



INFLUENCE OF MEAT MATURATION TO THE PRESENCE OF COLIFORM BACTERIA

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ABSTRACT

The aim of our study was detection of coliforms bacteria and pH changes in the process of beef maturation. The number of coliforms bacteria were lower as 1 log cfu.g⁻¹ in four samples and the highest coliforms bacteria count was 3,1 log cfu.g⁻¹ after 1st week of meat maturation. Average number of coliforms bacteria was lower as 1,43 log cfu.g⁻¹. The pH values of meat varied from 5,5 to 6,1 after 1st week. Average value of pH was 5,75. The number of coliforms bacteria were from 2,61 log cfu.g⁻¹ to 3,35 log cfu.g⁻¹ after 2nd week of meat maturation. Average number of coliforms bacteria was 3,17 log cfu.g⁻¹. The pH values of meat were from 6,0 to 6,2 after 2nd week of meat maturation. Average value of pH was 6,05.

Keywords: meat, coliform bacteria, beef maturation

INTRODUCTION

Meat is defined as the flesh of animals used as food. The term 'fresh meat' includes meat from recently processed animals as well as vacuum-packed meat or meat packed in controlled-atmospheric gases, which has not undergone any treatment other than chilling to ensure preservation (Storia et al., 2008). The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens. It is therefore essential that adequate preservation technologies are applied to maintain its safety and quality (Aymerich et al., 2008). The processes used in meat preservation are principally concerned with inhibiting microbial spoilage, although other methods of preservation are sought to minimise other deteriorative changes such as colour and oxidative changes (Tume et al., 2010).

Microbial contamination of meat starts during processing on the slaughter line. First, the microorganisms reach the carcass surface from where they penetrate into deeper layers of the meat. Reducing this primal surface contamination and avoiding or limiting the microbial growth, we can considerably prolong the shelf life of carcasses. Reducing surface contamination would improve food safety and extend shelf life.

Microbial pathogens of current concern that need to be controlled in fresh meat include *Salmonella*, *Campylobacter*, enterohaemorrhagic *E. coli* including serotype O157:H7, as well as other enteric pathogens. Even though progress is being made in their control, some of these pathogens will continue being of concern well into the future, considering that some of them (e.g., *Salmonella*) have been the target of control efforts for

many decades and they are still involved in large numbers of illnesses (Bacon, Sofos, 2003).

Salmonella is one of the most prevalent foodborne pathogens and infects over 160,000 individuals in the EU annually, with an incidence rate of 35 cases per 100,000. The annual cost of foodborne *Salmonella* is believed to reach up to €2.8 billion per year. Reports from the World Health Organisation surveillance programme for control of foodborne infections and intoxications in Europe, revealed the majority of outbreaks, where causative agents were reported, were caused by *Salmonella* serotypes (McGuinness et al., 2009).

Salmonellae are most often associated with any raw food of animal origin which may be subject to faecal contamination, such as raw meat, poultry, fish/seafood, eggs and dairy. *Salmonella* testing in the slaughter environment is important as intestinal pathogens are carried into the abattoir in the bowels and on the skin of the animals (Wray, 2000). Although total viable counts (TVC) and *Enterobacteriaceae* testing are routinely performed on fresh meat carcasses, there was no requirement to test for *Salmonella* contamination prior to 2006 (McGuinness et al., 2009).

Good hygiene practice (GHP) and a hazard analysis critical control point (HACCP) system must be employed to ensure minimal microbial contamination of meat carcasses during slaughter (Bolton et al., 2002).

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MATERIAL AND METHODOLOGY

Occurrence of coliform bacteria and pH changes were examined in beef during maturation.

Determining the number of coliforms bacteria

Six samples of meat were examined. Swabs were collected from the surface of the meat that was stored at 4 °C. Swab swere taken after 1st week of storage and after 2nd week of storage. Dilution plating method was used to determine the number of coliforms bacteria. Dilutions of 10⁻¹ and 10⁻² were used to determine the number of coliforms bacteria. Inoculation was performed with a sterile pipette, 1 ml of triple repeats (parallel to the three Petri dishes) for each dilution used. Plates were embedded by VRBL agar (VIOLET RED BILE AGAR) for determination of coliforms bacteria. Agar was cooled to temperature 50 °C. The plates were cultivated upside down in a thermostat at 37 °C for 24 hours. Grown colonies were counted after incubation. The number of microorganisms in 1 g samples (N) were calculated using the following formula:

$$N = \Sigma C / [(n_1 + 0,1n_2) \cdot d]$$

ΣC – sum of characteristic colonies on selected plates,

n_1 – number of dishes from 1. dilutions used to calculate,

n_2 – number of dishes from 2. dilutions used to calculate,

d – dilution factor identical with 1. used dilution.

The number of coliforms bacteria were compared with Commission regulation 2073/2005.

Measure the pH of meat

Meat pH was measured using a pH meter– Gryf 259

Statistics

For statistical analysis was used program STATGRAPHICS and differences was analysed by t-test. MiniCyclerTM, MJ Research, Watertown USA).

RESULTS AND DISCUSSION

Coliforms, especially *Escherichia coli* are microorganisms of concern in almost every food product, since high counts of coliforms and presence of *E. coli* in foods usually reflect unhygienic handling during production process, improper storage conditions and post-process contamination (Blood, Curtis, 1995; de Sousa et al., 2002; Gonzalez et al., 2003).

Six samples of meat were examined for the presence of coliforms bacteria. The number of coliforms bacteria were lower as 1 log cfu.g⁻¹ in samples no. 2, 3, 5 and 6 after 1st week of maturation. The number of coliforms bacteria was 1,47 log cfu. g⁻¹ in sample number 1 and 3.1 log cfu.g⁻¹ in sample number 4. Average number of coliforms bacteria was lower as 1,43 log cfu.g⁻¹.

The values of pH were from 5,5 (sample 2) to 6,1 (sample 4) after 1st week of maturation. Average value of pH was 5,75. This value is typical for *rigor mortis*.

Total coliform bacteria are used most frequently as indicator microbes (Turner et al., 2000). Their presence is indicative of external contamination (Gill et al., 2001). They are defined as rod-shaped Gram-negative non-spore forming organisms that ferment lactose with the production of acid and gas when incubated at 35–37 °C. Coliforms are abundant in the feces of warm-blooded animals, but can also be found in the aquatic environment, in soil and on vegetation. In most instances, coliforms themselves are not the cause of sickness, but their presence is used to indicate that other pathogenic organisms of fecal origin may be present.

The number of coliforms bacteria were from 2,61 log cfu.g⁻¹ in sample a to 3,35 log cfu.g⁻¹ in sample 5 after 2nd week of meat maturation. Average number of coliforms bacteria was 3,17 log cfu.g⁻¹.

Table 1 Number of coliform bacteria and pH values after 1st week

Samples	log cfu.g ⁻¹	pH
1	1,47	6
2	< 1,00	5,5
3	< 1,00	5,6
4	3,1	6,1
5	< 1,00	5,7
6	< 1,00	5,6

Table 2 Number of coliform bacteria and pH values after 2nd week

Samples	log cfu.g ⁻¹	pH
1	2,61	6,2
2	3,3	6,0
3	3,27	6,0
4	3,26	6,1
5	3,35	6,0
6	3,27	6,0

Table 3 Summary statistics of coliform bacteria number and pH value after 1st and 2nd week of meat maturation

	CB after 1 week	CB after 2. week	pH after 1. week	pH after 2. week
n	6	6	6	6
x	1,43	3,17	5,75	6,05
s	0,84	0,28	0,24	0,08
s _x	0,34	0,11	0,09	0,03
v%	58,82	8,80	4,22	1,38
t-test	+++		+	

CB – number of coliforms bacteria, n – samples, x – average, s – standard deviation, s_x – standard error, v % - coefficient of variation, + P_≥ 0,05; +++ P_≥ 0,001.

The values of pH were from 6,0 to 6,2 after 1nd week of maturation. Average value of pH was 6,05. This value is typical for stadium of matured meat.

For coliforms, although they can be effectively destroyed at pasteurizing step, coliforms can still be found occasionally in products after cooking even though GMP and HACCP programs are implemented. As contamination can come from various sources in the processing environment, identification of these sources is necessary, in order to establish effective control measures and strengthen the GMP and HACCP programs (Kochhar, Evans, 2007).

Differences between number of coliforms bacteria after 2nd week was significantly higher in compare with 1st week.

The safety of meat has been at the forefront of societal concerns in recent years, and indications exist that challenges to meat safety will continue in the future. Major meat safety issues and related challenges include the need to control traditional as well as “new,” “emerging,” or “evolving” pathogenic microorganisms, which may be of

increased virulence and low infectious doses, or of resistance to antibiotics or food related stresses (Thomas, Noppenberger, 2007).

On the base of correlation analysis was found out positive correlation between CB1 and pH1 (0,8354), value of pH1 and pH2 (0,8365) and negative correlation between CB2 and pH2 (-0,897).

Other microbial pathogen related concerns include cross-contamination of other foods and water with enteric pathogens of animal origin, meat animal manure treatment and disposal issues, foodborne illness surveillance and food attribution activities, and potential use of food safety programs at the farm (Doyle, Erickson, 2006).

Chilling is critical for meat hygiene, safety, shelf life, appearance and eating quality. Chilling in air reduces carcass surface temperature and enhances carcass drying; both of which reduce the growth of bacteria. An increase in air velocity and/or a decrease in temperature (both controllable) decrease chilling time. A limiting factor, however, is the difficulty in removing heat quickly from the deeper tissue of carcasses (Ockerman, Basu, 2004).

CONCLUSION

We determined the number of coliform bacteria and pH of the meat during two weeks of maturation. Veal has a higher water content. We recommend to reduce the time maturation of meat for one week, because the number of coliforms bacteria was higher as authorized Commission regulation 2073/2005 after two weeks of maturation.

Despite all efforts targeted on the maintenance of good hygiene practices during meat production, contamination of carcasses with meat-borne pathogens cannot be completely prevented. Efforts to control pathogens of biological origin associated with meat consumption will continue being one of our major goals well into the future.

REFERENCES

- AYMERICH, T., PICOUE, P. A., MONFORT, J. M. 2008. Decontamination technologies for meat products. In *Meat Science*, vol. 78, 2008, p. 114-129.
- BACON, R. T., SOFOS, J. N. 2003. Food hazards: biological food; characteristics of biological hazards in foods. In *Food Safety Handbook*, 2003, p. 157-195.
- BLOOD, R. M., CURTIS, G. D. W. 1995. Media for total *Enterobacteriaceae*, coliforms and *E. coli*. In *International Journal of Food Microbiology*, vol. 26, 1995, p. 93-115.
- BOLTON, D. J., PEARSE, R. A., SHERIDAN, J. J., BLAIR, I. S., MCDOWELL, D. A., HARRINGTON, D. 2002. Washing and chilling as critical control points in pork slaughter hazard analysis and critical control point (HACCP) systems. In *Journal of Applied Microbiology*, vol. 92, 2002, p. 893-902.
- Commission regulation 2073/2005 on Microbiological criteria for foodstuffs.*
- DE SOUSA, G. B., TAMAGNINI, L. M., OLMOS, P. D., GONZALEZ, R. D. 2002. Microbial enumeration in ready-to-eat foods and their relationship to good manufacturing practice. In *Journal of Food Safety*, vol. 22, 2002, p. 27-38.
- DOYLE, M. P., ERICKSON, M. C. 2006. Emerging microbiological food safety issues related to meat. In *Meat Science*, vol. 74, 2006, p. 98-112.
- GILL, C. O., MCGINNIS, I. C., BRYANT, J. 2001. Contamination of beef chucks with *Escherichia coli* during carcass breaking. In *Journal of Food Protection*, vol. 64, 2001 p. 1824-1827.
- GONZALEZ, R. D., TAMAGNINI, L. M., OLMOS, P. D., DE SOUSA, G. B. 2003. Evaluation of a chromogenic medium for total coliforms and *Escherichia coli* determination in ready-to-eat foods. In *Food Microbiology*, vol. 20, 2003, p. 601-604.
- KOCHHAR, H. P. S., EVANS, B. R. 2007. Current status of regulating biotechnology-derived animals in Canada – Animal health and food safety considerations. In *Theriogenology*, vol. 67, 2007, p. 188-197.
- MCGUINNESS, S., MCCABE, E., O'REGAN, E., DOLAN, A., DUFFY, G., BURGESS, C., FANNING, S., BARRY, T., O'GRADY, J. 2009. Development and validation of a rapid real-time PCR based method for the specific detection of *Salmonella* on fresh meat. In *meat science*, vol. 83, 2009, no. 3, p. 555-562.
- OCKERMAN, H. W., BASU, L. 2004. Carcass chilling and boning. In *Encyclopedia of Meat Sciences, Elsevier*, 2004, p. 144-149.
- STORIA, A. L., ERCOLINI, D., MARINELLO, F., MAURIELLO, G. 2008. Characterization of bacteriocin-coated antimicrobial polyethylene films by atomic force microscopy. In *Journal of Food Science*, vol. 73, 2008, p. 48-54.
- THOMAS, J. K., NOPPENBERGER, J. 2007. Avian influenza: A review. In *American Journal of Health-System Pharmacy*, vol. 64, 2007, no. 2, p. 149-165.
- TUME, R. K., SIKES, A. L., SMITH-ENRICHING, S. B. 2010. *M. sternomandibularis* with alpha-tocopherol by dietary means does not protect against the lipid oxidation caused by high-pressure processing. In *Meat Science*, vol. 84, 2010, p. 66-70.
- TURNER, K. M., RESTAINO, L., FRAMPTON, E. W. 2000. Efficacy of chromocult coliform agar for coliform and *Escherichia coli* detection in foods. In *Journal of Food Protection*, vol. 63, 2000, no. 4, p. 539-541.
- WRAY, A. 2000. *Salmonella in domestic animals*. CABI Publishing, Wallingford and New York, 400 p.

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