Pasteurization of Shell Eggs using Radio Frequency Heating

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Abstract

The USDA-FSIS estimates that pasteurization of all shell eggs in the U.S. would reduce the annual number of illnesses by more than 110,000. However, less than 3% of shell eggs are commercially pasteurized. One of the main reasons for this is that the commercial hot water process requires as much as 60 min to complete. In the present study, a radio frequency (RF) apparatus was constructed, and a two-step process was developed that uses RF energy and hot water, to pasteurize eggs in less than half the time. In order to select an appropriate RF generator, the impedance of shell eggs was measured in the frequency range of 10 – 70 MHz. The power density within the egg was modeled to prevent potential hotspots. *Escherichia coli* (ATCC 35218) was inoculated in the yolk to approximately 7.5 log CFU/ml. The combination process first heated the egg in 35.0 °C water for 3.5 min using 60 MHz RF energy. This resulted in the yolk being preferentially heated to 61 °C. Then, the egg was heated for an additional 20 min with 56.7 °C water. This two-step process reduced the population of *E. coli* by 6.5 log. The total time for the process was 23.5 min. By contrast, processing for 60 min was required to reduce the *E. coli* by 6.6 log using just hot water. The novel RF pasteurization process presented in this study was considerably faster than the existing commercial process. This should lead to an increase in the percentage of eggs being pasteurized, as well as a reduction of foodborne illnesses.

Keywords

Radio frequency; dielectric heating; shell eggs; pasteurization; impedance; finite element modeling

Introduction

Shell eggs and egg products were ranked the second riskiest food regulated by the FDA, and were linked to 352 outbreaks from 1990 to 2006 (Klein et al., 2009). *Escherichia coli*, including extraintestinal pathogenic *E. coli* (ExPEC), has been isolated from eggs (Englmaierova et al. 2014; Mitchell et al. 2015). The USDA-FSIS estimates that pasteurization of all shell eggs in the United States (i.e., reducing the bacterial population by 5 log) would decrease the annual number of illnesses by more than 110,000 (Schroeder et al., 2006).

Currently, only two companies in the U.S. pasteurize shell eggs. They both use hot water immersion whereby the eggs are submerged in water at an elevated temperature for approximately 60 min (Day, 2010). There are two reasons for the lengthy time required for the commercial hot water process. The first is the limiting temperature of the water. The foaming power of albumen begins to decline when momentarily subjected to a temperature of 57 °C (Cunningham, 1995). Van Lith et al. (1995) immersed shell eggs in hot water and determined that 57 °C is the maximum water temperature that can be used without coagulating the albumen. Pathogenic bacteria are not quickly inactivated at 56.7 °C. The U.S. Department of Agriculture's required minimum holding times for pasteurizing liquid yolk are 6.2 and 3.5 min at 60.0 and 61.1 °C, respectively (USDA, 1969). At a temperature of 56.7 °C, the pasteurization holding time is estimated to be 35 minutes (USDA 1969). Based on the results of Humphrey et al. (1990), who determined the *D*-values of *Salmonella* in egg yolk at 55 and 60 °C, the *D*-value at 56.7 °C is approximately 7.4 min (Geveke et al. 2016). The second reason for the protracted hot water process is that the heat must first be transferred from the water to the shell, and then, by conduction, from the shell to the heat-sensitive albumen, and finally to the yolk. The come-up time (i.e., the time required to raise the yolk temperature to 57 °C) is 20 min or longer (Cox et

al., 1996; Geveke et al., 2016; Park and Cho, 2006; Stadelman et al., 1996). The lengthy hot water immersion process contributes to the cost of commercially pasteurized eggs. In some cases, adding more than \$1 to the price of a dozen eggs (Sinclair, 2012).

Alternative techniques for pasteurizing shell eggs have been researched, but only one of these has been commercialized. This process uses microwave energy and is practiced only in South Africa (Wiid, 2008). Microwave energy can penetrate into the shell egg and heat up the yolk faster than hot water. However, the penetration depth of microwaves (915 and 2450 MHz) in foods can be as low as 0.3 cm (Tang et al., 2004). This means that very little microwave energy may make it to the yolk. The patent, which forms the basis of the South African microwave process, indicates that the method takes approximately 30 to 40 min and results in a bacterial reduction of between 2 and 5 log (Erasmus and Rossouw, 2008). In addition, the process causes a slight haziness of the albumen and a reduction in whipping ability (Wiid, 2008).

Penetration depth of electromagnetic energy is inversely dependent on the frequency. Thus, radio frequency (RF) energy, which ranges from 10 to 300 MHz (Orfeuil, 1987), can have a penetration depth ten times greater than that of microwave energy (Tang et al., 2004). The deeper penetration of RF energy should heat the yolk better than microwave energy and prevent loss of albumen quality. The use of RF energy to pasteurize shell eggs is discussed very little in the literature. Hamid-Samimi et al. (2002) reveal the use of RF energy to rapidly pasteurize egg products. However, the majority of their patent deals with processing liquid egg products and scant information is given regarding shell eggs. More recently, Dev et al. (2012) modeled the heating of shell eggs in a parallel plate RF applicator. The modeling showed that heating would be highly nonuniform and may lead to the generation of hot spots in the albumen. The modeling also indicated that rotating the eggs would reduce the nonuniformity; however, these results were

not experimentally validated. Kannan et al. (2013) heated eggs with a parallel plate RF applicator to 56 °C. Coagulation of the albumen was avoided, but only by prolonging the heat-up time to 45 min which approaches the time required for conventional hot water processing. In addition, no microbiological testing was performed.

RF energy, applied with a unique converged applicator, has been used to pasteurize apple cider and orange juice with minimal effects on quality (Geveke et al., 2007; Geveke and Brunkhorst, 2008). Research is needed to determine if shell eggs can be pasteurized using RF energy, applied in a more precise manner rather than by a simple parallel plate applicator. The present study developed a RF applicator that placed the egg in direct contact with curved electrodes. The equivalent circuit of the egg and electrodes is a series RC circuit. The resistance (R) is composed of the conductive albumen and yolk, while the capacitance (C) is due to the egg shell acting as a dielectric. Application of RF to the electrodes creates electric fields in both the resistive and capacitive portions of the egg. Due to the conductive nature of the albumen and yolk, power is dissipated ohmically. By closing the gap between the electrodes and the shell, the capacitance of the RC circuit is increased. Thus, a lower voltage at the electrodes is required to maintain the same degree of heating, and the potential for hotspots is minimized. Most commercial RF power systems operate at a source impedance of 50 Ω . For maximum power transfer, the load impedance should match the source impedance. The optimum frequency for impedance matching would be where the series impedance of the egg/electrode circuit would be closest to a value of 50 + j0 Ω . The intention of using this RF applicator is to gently pasteurize shell eggs in less time than using the conventional hot water immersion method with the aim of increasing the percentage of eggs pasteurized in the U.S. and reducing foodborne illness.

The major goals of this study were to: (1) design and assemble an apparatus to apply RF energy to a shell egg in a controlled fashion; and (2) develop a process for pasteurizing shell eggs using RF energy. To these ends, additional objectives included measuring the impedance of a shell egg and modeling the power density within the egg during RF processing.

Materials and Methods

Escherichia coli (ATCC 35218), a surrogate for *Salmonella*, was maintained on tryptic soy agar (TSA; Becton, Dickinson and Company, Franklin Lakes, NJ) at 4 °C. The *E. coli* was cultured in tryptic soy broth (Becton, Dickinson and Company) with shaking at 37 °C for 16-18 h.

Shell eggs were obtained from a local commercial egg producer. Eggs were sorted to obtain eggs weighing 61 to 64 g each and were stored overnight at room temperature (23 °C) prior to being inoculated.

The large ends of the eggs were first perforated by hand with an 18 gauge sterile needle (Becton, Dickinson and Company). Following shell perforation, eggs (with the large end up) were placed in an inoculation device (designed and assembled in-house) that precisely injected the *E. coli* inoculum into the geometric centers of the yolks. The device was comprised of a Hamilton, Gastight model 1725LT, luer tip, autoclavable, 250 µl glass syringe (Hamilton Co., Reno, NV) and a 16 gauge, 3.8 cm needle (Beckton-Dickson) that were clamped to a low-speed, battery-powered actuator. The glass syringe was filled with the inoculum and centered over the hole in the large end of the egg. The actuator slowly pushed the tip of the needle through the hole to a depth of 3.2 cm and into the center of the yolk. Eggs were then slowly injected with 50 µl of inoculum. This was followed by a 30 s delay to permit pressure equilibration within the egg to

prevent inoculum leakage from the yolk. The actuator then slowly retracted the needle and the egg hole was sealed with a drop of fast-curing epoxy gel (1 Minute™ #14277, ITW Devcon, Danvers, MA) and allowed to cure for at least 30 min before thermal treatments. The population of *E. coli* in the whole egg was measured at approximately 7.5 log CFU/ml (positive control).

Preliminary trials using a dye technique were done to confirm that cultures were inoculated into the geometric center of the yolks (Brackett et al., 2001). This technique consisted of injecting 50 µl of dye into the egg followed by a standard hard-boiling procedure to demonstrate consistent placement of the dye near the center of the yolk with no discernible drift (as shown in Figure 1).

For treatment with RF, a unique apparatus was designed and assembled in-house. The apparatus comprised an electrode that was attached to the large end of the egg and another to the small end (Fig. 2). The electrodes consisted of copper mesh (woven wire cloth, wire diameter 0.028 cm, open area 67.9%, McMaster-Carr, Elmhurst, IL) that was held in place with a retaining ring of zinc plated steel (for 4.1 cm shaft diameter, McMaster-Carr). A mesh was selected because the wire structure provided excellent electrical conductivity. The spaces between the wires allowed heat to freely transfer from the shell outwards, thus preventing any localized hot spots directly below the shell in the albumen. A clamp, made of high-strength electrical insulating material (Ultem, McMaster-Carr), applied a slight force to the electrodes which resulted in good contact between the electrodes and the egg. The egg and RF apparatus electrodes were then submerged in 35 °C deionized water by placing them in a 2.5 l plastic water bath. The purpose of the water was to cool the egg shell and protect the albumen from overheating. Thus, the RF energy preferentially heated the yolk.

The electrodes were connected via a coaxial cable to a RF power supply (Model CPS1000/60, Comdel, Gloucester, MA). The power supply produced up to 1 kW at an output resistance of 50 Ω and a frequency of 60 MHz. This frequency was selected based on measurements made on a room temperature shell egg with a vector impedance analyzer (model TE3000, Trewmac Systems Pty Ltd., Scott Creek, South Australia, AU). The impedance was measured across the egg between the large and small ends using the RF treatment apparatus shown in Figure 2. Because the impedance results indicated 60 MHz would provide a fairly good match, a 60 MHz power supply was purchased. An impedance matching network was designed and assembled in-house and installed into the RF circuit to adjust the resistance to 50 Ω . This ensured maximum coupling of energy from the power supply to the egg. The power supply included meters that measured forward and reflected power. Because of excellent matching, the reflected power was never more than 5 W. The net power (i.e., forward minus reflected) is reported throughout.

The experimental procedure consisted of two parts. In the first part, RF energy was applied to the shell egg. The RF power was applied in segments. Between these applications, the egg was manually rotated to improve the temperature uniformity, as discussed in the Results and Discussion section. The egg was rotated around the axis running from the large end to the small end. Each rotation step was completed in 10 s. The RF power was reduced in the latter segments (from 50 W to 25 W) to further prevent damaging the heat sensitive albumen. This was compensated for by increasing the treatment times (from 45 s to 60 s). The step by step method was to apply 50 W to an egg for 45 s, rotate the egg 180° in 10 s, apply an additional 50 W for 45 s, rotate 90° in 10 s, apply 25 W for 60 s, rotate 180° in 10 s, and finally apply an additional 25 W for 60 s.

In the second and final part of the experimental procedure, the egg was disconnected from the RF apparatus and placed in a circulating water bath (model Isotemp 3016S, Fisher Scientific, Pittsburgh, PA) at 56.7 °C for 20 min. The temperature of the water bath was measured with a type K thermocouple connected to a data logger (model HH309A, OMEGA Engineering, Stamford, CT).

In some instances, eggs were not processed with RF energy (eliminating the first part), but were only placed in the 56.7 °C water bath for 20 or 60 min. This was done to compare the antimicrobial efficacy of hot water immersion alone to that of the combined RF and hot water process.

Eggs were sampled by first aseptically cracking the contents into a stainless steel Waring Mini-Sample Blender Container (model MC2, Waring Products, Torrington, CT), and blending on medium speed with a Waring model LB10G variable speed blender for 1 min. The contents were then serially diluted with sterile 0.1% peptone solution and plated onto Petrifilm Aerobic Count Plates (3M, St. Paul, MN). Plates were incubated at 37 °C for 24 h before enumeration.

In order to determine the temperatures of the albumen and yolk following RF treatment, the contents of an egg were immediately placed in a Petri dish. Then, the temperatures of the albumen and yolk were measured with a type K thermocouple.

Each experiment was performed in triplicate with duplicate plating. The standard deviation was calculated using Microsoft Excel statistical analysis algorithms.

The anisotropic alternating current (AC) flow within a shell egg was modeled with the finite element analysis software QuickField™ (version 5.0, Tera Analysis Ltd, Svendborg, Denmark). The model provided the power density distribution, in W/m^3 at 100 V_{rms} at 60 MHz, within the egg. The shell egg consisted of the following components: air sac, albumen, yolk, and shell. The

proportions and geometry are inexact; however, the model does serve by showing the effect of the air sac and the difference in properties of the various egg components. The following electrical properties of the egg were used in this model: dielectric constant of albumen, yolk, and shell of 80, 60 and 3.5, respectively, as well as conductivity of albumen, yolk, and shell of 0.78, 0.25, and 0.0 S/m, respectively (Dev et al., 2008; Ragni et al., 2007).

Results and Discussion

For comparison to the radio frequency pasteurization process, shell eggs were pasteurized using a hot water immersion method, which was representative of the commercial processes used in the United States. When inoculated shell eggs were immersed in a circulating water bath at 56.7 °C for 20 min, the reduction of *E. coli* was 1.8 ± 0.3 log CFU/ml. When the water bath treatment was extended to 60 min, the inactivation increased to 6.6 ± 0.8 log CFU/ml. These inactivation results agree with those of others who have studied hot water processing of inoculated shell eggs. Immersing shell eggs in a 57 °C circulating water bath for 25 min gave reductions in *Salmonella* Enteritidis (SE) of about 3 log (Hou et al., 1996). The population of SE in shell eggs was reduced by greater than 5.8 log after a 65 min treatment in a 57 °C circulating water bath (Schuman et al., 1997) and by 4.5 log after a 60 min treatment in a 56.7 °C circulating water bath (Geveke et al., 2016). The maximum water temperature that will not affect the quality characteristics of the eggs is 57 °C (Van Lith et al., 1995).

The application of radio frequency (RF) heating could substantially reduce the total time to pasteurize shell eggs. This is because, unlike hot water which only heats the shell, RF energy has the potential to immediately penetrate into the egg. Therefore, the authors designed and assembled an apparatus (shown in Figure 2) that applies RF energy directly to a shell egg. As not

all frequencies may provide a good electrical match with the egg, impedance measurements were performed to determine which frequency range would be most suitable. The results are shown in Table 1, and are the first of their kind, as far as the authors know. The resistance at 60 MHz was 53.6Ω and was the closest to 50Ω of all frequencies tested. Most RF generators prefer to be driven in to a 50Ω load. In addition, the reactance at this frequency was 3.9Ω which was one of the closest to 0Ω of all frequencies tested (Table 1). A reactance of 0Ω indicates an excellent match between the load (egg) and RF generator. Based on the impedance results, a 60 MHz RF generator was used for the shell egg pasteurization experiments.

Table 1. Impedances of a shell egg, at various frequencies, at the contacts at the small and large ends of the egg.

Frequency, MHz	Impedance, Ω
10	$40.7 - j134$
20	$32.0 - j62.8$
30	$34.1 - j36.0$
40	$35.9 - j18.1$
50	$42.1 - j5.11$
60	$53.6 + j3.92$

$$70$$

$$71.2 + j4.33$$

The anisotropic AC flow within the egg was modeled to determine the power distribution within the egg as described in the Materials and Methods section. The power density is the product of conductivity times the square of the electric field strength. The RF current flow within the egg is nonuniform, due to the geometry of the egg and electrodes, and the difference in conductive properties of the various egg components. Figure 3 presents the model's results for an applied $100 V_{\text{rms}}$ at 60 MHz. The highest power densities within the egg are located directly below the shell in the vicinity of the electrodes at either ends of the egg. Furthermore, the power densities are much higher in the albumen than in the yolk. These areas of elevated power density led to hot spots and damage to the albumen near the electrodes when eggs were heated with RF energy in air. Therefore, the RF treatment chamber was bathed in circulating water at 35.0°C to cool the shell and albumen. As an additional precaution to prevent the occurrence of hot spots, the egg was rotated around its long axis. This was done because both the eggs and the RF treatment chamber are not perfectly symmetrical. These asymmetries could lead to an additive effect; that is, more power could be applied along one side of the egg than the other. By maintaining the position of the RF chamber and rotating the egg, the odds of creating a hot spot are decreased. Visual observation of the egg contents indicated that the combination of applying cooling water and rotating the eggs eliminated damage to the albumen.

The goal of the present study was to develop a faster pasteurization method for shell eggs as an alternative to the conventional hot water method. Consequently, a two-step RF/hot water immersion process was developed. In the first step, a shell egg was placed in a custom-built RF

treatment apparatus, as shown in Figure 2. The RF device was placed in a 35 °C water bath and 60 MHz RF was applied for 3.5 min using the method given in the Materials and Methods section (above). In the second step, the egg was removed from the RF applicator and placed in a 56.7° C water bath for 20 minutes. Following this two-step procedure, the population of *E. coli* was reduced by 6.5 ± 0.1 log CFU/ml. The appearances of the albumen and yolk were good with no visible damage. Similar results have been obtained at slightly different combinations of cooling water temperature, RF power and duration than those used in this study (data not shown).

The maximum temperatures of the albumen and yolk were 50 and 61 °C, respectively, after the 3.5 min RF treatment (at the end of the first step of the process). The maximum albumen temperature was well below 57 °C, the temperature at which the foaming power of albumen begins to rapidly decline (Cunningham, 1995). Furthermore, the maximum yolk temperature indicates that at least some part of the yolk was at a temperature where the pasteurization holding time is estimated to be only 3.7 min (USDA 1969). The duration of the entire process was 23.5 min and resulted in a reduction of *E. coli* of greater than 6.8 log. By comparison, the reduction of *E. coli* using just hot water for 60 min was 6.6 log.

Future research plans include developing a RF treatment apparatus that automatically rotates the shell egg without the need for manually doing so. This will facilitate commercialization of the RF process and may be accomplished by placing the egg on rollers, similar to those used for washing eggs, and perhaps changing the location of the electrodes. Furthermore, *Salmonella* in addition to *E. coli* will be inoculated into eggs and processed with the RF technology. Additionally, egg quality testing will be performed to determine the effect of RF pasteurization on functionality.

Conclusions

A novel method to pasteurize shell eggs using RF energy was developed which substantially reduces the treatment time. Whereas the conventional pasteurization method, using only hot water, needs approximately 60 minutes to complete, the new combination method, using RF heating and hot water, requires only 23.5 minutes. Thus, the pasteurization time has been reduced by 60%.

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Figure 1

Fig. 1. Cross-section of a hard-boiled egg that had been injected with dye, to confirm the placement of inoculum at the center of the yolk.

Figure 3

Figure 3. Modeled active power density (Q_{active}) within a shell egg, including albumen, yolk and air cell. The scale (at the right) has units of W/m^3 .

Figure 2

Fig. 2. RF treatment apparatus showing electrodes clamped to an egg. The electrodes consisted of copper mesh. The clamping device was made of Ultem high-strength electrical insulating material.