Meat Storage and Product Safety

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ABSTRACT

The growth of spoilage bacteria and the survival, or growth, of specific meat-borne pathogens during chilled storage in vacuum and saturated carbon dioxide atmosphere packagings are discussed in relationship to product safety. The microbiological safety of chilled meat remains a matter of ongoing concern, since the suppression of spoilage microflora development necessary to extend the storage-life may allow pathogen growth unrestrained by competing organisms while concomitantly eliminating any organoleptic warning that excessive microbial growth may have occurred. Safety may also be compromised by the formation and accumulation of toxic microbial metabolites during storage.

Most pathogens and toxigenic microorganisms likely to be present on fresh raw meat are mesophilic and so are unable to proliferate during chilled storage. Adequate refrigeration, -1 °C, will control the growth of the principal meat-borne psychrotrophic pathogens, *Aeromonas hydrophila, Clostridium botulinum, Listeria monocytogenes* and *Yersinia enterocolitica*, in both vacuum and carbon dioxide packs. Well-controlled chilled storage is not the weak link in the food safety chain from producer to consumer. Lowering of meat-borne disease morbidity will be more effectively and economically achieved by introducing HACCP principles into home and commercial kitchens than by targeting chilled meat storage practices.

INTRODUCTION

Microbial contamination of the tissue destined to become meat is an undesirable but currently unavoidable consequence of the process by which live animals are converted into meat for human consumption. Fresh meat is a near ideal substrate for the growth of many microorganisms, providing a moist, amiable pH environment that is rich in nitrogenous nutrients, minerals and accessory growth factors. Therefore, most contaminating bacteria will be able to proliferate rapidly if conditions, particularly temperature and the gaseous environment, remain favourable. Such microbial growth will cause product spoilage and, depending on the type of organisms present, may also pose a hazard to health. Consequently, if meat is to be stored, conditions must be created that are unfavourable for the survival and growth of contaminating microorganisms, including any pathogens present.

The traditional methods of meat preservation include drying, smoking, salting, curing, pickling, fermenting, chilling, freezing and canning. These processes either make meat a less favourable medium for microbial growth by removing water, adding toxic substances, increasing acidity, or lowering temperature; or they eliminate the contaminating microflora. All these processes will, with the exception of temperature reduction, significantly change the sensory characteristics of the meat. The New Zealand and Australian export meat industries historically and currently supply world markets principally with meat whose sensory attributes have not been compromised through preservative practices. These industries do this by relying on appropriate preservative packaging technologies and temperature reduction to assure the wholesomeness of product delivered to distant markets.

In the context of supplying distant markets, storage (which includes transportation and distribution) may theoretically increase, cause no change in, or decrease the safety hazard posed by meat. Storage of meat at temperatures that retard rather than prevent microbial growth has in the recent past been perceived as a highly dangerous practice, as it potentially provides ample opportunity for compromising product safety both through the accumulation of toxic microbial metabolites and through the survival or, generally more seriously, through the proliferation of specific pathogens. Nevertheless, although no longer the cause of regulatory paranoia, the microbiological safety of chilled meat on which microbial growth is not precluded remains a matter of ongoing concern. That concern centres on the possibility that the suppression of spoilage microflora development necessary for extension of storage-life: (a) may allow unrestrained growth of pathogenic or toxigenic microorganisms, and (b) may eliminate any organoleptic warning that excessive microbial growth has occurred. The review of microbial safety that follows discusses the growth of spoilage bacteria, and the survival, or growth, of specific pathogens during chilled storage in vacuum and carbon dioxide packs. In vacuum packs, storage life is extended through the ecological changes produced by maintaining an oxygen deficient environment around the product. In saturated carbon dioxide atmosphere packs, the preservative effect is achieved by a combination of an oxygen deficient environment and the antimicrobial effects of a high partial pressure of carbon dioxide (Gill, 1989).

INTOXICATIONS

Intoxication food poisonings result from the ingestion of food in which toxic microbial metabolites have accumulated as a consequence of microbial growth in that food. Toxic metabolites may either be breakdown products released by the "non-malicious" metabolism of particular precursor molecules present in foods by an otherwise benign spoilage microflora; or they may be "designer compounds", often secondary metabolites, produced by specific microorganisms through dedicated pathways. The first group of metabolites is associated with spoilage onset and the loss of wholesomeness while the second group, microbial toxins, is associated with the growth of specific pathogens (Table 1).

Table 1.
Growth characteristics (ICMSF, 1990 and other literature data) of intoxicating microorganisms and their toxic microbial metabolites that compromise the safety of meat and meat products.

Toxic metabolite		Microorganism					
Identity	Heat sensitivity	Identity	Oxygen requirement	Temperature requirement	pH requirement	CO_2 sensitivity	Minimum growth temperature
Tyramine	Stable	Lactic acid bacteria	Anaerobe	Psychrotrophic or mesophilic	None	Low	-1.5°C†
		Enterobacteriaceae	Facultative	Psychrotrophic or mesophilic	Little anaerobic growth below 5.8	Moderate	-1.0°C†
Enterotoxin (diarrhoeal)	Labile (56°C)*	*Bacillus cereus*	Facultative	Mesophilic	Little growth below 5.0	Moderate	7.0°C
Neurotoxin	Labile (85°C)	*Clostridium botulinum*	Anaerobe	Mesophilic or psychrotrophic	No growth below 4.8	Low	3.3°C†
Enterotoxin	Labile (60°C)	*Clostridium perfringens*	Anaerobe	Mesophilic	No growth below 5.0	Low	6.0°C (severely restricted below 15°C)
Enterotoxin	Stable	*Staphylococcus aureus*	Facultative	Mesophilic	No growth below 4.0	Moderate	7.0°C (no toxin production below 10°C)

* Temperature inactivating the toxin in 5 minutes
† Strain and substrate dependent

Loss of wholesomeness

The onset of putrefactive spoilage is coincident with the subjective assessment of a "loss of wholesomeness". Historically, putrefaction *per se* and food poisoning were erroneously causatively linked, with this misapprehension becoming widely articulated after Francesco Selmi, an Italian toxicologist, introduced the term "ptomaine poisoning" in the 1870s. Derivation of the word "ptomaine" from the Greek *ptoma* meaning corpse, was impeccable, as decomposing carcasses are indeed rich sources of the basic compounds that today are collectively known as the biogenic amines. In 1884 Vaughan isolated a substance from cheese that had produced poisoning symptoms. This substance was closely allied to the meat ptomaines, and was given the name "tyrotoxicon". Tyrotoxicon notwithstanding, the major piece of circumstantial evidence advanced in the first quarter of the present century to refute the link between putrefaction and toxigenesis was the safe consumption of the wholesome but olfactorily challenged cheese known as Limburger. Epicureans may, however, be exempt from the mid-century opinion that *"anyone eating a putrefied food is negligent in a legal sense and erratic in an esthetic way"* (Jensen, 1942).

The complete rejection of the concept of ptomaine poisoning (Savage, 1921) may have been premature in the light of what is now known about the health hazard posed by the ingestion of some biogenic amines. The symptoms of amine toxicity include nausea, sweating, migraine and hyper- or hypo-tension. The most toxic biogenic amines, histamine and tyramine, are associated with scombroid fish poisoning and cheese poisoning, respectively. In normal, healthy individuals, biogenic amines are rapidly degraded on ingestion by the enzymes monoamine oxidase and diamine oxidase. Consequently, for these individuals to experience amine intoxication requires the ingestion of a large amount of a toxic amine, as would be the case with scombroid poisoning. For more modest doses to cause intoxication a genetic, or pharmacologically induced, deficiency of the natural mechanisms for the catabolism of ingested amines is needed.

Recent studies (Smith *et al.*, 1993; Krizek *et al.*, 1995) have shown that potentially toxic levels of tyramine can accumulate in vacuum packaged beef during prolonged storage at between -2°C and 2°C. Ongoing studies in New Zealand (S. Buncic, pers. comm.) have demonstrated that decarboxylating enzyme systems capable of converting the amino acid tyrosine to tyramine are present in many of the putatively benign lactic acid bacteria that typically predominate in the spoilage microflora developing on vacuum packaged meat during chilled storage. The limited literature data suggest that amines produced on chilled meat are generally thermostable and diffuse from the surface, where they are formed, into deep tissue and are therefore not completely removed by washing. In other words, biogenic amines once formed cannot be eliminated by judicious trimming, washing or cooking. It is, however, pertinent to emphasise here that any adverse effects of biogenic amine ingestion would be more reasonably attributed to the red wine taken with, and the cheese and chocolate eaten after, a steak, than to the steak itself.

Microbial toxins

The temperature requirements for growth and toxin production by *Bacillus cereus, Clostridium perfringens* and *Staphylococcus aureus* effectively preclude their further consideration in respect to prejudicing the safety of chilled meat. Some discussion is, nevertheless warranted, as raw meat and poultry not infrequently serve as the immediate source of contamination of cooked product. Furthermore, both *B. cereus* and *Cl. perfringens* are likely to survive the cooking processes normally applied to meats and thereby can threaten the safety of cooked products. *Clostridium perfringens* does not, however, produce a classical intoxication, as the toxin is not present in the ingested food. Instead, high numbers of viable cells are ingested and those surviving passage through the stomach typically sporulate and produce toxin on reaching the intestine.

The heat resistant *B. cereus* and *Cl. perfringens* spores survive cooking. If the cooked meats or meat products are then either inadequately cooled or are held warm rather than hot, these spores germinate and grow to large numbers. Both these types of poisoning are readily avoidable if the

product temperature is maintained above 60°C or below 4°C. Thus, during either cooling or heating, product should rapidly pass through the intermediate range of temperatures that are conducive to pathogen growth. *Bacillus cereus* produces two enterotoxins, emetic and diarrhoeal. The former tends to be associated with carbohydrate-rich foods such as cooked rice and pasta whereas the diarrhoeal syndrome is associated with a wide range of foods including cooked meats. The diarrhoeal syndrome is characterised by abdominal cramps and profuse diarrhoea with watery stools and rectal tenesmus, with symptoms commencing 8 to 16 hours after ingestion of the offending food. Recovery is rapid, usually within 24 hours. Perfringens poisoning, on the other hand, is characterised by abdominal pain, diarrhoea and nausea with recovery occurring within one to two days.

Staphylococcus aureus food poisoning most frequently results from post-processing contamination of foods that are then held at inappropriate temperatures for significant periods before consumption. The source of *S. aureus* contamination is usually traced to food handlers. This type of poisoning is not infrequently associated with large social events such as weddings, where the quantity of food required may vastly exceed the refrigerated storage capacity of the catering establishment. Staphylococcal food poisoning is self-limiting, usually lasting less than 24 hours. Characteristically the symptoms, nausea, retching and vomiting, sometimes accompanied by diarrhoea, appear two to four hours after consumption of the contaminated food. Although meat and meat products are often implicated in outbreaks of staphylococcal food poisoning, the presence of *S. aureus* on raw meat is of little direct consequence since the organism is, because of its generally poor competitive performance, unable to grow on naturally contaminated substrates (McCoy and Farber, 1966). Consequently, *S. aureus* is an inappropriate indicator organism for raw meat safety but is highly appropriate for cooked meats and meat products.

Clostridium botulinum, because of the existence of psychrotrophic strains capable of growth at chill temperatures, remains a matter of regulatory concern, especially in respect to the use of anaerobic modified atmospheres for the packaging of fish. Meat products that have been implicated in botulism outbreaks are frequently inadequately processed canned goods stored at room temperature. Within 12 to 72 hours of ingestion of the botulism toxin, symptoms of neurological disturbance, including muscular weakness; double vision; speech impairment; headaches; dizziness and dryness of the mouth, throat and skin become evident. Unlike other food poisonings, intestinal symptoms of botulism are characterised by severe constipation accompanied by nausea and vomiting.

In the event of gross temperature abuse, where product temperature no longer controls *Cl. botulinum* growth, the safety of anaerobic modified atmosphere packs of cooked or partially cooked foods, e.g. *sous vide* packs, although safe when stored below 5°C, may be compromised. To the best of the author's knowledge no cases of botulism have been associated with chilled raw vacuum or carbon dioxide packaged meats. However, the increasing number of reported incidences of spoilage of vacuum packed raw meats caused by psychrotrophic clostridial species such as *Cl. laramie*, *Cl. estertheticum* and *Cl. algidicarnis* may be a matter of concern. Although, to date, none of these organisms has been shown to be toxigenic, the relatively close phylogenetic relationship between these organisms and other members of the butyric group (Collins *et al*, 1994), which includes *Cl. botulinum* strains, argues against complacency.

INFECTION

Unlike the true intoxications discussed in the previous section, food-borne infection requires the ingestion not of preformed toxins but of viable cells able to transiently colonise or otherwise become established within the host's intestinal tract. The bacteria most commonly associated with meat-borne infections are given in Table 2. This group of bacteria can be classified with respect to the safety of chilled meat as being mesophiles unable to grow, or psychrotrophs able to proliferate at commercial chilled storage temperatures.

Mesophilic pathogens

As normal chilled storage temperatures are below the minimum for growth of these organisms, carbon dioxide packaging should not afford any safety advantage over vacuum packaging. However, where temperature abuse during storage, transport or distribution may prejudice product safety, the elevation of minimum growth temperatures associated with carbon dioxide packaging atmospheres (Gill & De Lacy, 1991) may afford an extended margin of safety. The use of carbon dioxide packaging should not be regarded, or advocated, as an acceptable solution to inadequate temperature control, however.

Notwithstanding in inability of mesophilic enteropathogens to proliferate on chilled meat salmonellosis and campylobacteriosis vie worldwide for the position as the most common foodborne disease. Clinical symptoms of campylobacteriosis appear after a two to 11 day incubation period; firstly, as an influenza-like prodromal condition followed by intestinal envolvement producing abdominal pain, nausea, vomiting and later diarrhoea. Diarrhoea may be either secretory; profuse watery bile stained and odiferous; or dysentery-like; blood stained and indicative of colonic infection. Salmonellosis has a generally shorter incubation period of between 12 and 36 hours, but may last for several days before enteric infection becomes manifest through abdominal pain, vomiting and diarrhoea. *Escherichia coli* as an enteropathogen has progressed from being a non-specific cause of 'travellers diarrhoea' to being recognised as a specific and potentially highly virulent pathogen. Today, five types of diarrhoea-producing strains of *E. coli* are recognized. Of these the enterohaemorrhagic (EHEC) strains, in particular, serotype O157:H7 have a strong epidemiological link with undercooked beef. Unlike salmonellosis and campylobacteriosis the infective dose of *E. coli* O157:H7 is typically extremely low which creates problems for its effective control during meat processing.

As any laboratory microbiologist can attest, subcultures made from refrigerated stock cultures initially appear to grow more slowly than those made from actively growing cultures. This phenomenon is generally attributed to loss of viability and to an extended lag phase in those cells that remain viable. It is not unreasonable to speculate that a similar reluctance towards growth might afflict mesophilic pathogens present on long

stored chilled meat. Continuing this line of argument, is it not then reasonable to conclude that the consumption of, for example, raw meat contaminated with *Salmonella* or *Campylobacter* should be safe, provided the time between removal from refrigerated storage and elimination *per anum* does not exceed the lag phase resolution? This concept, which is of course seriously flawed, would certainly give a new meaning to the term "Fast Food".

Table 2.
Growth characteristics (ICMSF, 1996 and other literature data) of bacteria associated with meat-borne infection.

Organism	Oxygen requirement	pH requirement	CO_2 sensitivity	Minimum growth temperature
Campylobacter spp.	Microaerophilic	Sensitive to pH below 6.5	Low	25 to 30°C*
Escherichia coli	Facultative	No anaerobic growth below 5.8	Moderate	7°C*
Salmonella spp.	Facultative	Sensitive to pH below 4.5	Moderate	5 to 7°C*
Aeromonas hydrophila	Facultative	Sensitive to pH below 6.0	High	0 to 4°C*†
Listeria monocytogenes	Facultative	Sensitive to pH below 5.0	Moderate	0 to 4°C*†
Yersinia enterocolitica	Facultative	No anaerobic growth below 5.8	High	0 to 4°C*†

* Strain and substrate dependent
† Growth below 0°C has been reported

The hazard posed by the survival of mesophilic pathogens, e.g. *E. coli* O157:H7, on long stored chilled meat cannot be ignored and should not be trivialised. However in theory, it can be readily managed through the application of effective food hygiene and cooking practices. While the exporter is not exempted from the responsibility of supplying safe product, that is product which is perhaps not pathogen-free, but certainly product that complies with current meat hygiene standards, including appropriate packaging and temperature control; the critical food safety control point is to be found in the domestic or commercial kitchen in which that meat is prepared and cooked prior to consumption. Appropriate control measures include prevention of cross-contamination between raw meat and other foods, particularly cooked foods or foods to be eaten with no cooking, adequate cooking of the meat itself and appropriate post-cooking holding temperatures for cooked products.

Psychrotrophic pathogens

The growth characteristics of the three psychrotrophic pathogens *A. hydrophilia, L. monocytogenes* and *Y. enterocolitica* under conditions of chilled storage are shown in Table 2. Adequate refrigeration will control the growth of all three psychrotrophic pathogens; however, other variables such as substrate pH and gaseous environment markedly influence the minimum temperature at which growth can occur. As a general rule, minimum growth temperature increases as substrate pH falls or carbon dioxide concentration in the packaging atmosphere increases.

Aeromonas hydrophila

Aeromonas hydrophila and other motile aeromonads are recognised pathogens of fish, amphibians and reptiles. However, their role as human enteropathogens remains equivocal. To date, the problem of linking *Aeromonas* to diarrhoeal disease lies in the failure of feeding trials using human volunteers to confirm epidemiological links. Irrespective of the doubt cast on epidemiological findings, cholera-like and dysentery-like illnesses are reported to occur in about 75% and 25%, respectively, of *Aeromonas*-associated gastrointestinal infections.

Notwithstanding its suspect pedigree as an enteric pathogen, *A. hydrophila* growth is similar under aerobic and anaerobic conditions. Therefore, vacuum packaging provides no safeguard against the growth of this organism. However, depending on storage temperature, growth is retarded or numbers fall in carbon dioxide packs. For example, during storage at -1.5°C, numbers of *A. hydrophila* declined on roast beef in carbon dioxide packs but increased in vacuum packs (Hudson *et al.*, 1994). At 3°C, *A. hydrophila* grew in both packagings but growth was considerably slower under carbon dioxide.

Listeria monocytogenes

As with *A. hydrophila*, vacuum packaging provides no safeguard against the growth of this microorganism. The use of saturated carbon dioxide packaging, however, confers a considerable advantage over vacuum packaging in that growth is delayed, or numbers may decline during chilled storage (Hudson *et al.*, 1994). Whether growth is delayed or numbers fall appears to be determined by an interaction between substrate pH and storage temperature. Growth is favoured by high temperature and neutral pH. Gill and Reichel (1989) found that *L. monocytogenes* grew on high ultimate pH (pH>6.0) beef in carbon dioxide packs during storage at 10°C but not at 5°C. Avery *et al.* (1994) report that on normal pH beef (pH 5.3 - 5.5), not only did *L. monocytogenes* fail to grow in saturated carbon dioxide packs at 10°C, but numbers actually decreased. Not satisfied with this result, some safety officials then postulated that removal from a carbon dioxide atmosphere, as occurs during preparation for retail display, could trigger explosive proliferation of those *L. monocytogenes* cells that survive chilled storage. Work done to investigate this claim (Avery *et* al., 1995) showed that the inhibitory effect of carbon dioxide packaging or *L. monocytogenes* growth persists during retail display after removal of the meat from the carbon dioxide packaging. It should be noted that in respect to prolonged storage, both 5°C and 10°C are abusive temperatures and reflect experimental conditions rather than usual commercial practice. Under a temperature regime compatible with a prolonged storage life, ideally -1.5 ± 0.5°C, *L. monocytogenes* would not grow on either high or normal pH beef packaged under carbon dioxide.

Under storage conditions where growth does not occur, the pathogenicity of *L. monocytogenes* for chick embryos may be attenuated (Buncic *et al.*, 1996). Conversely, prolonged chilled storage where growth of *L. monocytogenes* occurs may result in enhanced intravenous but not enhanced intragastric pathogenicity for mice (Czuprynski *et al.*, 1989). Animal studies (Schlech *et al.*, 1993) suggest that gastric acidity provides an effective barrier to small inocula of ingested *L. monocytogenes*. Prolonged chilled storage of raw meats therefore appears unlikely to increase the safety hazard posed by *Listeria* and any such increase is of concern only if that meat were to be consumed raw, e.g. as steak tartare. The major vehicles of meat-borne listeriosis are chilled ready-to-eat products that are either lightly processed, e.g. pâté, which allows *L. monocytogenes* survival, or adequately cooked products that become recontaminated prior to packaging. In healthy people listeriosis does not develop beyond a mild influenza-like illness characterised by slight fever, malaise and diarrhoea. However, in susceptible patients the organism becomes widely disseminated throughout the body, producing a range of disease conditions including abortion, meningitis and septicaemia. In the case of at-risk individuals, e.g. pregnant women, *Listeria*-induced abortion is most effectively prevented by avoidance of high risk foods.

Yersinia enterocolitica

Yersinia enterocolitica is a typical member of the *Enterobacteriaceae* and as such its growth under anaerobic conditions is sensitive to substrate pH. Growth on cooked beef, pH 5.8, in vacuum packs was reduced compared to that in aerobic packs at both 5 and 10°C (Hudson and Mott 1993). At 3°C growth on sliced roast beef in carbon dioxide packs was retarded compared to that occurring in vacuum packs, and at -1.5°C numbers declined under carbon dioxide but increased in vacuum packs (Hudson *et al.*, 1994).

Symptoms of *Y. enterocolitica* infection vary considerably according to strain, dose, and the age and health of the host. The predominant symptoms are abdominal pain and diarrhoea. The pain associated with yersinial gastroenteritis can be so severe as to be mistaken for appendicitis resulting in unnecessary surgical intervention. Despite the frequent association of *Y. enterocolitica* with pigs, the only retail pork cuts from which the human pathogenic serovar 03 has been consistently isolated is fresh pork tongue. Consequently, lightly processed pork products derived from head meats may pose a direct hazard in respect to yersiniosis. Post-processing contamination, perhaps through contaminated water on work surfaces, is the most likely route of *Y. enterocolitica* introduction into meat products. However, the food safety significance of the presence of these predominantly environmental serovars in food products remains uncertain.

Growth of psychrotrophic pathogens on meat during chilled storage, except in the case of ready-to-eat products, does not create a health hazard that cannot be managed by effective kitchen hygiene practices. Furthermore, the extension of chilled storage life achieved through the use of carbon dioxide packaging over that attained by vacuum packaging does not produce a concomitant increase in health hazard in respect to either mesophilic or psychrotrophic pathogens. Well-controlled chilled storage in which product temperature is maintained between -1°C and 0°C is clearly not the weak link in the food safety chain between producer and consumer.

CONCLUSION

None of today's inspection and meat processing systems can assure the absence of either microorganisms in general, or pathogens in particular, from fresh meat (Bell, 1993). Therefore, raw meat must be assumed to be carrying both spoilage and pathogenic microorganisms and treated accordingly. The consumer must accept the major portion of the responsibility for safe food preparation and eating practices. With few exceptions raw fresh meats receive a bactericidal treatment, cooking, before consumption. Therefore, why, we must ask, do meat and meat products hold such unenviably high placings in the "league tables" of food poisoning vectors?

Analysis of food-borne illness, including protozoal and parasitic diseases and infestations which have not been considered in this review, attributable to meat and poultry or products manufactured from them shows that most illness is associated with one or more of the following: (a) consumption of raw product, (b) consumption of undercooked product, (c) inadequate cooling or warm holding of cooked product, and (d) inappropriate handling of product allowing cross-contamination or post cooking recontamination. This being the case, meat-borne disease morbidity would be more significantly reduced by the introduction of Hazard Analysis Critical Control Point (HACCP) programmes into the home and catering establishments than by targeting chilled meat storage practices. Refrigeration and packaging technology contribute significantly to the safety of chilled meat, but the ultimate responsibility must rest with the consumer, who, it appears, cannot be mandated to practice "safe eating", as the continued demand for Fugu and rare hamburger attests.

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