ANALYSIS OF PARAMETERS AFFECTING THE SHELF LIFE OF LIQUID WHOLE EGG

Csaba Németh, Zoltán Németh
Capriovus Kft.
H-2317 Szigetcsép, Duna sor 073/72 hrsz., Hungary
e-mail:nemeth.csaba@capriovus.hu

ABSTRACT

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.

1. INTRODUCTION

The shelf life of liquid eggs is relatively short since the proteins responsible for microbial resistance of shell egg is denatured during pasteurization (Baron et al., 1999), and in case of the mixture of white and yoke of egg provides a medium of excellent composition for microbial growth. Therefore, liquid egg production plants use various preservatives to increase the shelf life of their products. Such substances include citric acid and other additives according to the Hungarian Codex Alimentarius that approves these products such as sodium benzoate and potassium sorbate. The total concentration of the two substances together can be up to 5000mg/l.

In the selection of the amount of citric acid the pH sensitive proteins of egg represent the main limitation; these proteins are denatured at a relatively high rate at the pH lower than 5. However, the adjusted acidity highly effects on the efficiency of the preservatives that can be used in liquid egg products. But potassium sorbate and sodium benzoate do not have appropriate effect at nearly neutral pH values (Marín et al. 2003).

Sodium benzoate and potassium sorbate can be added to liquid egg products at any portions up to a concentration of 5000 mg/l (one of them can even be omitted); while the experiments with foods prepared from egg showed that they can reduce microbial growth significantly only in combinations (Wind és Restaino 1995).

However, it should be noted that in addition to correct selection of preservatives, adequate storage temperature and microbial contamination and composition of the fresh product from the production line also significantly effect on the shelf life of products.
Our purpose with this work was to determine how the total live germ count changes in liquid whole eggs during storage in refrigerator depending on the storage temperature, pH value of samples and their preservative content.

2. MATERIALS AND METHODS

The liquid egg white samples ($pH=7.1\pm0.1$) were obtained for this experiment from a Hungarian egg processing plant. Samples were raw liquid egg samples without heat treatment. Liquid egg samples were obtained from the production line in the evening before the

experiment, and were stored for maximum 24 hours at 4 °C in a refrigerator until starting the tests.

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 live germ count (N_0) of all samples was measured and found to be nearly similar, approximately 2.68×10^3 ($\lg N_0 = 2.43 \pm 0.19$). After adjustment of the values the samples were stored at 4 to10°C in a refrigerator in accordance with the test requirements.

The central complex rotation design (CCRD) (Box and Draper 1987) was used for the tests. The response surface method (RSM) was applied to analyze the effect of each variable (pH, storage temperature, storage time and preservative content) on the increase of the live germ count. Tables 1 and 2 show the design of the experiment and the factor levels. The main advantage of this experimental approach is that the number of the tests to be performed decreased. However, sufficient information was available for acceptable statistically results. We used the response surface method for approximation with polynomial model of second order. Experiments were conducted in random order and data were analyzed by a software (Unscrambler v 9.1 (CAMO PROCESS AS, OSLO, Norway). In the general form of the second order polynomial model used in this study there were three X variables:

$$Y = \beta_{11} + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} \cdot X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} \cdot X_4^4$$
$$+ \beta_{12} \cdot X_1 \cdot X_2 + \beta_{13} \cdot X_1 \cdot X_3 + \beta_{14} \cdot X_1 X_4 + \beta_{23} \cdot X_2 \cdot X_3 + \beta_{24} \cdot X_2 \cdot X_4 + \beta_{34} \cdot X_3 \cdot X_4$$

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. 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 live germ count). β_1, β_2, β_3, β_{11}, β_{22}, β_{33}, β_{12}, β_{13}, β_{23} expressions are the regression coefficients of the model (Table 3).

Table 1. Trial design and factor levels in encoded values

	Encoded factor	-1.682	-1	0	1	1.682
pH	X1	4.0	4.5	5.0	5.5	6.0
Preservative concentration, mg/kg	X2	0.0	0.1	0.3	0.5	0.7
Storage temperature, °C	X3	4	6	8	10	12
Storage time, day	X4	1	4	7	10	13

Testing viable cell count

1 gram of liquid egg samples were homogenized by continuous stirring and were diluted with sterile water for testing the viable cell count. From these test samples $1.0 - 10^{-8}$ g quantities (approximately tenfold of the usual dilution) were transferred into meat liquid agar medium by covered plate pouring technique. The prepared samples were incubated at 30 °C for 24 hours and the characteristic colonies grown were counted for each Petri dish (according to the standard). The colony counting was always performed with 3 samples. Dishes having less than 30 colonies were not included in the evaluation of results.

3. RESULTS AND DISCUSSION

The change of microbial count in samples treated and stored variously is 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 different tests were varied 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 storage at different temperatures after storage for 7 days 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 around 8 orders of magnitude between the results of Test 5 and Test 6.

We did not find significant differences between the results when the quantity of the preservatives was added according to upper and lower limits (Tests 3 and 4). After 7 days of storage of samples having higher pH 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 the 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 difference 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 tome 10 days.

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, 26, 27	5	0.3	8	7	1.64±0.11

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

Table 3. Regression coefficients of the secondary polynomial model for response analysis with encoded units (* significant effect demonstrated)

	B-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*
AB	0.328	2.02	2.53	0.14
AC	0.814	12.45	15.62	0.00*
AD	0.311	1.81	2.27	0.16
BC	0.317	1.89	2.37	0.15
BD	-0.12	0.27	0.35	0.57
CD	0.276	1.40	1.79	0.21
AA	0.389	3.78	4.75	0.05*
BB	-0.105	0.28	0.35	0.57
CC	0.497	6.188	7.763	0.02*
DD	-0.109	0.295	0.37	0.55

The storage time had significant effected on the changes in the viable cell count. After storage for 1 and 13 days (as the upper and lower limits) under similar conditions (pH=5, 8 °C, 0.3 g/kg preservative) applied to test this we measured a difference of around 2.5-3 orders of magnitude in the change in viable cell count (Tests 7 and 8).

There is a close correlation($r=0.97$) between predicted and measured $\lg(N/N_0)$ when taking into account the effect of the four variables. Therefore, there is a close correlation between the predicted and measured viable cell count in liquid egg samples.

4. CONCLUSIONS

In general pH and storage temperature of liquid eggs significantly affects the change in viable cell count during storage. However, our measurements did not clearly demonstrate that the mixture of Na benzoate and K sorbate added to liquid egg at the approved concentration range would significantly inhibit microbial growth.

We introduced storage time into our experiments as the fourth variable. This we could obtain a model closely correlated with our results ($r^2=0.97$) by which the storage time to a specific increase in viable cell count in various liquid whole egg products can be calculated with good approximation.

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