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Analysis of parameters affecting the shelf life of liquid whole egg

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SUMMARY

In our measurements we tested the changes in viable cell count in liquid whole eggs. Central complex rotation design was used in planning our experiments, and response surface method (RSM) was applied to analyze the effect of each parameter (pH, storage temperature, storage time and preservative content) on the viable cell count.
Based on our measurements, in addition to the storage time, the pH value and storage temperature of liquid egg samples significantly affect ($p < 0.01$) the viable cell count, but any inhibitory effect of preservatives (Na benzoate, K sorbate mixture) on microbial growth could not be clearly detected.
Using the secondary polynomial model which was adjusted to our data, the measurements were defined very well; therefore it is hoped that our results will afford real help in estimation of the microbiological condition of liquid whole egg products which are preserved by various methods.
Keywords: liquid egg, preservative, pH, storage temperature.

INTRODUCTION

Shelf life of liquid egg products is relatively short, since proteins responsible for the microbial resistance of eggshell are denatured during pasteurization (*Baron et al.* 1999),

and the mixture of egg white and yolk provides an excellent medium for microbial growth (*Powrie* and *Nakai* 1985). Therefore, liquid egg production plants use various preservatives to increase the shelf life of their products. Such substances include citric acid and other additives which adhere to the Hungarian Codex Alimentarius, such as sodium benzoate and potassium sorbate. Total maximum allowable concentration of these two preservatives together is 5000 mg/l (*Codex Alimentarius Hungaricus* 1995).

The main limitation in the selection of the amount of citric acid is the pH sensitive proteins of egg; these proteins are denatured at a relatively high rate, a pH lower than 5 (*Ferreira et al.* 1997). The adjusted acidity strongly influences the efficiency of preservatives that can be used in liquid egg products. To complicate matters further, potassium sorbate and sodium benzoate do not have the appropriate effect at nearly neutral pH values (*Marín et al.* 2003).

Sodium benzoate and potassium sorbate can be added to liquid egg products in any amounts up to a concentration of 5000 mg/l (one of them can even be omitted); however, experiments with foods prepared with eggs have shown that these substances can significantly reduce microbial growth only when present in combinations (*Wind* and *Restaino* 1995).

It should be noted that besides correct selection of preservatives, there are three other factors which also significantly affect the shelf life of products: adequate storage temperature (*Schoeni et al.* 1995, *McQuestin et al.* 2010), microbial contamination, and composition of the fresh product from the production line (*Petrak et al.* 2000).

Our purpose with this work was to determine how the total viable cell count changes in liquid whole eggs under refrigeration, depending on the storage temperature, the pH value of samples, and their preservative content.

Materials and methods

Samples and storage

Liquid egg white samples (pH = 7.1±0.1) were obtained from a Hungarian egg processing plant. Samples were raw liquid egg which had not been subjected to heat treatment. Liquid egg samples were collected from the production line the evening before the experiment, and were refrigerated at 4 °C for a maximum of 24 hours until the tests were started.

The pH value of samples was adjusted with citric acid, and we used a mixture of sodium benzoate and potassium sorbate in 1:1 ratio as preservative. After the adjustment of pH and preservative content, the baseline of total viable cell count (N_0) of all samples was measured and found to be nearly identical, 2.68×10^3 ($\lg N_0 = 3.43 \pm 0.19$). After adjustment of the values the samples were stored at 4 to 10 °C in a refrigerator in accordance with the test requirements.

Test design, data analysis

The central complex rotation design (CCRD) (*Box* and *Draper* 1987) was used for the tests. The response surface method (RSM) was applied to analyze how each variable (pH, storage temperature, storage time, and preservative content) influenced the viable

cell count. *Tables 1.* and *2.* show the design of the experiment and the factor levels. The main advantage of this experimental approach is the decreased number of the tests to be performed. However, sufficient information was available for acceptable statistical results.

Table 1. Trial design and factor levels in encoded values

	Encoded factor	**–2**	**–1**	**0**	**+1**	**+2**
pH	X_1	4.0	4.5	5.0	5.5	6.0
Preservative concentration (g/kg)	X_2	0.0	0.1	0.3	0.5	0.7
Storage temperature (°C)	X_3	4	6	8	10	12
Storage time (day)	X_4	1	4	7	10	13

Table 2. Trial design and factor levels (%) in actual values and test

Test no.	**pH**	**Preservative concentration** (g/kg)	**Storage temperature** (°C)	**Storage time** (day)	**lg(N/N$_0$)**
1	4	0.3	8	7	0.05±0.01
2	6	0.3	8	7	7.52±0.38
3	5	0.0	8	7	1.69±0.20
4	5	0.7	8	7	1.60±0.32
5	5	0.3	4	7	0.24±0.09
6	5	0.3	12	7	8.26±0.42
7	5	0.3	8	1	0.24±0.14
8	5	0.3	8	13	3.02±0.55
9	4.5	0.1	6	4	0.14±0.07
10	5.5	0.1	6	4	0.79±0.19
11	4.5	0.5	6	4	0.60±0.09
12	5.5	0.5	6	4	0.77±0.13
13	4.5	0.1	10	4	0.70±0.25
14	5.5	0.1	10	4	2.79±0.34
15	4.5	0.5	10	4	0.66±0.09
16	5.5	0.5	10	4	6.36±0.54
17	4.5	0.1	6	10	0.36±0.05
18	5.5	0.1	6	10	1.98±0.23
19	4.5	0.5	6	10	0.14±0.05
20	5.5	0.5	6	10	1.95±0.13
21	4.5	0.1	10	10	1.76±0.24
22	5.5	0.1	10	10	5.87±0.61
23	4.5	0.5	10	10	1.66±0.26
24	5.5	0.5	10	10	8.12±0.54
25	5	0.3	8	7	1.64±0.11
26	5	0.3	8	7	1.65±0.20
27	5	0.3	8	7	1.68±0.29

N_0 – baseline total viable cell count
N – total viable cell count measured in the test

We used the response surface method for approximation with a polynomial model of second order. Experiments were conducted in random order and data were analyzed with software (Unscrambler v 9.1 (CAMO PROCESS AS, OSLO, Norway). There were four

X variables in the general form of the second order polynomial model used in this study:

$$Y = \beta + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2$$
$$\beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4$$

that provide with linear X_1, X_2, X_3, X_4 expressions and quadratic X_1^2, X_2^2, X_3^2, X_4^2 expressions. The X_1 variable represents the pH adjusted with citric acid, X_2 represents the preservative concentration, X_3 represents the storage temperature, and X_4 represents the storage time. Y is the independent variable to be determined by the model (change in viable cell count). β is the intercept, and β_1, β_2, β_3, β_4, β_{11}, β_{22}, β_{33}, β_{44}, β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{34} expressions are the regression coefficients of the model.

Testing viable cell count

For testing the viable cell count, 1 gram of liquid egg samples were homogenized by continuous stirring and diluted with sterile water. A tenfold dilution series was prepared and from these test samples 1.0–10^{-8} g quantities were transferred into meat liquid agar medium by means of the covered plate pouring technique. The samples were incubated at 30 °C for 72 hours and the colonies grown in each Petri dish were counted. The colony counting was always performed three times. Dishes having less than 30 colonies were not included in the evaluation of results.

Results and discussion

Changes of microbial count in samples variously treated and stored are shown in *Table 2*. The effect of the different variables on the viable cell count can be observed even without analysis of the model. For example, in cases when the tests differed only in the pH adjustment (storage time 7 days at 8 °C with addition of 0.3 g/kg preservative), we observed differences of around 6 orders of magnitude between the samples adjusted to pH = 5.0 and pH = 6.0 (Test 2 and Test 26). Considerable differences were found with 7 days' storage at different temperatures, in terms of the change in viable cell count in samples stored at the lowest (4 °C) and at the highest (12 °C) temperatures. In this case, we measured a difference of 8 orders of magnitude between the results of Test 5 and Test 6.

No significant differences appeared among the results, when the quantities of the preservatives were added according to the upper and lower limit (Tests 3 and 4). After 7 days of storage of samples having pH higher than 5.0, an increase in viable cell count of 1.69±0.20 orders of magnitude was observed in samples stored at 8 °C without added preservative, while an increase of 1.60±0.32 orders of magnitude was observed with 0.7 g/kg preservative concentration. We did not observe significant antimicrobial effect of the preservatives added to liquid egg even at lower pH values (pH = 4.0–4.5). In Tests 21 and 23 we did not find any differences between samples containing the preservatives at 0.1 or 0.5 g/kg concentration after storage when the pH was 4.5, the temperature 10 °C and storage time 10 days.

Storage time had a significant effect on the changes of the viable cell count. After storage for 1 and 13 days (as the lower and upper limits) under similar conditions (pH = 5, T = 8 °C, c = 0.3 g/kg preservative) there was a difference of 2.5–3.0 orders of magnitude in the change of viable cell count (Tests 7 and 8).

Based on the statistical analysis of the mathematical model (summarized in *Table 3.*) among the four single variables only the preservative content proved not to be significant (p = 0.22).

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

	β-coefficients	**MS**	**F-ratio**	**p-value**
Intercept	1.657	8.24	10.34	0.01*
pH (A)	3.13	58.77	73.74	0.00*
preservative (B)	1.18	1.34	1.68	0.22
storage temperature (C)	0.775	57.65	72.33	0.00*
storage period (D)	0.203	8.86	11.11	0.01*
A x B	0.328	2.02	2.53	0.14
A x C	0.814	12.45	15.62	0.00*
A x D	0.311	1.81	2.27	0.16
B x C	0.317	1.89	2.37	0.15
B x D	–0.12	0.27	0.35	0.57
C x D	0.276	1.40	1.79	0.21
A x A	0.389	3.78	4.75	0.05*
B x B	–0.105	0.28	0.35	0.57
C x C	0.497	6.188	7.763	0.02*
D x D	-0.109	0.295	0.37	0.55

* significant effect demonstrated

The significant interactions ($p < 0.05$) were as follows: (pH x storage temperature), $(pH)^2$ and (storage temperature)^2.

Figure 1. shows that there was a close correlation (R = 0.97) between predicted and measured $lg(N/N_0)$ taking into account the effects of the four variables and the interactions between them.

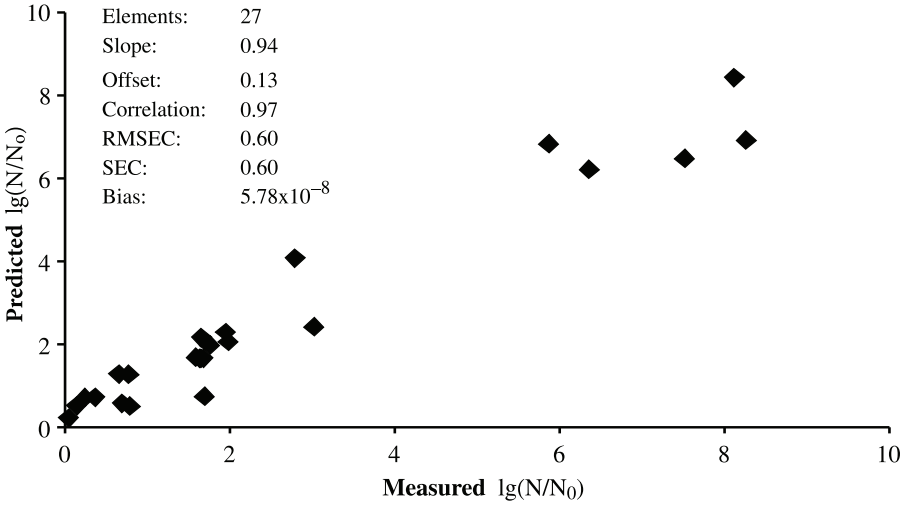

Figure 1. Predicted versus measured viable cell count

In *Figure 2*. the changes of viable cell counts are plotted against one variable while the others are constant. Five readings analyzed at 3 measurement points were revealed by keeping the other 3 variables constant. Three of the 5 readings were in the center of the measurement range (pH = 5.5, preservative concentration = 3.0 g/kg, storage time = 7 days, storage temperature = 8 °C), while one was at the –2 factorial level and the other one at the +2 factorial level. The constant value of the other three variables was the median value of each. It can be seen on the graphs that the results at changing temperature (A), pH (B) and storage time (C) fit quite well with the values described by the model. In the case of preservative (D) the model did not show such a close match to the measurement points. However, observing the scale, we can conclude that the variation during the 7 days of storage was approximately 1 log unit (below 0.2 g/kg preservative concentration).

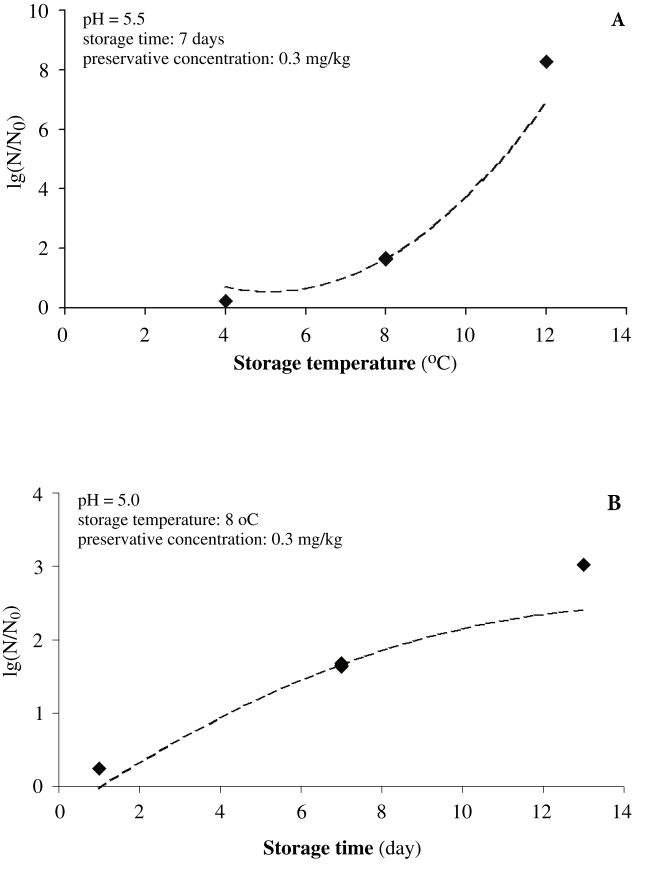

Figure 2A–2B. The changes of the viable cell count in the function of storage temperature (A), storage time (B), ◆ measured points; – – model curve

Figure 2A shows the microbial growth at different temperatures. It can be seen that the microbial growth increased at elevated temperatures, which corresponds to the Ratkowsky equation ($k = b*(T–Tmin)^2$, in other words, the microbial growth rate showed a quadratic correlation with the temperature (*Adams* and *Moss* 1995).
Figure 2B shows the effect of storage on the increase of viable cell count. It can be seen that under constant environmental conditions the microbial growth rate was constant as well.

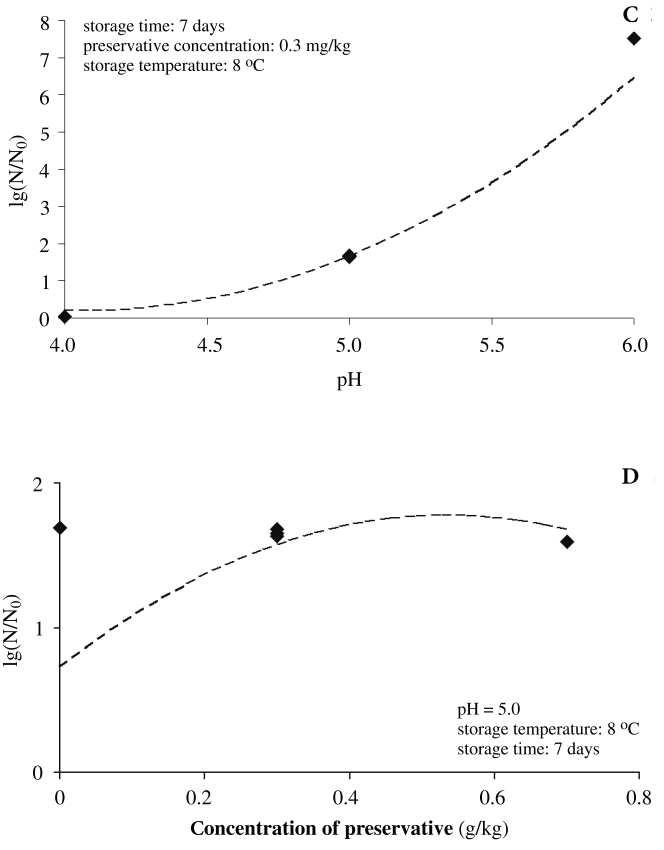

Figure 2C–D The changes of the viable cell count
in the function of pH (C) and concentration of preservative (D)
◆ measured points; – – model curve

Figure 2C shows the effect of the pH on the changes in viable cell count. Below the optimal pH level, the growth rate coefficient related almost quadratically to the pH. In the literature this is described as the expanded Ratkowsky equation $k = b*(pH–pHmin)^2$. The effect of the concentration of preservatives on the changes in viable cell number is presented *in Figure 2D* No significant correlation is shown. This can be explained by the pH level

applied, since at this pH level the undissociated ratio of benzoic acid is 13% and that of sorbic acid is 30% (*Deák* 2006) which is not strong enough to inhibit the microbial growth. *Figure 3.* shows the 3D surface of the changes in the viable cell count in liquid egg as the function of preservative concentrations and pH values. *Figure 3.* demonstrates that the concentration of the preservatives had no significant effect on the changes in the viable cell count.

In terms of plotting the changes in viable cell count as a function of the preservative concentration and storage temperature *(Figure 4.)* or storage time *(Figure 5.),* the preservative concentration obviously had less effect on $lg(N/N_0)$ compared to the other two variables.

While in the case of storage below 8 °C for 7 days the changes in viable cell counts were not significant, at 10 °C we experienced an increase of approximately 4 log units in the viable cell counts *(Figure 4.).*

Figure 5. shows the changes in the viable cell counts as a function of the storage time and the preservative concentration. It can be seen that the preservative had no positive effect on the inhibition of microbial growth. The $lg(N/N_0)$ value increased relatively continuously. When the changes in microbial count were plotted as a function of any 2 elements: pH, storage temperature, and storage time *(Figures 6–8)*; we observed that each variable significantly affected the microbial growth and all at similar levels. *Figures 5–7.* show that the microbial growth was appropriately inhibited at the lower limits of the tested range of pH and storage temperature (pH = 4.0 or T = 4 °C). Therefore, based on the model, in samples acidified to pH = 4.0 there is no reason to anticipate significant changes in the viable cell count even at temperatures around 10 °C. These results correspond with the growth ranges tested in micro-organisms typically present in egg (*Schoeni et al.* 1995, *Hänninen et al.* 1984).

When we combined the appropriate storage temperature (4–6 °C) with the low pH (below 5.0), we did not observe changes in the viable cell counts *(Figure 6.)*. However, we detected rapid increase in the viable cell counts for any changes in conditions when the storage temperature was above 8 °C and the pH was higher than 5.0.

The data presented in *Table 3.* show that among all the variables under consideration, only the interaction of (pH x storage temperature) and the quadratic parameters of $(pH)^2$ and $(\text{storage temperature})^2$ had significant ($p < 0.05$) effects on the viable cell count. This corresponds to the literature, and is explained by the Ratkowsky equation and experience with the combined preservation techniques. That is because the suboptimal culture conditions increase the demand of microbes on other environmental factors being optimal (*Adams* and *Moss* 1995).

When we plotted the changes in viable cell counts as a function of storage temperature and pH *(Figure 6.)* it appeared that below pH 4.5 there were no significant changes in viable cell count during the time interval of the experiments (4–10 days). At elevated pH values the increase of viable cell counts was significantly higher. At pH = 6.0 after 10 days of storage we observed a considerable change of 8 log units in the viable cell count, much higher s than at pH 4.5.

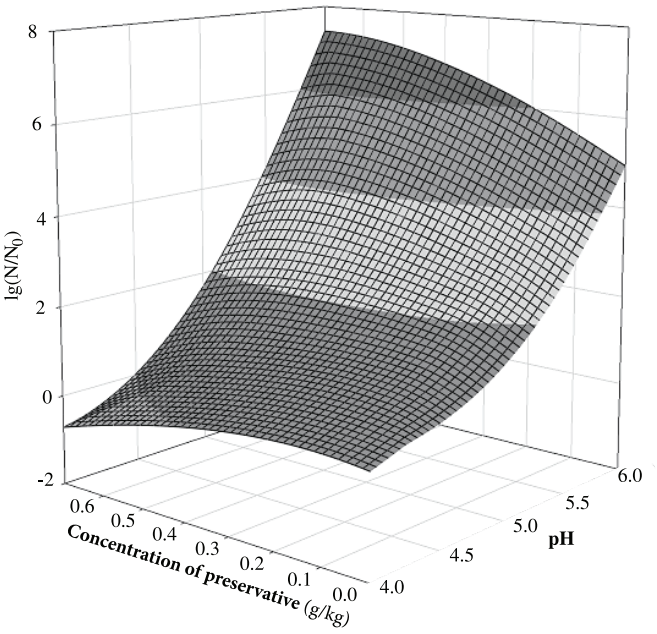

Figure 3. Viable cell count as the function of preservative concentration and pH (storage temperature: 8 °C; storage time: 7 days)

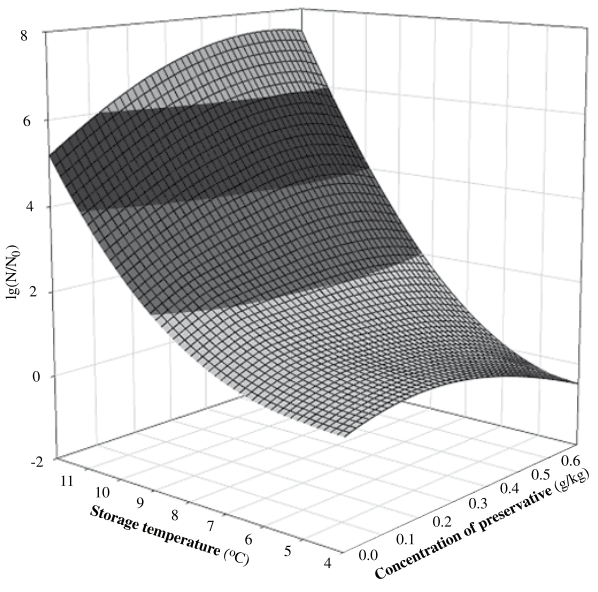

Figure 4. Viable cell count as the function of storage temperature and preservative concentration (pH = 5.0; storage time: 7 days)

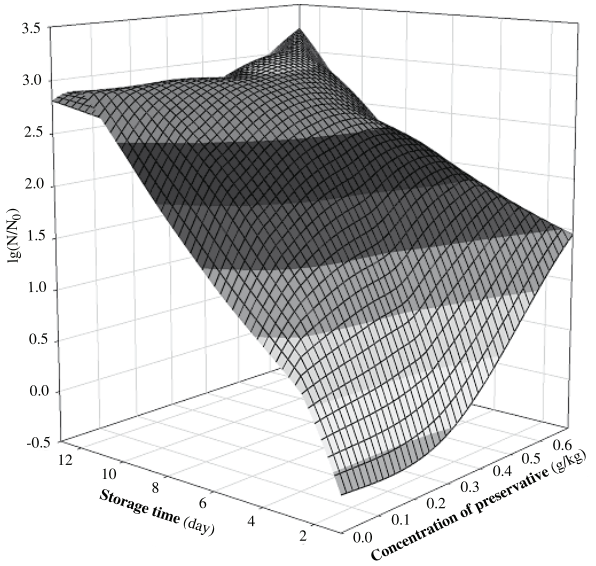

Figure 5. Viable cell count as the function of storage time and preservative concentration (pH = 5.0; storage temperature: 8 °C)

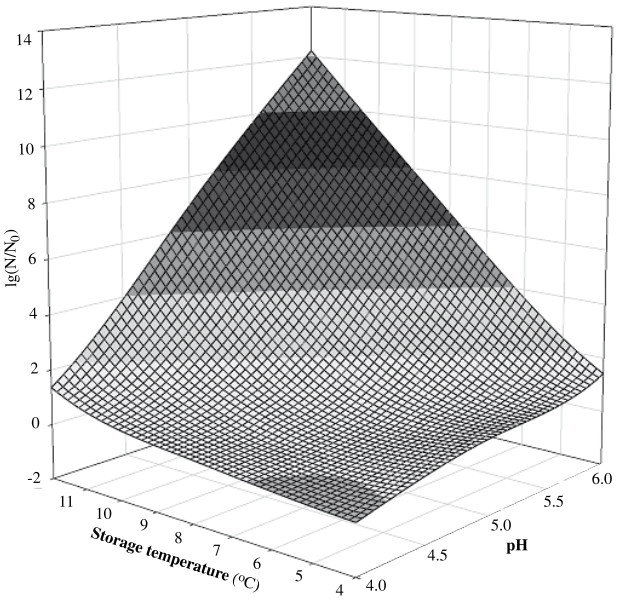

Figure 6. Viable cell count as the function of storage temperature and pH (preservative concentration: 0.3 g/kg; storage time: 7 days)

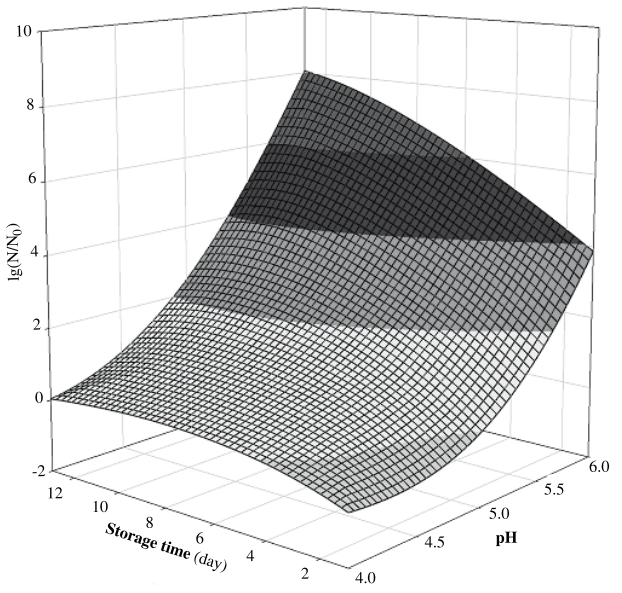

Figure 7. Viable cell count as the function of storage time and pH (preservative concentration: 0.3 g/kg; storage temperature: 8 °C)

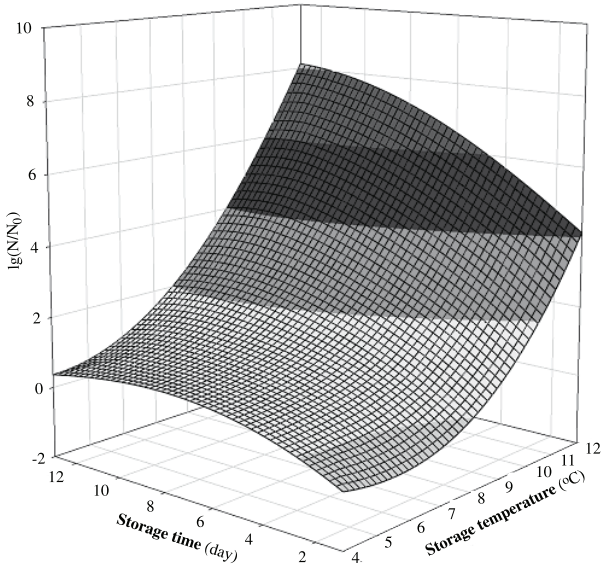

Figure 8. Viable cell count as the function of storage time and storage temperature (pH = 5.0; preservative concentration: 0.3 g/kg)

Conclusions

The pH and the storage temperature of liquid eggs significantly affected the change in viable cell count during storage. However, our measurements did not clearly demonstrate that the mixture of sodium benzoate and potassium sorbate added to liquid egg at the approved concentration range would significantly inhibit microbial growth.
Storage time was introduced into the experiments as the fourth variable. This allowed us to obtain a model highly correlated with our results ($r = 0.97$) by which it was possible to calculate quite accurately the storage time as it related to a specific increase in viable cell count in various liquid whole egg products.
Microbial contamination of liquid whole egg products may vary, thus our purpose in the future will be to analyze microbial composition and its effects on the shelf life of liquid egg products.

Teljes tojáslé eltarthatóságát befolyásoló paraméterek vizsgálata

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Összefoglalás

Méréseink során a teljes tojáslevekben tárolás során bekövetkező élősejtszám-változást vizsgáltuk. Kísérletünk megtervezésénél központi összetett rotációs elrendezést (CCRD) alkalmaztunk, és az egyes változók (pH, tárolási hőmérséklet, tárolási idő és tartósítószer-tartalom) élősejtszám-növekedésre gyakorolt hatásának elemzéséhez a válaszfelület-módszert (RSM) használtuk.
Méréseink alapján a tárolási idő mellett a tojásléminták pH-értéke, illetve tárolási hőmérséklete is szignifikánsan ($p < 0,01$) befolyásolja az élősejtszám alakulását, ugyanakkor a tartósítószerek (Na-benzoát, K-szorbát keverék) hozzáadásának mikrobaszaporodást gátló hatását nem tudtuk egyértelműen kimutatni.

A mérési eredményeinkre illesztett másodfokú polinomiális modellel jól leírhatóak voltak az eredményeink, így eredményeink remélhetőleg tényleges segítséget nyújthatnak a különböző módon tartósított teljestojáslé-termékek mikrobiológiai állapotának megbecsüléséhez.
Kulcsszavak: tojáslé, tartósítószer, pH, tárolási idő.

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