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Changes in spoilage pattern as a result of irradiation.

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Any particular food is normally spoiled by a characteristic association of micro-organisms, whose activities ultimately produce typical changes in appearance, odour or taste; and experience has taught the consumer to recognise these changes as signs that the food is no longer fit to use. Irradiation alters these characteristic patterns, and so removes the normal warning signs. Hence it is important to investigate the nature of the alterations.

Such investigations are laborious, and ours have so far dealt with only one set of circumstances, which merit brief explanation.

a) We have worked only with chicken meat, because this suffers relatively little organoleptic damage on irradiation.

b) We have used doses insufficient to sterilse, because sterilising doses - even with chicken - produce such damage to an undesirable degree.

c) We have stored at temperatures below 5°C because, at higher temperatures, the possibility that pathogenic organisms might develop would necessitate a degree of control which we cannot yet excercise.

Within these limits, we have studied two broadly different systems.

i) The chicken meat from all of the carcases, without bones or skin, was minced and packed under nitrogen in shallow aluminium cans, depth 1 cm. capacity 30 g. In this system, micro-organisms are distributed throughout the mass; and conditions probably become anaerobic rapidly, through the consumption of residual oxygen by the tissues - the samples were stored at 0° overnight before irradiation, to allow this to take place. The organoleptic results of this treatment have already been described.

ii) Eviscerated whole carcases were used. Here spoilage is caused by the development on the surfaces of a slime of aerobic or facultatively anaerobic bacteria, chiefly derived - as with other meats - from surface contamination during handling. The carcases were wrapped in sealed but loose polythene bags, to prevent misleading contamination subsequently; this created conditions of high humidity, and may have partly restricted access of oxygen.

The canned minced chicken will be considered first.

Control material contained about $10^5$ bacteria/g. - a comparatively small number for minced meat, since this was prepared in a hygienic manner, in the laboratory. After 14 days at 5°C, numbers had increased to about $10^8$/g. and the meat stank, behaviour similar to that of other meats. The nature of the bacteria will be described in a moment.

The numbers of bacteria at the outset of storage were reduced by irradiation, the diminution being roughly exponential with dose over the range used, which is the classical picture (Slide 1). There was moreover no clear evidence that the radiation exerted any selective action between the groups of bacteria which were distinguished. (Slide 2). If the samples were frozen at about -60° for irradiation, the dose needed to achieve a given kill was increased $2\frac{1}{2}$ times - this factor is roughly the same as for organoleptic changes.

The storage life at 5° increased progressively with the dose of radiation, though with 100 Kr the increase was trifling - about 3 days (Slide 3). With 175 Kr, different cans spoiled at very different times, some still being sound after 3 weeks. With 250 Kr, little change occurred during 3 weeks, but the bacteria had begun to develop in some cans after 6 weeks, and after 43 weeks only one can out of 6 showed little change.

This is shown in Slide 4, which reviews the analysis of the nature of the bacteria. In the control meat, the striking feature is the replacement of the initial flora by a spoilage flora consisting predominantly of lactobacilli and Gram-negative rods, with some faecal streptococci, a reflection of the near anaerobic conditions prevailing: the absence of strict anaerobes may be ascribed to the low temperatures. With the highest dosage 250 Kr, the Gram-negative rods had apparently been completely eliminated; and the lactobacilli almost so, for they were absent from 2 of the 3 cans examined towards the end of their storage life, though dominant in the one can where they were present. These lactobacilli appearing on storage in the irradiated samples were somewhat different from those in the controls: they belong to the genus Microbacterium and resemble organisms isolated at the American Meat Institute from irradiated minced beef.

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The odours of the spoiling irradiated samples are not noticeably different from those of controls - described as putrid or faecal (besides the irradiation odour); which is perhaps not surprising as rather similar lactobacilli predominated in most cases. Two points may be mentioned: the same odour was several times detected in cans with comparatively low bacterial counts (order of $10^4$ - $10^5$/g.); and nothing unusual was noted in the two cans (250 Kr, 21 days) where streptococci predominated. No greening was observed in any of these samples.

Let us turn now to the whole chickens. The increases in storage life following different doses of irradiation are shown in Slide 5: with the higher doses the increase is substantial, greater than has been attained with CTC alone. The effects of CTC and irradiation were approximately supplementary. The length of the storage life was determined by briefly opening the bags and smelling the birds, taking care not to introduce contamination.

The flora on control birds consisted of a mixture of green fluorescent Pseudomonas with similar but non-pigmented organisms (which used to be, and by many French workers still are, called Achromobacter). During storage at +3°, the latter group became almost wholly predominant, (Slide 6).with the development of a typical putrid smell. This situation is broadly the same as with other meats.

Irradiated samples behaved differently. In our first experiment they developed a quite different odour - sweetish, not so strong, and developing gradually so that it was more difficult to decide precisely when a sample should be adjudged spoiled. The spoilage flora consisted almost wholly of Achromobacters (Slide 6). This agrees with American suggestions that Pseudomonas are unusually readily killed by irradiation. It also illuminates two other features of the situation. First, a reason why CTC supplements irradiation is probably that the important organisms resisting irradiation are sensitive to tetracyclines. Second, subsequent work has suggested that Achromobacters are less active than Pseudomonas in producing amine-like compounds, especially with restricted access of air; which may be why, as agents of spoilage, they produce less putrid smells.

A similar experiment, with a rather higher dose, gave an essentially similar result - a preponderance of Achromobacter on the irradiated birds (Slide 7),

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and sweetish odours on spoilage. There were some differences: the flora on the control birds was more diverse including a significant Gram-positive element. Correspondingly, lactobacilli occurred on some of the irradiated birds: from the predominance of similar organisms under anaerobic conditions, this might be related to partial restriction of air supply by the wrappers.

A further experiment, still incomplete, is exploring the effects of somewhat higher doses, in more detail. At doses exceeding 250 Kr, the survival curve ceased to be exponential with dose, indicating the survival of a somewhat more resistant group of organisms (Slide 8). Detailed examination revealed that, as before, the Pseudomonas were the first to be eliminated by small doses below 250 Kr, and that the population surviving 500 Kr consisted mostly of yeasts (Slide 9). The predominance of yeasts was however only temporary, for they had evidently been overwhelmed by bacteria in the final spoilage flora. (This does not happen in the presence of CTC, which the yeasts resist better than the bacteria). A striking feature of this experiment was the predominance of non-pigmented Pseudomonas in the spoilage flora of the irradiated samples, though Achromobacters were common around the 250 Kr level; while at 825 Kr pigmented Pseudomonas appeared in quantity. Though the reasons for such differences from the earlier experiments are unknown (these carcases came from a different slaughterhouse), the predominance of Pseudomonas was signalled - with control and irradiated birds alike - by putrid smells resembling those in the earlier experiments.

One naturally hopes to interpret the behaviour of different organisms during spoilage in terms of their physiological peculiarities. For example, the behaviour of the yeasts in the last experiment is readily understood because they are more resistant to radiation than bacteria, but have much lower rates of cell division. Again, the near elimination of non-pigmented Pseudomonas by modest doses of irradiation, with their rapid assumption of dominance during cool storage, are consistent with the known properties of such organisms and their presumed susceptibility to radiation. We naturally wish to know whether our Achromobacters are in general more resistant, whether the pigmented Pseudomonas prominant on the 825 Kr samples are exceptions to this

rule, whether the Microbacteria and Streptococci of the minced chicken are unusually resistant, and so on. Our experiments for this purpose are in progress now; and the only indication so far is that the Achromobacters of the irradiated birds do indeed possess a greater resistance than the other organisms involved.

The most important practical aspect of such investigations is their bearing on the safety or otherwise of meats treated in this manner. As regards the eviscerated whole carcases, nothing untoward has yet appeared. The spoilage flora of the irradiated birds has consisted of non-pathogenic organisms similar to those which occur commonly on normal meats, under refrigeration, and the chief peculiarity is an unfamiliar smell towards the end of the storage life. With the canned minced meat, on the other hand, the prevalence of faecal streptococci - not signalled by any obviously unusual feature - raises some doubts; for where organisms of this group have grown on meat it has sometimes been suspected that food poisoning has ensued. This situation requires further study, but a desirable preliminary is a greater degree of precision in identifying the food poisoning streptococci.

It should be obvious that the above considerations would not apply to partially sterilised meats stored at higher temperatures. Without refrigeration there is the possibility that dangerous salmonellas, staphylococci, and clostridia might grow. Hence extensive investigations, of a kind similar to those just described, will be necessary before radio-pasteurisation without refrigeration can be recommended for meats.

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