

INFLUENCE MATURATION OF VEAL ON THE MICROBIOLOGICAL AND PHYSICAL INDICATORS

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ABSTRACT

The aim of the present study was to evaluate microbial and pH changes in veal meat during maturation. Total viable counts, coliform bacteria and pH changes in chilling meat were evaluated after 24 hours, 7 days and 14 days of meat maturation. There were analysed 8 samples of veal meat. Results of microbiological analysis were compared with Commission regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Total viable counts (TVC) in samples after 24 hours of chilling ranged from 2.02 log CFU.cm⁻² to 4.21 log CFU.cm⁻² (1.64 · 10⁴ CFU.cm⁻²). The average number of TVC after 24 hours of meat maturation was 3.27 log CFU.cm⁻², coliform bacteria (CB) after 24 hours were lower than than 1 log cfu.cm⁻² in five samples, and the highest number of coliform bacteria was 1.65 log cfu.cm⁻², average number of CB was 1.13 log cfu.cm⁻². pH values in samples after 24 hours of maturation were in range from 6.6 to 7.0, average pH value was 6.8. TVC in samples after 7 days of chilling ranged from 3.09 log CFU.cm⁻² to 4.01 log CFU.cm⁻², the average number of TVC after 7 days of storage was 3.39 log CFU.cm⁻². CB after 7 days of meat maturation were lower than 1 log CFU cm⁻² in three samples, the highest value of CB was 2.07 log CFU cm⁻², the average value of CB in samples after 7 days of meat chilling was 1.03 log CFU cm⁻². pH values of meat after 7 days of maturation ranged from 5.5 to 6.1. The average pH value of samples after 7 days of storage was 5.73 pH values of meat after 14 days of maturation ranged from 6.0 to 6.4. The average pH value of samples after 14 days of storage was 6.16.

Keywords: veal meat, maturation, total viable count, coliform bacteria, pH value

INTRODUCTION

Beef quality is affected by several factors related to livestock production and industrial processing, which may determine either greater or lesser acceptance of meat by consumers. The pre-slaughter stress, pH decline, the method and rate of chilling, meat aging and electrical stimulation, among other factors, affect the final quality of the meat (Hwang *et al.*, 2003; Bekhit *et al.*, 2007 and Mach *et al.*, 2008).

Meat is recognized as one of the most perishable foods. This is due to its chemical composition that favours microbial growth to unacceptable levels contributing significantly to meat deterioration or spoilage. When large numbers of microorganisms are present in raw meat, there will be changes such that it becomes unappealing and unsuitable for human consumption (Fung, 2010).

Storage temperature is considered the most important factor affecting meat spoilage by affecting the lag phase duration, the maximum specific growth rate and the final cell numbers (Mataragas *et al.*, 2006). Although most countries have established regulation with maximum temperature limits for refrigeration storage, it has been shown that temperature conditions higher than 10 °C are not unusual (Koutsoumanis *et al.*, 2006).

Meat freshness is a rather complex concept, which includes different microbiological, physicochemical and biochemical attributes and that is related with two different processes. One, desired, is known as aging that is determined by the period of storage that meat (especially beef meat) needs in order to reach the optimum state of consumption; whereas the other, also related with storage, deals with meat spoilage due to bacterial growth and autolysis (Yano *et al.*, 1996).

Meat spoilage is a complex event, in which a combination of biological and chemical activities may interact and render the product unacceptable for human consumption (Gram *et al.*, 2002). Besides lipid oxidation and autolytic enzymatic reactions, spoilage of meat can be considered the result of microbial

activity of a wide variety of microorganisms because meat nutrient composition, pH (5.5–6.5) and high moisture content allow the growth and survival of a large range of microorganisms (Nychas *et al.*, 2008; Doulgeraki *et al.*, 2012).

The aim of the present study was detection of total viable counts and coliform bacteria and pH changes during chilling of veal meat.

MATERIAL AND METHODS

The aim of this article was to determine the total viable counts (TVC), number of coliform bacteria (CB) and the pH values of veal during chilling of meat at 3 °C. The microbiological and physical parameters were performed after 24 hours, 7 days, and then finally after 14 days of ripening of meat.

Preparing and taking of samples

There were used 8 samples of veal (300 g) of Holstein-Friesian breed to experiment. After slaughter of animals, carcasses were chilled in ripening cellars at temperature 3 °C and relative humidity 73 %. Swabs from meat surface were performed for microbiological examination. Swabs were collected from the surface of the meat that was stored at 3 °C. Swabs were taken after 1st day, 1st week of storage and after 2nd week of storage of meat.

Determination of cfu counts

Dilution plating method was used to determine the microorganisms. Inoculation was performed with a sterile pipette, 1 ml of triple repeats (parallel to the three Petri dishes) for each dilution used. Dilutions 10⁻⁴ and 10⁻⁵ were used to determine of TVC. Plate Count Agar was used for determine of Total Viable Counts in samples. Petri dishes were cultivated upside-down in a thermostat at 30 °C for 48-72 hours under aerobic conditions. Dilutions of 10⁻¹ and 10⁻² were used to

determine the number of coliform bacteria. Violet red bile agar was used for determine of Coliform Bacteria in samples. Petri dishes were cultivated upside-down in a thermostat at 37 °C for 24 -48 hours.

The number of microorganisms were calculated using the following formula:

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

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.

Evaluation of CFU counts

CFU counts were recalculated at 1 cm² of sample. Number of microorganisms were compared with the requirements of Commission regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.

Reagulation No 2073/2005 provides:

- maximal limit of TVC for carcasses of cattle – 3.5 log CFU.cm⁻²,
- maximal limit of CB for carcasses of cattle – 1.5 log CFU.cm⁻².

Measurement the pH of meat

pH value of meat was measured using a digital portable battery powered pH-meter Gryf 259.

Statistical Analysis

Mathematical and statistical analysis (arithmetic mean, standard deviation, standard error, coefficient of variation) were performed using the program system Statgrafic.

RESULTS AND DISCUSSION

Determination of microorganisms in meat during maturing

Raw meat is one of the most perishable foods: its rich physic-chemical composition favours the microbial growth to unacceptable levels (Gram et al., 2002). In an intact piece, microorganisms are usually on the surface, while the interior is sterile. The initial bacterial load of meat depends on the physiological status of the animal and the spread of contamination into slaughterhouses and during processing (Bae et al., 2010). The survival and growth rates of specific spoilage microorganisms can be affected by different factors, including meat constituents and enzymes, temperature, pH, oxygen, light, moisture and other competing microbes (Nychas et al., 2008).

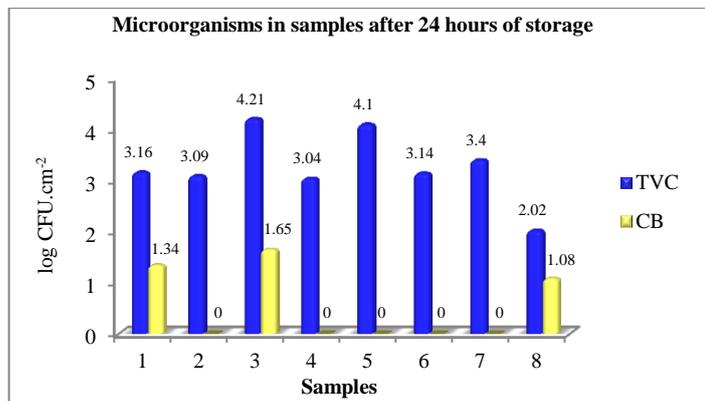


Figure 1 Determination of TVC and CB after 24 hours of meat maturation

Values of TVC in samples after 24 hours of meat maturation were in range from 2.02 log CFU.cm⁻² (1.05×10² CFU.cm⁻²) in sample no. 8 to 4.21 log CFU.cm⁻² (1.64×10⁴ CFU.cm⁻²) in sample no. 3 (fig. 1). The average number of TVC after 24 hours of meat maturation was 3.27 log CFU.cm⁻² (tab. 1). Values of TVC in samples no. 3 and no. 5 were higher than 3,5 log CFU.cm⁻², so they were not in accordance with Commission Regulation no. 2073/2005.

According to the literature, genera occurring on freshly cut meat frequently are *Acinetobacter*, *Pseudomonas*, *Brochothrix*, *Flavobacterium*, *Psychrobacter*, *Moraxella*, *Staphylococcus*, *Micrococcus*, lactic acid bacteria (LAB) and different genera of the family of *Enterobacteriaceae* (Dainty and Mackey, 1992). The environment then enforces a selection pressure on the bacterial community, and those groups of bacteria best adapted to the environment will outgrow the others, become dominant, and reach high numbers. Thus, the survival, growth and succession of specific spoilage bacteria can be affected by a diversity of ecophysiological factors in the physical and chemical environment.

These factors, including meat constituents, temperature, pH, oxygen or carbon dioxide (packaging atmosphere) and competing microbiota are important in maintaining meat quality over time (Koutsoumanis et al., 2006).

Values of CB in samples after 24 hours of storage were lower than 1 log CFU.cm⁻² (<10 CFU.cm⁻²) in samples no. 2, 4, 5, 6 and 7. Number of CB was 1.34 log CFU.cm⁻² (2.27×10¹ CFU.cm⁻²) in sample no. 1, 1.65 log CFU.cm⁻² (4.5×10¹ CFU.cm⁻²) in sample no. 3 and 1.08 log CFU.cm⁻² (1.2×10¹ CFU.cm⁻²) in sample no. 8 (fig. 1). The average number of CB in samples after 24 hours of meat maturation was 1.13 log CFU.cm⁻² (tab. 2). Value of CB in sample no. 3 were not in accordance with Commission Regulation no. 2073/2005.

Several authors have detected many members of the *Enterobacteriaceae* on raw beef, lamb, pork, and poultry products, as well as on offal meats (Garcia-Lopez et al., 1998). However, the genera *Serratia*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Proteus* and *Hafnia*, often contribute to meat spoilage (Nychas et al., 1998). With regard to their meat spoilage potential, the most important *Enterobacteriaceae* are the species *S. liquefaciens*, *Hafnia alvei* and *Enterobacter (Pantoea) agglomerans* (Samelis, 2006). Among the *Enterobacteriaceae*, *Serratia* spp. is the most commonly found genus in meat.

Table 1 Basic statistical characteristics of TVC and after 24 hours, 7 days and 14 days of meat maturation

Parameter	TVC after 24 hours	TVC after 7 days	TVC after 14 days
n	8	8	8
x	3.27	3.39	3.79
s	0.64	0.32	0.58
v%	19.57	9.43	15.30

n – number of samples, x – average, s – standard deviation, v% – coefficient of variation

It has been noted that the storage temperature can affect the spoilage potential of different bacteria (Stanbridge and Davies, 1998) and species belonging to the same bacterial group do not necessarily grow at the same temperature (Doulgeraki et al., 2010). Thus, differences on bacterial species/strains were observed for beef (Doulgeraki et al., 2011 and Fontana et al., 2006) during storage at different temperatures.

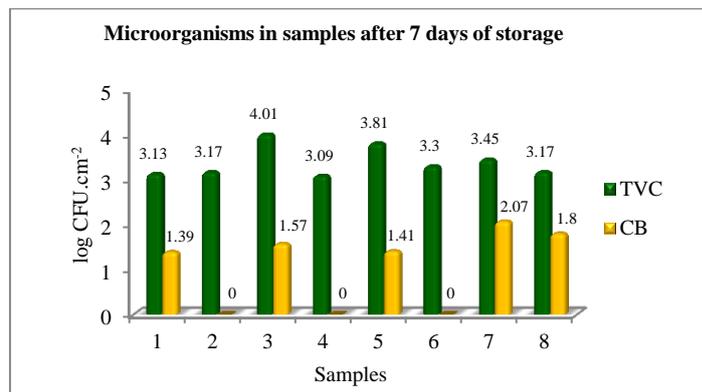


Figure 2 Determination of TVC and CB after 7 days of meat maturation

Microbial quality of raw meat results from the physiological status of the animal at slaughter, processing, transportation, preservation and storage conditions (Nychas et al., 2008).

Gill (2003) determined total viable counts in meat during storage. The microbial growth experienced a significant increase from 3.7×10³ CFU.g⁻¹ at the beginning of the experiment to values larger than 10⁹ CFU.g⁻¹ on day 9. A healthy and freshly slaughtered animal has its muscle sterile. After a period of time, the duration of which depends mainly on temperature, meat pH and slaughtering management, microorganisms experiment an exponential growth.

The lowest value of TVC after 7 days of chilling of meat was 3.09 log CFU.cm⁻² (1.25×10³ CFU.cm⁻²) in sample no. 4 and the highest value of TVC was 4.01 log CFU.cm⁻² (1.04×10⁴ CFU.cm⁻²) in sample no. 3 (fig. 2). The average number of TVC after 7 days of storage was 3.39 log CFU.cm⁻² (tab. 1). The values of TVC in sample no. 3 (4.01 log CFU.cm⁻²) and in sample 5 (3.81 log CFU.cm⁻²) were not in accordance with Commission Regulation no. 2073/2005.

The values of CB after 7 days of meat maturation were lower than 1 log CFU cm⁻² (10 CFU cm⁻²) in samples no. 2, 4 and 6. The highest value of CB was 2.07 log CFU cm⁻² (1.20×10² CFU cm⁻²) in sample no. 3 (fig. 2). The average value of CB in samples after 7 days of meat chilling was 1.03 log CFU cm⁻² (tab. 2). The values of CB in samples no. 3, 7 and 8 were higher than 1.5 log CFU cm⁻², these values were not in accordance with legislation.

The spoilage potential of a microorganism is determined by its ability to produce the metabolites that are associated with the spoilage. However, it is also important to consider the interaction between microbial growth and enzyme activities (Nychas et al., 2008). The microbial ecology associated to the spoilage

of meat in different storage conditions has been recently reviewed. The microbial populations associated with the meat environment are known as belonging to the groups of *Enterobacteriaceae*, lactic acid bacteria (LAB), *Brochothrix thermosphacta*, pseudomonads and some clostridia (Doulgeraki et al., 2012).

McEvoy et al. (2004) found that The Enterobacteriaceae count ranged from 0.52 to 6.98 log CFU.g⁻¹ with a mean range of 2.20–4.64 log CFU.g⁻¹. Studies conducted in Irish beef abattoirs have reported numbers of *Enterobacteriaceae* on beef carcasses at mean counts ranging from – 0.02 to 1.63 log CFU.cm⁻².

Cagney et al. (2004) investigated the prevalence and numbers of *E. coli* O157:H7 in minced beef products and reported sporadic high counts of *E. coli* O157:H7 with a range count of 0.52 – 4.03 log CFU.g⁻¹ and an average log count of 0.91 log CFU.g⁻¹.

Table 2 Basic statistical characteristics of CB and after 24 hours, 7 days and 14 days of meat maturation

Parameter	CB after 24 hours	CB after 7 days	CB after 14 days
n	8	8	8
x	1.13	1.03	1.99
s	0.22	0.82	0.77
v%	19.47	79.61	38.69

n – number of samples, x – average, s - standard deviation, v% - coefficient of variation

Bolton et al. (2002) estimated bacterial numbers (total viable counts) and the incidence of *Salmonella* at three surface locations (ham, belly and neck) on 60 animals/carcasses processed through a small commercial abattoir (80 pigs). A significant increase (P < 0.05) in bacterial numbers was observed after pre-evisceration washing. Final washing increased the bacterial counts to between 3.6 and 3.8 log CFU cm⁻² while chilling effected a small but statistically significant (P < 0.05) increase to between 4.5 and 4.7 log CFU cm⁻². The incidence of *Salmonella* on pigs at the farm was 27 %, decreasing to 10 % after preslaughter washing. However, stunning and bleeding effected a considerable increase in *Salmonella* contamination and the incidence after these operations was 50 %, which was reduced to 0 % during the scalding-dehairing process.

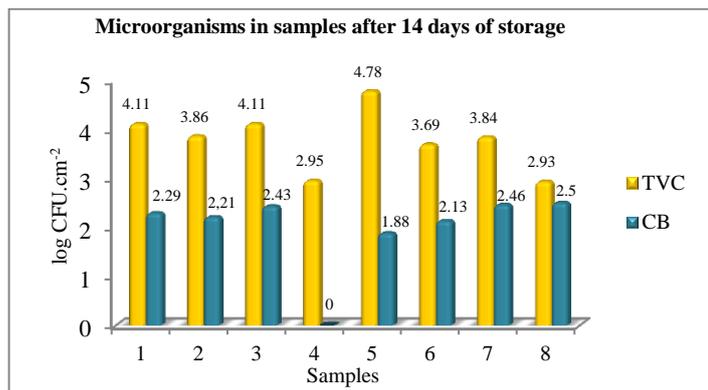


Figure 3 Determination of TVC and CB after 14 days of meat maturation

The values of TVC after 14 days of meat maturation were in range from 2.93 log CFU cm⁻² (8.63×10² CFU cm⁻²) in sample no. 4 to 4.78 log CFU.cm⁻² (6.13×10⁴ CFU cm⁻²) in sample no. 5 (tab. 3). The average value of TVC after 14 days of storage was 3.79 log CFU cm⁻² (tab. 1). Only samples no. 4 and 8 meet requirements of Commission Regulation no. 2073/2005.

The values of CB ranged from lower than 1 log CFU.cm⁻² (10 CFU.cm⁻²) in sample no. 4 to 2.50 log CFU.cm⁻² (3.22×10² CFU.cm⁻²) in sample no. 8 (fig. 3). The average value of CB in sample after 14 days of storage was 1.99 log CFU.cm⁻² (tab. 2). Only sample no. 4 was in accordance with requirements of Commission Regulation no. 2073/2007.

Determination of pH value

pH values of meat after 24 hours of maturation were in range from 6.6 (samples no. 8) to 7.0 (samples no. 3 and 5) (tab. 3). The average pH value of meat after 24 hours of storage was 6.8 (tab. 4).

Gill et al. (2003) found, that pH values decreased of the concentration of protons as a function of time from 5.6 (first day) to 6.27 (day 10th).

Table 3 pH value of meat

Sample	pH value after 24 hours	pH value after 7 days	pH value after 14 days
1	6.8	5.7	6.4
2	6.8	5.6	6.2
3	7.0	6.1	6.4
4	6.7	5.6	6.0
5	7.0	5.8	6.3
6	6.7	5.5	6.0
7	6.8	5.8	6.0
8	6.6	5.7	6.0

pH values of meat after 7 days of maturation ranged from 5.5 (sample no. 6) to 6.1 (sample no. 3) (tab. 3). The average pH value of samples after 7 days of storage was 5.73 (tab. 4).

Ruiz de Huidobro et al. (2003) found a significant decrease in pH in beef meat during the very first hours of storage. Values vary from 6.5 at 45 min post mortem to 5.5 at 24 h. This value remains stable during the next five days ageing.

Table 4 Basic statistical characteristics of pH values after 24 hours, 7 days and 14 days of meat maturation

Parameter	pH value after 24 hours	pH value after 7 days	pH value after 14 days
n	8	8	8
x	6.8	5.73	6.16
s	0.13	0.17	0.17
v%	1.91	2.97	2.76

n – number of samples, x – average, s - standard deviation, v% - coefficient of variation

pH values of meat after 14 days of maturation ranged from 6.0 (samples no. 4, 6, 7 and 8) to 6.4 (samples no. 1 and 3) (tab. 3). The average pH value of samples after 14 days of storage was 6.16 (tab. 4).

After slaughter, temperature and pH decrease. Final values have been attained 24 h post mortem, and no differences were found between heifers and bulls. pH values were similar to those reported by other authors and they were in the normal range (Silva et al., 1999). Zamora et al. (1996) reported that pH value at 15 min was 6.76; and at 24 h was 5.6. Other authors reported that pH in bovine meat at 24 h in *m. longissimus thoracis et lumborum* was 5.5±0.06.

CONCLUSION

Total viable count, number of coliform bacteria and pH of the veal meat were determined during two weeks of chilling. The microorganisms that can colonize the fresh meat depend highly on the characteristics of meat and way it is processed and stored. Despite all efforts targeted on the maintenance of good hygiene practices during meat production, contamination of carcasses with microorganisms cannot be completely prevented.

From a sanitary point of view, meat has to be chilled as soon as possible after slaughter. The rate of chilling is very critical; too slow or too fast chilling of beef can result in an inferior meat quality. Increase of temperature and decrease of relative humidity during chilling of the meat are important parameters to achieve of optimal conditions in terms of microbiological quality and of the maturation of meat.

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