Inactivation of the parasite *Anisakis* by Pulsed Electric Fields (PEF)

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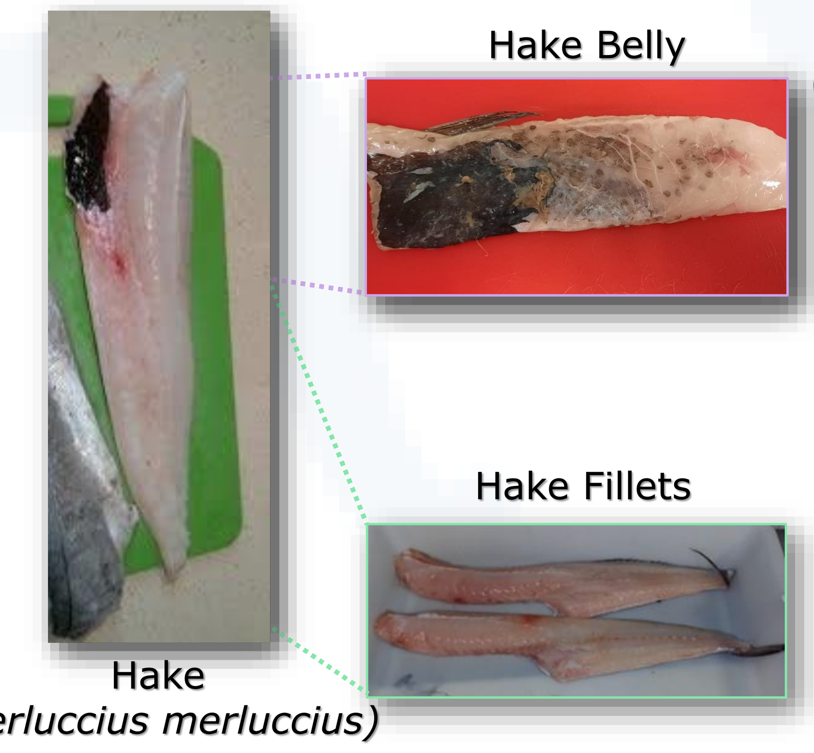
1 INTRODUCTION

Larvae of the nematode family *Anisakidae* are capable of causing zoonotic parasitic infections in humans associated with the consumption of fishery products, leading to intestinal syndromes and allergic reactions[1]. *Anisakidae* larvae are widely distributed geographically with rates of parasitism close to 100% in species like hake [2]. Currently Regulation (EC) No. 2074/2005 requires freezing fishery products to be consumed raw or undercooked [3]. This technology affects the quality of the meat because ice crystals formed cause dripping and softening of the meat when thawed [4]. Due to this, new strategies are required to inactivate the parasite without affecting fresh fish quality. Pulsed Electric Fields (PEF) could be a possibility.

2 OBJECTIVE

Evaluate PEF technology as a possibility to inactivate *Anisakis* in hake fillets and to determine the impact of PEF in hake quality.

3 MATERIALS & METHODS



***Anisakis* spp. inactivation** → **Extraction of *Anisakis* from hake** →

- ***Anisakis* inactivation in SALINE SOLUTION** — **10 *Anisakis* in the treatment chamber with saline solution (1 mS/cm)**
- ***Anisakis* inactivation in HAKE (VALIDATION)** — **Artificial parasitation of hake: 10 *Anisakis* in a piece (4.5 cm x 2.5 cm) of hake**

PEF treatment ranges:
- Field strength: 1 to 3 kV/cm
- Pulse width: 3 to 100 µs
- Specific energy: 3 to 50 kJ/kg

PEF Generator: EPULSUS-PM-10, 2 kW, Energy Pulse System, Lisbon, Portugal

Quality tests: Moisture; Water holding capacity; Cook-loss

Samples: Hake fillets: Control sample 4 ºC (fresh hake); PEF sample (3 kV/cm; 50 kJ/kg; 30 µs); Freeze 5 days -18 °C and thawed

4 RESULTS

Anisakis inactivation

Figure 1 shows how *Anisakis* inactivation increases with the field strength, and the specific energy. Pulse width only affected at low field strengths. A central composite design (CCD) (**Table 1**) was defined to investigate the effects of electric field strength, specific energy, and pulse width on the viability of *Anisakis* after PEF treatments. With the obtained results, a mathematical equation was developed describing the *Anisakis* lethality of PEF treated in aqueous solution:

$$S(\%)=59.29+1.900*W+0.515*P+8.388*E^2-2.210*E*W-0.482*E*P-0.02415*W*P+0.01429*E*W^2+0.01427*E*W*P;$$
$$(R^2=0.995;R^2adjusted=0.989;RMSE=1.917)$$

The equation allows predictions of inactivation of this parasite according to the treatment applied within the ranges of the equation (**Figure 3**). Predictions of the equation were validated in hake pieces artificially parasitized with *Anisakis*. A PEF treatment of 3 kV/cm, and 40-50 kJ/kg applying square wave pulses of 30 µs inactivated 90-100% of the parasites present in the fish pieces (**Figure 4**).

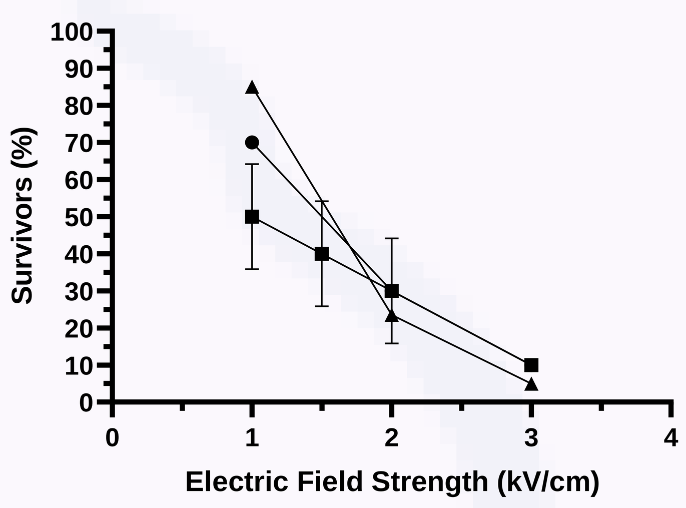


Figure 1. Influence of the electric field strength on the percentage of survivors after PEF treatments of different widths and specific energies: 40 kJ/kg and 3 µs (●), 50 kJ/kg and 3 µs (▲), 50 kJ/kg and 50 µs (■).

Table 1. Central Composite Design evaluating the survivability of *Anisakis* L3 larvae after 24 hours of PEF treatments of distinct field strength, pulse width and specific energy.

Field Strength (E)(kV/cm)	Specific Energy (W)(kJ/kg)	Pulse Width (P)(µs)	Survivability (%)
1	9	3	65
1	9	100	60
1	30	50	60
1	50	3	85
1	50	100	40
2	9	50	50
2	30	3	40
2	30	50	30
2	30	50	30
2	30	100	10
2	50	50	30
3	9	3	95
3	9	100	20
3	30	50	10
3	50	3	5
3	50	100	5

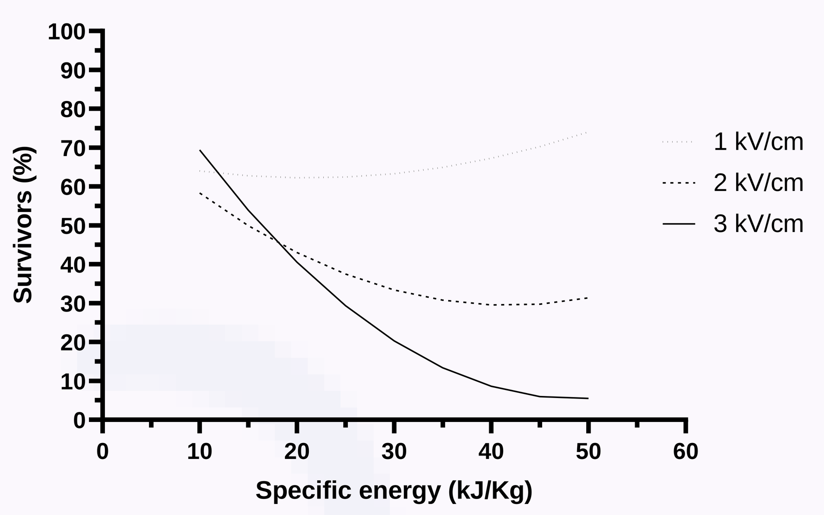


Figure 2. Influence of the specific energy and the electric field strengths on the estimated survivability of *Anisakis* larvae L3 when applying pulses of 30 µs in saline solution.

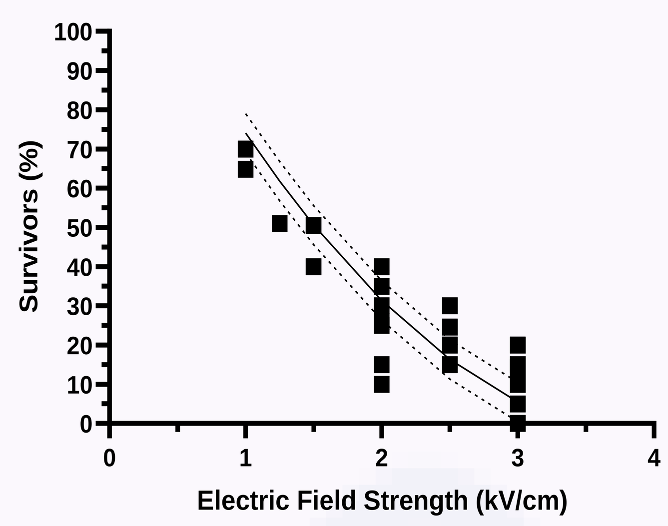


Figure 3. Survivability of *Anisakis* L3 larvae after applying 30 µs-pulses of 50 kJ/kg at different field strengths (square points) to pieces of hake meat and that estimated with the equation obtained in water solution (continued lines; the dotted lines correspond to the 95% confidence intervals).

Quality tests

Figure 4 shows the results for moisture, water holding capacity and cook-loss for control, PEF and frozen/thawed samples. Evaluated quality parameters were not significantly affected by PEF compared to control samples and resulted better than that of frozen/thawed samples.

The tested quality parameters indicated that PEF, as a treatment technology, could be a promising alternative to freezing because PEF-treated samples would be of superior quality than frozen samples.

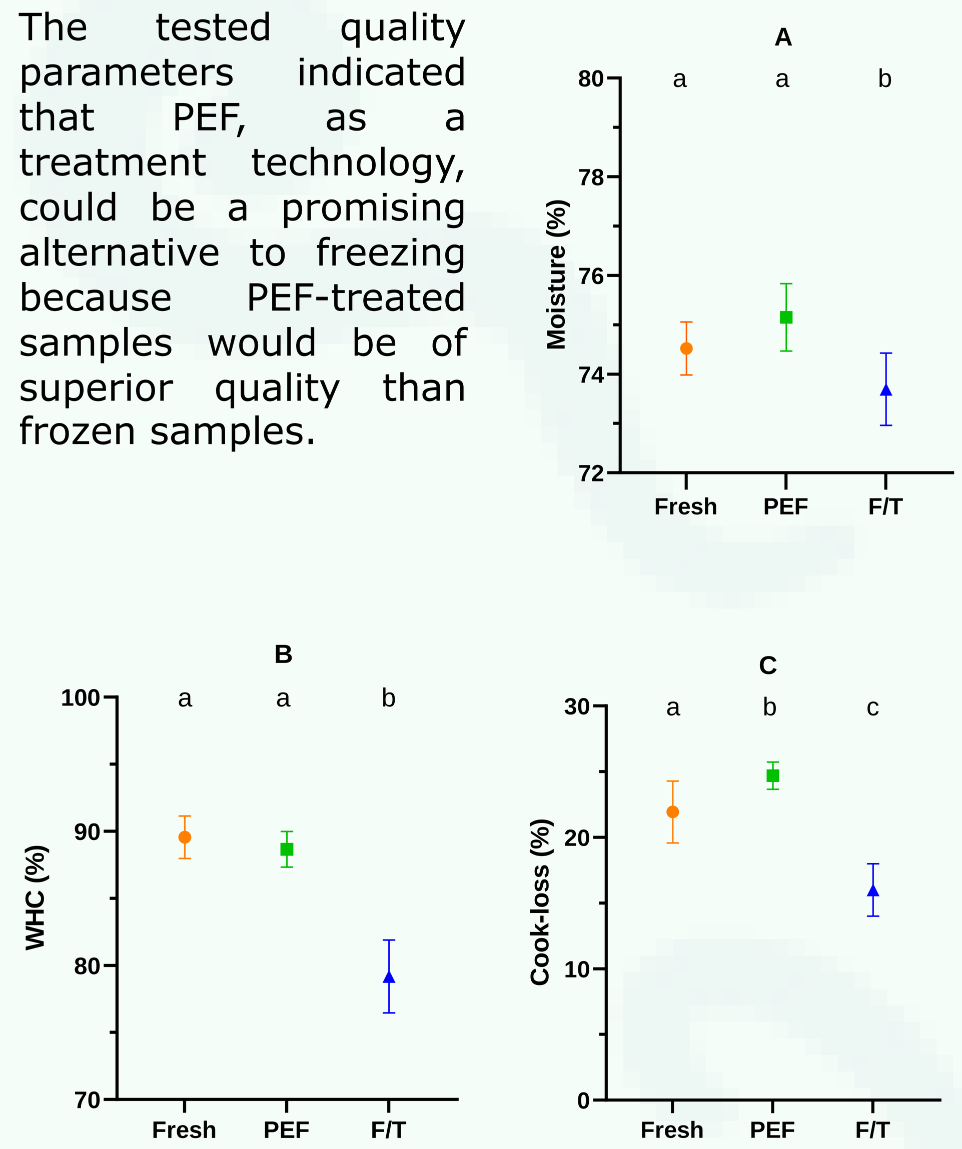


Figure 4. Moisture (4A), water holding capacity (4B) and cook-loss (4C) of control (●), PEF-treated (3 kV/cm, 50 kJ/kg and 30 µs) (■) and frozen/thawed (F/T) hake fillet samples (▲). Different letters indicate statistically significant differences among treatments (p=0.05).

5 CONCLUSION

The lethality of *Anisakis* spp. was highly dependent on the PEF applied parameters, mainly field strength and specific energy. Quality parameters indicate that PEF could be a technological alternative to freezing as it does not affect the quality of the fish.

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