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Pulsed Electric Fields (PEF) applications in the inactivation of parasites in food

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ABSTRACT

Background: Parasites are concerning food-borne pathogens. Some of them are currently not being routinely controlled in food, probably because their burden on public health is underestimated and the relative importance of different transmission routes is not completely known. Parasitic incidences could be avoided if preventive technologies were applied during food processing. Effective inactivation treatments are currently based on heat or freezing, but their side effects collide head-on with current consumer trends and new culinary habits.

Scope and approach: This review describes the potential application of Pulsed Electric Field (PEF) technology in the control of food-borne parasites, with the aim of reducing the viability and infectivity of parasite transmission stages without affecting food quality. Results of published studies performed on different media are critically analyzed and factors affecting the outcomes are examined.

Key findings and conclusions: Recent studies on the topic demonstrate the feasibility of PEF as an alternative to traditional freezing processes for the inactivation of *Anisakis* in fish. The development of new PEF equipment is advancing at a rapid pace, allowing for food treatment at a scale that would have been unimaginable some years ago. A review of more basic-science studies carried out on buffer media would contribute to progress in addressing the underlying drawbacks that remain to be solved. Thoroughly different fields (parasitology, physics, food engineering, water sanitation, etc.) should converge to achieve the industrial implementation of PEF for the inactivation of food-borne parasites.

1. Zoonotic parasites infecting humans through food consumption

Parasites are organisms that derive nourishment and protection from other living organisms known as hosts; they are occasionally found in food (EFSA, 2022). Although foodborne parasites (FBPs) are becoming increasingly recognized as important foodborne pathogens, they remain relatively neglected in comparison with bacterial and viral foodborne pathogens (Trevisan et al., 2019). Despite recent research advances, FBPs remain an important concern for public health in the 21st century (Torgeson et al., 2011; Mangen et al., 2015). Trevisan et al. (2019) described the importance of five **growing trends** regarding FBPs: globalization of the food supply, changing culinary habits and human behavior towards raw food, gaps in surveillance and control, the role of water, and lack of awareness on the part of public agencies. It is known that foodborne disease outbreaks today are less limited geographically; **worldwide outbreaks** are becoming more frequent (Robertson et al., 2014). The introduction of new, exotic FBPs to unprotected populations and to regions where health professionals are unfamiliar with them has posed serious challenges to diagnosticians (Kuchta et al., 2021; Liu et al., 2020). Consumption of raw and undercooked foods is increasing, and with it the opportunities for exposure to FBPs (Robertson et al., 2014; EFSA BIOHAZ, 2018). New **culinary trends** such as raw (sushi, sashimi), undercooked, pickled (anchovies marinated in vinegar in Spain) or smoked fish meat, and a growing consumption of crustaceans have resulted in increased transmission of a number of nematodes (i.e., *Anisakis simplex, Pseudoterranova* spp.), cestodes (*Diphyllobothrium* spp.), trematodes (*Paragonimus* spp., *Clonorchis* spp., *Opisthorchis* spp.), Myxozoa (f.e. *Kudoa, Unicapsula*), and Acanthocephala (f.e. *Bolbosoma, Corynosoma*) (Shamsi, 2019; Trevisan et al., 2019). Particularly in the case of seafood, the presence of parasites is an enormous problem because such foods are increasingly being consumed raw or only mildly

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processed, even in Europe (Kulawik et al., 2022). Regarding meat production, an increasing concern for **organic food** and animal welfare can be noted in European societies. The growing trend towards raising animals outdoors entails a greater possibility of infection with pathogens such as *Toxoplasma gondii*, the reintroduction of *Trichinella* spp., or even *Taenia* spp. (Meester et al., 2019; Noeckler et al., 2019). Since less time is allocated to meal preparation, the general trend increasingly tends toward fast food including ready-to-eat salads, another potential source of FBPs (Robertson, 2018). On the other hand, although public health **surveillance** is a pre-requisite for control, EU-level regulations only exist for certain FBPs such as *Trichinella* spp. and *Taenia* spp. For other FBPs, as is the case of *T. gondii*, surveillance systems have not been established and substantial gaps are evident: no country or regulatory authority requires meat inspection for this parasite. The ubiquity of *T. gondii* and the lack of simple detection methods in the slaughterhouse probably explain why the extent of its occurrence in meat products is not completely known (Belluco et al., 2016; Dámek et al., 2023). In addition to food itself, production water is also a source of parasite infection (irrigation, washing and processing of fresh produce). Changes in food production practices and exponential population growth lead to the re-utilization of wastewater, thereby providing a greater number of possibilities for contamination with parasites transmitted via fecal-oral route such as *Cryptosporidium* spp. and *Giardia* spp. (Robertson, 2018). Sanitizers are used in vegetable wash-water with the frequent intention of reducing bacterial contamination, but they are probably ineffective against FBPs (Gil et al., 2009). Assessing the extent to which water may lead to contamination of food with FBPs is worth investigating, in order to implement suitably targeted new treatments. Furthermore, parasitic diseases are often chronic, with **long-term sequelae**. Human echinococcosis, for example, results in morbidity and mortality several years, or sometimes decades, after infection (Torgerson & Macpherson, 2011). The cystic variety of this disease remains a substantial burden in certain EU countries such as Bulgaria or Germany, which accounted in both cases for 32% of reported *E. granulosus* cases in the EU (Casulli et al., 2022; EFSA, 2021).

The FBPs for which it would be worth applying technologies to reduce infectivity along the food chain are those that are currently not being routinely controlled and thus food is not inspected and discarded if the parasite is present. Additionally, some zoonotic diseases such as trichinellosis today remain responsible for costly inspection regimens. In a recent scientific opinion, EFSA identified the three potential FBPs of importance that are not regularly controlled and whose occurrence in food is not notifiable in most of EU member states: *Cryptosporidium* spp., *T. gondii*, and *Echinococcus* spp (EFSA BIOHAZ, 2018). Their complex life cycles, varied transmission routes, and prolonged incubation periods to trigger clinical signs after infection all entail that the public health burden is often difficult to assess. In our opinion, the processing of food to prevent transmission of FBPs should focus, apart from the macroscopic pathogen *Anisakis*, on the three above-mentioned parasites. However, the challenges involved in detection, the uncertainty regarding these parasites' occurrence, and the inexistence of standard methods to assess infectivity still continue to pose obstacles to the development of technological barriers against their transmission.

2. Determining parasite viability *in vitro* and *in vivo* (infectivity assessment)

Difficulties in detecting viable parasites in food have hampered potential progress in tackling them, and have probably somewhat downplayed their perceived importance (Trevisan et al., 2019). To arrive at implementing control measures along the food chain, two major issues need to be addressed: **(1) identifying appropriate methods for evaluating parasite viability/infectivity and (2) taking into account the different infective stages along the life cycle**. For example, not only bradyzoites present in meat and tachyzoites in dairy products, but also oocysts released with feces and contaminating fresh vegetables, marine products, or processing water can transmit *T. gondii* via food (Marín-García et al., 2022). This means that before developing technologies designed to inactivate a particular parasite stage in food, a proper methodology for the assessment of that parasite's infectivity and its occurrence in the matrixes needs to be conceived. When FBPs are detected in food, the question arises whether they are capable of causing infection. For some, such as *Anisakis* larvae, clearly discernible traits (motility) indicate viability of infective stages (Klapper et al., 2021). However, it is more difficult to determine the viability of parasites in infective stages that are not motile (oocyst, muscle cysts). Methods rely on *in vivo* animal models that are slow, expensive, and ethically controversial. For example, none of the current methodologies is ideal for a reliable assessment of the viability of protozoa (*Giardia, Cryptosporidium,* and *Toxoplasma*) contaminating fresh products (Rousseau et al., 2018). Whereas degrees of inactivation for most other foodborne pathogens, viruses, and bacteria are often quantified by log reduction, this is less clear for parasites due to variations in terms of units of infection (Franssen et al., 2019). On the other hand, as FBPs do not replicate during food storage, a two or three log reduction, which would be inadequate for bacteria, may be sufficient for FBPs (Franssen et al., 2019; Gerard et al., 2019).

3. Processing methods for the inactivation of parasites in food

Although on-farm measures that reduce the likelihood of contamination can be a more direct control method than post-harvest interventions, **food processing is a key step**. Traditional preservation methods (i.e., smoking, fermenting, drying, and freezing) or cooking may have different effects on the survival of parasites in meat and fish, whereas other novel methods (such as high-pressure processing or ionizing radiation processes) are variable in terms of effectiveness (Franssen et al., 2019). Heat treatments, such as pasteurization (juice and milk), adequately steaming shellfish, and cooking meat and fish thoroughly are generally sufficient for the inactivation of parasite transmission stages. Conversely, the long freezing of meat inactivates *T. gondii* tissue cysts, but may be not sufficient to inactivate fecal contaminant transmission stages on fresh products. These parasites, which are associated with fresh produce (helminth eggs/larvae/metacercaria and protozoan cysts/oocysts), are particularly resistant to inactivation by freezing. Furthermore, temperature-based treatments may substantially affect sensory qualities and therefore tend to be avoided in fresh vegetables, which are the fresh produce most commonly contaminated with these parasite stages (Gerard et al., 2019). According to the EFSA review, the optimization of inactivation technologies for *Echinococcus*, *Cryptosporidium,* and *Toxoplasma* remains an unfulfilled need; studies in this field are thus recommended (EFSA BIOHAZ, 2018). As mentioned above, information on the survival of these parasites as contaminants in food is largely lacking, due to methodological limitations and absence of official control. However, the transmission stages of all three parasites are known to be very robust. ***Echinococcus multilocularis*** eggs, in particular, can survive heating up to 65 °C for 120 min and freezing at −18 °C for several months. Eggs can be detected in the wash water after intense washing of vegetables; the proportion of contaminating eggs removed by this process thus remains unknown (Federer et al., 2016). The physical conditions that are known to inactivate these eggs are relatively intense, and usually not suitable for the food item in question: heating of food for at least 3 h at 65 °C (Federer et al., 2015) or deep freezing at −80 °C for a minimum of 24 h (Eckert et al., 2001, pp. 238–247). Similarly, *Cryptosporidium* spp. and *T. gondii* oocysts survive in moist environments at ambient temperatures for many months; *T. gondii*, in particular, survives even for weeks at freezing temperatures. Molluscan shellfish are contaminated by these two parasites at occurrence rates of 20–40% (EFSA BIOHAZ, 2018). During processing, ***Cryptosporidium*** contamination in food may arise from poor quality water used in washing (of fresh produce) or in dilution (of fruit juice), or from cross-contamination among food items, or from water

used in processes such as cooling. For example, the water used to wash salad leaves destined for bagged packaging is a recirculating system that may promote the spread of oocysts. Inadequate dairy hygiene can fail to prevent fecal contamination of milk; meats may be exposed to contaminated water or cross-contamination during curing processes (Robertson & Huang, 2012). Preservation treatments for fruit juice include flash pasteurization, high hydrostatic pressure (HHP), UV-C, ozone, organic acids, H_2O_2 and even PEF (Deng & Cliver, 2001; Hanes et al., 2002; Kniel et al., 2003; Slifko et al., 2000). In the case of milk, pasteurization is an effective control measure and the hardening of milk ice cream (−20 °C for 24 h) resulted in unviable oocysts, as determined by vital dyes (Deng & Cliver, 1999; Harp et al., 1996). Regarding cured meat, *Cryptosporidium* might be inactivated by controlling salinity and maintaining low water activity, but these are assumptions from other investigations, as no viability or infectivity studies have been conducted on the cured meat matrix (Robertson & Huang, 2012). In beef muscle, oocysts have been rendered non-infectious after heating (60 °C for 45 s/75 °C for 20 s), indicating that adequate cooking would be an effective control measure (Moriarty et al., 2005). However, domestic cooking temperatures are usually uncontrolled, and the widespread habit of consuming rare meat, or *tartar* and *carpaccio* could increase the risk. Other novel technologies such as cold plasma (CP) have been studied in the inactivation of *C. parvum* oocysts (Craighead et al., 2020). Authors noted 0.8–2 $\log_{10}$ cycles of inactivation on coriander leaves after 0.5–3 min treatment. Although recognizing that CP can affect parasite viability, they added that the treatment's effect might be lower than that of HHP; further research is needed to adjust parameters. On the subject of ***Toxoplasma***, no control methods for their detection during meat inspection are currently available, although meat is reported to account for almost 60% of *T. gondii* infections in humans (WHO, 2014, pp. 1–15). Visual meat inspection cannot detect tissue cysts of infected animals, as they are normally only identifiable by microscopy. The correlation between serological testing of slaughtered animals and the presence of muscle cysts is high in pigs, sheep, and chickens, but routine tests are not carried out (Opsteegh et al., 2016). Tissue cysts were also described in 5% of seronegative pigs; the serological testing technique is thus not perfect. In any case, estimations indicate that seroprevalence can reach over 80% depending on the husbandry system (EFSA BIOHAZ, 2018). Estimates based on direct detection of *T. gondii* in meat in Europe indicated prevalence values of 2.2% for cattle and 8.7% for pigs (Belluco et al., 2016). Nevertheless, quantitative risk assessment in the Netherlands and Italy has shown that beef contributes 1.8 times more than pork consumption to human infections because it is frequently eaten raw (*carpaccio*, *steak tartare*, lightly cooked beefsteak …) (Belluco et al., 2018). A high prevalence in pork and a high risk in beef indicate that the inactivation of tissue cysts within meat products might be the only practical possibility. Several methods for the decontamination of meat containing bradyzoites have been studied (Franssen et al., 2019). Tissue cysts can be rendered nonviable by freezing meat at −12 °C for 2 days, as well as by applying ionizing radiation (effective doses varying from 0.4 to 0.7 kGy have been reported), and by HHP processing at 300 MPa or more (Lindsay et al., 2006). Gracia et al. (2020) evaluated the efficacy of HHP on the viability of bradyzoites in ham from pigs using bioassay in mice followed by qPCR. In raw ham, 100–400 MPa/1 min did not inactivate *T. gondii*, whereas 600 MPa/20 min was effective. In dry-cured ham, 600 MPa for 3 or 10 min were not effective and a 20-min treatment was required to render the bradyzoites non-infectious for mice. Adequate cooking is the primary control factor for prevention of *T. gondii* infection in meat consumption, although cooking times will vary with the thickness and type of meat. Overall, meat should be cooked thoroughly until the internal temperature has reached 67 °C (Mirza Alizadeh et al., 2018). Quantitative risk assessment models have reported that cooking temperature and bradyzoite concentration in muscle have the highest impact on the risk of transmission to humans (Condoleo et al., 2018). Microwave cooking is considered unreliable for the inactivation of viable tissue cysts because of hot and cold spots due to the physics of microwaves (Lundén & Uggla, 1992). It should be noted that apart from efficacy of the different methods, the consumer acceptance may be a problem because of effects on the color, texture, and taste of meat after treatments. In addition, the use of irradiation and HHP processing may be restricted by legislation and may incur high costs (Opsteegh et al., 2015). Salting, curing, and smoking can reduce the viability of tissue cysts, but domestic conditions vary too widely, and different types of products on which these methods have been applied are not completely safe (Dubey, 2021). Reports indicate that salt-cured meat reduces the risk of *T. gondii* infectivity despite variability in manufacturing parameters: risk reduction is associated with a more extended curing period (Pott et al., 2013). However, another study found viable *T. gondii* in dry-cured ham samples (Gomez-Samblas et al., 2015). Regarding oocyst contamination, no specific testing or control measures exist for the production of *T. gondii*-free fresh vegetables; likewise, there are no specific guidelines regarding safe depuration times for shellfish. Ware et al. (2010) evaluated the effect of UV exposure on *T. gondii* oocysts by three approaches: mouse bioassay (gold standard), an *in vitro* plaque assay, and RT-qPCR. The results from the animal bioassay showed that 1- and 3-$\log_{10}$ cycles of inactivation were achieved with 4 mJ/cm^2 and 10 mJ/cm^2 low-pressure UV-C lamps, respectively. Plaque assay results, but not RT-qPCR results, correlated well with *in vivo* bioassay, thus making them a promising alternative to mouse tests. These advances in viability assessment would facilitate further research in the area of inactivation technologies.

In addition to the three parasites mentioned by EFSA, ***Anisakis*** deserves attention as it is highly prevalent in fish, and is capable of generating intestinal syndromes and allergic reactions. Up to 80% of captures that reach the fish markets are infested with *Anisakis* (Aibinu et al., 2019). Regulation (EC) No. 2074/2005 establishes that fishery products destined to be consumed raw or virtually raw (including cold-smoked, salted, or pickled products) must be frozen for at least 24 h at a temperature of −20 °C or below, or for at least 15 h at a temperature of −35 °C or below. The disadvantage of freezing/thawing is the impact it has on fish quality because the ice crystals formed during freezing and storage cause dripping and softening of the meat when thawed (Nakazawa & Okazaki, 2020). In addition, freezing alone is not always sufficient to ensure inactivation (Podolska et al., 2019) and is very energy-consuming. HHP seems effective in the inactivation of *A. simplex*, although treatment times lasting 5 min or longer and pressures of at least 300 MPa are often required to obtain full inactivation (Kulawik et al., 2022), and such parameters would affect fish quality, especially if intended for sushi. New processing techniques are under research, such as microwaves, ohmic heating, ozone, ionizing radiation, and chemicals such as essential oils, apart from salt, acetic acid, and other marinades. Although these can be regarded as alternatives to traditional processes, they still affect the product's sensory quality in one way or another (Abad et al., 2023).

4. Pulsed Electric Fields (PEF) as a technology for parasite inactivation

This review describes the potential of **Pulsed Electric Fields (PEF)** technology for the inactivation of parasites in food. Although the use of PEF is widespread for the nonthermal inactivation of microorganisms in foods (Raso et al., 2022), this technology has hardly been applied for the inactivation of zoonotic parasites, apart from the two studies reported in this review (Abad et al., 2023; Onitsuka et al., 2022) and the recently published patent for paralyzing parasites by PEF in food (EP4039096A1). PEF treatments consist of subjecting a product placed between two electrodes, usually immersed in an aqueous solution, to high-intensity electric fields (between 0.5 and 30 kV/cm) by applying intermittent pulses of short duration (microseconds to milliseconds) without increasing the product's temperature. If the electric field strength is sufficiently intense, a phenomenon called electroporation occurs, which consists in the increment of the permeability of the

cytoplasmic membrane to the passage of ions and macromolecules (Kotnik et al., 2012). PEF is commercialized for fried potato processing and cold pasteurization of liquid foods such as fruit juices (Barba et al., 2015). Furthermore, several studies have already demonstrated the feasibility of PEF for a series of different applications in the food industry, such as extraction of compounds of interest (Martínez et al., 2020), as well as the improvement of drying, freezing, or osmotic dehydration (Nowosad et al., 2021). The International Organisation of Vine and Wine (OIV) has admitted the implementation of PEF to grapes with the aim of improving the extraction of polyphenols and other desirable substances (OIV-OENO 634–2020).

When electroporation occurs – and depending on treatment intensity – the cell can still be capable of resealing the pores (reversible electroporation), or, on the contrary, pores become permanent (i.e., irreversible electroporation), thereby leading to the inactivation of vegetative cells of bacteria and yeast (Martínez et al., 2016; Saldaña et al., 2009). Regardless of the medium or matrix, the objective of the studies described in the present review is to attain irreversible electroporation of cell membranes, thereby compromising parasite physiology to the extent that inactivation occurs.

4.1. PEF for the inactivation of parasite stages in water sanitation (protozoa trophozoites/cysts/oocysts and nematode eggs)

It is known that the matrix or environment where the parasite is processed can have a considerable influence on the outcome after the PEF treatment. However, the scarcity of literature on PEF parasite inactivation in food points to the need for analysing results in other fields such as water sanitation. The most significant results and the conditions applied are illustrated in Table 1. Haas & Aturaliye (1999a) characterized electroporation-assisted disinfection processes for inactivation of *Giardia* cysts and *Cryptosporidium* oocysts. They studied the viability of these protozoa alone and in the presence of free chlorine, combined chlorine, hydrogen peroxide, and potassium permanganate. Although the combination of an electrical and a chemical treatment produced a certain degree of inactivation, electroporation itself had only a minor effect on survival. The same authors (Haas & Aturaliye, 1999b) developed a kinetics model of electroporation-assisted chlorination of *Giardia muris*. PEF (3.2–7.5 kV/cm; 28–32 kJ/kg) allowed for the reduction of the chemical doses required for cyst inactivation, although the technology scarcely achieved inactivation on its own. According to the model, electroporation would allow for a reduction of the chlorine dose required to achieve a given level of inactivation (30–40% savings in chemicals). Vernhes et al. (2002) investigated the effects of PEF on the inactivation of the trophozoite stage of the potential pathogen parasite amoeba *Naegleria* spp. in batch and flow processes. A nonpathogenic species (*N. lovaniensis*) was used instead of the pathogenic *N. fowleri* for safety reasons. Massive destruction (i.e., viability lower than 5%) was observed at field strengths >1.5 kV/cm. The degree of inactivation was modulated by pulse parameters, composition of the treatment medium, and physiological state of the cells. Authors mentioned the possibility of processing large-scale volumes of water directly in a flow process for the inactivation of parasites, with the advantage that this technique is not toxic to the environment and the energy cost is low compared to other

Table 1
Studies on PEF used in the inactivation of parasites in water sanitation.

Parasite and cycle stage	Matrix/media	Pulse (shape, width, number)	Input Voltage/ Electric Field	Energy	Evaluation	Result	References
Giardia muris cysts	PBS	Square, 1–99 μs, 20 pulses	1–3 kV in 0.8 mL cuvettes	31–87 kJ/kg	Excystation and counting	Combined with free chlorine 2 mg/L: survival ratio 35%. Combined with potassium permanganate 4 mg/L: survival ratio 27%.	Haas and Aturalie (1999 a).
Cryptosporidium parvum oocysts	0.1 PBS	Square, 1–99 μs, 20 pulses	1–3 kV in 0.8 mL cuvettes	22–87 kJ/kg	Excystation and counting	Combined with hydrogen peroxide 10 mg/L: survival ratio 27%. Combined with potassium permanganate 4 mg/L: survival ratio 30%.	
Giardia muris cysts	PBS, river water	Square, 1–99 μs, Up to 99 pulses	Mean/ maximum: 3.2/7.5 kV/ cm	28–32 kJ/kg	Excystation and counting	Combined with chlorine 1.26–2 mg/L: 1 log transformed survival ratio	Haas and Aturalie (1999) b)
Trophozoite of *Naegleria lovaniensis*	River water	Square, variable duration up to 10 ms, up to 10 pulses	>0.25 kV/cm	2–5 kJ/kg for ms pulses and 2–3 kJ/kg for μs pulses	Crystal violet staining and FDA (metabolic functionality)	Electric field strengths above 1.5 kV/cm allowed less than 5% survival	Vernhes et al. (2002)
Ascaris suum eggs	Saline (0.9%), urine mixture, simulated ceramic toilet	Square, 2–10 μs, 5–10 min, 70–100 Hz (21.000–60.000 pulses)	60–100 kV/ cm	Not provided	Direct smear for microscopic examination after a month	When pulsed voltage is higher than 60 kV/cm, the death rate is always higher than 80% despite the remaining factors	Zhang et al. (2012)
A. suum eggs	PBS	Square, 100 μs, 60–480 pulses	1.5–2 kV/cm	Not provided	PI uptake	Eggs exhibited fluorescence across all voltage parameters investigated	Niven et al. (2018)
Caenorhabditis elegans eggs	PBS	Square, 100 μs, 60–480 pulses	1.5–2 kV/cm	Not provided	PI uptake	Fluorescent images of eggs electroporated for 2 min at > 1.5 kV/cm	Dryzer et al., 2019 a)
C. elegans and A. suum eggs	Polished blackwater	Square, 100 μs, 60–480 pulses	1.5–2 kV/cm	Not provided	PI uptake	Eggshell permeability to PI was increased by PEF both in saline and blackwater media.	Dryzer et al., 2019 b)
A. suum eggs	PBS	Square, 100 μs, 60–480 pulses	1.5–2 kV/cm	Not provided	PI uptake Microscope observation after 28 days (developed larvae)	Eggs exhibited fluorescence across all voltage parameters investigated PEF alone (2 kV/cm) left 37% viable PEF (2 kV/cm) + 10 mg/L free chlorine resulted in 100% inactivation	Niven et al. (2020)

physical procedures. Zhang et al. (2012) evaluated the destruction of *Ascaris suum* eggs using very high electric fields (60–100 kV/cm) in different media (buffer, urine mixture, and a simulated ceramic toilet) with different chamber configurations. The death rate increased with the increase of electric field and acting frequency, but no influence of acting time was observed. When pulsed voltage was fixed and higher than 60 kV/cm with pin-pin electrodes, the death rate was always higher than 80%. More recently, interest has returned to the effects of PEF on water parasites. Niven et al. (2018) demonstrated the effectiveness of PEF (1.5 kV/cm) for the permeabilization of enteric helminth eggs to the fluorochrome propidium iodide (PI), using *A. suum* ova (recommended per the PC 305 ISO standard) as a back-end blackwater treatment process for helminth remediation. However, the developmental stage of the embryo/eggshell appeared to impact the efficacy of eggshell poration. Another team of authors who studied the potential efficacy of PEF on helminth eggs were Dryzer et al. (2019 a), who applied the same PEF equipment and processing conditions to cuvettes containing *Caenorhabditis elegans* eggs, often used by parasitic nematodes as a surrogate. Eggs showed increased permeability to PI after PEF treatment. However, no obvious changes in the geometric size or shape of the eggs were observed under all conditions evaluated, suggesting that the pores were selective to existing eggshell strata, thereby not compromising structural integrity. Eggshell electroporation kinetics showed similarity to the kinetics of cellular electroporation; the authors suggested that, similarly to the cell's phospholipid bilayer, modifications may occur within the lipid-rich nematode eggshell permeability barrier. Dryzer et al. (2019 b) reported electroporation to PI of both *C. elegans* and *A. suum* eggs without supplementary methods (i.e., chlorination or oxidation), not only in saline water but also in blackwater media. Niven et al. (2020) reported the deactivation of *A. suum* eggs using electroporation and sequential chemical disinfection. Eggs developing larval structures after 28 days of incubation were considered viable. While 2 kV/cm treatment left 37% of the eggs viable, a combination of electroporation (2 kV/cm) with chemical exposure, using low concentrations of commercially available disinfectants (10 mg/L free chlorine), achieved total inactivation. PEF would allow for oxidizing agents to pass through the complex strata of the *A. suum* eggshell, specifically reaching the innermost embryonic environment, thereby leading to successful deactivation as compared with either method used separately.

4.2. PEF for the medical ablation of parasites

Another field in which PEF has been studied on parasites is medicine (Table 2). The number of cases of hepatic cystic echinococcosis that require interventional treatments is high, but the chemicals or high temperatures used in these methodologies cause biliary complications, thereby limiting their clinical applications (Casulli et al., 2022; Zhang et al., 2017). Zhang et al. (2017) studied nanosecond PEF (nsPEF) for the treatment of hepatic *Echinococcus* cysts isolated from humans. The treatment parameters they selected were optimized *in vitro*. Fresh protoscolices were exposed to 300 ns pulses with different field strengths (0, 7, 14, 21, and 29 kV/cm) and pulse numbers (50 and 100 pulses); viability was followed up by H&E staining and scanning electron microscopy. Treatment induced significant lethal damage with 50 pulses at 21 or 29 kV/cm and with 100 pulses at 14, 21, or 29 kV/cm, accompanied by morphological destruction and increased levels of alkaline phosphatase (AP) and gamma-glutamyl-transpeptidase (GGT) membrane enzymes. Authors hypothesized that the mechanism may involve direct damage to the membrane structures of the protoscolices, promoting enzyme exhaustion and disruption of metabolism. This phenomenon of enzyme triggering has also been observed in different microorganisms (yeast and microalgae) after PEF treatment (in this case μs pulses) (Martínez et al., 2019, 2020). Chen et al. (2017) studied this alternative for benign hepatic hydatid cysts *in vivo* in mice. Cysts were induced in the liver by injecting protoscolices in the portal vein, then treated with nsPEF with different intensities and monitored over time in comparison to the standard surgery. Ultrasound diagnosis, gross anatomy, and pathology with H&E stain was monitored after euthanasia. Significant inhibition of parasite growth was seen in high nsPEF intensity group (14 and 21 kV/cm) as compared with control group ($P < 0.05$). Pathological analysis confirmed inhibition of parasite growth and destruction of hydatid cysts with sharp demarcation defined by the electrodes. After that, the same author (Chen et al., 2018) studied the treatment on human hydatid cysts *ex vivo*. The influence of nsPEF on macromembrane structure, permeabilization, and biochemistry was studied in this independent multimembrane parasite. The screening found that nsPEF (300 ns) can damage hydatid cyst effectively when field strength is greater than 14 kV/cm. When nsPEF is over 29 kV/cm, nsPEF destroys hydatid cysts completely by collapsing the germinal layer, destroying protoscolices, and exhausting its nutrition.

4.3. PEF for parasite inactivation in food

Despite the trials carried out in water sanitation and medicine, the effect of PEF on parasites in food matrices has only recently begun to be explored. To the best of our knowledge, only two articles have been published on the use of PEF for the inactivation of FBP (Table 3). The matrix in both cases was fish, and the target parasite *Anisakis* spp. These larvae are widely distributed geographically, with rates of parasitism close to 100% in certain fish species. As mentioned above, it is necessary to establish methods for their inactivation and elimination, especially in fishery products that are to be consumed raw, pickled, or salted, or which have been insufficiently treated to kill the parasite. Onitsuka et al. (2022) applied pulsed power with high electric voltage, current, and power (15 kV, 6 kA and 100 MW respectively) during 380 μs to fish filets (horse mackerel) containing *Anisakis*. The method was introduced as an alternative to freezing to avoid a decrease in quality when eaten as sashimi. The fish meat was treated in a plastic mesh basket; buffer

Table 2
Studies on the use of PEF for the medical ablation of parasites.

Parasite and cycle stage	Matrix/ media	Pulse (shape, width, number)	Input Voltage/ Electric Field	Energy	Evaluation	Result	References
Echinococcus granulosus Protoscolices from naturally infected human liver	Saline solution	Square, 300 ns, 50–100 pulses	7, 14, 21 and 29 kV/cm	Not provided	Morphological and ultrastructure changes, H&E staining and scanning electron microscopy, enzymatic activity	Significant lethal damage with 50 pulses at 21 or 29 kV/cm and with 100 pulses at 14, 21, or 29 kV/cm, accompanied by morphological destruction and increased enzymes	Zhang et al. (2017)
Liver hydatid cysts *(E. granulosus)*	*In vivo* in mouse	Square, 300 ns, not provided	7, 14 and 21 kV/cm	Not provided	Cyst growth monitored by ultrasound; gross anatomy and pathology by H&E stain.	Anti-hydatid effect is associated with electric field strength. Only 21 kV/cm inhibited parasite cyst growth.	Chen et al. (2017)
Hydatic cyst (*E. granulosus*) *ex vivo*	Culture medium	Square, 300 ns, 50–100 pulses	14, 21 and 29 kV/cm	Not provided	Pathological changes and biochemistry of cyst fluid after 7 days	Cyst damaged after treatment of 14 kV/cm and complete destruction of cyst after 29 kV/cm	Chen et al. (2018)

Table 3
Studies on the use of PEF for the inactivation of parasites in food.

Parasite and infective stage	Matrix/media	Pulse (shape, width, number)	Input Voltage/ Electric Field	Energy	Evaluation	Result	References
Anisakis pegreffii larvae L3	Horse mackerel	Exponential decay, 380 μs, up to 500 pulses	1.36 kV/cm	Energy input between the electrodes: 7 kJ per pulse	Immobility ratio of larvae after 24 and 48 h	Highest immobility ratio at 5 mS/cm conductivity 500 shots resulted in 100% immobility ratio	Onitsuka et al. (2022)
Anisakis larvae L3	Saline solution and hake meat	Square, 3–100 μs in solution, 30 μs in hake	1–3 kV/cm	3–50 kJ/kg	Post-puncture motility of larvae after 3 h of incubation	Viability was highly dependent on field strength and specific energy 3 kV/cm, 50 kJ/kg capable of almost 100% inactivation	Abad et al. (2023)

saltwater was pumped in to avoid overheating during the process caused by the high energy input. Survival was judged on the basis of whether the parasites moved spontaneously or not after stimulation. The immobilization rate was highest when the buffer saltwater was 5 mS/cm. The proportion of inactivated parasites augmented as the number of pulses increased. Sensory evaluation of the fish meat after the pulse treatment confirmed that it retained its quality as sashimi to a large extent. Breaking tests and color measurements did not find differences in treated samples in comparison to control. After that, Abad et al. (2023) focused on the evaluation of PEF in the inactivation of *Anisakis* spp. larvae in saline solution and hake meat in terms of electric field strength, specific energy, and pulse width; they also conducted a follow-up evaluation of fish quality. Results showed that viability of *Anisakis* was highly dependent on field strength and specific energy, while pulse width only exerted a notable influence at the lowest field strengths tested (1 kV/cm). Central composite design helped to define a PEF treatment of 3 kV/cm and 50 kJ/kg as the one capable of inactivating almost 100% of *Anisakis* larvae in pieces of hake, while affecting the investigated quality parameters (moisture, water holding capacity, and cooking loss) to a lesser extent than freezing and thawing. Apart from scaling-up issues and the adjustment of parameters, the results of these two studies (Abad et al., 2023; Onitsuka et al., 2022) point to an optimistic scenario in which PEF could serve as an alternative to traditional freezing processes for the inactivation of *Anisakis* in fish.

5. Theoretical influence of electrical parameters on the shape and size of FBP

As a simplified approximation, the cytoplasmic membrane of cells is two phospholipid molecules thick (about 5 nm) with embedded proteins and displaying very low electrical conductivity, whereas the extra- and intracellular media surrounding the membrane are aqueous, highly conductive electrolyte solutions. Thus, the structure formed by the extracellular medium, the lipid bilayer, and the intracellular medium is a conductor–dielectric–conductor that behaves like a capacitor (Ivorra, 2010). The system of ion transport through the healthy cell membrane leads to an irregular distribution of positive and negative ions on both sides, thus generating a difference in electrical potential called resting transmembrane voltage (RTV). However, a reorganization of charges occurs when a cell is subjected to a high electric field strength: the charges accumulate on both sides of the membrane acting as a capacitator. This phenomenon supposes an increase in transmembrane voltage, the value of which is designated as induced transmembrane voltage (ITV) (Tsong, 1991). When ITV reaches a determined threshold, electroporation of the cytoplasmic membrane takes place (Zimmermann et al., 1974). The ITV that occurs prior to the electroporation phenomenon depends on the electric field strength applied, as well as the size and shape of the cell. For a single spherical cell with a nonconductive plasma membrane, the Laplace equation is solved in the spherical coordinate system, yielding the expression often referred to as the steady-state Schwan equation (Schwan, 1957) (Eq. (1)):

$$\mathrm{ITV} = \frac{3}{2}|\mathrm{E}|\ \mathrm{r}\cos\theta$$

where ITV is the induced transmembrane voltage, E is the electric field strength applied, r is the cell radius, and θ is the angle measured from the center of the cell with respect to the direction of the field. Therefore, the external electric field strength required to reach the transmembrane voltage threshold is inversely correlated to cell size. Consequently, the electric field strength required to induce electroporation in microbial cells (bacteria, yeast; 1–10 μm) is greater (>10 kV/cm) than that required for eukaryotic plant cells (40–200 μm; <5 kV/cm) (Donsì et al., 2010). Martínez et al. (2020) compared the electric field strength and total specific energy required to electroporate a certain percentage of different types of cells, showing the association between those parameters and cell diameter. However, this is merely a mathematical simplification for a single spherical cell; although bacteria or yeast are quite homogeneous regarding shape, FBP comprise a number of very different and complex organisms. To perniciously affect the viability of parasites, the pores formed in the cell membrane during treatment should persist in the absence of the electric field (irreversible electroporation). Then, once the membrane is eventually disrupted, intracellular contents leak out with the consequent loss of cell metabolic activities (Chauhan & Unni, 2015). PEF lethal impact will depend on different factors, such as electric field strength and treatment time, as well as on the microorganism itself, treatment temperature and the characteristics of the medium (Barba et al., 2015). Augmenting treatment intensity, either via an increase in electric field strength or in treatment time, increases the pores' irreversibility (Ivorra, 2010), since the larger the size of the pore created, the longer it will take it to close once the electric field strength has ceased (Saulis et al., 1991; Jhoshi & Hu, 2012). Increasing the electric field amplitude and pulse duration generally increases cell inactivation, but also affects the amount of energy required and the temperature of the medium during the process (Delso et al., 2022). Thus, these parameters are limited because food matrices susceptible to PEF treatment for parasite inactivation are especially sensible to temperature increases (salads, fish to be consumed as sushi, cured ham, etc.).

As mentioned above, FBP include eukaryote organisms of a variety of shapes and sizes, with complex life cycles and different transmission stages, thus making them difficult to discuss as a whole. In the joint analysis in this section, we will describe several examples of parasitic stage morphologies and one example of additional structures that protect the cytoplasmic membrane. The simplest case would be a parasite with a single transmission stage. *Giardia*, a flagellated protozoan, spreads easily through food by means of ellipsoidal cysts that are 8–12 μm long by 5–8 μm wide. Other protozoa such as *Cryptosporidium* can contaminate process water and vegetables with oocysts that are spherical in shape and measure 4.2–5.4 μm in diameter. Apart from differences in terms of cell type and extra layers, the size and shape of these parasites are quite similar to those of yeasts, on which a great number of studies have been published (Martínez et al., 2020). Certain parasites,

however, have various infective stages with complicated structures that would make it difficult for the cell and the extracellular medium to behave as a capacitor, i.e., complying with the properties that would make them susceptible to electroporation. That is the case of the protozoan *T. gondii*. Their oocysts can contaminate vegetables and water and are almost spherical in shape and 10–12 μm in diameter (Foreyt, 2013). These resistant stages have two walls with several layers that protect them from environmental threats. The outer oocyst wall layer (20 nm thick) contains cysteine-rich and tyrosine-rich proteins, forming robust polymeric structures through disulphide or dityrosine cross-linking. Additional PAN-domain-containing proteins contribute to the stabilization of the architecture of the outer oocyst wall layer through disulfide bridging. Acid-fast lipids coat the oocyst surface and render the oocyst wall almost impermeable to water-soluble molecules. The inner oocyst wall layer (30–70 nm thick) contains crosslinked Tyr-rich proteins and fibrils of β-1,3-glucans that improve structural resistance. In addition to this complex framework, several glycoproteins located in both walls accompany the structure. Furthermore, this is just the first level of protection, because to reach the sporozoite nucleus it is also necessary to surpass the sporocyst wall, which is formed again by two layers. The outer sporocyst wall layer (15–20 nm thick) resembles the outer oocyst wall in structures and molecular composition, while the inner sporocyst wall layer (40–50 nm) is made of four curved plates joined together by thick sutures that provide additional mechanical resistance (Shapiro et al., 2019). Thanks to this polymeric nature, the oocyst walls are very resistant to mechanical perturbations (Dumetre et al., 2013) and almost hermetic to chemical inactivation agents, in particular strong acids, detergents, and chlorinated disinfectants (Jones & Dubey, 2010). For instance, diluted household bleach solutions can destroy the outer oocyst wall but do not significantly alter the structure, mechanics, and permeability of the inner oocyst wall and the sporocyst walls, nor do they significantly affect the sporozoite's infectivity (Dumetre et al., 2013). Oocyst tolerances to other environmental stressors including salinity and enzymatic digestion also rely on its robust structure. The nature, molecular content, and lack of permeability of the walls protecting the cytoplasmic membranes can prevent the accumulation of charges on both sides of the membrane and thereby hinder electroporation. On the other hand, bradyzoites found within intracellular tissue cysts (muscle and neural tissue) are 7 × 1.5 μm, crescent-shaped, and are also protected by additional structures not covered in electroporation theories.

Conversely, other FBPs belonging to the *Animalia* Kingdom are larger, such as *Echinococcus* spp, a cestode whose eggs are spherical in shape and have a diameter about 30–40 μm. The nematode *Ascaris* spreads round eggs either ranging from 45 to 75 μm when fertilized, or which are elongated and larger (up to 90 μm) when unfertilized. Other nematodes have transmission stages as larvae instead of eggs because they are viviparous. *Trichinella* is the smallest known nematode parasite in humans: its larvae encysted in muscle are 1 mm long. This stage encysted in the meat is worm-shaped with a coiled, spiral appearance, far removed from the spherical morphology and simplicity of unicellular organisms. Finally, *Anisakis* spp. share the common features of all nematodes: a vermiform body plan, a round cross section, and a lack of segmentation. The squamous epithelium secretes a layered cuticle to protect the corpus from digestive acids, and which probably would protect it from electroporation as well. In the stage which infects fish (L3), *Anisakis* larvae are found in a distinctive "watch-spring coil" shape. They are roughly 2 cm long when uncoiled, thus again quite different from the type of microorganisms usually studied in PEF inactivation. Equations similar to the one described above can be derived for nonspherical cells, provided that they resemble a regular geometrical body such as a cylinder, an oblate spheroid, or a prolate spheroid (Kotnik & Miklavčič, 2000). However, the effect of PEF on such widely varying multicellular organisms should be studied case by case, and parameter optimization would require specific laboratory experiments (although mathematical modeling would be of help as a supplementary tool).

6. Side effects in vegetables contaminated by FBP and possible affectation of muscle (fish and meat) where parasite is located

The aim in treating by PEF food against FBP would be to electroporate the parasite cell membranes without negatively affecting the tissue (meat, fish, vegetable, etc.). However, certain negative effects on food quality can be expected depending on the PEF treatment and specific product. Additionally, some positive effects of PEF in particular foodstuffs such as the improvement of meat tenderization can occur in parallel to parasite inactivation, thus resulting beneficial on two vertices (safety and quality). Analysing the impact of the electric field on food matrixes is more complex in multicellular systems that are composed of different cell types, featuring numerous properties within a variety of tissues. Permeabilization influences the turgor of the cell. Thus, turgor and texture measurements, such as stress deformation and relaxation assays of complex tissue, may likewise reflect the degree of tissue damage and cell rupture (Lebovka et al., 2004). Plant cell membranes are significantly altered after PEF, and a disruption of compartmentalization can affect overall fruit and vegetable quality (Jäger, 2013). On the other hand, as mentioned above, although PEF is a non-thermal food processing technology, food temperature may rise considerably during high intensity PEF treatments, thereby compromising sensitive compounds such as proteins (Jäger, 2013). The side effects of PEF depend on processing parameters and treatment conditions; mild treatments can minimize problems associated with temperature increase or electrochemical reactions. To prevent damage to pigments, flavour compounds, and vitamins, and in order to consequently avoid the degradation of certain sensory characteristics and of the nutritional value of food, PEF parameters need to be optimized. Treatments of high voltage and of very short duration are usually preferred, in order to inactivate microorganisms without losing food quality (Ohshima et al., 2007). The specific energy input of the process (which is expressed in kJ/kg and depends on the input voltage, the processing time, and the ohmic resistance of the treated product) is closely associated with the temperature increment and proves useful in comparing efficacy and eventual losses of quality. However, the information provided in the articles published on this subject is not always sufficient to calculate this parameter; moreover, the scarcity of studies on parasite species makes it difficult to carry out a systematic analysis of the correlation between electric parameters and inactivation rates. Also related quality changes; process-product interactions should be studied.

Apart from possible side effects, certain studies have reported that the application of PEF to muscle before aging can improve the meat tenderization process due to an increased rate of proteolysis (Faridnia et al., 2015). Meat, however, is a complex set of different tissues (adipose, vascular, nervous, and muscle cells); moreover, further factors such as species, breed, age, environmental conditions, history of physical activity, and nutrition will determine the degree of modifications. Other authors have used PEF to improve meat drying in curing processes (Astrain-Redin et al., 2019). An optimal PEF treatment (1 kV/cm, 200 μs of pulse width, 28 kJ/kg) was used to electroporate meat cells, improving water content reduction by 60.4% in samples of treated meat dried at 4 °C. After the curing process, the application of PEF to sausages stuffed into gauze reduced drying time from 17 to 9–10 days. On the contrary, the generation of pores and the facilitation of water movement is a disadvantage if meat is destined to be braised rather than dried, due to reduction of water-holding capacity (Faridnia et al., 2015). PEF processing also affects muscle cell membranes that exert an influence on the interaction between fatty acids and membrane phospholipids with prooxidant effect in meat (Faridnia et al., 2015). Such interactions can give rise to undesirable compounds, which, in turn, decompose into secondary products that can cause off-flavours and odors in meat, thereby reducing its sensorial and nutritional quality. However, this all depends on the chosen PEF parameters as well as on the species (Gomez

et al., 2019). Regarding meat colour, high PEF intensities and numbers of PEF repetitions may lead to negative effects on the appearance of meat, as the temperature of the product increases and promotes myoglobin oxidation; mild PEF conditions are thus preferable (Alahakoon et al., 2016). Khan et al. (2017) demonstrated that an intense PEF treatment (0.68 kV/cm, 149.8 kJ/kg, 20 μs pulse width) can negatively influence the quality of beef as compared with the effects observed for low PEF treatments (0.23 kV/cm, 12.4 kJ/kg, 20 μs pulse width). Specifically, the parameters that were negatively affected by the intense PEF treatment were shear force, color, lipid oxidation, and mineral levels (P, K, and Fe). The same authors (Khan et al., 2018) found changes in element concentrations after PEF and, interestingly, differential effects depending on the treated meat material (beef or chicken). Furthermore, certain increased mineral contents suggested that there had been potential migrations from the electrodes, especially at high PEF. However, different outcomes can be observed when minerals are analyzed in meat after PEF. Bhat et al. (2019) did not observe negative effects on minerals such as Fe, K, P, Ca, Na, and Mg in PEF-treated meat after digestion. Conversely, significantly higher amounts of those minerals were detected in PEF-treated samples in comparison with control, which might be attributed to a greater release of minerals due to the membrane's increase in permeability after PEF treatment.

The potential of PEF as a non-thermal alternative for the **fish industry** is gaining increased attention, since PEF can have less impact on the micro-structure of muscle food compared with emerging heat-based processes such as ohmic (Gavahian et al., 2019) and microwave heating (Gomez et al., 2019), thus having less impact on product quality. Several authors have indicated this technique's utility in maintaining the final product's (meat and fish) physical, organoleptic, and functional characteristics, introducing only minimal changes in flavour as well as in terms of vitamins and other nutrients (Gomez et al., 2019). Onitsuka et al. (2022) applied high PEF energy levels to inactivate *Anisakis* larvae; their samples retained a texture (elastic modulus and breaking load) closer to that of the control than to that of the freeze/thawed product. The sensory evaluation of the PEF-treated samples was slightly worse than the control but very close to neutral, indicating that the quality of the sashimi was mostly maintained. Abad et al. (2023) evaluated the effects of a PEF treatment capable of killing *Anisakis* (3 kV/cm; 50 kJ/kg; 30 μs pulse width) on the following quality parameters in pieces of hake: moisture, water holding capacity (WHC), cooking loss (CL), and colour. PEF-treated fish was compared to frozen fillets that had been stored for 2 days and subsequently thawed. Neither water content nor WHC were affected by PEF treatments, but the same parameters were significantly reduced in the case of the frozen specimens. The electroporation achieved in muscle cells does not reduce moisture or the capacity to retain water to the same extent as freezing-thawing. On the other hand, CL indicated a slight effect on the proteins most sensitive to denaturisation, despite the fact that the final temperature of the fish meat after PEF treatments never exceeded 15 °C. Although the freezing/thawing process affected fish quality to a greater extent than PEF, further research would be required to clarify the effect of PEF on protein denaturisation at low temperatures. It is crucial to maintain the appearance of raw fish in sushi or sashimi. Both research teams (Abad et al., 2023; Onitsuka et al., 2022) analyzed colour as well. However, the variability among species, batches and even among parts of the fillet is high; we thus lack sufficient data to discuss this parameter in further detail.

7. Concluding comments

New tools for detecting FBPs in food matrices will require further development and improvement, coupled with approaches designed to assess infectivity and viability of different FBP transmission stages. Determining which measures are most appropriate to minimize the risk of FBP transmission will require careful consideration. The implementation of a processing step in the food industry that would reduce the infectivity of FBPs will have a considerably positive impact on public health. So far, traditional cooking, heat-based emerging technologies, and the intense freezing methods proposed for this objective collide head-on with new culinary habits and growing consumer demands for high-quality food. On the other hand, the highly heterogeneous array of existing FBPs with different complexities, morphologies, structures, and sizes makes it difficult to achieve successful inactivation. The wide variety of food matrices and the difficulty of executing and scaling up PEF treatments (chamber and treatment zone design) to achieve an industrially competitive flow process leaves room for substantial research efforts. Treatment of solid products (fish fillets or entire fishes, meat cuts, vegetables, etc.) may entail operational problems such as the presence of bubbles, the need to design new treatment chambers, and the absence of sufficiently powerful equipment; these subjects lie outside the scope of this review. A precise, optimal balance among electrical parameters needs to be achieved, particularly in order to avoid overheating, apart from avoiding further reactions that would impact high quality. It would be necessary to conduct a case-by-case analysis of PEF efficiency in each FBP stage in each one of the matrices. If total inactivation cannot be achieved, the prospect of achieving synergistic effects by combining PEF with other processing techniques makes it possible to envision a situation in which FBPs could be prevented from reaching the consumer.

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Data availability

Data will be made available on request.

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