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DESTRUCTION OF SALMONELLA ENTERITIDIS IN LIQUID EGG WHITE AS THE FUCTION OF TREATMENT TEMPERATURE AND HEATING RATE

NÉMETH CSABA, NÉMETH ZOLTÁN, DALMADI ISTVÁN FRIEDRICH LÁSZLÓ, SURÁNYI JÓZSEF, BALLA CSABA

Department of Refrigeration and Livestock Products Technology, Faculty of Food Science, Corvinus University of Budapest, H-1118 Budapest, Ménesi út 43-45, Hungary
csaba.nemeth@uni-corvinus.hu

ABSTRACT – Destruction of Salmonella *enteritidis* in liquid egg white as the function of treatment temperature and heating rate
In our study we investigated the effect of heat treatment temperature and heating rate on heat resistance of *Salmonella enteritidis*. The samples were heated from 4 °C to 48,96-56,04 °C by a heating rate 0,76-9,24 °C·min^{-1} and the changes of the colony counts were determined at given time by plating to XLD agar with overlay.
We used Central Composite Rotatable Design (CCRD) in our experiment and Response Surface Method (RSM) was used to evaluate the data. Our results pointed out that beside the temperature of heat treatment the heating rate have also an effect on the heat destruction of *Salmonella enteritidis*. In case of heating rate 9,24 °C·min^{-1} the $D_{52,5}$-value was 2.32 min, however at heating rate 0,76 °C·min^{-1} the $D_{52,5}$-value was 19.23 min.
In our measurements the samples were heated with linear heating rate in laboratory scale. so further studies are necessary to describe the heat resistance changes of *Salmonella enteritidis* under parameters which model the industrial heating circumstances.

Key words: *Salmonella*, liquid egg, heat treatment, heating rate

INTRODUCTION

Nowadays, the food industry uses pre-processed egg products as raw materials instead of shell eggs. These are commercialized as liquid egg products or egg powders, which are produced by breaking the shell eggs followed by a pasteurization step, moreover when the customer needs only the egg white, he can purchase it separately (FRONING at al., 2002). Most customers prefer liquid egg products because these well preserve the original properties of native egg, however the beneficial microbial growth inhibition properties of native egg does not prevail in liquid egg white (PARK *et al*., 2006; BOARD & FULLER, 2008). Pathogenic microbes can occur in liquid egg products even after the extensive disinfection of egg shell since the *Salmonella enteritidis* can infest egg in the oviduct (T. J. HUMPHREY, 1994). For this reason the raw liquid egg white must be pasteurized below the temperature 60 °C, since the konalbumin, which is the most heat sensitive protein of the egg, denaturates at higher temperatures (FERREIRA *et al*., 1997).
Some studies showed the general pasteurization procedure is inefficient in some cases and the heat treated product proved to contain pathogenic microbes (PETRAK *et al*. 2000) which can proliferate at improper storage conditions (SCHOENI *et al*. 1995; MCQUESTIN *et al*., 2010). During the last decade several new liquid egg preservation technologies have been investigated but most of them are hardly feasible in industrial scale or their application is limited through the heat sensitivity of the egg proteins (SCHWARZEL & PALANIAPPAN, 1997; HAMID-SAMIMI, 2000; ANDRASSY *et al*., 2006). Such a new procedure is the long time (6-24 h) heat treatment at lower temperature (50-55 °C) of liquid egg which we studied in case of packed products (NÉMETH *et al*.,

2010). In these studies we investigated the heat destruction of *Salmonella* and we experienced much higher heat tolerance (D_{55}=47,4) than earlier researchers (D_{54-52} = 1,51-6,12 min) (SÖRQVIST *et al*. 2003; Jin *et al*., 2008). Some microbes such as *Salmonella* show an increasing resistance after heat shock (MAÑAS *et al*., 2003; CEBRIÁN *et al*., 2009). Such a heat shock could be the heating of refrigerated liquid eggs to the temperature of long time heat treatment.
The aim of our work was to investigate the extent of heat resistance changes of *Salmonella enteritidis* in liquid egg products during heating from 4°C to 50-55 °C in 5-60 minutes.

MATERIALS AND METHODS

Samples
The liquid egg white (pH=8.9±0.1) was purchased from a Hungarian egg processing factory. The samples were unpasteurized liquid egg white. Samples were taken from the production line 8 hours prior to the measurements and until the tests they were stored at 4 °C in a refrigerator. The total aerobic colony counts were below 10^3 CFU·ml^{-1}.

Inoculation of samples
1000 g sample was inoculated with overnight culture of *Salmonella enteritidis* NCAIM B2052. 10 loops of overnight cultures from the surface of three plates of modified GPM agar (18 g·l^{-1} agar, 5 g·l^{-1} peptone, 5 g/l glucose, 3 g·l^{-1} meat extract, 0,5 g·l^{-1} sodium chloride) were transferred into 10 ml of sterile water. This was used to set the initial cell number of liquid egg white to ~10^8 CFU·ml^{-1}.

Experimental set up, data analysis
We used Central Composite Rotatable Design (CCRD) in the experiment (BOX & DRAPER, 1987). Response Surface Method (RSM) was used to assess the effect of variables (heating rate, holding temperature) on the decimal reduction time (D-value). *Table 1* and *Table 2* contains the experimental set up and the level of factors. The main advantage of this kind of experimental approach was, that lower number of experiments was necessary to get statistically acceptable information. We used the response surfaces provided by the quadratic polynomial model. The experiments were processed in random order, and the data were analyzed using software (SPSS for Windows, v. 8.0. SPSS Inc., Chicago, IL). Our present study used a general form of cubic polynomial model included two variables X:

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

which contains linear terms X_1, X_2, and quadratic terms X_1^2, X_2^2. Variable X_1 means the heat treatment temperature, variable X_2 means the heating rate. The independent variable for modelling is Y. The β_1, β_2, β_{11}, β_{22}, β_{12} are the regression coefficient expressions of the model (*Table 3*).

Table 1. The experimental set up and the level of factors with coded values

Variable	Factor	-1,4142	-1	0	+1	+1,4142
Temperature (°C)	X1	48.96	50.0	52.5	55.0	56.04
Heating rate (°C·min^{-1})	X2	0.76	2.0	5.0	8.0	9.24

Table 2. Experimental set up and the level of factors (%) with real values

Test	The real value of factors	
	Temperature (°C)	Heating rate (°C·min^{-1})
1	48.96	5.00
2	52.50	5.00
3	55.00	8.00
4	52.50	9.24
5	50.00	8.00
6	52.50	0.76
7	52.50	5.00
8	50.00	2.00
9	56.04	5.00
10	55.00	2.00
11	52.50	5.00

Sample treatment
Prior to the experiment the liquid egg white samples inoculated with *Salmonella enteritidis* were placed into sterile adjustable-temperature thermostate. The samples were stirred during the experiment. The heating rate was linear: 0.76; 2; 5; 8 and 9.24 °C/min to the final temperature 48.96; 50.0; 52.0; 55 and 56.04 °C. The samples were held at the final temperature for 30 min and the changes of the colony counts were determined.

Determination of colony counts
The viable cell number of *Salmonella enteritidis* was determined by counting colony forming unites when the temperature reached the holding temperature and after 2, 5, 7, 10, 15, 20, 25 and 30 minute. The stir homogenized samples were decimal-diluted in sterile water and XLD agar (Merck) plates were poured with overlay. The plates were incubated at 37 °C for 24 h and the typical colonies were counted (MSZ 3640/21-83). Three parallel samples were counted. Plates with less than 30 colonies were not counted.

RESULTS AND DISCUSSION

Changes in the heat resistance of Salmonella enteritidis
The heat destruction of Salmonella *enteritidis* was different at the same holding temperature (50; 52.5; and 55 °C) when the applied heating rate of the refrigerated was different (Figure 1). When the heating rate of the refrigerated liquid egg white was 9.24 °C·min^{-1} and 5 °C·min^{-1} the $D_{52.5}$ value ranged from 1.88 to 2.42. In case of lower

heating rate (0.76 °C·min^{-1}) when the time of the heating to the holding temperature was 62.5 minute the $D_{52.5}$ value was 19.23. At 50 and 55 °C at which temperatures only 2-2 experiments were done with different heating rate the decimal reduction time was significantly (P<0.05) higher at lower heating rate like in the case of 52.5 °C (*Table 3*).

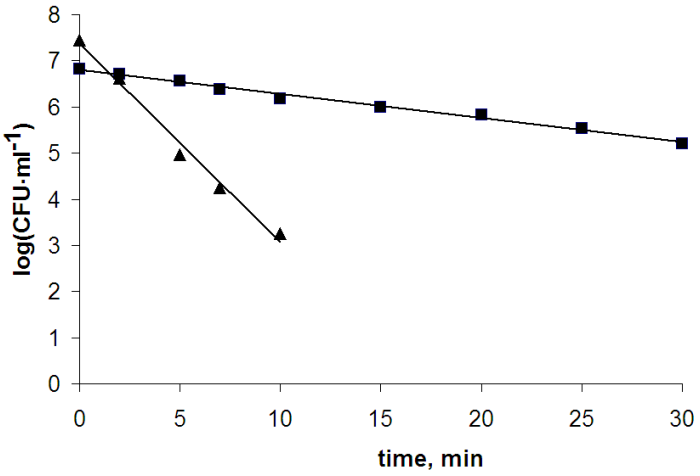

Figure 1. Heat destruction of *Salmonella enteritidis* at 52.5 °C with warming from 4 °C with a heating rate of 0.76 °C·min^{-1} (■) and 9.24 °C·min^{-1} (▲)

Table 3. Heat destruction of *Salmonella enteritidis* in different tests

Test	n	R^2	Stand. error	D-value	
				Mean	Conf. Interval (95%)
1	9	0.993	0.125	11.05	10.87 - 11.23
2	5	0.978	0.326	2.34	2.12 – 2.56
3	5	0.940	0.195	1.88	1.65 – 2.11
4	5	0.989	0.403	2.32	1.97 – 2.67
5	6	0.995	0.080	6.54	6.23 – 6.85
6	9	0.992	0.261	19.23	18.77 – 19.69
7	5	0.972	0.340	2.42	2.13 – 2.71
8	9	0.989	0.218	9.60	9.19 – 10.01
9	5	0.957	0.267	2.10	1.94 – 2.26
10	6	0.985	0.386	3.29	3.01 – 3.57
11	5	0.968	0.177	2.35	2.11 – 2.59

Model development

Based on the statistical analysis of the data we can conclude that the heating rate has a significant (P < 0.05) effect on the heat resistance of *Salmonella* cells in the studied temperature range. The above mentioned heat shock effect (Mañas *et al*.. 2003; Cebrián et al. 2009) could be the reason for this behaviour so certain microorganisms may increase their resistance toward different preservation technologies like heat treatment if they are incubated under sub lethal conditions.

Figure 2 shows the response surface concerning D-values of different holding temperatures and the heating rates. The decimal reduction time decreases with increasing temperature and with the decreasing heating rate. The statistical analysis established that the studied parameters have a significant effect on the D-value.

The *Table 4* shows parameters of the quadratic polynomial model fitted on the results. The model correlated. The model has a relatively good (R^2=0.904) correlation with the results.

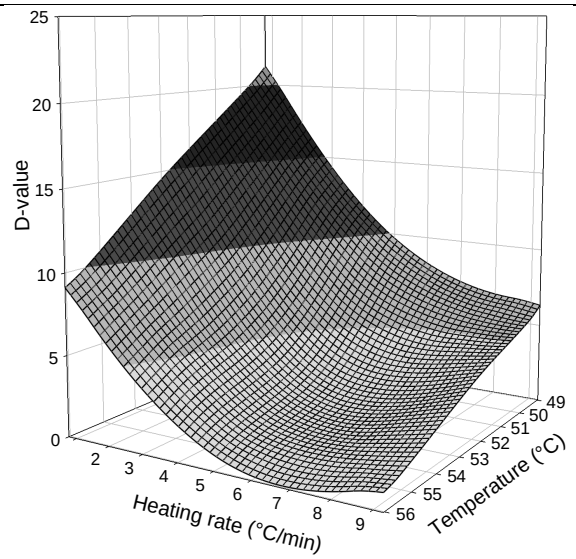

Figure 2 The effect of heating rate and holding temperature on the heat resistance of Salmonella

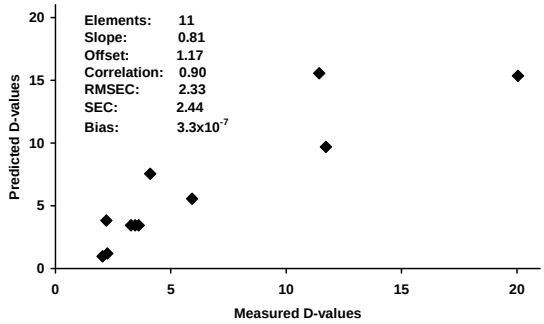

Figure 3 The correlation of the model and the results

Table 4. The regression coefficient of the quadratic polynomial model for the RSM with the coded parameters

Factors	β-coefficient
Constant	3.45
H	-1.235
T	-1.358
H^2	0.5445
T^2	0.0887
H × T	0.6201
R^2	0.904
F-rate	4.44
Likelyhood of *F*	$P \leq 0.1$

H-heating rate (°C·min^{-1})
T- temperature (°C)

CONCLUSIONS

We can conclude that the heating rate and the holding temperature have an effect on the heat resistence of *Salmonella enteritidis* in liquid egg white. This should be considered particularly in case of technologies where the refrigerated liquid egg white is heated to heat treatment temperature for a relatively long time.
In our measurements the samples were heated with linear heating rate in laboratory scale so further studies are necessary to describe the heat resistance changes of *Salmonella enteritidis* under parameters which model the industrial heating circumstances.

REFERENCES

ANDRASSY E.; FARKAS J.; SEREGELY ZS.; DALMADI I.; TUBOLY E.; LEBOVICS V., (2006). Changes of hen eggs and their components caused by non-thermal pasteurzing treatments. Acta Aliment. 35. 305-318.

BOARD. R.G.; FULLER. R.. (2008). Non-specific antimicrobial defences of the avian egg. embryo end neonate. Biol. Rev.. 49. 15-49.

BOX. G.E.P.; DRAPER. N. R.; (1987). *Empirical Model-building and Response Surfaces*. NY: John Willey and Sons Inc.

CEBRIÁN. G.; CONDÓN. S.; MAÑAS. P., (2009). Heat-adaptation induced thermotolerance in *Staphylococcus aureus*: Influence of the alternative factor σ^B. Int. J. Food. Microbiol.. 135. 274-280.

FERREIRA. M.; HOFER. C; RAEMY A., (1997). A calorimetric study of egg white proteins. J. Therm. Anal. Calorim.. 48. 683-690.

FRONING. G.W.; PETERS. D.; MURIANA. P.; ESKRIDGE. K.; TRAVNICEK. D.; SUMNER. S.S., (2002). International egg pasteurization manual**.** United Egg Association. Alpharetta. GA

HAMID-SAMIMI. M. H., (2000). Process for producing pasteurized liquid egg products. US 6024999

HUMPHREY. T.J., (1994): Contamination of egg shell and contents with *Salmonella enteritidis*: a review. *Int. J. Food. Microbiol.*. 21. 31-40.

JIN. T.; ZHANG. H.; BOYD. G.; TANG. J., (2008). Thermal resistance of *Salmonella enteritidis* and *Eschericha coli* K12 in liquid egg determined by thermal-death-time disk. J. Food Eng., 84. 608-614.

MCQUESTIN. O.J.;MUSGROVE. M.T; TAMPLIN. M.L., (2010). Kinetics of growth and inactivation of *Salmonella enterica* serotype Typhimurium DT104 in pasteurised liquid egg products. Food Microbiol.. 27. 396-402.

MAÑAS. P.; PAGÁN. R.; ALVAREZ. I.; USÓN. S.C., (2003). Survival of *Salmonella senftenberg* 775 W to current liquid whole egg pasteurization treatments. Food Microbiol.. 20. 593-600

NÉMETH CS.; FRIEDRICH L.; BALLA CS.; SUHAJDA Á., (2010). Effect of changes in heat resistance of *Salmonella spp.* during pasteurization on the efficiency of long term heat treatment at 55 °C. Acta Agronomica Óváriensis. 52. 3-10

PARK. S.-I.; DAESCHEL. M.A.; ZHAO. Y., (2006). Functional properties of antimicrobial lysozyme-chitosan composite films. J. Food. Sci.. 69. 215-221

PETRAK. T.; MEDIC. H.; NOVAKOVIC. P.; BOTKA-PETRAK K., (2000). Bacteriological contamination of egg products after thermal preservation processes. Acta Aliment.. 29. 315-322

SCHOENI. J.L.; GLASS. K.A.; MCDERMOTT. J.L.; WONG. A.C.L., (1995). Growth and penetration of *Salmonella enteritidis*, *Salmonella heidelberg* and *Salmonella typhimurium* in eggs. Int. J. Food. Microbiol.. 24. 385-396

SÖRQVIST. S., (2003). Heat resistance of liquids of *Enterococcus spp.. Listeria spp.. Escherichia coli. Yersinia enterocolitica, Salmonella spp. and Campylobacter spp*.. Acta Vet. Scand., 44. 1-19

SWARTZEL. K. R.; PALANIAPPAN. S., (1997). Method for pasteurzing liquid whole egg products. US 5670199