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Synergistic effect of temperature and pulsed electric field on inactivation of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in liquid egg yolk

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Abstract

The effects of temperature, treatment time and electric field strength on inactivation of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in liquid egg yolk by pulsed electric field (PEF) processing were examined using the reaction kinetics approach. Egg yolk, inoculated with $\sim 10^8$ CFU mL^{-1} of *E. coli* O157:H7 or *S. enteritidis*, was treated in a continuous flow process at 20, 30 and 40 °C in combination with pulsed electric field intensities of 20 and 30 kV cm^{-1}. A biphasic instant reversal PEF waveform with a 2 μs pulse width was used and a maximum of 105 pulses were applied. Increasing the applied electric field intensity, treatment time and process temperature resulted in increased bacterial inactivation. At 30 kV cm^{-1} and 40 °C, the populations of *E. coli* O157:H7 and *S. enteritidis* were reduced by ~5 logs. The inactivation rate constants increased from 0.004 to 0.098 μ s^{-1} for *S. enteritidis* whereas for *E. coli* O157:H7 the constants increased from 0.009 to 0.039 μs^{-1} as processing temperature increased from 20 to 40 °C. *S. enteritidis* was more resistant to PEF inactivation than *E. coli* O157:H7 at lower processing temperatures.

Keywords: Pulsed electric field; Egg yolk; *Escherichia coli* O157:H7; *Salmonella enteritidis*; Microbial inactivation; Reaction kinetics

1. Introduction

The Canadian Food Inspection Agency (CFIA) requires that egg yolk be heated to at least 61 °C for 3.5 min in order to inactivate food spoilage and pathogenic microorganisms (Personal Communication). However, conventional heat pasteurization may adversely affect the functional properties, such as emulsification, foaming and gelatinisation of liquid egg yolk. In order to retain qualities of liquid egg, non-thermal technologies, such as pulsed electric field (PEF), may be used as an alternative or supplement to conventional heat pasteurization. PEF involves the application of high-power electric field pulses for short-duration (in the order of 1 μs) using electric field strength in the range from 20 to 80 kV cm^{-1} (Barbosa-Cánovas & Rodríguez, 2002; Rasgoti, 2003). Several studies have reported successful PEF-inactivation of pathogenic and food spoilage microorganisms as well as selected enzymes, resulting in better retention of flavours and nutrients and fresher taste compared to heat pasteurized products (Barbosa-Cánovas & Rodríguez, 2002; Barbosa-Cánovas, Góngora-Nieto, Pothakamury, & Swanson, 1999; Barsotti, Dumay, Mu, Diaz, & Cheftel, 2002; Bendicho, Barbosa-Cánovas, & Martín, 2002; Espachs-Barroso, Barbosa-Cánovas, & Martín-Belloso, 2003; Ho & Mittal, 2000; Jeyamkondan, Jayas, & Holley, 1999; Rasgoti, 2003; Van Loey, Verachtert, & Hendrickx, 2002).

The microbial lethality of PEF normally increases with electric field intensity (Bazhal, Ngadi, & Raghavan, 2006;

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Hülsherger, Potel, & Niemann, 1981; Wouters, Dutreux, Smelt, & Lelieveld, 1999). Synergistic interactions between PEF and thermal treatments have been reported at both lethal and non-lethal processing temperatures ranges (Bazhal et al., 2006; Hermawan, Evrendilek, Zhang, & Richter, 2004; Wouters et al., 1999). However, few studies have examined the combined effects of PEF and heat on inactivation of *Escherichia coli* O157:H7 or *Salmonella enteritidis* in liquid egg products. Using a static treatment chamber, Bazhal et al. (2006) reported an additional two log reduction for *E. coli* O157:H7 in liquid whole egg treated with PEF at low field intensities ranging from 9 to 15 kV cm^{-1} in conjuction with thermal treatment at 60 °C compared to thermal treatment alone. Hermawan et al. (2004) reported a maximum of 4.3log reduction in *S. enteritidis* counts after subjecting inoculated, pre-warmed (55 °C) liquid whole egg to a 25 kV cm^{-1} treatment at 200 Hz with 2.12 μs pulses totalling 250 μs.

Various models have been proposed to describe the kinetics of microbial inactivation by PEF. An early model suggested a linear relationship between the logarithm of the survival fraction and electric field strength, as was the case for the logarithm of the survival fraction and the logarithm of treatment duration (Hülsherger et al., 1981). Martín-Belloso et al. (1997) considered first-order kinetics by considering the exponential decay behaviour of the *E. coli* survival fraction with treatment time. Bazhal et al. (2006) also used an exponential decay model to predict the survival fraction rate of *E. coli* O157:H7 during PEF application with different pulses (0–138 pulses), field intensities (9–15 kV cm^{-1}) and treatment temperatures (50, 55 and 60 °C). The kinetic rate constant of the combined treatment varied from 0.025 to 0.119 $pulse^{-1}$ and from 0.034 to 0.228 $pulse^{-1}$ for 55 and 60 °C, respectively. The authors reported a synergistic effect of temperature with electric field on the inactivation of *E. coli* O157:H7 within a given temperature range (Bazhal et al., 2006).

The aim of this study was (i) to investigate the synergistic interaction of PEF and heat treatment on the inactivation of *E. coli* O157:H7 and *S. enteritidis* in liquid egg yolk and (ii) to model the inactivation kinetics using exponential decay model.

2. Materials and methods

2.1. Egg yolk

Large hen's eggs (GradeA, ~65 g) were obtained fresh from a commercial producer (Burnbrea Farm, Lyn, ON, Canada) and transported to our laboratory under refrigerated conditions (4 °C). Egg yolk samples were prepared in the laboratory by breaking the eggs under aseptic conditions and carefully separating the yolk from the egg white and chalazae. The separated egg yolk was then manually homogenized by gentle stirring and stored in sterile 250 mL Erlenmeyer glass flasks. Samples of 150 mL were aseptically withdrawn for experimental treatments.

2.2. Preparation of inoculum and inoculation

E. coli O157:H7 (ATCC 43894) and *S. enteritidis* (ATCC 13076), were used throughout this study. Cultures were grown to the early stationary phase in 50 mL of Brain Heart Infusion Broth (BHIB, DIFCO, 0037-17-8) incubated at 37 °C for approximately 12 h. Cells were harvested by centrifugation at 10,000*g* for 10 min (4 °C) and the cell pellets were washed three times by re-suspension in 10 mL of sterile distilled water. The washed pellets were finally re-suspended in 125 mL of liquid egg yolk to obtain an initial cell concentration of ~10^8 CFU mL^{-1}.

2.3. Bacterial enumeration

Counts of *E. coli* and *S. enteritidis* were evaluated, in triplicate, by plating appropriate dilutions of egg yolk in 0.1% peptone water onto a non-selective agar (TSA) and then overlaying with Violet Red Bile Agar (VRBA, DIFCO, 0012-17) and *Salmonella-Shigeilla* Agar (SSA, BD, 274500) before (control) and after PEF treatments. Prior to plating on the selective media, the processed egg samples were maintained at 4 °C for about 4 h to repair any injured cells. This method has been shown to be an effective technique for resuscitation of injured cells compared to plating on a non-selective medium and followed by overlaying with the appropriate selective media (Mussa, Ramaswamy, & Smith, 1999). Plates were incubated at 37 °C for 24 h and colonies were counted using a dark-field Quebec colony counter (Model 3327, AO Scientific Instruments, Keene, NH). All counts were expressed as Colony Forming Units per mL (CFU mL^{-1}).

2.4. Pulse treatment apparatus

A 1.45 mL continuous treatment chamber consisting of two parallel stainless steel electrodes separated by a 50 mm long Polyoxymethylen Derlin® chamber with a 290 mm^2 surface area was used to treat the samples (Fig. 1). A 30 kV pulse generator (TG-01, Samtech Ltd., Glasgow, UK) with a matched output impedance of 100 Ω was used. The output voltage profile followed a biphasic instant reversal square waveform with a pulse duration of 2 μs. The voltage and current across the treatment chamber were captured simultaneously using a two-channel digital oscilloscope (TDS3000, Tektronix, Wilsonville, OR).

Factorial $3 \times 2 \times 5$ experiments were run by feeding yolk continuously to the treatment chamber (Fig. 1) at 20, 30 and 40 °C and 20 and 30 kV cm^{-1} at a pulse frequency of 2 Hz. Samples of 2–3 mL were withdrawn aseptically at 0, 30, 60, 90 and 105 pulses and transferred to sterile test tubes and stored in an ice–water bath for the duration of the procedure. The entire volume of yolk (about 50 mL) recirculated throughout the system once every 15 pulses. The flow rate (F) was maintained at 12 mL min^{-1} by a peristaltic pump (Masterflex 77521-40, Cole-Parmer Instruments Co., Vernon Hills, IL) and a cooling system

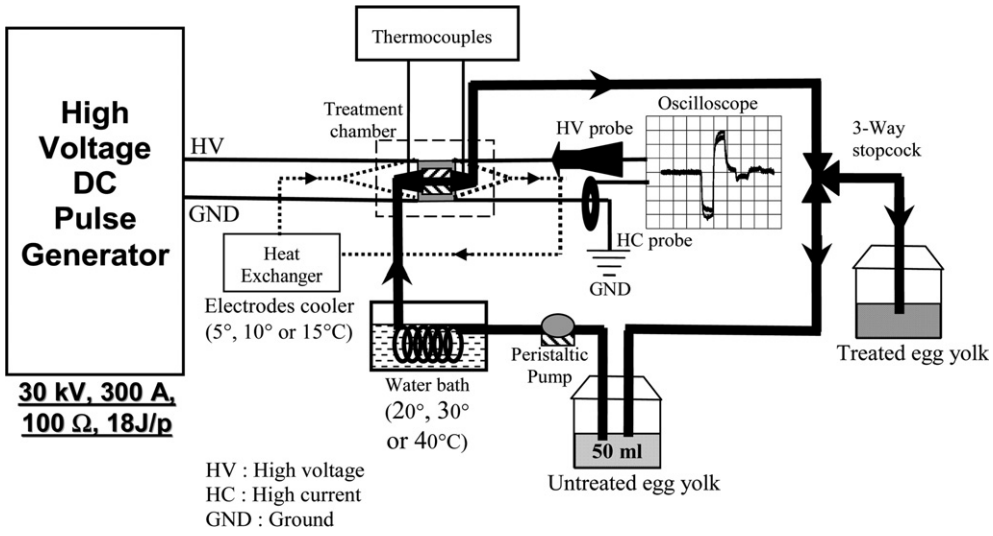

Fig. 1. Diagram of experimental continuous-flow PEF treatment apparatus.

was used to maintain constant temperature during the treatment.

$$F = \frac{f \cdot v}{n} \quad (1)$$

where, v is the volume of the treatment chamber (mL), f is the pulse frequency (Hz) and n is the applied pulse number.

Heat exchange with cold water varying from 5 to 15 °C depending on the treatment conditions was used to maintain constant temperature during the treatment. The pre- and post-PEF exposure temperatures (T_{inlet}–T_{outlet}) at the inlet and outlet of the treatment chamber were measured using a K-type thermocouple (OMEGA, Stamford, CT). The maximum average elevation temperature ($\Delta T = T_{outlet} - T_{inlet}$) during the PEF treatments was only 3 ± 1 °C. The apparatus was thoroughly cleaned with 70% ethyl alcohol and rinsed with sterile distilled water after each experimental run.

2.5. Inactivation kinetics modeling

The reduction of bacterial survival fraction as a function of treatment time at each electric field treatment may be expressed by a first-order kinetic model (Eq. (2)). The rate constants are dependent on electric field strength and temperature. The effect of temperature was described using the Arrhenius model (Eq. (3)):

$$s(t) = e^{-k_T(E)\cdot t} \quad (2)$$

$$k_T(E) = k_0 \cdot e^{-\frac{E_a}{RT}} \quad (3)$$

where $s(t)$ is the fraction of total survivors, t is the treatment duration (μs), $k_T(E)$ is the kinetic rate constant (μs^{-1}), T is the treatment temperature (K), E_a is activation energy (J mol^{-1}), R is gas constant (8.31 J K^{-1} mol^{-1}), and k_0 is a constant (μs^{-1}).

2.6. Data analysis

Regression analyses were conducted using Sigmaplot software (Sigmaplot, Version 6.00, 2000, SPSS Inc, Chicago, Illinois). Analysis of variance (ANOVA) was performed using the General Linear Models procedures (GLM) of the Statistical Analysis System (SAS, Version 8.02, 2001, Cary, NC, USA). Experiments were duplicated and the means of the two data sets are presented.

3. Results and discussion

3.1. Microbial inactivation

Pulsed electric field treatments had a significant effect ($P < 0.05$) on counts of *E. coli* O157:H7 and *S. enteritidis* in liquid egg yolk. As expected, process temperature and time had the most profound effect on microbial inactivation. The influence of temperature, duration of PEF exposure and strength of the applied electric field on bacterial inactivation in different egg products has been reported in other studies (Barbosa-Cánovas & Rodríguez, 2002; Barbosa-Cánovas et al., 1999; Barsotti et al., 2002; Bazhal et al., 2006; Espachs-Barroso et al., 2003; Rasgoti, 2003). Although increasing the electric field strength from 20 to 30 kV cm^{-1} increased the inactivation of *E. coli* O157:H7 and *S. enteritidis* in this study, these increases were only marginal and not significant at the 5% level. Hermawan et al. (2004) also observed marginal but non-significant effect (at the 5% level) on the inactivation of *S. enteritidis* that was inoculated into liquid whole egg using electric field strength in the range from 20 to 25 kV cm^{-1}, treatment time of 250 μs and temperature of 38 °C.

The survival fraction of *E. coli* O157:H7 after treatment using the electric field strength of 20 kV cm^{-1} for 210 μs, decreased by 1.4, 2.3 and 3.7 logs at the processing

temperatures of 20, 30 and 40 °C, respectively as shown in Fig. 2. Treatment using 30 kV cm^{-1} for the same 210 μs treatment time, resulted in a survival reduction of 1.9, 2.3 and 4.9 log at 20, 30 and 40 °C, respectively. Similar inactivation trends were obtained for *S. enteritidis* (Fig. 3). PEF treatments using 20 kV cm^{-1} resulted in survival ratio reductions of 0.5, 2.5 and 3.9 logs whereas the reductions at 30 kV cm^{-1}, were 0.8, 2.8 and 4.8 logs for *S. enteritidis* at temperatures of 20, 30 and 40 °C, respectively. Although it may be problematic to directly compare results from other studies (due to different PEF equipment specifications, products, etc.), the inactivation level reported by Hermawan et al. (2004) for *S. enteritidis* in whole liquid egg using electric field strength of 20 kV cm^{-1} at 38 °C compare well with the results obtained in this study using a similar electric field and temperature. Amiali, Ngadi, Smith, and Raghavan (2004) obtained a 3 log reduction in counts of *E. coli* O157:H7 in liquid egg yolk at 0 °C using PEF treatment of 15 kV cm^{-1}, but much longer treatment times of 500 pulses and 200 μs pulse width. The results show that inactivation rates at each electric field strength increased with increasing temperature. Microbial inactivation normally increases with an increase in treatment temperature (Jeantet, Baron, Nau, Roignant, & Brulé, 1999; Liang, Mittal, & Griffiths, 2002; Liu, Youcef, & Chism, 1997; Vega-Mercado, Martín-Belloso, Chang, Barbosa-Cánovas, & Swason, 1996). This increase in inactivation rate was higher at the higher electric field strength indicating synergy between temperature and electric field strength. Other authors (Bazhal, Ngadi, & Raghavan, 2003, 2006; Hermawan et al., 2004) have reported a synergistic effect of temperature and electric fields on bacterial inactivation in whole egg. The effect of processing temperature has been attributed to its influence on the fluidity of cellular membranes (Beney & Gervais, 2001). With increasing temperature, cellular membranes become more fluid and their mechanical resistance decreases (Zimmermann, Pilwat, & Riemann, 1974). Thus, higher field intensities are needed at lower temperatures to induce membrane rupture.

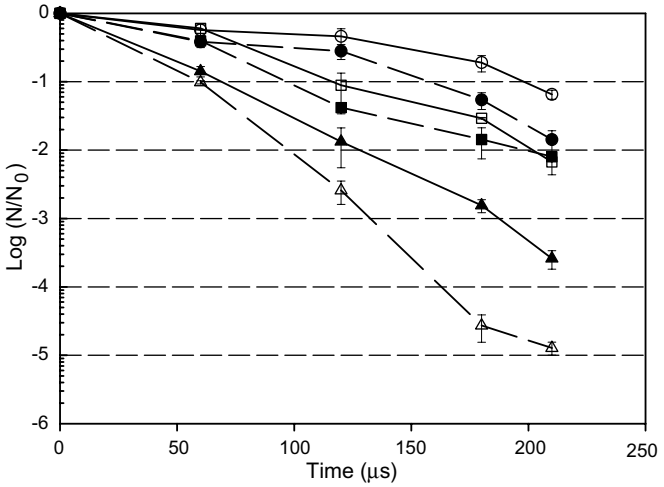

Fig. 2. Survival fraction of *E. coli* O157:H7 as a function of PEF treatment time, electric field strength and temperature. (○) 20 kV cm^{-1} and 20 °C; (●) 30 kV cm^{-1} and 20 °C; (□) 20 kV cm^{-1} and 30 °C; (■) 30 kV cm^{-1} and 30 °C; (△) 20 kV cm^{-1} and 40 °C; (▲) 30 kV cm^{-1} and 40 °C.

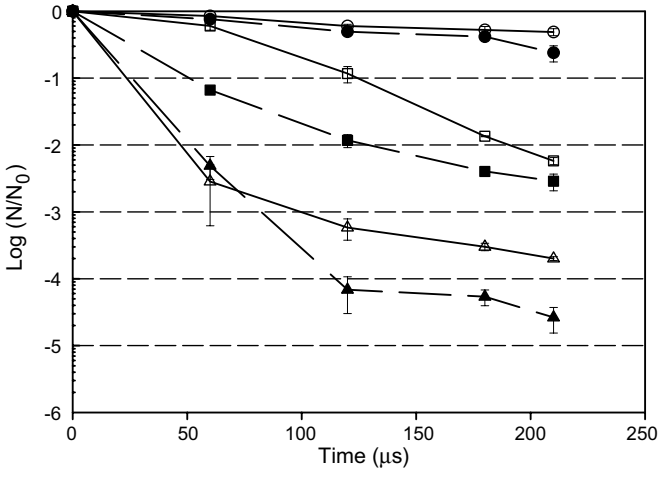

Fig. 3. Survival fraction of *S. enteritidis* as a function of PEF treatment time, electric field strength and temperature. (○) 20 k V cm^{-1} and 20 °C; (●) 30 kV cm^{-1} and 20 °C; (□) 20 kV cm^{-1} and 30 °C; (■) 30 kV cm^{-1} and 30 °C; (△) 20 kV cm^{-1} and 40 °C; (▲) 30 kV cm^{-1} and 40 °C.

3.2. Inactivation kinetics

The first-order kinetic equation adequately described the survival fraction of *E. coli* O157:H7 and *S. enteritidis* in liquid egg yolk for the treatments used in this study. The coefficient of determination (R^2) obtained for the different treatment levels varied from 0.947 to 0.999. The values of kinetic rate constants for the two pathogens are presented in Table 1. The rate constants increased from 0.009 to 0.039 μs^{-1} for *E. coli* O157:H7 and from 0.004 to 0.098 μs^{-1} for *S. enteritidis* within the range of process conditions used in the study. Martín-Belloso et al. (1997) obtained rate constants ranging from 0.108 to 0.178 pulses^{-1} (that is from 0.054 to 0.089 μs^{-1}, respectively for 2 μs pulse width, 26 kV cm^{-1}) for PEF inactivation of *E. coli* in whole liquid egg at 37 °C. Bazhal et al. (2006)

Table 1
Kinetic model rate constant (k_T) for inactivation of *E. coli* O157:H7 and *S. enteritidis* in liquid egg yolk by pulsed electric field at different electric field intensities and temperatures

Electric field (kV cm^{-1})	Temperature (°C)	*E. coli* O157:H7		*S. enteritidis*	
		$k_T \times 10^3$ (μs^{-1})	R^2	$k_T \times 10^3$ (μs^{-1})	R^2
20	20	8.8 ± 0.2	0.959	3.6 ± 0.6	0.976
	30	13.8 ± 0.9	0.947	13.7 ± 1.3	0.951
	40	32.8 ± 2.9	0.999	97.7 ± 1.5	0.999
30	20	13.9 ± 1.1	0.979	5.8 ± 0.2	0.975
	30	18.3 ± 3.4	0.998	44.9 ± 0.1	0.999
	40	38.9 ± 2.9	0.999	88.7 ± 6.4	0.999

reported inactivation kinetic rate constant of 0.119 pulse^{-1} (that is about 0.06 μs^{-1}) for *E. coli* O157:H7 using 15 kV cm^{-1} at 55 °C. The differences between the values reported in the literature and those obtained in this study may be attributed to differences in treatment parameters (e.g. medium, treatment chamber, energy delivered to samples, etc.).

Change in kinetic rate constants with respect to temperature was modeled using the Arrhenius equation. The data for *E. coli* O157:H7 yielded Eq. (4):

$$k_T = 2.46 \times 10^6 \cdot e^{\left[\frac{-47}{8.31\times10^{-3}\cdot T}\right]} \quad (4)$$

whereas, Eq. (5) was obtained for *S. enteritidis*,

$$k_T = 9.24 \times 10^6 \cdot e^{\left[\frac{-48.3}{8.31\times10^{-3}\cdot T}\right]} \quad (5)$$

The results show that the rate constants increased with increasing temperature as expected. Lower values of inactivation rate constants were obtained at the lower temperature of 20 °C for *S. enteritidis* compared to *E. coli* O157:H7. However, at the higher temperature of 40 °C, the rate constant for *S. enteritidis* was considerably higher than for *E. coli* O157:H7. The values of activation energies obtained for *E. coli* O157:H7 and *S. enteritidis* were 47 and 48.3 kJ mol^{-1}, respectively. The rate of change of kinetic rate constant with respect to temperature was lower for *E. coli* O157:H7 than for *S. enteritidis*. Apparently, *S. enteritidis* was more resistant at lower temperature but more susceptible to PEF inactivation at higher temperatures. This may be attributed to physiological difference the bacteria cells. Data on comparative inactivation resistances of *E. coli* O157:H7 and *S. enteritidis* is scarce in the scientific literature.

4. Conclusion

PEF treatment inactivated *E. coli* O157:H7 and *S. enteritidis* in fresh liquid egg yolk with the inactivation rate being a function of treatment time and temperature. The first-order inactivation kinetic model adequately described survival fraction of the pathogens as a function of treatment time. *S. enteritidis* was more resistant than *E. coli* O157:H7 at lower temperature whereas at higher temperature, *E. coli* O157:H7 was more resistant. This study has also shown that a maximum 5log reduction of both pathogens could be obtained using PEF treatment of 30 kV cm^{-1} at 40 °C. However, the residual inoculum (approximately 3×10^3 CFU mL^{-1}) could grow, particularly under mild to moderate temperature abuse conditions, to levels of public health concern in yolk. Therefore, PEF processing must be used in conjunction with strict refrigerated temperature to ensure the safety of processed egg products. Further studies are now underway to monitor the growth of this residual inoculum at refrigerated, mild (8 °C) and moderate (12 °C) temperature abuse conditions and the quality changes during storage.

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