FRESH RED MEAT PACKAGING AND MEAT QUALITY

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Summary

The extension of the storage life of fresh red meat is influenced by the mode of packaging. Among the quality attributes, the maintenance of the bright-red colour, interpreted as an indication of wholesomeness, is of primary importance. This point will be particularly examined. Solutions to slow the development of off-flavors, largely linked to the lipid oxidation, and the growth of spoilage bacteria, which limit the storage life, will be proposed. The advantages and drawbacks of the different packaging modes (air, modified atmospheres, vacuum) are presented and the key roles of temperature control and oxygen pressure underlined. For the longest periods of raw meat storage, the advantages of oxygen-free CO_2 atmosphere, compared to vacuum, will be examined.

Introduction

Today meat is displayed in an attractive, hygienic, and convenient form in plastic materials which permit consumer evaluation. The methods used for retail packaging are: overwrapping with permeable film, (low-high-O_2) modified atmospheres and under vacuum. The colour of pre-packaged fresh meat, and more particularly its colour stability, is the most important quality attribute contributing to the shelf-life. Consumers relate the bright red colour of meat to freshness and discoloration of packaged fresh meat, known as loss of bloom, is sometimes related to bacterial growth. Moreover, discoloration which normally occurs more rapidly than other deteriorative changes, may prevent the successfull development of prepackaging of fresh meat.

Overwrapping meat is a cheap means of meat storage and often used in many countries, but the main drawback is its short shelf-life due to the deterioration of its bright red colour. Vacuum-packaging of beef is the standard method in industry: removing of oxygen from the packaging prevents the growth of aerobic organisms responsible for spoilage and allows storage of meats for many weeks. Nevertheless, the purple colour of reduced myoglobin in vacuum-packaging leads to rejection by the consumer. As the consumer associates the bright-red meat of oxygenated myoglobin with good eating quality, new packaging systems for self-service retailing, maintaining a bright red colour of meat, have been investigated. Controlled/modified atmosphere packaging (CA/MAP) is such a technology which has been particularly developed in France and elsewhere in Europe. The advantage of CA/MAP is the formation of the red colour, but the main drawbacks are a shorter shelf-life than in vacuum-packaging. The introduction of limited quantities of carbon dioxide inhibit the growth of aerobic microorganisms for several days. Especially in the U.S., CA/MAP packaging of meat is not so widespread because it may not guarantee complete control of pathogenic organisms. Finally, with high concentrations of carbon dioxide in packaging, it is now possible to envisage storage (low O_2-MAP) of fresh raw meat for many months and maintenance of good eating qualities.

Old and new techniques to extend the display life of fresh red meat will be presented in this paper, and the consequences on the main quality attributes such as colour, flavour (lipid oxidation), drip loss, microbial spoilage/safety will be presented.

Meat colour

The colour of fresh meat is due to the relative amounts of the three derivatives of myoglobin. Reduced myoglobin (Mb) is the pigment deep inside the muscle and of the vacuum-packaged meat and is dull red or purplish. On exposure to the air, myoglobin combines with oxygen to form bright red oxymyoglobin (MbO_2), which is synonymous with freshness (*bloom*), and considered attractive by the consumer. The oxymyoglobin form exists on the surface of fresh meat when it is placed on retail display with an overwrap of oxygen permeable film, or in modified atmospheres where concentrations of oxygen are more than 50% in the package. Finally, contact with oxygen leads to oxidation which results in the formation of the oxidized form, metmyoglobin (MetMb), which is brown and unattractive. When overwrapped packaged meat is exposed to air for long periods of time or when the oxygen concentration in vacuum packaging or in CA/MAP is very low, metmyoglobin formation can be extensive.

The principal factors which can influence meat discoloration are of biological and technolgical nature. Among the biological factors, the more important are: species, sex, age, breed, muscle type and pH. *Ante-mortem* treatment of animals, such as pre-slaughter exercise; treatments of carcasses, such as chilling/boning methods (pH/temperature); and mechanical separation of meat can influence the colour of the fresh red meat.

Among the technological factors during meat retail display, the effects of temperature and partial pressure of oxygen will be paticularly important. With different packaging modes, the complex relationships between colour stability, lipid oxidation and bacteriology will be underlined. In meat, no factor acts independently but, for ease of presentation, they will be treated separately.

Muscle Type

In fresh packaged meat under different conditions, the oxidation to metmyoglobin, which reduces saleability on display to the consumer, is essentially affected by:

- oxygen availability (oxygen penetration and tissue respiration);
- rate of oxymyoglobin autoxidation; and
- metmyoglobin reducing activity of the muscle (Renerre, 1990).

The influence of biological factors on colour characteristics can be largely explained by specific properties of different muscle metabolic types. Several reports (Ledward, 1971; Hood, 1980; O'Keefe and Hood, 1982; Renerre, 1982, Renerre and Labas, 1987a; 1987b) have shown that meat colour stability is muscle-dependant. Trials in our laboratory (Renerre, 1984) have classified nine muscles, from beef carcasses of different age, sex and breed into 3 main groups. After overwrapping of the meat, muscles *longissimus dorsi*, *obliquus externus* and *tensor fasciae latae* are the most stable; *m.semi-membranosus* being of intermediate stabiltiy and *m.gluteus medius*, *supra-spinatus*, *triceps brachii caput longum*, *psoas major* and *diaphragma* have least stabiltiy. The percentage of MetMb between the muscles varied from 25% to more than 50%. It is interesting to note that the most expensive muscles are often the least stable. More recently, other researchers have indicated that the colour of *gluteus medius* was less stable than *longissimus dorsi* (Lanari and Cassens, 1991).

Although Ledward (1985) believes that enzymic reduction capacity occurs in aerobic (ARA) and in anaerobic conditions (MRA), and it is the most important factor affecting rate of MetMb formation in different muscles, other workers (O'Keefe and Hood, 1982; Renerre and Labas, 1987a; 1987b) claimed that the rates of oxygen consumption and myoglobin autoxidation were the main factors controlling colour stabiltiy in aerobic storage. Moreover, Faustman and Cassens (1991) and Gatellier *et al.* (1992) showed that differences in colour stability among beef muscles were dependent on inherent metabolic differences and the extent of lipid oxidation. They also suggested that differences in oxygen consumption rates of mitochondria may account for the rates of discoloration in different muscles and breeds. In our laboratory, it was also observed that the most unstable beef muscles, from the colour point of view, showed the highest myoglobin content, cytochromes $a+a_3$ content, and autoxidation rates but also the highest reducing activities (Renerre and Labas, 1987a; 1987b; Echevarne *et al.*, 1990).

The rate of oxymoglobin autoxidation, is increased by the presence of concentrated salts and heavy metals and by low pH, high temperature and low oxygen partial pressure. Autoxidation is highly temperature-dependent, with a Q10 of about 3.5 and an activation energy of about 23 kcal mol_{-1} (Renerre *et al.*, 1992); myoglobin denatures below pH 5.0. Also, during autoxidation, the oxygenated myoglobin separates into ferrimyoglobin, superoxide anion ($O2^{\circ}$-) and hydrogen peroxide. We will see later how these oxy-radical products will play a key role in myoglobin and lipid oxidation processes during meat storage and how the conditions which favour MetMb formation also enhance lipid peroxidation.

The system, which could reduce MetMb to MbO_2 during meat storage, is enzymic in nature (Arihara *et al.*, 1990) and there are significant differences in enzyme activity among beef muscles (Reddy and Carpenter, 1991). The physiological importance of these enzyme activities in colour stability has been questioned by various authors (Livingston and Brown, 1981; Renerre, 1990). Loss of reducing activity in meat during storage is due to the combination of factors including fall in pH, depletion of the substrates, co-factors as NADH (Renerre, 1984) and, ultimately complete loss of structural integrity and functional properties of the mitochondria (Giddings, 1974). It has been established (Renerre and Labas, 1987a; 1987b; Lanari and Cassens, 1991), that the least stable muscles had the highest reducing activities and we disagreed with the conclusions of Ledward (1985) about the effectiveness of the reducing system in the MetMb apparition rate. However, from a technological point of view, as MetMb reducing activity seems more active in anaerobic conditions and high temperatures, this mechanism could play a role in colour stability during vacuum packaging of hot-deboned meats (Ledward, 1985; Renerre and Bonhomme, 1991) but more research is needed in this area. Cassens *et al.* (1988), have shown that a crude extract of cardiac MetMb reductase and a cytochrome b5 preparation from bovine liver reduce MetMb in model systems.

Inter-animal variability is another intrinsic factor which can significantly influence the rate of discoloration of the meat between small groups of animals and this genetic factor is probably the least understood. From birth to about 24 months of age, a three phase increment in myoglobin level has been found, depending essentially on breed and muscle examined (Lawrie, 1985), and in many cases, a parallel enhancement of the capacity for aerobic metabolism. With young

Frisian bulls, we have observed that age negatively affects the colour of short shelf-life chilled muscles e.g., *m.gluteus medius* (Renerre, 1982). We will see later how the myoglobin environmental factors can play a role in these changes. Biological factors other than age, for example sex and breed, can influence the colour stability. Faustman and Cassens (1991) indicated that the rate of MetMb accumulation in Holstein beef was higher than in crossbreeds.

pH Effect

Because the rate of oxymyoglobin autoxidation increases with decreasing pH (Renerre *et al.*, 1992), whilst the enzymic reduction is much reduced at low pH, it is not surprising that, in general, muscles of low pH discolour more rapidly than those of high ultimate pH (Ledward, 1985). But we think, as Hood (1980), that there is no important effect of ultimate pH on the variability of discoloration among different beef muscles.

If ultimate pH has a limited effect on meat colour and rate of discoloration, the rate and extent of pH fall (influenced by biological as extrinsic factors) can play a more marked role. Heat and low pH enhance autoxidation of myoglobin and are responsible for the colour fading observed in PSE (pale, soft, exudative) pig meat. In other species, such as turkey, the onset of rigor mortis can be extremely rapid with negative consequences on the colour of the packaged meat (Santé *et al.*, 1992). In veal, the importance of pH fall on the meat colour is muscle dependent (Guignot *et al.*, 1992).

In beef, pre-slaughter stress may result in dark-cutting beef (DFD) which is translucent and more susceptible to bacterial spoilage particularly under vacuum. Moreover, the high pH will also accelerate respiratory activity of the tissue and the formation of purple reduced Mb. As it is known that Mb is more susceptible to oxidation than MbO_2, DFD meat can autoxidize more rapidly than normal meat. Because the packaging of high-pH meat is forbidden in France, we will see how high CO_2 atmospheres can offer very interesting solutions to overcome this problem.

Among the many pre-slaughter treatments which can influence colour stability, electrical stimulation (ES) of the carcasses was one of the most studied. ES of beef carcasses was used to increase the rate of pH drop and to obviate the risk of cold-shortening and subsequent toughening of meat. Many studies in the U.S. and in Europe, have shown that ES reduces the incidence of *heat-ring* and the meat *blooms* more rapidly with a brighter colour. However, the action of ES on meat colour stability during retail storage is still uncertain and the results are contradictory (Tarrant and Castels, 1983; Renerre and Dantchev, 1987).

Hot-boning (HB) offers some economic and technological benefits, but, with potentially increased microbiological contamination, more rapid cooling is necessary. To avoid the risk of cold-shortening and damage to colour, ES may be employed before HB. Generally, HB is followed by ageing the portions in vacuum-packs under refrigerated conditions before retail preparation. Some research has been conducted to establish if the changes in temperature/pH regimes during glycolysis (ESHB meat) modify the perceived colour (Renerre and Bonhomm, 1991; Faustman and Cassens, 1990).

Temperature effect

High temperatures are known to decrease colour stability and to promote bacterial growth and enhance myoglobin oxidation. So, temperature control is very important because we want to reduce the rates of the undesirable chemical reactions whatever the packaging. The rate of discoloration with temperature is muscle dependent and, in aerobic conditions, the rate is two to five-fold higher at 10°C than at 0°C (O'Keefe and Hood, 1980-81). High temperatures favour greater scavenging of oxygen by residual respiratory enzymes and other oxygen consuming processes such as fat oxidation, leading to low oxygen tension which facilitates autoxidation of myoglobin. In contrast, low temperatures promote penetration of oxygen into the meat surface and oxygen solubility in tissue fluids. This maintains myoglobin in its oxygenated form which is more stable than reduced myoglobin. Other factors regulating meat colour stability, such as the gaseous environment in modified atmosphere packaging, are affected by temperature. Temperature control is also fundamental to reduce the growth of spoilage bacteria and pathogen organisms (by increasing the lag phase), in all packaging methods. With controlled atmosphere packaging (CAP) (oxygen-free CO_2 packaging), pathogen proliferation at <10°C is severely restricted and at <5°C entirely prevented (Gill, 1990), but this is not true for vacuum or modified atmosphere (MAP) packaged meat.

Oxygen pressure

The bright red colour of fresh meat depends on the depth of MbO_2 which is determined by the rate of O_2 diffusion, O_2 consumption and by the partial pressure of O_2 at the meat surface (Giddings, 1974). At low temperatures, consumption of oxygen by mitochondria is low and solubility is high, therefore, penetration of oxygen is deep and the colour is red (Taylor, 1985). In air, during the first days of storage, the depth of oxygen penetration in meat increases, due to a decrease in tissue oxygen consumption, and a better O_2 diffusion (Bendall and Taylor, 1972). But after some days, the MetMb layer is nearer the surface which is becoming brown. Aerobic bacterial on the surface of the meat may also consume oxygen and produce carbon dioxide.

The maximum rate of MetMb formation has been reported for oxygen partial pressures of 6-7.5 mm Hg depending on pH and temperature (Georges and Stratman, 1952). Consequently, prevention of myoglobin autoxidation and spoilage by aerobic bacteria can be achieved by vacuum packaging in an O_2-impermeable wrap which reduces the effective O_2 tension to nearly zero. In vacuum packaging, with a high barrier film, the meat colour is purple-red which can be maintained for long periods, but colour is unacceptable to the consumer.

With modified atmosphere packaging (MAP), the ratio of MbO_2 to MetMb depends on the amount of oxygen present. High concentrations, such as in high O_2-MAP, favour production of MbO_2 and autoxidation is minimized in the first days, and does not depend on the partial pressure. In low O_2-MAP, brown MetMb will rapidly dominate and will severely reduce the shelf-life.

Lipid oxidation is increased by the high concentrations in oxygen, produces increased rancidity, and is one of the primary causes of quality loss in meat during storage. Aerobic bacteria have been associated with brown discoloration which

occurred during their logarithmic growth phase. As noted by Seideman and Durland (1984), *pseudonomas* caused MetMb formation by reducing the O_2 tension at the meat surface, whereas *Lactobacilli sp.*, which develops in vacuum and in low O2-MAP, did not cause this discoloration.

Type of lighting

The problem of discoloration caused by light exposure is important with packaged meat stored in well lit retail display conditions. Light plays a critical role in pigment *photo* oxidation and its energy catalyses MetMb formation. If temperature enhancement may often be the cause of discoloration (Hood, 1980), type of display lighting (incandescent, tungsten filament, fluorescent, UV radiation) on product appearance could also result from a photochemical effect which might initiate or catalyse adverse reactions. Light rendering can also be important because of different spectral energy distributions (Kropf, 1980). White fluorescent light doesn't generally cause discoloration of meat to any appreciable extent but exposure to UV light is quite spectacular causing initially spectacular desication of wrapped meat, myoglobin oxidation and the meat turns brown after only a short exposure. When certain molecules are illuminated with light of a given wavelength, they absorb it and the energy raises the molecule into an *excited stage* and singlet oxygen can formed. Singlet oxygen could initiate lipid peroxidation and this photosensitizer molecule can oxidize haeminic proteins (Kanner *et al.*, 1992). Plastic films exist to stop the negative effects of UV radiation. Metallized films, which are opaque, also achieve higher barrier properties (Gill and Penney, 1986). These high barrier films limit the amount of oxygen in the package and so the light will be less destructive. However, it must not be forgotten that UV light, similar to ozone treatment directly on carcasses, also has bactericidal properties; nevertheless, the oxidative processes on the fat and on the pigment are generally accelerated.

Microbiology

Before considering the microbiology of the meat, it is useful to remember that the microbial population is also affected by many factors such as species, health and handling of the live animals, slaughtering practices, chilling of the carcass and the sanitation during fabrication (Young *et al.*, 1988).

During storage, the importance of the bacterial flora depends largely on the packaging method. The cheapest meat packaging method is the overwrapping in transparent plastic film. High oxygen and humidity content make the colour deteriorate as soon as the aerobic flora reach the exponential phase of growth which may occur in a few hours, and consequently, aerobic storage must be limited to retail. Vacuum packaging is an excellent alternative for prolonged storage period but the purple red colour is a drawback for the retail shelf-life. CO_2 accumulation around the meat leads to a complete change in the composition of the flora. Anaerobic or facultative anaerobic flora grow rapidly and lactic acid bacteria dominate rapidly. If the temperature is near 0°C, and the meat pH, storage is possible for several weeks without microbial hazard. The use of modified atmospheres (CA/MAP) was developed to preserve the red colour of the meat, to prevent anaerobic spoilage and to slightly inhibit the growth of aerobic bacteria. Nevertheless, with high oxygenated atmospheres (80%) and low CO_2 content (20%), aerobic spoilage is rapidly limiting. We will see how atmospheres containing pure CO_2, with the lactic acid bacterial growth, will be very efficient

for maintaining storage life. As noted by Faustman and Cassens (1990), and we concur, it is surprising that the specific role of bacterial in meat discoloration has received little research attention. For example, and more particularly in modified atmosphere packaging (MAP), there is a need to ensure that bacterial contamination is not masked by colour enhancement processes.

If one of the best ways to improve the aerobic storage life of meats (spoilage and pathogenic flora), is the control from slaughter to conditioning, it is also possible to use different treatments (on carcass and/or cuts) to slow the contamination. Apart the use of some acids (sorabic, ascorbic) or reductants (such as sodium ascorbate, erythrobate) to maintain fresh meat colour and delay lipid oxidation (Manu-Tawiah *et al.*, 1991), antibiotics and chemical preservatives can be used, either perfused into or sprayed on cuts. Greer and Jones (1989) have studied effects of ozone on carcasses and concluded that it did not reduce the bacterial growth on retail steaks but did not affect the retail case life. Others have studied the effect of irradiation on meat quality which is a powerful technique for the destruction of parasites and microorganisms in meats (Shary *et al.*, 1988). Doses of 2-5kGy are effective in reducing the populations of pathogens and spoilage organisms. This treatment is beneficial essentially to vacuum packaged pork and sheep meats which otherwise may have too short a shelf-life to be transported around the world. But irradiation causes changes in flavour, aroma and colour. Its influence on myoglobin is not clear, but ionizing radiation favours the oxidation of the lipid fraction and is conductive to the formation of *off-flavours* by the potential formation of radicals. Irradiation in the frozen state, and after the removal of oxygen, can be advantageous (Lawrie, 1985). In France, only mechanically deboned poultry meat is irradiated to control contamination by *Salmonella*. In the U.S., the FDA has opened a dialogue on recommendations for such processing and the consumer will ultimately determine the acceptability of this process in the future.

Lipid oxidation

Lipid oxidation is one of the primary causes of loss of quality in meat during storage. The primary quality deterioration during lipid oxidation, and particularly after cooking, is the production of off-flavours and off-odours. Oxidation of fatty acids also affects adversely the colour, with which it is frequently linked, and, sometimes, the texture, the nutritional quality and safety of meat. Many authors have shown that lipid and pigment oxidation were closely coupled in beef but it was not always possible to deduce whether or not the pigment oxidation caused the lipid oxidation. If lipid oxidation in muscle was attributed to haeminic compounds, such as myoglobin, recent studies imply that non-heme iron is the main catalyst. In raw meat, *free* heavy metals, such as iron and protein-bound iron, which may be haeminic or non-haeminic, such as ferritin, transferrin, hemoglobin and myoglobin, may play an important role in the catalysis of lipid oxidation (Kanner *et al.*, 1992; Decker and Hultin, 1990). Conversely, other authors have shown that lipid oxidation was a promoter of myoglobin oxidation (Lin and Hultin, 1997) and colour stability was enhanced by the addition of antioxidants to meat (Greene, 1969). As reported by Gray and Pearson (1987), many factors affect lipid peroxidation in animal tissues such as species, anatomical location, diet, temperature, sex, age, phospholipid composition (Genot *et al.*, 1991) and content. It is generally accepted that it is the polyunsaturated fatty

acids from polar phospholipids, present in the subcellular membranes (microsomes, mitochondria), rather than triglycerides, which are responsible for the initial development of off-flavours in raw and cooked meats during the storage (Gray and Pearson, 1987). There are also controversial results which indicate that ferrous ions have a greater pro-oxidant activity than ferric ions.

Concerning the mechanisms, the main unanswered questions are: What is the main source of the primary catalysts which initiate lipid peroxidation in raw tissue? Is lipid peroxidation in meat catalysed by enzymic or non-enzymic peroxidation systems or a combination of both? And, particularly in the beef meat, do enzymic lipid peroxidation systems exist in all muscle membranes as they do in the microsomal system (Asghar *et al.*, 1988; Anton *et al.*, 1993)? Lipid peroxidation, which consists of one or more initiation, propagation and termination reactions, forms free radicals (oxy- and lipid-radical) as superoxide anion, hydrogen peroxide, hydroxyl radical and porphyrin cation radical (Harel and Kanner, 1985; Rhee *et al.*, 1987; Anton *et al.*, 1992). The production of free radicals in muscle could also be more pronounced in unstable muscle than in stable muscle (Anton *et al.*, 1991).

Both lipid and myoglobin oxidation might be controlled by antioxidants from natural (e.g., tocopherol, ascorbic acid, citric acid, polyphenolic substances, Maillard reaction products) or synthetic (e.g., BHA, BHT, propylgallate) compounds but the use of synthetic compounds is always restricted. The latest results of Faustman and Cassens (1989) on beef, of Asghar *et al.* (1991b) on pork, and of Santé *et al.* (1992) on turkey, showed that the addition of vitamin E to diets of animals, by stabilizing the membrane lipids against peroxidation, improves, more or less successfully, both pigment and lipid stability of meat. In meat, many radicals may participate directly or indirectly in oxidative processes but many questions have to be resolved. In meat, there are many compounds of different origins acting to stimulate and/or inhibit, these lipid peroxidation processes. There are preventive antioxidants of enzymic nature and chain-breaking antioxidant lipid-soluble. In our laboratory, some research is in progress to examine the relationships between lipid oxidation, antioxidant enzyme activities (SOD, catalase, Glutathion peroxidase) and colour stability in raw beef during aerobic storage (Gatellier *et al.*, 1992).

Processing of raw meat such as the method of deboning of carcasses, mechanical separation, restructuring and grinding can all increase the lipid peroxidation. This is due to the disruption of muscle membrane which alters the compartmentalized cellular systems and facilitates the formation of free radicals (Buckley and Morissey, 1992). Increase in temperature, UV lighting, presence of metal ions and ionizing radiation can also influence greatly the rate of lipid peroxidation (Kanner *et al.*, 1992). Apart from temperature, other biological or environmental factors of the meat such as pH and/or molecular oxygen can greatly affect lipid peroxidation (Hultin, 1992).

All these factors must be taken into account if we want to find effective solutions to increase the shelf-life of chilled meat with different atmosphere packaging. In overwrapped packaged meat, the lipid peroxidation is largely due to the increase of the aerobic spoilage organisms e.g., *pseudomonas* (Dainty *et al.*, 1981) and the production of esters and sulphur-containing compounds. *Fruity* odours were attributed to ethyl and/or methyl esters of different fatty acids. To slow the appearance of *off-flavours*, the creation of anaerobic conditions is the simplest way to control reactions with molecular oxygen. After vacuum packaging, Taylor

et al. (1990), showed that off-odours were absent until 18 days in pork and 28 days in beef. In vacuum, several studies have also reported the development of off-odours described as *sour, buttermilk, sulphur-like, and hydrogen-sulphide* (Jackson *et al.*, 1992; Gill and Penney, 1986). Nevertheless, beef vacuum packaged in good conditions, contains predominantly lactic acid bacteria (the other bacteria growing slowly, it at all) resulting in a long odour-free shelf-life. Taylor *et al.* (1990), found that off-odours developed more rapidly in MAP packs, rich in oxygen (60%), than in vacuum packs. This may be attributed in part to the higher numbers of *B. thermosphacta* in MAP packs, allied to the fact that this organism produces odorous end-products under aerobic conditions. Many authors have shown that compounds with strong off-odours were less developed in vacuum and in 100% CO_2 than in MAP (CO_2/O_2). For Gill (1990), high concentrations of CO_2 may prevent offensive odours and flavour components.

Although the identity of many volatile compounds produced by common aerobic spoilage bacteria on beef has been established (Dainty *et al.*, 1984), little information exists on the volatile compounds produced by bacteria present on vacuum and modified atmosphere packaged red meats (Jackson *et al.*, 1992). These authors have identified the volatile compounds associated with beef that has been stored under vacuum or modified atmospheres and evaluated the contribution of the microbial population with storage. Among 30 volatile compounds identified, nonanal was present in the headspace of all of the samples whatever the method of packaging but many volatile compounds also originated from the packaging materials.

Warmed-over flavour (WOF) describes the rapid development of oxidized flavours in refrigerated cooked meat, in which a rancid or stable flavour becomes apparent within 48 hours when stored at 4°C. WOF differs from the normal rancidity encountered in raw meats and fatty tissues, in that it is not apparent until prior to storage.

To measure lipid oxidation, the thiobarbituric acid test (TBA), sensory evaluation and head-space analysis can be used. For many authors, lipid oxidation is the major causative factor in the development of off-flavour in most foods as in meat and the determination of TBA-RS has long been accepted as an indicator of WOF (Pearson *et al.*, 1983). In different bovine muscles, strong relationships were found between TBA-RS and MetMb% at the meat surface during an aerobic storage (Faustman and Cassens, 1991; Anton *et al.*, 1992). St. Angelo *et al.* (1988) utilized a multidisciplinary approach consisting of chemical, instrumental analyses (as GC) and sensory evaluation. With ground cooked beef, of the many compounds that increased during storage, the most obvious was hexanal, a secondary reaction product of linoleic oxidation. Although other products develop during storage (e.g., propanal, 2-3 octanedione, nonanal), it is not clear if any of these compounds could be used to follow the development of WOF. Conversely, in other laboratories, hexanal (and total volatiles) are considered good indicators of WOF development (St. Angelo *et al.*, 1988) and a good correlation was obtained between sensory score and total volatiles, TBA, hexanal and 2-3 octanedione. Bailey (1988) found that approximately 23 different volatile compounds increased in concentration during storage of heated meats. Spanier *et al.* (1992) have used antioxidants and chelators as tools to examine the relationships among the sensory and chemical attributes of vacuum packaged meat subjected to various treatments.

On heating, there is some cleavage of the porphyrin ring and a subsequent release of the iron which can then enhance lipid oxidation and the development of oxidative rancidity. Heat could also disrupt the muscle membrane and break lipoprotein complexes, exposing lipids to atmospheric oxygen and to catalysts which make them more susceptible to oxidation (Fox and Benedict, 1987). It is also well noted that when meat is heated, browning compounds (Maillard reaction products) are formed and are responsible for the anti-oxidant activity.

In addition to affecting palatability factors, such as meat flavour and colour, lipid peroxidation produces compounds which may have adverse biological effects. During the process of lipid peroxidation, free radicals are generated which oxidize several vitamins, lipids and proteins. Unsaturated fatty acids are especially vulnerable to oxidation and they produce various oxidation products, such as malonaldehyde, associated with rancidity. Many studies have shown that malonaldehyde can play a role in toxicity, mutagencitiy and carcinogenicity (Pearson *et al.*, 1983). Different studies have also indicated that PUFA may increase the risk of cancer, apparently because of their greater propensy for autoxidation. These oxidative processes could oxidize cholesterol present in lipid fraction and membranes. The possible cardiotoxicity of cholesterol oxidation products has been suggested as playing as role in coronary heart disease. Pearson *et al.*, (1983) have examined how malonaldehyde and cholesterol oxidation products have potential toxicity in the diet of man. But Buckley and Morissey (1992) concluded that the health risks associated with dietary intake of lipid and cholesterol oxidation at the levels present in meats remain undefined. Increasing the alpha-tocopherol content of muscle by dietary means, may provide an effective method of reducing lipid and cholesterol oxides, and optimize the safety and healthfulness of meats. Developments of this research in many countries are in progress.

Drip loss

The water activity of chilled meat is very high and it is almost certain to lose weight by evaporation. Further weight loss occurs when the meat is cut. In most cases, uncovered meat loses weight by evaporation of moisture which dries and darkens the surface (Taylor, 1985). The water holding capacity can be affected by treatments of the carcasses, such as variations of pH-temperature profiles during ESHB, and by technological changes on the meat cuts such as freezing-thawing or grinding. Although efficient chilling can reduce the quantity of exudate, a certain amount will be present when meat cuts are held for retailing and large exudates in packaged meat affect quality as perceived by the consumer. Pads are sometimes included to absorb the exudate in the tray. In all these cases, the choice of packaging is very important because packages reduce meat contamination, are moisture barriers and prevent weight loss by evaporation. Conventional fresh meat packaging films are highly permeable to oxygen but only minimally permeable to moisture, and thus retard surface dessication (Faustman and Cassens, 1990). In MAP, meat is retailed in trays of rigid or expanded plastic which are than overwrapped with a clear plastic film (vinyl or polyethylene derivative) and heat sealed. This film can decrease the release of fluids when combined with good refrigeration. With high-O_2 MAP, drip loss is higher than in vacuum (Taylor *et al.*, 1990). Although high CO_2-atmosphere packaging may lead to identical drip loss to that using vacuum packaging (Lee *et al.*, 1985), less drip loss in CO_2 packaging may occur (Rousset and Renerre, 1991). Moreover,

Gill (1988) observed that high CO_2 concentration gives a more viscous exudate than is usual and recommends appropriate absorbents.

Type of packaging

Air storage

In self-service retailing, and in most cases after vacuum packaging in meat plants and distribution, meat is retailed on rigid or expanded plastic trays and overwrapped with clear plastic films. These films are composed of polyvinyl chloride (PVC), are very thin and present a high oxygen permeability (8000-12000 cc/m2/day-atm) and a low moisture permeability. Under these conditions, the red colour (*bloom*) is maintained. Overwrapped meat is acceptable microbiologically for up to one week in many countries as it is in France (Renerre, 1988), when bacteria reach about 10^{7-8} bacteria/cm^2 and when formation of Met Mb at the surface becomes important. This storage method in PVC film is the simplest and the cheapest means of meat storage and it represents the greatest production for retailing in France. Many papers pointed out the importance of microbiology on the shelf-life of aerobically stored meats. As *pseudomonas spp. (P. fragi, P. fluorescens, P. undensis),* which dominate in meats, are psychrotrophic, their proportion within the flora will increase with the time of storage and largely determine the shelf-life of the overwrapped meat. With this packaging method, other bacteria could dominate the flora i.e. *Brochotrix thermosphacta, Acinetobacter sp, Moraxella sp, Enterobacteriaceae* but their respective influence on storage life largely depends on the type and the pH of the meat and their respective proportions in the initial flora (Dainty *et al.,* 1981). For example, *B thermosphacta* is more frequently associated with spoilage of the pork and lamb because it is dominant in the initial flora (Dainty *et al.,* 1981; Talon and Montel, 1986) and because it grows much more rapidly at low temperature.

Vacuum packaging

The distribution and the storage of beef under vacuum is the greatest innovation in meat handling during the last 25 years. In France for industrial markets, nearly half of the beef is handled in this way. Used commercially, vacuum packaging could keep meat for about three to four weeks. Taylor (1985) has described the different vacuum packaging methods: Cryovac, Chamber and Thermoforming. With this last method, meat is placed in the trays and an upper web of plastic is heat-sealed under vacuum to form a lid. In France, meat with bone such as poultry, and many meat products, are vacuum packaged commercially. There is also the possibility of using vacuum-skin packaging with a peeleable O_2 barrier layer and an O_2 permeable skin-layer which remains after storing. Nevertheless, for retail packaging of beef, vacuum packaging is rarely used compared to modified atmospheres, because of the purple red colour which is the greatest drawback to the retail sale. Contrary to the U.S. situation, where some ranking studies have shown that the consumer is likely to prefer vacuum-packaging despite its adverse effect on colour (Lynch *et al.,* 1986), in France there is no extensive promotion of this for the consumers. However, in limited quantity, beef cuts are conditioned now in vacuum-skin packaging and an opaque aluminum film (with a photo of the product) and this new product seems to be beginning to attract consumers.

The shelf-life of vacuum-packaged meat depends to a very great extent on the bacteriological status of the meat at the time of packaging. When meat is packaged in this way, residual oxygen is rapidly consumed by the metabolic activity of enzymes present in the meat and carbon dioxide is produced as the end product of respiration. These anaerobic conditions, with an elevated partial pressure of carbon dioxide in the residual atmosphere, eliminate the growth of aerobic spoilage bacteria. In these conditions, the growth of *pseudomonas* responsible for spoilage of meat in air is limited and lactic acid bacteria become the dominant organisms (Taylor, 1985). It is possible to accumulate 20% carbon dioxide in these conditions, within only a day or two of packing and nearly 70% after 14 days of packing.

The most effective way of vacuum packaging is to hold the meat at allow temperature, about 0°-2°, i.e., just above the freezing point of the meat. Hermansen (1983) has shown that low temperatures favoured more the Lactic acid bacteria than other bacteria such as *Brochotrix thermosphacta* and *Enterobacteriaceae* which grow also in anaerobic conditions. In vacuum, the other aerobic bacteria such as *Acinetobacter sp, Moraxella sp,* are more or less rapidly inhibited by an increase of the CO_2 content in the packs (Enfors and Molin, 1981). We will examine how CO_2 evolution will play a fundamental role in extending the shelf-life of vacuum-packaged fresh meat. Among different species, *Lactobacillus sake* and *Lactobacillus carnis* largely dominate on beef (Montel *et al.,* 1991; Shaw and Harding, 1989) whereas *Carnobacterium* species are much more frequently isolated from pork. Although *Lactobacillus* species growing on beef meat may inhibit the growth of other bacteria, *Carnobacterium spp* growing on pork would be less of an inhibitor than *Lactobacillus spp*.

Oxygen availability within the packs is also a crucial parameter for controlling bacterial contamination. In vacuum, the growth of *B. thermosphacta* is largely dependent on the permeability of the film used and the growth of *Lactobacilli* decreases with the increasing permeability of the film (Egan and Grau, 1981). Taylor and Shaw (1977) showed that approximately 1% oxygen may remain after prolonged storage. When oxygen partial pressures are of 6-7.5mm Hg, the autoxidation of oxymyoglobin is favoured in the package with the formation of a brown MetMb layer near the meat surface (Ledward, 1970). So films used in vacuum packaging must have a high degree of impermeability of oxygen but also to water vapour to avoid surface dessication.

Examples of films used in Franc are PES/aluminum/PES/PP; Saran/EVA /Saran/EVA and PE/Saran/PE with O_2 permeability between 0.2 and 40ml/m2/atm/day (Renerre, 1988), but now, EVOH is more and more used instead of Saran. In practice, most of the plastics used for vacuum packaging have permeabilities below 10ml/m2/atm/day. In Ireland (Hood, 1983), films used in vacuum packaging can be coexturded EVA/PVDC/irradiated AEVA shrink bags, polyvinylidene chloride (Saran) or nylon/low density PE and nylon/surlyn laminates. In New Zealand, use of films with very low gas permeability have tested and, particularly, vacuum packs with foil laminate films exist and may allow a shelf-life of meat from different species for many weeks, even for high-pH meats (Gill and Penney, 1986).

In laboratory conditions, normal pH beef can be stored under vacuum for periods for up to 12 weeks (Hood, 1983) but is shorter with high pH beef because vacuum packaged meat with a high pH value spoils very rapidly by growth of *Alteromonas putrefaciens* (Newton and Gill, 1979). In high pH meats, the principal

psychrotrophic species isolated belong to the *Serratia Liquefaciens* species and, to a lesser extend, to *Hafnia sp* and *Enterobacter sp.* The onset of spoilage by these bacteria is detected by the greening of the meat surface (Fournaud and Valin, 1978) and off-odours dominated by sulphur containing compounds (Gill, 1983; Dainty, 1986). Egan *et al.* (1989) have shown that spoilage by H_2S-producing *Lactobacillus sake* is more rapid when pH is high and that it is dependent on the film permeability. H2S react with unstable reduced myoglobin present in vacuum-packaging to produce sulphomyoglobin or cause degradation beyond porphyrins to bile pigments. In our laboratory, on beef packaged under vacuum, *Enterobacteriaceae, B. thermosphacta* and *Pseudomonas* numbers were 10- to 100-fold greater on high than on normal -pH meat. Moreover, the R630-R580 value, which is positively correlated with the percentage of oxymyoglobin at the surface (Renerre and Mazuel, 1985), decreases rapidly after 28 days storage under vacuum in high pH meat at 2°C (Rousset and Renerre, 1991). When the pH of beef is higher than 6.0, it will not be kept much beyond two to three weeks at 1°C and such beef should not be vacuum packed (Taylor, 1985).

Although vacuum packaging is a good method for normal pH beef, it is not so for lamb and pork. In England, Taylor (1985) recommended that vacuum packed lamb and pork should not be stored for more than four weeks. Apart from the greater proportion of pork and lamb of high initial pH than in beef, it is more critical to control and determine the exact oxygen content in the packs. As noted by Gill and Penney (1985), packaged lamb with an important adipose tissue and a high pH, shows an heterogeneous environment for microbial growth. These authors demonstrated that by using film with immeasurably low gas permeability (foil laminate) and temperatures near 0°C, it was possible to extend the commercial life of high pH lamb meats in vacuum to about 12 weeks, instead of the normal six to eight weeks. To better explain the differences between species, it is necessary to have a better knowledge about the physiology of bacteria and the interactions between bacteria and meats. Even if the bacteria growing on meats are well defined, and the used substrates identified, it is likely that bacterial ecology, physiology and muscle metabolism will be insufficiently understood.

Many experiments have shown also that the inoculation of *lactobacillus* strains in vacuum-packaged beef may lead to a slight inhibitory effect on spoilage bacteria without green discoloration even if *off-aroma* can be produced with some strains. In our laboratory, we have observed that the inoculation of a selected strain of *Lactobacilli* has a slightly inhibitory effect on *B. thermosphacta*, but not on gram negative bacteria, and a slight positive effect on meat colour after retail display (Renerre and Montel, 1986). Recently Arihara *et al.* (1993), screened Lactic acid bacteria for their ability to improve meat colour. The conversion was possible with *Chromobacterium violaceum, Kurthia sp.*, and, more interestingly, by *Lactobacillus fermentum.* This last strain generated nitric oxide which gives nitric oxide nitric oxide myoglobin of red colour, and the first two strains produce oxymyoglobin. Schillinger and Lucke, (1987) found that the shelf-life of meat was not dependent on the composition of the inoculated Lactobacilli (*L. sake, L. curvatus, L. bavaricus*). Conversely, Faustman *et al.* (1990), showed that beef homogenates at 4°C, inoculated with high levels of fluorescent *pseudomonas sp.*, which consume oxygen, caused a decrease in MetMb and a change from brown to red. Bacterial metabolites or intracellular components freed from bacterial cells were presumed responsible for Met Mb conversion but the exact nature of the reddish pigment was not determined. With pork and veal, as well as for poultry, the differences in colour between oxygenated and reduced myoglobin is not so

pronounced, and consequently vacuum packaging could be well accepted by the consumer. In France, the colour stability of turkey meat, now an important commodity, is of increasing economic importance. When the colour stability was good under vacuum, microbial contamination, essentially by *B. thermosphacta,* was high (Sante *et al.,* 1993).

More importantly in vacuum packaged meats is the possible growth of well know psychrotrophic pathogens such as *Yersinia enterocolitica, Listeria monocytogens* and *Aeromonas hydrophyla.* Below pH 6.0, L. *monocytogenes* grows very slowly (Gouet *et al.*, 1978) and this poor growth is linked to the undissociated lactic acid content of meat, as it was demonstrated for *B. thermosphacta,* which is closely related to the *Listeria* group (Talon *et al.,* 1990). For some authors such as Gill and Reichel (1989), growth of the cold-tolerant pathogens will be better controlled in CO_2-packaged meat than in similar vacuum packaged product.

As a matter of fact, most of the lactic acid bacteria isolated from eats produced bacteriocins (Ahn and Stiles, 1990) which are particularly effective on closely related bacterial species and groups. However the influence of meats as culture median on these productions is largely unknown. Many recent reports (Ahn and Stiles, 1990; Greer and Dilts, 1992) have shown that these bacteriocins, which may be antimicrobial proteins and which act by unknown mechanisms, are produced by Lactic acid bacteria (*Lactobacillus, Carnobacterium, Leuconostocs*). These bacteriocins seem to have an effect on mesophilic and spoilage bacteria but also on cold tolerant pathogens (Listeria).

As a combination of factors are critical in may spoilage problems (Newton and Gill, 1978; Erichsen and Molin, 1981; Egan *et al.,* 1989; Cantoni *et al.,* 1991) such as low residual oxygen, elevated CO_2 concentration, pH, low storage temperature and antimicrobial activities of *Lactobacilli,* a better understanding of these synergistic or additive interactions of these factors is required if we are to comprehend the ecological relationships between meats and microorganisms.

Modified atmospheres

Gases used to modify atmospheres in packages have included nitrogen, carbon dioxide and oxygen. Gas packaging can be defined as the alteration of the proportional volumes of the gases which comprise a normal atmosphere. After the removal of air, the pack is flushed with a combination of gases and hermetically sealed. A high oxygen concentration maintains the oxymyoglobin bloom of red meat and carbon dioxide inhibits the growth of gram-negative psychotrophic organisms responsible for the spoilage of meat. During storage, the percentages of the gases change in particular due to the consumption of oxygen by the mitochondria, the metabolic activity of the bacteria, lipid oxidation and may limit meat qualities.

Controlled atmosphere (CA) packaging is a system whereby the gases are added or removed to maintain a desired balance. Modified atmosphere packaging (MAP) is a one-time alteration of the gases surrounding the product during the storage (Hotchkiss and Galloway, 1989). The aim of this packaging method is firstly to extend the keeping quality, by reducing microbial growth and influencing the types of microorganisms present, and secondly, to preserve the bright red colour of the oxymyoblobin. CAP may also prevent collapse of the package and aid moisture retention. The packaging material must possess good gas-barrier properties to keep the gases within the package and special machines for the

heat-sealing must be used. To maintain the atmosphere during storage, in MAP, a ratio of the pack to the meat of three to one is recommended, although not observed in practice. Consequently, this packaging method takes up more space during distribution than other types of packaging. For all these reasons the costs of MAP for the manufacturer are relatively high (Renerre, 1988). Compared with the vacuum method, the bright-red colour, synonymous with freshness of meat, and expected by the consumer, is preserved but the shelf-life of meat in MAP is shorter usually between seven and 14 days in chill.

Nitrogen is an inert gas and its effect is similar to that of vacuum packaging, but its use, unlike vacuum packaging, avoids internal pressure on the meat and does not distort the shape of the packaged meat. This gas finds little commercial use with fresh meat and is commonly used for cured meat products. Added to other gases, nitrogen would be more useful in extending the shelf-life of white meats where the colour problem is not so important. Nevertheless, this gas has no antimicrobial properties. With nitrogen (Taylor, 1985), residual oxygen is diluted and MetMb formation on the surface should be less pronounced than in vacuum. Use of nitrogen sometimes presents advantages for organoleptic quality, and its use with oxygen, but without CO_2, could lead to the growth of *pseudomonas*. O'Keefe and Hood (1980-81) showed the meat in nitrogen had less exudate than similar cuts in vacuum packs. Steaks stored in 100% N_2 have the same discoloration as in vacuum or in 100% O_2 (Seideman and Durland, 1984) and beef stored in N2 atmosphere gave better results than in vacuum by retarding the development of discolouration and by minimizing the exudate losses (Lee *et al.*, 1985). Vacuum packaging give better results than $CO_2 + N_2$ because the CO_2 concentration increased in the package during storage (Smith *et al.*, 1983).

With high concentrations of *oxygen* in the package, it is possible to prolong the bright red colour during storage because there is the development of a thick surface layer of oxymyoglobin, particularly with more than 50% oxygen. But, when used alone, oxygen does not inhibit microorganism growth. Moreover, with high oxygen concentrations, there are off-odours and poor colour stability due to lipid oxidation and/or aerobic spoilage. Consequently, oxygen is not used alone and we will see that, even when present in very small quantities, as part of CO_2 packaging, oxygen may have a detrimental effect on meat colour stability.

To maintain an attractive colour to the meat, *carbon dioxide* was used with *oxygen*. Various research workers such as Clark and Lentz (1972), Taylor and MacDougall (1973) have shown that a mixture $CO_2 + O_2$ improves shelf-life by enhancing colour and by bacteriostatic effects. Many experiments, cited by Cole (1986), have shown that 20% CO_2 was sufficient to prevent bacterial growth and that higher concentrations of CO_2 can cause a detrimental change on the colour of the meat. In these conditions, the oxygen concentration must be 80%. Patents in Great Britain have also indicated that lower concentrations of either gas by dilution with nitrogen were less effective for colour or microbial growth (Seideman and Durland, 1984; Taylor 1985).

With 80% O_2 + 20% CO_2, and at temperature close to 0°C, meat remains red for up to 15 days with an acceptably low level of microbial spoilage. Shay and Egan (1990), showed that meat stored in this mixture has a storage life up to 3 times that of meat from the same muscle stored in aerobic conditions. Nevertheless, the low efficacy of this atmosphere is limited because there is little accumulation of CO_2 in the pack after 14 days. Mixtures containing more than 20% of CO_2 change

the composition of the conventional flora to one with an increasing proportion of Lactic acid bacteria. CO_2 primarily inhibits the growth of carbon dioxide sensitive aerobic bacteria (King and Nagel, 1975). However, at the end of the shelf-life, meats could be dominated by *Pseudomonas, B. thermosphacta* or Lactic acid bacteria (Jackson *et al.* 1992). Such differences are explicable by the permeability of the films, the initial bacterial load, the nature of the initial flora, the storage temperature and the type of meat used (Fu *et al.*, 1992). Others workers, cited by Taylor (1985), have also concluded that mixtures of 75-85% O_2 with 25-15% CO_2 are most effective, but ther hav also been contradictory reports of off-odours and rancidity in meats stored in high concentrations of oxygen. Taylor *et al.* (1990) have indicated that 25% CO_2 + 75% O_2 was not as efficient as vacuum packaging using the "Darfresh" process.

Many experiments have also indicated that the mixtures of $CO_2 + O_2 + N_2$ were less effective at maintaining colour stability even though, in France for example, this gas is recommended for commercial reasons. To maintain a good colour with this mixture, the oxygen concentration must be higher than 60%. With a mixture of O_2 (70) CO_2 (20) N_2 (10), we have had good results for microbial growth and colour (Renerre, 1986). However, the effectiveness of MAP depends on the meat species. For example, we have observed that, with horse meat packed in a CO_2 (20) + O_2 (80), the shelf-life was much shorter than that of beef meat (unpublished results). The reasons are not yet clear but the quality of the lipid fraction is implicated. More 'exotic' species will be tested using MAP in our laboratory in the future.

The influence of MAP on sensory qualities is not clearly established but many results seem to show that gas mixtures rich in oxygen may accelerate deterioration of flavour. Recently, Taylor *et al.* (1990) using beef and pork in MAP, showed that off-odours and rancidity developed more rapidly than in vacuum. Prepackaging in vacuum and storage in MAP gave a rapid discoloration, higher bacterial counts and off-odours compared to a shorter ageing treatment in air (Nortje and Shaw, 1989).

It is known that *carbon monoxide* at low levels increases colour and odour shelf-life to long periods and that CO can counteract the discoloration often observed with CO_2 packaging but relatively little is known about the effects of CO on the growth of microorganisms. Our results (Renerre, 1986), showed a mixture of CO_2 (20) CO (2) and N_2 (78), under industrial conditions, caused a great improvement in beef colour stability for up to 35 days by formation of carboxymyoglobin but the colour was judged *too artificial* by the panel. CO can increase the reduction potential of the muscle and slow the apparition of MetMb (Wolfe, 1980). Nevertheless, there were few significant differences in microbial counts between the two MAP methods and aerobic psychrotrophic microflora is not inhibited by a low level of CO. With high levels of CO, it is possible to inhibit the growth of *E. Coli* and *P. fluorescens* (Gee and Brown, 1980). However, despite the favourable trials, the addition of CO is forbidden because of its potential toxicity and health hazard to the people using CO.

In France, in 1991, the proportion of manufactured MAP in retail distribution (individual portion) represents about 20% of the total, the greater quantity being overwrapped with permeable film. Many authors have shown the principal benefits and drawbacks of this mode of packaging (Renerre, 1988; Young *et al.*, 1988; Hotchkiss and Galloway, 1989). There are many variables to be considered in MAP. The product itself, mixture of gases, type of packaging, temperature and

additives. As we have seen it earlier, low temperature slows the growth of microorganisms and increases the solubility of CO_2 in the meat and is of paramount importance.

Another feature of MAP meats is that spoilage is often less evident at equivalent total aerobic or anaerobic counts than in aerobic conditions (Dainty, 1986). For examples, 10^{6-7} bacteria/cm^2 often does not spoil the products in equivalent conditions as those noted in air stored meats. It seems that metabolism leading to spoilage is more or less inhibited under CO_2 containing atmospheres. The major safety question (Hotchkiss and Galloway, 1989) for CA/MAP is whether or not pathogenic organisms will proliferate while the normal organism, that serve to indicate spoilage, are inhibited. This is the most hazardous of all situations because spoilage often warns consumers that a product might be unhealthy (Hotchkiss and Galloway, 1989; Manu-Tawiah *et al.*, 1991). Combining MAP with low-dose irradiaiton can delay toxin production by *Clostridium botulinum* in pork meat (Lambert *et al.*, 1991).

Carbon dioxide alone has been used in studies several years ago but its use is becoming more and more common because of its ability to inhibit the growth of spoilage bacteria, by extending the lag phase and generation time (Clark and Lentz, 1972). As shown by many authors, Gram-negative spoilage flora are especially sensitive to carbon dioxide while lactic acid bacteria are less affected (Young *et al.*, 1988). The inhibitory effects of CO_2 have been attributed to alteration of the permeability of the bacterial cell, pH and to enzymatic inhibition (King and Nagel, 1975). In vacuum packaging, CO_2 is generated, eliminating the growth of aerobic bacteria and favouring the Lactobacilli species. In the last decade, several experiments were conducted to exploit the preservative effect of CO_2. As shown by Erichsen and Molin (1981) and by Gill and Penney (1986), irrespective of the muscle tissue pH, beef packaged under CO_2 can remain unspoiled for periods exceeding 15 weeks. Growth of Gram-negative organisms, such as *Enterobacteriaceae* and *Alteromonas putrefaciens*, can be suppressed on high pH meat. Although this CO_2 packaging gave better results than vacuum for spoilage organisms in beef, it was difficult to obtain this storage life with lamb. Research was conducted in New Zealand on chilled lamb shipped to Europe. The *Captech* system uses packs, with a layer of aluminum foil, which are vacuum packaged and inflated with CO_2 and stored at -1.5 to 0.5°C. Under these conditions, lamb will stay fresh for up to 16 weeks (Gill and Penney, 1986). These authors have shown the precise conditions in terms of the gas impermeability of the film, the temperature, the initial gas volume to meat, the solubility of the CO_2 (Gill and Penney, 1988). With one to two litres CO_2 per kilo of meat, spoilage of normal pH beef meat is delayed until 21 weeks. Other workers have shown that pork meat packaged under CO_2 was also a good alternative to vacuum with a longer shelf-life when the temperature was maintained as low as -1.5°C.

Lamb meat, packaged in CO_2, gave a better display life than fresh meat. On the contrary, with venison, Seman *et al.* (1989) indicated that vacuum- packaging resulted in a lower incidence of off-odours and higher colour acceptability scores than did CO_2 flushed packaging systems.

Although many experiments have given good results for spoilage organisms, it is not clear whether or not high concentration of CO_2, reduce or extend meat colour stability during its display life. In the literature, there are conflicting data on the

effect of CO_2 on colour. Clark and Lentz (1969), Ledward (1970) and Taylor (1971) showed that high levels of CO_2 discolour the meat while others (Seideman *et al.,* 1979) do not agree (see Young *et al.,* 1988: Seideman and Durland, 1984). With the Captech system (Gill, 1988), there may be no risk of discoloration, but it is well known that one problem with the use of CO_2 packaging systems is to prevent any O_2 introduction. Many years ago, it was established that very low concentrations of O_2 in packaging cause discoloration of fresh meat (Georges and Stratman, 1952; Ledward, 1970; O'Keeffe and Hood, 1980-81, Seideman and Durland, 1984). Small amounts of residual oxygen in high CO_2 packs gave discoloration (Finne, 1982), but not (Moore and Gill, 1987) when using an aluminum foil laminate. To study this problem, we have done experiments by comparing three methods to prevent the low residual O_2 concentration (Rousset and Renerre, 1990). We have established that, when beef was vacuum-packaged in a tray filled with 100% CO_2, at 2°C, and with a very low permeability film, a high rate of meat discoloration was measured. After only eight days of packaging and display, the browning at the surface was significant. Conversely, when an oxygen scavenger (*ageless*) was added to the tray filled with 100% CO_2, and even after seven weeks, the colour after a display life of 48h was highly acceptable. With packaging under 100% CO_2 and an O_2 scavenger, the residual O_2 concentration was reduced very rapidly to less than 0.1%, remained at this level and was the main reason for the extended colour stability. Under these conditions, we think that the problem of meat discoloration with low O_2 / high CO_2 atmospheres can be resolved (Rousset and Renerre, 1990;1991). Penney and Bell (1993), have recently shown that CO_2 packaged beef and lamb became noticeably brown in packs containing more than 0.15% of residual oxygen. O'Keeffe (1988) has observed that by using a catalytic system, less than 0.1% oxygen was necessary to obtain a better red colour.

Packaging under 100% CO_2, with an oxygen scavenger, was also compared to vacuum, using normal and high pH beef meat stored for long periods. It was shown that MetMb % increased after 28 days under vacuum irrespective of the meat pH (Rousset and Renerre, 1991). Conversely, packaging high pH with 100% CO_2 gave a shelf-life up to 42 days, growth of *Pseudomonas, Enterobacteriaceae* and *B. Thermosphacta* was completely inhibited and only Lactic acid bacteria were present. These interesting results accord with those of the literature (Ericksen and Molin, 1981) which show that by using 100% CO_2, only Lactic acid bacteria are present at 51 days for both normal and DFD meats. According to Gill and Penney (1988), it is noteworthy that, in our experimental conditions, CO_2 suppresses the growth of *Enterobacteriaceae* while there is an important number in vacuum packaging with the same meat cuts (Rousset and Renerre, 1991). It was also observed that the acidification of high-pH meat was important compared to normal-pH meat. Gill (1988) has shown that solubility of CO_2 increased with increasing pH and with decreasing temperature. Moreover, the colour stability of the high pH meat after a display life of 48 hours was better than that of normal pH. A pH of 5.85 can have a positive effect on the oxidation-reduction potential of the myoglobin by lowering the MbO_2 autoxidation rate and/or enhancing MetMb reducing activity (Renerre, 1990).

With 100% CO_2 and an oxygen scavenger, we have observed that the atmosphere was invariant during seven weeks storage, and, consequently, this MAP could almost be considered a CAP allowing longer storage lifes. Because of the benefits of colour stability and presence on only Lactic acid bacteria, it is evident that this process may be used to store beef for longer periods. After seven weeks storage in CO_2, the display life at 48 hours for colour stability was good, and its evolution

makes us think that a longer display-life is possible and could be comparable to that of fresh overwrapped meat. Works on other products using this packaging method is in progress in the laboratory.

Furthermore, Gray *et al.* (1983) and Gill and Reichel (1989) showed that cold-tolerant pathogens such as *Listeria monocytogenes, Yersinia enterocolitica* and *Aeromonas hydrophila* did not grow on high-pH beef under CO_2 at 2°C; at 5°C, only *Y. enterocolitica* grew. But these microorganisms were able to grow at 0°C on DFD meat packaged under vacuum. All these results indicate that packaging in pure CO_2 with an oxygen scavenger is promising. Further experiments are needed to evaluate the exact effect of 100% CO_2 on other quality attributes, such as flavour and texture of the meat. We have used this packaging technique with pork meat with interesting results although the extension of shelf-life was less than with beef.

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