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A RAPID METHOD FOR THE BACTERIOLOGICAL EXAMINATION OF SLICED MEATS

S.M. Herschdoerfer, E.J. Dyett.

With the development of aseptic manufacturing techniques, the numbers of viable bacteria present in freshly prepared sliced cured meats may be very low. Sliced ham produced by one such process which the authors have investigated was found to have regularly Total Counts of the order of 10 to 100 organisms per gram. This product is sold in transparent laminated film under vacuum, and it is considered necessary to obtain a low initial Total Count of the order mentioned. The method of manufacture must be constantly checked and the sources of contamination must be tracked down and eliminated. The distribution of the organisms in the slices can give a valuable pointer to their source.

Leistner, Grau and Mirna [1] have described a method which involves pressing a metal plate first to the surface of the meat, and then to a poured Agar. Incubation of the Agar, and the consequent development of colonies not only gives a guide to the numbers of organisms present, but also to their distribution. The method becomes rather cumbersome if fairly large areas are to be tested. Like most colony counting techniques, this method requires an incubation period of two or three days.

Dye reduction methods have been in vogue for many years as techniques for a more rapid estimation of bacteriological quality of foodstuffs, and during the past ten years tetrazolium compounds have been recommended for many uses in this role. Ciblis and Wegener [2] have recommended the use of paper strips impregnated with triphenyl tetrazolium chloride (T.T.C) for testing the cleanliness of equipment, by pressing moistened strips onto the surfaces of equipment and then incubating the papers for a few hours. Reduction of the T.T.C. to the red formazan occurs in the vicinity of the developing colonies, and red spots on the papers can be seen and counted long before the colonies become visible.

Triphenyl tetrazolium chloride is used in the method here described. Bradshaw [3] examined a number of brands of the compound and stated that the one manufactured by Mersey Chemicals Ltd gave the most rapid reaction. This is the brand used in the described method. The only reagent required is an 0.5% solution of T.T.C. in distilled water, sterilised by autoclaving for 20 minutes at 10 lb. pressure. The solution has kept well when stored in an amber bottle for one month. The reagent is applied directly to the surface of the slice by spraying with an ordinary throat spray to give a uniform application. About 0.5 ml., per 300 sq. cm. of surface has been found to be sufficient.

Routine production samples that are expected to have very low Total Counts are incubated for 17 hours at 30°C or 37°C in their film bags. Samples returned from the shops, or which for some reason are expected

to have higher Total Counts, need no preliminary incubation. The slice to be examined is suspended aseptically in a clip and both sides are sprayed with the reagent. A film bag normally used for marketing the product is held around and below the slice, the clip is released, and the slice falls into the bag. The bag is then heat sealed, or the open end folded over. It has been our experience that the bags as supplied by the manufacturer are virtually sterile inside, and no preliminary sterilisation process is necessary. The bag with the sample is incubated for 15 - 30 minutes at 37°C and the slice examined.

Red areas develop on the slice where bacterial activities are in progress. Direct microscopical smears have confirmed that there are far more bacteria in these areas than in the areas where formazan production is not apparent. The colour can be detected quite easily against the normal pink colour of the ham. Experiments made with samples of known history have given the following information. Ham with high Total Counts due to undercooking tends to give a fairly uniform development of colour. Post-cooking contamination tends to give spots, or sharply defined areas of formazan formation that can often be related to the use of specific pieces of equipment. Thus, for example, the four spikes on the machine that transfers the slice away from the cutter will, if contaminated, cause four well defined spots to develop in the positions where the spikes pierced the slice.

In general we have found that the Micrococci, Streptococci, and the sporing Bacilli tend to give a rapid and deep production of formazan. Lactobacilli also act rapidly. Coliforms, Proteus and Salmonellae with which we have experimented tend to form the formazan rather more slowly. As a rough generalisation it can be said that, in ham, the Gram positive organisms tend to react more rapidly than the Gram negative organisms. This is also generally true in the reduction of Methylene Blue in the Milk and Ice Cream tests.

Although most of our experiments have been made on sliced ham, we have also tested the method with sliced bacon, with satisfactory results.

The advantages of the method are (a) its simplicity, (b) its speed, and (c) its ability to locate areas of contamination.

The commercial packs of sliced meat normally contain a number of slices. If some of the slices are treated with the described technique, and others with the routine Total Count method, it is possible, not only to give a value to the numbers of spoilage organisms present, but also to suggest in what areas they predominate, and therefore to suggest a source of contamination.

1. L. Leistner, R. Grau, A. Mirna. Bundesforschungsanstalt für Fleischwirtschaft Kulmbach 1955 (65).

2. E. Ciblic and K. Wegener. Dtsch Molkereiztg 79 (8) 237-240 (1958).

3. N.J. Bradshaw. Private communication.