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DETERMINATION OF THE TOTAL NUMBER OF AEROBIC MEZZOPHILIC BACTERIA, *ENTEROBACTERIACEAE* AND POTENTIAL PATHOGENES IN THE SLAUGHTERHOUSE BY MEANS OF STANDARD AND 3M PETRIFILM™ METHODS

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ABSTRACT. This paper contains the observations which where found during the study of microbiological risks in two phases of technological process in production of pork meat (2 hours post-mortem, before going into the chamber, and 24 hours post-mortem, immediately after leaving the chamber). These two markers are viewed as possible CCP and/or CP as part of the project of implementation of HACCP in this meat industry.

The total number of aerobic mezzophilic bacteria on a carcass 2 hours post-mortem is on average 120/cm^2, expressed as an average value of the total number of aerobic mezzophilic bacteria which were got by means of standard and quick methods, while in the second marker 24 hours post-mortem it is 70/cm^2; the total number of *Enterobacteriaceae* 2 hours post-mortem is on average is 4/cm^2, while in the second marker 24 hours post-mortem it is 3/cm^2; the frequence of *Salmonellae* is 12,5%, and Escherichia 7,5% on the total number of samples. A bit larger number of aerobic mezzophilic bacteria is justified since thermically non-processed meat was observed while the presence of pathogenes can be a marker of insufficiently and irregularly applied hygienic and technical-technological aids of protection.

INTRODUCTION

All the efforts to advance health protection against alimentary poisonings inconditions of modern food production are directed towards removal of all those factors which contribute to the occurence of poisoning. By preventive hygienic-sanitary measures and tehnological inovations, experts have tried to eliminate and reject biological contamination of food in the process of production, and by using effective and, for the consumers, acceptable actions of decontamination – to destroy or reduce contaminated microflora to the level at which it does not endanger health. Preventive measures exact systematical, effective and continuous work on realization of sanitary and hygienic principles in the course of food production from the farm to the dining table;

consequential application of technological prescriptions and effective control of those activities [1]. In order to achieve these goals, certain concepts are suggested, such as HACCP (Hazard analysis critical control point), Good Manufacture Praxis (GMP) and others [21, 22, 23, 24]. A precondition for their application is effective routine diagnosis, and that is the reason why experts work on introduction of more modern actions into the practice which aside from quick getting of the results, make sure that they are corect .

In the course of slaughtering animals, bacteria can get on the meat from the surface of the carcass and that meat, for this reason, becomes health hazard. Contamination is affected by many factors: species, age, background, breeding, nutriment, transport and manipulation, hygiene of slaughtering, treatment and distribution of meat [18].

Aside from inspection of meat, it is also necessary to monitor all the factors which represent risk of contamination in all phases of process in the slaughtering section. Since meat can be primarily contaminated, from various sources, WHO through their committee ICMSF (1982-1988) recommend [20], as the most effective way of control,
HACCP programme (Hazard analysis critical points) – the analisys of risk by controling critical points [22].

The key element of HACCP system is usage of quick methods for monitoring control at all critical points. The establishing of microbiological status of meat and meat products is generally an unavoidable act for determination of their general quality and suitability for further treatment and consumption. In order to obtain the necessary data for determination of microbiological quality of raw materials and products, it is necessary to applay suitable acts of inspection which will give us the most accurate presentation of the circumstances in a short period of time. When using standard procedures, the results are obtained fairly late, when they practically cannot be used anymore [8]. That is why the experts have lately been working on introduction of more modern actions into practice, which will, aside from quick getting of the results, make sure that they are correct .

This paper was done as a part of the project „Application of HACCP in the production of tinned pork ham", and with the purpose of defining possible critical control point (CCP), and also the points by whose control we can only reduce the risk and bring it to the acceptable level (CP) critical point.

MATERIAL AND METHODS

The sampling was carried out in the Meat industry Neoplanta AD Novi Sad, which is a slaughterhouse of bigger capacity with daily capacity of, in average, 900 pigs The pigs are hybrids, which originate from crossing of Landras, Yorkshire and Durok genus (F1, F2, F3) and were bred on the Čenej farm.

The sampling was carried out in the slaughter section 2 hours post-mortem, before entering the chamber and 24 hours post-mortem, immediately after leaving the chamber. These two points are viewed as possible CCP and/or CP. Twenty carcasses were sampled on two different occasions (the total number of forty animals).The samples are taken from the places which are assumed to be most exposed to contamination.

In the alredy mentioned phases of technological process, the carcass hangs from the tracks, and 2 hours post-mortem and 24 hours post-mortem, a swab is taken from the leg area on both sides in this way:

First put the metal model on the leg area and then with a steril glass stick, with cotton-wool at one and soaked in physiological solution make five strokes in horizontal and then five strokes in vertical direction [19]. Two swabs are taken from each leg, so that they represent an average description of the inspected surface.

In this paper, in particular, the total number of aerobic mezzophilic bacteria i *Enterobacteriaceae* was determined by the method of taking a swab from the carcasses in the slaughterhouse, 2 hours post-mortem, before entering the chamber (phase 57. of the suggested HACCP plan), 24 hours post-mortem(phase 58. and 59. of the suggested HACCP plan) after slaughter and after leaving the cooling chamber [21]. The total number of aerobic mezzophilic bacteria and *Enterobacteriaceae* was determined by ways of paralel spreading in two ways: by standard methods [20] and by using so-called quick methods- spreading on 3M Petrifilm ^{TM}[26]

Counting of developed colonies is done by an automatical counter FUNKE GREBER .

RESULTS

The results are presented in the tables 1-2 and they are in accordance with the Regulation [22]. All values are calculated for basic rarefied, a then for 1cm^2, according to which: a) the total number of bacteria must not be above 100 per 1cm^2, b) pathogenic bacteria must not be found .

Total number of aerobic mezzophilic bacteria has changed very slightly during the two visit. During the first visit, the total number of aerobic mezzophilic bacteria was 130/cm^2, and it is was the same during the second visit, at the first point, or to be more precise, 2 hours after slaughtering .

The same may be observed for the total number of *Enterobacteriaceae:* the number for the first visit is 6/cm^2, and for the second 2/cm^2 .

As far as *Salmonellae* and *Escherichiae* on HiChrom are concerned, 3 positive findings on *Salmonellae* and 2 on *Escherichiae* were observed during the first visit, while during the second 2 positive findings for *Salmonellae* and 1 for *Escherichia*.

If we observe the obtained results for the second point, we may see that the total number of aerobic mezzophilic bacteria on the first visit was 70/cm^2, and on the second 60 /cm^2.

The total number of *Enterobacteria* on the first visit was 3/cm^2, and on the second 5/cm^2.

If compare the obtained results of samples which were spread on 3M Petrifilm^{TM}, we will observe the following:

Sampling 2 hours post-mortem:

- the total number of aerobic mezzophilic bacteria at the first sampling 120/cm^2, and at the second 130/cm^2 .
- the total number of *Enterobacteriaceae* at the first sampling 6/cm^2, and at the second 2/cm^2.
- No positiv finding on 3M Petrifilm^{TM} for Coliforms.

If we observe the obtained results for the second point, we may see that the total number of aerobic mezzophilic bacteria on the first visit was 70/cm^2, and on the second 60 /cm^2 .

The total number of *Enterobacteriaceae* on the first visit was 3/cm^2, and on the second 5/cm^2.

On HiChrome 1246 for *Salmonellae* and *Escherichia* there is no positive finding.

Sampling 24 hours post-mortem:

- the total number of aerobic mezzophilic bacteria at first sampling 90/cm^2, and at the second 60/cm^2 .
- No positiv finding on 3MPetrifilm^{TM} for Coliforms.

Tab. 1. The surface of the carcass at the end of slaughter (2 hours post mortem)

Ser.nr.	Standard methods				3M Petrifilm^{TM} – Quick methods		
	Total number of aerobic mezzophilic bacteria	Total number of *Enterobacteriacae*	*Salmonellae*	*Escherichia*	Total number of aerobic mezzophilic bacteria	Total number of *Enterobacteriacae*	Coliform
1	6.0x10^3	-	-	-	5.0x10^3	2.0x10	-
2	2.0x10^2	4.0x10	-	-	5.0x10^2	2.0x10	-
3	4.0x10^3	-	-	-	3.0x10^3	-	-
4	2.0x10^3	6.0x10	-	-	1.0x10^3	8.0x10	-
5	9.0x10^2	7.0x10	-	-	1.0x10^3	1.0x10	-
6	2.0x10^3	4.0x10	-	-	1.0x10^3	4.0x10	-
7	5.0x10^2	4.0x10	-	-	5.0x10^2	4.0x10	-
8	2.0x10^3	1.0x10^2	-	-	1.0x10^3	8.0x10	-
9	6.0x10^2	7.0x10	-	-	5.0x10^2	4.0x10	-
10	2.0x10^3	1.0x10^2	-	-	2.0x10^3	7.0x10	-
11	2.0x10^3	6.0x10	-	-	2.0x10^3	1.0x10^2	-
12	1.0x10^3	4.0x10	-	-	1.0x10^3	3.0x10	-
13	4.0x10^3	7.0x10	-	-	3.0x10^3	8.0x10	-
14	1.0x10^3	2.0x10^2	-	-	1.0x10^3	1.0x10^2	-
15	7.0x10^3	1.0x10^2	-	-	8.0x10^3	5.0x10	-
16	9.0x10^3	9.0x10	-	-	8.0x10^3	9.0x10	-
17	8.0x10^2	1.0x10^2	-	-	8.0x10^2	6.0x10	-
18	5.0x10^3	2.0x10^2	-	-	5.0x10^3	1.0x10^2	-
19	3.0x10^3	5.0x10	-	-	2.0x10^3	1.0x10^2	-
20	3.0x10^3	5.0x10	-	-	2.0x10^3	2.0x10^2	-
	6.0x10^4	2.0x10^3			5.0x10^4	1.0x10^4	
	120	4			120	4	

As we can see (Tab.1), the average number of total aerobic mezzophilic bacteria is, at the first point 2 hours post-mortem, by using standard methods, the spreading on nourishing agar 130/cm^2, while after spreading on 3M Petrifilm^{TM} average number for total aerobic mezzophilic bacteria is 120/cm^2. On average at the first point, the total number of aerobic mezzophilic bacteria is 120/cm^2.

The average number for bacteria from the *Enterobacteriaceae* family is 4/cm^2 on endo-agar for the first point while on 3M Petrifilm^{TM} it is 3/cm^2. Total number in the second point is 3/cm^2 of colonies.

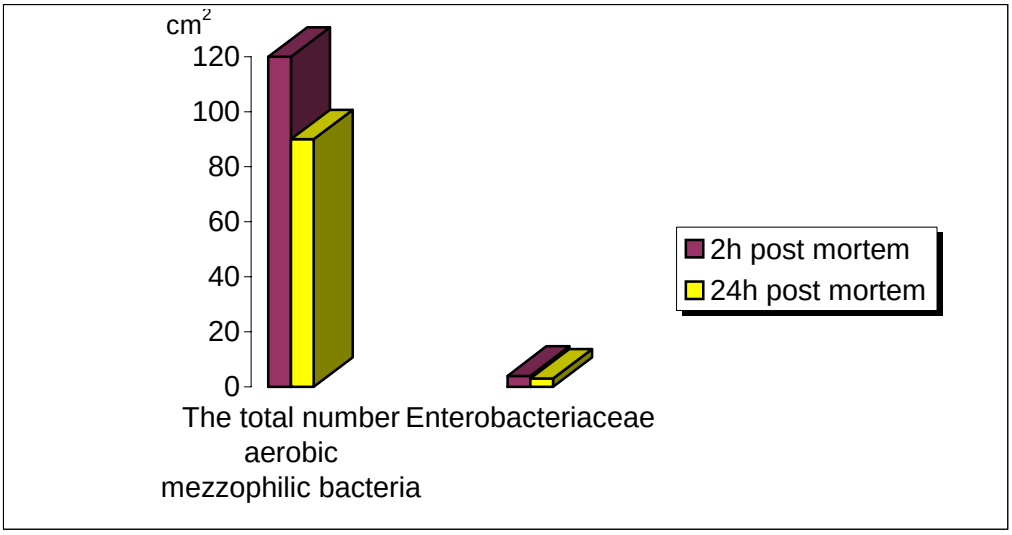
If we observe the second point (24 hours post-mortem) average number of aerobic mezzophilic bacteria is by using standard methods, spreading on nourishing agar 60/cm^2, as it is on 3M Petrifilm^{TM} . The total of 60/cm^2 of colonies (Tab.2).

The total number of *Enterobacteriaceae* is in the second point on standard spreadings 4/cm^2, while on 3M Petrifilm^{TM} it is 3/cm^2. The total of 3/cm^2 of colonies.

Regarding pathogenes (*Salmonellae* and *Escherichia*) the number of positive findings for the first point is 12,5% for *Salmonellae*, and 7,5% for *Escherichia* when observed according to the total number of samples .

Tab. 2.The surface of the carcass in the chamber (24 h post-mortem)

Ser.nr..	Standard methods				3MPetrifilm™ - Quick methods		
	Total number of aerobic mezzophilic bacteria	Total number of *Enterobacteriacae*	*Salmonellae*	*Escherichia*	Total number of aerobic mezzophilic bacteria	Total number of *Enterobacteriacae*	Coliform
1	$5.0x10^3$	$1.0x10^2$	-	-	$6.0x10^3$	$1.0x10^2$	-
2	$3.0x10^2$	-	-	-	$2.0x10^3$	5.0x10	-
3	$3.0x10^3$	-	-	-	$3.0x10^3$	5.0	-
4	$1.0x10^3$	8.0x10	-	-	$2.0x10^3$	3.0x10	-
5	$3.0x10^2$	8.0x10	-	-	$3.0x10^2$	6.0x10	-
6	$1.0x10^3$	6.0x10	-	-	$2.0x10^3$	4.0x10	-
7	$1.0x10^3$	6.0x10	-	-	$3.0x10^3$	4.0x10	-
8	$1.0x10^3$	6.0x10	-	-	$1.0x10^3$	$1.0x10^2$	-
9	$5.0x10^3$	8.0x10	-	-	$4.0x10^3$	4.0x10	-
10	$4.0x10^3$	$1.0x10^2$	-	-	$4.0x10^3$	$1.0x10^2$	-
11	$4.0x10^2$	-	-	-	$2.0x10^2$	-	-
12	$7.0x10^2$	-	-	-	$5.0x10^2$	2.0x10	-
13	$3.0x10^2$	4.0x10	-	-	$2.0x10^2$	3.0x10	-
14	$4.0x10^2$	2.0x10	-	-	$1.0x10^2$	4.0x10	-
15	$1.0x10^2$	-	-	-	$1.0x10^2$	-	-
16	$3.0x10^2$	4.0x10	-		$1.0x10^2$	5.0x10	-
17	6.0x10	-	-	-	$1x10^2$	-	-
18	$3.0x10^2$	8.0x10	-	-	$4.0x10^2$	4.0x10	-
19	$2.0x10^2$	2.0x10	-	-	$3.0x10^2$	1.0x10	-
20	$2.0x10^2$	1.0x10	-	-	2.0x10	-	-
	$3.0x10^4$	$9.0x10^2$			$3.0x10^4$	$8.0x10^2$	
	70	3			70	3	



Graf.1. The relationship of total aerobic mezzophilic bacteria and Enterobacteriaceae 2 hours and 24 hours post-mortem in the slaugterhouse

DISCUSSION

Hygiene as a condition for obtaining microbiologically acceptable food is applied through appropriate sanitation and maintenance of hands and tools, equipment, correctly done technnological operations [6, 7, 10]. The control of conducting so-called good hygienic practice, should provide for the created technical-technological conditions to give positive result, and that result is hygienically acceptable meat [2, 12]. Microbiological research is only an objective conformation of the efficacy of that control. Since standard methods are not suitable for a routine check-up when there is a big number of samples, various methods have been developed with the goal to define microbiological status of meat and meat products more quickly [8, 21, 26].

Quick methods should be more and more applied in industrial production of food products, especially if we talk about implementation of HACCP, which itself requires quick observation of all possible hazards, in this case microbiological, so that we can react on time if any unwanted situation should occur [3, 4].

This paper deals with quantity and quality makeup of microorganisms which occur in meat, with special attention payed to aerobic mezzophilic bacteria and the representatives of the *Enterobacteriaceae* family, with a partial retrospective of pathogenes *Salmonellae* and *Escherichia* by means of standard tests, just like Coliform, as potential cause of fecal contamination [5, 17].

It is impossible to totally observe the problem of the state of meat only through quantity and quality makeup of microorganisma, but we also have to take into consideration all those parametres which can directly affect meat most importantly the state of the surroundings. Analysis of the surroundings must be done regularly, to be more precise – of all the subjects which are in contact with the raw materials. The methods were determined by internal documents which are made according to the Regulations and ISO standards 9001 [20, 22].

The basic goal of our research was to interrogate the presence and frequency of aerobic mezzophilic bacteria, bacteria from the *Enterobacteriaceae* family, and also some of the provokers of alimentary intoxications in the samples of raw meat – carcasses [13]. Coliforms are pretty useful as indicators of bad hygiene or bad treatment of food products, and their presence in large number marks a big possibility of their multiplication, including multiplication of other pathogens [9].

The total number of aerobic mezzophilic bacteria in meat and meat products is one of the most useful indicators of microbiological status of meat and meat products. A large number of living microorganisma often shows that the raw material was contaminated, that the hygiene during treatment was unsatisfactory, that the temperature during production or storing was unsatisfactory or that a combination of these or other factor occurred. A large number of living microorganisms also indicates thepossibility that meat can quickly become rotten, so, the total number of aerobic mezzophilic bacteria can be observed as the indicator of hygienic state, even thought it is not reliable enough [16]. Counting of aerobic mezzophilic bacteria can be done, for example if we want to determine how long we can preserve some product in the refrigerator wagon or some other warehouse.

The development of microorganisms can be slowed down or even prevented [17] with temperatures just above the point of freezing. It is widely known that by lowering the temperature under optimal values we can slow down or even totally prevent the multiplication of microorganisms. The development of most microorganisma stops at temperatures above the point of freezing. Microorganisms in cold meat are usually representatives of psychrofils, especially psychotrops (*Pseudomonas, Acinetobacter, Alcaligenes, Flavobacterium)*, yeast and mould. The cooling of meat ($0 – 7^0$C) does not eliminate rotting of meat totally, because microbe processes last even at $–10^0$C, but meat cooled on 7^0C does not allow multiplication of microorganisms which endanger health [2]. The temperature in the depth of cold meat should be under 7^0C.

How long will cold meat hold also depends on contamination, the speed of cooling meat will hold longer if it is less contaminated.

CONCLUSION

As a part of this paper, microbiological analysis of meat was done, or to be more precise – analysis of carcasses 2 hours post-mortem, in the phase of draining and conditioning, and 24 hours post-mortem, immediately after leaving the cooling chamber Based on the obtained results, we can conclude the following :

- the total number of aerobic mezzophilic bacteria on the carcass 2 hours post mortem is $120/m^2$, which is justified because meat wasn't treated thermically;
- the total number of *Enterobacteriaceae* is $3/cm^2$, which is not a big discrepancy because it is a phase of technological process when meat is still relatively warm and wet, although it can indicate partial contamination from the outside surroundings;
- 12,5% of samples, taken from the carcass 2 hours post-mortem, contained *Salmonella*, which indicates contamination from the outside;
- 7,5% of samples was positive to *Escherichia*, which also indicates contamination from the outside;
- the total number of aerobic mezzophilic bacteria samples taken 24 hours post-mortem is $60/cm^2$, which indicates a positive effect of low temperature, which inhibits mezzophiles;

- the total number of *Enterobacteriaceae* is 3/cm^2, which can indicate contamination of meat;
- usage of modern tests 3M Petrifilm^{TM} enabled quicker and more accurate obtaining of results, these test can be used to monitor the whole technological process;
- it is very important to respect hygienic and technicaly-technological measures of protection in order to avoid postmortal contamination;
- the temperature in the depth of the leg of the carcass was 5^0C, which is low enough to make growth of microorganisms hazardous to health impossible;
- temperature is a very important factor which can be helpful for determining CCP;
- cooling as a method of preserving can be suggested as CCP.

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