COMPARISON OF THE METHODS FOR ENUMERATION OF AEROBIC MICRO- ORGANISMS IN MEAT AND MEAT PRODUCTS

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SUMMARY

During amendment procedure for methodology of enumeration the total count of aerobic micro-organisms in meat and meat products, conducted by the ISO, three media were compared: PCA, APT-agar, TGE-agar. The influence of the media composition, plating technique (surface and pour plating) and time of incubation at 25°C (3, 5, 7 and 10 days) on recovery of micro-organisms was tested. The tests were performed on 22 samples of meat and meat products, differing in quality and quantity of microflora. The tests proved that the acceptable results could be obtained on 3-rd day of incubation using the TGE-agar and surface plating technique or using PCA and pour plating technique.

Introduction

The total count of aerobic mesophilic micro-organisms is one from important parameters characterizing the microbiological quality of meat and meat products. It indicates the sanitary-hygienic condition of applied technological process and ready to eat end-product. This parameter allows to evaluate the durability of end-product and estimate the optimal safe store time. That enables to minimize the danger of food poisoning in consumers.

The aim of the study conducted on the initiative of ISO was the optimization of the methods for estimation of this parameter in meat and meat products.

Materials and methods

1. Media

a) - Plate Count Agar (PCA) (Oxoid)

b) - All Purpose Tween Agar (APT-agar)

Composition:

Tryptone (Oxoid)	10g
Yeast extract powder (bioMerieux)	7,5g
Glucose	10g
Tween 80	0,2g
Sodiumchloride (NaCl)	5g
Dipotassiumhydrophosphate (K_2HPO_4)	5g
Trisodiumcitrate ($Na_3C_6H_5O_7 \times 5,5\ H_2O$)	5g
Magnesiumsulphate ($MgSO_4 \times 7\ H_2O$)	0,8g
Manganese(II)chloride ($MnCl_2 \times 4\ H_2O$)	0,14g
Iron(II)sulphate ($FeSO_4 \times 7\ H_2O$)	40mg
Sodiumcarbonate (Na_2CO_3)	1,25g
Agar	15g
Water	1000ml
pH	6,70,2

c) - Tryptone Glucose Extract Agar ("Count agar for foodstuffs" - TGE-agar)

Composition:

Beef extract (Difco)	3,0g
Tryptone (Oxoid)	5,0g
Glucose	1,0g
Agar	15,0g
Water	1000ml
pH	7,00,2

All media were sterilized in autoclave at temperature 121°C 1°C for 20 minutes.

2. Samples

The study was done on 22 samples of meat and meat products differing in quality and quantity of microflora:

- raw meat,
- cooked meat products,
- fermented sausages (salami).

3. Method

a) Preparation of the test sample

A representative sample was taken according to the norm ISO 3100-2 and blended in Stomacher. The examination was started immediately after the preparation.

b) Preparation of the initial suspension and dilutions

The initial suspension and dilutions were prepared according to the norm ISO 6887.

c) Inoculation and incubation

- Pour plating

1ml of initial suspension was transferred to both of two plates, 15ml of the medium at temperature 45°C1° C was added and carefully mixed. The procedure was repeated with further decimal solutions.

- Surface plating

To two plates with dried medium (about 15ml of medium) 0,1ml of initial suspension was transferred and spreaded with a spreader.

All plates were incubated for 3, 5, 7 and 10 days at temperature 25°C 1°C. Simultaneously plating on PCA, TGE - agar and APT - agar was done.

4. Expression or results

Only dishes containing from 15 to 300 colonies were considered. The results were counted according to the equation included in the norm ISO 7218.

5. Statistical analysis

The parameters of the percentage increase of the amount of micro-organisms per gram as compared to the shortest time of incubation (3 days) and to both techniques of plating to not discriminate any of them, were counted according to the following equation:

a - the count of micro-organisms per gram of the test sample for one medium, one plating technique, one incubation time,

b - the sum of micro-organisms per gram of the sample for three media, both plating techniques and 3 days of incubation (the sum of six results).

The diagram was made using the method "Notched Box and Whisker Plot" (Tukey 1977, Tukey and Tukey 1982).

Results and discussion

In Table 1 the list of examined products and levels of their contamination based on results of surface plating on PCA, TGE-agar, APT-agar after 3 days of incubation at temperature 25°C are presented. In the table it can be seen that the level of contamination was very variable - from low (10^3 / g) in the case of fresh cooked meat products (the dominated microflora were Gram-positive spore-forming bacteria) to high (10^7 - 10^8 / g) in the case of stored fresh meat (mixed microflora) and fermented sausages (the dominated microflora were lactic acid bacteria).

At figure 1 are shown:

- upper and lower lines limiting boxes indicate the borders between 1 and 2, 3 and 4 quartiles. Distances between them represent 50% of results in the group,
- notches in boxes indicate confidence limits for the median calculated for probability 95%,
- whiskers expanded down and up for one and a half of interquartile distance from the median,
- medians are represented in forms of horizontal lines in central parts of the boxes.

The x-axis depicts the data in form of the codes. Combinations of media types, plating techniques, incubation periods and corresponding codes are presented in Table 2.

Figure 1 shows that the highest values of medians correspond with successive numbers: 1, 4, 7, 10, 14, 17, 20 and 23, which in turn correspond with PCA medium and pour plating or TGE medium and surface plating. There are no differences in confidence limits for probability 95% between medians representing different periods of incubations time for PCA medium and pour plating or TGE medium and surface plating. Therefore it is better to choose shorter period for analysis. Because there are also no statistically significant differences between plating on PCA medium with 3 days of incubation and surface plating on TGE medium with the same incubation time, a type of method can be chosen freely.

Conclusions

Optimal methods for estimation of the total count of aerobic mesophilic micro-organisms in meat and meat products are pour plating on PCA medium or surface plating on TGE medium and 3 days of incubation at temperature 25°C.

Reference

ISO Standard No.3100-2, (1988). Meat and meat products. Sampling and preparation of test samples. Part 2: Preparation of test samples for microbiological examinations.

ISO Standard No.6887, (1983). Microbiology. General guidance for the preparation of dilutions for microbiological examination.

ISO Standard No.7218, (1985). Microbiology. General guidance for microbiological examination.

Tukey, J.W., (1977). Exploratory data analysis. Reading. Mass: Addison - Wesley.

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List of figures and tables

Table 1. Number of micro-organisms per gram in meat and meat products (surface plating, 3 day incubation at 25°C)

Table 2. Explanation to codes number to Figure 1

Figure 1. Percentage increase of cfu/g in relation to 3 days of incubation