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5-1 MICROBIOLOGY OF MEAT AND MEAT PRODUCTS

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In this report recent points of view with respect to the microbiology of meat and meat products will be reviewed. The commodities have been arranged, in principle, in order of increasing stability in the sense of resistance to microbial colonization.

The field is a very vast one. Consequently priorities had to be set. It was decided that emphasis be placed on attaining and monitoring of safety, quality and acceptability of meat and meat products

CARCASS MEAT

Ecological Introduction

All meat animals carry large numbers of many different types of micro-organisms in their intestines and on the skin, particularly of the legs. Of these, only a few are of public health significance and only a minor fraction will be involved in spoilage when the meat is stored, as is mostly the case, at chill temperatures, i.e. at -2 to 5° C (Mossel, 1983). Almost all contamination is found on the surface of carcass meat, whereas the deep muscle of healthy animals generally contains few or no viable organisms. (Hone et al., 1975; Labadie et al., 1977; Gill, 1979).

The few bacteria occurring in the depth of meat partly originate from lymph nodes, which frequently harbour organisms in the living animal. They might also have been introduced via the blood stream by contaminated captive bolt pistols, pithing rods or sticking knives. Finally a few bacteria might enter the blood stream from the gut, during agony or just after death. The low numbers of viable organisms normally found in the depth of meat may also result from residual anti-

microbial defence mechanisms, which show some activity up to about 24 hours post mortem (Gill and Penney, 1979).

Viscera are generally more suceptible to spoilage than muscular tissue (Bijker et al., 1985). This is the result of (i) their severecontamination by the mechanisms listed above (Mackey and Derrick, 1979); (ii) being nearer to the gut; (iii) their initial pH is, as a rule, higher than that of meat: for example, liver has a pH of about 6.3 (Shelef, 1975) compared to the normal pH 5.4-5.8 for muscle (Ingram, 1948; Van Logtestijn, 1965).

In slaughter houses and processing plants the main aim is to minimise contamination. Tremendous efforts have been made to achieve this (Snijders et al., 1985). Monitoring per se will, clearly, be totally ineffective to improve hygiene, since no act of inspection but rather intervention only, can attain this (Mossel and Kampelmacher, 1981; Kayser and Mossel, 1984). Nonetheless, regular examination of meat for numbers of colony forming units (cfu) per cm^2 (Snijders et al., 1984b) of psychrotrophic bacteria and thermotrophic Enterobacteriaceae (Mossel et al., 1986) is most useful to maintain high standards of hygiene, since it enables to detect excessive increases in bacterial loads at an early stage andthus allows prompt rectification of defective practices (Snijders et al., 1984a).

Meat spoilage

The psychrotrophic organisms found on processed carcasses include species of Pseudomonas, Moraxella, Flavobacterium, Acinetobacter, psychrotrophic Enterobacteriaceae, Lactobacillaceae and Brochothrix termosphacta, as well as certain yeasts and moulds. Unlike pathogens occurring on meats (vide infra), these organisms do not originate from the gut, but stem mainly from the outside of the animal and the environment of chill rooms and the water supply. Of all these organisms the non-pigmented pseudomonads are the most frequently involved in the spoilage of meat under normal chill conditions. On freshly slaughtered meat the proportion of these pseudomonads may not be exceeding some 10 %, whereas this increases to over 80 % when meat reaches the consumer (Gill, 1983; Shaw and Letty, 1984; Eribo and Jay, 1985).

Close attention to hygiene during the processes of slaughtering, bleeding, evisceration and skinning or dehairing can significantly reduce the microbial load on carcasses (Roberts, 1980). However, even the most faithfully followed, sophisticated systems of hygiene cannot completely control contamination (Gerats et al., 1981). Consequently treatment of carcasses by dipping into, or spraying with lactic acid solutions of the appropriate concentration and pH (Snijders et al., 1985a; Woolthuis and Smulders, 1985; Smulders and Woolthuis, 1985) is a recommendable measure for substantially reducing numbers of colony forming units of psychrotrophic organisms. Besides it will virtually eliminate Enterobacteriaceae, including salmonellae, campylobacters and an occasional more infrequent pathogen (Van Netten et al., 1984).

The temperature of storage is probably the single, most important factor in affecting the types

and numbers of micro-organisms present on meat at retail outlets. Amongst the parameters of secondary significance are pH and a_W of the meat surface and mode of packaging, particularly the gaseous environment (Woolthuis et al., 1984).

The pH of themeat of cattle, sheep and pigs under optimal conditions falls to about 5.5 post mortem (vide supra); if, however, the pH remains above c. 6.0 for pshysiological reasons, Alteromonas putrefaciens can multiply and produce extremely unpleasant offodours (Gill and Newton, 1979; Seeleye and Yearbary, 1979). High pH meat tends also to spoil more rapidly because of the regular psychrotrophic association (vide supra), since they can colonize the meat surface more rapidly. Also at this higher pH certain clostridia can proliferate in the deep muscle (Ingram, 1952; Nottingham, 1960; Gill et al., 1984).

Whole carcass meat is normally stored at -2 to +5 °C under conditions of reduced relative humidity. Cuts are often further protected by an atmosphere containing 10 - 20 % carbon dioxide - either fortuitously formed as a result of vacuum packaging, or deliberately added. Under these conditons meats will keep for many weeks, provided the initial contamination is kept low (Ingram, 1949; Pierson et al., 1970; Gill and Tan, 1980). The reduced environmental humidity dries out the surface of meats, where almost all contaminating organisms are located. The only organisms colonizing meat surfaces will be psychrotrophs tolerating a_W values below c. 0.95. Under these conditions fungal spoilage dominates. It is due most often to moulds such as Thamnidium, Mucor, Penicillium and Rhizopus species, with Cladosporium herbarum, which causes "black spot" and Sprotorichum carnis, the organism of "white spot" (Gill et al., 1981). Yeasts including species of Candida, Torulopsis and Rhodotorula may also grow (Hsieh and Jay, 1984); they tend to be more tolerant than moulds of elevated CO_2 concentrations. Growth of all fungi is confined to the surface layer and, because these organisms are nonmotile, colonization will be spotty.

Under customary conditions of processing, packaging and distribution of carcass meats, deep spoilage will not occur. The reason is, that any organisms present there are usually mesophilic rather than psychrotrophic, which follows logically from their original habitat: the animals' gut.

Public Health Aspects

The most common pathogens transmitted by fresh meat include salmonellae, Campylobacter jejuni, Staph. aureus, Clostridium perfringens (Mossel, 1984) and species of parasitic worms and protozoa (Beaver et al., 1984). A recent acquisition to the latter is Cryptosporidium parvum (Gray, 1985). One of the aims of veterinary meat inspection is to detect these pathogens by carcass inspection and examination. However, there is no way of completely preventing the occurrence of the bacterial pathogens and even of the parasites in the meat supply. There is absolutely no reason why this conclusion should be difficult to accept. In spite of immense preventive efforts it has, similarly not proved possible to supply raw milk which is consistently bacteriologically safe (Mossel, 1984).

Also in principle, as emphasized before, meat examination is an act of inspection, not of control. Consequently, contamination of meat and its sequel: infection of the consumer is best avoided by intervention (Mossel and Kampelmacher, 1981; Kayser and Mossel, 1984).

This should include (Edel et al., 1973; Oosterom and Notermans, 1983): (i) at the farmer's level: acquisition of healthy young animals, design of effective housing and logistics ("all in/all out") and the use of decontaminated feed and water of drinking water quality only; (ii) during transportation to the abattoir: provision of clean lairage, allowing sufficient resting and cleanliness of animals; (iii) along the processing line: carefully avoiding spillage of gut contents during evisceration, cleaning and disinfection of knives and other equipment (Snijders et al., 1984) and terminal decontamination of carcasses by a suitable treatment (Eustace, 1980) to eliminate the few organisms of health significance which hygiene programmes cannot possibly control; (iv) at consumers' level: in spite of all precautions taken by the industry nonetheless only eating cooked meats and preventing cross contamination of cooked from raw meat - e.g. by not cutting up cooked items on surfaces contaminated previously by the meat while it was raw.

MINCED ("GROUND") MEAT

Mincing of meat distributes the micro-organisms that were originally present only at the surface throughout the product. Spoilage is therefore accelerated and shelf-life of minced meat consequently shorter than for whole raw meat. In addition, initial contamination, including the incidence of pathogenic organisms is higher, probably because lower-grade meats tend to be used and remnants also be incorporated.

In spite of the higher risk from pathogens, minced meat, for instance in the form of hamburger or steak tartare, is often eaten raw. In order to minimize the risk, colony counts at 30 °C not exceeding 10^4 g^{-1} and Enterobacteriaceae cfu's below 10^3 g^{-1} have been suggested (Mossel et al., 1975; Beumer et al., 1983). Effective protection of the consumer cannot, as emphasized repeatedly, be attained by such an inspection procedure. Unless minced meat is systematically terminally decontaminated by radicidation (Kampelmacher, 1983; Dempster, 1985; Mossel and Stegeman, 1985) or an alternative, effective procedure, consumers have to protect themselves by measures of domestic hygiene recommended before.

Low temperature storage of minced fresh meat in vacuum packs that have low permeability for oxygen and carbon dioxide prolongs shelf life markedly; vide supra. Numbers of non pigmented pseudomonads are much reduced, because of the CO_2 that accumulates and they are replaced by lactobacilli and the related psychrotrophic Gram positive bacteria of fresh meats mentioned earlier. Because the Gram positive psychrotrophs do not produce metabolites that render the product unacceptable to the consumer, shelf life is substantially prolonged when they predominate in the microbial community structure (Smulder and Woolthuis, 1985).

FROZEN BONELESS MEATS

Deboning is often done without application of sufficient refrigeration as well as under insanitary conditions. Consequently there is an increasing tendency to require certificates testifying adherence to Good Practices, and, moreover, monitor imported frozen boneless meats. Colony counts of psychrotrophic as well as mesophilic bacteria and Enterobacteriaceae, in addition to a Presence-or Absence test for salmonellae have been suggested as a means of verifying that proper hygienic and handling practices have indeed been followed during manufacture (Mossel et al., 1972). Where in spite of these precautions further intervention seems required, radicidation may be a convenient tool to assure consumer protection (Mossel and Stegeman, 1985).

In an attempt to save energy and to increase yield, a start has been made with deboning carcasses in an early post-mortem stage - so-called hot (de)boning. In the case of beef, mutton and lamb, carcasses are usually stimulated electrically before boning, to accelerate glycolysis and thus avoid excessive shortening (Williams, 1978). The lower pH values resulting from stimulation will extend the lag phase of bacteria (Walker, 1982); but, on the other hand, high carcass temperatures during cutting and the sticky nature of hot meat favour bacterial colonization (Van Logtestijn et al., 1983). The latter can be effectively controlled by (i) a high level of plant hygiene, including hot boning from the hanging carcass (Buchter, 1982; Smulders and Woolthuis, 1983); (ii) securing an uninterrupted cold chain. Hot deboned meat will then show improved storage properties because of the low initial levels of psychrotrophic spoilers (Smulders and Woolthuis, 1985). Although in essence this would allow hot boned, vacuum packaged meat to be slowly cooled, particularly fatty tissues will thus be subject to bacterial growth, unless temperature and a_w are effectively controlled (Walker, 1982).

Where hot boning is not preceded by electric stimulation, high temperature conditioning is required to avoid loss of sensory quality. This procedure may, however, lead to growth of mesophilic bacteria, including Enterobacteriaceae. This calls for strict temperature control supported by studies on the fate of mesophiles during the process (Herbert and Smith, 1980; Smulders et al., 1984).

SEMI-PRESERVED MEAT PRODUCTS

Definition and inventory of the group

From the ecological point of view, all products which keep well for a number of weeks at +2 to +5 °C, although spoiling in a few days if kept at ambient temperature, could well be classed as semi-preserved commodities. In practice, however, this term is usually limited to only two groups of food. In the field of meat technology these include, in increasing order of intrinsic stability: (i) raw ham, bacon and various types of sausages; (ii) canned cured meat products which have been given pasteurization rather than a more severe heat treatment, because the latter would affect their

organoleptic quality; this applies particularly to larger size hams and a few other meat products.

Raw meat products

In addition to ham and bacon this group comprises "British fresh sausage" and fermented sausages e.g. salami. The preservatives used in ham and bacon include sodium chloride and sodium nitrite and sometimes smoking besides. British type fresh sausage is usually preserved with sulphite only (Dowdell and Board, 1968) though this is prohibited in most European countries. Fermented products generally also contain curing salts but are moreover preserved by lactic acid, produced by Lactobacteriaceae which are responsible for the fermentation. Salami types of sausage are often also dried to some extent, in appropriate machines.

In all these products the normal putrefactive Gram negative spoilage association is inhibited due to the reduced a_w and/or preservatives. It is replaced by lactic acid bacteria and sometimes M. thermosphacta together with yeasts and moulds (Gill and Tan, 1980). Micrococci can sometimes also be found, but Staph. aureus does not usually occur in high numbers in uncooked cured products, because of inhibition resulting from competition from other components of the microbial community structure (Mossel, 1983). Staph. aureus (Daly et al., 1973) as well as salmonellae (Smith et al., 1975) have, nevertheless, occasionally caused outbreaks of food poisoning from fermented sausage, i.e. when the fermentation process has been delayed, resulting in a slower than normal drop in pH. Even though the staphylococci may subsequently die out, their enterotoxins will persist. Monitoring the final product is of little use here as elsewhere; rather should the course of fermentation and the fate of suitable marker organisms mimicking the behaviour of pathogens be followed carefully (Erichsen, 1983).

Cured, raw meat products may also present a risk of botulism. This applies particularly to commodities with a relatively high a_w, or where the curing slats are unevenly distributed, allowing germination of spores of Clostridium species at sites with elevated a_w. Home-cured hams are prone to this event (Famerée et al., 1975; Colardyn et al., 1976; Billon, 1984).

More perishable cooked meat products

These are the most popular and therefore the most important of the more perishable semi-preserved meat products. When contained in the intact casing wherein they were heat-processed, these products are quite stable under refrigeration, because they are protected by their reduced a_w, nitrite content and mostly slightly lowered pH. All bacterial endospores will survive to a certain extent the "cooking" of sausages (Mol and Timmers, 1970). In addition such products mostly contain some viable streptococci of Lancefield's group D, which are rather heat resistant so that there is little that there can be done to eliminate them entirely (Bell and De Lacey, 1984). Reference values for Dutch types of cooked sausages have been elaborated from surveys on commercially marketed brands, which were previously validated for following good manufacturing and distributing practices. They are presented in Table 1 (Mossel, 1961).

An other very popular commodity are sliced, vacuum packaged sausages. During slicing they are subject to recontamination by lactic acid forming bacteria and micrococci, that are absent immediately after cooking. This can be controlled by meticulous application of hygienic principles during slicing as well as packaging. Nonetheless these products eventually spoil due mainly to colonization by psychrotrophic micrococci, lactic acid bacteria, B. thermosphacta and streptococci of the D and N groups (Mol et al., 1974; Eagan, 1983; Gardner, 1983). Many of these organisms being catalase negative, they can produce hydrogen peroxide; this may attack the red cured-meat pigment, producing choleomyoglobin, which is a green compound. Besides surface greening in sliced meat products, this may cause core greening in whole, cooked sausages, i.e. when neating has been insufficient to completely eliminate catalase negative organisms (Niven et al., 1949; Gardner, 1983).

In cooked cured meats Staph. aureus is a definite hazard. Because the competitive flora has been eliminated by cooking, an occasional recontaminant may develop freely and form enterotoxin, unless the temperature at which the product is stored precludes this. It is for this reason that cooked sliced cured meat products should be labeled: "keep refrigerated until use" and, of course that the consumer should follow this advice (Oblinger and Kennedy, 1980).

Because of their big sales volume, sliced cooked sausages are often monitored to validate GMP and identify an occasional case of process failure. For this purpose the products are stored at 10 °C until the final date for consumption occurring on the label. Obvious spoilage, including greening, should notoccur. Testing for Enterobacteriaceae, Staph. aureus and Gram positive spoilers has been recommended; Reference Values found attainable are 10^2, 10^2 and 10^3 g^{-1} respectively (Mossel and Ratto, 1973).

Meat pies

These are fully baked products made from meat or poultry. A few spores along with an occasional fat-entrapped Micrococcus is all that should survive, and their growth should be inhibited by a properly adjusted salt and nitrite content. The low water activity of the crust is such, that no bacteria will grow there, but moulds will.

After baking, sometimes gelatin is added to pies. This has led to outbreaks of salmonellosis, because the gelatin was contaminated (Jardin, 1966). Such experience has prompted testing pies by challenging them for c. 5 days at 20-25 °C and at the end of this time checking their organoleptic properties and assessing cfu's of the most hazardous organisms : Enterobacteriaceae, Staph. aureus, C. perfringens and B. cereus (Mossel and Ratto, 1973) in the most vulnerable part, i.e. gelatin. None of these counts have been found to exceed the order 10^2 g^{-1} in pies manufactured and distributed according to GMPs.

Canned large size hams and similar products

Provided these commodities have been manufactured according to GMPs and are uninterruptedly stored as instructed on the label, i.e. at refrigeration temperature, their spoilage association consists of psychrotrophic bacteria that are relatively heat resistant. These are mainly Lancefield group D streptococci and the occasional psychrotrophic Bacillus. Canned hams have a good record with regard to the incidence of pathogenic organisms, particularly Cl. botulinum (Pivnick et al., 1969; Robinson et al., 1982). The most likely types to occur are Staph. aureus and salmonellae, their presence resulting as a rule from inadequate heat treatment and occasionally because of post-process recontamination (Buttiaux, 1953; Ingram, 1955).

For the testing of these products there is little point in examining them immediately after manufacture. Suitable conditons of time and temperature for a realistic challenge have been discussed for many years. An ecologically sound approach is similar to that which is currently used for testing pasteurized milk. The product is incubated at a temperature sufficiently high to accelerate growth of psychrotrophic bacteria, without exceeding the maximum growth temperature of the most psychrophilic types. Investigations relying on samples processed under guaranteed GMP, wherein microbial growth was studied as a function of time have shown that 17 °C is a temperature which fulfills these requirements (Mossel and Ratto, 1973). Three days at this temperature is well-tolerated by first class semi-preserved canned meat products; their contents subsequently appeared to meet the Reference Values for cooked meat products, collected in Table 1.

MORE FULLY HEAT PROCESSED MEATS PACKED IN HERMETICALLY SEALED CONTAINERS

Most containers used in food preservation are cans, but sealed pouches are also becoming more common. Both groups of heat-treated foods can be divided in two categories: those which are sterile and those that contain low numbers of viable, though dormant bacterial spores. The latter type of products are known as appertized (Goresline et al., 1964) or, less appropriately "commercially sterile" foods.

Appertized foods are those which are safe and stable, provided they are not stored at temperatures above 40 °C. Organisms which affect the safety of these packs include (i) spores of Cl. botulinum which may survive if the heat treatment is inadequate and grow out subsequently if there is insufficient intrinsic preservation; (ii) post-process recontamination usually due to Staph. aureus or Enterobacteriaceae, caused by seam faults and/or contamination of the cooling water or transportation lines. Both deficiencies occur so sporadically that monitoring of the manufactured product is of little use in protecting the consumer. Control lies in carefull supervision of the processing lines and intensifying intervention where required. Where checks are required, for instance upon importation, an extensive survey of appertized meat products manufactured according to GMP has shown, that, after challenging for a few weeks at about 30 °C aerobic and anaerobic colony counts can easily attained which (a) consist entirely of spores; and (b) never exceed the order $10\ g^{-1}$ (Mossel, 1956). Colony counts substantially over this level are not acceptable, because they point to one of the following intrinsic defects: (i) a correct, i.e. infinitesimally low spore count of the freshly processed commodity, but increasing as a result of the incubation challenge, i.e. product with insufficient stability; or (ii) an initial colony count of at least $10\ g^{-1}$ increased or not during incubation, corresponding to inadequate processing or post-process recontamination.

Fully sterilized packs should of course contain no viable organisms whatever. So-called 'thermophilic' Bacillaceae, i.e. sporing organisms with a heat resistance well over that of the Cl. botulinum group and quite often with a rather high optimum temperature of growth, are the most likely micro-organisms to survive heat-treatment. Hence monitoring concentrates on detecting these. Routine testing, should, however, also be carried out for recontaminants, particularly by examining the outside part of solid pack products. There is, once more, no point in searching directly for the few spores of thermophilic Bacillaceae that may have survived in the food or the sporadic recontaminant - these organisms should be allowed to germinate and multiply first, as indicated in the testing of appertized products. The optimal temperature for challenge testing has been subject to much debate. The cardinal temperatures for the thermophiles and the customarily encountered leakage organisms (Enterobacteriaceae, pseudomonads and micrococci) indicate that 45 °C for the former and 30 °C for the latter are suitable challenge temperatures. None should be detected in fully sterilized foods; hence the usual enrichment tests using appropriate liquid enrichment media have to be applied, obviously with the usual precautions to avoid contamination as a result of the use of an inappropriate examination technique (Mossel and Visser, 1960).

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TABLE 1

Bacteriological reference values for cooked sausages of standard Dutch quality Sampled when leaving the factory, all with a core temperature not exceeding 7 °C

	cfu g^{-1}
Enterobacteriaceae*	10
Staph. aureus*	10^2
Lancefield group D streptococci*	10^3
Clostridium spp*	10^3
Aerobic mesophilic colony count*	10^5
Anaerobic mesophilic colony count*	10^5

* In all instances the usual "three class tolerances" apply, i.e. the values aimed at, as recorded, may be exceeded by a maximum of 2 out of 10 samples, but none of these should show a cfu g^{-1} value over 10 times the reference value