Scientific Papers Series Management , Economic Engineering in Agriculture and Rural Development
Vol. 14, Issue 1, 2014
PRINT ISSN 2284-7995, E-ISSN 2285-3952

THE INFLUENCE OF MICROBIOLOGICAL POLLUTION FACTORS ON THE QUALITY OF CONSUMPTION EGGS

Ionut Silviu BEIA[1], Violeta BEIA[2]

[1] University of Agricultural Sciences and Veterinary Medicine Bucharest, 59 Marasti, District 1, 11464, Bucharest, Romania, Phone/Fax: 00 40 723468301, Email: beiaionut@yahoo.com
[2]National Sanitary Veterinary Authority for Food Safety, 1, Presei Libere Square,Building D1, District 1, Zip code 013701, Phone: 0040766335246, Email: beia.violeta@ansvsa.ro

Corresponding author: beiaionut@yahoo.com

Abstract

Eggs' microbiological status intended for human consumption represents a determining factor of products' quality and safety. The possible contamination of eggs with pathogene microorganisms presents a major risk for food safety. In comparison with meat and milk, eggs have a better capacity of conservation and therefore they are more resistant to the aggression of damaging factors. However it is a perishable product and its age as food may cause affections to consumers. One cannot find out the defficiencies and prevent the risks of food safety without the awareness and knowledge of microbiological, enzymatic and biochemical mechanisms that occur in the process of eggs' becoming old. Thus, one can establish the critical limits in which eggs as food may endanger consumers' health status.

Key words: *egg, eggs' microbiological status, food safety, risk*

INTRODUCTION

1. Importance and purpose of study

The microbiological status of consumption eggs stands for a determining factor on products' quality and safety. The possible contamination of eggs intended for human consumption with pathogen microorganisms presents a major risk to public health.

In comparison to meat and milk, eggs have a better preservation capacity, therefore it is more resistant to aggressivity of damaging factors. However, eggs are perishable products that can cause diseases to consumers according to their different stages of staleness and alteration . [6, 8].

One can hardly detect defficiencies in the absence of microbiological, enzimatic and biochemical mechanisms that occur during eggs' alteration processes, therefore, risks cannot be prevented.

The physic -chemical modifications produced by the enzimes in eggs' whites and yolks are intensified by the micro-organisms that may penetrate inside eggs. Although eggs have natural protection means (shells, membrane, cuticles and whites' richness in lisozim substance), they are exposed to micro-organisms' invasions. Eggs' staining with faeces, surface moisture and preservation under normal temperature conditions are important factors of eggs' polution.

Certain investigators found out the degree of cleanliness of eggs' shells is revealed in the microbial plate count at eggs' surface and inside. Bacterias' penetration eggs' shells is closely related to shells' thickness, pores' sizes and preservation temperature. Among the isolates of germs there prevailed bacteria of Pseudomonas species, followed by Proteus, Staphylococcus, Eschirichia coli species [5].

Eggs can bear pathogen germs. The difficulty in the detection or suspicion of their presence consists in the fact that most of the times, the presence of pathogen germs is not accompanied by the modification of eggs' layers that can only be perceived by organoleptic and physic - chemical examination [2, 7].

Eggs' contamination can occur either in the bodies of birds, or during and after their eggs' laying. At ratites Salmonella enteritidis [5 6], NDV [4] and S. pullorum [10] one can convey by vertical level. Considering the large spread

of Salmonella in birds, especially in ducks' eggs that are often contaminated with these microorganisms, producing serious food borne diseases to humans, by consumption.
In order to grant the warranty of an appropriate food value, it is necessary that eggs and eggs' products shall be subject to microbiological examinations that enable the quality of salubrity and freshness.

MATERIALS AND METHODS

In order to follow the influence of microbiological pollution on the quality of consumption eggs according to its validity term and preservation temperature there have been made up two experimental models as follows:
- The microbiological study of eggs that were continuously preserved at the temperature of 4°C. They were analysed on the first day of laying and then at 6, 10, 20, 30 and 40 days. This model refers to the eggs preserved under the temperature conditions of the warehouse of establishment (group A).
- The microbiological study of eggs preserved under the temperature of 23-25 °C.
They were analysed after 1, 6, 10, 20, 30 40 preservation days. This model refers to eggs within the commercial network of turning into account during summer (group B).
The purpose of the study is to establish by means of comparison the dynamics of the evolution of the main group of bacteria according to validity term and preservation temperature for the two experimental pattern groups.
There have been analysed 360 eggs from the following 6 categories of different validity terms: 1 day, 6 days, 10 days, 20 days, 30 days and 40 days (summing up to 180 eggs).
There has been followed the microbiological quality according to the validity terms of eggs and the modifications that may occur as a result of growth and proliferation of different germs.
The 60 eggs of each category were scatterred in 6 groups made up of 10 eggs, thus rising up to 36 groups. The microbiological analyses were carried out in each group.
There has been investigated the microbiological quality of eggs' shells' surfaces and contents and the following parametres were observed : NTG, B coliform, E. coli, Pseudomonas, Micrococcus, Proteus, Salmonella, yeasts and moulds. The results refer to 1 cm2 shells and to 1 g contents. Salmonella represents the exception, as the results refer to the whole surface of eggs and to their entire contents (approximately 50g).
The detection of the total plate count of coliform bacteria and of Escherichia coli species relied on the presumption of an important biochemical property –namely the fermentation of lactose producing gas [3, 4, 10].
For this purpose, there have been used plate count environment that contain lactose and inhibitory substances for the plate count related to them.
-environment of selective enrichment: tryptose broth and both simple and doubly concentrated lauril sulphate
-environment for isolation, identification and confirmation: brilliant green lactose bile broth, Lactose Agar, Eosin-methylene blue (Levine), tryptone water, inclined nutritive agar, Kovacs reagent
There has been followed the establishment of the most probable total plate count –MPN on a growth environment, by using three tubes for each dilution,
There has been inoculated 1 ml from the homogenized product and from each dilution, in series of three tubes per dilution that contained one of the growth environment mentioned above.
The supports with the inseminated environments were introduced in the thermostat at a temperature of 37°C for 24 - 48 hours;
- After 24-48 hours since incubation, there have been checked the tubes with the inseminated environment and there have been noted for each series the tubes in which there was noticed the presence of gases in the fermentation tube (there was considered a positive reaction the presence of gases in at least 1/10 of the height of the tube);

According to the number of positive tubes from each dilution (confirmed in brilliant green lactose bile broth), there has been reported the most probable bacterial plate count of coliform bacteria per g (ml) product, according to Mc Crady table.

For confirmation, from two or more characteristic colonies of E coli, there has been inseminated the bacterial plate in three tubes with the following environments:

- one tube with BBLV (or lauril suphate) and a fermentation tube;
- one tube with tryptone water, heated at 45°C;
- one tube with inclined nutritive agar;

The tubes with inseminated bacterial plates were imAveragetely incubated within 24 hours. The 45°C temperature acted selectively, favouring the germ competing with the associated microflora;

After incubation, there were examined the tubes regarding the presence of gases that occurred in Durham tube from the tube with BBLV environment (lauril sulphate) and of the presence of indole in the tube with inseminated and incubated trypton water , by adding some drops of Kovacs reactive (red ring at the surface of environment). When bacterial plate reacted positively, there was considered E-coli confirmation ;

During the process of isolation and identification of germs of Salmonella type, there have been used several bacterial plate count:

- environments of non-selective pre-enrichments - peptonated water used for the revivification of Salmonella species present in eggs.
- environments of selective enrichment – malachite green and magnesium chloride broth (environment RV = Rappaport-Vassiliadis) and selenite-cistine broth
- selective, solide and identification isolation environments – phenol red and brilliant green agar (Edel şi Kampelmacher), a strongly inhibitory environment and a less inhibitory one- bile agar (Istrati-Meitert)
- identification environments: agar with iron citrate, trizaharides (TSI), environment for the hydrolysis of urea (Christensen), environment for the decarboxylation of L-lysine (LIA agar), etc..

For the determination of germs of Proteus type there was used inclined nutritive agar, freshly prepared- as plate count, with condensation liquid and humid surface without water condensation as drops. After the insemination of tubes and their incubation at 35º - 37 ºC, the development with inclined agar of a plate count that invaded as concentratic waves or as fine, whitish film, consisting of negative Gram small bacilli, there was considered the presence of Proteus type bacteria.

The method of determination of the total number of microorganisms and of yeasts and moulds was based on the inclusion of a certain quantity of the investigated sample in an appropriate nutritive environment (Plate count agar and respectively, agar with gelosis and potatoes), in Petri plaques, in which , after incubation under convenient temperatures (35^oC), there grew visible colonies accessible to eye sight, out of each microorganism or piles of microorganisms.

All sanitary microbiological indicators that were subject to analysis provided data on the state of contamination of the examined product.

RESULTS AND DISCUSSIONS

Table 1 summaries the results of microbiological analyses that were carried out on eggs originated from Group A according to their age, on the basis of which one can establish a series of observations.

The contents of eggs older than 10 days was free of mesophilic aerobic plate, including coliform B, E coli, Pseudomonas, Proteus, Micrococcus and Salmonella, as well as yeasts and moulds, therefore, one can be considered sterile.

This can be explained by the fact that eggs are protected against microorganism attacks by a series of physical, chemical factors and the efficiency of this defence factors depends on the integrity of and the freshness degree.

In the microbiological analysis of the contents of eggs which are older than 10 days, there

have been identified bacteria of the following types as follows:

- total plate count (N.T.G.) – varied from 5 ufc /g up to 25 ufc / g, only for the eggs older than 30 days;

Coliform bacteria - 2,9 x 10 ufc / g and respectively 1,1 x 103 ufc / g for the eggs older than 40 days..

-Escherichia coli was identified only in the case of the eggs older than 40 days., respectively of 1 and 1,1 x 10 ufc / g contents;

Bacteria of Pseudomonas type – for the eggs older than 30 and 40 days

The penetration of microbes through the pores of the shells does not occur under normal conditions, as cuticles that covers the surface of shells obstruct the external opening of pores.

This may be the situation of fresh eggs up to 10 days. In eggs which are older than 10 days, the cuticles covering the external surface of shells alters, so that they do not offer an efficient protection against microbes any longer.

The total plate count on the surface of eggs varied as follows:

- under 3,1 x 103 ufc / cm 2 in eggs from 1 day and 6 days;
- under 8,1 x 103 ufc / cm 2 in eggs from 10 days;
- under 4,6 x 105 ufc / cm 2 in eggs from 20 days;
- under 5,9 x 105 ufc / cm 2 in eggs from 30 days;
- under 5,4 x 105 ufc / cm 2 in eggs from 40 days.

At 26 groups of eggs, B. Coliform were not isolated from shells and in 30 groups, E. Coli neither. In 13 groups of eggs, Pseudomonas was not isolated, and neither Micrococcus.

But Proteus was present in the shells of eggs only in the case of eggs of 2 groups older than 40 days and the number of yeasts and moulds was relatively low, under 50 ufc / cm^2.

Table 1.Results obtained following eggs' microbiological analysis of group A

No. crt.	Eggs' age	Groups made up of 10 eggs	N.T.G.		B.coliforme		E.coli		Pseudomonas		Micrococcus		Proteus		Salmonella		Yeasts and moulds in shells / cm^2
			ucf /g	ucf /cm^2	ucf /g	ucf /cm^2	ucf /g	ucf/cm^2	ucf /g	ucf /cm^2	ucf /g	ucf /cm^2	ucf /g	ucf /cm^2	ucf /g	ucf /cm^2	
1	1 day	1	Abs.	2,7 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	6
2	1 day	2	Abs.	1,65 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	4
3	1 day	3	Abs.	3,1 x 10^3	Abs.	1,7 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	10
4	1 day	4	Abs.	2,1 x 10^3	Abs.	10	Abs.	1	Abs.	1,3 x 10	Abs.	1,3 x 10^2	Abs.	Abs.	Abs.	Abs.	12
5	1 day	5	Abs.	1,7 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	10
6	1 day	6	Abs.	2,1 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	1,4 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	4
Average			0,00	2225,00	0,00	30,00	0	0,17	0,00	25,50	0	21,67	0	0,0	0,0	0,0	7,7
7	6 days	1	Abs.	6,9 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	1,1 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	3
8	6 days	2	Abs.	1,3 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	1,3 x 10^2	Abs.	Abs.	Abs.	Abs.	5
9	6 days	3	Abs.	1,4 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	1,6 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	6
10	6 days	4	Abs.	2,2 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	1,2 x 10	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	10
11	6 days	5	Abs.	1,7 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	1,2 x 10^2	Abs.	Abs.	Abs.	Abs.	8
12	6 days	6	Abs.	1,4 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	2,1 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	5
Average			0,00	1448,33	0,00	0,00	0	0,00	0,00	82,00	0	41,67	0	0,0	0,0	0,0	6,2
13	10 days	1	Abs.	7.3 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	2,1 x 10^2	Abs.	Abs.	Abs.	Abs.	10
14	10 days	2	Abs.	1,1 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	1,8 x10	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	10
15	10 days	3	Abs.	1,3 x 10^3	Abs.	1,7 x 10^2	Abs.	10	Abs.	Prez.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	5
16	10 days	4	Abs.	2,1 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	4,3 x 10	Abs.	Abs..	Abs.	Abs.	Abs.	Abs.	8
17	10 days	5	Abs.	8,1 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	4,9 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	24
18	10 days	6	Abs.	1,4 x 10^3	Abs.	1,1 x 10	Abs.	5	Abs.	2,4 x 10	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	10
Average			0,00	3550,00	0,00	30,17	0,00	2,50	0,00	115,00	0,00	35,00	0,00	0,00	0,00	0,00	11,17

Table 1.Results obtained following eggs' microbiological analysis of group A (continuation)

N. crt.	Eggs' age	Groups made up of 10 eggs	N.T.G.		B.coliforme		E.coli		Pseudomonas		Micrococcus		Proteus		Salmonella		Yeasts and moulds in shell cm^2
			C/g	C/cm^2	C/g	C/cm^2	C/g	C/cm^2	C/g	C/cm^2	C/g	C/cm^2	C/g	C/cm^2	C/g	C/cm^2	
19	20 days	1	Abs.	6,1 x 10^4	Abs.	Abs.	Abs.	Abs.	Abs.	3,8 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	7
20	20 days	2	Abs.	3,4 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	1,1 x 10	Abs.	2,2 x 10^2	Abs.	Abs.	Abs.	Abs.	20
21	20 days	3	Abs.	4 ,1x 10^5	Abs.	1,3 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	30
22	20 days	4	Abs.	3,5 x 10^4	Abs.	Abs.	Abs.	Abs.	Abs.	2,5 x 10^2	Abs.	3,5 x 10^3	Abs.	Abs.	Abs.	Abs.	10
23	20 days	5	Abs.	3,1 x 10^4	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	2,5 x 10^3	Abs.	Abs.	Abs.	Abs.	12
24	20 days	6	Abs.	4,6 x 10^5	Abs.	1,3 x 10^3	Abs.	10	Abs.	1,6 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	10
Average			0,00	166733,33	0,00	238,33	0,00	1,67	0,00	133,50	0,00	1036,67	0,00	0,00	0,00	0,00	14,83
25	30 days	1	Abs	4,2 x 10^4	Abs.	Abs.	Abs.	Abs.	Abs.	1,5 x 10^2	Abs.	2,5 x 10^4	Abs.	Abs.	Abs.	Abs.	20
26	30 days	2	Abs	4,8 x10^5	Abs.	Abs.	Abs	Abs	Abs	1,9 x 10^3	Abs	Abs	Abs	Abs.	Abs	Abs	25
27.	30 days	3	Abs	5,9x10^4	Abs.	Abs.	Abs.	Abs.	Abs.	2,1 x 10^2	Abs.	Abs	Abs.	Abs.	Abs.	Abs.	20
28.	30 days	4	5	5,2x10^4	Abs.	3,8 x 10^2	Abs.	Abs.	1,1 x 10	3,8 x 10^3	Abs.	1,1 x 10^4	Abs.	Abs	Abs.	Abs.	45
29.	30 days	5	Abs	4,6x10^5	Abs.	Abs.	Abs	Abs.	Abs.	4,3 x 10	Abs	4,8 x 10^3	Abs	Abs.	Abs	Abs	15
30.	30 days	6	Abs	3,9x10^5	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs	Abs.	Abs.	Abs.	Abs.	20
Average			0,83	247166,67	0,00	63,33	0,00	0,00	1,83	1017,17	0,00	6800,00	0,00	0,00	0,00	0,00	24,17
31.	40 days	1	Abs	4,4x10^4	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs	Abs.	Abs.	Abs.	Abs.	35
32.	40 days	2	Abs	5,2x10^5	2,9x10	2,1 x 10^2	1	1,2 x 10	1,3 x 10^2	4,9 x 10^4	Abs	2,5 x 10^5	Abs	1,1 x 10^2	Abs	Abs	30
33.	40 days	3	15	3,2x10^5	Abs.	4,3 x 10^3	Abs.	Abs.	1,1 x 10^2	2,3 x 10^4	Abs.	3,8 x 10^4	Abs.	Abs.	Abs.	Abs.	20
34.	40 days	4	Abs	5,4x10^5	Abs.	Abs.	Abs.	Abs	Abs.	2,8 x 10^3	Abs.	Abs	Abs.	Abs.	Abs.	Abs.	20
35.	40 days	5	Abs	6,3x10^4	Abs.	Abs.	Abs	Abs.	Abs.	Abs.	Abs	Abs	Abs	Abs.	Abs	Abs	25
36.	40 days	6	25	5,4x10^5	1,1 x 10^3	7,5 x 10^3	1,1x 10	1,3 x 10^2	2,1 x 10^2	2,1 x 10^5	Abs.	Abs.	Abs.	2,5 x 10^2	Abs.	Abs.	50
Average			6,67	337833,33	23,17	2001,67	2	23,67	150,00	474667	0	48000,00	0	60,0	0,0	0,0	30,0

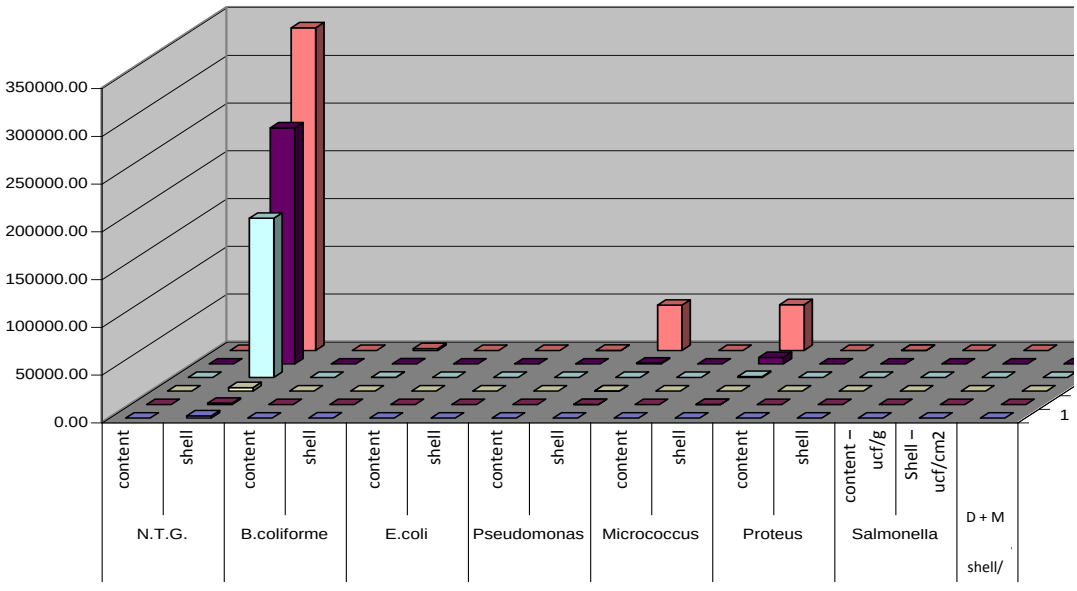

Fig.1.The dynamics of the evolution of average values of total total platecount according to the age of eggs of group A

Table 2.Results obtained following eggs' microbiological analysis of group B

Nr. crt.	Eggs'age	Groups made up of 10 eggs	N.T.G.		B.coliforme		E.coli		Pseudomonas		Micrococcus		Proteus		Salmonella		Yeasts and moulds in shells / cm^2
			ucf /g	ucf /cm^2	ucf /g	ucf /cm^2	ucf /g	ucf /cm^2	ucf /g	ucf /cm^2	ucf /g	ucf /cm^2	ucf /g	ucf /cm^2	ucf /g	ucf /cm^2	
1	1 day	1	Abs.	2,5 x 10^3	Abs	Abs.	Abs	Abs.	Abs.	Abs.	Abs.	1,3 x 10^2	Abs.	Abs.	Abs.	Abs.	8
2	1 day	2	Abs.	1,6 x 10^3	Abs	Abs.	Abs	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	14
3	1 day	3	Abs.	3,3 x 10^3	Abs	1,7 x 10^2	Abs	Abs.	Abs.	1,3 x 10	Abs.	Abs.	Abs.	2,1 x 10	Abs.	Abs.	12
4	1 day	4	Abs.	2,1 x 10^3	Abs	10	Abs	1	Abs.	Abs.	Abs.		Abs.	Abs.	Abs.	Abs.	12
5	1 day	5	Abs.	2,7 x 10^3	Abs	Abs.	Abs	Abs.	Abs.	1,4 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	10
6	1 day	6	Abs.	2,1 x 10^3	Abs	Abs.	Abs	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	4
Average			0,00	2383,33	0,00	30,00	0,00	0,17	0,00	25,50	0,00	21,67	0,00	35,00	0,00	0,00	10,00
7	6 days	1	Abs.	7,,9 x 10^2	Abs	Abs.	Abs	Abs.	Abs.	2,1 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	13
8	6 days	2	Abs.	1,2 x 10^4	Abs	Abs.	Abs	Abs.	Abs.	1,2 x 10	Abs.		Abs.	1,3 x 10	Abs.	Abs.	15
9	6 days	3	Abs.	1,5 x 10^4	Abs	Abs.	Abs	Abs.	Abs.	1,6 x 10^3	Abs.	Abs.	Abs.	1,6 x 10	Abs.	Abs.	26
10	6 days	4	Abs.	1,2 x 10^3	Abs	Abs.	Abs	Abs.	Abs.	Abs.	Abs.	1,3 x 10^2	Abs.	Abs.	Abs.	Abs.	20
11	6 days	5	Abs.	1,5 x 10^4	Abs	Abs.	Abs	Abs.	Abs.	3,1 x 10^2	Abs.	2,2 x 10^3	Abs.	1,1 x 10	Abs.	Abs.	28
12	6 days	6	Abs.	2,4 x 10^3	Abs	Abs.	Abs	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	25
			0,00	9531,67	0,00	0,00	0,00	0,00	0,00	355,33	0,00	388,33	0,00	6,67	0,00	0,00	21,17
13	10 days	1	Abs.	5.3x10^4	Abs	Abs.	Abs	Abs.	Abs.	Abs.	Abs.	2,1 x 10^3	Abs.	Abs.	Abs.	Abs.	55
14	10 days	2	Abs.	1,2 x 10^4	Abs.	Abs.	Abs.	Abs.	Abs.	1,8 x10	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	40
15	10 days	3	Abs.	2,3 x 10^5	Abs.	1,7 x 10^2	Abs.	10	Abs.	Abs.	Abs.	Abs.	Abs.	1,7 x 10^2	Abs.	Abs.	65
16	10 days	4	Abs.	3,1 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	4,3 x 10^3	Abs.	3,3 x 10^2	Abs.	Abs.	Abs.	Abs.	28
17	10 days	5	5	8,2 x 10^5	3	1,5 x 10^2	Abs.	Abs.	Abs.	4,9 x 10^2	Abs.	Abs.	Abs.	1,5 x 10^3	Abs.	Abs.	64
18	10 days	6	Abs.	2,4 x 10^4	Abs.	1,1 x 10	Abs.	5	Abs.	2,4 x 10	Abs.	3 x 10^2	Abs.	Abs.	Abs.	Abs.	70
			0,00	189885,00	0,60	55,17	0,00	2,50	0,00	805,33	0,00	455,00	0,00	278,33	0,00	0,00	53,67
19	20 days	1	5	3,1 x 10^5	Abs.	Abs.	Abs.	Abs.	Abs.	3,8 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	87
20	20 days	2	Abs.	2,4 x 10^6	5	2,1 x 10^3	Abs.	Abs.	Abs.	1,1 x 10	Abs.	2,2 x 10^2	Abs.	Abs.	Abs.	Abs.	60
21	20 days	3	15	2 ,1x 10^4	1,1 x 10	1,3 x 10^2	3	10	Abs.	Abs.	Abs.	Abs.	Abs.	1,1 x 10	Abs.	Abs.	90
22	20 days	4	Abs.	2,5 x 10^6	Abs.	Abs.	Abs.	Abs.	Abs.	2,5 x 10^2	Abs.	2,5 x 10^3	Abs.	Abs.	Abs.	Abs.	40
23	20 days	5	5.	2,1 x 10^5	Abs.	2,7 x 10^3	Abs.	1,4 x 10	Abs.	Abs.	Abs.	2,5 x 10^4	Abs.	Abs.	Abs.	Abs.	80
24	20 days	6	Abs.	3,6 x 10^5	Abs.	1,3 x 10^3	Abs.	10	Abs.	1,6 x 10^2	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.	210
			4,00	966833,33	2,67	1038,33	0,50	5,67	0,00	133,50	0,00	4620,00	0,00	1,83	0,00	0,00	94,50
25	30 days	1	Abs	2,2 x 10^6	10	1,4 x 10^3	Abs.	Abs.	Abs.	2,5 x 10^3	Abs.		Abs.	Abs.	Abs.	Abs.	120
26	30 days	2	15	4,8 x10^6	Abs.	Abs.	Abs	Abs	Abs	3,9 x 10^3	Abs	Abs	Abs	Abs.	Abs	Abs	135
27.	30 days	3	Abs	4,9x10^6	5	2,3 x 10^2	Abs.	1,2 x 10	1,1 x 10	2,1 x 10^2	Abs.	Abs	1,3 x 10	2,1 x 10^3	Abs.	Abs.	140
28.	30 days	4	25	2,2x10^4	1,2 x 10	3,8 x 10^2	5	1,4 x 10	Abs.	2,8 x 10^3	Abs.	1,1 x 10^4	Abs.	1,5 x 10^2	Abs.	Abs.	45
29.	30 days	5	Abs	4,6x10^5	Abs.	Abs.	Abs	Abs.	Abs.	2,3 x 10^2	Abs	2,5 x 10^5	Abs	Abs.	Abs	Abs	85
30.	30 days	6	20	1,9x10^7	Abs.	2,5 x 10^3	Abs.	Abs.	Abs.	Abs.	Abs.	4,8 x 10^4	Abs.	2,1 x 10^2	Abs.	Abs.	120
Average			10,00	5230333,33	4,50	751,67	0,83	4,33	1,83	1606,67	0,00	61800,00	2,17	410,00	0,00	0,00	107,50
31.	40 days	1	20	2,4x10^7	Abs.	Abs.	Abs.	Abs.	1,3 x 10^2	Abs.	Abs.	1,5 x 10^5	Abs.	Abs.	Abs.	Abs.	175
32.	40 days	2	35	3,2x10^5	2,9x10	2,1 x 10^2	1	1,2 x 10	Abs.	4,9 x 10^4	Abs		Abs	1,1 x 10^2	Abs	Abs	145
33.	40 days	3	15	2,2x10^7	Abs.	4,3 x 10^3	Abs.	Abs.	1,2 x 10^2	2,3 x 10^5	Abs.	2,8 x 10^6	Abs.	Abs.	Abs.	Abs.	120
34.	40 days	4	Abs	5,4x10^6	15	2,3 x 10^4	4	1,3 x 10	Abs.	3,8 x 10^5	Abs.	Abs	1,4 x 10	1,5 x 10^3	Abs.	Abs.	230
35.	40 days	5	15	4,3x10^7	Abs.	Abs.	Abs	Abs.	Abs.	Abs.	Abs	2,3 x 10^5	Abs	Abs.	Abs	Abs	135
36.	40 days	6	25	5,4x10^6	1,1 x 10^3	7,5 x 10^3	1,1x 10	1,3 x 10^2	1,1 x 10^2	1,1 x 10^6	Abs.	Abs.	Abs.	2,5 x 10^3	Abs.	Abs.	250

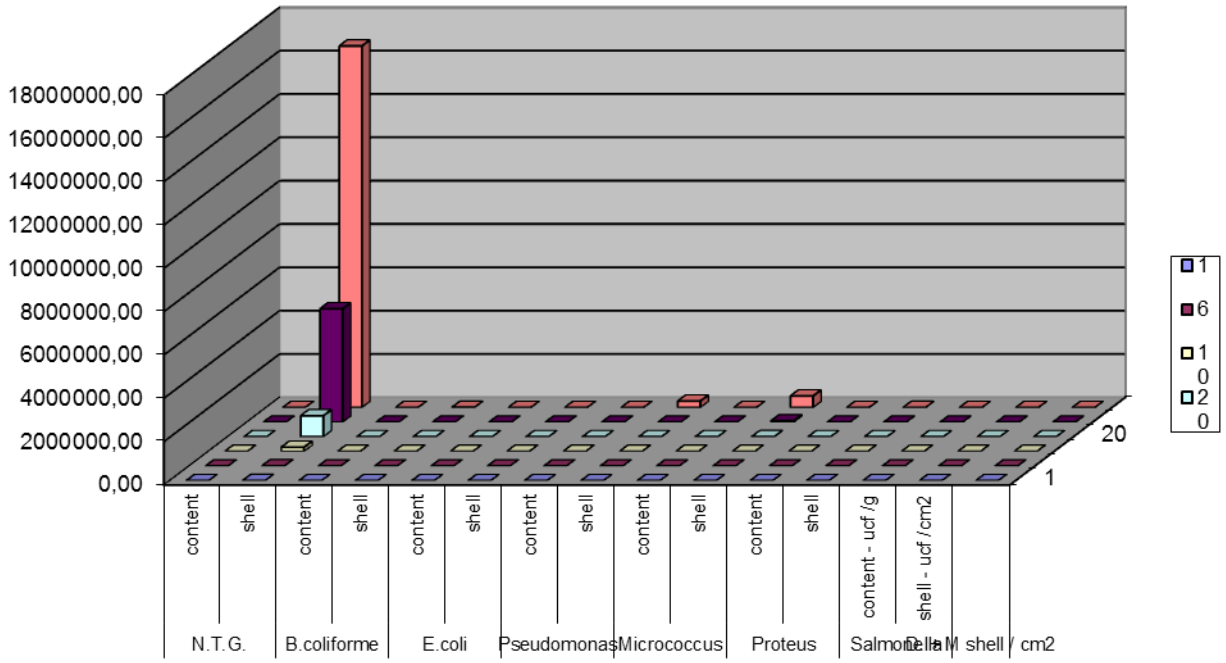

Fig.2.The dynamics of the evolution of average values of total total plate count according to the age of eggs of group B

The absence of Salmonella in the surface of all eggs is particularly important. This proves that the laying hens were free of these germs and the absence of further contamination (from other sources), proves that the keeping and handling of eggs were performed in better hygiene conditions.

Figure 1 presents the dynamics of evolution of average values of total plate count from the contents and surface of shells analysed in group A according to their age. One can notice that the total plate count on the surface of shells grows directly with the time of preservation time, being correlated as well with the NTG found out in the contents.

One can notice that the yeasts and moulds as well as the bacteria of Micrococcus type were present in a high degree, their number growing according to the preservation time.

The bacteria of Pseudomonas type were present on the surface of shells of one day eggs, this multiplying as eggs grow old and recording at the same time average values which are high enough.

In table 2 there are summarized the results obtained for group B following the microbiological analyses from which one can infer several observations.

As regards the total plate count from the contents, the results show that only in eggs older than 20 days there were identified mesophilic anerobic bacteria.

The coliform bacteria were identified in 7 groups of eggs older than 20 days, recording values ranging from 5 – 2,9 x 10 ufc / g contents.

The total plate count of aerobic bacteria detected in the contents of eggs older than 20 days varied in the range 5 - 35 ufc / g.

The largest number of plate count making up colonies was decisive for the bacteria of Pseudomonas type in eggs older than 40 days, being as well the most frequent bacteria negatively Gram determined in its contents.

If one refers to the total plate count on the surface of eggs, one can find out that its value is very different from one sample to another one. The contamination of eggs'shells was deeper for the eggs aged over 30 days, this

being 1000 higher than one day eggs. The progressive growth of NTG cannot be due to the initial pathogens available on eggs'shells, as on dry layers they cannot grow. It involves the atmospherical contamination whose intensity is proportionate with the preservation type and with the hygienic quality of the development area.

Coliform bacteria were isolated on shells in 14 groups of eggs unlike Escherichia coli which was isolated in 11 samples.

In 13 groups of eggs, Pseudomonas was not isolated and in 16, Mircococcus neither. But Proteus was present in the shells of eggs of 12 groups with an age over 40 days and the total number of yeasts and moulds ranged between 4 and 250 ufc / cm^2.

Like in the case of the first group, the bacteria of Salmonella type were neither identified in eggs' contents, nor on the external surfaces of eggs. This provides a reason for the fact that the laying hens were free of these germs and the absence of further contamination from other sources highlights that the maintenance and handling of eggs was performed under good hygiene conditions.

In figure 2, one can follow the dynamics of the evolution of average values of the total palte count from the contents and surface of eggs' shells which were analysed in group B according to their age.

On a whole analysis, the total plate count shall be assessed as being proportionate with the preservation time of eggs.

As one can notice in figure 2 in the analysis of evolution of environments of germs making up colonies / cm^2, within each age category, one can find out that the average number of total plate count is approximately 100 higher than the average recorded for the other types of bacteria.

For one day fresh eggs , the average negative Gram bacteria was not higher than 35 ufc/$cm^{2.}$

For the group of 6 days old eggs one can notice that the average of coliform bacteria was very low, while the environments for *Pseudomonas* and *Micrococcus* recorded a high growth, a tendency which was kept for the category of 10 days' old eggs.

For the eggs older than 20 days, one can notice the presence of a higher number of coliform bacteria and of Micrococcus type as compared with Pseudomonas that record a decrease of the averge in comparison with that of 10 days old eggs. As it is shown in the average values for the group of 40 days old eggs, one can settle that in the plate count of the eggs, there were found out all types of analysed bacteria.

One cand find out a significant increase of the number of moulds –filamentous fungi and of unicellular fungi in the case of eggs which were preserved under high temperatures (23-25ºC) and a relatively low humidity, in comparison with the degree of development of yeasts and moulds which were identified in the case of eggs preserved under opposite conditions (figure 3).

One can notice the fact both for group A and for group B, there were isolated and identified yeasts and moulds on the basis of cultural and morphological characters . The increased number of microscopic fungi to get developed optimally under temperatures of 20-30°C.

In this case, the isolation of filamentous fungi-moulds was most of the times difficult due to the invasive growth of colonies, which led to the cross-contamination and their superposing. The development of a low number at temperatures of 4°C could be explained by the availability of a psychotrophic that can grow as well under negative temperatures.

In comparison to other micro-organisms, they need little water in order to develop a metabolic activity and that's why, they were able to grow on eggs with a reduced water contents. It is a known fact that their growth and multiplication is favoured by a humid environment.

Coliform bacteria and microorganisms of Escherichia type identified both in the contents and on the surface of eggs'shells – situation encountered in both groups but with significant differences regarding proportions and sizes – stood for the most important sanitary veterinary indicator of eggs in the undertaken study.

Taking into account the fact that the prevalence of category of E coli –biotype 1 in the total plate count found out in the faeces of birds, one can say that the contamination of eggs could have occurred during and also after their laying (secondary contamination).

This contamination involves only the surface of eggs' shells in the beginning. One is known that the first contamination of shells takes place in the moment of laying in a low degree., by the contact with the cloacal mucosis neighbouring the opening of oviduct.

The actual contamination of shells occur by means of microflora on the surfaces and objects that come into contact with them, that explains the presence in a high number of coliform bacteria and of the total plate count on the surface of eggs. In a parallel analysis of the two groups, one can estimate the higher number of coliform bacteria and live microorganisms, both in the contents and in the shells of eggs of group B (kept under temperatures of 25°C)

This can be explained by the capacity of these bacteria to grow well under high temperatures (37°C). There was found out that the total aerobic bacterial count on the surface of shells was very different in groups and categories, according to eggs' age and preservation conditions.

The bacteria of Pseudomonas type registered higher values both in the contents and the surface of shells, in the case of eggs preserved at temperatures of 4°C, in comparison to the eggs of group B., whose preservation conditions were exemplified by temperatures of 23 - 25°C and **humidity.**

This is explained relying on the properties of bacteria of Pseudomonas type that, owing to their psychotrophyic, they can normally grow at temperatures of 4°C, being considered the main agents of alteration of eggs kept under refrigerated conditions. The isolation of pseudomonadae on the contents and shells of eggs of group B in a sufficient proportion in order to catch attention is due to the capacity of most of the species to develop under temperatures ranging between 25 - 30°C.

In the case of the two groups, one can notice a gradual growth of microorganisms from different bacteria types on both eggs' shells and contents according to the age and the preservation conditions, reaching a contamination peak of 30 days. It is well known that during eggs' preservation, the proteolitical enzymes of whites and yolks develop its activity of simplifying their specific layer.

The intensity of this activity is conditioned by a special temperature and humidity, responsible factors for the multiplying of microorganisms. Upon a whole analysis, one can make up a correlation between the microbial plate count from the surface of shells and the contents' total plate count.

The advanced contamination of eggs from both groups with an age of 30-40 days under different preservation conditions is due to proteolitical and aminoacidolitical enzymes that are available that are used by most of bacteria that penetrate eggs, resulting the liquefying of proteins of whites and yolks, and alteration of **antimicrobial structures**, such as follows:

- membrana of shells contain some bacteriolitical enzymes (eg. lisodaym and N acetil glucozaminidase / N-acetyl glucosaminidase) and other membrane components that can alter thermal resistance of pathogene bacteria- positive Gram and negative Gram (Salmonella Enteritidis, Escherichia coli 0157: H7, Listeria monocytogenes and Staphylococcus aureus).)

Nevertheless, the presence of organic matter significantly reduces the thermoresistance capacity, by reducing the capacity of membranae. A decrease of thermoresistance capacity of microorganisms may result in the use of several preparation processes (low processing temperatures, reduces operation time), with an improvement of products' quality, exposing *Salmonella Typhimurium* to

- shells' membranae under an increased organic plate count (eg.: skim milk), significantly reduced the activity of membrana against *Salmonella Typhimurium*.

A possible explanation for the presence of different bacteria speciaes of different ages of

eggs can be allotted to their pH. It is well known that immediately after laying, the whites of eggs have the pH-ul of 7,6 -7,9. as J. A. Garibaldi states, and during the storage og eggs, the pH of whites decreases by a value according to temperature to the maximum value of 9,7. Sharp and Whitaker proved that after 3 days of eggs' storage at a temperature of 3°C, the pH of whites reaches a peak of 9,18 and after 21 days, to 9,4, this being considered an explanation for the microbial plate which was found out in the eggs analysed in this study.

Besides the alacaline reaction, the most important role in antimicrobial defence is represented by lisosyme and conalbumin. The high level of negative Gram bacteria can be accounted for the antimicrobial activity of conalbumin (Valenti and col., 1983).

Ibrahim (2000) stressed the fact that the lack of ferum available at the level of membranaer and of whites blocked in conalbumin represents an intrinsic factor of utmost importance in selecting the microbial association developing in the contents of eggs. The conalbumin shall be in excess in order to inhibit the microbial growth, an action that intensifies as the concentration of hydrogen ion decreases.

The high sensitivity towards lysozym of celullar wall of some positive Gram bacteria which is rich in mucopeptides [10] may account for the reason for which the alteration of eggs occur mostly by means of Gram negative bacteria.

The results obtained in this study are in conformity with those obtained by de V. Beia and col., (2005) [8] that prove that the storage of eggs at a temperature of 4 ºC limit the multiplying of microorganisms both in the contents and shells of eggs, in comparison to the storage of eggs at a temperature of 25ºC.

The detection of the presence of Pseudomonas bacteria in both storage conditions (temperatures of 4ºC and 25ºC) in relatively similar proportions is in conformity with the data in the literature describing the proteolitical activity of different *Pseudomonas* subtypes in eggs and egg products at temperatures of 10º and 25ºC .

CONCLUSIONS

1.Following the examination of eggs' contents there has been detected the absence of aerobic mesophilic germs, of Micrococcus and Salmonella types for two analysed batches irrespective of eggs'age and preservation conditions.

2. In the case of eggs ober 30 days kept at a temperature of 4º C, besides NTG, there have been detected some germs of *Pseudomonas* type in the contents of eggs and after 40 days there have been found out coliform bacteria as well as *Escherichia coli.*

3. In the case of eggs of group B (kept at a temperature of 25ºC), the microbial plate present both in the contents and in shells was higher and of a younger age in comparison to the first groups.

4. Following the examination of eggs'surface there have been found out the following: the presence of bacteria of Proteus and Pseudomonas ttypes on the shells of eggs., the absence of Salmonellas, a very low number (under 40 ufc /cm^2) of yeasts and moulds, a progressive rise of NTG corellated with the age of eggs that can only be caused by the atmospheric contamination.

5. If one refers to the total plate count, one can notice that the average of values found out for batch A is lower than the average of values found out for batch B, a situation in which the values were more homogenous in comparison to batch B, in which the amplitude of values was higher.

For batch A, the total microbial plate count of coliform bacteria had a more intense activity, unlike group B, in which there were obtained less positive samples of the type, of lower intensity.

Pseudomonas type stands out by higher values of the units making up colonies per cm^2 of shells for group A, in comparison to group B.

The values recorded for Micrococcus type bacteria were close in similarity.

The comparative analysis of the total plate count of yeasts and moulds shows that the two batches differ significantly.

By comparing the results obtained for the two batches analysed, one can generally notice the same trend of progressive rise in the number of microorganisms as the process of eggs'growing old.

REFERENCES

[1]Abola, J.E., Wood, M.C., Chwen, A., Abraham, D., Pulsinelli P.D.,1982, In: The Biochemistry and Physiology of Iron. Saltman P and Hegenarer J (Eds). Elsevier

[2]Albright, C.D., Lui, R., Bethea, T.C., Da Costa K.A., Salganik, R.I., Zeisel, S.H., 1996, Choline deficiency induces apoptosis in SV40-immortalized CWSV-1 rathepatocytes in culture. FASEB Journal 10:510-516

[3]Al-Soudi, K.A., Al Fayadh, H.A., Al-Khazrje, A.K., Mehdi, A.W., 1988, Preliminary study effects of feeding ethyl mercury chloride on four breeds of chickens, Poul. J., 425(12):146-150

[4]Anon, 1998, Dietry referenc eintakes for folate, thiamin, riboflavin, niacin, vitamin B12,panthothenic acid, biotin and choline. Institute of Medicine and National Academy of Sciences USA. National Academy Press ,Washington DC

[5]Baker, R. C., 1983, Survival of Salmonella typhimurium and Staphylococcus aureus in Eggs Cooked by Different Methods, Poult. Sci., 62 1211-1216

[6]Baron, F., Gautier, M., Bmle, G., 1997, Factors involved in the inhibition of growth of *Salmonella enteritidis* in liquid egg white. J. Food Prot. 60: 13 18-1323

[7]Bărzoi, D, Apostu, S., 2002, Microbiology of Food, Risoprint Publisher Cluj-Napoca, ISBN 973-656-191-7

[8]Beia Violeta, Savu, C., Beia, S. I., Florea, C., Beia Daniela, 2005, The importance ofthe study on microbiological assayson eggsand egg products, International Scientific Symposium" Achievements and Perspectives in Agriculture" State Agrarian University of Moldova, Chisinau

[9]Beia Violeta, Savu, C., Petcu Carmen, Milca Irina, Beia, S. I., 2005, Study onthe microbiological quality ofeggs for consumption, USAMV Scientific Symposium, Faculty of Veterinary Medicine-Bucharest.

[10]Ibrahim, H.R., Sugimoto, Y., Aoki, T., 2000, Ovo transferin antimicrobial peptide (OTAP-92) kills bacteria through a membrane damage mechanism. Biochimica et Biophysica Acta 1523:196-205

[11]Savu, C., Georgescu Narcisa, 2004, Food safety - risks and benefits –Semne Publisher, Bucharest

[12]Savu, C., Petcu, C., 2002, Hygiene and Control of Animal Products, Semne Publisher

[13]Takahashi, K., Lou, X.F, Ishii, Y., Hattori, M., 2000, Lysozyme-glucose stearic acid monoester conjugate formed through the Maillard reaction as an antibacterial emulsifier. Journal of Agricultural and Food Chemistry 48:2044-2049

[14]Valenti, P., Antonin, G., Von Hunolstein, C., Visca, P., Ors,i N., Antonini, E., 1983, Studies of the antimicrobial activity of ovo transferrin. International Journal Tissue Reactions 1:97-105