INFLUENCE OF STORAGE TIME AND MICROCLIMATE FACTORS ON QUALITATIVE MICROBIOLOGICAL FACTORS OF EGGS FOR PUBLIC CONSUMPTION

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Abstract

The aim of this paper is to highlight the types of microorganisms found in egg and to comment on their origin. It is known that an egg is generally sterile just after laying and the fact that the microbiological invasion appears after laying and in storage condition. For this, we decided to study a part of the microbiological indices of eggs storage in two different conditions. The fist batch was storage at +4°C and U.R. = 90% and the second oane at +25°C and U.R. = 55%. We study the evolution of total number of germs on the shell and the mixture, the evolution of coliform bacteria, identification of the presence and evolution of bacteria from Salmonella type and identification of the presence and evolution of staphylococcus coagulant positives.

Key words: eggs, microbiologic, storage, quality.

INTRODUCTION

Eggs are an important source of energy, proteins and other substances good for human health and their rational consumption stimulate metabolic functions of the organism and determine an increasing resistance at illness and a good function of nervous system (Apostu, S. 2006).

During years were made multiple experiments to increase the preservation period of eggs for public consumption without depreciate the quality of their component parts (Clavijo et al. 2006). When we speak about the quality of consumption eggs we have in mind the complex character of quality which is given by various groups of technological and technical characters, psycho-sensorial, sanitary, aesthetic, nutritional, economical features which must be evaluated for receiving the quality certificate (Ahlboorn G and Sheldon BW, 2005; De Ketelabere et al. 2004).

During the present research were investigated a series of quality microbiological indexes both on shell and also on the obtained mix from the hen eggs stored in different microclimate conditions (Fromm and Margolf., 1958).

MATERIAL AND METHOD

To realise the current study were used a number of 180 eggs, uniform as weight and shape, which were divided in two batches, respectively Lc and Lexp, the analysed eggs were gathered from ISA BROWN hybrid with an age of 29 weeks.

The eggs from the two batches were stored during 90 days in different microclimate conditions as follows: the ones from batch Lc were stored in refrigeration conditions (+4°C and R.M = 90%); the eggs from Lexp batch were kept at the environmental temperature (+25°C and R.M = 55%).

Determination of microbiological indexes was realised in the day of laying (fresh eggs) and after that at each 30 days (tab. 1).

Table 1

Experimental scheme

Control day	Batches	Assured microclimate factors	Indicators
First day	Lc	+4°, R.M =90%	▪ Evolution of total number of germs (on mineral shell and from mix); ▪ Evolution of coliform bacteria (on mineral shell and from mix); ▪ Identification of the presence and evolution of bacteria from Salmonella type (from the mix); ▪ Identification of the presence and evolution of staphylococcus coagulant positives (from the mix).
	Lexp	+25°, R.M =55%	
30 days	Lc	+4°, RM. =90%	
	Lexp	+25°, R.M =55%	
60 days	Lc	+4°, R.M =90%	
	Lexp	+25°, R.M =55%	
90 days	Lc	+4°, R.M =90%	
	Lexp	+25°, R.M =55%	

- **NTG** was established through decimal dilutions method. From each established dilution for insemination 1ml was placed in the centre of two Petri plates. To realize the inoculums in the plates are poured 15ml culture environment melted and chilled at 45-50ºC, after that inoculums is mixed with Plate Count gelose, by moving up and down and from right to left. After solidification of the environment, Petri plates are placed in a thermostat, with the lid down at the temperature of 30ºC (Fromm, D. and Margolf, P.H. 1958).
- **Determination of coliform bacteria.** Also in this case was used the decimal solution method, the presence of coliform bacteria at a certain quantity of product (1, 0.1g) being determinate through insemination of one ml from homogenized product and from each dilution, in tubes which contain 10ml broth sodium lauryl sulphate and gas collecting tube (Durhan). After insemination is made a thermo-station at 30-37ºC for 48 hours. At each 24 hours of incubation the tubes are examined and are noted the ones in which is observed the presence of gas in collecting tube (at least 1/10 from tube height).

- **Determination of the presence of bacteria from Salmonella type**.

Methodology of isolation of salmonella from foods must assure environment conditions which permit a normal replay of growing processes and at the same time to inhibit the concurrent flora (Karem A. and H. Mattar, 2001).

Colonies identified are inseminated on gelose and nutritive broth and thermo-stated for 24 hours at 37ºC. For confirmation by fast agglutination on blade are used anti-Salmonella multi-purpose serums ,,O" and ,,O" group. Serums of ,,O" group are: serum anti-S. paratiphy A; serum anti-salmonella B; serum anti-salmonella C, serum anti-salmonella D; serum anti-salmonella E (Anjum, M.F et al. 2005).

- **Determination of the presence of staphylococcus coagulant positive by enriching the inoculums**

Determination of the possible number of staphylococcus coagulant positives are realised by insemination from the homogenized product and from each dilution of 1 ml in three tubes, with enrichment environment; for each sample will be inseminated at least three successive dilutions. Inseminated tubes are incubated for 48 hours at a temperature of 30-37ºC**.**

From each tube with hyper chlorinate environment are made passing with loop on solid hyper chlorinate environment (Chapman) or on ETGPA-Baird Parker environment, with a well dried surface, so to be able to obtain isolated colonies. Petri boxes with selective isolation environment are incubated at 30-37ºC for 24 hours.

Selection environment for staphylococcus coagulant and manito-positives is Vogel-Johnson environment. Colonies of staphylococcus coagulant-positives are small, black and if disintegrates manita, are surrounded by a yellow zone on a red environment.

Two or more typical colonies are inseminated into a hemolyse tube which contains 0.5 ml broth brain-cord or nutritive broth; those ones are incubated for 18-24 hours at 37ºC and will serve at the research of coagulase (Andrews M.F., 1996).

RESULTS AND DISCUSSIONS

Determination of NTG existed on the eggs' mineral shell

In the case of the eggs studied by us, in the first day of storage was observed at batch Lc a total number of germs with a maximum value of 92.15 ufc/cm^2 and a minimum value of 90.89 ufc/cm^2; as regarding the mean its value was of 91.98±0.49 ufc/cm^2, variability being of 5.12%, fact which enlightened a very homogenous character inside the batch. For batch Lexp, at the first realised control the maximum obtained value was of 93.11 ufc/cm^2 and the minimum one of 89.97 ufc/cm^2, resulting a mean of 91.90±0.57 ufc/cm^2. As regarding the value of variation coefficient for the

eggs from this batch was of 5.93%, character being also in this case very homogenous (tab. 2).

Table 2

Identification and evolution of the total number of germs on the eggs; mineral shell

Storage time	Batch	n	Statistical estimators			
			$\overline{X} \pm s_{\overline{X}}$ ufc/cm^2	V%	Min ufc/cm^2	Max ufc/cm^2
First day	Lc	5	91.98±0.49	5.12	90.89	92.15
	Lexp	5	91.90±0.57	5.93	89.97	93.11
30 days	Lc	5	97.03±0.95^a	9.36	96.11	98.42
	Lexp	5	136.44±1.51^c	10.53	134.77	138.08
60 days	Lc	5	102.09±1.32^a	12.32	100.11	103.08
	Lexp	5	192.56±3.11^d	15.33	190.53	194.03
90 days	Lc	5	107.16±1.70^a	15.11	106.18	107.47
	Lexp	5	258.77±5.32^d	19.51	254.49	261.33

Statistical signification (ANOVA): exponents from the same column, for each batch and control day:

Without exponent = insignificant differences between means

^{ab} = significant differences, $\hat{F} > F_{\alpha}(0.05)$ at 1; 8 GL.

^{ac} = distinct significant differences, $\hat{F} > F_{\alpha}(0.01)$ at 1; 8 GL.

^{ad} = very significant differences, $\hat{F} > F_{\alpha}(0.001)$ at 1; 8 GL.

From statistical point of view at the first effectuated control for identification of the total number of germs existed on the mineral shell of the eggs gathered from commercial hybrid ISA BROWN, weren't identified distinct statistical significations.

After 90 days of storage in specific microclimate conditions for each batch was observed an excessive increasing of the total number of aerobian germs, especially in the case of batch Lexp which was kept at temperatures of +25ºC and air relative moisture of 55%. So the average calculated value was of 258.77 ufc/cm^2, minimum being of 254.49 ufc/cm^2 and maximum of 261.33 ufc/cm^2. Eggs from batch Lc (were kept in refrigeration conditions) recorded an average value of 107.16±1.70 ufc/cm^2, minimum being of 106.18 ufc/cm^2 and maximum of 261.33 ufc/cm^2. As regarding the value of the variation coefficient this one varied between 15.11 – 19.51%, fact which enlightened the lack of homogeneity of the character.

Determination of the NTG existed in the eggs' mix

From batches Lc and Lexp after each storage period (fresh eggs, 30 days, 60 days and 90 days) was made an egg mix (albumen + yolk).

The charge of total number of germs obtained at batch Lc in the first day of storage had a maximum value of 3.11 ufc/g and a minimum value of 2.01 ufc/g, average being at 2.21±0.009 ufc/g. At the mix obtained from the eggs belonging to batch Lexp, minimum value of NTG was placed at the level of 2.01 ufc/g while the maximum one was 3.17 ufc/g, average calculated value being of 2.35±0.010 ufc/g. Variation coefficient have values between 4.22 – 4.56%, fact which show a very homogenous charcter inside the batches at the effectuated control on fresh eggs (tab. 3).

Table 3

Identification and evolution of total number of germs from eggs' mix

Storage period	Batch	n	Statistical estimators			
			$\bar{x} \pm s_{\bar{x}}$ ufc/g	V%	Min ufc/g	Max ufc/ g
First day	Lc	5	2.21±0.009	4.22	2.01	3.11
	Lexp	5	2.35±0.010	4.32	2.01	3.17
30 days	Lc	5	5.17±0.033[a]	6.19	4.75	6.12
	Lexp	5	9.55±0.091[b]	9.12	7.48	11.32
60 days	Lc	5	7.21±0.070[a]	9.32	6.51	8.78
	Lexp	5	14.47±0.201[d]	13.24	12.01	16.12
90 days	Lc	5	9.11±0.116[a]	12.15	8.34	10.23
	Lexp	5	19.31±0.332[d]	16.33	17.68	21.31

Statistical signification (ANOVA): exponents from the same column, for each batch and control day:

Without exponent = insignificant differences between means

[ab] = significant differences, $\hat{F} > F_{\alpha}(0.05)$ at 1; 8 GL.

[ac] = distinct significant differences, $\hat{F} > F_{\alpha}(0.01)$ at 1; 8 GL.

[ad] = very significant differences, $\hat{F} > F_{\alpha}(0.001)$ at 1; 8 GL.

Also like in the case of mineral shell, during storage, the total number of germs from eggs' mix increased. After 30 days of storage the minimum value recorded for batch Lc was of 4.75 ufc/g, and the maximum one of 6.12 ufc/g, average being at 5.17±0.033 ufc/g. For batch Lexp the average was of 7.21±0.070 ufc/g, minimum being of 7.48 ufc/g and maximum of 11.32 ufc/g. Variation coefficient recorded higher values that the ones from previous control its values being between 6.19-9.12%, studied character being also in this case quite homogenous (tab. 3). After 3 months of storage (90 days), the assured microclimate factors, offered conditions for the development of germs from eggs' mix reaching a maximum value of 21.31 ufc/g and a minimum one of 16.33 ufc/g for batch Lexp, with a mean of 19.31±0.332 ufc/g. Variation coefficient was placed at a level of 16.33%, fact which show that the studied character had a mean variability inside the batch. The lowest values were founded at the mix obtained from the eggs of batch Lc, due to the fact that the conditions of storage were the best ones, so the development of micro-organisms was dramatically decreased; so the minimum have the value of 8.34 ufc/g, and the maximum was up to 10.23 ufc/g, the calculated mean being of 9.11 ufc/g.

Evolution of coliform bacteria

As regarding the presence of coliform bacteria on mineral shell, was identified both at eggs from batch Lc and also at the ones from batch Lexp, after making serial dilutions at level 10^{-1}, 10^{-2} and absent at dilutions 10^{-3} and 10^{-4} (tab. 4.).

After 30 days of storage due to the low temperature and air moisture content (t = 4ºC, R.M = 90%), presence of these bacteria was enlightened in the case of mineral shell from the eggs from batch Lc only on first dilution (10^{-1}), in rest being absent. At batch Lexp where the storage temperature of eggs was higher and moisture content lower (t = +25ºC, R.M

= 55%), being a favourable environment for keeping those bacteria, its identification was possible also in the first dilution (10^{-1}) and on the second one (10^{-2}).

Table 4

Identification of the presence and evolution of coliform bacteria on mineral shell and eggs' mix

Coliform bacteria			I(10)	II(100)	III(1000)	IV(10000)
First day	Lc1	Shell	+	+	-	-
		Mix	-	-	-	-
	Lexp1	Shell	+	+	-	-
		Mix	-	-	-	-
30 days	Lc1	Shell	+	-	-	-
		Mix	-	-	-	-
	Lexp1	Shell	+	+	-	-
		Mix	-	-	-	-
60 days	Lc1	Shell	+	-	-	-
		Mix	-	-	-	-
	Lexp1	Shell	+	+	-	-
		Mix	-	-	-	-
90 days	Lc1	Shell	-	-	-	-
		Mix	-	-	-	-
	Lexp1	Shell	+	+	-	-
		Mix	-	-	-	-

+ = present; - = absent.

In the last day of storage coliform bacteria were absent at all the effectuated dilutions for mineral shell and also for eggs' mix from batch Lc. Could be noticed their absence from the eggs' mix from batch Lexp. But for dilutions effectuated for mineral shell, batch Lexp, was enlightened the presence of coliform bacteria also for dilution 10^{-1} and also for dilution 10^{-2} being absent in dilutions 10^{-3} and 10^{-4}.

Identification of the presence and evolution of bacteria from Salmonella type

The only pathogen micro-organisms which could infest eggs and which present signification for consumers health are Salmonella, which could reach in the egg by inner-vital contaminations or the ones which are on the shell to enter inside egg.

At the eggs studied by us, after making the microbiological examination in the first day from laying and also at 30, 60 and 90 days in eggs mix weren't identified the presence of pathogen micro-organisms

Tabele 5.

Identificarea prezenţei şi evoluţia bacteriilor din genul Salmonella din melanjul ouălor

Storage period	Lotul	Serial dilutions		
		I(10)	II(100)	III(1000)
First day	Lc1	-	-	-
	Lexp2	-	-	-
30 days	Lc1	-	-	-
	Lexp2	-	-	-
60 days	Lc1	-	-	-
	Lexp2	-	-	-
90 days	Lc1	-	-	-
	Lexp2	-	-	-

+ prezent; - absent.

Identification of the presence and evolution of staphylococcus coagulant positives from eggs' mix

For identification of staphylococcus were used three serial dilutions respectively (10^{-1}, 10^{-2}, 10^{-3}). As it is show in table 6, like in the case of enlightening the salmonella, weren't identified the presence of staphylococcus in the eggs' mix for none of the periods for microbiological analyses.

Table 6

Identification of the presence and evolution of staphylococcus coagulant positive from eggs' mix

Storage period	Batch	Serial dilutions		
		I(10)	II(100)	III(1000)
First day	Lc1	-	-	-
	Lexp1	-	-	-
30 days	Lc1	-	-	-
	Lexp1	-	-	-
60 days	Lc1	-	-	-
	Lexp1	-	-	-
90 days	Lc1	-	-	-
	Lexp1	-	-	-

+ present; - absent.

CONCLUSIONS

Quality microbiological indexes which were determinate both on the surface of mineral shell and also on eggs' mix (albumen + yolk) modified their initial values, the main reason being temperature and air relative moisture from the eggs' storage spaces.

NTG which was identified on the mineral shell of eggs at the beginning of storage (fresh eggs) have a value of 91.98±0.49 ufc/cm^2 at batch Lc and of 91.90±0.57 ufc/cm^2 at batch Lexp, reaching at the end of determinations at values between 107.16-258.77 ufc/cm^2.

As regarding the evolution of coliform bacteria those ones were identified at the surface of mineral shell at the beginning at storage period at

both batches but at the end of storage period at batch Lc, which was kept at a temperature of +4ºC and an air relative moisture of 90% those ones disappear due to the fact that low temperature inhibits their development.

For the determination regarding the evolution of Salmonella type bacteria and staphylococcus coagulant positive from eggs' mix, as was showed these ones were absent during the whole period of research.

Acknowledgments

This work was a part of a research project supported by contract POSDRU/88/1.5/S/52176. We thank also to S.C. CONDOR S.A. Matca, the farm theat give as the biological material.

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