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Full Length Research Paper

Pasteurization of liquid egg by high hydrostatic pressure (HHP) treatment

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In our tests, we artificially infected the liquid whole egg samples with *Salmonella Enteritidis*, *Listeria monocytogenes,* and *Staphylococcus aureus* bacteria, and then treated the samples in "Food Lab900" high hydrostatic pressure instrument for 3 – 17 min at 200 – 400 MPa. Subsequently, the change of the viable cell count of the specific bacteria has been tested. In addition to the samples infected with various bacteria, non-infected samples were also treated in each test and the change in viable cell count of the samples upon the effect of the treatment. In summary, it can be concluded that in each test of our investigations the viable cell count of *S. Enteritidis* critical for egg products is reduced significantly while the reduction of the total viable cell count was around 2 magnitudes. In addition, based on our results microbial destruction was significantly affected by the pressure level only ($p<0.05$).

Key words: Liquid egg, high hydrostatic pressure (HHP), *Salmonella*, *Listeria*, *Staphylococcus*.

INTRODUCTION

Numerous researches have been conducted today to develop procedures replacing conventional liquid egg pasteurization technologies (heat treatment at 60-65°C for 5-10 min). One such procedure includes treatment of liquid egg products at high hydrostatic pressure (HHP) (Farr, 1990; San Martín et al., 2002). Previous research has shown that HHP technology is suitable for destruction of numerous pathogenic micro-organisms in egg products (Ponce et al., 1998, 1999; Jankowska et al., 2005).

Use of HHP technology allows better preservation of native characteristics of food raw material with similar antimicrobial efficacy as heat treatment (Lund 1977), and its beneficial effect has been demonstrated with many foods sensitive to heat treatment (Seregély et al., 2007; Oey et al., 2008). An additional advantage includes that in HHP procedure liquid foods are treated in the packing material to avoid potential post-infection of the product. Furthermore, for pasteurization of bulk material (exceeding 1 kg) in contrast to heat treatment, no heat shock effect (Lindquist, 1986) induced by low warming-up ate is expected in case of HHP procedure since antimicrobial effect occurs momentarily at the same time at all points of food (Lechowich, 1993).

For treatment of liquid whole egg in addition to the purpose of achieving the satisfactory microbiological condition, it is important that the product should preserve its beneficial organoleptic and functional features. Deterioration of such properties such as viscosity of product is related to coagulation of specific egg proteins induced by high hydrostatic pressure (Ahmed et al., 2003).

In this work, our purpose was to investigate the effect of treatment at high hydrostatic pressure not significantly deteriorating the calorimetric properties (treating pressure 500 MPa) (Andrássy et al., 2006) on the microbiological and physical characteristics of whole liquid egg.

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Table 1. Trial design and factor levels in encoded values.

Variable	Encoded factor	-1,4142	-1	0	+1	+1,4142
Treatment time (min)	X1	3	5	10	17	20
Treatment pressure (MPa)	X2	200	230	300	370	400

Table 2. Specific test parameters and corresponding results.

Test	P	T	Results, mean (±SD)			
			lg(N/N$_0$), [CFU/ml]			Total germ count
			S. Enteritidis	*L. monocytogenes*	*S. aureus*	[CFU/ml]
1	300	10	5.28(±0.23)	1.51(±0.12)	2.01(±0.19)	2.37(±0.21)
2	200	10	4.89(±0.17)	0.90(±0.18)	1.84(±0.17)	2.41(±0.15)
3	300	17	5.75(±0.18)	1.67(±0.19)	2.28(±0.23)	2.21(±0.16)
4	230	5	4.91(±0.22)	0.94(±0.15)	1.83(±0.18)	2.40(±0.20)
5	370	5	5.96(±0.30)	1.95(±0.17)	2.34(±0.32)	1.99(±0.17)
6	230	15	5.00(±0.13)	0.97(±0.18)	2.00(±0.16)	2.35(±0.21)
7	300	10	5.31(±0.23)	1.65(±0.12)	1.93(±0.18)	2.32(±0.21)
8	400	10	5.31(±0.25)	1.98(±0.20)	2.63(±0.26)	1.36(±0.12)
9	300	3	6.41(±0.31)	1.41(±0.13)	0.92(±0.09)	2.34(±0.17)
10	300	10	4.99(±0.19)	1.59(±0.14)	1.95(±0.17)	2.25(±0.19)
11	370	15	6.11(±0.20)	1.96(±0.19)	2.47(±0.18)	1.65(±0.11)

P: treatment pressure [MPa]; T: treatment time [min].

MATERIALS AND METHODS

Samples and structure of experiments

In our measurements we tested liquid whole egg samples and for each test we artificially infected the liquid whole egg samples with *S. Enteritidis*, *L. monocytogenes,* and *S. aureus* bacteria to achieve 10^8 CFU/ml viable cell count in the samples. 50ml of each sample was treated in "Food Lab900" high hydrostatic pressure instrument (model S-FL-850-9-W, STANSTED Fluid Power Ltd., UK) for 3 – 17 min at 200 – 400 MPa by using Central Complex Rotation Design.

The designs of the experiment and factor levels are shown in Tables 1 and 2. The main advantage of this experimental approach is that we had to perform less experiment to obtain information sufficient for statistically acceptable results since only 17 tests are needed to establish 1 model. For approximation we used the response surface obtained based on secondary polynomial model.

Experiments were conducted in random order and data were analyzed by The Unscrambler v 9.1 (CAMO PROCESS AS, OSLO, Norway) software. In the general form of the secondary polynomial model used in this study there were two X variables that comprises linear X_1, X_2 expressions and quadratic X_1^2, X_2^2 expressions.

$$Y = \beta_{11} + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} \cdot X_1^{\,2} + \beta_{22} X_2^{\,2} + \beta_{12} \cdot X_1 \cdot X_2 \quad (1)$$

X_1 variable represents the treatment time and X_2 represents the treatment pressure. Y is an independent variable to be modelled. β_1, β_2, β_{11}, β_{22}, β_{12} expressions are the regression coefficients of the model (Table 3).

The heating rates and corresponding heat treatment temperatures used in each test are included in Table 2.

Viable cell count test

In our tests we tested the effect of treatment time and treatment pressure on the total viable cell count and *S. Enteritidis*, *L. monocytogenes*, and *S. aureus* viable cell count within the tested range. Dilution plate pouring was performed for each testing to measure viable cell count.

Tenfold serial dilution was performed from the samples by using sterile water and then microbial count of samples was measured by plate pouring with Nutrient agar (Brain Heart agar for *L. monocytogenes*). Plates were incubated at 37°C (30°C for total viable cell count) for 48 h and the colony grown was counted by a colony counter.

RESULTS AND DISCUSSION

Table 2 shows the reductions of each bacterial count and the total germ count in various tests. Comparing the test result many times significant differences can be seen in effect of HHP treatment with various levels and times. Effect of pressure rate and time can be observed in comparison of tests in which one variable was included with the minimum and maximum value of the test range but the other variable is the same in the two tests. For example comparing Test 2 (treatment pressure (P): 200 MPa, treatment time (T): 10 min) and Test 8 (P: 400 MPa,

Table 3. Regression coefficients of the secondary polynomial model for response analysis with encoded units.

Variable	lg (N/N0), [CFU/ml]						Total germ count [CFU/ml]	
	S. Enteritidis		*L. monocyt.*		*S. aureus*			
	β coeff.	p	B coeff.	p	B coeff.	p	B coeff.	p
Constant	5.192	0.00	1.583	0.00	1.965	0.00	2. 315	0. 00
P	0.005	0.03	0.006	0.00	0.004	0.05	-0. 005	0. 00
T	-0.017	0.49	0.010	0.24	0.055	0.06	-0. 014	0. 06
P×T	0.010	0.95	-0.004	0.93	-0.010	0.94	-0. 057	0. 13
P^2	-0.055	0.64	-0.064	0.13	0.157	0.18	-0. 168	0. 00
T^2	0.336	0.03	-0.023	0.55	-0.097	0.38	-0. 012	0. 68
M(r^2)	0.89		0.98		0.88		0.99	

P: treatment pressure [MPa]; T: treatment time [min]; M(r^2): correlation between results measured and calculated with the model.

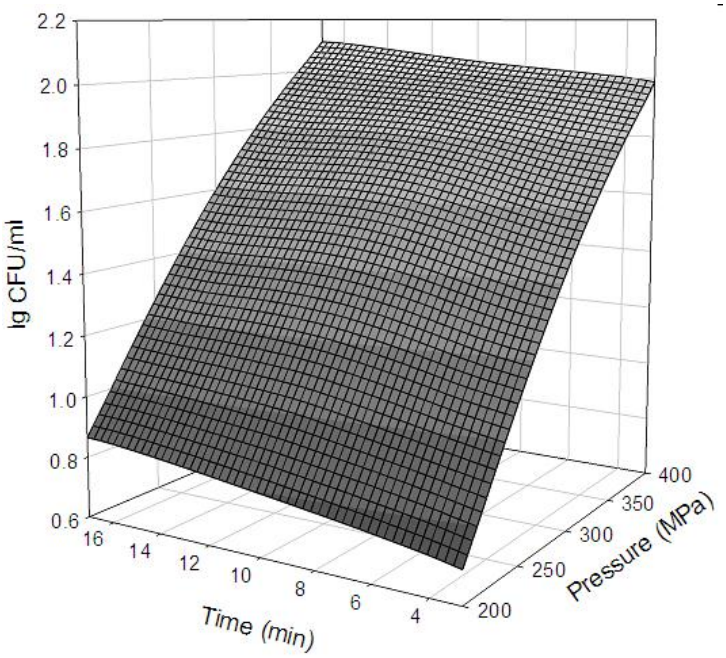

Figure 1. Changes in reduction of *Listeria monocytogenes* viable cell count in function of treatment pressure and treatment time.

T: 10 min) it can be seen that changes in pressure result in more the 1 magnitude difference in reduction of viable cell count of all three tested bacteria. The similar test of pressure treatment time (Tests 3 and 9) also demonstrated differences in microbiological results; however, no definite conclusions could be drawn from these results. For example reduction of *L. monocytogenes* count was not enhanced by the increased treatment time.

By evaluation of secondary polynomial model created for our test results the effects of each variable can also be mathematically analyzed. It is clearly seen that majority of the models fit relatively well to the test result; r^2 did not exceed 0.9 only for the testing of reduction of *S.* Enteritidis and *S. aureus* count. However, the correlation of measured and calculated values were very high for reduction of *L. monocytogenes* viable cell count (r^2=0.98) and total viable cell count after treatment (r^2=0.99).

Analysis of p-values for various β coefficients demonstrates (Table 3) that destruction of various microorganisms was significantly ($p<0.05$) affected only by the pressure rate.

By using these models we can measure the approximate microbiological changes in liquid whole egg in the experimental range with the specified treatment parameters. Graphical illustration of models clearly shows the effect of each parameter. Figure 1 shows the reduction of *L. monocytogenes* viable cell count in liquid whole egg induced by HHP treatment based on the

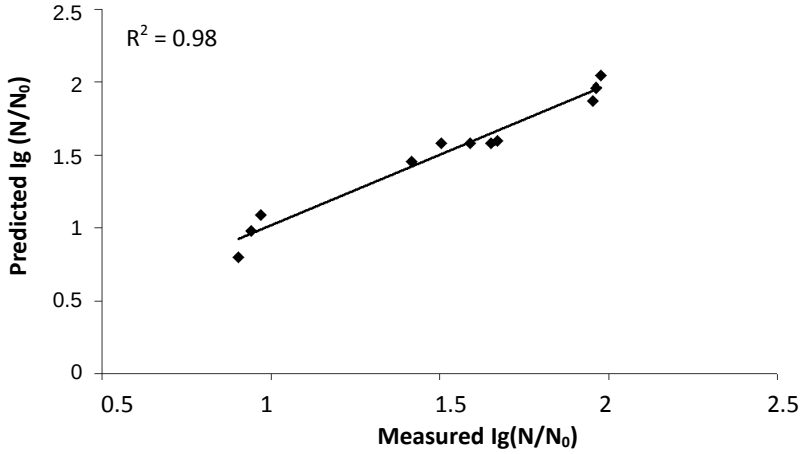

Figure 2. Correlation between the model for reduction of *Listeria monocytogenes* viable cell count and the measured results.

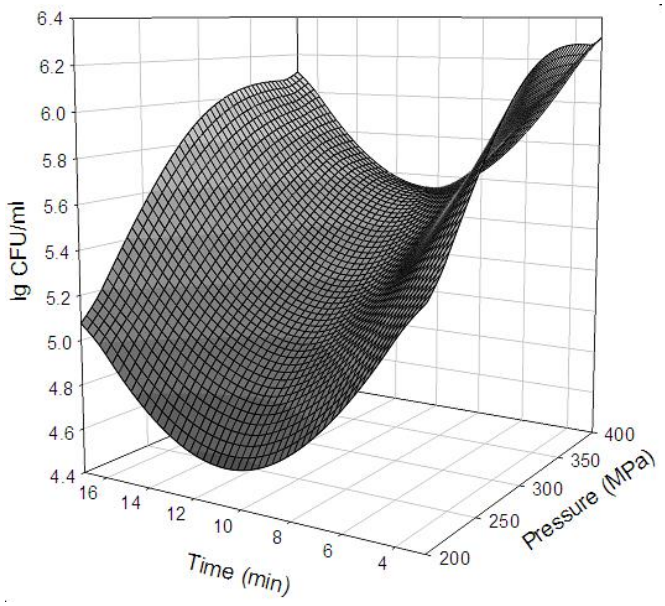

Figure 3. Changes in reduction of *Salmonella Enteritidis* viable cell count in function of treatment pressure and treatment time.

model calculated in function of the treatment pressure rate and time. It can be observed on the figure and primarily the pressure affected the reduction of *L. monocytogenes* viable cell count.

Figure 2 shows the measured and calculated results for the model. The high correlation between the measured and calculated results is clearly seen as it was shown in Table 3. Figure 3 shows reduction of *S. Enteritidis* viable cell count in 3D model in function of treatment time and treatment pressure. The graph clearly shows that efficiency of HHP treatment is not definitely enhanced by the increase in treatment time, within the test range. In contrast, perhaps because of measurement errors, graph shows that after some time $lg(N/N_0)$ value starts to decrease with the increase of treatment time. However, it should be noted that the model demonstrating the reduction of *S*. Enteritidis viable cell count reflects the test results less clearly as the correlation between the results calculated with the model and measured was much lower compared to that for *L. monocytogenes*.

Conclusions

In each test of our investigations the viable cell count of S. *Enteritidis* critical for egg products is reduced significantly.

Reduction of *S. Enteritidis* count was at a level of 5 magnitudes or above even with the treatment pressure of 200 MPa. The other tested micro-organisms were somewhat more stable. However, the viable cell count of *L. monocytogenes* occurring rarely in egg products but being a significant pathogen from food safety aspects can be reduced to zero at pressures above 350 MPa, and with HHP treatment at pressures above 200 MPa for 3-5 min the viable cell count can be reduced by 2 magnitudes.

In our studies we obtained models showing good correlation with our measured results. In conclusion of these results microbial destruction caused by the treatment at high hydrostatic pressure is significantly affected by the pressure level only ($p<0.05$) within the test range.

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