THE DYNAMICS OF THE MICROBIAL LOAD OF THE EDIBLE OFFALS OF POULTRY STORED UNDER REFRIGERATED CONDITIONS

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Abstract

Food contamination with pathogens is an important public health issue because it can lead to human food poisoning.

This work is an investigation of the microbiological quality of the edible offal of chicken marketed in Romania. The bacteriological analysis was performed on 5 samples from every organ studied, harvested both in the first day they were obtained,as well as during storage at +3ºC. Tests were performed to detect the presence or absence of microorganisms, conducted according to standards in use, and the results obtained indicated that both the total number of germs (TNG) as well as Enterobacteriaceae were recorded higher in the samples of gizzard. The dynamic analysis of the TNG and bacteria of the Enterobacteriaceae family revealed a growth from the contol stage to the other stages. Thus, from a statisticall standpoint it was observed that TNG of the gizzard has recorded the first statistically significant differences at day 3, while the liver and heart had highlighted differences in the fourth day. The Enterobacteriaceae bacteria recorded the first differences in the 3rd day from the samples of liver and gizzards, respectively in the 4th day from the hearts. The bacteria Escherichia coli and Clostridium perfringens were absent in all samples analyzed.

The results of this study emphasizes the constant need for microbiological evaluation of edible poultry offal.

Key words: offal, quality, microbiological, chilling

INTRODUCTION

Food poisoning is a current concern both for consumers as well as for the food industry, despite the use of different methods of preservation.

Microorganisms, by nature that they hold, can lead to reducing quality of these organs, or make them unpalatable, both through their pathogenic action, and by the deterioration and production of toxic metabolites.

The use and value of edible offal depends entirely on the culture and tradition of the country concerned; they can be considered waste material but can be perceived as delicacies and sold at high prices [12]; in Romania, heart, liver and gizzard of the poultry are consumed at a high level.

The edible offal of chicken have a high content of water and protein and contain other water-soluble constituents, which leads to the the creation of a suitable environment for the growth and multiplication of microorganisms.

The purpose of this study was of investigating microbiological indicators of chicken edible offal both for the first fresh product, as well as during in the 9 days of storage; being perishable products the conservation plays a key role in the extension of shelf life.

The most frequently used indicator organisms to reflect the the microbiological quality of poultry offal in relation with their durability for use and to assess the safety and wholesomeness them are represented by total number of germs (TNG), *Enterobacteriaceae*, *Clostridium perfringens* and *Escherichia coli.*

TNG and *Enterobacteriaceae* are considered the quality indices that provides

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information on the hygiene measures during the manufacturing process. *Clostridium perfringens* is an important pathogen in the gastrointestinal tract of broilers, which produces a broad spectrum of diseases [6, 9], while the *Escherichia coli* is a public health hazard causing the food poisoning.

MATERIALS AND METHODS

The sample studied consisted of samples of liver, chicken gizzards and hearts that have been purchased at a manufacturer zonal obtained from chickens breed "Cobb 500" slaughtered at 42 days. After harvesting edible offal were packed in polystyrene trays and stretch film and transported under appropriate conditions (cooler). To observe the absence or presence of microbiological waste and track during storage microbiological stability of the 3 bodies it was agreed that they shoult be kept according to manufacturers recommendations (at 0...+ 4ºC for 6 day-period of validity) and 3 days after their expiry. Thereby, in order to determine of microbial load have been achieved sanitation tests which allowed the determination the total number of germs - TNG and tests for the determination *Enterobacteriaceae*, *Escherichia coli* and *Clostridium Perfringens*.

Bacteriological criteria were analyzed as follows: TNG was determined on the standard plate, the medium PCA, at 37ºC for 72 hours [14]. The number of *Enterobacteriaceae* was determined on medium VRBG for 24 hours at 37ºC [15], the *Escherichia coli* on medium TBX at 44ºC for 24 hours [16] and the *Clostridium perfringens* on the medium TSC for 24 hours at 37ºC [17]. All samples were serially diluted up to 10^{-6}.

RESULTS AND DISCUSSIONS

TNG. The dynamic analysis of the TNG value for liver, gizzard and heart poultry revealed an increase from one stage of to the other control under influence of the temperature and duration of storage.

If the product fresh liver, TNG had a value of 3.370±0.0041 $\log_{10}$cfu/g. Different results were obtained of Cox I. A et al. (1983) [1], which have showed values ranging from 3.8 and 5 $\log_{10}$cfu/g. Significantly higher results were presented by Mead G. C. and Adams B. W. (1980) [7] who have identified values ranging from 4.2 and 4.7 $\log_{10}$cfu/g (tab. 1).

Table 1 The dynamics of TNG in poultry livers

Day of storage	Statistical estimators				
	n	$\bar{X} \pm s_{\bar{x}}$	V%	Minimum	Maximum
0	5	3.370±0.0041	0.2772	3.361	3.380
1	5	3.372±0.0035	0.2356	3.361	3.381
2	5	3.380±0.0033	0.2189	3.370	3.390
3	5	3.382±0.0033	0.2243	3.373	3.391
4	5	3.390±0.0040	0.2678	3.381	3.401
5	5	3.410±0.0068	0.4520	3.390	3.428
6	5	3.440±0.0100	0.6520	3.412	3.459
7	5	3.503±0.0064	0.4124	3.491	3.519
8	5	3.546±0.0091	0.5764	3.518	3.568
9	5	3.658±0.0088	0.5420	3.363	3.368
Significance of differences between the values					
Day_0 vs Day_1 = n.s.; F_α (5.137)> $\hat{F}$ (0.114) pt. 1:8 GL Day_0 vs Day_2 = n.s.; F_α (5.137)> $\hat{F}$ (3.182) pt. 1:8 GL Day_0 vs Day_3 = n.s.; F_α (5.137)> $\hat{F}$ (4.577) pt. 1:8 GL Day_0 vs Day_4 = *; F_α (11.258)> $\hat{F}$ (10.996) pt. 1:8 GL Day_0 vs Day_5 = **; F_α (25.414)> $\hat{F}$ (23.769) pt. 1:8 GL			Day_0 vs Day_6 = ***; F_α (25.414)< $\hat{F}$ (40.670) pt. 1:8 GL Day_0 vs Day_7 = ***; F_α (25.414)< $\hat{F}$ (296.440) pt. 1:8 GL Day_0 vs Day_8 = ***; F_α (25.414)< $\hat{F}$ (304.025) pt. 1:8 GL Day_0 vs Day_9 = ***; F_α (25.414)< $\hat{F}$ (891.116) pt. 1:8 GL		

ANOVA: ns= not significant (F <0.05); *= significant (F <0.01); **= distinguished significant (F <0.001); ***= highly significant (F > 0.001)

After calculating the statistical significance between the values of samples analyzed during storage first statistical differences were observed on day 4, after which they have changed every day remarking at day 5 as a distinctly significant, as a the final, in day 6 to present themselves as highly significant.

Regarding TNG samples analyzed gizzard it recorded values which grew from one day to another control day. Thus, after determinations on the fresh product, TNG have registered the values of 4.993±0.0010 log_{10}cfu/g. Results similar with those obtained were mentioned by Cox I. A (1983) [1], which showed values between 4.4 and 5.0 log_{10}cfu/g. A lower value had been obtained by Smith D. P. and Berrang M. E. (2006) [10], respectively 2.9 log_{10}cfu/g, while the Saikia P. and S. R. Joshi (2010) [8], have highlighted a level of microbial load of 5.0 × 10^5 cfu/g (5.69 log_{10}cfu/g).

From a statistical standpoint, after the determinations between the difference between the values of the analised samples significant differences had been observed in the 3rd day of storage, which then became highly significant in the 6th day (tab. 2).

Table 2 The dynamics of TNG in poultry gizzard

Day of storage	Statistical estimators				
	n	$\bar{X} \pm s_{\bar{x}}$	V%	Minimum	Maximum
0	5	4.993±0.0010	0.0483	4.991	4.996
1	5	4.995±0.0009	0.0412	4.993	4.998
2	5	4.996±0.0008	0.0363	4.994	4.998
3	5	4.997±0.0008	0.0363	4.995	4.999
4	5	4.998±0.0008	0.0363	4.996	5.000
5	5	4.999±0.0007	0.0334	4.997	5.001
6	5	5.003±0.0003	0.0141	5.002	5.004
7	5	5.031±0.0018	0.0807	5.025	5.036
8	5	5.506±0.0284	1.1570	5.412	5.578
9	5	6.086±0.0366	1.3453	5.949	6.146
Significance of differences between the values					
Day_0 vs Day_1 = n.s.; F_α (5.137)> $\hat{F}$ (1.358) pt. 1:8 GL Day_0 vs Day_2 = n.s.; F_α (5.137)> $\hat{F}$ (3.500) pt. 1:8 GL Day_0 vs Day_3 = *; F_α (11.258)> $\hat{F}$ (6.816) pt. 1:8 GL Day_0 vs Day_4 = *; F_α (11.258)> $\hat{F}$ (11.227) pt. 1:8 GL Day_0 vs Day_5 = **; F_α (25.414)> $\hat{F}$ (19.007) pt. 1:8 GL			Day_0 vs Day_6 = ***; F_α (25.414)< $\hat{F}$ (65.798) pt. 1:8 GL Day_0 vs Day_7 = ***; F_α (25.414)< $\hat{F}$ (308.641) pt. 1:8 GL Day_0 vs Day_8 = ***; F_α (25.414)< $\hat{F}$ (322.851) pt. 1:8 GL Day_0 vs Day_9 = ***; F_α (25.414)< $\hat{F}$ (889.989) pt. 1:8 GL		

ANOVA: ns= not significant (F <0.05); *= significant (F <0.01); **= distinguished significant (F <0.001); ***= highly significant (F > 0.001)

Following the analysis of the TNG present in the heart of the poultry there can be seen an increase of the load from 3.565±0.043 log_{10}cfu/g, value obtained in the first day of storage up to 3.789±0.033 log_{10}cfu/g (the 6th day of storage) and respectively 4.486±0.105 log_{10}cfu/g (the 9th day). Similar results had been obtained by Cunning F. E. and Cox N. A., (1987) [2], they have obtained values between 3.5 and 4.5 log_{10}cfu/g. From the statistical analysis of the difference between the values of the samples it can be observed the presence of significant difference from the 4th day and highly significant in the 6th day of control (tab. 3). Following this study, during the six days of freshness there have been recorded values under the threshold level of the TNG, below 1x 10^6 cfu/g (6.0 log_{10}cfu/g) [13].

French manufacturers specify that organs separated from the housing have a lower valability period than those still attached to the carcass because of cross contamination. Thus, using the value of freshness period of the heart and liver, kept in the carcass, can be estimated at nine day after slaughter of the poultry [4,11]. The same value has been reported in the liver of the poultry kept in aerobe conditions at 4±0.5°C, for three day [3].

Table 3 The dynamics of TNG in poultry heart

Day of storage	Statistical estimators				
	n	$\overline{X} \pm s_{\overline{x}}$	V%	Minimum	Maximum
0	5	3.565±0.043	0.270	3.556	3.579
1	5	3.577±0.006	0.424	3.556	3.591
2	5	3.585±0.009	0.562	3.568	3.612
3	5	3.629±0.028	1.762	3.579	3.707
4	5	3.660±0.029	1.786	3.591	3.732
5	5	3.682±0.026	1.583	3.621	3.740
6	5	3.789±0.033	1.986	3.732	3.875
7	5	3.880±0.036	2.118	3.740	3.934
8	5	4.015±0.040	2.235	3.875	4.113
9	5	4.486±0.105	4.881	4.490	5.079
Significance of differences between the values					
Day_0 vs Day_1 = n.s.; F_α (5.137)> $\hat{F}$ (0.083) pt. 1:8 GL Day_0 vs Day_2 = n.s.; F_α (5.137)> $\hat{F}$ (3.364) pt. 1:8 GL Day_0 vs Day_3 = n.s.; F_α (5.137)> $\hat{F}$ (4.532) pt. 1:8 GL Day_0 vs Day_4 = *; F_α (11.258) > $\hat{F}$ (9.854) pt. 1:8 GL Day_0 vs Day_5 = **; F_α (25.414)> $\hat{F}$ (18.919) pt. 1:8 GL			Day_0 vs Day_6 = ***; F_α (25.414)< $\hat{F}$ (42.733) pt. 1:8 GL Day_0 vs Day_7 = ***; F_α (25.414)< $\hat{F}$ (71.572) pt. 1:8 GL Day_0 vs Day_8 = ***; F_α (25.414)< $\hat{F}$ (123.038) pt. 1:8 GL Day_0 vs Day_9 = ***; F_α (25.414)< $\hat{F}$ (145.887) pt. 1:8 GL		

ANOVA: ns= not significant (F <0.05); *= significant (F <0.01); **= distinguished significant (F <0.001); ***= highly significant (F > 0.001)

Enterobacteriaceae. Following the microbiological exam, in the liver there has been reported a value of the *Enterobacteriaceae* bacteria of 1.586 ± 0.0027 log_{10}cfu/g. Higher values had been observed by Saikia P. and Joshi S. R. (2010) [9], respectively 1.0×10^4 cfu/g (4 log_{10}cfu/g). From the statistical analysis there had results significant differences between the 3rd day, which then became more obvious in the following day and highly significant in the 6th day (tab. 4).

Table 4 The dynamics of *Enterobacteriaceae* in poultry livers

Day of storage	Statistical estimators				
	n	$\overline{X} \pm s_{\overline{x}}$	V%	Minimum	Maximum
0	5	1.586±0.0027	0.381	1.580	1.591
1	5	1.595±0.0044	0.618	1.580	1.602
2	5	1.659±0.0323	4.364	1.602	1.778
3	5	1.718±0.0486	6.324	1.602	1.832
4	5	1.811±0.0529	6.532	1.678	1.995
5	5	1.842±0.0509	6.182	1.778	2.041
6	5	1.968±0.0536	6.099	1.845	2.114
7	5	2.161±0.0453	4.688	2.041	2.278
8	5	2.437±0.0375	3.439	2.301	2.505
9	5	2.622±0.0455	3.884	2.505	2.716
Significance of differences between the values					
Day_0 vs Day_1 = n.s.; F_α (5.137)> $\hat{F}$ (2.885) pt. 1:8 GL Day_0 vs Day_2 = n.s.; F_α (5.137)> $\hat{F}$ (5.035) pt. 1:8 GL Day_0 vs Day_3 = *; F_α (11.258)> $\hat{F}$ (7.333) pt. 1:8 GL Day_0 vs Day_4 = **; F_α (25.414)> $\hat{F}$ (18.060) pt. 1:8 GL Day_0 vs Day_5 = **; F_α (25.414)> $\hat{F}$ (25.180) pt. 1:8 GL			Day_0 vs Day_6 = ***; F_α (25.414)< $\hat{F}$ (43.960) pt. 1:8 GL Day_0 vs Day_7 = ***; F_α (25.414)< $\hat{F}$ (160.101) pt. 1:8 GL Day_0 vs Day_8 = ***; F_α (25.414)< $\hat{F}$ (512.535) pt. 1:8 GL Day_0 vs Day_9 = ***; F_α (25.414)< $\hat{F}$ (514.871) pt. 1:8 GL		

ANOVA: ns= not significant (F <0.05); *= significant (F <0.01); **=distinguished significant (F <0.001); ***= highly significant (F > 0.001)

Analysis regarding the identification of germs from the *Enterobacteriaceae* genre in the chicken gizzard, ultra fresh product, have highlighted a value of 1.836±0.0015 $\log_{10}$cfu/g.

Studies of Saikia P. and Joshi S. R. (2010) [8] had shown higher contamination levels, respectively 2.3 x 10^4 cfu/g (4.361 $\log_{10}$cfu/g). By analysing the significance of the difference between the values of the 9 days of control there have been no significant recorded difference in the first two days and became significant in the 3rd day and highly significant in the 6th day (tab. 5).

Table 5 The dynamics of *Enterobacteriaceae* in poultry gizzard

Day of storage	Statistical estimators				
	n	$\bar{X} \pm s_{\bar{x}}$	V%	Minimum	Maximum
0	5	1.836±0.0015	0.1891	1.832	1.838
1	5	1.838±0.0028	0.3423	1.832	1.845
2	5	1.840±0.0014	0.1809	1.837	1.846
3	5	1.842±0.0021	0.2613	1.838	1.848
4	5	1.843±0.0022	0.2696	1.839	1.849
5	5	1.845±0.0022	0.2694	1.840	1.850
6	5	1.856±0.0353	0.4261	1.845	1.863
7	5	1.960±0.0218	2.4930	1.878	2.000
8	5	2.375±0.0852	8.0288	2.196	2.612
9	5	3.173±0.0477	3.3610	3.029	3.298
Significance of differences between the values					
Day_0 vs Day_1 = n.s.; F_α (5.137)> $\hat{F}$ (0.604) pt. 1:8 GL Day_0 vs Day_2 = n.s.; F_α (5.137)> $\hat{F}$ (3.723) pt. 1:8 GL Day_0 vs Day_3 = *; F_α (11.258)> $\hat{F}$ (6,166) pt. 1:8 GL Day_0 vs Day_4 = *; F_α (11.258)> $\hat{F}$ (7,492) pt. 1:8 GL Day_0 vs Day_5 = **; F_α (25.414)> $\hat{F}$ (12,181) pt. 1:8 GL			Day_0 vs Day_6 = ***; F_α (25.414)< $\hat{F}$ (26.149) pt. 1:8 GL Day_0 vs Day_7 = ***; F_α (25.414)< $\hat{F}$ (32.039) pt. 1:8 GL Day_0 vs Day_8 = ***; F_α (25.414)< $\hat{F}$ (39.943) pt. 1:8 GL Day_0 vs Day_9 = ***; F_α (25.414)< $\hat{F}$ (758.043) pt. 1:8 GL		

ANOVA: ns= not significant (F <0.05); *= significant (F <0.01); **=distinguished significant (F <0.001); ***= highly significant (F > 0.001)

Table 6 The dynamics of *Enterobacteriaceae* in poultry heart

Day of storage	Statistical estimators				
	n	$\bar{X} \pm s_{\bar{x}}$	V%	Minimum	Maximum
0	5	1.755±0.0024	0.306	1.748	1.763
1	5	1.778±0.0171	2.153	1.748	1.845
2	5	1.865±0.0501	6.019	1.756	2.000
3	5	1.921±0.0277	8.672	1.845	2.219
4	5	2.048±0.0381	9.538	1.954	2.397
5	5	2.211±0.0909	9.201	2.041	2.518
6	5	2.322±0.1026	9.888	2.147	2.708
7	5	2.474±0.1070	9.667	2.230	2.716
8	5	2.604±0.0959	8.238	2.380	2.857
9	5	3.069±0.0877	6.394	2.857	3.255
Significance of differences between the values					
Day_0 vs Day_1 = n.s.; F_α (5.137)> $\hat{F}$ (1.661) pt. 1:8 GL Day_0 vs Day_2 = n.s.; F_α (5.137)> $\hat{F}$ (4.489) pt. 1:8 GL Day_0 vs Day_3 = n.s.; F_α (5.137)> $\hat{F}$ (4,908) pt. 1:8 GL Day_0 vs Day_4 = *; F_α (11.258)> $\hat{F}$ (11.188) pt. 1:8 GL Day_0 vs Day_5 = **; F_α (25.414)> $\hat{F}$ (25.009) pt. 1:8 GL			Day_0 vs Day_6 = ***; F_α (25.414)< $\hat{F}$ (30.380) pt. 1:8 GL Day_0 vs Day_7 = ***; F_α (25.414)< $\hat{F}$ (45.231) pt. 1:8 GL Day_0 vs Day_8 = ***; F_α (25.414)< $\hat{F}$ (78.205) pt. 1:8 GL Day_0 vs Day_9 = ***; F_α (25.414)< $\hat{F}$ (223.823) pt. 1:8 GL		

ANOVA: ns= not significant (F <0.05); *= significant (F <0.01); **=distinguished significant (F <0.001); ***= highly significant (F > 0.001)

Taking into consideration the data presented in table 6 bacterias from the *Enterobacteriaceae* genre had been found in all samples from the heart, the fresh product having a level of 1.755±0.0024 $\log_{10}$cfu/g.

From the statistical analysis of the difference of the values there have been observed no significant differences in the first three days of storage and as the time passed by these became highly significant (in the 6th day).

Clostridium perfringens and ***Escherichia coli.*** From the analysis of the results there had been observed the absence of *Clostridium perfringens* and *Escherichia coli* bacteria from all the samples. The liver, gizzard and hearts that had been analises were good for human consumption.

CONCLUSIONS

The results of this study show the need of constant evaluation of the organs of the chicken available in commerce, so that microbiological indicators can be maintained in established values for the health of the consumer.

The microbiological load, dynamically speaking, from the broilers organs, during the nine days, presents on ascending trend, from the TNG point of view and from the germ from the *Enterobacteriaceae* species; the variations of the microbiological parameters is within the allowed limit.

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