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Combined inhibitory effect of milk fat and lactose for inactivation of foodborne pathogens by ohmic heating

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ABSTRACT

Numerous dairy products are produced with reduced fat and/or lactose content as consumer demand for foods of modified nutritional content has increased recently. The combined inhibitory effect of milk fat and lactose on the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* by ohmic heating was investigated in the present study. Response surface methodology with central composite design was used to analyze the inactivation of pathogens and develop a predictive model. Both lactose and fat had an inhibitory effect on the inactivation of all three pathogens by ohmic heating. Inactivation of *E. coli* O157:H7 has a quadratic relationship with lactose and fat, whereas the cross product of treatment time with fat or lactose has a significant effect on the inactivation of *S.* Typhimurium and L. *monocytogenes*. The developed model predicted the inactivation of all three pathogens well within the range of experimental conditions. Color change and lipid oxidation were not observed following ohmic heating, while pH values slightly decreased. Therefore, treatment conditions of ohmic heating should be decided carefully considering the lactose and fat content when using this method to pasteurize milk products.

1. Introduction

Foodborne illnesses have increasingly been reported worldwide, especially in developing countries due to lack of food safety management. Consuming food containing physical, chemical, and biological hazards can result in foodborne illness, hospitalizations, and deaths. Pathogenic bacteria are a major cause of hospitalizations, which account for 64% of hospital cases caused by contaminated food eaten in the United States (Scallan et al., 2011). In particular, *Escherichia coli* O157:H7, *Salmonella enterica* Serovar Typhimurium, and *Listeria monocytogenes* are representative foodborne pathogens which cause hemolytic uremic syndrome, salmonellosis, and epidemic listeriosis, respectively. Several preservation methods, including physical, chemical, and biological treatments, have been developed to control these foodborne pathogens. Even though chemical treatments have a powerful antibacterial effect, consumers avoid chemically treated food due to the possibility of toxicity. Biological treatments such as bacteriophage application are being investigated nowadays, but the host range is too narrow to apply this method to food production (Hudson et al., 2013). Therefore, physical treatments are still used widely to inactivate foodborne pathogens.

Physical treatments can be divided into thermal and non-thermal treatments. Non-thermal treatments such as pulsed electric field, LED-UV, and cold plasma have been investigated recently for inactivation of foodborne pathogens (Shin, Kim, Kim, & Kang, 2016; Timmermans et al., 2014; Yong et al., 2015). Even though quality degradation of food can be minimized by non-thermal treatment, only the food surfaces can be treated with interventions such as LED-UV and cold plasma. Quality degradation by severe heat is a major limitation of thermal treatment, which has long been used for food processing. Therefore, alternative thermal treatments such as 915 MHz microwave, radio frequency, and ohmic heating have been developed. Rapid heating by these alternative thermal treatments reduces quality degradation while inactivating foodborne pathogens (Jeong & Kang, 2014; Song &

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Kang, 2016). In particular, ohmic heating has an advantage in heating uniformity compared to 915 MHz microwave and radio frequency heating (Kim, Sung, et al., 2016).

Inactivation of foodborne pathogens by ohmic heating has been investigated recently (Kim & Kang, 2017; Kim, Choi, & Kang, 2017). The efficacy of ohmic heating is affected by intrinsic and extrinsic factors. Extrinsic factors such as voltage and frequency have a significant effect on the inactivation of pathogens. For example, accelerated heating rate by means of increased voltage and frequency results in rapid inactivation of foodborne pathogens (Baysal & Icier, 2010; Lee, Ryu, & Kang, 2013). Reduction of pathogens is also significantly affected by intrinsic factors. In particular, nutritive components such as fats, protein, and carbohydrates not only change the electrical conductivity of food but also have a protective effect on the inactivation of pathogens (Kim & Kang, 2015).

Consumer demand for foods of modified nutritional content has been increasing recently, and accordingly, such foods have appeared in the market place. Particularly, various milk products are being produced which have reduced fat and/or lactose content. Fat content of whole milk, low fat milk, and skimmed milk are 3–4, 1–2, and 0–0.5%, respectively. Low fat or skimmed milk is preferred by some consumers who worry about obesity. Lactose content is about 4–5% in whole milk, which can cause lactose indigestion in lactose-intolerant consumers (Scrimshaw & Murray, 1988). Therefore, lactose-free milk is preferred by some consumers who have trouble digesting this sugar. The level of fat and/or lactose can influence inactivation of foodborne pathogens by thermal or non-thermal treatments. The inactivation levels of *S.* Typhimurium DT 104 and *Listeria innocua* decreased with increasing fat content when treated with a shaking water bath (Juneja & Eblen, 2000) and oil bath (Bermúdez-Aguirre & Barbosa-Cánovas, 2008), respectively. Ramaswamy, Jin, and Zhu (2009) reported that casein and lactose are important factors affecting the baro-protection of *E. coli* in milk during high-pressure treatment. Even though ohmic heating has been used to process milk products, to the best of our knowledge, research about the combined effect of milk fat and lactose on the inactivation of pathogens is very limited.

In the present study, the combined effect of milk fat and lactose on the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was investigated. First, reductions of the three pathogens subjected to ohmic heating in milk of various fat and lactose content were analyzed by response surface methodology (RSM). Secondly, a predicted model developed by RSM was verified within the range used in the experiments. Finally, quality aspects including pH, color, and lipid oxidation were assessed.

2. Materials and methods

2.1. Bacterial cultures and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104), and *L. monocytogenes* (ATCC 19111, ATCC 19115, ATCC 15313) were obtained from the bacteria culture collection of Seoul National University (Seoul, Korea). Stock and working cultures were prepared according to a previously described method (Kim & Kang, 2015a). A single colony cultivated from frozen stocks on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) was inoculated into 5 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD), incubated at 37 °C for 24 h, collected by centrifugation at 4000 × g for 20 min at 4 °C, and washed three times with 0.2% peptone water (PW; Bacto, Becton, Dickinson, Sparks, MD). The final pellets were resuspended in 0.2% PW. Afterwards, suspended pellets of the three pathogens were combined to comprise a mixed culture cocktail containing approximately equal numbers of cells of each strain of *E. coli* O157:H7 (10^9 CFU/ml), *S.* Typhimurium (10^9 CFU/ml), and *L. monocytogenes* (10^8 CFU/ml).

2.2. Sample preparation and inoculation

Pasteurized lactose free and low fat (1.5%) milk (pH 6.9) and sterilized cream containing 37% fat and emulsifier were purchased at a local grocery store (Seoul, South Korea). Milk and cream were stored under refrigeration (~4.0 °C) until used for experiments. Cream and lactose (Difco) were added to milk to achieve fat contents of 1.5, 2.5, 3.5, 4.5, or 5.5% and lactose content of 0, 1, 2, 3, or 4%. Fat and lactose content were calculated on the basis of manufacturer's declarations. Samples were mixed using a magnetic stirrer and stir bar. Mixed-culture cocktail (0.2 ml) was inoculated into 50 mL of prepared sample.

2.3. Experimental design

The effect of lactose, fat, and treatment time on the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was identified using RSM. Lactose and fat levels ranged from 0 to 4% and 1.5–5.5%, respectively, depending on food products. Treatment time was determined to be 80–120 s based on our preliminary studies. A 3-factor Central Composite Design (CCD) was used and five levels for each factor were coded as −2, −1, 0, +1, +2, respectively (Table 1). The 16 experiments were performed in random order.

2.4. Ohmic heating treatment

Ohmic heating treatments were carried out in a previously descried apparatus (Kim & Kang, 2015b). The ohmic heating system consisted of a function generator (catalog number 33210A; Agilent Technologies, Palo Alto, CA), a precision power amplifier (catalog number 4510; NF Corp., Yokohama, Japan), a two-channel digital-storage oscilloscope (catalog number TDS2001C; Tektronix, Inc., Beaverton, CO), a data logger (catalog number 34970A; Agilent Technologies), and an ohmic heating chamber. The distance between the two electrodes was 4 cm, and the cross-sectional area was 60 cm^2. Prepared and inoculated samples were subjected to pulsed ohmic heating (0.3 duty ratio, 10 kHz) with fixed electric strength of 18.2 V_{rms}/cm. Samples were taken after each treatment and populations of surviving microorganisms were enumerated.

2.5. Bacterial enumeration

For microbial enumeration, each treated 50 mL sample was immediately transferred into a sterile stomacher bag (Labplas, Inc., Sainte-Julie, Quebec, Canada) containing 100 ml of sterile 0.2% peptone water (4 °C) and homogenized for 2 min using a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1 ml samples were 10-fold serially diluted with 9 ml of sterile 0.2% peptone water and 0.1 ml of stomached or diluted samples were spread-plated onto each selective medium. Sorbitol MacConkey (SMAC) agar (Difco), xylose lysine deoxycholate (XLD) agar (Difco),

Table 1
Variables and levels used for the central composite design.

Xi	Independent variables	Levels				
		−2	−1	0	+1	+2
X_1	Lactose content (%)	0	1	2	3	4
X_2	Fat content (%)	1.5	2.5	3.5	4.5	5.5
X_3	Time (s)	80	90	100	110	120

and Oxford agar base (OAB; Difco) with antimicrobial supplement (Bacto Oxford antimicrobial supplement; Difco) were used as selective media for enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. All plates were incubated at 37 °C for 24–48 h before counting colonies characteristic of the pathogens.

2.6. Mathematical modeling and verification

The response was measured as the log reduction of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. Mathematical modeling was performed using the statistical analysis system (SAS, version 9.3, Cary, NC, USA). The following second order polynomial equation was used to develop a predictive model for the inactivation of each pathogen by ohmic heating treatment.

$$\text{Log reduction} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{23} X_2 X_3 + \beta_{13} X_1 X_3$$

Where β_i are constant regression coefficients (β_0: constant term; β_1, β_2, β_3: linear effect; β_{11}, β_{22}, β_{33}: quadratic effect; β_{12}, β_{13}, β_{23}: interaction effect); X_1, X_2, and X_3 are lactose content (%), fat content (%), and treatment time (s), respectively. A stepwise regression was performed using the PROC REG procedure with 'selection = backward' option to include only the significant ones ($p = 0.1$). Four treatment conditions were selected within the range of experimental conditions to verify the accuracy of the developed predictive models. The accuracy factor (A_f) and bias factor (B_f) were used to validate the predictive model. The A_f and B_f values were calculated using the following equations (Ross, 1996)

$$A_f = 10^{\frac{\sum |\log(predicted/observed)|}{n}}$$

$$B_f = 10^{\frac{\sum \log(predicted/observed)}{n}}$$

Where n is the number of observations. A_f represents how absolutely close, on average, the predictions are to the observations. The larger the value, the less accurate is the average estimate. B_f indicates by how much, on average, a model over-predicts or under-predicts the observed data. Perfect agreement between predictions and observations will lead to a bias factor of 1.

2.7. Color, pH, and TBARS measurement

For color, pH, and TBARS measurements, each heat treatment time was calculated from the predicted model to achieve 5 log reductions for all three pathogens. The pH of treated and untreated samples was measured with a Seven Multi 8603 pH meter (Mettler Toledo, Greifensee, Switzerland). The color of treated and untreated samples was measured using a Minolta colorimeter (CR400; Minolta Co., Osaka, Japan). Color values for L^*, a^*, and b^* (lightness, redness, and yellowness, respectively) were recorded to evaluate color changes of treated and untreated samples. Lipid oxidation was determined by measuring the levels of 2-thiobarbituric acid reactive substances (TBARS) values in the samples (Jung, Nam, & Jo, 2016). Samples (3 mL) were vortexed with 9 ml of 7.5% TCA solution and 50 μL of butylated hydroxytoluene (7.2%). The homogenate was centrifuged at 2090 × g for 15 min, and filtered through a Whatman No.4 filter paper (Whatman, Maidstone, UK). One mL of filtrate was transferred into a test tube, and 1 mL of a 20 mM 2-Thiobarbituric acid (TBA) was added. The tubes were heated in water bath at 90 °C for 30 min and cooled in tap water for 10 min. Absorbance was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at 532 nm. The concentration of malondialdehyde (MDA) in a sample was expressed in milligrams of MDA per kilogram.

2.8. Statistical analysis

All experiments were replicated three times. All experimental data were analyzed by the analysis of variance (ANOVA) procedure and mean values were separated using Duncan's multiple-range test. Significant differences in the processing treatments were determined at a significance level of $p = 0.05$.

3. Results and discussion

3.1. Reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to ohmic heating of milk products of various lactose and fat content

The combined inhibitory effect of lactose and milk fat on the inactivation of foodborne pathogens by ohmic heating was observed in the present study. Even though lactose and fat content vary for different milk products, research investigations reporting the effect of lactose and fat content on ohmic heating have been limited. Reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were significantly different corresponding to lactose content, fat content, and treatment time in the present study (Table 2). In general, reductions of pathogens increased as lactose and fat content decreased and treatment time increased. At a fixed treatment time of 100 s, lactose and fat had a significant inhibitory effect on the inactivation of pathogens. Reductions (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* increased by 1.19, 2.35, and 1.51, respectively as lactose content decreased from 4 to 0% (Trials 11 and 12). Similarly, reductions (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* increased 2.14, 1.46, and 0.80, respectively as fat content decreased from 5.5 to 1.5% (Trials 13 and 14). Increased lactose or fat not only decreased the electrical conductivity of milk but also protected these foodborne pathogens from thermal damage. Decreased electrical conductivity leads to reduced thermal efficiency of ohmic heating, which results in the survival of pathogens (Bozkurt & Icier, 2010). Moreover, an effect of nutritive components protecting foodborne pathogens from thermal treatment has been reported. Espina, Somolinos, Pagán, and García-Gonzalo (2010) reported a small protective effect of apple juice on thermal inactivation (54 °C) of *E. coli* O157:H7. The contribution of food components such as salts, sugars, proteins, and fats on increased bacterial heat resistance was discussed in this same study. Juneja and Eblen (1999) also reported that increasing the sodium chloride concentration (0–6%) protected *L. monocytogenes* against heat treatment (55–60 °C). Therefore, more foodborne pathogens can survive in the milk product of higher fat or lactose content when subjected to ohmic heating with fixed treatment time.

3.2. Predictive modeling based on RSM

RSM is a useful tool for analyzing and predicting the effect of several factors on the inactivation of foodborne pathogens (Bover-Cid, Belletti, Garriga, & Aymerich, 2012; Kwak, Kim, & Rhee, 2011). In the present study, regression analysis of the experimental data produced the following predictive equations for *E. coli* O157:H7 [1], *S.* Typhimurium [2], and *L. monocytogenes* [3], respectively.

E. coli O157:H7

Table 2
Inactivation (log N/N_0) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in milk products of varying fat and lactose content subjected to ohmic heating of varying time intervals.[b,c]

Trial	Lactose (%)	Fat (%)	Time (s)	Inactivation log (N/N_o)[a]		
				E. coli O157:H7	*S.* Typhimurium	*L. monocytogenes*
1	1.0	2.5	90	0.49 ± 0.77 ABa	0.80 ± 0.05 ABa	0.65 ± 0.41 ABa
2	1.0	2.5	110	2.48 ± 0.36 DEa	3.30 ± 0.28 Fa	2.73 ± 0.83 Fa
3	1.0	4.5	90	0.41 ± 0.20 ABa	0.70 ± 0.37 ABa	0.37 ± 0.19 Aa
4	1.0	4.5	110	2.27 ± 0.35 DEa	2.59 ± 0.80 EFa	1.99 ± 0.18 CDEFa
5	3.0	2.5	90	0.72 ± 0.13 ABa	0.87 ± 0.22 ABa	0.70 ± 0.20 ABa
6	3.0	2.5	110	2.31 ± 0.22 DEa	2.72 ± 0.37 EFa	2.24 ± 1.05 DEFa
7	3.0	4.5	90	0.25 ± 0.42 Aa	0.47 ± 0.50 Aa	0.31 ± 0.24 Aa
8	3.0	4.5	110	2.13 ± 0.09 DEa	2.48 ± 0.47 DEa	1.65 ± 0.54 BCDEa
9	2.0	3.5	100	1.82 ± 0.38 CDa	1.43 ± 0.14 BCa	1.29 ± 0.47 ABCDa
10	2.0	3.5	100	2.06 ± 0.15 Da	1.80 ± 0.67 CDa	1.42 ± 0.49 ABCDa
11	0.0	3.5	100	2.34 ± 0.41 DEa	3.33 ± 0.33 Fa	2.57 ± 0.81 EFa
12	4.0	3.5	100	1.15 ± 0.71 BCa	0.98 ± 0.34 ABa	1.06 ± 0.52 ABCa
13	2.0	1.5	100	2.87 ± 0.41 Ea	2.48 ± 0.98 DEa	1.88 ± 0.48 CDEFa
14	2.0	5.5	100	0.73 ± 0.54 ABa	1.02 ± 0.30 ABa	1.08 ± 0.24 ABCa
15	2.0	3.5	80	0.20 ± 0.50 Aa	0.65 ± 0.14 ABa	0.75 ± 0.48 ABa
16	2.0	3.5	120	3.58 ± 0.18 Fa	5.45 ± 0.05 Gb	4.69 ± 0.71 Gb

Mean values ± standard deviation.
[a] Detection limit: 0.48 log CFU/ml.
[b] Values in the same column followed by the same uppercase letter are not significantly different ($p > 0.05$).
[c] Values in the same row followed by the same lowercase letter are not significantly different ($p > 0.05$).

$$Y = -6.655 + 0.38375X_1 - 0.44125X_2 + 0.1035X_3 - 0.04875X_1^2 - 0.035X_2^2 - 0.000125X_3^2 - 0.025X_1X_2 + 0.004X_2X_3 - 0.00275X_1X_3 \quad (1)$$

S. Typhimurium

$$Y = 25.59125 - 0.29875X_1 + 0.01063X_2 - 0.573X_3 + 0.02125X_1^2 - 0.00662X_2^2 + 0.00359X_3^2 - 0.00563X_1X_2 + 0.03375X_2X_3 + 0.135X_1X_3 \quad (2)$$

L. monocytogenes

$$Y = 23.39656 + 0.306250X_1 + 0.37125X_2 - 0.54275X_3 + 0.115X_1^2 + 0.03125X_2^2 + 0.003413X_3^2 + 0.005X_1X_2 - 0.00825X_2X_3 - 0.01025X_1X_3 \quad (3)$$

The analysis of variance for the response variable indicated that the developed models were significant for all three pathogens (Table 3). A response surface 3D contour plot was described to identify the combined inhibitory effect of lactose and fat on the inactivation of pathogens when treatment time was fixed at the coded 0 level (Fig. 1). The contour plot of *E. coli* O157:H7 has a convex upward surface, while those of *S.* Typhimurium and L. *monocytogenes* have a convex downward surface. The inhibitory effect of fat was more significant than that of lactose for inactivation of *E. coli* O157:H7 (Fig. 1A). On the other hand, the inhibitory effect of lactose was similar or more significant than that of fat for inactivation of *S.* Typhimurium and L. *monocytogenes* (Fig. 1B and C. It seems that the protective effect of fat on the inactivation of *E. coli* O157:H7 is more significant than for *S.* Typhimurium and L. *monocytogenes* as described in one of our previous research investigations (Kim & Kang, 2015). As indicated in this previous study, regions containing high fat and lactose content may heat more slowly than their surroundings due to lower electrical conductivity. Therefore, pathogens present within the fat or lactose phase receive less thermal damage than their surroundings. Further study is needed to identify why the protective effect of fat is more significant for *E. coli* O157:H7 than for *S.* Typhimurium and L. *monocytogenes*.

Table 3
Analysis of variance of inactivation models of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to ohmic heating ***1% and **5% probability level.

Pathogen	Analysis of variance				
	DF	Sum of squares	*F-value*	*p-value*	R^2
E. coli O157:H7					
Total model	9	14.47	5.67**	0.0235**	0.8947
Linear	3	14.40	16.91***	0.0025***	0.8904
Quadratic	3	0.05	0.05	0.9818	0.0029
Cross product	3	0.02	0.03	0.9930	0.0015
Lack of Fit	5	1.67	11.63	0.2189	
S. Typhimurium					
Total model	9	25.61	8.70***	0.0080***	0.9288
Linear	3	23.03	23.48***	0.0010***	0.8354
Quadratic	3	2.51	2.56	0.1508	0.0911
Cross product	3	0.06	0.07	0.9763	0.0023
Lack of Fit	5	1.89	5.53	0.3116	
L. monocytogenes					
Total model	9	17.23	7.76**	0.0107**	0.9209
Linear	3	14.81	20.01***	0.0016***	0.7916
Quadratic	3	2.28	3.08	0.1119	0.1219
Cross product	3	0.14	0.19	0.9012	0.0074
Lack of Fit	5	1.47	34.83	0.1279	

3.3. Validation of the predictive model

The predictive model equations (4)–(6) were reduced with stepwise backward selection (Table 4). A stepwise regression of the experimental data produced the following predictive equations including only the significant factors for *E. coli* O157:H7 [4], *S.* Typhimurium [5], and *L. monocytogenes* [6], respectively.

E. coli O157:H7

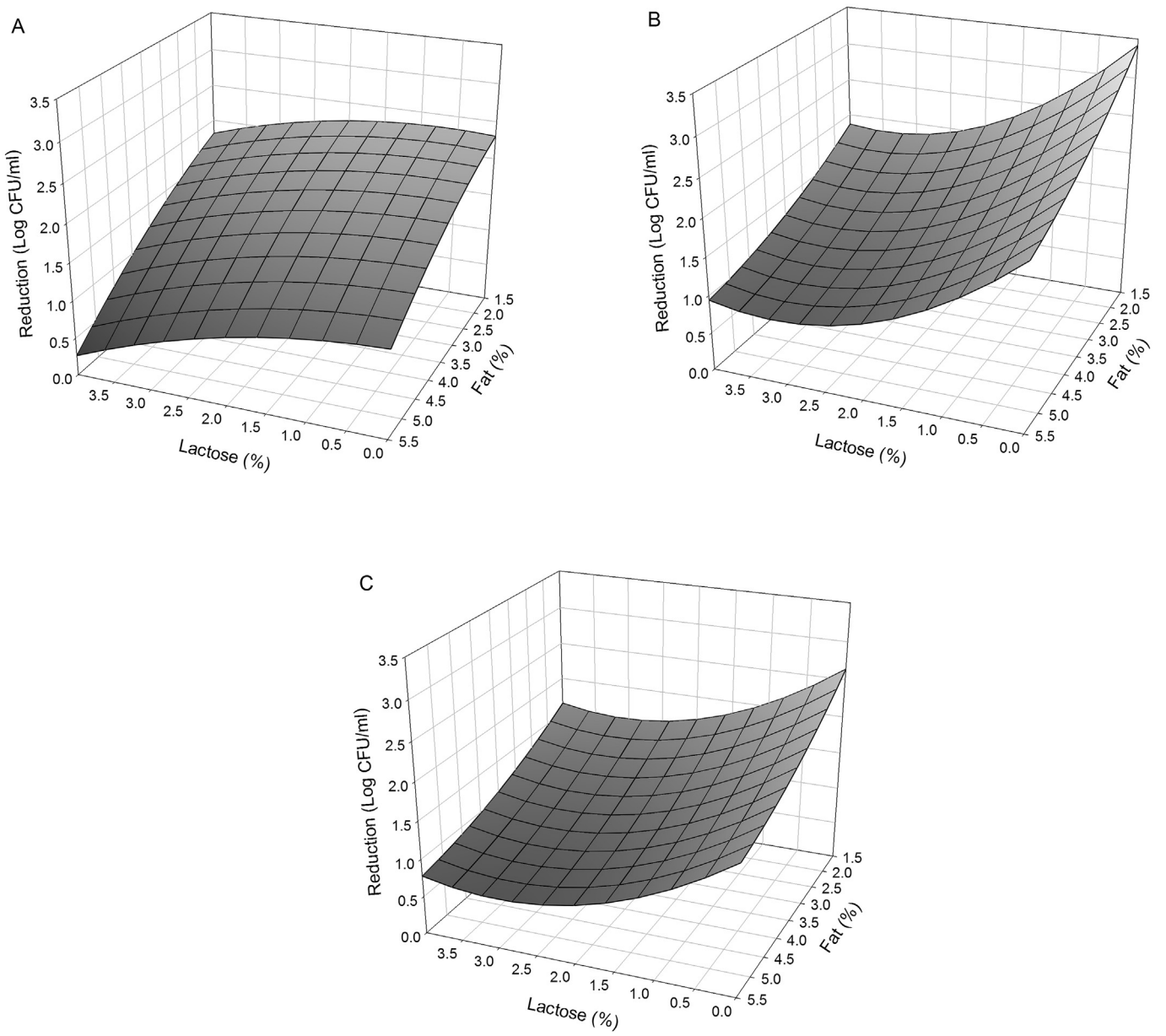

Fig. 1. Response surface 3D contour plot indicating the effect of lactose and fat content on inactivation of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) subjected to ohmic heating for 100 s.

Table 4
Estimated regression coefficients of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* with significant values.

	E. coli O157:H7		*S.* Typhimurium		*L. monocytogenes*	
	Estimate	p-value	Estimate	p-value	Estimate	p-value
Intercept	−6.22835	<0.0001	20.73312	0.0477	21.50625	0.0237
X_3	0.087	<0.0001	−0.47686	0.0269	−0.48174	0.0138
X_1X_3	–	–	−0.00348	0.0109	−0.00245	0.0328
X_2X_3	–	–	−0.00275	0.0346	−0.00228	0.0443
X_1^2	−0.04389	0.0776	–	–	–	–
X_2^2	−0.04748	0.0070	–	–	–	–
X_3^2	–	–	0.00303	0.0078	0.00292	0.0045

$$Y = -6.22835 + 0.087X_3 - 0.04389X_1^2 - 0.04748X_2^2 \quad (4)$$

S. Typhimurium

$$Y = 20.73312 - 0.47686X_3 + 0.00303X_3^2 - 0.00275X_2X_3 - 0.00348X_1X_3 \quad (5)$$

L. monocytogenes

$$Y = 21.50625 - 0.48174X_3 + 0.00292X_3^2 - 0.00228X_2X_3 - 0.00245X_1X_3 \quad (6)$$

Inactivation of *E. coli* O157:H7 has a quadratic relationship with lactose (X_1) and fat (X_2), whereas the cross product (X_1X_3 and X_2X_3) has a significant effect on the inactivation of *S.* Typhimurium and L. *monocytogenes*. These results indicate that the effect of fat and lactose on the reduction of *E. coli* O157:H7 is more significant at higher than at lower levels, and the effects on *S.* Typhimurium and L. *monocytogenes* become more significant as treatment time increases. Chhabra, Carter, Linton, and Cousin (1999) also reported

Table 5
Predicted and observed reduction of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to ohmic heating.

Lactose (%)	Fat (%)	Reduction (Log CFU/ml)					
		E. coli O157:H7		*S.* Typhimurium		*L. monocytogenes*	
		Predicted	Observed	Predicted	Observed	Predicted	Observed
0	1.5	2.36	2.77 ± 0.55	2.93	2.45 ± 0.46	2.19	1.63 ± 0.33
0	5.5	1.03	1.57 ± 0.25	1.83	2.06 ± 0.40	1.28	1.23 ± 0.35
4	1.5	1.66	1.33 ± 0.57	1.54	1.38 ± 0.10	1.52	0.99 ± 0.68
4	5.5	0.33	0.49 ± 0.03	0.44	0.49 ± 0.51	0.30	0.25 ± 0.24

Table 6
Accuracy factors (A_f) and bias factors (B_f) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*.

	Verification value	
	A_f	B_f
E. coli O157:H7	1.35	0.83
S. Typhimurium	1.14	1.02
L. monocytogenes	1.27	1.27

Table 7
Color, pH, and TBARS values of untreated or treated samples of different lactose and fat content.[a]

Lactose (%)	Fat (%)	Treatment	pH	Color			TBARS (mg malondialdehyde/kg)
				L*	a*	b*	
0	1.5	Untreated	6.99 ± 0.04 A	68.86 ± 0.91 A	−3.04 ± 0.09 A	1.24 ± 0.21 A	0.74 ± 0.26 A
		Treated	6.91 ± 0.01 B	68.01 ± 2.15 A	−3.01 ± 0.14 A	1.25 ± 0.08 A	0.86 ± 0.31 A
4	5.5	Untreated	6.96 ± 0.02 A	69.57 ± 1.27 A	−2.93 ± 0.13 A	1.78 ± 0.30 B	0.71 ± 0.27 A
		Treated	6.89 ± 0.02 B	68.85 ± 1.61 A	−2.90 ± 0.06 A	1.73 ± 0.33 B	0.76 ± 0.20 A

Mean values ± standard deviation.
[a] Values in the same column followed by the same letter are not significantly different ($p > 0.05$).

that inactivation of *L. monocytogenes* is affected by milk fat (0–5%) and temperature (55–65 °C) interaction. Moreover, a similar tendency was observed in our preliminary experiment, which indicated that inactivation of *E. coli* O157:H7 has a quadratic relationship with fat whereas that of *S.* Typhimurium and L. *monocytogenes* has a linear relationship with fat (data not shown). The reduced model including only the significant factors was verified under four treatment conditions which are within the range of experimental conditions. The observed reductions of pathogens did not significantly differ from predicted levels (Table 5). The accuracy factor (A_f) and bias factor (B_f) were used to validate the predictive model. For all three pathogens, A_f values did not exceed 1.35, and B_f values ranged from 0.83 to 1.17 (Table 6). Because the accuracy factor (A_f) and bias factor (B_f) were close to 1, we concluded that the developed model reliably predicted the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. Several previous research investigations also reported that models developed by RSM predicted inactivation of pathogens well (Skandamis & Nychas, 2000; M.; Song, Kim, & Rhee, 2016).

3.4. Color, pH, and TBARS values

Color, pH, and TBARS have been used as quality indicators of milk products (Kim et al., 2015). Changes in color, pH, and TBARS in samples of low (0% lactose and 1.5% fat) and high (4% lactose and 5.5% fat) nutrient levels were observed in the present study. Color and TBARS values were not significantly degraded ($p > 0.05$) in samples of both low and high nutrient levels, whereas pH values decreased significantly ($p < 0.05$) in both types of samples (Table 7). However, the degree of pH change was not great for either type of samples. pH values of samples having low levels of nutrients decreased from 6.99 to 6.91 after ohmic heating, and those containing high levels decreased from 6.96 to 6.89. Even though color and TBARS values of ohmic heated samples were not significantly degraded ($p > 0.05$) in the present study, more treatment time is needed to satisfy the pasteurization standards for milk products. These standards are based on the destruction of *Coxiella burnetii*, which is the most heat-resistant milkborne pathogen (Claeys et al., 2013). Therefore, further study is needed to identify quality changes occurring in milk containing high and low levels of nutrients subjected to conditions necessary to inactivate *C. burnetii*.

4. Conclusion

The combined inhibitory effect of lactose and milk fat on the inactivation of foodborne pathogens by ohmic heating was identified in the present study. RSM was used to analyze the inactivations and develop predictive modeling. The inhibitory effect of fat was more significant in *E. coli* O157:H7 than for *S.* Typhimurium and L. *monocytogenes*. Predictive models developed using stepwise backward selection showed that inactivation of *E. coli* O157:H7 has a quadratic relationship with lactose (X_1) and fat (X_2) content, whereas the cross products (X_1X_3 and X_2X_3) have a significant effect on the inactivation of *S.* Typhimurium, and *L. monocytogenes*. Developed predictive models were verified under four treatment conditions, and observed values were not significantly different from those predicted. Color change and lipid oxidation were not observed, while pH values slightly decreased following ohmic heating treatment. Therefore, treatment conditions for ohmic heating pasteurization should be decided considering lactose and fat content, and models developed by RSM could be used effectively to predict inactivation of pathogens.

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