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TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Effect of electric field pulses on microstructure of muscle foods and roes

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The effect of pulsed electric fields (PEF) and combination of PEF and high pressure were studied on microstructure of salmon, chicken and lumpfish roes. The results showed that PEF treatment with low field strength (less than 2 kV/cm and 20–40 pulses) had considerable effect on the microstructure, i.e. the muscle cells decreased in size and gaping occurred. PEF treatment had greater effect on salmon than chicken samples. However, roes seem to tolerate up to 18.6 kV/cm and seven pulses without visible effect. Reduction of microorganisms was still not sufficient at the same time. Combination of PEF and high pressure (200–300 MPa) had more detrimental effect on microstructure than PEF treatment alone.

Introduction

The possible uses of pulsed electric fields (PEF) as a food preservation method has been investigated for number of years. The main focus has been on the effect of electric pulses on inactivation of different types of microorganisms in different states such as lag-phase, growth phase or as spores (Castro, Barbosa-Cánovas, & Swanson, 1993; Hülsheger & Niemann, 1980; Sale & Hamilton, 1967; Wouters, Dutreux, Smelt, & Lelieveld, 1999). Studies on electric permiabilization have also been done to improve the yield of apple juice (Flaumenbaum, 1968) or carrot juice (Knorr, Geulen, Grahl, & Sitzmann, 1994) in juice production. It is also of great importance to investigate the effects of pulsed electric fields on microstructure and texture of foods. Basically PEF is a non-thermal preservation method like high-pressure treatment and other processes like irradiation, oscillation magnetic fields, light pulses and modified atmosphere packaging that are expected to keep the food as fresh as possible (Barbosa-Cánovas, Pothakamury, Palou, & Swanson, 1998; Davies, 1995; Karel, 1975; Knorr, 1995; Knorr, Heinz, Un-Lee, Schlüter, & Zenker, 1998). One of their advantages is a minimum alteration of food structure due to low temperature increase during the process. The consumer acceptance of food is to a large degree based on sensoric experience where texture plays a large role and therefore the food need to be as fresh as possible before consumption or cooking. It is known that high-pressure treatment affects texture and microstructure of meat and fish at pressures between 200 and 400 MPa depending on the type of product (Ledward, 1998). At these pressures, depending on temperature and time, the microorganisms are inactivated to some extend, e.g. *Listeria innocua* for four log cycles at 300 MPa, 30°C and 2000 s (Knorr *et al.*, 1994). All traditional processes for meat and fish product such as frozen storage, drying, salting and canning have from moderate to severe effect on the microstructure of the product compared to fresh product (Bello, Luft, & Pigott, 1982; Chu & Sterling, 1970; Connell, 1964; Duerr & Dyer, 1952; Dunajski, 1980; Fennema, 1990; Greaser & Perason, 1999; Mackie, 1993; Sikorski & Kotakowska, 1994). The only processes that do not affect the microstructure to any great extend are chilling and modified atmosphere packaging but then the product has limited shelf life compared to the traditional processes (Brody, 1989; Davies, 1995; Fennema, 1975). There are only limited researches on the effect of PEF treatment on microstructure (Barsotti, Merle, & Cheftel, 1999; Fernandez-Diaz, Barsotti, Dumay, & Cheftel, 2000) and none is available on muscle food. Prior to any investigations, it was considered a possibility that PEF process could have less impact on microstructure of muscle food compared to heat-based processes or other non-thermal processes.

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Researches in that area are therefore necessary and in this research the effect of PEF treatment on microstructure of fish and meat has been investigated. PEF treatment can also be used in combination with other processes and in this research it was studied in combination with high-pressure treatment. The idea was to estimate if hurdle effect could be achieved with relatively mild PEF and high-pressure treatments as has been described for other technologies (Leisner, 1995).

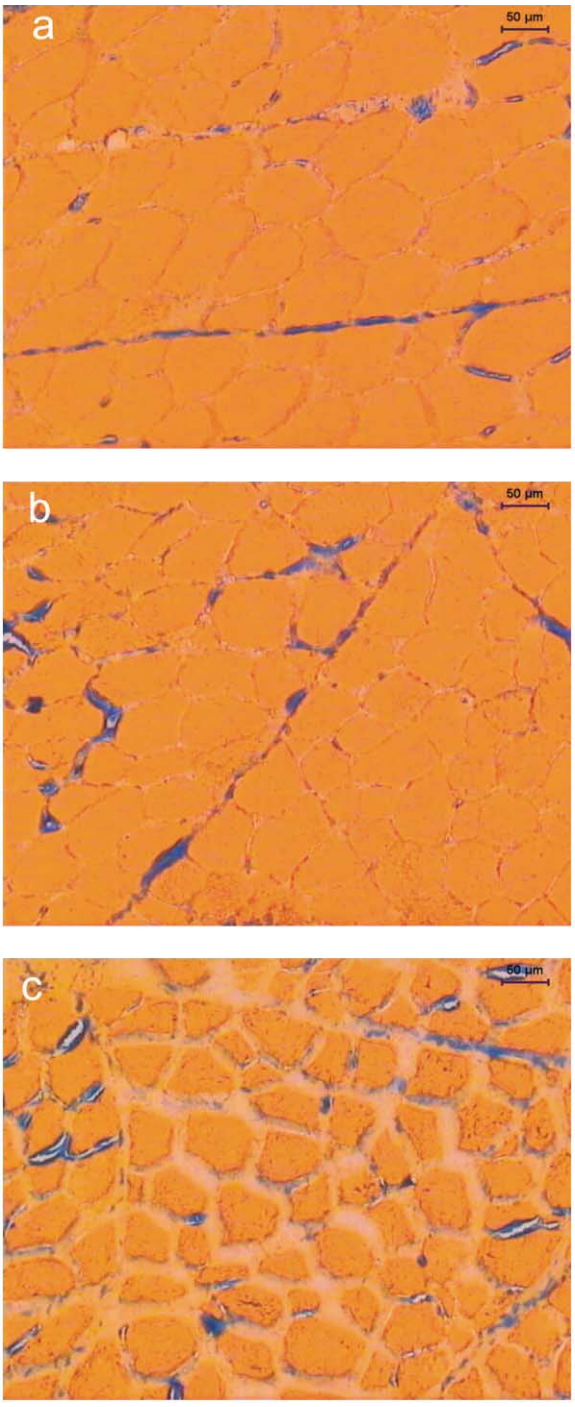

Fig. 1. Chicken muscle samples. Muscle proteins are stained orange and collagen is stained blue: (a) untreated muscle; (b) 1.36 kV/cm and 40 pulses (each pulse is 2 µs width); (c) high-pressure 300 Mpa.

Effect on chicken meat and salmon

The effect of a high electric field on cells has been explained by the dielectric rupture theory (Zimmerman, 1986; Zimmerman, Pilwat, Beckers, & Riemann, 1976) where the external electric field induces electric potential over the membrane, which in return causes charge separation in the cell membrane. When the transmembrane potential exceeds a critical value of 1 V it causes a

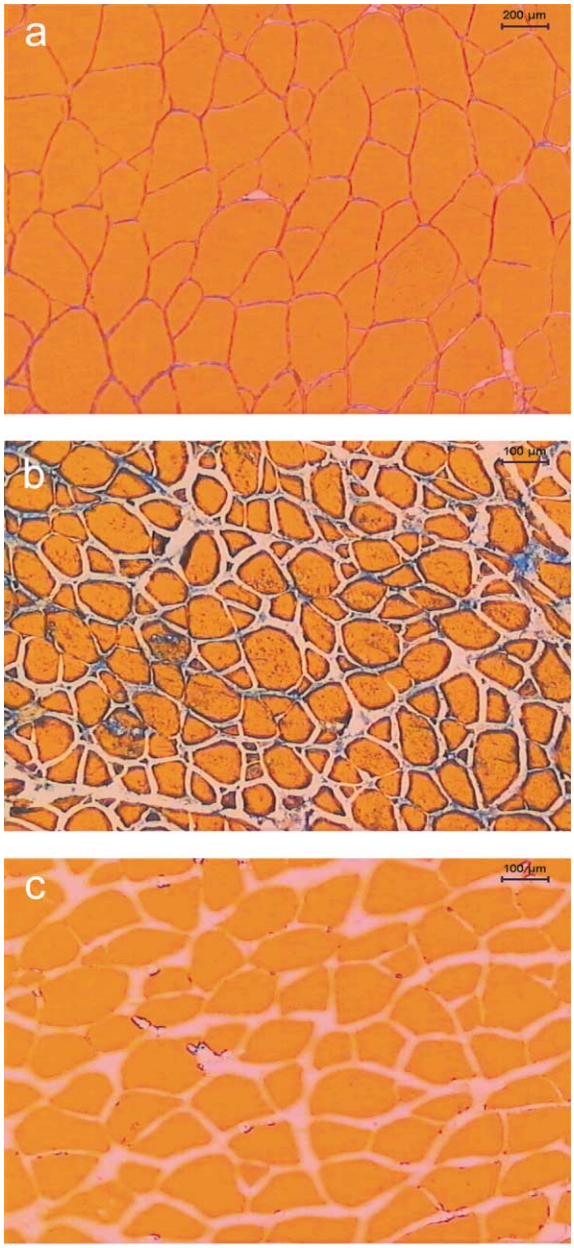

Fig. 2. Salmon sample. Same staining as fore Fig. 1: (a) untreated; (b) 1.36 kV/cm/40 pulses; (c) 300 MPa (−10°C).

formation of pores. This rupture of cell membranes can either be reversible or irreversible depending on the voltage of the field (Mertens & Knorr, 1992). Changes in microstructure and texture can be expected as a consequence of the permiabilization that causes changes in water-holding properties of the muscle.

Figure 1 shows PEF treatment on chicken meat. The control sample showed cells that were intact and no gaping was visible. The PEF treatment was performed with an electric field of 1.36 kV/cm and 40 pulses at room temperature and stained orange for protein and blue for collagen. The PEF treatment caused a reduction in size of the cells but without visible gap between the cells. In comparison, high-pressure treatment of 300 MPa reduced the size of the cells and gaping was visible. High-pressure treatment between 100 and 300 MPa is known to denature myosin and actin (Anguspanich & Ledward, 1998). It has also been shown (Dransfield, 1994) that high pressure can damage membranes in the muscle that result in release of calcium ions which

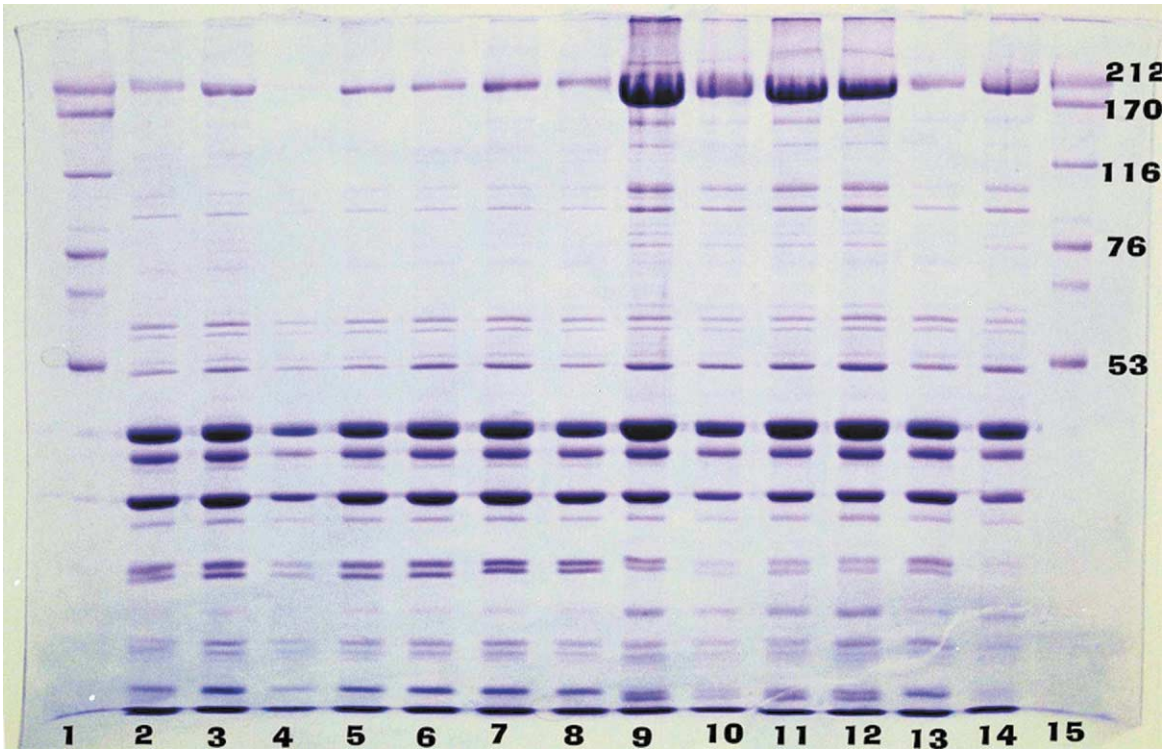

Fig. 3. Electrophoresis of cod proteins from whole muscle with electric pulses or combination of electric pulses and high pressure. Rows 1 and 15 are standard proteins with highest molecular weight at the top. Samples 2–8 are cod proteins treated with combination of electric pulses from 3 to 7 kV/cm, 13 to 39 pulses and 200 MPa. Samples 9–12 are cod proteins treated with electric pulses (18, 15, 12.5 and 10.6 kV/cm, respectively, and 7 pulses) and 13 and 14 are untreated control samples.

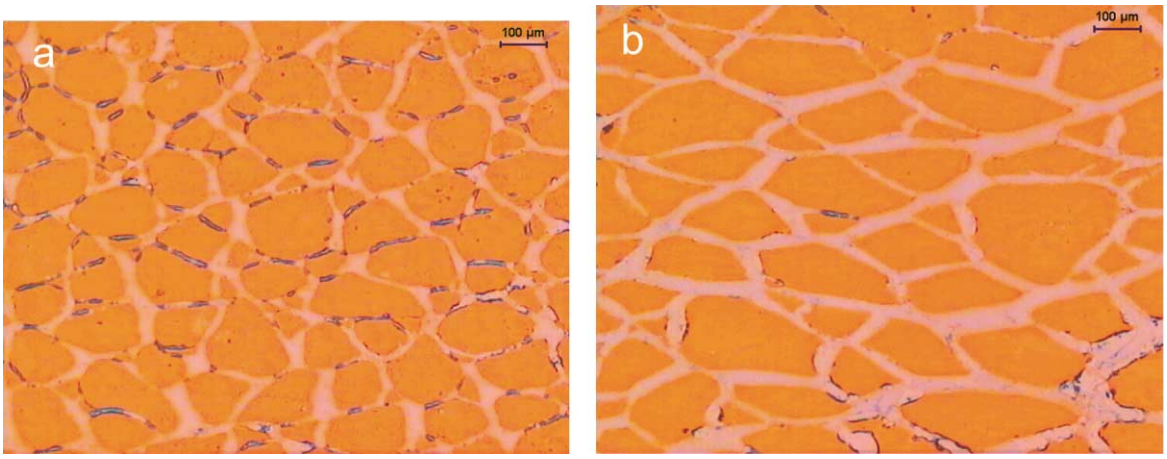

Fig. 4. Chicken muscle: (a) treated with 0.35 kV/cm and 60 pulses (each pulse is 2 µs width) and then 200 MPa pressure; (b) reverse order of treatment.

eventually can tenderise the meat. High pressure could probably also cause gaping if the treatment is severe. The PEF treated chicken muscle is very similar in appearance to untreated chicken and different from high-pressure treatment.

In Fig. 2, the PEF treated and high-pressure treated Salmon samples are compared to the control sample. The same staining technique is used for salmon as the chicken muscle and the PEF parameters are the same.

In contrast to chicken muscle, the PEF treatment produces gaping in salmon samples. The collagen leaked into the extra cellular space and was stained there in the gap. Treatment with high pressure at 300 MPa also produced gaping but the collagen did not stain in the gap either because there is no leakage or the collagen has disintegrated. Fishes including salmon contain much less connective tissue (0.66%) (Dunajski, 1980; Eckhoff, Aidos, Hemre, & Lie, 1998) than the chicken meat (2%) (Baily & Light, 1989). That could explain why salmon was susceptible for gaping with PEF treatment, as less energy is needed to take a fish muscle apart than other muscle food.

An electrophoresis of cod samples is shown in Fig. 3. The pulsed electric field up to 18.6 kV/cm and seven pulses did not affect the fish proteins to any extent, as the same protein bands were visible as in the control sample. However, if high pressure of 200 MPa was also used, some high molecular bands disappeared or were less strong. An electric field as high as 18.6 kV/cm did not seem to affect the primary structure of the fish proteins. It has also been shown that PEF treatment of 27–33 kV/cm and 50–400 pulses did not affect ovalbumin notably (Fernandez-Diaz *et al.*, 2000). It can be concluded that the impact of PEF treatment on the microstructure of fish and chicken meat was not due to protein denaturation but involved punctuation of the cell membranes causing leakage of cell fluids into extra cellular space.

Combination of PEF and high pressure treatment

Figure 4 showed the effect of combination of PEF treatment at 0.35 kV/cm and 60 pulses and high pressure at 200 MPa compared to no treatment on chicken meat. This process was either performed with PEF treatment first and then the high-pressure or reverse treatments. These two combination treatments look similar, though the combinations with PEF introduced first showed more visible gaping. In both cases, the gaping was stained blue which indicated leakage of collagen into the extra cellular gap.

Figure 5 shows the effect of the combination of PEF and high pressure on salmon samples. Gaping was visi-

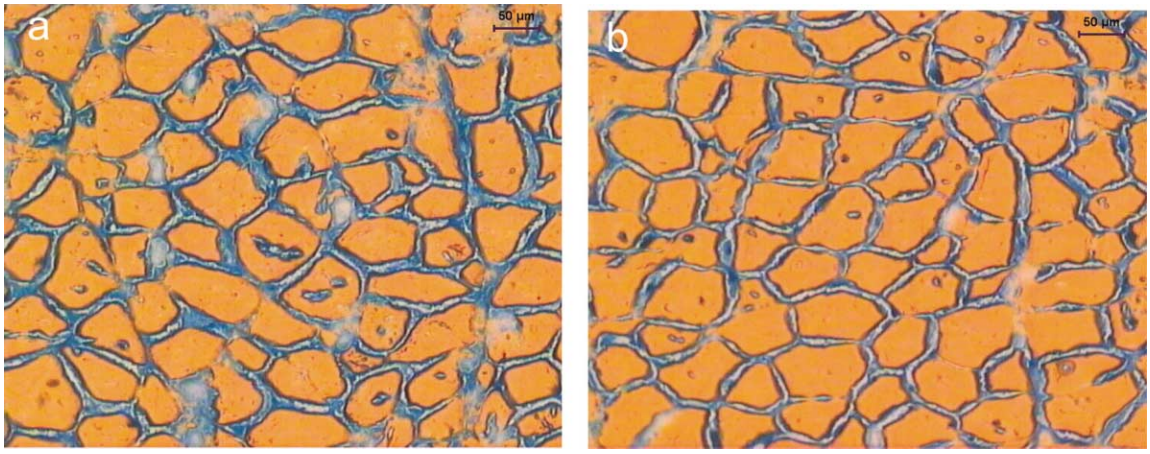

Fig. 5. Salmon sample: (a) 0.36 kV/cm/60 pulses then 300 Mpa; (b) reverse order.

Table 1. Average cell area (µm²) of chicken and salmon samples after treatment

Samples	Control	300 Mpa	1.36 kV/cm/ 60 pulses	300 MPa/ 0.35 kV/cm	0.35 kV/cm/ 300 MPa
Chicken	3600[a] (100%)	2340[b] (65%)	2190[b] (61%)	2390[b] (66.4%)	2170[b] (60.2%)
Salmon	13200[a] (100%)	10100[b] (77.3%)	4480[c] (33.9%)	8000[d] (60.2%)	6600[e] (50%)

[a–e] Different letters a, b, c, d and e mean that treatments are significantly different at $P < 0.05$ within each sample. The same letter means that samples are not different.

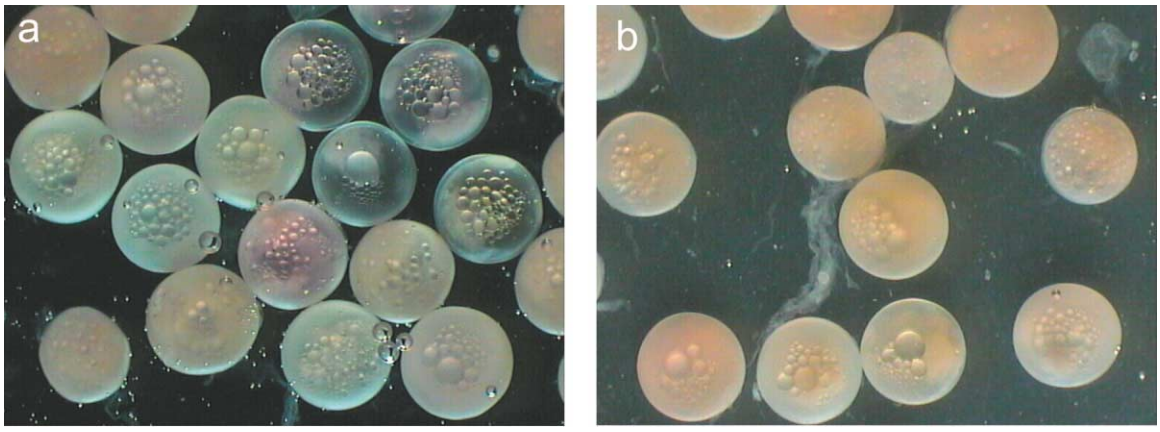

Fig. 6. (a) Control, fresh lumpfish roes; (b) treated roes, 12 kV/cm and 12 pulses.

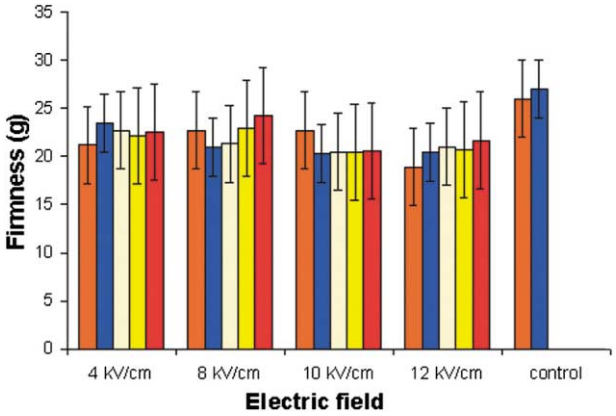

Fig. 7. Firmness of roes after PEF treatment compared to control. The order of columns indicate treatment with 2, 4, 6, 10 and 12 pulses, respectively, except for the two control samples.

ble and there was a size difference of cells between samples depending on which treatment was introduced first.

Table 1 shows the average cell area in square micrometers as a result of the different treatments. The area was calculated with the help of image analysis on 150–200 cells. As shown in Table 1, all the treatments decrease the cell size compared to the control sample for both the chicken meat and the salmon. The average cell size of chicken meat was not significantly different between the different treatments or between 60 and 66% of the original size, despite the different appearance of the images. However, the result for salmon is different as when the PEF treatment is used alone it reduces cell size more than any other treatment. It was also clear from the combination treatments that applying PEF treatment first for the salmon sample decreases the cell size more than if the reverse order was applied. This was not evident for the chicken sample.

If the order of treatment matters, one can only speculate at this moment why it should matter. Perhaps the PEF treatment punctuates the cells and forms microscopic pores. The cells will eventually leak some of their fluid to the extra cellular space and the high-pressure treatment will then help to squeeze even more fluid out of the cells. However, when high pressure is used first, the cells will decrease but without any pore formation. When PEF treatment is then used, there is no additional pressure to squeeze out the fluid and less fluid leaks out.

It is evident from these results that even mild PEF treatment causes changes in the microstructure of fish and meat tissues, which do not affect microorganisms. To have bactericidal effect, the electric field voltage needs to be considerably higher (Castro *et al.*, 1993; Hülsheger, Potel, & Niemann, 1981; Sale & Hamilton, 1967). The consequence is that PEF or the combination treatment is not suitable as a preservation method for fish or muscle food.

Effect on lumpfish roes

Lumpfish roes are used in many kinds of fish roe- and caviar-like products. The roe membrane is made out of three main layers that give the roe its strength, but the dry matter is mainly protein and polysaccharides (Grierson & Neville, 1981; Kobayashi, 1982; Riehl, Brunegger, & Jakopic, 1980). Inside is the yolk that is mainly protein-rich fluid that also contains some lipid (Jared & Wallace, 1968). Figure 6 shows the effect of 12 kV/cm and 12 pulses on the appearance of fresh lumpfish roes. The roes were intact after the treatment except for very few roes and that gave an indication that the lumpfish roes can tolerate relatively strong electric pulses without damage. This was further supported by measurements on firmness of PEF treated roes made with a compression test as seen Figure 7. The PEF treatment only marginally affected the firmness of the roes. In comparison, frozen and then thawed salmon roes needed 46% less energy to be ruptured than fresh roes measured with a similar compression test (Craig & Powrie, 1988).

Figure 8 shows the damage percentage of roes after PEF treatment. The roes were only damaged to a small

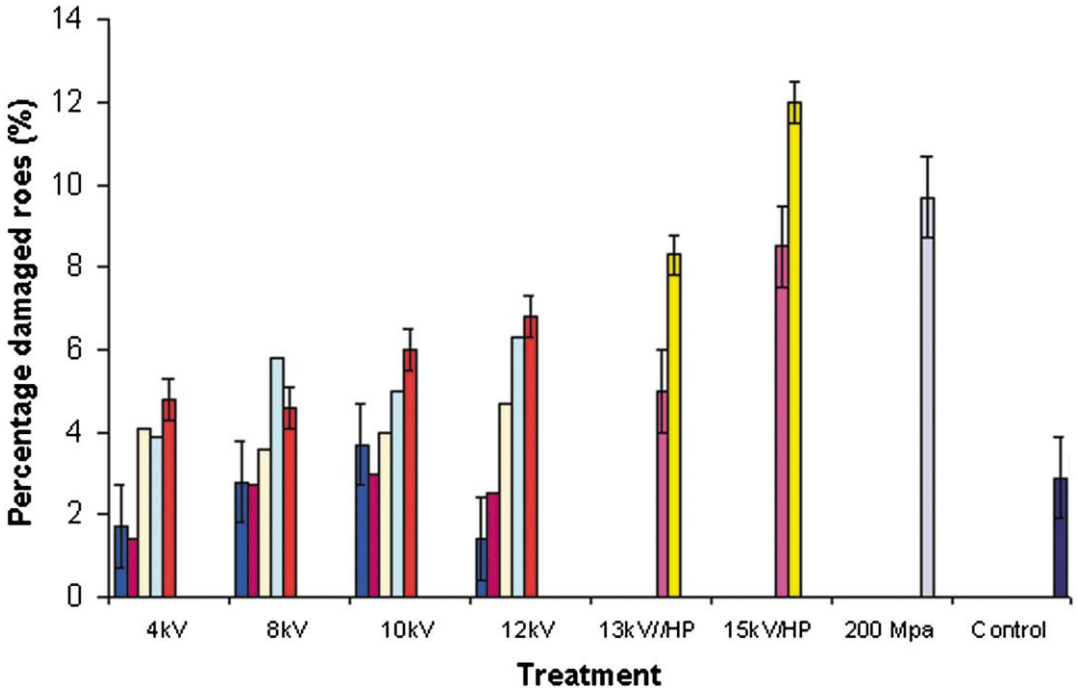

Fig. 8. Percentage of damaged roes after treatment compared to control sample. Different coloured columns had different amount of pulses from 2, 4, 6, 10 and 12 for PEF treatment and 5 and 10 pulses in combination treatment.

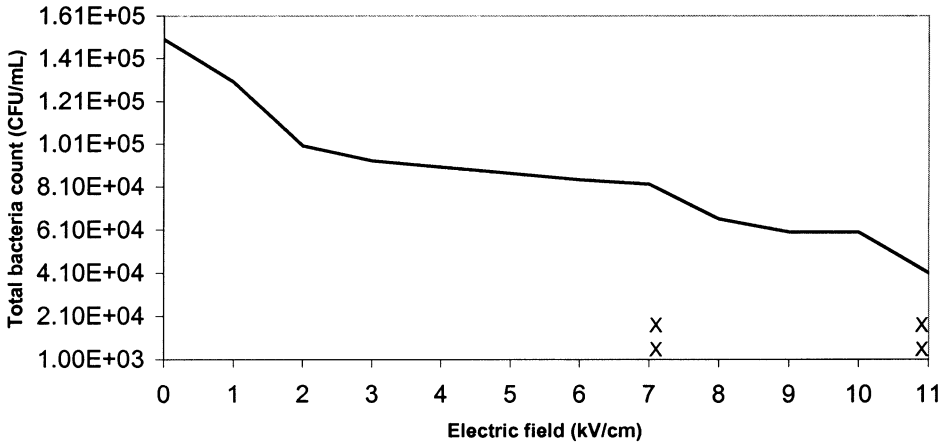

Fig. 9. Total bacteria count as function of electric field and combination treatment. The crosses are combination treatment of 7 and 11 kV/cm and 200 and 300 MPa pressure.

extent or up to 6% compared to 1.5% for the control sample. The results from the bacteria count are shown in Figure 9. The reduction was one log cycle at 11 kV/cm and 12 pulses and two log cycles for the combination of PEF treatment and high pressure at 13 kV/cm and 200 MPa.

In order to use PEF treatment as a preservation method for roes, one needs to increase the PEF treatment considerably above 13 kV/cm to have an effect on the micro-organisms.

Future research needs

It can be said that, as far as meat and fish are concerned, the PEF treatment is not suitable for preservation because it affects the texture and microstructure at lower field voltage than effectively reduces the growth of bacteria. However, roes seem to tolerate a high level of PEF treatment without a visible effect on the microstructure or texture. PEF treatment could therefore be valuable as a pre-treatment for roes. This needs to be further investigated to set the upper limits of the electric

field that can be used for roes. Other potential uses of PEF treatment on fish and meat products have not been considered but it could be possible to use PEF treatment in processes that extract substances from waste material of fish and meat industries, e.g. enzymes.

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