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MANAGEMENT OF MICROBIOLOGICAL QUALITY OF MEAT IN THE CONTEXT OF RAPID TENDERIZATION

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ABSTRACT: This paper focuses on the study of the microbiological load of pork and beef meat, in the context of utilising fast methods of tenderizing them. The study highlights, through both classic and fast methods, the microbial load of fresh, salted and smoked pork and beef meat. It has been established that fresh pork is at around $7,2x10^4$ -$8,5x10^4$ CFU/g, whereas fresh beef sits at around $2,3x10^3$ CFU/g. For salted, smoked and classically tenderized, these values are between $3,3x10^4$ CFU/g and $4,7x10^4$ CFU/g for pork and $6,1x10^2$ CFU/g for beef. The method of rapid tenderizing leads to highlighting a decreased microbial load: $2,2x10^3$ CFU/g-$5,4x10^3$ CFU/g for pork, $2,8x10^2$ CFU/g for beef, which constitute a huge advantage in the value and management of these products. From the point of view of contaminated pathogenic bacteria, the Vidas tests have shown their complete absence. As a conclusion, it can be said that fast methods of tenderization lead to a reduced development of microorganisms, which undoubtedly contributes to their microbiological quality.

1. INTRODUCTION

Meat is a type of food that contains water, valuable protein, fat, extremely small quantities of carbohydrates (glycogen), vitamins and mineral salts. From a microbiological standpoint, meat is extremely important and needs to be stored and sold according to health and safety standards. Its structure and origin may determin an unwanted microbial alteration or the presence of pathogen bacteria might lead to food poisoning (Apostu et al. 2001, Banu 2008, Oprean et al. 2014). Meat is an extremely important type of food for nutrition, as it contains sources of carbon and energy (glycogen, lactic acid that results through glycolysis), sources of nitrogen (digestible protein), mineral salts, vitamins, free water around 67% (beef), 71% (chicken), which represents a favorabe environment for the development of microorganisms, especially putrefying bacteria (Marshall et al. 2001, Oprean et al. 2014).

After the animal is sacrificed, the meat can be involved in aseptic processes which take place in the presence of enzymes in the muscle tissue, which can enhance the value of the meat through maturation or there can be an unwanted microbiological effect, such as the alteration of meat or food poisoning (Jay et al. 2003, Leveau et al. 2003).

A living and healthy animal has in the composition of its muscles very few microorganisms (they can be absent or, at most, one cell per 100 g.). If the animal is tired or sick before being sacrificed, phagocytes cannot eliminate the microorganisms (which are now present in blood) and can accumulate in certain organs: kidney, liver, spleen. If the animal is sick, existing pathogen bacteria contaminate the meat which will be consumed, further passing the microorganisms. The modifications to bacteria contaminating the meat depend on intrinsic factors (composition, aw, pH, rH) and extrinsic (storage temperature).

Meat is a reservoir for the multiplication of germs, even microorganisms, which need the highest values. By way of eliminating a part of the water in the meat of the carcasses, values are no longer appropriate, except for the development of xerophiles, molds (Larpent et al. 2005, Koutsoumanis et al 2008, Leyral et al. 2001).

In living tissue and immediately after an animal is sacrificed, the potential of redox has a value of over 0 which determines the development of aerobic microbiota. In 4 to 6 hours after the animal is sacrificed, when oxygen is no longer produced through blood flow, the potential of reduction-oxidation becomes negative (-50 mV) and the appropriate environment for the multiplication of putrefaction anaerobic germs is created.

The pH of the meat can also lead to its alteration. pH = 6,5 – 7 is the normal pH of the meat, which favors the multiplication of putrefaction bacteria. After being sacrificed, a healthy animal tends to become rigid, due to the glycolysis which takes place in the presence of enzymes in the tissue, resulting in lactic acid, while phosphate groups result from adenylic acids. Rigid complexes appear, which determine a decrease in pH to 5,5 – 5,7, which results in preventing development of germs. The rigidity period can extend to several hours and during this time a biochemical maturation can take place in the presence of proteolytic enzymes in the tissue, when more soluble, easily assimilable protein appear and, as a result of deamination, the pH can increase, reaching 6 – 6,5, which is the appropriate environment for the development of putrefaction germs (Ahmed et al. 2003, Oprean et al. 2014, Pennacchia et al. 2011).

An important role in adequate conservation of the meat is taken by the temperature, which is an extrinsic factor. If the meat is cooled immediately after the animal is sacrificed and if it is stored according to standard, the process of development of bacteria and bacteria toxins are reduced. If meat is stored at a temperature of +10ºC, the process of creating toxins for species of the Clostridium genus is stopped, while for storage at a temperature lower than +3ºC, toxin synthesis is prevented for all toxigenic germs. Standard recommendation is storing the meat and keeping it at a temperature of 0ºC, in order to stop the development of putrefaction germs (in a vacuum environment), and at temperatures under -18 ºC the development of bacteria in meat and meat products is completely stopped (Oprean et al. 2014, Muşat et al. 2007, Tofan 2004).

Surface alteration – meat is kept at temperatures between 0 and 10ºC, takes place over a longer period of time, due to lower temperatures, while the bacteria development process is slowed down, which determines a decrease in the alteration speed, which depends on the humidity of the storage space. If the humidity levels are bigger than 80-90% and the surface of the meat is wet, then it becomes an appropriate environment for the development of psychofile and psychotrofe microorganisms, of the Pseudomonas genus.

Microbial alterations of meat depend on the type and number of bacteria, of the type of meat, of the storage space humidity and of the storage temperature. Alteration of meat is specific and, due to previously-mentioned factors, alteration is produced by 1 to 4 species, even if in the meat we can find an extremely complex microbiota. Alteration can be classified as such:

When the number of microorganisms reached values of over 10^7 /g, a strong putrefaction smell is felt, while at a value of over 10^8 / g, alongside that smell, mucus is formed as well. Mucus is the result of the combination or coalescence of microorganism colonies and change in structure of surface protein. Bacteria which secrete mucus are those described under the Pseudomonas genus, gram-negative germs, aerobic, with a lipolytic and proteolitolytic activity, such as the species *Pseudomonas fleurescens, Pseudomonas ambigua, Pseudomonas fragi, Pseudomonas putida*, and genera: Aeromonas, Micrococcus (Brukner et al. 2012, Borch et al. 1996).

If the meat is stored in a space with air humidity under 75% and the superficial area of is less wet, alteration can occur due to yeasts and molds (Dan 2001, Doulgeraki et al. 2012, Clemens et al. 2010).

Molding can be noticed after 1 or 2 weeks of storage, when the awr is small enough so that development of bacteria does not take place. Initially molds can be eliminated through washing, but if there are spores, hyphae pass to the meat, if these are washed away, unpleasant stains can still be seen and this process decreases the value of the meat. The molds which form on the meat through refrigeration are: *Clodosporium herbarum, Sporotrichum carnis* (which can appear on the meat at a temperature of -5-7 ºC), *Thamnidium elegans*, species of the Penicilium genome. Molding can be accompanied by the appearance of yeasts such as: Candida (these can produce lipases which are catalysts for molds), Rhodotorula, Debaryomyces.

Surface and deep alterations can take place by storing the meat at temperatures ranging from 10 to 25ºC (delivery network). This alteration can also take place if the meat is being cooled in a long time after being sacrificed and is being kept at room temperature. This can be evidenced 2 or 3 days after and is the result of multiplication of psychotrofic and mesophilic putrefaction aerobic germs, part of the following genera: Psudomonas, Lactobacillus, Coliforme, (OIE 2010, Al-Nabulsi et al. 2007).

Outside of superficial alteration, at the end there can also be a deep alteration, especially behind the carcas, as the cools down slower and germs from the genera Bacillus and Clostridium (*Clostridium perfringens*) often develop there. Spoiled meat has a gay-green coloration to it, as the bacteria form oxigenated water which reacts to the pigments of the meat, resulting in choleglobin or H_2S through putrefaction, which mediates the passing of oxyhemoglobin in a green-colored sulfhemoglobin (ICMSF 1996, Feiner 2006, Eckhaut et al. 2012).

If the number of germs belonging to the *Bacilus* genus with the species: *Bacillus megatherium, Bacillus subtilis-mesentericus* is superior to the value of 10^3CFU/ g, by way of the production of proprionic acid and by forming fatty acids through hydrolysis of fats in the fatty tissue, the meat takes on a sour smell.

Deep alteration is produced in the meat which was contaminated internally at temperatures ranging from 20 to 45ºC. Deep alteration takes place when the sacrificed animal is not cooled and the meat is stored in inadequate places. Alteration is observed over the course of 4 to 8 hours, is produced when the animal is not gutted immediately after being sacrificed and due to anaerobic germs of the *Clostridium* genus. Initially this develops in order to feed the glycogen, an example being the *perfringens* species; at this stage a disgusting smell is not yet felt, however due to CO2 and H2 gases being created through fermentation, the tissue takes on a spongy aspect. In the second phase, anaerobic putrefaction germs start their activity: *Clostridium sporogenes, Clostridium perfringens*, which determine the modification of the structure of protein in the meat and the development of potentially toxic amines and some substances which give off an unpleasant, characteristic smell.

Borch et al. (1996) establishes that the influence of environmental factors (composition of product and storage conditions) over the selection, growth rhythm and metabolic activity of the bacteria's flora is important for pork and beef alike. Bacteria predominantly associated with the decay of beef and pork are *Pseudomonas spp. Brochothrix thermosphacta, Leuconostoc spp., Carnobacterium spp., Enterobacteriaceae, Lactobacillus spp., şi Shewanella putrefaciens*. The main flaws of meat are smells and aromas, but discoloration and gas also appear. Bacteria associated with the decay of refrigerated meat products, which cause effects such as abrasive aromas, discoloration, gas and a decrease in pH, are materialized in *Campylobacter spp*, *Carnobacterium spp, B. termophacta, Leuconostoc spp. , Listeria spp., Lactobacillus spp, and Weissella* spp. (Codex, 2009, Allos et al. 2010).

2. MATERIALS AND METHODS

In order to evaluate the microbial load of the types subjected to the study, determinations were made both on the fresh pork and beef, as well as on the salted-smoked one, by way of comparison of the total number of germs, taking into account the tenderization time.

2.1. Materials and methods

Pork (pork chop, pork collar)

Beef (pastrami)

The standard cultural method of counting living cells in plates with an appropriate environment (Standard plate count – SPC) is the most widespread technique of counting living cells or colony-forming units (CFU) of determining the total number of mesophilic aerobic bacteria. Decimal dillutions for analysis are performed, which is why expressing in CFU is better (Oprean et al. 2014, Mershall et al. 2001, Muşat et al. 2007).

When determining the living cells in a food product, the precision of results stems from the following: method applied when collecting the sample, the distribution of microorganisms in the collected sample; the nature of the product's microbiota; if the nutritional environment used is adequate for the microorganisms; time and regulating temperature; pH; a_w; type of diluting agent; relative number of microorganisms inside the sample; possible antagonistic relationships. As such, when the microbiota of the product is unknown, there is no precise cultural method of determining the total number of living cells.

Mesophilic aerobic bacteria were identified on the TEGA environment (tryptone, yeast extract, glucose, agar, Sharlau, Barcelona, Spain) with a 30°C incubation and expressed in CFU/g. Pathogen contamination bacteria were monitored with the help of specific Vidas tests, in order to identify the following species: *Salmonelella tiphy, Listeria monocitogenes, Escherichia coli O157, Campylobacter jejuni.*

Yeasts and molds were identified through cultivation on agarized malt mold (AMM, Sharlau, Barcelona, Spain), with incubation at 22°C for 5 days.

3. RESULTS AND DISCUSSION

As we can see in table 1, the identified microorganisms in the samples subjected to the study have presented oscillating values, based on the moment of harvest. We can ascertain that for fresh pork the values are between $7,2x10^4$ CFU/g and $8,5x10^4$ CFU/g, while for beef we have a value of $2,3x10^3$ CFU/g. With regards to the total number of yeasts and molds, these reach up to $1,2x10^2$ CFU/g - $1,7x10^2$ CFU/g. These values are admissible through the fact that, due to sacrificing, butchering and storing it, the meat itself comes into contact, in one way or another, with various spores or microorganisms, especially mesophiles. Salting and smoking contribute to the partial breakdown of various microorganisms, but as seen in table 1, this also depends on time. In case the classic method of tenderizing for 21 days, the values we see regarding the microbial load of the meat is superior to using the method of rapid tenderizing. Many types of microorganisms can form a sort of imunity from used compounds such as sodium chloride and smoke and a longer exposure leads to their revitalization. We can also observe a significant drop in bacteria, in the case of pork, with values starting from $3,3x10^4$ CFU/g-$4,7x10^4$ CFU/g for salted meat and 2,2x103 CFU/g- 5,4x103 CFU/g for classic, smoked tenderization. For beef the drop is 50%: from $6,1x10^2$ CFU/g to $2,8x10^2$ CFU/g.

Table 1. Results regarding the microbial load of pork and beef, subjected to the study

		NTG (CFU/g)	total number of yeasts and molds (CFU/g)
pork chop	fresh	$7,2x10^4$	1212
	Salting and smoking classic method of tenderizing	$3,3x10^4$	476
	Salting and smoking, rapid tenderizing	$2,2x10^3$	93
pork collar	fresh	$8,5x10^4$	1340
	Salting and smoking classic method of tenderizing	$4,7x10^4$	359
	Salting and smoking, rapid tenderizing	$5,4x10^3$	27
Beef pastrami	fresh	$2,3x10^3$	35
	Salting and smoking classic method of tenderizing	$6,1x10^2$	22
	Salting and smoking, rapid tenderizing	$2,8x10^2$	15

With regards to the total number of yeasts and molds, these were in reduced numbers, decreasing from 476 CFU/g- 359 CFU/g to 93 CFU/g -27 CFU/g for pork and from 22 CFU/g to 15 CFU/g for beef. Molding can be seen after 1-2 weeks of storage, when the awr is low enough so that there isn't a bacteria development anymore. Initially molds can be eliminated through washing, but if there are spores, hyphae pass to the meat, on a distance of 1-2 cm and if these are washed away, unpleasant stains can still be seen and this process decreases the value of the meat. The molds which form on the meat through refrigeration are: *Clodosporium herbarum, Sporotrichum carnis* (which can appear on the meat at a temperature of -5-7 ºC), *Thamnidium elegans*, species of the Penicilium genome. Molding can be accompanied by the appearance of yeasts such as: Candida (these can produce lipases which are catalysts for molds), Rhodotorula, Debaryomyces.

The Vidas tests used were negative, as they did not detect any pathogen microorganisms in the samples.

4. CONCLUSION

The total number of identified germs, yeasts and molds correspond to values highlighted in specialty literature as being normal for a raw material such as meat. The Vidas tests, used to rapidly identify pathogen germs, were negative, which leads us to the conclusion that both pork and beef were of good quality, kept in hygienic conditions and to adequate technological parameters. It has been observed that the fast method of tenderizing the meat led to a decrease in the number of microorganisms compared to the classic method of tenderizing, which also recommends it from a microbiological quality point of view.

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