Effect of ohmic heating application on *Salmonella* Enteritidis in liquid whole egg

Research Article

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ABSTRACT

Liquid eggs have high nutrient levels and are prone to spoilage. Although pasteurization can reduce the risk of pathogenic microorganisms in liquid eggs, high temperatures can damage the egg's basic components and structure. Ohmic heating (OH) is a food processing technique that is applied to inactivate food pathogens and causes less change in the nutritional profile. This study aimed to investigate the inactivation level of *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S.* Enteritidis) in liquid eggs by different voltage gradients of ohmic heating. Inoculated liquid egg samples with *S.* Enteritidis PT4 (NCTC 13349) were exposed to OH at 5V/cm, 10V/cm, and 20 V/cm for 5 minutes. The results showed that OH at 20 V/cm reduced *S.* Enteritidis counts by about 4 logs (65.6% reduction) in 4 minutes without coagulation, while OH at 5V/cm and 10 V/cm had no significant impact. In conclusion, the effectiveness of OH for inactivating pathogens in liquid eggs depends on the electric field intensity and the duration of treatment. Therefore, the best OH conditions should be chosen carefully to ensure food safety and quality.

Keywords: Foodborne pathogens, inactivation, liquid egg, ohmic heating, *Salmonella* Enteritidis

INTRODUCTION

The egg is a food with high biological value due to the exogenous amino acids, vitamins, and minerals it contains, and it has an essential place in human nutrition. Egg and egg products are used in the production of pasta, mayonnaise, confectionery, and ice cream for functions such as coagulation, emulsification, adding flavor color to the product, and increasing its nutritional value (Yüceer, 2019). Since eggs are widely used in producing of many food products and provide a suitable environment for the growth of microorganisms due to their high biological value, they can be contaminated with various pathogens during production and processing (Doğruer et al.,2015). Due to the significant health risks, eggs should not be consumed raw and should be subjected to heat treatment before use. It should be noted that especially dirty, cracked, broken, or insufficiently heat-treated eggs and egg products can cause Salmonella infection (Anonymous 2001; Braden 2006). The most common serotype isolated from egg, eggshell, and egg-borne outbreaks is *S.* Enteritidis (Martelli and Davies, 2012, Keyvan et al., 2023). Since the early 1980s, *S.* Enteritidis phage type 4 (PT4, multidrug-resistant forms) has grown significantly more common in poultry and humans (Głośnicka and Dera-Tomaszewska, 1999).

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Due to the role of eggs in foodborne illnesses, pasteurized liquid egg products that are microbiologically safer and easier to use are demanded by food producers (Nemeth et al., 2011). The pasteurization aims to reduce the risk of pathogenic microorganisms in the product and preserve liquid eggs' physical and functional properties. In the heat treatments applied for pasteurization, the highest possible temperature is tried to be used to reduce the risk of pathogens. These temperature degrees are 58–65.5°C for 2.5–5.0 min for a liquid whole egg, 58–63°C for 2.5–4.0 min for a liquid egg yolk, and 55–57.2°C for 1.0–8.0 min for egg white (Liang, 2007).

Nowadays, alternative processing methods that allow safe products to be obtained by preventing all the negative effects caused by heat treatment are becoming increasingly important. In addition, with the increasing demand for healthy and safe food by conscious consumers, the food industry has entered into new searches in food processing technologies. Ohmic heating (OH), an innovative food processing technique, is based on the principle that the food placed between two electrodes heats up due to the resistance it shows against the applied electric current (Baysal et al., 2011). Although there are many publications on the applicability of OH in different solid and liquid media (Balpetek and Gürbüz, 2015; İçier and Bozkurt, 2011; Kim and Kang 2015; Park and Kang, 2013; Tian et al., 2019), there is no study has been found on the inactivation of *Salmonella* Enteritidis in liquid eggs by OH. This study aimed to evaluate the effectiveness of OH applied at different electric field intensities on the inactivation of *S.* Enteritidis, which poses a health hazard in liquid eggs.

MATERIALS AND METHODS

Sample preparation

In the research, pasteurized liquid whole eggs obtained from the local markets of Burdur province were used**.** For sterility control of the liquid eggs used, 25 mL sample was taken and placed in 225 mL buffered peptone water (Oxoid, CM0509) and incubated at 35 ± 2°C for 24 ± 2 hours (Berrang et al., 1991). Then, 0.1 mL bacterial culture was incubated in 10 mL Rappaport-Vassiliadis broth (Oxoid, CM0669) at 42 ± 0.2°C for 24 ± 2 hours. After that, it was checked whether it was contaminated with *Salmonella* by spreading it on XLD agar (Merck 1.05287) using the spread plate method (Andrews et al.,2016). The *S.* Enteritidis PT4 (NCTC 13349) was inoculated into a 10 mL Tryptic Soy Broth (TSB) (Merck 1.05459) and incubated at 37°C for 18 hours. Then, it was centrifuged at 5000 g for 20 minutes to remove the supernatant. Pellets were washed twice with sterile 0.9 % NaCl and the pellet suspend into a 0.1% peptone suspension. To enumerate bacterial cells, they were serially diluted in 0.1% peptone water and sprouted on XLD agar (Merck, Germany). The plates were then incubated at 37°C for 24-48 hours. The final concentration of *S.* Enteritidis cells in liquid egg was confirmed to be approximately 10^7 CFU/mL using the spreading plate technique.

Experimental equipment

The OH unit used in the experiment was based on a previous study by Özkale and Kahraman (2023). The OH device consisted of 304 L stainless steel electrodes, a K-type thermocouple, a microprocessor, a personal computer, a power supply (AC, 50Hz, 10 A, 0-250 V), a magnetic stirrer and a heating unit. During the heating process, time and temperature changes were recorded using a microprocessor connected to a personal computer. Two hundred mL liquid whole egg samples inoculated with *S.* Enteritidis PT4 (1 mL) were subjected to 5V/cm, 10V/cm and 20 V/cm in the OH treatment. All experiments began at 23.5°C and were continued until the temperature at the core of the liquid egg reached 57°C. This temperature was regarded as the end of the heating.

Enumeration of cells

A mL of liquid egg sample cooled with ice was serially diluted in 0.1% peptone water. The dilutions were plated onto XLD agar to count the viable ones and onto XLD+TSA (XLD-TSA) to count both injured and uninjured bacterial cells. All plates were incubated at 37°C for 24-48 hours before counting the colonies. The sub-lethal rate (%) was calculated according to Tian et al. (2019).

Statistical analysis

The experiments were carried out in triplicate. The data was analyzed using one-way ANOVA and the T-test with SPSS software (Version 21.0; SPSS Inc., IBM Corporation, USA). Duncan's multiple range test ($p<0.05$) was used to determine significant differences.

RESULTS

This study investigated the effect of different electric field intensities of OH on the inactivation of *S.* Enteritidis in pasteurized liquid egg. Pasteurized liquid egg samples in 200 mL sterile glass beakers were inoculated with 1 mL (10^7 log CFU/mL) bacteria. OH was applied at 5 V/cm, 10 V/cm and 20 V/cm electric field intensities using stainless steel electrodes. The OH process began when the liquid egg temperature was around 23.5 °C and samples were collected with a sterile syringe at 0, 1., 2., 3., 4. and 5. minutes of heating. After the five-minute process, the liquid egg temperature reached 57 °C in about 2 minutes in the group with 20 V/cm electric field intensity, while this temperature was not achieved in the groups with 5V/cm and 10 V/cm electric field intensity.

Table 1. Colony counts of *S.* Enteritidis (log CFU/mL) in liquid egg samples treated with 5V/cm, 10V/cm and 20V/cm voltage gradient

Experiment time (min)	5 V/cm	10 V/cm	20 V/cm
0	6.69±0.16	6.69±0.23	$6.74±0.10^{A}$
1	6.55±0.22	6.94±0.04	$6.59±0.13^{A}$
2	$6.74±0.29^{a}$	$6.68±0.13^{a}$	$4.86±0.96^{Bb}$
3	$6.60±0.22^{a}$	$6.58±0.24^{a}$	$3.65±0.11^{Cb}$
4	$6.76±0.18^{a}$	$6.60±0.13^{a}$	$2.77±0.02^{Db}$
5	$6.65±0.09^{a}$	$6.67±1.11^{a}$	$2.32±0.01^{Db}$

Values were means ± standard deviation of three replicates. $^{a-b}$: Values with different superscripts within rows differ significantly ($p<0.05$) $^{A-D}$: Values within a column with different letters are significantly different ($p<0.05$)

Table 1 presents the change in *S.* Enteritidis count in liquid egg after OH with different electric field intensities (5V/cm, 10V/cm and 20 V/cm). There was a significant difference in *S.* Enteritidis count between the groups after 2 minutes of OH ($p<0.05$). The group with 20 V/cm electric current had a greater reduction of *S.* Enteritidis count than the other two groups ($p<0.05$). Moreover, the group with 20 V/cm electric field intensity showed a significant decrease in *S.* Enteritidis count from the initial level from the 2nd minute of the process and this decrease lasted until the 4th minute ($p<0.05$). The groups with 5 V/cm and 10 V/cm electric field intensity did not significantly decrease the *S.* Enteritidis count from the initial level. The group with 20 V/cm electric field intensity reached a *S.* Enteritidis count of 2.32±0.01 log CFU/mL at the 5th minute of the process, which was about 4.42 log lower (65.6 % reduction) than the initial *S.* Enteritidis count (Table.1; $p<0.05$).

Figure 1 shows the percentage of sub-lethally injured cells during the OH process. The number of sub-lethally injured cells increased progressively in all three groups as the processing time increased. This increase was statistically significant for all groups except for the one with 10 V/cm electric field intensity

(p<0.05). The percentage of sub-lethally injured cells in a group with 20 V/cm was significantly higher than after the 2nd minute of the OH process. The groups with 20 V/cm, 10 V/cm, and 5 V/cm electric field intensity had the highest and lowest percentage of injured *S.* Enteritidis cells (%) during the process, respectively (p<0.05).

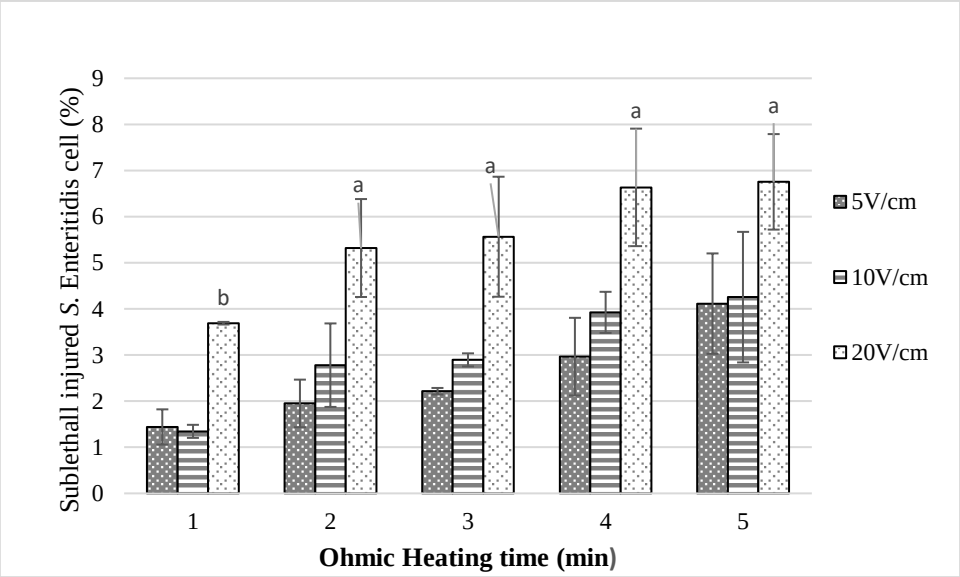

Figure 1. Sublethal injury levels (%) of *Salmonella* Enteritidis in liquid egg samples by 5V/cm, 10 V/cm and 20V/cm OH treatments. Values with different superscripts (a-b) differ significantly (p<0.05).

DISCUSSION

The mechanism of microbial inactivation in OH is due to the thermal effect generated during the process and the electroporation caused by the alternating current in the bacterial cells (Jeager et al., 2016). The electric current increases the membrane permeability of microbial cells, causing the formation of pores in the cell membrane and reducing the resistance of bacteria to heat (Cappato et al., 2017; Yıldız-Turp et al., 2013). In a study investigating the inactivation of *Streptococcus thermophilus* inoculated into milk by ohmic heating, Sun et al. (2011) stated that OH caused non-thermal damage to *S. thermophilus* cell membrane by increasing the permeability of bacterial cell membrane. According to our results, we assume that this electroporation effect generated during the OH process is the reason for the inactivation of the *Salmonella* Enteritidis PT4.

Alamprese et al. (2019) stated that OH is a suitable alternative to conventional pasteurization in eggs, and that low temperature applications should be preferred to avoid rheological problems caused by protein denaturation. Furthermore, due to low processing temperatures, the standard pasteurization procedure may not entirely eliminate *Salmonella* in products (Nemeth et al., 2011). *Salmonella* cells that survive the low-temperature pasteurization process can still cause infection. (Braden, 2006; Noble et al., 2012; Sasaki et al., 2011). However, high temperatures reduce the foaming ability of liquid eggs and cause protein coagulation. (Hamid-Samimiet al., 1984). In general, methods used at low temperatures to eradicate pathogens take more processing time, but processes used at high temperatures can harm food quality. As a result, balancing the processing time and temperature of classical pasteurization is difficult. For this reason, the results we obtained from our study show that OH provides pathogen inactivation at lower temperature and in a shorter time without

damaging the general properties of the liquid egg.

Although there are many studies on the OH application in liquid eggs (Alamprese et al., 2019; Darvishi et al., 2012; İçier and Bozkurt, 2011), the number of studies on the inactivation of pathogens in liquid eggs is quite few. Martín-Belloso et al., (1997) conducted research on the inactivation of *E. coli* in a liquid egg with a pulsed electric field which resulted that a 60% reduction for viable *E. coli* using a pulsed electric field without coagulation. The results of this study demonstrate the similarity to our results in the reduction rate of bacteria. In another study investigating the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* inoculated into orange and tomato juice with OH at 10-20 V/cm electric field strength and 540 s exposure to electric current, Sagong et al. (2011) reported that *L. monocytogenes* population decreased to 3.76 log CFU/mL after 180 s application of 15 V/cm current in orange juice. They also reported that the same electric field strength decreased *L. monocytogenes* count below the detection limit (1 log) after 210 s application. They observed that 20 V/cm electric field strength applied for 90 s reduced three pathogens below the detection limit (<1 log) of all in tomato juice, while 15 V/cm electric field strength applied for 180 s and 150 s reduced the detection limit of all three pathogens They also reported that the same pathogens were reduced below the detection limit after applying 10 V/cm electric field strength for 480 s and 420 s. These findings indicate that OH can be used to inactivate *E. coli* O157:H7, *S.*Typhimurium, and *L. monocytogenes*, and that the effectiveness of inactivation is dependent on the applied electric field intensity, application time, pathogen, and type of food.

Lee et al., (2013) investigated the inactivation level of *Escherichia coli* O157: H7 and *Salmonella* Typhimurium in salsa by OH. They concluded that when the frequency increased, the time required to reduce *Escherichia coli* O157:H7 and *Salmonella* Typhimurium to below the detection limit (1 log CFU/g) decreased. Our results confirm that the effectiveness of OH in pathogen inactivation depends on the applied electric field intensity and the application time and are in a line with the results of Lee et al, (2013), Martin-Belloso et al., (1997), and Sagong et al. (2011).

CONCLUSION

Salmonella is an important burden for the egg industry. Alternative methods are required to ensure food safety with the least detrimental impact on the nutritional content of egg products. Due to the short processing time and rapid microbial inactivation provided by ohmic processing technology, reliable products requiring less processing can be produced for the egg industry by selecting appropriate electric field strength and processing time.

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