

Study of Long Term Post-Treatment of Whole Egg Powder at 50–55°C

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In our experiment whole egg powder samples (infected with *Salmonella* Enteritidis for microbiological tests) were treated at 50.38–54.62°C for 6.41–24.09 h and tested for decrease in *Salmonella* count or changes in their solubility and colour. Central Composite Rotational Design (CCRD) was used in our tests. In all tests performed within the testing range we achieved a decrease in *Salmonella* count of at least 5 magnitudes and in cases when the samples were treated at min. 52.5°C for at least 9 h no viable *Salmonella* could be detected in the whole egg samples at the end of treatments. Our measurements have shown that deterioration of solubility and discolouration was significantly affected only by the temperature change ($p < 0.05$).

INTRODUCTION

Egg products in the European Union with 27 member states has reached 6.4 millions metric tones in 2008 which is a little more than 10% of world production. Despite the increase in egg production and consumption in the world in the last ten years, egg production stagnates in the European Union. Meanwhile the layer sites and egg processing plants underwent significant development in accordance with the consumers' needs for protection of animals and regarding various products and as a result of implementation of the EU regulation on protection of animals. The average annual consumption is 240 eggs per year which varies between countries, and egg consumption shows an increasing trend compared to the total consumption. These facts as well as the European regulations (animal protection, environmental protection, hygienic requirements), fluctuation of feed prices, changes in consumers' preferences and finally the results of WTO discussions suggest that egg products (mainly egg powder due to transportation considerations) from developing countries will have to be considered as well [Magdelaine & Braine, 2010].

It should also be taken into account that besides the fact that pathogenic bacteria may be present in the European liquid egg and egg powder products [Petra^k *et al.*, 2000], this

may apply even more to products from non-EU countries. Furthermore, their inactivation is not sure even under the unfavourable conditions of the powders (low water activity, possible refrigeration) [Brackett & Beuchat, 1991]. Rapid growth after getting into the human body or reconstitution of powder may cause serious problems [Baron *et al.*, 1999]. That is why egg distributors/egg processing plants are seeking for new procedures to improve the microbiological status of egg powders that underwent insufficient heat treatment, and to eliminate *Salmonella*.

Such procedures may include posterior heat treatment of egg powders at 45–50°C that has been applied successfully for a long time for skimmed milk powders. In case of such technologies it should be considered that the highest heat stability of microorganisms can be observed in powdered food products with various water activities [Laroche *et al.*, 2005], thus higher than the expected heat stability can be observed in case of some critical microorganisms.

In addition to the microbiological status, sensory and functional characteristics of egg powders are also important. It is important that deterioration of egg during spray-drying [Lechevalier *et al.*, 2007] should not be increased by the effects of treatments at excessive temperatures or too long incubation [Talansier *et al.*, 2009].

Response surface methodology (RSM) explores the relationships between several explanatory variables and one or more response variables. This statistical method reduces

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the factorial experiments from a large number of parameter combinations to a far less number without losing any information including quadratic and interaction effects [Nwabueze, 2010]. Granato *et al.* [2010] used this method to evaluate the sensory properties of soy-based dairy free products.

The purpose of our tests was to find out how the incubation for more than 6 hours but less than 24 hours at the temperature range of 50–55°C affects the microbiological status and some physical properties (colour, solubility) of whole egg powder.

MATERIALS AND METHODS

Samples

We used fresh, un-pasteurised but homogenised liquid whole egg samples from a Hungarian egg processing plant for our test. Liquid samples were taken from the production line on the day before the experiment and the samples were stored in a refrigerator at 4°C until the testing.

On the day of experiments liquid egg samples were inoculated with pure 24-h culture of *Salmonella enterica subsp. enterica*, serotype Enteritidis strain NCAIM B2052 in order to achieve a concentration of *Salmonella* Enteritidis in the liquid egg of 10^{10} CFU/mL. The resulting liquid whole egg samples were dried in a direct flow laboratory spray drier (temperature of drying: 220°C). In the experiments, incubation tests were performed with the whole egg powder samples obtained with this method (*Salmonella* content: $\sim 1.58 \times 10^9$ CFU/g, moisture content $4.7 \pm 0.12\%$).

Experiment design, data analysis

In our experiment we used Central Composite Rotational Design (CCRD) [Box & Draper, 1987]. For analysis of the effect of specific variables (treatment temperature, treatment time) on the changes in *Salmonella* Enteritidis count ($\lg(N/N_0)$), solubility and colour-difference we used the Response Surface Method (RSM). The design of the experiment and factor levels are shown in Table 1. The temperature levels were selected based on our previous investigation on the calorimetric changes of egg products and the thermal resistance of *Salmonella* Enteritidis [Németh *et al.*, 2010; 2011]. Briefly, we experienced the denaturation of conalbumin at higher temperatures (above 55°C), and this temperature range was efficient for the inactivation of *Salmonella* Enteritidis. The main advantage of this experimental approach was that we had to perform less experiment to obtain information sufficient for statistically acceptable results. For approximation we used the response surface obtained based on the secondary polynomial model. Experiments were processed in random order, and the data were analysed by specific software (SPSS for Windows, v. 8.0. SPSS Inc., Chicago,

IL). In the general form of the polynomial model used in this study there were two X variables.

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

that comprises linear X_1, X_2 expressions and quadratic X_1^2, X_2^2 expressions. X_1 variable represents the treatment temperature and X_2 represents the heating rate. Y is an independent variable to be modelled. $\beta_1, \beta_2, \beta_{11}, \beta_{22}, \beta_{12}$ expressions are the regression coefficients of the model (Table 4).

Measurement of *Salmonella* Enteritidis count

For *Salmonella* Enteritidis count testing 1 gram of liquid egg samples was homogenised with sterile water used as a diluent. From this prepared test sample quantities corresponding to $1.0 \text{--} 10^{-8}$ g (by tenfold serial dilution) from three different weighings were transferred into XLD medium by the covered plate pouring technique. The samples from three different weighings were decimal-diluted in sterile water and XLD agar plates were poured with overlay. The prepared plates were incubated at 37°C for 24 h and the characteristic colonies grown were counted on each Petri dish (MSZ 3640/21–83). The colony count was always determined based on testing of 3 parallel samples. Plates with less than 30 but more than 300 colonies were not included in the evaluation of results.

Solubility testing

Five grams of the test sample (we used 3 replicates for each test) were weighed with an analytical balance with 0.0001 g accuracy and rubbed on a friction board with a few mL of distilled water, and then left for 15 min (with several rubbing meanwhile). Then, the sample was completely rinsed into a volumetric flask (200 mL) by using a funnel. Subsequently, the flask was filled up with water to the level, sealed with a plug and left for 10 min with several mixing. Then the flask content was centrifuged for 20 min (110 xg) to separate the insoluble particles of the product. Then, 20 mL of the supernatant were pipetted into the previously dried (at 105°C) and weighed Petri dish. This Petri dish with the supernatant was placed into a drying chamber at 105°C for evaporation of the water and then the residue was dried at the same temperature until final mass stability. The sample was subsequently cooled to room temperature in an exsicator and weighed with 0.0001 g accuracy by using an analytical balance.

Solubility (O) was calculated using the following formula:

$$S = (G \cdot 10^5) / [a \cdot (100 - b)] \quad (2)$$

where: S – mass of the dry residue after evaporation and drying of 20 mL supernatant phase (g); a – mass of the initially measured egg powder (g); and b – moisture content of egg powder (%).

Colour analysis

Colour analysis was performed by using Minolta Chroma Meter CR-200 model tristimulus colour analyser for measuring reflected-light colour. In the CIELAB system L^* is lightness (black point $L^*=0$, white point: $L^*=100$), a^* is characteristic to red-green colour, and b^* is the blue-yellow colour (sign: $+a^*$ red, $-a^*$ green, $+b^*$ yellow, $-b^*$ blue).

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

Variable	Encoded factor	-1.4142	-1	0	+1	+1.4142
Treatment temperature (°C)	X1	50.38	51.0	52.5	54.0	54.62
Treatment time (°C/min)	X2	6.41	9.0	12.25	21.5	24.09

The CIELAB colour stimulus space uses vector metrics to characterise chromaticity. The colour-difference is the most important value of the CIELAB system. Colour-difference (ΔE^*_{ab}) for comparison of the samples and the control was calculated with equation 3.

$$\Delta E^*_{ab} = \sqrt{(\Delta a^2 + \Delta b^2 + \Delta L^2)} \quad (3)$$

There can be ranges by which the numerical values of the resulting colour stimulus can be assigned to the level of human perception (Table 2).

Since the amount and nature of samples did not allow direct analysis of the colour status of egg powders, this measurement was performed through a plastic foil. We tried to minimise the resulting difference by performing the preliminary calibrations not simply with the white test piece (ceramic sheet) but we placed a clean plastic foil be-

TABLE 2. Evaluation of colour difference of the average human eyes.

ΔE^*			Detected difference
0.0	0.5	Non-detectable
0.5	1.5	Hardly detectable
1.5	3.0	Detectable
3.0	6.0	Clearly visible
6.0	12.0	Great

low the measuring head. Ten replicates were measured with the sample of each test.

RESULTS AND DISCUSSIONS

Table 3 shows the actual values of the various tests as well as our measuring results. Worthy of notice is that *Salmonella* Enteritidis could not be detected at the end of most of the various tests. In all tests performed within the testing range we achieved a decrease in *Salmonella* count of at least 5 orders of magnitude, and in cases when the samples were treated at min. 52.5°C for at least 9 h no live *Salmonella* could be detected in the whole egg samples at the end of treatments.

Solubility of powdered whole egg powders without post-treatment was 72%. As it is shown in the table this value did not decrease below 69% in the majority of cases. Deterioration was 5% or worse only in cases when the temperature of post-treatment was 54°C (Tests 6 and 8) or higher (Test 2) and the time of incubation was longer than 24 h (Test 4).

The colour differences observed with post-treatment of egg powders were not visible with human eyes in most of the tests, i.e. ΔE^* value did not exceed 0.5. In these tests, higher values were measured only in tests performed at 54°C (Tests 6 and 8) and higher (Test 2). Colour difference of control and treated samples did not reach the “hardly visible” range ($\Delta E^* < 1.5$).

Table 4 shows the regression coefficients of the quadratic polynomial model for response analysis with encoded units.

TABLE 3. Experiment design and factor levels (%) with actual values, and measuring results.

Test	Treatment temperature (°C)	Incubation time (h)	Decrease in <i>Salmonella</i> count (lgN/N ₀)	Solubility (%)	ΔE^*
1	50.38	15.25	9.2±0.0*	71.2±1.3	0.12±0.01
2	54.62	15.25	9.2±0.0*	59.0±0.6	1.02±0.08
3	52.50	6.41	4.8±0.1	71.8±1.1	0.28±0.01
4	52.50	24.09	9.2±0.0*	67.1±0.4	0.45±0.02
5	51.00	9.00	5.1±0.2	71.1±0.6	0.24±0.01
6	54.00	9.00	9.2±0.0*	67.0±0.7	0.53±0.02
7	51.00	21.50	9.2±0.0*	69.7±1.0	0.32±0.00
8	54.00	21.50	9.2±0.0*	64.2±0.6	0.68±0.03
9	52.50	15.25	9.2±0.0*	70.2±0.3	0.37±0.01
10	52.50	15.25	9.2±0.0*	68.8±1.2	0.38±0.02
11	52.50	15.25	9.2±0.0*	69.2±0.9	0.35±0.01

* *Salmonella* count decreased below the detection limit in each replicate measurement.

TABLE 4. Regression coefficients of the quadratic polynomial model for response analysis with encoded units.

Factors	lg(N/N ₀)		Solubility		ΔE^*	
	β coefficient	p-value	β coefficient	p-value	β coefficient	p-value
Constant	9.20	0.00*	69.33	0.00*	0.37	0.00
A	0.34	0.10	-2.25	0.00*	0.16	0.00
B	0.21	0.00*	-0.22	0.03	0.01	0.16
A×B	-0.82	0.04*	-0.40	0.49	0.01	0.74
A×A	0.02	0.95	-1.58	0.02*	0.08	0.07
B×B	-0.87*	0.02*	0.22	0.65	-0.01	0.87

A - Treatment temperature (°C); B - Incubation time (h); * - significant effect (p<0.05).

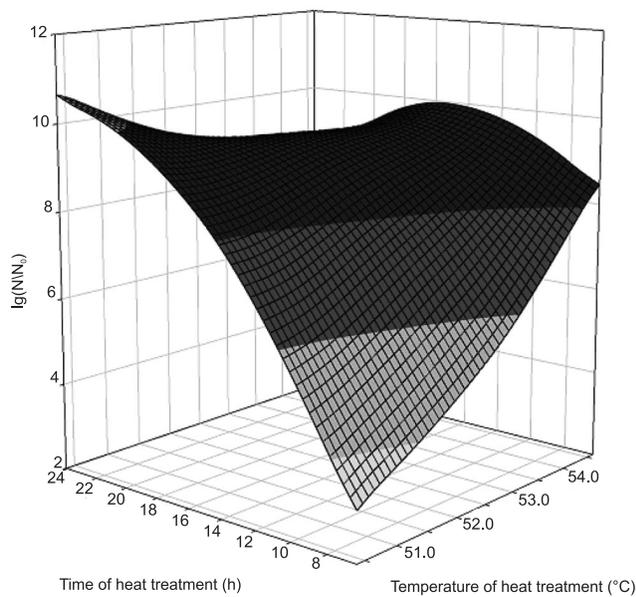


FIGURE 1. Change in *Salmonella* count in function of time and temperature of treatment.

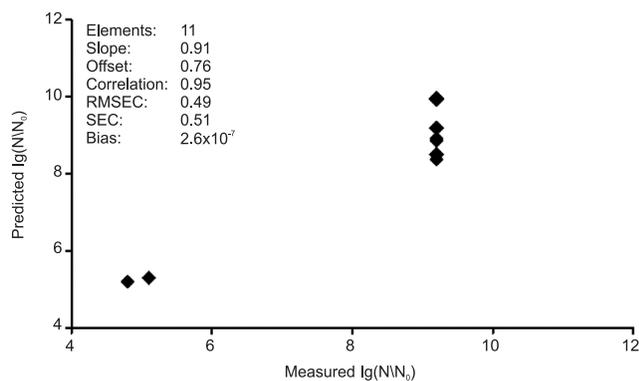


FIGURE 2. Correlation of the obtained model and the measured results for the change in *Salmonella* count.

As it is shown in the table, Figure 1 also clearly shows that in the tested range mainly the treatment time had a significant effect on the change in *Salmonella* count ($p < 0.01$). It is demonstrated that *Salmonella* count decreased by more than 8 orders of magnitude with 12-h incubation even at the lowest treatment temperatures of the tested temperature range.

The correlation of our model with the measuring points ($r^2 = 0.9$) is shown in Figure 2. However, it should be noted that we could get a more accurate picture of the change in the effect of the treatment on the decrease in *Salmonella* count by narrowing the testing range (temperatures below 52.5°C , incubation for not more than 12 h), because the number of *Salmonella* Enteritidis cells decreased below the detectable limit in large portion of our testing range.

These results are consistent with literature data since similar results were obtained in a study performed with products similar to egg, e.g. in skimmed milk powder [Licari & Potter, 1970]. However, it should be considered

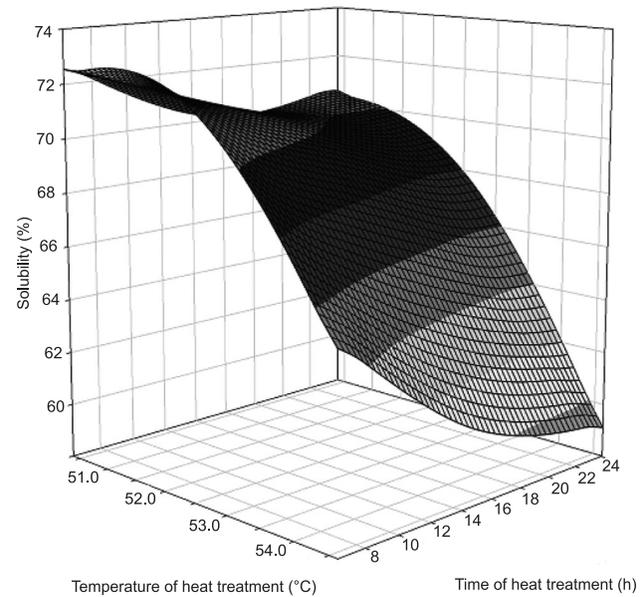


FIGURE 3. Solubility of whole egg powder in function of time and temperature of treatment.

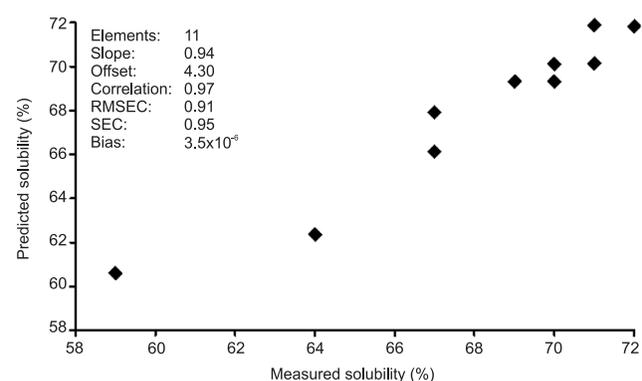


FIGURE 4. Correlation of the obtained model and the measured results for the change in solubility of whole egg powder.

that this type of post-treatment at the edges of the measuring range (54.62°C , 24.09 h) might be insufficient against specifically resistant *Salmonella* strains [Jung et al., 1999].

According to the obtained model, the treatment temperature had a significant effect on the solubility of whole egg powder ($p < 0.01$). It can also be seen in Figure 3 that, although extension of incubation time from 6 h to 24 h may cause up to 3–5% difference in solubility at (higher) temperatures above 54°C , this difference does not reach 2% at lower temperatures.

The correlation between the measured values and the values predicted by the model (Figure 4) was even stronger in this case ($r^2 = 0.94$).

Based on the estimated model treatment, the temperature had a significant effect on colour difference ($p < 0.01$). It is seen from the model and in Figure 5 that the effect of incubation time was significantly more negligible in this case compared to the change in solubility ($p = 0.16$).

The model for colour difference also well correlated ($r^2 = 0.92$) with the measured results (Figure 6).

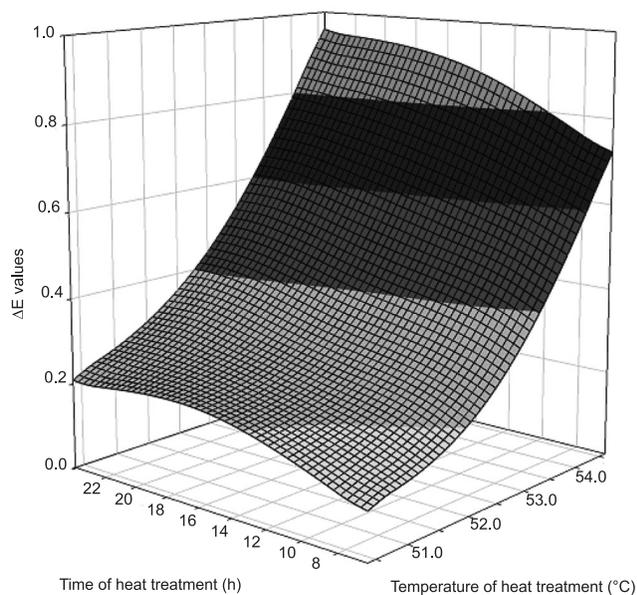


FIGURE 5. Colour change due to incubation of whole egg powder at a specific temperature in function of the time and temperature of treatment.

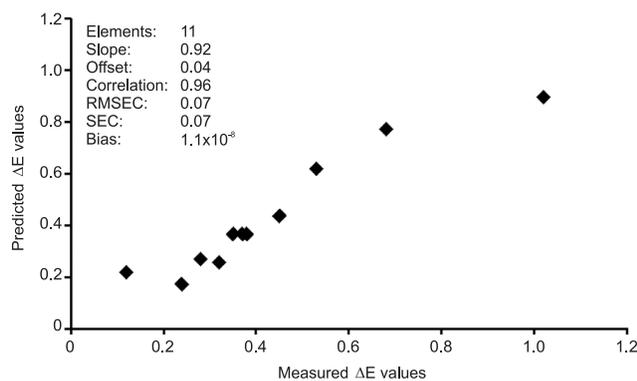


FIGURE 6. Correlation of the obtained model and the measured results for the colour change due to incubation of whole egg powder at a specific temperature.

CONCLUSIONS

Based on our results, the viable cell count of *Salmonella* Enteritidis in whole egg powder can be significantly reduced by post-treatment at 50–55°C for 6–24 h. Considering the actual infection characteristic to egg powders, this procedure is found suitable for the elimination of *Salmonella* from the infected lots.

In our measurements, the effects of heat treatment parameters on the solubility and colour change were tested. In each case we could find the models well correlating with the results that may help with optimisation to achieve maintenance of appropriate physical properties in addition to the efficacy of the procedure to decrease microorganism count.

Based on our study it can be concluded that the applied heat treatment can enhance the safety properties of egg powders without the significant deterioration of their physical properties.

REFERENCES

1. Baron F, Gautier, M., Brulé, G., Rapid growth of *Salmonella* Enteritidis in egg white reconstituted from industrial egg white powder. *J. Food Protect.*, 1999, 62, 585–591.
2. Box, G.E.P., Draper, N.R., *Empirical Model-Building and Response Surfaces*. 1987, John Wiley and Sons Inc. NY.
3. Brackett R.E., Beuchat L.R., Survival of *Listeria monocytogenes* in whole egg and egg yolk powders and in liquid whole eggs. *Food Microbiol.*, 1991, 8, 331–337.
4. Granato D., De Castro I.A., Ellendersen L.S.N, Masson M.L., Physical stability assessment and sensory optimization of a dairy-free emulsion using response surface methodology. *J. Food Sci.*, 2010, 75, 149–155.
5. Jung Y.S., Beuchat L.R., Survival of multidrug-resistant *Salmonella* typhimurium DT104 in egg powders as affected by water activity and temperature. *Int. J. Food Microbiol.*, 1999, 49, 1–8.
6. Laroche C., Fine F., Gervais P., Water activity affects heat resistance of microorganisms in food powders. *Int. J. Food Microbiol.*, 2005, 97, 307–315.
7. Lechevalier V., Jeantet R., Arhaliass A., Legrand J., Nau F., Egg white drying: Influence of industrial processing steps on protein structure and functionalities. *J. Food Eng.*, 2007, 83, 404–413.
8. LiCari J.J., Potter N.N., *Salmonella* survival during spray drying and subsequent handling of skim milk powder. III. Effects of storage temperature on salmonella and dried milk properties. *J. Dairy Sci.*, 1970, 53, 877–882.
9. Magdelaine P., Braine A., International and European overview of egg production and consumption., *Productions Animales*, 2010, 23, 111–121.
10. Németh Cs., Horváth K., Drobecz Á., Friedrich L., Pásztor-Huszár K., Balla Cs., Calorimetric study of changes induced by preservatives in liquid egg products. *Pol. J. Food Nutr. Sci.*, 2010, 60, 347–352.
11. Németh Cs., Mráz B., Friedrich L., Suhajda Á., Janzso B., Balla Cs., Microbiological measurements for development of a new preservation procedure for liquid egg., *Czech J. Food Sci.* (in press).
12. Nwabueze T.U., Review article: Basic steps in adapting response surface methodology as mathematical modelling for bioprocess optimisation in the food systems. *Int. J. Food Sci. Technol.*, 2010, 45, 1768–1776.
13. Petrak T., Medic H, Novakovic P., Botka-Petrak K., Bacteriological contamination of egg products after thermal preservation processes. *Acta Aliment.*, 2000, 29, 315–322.
14. Talansier E., Loisel C., Dellavalle D., Desrumaux A., Lechevalier V., Legrand J., Optimization of dry heat treatment of egg white in relation to foam and interfacial properties. *Food Sci. Technol.-Lebensm. Wiss.*, 2009, 42, 496–503.

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