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Design of prototype device for killing anisakid larvae using pulsed power technology

Chinari Onitsuka [1,2], Kengo Nakamura[2], Douyan Wang[3], Mikiya Matsuda[4], Akihiko Ogasawara[3], Ritsuo Tanaka[3], Yoichi Inoue[2], Masayoshi Kouduma[5], Kenji Negoro[6], Takayasu Negoro[6], Takao Namihira[3, *]

[1] Graduate School of Science and Technology, Kumamoto University, Japan
[2] Japan Seafoods Co. Ltd., Japan
[3] Institute of Industrial Nanomaterials, Kumamoto University, Japan
[4] Technical Division, Kumamoto University, Japan
[5] Maycraft, Japan
[6] Pultec Electronics Co., Ltd., Japan

* Corresponding author: namihira@cs.kumamoto-u.ac.jp (Takao Namihira)

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Abstract

Anisakid nematode larvae are found in fish and cephalopods and can cause anisakiasis in humans when raw or undercooked seafood is consumed. An estimated 20,000 cases of anisakiasis occur annually in Japan. The only practical way to kill anisakis apart from heating has been freezing, but freezing can damage the texture and color of the fish meat. As an alternative to freezing, we have shown that anisakis in fish can be killed through the repeated application of pulsed high current through the fish meat, and that the quality of pulse-treated fish meat is almost the same as that of untreated meat. The following includes a description of a prototype device used to kill anisakid larvae using pulsed power technology. High temperatures can adversely affect fish meat quality. In order to apply the pulsed current while keeping the temperature of the fish meat as low as possible, we created a treatment tank filled with 180 liters of buffer saltwater, placed the fish meat between plane-to-plane electrodes in the treatment tank, and applied pulsed current while using pumps to agitate the water. Parameters such as saltwater conductivity, charging voltage, and capacitance necessary for killing anisakid larvae were studied using Taguchi methods, and the performance of the device was analyzed. To ensure worker safety, all parts of the device that may be touched by workers were grounded. The device was installed at a seafood processing factory where tests were conducted showing that all test larvae were killed. Currently, the company sells horse mackerel fillets treated with this device to some of its customers, and it is highly regarded for its safety and low impact on meat quality.

Keywords: Anisakis, Anisakid larvae, parasite, pulsed current, pulsed power.

1. Introduction

Anisakid nematode larvae live in the internal organs of various marine organisms. When humans consume raw or undercooked seafood, the presence of live anisakid larvae can cause a zoonotic disease called anisakiasis. As consumption of seafood increases worldwide [1], cases of anisakiasis are also increasing [2, 3]. In Japan, where people traditionally eat a large quantity of raw fish such as sushi and sashimi, there are approximately 20,000 cases of anisakiasis per year [4]. *A. simplex* sensu stricto and *A. pegreffii* are the most common causes of human infections in Asia and Europe [4, 5].

In addition to heating, a common method for killing anisakid larvae is freezing. Freezing requires considerable time to effectively kill larvae [6, 7]. Storage over at least 24 hours at −20 °C is required to kill anisakid larvae. The FDA provides guidance to processors of fish and fishery products on freezing and storage

processes for raw fish [8] and Europe mandates the freezing of seafood for raw consumption [9]. However, freezing affects the quality of the fish meat due to the formation of ice crystals and denaturation of protein during freezing that damage fish meat tissue. The formation of myoglobin during frozen storage also causes the meat to brown [10, 11].

Pulsed power technology is a promising alternative for killing anisakid larvae. Although there have been many studies on pasteurization using pulsed electric fields [12], there have been few on killing zoonotic parasites, with *Echinocossus granulosus* and *Ascaris suum* being the only examples [13, 14]. Recently, it was confirmed that pulsed high current can render anisakis inactive inside fish meat and that it is possible to kill anisakid larvae with less effect on fish meat quality than freezing at comparable quality to untreated fish meat [15, 16]. We built a prototype pulsed power device to kill anisakid larvae, and this paper is an analysis of the effectiveness of this device.

2. Development

2.1 Fish to be treated

The device was intended to be used at a Japan Seafoods Co., Ltd. processing factory to process horse mackerel, which is the main product of the company. The edible portion of horse mackerel is about 35% of the whole fish. Treating the entire fish would waste electricity as the inedible portions would be treated as well. Therefore, the equipment was designed to treat horse mackerel fillets. The factory produces about 4 tons of horse mackerel fillets per day, and the first goal was to treat 200 kg (5%) in batches of 3 to 7 kg, which is the normal basket size for fillets used at the factory.

2.2 Verification of anisakid larvae death

Previous studies have confirmed that anisakid larvae can be killed by placing fish meat containing larvae between plate-to-plate electrodes in a saltwater media and repeatedly applying pulsed current [15,16]. Since it is difficult to find fish meat with naturally occurring anisakid larvae, larvae were artificially inserted into fillets and subjected to pulse treatment for the experiments. Anisakid larvae were collected from chub mackerel caught off the coast of Nagasaki, Japan. Representative larvae used in the experiments ($n = 32$) were confirmed to be *A. pegreffii* by PCR-restriction fragment length polymorphism (PCR-RFLP) analysis [17].

The method for artificially inserting larvae in the fillets was the same as in a previously reported paper [15]. Larvae were placed on horse mackerel fillets that had been partially cut, and transglutaminase (Activa, Ajinomoto) was used to glue the horse mackerel meat to trap the larvae inside. There is currently no way to reliably confirm that anisakid larvae are capable of infecting a human [9]. Japanese analysis standards for food safety regulation state that the criterion for determining parasite survival is spontaneous movement [18]. Therefore, anisakid larvae were stimulated with tweezers to check for movement, and those that did not move upon stimulation were defined as "immobile." Evaluations were conducted 24- and 48-hours post-treatment and larvae that were immobile at both evaluation times were judged as dead.

2.3 Reducing temperature increase in fish meat

Pulsed power produces a very high peak power, but the current is instantaneous and any subsequent rises in temperature can be offset by using the time between pulses as a cooling period. The temperatures of the buffer saltwater and fish meat between the electrodes, however, still rise due to Joule heating. It is important to keep the temperature of the fish meat as low as possible as increases in temperature damage the meat. For this reason, the electrodes are placed in adequately cycled buffer saltwater so that warm saltwater is continuously replaced with cold saltwater. The goal was to keep the temperature of the fish meat below 20 °C after treatment.

The Japanese Food Sanitation Act requires that electrodes that apply electric current to food be made of either iron, aluminum, platinum, or titanium [19]. Because this device uses saltwater, titanium was chosen as the electrode material due to its resistance to rust and its cost. The electrodes were circular titanium plane-to-plane electrodes with a diameter of 40 cm and a distance of 11 cm between electrodes, with a buffer saltwater

volume of 13.8 L between the electrodes and 180 L outside the electrodes. A plastic mesh basket containing fillets was placed between the electrodes and the current was applied through the basket.

2.4 Safety

This device is to be installed in a wet area of a fish processing factory. Since high voltages are involved, worker safety must be a top priority. To prevent exposure to high-voltage components, the device was designed for batch processing (pulses are applied in enclosed areas) and all parts that may be touched by workers are grounded.

2.5 Determination of specifications

The device consists of a fillet treatment tank and a pulse generator. The simplest capacitor-bank-type circuit was used as the pulse generator. A parameter design conformed to the ISO standard 16336:2014 based on Taguchi methods, aiming at minimized variability of product's function under various noise conditions. 2 levels of 10 factors (electrode polarity, capacitance, initial saltwater temperature, saltwater conductivity, fillets weight, charging voltage, operating frequency, pump flow rate, fillet size, number of pump spouts) were selected by preliminary investigations and assigned to a L_{12} orthogonal array experiment, which can statistically identify optimal levels for each factors efficiently through 12 experiments (effects of shot number of pulsed power as well as electrocution resistance of each anisakis were also considered in the experiments). The process details of this parameter design will be published in a separate paper [20].

3. Prototype design

3.1 Deciding treatment tank specifications

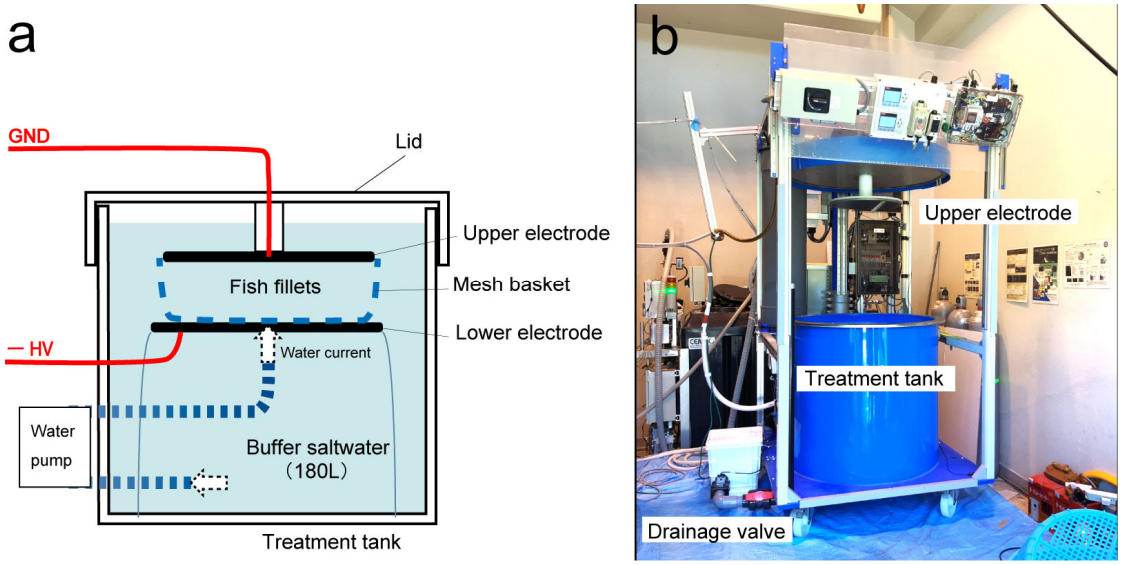

Fig. 1. Schematic diagram of treatment tank (a) and photo of treatment tank with lid open (b).

A schematic diagram and a photograph of the treatment tank are shown in Fig. 1. The treatment tank was designed with a lid so that the area exposed to high voltage was enclosed during treatment. The lower electrode is installed inside the treatment tank, and the upper electrode is attached to the lid, which moves up and down with the lid. A crane is used to raise and lower the lid. The treatment tank is a plastic circular tank surrounded by a cylindrical metal plate (ground), which was further covered with plastic.

In order to kill anisakid larvae, an electric current needs to be applied to the larvae, i.e., a fillet where larvae are present. The frequency dependence for fillet conductivity was measured by amplifying the voltage from a function generator with a high-speed bipolar amplifier, applying the voltage to the fillet between parallel plate

electrodes, and measuring the voltage and current waveform with an oscilloscope. At any frequency, there was no phase difference, so it consists only of resistive components. The results are shown in Fig. 2. The conductivity of the horse mackerel fillet varied individually by several mS cm^{-1} and increased as frequency increased, which is consistent with commonly known properties of biological tissues [21]. In the capacitor bank circuit used, the major frequency component of the output current was about 100 kHz and the average conductivity of the horse mackerel fillets at that frequency was about 8 mS cm^{-1}.

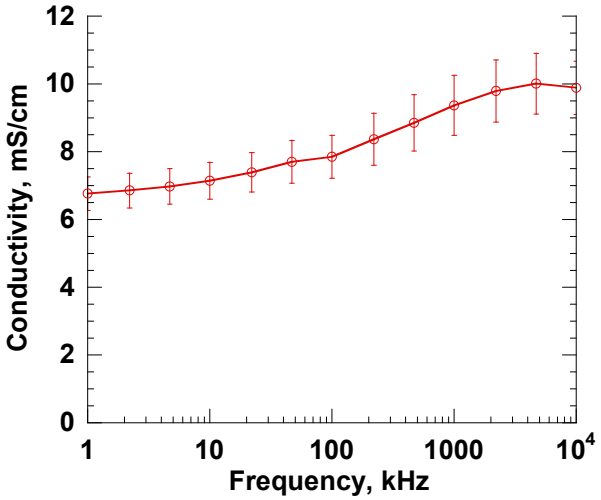

Fig. 2. Average conductivity of horse mackerel fillets (n = 8) at different frequencies.

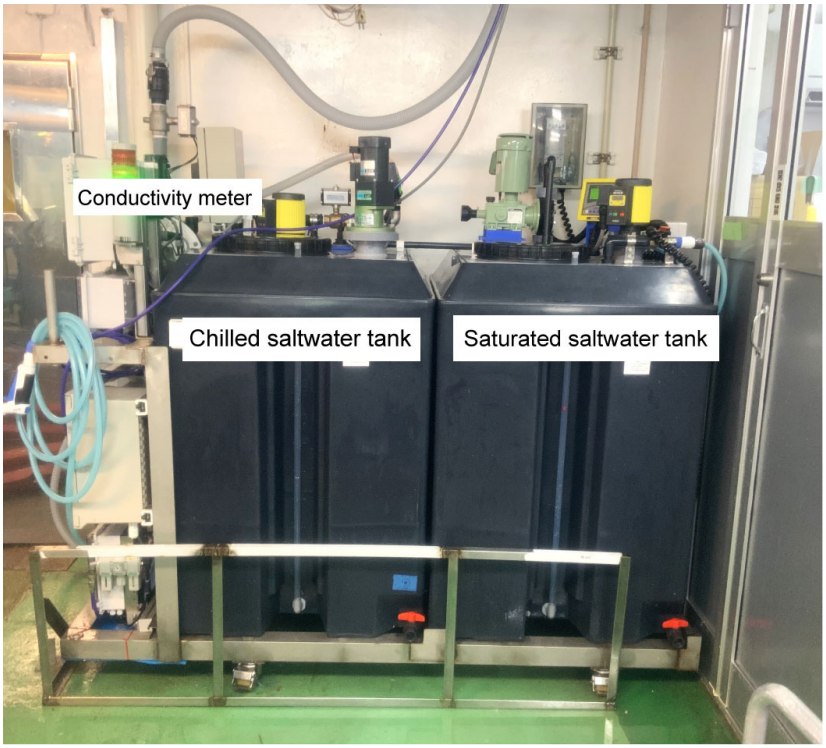

Fig. 3. Equipment for making saltwater with precisely adjusted conductivity.

Previous studies have shown that buffer saltwater has an optimal conductivity for killing anisakid larvae and that larvae are most effectively killed when the saltwater conductivity is about 5 mS cm^{-1} with 40 cm diameter electrodes separated by 11 cm [15]. When saltwater conductivity is high, the current flows preferentially through the saltwater rather than the fillet, making it difficult to kill larvae. When saltwater

conductivity is low, the current flows more easily through the fillet than through the saltwater, but the total current value is low because the impedance between the electrode and the fillet is high. Since 5 mS cm^{-1} equates to a salt concentration of about 0.3% and there were no devices on the market that could make saltwater at such low concentrations, we built equipment that could make saltwater at the set conductivity with an accuracy of ±5%. This equipment was designed to make saturated saltwater in one of two tanks and store water at approximately 4 ℃ in the other tank. Saturated saltwater was gradually added to the chilled water to adjust the conductivity as measured by a conductivity meter (Fig. 3). The chilled saltwater produced by this equipment is sent to the treatment tank to be used as buffer saltwater. Float switches were attached inside the treatment tank to control the water level. When pulse treatment is complete, a portion of the buffer saltwater in the treatment tank is drained and chilled saltwater is added to keep the temperature of the buffer saltwater in the treatment tank low. The amount of water drained can be set in advance, and all processes are done automatically.

Pump spouts were installed on the lower electrode to circulate cold saltwater and keep the fillets cold. The pump circulates buffer saltwater between the electrodes when the pulses are active. If the fillets are distributed unevenly between the electrodes, the path of the electric current does not run evenly through all the fillets due to the difference in conductivity between the fillets and the saltwater. Therefore, the water flow agitated the fillets so that the current would be applied uniformly. In order to adequately agitate the fillets in the basket, an upward spout was placed in the center of the electrode, and three spouts were placed around it at an angle of 45 degrees in a circle to circulate the water and rotate the basket containing the fillets, thereby agitating the fillets in the basket (Fig. 4). The pump flow rate was 72 L min^{-1} per pump, which means that the saltwater between the electrodes is replaced every 2.9 seconds (13.8 L ÷ 72 × 4 L min^{-1}). Fillets were treated in 3 kg batches, because larger volumes of fillets often resulted in poor agitation and thermal denaturation of fillets that clumped together.

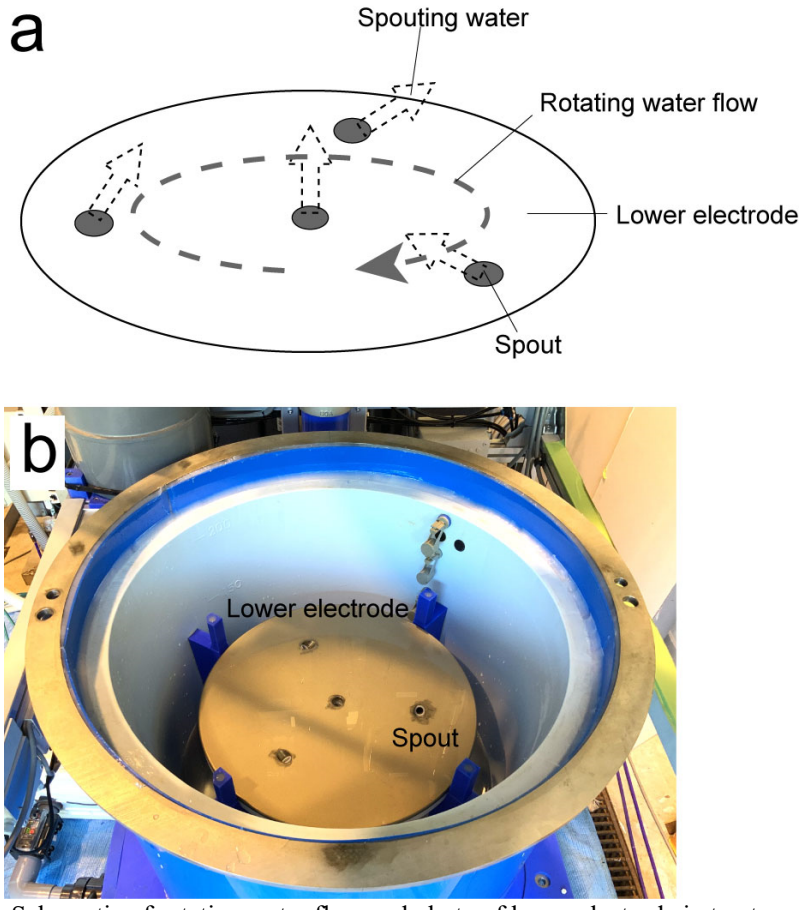

Fig. 4. Schematic of rotating water flow and photo of lower electrode in treatment tank.

3.2 Determination of pulsed power generator specifications and application conditions

3.2.1 Charging voltage

The dielectric breakdown strength of air is 30 kV cm^{-1}. Considering the electric field concentration coefficient of metal parts such as wires and electrodes, which are subject to high voltages, the upper charging voltage limit was set at 15 kV in order to keep the electric field strength on metal surfaces with high potential from the pulsed power generator to the electrodes below 30 kV cm^{-1}.

3.2.2 Operational parameters

The electrode polarity, capacitance, initial buffer saltwater temperature, saltwater conductivity, fillet weight, charging voltage, operating frequency, pump flow rate, fillet size, and number of pump spouts were optimized with L_{12} orthogonal array so that immobility ratio increases proportionally with increasing applied pulse numbers, where the proportionality investigated at 24 h and 48 h after pulse application.

Fig. 5 shows the results of an experiment conducted under the optimized conditions to maximize the anisakis inactivation (upper electrode polarity: positive, capacitor: 80 μF, initial saltwater temperature: 6 ℃, saltwater conductivity: 5 mS cm^{-1}, fillets weight: 4 kg, charging voltage: 15 kV, operating frequency: 0.5 Hz, pump flow rate: 150 L min^{-1}, fillet size: small, number of pump spouts: 1). The immobility ratio depends on shot number, with results indicating that 300 shots are required for 100% immobility. Since the inactivation ratio of anisakis larvae increases as the number of shots increases, it is thought that damage to anisakis accumulates and the damage reaches a lethal dose, leading to death, but the detailed mechanism is unclear and is a future theme. The charging voltage and capacitance of the prototype device were determined to be 15 kV and 80 μF, based on these conditions. The typical pulse waveform used in this device is the same as in Fig. 3 of the previous paper [15]. The peak values of voltage, current, and power are 15 kV, 6 kA, and 100 MW, respectively, and the time duration (10% of the peak) was about 380 μs. The energy input between the electrodes was 7 kJ, which means that the energy transfer efficiency from the capacitor to the load was 78%.

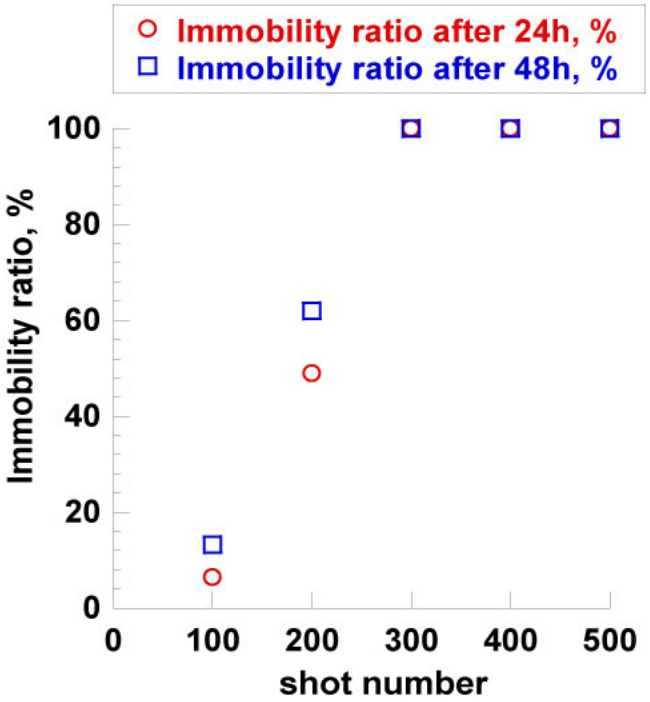

Fig. 5. Number of pulses applied and immobility ratio under conditions that maximize inactivation.

The main parameters for the pulsed power generator are the charging voltage, capacitance, and operating frequency. The charging voltage and capacitance were determined as described above. The effect of operating frequency on inactivation efficiency is small. When the operating frequency is low, the treatment time becomes

longer and the weight of fillets that can be treated per hour decreases. Also, the fillets spend more time immersed in saltwater and become damaged by osmotic pressure, although the time between pulse applications (fillet cooling period) is longer and temperature increase is suppressed. In practical use, a shorter treatment time is better, but raising the operating frequency increases the cost of the pulsed power generator, so the prototype was designed to run at 2.4 Hz. In a volume of 13.8 L (approximately 13.8 kg) between the electrodes, 3 kg of fillets are contained, and about 75% of the fillet component is water. Most of the material between the electrodes is water, and the specific heat of water is 4.2 kJ $kg^{-1} \cdot K^{-1}$. Calculating from this, the temperature rise of saltwater between the electrodes by single pulse is about 0.12°C (7 kJ/13.8 kg/4.2 kJ/(kg·K)). While applying 500 pulses, continuous circulation replaces the saltwater between the electrodes with cold buffer saltwater. As a result, the temperature of the fillet immediately after the treatment remains below 20 °C.

For the switches, semiconductor switches were selected for ease of maintenance and longevity. In order to ensure that the predetermined process was performed, a log of the applied voltage and current values was recorded, and the program was built to apply a preset number of pulsed currents with peaks within a set range. A photograph of the pulsed power generator is shown in Fig. 6.

Fig. 6. Pulsed power generator.

3.3 Treatment Process

The treatment process of the prototype device is described below:
1. The mesh basket containing the fillets is placed on the lower electrode.
2. The worker presses the start button.
3. The safety door is closed.
4. The treatment tank is filled with saltwater.
5. The upper electrode is lowered.
6. The pulses are applied.
7. The upper electrode and safety door are raised and the saltwater in the tank is drained.

The following safety measures were implemented:
- All surfaces of the device are grounded. As an additional precaution, a safety door was installed in front of the treatment tank to prevent workers from touching the tank during pulse application (Fig. 7).
- If there is air between the fillets when the basket containing fillets is placed in the treatment tank, abnormal discharge may occur when pulses are applied. Therefore, after the basket is placed in the treatment tank, the pump is run at low power to dislodge any air pockets, and then the upper electrode is lowered.

- We programmed the system to start applying pulses only after confirming that the saltwater in the treatment tank is full and the lid and safety door are closed.
- At the beginning of the pulse application, a low voltage pulse (1 kV) is applied to confirm the conductivity of the saltwater is within an appropriate range based on the current value before the main pulses begin.
- No water is added or drained during pulse application but all the saltwater in the treatment tank is at high potential during application. For this reason, the water supply and drainage pipes were designed to insulate the saltwater in the tank from the outside. Specifically, when saltwater is added, it is poured in from above to fill the treatment tank and, when the saltwater is drained, it is done so via a drain valve under the tank, thereby preventing saltwater from coming in contact with any parts other than the treatment tank.

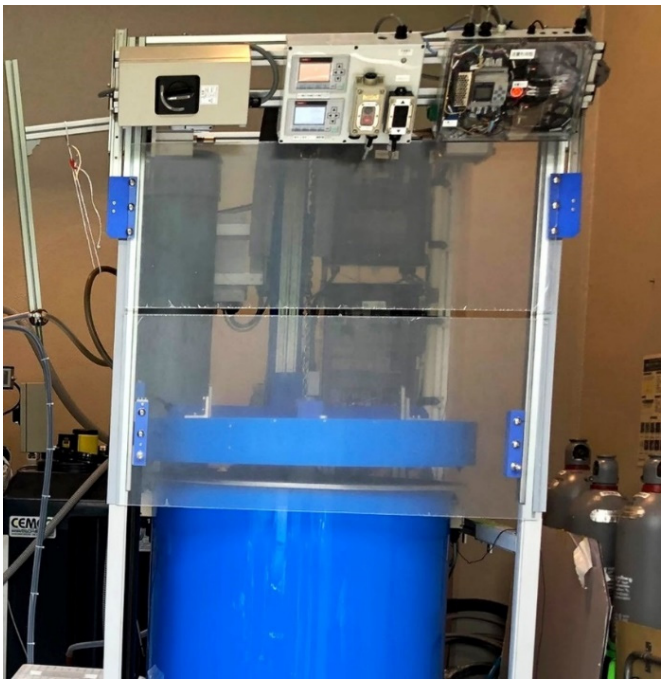

Fig. 7. Safety door at front of treatment tank.

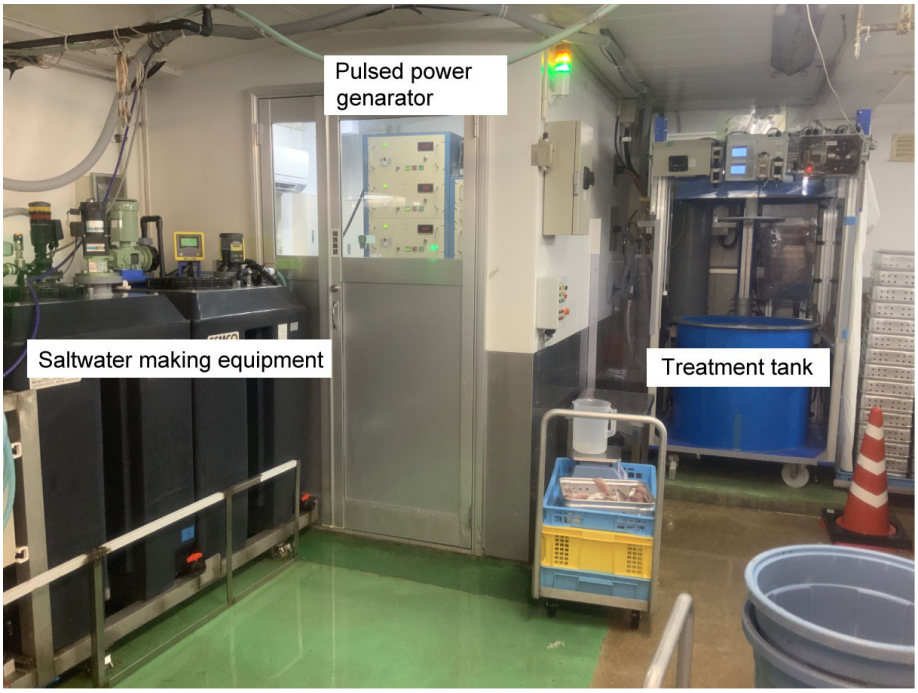

Fig. 8. Photo of the prototype installed at the factory.

3.4 Final prototype specifications

A photo of the device installed in the factory is shown in Fig. 8. The pulsed power generator is located in a separate room to guard against moisture. The final specifications and treatment conditions are listed in Table 1. The treatment time for one batch, including water drainage, water fill, lid raising, and lid lowering, is approximately 7 minutes.

Table 1. Final specifications of prototype.

Parameters	Value
Upper electrode polarity	Positive
Capacitance	80 μF
Initial saltwater temperature	6 °C
Saltwater conductivity	5 mS cm^{-1}
Fillet weight	3 kg
Charging voltage	15 kV
Frequency	2.4 Hz
Pump flow rate	72 L min^{-1}
Number of pump spouts	4
Shot number	500

3.5 Performance evaluation and field tests

3.5.1 Tests for killing anisakid larvae

Tests were conducted to confirm the inactivation of anisakid larvae by treating fillets artificially embedded with larvae. The fish size and meat properties of the fillets varied, and there were also individual differences among the fillets. To confirm that anisakid larvae can be killed regardless of fillet type, multiple tests were conducted using various types of fillets. A total of 500 pulses were applied per batch. Twelve tests were conducted in which 5 fillets containing 10 larvae per fillet were put into 3kg batches of fillets (10 larvae/fillet × 5 fillets × 12 tests = 600 larvae), and 2 tests in which 200 fillets containing one larva per fillet were put in each batch (1 larva/fillet × 200 fillets × 2 tests = 400 larvae). All 1,000 larvae were determined to be dead, equating to a kill ratio of greater than 99.9%. A total of approximately 10 tons of pulse-treated horse mackerel fillets have been sold, with no complaints of anisakid larvae, suggesting that this system reduces the risk of anisakiais with horse mackerel fillets.

3.5.2 Confirmation of safety of pulse-treated fillets

Because electric current is run between the electrodes in the saltwater, it is possible that ions of electrode material are eluted. To test this, the titanium content in the saltwater was measured using an ICP optical emission spectrometer after applying 5,000 pulses (10 times more than in the regular treatment). The results showed that concentrations were below the detection limit (less than 1 ppb). In order to confirm the food safety of pulse-treated horse mackerel fillets, microbiological and histamine tests were performed on the fillets immediately after pulse treatment and on the fillets stored at 5°C for 4 days after pulse treatment. Both showed a general viable count of less than 300 bacteria g^{-1}, a negative coliform group count (less than 10 bacteria g^{-1}), and a histamine level of less than 2.5 ppm, which was the same as the untreated fillets. This confirms that pulse treatment does not increase the risk of microorganisms and histamine levels.

6. Conclusion

The prototype device developed in this study is installed at a Japan Seafoods Co., Ltd. processing factory. Test results confirmed that all 1000 anisakid larvae were killed with treatment (a kill ratio over 99.9%). Horse mackerel fillets treated with this device are currently being test marketed to a number of customers and are praised for their quality and safety. After our press release, the technology was picked up by many media outlets (TV, radio, newspapers, magazines, etc.) in Japan and is attracting a great deal of attention. If this technology becomes widely available, we believe it will eliminate the risk of anisakiasis and protect the culture of consuming raw fish. The ability to safely eat raw fish without freezing could lead to an increase in fish consumption throughout the world. One issue is that the capacity of this prototype device is insufficient for the factory, and there is a need for a device that can treat large quantities of fish while using less energy. We are currently developing a conveyor device that applies pulses while the fillets are moving along a conveyor, which should allow us to treat more fillets in a shorter time.

Acknowledgment

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