

## Energy analysis of liquid whole egg pasteurized by pulsed electric fields

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### Abstract

Non-thermal preservation of liquid whole egg (LWE) with pulsed electric fields (PEF) is an attractive alternative to thermal processing where protein coagulation is of concern. The objective of this study was to evaluate the energy applied under different PEF processing conditions and its effect on the microbial quality of LWE during refrigerated storage. The LWE was stabilized with citric acid (CA) at 0.15% and 0.5% to prevent color darkening. Inside a pilot plant-size PEF chamber, the peak values of the electric field traces at the high-voltage electrode, middle gap, and low-voltage electrode were 37, 30, and 25 kV/cm, respectively. The pulse width was 1.84  $\mu$ s, with energy density at 11.9 J/ml per pulse. The total treatment time varied from 54 to 478  $\mu$ s (corresponding with 30–266 slightly underdamped pulses). The microbiological quality of the LWE was monitored weekly while under refrigerated storage at 4 °C. The CA not only acted as a color stabilizer but also increased the effectiveness of PEF treatment.

The maximum shelf-life sustained at 4 °C of LWE with 0.15% CA was 20 days, with PEF treatments up to 489  $\mu$ s (266 pulses) at an average electric field of 30 kV/cm. The total processing energy delivered to this product was 6331 J/ml. LWE with 0.5% CA had a shelf-life of almost 30 days at 4 °C, using a maximum PEF energy expenditure of 357 J/ml (30 pulses or 55  $\mu$ s of 30 kV/cm).

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### 1. Introduction

Processing whole egg products with additives, under current USA commercial pasteurization standards, involves subjecting the product to a temperature of 61.1 °C for 3.5 min (CFR 590.570, 2000). Based on this critical time–temperature relationship, it follows that a lower than specified temperature will decrease the efficiency of pasteurization, while overheating may result in coagulation of the egg and formation of a film on the heat exchanger surface (Powrie & Nakai, 1985). Pasteurized liquid egg products routinely contain less than 1000 organisms per gram. *Salmonella* tests are run regularly by the egg product industry, because only *Salmonella*-negative products can be sold. Although the normal presence of *Salmonella* spp. in unpasteurized egg is less than one per gram, current pasteurization pro-

cedures produce at least six log reductions (Vanderzant & Splittstoesser, 1992).

Pulsed electric fields (PEF) technology has been shown to be an attractive alternative to thermal treatments. However, this emerging technology must also maintain or surpass the main purpose of egg pasteurization, which is to yield a wholesome product by eliminating pathogenic bacteria. Primary concern has been *Salmonella* spp. because this organism is commonly found in eggs and egg products (Cunningham, 1986). Jeantet, Baron, Nau, Roignant, and Brule (1999) evaluated the inactivation of *S. enteritidis* in egg white, achieving a 3.5-log reduction in viable cells after nine exponential decay pulses of 35 kV/cm were applied. With a PEF treatment of 180  $\mu$ s at 28 kV/cm (in co-field treatment chamber), Sensoy, Zhang, and Sastry (1997) inactivated 4 log cycles of *S. dublin* (ATCC 15480) inoculated in a KCl solution with electrical conductivity at 0.47 S/m; and 5 log cycles when the microorganism was suspended in a KCl solution with 0.9669 S/m (liquid whole egg (LWE) has 0.6 S/m). Ohshima, Sato,

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Terauchi, and Sato (1997) studied the inactivation of *S. typhimurium* under controlled temperatures with and without PEF treatment (at 32 kV/cm) in a recirculation system of parallel plates, and found that the survival ratios at 50 °C with and without PEF were  $10^{-4}$  and  $10^{-1}$ , respectively. Alvarez, Raso, Palop, and Sala (2000) reported the inactivation of *S. senftenberg* suspended in a MacIlvaine citrate–phosphate buffer, treated for 600  $\mu$ s with 22 kV/cm in a parallel treatment chamber using square pulses of 2  $\mu$ s. The presence of coliforms is indicative of poor handling conditions, and thus a high risk of *Salmonella* presence. Martin-Belloso et al. (1997) achieved 6-log reductions of *E. coli* ATCC 11229 inoculated in LWE with a PEF treatment of 400  $\mu$ s at 26 kV/cm using a continuous coaxial treatment chamber and exponential decay-like pulses (pulse width 4  $\mu$ s), in a recirculation mode. Ma, Chang, Barbosa-Cánovas, and Swanson (1997) showed that by using the same system a stepwise process is more effective than the recirculation mode. Ma et al. (1997) inoculated *E. coli* ATCC 11775 on LWE and achieved a 6-log reduction in five steps with a total of 20 pulses of 2.5  $\mu$ s pulse width at 36 kV/cm.

*Pseudomonas* spp. is a common spoilage organism found in eggshells. Since most bacteria in egg products comes from the shell, *Pseudomonas* spoilage is common in egg products stored for extended periods (Vanderzant & Splittstoesser, 1992). Some important species of relevance in foods are *P. fluorescens*, *P. aeruginosa*, *P. putrida*, and *P. fragi*. Since each one is psychrotrophic, growth can occur at 5 °C and below, and multiply quite rapidly between 10 and 25 °C. Many foods are stored under aerobic, refrigerated conditions, providing an ideal environment for growth of these spoilage flora. In general, *Pseudomonas* spp. can taint milk, liquid eggs, fruit, vegetables, fish, and meats by contamination both before and after processing. It will cause detectable flavor defects at populations of  $10^6$  colony forming units (CFU) per milliliter, and under favorable conditions, may grow from  $10^1$  to  $10^6$  CFU/g in 12 days at 2 °C (Jay, 1996). Ho, Mittal, Cross, and Griffith (1995) studied the microbial reduction of *P. fluorescens* following PEF treatment in five media (distilled water, peptone, sucrose, Xanthan gum, and NaCl), each with different conductivity and rheological properties. “Instant” reverse pulses were delivered to a static PEF chamber containing stainless steel parallel disk electrodes, derlin as the insulator, and variable gaps of 0.3, 0.6, and 0.9 mm. The killing rate was found to be either extremely high or drastically low. With a gap of 0.3 mm, the microbial reduction was between 6.6 and 7.3 log cycles; at higher gaps the microbial inactivation was less than 1 log cycle. In both cases, no significant effects due to electric field strength (10–45 kV/cm), pulse period (2 and 4 s, pulse width of 2  $\mu$ s), or number of applied pulses (10–30 pulses at 0.3 mm gap and 10–100

pulses at 0.6 and 0.9 mm gaps) were observed. These extreme inactivation results can be explained by the differences in pulse waveforms caused by the gap differences, given that under the same fluid medium and electrical conditions, a decrease in electrode distance (to 0.3 cm) produces sudden reversal voltages or spikes. Góngora-Nieto et al. (2001) studied the inactivation, with PEF treatment, of the three *Pseudomonas* strains (ATCC 17400, ATCC13252 and WSU-07) suspended in LWE. The authors reported significant difference among the inactivation rates of the three strains, using continuous recirculation and a coaxial treatment chamber that delivered slightly underdamped pulses (pulse width 1.97  $\mu$ s). Experimental results showed that an isolated strain of *P. fluorescens* from spoiled LWE previously treated with PEF (*P. fluorescens* WSU-07) undergoes a total microbial reduction of 0.95 log cycles after 230  $\mu$ s of 48 kV/cm, while an ATCC 17400 strain is reduced by more than 3.5 log cycles. The third strain, ATCC 13525, proved intermediate in resistance, in comparison to the former strains, with a final microbial reduction of more than 2 log cycles. Even though research groups find the inactivation studies of certain species difficult to compare due to the difference in PEF systems, pulse shapes, and treatment chambers; their results offer good insight into the pasteurization levels achievable with PEF.

High quality and microbial stable LWE, immediately after PEF treatment and during refrigerated storage, has also been reported. A study conducted by Dunn and Pearlman (1987), in which fresh LWE was treated in a static parallel electrode PEF chamber 2 cm high, using 25 exponentially decaying pulses with peak voltages close to 36 kV, showed a final count around 2.7 log CFU/ml of naturally occurring flora. Refrigeration of PEF-treated LWE at 10 °C maintained a low count for four days, but at 4 °C, the initial count lasted for up to 10 days. This is especially significant since the control samples were unable to hold their microbial limits for more than a few hours.

Using PEF technology, Qin et al. (1995) reported the preservation of LWE that has been aseptically removed from the shell and stabilized with 0.15% citric acid (CA). A stepwise (three steps) approach was used to apply 20 pulses to the LWE in an electric field of 35 kV/cm. The treated liquid eggs were then aseptically filled into packaging bags for shelf-life study. Microbial and chemical analyses were conducted during the refrigerated storage period. The shelf-life was four weeks. There were no apparent changes in most of the eggs’ physical and chemical properties except for a drop in viscosity and an increase in color. Also, a sensory panel did not find detectable differences between the sensory attributes of scrambled eggs made with fresh and PEF-treated eggs. The quality attributes and long shelf-life extension achievable with PEF is very promising, because

refrigerated LWE products that have been thermally treated must be maintained unopened below 4 °C for 2–6 days maximum, depending on the microbial quality of the product (American Egg Board, 2000).

Ma, Chang, Góngora-Nieto, Barbosa-Cánovas, and Swanson (2000) compared the independent effects of PEF, high hydrostatic pressure, and thermal processing on the sensory, physical, chemical, and microbiological attributes of LWE. The results led to the conclusion that PEF (40  $\mu$ s at 48 kV/cm) is suitable for industrial implementation, since the quality attributes of LWE treated by this method are higher compared to other methods studied. Fernández-Díaz, Barsotti, Dumay, and Cheftel (2000) evaluated the effects of PEF on egg white ovalbumin solutions. A treatment of 200 (0.9  $\mu$ s) pulses at 30 kV/cm only slightly reduced the gelling properties of egg white.

Few inactivation studies report the energy delivered by PEF treatment and even fewer correlate the energy applied with the efficacy of PEF (Barbosa-Cánovas, Góngora-Nieto, Pothakamury, & Swanson, 1999; Evrendilek et al., 2000; Giner et al., 2000; Heinz, Phillips, Zenker, & Knorr, 1999; Ma et al., 1997; Martin-Belloso et al., 1997; Ohshima et al., 1997; Zhang, Monsalve-González, Qin, Barbosa-Cánovas, & Swanson, 1994). The energy stored in the capacitor of the PEF system is reported in some of these studies, and assuming a perfect RC circuit with this energy, is defined as follows:

$$Q = 0.5CV^2, \quad (1)$$

where  $Q$  is the energy per pulse,  $C$  the capacitance, and  $V$  the voltage at which the capacitor is charged. Due to energy loss in PEF systems, it is unlikely that Eq. (1) defines the energy the sample receives, even if  $V$  is considered as the peak voltage delivered to the treatment chamber. The energy delivered to an element of an electrical circuit at time  $t_1$  is given by the integral over time of the power, which by definition is the product of the voltage across the element and current through it

$$En(t_1) = \int_0^{t_1} W(t) dt; \quad W(t) = V_0(t)I(t), \quad (2)$$

where  $W(t)$  is the power as a function of time,  $V_0(t)$  the differential voltage as a function of time, and  $I(t)$  the current as a function of time. By allowing  $t_1$  to become much larger than the pulse width ( $t_1 \gg \tau$ ), the energy resulting from Eq. (2) can be used to calculate the energy density (ED) of the applied treatment:

$$ED = En(t_1)N/Vol, \quad (3)$$

where  $N$  is the number of pulses received by the product, and  $Vol$  the effective volume of the PEF treatment chamber. PEF processing of relatively highly conductive products such as LWE leads to ohmic heating of LWE, and thus also requires some cooling energy.

The objective of this study was to evaluate the energy applied under different PEF processing conditions, including the cooling energy and its effect on the microbial quality of LWE under refrigerated storage.

## 2. Materials and methods

### 2.1. Preparation of liquid whole egg

Fresh eggs from a local supermarket were inspected for shell integrity and washed with chlorinated water (200 ppm). The contents (whites and yolks) of selected eggs were removed under aseptic conditions, and collected in a sterilized container. The eggs were then beaten using an electric blender for 10 min at minimum speed. During mixing, CA was added to the product at the desired concentration level (0.15% or 0.5% respectively, by weight in volume), thus stabilizing the color of the product. The pH of the LWE samples with 0.15% CA was 7.3, while the sample with 0.5% was 7.1. The pH was registered by an Orion pH meter model 420A (Orion™ Research Co., Boston, MA). The conductivity of the LWE ranged between 0.6 and 0.7 S/m for temperatures between 9 and 35 °C, and was registered by a temperature controlled conductivity meter model CON 500 (Cole Parmer Instrument Co., Singapore). The homogenized and stabilized LWE was transferred into sterile glass containers and the above procedure repeated for each experiment.

### 2.2. Treatment of liquid whole egg

Stepwise and continuous recirculation modes were used to process the LWE. Although using several steps (stepwise) was the best approach to emulate the effects of multiple chambers, treatments involving more than four steps were operationally inconvenient. Thus, a recirculation mode was also used. In the stepwise mode, a batch is PEF processed, collected, and then retreated for as many steps as desired, cleaning with chlorinated water (rinsed by sterile water) in between steps. In the recirculation mode, the LWE is continuously pumped through the system until the desired number of pulses has been applied. A total volume of two liters per sample of 0.15% CA LWE were treated in continuous recirculation at a flow rate of 30 l/h, until 30, 89, 92, 172, and 266 pulses were reached. The stepwise processing of 0.15% CA LWE involved three to four consecutive steps at 10 pulses each (also at a 30 l/h flow rate). In preliminary studies, the treatment of LWE at 30 l/h presented some arcing (electrical sparking) problems. This was attributed to a deposition of LWE on the surface of the high-voltage electrode and the presence of air bubbles introduced during preparation. For this reason, a higher flow rate and a number of steps (to achieve the same

treatment delivery) were selected for the LWE samples with 0.5% CA. This solved the arcing problem and eliminated most of the protein deposition.

A total volume of two liters per sample of 0.5% CA LWE was continuously recirculated at 39 l/h, until 30 pulses were reached, or by using four steps to achieve a total of 30 pulses. Between treatment runs of all LWE products, a complete disassembly and thorough cleaning of the treatment chamber and fittings was conducted using chlorinated water (200 ppm), followed by a thorough rinse with sterilized water. Two cooling systems maintained the treatment temperature below 35 °C (one before and one after the PEF treatment chamber). The temperatures at the entrance and exit of the chamber were recorded with two digital thermocouples (DigiSense™, Cole Parmer Instrument Co., Veron Hills, IL). In both treatment modes the LWE was cooled to 7 °C immediately after PEF treatment. Twenty control and 20 PEF-treated samples were individually packaged in sterile polypropylene tubes (50 ml capacity) and stored in refrigerated rooms at 4 °C until needed for shelf-life evaluation.

### 2.3. Pulsed electric fields system

A PEF pilot plant pulsing system manufactured by Physics International (San Leandro, CA) was used to conduct the treatment. The PEF system charges a 0.5 μF capacitor to 40 kV and delivers a slightly underdamped pulse to a coaxial chamber with a 0.73 cm gap, and 28 ml of volume. The capacitor was charged and discharged at a repetition rate of 3 Hz.

### 2.4. Microbial analysis

Triplicate samples of LWE were taken before and after each PEF treatment for microbial plate counts. Samples were serially diluted with 0.1% sterile peptone solution. Dilutions were plated in replicate in PCA, PDA, and VRB agars. The plates were incubated at 37 °C for 24 h for VRB, 48 h for PCA, and 7 days at room temperature for PDA. Colonies in the plated agars were reported only if between 25 and 250 CFUs per plate existed. *Salmonella* contamination was determined by isolation from the LWE samples, following the approved method in the Bacteriological Analytical Manual as described by Vanderzant and Splittstoesser (1992).

Microbial stability during refrigerated storage at 4 °C was evaluated at 7, 14, 18, 21, 25, and 31 days. Shelf-life was established using the recommended microbial limits for liquid egg products: 25,000 CFU/ml of aerobic plate count (APC) with <10 CFU/ml coliforms, <10 CFU/ml yeasts and molds, and negative *Salmonella* (Vanderzant & Splittstoesser, 1992).

### 2.5. Treatment delivery and energy evaluation

The electrical parameters that define treatment delivery, including electric field, pulse width, and energy density, were determined by a computerized metrology system developed and validated by Góngora-Nieto, Younce, Hyde, Pedrow, Barbosa-Cánovas, and Swanson (2000). The metrology system consists of one current probe, two high-voltage probes, one four-channel oscilloscope, and a graphical monitoring program run by a desktop computer. The current probe was a Pearson 310X (Pearson Electronics™ Inc., Palo Alto, CA) with a divider ratio of 0.005 V/A (200 A/V), in a 50 Ω terminator. The high-voltage probes were a matched pair of PVM5™ (Northstar Research Corporation, Albuquerque, NM) with a divider ratio of 5000:1 and a 50 Ω double-shielded cable. The windows monitoring program via a GPIB card controlled the digital oscilloscope (Combiscope™ 3394B, Fluke, Everett, WA).

The monitoring program of the metrology system reports the differential voltage and current traces of PEF pulses, determining peak values and pulse width. From these traces and the dimensions of the chamber, the program calculates the peak values of the minimum, average, and maximum electric field experienced by the food in the treatment gap of the PEF chamber:

$$E_r(r_i) = \frac{V_D}{r \ln(R_{LV}/R_{HV})}; \quad R_{HV} \leq r_i \leq R_{LV}, \quad (4)$$

where  $V_D$  is the differential voltage across the chamber gap,  $r$  the radial position within the chamber gap (0.73 cm), and  $R_{LV}$  and  $R_{HV}$ , the radii of the low- and high-voltage electrodes, respectively. Coaxial treatment chambers can be designed to have a uniform electric field profile, yet with a non-uniform electric field. Across the gap of a very long coaxial geometry, there is a gradient of electric fields determined by Eq. (4). Although a PEF coaxial chamber is not very long, this is a convenient approximation. Electric fields calculated with Eq. (4) have a 99% agreement with mathematical simulation results for this particular PEF chamber (Góngora-Nieto, Pedrow, Barbosa-Cánovas, & Swanson, 2000). The monitoring system also calculates the energy per pulse and energy density using Eqs. (2) and (3).

The temperature readings registered by two digital thermocouples placed at the inlet and outlet of the PEF chamber were used to evaluate the energy removed in cooling the LWE after PEF:

$$E_d = (T_{out} - T_{in})C_p\rho_p, \quad (5)$$

where  $T_{out}$  and  $T_{in}$  are the outlet and inlet temperatures of the LWE at the two ports of the PEF treatment chamber during processing,  $C_p = 3.8$  J/kg °C is the specific heat of the product, and  $\rho_p = 1$  kg/l is the density of the product.

### 3. Results and discussion

#### 3.1. Microbial stability during refrigerated storage at 4 °C

The aseptic preparation of the LWE prior to PEF yielded a control product with less than 1 CFU/ml of yeast and mold, 20 CFU/ml or less of coliforms, 1000 CFU/ml or less of APC, and negative *Salmonella*. PEF treatment of LWE for any duration (number of pulses • pulse width) yielded a product with no detectable coliforms immediately after or during refrigerated storage. The APC counts of the different processed products under refrigerated storage are presented in Fig. 1.

For equal treatment times, stepwise treatment of LWE was slightly more effective in samples containing 0.15% CA and significantly more effective in samples containing 0.5% CA, than with continuous recirculation to maintain the microbial stability of LWE under refrigerated storage (Fig. 1). Thirty pulses applied to 0.15% CA LWE in the stepwise mode (Fig. 1: 30 P-S) achieved a shelf-life of almost one day more compared to the recirculation treatment (Fig. 1: 30 P-R) of the same product. At 0.5% CA, with 30 pulses applied in the stepwise mode (Fig. 1: 30 P-S 0.5% CA), the product shelf-life increased by approximately five days compared to the recirculation treatment (30 P-R 0.5% CA) yielding a total shelf-life of almost 30 days (Table 1).

The addition of CA verified its effectiveness in favoring a multiple hurdle approach when combined with PEF, achieving with as little as 30 pulses between 22 and 26 days of shelf-life (recirculation and stepwise, respectively). Previous shelf-life studies reported four weeks after applying an average electric field between 35 and 48 kV/cm (Ma et al., 1997; Qin et al., 1995). However, in this research a lower average electric field (30 kV/cm) was used.

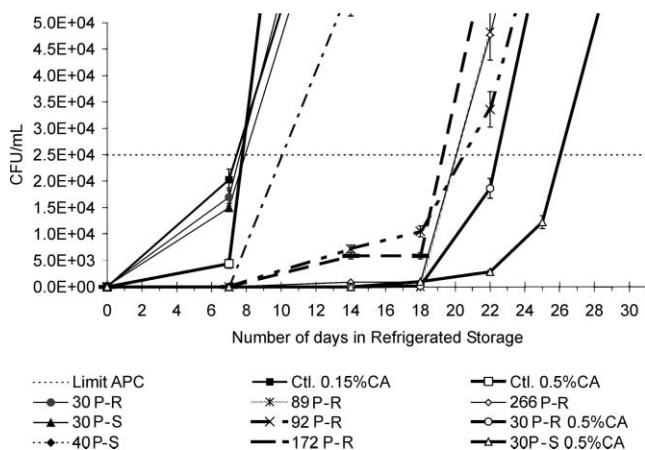


Fig. 1. Microbial quality expressed as APCs, of LWE during refrigerated storage at 4 °C: P, pulses; R, recirculation mode; S, stepwise mode; CA, citric acid. For products where no % CA was indicated, samples contained 0.15% CA.

Table 1

Total energy density delivered with PEF treatment and shelf-life extension of LWE

Product	Shelf-life at 4 °C (days)	PEF energy (kJ/l)	PEF plus cooling energy (kJ/l)
Ctl. 0.15% CA	7	0	0
Ctl. 0.5% CA	7.3	0	0
30 P-R <sup>a</sup>	7.1	357	714
30 P-S <sup>a</sup>	7.5	357	714
40 P-R <sup>a</sup>	10	476	952
89 P-R <sup>a</sup>	20	1059	2118
92 P-R <sup>a</sup>	20.5	1095	2190
172 P-R <sup>a</sup>	19	2047	4094
266 P-R <sup>a</sup>	20	3165	6331
30 P-R 0.5% CA	22.5	357	714
30 <sup>b</sup> P-S 0.5% CA	26	357	714

Ctl., control; CA, citric acid; P, number of pulses; R, recirculation mode; S, stepwise mode. i.e., 40 P-R = 40 pulses in recirculation mode.

<sup>a</sup> Products with 0.15% CA.

<sup>b</sup> Optimum processing condition.

In general an increase in treatment time yield more effective treatments, although it was noticed that PEF treatments beyond 89 pulses under recirculation of LWE with 0.15% CA did not significantly increase the refrigerated shelf-life of the samples (Fig. 1). Since the energy delivered by PEF treatment is proportional to the number of pulses, based on the shelf-life data, PEF treatments beyond 89 pulses would be a waste of both PEF and cooling energies (Table 1).

The undetectable microbial counts at the beginning of most of the treatments (Fig. 1) and the slow microbial growth followed by sudden microbial growth could indicate that very few cells survived PEF treatment and some remained injured for several days. This could also explain why treatments beyond 89 pulses did not significantly increase the shelf-life of LWE with 0.15% CA. It was observed that as storage time increased, variability in the APC of samples drawn to evaluate microbial quality during refrigerated storage also increased. To explain this, it was assumed that the treated product contained undetectable levels of injured cells that were non-homogeneously divided among the individually packaged samples. During refrigerated storage of LWE, the injured cells had the opportunity to recover and multiply, thus becoming detectable with plate count methods. If some samples were contaminated with slightly more injured cells than others, then as these cells began to recover, the colony plate counts of these samples became significantly higher.

#### 3.2. Treatment delivery and energy analysis

The differential voltage, current, and energy per pulse traces delivered by the PEF pulser were registered by the metrology system, as shown in Fig. 2. Since the presence

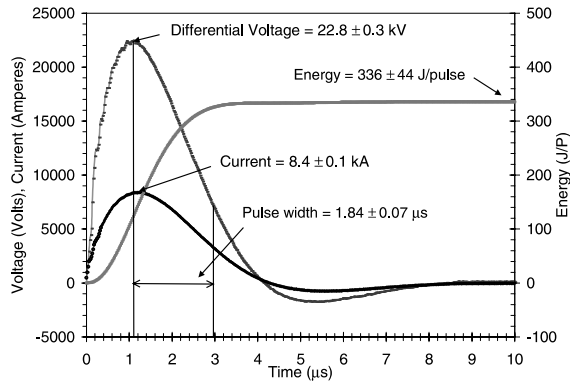


Fig. 2. Pulse traces delivered to a resistive coaxial treatment chamber, processing LWE by 0.5  $\mu\text{F}$  capacitor charged to 40 kV.

of CA had a negligible effect on the conductivity of the LWE, the electrical processing factors were the same for both products.

The peak differential voltage ( $22.8 \pm 0.3$  kV), peak current ( $8.4 \pm 0.1$  kA), peak energy per pulse ( $336 \pm 44$  J/pulse), and pulse width ( $1.84 \pm 0.07$   $\mu\text{s}$ ), indicated in Fig. 2, correspond to the average of 54 pulse traces sampled by the metrology system over three replicate runs. The pulse width was defined as the time difference between the peak voltage and  $1/e$  (37%) of the peak.

Fig. 3 illustrates the minimum, maximum, and average electric fields to which the LWE was subjected in the coaxial chamber. The minimum, maximum, and average peak values of the electric fields were 25.5, 30.4, and 37.63 kV/cm, respectively. Since this electric field distribution within the treatment gap is rarely mentioned, it could explain the inconsistent results when comparing studies by various research groups on different PEF systems and chambers.

Table 1 indicates the shelf-life extension achieved with each PEF treatment considering the shelf-life limit

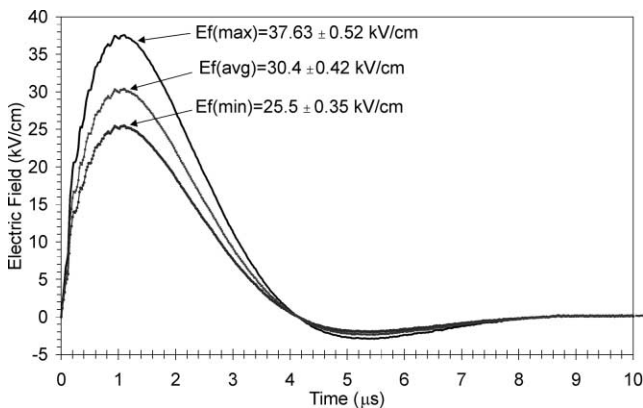


Fig. 3. Electric field profile of coaxial treatment chamber.

at 25,000 CFU/ml (Fig. 1) as well as the energy density delivered during different PEF treatments. At a flow rate of 30 l/h, a pulsing frequency of 3 Hz, and a chamber volume of 28 ml, the product receives 10 pulses with every pass through the PEF chamber. With a PEF energy per pulse of 336 J/pulse (Fig. 2), the total energy density per pulse is  $\sim 120$  J/ml. Due to the uncertainty propagation in evaluating PEF energy, the reported energy values have an uncertainty of 13% (Góngora-Nieto & Younce et al., 2000), and the energy density is closer to  $120 \pm 15$  J/ml. The average input temperature of the LWE was  $3.7 \pm 0.5$   $^{\circ}\text{C}$ , the average exit temperature  $35 \pm 1$   $^{\circ}\text{C}$ , and the average temperature rise  $31 \pm 2$   $^{\circ}\text{C}$ . This temperature rise is equivalent to  $119 \pm 8$  J/ml, quite close to the energy delivered per pass  $120 \pm 15$  J/ml (10 pulses). Accordingly, the required cooling energy is equivalent to that delivered by the PEF treatment, and a rough approximation of the minimum total energy—PEF plus cooling—would be twice that. Thus, if at least 357 kJ/l are delivered by a 30-pulse treatment, the—PEF plus cooling—energy will be at least 714 kJ/l (see Table 1: 30P-R, 30P-S).

In the PEF recirculation mode studies as the energy expenditure increased the shelf-life extension of LWE with 0.15% CA reached a plateau for treatments with pulses ranging from 89 to 266. This indicates unnecessary energy expenditure after an optimum point, suggesting that for process optimization minimum processing conditions with maximum shelf-life extension should be considered. In this particular case optimum processing conditions identify a 0.5% of CA concentration in the LWE, a maximum treatment of 30 pulses ( $\sim 55$   $\mu\text{s}$ ) in a stepwise mode or using a multiple chamber configuration, with a PEF energy expenditure of 357 kJ/l (Table 1), and requiring at least 714 kJ/l to PEF process and cool the product. It is worth mentioning that not all energy delivered by a decaying pulse is effectively used to inactivate the microorganisms present; some energy is only transformed into ohmic heating. Thus, if a correlation between the energy density delivered by PEF treatment and its efficacy to extend shelf-life and reduce microbial load is to be drawn, the effective energy must first be estimated. Furthermore, once the efficient energy is determined, it might be possible to find equivalencies among different processing pulse shapes.

#### 4. Conclusion

The energy applied in PEF pasteurization showed an optimum level of approximately 357 kJ/l, which yielded an LWE shelf-life of around 26 days; energy inputs beyond this value negligibly extended the product's shelf-life. A convenience product with a shelf-life close to 30 days could be a strong competitor of shelled eggs,

which should not be sold if 30 days old (American Egg Board, 2000).

The cooling energy was a direct function of the energy delivered by the pulses. Thus, if energy expenditure were to be minimized, a PEF treatment with minimum processing time would be ideal. Another way to reduce the energy density per pulse would be to reduce the energy per pulse, which has an inverse relationship with the PEF chamber resistance. Since the electrical conductivity of LWE is relatively high, the resistance of the coaxial treatment chamber decreases, generating an increase in current, power and energy delivered by each pulse. Hence, strategies to increase chamber resistance or different chamber configurations need to be evaluated.

Due to the electrical properties of LWE, the cooling energy required for the PEF process to maintain product temperature below 40 °C (low enough for non-thermal treatment and to prevent protein coagulation), is also significant, and optimal processing conditions must always be determined to assure efficient energy utilization. To minimize cooling energy, a sound approach would be the use of regenerative heat exchange between the LWE inlet cold stream and the heated stream following PEF. Without regenerative heat exchange, a minimum expenditure of 714 kJ/l (PEF plus cooling) is needed to obtain a microbiologically stable LWE product for 26 days under refrigerated storage. Optimization of processing conditions based on the minimum energy expenditure, to achieve a desired shelf-life, could be a sensible approach to minimizing the overall energy requirement of the PEF operation. This overall energy requirement should take into account the efficiency of the chillers used and the energy consumed by the power supply and circuitry needed to run the PEF system. A relatively high energy consumption could be justified by lower coagulation risk, superior quality (Ma et al., 1997; Qin et al., 1995), and extended shelf-life under refrigerated storage of LWE processed by PEF, as compared to thermally treated products.

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