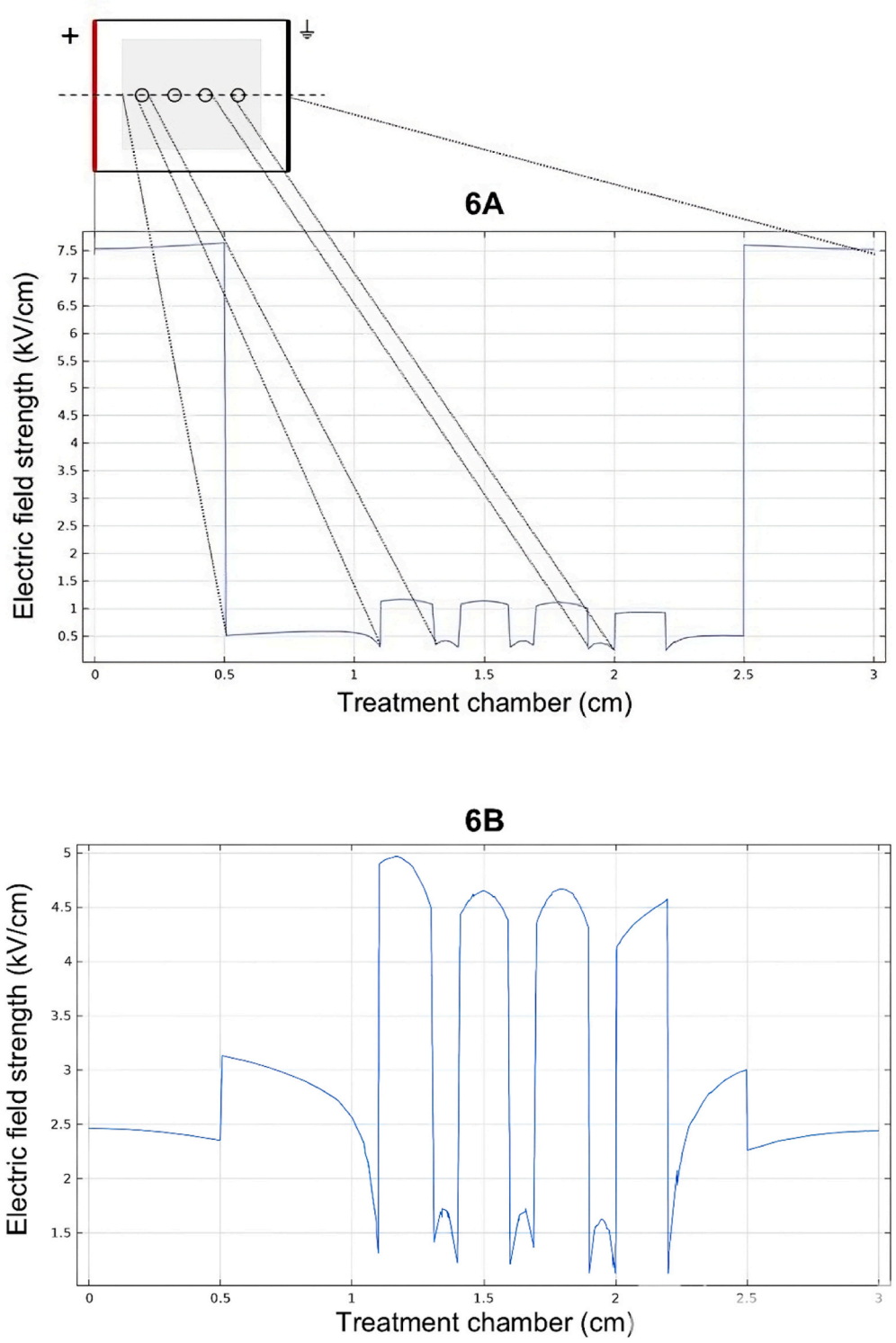

Fig. 6. Electric field strength on the central axis of a 3-cm-gap treatment chamber for a PEF treatment of 9 kV with two different electrical conductivities of the treatment medium including *Anisakis* inside of a piece of hake fish: 0.4 mS/cm (Fig. 6A) and 8 mS/cm (Fig. 6B). To clarify results, a scheme of the treatment chamber has been included.

have been carried out above corresponded to simplified systems since the same electrical conductivity has been considered for the whole piece of fish or the whole parasite. For example, *Anisakis* exhibits a differentiated anatomical organization, which includes an outer cuticle, hypodermis, muscle tissues, and internal systems such as the nervous, excretory, and digestive systems. These components likely present distinct dielectric properties. In particular, the cuticle, which serves as a protective barrier against external stresses such as gastric acid, is composed of several layers with different compositions, including a superficial lipid layer, an intermediate collagen-keratin layer, and an internal matrix rich in proteins, carbohydrates, and minor amounts of lipids and esterase enzymes; or internal tissues which are crucial for the parasite survivability (Gago, García, Fernández, & González, 2007). These structural differences could result in a non-uniform distribution of

the electric field within the *Anisakis*, potentially leading to localized field concentrations in specific regions which could make effects for localized damage.

Considering the numerically simulated effect of electrical conductivity on field strength applied to the parasite when located in the fish meat, several scenarios similar to those shown in Fig. 6B were simulated by varying the theoretical field strength in the treatment chamber (voltage applied to the 3-cm-gap treatment chamber) and the electrical conductivity of the treatment medium, while estimating the field strengths achieved in the parasite and in the fish meat. Fig. 7 shows the estimated field strength that would be achieved in the nematode (7A and 7B) and in fish (7C) when applying different field strengths (1–5 kV/cm) in saline solution (7A) or in fish immersed in saline solutions (7B and 7C) of varying electrical conductivities. The estimated field strength increased along with electrical conductivity as applied to *Anisakis* larvae (7A and 7B) and to hake meat (7C). Also, the estimated field strength was consistently higher in the nematode (7B) than in fish meat (7C) when comparing the same PEF treatment condition. Comparing the results in Fig. 7B and C, the maximum estimated field strength reached in the fish would be 4.9 kV/cm when selecting 5 kV/cm in the PEF system (Fig. 7C), whereas in the nematodes, it would be of 8.1 kV/cm (Fig. 7B) for the highest intensity (5 kV/cm, 10 mS/cm).

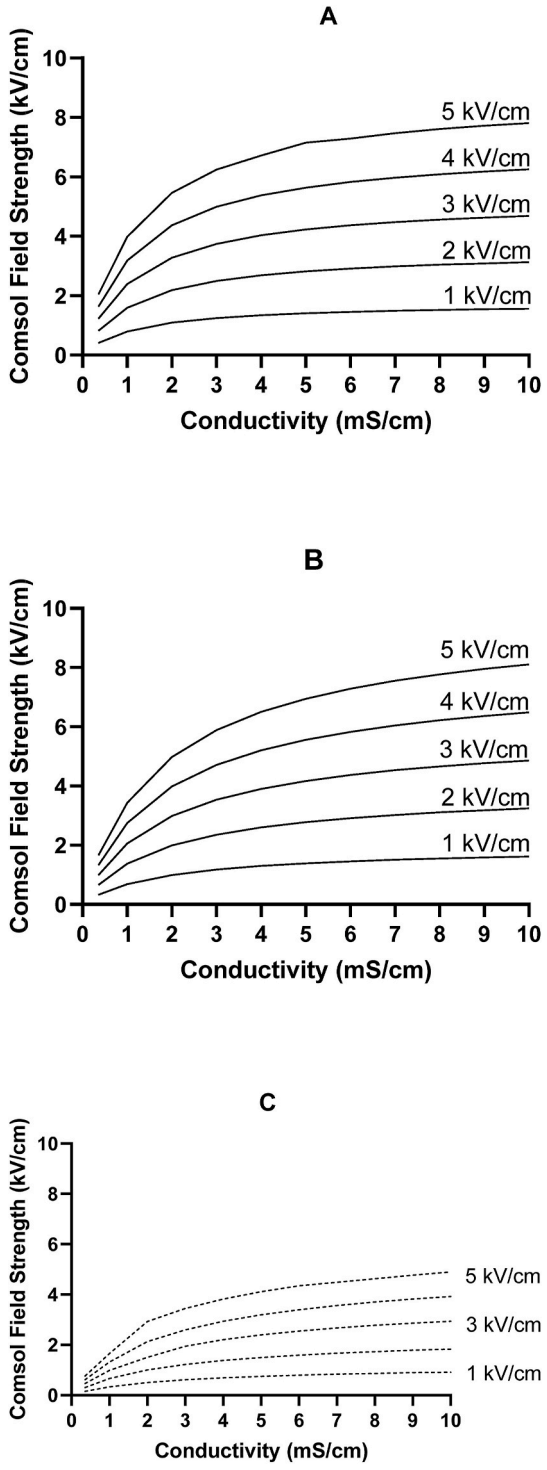

Fig. 7. Electric field strength estimated by numerical simulation of *Anisakis* nematodes (7A and 7B) and hake (7C) when treating in saline solution (7A) or in fish meat (7B and 7C) while selecting different theoretical field strengths (1–5 kV/cm) and varying the electrical conductivity of the treatment medium.

Based on the estimations shown in Fig. 7A and applying multiple regression, Equation (6) was obtained (Table 3) that permitted to estimate the electric field strength to which *Anisakis* would be exposed based on numerical simulation if PEF treatments of different selected field strengths (1–5 kV/cm) were applied in saline media of varying electrical conductivities (0.4–10 mS/cm):

$$\textit{Anisakis}\text{ electric field strength} = -0.9041 + 0.5622{*}\sigma + 0.7754{*}E + 0.0971{*}\sigma{*}E - 0.0551{*}\sigma^2 \quad \text{(Equation 6)}$$

where E is the selected field strength, and σ the electrical conductivity.

Similarly, using the estimations shown in Fig. 7B and by applying multiple regression, Equation (7) was obtained (Table 4), enabling to estimate the electric field strength received by *Anisakis* in hake belly when applying PEF treatments in media of different electrical conductivities (σ) and electric field strengths selected in the PEF system (E):

$$\textit{Anisakis}\text{ electric field strength} = -0.8869 + 0.5539{*}\sigma + 0.6605{*}E + 0.1148{*}\sigma{*}E - 0.430{*}\sigma^2 \quad \text{(Equation 7)}$$

The fits were good both for equations, based on the calculated R^2, R^2-adjusted, and RMSE. Using these two equations, Fig. 2 was rebuilt into Fig. 8, now including the field strength estimated with Equations (6) and (7), depending on the electrical conductivity of the media and the selected field strength in the PEF system, that would be achieved in *Anisakis* when treated in saline solution (circles) and in fish meat (squares). Fig. 8 shows that PEF treatments would achieve a higher degree of inactivation if the parasites were treated directly in the saline solution. However, in Fig. 2, the observed difference in inactivation between saline solution and fish was 30–40 %, whereas in Fig. 8, it is only 20 ± 5 %. This could indicate that the pulsed electric fields received by the *Anisakis* nematodes would indeed have an effect but that the matrix would exert a certain effect as well. Specifically, the manner in which the nematodes are encased in the fish tissue (encysted, wrapped in a cuticle, etc.) would offer a certain extent of additional protection against inactivation. Thus, even when nematodes are receiving the same treatment, the degree of inactivation in water is more pronounced. This

Table 3
Polynomial equation (Equation (6)) describing the electric field strength received by *Anisakis* larvae in saline solution when applying PEF treatments in media of different electrical conductivities (σ) and electric field strengths as parameters selected in the PEF system (E). Statistical significance (p value) and the 95 % confidence limits (CL) of each parameter are included.

Anisakis electric field strength = $b_0 + b_1 \times \sigma + b_2 \times E + b_3 \times \sigma \times E + b_4 \times \sigma^2$
$R^2 = 0.9734$; R^2 adjusted = 0.9713; RMSE = 0.3502

	Coefficient	p value	CL (-95 %)	CL (+95 %)
b_0	−0.9041	0.0004	−1.3843	−0.4238
b_1	0.5622	<0.0001	0.4203	0.7042
b_2	0.7754	<0.0001	0.6429	0.9079
b_3	0.0971	<0.0001	0.0747	0.1195
b_4	−0.0551	<0.0001	−0.0670	−0.0432

Table 4
Polynomial equation (Equation (7)) describing the electric field strength received by *Anisakis* larvae in hake belly when applying PEF treatments in media of different electrical conductivities (σ) and electric field strengths as parameters selected in the PEF system (E). Statistical significance (*p* value) and the 95 % confidence limits (CL) of each parameter are included.

Anisakis electric field strength = $b_0 + b_1 \times \sigma + b_2 \times E + b_3 \times \sigma \times E + b_4 \times \sigma^2$
$R^2 = 0.9794$; R^2 adjusted = 0.9777; RMSE = 0.3165

	Coefficient	*p* value	CL (-95 %)	CL (+95 %)
b_0	−0.8869	0.0001	−1.3210	−0.4529
b_1	0.5539	<0.0001	0.4256	0.6822
b_2	0.6605	<0.0001	0.5407	0.7803
b_3	0.1148	<0.0001	0.0946	0.1351
b_4	−0.5430	<0.0001	−0.0650	−0.0436

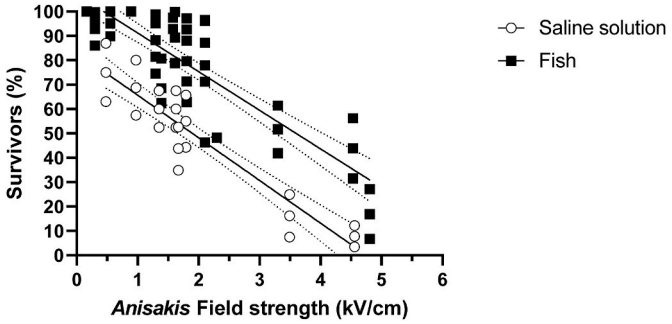

Fig. 8. Representations of survivor counts based on data shown in Fig. 2 but including the field strength theoretically achieved in *Anisakis* nematodes subjected to PEF treatments in saline solution and in fish meat, as estimated by Equations (6) and (7).

discrepancy found between experimental and simulated results highlights the need for further studies to explore the influence of these factors related to the fish matrix and parasite encystation on the larvae's resistance to PEF, besides simulating more complex scenarios including other electrical conductivities of the different parts of the parasite, fish, etc., as it has been indicated above.

3.3. Evaluation of fish microbiota after PEF treatments

To assess the effect of PEF treatment in saline media of differing electrical conductivities on the microbial quality of hake fillets, microbiological analyses were performed on both control and PEF-treated samples (0.4 mS/cm and 8 mS/cm) stored at 4 ± 0.5 °C under modified atmosphere packaging (MAP) consisting of 50 % CO_2 and 50 % N_2. The microbial groups evaluated included Aerobic Psychrotrophes (Fig. 9A), Anaerobic Psychrotrophes (Fig. 9B), *Pseudomonas* (Fig. 9C), *Shewanella* (Fig. 9D), lactic acid bacteria (Fig. 9E), and Enterobacteriaceae (Fig. 9F). The microbial evolution observed in the present study showed similar trends across all experimental conditions, with no noticeable differences between control samples and those subjected to PEF treatment, regardless of the conductivity of the saline medium used. Aerobic and Anaerobic Psychrotrophes exhibited similar growth patterns, characterized by a progressive increase throughout the storage period. *Pseudomonas* populations remained practically stable throughout storage, which is attributed to their strict aerobic metabolism and the inhibitory effect of the oxygen-depleted atmosphere. *Shewanella* and lactic acid bacteria demonstrated moderate growth, while Enterobacteriaceae populations were effectively controlled under the applied MAP storage conditions. All these results demonstrate microbial growth patterns and counts comparable to those reported by Abad et al. (2024) and in some cases, slightly lower than those reported by Antunes-Rohling et al. (2019).

Overall, the microbial dynamics observed in this work closely aligned with those described in previous studies, and no significant influence of the saline medium's electrical conductivity was detected on microbial evolution following PEF treatment. These results suggest that electrical conductivity is not a critical factor influencing microbial growth kinetics under the evaluated conditions and in extension with the quality properties of the treated fish. Additionally, the sensorial evaluation performed by trained personnel of a fishery company collaborating in this work (Scanfisk Seafood S.L.) was unable to distinguish PEF treated and un-treated hake fillets**.** These findings would indicate that the application of PEF under the tested conditions did not produce detectable changes in the organoleptic properties of the product, supporting the suitability of this technology for preserving fish quality without altering its sensory characteristics. However, more specific sensorial analysis should be done to evaluate this aspect in more detail.

4. Conclusions

The electrical conductivity of the treatment medium resulted a crucial factor in the effectiveness of PEF treatments designed to inactivate *Anisakis*. Whether the larvae were suspended in saline solution or embedded in naturally parasitized hake belly fillets made a difference. The higher the electrical conductivity, the greater the inactivation achieved with the same PEF treatment in saline solution or in meat fish. The lethality of PEF was reduced when nematodes were located inside the fish compared to water. For the first time, it has been developed an equation that would permit researchers to determine the survivability of *Anisakis* in hake belly (which is a worst-case scenario, as this portion of hake is extremely parasitized) after PEF treatments, taking the effect of the electrical conductivity of the treatment medium into account. This equation incorporates the factor of electrical conductivity in addition to the standard factors habitually defined in a PEF treatment: electric field strength, specific energy, and pulse width.

Numerical simulations demonstrated that, for the same PEF treatment, increasing the electrical conductivity of the medium would tend to enhance the degree of inactivation of *Anisakis* nematodes, as the electric field strength to which they were subjected would be greater. This effect would be more noticeable when nematodes were inside the fish than in saline solution due to the differences between the electrical conductivity of the fish meat cells and that of the nematode's own cells. However, other factors could be affecting the larvae's PEF resistance when located in the fish in terms of matrix effect, parasite encystation, electrical properties of the parasite's tissues, etc., thus indicating that there is a need for further studies to explore the influence of those other factors.

CRediT authorship contribution statement

V. Abad: Writing – original draft, Validation, Software, Investigation. **A. Ruíz:** Investigation. **J. Grasa:** Writing – review & editing, Software, Data curation. **B. Calvo:** Writing – review & editing, Formal analysis. **N. Escursell:** Project administration. **T. Peiro:** Validation. **J. Raso:** Supervision, Investigation. **G. Cebrián:** Supervision, Methodology. **I. Álvarez-Lanzarote:** Writing – review & editing, Visualization, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ignacio Alvarez-Lanzarote reports financial support was provided by Departamento de Ciencia, Universidad y Sociedad del Conocimiento and Fondo Social Europeo-Gobierno de Aragón. Ignacio Alvarez-Lanzarote reports financial support was provided by Cátedra SAMCA de Desarrollo Tecnológico de Aragón. Vanesa Abad reports financial support was

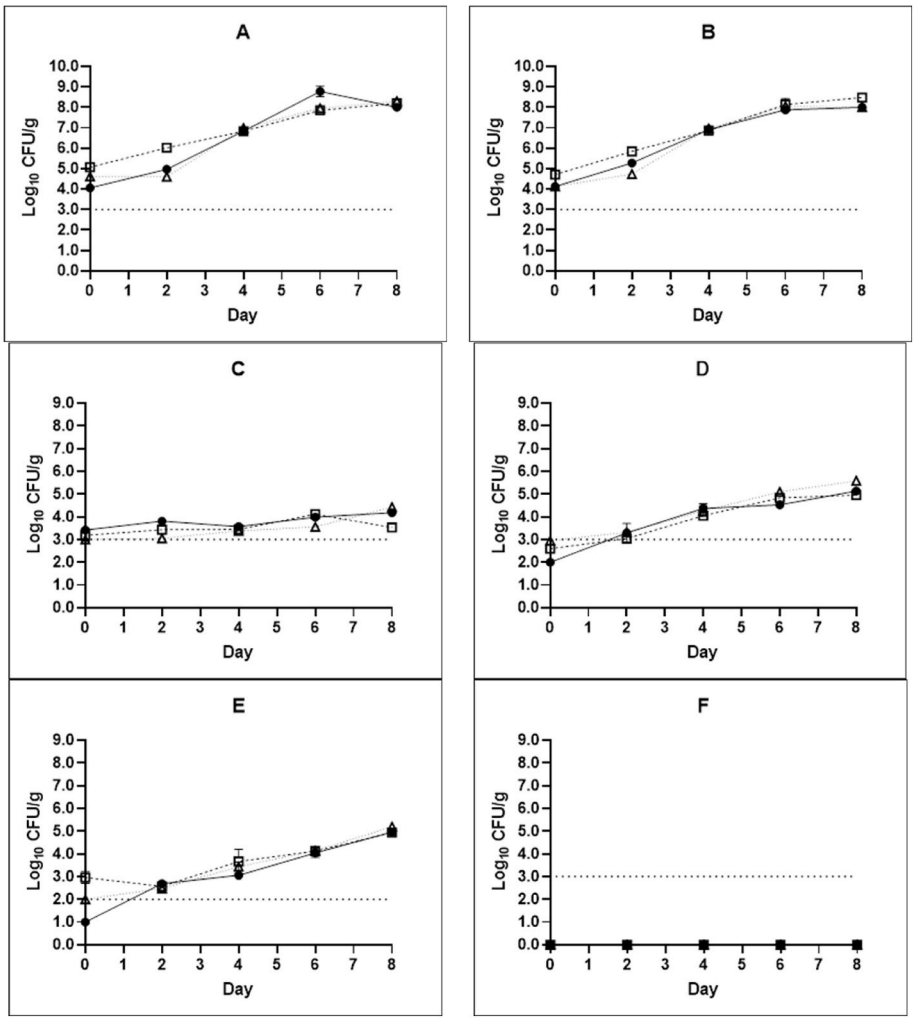

Fig. 9. Growth curves of Aerobic Psychrotrophes (9A), Anaerobic Psychrotrophes (9B), *Pseudomonas* (9C), *Shewanella* (9D), Lactic Acid Bacteria (9E) and Enterobacteriaceae (9F) in control (●), and PEF samples (3 kV/cm, 20 kJ/kg, and 7 μs, with electrical conductivity of saline solution: 0.4 mS/cm (□) and 8 mS/cm (△)) stored under MAP (50 % CO_2 – 50 % N_2) at 4 °C.

provided by Gobierno de Aragón. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Abad, V., Alejandre, M., Hernández-Fernández, E., Raso, J., Cebrián, G., & Álvarez-Lanzarote, I. (2023). Evaluation of pulsed electric fields (PEF) parameters in the inactivation of *Anisakis* larvae in saline solution and hake meat. *Foods, 12*(2), 264. https://doi.org/10.3390/FOODS12020264, 2023, Vol. 12, Page 264.

Abad, V., Martínez, J. M., Mañas, M. P., Raso, J., Cebrián, G., & Álvarez-Lanzarote, I. (2024). Inactivation by pulsed electric fields of *Anisakis* in naturally infected hake meat. *Lebensmittel-Wissenschaft und -Technologie, 208*, Article 116714. https://doi.org/10.1016/j.lwt.2024.116714

Aibinu, I. E., Smooker, P. M., & Lopata, A. L. (2019). *Anisakis* nematodes in fish and Shellfish- from infection to allergies. *International Journal for Parasitology. Parasites and Wildlife, 9*, 384–393. https://doi.org/10.1016/J.IJPPAW.2019.04.007

Ángeles-Hernández, J. C., Gómez-De Anda, F. R., Reyes-Rodríguez, N. E., Vega-Sánchez, V., García-Reyna, P. B., Campos-Montiel, R. G., Calderón-Apodaca, N. L., Salgado-Miranda, C., & Zepeda-Velázquez, A. P. (2020). Genera and species of the anisakidae family and their geographical distribution. *Animals: An Open Access Journal from MDPI, 10*(12), 1–23. https://doi.org/10.3390/ANI10122374

Antunes-Rohling, A., Calero, S., Halaihel, N., Marquina, P., Raso, J., Calanche, J., Beltrán, J. A., Álvarez, I., & Cebrián, G. (2019). Characterization of the spoilage microbiota of hake fillets packaged under a modified atmosphere (MAP) rich in CO_2 (50% CO_2/50% N_2) and stored at different temperatures. *Foods, 8*(10). https://doi.org/10.3390/FOODS8100489

Astráin-Redín, L., Raso, J., Álvarez, I., Kirkhus, B., Meisland, A., Borge, G. I. A., & Cebrián, G. (2023). New pulsed electric fields approach to improve the blanching of carrots. *Lebensmittel-Wissenschaft und -Technologie, 189*, Article 115468. https://doi.org/10.1016/j.lwt.2023.115468

Baranyi, J., Pin, C., & Ross, T. (1999). Validating and comparing predictive models. *International Journal of Food Microbiology, 48*(3), 159–166. https://doi.org/10.1016/S0168-1605(99)00035-5

Broekaert, K., Heyndrickx, M., Herman, L., Devlieghere, F., & Vlaemynck, G. (2011). Seafood quality analysis: Molecular identification of dominant microbiota after ice storage on several general growth media. *Food Microbiology, 28*(6), 1162–1169. https://doi.org/10.1016/J.FM.2011.03.009

Chai, J. Y., Murrell, K. D., & Lymbery, A. J. (2005). Fish-borne parasitic zoonoses: Status and issues. *International Journal for Parasitology, 35*(11–12), 1233–1254. https://doi.org/10.1016/J.IJPARA.2005.07.013

Chen, Y., Llave, Y., Jiao, Y., Okazaki, E., Sakai, N., & Fukuoka, M. (2022). Ohmic tempering using a high frequency ohmic heating and model food of minced tuna based on allaska pollock surimi – Evaluation of electrical conductivities. *Innovative Food Science and Emerging Technologies, 76*. https://doi.org/10.1016/j.ifset.2022.102940

Choudhury, G. S., Jenks, W. G., Wikswo, J. P., & Bublitz, C. G. (2002). Effects of parasite attributes and injected current parameters on electromagnetic detection of parasites in fish muscle. *Journal of Food Science, 67*(9), 3381–3387. https://doi.org/10.1111/j.1365-2621.2002.tb09594.x

Daschner, A., Alonso-Gómez, A., Cabañas, R., Suarez-de-Parga, J.-M., & López-Serrano, M.-C. (2000). Gastroallergic anisakiasis: Borderline between food allergy and parasitic disease—Clinical and allergologic evaluation of 20 patients with confirmed acute parasitism by *Anisakis simplex*. *Journal of Allergy and Clinical Immunology, 105*(1), 176–181. https://doi.org/10.1016/S0091-6749(00)90194-5

ELIKA. (2023). Anisakis. Retrieved from https://seguridadalimentaria.elika.eus/fichas-de-peligros/anisakis/. (Accessed 12 June 2025).

European Food Safety Authority (EFSA). (2010). Scientific opinion on risk assessment of parasites in fishery products. *EFSA Journal, 8*(4). https://doi.org/10.2903/j.efsa.2010.1543

Grabda, J. (1976). Studies on the life cycle and morphogenesis of *Anisakis* simplex (Rudolphi, 1809) (Nematoda: Anisakidae) cultured in vitro. *Acta Ichthyologica et Piscatoria, 6*(1), 119–141. https://doi.org/10.3750/AIP1976.06.1.08

Herrador, Z., Daschner, Á., Perteguer, M. J., & Benito, A. (2019). Epidemiological scenario of anisakidosis in Spain based on associated hospitalizations: The tip of the iceberg. *Clinical Infectious Diseases, 69*(1), 69–76. https://doi.org/10.1093/cid/ciy853

Hochberg, N. S., & Hamer, D. H. (2010). Anisakidosis: Perils of the deep. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America, 51*(7), 806–812. https://doi.org/10.1086/656238

Lanfranchi, A. L., & Sardella, N. H. (2010). *Anisakids* survival after microwaving, freezing and salting fish from Argentina. *Food Science and Technology Research, 16*(5), 499–504. https://doi.org/10.3136/fstr.16.499

Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science, 91*(2), 93–98. https://doi.org/10.1016/J.MEATSCI.2012.01.013

Moya, J., Astráin-Redín, L., Grasa, J., Cebrián, G., Calvo, B., & Álvarez, I. (2022). A numerical approach to analyze the performance of a PEF-Ohmic heating system in microbial inactivation of solid food. *Frontiers in Food Science and Technology, 2*. https://doi.org/10.3389/frfst.2022.880688

Oh, S.-R., Zhang, C.-Y., Kim, T.-I., Hong, S.-J., Ju, I.-S., Lee, S.-H., Kim, S.-H., Cho, J.-I., & Ha, S.-D. (2014). Inactivation of *Anisakis* larvae in salt-fermented squid and pollock tripe by freezing, salting, and combined treatment with chlorine and ultrasound. *Food Control, 40*, 46–49. https://doi.org/10.1016/j.foodcont.2013.11.023

Onitsuka, C., Nakamura, K., Wang, D., Matsuda, M., Tanaka, R., Inoue, Y., Kuroda, R., Noda, T., Negoro, K., Negoro, T., & Namihira, T. (2022). Inactivation of *Anisakis* larva using pulsed power technology and quality evaluation of horse mackerel meat treated with pulsed power. *Fisheries Science, 88*(2), 337–344. https://doi.org/10.1007/S12562-022-01593-2/FIGURES/7

Onitsuka, C., Nakamura, K., Wang, D., Matsuda, M., Tanaka, R., Inoue, Y., & Namihira, T. (2024). Dependence of *anisakid* larva inactivation by pulsed power on various parameters. *Journal of Food Engineering, 360*, Article 111715. https://doi.org/10.1016/j.jfoodeng.2023.111715

Pascual, S., Antonio, J., Cabo, M. L., & Piñeiro, C. (2010). *Anisakis* survival in refrigerated fish products under CO_2 modified-atmosphere. *Food Control, 21*(9), 1254–1256. https://doi.org/10.1016/j.foodcont.2010.03.002

Raso, J., Heinz, V., Alvarez, I., & Toepfl, S. (Eds.). (2022). *Pulsed electric fields technology for the food industry*. Springer International Publishing. https://doi.org/10.1007/978-3-030-70586-2.

Smith, J. W. (1983). *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) (Nematoda: Ascaridoidea): Morphology and morphometry of larvae from euphausiids and fish, and a review of the life-history and ecology. *Journal of Helminthology, 57*(3), 205–224. https://doi.org/10.1017/S0022149X00009512

Sugiyama, H., Shiroyama, M., Yamamoto, I., Ishikawa, T., & Morishima, Y. (2022). Anisakiasis annual incidence and causative species, Japan, 2018–2019. *Emerging Infectious Diseases, 28*(10), 2105–2108. https://doi.org/10.3201/eid2810.220627

Zhang, Q., Barbosa-Cánovas, G. V., & Swanson, B. G. (1995). Engineering aspects of pulsed electric field pasteurization. *Journal of Food Engineering, 25*(2), 261–281. https://doi.org/10.1016/0260-8774(94)00030-D

Gago, L., García, E., Fernández, J. L., & González, J. M. (2007). Detection and Inactivation Methods of Anisakis simplex and Diseases that this parasite produces. Biotechnology Innovation Circle. https://Pesca.Elika.Eus/Metodos-Para-La-Deteccion-e-Inactivacion-de-Anisakis-Simplex-2007/. (Accessed 17 June 2025).