

Announcements for the Percy Gitelman Memorial Scholarship will soon be available. This scholarship is provided by the CMSA to encourage and assist students pursuing graduate studies in meat science.

The CMSA website (<http://cmsa.ca/>) is being maintained very effectively by Gabriel Piette and Luc Jacques. Through the Website the CMSA is seen, not only by members, but by the rest of the world. It has a very different function from the Newsletter, which is only circulated to members. The website will become our most powerful communication tool, and over the next few years, much attention will be given to its mandate.

I leave you a parting thought. Does the speed at which we move in circles have any bearing on the time we require to get from one point to another? Perhaps we should take a lesson from Ghandi who suggested that "There is more to life than increasing its speed."

I sincerely wish you all a very enjoyable, safe, productive summer. And "productive" does include family and friends.

Sincerely,

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UNDER THE MICROSCOPE:

MEAT SAFETY: CHALLENGES FOR THE FUTURE

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Biography



Linda Saucier is currently a junior scientist at the Food Research and Development Center of the Ministry of Agriculture and Agri-Food Canada. Since

her appointment in January 1997, she has been developing a research program on microbial ecology and microbial quality of meat. Prior to her Ph D study, she worked for three years in the dairy industry, with Group Lactel, as supervisor of the quality control laboratory and as food scientist in research and development.

This article is the winning essay for the 1999 International Meat Secretariat Prize for meat science and technology and human nutrition. The Prize is worth \$10,000 and the winner is expected to give a lecture based on the essay to the World Meat Congress, which met this year in Dublin, Ireland in May. The next competition will be for the millennium meeting to be held in Brazil. Details of the subject for the Prize and the rules of entry and text format may be obtained by e-mailing IMS.Prize@dia1.pipex.com or consulting the website: <http://www.meat-ims.org>

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MEAT SAFETY: CHALLENGES FOR THE FUTURE

Summary

Meat and meat products are part of the daily diet of many individuals and constitute an important economic sector in the agricultural activities of several countries. Meat safety can be challenged in various ways including chemical residues (e.g., pesticides, antibiotics), disease in animals (e.g., transmissible spongiform encephalopathy) but, most importantly, by microbial contamination with pathogenic microbes or their toxins. Microorganisms evolve constantly and their capacity to adapt to the inhibitory processes used to control them make meat safety a perpetual issue. On the verge of the new millennium, with the world population still rising and with the increasing market globalization where meats will travel greater distances and for longer time periods, a concerted effort among regulatory agencies, scientists and industry is necessary in order to develop novel alternatives for food preservation and to identify new investigation and detection methods for causative agents of meatborne illnesses.

With all the elaborate technologies surrounding us, one would assume that science would have by now conquered human food plagues such as famine, malnutrition, food poisoning and so forth. Unfortunately, much remains to be done to overcome those scourges. Despite all our efforts to develop efficient surveillance programs, more than half the outbreaks reported remain of unknown etiology. This reflects our perpetual needs to seek better investigative and experimental methods to identify the causative agents of foodborne diseases. The World Health Organization statistics indicate that foodborne diseases may be 300 to 350

times more frequent than the reported cases reveal. Estimates suggest that hundreds of millions of people worldwide suffer from diseases contracted through food consumption. Developing countries suffer the most, but the incidence of foodborne diseases in a number of industrialized countries has increased in recent years. No less than five to ten percent of the population is believed to be affected annually, generating substantial economic losses. The safety and preservation of our food supply were, are, and always will be a crucial concern in order to feed the growing population adequately.

Why eat meat in the first place?

The most accessible food supply available to our ancestor was of plant origin. Gathering and plant picking were rather secure and less risky ways to obtain food compared to hunting. Nonetheless, hunting wild animals must have provided some advantages that outweighed the risks of injury. A more carnivorous diet was better suited to fulfill the high energy demands of that particular life style; it was, back then, a matter of survival. However, some may argue that an ecological imbalance due to the over-utilization of the available resources brought on the need to search for new alternatives.

Domestication of herbivores permitted mankind to rely on a more sustainable and readily available source of meat. The statistics available at the FAO indicate that animal farming generates over two billions metric tons of meat in terms of dressed carcass weight worldwide each year. Whether people decide to eat meat, or not, for health, price or cultural reasons, meat production remains a huge economical sector of activity that is here to stay.

The challenge of meat preservation

Meat and meat products are an excellent source of protein. One hundred grams of lean meat can provide up to half our daily protein requirement. It is also a good source of vitamin B-complex and minerals, especially iron, which is of good bioavailability in meat (1). Unfortunately, bacteria, yeasts and mold also find a suitable substrate for growth in meat; that is why it is highly perishable. Foodborne diseases of known etiology are mostly of bacterial origin and meat has been implicated in roughly one third of the foodborne outbreaks in North America. The pathogenic microorganisms representing the greatest risk with meat- and poultry- borne diseases are *Salmonella* spp., *Campylobacter* spp., verotoxigenic *Escherichia coli*, *Listeria monocytogenes* and *Toxoplasma gondii* (2).

Although the edible tissues of a healthy animal are either sterile or contain very low microbial populations (1), the surface of carcasses gets contaminated by organisms originating from the hide/skin of the animal, gut content, workers' hands and the slaughter environment (3). Fresh meat is a good culture medium allowing a broad range of microorganisms to develop because nutrients and moisture are present in sufficient quantity to support microbial growth, pH is close to neutral and the redox potential at the surface is higher than at the core. Low molecular-weight compounds (e.g., glucose, glycogen, amino acids) are readily available and can sustain the growth of indigenous flora up to 10^9 cells per gram.

Microbial evolution and adaptation

We are facing an everlasting challenge to control spoilage and pathogenic microflora. Microbes can modify their genetic pool either by spontaneous mutation or by acquiring foreign DNA fragments. Mutations occur

either naturally (e.g., mistakes made during DNA replication) or after being exposed to mutagens. Heterologous genes can be transferred through natural processes known as transformation (uptake of naked DNA in solution), conjugation (transfer of genes through sex pili) and transduction (transfer of genes after viral infection). Furthermore, we now know that resistance towards an inhibitory treatment, such as heat or acid, can be increased by a prior exposition to sublethal conditions. Stress proteins induced by a sublethal heat treatment have been identified in many microbial genera. The response associated with heat shock can also be induced by other factors (e.g., ethanol, UV, inhibitors of DNA gyrase) and many more proteins induced by various stresses are now identified. **The role, mode of action and impact of stress proteins on the efficiency of antimicrobial systems currently used by the food industries are still poorly understood.** Hence, the microbial world is in constant evolution, with basically infinite adaptive capacities towards the conditions that we will use to control it.

Intrinsic and extrinsic factors that inhibit microbial growth

It did not take long before *Homo erectus*, the pre-Neanderthal man (800 000 BC), realized that meat and fish preserved better when they were cooked. It soon became obvious that other means of preservation were necessary to extend the shelf life of food in order to survive periods of scarcity. Today, because of market globalization, food commodities may have to travel greater distances and for a longer period. New methods of food preservation must be investigated in order to improve product shelf life. Many intrinsic factors (naturally present in food; e.g., nature of constituents, pH and buffering capacity, redox potential, etc.) and extrinsic factors (applied on food systems; e.g., temperature,

preservatives, modified atmosphere, etc.) influence microbial growth to various extents. The use of these extrinsic factors in meat processing, especially in combination (i.e., hurdles technology), has increased meat shelf life from a few days to a few months in some instances.

Preservation using cold temperature

Microbial population of red meat carcasses can reach up to 10^4 bacteria/cm² after dressing. The initial microflora is mainly mesophilic. Under refrigeration temperature a psychrotrophic flora develops and prevails (3). The presence of cold-growing toxigenic strains of *Bacillus cereus*, *Listeria monocytogenes*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Escherichia coli* O157:H7 and *Clostridium botulinum* in food has raised considerable health concern.

L. monocytogenes and other *Listeria* are widespread in nature and their association with livestock makes their presence unavoidable in raw meat. Listeriosis occurs mainly in infants, the elderly and the immunocompromised, where mortality rates in these groups can reach 30%. *L. monocytogenes* is a recognized cause of meningitis and infection of the fetus that can lead to abortion. Proper cooking and handling seems to be the best way to control it.

B. cereus is found on the surface of meat but most probably due to soil contamination. Although swine are the principal reservoir of *Y. enterocolitica*, some cases are directly related to handling and consumption of pork (4). *A. hydrophila* is also associated with pork. Although it was originally considered as an opportunistic pathogen in immunocompromised individuals, we now know that it can cause gastroenteritis in normal hosts as well (4).

Preservation by heat treatment

C. botulinum got its name from the Latin word botulus, which mean sausage, the food it was first scientifically associated with in cases of foodborne illnesses. Its toxin is highly potent, a nanogram of toxin can be lethal to humans. The toxin is heat labile, but the spores are heat resistant. The spores of this obligate anaerobe will develop on food minimally or unprocessed by heat where the concentration of oxygen in the packaging atmosphere is limited.

Not all *E. coli* have been created equal. Most strains are innocuous, but some are pathogenic. In quality control, this organism is used as an indicator of the hygiene level. One strain in particular, the verotoxigenic (or enterohemorrhagic) O157:H7, is especially virulent. As few as ten cells can cause gastrointestinal disorder and, if not promptly diagnosed and treated, the infection can lead to severe renal problems (hemolytic uremic syndrome, thrombotic thrombocytopenic purpura), especially in children under the age of five. The disease is commonly referred to as the "hamburger disease" because it was often associated with ground meat, although fermented meats, cabbage, unpasteurized milk and cheese, water and apple cider have also been incriminated. Cattle are the main known reservoirs, but the intestines of other animals can be contaminated with this strain or other enterohemorrhagic ones. Detection of enterohemorrhagic *E. coli* in healthy farm animals and meats is sporadic and variable. During the slaughter processes, the surface of the meat gets contaminated with the organism, which spreads into the mass during grinding. The organism is psychrotrophic and quite acid tolerant but not particularly heat tolerant hence thorough cooking is the best way to control it; an internal temperature of 71 °C (160 °F) is recommended for ground meat. As simple as it may sound, it involves

the challenging task of educating the public on proper handling and cooking because ground meat is most often bought raw and processed at home. Because food preparation is a daily task, the level of confidence in its practice acquired through generations is high and habits are difficult and long to change.

Several alternatives are under investigation to reduce the incidence of *E. coli* O157:H7 in meat. Vaccines, competitive exclusion using nonpathogenic gut flora of healthy individuals, and probiotic preparations (i.e., substances that affect physiological functions to improve health status) are studied at the animal production level to modify the cattle's gut flora. Although consumers have been reluctant to accept the use of irradiation as a method of food preservation, this might change considering the severity of the disease caused by enterohemorrhagic *E. coli*, especially in children. Irradiation can reduce the incidence of the organism in the product to zero or near zero but limitations due to fat oxidation are to be expected. Lower doses would not completely eradicate the organism and hence the risk to develop an infection will be reduced but not eliminated, the necessity of thorough cooking will therefore remain. Equipment for carcass pasteurization has recently been introduced in processing plants. Meat pasteurization follows almost the same path that milk pasteurization has many decades ago. Pasteurization has allowed the control of several deadly diseases in milk including diphtheria, scarlet fever, tuberculosis and salmonellosis (4). The difference between the two is that milk is bottled to reduce post-pasteurization contamination but no such barrier yet exists for pasteurized carcasses. Although the reduction of total microflora reduces the risk of exposition to pathogenic organisms, it also provides an environment devoid of competition for any organism contaminating the meat surface after treatment. We are now

at a stage where we should look for ways to selectively eliminate the undesirable organisms and leave the desirable flora to develop, a strategy which could be referred to as "flora management".

Modified atmosphere packaging

In the meat industry, modified atmosphere packaging (MAP) is used to extend the shelf life of meats. Because of a better growth rate under aerobic conditions, *Pseudomonas* spp. prevail and constitute up to 50 to 90% of the overall microbial population. *Enterobacteriaceae* prevail under conditions of poor refrigeration (10 °C) and spoil the meat (3). When meat is placed in an anaerobic environment, a lactic microflora develops because they are more tolerant to carbon dioxide (CO₂) than the pseudomonads and the *Enterobacteriaceae*. CO₂ affects microbial growth by extending the lag phase and increasing the generation time. In vacuum packaged meat, the residual O₂ is used up through muscle and microbial respiration and a maximum concentration of 30% CO₂ can be reached. A minimum of 20% CO₂ is necessary for microbial growth inhibition (5). The extended storage life of meat under anaerobic conditions is due to the fact that the LAB that grow on meat cause spoilage only after maximum populations are reached, whereas the aerobic spoilage bacteria cause putrefactive odors earlier in the growth cycle. The defect caused by LAB is described as "souring" which is less offensive than the putrefaction developed aerobically (3). The prevalence of LAB exerts an inhibitory pressure on several foodborne pathogens and improves the safety of the product. However, the safety of vacuumed and MAP fresh meat was questioned when it was shown that *Clostridium* species could represent a significant portion of the prevailing microflora and when toxin production by *C. botulinum* was observed on

irradiated fresh pork packaged in MAP and stored at temperature of 15 °C (5).

Use of chemical preservatives

Salt has been used for both preservation and as a flavoring agent since ancient times. As a preservative, it reduces water activity. Nitrate was originally used in the Middle Age in the form of saltpeter and is transformed to nitrite and nitric oxide by naturally occurring bacteria. Nitrites and nitrates are ubiquitous in nature and are found in soil, water and food (e.g., cured meats, baked goods and cereals, vegetables). From a microbiological standpoint, nitrite is added to meat for its antimicrobial properties mainly to control the outgrowth of spores, especially of *C. botulinum*. However, the formation of N-nitrosamine from the reaction of nitrite with secondary or tertiary amines has raised public health concern when it was demonstrated that they were carcinogen and caused toxic hepatitis in animals. Fortunately, ascorbic acid, sodium ascorbate, sodium erythorbate and alpha-tocopherol have been recognized as inhibitors of nitrosamine formation. The use of these inhibitors and the optimization of nitrite concentration have greatly contributed to reduce the nitrosamine concentration in cured meats to a few parts per billion. The multitude of factors that influence the efficiency of nitrite and the formation of nitrosamines (e.g., pH, cooking methods) are forcing the industry to look at each product application as a case by case scenario (1,6).

Today, consumers demand foods that are minimally processed, as "natural" as possible, and yet still convenient to use. It will be difficult to completely eliminate the use of nitrite as there is no known substitute for it as a curing agent for meat. This small molecule can, at a relatively low concentration, stabilize colour, produce flavour, change texture, and act as antioxidant and

preservative. The use of nitrite requires a thorough risk-benefit analysis where exposure to the potential hazard of nitrosamine should be limited and the concentration used should not be reduced to the extent that protection against botulism is compromised. Nonetheless, this demand for fewer chemical additives in foods has put pressure on the industry and the scientific community to seek new alternatives. "Biopreservatives", such as enzymes (e.g., lysozyme), essential oils (e.g., horse radish) and bacteriocins (antimicrobial peptides produced by bacteria) have been investigated and a few are commercially available. Nisin, a bacteriocin produced by *Lactococcus lactis* subsp. *lactis*, like nitrite, can inhibit the outgrowth of *C. botulinum* spores. Unfortunately, it has no curing effect and is insoluble at high pH, which limits its application in meat systems.

Threats of nonmicrobial origins

Bovine spongiform encephalopathy (BSE): when a protein becomes our worst enemy

BSE, also referred to as Mad Cow Disease, is a transmissible fatal neurodegenerative disease that results in brain damage and death. Similar diseases also exist in other species such as scrapie in sheep and, kuru and Creutzfeld-Jakob disease (CJD) in human. The epidemic of BSE in the United Kingdom (UK) started in 1986 and, by August 1997, more than 168 578 cases were reported and more than a million cattle might have been infected. Recognition of the emergence of a new variant of the CJD (vCJD) roughly ten years, after the BSE epidemic started, could not be a mere coincidence considering the low probability of the appearance of a new variant for this particular type of disease (7). In October of 1997, two different teams with two different approaches provided evidence that the vCJD is caused by the same agent responsible for BSE. Hence, the disease is

not species specific as it was originally thought (7). The most probable hypothesis is that cattle acquired the disease through consumption of contaminated meat and bone meal. Since the 1988 ban preventing ruminant bone and meat meal to be fed to cattle, the incidence of the disease has been decreasing in the UK. The infectious agent is particularly resistant to all kinds of treatment and changes in the rendering process are considered among the factors responsible for the epidemic.

There are two hypotheses regarding the nature of the infectious agent. The 'protein- only' or 'prion' hypothesis stipulates that it is the conformational change of a normal protein (PrP) to an infectious form (PrP^{sc}) that is responsible for the disease. The normal function of the PrP protein is still unknown, but its involvement in the disease was demonstrated by gene disruption strategy. On the other hand, the 'unconventional virus' hypothesis maintains that a virus with usual properties is the responsible agent and that the PrP protein is probably required as part of the virus receptor, but such a virus has yet to be isolated. The main opposition to the 'protein-only' hypothesis comes from the diversity of the conformational change necessary to explain the large number of transmissible spongiform encephalopathies recognized today. Much credibility was given to the prion theory when the 1997 Nobel Prize in medicine was awarded to its promoter, Dr. S. B. Prusiner. The defenders of the 'unconventional virus' hypothesis are now facing a bigger challenge...

Our chance of eradicating the disease completely in the near future depends on how soon science can provide us with better knowledge of the disease. Infected cattle with no clinical signs and symptoms are not detected because the disease has a long incubation period (two to five years) and no

effective preclinical testing is currently available.

Chemical residues and antibiotics used as growth hormones

Statistics reveal that the most predominant hazards in food are of microbial origin. Even if media coverage of foodborne outbreaks caused by microbial contamination has increased consumer awareness of microbial hazards in food, consumers still perceive chemical residues of all kinds as the major food related threats to their health. Chemical residues found in meat, milk and eggs are mainly pesticides, growth hormones, antibacterial drugs, heavy metals, industrial chemicals and products of pyrolysis (e.g., polycyclic aromatic hydrocarbon and heterocyclic amines produced during smoking and broiling). These chemical residues are usually detected in trace amounts and do not constitute a major health concern. Incidences of foodborne disease have rarely been attributed to hazardous levels of these contaminants in milk and meat (8).

Antimicrobial drugs are used in farm animals for three main purposes: for therapeutic use to cure illness, to prevent possible infection, and to promote animal growth and hence increase farm productivity. To cure and prevent illness, the drug is prescribed and often administered by a veterinarian. In food animals reared in crowded environment, infections can easily spread if herd management is deficient. Producers will then have to rely on heavy usage of antibiotics to maintain the health status of their herd. In growth promotion, antimicrobials are added to animal feed in a concentration approximately 10 to 100 times lower than those used for therapeutic purposes. The mechanisms by which animal growth is promoted remain speculative. Use of growth promoting antimicrobials at this low level

presents limited toxicological risk to the meat consumer because of their low absorption rate and because they are eventually transformed into microbiologically inactive and less toxic forms (9). Nonetheless, the major concerns regarding the use of antimicrobial drugs in farm animals reside in the capacity of these compounds to modify the gut flora of animals. Some drugs will increase the enteric pathogen excretion and organisms in the intestinal tracts can acquire resistance towards these antimicrobial compounds. Even if different antimicrobial drugs are used in animals and humans, cross-resistance between different antimicrobials has been observed (e.g., cross-resistance to avoparcin used in animal feed and vancomycin used to treat infections in human). The presence of one or several resistance genes on mobile genetic elements (e.g. plasmid, transposon) eases the spread of resistance to normally nonpathogenic strains. Contamination of animal and meat by these drug resistant organisms (e.g., *Salmonella typhimurium* DT104) compromises the efficiency of antibiotics used in human against bacterial infections (9).

Meat, meat fat and metabolic diseases

Consumption of meat and more particularly of meat fat has been considered a risk factor in cardiovascular disease, hypertension, stroke, diabetes, obesity and various forms of cancer (10). Epidemiological studies have been at the origin of the debate but unfortunately they do not provide any answers regarding the mechanism by which meat components cause the disease. It is well accepted that any excessive fat intake over the caloric needs is not good for your health whatever the source may be. In this regard, total calories intake rather than the source is the determinant in tumor formation (10). Animal breeders have taken into account those facts and have already reduced the

adiposity of meat animals, particularly in swine.

There is good evidence that elevated plasma cholesterol is related to a greater risk of cardiovascular disease. Meats provide 34% of the dietary cholesterol in a typical American diet. It is a normal blood constituent needed for steroid production and tissue repair, that our body synthesizes, excretes, in the form of bile acids, and recycles. These reactions are tightly regulated to maintain cholesterol concentration within physiological limits. When the regulation mechanisms are deficient (e.g., due to genetic factors, life style), the individual develops hypercholesterolemia. About half the American population is at low risk and the other half is equally distributed between the moderate and high risk groups (10). The important question is whether we promote restriction of meat consumption to everyone when only a portion of the population is at risk? Meat is an important source of protein and other nutrients that should be part of a balanced diet in accordance to one's health, genetic background and life style.

In conclusion

The main hazard to meat safety is of microbial origin and bacteria are responsible for the majority of meatborne diseases. Their capacity to adapt to various stresses and environments, as demonstrated in the development of antibiotic resistance, indicates that they will represent an everlasting challenge, forcing us to seek novel preservation alternatives. Reducing the total numbers of bacteria in foods will certainly reduce the risk of getting sick, but it will leave no competition to the post-processing harmful contaminants. The desire to produce food with less preservative will lead us to find new ways of "flora management", where the

type of microorganisms and their numbers will be controlled according to the targeted shelf life. The Hazard Analysis and Critical Control Points (HACCP) systems have the potential to provide the meat industries with new tools to rationally control each processing step. It will do so as long as it does not become a load of policies and paper work done simply to ease our conscience. Meat and meat products are no longer produced and transformed in small-scale facilities. They travel great distances to reach different markets. This means that when problems occur, they have the potential to affect many peoples widely spread and they are therefore more difficult to confine. Modern medicine has enabled us to improve our life expectancy and has allowed people with various health problems to live a normal life, but these individuals (e.g., diabetics, HIV positive individuals, elders, patients under cancer therapy, etc.) are immunocompromised. They represent about 20% of the population and a challenge to feed due to their higher sensitivity to pathogens and other contaminants. These many challenges are for us - industry, scientists and government agencies - to face if we want to provide wholesome meat and meat products to the consumers.

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