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A comparison of bacterial growth on fresh and thawed meat

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INTRODUCTION:

In comparing the merits of fresh, chilled and frozen meat an important factor is whether the frozen meat, when thawed, is more perishable than comparable fresh or chilled meat. It has usually been assumed that thawed meat is in fact more perishable although experimental evidence for this view is lacking. Work done in Germany by Kallert (Berl. tierärzliche Wschr. (1928) 44 547) and Gressel & Gräfe (ibid (1929) 45, 430) in which minces made from fresh and thawed meat were allowed to go 'off' at room temperature yielded conflicting results. Kallert maintained that minced thawed meat ought to keep at least as long as minced fresh meat whereas Gressel & Gräfe thought this unlikely.

In 1952, Ingram (Proc. Soc. appl. Bact., 14, 243) expressed the view that, because thawed foods usually have a damaged texture and therefore exude fluid (drip), food in this state is more favourable to the development of bacteria than when fresh. In the same year, however, Salzbacher (Food Tech., (1952), 6,341) published results which indicated that, on the contrary, the effect of freezer storage (-23°C) for 15 days on minced meats inoculated with a psychrophilic spoilage bacterium was to add at least 2 days to their subsequent storage life at +7°C when compared with unfrozen controls. The effect was mainly on the lag period of the bacterium, which was prolonged by storage at -23°. The same organism frozen in nutrient broth, thawed, and then incubated at +7° did not exhibit the prolonged lag observed in minces.

In the work to be described, some of Sulzbacher's experiments have been repeated. A psychrophilic Aerobacter (formerly thought to be an Aeromonas resembling Pseudomonas multistriata)isolated as the predominant organism in slime on pork stored at 15°C, was used in our experiments.

Initial experiments with minced pork and horse meat produced no differences of practical significance between the growth of the bacterium in fresh and thawed samples. As we were more concerned about the surface spoilage of carcase meat than about the spoilage of minces tests were continued using slices of muscle.

Slices of the Sternocephalicus (neck muscle) of horse were sprayed with a broth culture of the bacterium and set out in pairs in Petri dishes. Half of the material was used as controls and incubated immediately at 10°C in an

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atmosphere with a relative humidity of 86%. The remainder was stored at -20° for 7, 28 or 56 days before being thawed and incubated at 10°C (RH 86%). Drop plate counts were made at intervals to determine the growth rate at 10°C.

A progressive decrease in the initial viable count (58% survival at 56 days) and a progressively longer lag period (up to 6 hours longer) were observed with increasing storage time at -20°. Nevertheless, together these changes made less than 10 hours difference between the fresh and thawed slices in the time taken to reach a population of 30 x 10^6/sq. cm. which is the critical value for the appearance of slime on meat (Haines (1933), J. Hyg., Camb., 33, 175).

Thus it was not possible, using either minces or slices of muscle, to demonstrate any beneficial effect of freezing on the subsequent storage life at +10°; nor, alternatively, was it found that growth was more rapid on thawed meat. Generation times at 10° on fresh and thawed material were not very different e.g. fresh pork mince 4.2 hr., thawed pork mince 4.3 hr., fresh slices of stomocephalicus 3.8 hr., and thawed slice 3.8-4.0 hr.

However, when strictly comparable tests were made with sprayed nutrient agar plates it was clear that prolonged storage at -20° increased the time at 10° required to reach a "slime level" of 30 x 10^6/sq. cm. 26.5 hours at 10° were sufficient for this level to be attained on the unfrozen agar, but 78 hours were required in the case of the material kept for 56 days at -20°. The difference of 51.5 hours between fresh and frozen samples was compounded of a reduced initial viable count (0.2% of the original sprayed load), a lag period increased by 13 hours, and a generation time of 3.8 hours as compared with 3.0 hours on unfrozen agar.

These results are quite the opposite of those of Sulzbacher. For a more detailed account of the above work see Kitchell & Ingram (Ann. Inst. Pasteur, Lille, (1956) 8, 121.). Some further work arising out of these initial experiments will now be described, although the work is, as yet, unfinished.

The use of Ps. geniculata.

The possibility that different results might be obtained with a different bacterium was tested by using Ps. geniculata. This organism was obtained from the American Meat Institute Foundation, Chicago. It is commonly found predominating in slime on meat (Felton, Buettner, & Niven (1954) "Utilization of gross fission products").

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No important differences were noticed between the growth on fresh and thawed slices of the sternocephalicus of horse. On agar, too, it behaved like Ps. multistriata. The time to reach "slime level" (30 x 10^6 per sq. cm.) was 36.5 hours greater in the sample stored for 56 days at -20°C than in the fresh material. This difference resulted mainly from a reduction of the initial count to 0.5% of the sprayed load and a lag period increased by 17 hours.

The free water content of fresh and frozen muscle.

Experience of the meat trade suggests that thawed meat may be more susceptible to spoilage than fresh meat. Because we had failed to demonstrate this, the conditions under which our experiments were made were re-examined.

The possibility that muscle slices were too thin to yield sufficient "drip", when frozen and thawed, to create conditions favouring more rapid growth was explored. Comparable pieces of horse muscle (hind quarter) weighing about 200 g. were trimmed to fit metal cans without end pieces. The meat surface exposed at one of the open ends was sprayed in the usual way with Ps. multistriata. Half of the material was frozen and thawed and the remainder was used as control. The fresh and thawed samples were suspended, sprayed-side down, at 20°C (R.H. 80%). No systematic differences were observed in the growth of the bacterium on the fresh and thawed meat.

It became desirable, therefore, to evaluate the water content of the surfaces of these blocks of muscle to see if the expected difference existed between the fresh and thawed meat. Four comparable pieces of horse muscle weighing about 200g. were split into 2 pairs. One pair was frozen at -20°C and thawed. The other pair were used as controls. The fresh and thawed samples were hung at 20°C in an atmosphere of relative humidity 80% with fan circulation.

The method of Grau and Hamm (Zeit. Lebensm. - Untersuch. Forsch. (1957) 105, 446) was used to determined the free water content at intervals over a period of 3 days. The weight losses due to evaporation were also followed.

Although the initial percentages of free water in fresh and thawed muscle blocks were 32 and 34% respectively, and differences only of similar magnitude probably prevailed subsequently, it seemed possible that such small differences could be important for the growth of bacteria. The actual weight of free water in the initial samples weighing 0.3 g. were 97.5 mg. and 111.8 mg. respectively

for fresh and thawed meat. Samples were not of identical surface area, but approximated to 0.5 sq. cm. Assuming the difference between 97.5 and 111.8 mg. to be due to "drip" accumulated at the surface of the thawed meat, the surface moisture on this material could exceed that on fresh meat by as much as 28 mg. per sq. cm., equivalent to a layer 0.25 mm. deep. Nevertheless, as stated above, it was not possible to demonstrate that Ps. multistriata grew faster on the thawed muscle

Further, an experiment in which the growth of this bacterium on ox muscle slices superficially dried with filter paper was compared with growth on slices sprayed with water before inoculation showed that increasing the surface moisture by 3 mg. per sq. cm. was without effect. The amount of water added to all samples with the bacterial inoculum was equivalent only to 0.16 mg. per sq. cm.

Growth of Ps. multistriata on ox muscle press juice and "drip".

Having considered the influence of water alone on the growth of bacteria on meat surfaces, a comparison was made of "drip" from thawed meat and juice expressed from fresh meat as substrates for bacterial growth. On the basis of chemical analyses of ox muscle fluids (Lawrie, in the press) it seemed possible that "drip" could be superior to juice as a medium for growth.

The longissimus dorsi and adjacent muscle were cubed and split into 2 batches. One lot was frozen slowly to -20°C, thawed rapidly, and the "drip" collected. The other was minced, placed under a weight in a Buchner funnel, and the juice drawn off. Both lots of fluid were Seitz filtered and then used to soak sterile filter papers laid in Petri dishes. They were sprayed with Ps. multistriata in the usual way, stored over water at 30°C, and sampled at intervals to determine the growth rate.

Presumably because of the pH of these fluids, 5.70, the lag period was rather long and the growth rate relatively slow. Apart from the suggestion of a slightly shorter lag period on "drip", there were no noteworthy differences in the growth on the two fluids.

Survival of bacteria frozen on the surface of agar.

Whatever the reasons for our failure to demonstrate differences between the growth of bacteria on fresh and frozen meat, there still remained, from the early experiments, the observation that the numbers of bacteria surviving prolonged storage at -20°C are substantially larger on meat than on agar.

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This has been investigated by making additions to culture media and observing their influence on survival and growth. Three media were used viz. our usual tryptic digest agar (T.D.A.), Difco brain heart infusion with the addition of 0.3% yeast extract (B.H.Y.), and a medium containing a Seitz filtered, cold aqueous extract of horse muscle (H.M.E.). The bacteria were sprayed on plates of these media which were then stored at -20°C. Samples were taken for counting at various intervals for 56 days. The growth rates at 10°C on those media of the unfrozen bacteria and those stored at -20°C for 56 days were also determined.

Whereas the nutritionally rich medium B.H.Y. was little better than ordinary T.D.A. in the matter of maintaining viability of the bacteria at -20°C, that containing undenatured meat protein (H.M.E,) was clearly superior. In the case of Ps. multistriata, no viable cells were recovered from either T.D.A. or B.H.Y. after 56 days. (cf. published data from tests with higher initial populations). However, from H.M.E. 0.2% of the initial population was recoverable after 56 days. At earlier intervals during storage it also gave higher counts than the other media.

Ps. geniculata was reduced to less than 1% of its original numbers on T.D.A. and B.H.Y., but 13% remained viable on H.M.E. after 56 days at -20°C.

As regards the influence of these media on the rates of growth of the unfrozen bacteria, the order of value to both was (1) H.M.E., (2) B.H.Y. and (3) T.D.A., but whereas their effect on Ps. multistriata was seen in decreasing lag periods, the generation time remaining roughly constant, with Ps. geniculata the effect was mainly upon generation time.

After storage at -20°C for 56 days, growth on transfer to 10°C was characterized by prolonged lag periods, but these were shortest on H.M.E. Generation times on H.M.E. were the same or shorter than before freezing, but on T.D.A. abd B.H.Y. those of Ps. geniculata were longer.

The evidence is, therefore, that unheated extracts of horse muscle will, when added to culture media, reproduce in part the protective effect during freezer storage of bacteria observed on slices of horse muscle and will also maintain their subsequent growth rate at 10°C. Whether the mechanism of action of the extract is physical or chemical yet remains to be elucidated.