

## **ABSTRACT**

This thesis entitled „**RESEARCH ON MICROBIOLOGY FRESH MEAT, CHILLED AND FROZEN POULTRY**” is supported scientifically and practically by the following reasons:

- the need of knowledge of the most important factors contributing to the quantitative and qualitative changes of microflora of poultry carcasses at poultry breeding until delivery carcasses of processing units to network marketing.
- Identify potential hazards through hazard analysis and indicating effective means to control them by establishing critical control points which are the main elements to be taken into account in any HACCP plan development. HACCP (Hazard Analysis Critical Control Point), with other specific programs (ISO, SSOP, GMP, GHP, etc..), plays an important role in ensuring the quality of poultry meat. The main role of this program is to prevent any potential problems that might, directly or indirectly be a threat for public health or food safety.

The thesis consists of 200 pages and is structured, in accordance with existing legal recommendation, in two main parts: the first part entitled, “**Actual state of knowledge**” representing 39.4% of the thesis and the second part, “**Personal researches**”, representing 60.6% of the thesis. The thesis includes 30 tables and 33 figures (graphs and photos), in order to a better presentation of the content. The references include 384 bibliographic titles found in domestic and foreign literature.

The first part, “**The current state of knowledge**”, contains four chapters, which are summarized, information from literature on the subject of the thesis, data were used to interpret and compare results obtained in the second.

The first chapter presents the potential sources of contamination of poultry with organisms and factors influencing them.

Chapter II entitled **“Microbiology of poultry”** presents, based on literature, the main microorganisms that interests the quality control of meat, namely, bacteria, yeasts and molds. Also, in this chapter are presented the main characteristics of the pathogen that may be isolated from/upon the poultry carcasses and which are capable of causing food poisoning in humans.

**“Influence of cold on poultry microbiology”** is presented in Chapter III. Described the effect of low temperatures on microorganisms that may be isolated from/upon the poultry carcasses and the spoilage of refrigerated and frozen poultry.

Chapter IV contains data concerning the control of microbiological hazards from/upon the poultry in all stages of poultry processing, storage, transport and retail of poultry. Are presented:

- current standards for microbiological control to ensure quality assurance and safety of poultry;
- risk management and processing of poultry meat getting it;
- traceability of poultry and poultry products from its role in managing risks, microbiological;
- implementation of good hygiene in poultry processing;
- sanitation in the poultry breeding farms and poultry processing plants;
- application of HACCP in poultry processing;

In part II, "Personal researches" comprises eight chapters and references, are exposed and discussed results of investigations.

In the fifth chapter, **“The natural, organizational and institutional environment in which investigations were performed”** are presented the circumstance of the conducted research. The research was conducted at a poultry farm, a poultry slaughtering unit and four units retailing of poultry meat. These units operate on concordance with actual legislation concerning poultry farming, production, marketing and sale.

In chapter VI, entitled **“Research on the influence of technology on the microbiology of poultry carcasses”** are presented the results of investigations conducted during 2006 - 2009 three halls of a poultry breeding farm from Vaslui County. The purpose of these researches was to monitor the microclimate (temperature, humidity and airflow speed) in the halls of growth and microbiota from the breeding facilities (samples from surfaces and air of shelter were analyzed) because the microclimate of poultry breeding farms affect their health and therefore the quality of poultry meat.

During study shelter growth microclimate was kept within relatively constant parameters. Temperature, humidity and air speed in the shelter of the poultry farm varied within narrow

limits because these parameters are monitored continuously microclimate and correct whenever it is found that values may adversely affect the performance of poultry. Temperature values recorded during the study period that ranged between 20.6 and 23.0°C, averaging  $20.9 \pm 0.27^\circ\text{C}$ . Relative humidity of housing as a factor of comfort was maintained between 53.4 and 64.4%, averaging  $57 \pm 3.88\%$ . Halls air speed was increased  $0.08 \pm 0.006$  ranging from 0.01 to 0.18 m/s.

Regarding housing microbiota, the highest values were recorded at floor level, where NTG ranged on average between 6.2 and 7.31 log UFC/cm<sup>2</sup> and the number of coliforms, between 4.07 and 5.0 log UFC/cm<sup>2</sup>. The high values of *Enterobacteriaceae* and *E. coli* load is normal in growing on the floor technology since chicken feces are rich in such microorganisms.

The presence of these groups of bacteria in large numbers shows that in the growth halls the litter is an important cause of cross-contamination. This is especially important because, as shown in the literature, many birds are carriers of microorganisms that may be dangerous to humans.

Also, high levels of microorganisms on the floor and bedding can cause accidental contamination of wounds and may lead to increased incidence of confiscation of carcasses at the slaughterhouse, also, could induce with increased frequency the dermatitis, that mainly affects skin of chest and of the limbs, two important regions in terms of recuperation of birds in slaughterhouses. High humidity of litter stimulates the development of microorganisms as occurring fermentation processes lead to increased concentrations of substances with negative effects on bird health.

The presence of microorganisms in air of poultry breeding facilities is a natural phenomenon, number and type of microorganisms being an important indicator of the status of compliance with good hygiene practice and the farming practices.

This study shows that poultry are continuously exposed to a large number of microorganisms in both air and the floor level and continuously measures to reduce the microbial load from farm must be implemented to improve the microbiological quality of poultry meat.

Chapter VII, entitled “**Research on the influence of transport of live birds, poultry carcasses on microbiology**” include the assessment of the transportation impact on the microflora of poultry. Using specific microbiological investigations, the microbiological parameters determined were: *total viable counts*, *coliforms*, *Enterobacteriaceae* and generic *E. coli*. These parameters were chosen as they reflect accurately the state of areas hygiene.

Vehicles and cages with chickens transported to the slaughterhouse, prepared (cleaned and decontaminated) had low values of microbiological monitored parameters, showing that decontamination was performed in good conditions.

Determinations made at the slaughterhouse, after unloading chickens, reveal higher values in all parameters analysed. While the total number of germs has increased by 2 to 4 log CFU/cm<sup>2</sup> in *Enterobacteriaceae* growth was 3.5 to 5 log CFU/g. Significant increases were observed in other specific parameters.

The main cause of these increases has been the contamination of crates used for transportation of birds with faeces from them.

Comparing the results of the evaluation of transport crates microflora and microflora of carcasses after bleeding in the four determinations carried out there was a positive correlation between these developments (corel. coef. 0.79; R<sub>2</sub> 0.0.63 for coliforms and 0.58 and 0.34 for NTG respectively) indicating that contamination during chickens transport contributes to microbial load of carcasses.

Chapter VIII, entitled “**Research on the influence of technology to slaughter on fresh poultry carcasses microbiology**” sought to evaluate microflora of carcasses, water, equipment and environment of spaces used in processing of poultry in slaughterhouse in connection with changes in temperature and humidity, in order to see the evolution of carcasses microflora and to identify the main causes of these changes.

Samples from poultry carcasses were collected during the four successive visits, on which occasion six carcasses at a time were randomly removed from the processing line from six different points as follows: immediately after bleeding, after defeathering, after evisceration, after washing the eviscerated carcasses after chilling carcasses and after packaging. In total, 144 were considered for poultry carcasses.

Water samples were collected from the main source of water, from the scalding basin and the water drained of the carcass before chilling, following after the procedure used for carcasses. Samples were collected in sterile glass containers, each 25 ml of water at each point above. In total, 56 samples were taken water (eight samples from primary source of water 24 from the scalding basin and 24 of rinse water).

Air samples were collected from the initial processing zone of slaughterhouse - at the bird conveyor hanging place, near the plucking facility, from the final processing zone and cooling the carcasses. Samples were collected using the MAS-100 apparatus (Merck KGaA) at 1.5 m above the floor, in three different zones of analysed space. In total 108 samples were collected from the air for each parameter microbiologically considered parameter.

Regarding the surfaces areas, the microflora of scalding tank, defeathering and evisceration facilities were evaluated. Following microbiological parameters were determined: total aerobic plate count mezofili, coliforms, *Enterobacteriaceae* and *E. coli*.

Highest bacterial load on the surface of examined carcasses was usually detected immediately after defeathering and after evisceration these two being the steps in which contamination with microorganisms was expressed very intensely. On the opposite side stood the results obtained after carcass washing stage, consecutive evisceration.

Depending on the number of processed carcasses, the comparison of the results, showed an increase in the number of microorganisms on the carcasses surface while increasing the number of processed carcasses. Microbial load detected three hours after the start of the processing was higher by about 0.7 to 0.8 log compared with values obtained one hour after the start of the process. The biggest difference was obtained for coliforms (0.83 CFU/g). Remarkable is the fact that at one hour after the start of the processing of poultry carcasses microbial load was quite high in this phase.

Quantitative assessment of air microflora in the area of the slaughterhouse revealed a total viable count of 6.73 log CFU/m<sup>3</sup> germ being the highest value found in this study. High level of microbial load in this area of the slaughterhouse has been found by other researchers (3, 360) and is the result of operating features facilities and plucked but hygiene shortcomings.

Qualitatively in the air around defeathering machine frequently Gram positive bacteria such as *Staphylococcus* (26.5%) and Gram negative group of *Enterobacteriaceae* (11.2%) were isolated. Gram positive bacteria were predominant (71.3%).

The presence of *Enterobacteriaceae* in high proportion in the air of this area of the slaughterhouse is the result of contamination with feces expelled due to pressure on the plucked carcasses. Another source of *Enterobacteriaceae* in this area is scalding tank water which is rich in this kind of bacteria.

Particularly in this study, air microflora in evisceration area contributed significantly to the quantitative development of microflora of poultry carcasses. Statistical correlation coefficients between evaluated microbiological parameters determined in both room air and the bird carcasses were 0.63 for TVC, and 0.81 for *Enterobacteriaceae* and coeficientul R<sub>2</sub> was 0.78 and 0.82 respectively. These figures confirm the importance of air microflora in poultry processing facilities to maintain acceptable levels of microbial load.

Evisceration as stage of birds processing in the slaughterhouse may be a critical control point for microbiological hazards that more so since in this stage was noticed a massive increase of *Enterobacteriaceae* on carcass, surfaces and air. Their presence in high numbers constitutes a big risk because some species of this family are pathogenic to humans.

The carcasses washing reduced the number of microorganisms on evaluated carcasses with approximately 1.5 log CFU/g, the largest reduction was found in the total viable count (2.02 log CFU/g).

Water used in abattoir proved acceptable microbiological quality with the average of total viable counts of 2.32 CFU/ml, the other investigated bacteria being not detected.

Depending on the samples harvest time was found an increase of the number of microorganisms on both the surface of carcasses and in wash water, but while growth on carcasses has been continuous, the increase in the rinse water was inconsistent.

The air microflora in the refrigeration areas, compared to that of evisceration spaces has been less with 0.4 log CFU/m<sup>3</sup> for TVC and 1 log CFU/m<sup>3</sup> for *Enterobacteriaceae* (average).

Air microflora increased with the number of carcasses processed being greater for areas of the final processing. One good explanation of this phenomenon is the higher air speed necessary in order to accelerate the cooling the carcasses. The high speed air currents take the microorganisms on surfaces making cross-contamination possible. This is why in the literature is considered that after air chilling of carcasses they have usually a higher microbial load than in case of carcasses chilled by water immersion.

As expected the microflora of refrigerated air tunnel is consisted of by many Gram positive organisms, resistant to low temperatures, the primary source of them being contaminants entering the slaughterhouse to the farm with breeding birds, and psychrotrophic bacteria that arrive here from the surface of carcasses. In these areas this group of bacteria can multiply and become sources of perpetual contamination of poultry carcasses.

Chapter IX, entitled **”Research concerning the influence of conditioning and storage on microbiology of chilled and frozen poultry carcasses”** aimed to qualitative and quantitative analyze of microflora of poultry meat in refrigeration and freezer area. Studies were conducted during 2007 - 2009 in a slaughterhouse in Vaslui County

In total 160 air samples from the freezing tunnel were collected, for each determined microbiological parameter and 250 samples from the surfaces of chilled and frozen poultry carcasses. The parameters followed were total number of psychrotrophic bacteria, *Enterobacteriaceae*, *Escherichia coli* and coliforms.

Chilling effectiveness on development of contamination microflora is influenced at the highest level by relative humidity and temperature of the atmosphere from stored poultry carcasses space. Same meat stored in an atmosphere with 85% humidity will spoil after five days at 4°C, and after 30 days at 0°C. Effectiveness of the two levels of temperature is much smaller than UR, and that the temperature of 0°C, similar to that of temperature of 4°C.

Statistical processing of recorded data showed that the indicators examined (total number of psychrophilic bacteria, *Enterobacteriaceae*, *E. coli* and coliforms) of refrigeration facilities had significantly higher values than the values obtained in the freezing spaces.

During freezing of poultry the destruction of microorganisms occurs but this destruction it is never complete and is only interested in a limited number of microorganisms, which may be higher or lower depending on type of microorganisms.

Regarding the microbial load on the surface of carcasses examined (total number of psychrophilic bacteria, *Enterobacteriaceae*, *E. coli* and coliforms), the value of all parameters, were bigger in areas of frozen carcasses at 24 h after freezing compared to 3 months.

Chapter X, entitled **”Research on the influence of transport and retail on microbiology of chilled and frozen poultry meat”** intended to analyze qualitatively and quantitatively the microflora of poultry in marketing units, with particular emphasis on isolation and identification of pathogenic bacteria, biological threats needed to be managed in order to prevent the occurrence of food poisoning to consumers.

Meat samples were collected between January 2007 - December 2009 from a total of four units from Vaslui County where poultry and poultry products processed in the unit that conducted the studies are retail.

Samples of poultry (whole carcasses, body parts and organs, chilled or frozen) were collected at the time of delivery to retail units and after a variable period of time, taking account of the life of the product. Samples were collected and kept in conditions that do not change their state heat until examination (frozen meat was kept in a freezer at  $-18^{\circ}\text{C}$  and chilled in the refrigerator ( $0-4^{\circ}\text{C}$ )).

Bacteriological samples collected were analyzed by determining the total viable count, identification and quantification of bacteria of the genus *Salmonella*, *Listeria monocytogenes*, *Staphylococcus spp*, *Enterobacteriaceae*, *Campylobacter spp*. and sulphite-reducing clostridia.

Total viable count in the samples examined ranged from 2.30 to 7.41 log CFU/g in samples of poultry analyzed. The values were higher in samples of breast boneless skinless poultry, with an average of  $6.36 \pm 0.32$  log CFU/g, compared with samples of chicken breast skin ( $6.17 \pm 0.49$  log CFU/g).

*Enterobacteriaceae* were found in 38.47% of the samples of skinless chicken breast, and 42.85% of chicken breast with skin. Average number of enterobacteria in skinless poultry breast was  $3.62 \pm 0.48$  log CFU / g, respectively and  $2.28 \pm 0.52$  log CFU / g chicken breast with skin.

These results are similar to those reported in the literature by other researchers (from 2.58 to 3.53 log CFU/g). In the current study, we can say that the average *Enterobacteriaceae* in poultry samples examined was lower than reported by the authors cited.

Average value level of coliforms contamination in the samples examined is similar to values published by other authors and is considered as part of the normal range for this type of meat. It may consist of a value greater than the number of coliforms in the case of organs and body parts that have supported cutting. This underlines the important role of hygiene conditions in which the meat processing and handling. Contact surfaces and worker hands contribute to microbial contamination of the carcass.

Following the analysis of bacterial colonies isolated from a total of 30 samples, based on morphological and biochemical characteristics, has been identified in poultry a diverse microflora, composed of bacteria belonging to the genera: *Enterococcus*, *Escherichia*, *Enterobacter*, *Klebsiela*, *Citrobacter*, *Proteus*, *Staphylococcus*, *Micrococcus*, *Shigella*, *Yersinia*, *Listeria*, *Salmonella*, *Aeromonas*, *Alcaligenes*, and *Bacillus*.

With greater frequency, in samples of poultry meat cut and offal were isolated bacteria of the genera: *Enterococcus*, *Enterobacter*, *Escherichia* and *Klebsiela* while on the carcasses were found, besides the genera listed, bacteria of the genera *Staphylococcus*, *Alcaligenes*, and *Bacillus*. With a lower frequency were identified a number of bacteria as those in genera *Aeromonas*, *Shigella* and *Yersinia*.

Of potentially pathogenic bacteria *Salmonella* spp. was found in 15.39% of the samples of boneless poultry breast skin and 9.52% of the samples of chicken skin breast. *Salmonella* spp. was isolated also from 10.53% in minced poultry meat samples frozen.

*L. monocytogenes* was isolated from 4.76% in samples of poultry breast skin and 5.26% in frozen minced meat samples.

Bacteria of the genus *Clostridium* (sulphite-reducing species) were isolated from a single sample of skinless poultry breast.

In general, bacteria *Salmonella* spp were found in 18.40% of poultry samples examined, *S. aureus* from 30.30% *Enterobacteriaceae* to 24.84% *L. monocytogenes* in 3.03% and *Clostridium* (sulphite-reducing species) were isolated from 1.5% of the samples examined. *Campylobacter* spp. was not identified in the samples analyzed.

Also, the average of total viable count was higher in samples of chicken breast without skin compared with samples of chicken skin. A greater number of mesophilic aerobic bacteria were found in minced meat, compared with cut meat.

The results of this study confirm the findings of other researchers that the processing of poultry carcasses in general and mincing and cutting, in particular, are important factors favoring meat contamination with microorganisms.

Chapter XI, entitled **”Using the results obtained in the implementation of control systems, microbiological hazards associated with poultry”** aimed to track the effectiveness of compliance with good manufacturing practice (GMP) which in connection with laboratory analysis of the final products does not always solve the problem of microbial contamination. The meat safety problem can be solved only by fully integrated application of the food safety management systems such as HACCP, which allow the identification and keeping under control of the identified hazards.

Since the current technologies for slaughter do not provide products free of pathogens, the developing and implementation of HACCP plans are compulsory in order to minimize contamination of poultry and poultry products. To this aim it has specifically created critical control points and risk analysis for stages of technological flux of processing poultry.

It was established that the most important stages in the contamination of poultry meat on the technological flow are scalding, defeathering, evisceration, cutting and boning.

In Chapter XII, **”Final conclusions and recommendations”** are exposed synthetically the most important conclusions that were made after analyzing data from investigations.

Slaughter poultry are exposed in breeding farms to the action of a rich and diverse microflora that can mostly be found on poultry carcasses after processing. Bacteria of *Enterobacteriaceae* family that includes an impressive number of species were often present in large numbers in the farming environment constituting a potential danger because some bacteria species of this family are pathogenic to humans. Their presence in large numbers of these species in housing environment is an indicator of the existence of carriers in bird flocks.

In order to reduce and even eliminate the carriers of human pathogenic microorganisms from bird flocks is necessary to improve the farming technologies. This can be achieved through applying integrated programs from hatching stations and continuing until delivery of birds to slaughterhouse, by controlling all the factors that may promote increased incidence of dangerous organisms to the consumer. Continuous monitoring of microclimate factors in poultry farms and immediate correction of any deficiencies, ensure microbiologically comfort and avoid the stress which lowers the birds resistance to diseases. Detailed studies are needed to look at the incidence and characteristics of human pathogens in poultry breeding farms because at this level can be achieved effectively prevention through the application of appropriate biosecurity measures.

Transport of poultry from farms to processing units has proved to be a contributing factor to quantitatively increase microflora of poultry carcasses because of the phenomenon of cross-contamination that cannot be eliminated at this stage of processing. During transport increase of values in all determined parameters (enterobacteriaceele, coliformii and *E. coli*) were established. Decontamination of equipment used for transport of birds, although not eliminate cross-contamination that occurs during transportation, is, when properly done (with modern technologies and substances with precise action spectrum), an effective means of reducing effects of pathogen contamination.

In slaughterhouses, the reception of birds is the major source of air pollution with microorganisms. Along the processing line of poultry slaughterhouse air microflora tends to decrease reaching the lowest values in the freezing tunnel. The results obtained (variation of *Enterobacteriaceae* and other Gram-negative bacteria) indicates that the slaughterhouse air microflora is supplied during the technological flow, and particularly in so called "risk stages" (defeathering and evisceration).

Lowering the contamination of carcasses through the air at the slaughterhouse can be done by mechanically isolation of facilities known as "bioaerosoli" generating pollutants and by restricting the free circulation of air as far as possible from risk areas and other spaces. Such measures reduce cross-contamination via air contamination but do not affect the circulation of air that occurs inside the premises. A measure to reduce contamination is to maintain the temperature of the working spaces of less than 10°C to prevent proliferation of microorganisms.

Scalding birds, as resulted from this study, contributes to cross-contamination of carcasses. Bacterial load of scalding water in the basin has increased steadily reaching three hours after the start of slaughtering process values of the total number of germs in the order of 10<sup>7</sup> CFU/ml. Scalding cannot be considered a critical control point, because the water temperature cannot be increased above certain limits (50-55°C) and exposure time cannot be increased because these modification would lead to depreciation of carcasses in subsequent phases of processing and increasing of economic losses. Reducing the number of microorganisms at this stage can be achieved by applying modern technology in which the bird passes successively through several tanks of scalding water moving against the current, or by adding water with bactericide substances such as acids or bases. The effect of these substances in scalding water is limited, bacteria being protected by skin crevices.

Defeathering as was found in this study was the stage from which carcass microbial load has greatly increased, this phenomenon was generated by operating features of defeathering machine that fuel cross-contamination of carcasses and spreading around of aerosol rich in

microorganisms. Reducing the intensity of contamination of carcasses in this stage could be achieved by continuous spraying of carcasses with heavily chlorinated water by combined microorganisms destruction effect (chemical and mechanical) exerted on the surface of carcasses.

Evisceration was the stage after which from the surface of carcasses were isolated the highest number of microorganisms. Frequent underlying cause of such results is the intestine rupture during evisceration. Even if the pollution with content of the digestive tract was not visible, the results have shown that such accidents occurred. This was confirmed by the large number of microorganisms isolated from active surface facility gutted. Also, the large amounts of *Enterobacteriaceae*, coliforms and *E. coli*, isolated from air, surfaces and carcasses in this area supports this assertion;

Final washing of carcasses (after evisceration) reduced the number of microorganisms from carcasses by the mechanical effect exerted by water. Drastic reduction in the number of microorganisms has shown once again that gutting the carcasses was the most important stage resulting in microbial contamination. Samples of washing water were evaluated and were found rich in microorganisms with enteritis origin. Effect of washing carcasses in the slaughterhouse could be potentiated by the addition of certain bactericide substances in water with, fact allowed, at least at principle, in the European Union since 2006;

Cooling of carcasses has an insignificant influence on carcasses microflora. It can say that cold acted selectively on microorganisms. Thus, while the total number of germs and coliformii grew *E. coli* and *Enterobacteriaceae* bacteria decreased. Variations were insignificant and are due to cross contamination that occurs and the action of low temperatures. Air microflora of cooling tunnel, reflects the action of low temperatures, being made in larger proportion of microorganisms that can grow at low temperatures and chilled products involved in the alteration.

It can be concluded that the microflora of poultry carcasses is originated mainly, at the farming place, transportation and further processing contributing to qualitative and quantitative equalization by processes of cross contamination. Carcass contamination with microorganisms other than those coming from the breeding farm is not excluded but rare. As a consequence due to the possibility of presence of some microorganisms that may possess a risk for consumer (*Salmonella spp*, *Campylobacter spp*, etc.) prevention and control measures must be applied from farm field.

Meat processing in retailing units plants increases the degree of contamination by microorganisms.

Decontamination of carcasses in processing units and/or modified atmosphere packaging can be effective alternatives to reduce the incidence of the pathogenic bacteria from poultry. Since it cannot give absolute guarantees regarding the pathogen free production of chilled and frozen poultry, consumer education play an important role in protecting against food poisoning.