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Original article

Ohmic heating application with different electric fields on inactivation of *Listeria monocytogenes* in protein-enriched cow milk

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Abstract

The aim of this study was to determine the effects of an ohmic heating (OH) process with different electric field intensities on *Listeria monocytogenes* inactivation in protein-enriched cow milk. Protein powder was added at rates of 2.5%, 5% and 7.5% in 1.5% fat content milk, and *L. monocytogenes* (ATCC 13932) strain was then inoculated into the samples. The OH process was carried out in a laboratory-type pilot unit created using stainless steel electrodes, a K-type thermocouple, a datalogger and power supply providing AC current at 0-250 V, 10 A. The inoculated milk samples were heated to 63°C by applying an electric field intensity of 10V/cm and 20V/cm. *L. monocytogenes* counts, pH, color measurement and hydroxymethylfurfurol levels were then determined. OH applied with an electric field intensity of 10 V/cm caused an average decrease of 5 logs in *L. monocytogenes* level in the samples containing 2.5% protein and decreased below the detection limit (<1 log) at the 9th minute ($p<0.05$). Similarly, application of an electric field intensity of 20 V/cm in milk containing 2.5% and 5% protein caused the *L.monocytogenes* level to decrease below the detection limit (<1 log) at 2 minutes 30 seconds ($p<0.05$). No change was observed in the L^* (brightness) values of the samples but it was determined that there was a slight increase in pH, a^* (redness) and b^* (yellowness) values compared to the control group. It was observed that the inactivation of *L. monocytogenes* by OH depends on the duration of the OH process, protein concentration in the milk and the applied voltage gradient.

Keywords: inactivation, *Listeria monocytogenes*, milk, ohmic heating, protein

Introduction

One of the most important foodborne pathogens is *Listeria monocytogenes*. These gram-positive bacteria can cause foodborne illness and are generally found in dairy products, meat products, and ready-to-eat foods. They can also be found ubiquitously in nature, farm environments, and food production areas. The ability to survive in such a wide range of habitats depends on regulating its gene expression and metabolic properties.

Developing technology and the demand for healthier products have led to the emergence of new heating methods to eliminate foodborne pathogens. Thus, various processing procedures have been developed in recent years to eradicate foodborne microorganisms. The undesired sensory and structural changes resulting from thermal processing have expedited the advent of OH (Ohmic heating) as an alternative to thermal processing approaches OH is a food processing technique applied to inactivate food pathogens and causes less nutritional profile change than other thermal processing methods. OH is generally used for pasteurized liquid egg production, thawing and cooking meat products, juice, baby food, and sports drinks etc. (Park and Kang 2013, Balpetek and Gürbüz 2015, Pereira et al. 2020, Pires et al. 2020, Wang et al. 2021). The practicality and cost-effectiveness of the OH system compared to other thermal heating methods have made it a promising application in the processing of dairy products (Bosi et al. 2013). Although there are many studies on the application of OH in different food matrices (Park and Kang 2013, Kim and Kang 2015, Cho et al. 2017, Tian et al. 2019, Pires et al. 2020, Özkale and Kahraman 2023), research on pathogen inactivation in protein-enriched milks is quite limited as far as we know. This study aimed to investigate the effects of the OH process applied with different voltage gradients on *L. monocytogenes* inactivation and some quality parameters in protein-enriched milks.

Materials and Methods

Sample preparation

A total of 30 samples of UHT cow milk with 1.5% fat content, packaged in 200 mL aseptic tetra pack containers, were purchased from a local market in the Burdur province of Turkey. The samples were stored in a refrigerator (+4°C) until the experiments (within 24 h). Protein powder (Yeşilmarka, Tekirdağ, Turkey) was added to the milk samples at rates of 2.5%, 5% and 7.5% and mixed well. The milk samples were then pasteurized by autoclaving at 80°C for 15 minutes. Before inoculation, the milk samples were spread on Nutrient Agar (105450, Merck) and incubated at 37°C for 24-48 hours, and no bacterial colony growth was detected. For bacterial inoculation, *L. monocytogenes* 4b (ATCC 13932) strain was cultured in Tryptic Soy Broth (Merck, Germany) at 37°C for 24 hours. The bacterial suspension was centrifuged at 5000 rpm for 5 minutes at +4°C. The pellets were washed twice with 0.9% saline and resuspended in milk. For counting, bacterial suspensions were diluted with 0.2% peptone water spread on Oxford agar (Merck, Germany) and incubated at 37°C for 24-48 hours. The final concentration of *L. monocytogenes* cells in the milk was confirmed to be approximately 10^7 CFU/mL, using the spread plate technique. Inoculation was performed by taking 1 mL from bacterial cells suspended in 9 mL of milk.

OH equipment

The OH unit used in the study is based on our previous research (Özkale and Kahraman 2023). The OH device consists of stainless steel electrodes, thermocouples, 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 with a microprocessor connected to a personal computer. Milk samples containing protein at different rates (2.5%, 5% and 7.5%) were inoculated with 1 mL (10^7 CFU/mL) *L. monocytogenes* and then subjected to the OH process at electric field intensities of 10V and 20 V/cm. The OH process started when all samples were at 23°C and continued until the core temperature of the milk reached 63°C. The time it took for each sample to reach the target temperature was recorded, and samples were taken at specified minutes.

Enumeration of *Listeria monocytogenes*

One mL samples were taken with a sterile syringe and cooled in ice water. Dilutions were made then with 0.2% peptone water and streaked onto Oxford Agar (Merck, Germany). All petri dishes were incubated at 37°C for 24-48 hours before colony counting (Kim and Kang 2015).

pH determination

The pH of the samples was measured before and after the ohmic experiment using a pH meter (Testo 205, Lenzkirch). The calibration was performed using standard buffers (pH 7.01 and 4.01) before the measurement.

Table 1. HPLC conditions and settings.

Conditions	Settings
Instrument	Agilent Technologies 1200 infinity
Detector	DAD 285/4 nm, REF; 360/100 nm
Column	C18, Generix 5C, 5 µm, (25x4,6 mm)
Column temperature	30°C
Mobile phase	A: Methanol (10%) B: H_2O (90%)
Injection volume	10 µL
Flow rate	1.0 mL/min

Hydroxymetil-furfurole analyses

The hydroxymetil-furfurole analysis was performed according to the method of Morales and Jimenez-Perez (2001), with some minor modifications. The first step involved digesting 2 mL of milk at 100°C for 1 hour with 1 mL of 0.3 N oxalic acid solution in securely stopped Pyrex containers. After a rapid cooling in ice, the mixtures were slowly de-proteinised using a trichloroacetic acetic acid (1 mL) solution (40%, w/v) and centrifuged at 11 000 rpm for 12 min at 4°C. The sample was then filtrated through a 0.45-µm acetate filter (13 mm, MSI Inc., Westboro, MA) and was ready for HPLC analysis. HPLC conditions and settings for the applied method are given in Table1.

Color measurement

The color measurements of the samples were conducted using a Konica Minolta CR-400 color measurement device (Konica Minolta, Japan). After thorough homogenization, the samples were subjected to measurement of *L** (lightness), *a** (greenness/redness), and *b** (blueness/yellowness) values. Three measurements were taken for each sample.

Statistical analysis

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

Results

In the current study, the OH process was started when the milk temperatures were, on average, 23°C and heating was carried out up to the pasteurization temperature of 63°C by applying electric field at intensities of 10 and 20 V/cm. Differences were observed in the time required for the samples to reach the desired temperature depending on the differences in the protein content of the milk. It was observed that the required time for samples containing 2.5% protein was 8 and 2.5 min, for samples containing 5% protein it was 10 and 2 min, and for samples containing 7.5% protein it was 10 and 2 min, depending on the electric field intensity of 10 and 20 V/cm applied to the samples, respectively. The changes in *L. monocytogenes* counts in milk with different percentages of protein after applying an electric field intensity of 10 V/cm are given in Table 2. It was determined that the decrease in *L. monocytogenes* count after applying an electric field intensity of 10 V/cm was significant in the 8th, 9th, and 10^{th} minutes in groups containing 2.5%, 5% and 7.5% protein, respectively (Table 2, $p<0.05$).

At the 8th minute of OH processing, a slight decrease occurred in the *L. monocytogenes* counts in milk containing 2.5%, 5% and 7.5% protein powder compared to the initial load, at levels of 2 $\log_{10}$ CFU/mL, 0.3 $\log_{10}$ CFU/mL and 0.56 $\log_{10}$ CFU/mL, respectively. At the 8th minute of OH, it was observed that the group containing 2.5% protein had significantly less bacterial count than the other two groups (3.16 $\log_{10}$ CFU/mL) while the *L. monocytogenes* counts of the 5% and 7.5% groups were similar (4.95 and 4.70 $\log_{10}$ CFU/mL, respectively) (Table 2, $p<0.05$). At the 9^{th} minute of OH processing, while the *L. monocytogenes* count in the group containing 2.5% protein decreased below the detection limit (<1 log), the groups containing 5% and 7.5% protein were 4.48 and 4.76 $\log_{10}$ CFU/mL, respectively, and this difference between groups was significant ($p<0.05$). At the 10^{th} minute of OH processing, there was a decrease of 4.09 log in the group containing 5% protein and a decrease of 2.29 log in the group containing 7.5% protein in comparison to initial bacterial load. At the 10^{th} minute of OH, the difference between the bacterial counts in 2.5% (<1 log), 5% (1.16 log) and 7.5% (2.97 log) groups was statistically significant (Table 2, $p<0.05$)

Table 2. Change in *Listeria monocytogenes* counts after applying 10V/cm OH in protein-enriched cow milk (log 10 CFU/mL, p<0.05).

Ohmic Heating Time (min)	*Listeria monocytogenes*		
	2.5%	5%	7.5%
0	5.23 ± 0.13^{A}	5.25 ± 0.04^{AB}	5.26 ± 0.07^{A}
1	5.16 ± 0.72^{AB}	5.29 ± 0.26^{AB}	5.13 ± 0.16^{A}
2	5.19 ± 0.06^{A}	5.08 ± 0.13^{BC}	4.98 ± 0.03^{ABCD}
3	5.10 ± 0.14^{AB}	5.01 ± 0.02^{BC}	4.94 ± 0.15^{ABCD}
4	5.21 ± 0.12^{A}	5.39 ± 0.37^{A}	5.07 ± 0.21^{AB}
5	5.23 ± 0.08^{A}	4.90 ± 0.09^{C}	5.03 ± 0.26^{ABC}
6	5.04 ± 0.04^{B}	4.92 ± 0.03^{C}	4.97 ± 0.07^{ABCD}
7	5.02 ± 0.08^{B}	4.93 ± 0.16^{C}	4.63 ± 0.30^{D}
8	3.16 ± 0.45^{Cb}	4.95 ± 0.17^{Ca}	4.70 ± 0.04^{CDa}
9	$<1^{Dc}$	4.48 ± 0.00^{Db}	4.76 ± 0.002^{BCDa}
10	$<1^{Dc}$	1.16 ± 0.27^{Eb}	2.97 ± 0.03^{Ea}

Values were means ± standard deviation of three replicates. a-c Values with different superscripts within rows differ significantly (p<0.05). A,B,C Values within a column with different letters are significantly different (p<0.05).

Table 3. Change in *Listeria monocytogenes* counts after applying 20V/cm OH in protein-enriched cow milk (log 10 CFU/mL, p<0.05).

Ohmic Heating Time (min)	*Listeria monocytogenes*		
	2.5%	5%	7.5%
0	5.10 ± 0.17^{A}	5.10 ± 0.17^{A}	5.21 ± 0.15^{AB}
0.5	5.11 ± 0.16^{A}	5.02 ± 0.26^{A}	5.20 ± 0.17^{AB}
1	5.11 ± 0.11^{A}	5.08 ± 0.11^{A}	5.24 ± 0.52^{A}
1.5	4.93 ± 0.14^{A}	4.94 ± 0.17^{A}	4.96 ± 0.22^{B}
2	4.42 ± 0.58^{Ca}	4.76 ± 0.20^{Bb}	4.96 ± 0.21^{Ab}
2.5	$<1^{Ca}$	$<1^{Da}$	1.16 ± 0.27^{Bb}

Values were means ± standard deviation of three replicates. a-c Values with different superscripts within rows differ significantly (p<0.05). A,B,C Values within a column with different letters are significantly different (p<0.05).

The results of the change in *L. monocytogenes* counts after applying 20V/cm electric field intensity to protein-added milk are given in Table 3. According to the results, it was determined that the *L. monocytogenes* count in the group containing 2.5% protein decreased from 5.10±0.17 $\log_{10}$ CFU/mL to 1.16±0.27 $\log_{10}$ CFU/mL at 2.5 min after OH treatment with 20V/cm electric field intensity. While the initial bacterial count in groups 5% and 7.5% was 5.10±0.17 and 5.21±0.15 $\log_{10}$ CFU/mL, respectively, both groups decreased below the detection limit (<1 log, Table 3) at 2.5 min after OH with 20V/cm (Table 3, p<0.05). There were significant differences in groups 2.5%, 5% and 7.5% in terms of bacterial counts after 20 V/cm OH at both 2 min (4.42 log, 4.76 log, and 4.96 $\log_{10}$ CFU/mL, respectively) and 2.5 min (<1 log, <1 log and 1.16 log CFU/mL) in 2.5%, 5% and 7.5% protein added milk groups. (Table 3, p<0.05). At the end of OH applied with an electric field intensity of 20 V/cm for 2.5 min in milk containing 2.5%, 5% and 7.5% protein in comparison to initial load, there was a decrease of approximately 5 log CFU/mL, 5 log CFU/mL and 4 log CFU/mL respectively (p<0.05). From the second minute of the process, there is a statistically significant difference between *L. monocytogenes* counts in 2.5%, 5% and 7.5% groups (Table 3, p<0.05). At the end of OH applied with an electric field intensity of 20 V/cm for 2.5 min, it was observed that *L. monocytogenes* was inactivated in groups containing 2.5% and 5% protein; at the same time, OH caused a significant decrease of about 4 log in the group containing 7.5% protein.

The changes in pH and HMF values of the samples at the end of the time required to reach the initial and desired pasteurization temperature are given in Table 4. The highest pH value was found in the 2.5% protein group and 20 V/cm electric current was applied (6.68±0.06), while the lowest pH value was detected in the control group where 7.5% protein was added (6.17±0.05; p<0.05). There was a statistically significant increase in hydroxymetil-furfurole (HMF) levels

Table 4. pH, HMF and color changes of the milk samples initially and after 63ºC was reached.

Protein Concentration		pH	HMF (ng/µL)	*L**	*a**	*b**
2.5%	Initial	6.46±0.11^{bc}	1.10±0.01^{Be}	86.34±0.57	1.62±0.01^{Cg}	5.20±0.08^{Ce}
	10V/cm	6.61±0.01^{ab}	4.95±0.33^{Ad}	85.90±0.78	1.88±0.08^{Bf}	5.82±0.09^{Bd}
	20V/cm	6.68±0.06^{a}	5.60±0.59^{Ac}	85.82±0.12	2.09±0.06^{Ae}	6.71±0.27^{Acd}
5%	Initial	6.36±0.01^{c}	1.15±0.07^{Be}	85.45±0.29	1.97±0.01^{Bef}	7.88±0.16^{abc}
	10V/cm	6.41±0.10^{c}	7.40±0.10^{Aa}	85.54±3.61	2.84±0.03^{Ac}	7.57±0.2^{abc}
	20V/cm	6.43±0.10^{c}	7.52±0.07^{Aa}	84.70±0.73	2.80±0.07^{Ac}	7.89±0.56^{abc}
7.5%	Initial	6.17±0.05^{d}	1.07±0.01^{Ce}	84.80±0.73	2.31±0.01^{Bd}	8.05±0.78^{a}
	10V/cm	6.30±0.08^{cd}	7.71±0.01^{Ba}	79.93±5.73	3.42±0.17^{Aa}	8.67±1.09^{ab}
	20V/cm	6.32±0.06^{cd}	7.98±0.30^{Aa}	80.7±3.38	3.08±0.08^{Aab}	8.69±0.37^{ab}

Values were means ± standard deviation of three replicates. a-c Values with different superscripts within rows differ significantly ($p<0.05$). A,B,C Values within a column with different letters are significantly different ($p<0.05$).

in samples after 10V/cm and 20 V/cm electric field intensity applications in each of the groups containing 2.5%, 5% and 7.5% protein compared to the control group (Table 4, $p<0.05$). While the lowest HMF values were detected in control groups (1.10 ng/µL, 1.15 ng/µL and 1.07 ng/µL, respectively), the highest HMF values were observed in groups with 5% and 7.5% protein and 10 V/cm and 20 V/cm ohmic treatment (7.40 ng/µL, 7.52 ng/µL, 7.71 ng/µL and 7.98 ng/µL, respectively). In general, it was observed that an increase in the protein content of the milk and the ohmic treatment voltage applied caused significant increases in HMF levels.

The color changes of the samples at the initial stage and after reaching 63°C are given in Table 4. There was no statistically significant difference between the groups in terms of *L** (brightness) values of the samples ($p>0.05$). It can be concluded that the protein amount added to the milk (2.5%, 5% and 7.5%) and different voltage values (10 V/cm and 20 V/cm) applied in OH did not cause any change in *L** values. While the highest *a** value was found to be 3.42±0.17 and 3.08±0.08 in the 7.5% protein group and 10 V/cm and 20 V/cm OH treatment was applied, respectively, the lowest *a** values was observed in the control group where 2.5% protein was added (1.62±0.01; $p<0.05$). The highest *b** value was detected in the 7.5% protein group and 20 V/cm OH was applied (8.69±0.37), while the lowest *b** value was found in the control group where 2.5% protein was added (5.20±0.08).

Discussion

Protein-enriched milk is an effective complementary nutrition source to support individuals in need when consumed in appropriate amounts as an addition to a regular diet. Among the groups that can consume protein-enriched milk are premature babies, young children, and middle-aged and elderly people. The demand for protein-enriched milk is increasing as it is predicted that meeting the protein requirement will be a fundamental problem in the future (Belford et al. 2020, Salas et al. 2022).

Pasteurization is one of the oldest methods used to produce microbial-safe products. The main purpose of pasteurization is to ensure microbiological safety with minimal loss in the nutritional value of the food. However, high-temperatures and the duration of exposure to these temperatures can cause significant losses in the food's color, flavor, and nutritional values. Due to the adverse effects caused by these traditional food processing techniques, the need for innovative technologies has increased (Awuah et al. 2007). Developing technology and the demands of individuals to consume healthier products have accelerated the emergence of OH, an alternative to thermal processing techniques. The OH method is thought to be more effective due to heating in a shorter time than traditional thermal methods, causing less loss of vitamins and minerals and providing rapid microbial inactivation. Many microorganisms can be inactivated using the OH method. *Listeria monocytogenes*, *Salmonella* Typhimurium, *Bacillus coagulans* spores, and *Geobacillus stearothermophillus* are among the microorganisms that have been inactivated using the OH method in the studies (Somavat et al. 2012, Somavat et al. 2013, Inmanee et al. 2019, Kim and Kang 2019).

This research determined the effect of OH applied with different electric field intensities on the inactivation of *L. monocytogenes* in protein-enriched milk. According to the results, while the number of *L. monocytogenes* in the group containing 2.5% protein decreased below the detection limit (<1 log) in the 9th minute of OH applied with an electric field intensity of 10V/cm, it was observed that the values in the groups containing 5% and 7.5% protein were 4.48 and

4.76 $\log_{10}$ CFU/mL, respectively. Similarly, it was observed that OH applied with an electric field intensity of 20 V/cm caused inactivation of *L .monocytogenes* in groups containing 2.5% and 5% protein in 2.5 minutes (<1 log), while it provided a significant decrease of 4.0 log in the group containing 7.5% protein. In the inactivation of microorganisms, temperature and electric current have significant effects. In a previous study, non-thermal damage occured in microorganisms exposed to electric fields with the application of OH. Therefore, the time required to reduce the number of existing microorganisms by 1.0 log (D value) is shortened (Knirsch et al. 2010).

Sagong et al. (2011) investigated the inactivation levels of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* in orange and tomato juice using OH. The *L. monocytogenes* population was found to fall below the detection limit (<1 log) after applying a voltage gradient of 15V/cm for 210 seconds in orange juice. The same researchers stated that a 20 V/cm voltage gradient for 90 seconds is required to inactivate all three pathogens in tomato juice, while 15 V/cm for 180 seconds and 10 V/cm for 420 seconds are required for orange juice. These results show that ohmic thermal processing is useful for the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* and that the inactivation effect depends on the applied electric field strength, application time, pathogen type, and food type. Similarly, Lee et al. (2015) investigated the effectiveness of OH in reducing *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in orange juice; the study showed that OH applied with higher electric field power and longer processing time was more effective on pathogen inactivation. Pereira et al. (2020) evaluated the inactivation kinetics of *L. monocytogenes* in dairy beverages with added whey protein using OH; they found that the OH process resulted in a decrease of 2.10 log CFU/mL in the *L. monocytogenes* count at 65°C. The present study demonstrates that the effectiveness of pathogen inactivation by OH depends on the applied electric field strength and application time and that the results obtained are consistent with Sagong et al. (2011) and Pereira et al. (2020). Accordingly, it has been observed that an increase in protein content in milk reduces the effectiveness of OH, increases the processing time required to reach the target temperature, and delays *L. monocytogenes* inactivation. Moreover, it has been determined that higher milk protein content leads to a lower decrease in *L. monocytogenes* count when applied with a low electric field intensity. Indeed, Kim and Kang (2015) state that the effectiveness of the OH process in pathogen inactivation is affected by internal (fat, protein, and carbohydrate content of food) and external (voltage and frequency) factors, and thus the electrical conductivity of foods can change and a protective effect can occur in pathogen inactivation.

In this study, the samples' pH values showed a slight increase compared to the control due to the ohmic process. In addition, it was observed that there were differences in the *a** (redness) and *b** (yellowness) values of samples but no change was observed in *L** (brightness) values. Similarly, Parmar et al. (2018) stated that the OH process caused a significant increase in redness (*a**) and yellowness (*b**) values but caused a significant decrease in the pH value and whiteness (*L**) of concentrated milk. The results showed that yellowness increased significantly over time due to the formation of Maillard reaction products. Casein micelles in milk are the components that contribute most to the white color of milk. It is stated that heat-induced denaturation of proteins and their aggregation to casein micelles, phosphorylation of casein, fragmentation of k-casein and precipitation of soluble calcium phosphate on casein micelles cause a decrease in the whiteness of milk (Parmar et al. 2018).

The initial HMF content of UHT milk groups significantly affects HMF content after the ohmic process. A study conducted by Urgu et al. (2017) showed that the HMF content of full-fat, semi-skimmed and skimmed UHT milk belonging to different brands was in the range of 315-1606 ng/µL. Morales and Jiménez-Pérez (1999) also stated that the total HMF content of commercial UHT milk samples with different fat contents was in the range of 436-725 ng/µL. In the present study, it was observed that although the OH process applied at different voltage gradients created high levels of HMF content compared to control samples, similarly to Parmar et al. (2018), the results obtained were not as high as mentioned in the studies above.

It has been shown that the rapid increase in temperature caused by OH creates the positive aspect of the system and that OH is more effective on pathogen inactivation. It has been reported that OH causes less deterioration in food's sensory and physical properties than classical heating methods of reducing pathogen activation (Coimbra et al. 2020). The most well-known method for eliminating unwanted microorganisms in foods is thermal processing applied at temperatures above 70°C. However, it has been reported that during this thermal application, there are significant losses in heat-sensitive compounds, there is unwanted taste and odor development, and Maillard reaction products occur due to the formation of browning in the product (Jaeger et al. 2010, Sakr and Liu 2014, Shi et al. 2015, Ahmad et al. 2019). OH is an alternative processing method of reducing all these harmful effects. In this study, it was determined that pathogen inactivation was

achieved in a shorter time with the application of OH at high electric field intensity and that the physico-chemical properties of the milk were not damaged. Moreover, as the protein content in milk increased, the required processing time to achieve the desired reduction in pathogen levels also increased due to the decrease in the milk's electrical conductivity. It was observed that the inactivation rate of *L. monocytogenes* with OH changed depending on the processing time, protein concentration and applied voltage gradient. It was concluded that high protein content may significantly inhibit pathogen inactivation with OH. As a result of the study, it was observed that OH is an effective method for *L. monocytogenes* inactivation in protein-enriched milks. Thanks to the rapid microbial inactivation provided by OH, it is thought that it will be more widely used in the future to produce safe foods in the food industry without losing quality properties.

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