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FRISKHEDSBESTEMMELSE-MIKROBIOLOGISK
Manuskript nr. 306 E

Some Quick Methods for The Quality Control of Meats x)

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Summary

The endeavours of the meat industry to avoid microbial spoilage of their products have resulted in a need for easy and quick methods, so that inferior quality of the raw materials can be detected at an early stage.

The enormous quantities which are handled, makes it impossible to carry out a secure quality control if traditional laboratory methods are employed, partly because they are laborious and, therefore, time consuming, and partly because they give the answer when it is nearly too late.

This paper describes two quick methods, one for evaluation of raw materials, and another for raw mixes. They have the great advantage that the results are obtained within one hour.

The principle of both methods is that meats which are heavily contaminated with microorganisms will cause a rapid conversion of certain dyes which change colour when this happens. This principle is well-known from the dairy industry, where the reductase test has been accepted and used widely for many years.

x) To be held at the 11th Meeting of European Meat Research Workers, Beograd, August 1965.

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Einige Schnell-Methoden für Qualitätskontrolle von Fleischprodukten

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Zusammenfassung

Durch die Bestrebungen der Fleischindustrie um Verderben ihrer Produkte durch Mikroben zu vermeiden ist die Notwendigkeit für eine leichte und schnelle Methode entstanden, damit man eine schlechtere Qualität des Rohmaterials früher entdecken kann.

Mit den traditionellen Labormethoden wird es unmöglich eine sichere Qualitätskontrolle der ungeheuren behandelten Quantitäten auszuführen. Erstens sind sie zu umständlich und nehmen zu viel Zeit, und zweitens bekommt man erst die Antwort, wenn es beinahe zu spät ist.

Das Paper beschreibt zwei Schnellmethoden, die eine für Schätzung der Rohmaterialien, die andere für rohe Farcen. Der grosse Vorteil ist, dass die Ergebnisse innerhalb einer Stunde vorliegen.

Das Princip beider Methoden ist, dass Fleischprodukte mit schwerer Infizierung von Mikroorganismen eine schnelle Einwirkung auf bestimmten Farbstoffen haben, und dass diese dadurch Farbe wechslen.

Das Prinzip ist schon aus der Industrie in Verbindung mit der Milchwirtschaft bekannt; hier ist die Reduktase-Probe schon lange akzeptiert, und sie ist schon viele Jahre verwendet worden.

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Quelques Méthodes Rapides pour le Contrôle de Qualité des Produits de Viande

de

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Résumé

Les essais effectues par l'industrie de viande pour eviter la déstruction microbienne des produits ont abouti au besoin d'une méthode facile et rapide qui peut donner en temps utile l'indication de mauvaises qualités des matières premières.

Comme il s'agit des quantités si énormes il est impossible d'accomplir un solide contrôle de qualité avec les méthodes traditionnelles de laboratoire, d'une part parce quèlles sont trop pénibles et prolixes, d'autre part parce qu'elles ne nous donnent la réponse que presque trop tard.

Cette dissertation-ci vous décrit deux méthodes rapides, la première concernant à l'évaluation des matières premières, la deuxième aux chairs à saucisses. En appliquant ces méthodes, nous connaissons les resultats au bout d'une heure.

Le principe de deux méthodes est que les qualités de viande fort contaminées par micro-organismes vont causer une rapide conversion de certains colorants qui changent de couleur quand celle-ci se passe.

Ce principe-ci est bien connu dans l'industrie laitière. Il y a beaucoup d'ans que cette industrie accepte et emploie dans une large mesure le test de réductase.

Through sanitation, chilling, suitable fixtures and utensils, and careful supervision, the meat industry is trying to endeavour that the microflora, which will unavoidably contaminate meats, will have only few possibilities to spoil the raw products. However, it is a fact that it is insufficient to aim at safety. It is also necessary to possess control methods which can show whether or not the precautions which have been taken have led to the desired results. In bigger plants laboratories have been introduced who control the raw materials, the processing, and the finished products. In smaller plants, however, this is impossible and even in bigger plants it may be difficult to overcome a thorough surveyance.

The need for simple and quick control methods, therefore, seems to be urgent, and one of our projects at the institute in Roskilde is to develop control methods. In particular, we are interested in working out quick methods which are simple and do not require any skilled laboratory work and which can be carried out using simple utensils at a small expense, i.e. methods which can also be used by small plants.

At the 10th Meeting of European Meat Research Workers Jørgen Baltzer of the Danish Meat Research Institute gave a preliminary information called "Simplified Bacteriological Control Methods in The Canning Industry". In this a method was described which evaluates the bacterial condition of raw materials and raw mixes.

The significance of quick methods is that it enables one to examine a larger number of samples. Thus, one gets a quick survey of the bacterial condition of raw materials which make up a production.

Of course, quick methods are not as exact as traditional laboratory methods. We do not consider quick methods a substitute for traditional methods, but we think they are a useful supplement, which in a short time can give a usable answer which is far to prefer to no answer.

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The principle in reductase tests is that certain dyes can act as artificial acceptors for the hydrogen, which is transported during the metabolism by certain enzymes - the dehydrogenases. These dyes are differently coloured in the oxidized and the reduced state. One of the dyes is resazurin.

From the knowledge with reductase tests used in the dairy industry, and among others Thunberg's work with dehydrogenases, we will list the following:

Short conversion time - much enzyme - many bacteria
Long conversion time - small amount of enzyme - few bacteria

The methods should be considered quick methods and <u>not short cut bacteriological methods</u>.

An absolute correlation between results of such a quick method and a real bacteriological method cannot be expected. Through cultivation one determines the numbers of "live bacteria" (i.e. bacteria which are able to propagate) and through a microscopic examination one gets the numbers of "live and dead bacteria". These live and dead bacteria consist of live bacteria, some of which are able to propagate, and some of them which are unable to do so, as well as the actual dead bacteria. All bacteria which can be dyed and can be recognized as bacteria in the microscope will thus go into the group "live and dead bacteria". What we register through the resazurin method is the activity of enzymes. The enzymes will not necessarily lose this ability bacause they are no more in a live bacterial cell. The enzymes of a dead bacterium will still have some activity and even bacterial cells which are autolyzed and thus are impossible to see in the microscope, also possess some activity. Therefore, it will easily be understood that one should not expect an absolute correlation between bacteriological examinations and quick methods based on this principle.

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It is also a fact that the decomposing activities of bacteria are not stopped the moment the bacteria are unidentifiable in the microscope, but may continue even after the cell has been broken up. What we very often register through the reductase method is the bacterial fate of the raw materials. Therefore, we find a better correlation between the result of the reductase test and the numbers of live and dead bacteria than when correlated with the numbers of live bacteria.

One would have expected that the enzymes of the meat would have influenced the result of the reductase test. Of course, they do so, but happily enough the activity of the muscle enzymes is far smaller than the bacterial enzymes. The basal metabolism of muscle cells is far smaller than that of bacterial cells.

The Methods

There are two methods: One evaluates the bacterial quality of trimmings, the other that of the raw mixes. For both methods we use resazurin.

Examination of trimmings

To examine trimmings we use filter paper impregnated with 0.01% resazurin dissolved in water. This examination requires three pieces of resazurin paper (this is made by saturating a piece of circular filter paper with a resazurin solution, drying, and cutting into sectors). From the portion of meat to be examined pieces of meat are picked out at random and transferred to a polyethylene bag. Frozen meat should be thawed prior to examination. The total weight of the sample should weigh about 1 kilogram. The bag is closed and kneaded, so that the pieces are mixed well and their surfaces rub one another. Thus, an even distribution of the bacteria is achieved.

The three pieces of resazurin paper are then removed from a small pouch in which they are stored, and wetted slightly in slowly running tap water. Any superfluos water is shaken off and the paper is placed on the meat in the big plastic bag. Great care should be taken so that good contact between paper and meat surfaces is obtained. The bag is turned so that the meat rests on the paper.

After one minute the papers are removed and placed in the small plastic pouch (avoid overlapping). The air in the small bag is carefully squeezed out. The meat is undamaged and can be used afterwards. It can easily be controlled whether or not the paper contacted the meat well. Looking at the paper as soon as it is put into the small pouch, one will observe that where it has not touched the meat it will have a clearer blue colour than elsewhere.

During the examination the pouch with the paper strips must be kept in the dark at 22-23°C (resazurin is spoiled by light, and the velocity of the reaction - like any other enzymatic reaction - is temperature dependent). The colour of the strips is controlled after 10, 30, and 60 minutes. If the pieces of meat have been sufficiently mixed in the big bag, the colour of the paper strips will be even and will turn gradually, and be well spread over the whole of the papers.

As time passes the original colour changes from azure blue to violet, red-violet, clear rose-red, and finally a decolouration takes place.

Results. Colour change to red or colourless:

in less than 10 minutes:	not acceptable
between 10 and 30 minutes:	just acceptable
between 30 and 60 minutes:	good quality
more than 60 minutes:	prime.

Examination of Raw Mixes

The natural colour of the raw mix makes it difficult to determine the time for the colour change of resazurin from blue to red. It is easy, though, to see when the blue colour has disappeared if after some time a new mixture of raw mix and resazurin is made up, and compare this with the first sample.

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Method. Equal parts of raw mix and lukewarm water are mixed in a plastic bag and are kneaded or shaken till they get well mixed without lumps. 5 ml each of the slurry is transferred to three test tubes. 20 drops of 0.005% resazurin dissolved in water is added at once to the first tube and it is mixed well by shaking. All tubes are placed in the dark at 22-23°C.

If ascorbic acid is added the raw mix, it must be taken into consideration whether there is more or less than 10 p.p.m. of ascorbic acid present.

In the case of raw sausage mix the addition of resazurin to tubes 2 and 3 is done according to the following table:

	Addition of resazurin solution		
Contents of ascorbic acid	1. tube	2. tube	3. tube
More than 10 p.p.m.	0 min.	15 min.	45 min.
less than 10 p.p.m.	0 min.	15 min.	55 min.

The colour of the three tubes is then compared immediately after the resazurin solution has been added to tube 3.

Results. The raw mix is of satisfactory quality if tube 2 has the same shade of blue colour as tube 3 and tube 1 is still somewhat blue. The raw mix is unacceptable if tube 2 is partly or completely decolourized and tube 1 has completely lost the blue shade.

Final Remarks

The described methods have been introduced to the Danish manufacturers of canned meats and many of them have started a routine control on the basis of these.

The work involved using these methods is not very time consuming, and the results are, even if not very accurate, very useful for a judgement of the bacterial standard of the products involved, and it helps to secure that the products will be stable and acceptable.

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The effect of the introduction of these simple control methods can be found in the fact, that an amazingly high decrease in the numbers of remarks about off-flavour from bacterial reasons has taken place. This decrease has been found through the thorough control which is carried out by the Danish authorities who inspect canned meats for export.

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