

Microbiological Analysis of Crude and Ready-to-Eat Meats

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Abstract: Different samples of raw and cooked meats were collected of two supermarkets and examined according to a standardized protocol. Comparison with published microbiological guidelines revealed that all crude and cooked samples were of acceptable microbiological quality and only 14% of ready-to-eat were of unsatisfactory/unacceptable quality, because of the presence of *E. coli*, *Salmonella*, and *Staphylococcus aureus*. Acceptable microbiological quality was associated with food hygiene. Poor microbiological quality was associated with lack of enabled workers or deficient hygiene. At the same time, the results, obtained from samples of the work surface and from the hands of the workers, indicated the presence of *E. coli* in the hands of some workers involved in the cooking process of the meat.

Key words: Microbiology quality, Hazards, Raw, Ready-to-eat food

Introduction

The foods are considered essential for life. However, if we did not take certain cares, they can cause diseases known like Foodborne Diseases. The development of this type of pathology is related to the ingestion of contaminated foods and/or water able to affect the health of the consumer.

The polluting agents can be classified in agents: biological (bacteria and/or their toxins, fungi, virus, etc.), chemical (fertilizing, poison, etc.) and physical (metals, glass, wood, etc.); being the contamination from bacterial agents the cause of greater frequency of Foodborne Diseases in the world. They origin numerous deaths, because the foods are environmental where they are dissolved great quantity of nutritious, and in this way the foods can act as culture media for growing of pathogenic microorganisms. Bacterial cells, saprophytic or not, can spread in more than one habitat: air, ground, hands, clothes, hair, mouth, nose, intestinal mucus, foods, etc. Considering the food industry, the contamination can be located in any step of the production chain, from the raw material to the storage process. The potentially pathogenic bacteria can be present in the food at the time of buying or arrive by later pollution; this transference of pathogenic microorganisms is known as cross-contaminating. The foods are not sterile products therefore they must be manipulated, be stored and cook of suitable way to avoid cross-contaminating. An adequate process can be reach by washing hands, utensils, and cutting boards after they have been in contact with raw meat and before they touch another food. Put cooked meat on a clean platter, rather back on one that held the raw meat crossed contamination. Very simple preventive measures protect the consumers to become ill by negligence in manipulation of foods. The application of Good Manufacturing Practice (GMP) and the Operational Procedures Standard of Sanitation (OPSS) are pre-requisites for the development and implementation of a quality system, HACCP (Hazard Analysis Critical Control Points) (Notermns and Mead, 1996). During the last years a considerable increase of sales of fast foods was registered, for this reason it is very important, to focus subject of research in the way to assure innocuous conditions for consumers. At the present some bacteria are used as microbiological quality indicators (ICMSF, 1990); and within this group we can mention coliform bacteria. Specifically, *Escherichia coli* is used as indicator of fecal contamination and *Staphylococcus aureus* like worker contamination. *Salmonella* is widespread in the intestines of birds, reptiles and mammals and it can spread to humans via a variety of different foods of animal origin. The consumption of fast foods is included into our nutritious habits, and then we consider useful the study of real risks, for beginning to know the degree of contamination that the population is exposed. The objective of this work was to study the degree of contamination of meat foods, by analysis of the levels of indicating microorganisms of contamination; and to establish the possible dangers and corrective measures during the elaboration of these products, tending to the development of a HACCP system.

Materials and Method

Hazards Analysis and GMP Evaluation: In order to be able to make identification of the possible hazards that affect the quality of foods, check list was made studying different aspects related to the GPM: temperatures of gondolas, hygiene of the work surfaces and personnel, etc. Different samples were taken from two supermarkets of the city of Tucumán (Argentina) (foods, surfaces of work and hands of the personnel) to detect the presence of

microorganism pathogens.

Samples of Work Surfaces: The samples of surfaces were taken with sterile sponge Whirl-Pak type, with buffered extender on 100 cm² during the evaluated cycles of production. The samples were analyzed following the recommendations of method described by McClain and col. (McClain, 1898) modified by USDA-FSIS (Microbiology Lab Guid, 1998), adapting it for presence determination of *Salmonella* and *E. coli*.

Hands Samples of the Personnel: The samples from hands of the personnel related to elaboration and sale of foods were collected with sterile swabs shrunk in buffered diluent's solution. The presence of *Salmonella* and *E. coli* were determined by spread of swabs on plates containing SSA agar and CHROM agar-coli, respectively.

Food Samples: Trays meats, cooked and crude, were purchased from two supermarket (Tucumán, Argentine), and stored at 7°C until analyzed in the laboratory. Ten grams of samples were homogenized using a Stomacher *Lab-Blender* 400 with 90 ml of peptone water (PW) in sterile stomacher bags for 2 min, and then progressive dilutions (ten-fold) were prepared in PW.

Bacterial Isolation: For the count of microorganisms, suitable dilutions and different culture media were used. In all cases the inoculations were made by duplicate. Total bacterial count was determined by using Plate Count Agar (PCA; Merck). The plates were incubated at 30°C during 24- 48 hours; for enterobacteria McConkey agar (Merck) was used. For total coliforms (TC), suitable dilutions were inoculated in Violet red bile Lactose agar (Britania), incubated at 36°C during 1 day; and CHROM agar-coli were used to *E. coli*. The identity of typical colonies of *E.coli* was confirmed by production of indol after addition of Kovacs reagent (Anonymous, 2000). One gram of samples was also placed into Tetrathionate Selenite Enrichment broth and restreaked on Salmonella-Shigella Agar (SSA) after 24 h of incubation to select for pathogenic. CHROM agar-aureole was used to select staphylococci.

Results and Discussion

This work can be described as a previous analysis of preventative quality control to identify, evaluate, and control hazards of significance to food safety. Over the past twenty years microorganisms has emerged as a significant foodborne pathogen. Under certain conditions, many of these organisms present significant food safety hazards. The food industry bears the responsibility of raising, transporting, processing, and preparing foods that present the minimum level of risk from foodborne hazards, including pathogenic microorganisms. When analyzing the results obtained from different performed check lists, we can frequently observe that some activities focused to take care of and to improve the quality of raw and/or cook foods, were not carried out.

Some mistakes detected in the different stages of the process are detailed in Table I. It is very important, at any moment, to maintain 1) the suitable temperature for conservation of crude and/or processed products, 2) hygiene of the site and the personnel, 3) adequate clothes according to the responsibility of each worker. All these errors will allow the development of pathogenic microorganisms in foods, being able to produce gastrointestinal disorders well-known as Foodborne Diseases. The recommendations are related to the behavior of the personnel and to the internal management of the commerce.

On the other hand, the specific measures aim to assure the hygienic development of the process in a focused stage. With respect to the personnel, it is very important to consider that as much the employees as their activities are contamination sources. For this reason the qualification of the personnel about the dangers is considered the first point to implement.

In order to avoid crossed contamination, it is fundamental that each worker knows the importance of conducting the operations in the site and of the suitable way. For example the use of a thermometer to measure the internal temperature of meat is a good way to be sure that it is cooked sufficiently to eliminate microorganisms.

The industry must have divisions or sections where to make the different tasks. The objective of these divisions is not exposing the product to the potential contaminations derived from the reception of raw material, the cleaning tasks, and the products, packages and cleanings' utensil storage.

Finally, between the topics on which there are to exert more attention are the remainders and the by-products and to avoid contaminations, they must be suitably stored and must retire periodically of the processing zone.

The limits of acceptability for meat foods (crude and prepared) established in Argentine by the National Institute of Foods and by the National Service of Health Animal (SE.NA.SA.) are: <10⁶ CFU mesophilic bacteria/g; <10² CFU enterobacteria/g, 5x10³ CFU Total microorganisms/g, Absence of *E coli* in 0.1 g of food; <10² CFU staphylococci/g and Absence of *Salmonella* in 1g of food. The analyzed meat samples showed high percentage of presence of enterobacteria, but not the other microorganisms (Tables 2 and 3). The samples of cook meat foods taken on the fifth week showed a significant microbial increase, but this situation was not observed from the samples of raw meat foods obtained in the same period. This fact would be indicating that a contamination took

place, possibly crossed, during the steps of cooking food and/or their gondola exhibition. It is important to emphasize the increase in the number of *E. coli* in this period. This problem can be explained by the previously mentioned reasons such as lack of enabled workers or deficient hygiene. At the same time, the results, obtained from samples of the work surface and from the hands of the workers, indicated the presence of *E. coli* in the hands of some workers involved in the cooking process of the meat (Table 4).

Comparison with published microbiological guidelines revealed that all crude and cooked samples were of acceptable microbiological quality and only 14% of ready-to-eat were of unsatisfactory/unacceptable quality, because of the presence of *E. coli*, *Salmonella*, and *Staphylococcus aureus*.

Table 1: Mistakes in the different steps of cooking foods.

Raw meat		
Reception of raw material of the	Lack of control and registry of entrance temperature.	Inadequate clothes personnel
	Re-freezing of raw material	Do not consider expiration date
Packaging of crude meat accessories	Deficient hygiene in surface and material of work	Lack of protective
Exhibition in gondolas	Fluctuating temperatures in refrigerators	Expired products
Cook meat		
Cooking	Lack of control of internal temperature	Deficient hygiene in kitchen area
Exhibition in gondolas	Fluctuating temperatures in refrigerators	Expired products

Table 2: Bacterial counts in crude meats (log CFU g⁻¹)

Samples	Mesophiles	Total Coliforms	Salmonella	<i>E. coli</i>	<i>Staphylococcus aureus</i>
Week 1	6.35 ± 0.17	3.28 ± 0.08	(-)	(-)	< 10 ²
Week 2	6.59 ± 0.10	4.29 ± 0.13	(-)	(-)	< 10 ²
Week 3	5.05 ± 0.09	3.47 ± 0.24	(-)	(-)	< 10 ²
Week 4	6.59 ± 0.18	4.59 ± 0.20	(-)	(-)	< 10 ²
Week 5	6.61 ± 0.11	4.61 ± 0.28	(-)	(-)	< 10 ²
Week 6	6.58 ± 0.25	4.60 ± 0.20	(-)	(-)	< 10 ²
Week 7	6.47 ± 0.23	3.17 ± 0.40	(-)	(-)	< 10 ²
SENASA	6	2.7	Absence 25 g	Absence 0.1 g	< 10 ²

Table 3: Bacterial counts in raw meats (log CFU g⁻¹)

Samples	Mesophiles	Total Coliforms	Salmonella	<i>E. coli</i>	<i>Staphylococcus aureus</i>
Week 1	6.30 ± 0.10	2.61 ± 0.20	(-)	(-)	< 10 ²
Week 2	5.35 ± 0.29	2.31 ± 0.16	(-)	(-)	< 10 ²
Week 3	3.62 ± 0.16	2.66 ± 0.38	(-)	(-)	< 10 ²
Week 4	6.65 ± 0.30	6.64 ± 0.27	(-)	(-)	< 10 ²
Week 5	9.25 ± 0.19	7.06 ± 0.09	(+)	5.07 ± 0.12	5.09 ± 0.08
Week 6	6.63 ± 0.22	5.61 ± 0.21	(-)	(-)	< 10 ²
Week 7	6.65 ± 0.37	3.30 ± 0.11	(-)	(-)	< 10 ²
SENASA	< 1x10 ⁵	2.3	Absence 25 g	Absence 0.1 g	< 1x 10 ²

Table 4: Bacterial counts in work surface and hands of workers

Samples	Work Surface	Hands of workers
Week 1	-	-
Week 2	-	-
Week 3	-	-
Week 4	-	-
Week 5	-	+
Week 6	-	-
Week 7	-	-

Elson *et al.* (2004) showed that most ready-to-eat meat samples (75%) were of satisfactory/acceptable microbiological quality and 25% were of unsatisfactory/unacceptable quality. Scholossner *et al.* (2000) collected and analyzed a total of 188 broiler samples and 56 water overflows from two chillers; the overall prevalence of *Salmonella* was 24.6% (60 of 244 samples). de Sousa *et al.* (2003) determined in food store a high percentage

of *E. coli* in cooked food (46%), in raw food (31%), in surfaces (37%) and in hands (21%).

Acceptable microbiological quality was associated with premises where the management was trained in food hygiene and those that had hazard analysis in place. Poor microbiological quality was associated with storage above 8 degrees C, presliced meats, infrequent cleaning of slicing equipment and poor control of practices that may lead to cross contamination. (Elson et al., 2004). Ingham et al. (2004) studied the ham cold point in uncooked surface and uncooked ground interior ham inoculated with a three-strain *Staphylococcus aureus* mixture, exposed to simulated surface and interior slow-cook conditions, *S. aureus* numbers increased by no more than 0.1 and 0.7 log units, respectively.

During the last years, there was radical change in regulation of meat and poultry hygiene were developed for each sector of the meat industry. Systems for industry/government co-regulation and company-employed meat inspection were introduced based on company HACCP programs. For red meat, the same serovars were prominent among the top 10 isolates both before and after regulation, and there was little linkage with salmonellosis. For poultry, frequently isolated serovars differed pre- and post-regulation, however, in both periods there was some linkage between serovars isolated from poultry and those causing salmonellosis. Using published and unpublished survey data, it was concluded that there had been improvements in microbiological quality of red meat and poultry over the same timeframe as regulatory changes. That these improvements apparently have not carried through to reduced case-rates for salmonellosis may be due to numerous causes, including lack of control in the food processing, food service and home sectors. (Sumner et al., 2004)

A good hygiene demands an effective and regular cleaning of the establishments, equipments and workers to eliminate the dirt and the remainders which can contain deleterious microorganisms able to affect the food quality. A preventive action to avoid crossed contamination during the storage, includes the prohibition to store simultaneously in a same refrigerator chamber products, by-products and raw meats of different species animals. The operation of load and the transport are stages of extreme importance in which it refers the preservation of product quality, because the temperatures of storage must be maintained.

Each segment of the food industry must provide the best conditions to protect food while it is under their control. This has traditionally been accomplished through the application of Good Manufacturing Practices (GMPs). These conditions and practices are now considered a prerequisite to the development and implementation of effective HACCP system, which provide the necessary basic environmental and operating conditions able to reach a safety production, wholesome food (Herrera, 2004). A critical control point (CCP) is defined as a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or to reduce it to an acceptable level. The potential hazards that are reasonably likely to cause illness or injury in the absence of their control must be addressed in determining CCPs. CCPs are located at any step where hazards can be either prevented, eliminated, or reduced to acceptable levels. Examples of CCPs may include thermal processing, chilling, testing ingredients for chemical residues, product formulation control, and testing product for metal contaminants. The information developed during the hazard analysis is essential for the HACCP team in identifying which steps in the process are CCPs (Hogue et al., 1998; Vadhanasin et al., 2004). The crude foods undergo manipulation and will be under the same nonhygienic practices that the cooked foods. It will be interested study the factors that to allow development of indicating microorganisms of contamination in crude and cooked foods.

References

- Anónimo, 2000. Microbiology Methods. Merck. Darmstadt.
- Sousa, de G. B., L. M. Tamagnini and R. D. González, 2003. Indicadores de contaminación y su relación con la presencia de *E. coli* en alimentos listos para consumo. Rev. Argentina Microbiol., 35:86-90.
- Elson, R., D. Burgess, C. L. Little, R. T. Mitchell and Local Authorities Co-Ordinators of Regulatory Services and the Health Protection Agency. 2004. Microbiological examination of ready-to-eat cold sliced meats and pate from catering and retail premises in the UK. J Appl Microbiol., 96:499-509.
- Herrera, A. G., 2004. The hazard analysis and critical control point system in food safety. Methods Mol Biol., 268:235-80.
- Hogue, A. T., P. L. White and J. A. Heminover, 1998. Pathogen Reduction and Hazard Analysis and Critical Control Point (HACCP) systems for meat and poultry. USDA Vet Clin North Am Food Anim Pract., 14:151-64.
- ICMSF (International Commission on Microbiological Specification for Foods). 1990. Microorganisms in Food, Vol. I, Their significance and methods of enumeration, Univ. Press, Toronto.
- Ingham, S. C., J. A. Losinski, B. K. Dropp, L. L. Vivio and D. R. Buege, 2004. Evaluation of *Staphylococcus aureus* growth potential in ham during a slow-cooking process: use of predictions derived from the U.S. Department of Agriculture Pathogen Modeling Program 6.1 predictive model and an inoculation study. J Food Prot., 67:1512-6.
- McClain, D. and L. Wei Hwa, 1989. FSIS method for the isolation and identification of *Listeria monocytogenes* from processed meat and poultry products. USDA-FSIS, Microbiol., Division Beltsville, MD Laboratory

Communication N°57.

- Microbiology Laboratory Guidebook, 1998. Guidelines for environmental and chill water/brine sampling for *Listeria*. USDA-FSIS Third Edition, Washington DC, USA.
- Notermans, S. and G. C. Mead, 1996. Incorporation of elements of quantitative risk analysis in the HACCP system, *Internat. J. Food Microbiol.*, 30: 150-173
- Schlosser, W., A. Hogue, E. Ebel, B. Rose, R. Umholtz, K. Ferris and W. James, 2000. Analysis of *Salmonella* serotypes from selected carcasses and raw ground products sampled prior to implementation of the Pathogen Reduction; Hazard Analysis and Critical Control Point Final Rule in the US. *Int J Food Microbiol.*, 58:107-11.
- Sumner J., G. Raven and R. Givney, 2004. Have changes to meat and poultry food safety regulation in Australia affected the prevalence of *Salmonella* or of salmonellosis?. *Int J Food Microbiol.*, 92:199-205.
- Vadhanasin. S. A. Bangtrakulnonth and T. Chidkrau, 2004. Critical control points for monitoring salmonellae reduction in thai commercial frozen broiler processing. *J Food Prot.*, 67:1480-1483.