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Rapid method for estimation of microbiological quality of meat and meat products.

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It has been needed for a long time to have a rapid method for estimation of viable count of meat and meat products.

By using traditional methods the bacteriological results are available only after 48 hrs of incubation. Naturally, one has to make compromise in this respect between accuracy and rapidity, but a certain loss in accuracy is worth a great gain in time requirement. These rapid methods have a common characteristic: from a certain metabolic activity of a bacteria is deduced to viable count, i.e. the deduction is indirect. Most frequently the reductase enzyme activity of bacteria — as metabolic activity — has been used. The basis of this method is the hypothesis: the speed of color change of redox indicator is proportional to viable count. The literature of this field is rather extensive, and the rezazurin-method — as far as we know — is already in use in some countries for estimation of bacteriological quality of meats. At the same time publications can be found, which deny the usefulness of the method. As rezazurine has been proved the best among redox dyes, we made therefore some experiments with rezazurine, but our preliminary results failed to prove those close correlations found by others. It seemed therefore necessary to clear up this matter, so the following investigations were carried out.

EXPERIMENTS

The main aim of our experiments was to determine the relationship between viable count and reduction time of dye, and — on the basis of this knowledge — to determine how to estimate the »spoilage-tendency». In the preliminary investigations 1 g meat was minced and put in vial containing 9 ml pepton-water. (The pork and beef were taken from various batches, and were stored for various length of time.) It was then mixed thoroughly,

put in water-bath of 37 ° C. 1 ml was taken out for plate count determination, and 0,5 ml rezazurine (0,005 % w/v) was added to the residual 9 ml. The content of the vial was again thoroughly mixed, and put into thermostate (37° C).

The incubated vials were shaken and judged periodically. (Visual evaluation).

When using photometric evaluation, the following method was employed.

Since the suspension containing minced meat is not suitable for direct measurement, the formed color was extracted by ether-ethanol mixture. We found the proportion of 1: 1: 1,5 (suspension with rezazurine: ethanol: ether) as optimal. The ether-extractable dye was measured with filter S 61. In this case it was possible to measure the blue color exclusively. With the advancement of reduction the blue color-component decreases continuously. In our later experiments was proved, that the extraction by solventmixture can be replaced by alcoholic denaturation, filtration and by the measurement of the filtrate.

Evaluating the photometric method, we feel, that this instrumental evalution has reliability when the viable count of sample is low, consequently the reduction time is very long. In this case the photometric evaluation gives more exact results than visual evaluation.

RESULTS AND DISCUSSION.

The relationships between viable counts and reduction time of rezazurine are shown by the rank correlation coefficients in table 1.

Table 1. *Rank correlation coefficients between viable count and reduction time of samples investigated*

Material	*Rank corr. coefficients belonging to various rezazurin colors*			*No of samples*
	lilac	*pink*	*colorless*	
Beef	—0,926	—0,932	—0,911	18
Pork	—0,745	—0,749	—0,842	37
Bologna sausage	—0,88	—0,90	—0,96	23

It can be seen, that the correlation in case of beef is close, but not so much with Bologna sausage, and it is even less with pork.

The regression between viable count and reduction time of beef, and the standard error of estimate line are shown on fig. 1. 2. and 3.

Figure 1. *Regression between viable count and reduction time of rezazurine; standard error of estimate. Beef. Reduction: until lilac shade was formed.*

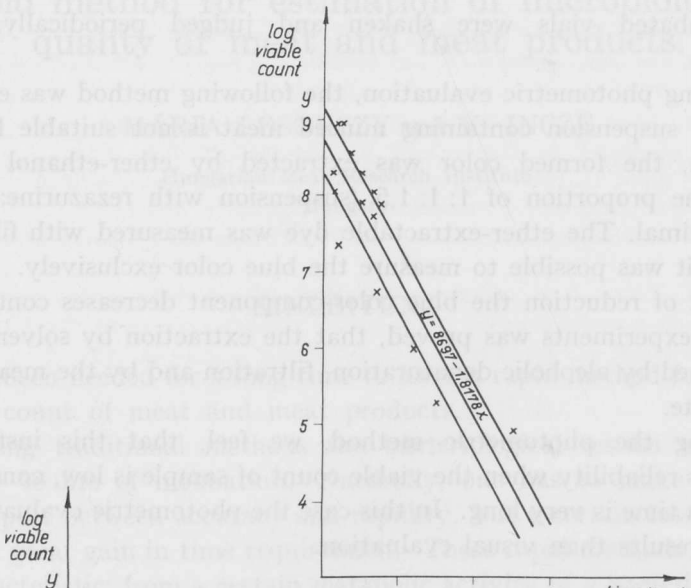

Fig. 1.

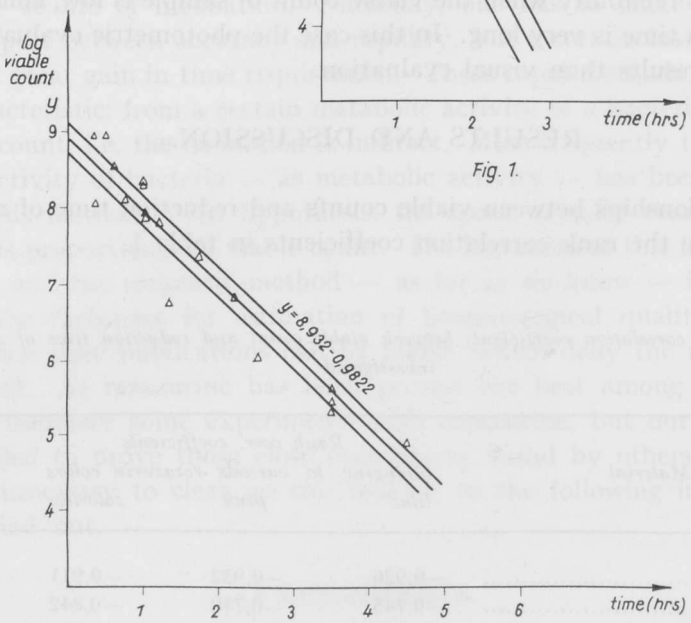

Fig. 2.

Figure 2. *Regression between viable count and reduction time of rezazurine; standard error of estimate. Beef. Reduction: until pink shade was formed.*

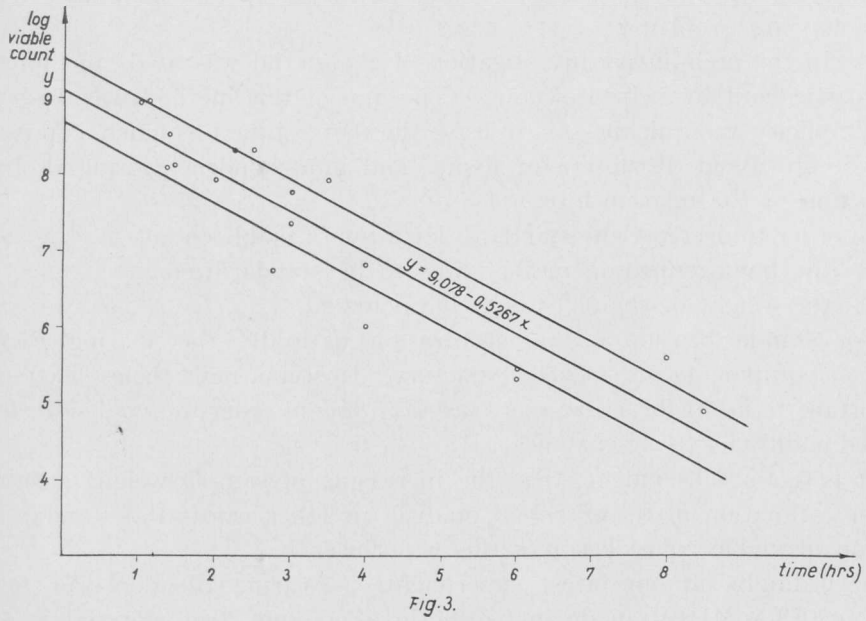

Figure 3. *Regression between viable count and reduction time of rezazurine; standard error of estimate. Beef. Reduction: until colorless.*

These data were calculated also for Bologna-sausage and pork, and the results are shown in table 2.

Table 2. *Standard error of estimate* (s_{xy})

Material	*Standard error ef estimate in case of various color shades*		
	lilac	*pink*	*colorless*
Beef	±0,40	±0,17	±0,43
Pork	±1,30	±1,27	±0,99
Bologna sausage	±1,32	±1,30	±0,89

Since the correlation between viable count and reduction time of rezazurine is in some cases rather weak (Table 1.) and the standard error is high (Table 2.), we found necessary to elucidate the factors influencing the reduction time and viable count, and to determine the degree of influence. If we knew all these, we could take these factors into consideration or even eliminate some of them during the test.

In order to clear up the above mentioned factors, we have to consider the following problems:

1) In the preliminary investigations 1 g material was used, and mincing was carried out by help of scissors. The aim of this method was — beside the simplicity and quickness — to leave the tissues intact as much as possible, in order to avoid disruption of tissues and mitochondria causing probably distortion of the relationships.

In order to decrease the standard deviation of viable count

a) the homogenization method was to be standardized

b) the weight of sample was to be increased.

For standardization of homogenization naturally the Waring Blender type of equipments are most satisfactory. It seems nevertheless extremely important to use it for a *very short period of time* in order to avoid side effects caused mainly by tissue-enzymes.

It is again self-evident, that the increasing of sample weight ensures a better estimation of its microbial quality, in other words the standard deviation of viable count becomes this way lower.

Accordingly, in our latest experiments a Waring Blender type homogenizer (ETA MIRA, made in Czechoslovakia) and 20 g material is used.

2) In case of raw fresh meats *reductase activity* of *muscle-tissue* has the greatest influence on reduction time of rezazurine. At the same time the reduction period depends also on pH changes taking place in post mortem muscle. It was thus found, that reduction of rezazurine took place very fast with fresh meat, in spite of its low viable count. We found further, that the reducing ability (reductase activity) of muscle tissue is in close correlation with the degree of homogenization.

3) The period of reduction of blue pink, and mainly of pink colorless transition is influenced by the consistency, inhomogenity of sample, in other words: by the presence of various phases and boundary surfaces. The presence of boundary surface has an influence on diffusion.

On the basis of our new experiments it can already be stated, that the rezazurine method is not reliable with fresh raw meats because of the high tissue-reductase activity. (Whether a meat is fresh or not can be judged by pH measurement and by organoleptical examination). Nevertheless, with refrigerated meat, where the viable count is rather high (10^6/g—10^8/g) consequently the reduction time is short, the relationship is close, thus the method works well.

The results of our latest investigations concerning the relationship between reduction time and storage, pH, degree of homogenization, and the significance of the time of homogenization as well will be reviewed at the conference more thoroughly.

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