Comprehensive REVIEWS in Food Science and Food Safety

Antimicrobial and Antioxidative Strategies to Reduce Pathogens and Extend the Shelf Life of Fresh Red Meats

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Abstract: The shelf life of packaged fresh red meats is most frequently determined by the activity of microorganisms, which results in the development of off-odors, gas, and slime, but it is also influenced by biochemical factors such as lipid radical chain and pigment oxidation causing undesirable flavors and surface discoloration. The predominant bacteria associated with spoilage of refrigerated meats are *Pseudomonas, Acinetobacter/Moraxella (Psychrobacter), Shewanella putrefaciens,* lactic acid bacteria, *Enterobacteriaceae,* and *Brochothrix thermosphacta*. The spoilage potential of these organisms and factors influencing their impact on meat quality are discussed. High O_2-modified atmosphere (80% O_2 + 20% CO_2) packaging (MAP) is commonly used for meat retail display but vacuum packaging remains the major MAP method used for meat distribution. Two-step master packaging (outer anoxic-20% CO_2 + 80% N_2/inner gas-permeable film) is used for centralized MAP distribution, but CO use (0.4%) in low O_2 packaging systems is limited by consumer uncertainty that CO may mask spoilage. Active packaging where the film contributes more than a gas/physical barrier is an important technology and has been studied widely. Its application in combination with MAP is very promising but impediments remain to its widespread industrial use. The influence of processing technologies including modified atmospheres on lipid oxidation and discoloration of meats are analyzed. Because both organic acids and antioxidants have been evaluated for their effects on microorganism growth, in concert with the prevention of lipid oxidation, work in this area is examined.

Introduction

Meat is animal flesh that is used as food. The term meat is generally used in commerce in a more restrictive sense—the flesh of mammalian species (cattle, pigs, lambs, and so on) raised and prepared for human consumption, except fish, poultry, and certain other animals. Shelf life is the length of time that the defined quality of food and other perishable products remains acceptable under expected (or specified) conditions of distribution, storage, and display (Gyesley 1991). Spoilage is the process by which food deteriorates to the point where it is considered nonedible by humans or its quality is reduced making it undesirable or unsuitable for sale or consumption.

Since meats have high water activity and high concentrations of readily available nutrients, they are susceptible to microbial growth, which results not only in off-odors and off-tastes, but also in texture changes and slime formation. Further, biochemical reactions such as lipid oxidation contribute to discoloration and meat rancidity. It is usually considered that surface contamination by microorganisms causes off-flavors and results in spoilage of meats, but chemical changes, which alter meat appearance, before microbial deterioration takes place can be important. In general, spoilage is a subjective judgment made by the consumer, which may be affected by sensory acuity, the intensity of the change, cooking traditions, and even religious and cultural considerations.

For the meat industry, retailers, and consumers, spoilage of raw meat represents a loss, which could be as high as 40% of production (Sperber 2010). To satisfy the demand for extending fresh meat shelf life and reduction of spoilage, anoxic preservative packaging techniques are widely used, but there is still a place for meat packaged with enhanced O_2.

The primary factors determining meat spoilage are related to its intrinsic properties (pH, free moisture) contamination during slaughter, packaging, and storage, as well as extrinsic influences including temperature and storage atmosphere. Usually meat spoilage is characterized by (a) bacterial growth and metabolism, which causes formation of objectionable compounds, including those causing off-odors, gas, and slime, and (b) oxidation of lipids and pigments to cause undesirable flavors and discoloration.

During chill storage, the numbers of microorganisms on fresh meat surfaces change following a typical pattern of microbial growth (García-López and others 1998) in response to temperature, pH, and oxygen availability, but only around 10% of the bacteria initially present are capable of growth during refrigerated storage of meat. The portion causing spoilage is even lower (Borch

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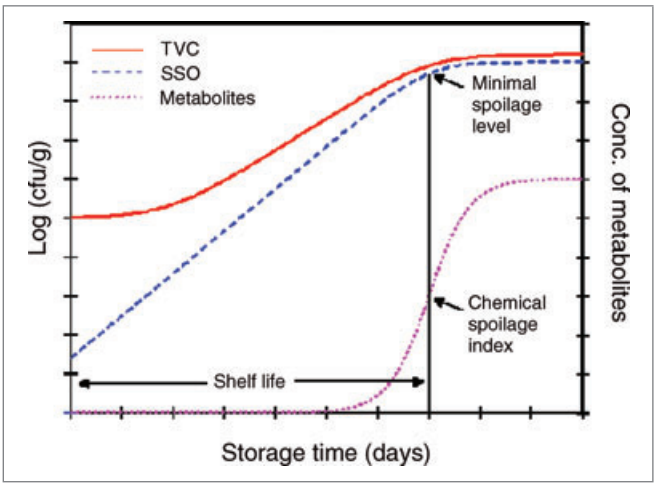

Figure 1–General pattern of microbial spoilage. TVC, total viable count; SSO, specific spoilage organisms; MSL, minimal spoilage level; CSI, chemical spoilage index (Source: Dalgaard [1993], as reproduced by Huis in't Veld [1996]).

and others 1996). The nature and extent of surface contamination of the cut meat will determine its potential shelf life.

In this review microorganisms causing meat spoilage are discussed, as well as factors such as temperature and packaging conditions that retard bacterial activity are presented. Biochemical issues, which sometimes act independently and result in lipid oxidation, pigment discoloration, and off-flavor production, as well as techniques used to inhibit bacterial spoilage organism growth and biochemical deterioration are discussed.

Microbial Meat Spoilage

Meat spoilage usually results from the metabolic activity of a variety of microorganisms. The microbial flora that spoils specific muscle tissue depends upon the characteristics of the meat (residual glucose, pH), the environment in which meat is stored (temperature, atmosphere), as well as the O_2 permeability of packaging material used, the number of bacteria initially present, and the ability of bacteria present to grow (García-López and others 1998).

The pattern of spoilage, which develops during meat storage is determined by the characteristics of specific spoilage organisms (SSOs) normally present in low numbers, and which represent a small fraction of the fresh meat microflora. During storage, SSOs generally grow faster than the other organisms present and produce metabolites leading to off-odor, off-taste, as well as visual slime development, and these finally result in sensory rejection (Huis in't Veld 1996). Prediction of shelf life based on growth of SSOs in model substrates was studied by Dalgaard (1995) who developed a general pattern of spoilage, which outlined relationships among total numbers of bacteria and the SSOs, as well as organoleptic and chemical indexes of microbial spoilage (Figure 1).

Spoilage-related microorganisms

Meat flora frequently consists of various fractions of spoilage bacteria, and the spoilage activities of some types are not proportional to their relative numbers (Gill and Greer 1993). Although there are exceptions for sensory changes to be noticeable, in general, a count of 10^6 to 10^9 CFU spoilage microorganisms/g meat is needed (Bruhn and others 2004; Gill and Gill 2005). Although all the results cited in this review are presented as colony forming units (CFU)/g, readers should be aware of the difference between CFU/g and CFU/cm^2 to prevent confusion because in some reports the data are presented as CFU/cm^2.

Huis in't Veld (1996) divided spoilage microorganisms into several broad categories: Gram-negative rod-shaped bacteria, Gram-positive spore-forming bacteria, lactic acid bacteria (LAB) plus other Gram-positive bacteria (for example, *Brochothrix thermosphacta*). Conditions necessary for growth of the main groups of spoilage bacteria and their spoilage potential are shown in Table 1.

García-López and others (1998) indicated that the Gram-negative bacteria have the greatest potential to spoil meats. Members of the genera *Pseudomonas, Acinetobacter, Psychrobacter*, and *Moraxella* have the most rapid growth rates and therefore the greatest spoilage potential when fresh meat is chill-stored aerobically. The shelf life of fresh meat stored in air is in the range of days before signs of spoilage (off-odors and slime) are evident, depending on temperature.

B. thermosphacta often dominate the microflora in packages when modified atmosphere or vacuum packaging are used where meat pH is $\geq$6.0 and a small amount of residual O_2 is present (Huis in't Veld 1996). Other types of Gram-positive organisms, such as *Micrococcus* spp. are able to grow in the presence of salt (Huis in't Veld 1996) and may be responsible for the spoilage of seasoned fresh meats following production of slime, souring, or pigmented growth.

Under normal conditions of cutting, packaging, and storage, especially at chill temperatures, bacteria are usually the primary organisms present on meat (Dillon 1998). Yeasts and molds can be involved in meat spoilage, but usually do not play important

Table 1–Basic characteristics and conditions for growth and the spoilage characteristics of the 7 main groups of chilled-meat spoilage bacteria.

	Gram reaction	Cell shape	Oxygen requirement	pH requirement	Spoilage potential	General remarks
Pseudomonas	Negative	Rod	Strict aerobe	None	High	Dominant in all spoilage flora
Acinetobacter	Negative	Cocci or coccobacilli	Strict aerobe	None	Low	Little significance for spoilage
Moraxella	Negative	Cocci or coccobacilli	Strict aerobe	None	Low	Little significance for spoilage
Enterobacteriaceae	Negative	Rod	Facultative anaerobe	No anaerobic growth below pH 5.8	High	Major spoilage organisms of VP, high-pH meat
Lactic acid bacteria	Positive	Rod and cocci		None	Low	Usually the dominant organisms of VP meat
Brochothrix thermosphacta	Positive	Rod	Facultative anaerobe	No anaerobic growth below pH 5.8	High	Major spoilage organism of some VP meats
Shewanella putrefaciens	Negative	Rod	Facultative anaerobe	No growth below pH 6.0	Very high	Major spoilage organism of some high-pH meat

Adapted from: Gill and Greer (1993).

Table 2–Expected shelf life under refrigerated storage, and growth potential of bacterial groups and specific bacteria on meat and meat products.

Product	Storage	Expected shelf life	Growth[a]			
			Pseudomonas spp	Enterobacteriaceae	Lactic acid bacteria	B. thermosphacta
Meat, normal pH	Air	Days	+++	++	++	++/+++
	High O_2-MA	Days	+++	++/+++	++/+++	+++
	Vacuum	Weeks-months	+	+/++	+++	++/+++
	100% CO_2	Months	+	+/++	+++	+
Meat, high pH	Vacuum	Days	+	++/+++	+++	++/+++
	100% CO_2	Weeks-months	+	+/++	+++	+
Meat products	Air	Days	+/++	+	++	+++
	Vacuum	Weeks	+	+	+++	++/+++
	CO_2+N_2	Weeks	+	+	+++	+

[a] +++, dominant part of the microflora; ++, intermediate part of the microflora; +, minor part of the microflora.
Source: Borch and others (1996).

roles. At pH values below 5.5, the growth of acid-tolerant yeasts is enhanced, but acid-tolerant bacteria normally predominate at chill temperatures above freezing. Mold can cause spoilage when temperatures cycle below and just above freezing, particularly with poultry.

On fresh meat under aerobic storage, pseudomonads will predominate and preferentially consume available tissue glucose. Upon glucose depletion, amino acids will be metabolized generating ammonia, amines, and organic sulfides, which are offensive even in small quantities and rapidly cause detectable spoilage in meat of normal pH (5.4 to 5.8) as soon as the threshold of glucose depletion is crossed (usually 10^8 bacteria/cm^2). However, on meat of high pH value (>6, dark firm, dry) or on fatty tissue, residual glucose is low and spoilage by the pseudomonads can occur at 10^6 bacteria/cm^2. In the absence of O_2 (<1%) pseudomonads are suppressed and the spoilage microflora consists almost entirely of the LAB, which eventually produce inoffensive acidic off-flavors (lactobacilli, leuconostocs, carnobacteria). Nonetheless, obligate aerobes such as *Pseudomonas* and *Acinetobacter* can still be detected in vacuum packages, but they do not contribute to spoilage. In anoxic packages where the vacuum is poor or the barrier film is inadequate to prevent O_2 ingress, if meat pH is >5.8 (and in the presence of a small amount of oxygen), a number of facultative anaerobes including *B. thermosphacta, Shewanella putrefaciens, Hafnia alvei*, or even the pathogen *Yersinia enterocolitica* may grow at chill temperatures and contribute to early meat spoilage. *Brochothrix* causes off-odors by its production of acetoin (sweaty socks smell) from glucose while the other facultative anaerobes utilize sulfur-containing amino acids to produce sulfide-like odors and flavors, which may interact with myoglobin and yield green or gray discoloration of the meat. If meat is stored at >9 °C without O_2, the *Enterobacteriaceae* may play significant roles in accelerating meat spoilage. Use of CO_2 at levels >20% are inhibitory to pseudomonads even in the presence of O_2, and concentrations of CO_2 (20% to 40%) are commonly combined with 60% to 80% O_2 to package fresh meat, giving a stable red color for 12 to 16 d at 4 °C. Use of MAP with 100% N_2 yields spoilage, which is similar to that seen with vacuum packaging. However, if 100% CO_2 is used (gas volume must be 2 to 3 times the meat mass to prevent package collapse due to CO_2 solubility), growth of bacteria other than LAB is prevented when stored at –1.5 °C, regardless of meat pH. It is the latter type of packaging, which yields the longest shelf life of fresh meat (Gill and Gill 2005).

Environmental effects on bacterial growth and shelf life of fresh meats

Environmental conditions including temperature, gaseous atmosphere, and pH interact to affect bacterial growth, and in concert with baseline levels of bacteria present, which are affected by sanitation and hygiene, they determine the shelf life of fresh meats. The expected shelf life of refrigerated meat and the ability of various bacteria to grow under different packaging conditions are shown in Table 2.

Temperature. It is understood that temperature is the most important factor that influences meat spoilage rate and its safety. The growing demand for fresh meat has resulted in more emphasis being placed on adoption of new technologies to improve the shelf life, safety, and quality of fresh meat.

Since low temperature effectively decreases the growth rate of microorganisms, good control of meat temperature during distribution has yielded enhanced shelf life performance. In the review by Nychas and others (2008), the modern meat chill-chain was characterized by 2 independent phases: primary and secondary chilling. Cooling meat carcasses after slaughter from body (38 °C) to refrigeration temperature (7 °C) represents primary chilling, and when done as quickly as possible, while respecting the negative effects of rapid chilling on meat tenderness under some conditions, shelf life can be maximized. Maintenance of primal and subprimal carcass cuts at 4 °C during further processing, especially during chopping or grinding to form emulsified batters for bologna or frankfurter manufacture, is considered secondary chilling. Maximum meat shelf life is attained at –2 °C, but its use requires strict thermostatic control to prevent fluctuation below this temperature.

Chiller storage at 4 °C inhibits the growth of mesophilic (cold-intolerant) species, and facilitates growth of the psychrotrophic (cold-tolerant) microorganisms that were originally present only as a small fraction of the bacterial population on the meat, and these become the spoilage flora. Small changes in refrigerator temperature can significantly alter the microbial profile. McMullen and Stiles (1994) stored fresh pork in 100% CO_2 at –1.5 °C and found that *Carnobacterium* spp. dominated the microbial flora, while at 4 °C and 7 °C homofermentative *Lactobacillus* spp. were the dominant organisms present.

According to Gill and Greer (1993), when fresh meat is frozen at temperatures that are too low for microbial growth to occur (–4 °C), changes caused by microbial action will not be evident.

Packaging. Packaging under vacuum or modified atmosphere is very effective in extending the refrigerated shelf life of fresh meat. Changing the atmosphere to which the bacteria are exposed, by both decreasing the concentration of oxygen and increasing carbon dioxide, tends to reduce the growth rate of most spoilage bacteria and extends shelf life (O'Keefe and Hood 1980–1981; Korkeala and others 1989; Gill and others 1990; McMullen and Stiles 1993; Skandamis and Nychas 2002). Rao and Sachindra (2002) have reviewed the effects of modified atmosphere and vacuum packaging on meat and poultry products.

Table 3–Modified atmosphere packaging.

Type	Gas	Pigment form	Use	Shelf life	Distribution	Pack format
Vacuum[a]	Residual O_2	Deoxy nitroso	Fresh cured	Better	Export	Single
Low O_2[b]	Residual O_2, $\geq$20% CO_2 balance N_2	Deoxy nitroso	Fresh cured	Good	Domestic	Single
High O_2[b]	$\geq$80% O_2 $\geq$ 20% CO_2	Oxy	Export	Short	Within cities[c]	Multiple
Controlled	O_2 absent 100% CO_2 or 100% N_2	Deoxy	Fresh	Best	Export	Multiple

[a]Vacuum used for primal, subprimals fresh meats; retail cuts, cured meats.
[b]Where legal $\leq$ 0.5% CO can be used (red color = carboxymyoglobin), fresh meat.
[c]Retail display-case ready.

Under anaerobic conditions. Once carcasses have reached 4 °C and are broken, primal and subprimal cuts of fresh meat are usually packed under vacuum. Following packaging the proportion of reduced myoglobin (deoxymyoglobin) in fresh red meat (beef, lamb, and pork) increases due to residual O_2 consumption by respiring meat tissue in packages, and the surface red color changes to purplish-red (Grobbel and others 2008). Vacuum packaging is uncommonly used for the retail sale of individual cuts of fresh meat since consumers consider its color undesirable (Gatellier and others 2001), although this is changing as vacuum-packed fresh pork, lamb, and beef roasts are occasionally available in the retail display case.

Vacuum packaging meat in a film of low oxygen permeability is the most common method of achieving anaerobic conditions (Gill and Greer 1993). The storage life of vacuum-packaged (VP) meat will be extended 4-fold or more beyond that possible during aerobic storage (Gill and Harrison 1989). Examples of the extension of shelf life possible using differently packaged products are shown in Table 3. With vacuum-packed meat, the level of *Enterobacteriaceae* ranges from 10^5 to 10^7 CFU/g and their role in the spoilage process is variable and genus-dependent (Bruhn and others 2004). At temperatures $\geq$9 °C their influence on product shelf life is significant. On meat *Enterobacteriaceae* are dominated by *H. alvei* and *Serratia* spp. (Borch and others 1996; Riddel and Korkeala 1997). Vacuum-packed meat (including beef) with extended shelf life was also observed to be spoiled by psychrotrophic *Clostridium* spp. (Dainty 1996). When stored at 3 °C after 2 wk and at –1.5 °C after 5 wk, VP pork cuts were grossly spoiled by *B. thermosphacta* (Gill and Harrison 1989), which indicated the packages contained residual O_2. In contrast, when stored under vacuum and 40% CO_2/60% N_2 at 3 °C, the dominant flora on beef loins was *Lactobacillus plantarum* (Jackson and others 1992).

Under anaerobic conditions, the spoilage flora of stored meat is usually dominated by LAB of the genera *Lactobacillus, Leuconostoc,* and *Carnobacterium*. Such LAB will usually be the only bacteria distinguishable among the anaerobic spoilage flora from muscle tissue of normal pH (5.3 to 5.8) (Gill and Greer 1993; Gill and Gill 2005). During storage at –1.5 and 3 °C, enterobacteria were reported on vacuum-packed pork (Gill and Harrison 1989), but this was related to uncertain hygienic practices prior to packaging. On vacuum-packed lamb, growth of *Enterobacteriaceae* has also been observed (Gill and Penney 1985). Growth of *Shewanella, putrefaciens, Alcaligenes* spp., *Aeromonas* spp., and other species of *Enterobacteriaceae* may cause spoilage when pH is $\geq$6 (García-López and others 1998).

Use of O_2 and CO_2 in modified atmosphere packaging (MAP). With O_2-containing atmospheres, MAP can extend fresh meat shelf life by 6 to 10 d (McMillin 2008). Early work investigated the use of high O_2 levels (70% to 80%) in gas mixtures to extend the shelf life of fresh meat products, but more recently its use has been restricted to short-term storage of retail-ready fresh meat (Sørheim and others 1997; Eilert 2005). Oxygen, either accidental as a residual in packages or deliberately added as part of the MAP gas mixture, can temporarily maintain an attractive ''bloomed'' color in red meats (Behrends and others 2003; Seyfert and others 2005). Extended exposure of red meat to a low (about 4 mm) partial pressure of O_2 (O'Keefe and Hood 1980–1981) accelerates deoxymyoglobin oxidation, which irreversibly forms brown metmyoglobin. This reaction overwhelms the energy-dependent, inherent metmyoglobin-reducing capacity of meat and leads to permanent discoloration, which is not reversed upon exposure to air (Tewari and others 2002). Since the growth of spoilage bacteria and lipid oxidation are supported by O_2, the shelf life of meat packed with O_2 is relatively short ($\leq$16 d) (Sørheim and others 1997). Pseudomonads are ideally suited to cause meat spoilage by proteolysis after glucose utilization in meat tissue stored at chill temperature in the presence of oxygen, and they determine meat shelf life under these conditions (Dainty 1996). Nonetheless, under conditions where there is good hygiene and temperature control ($\leq$4 °C) MAP systems containing 80% O_2 and 20% N_2 can be used for fresh retail-ready packed ground meat and cuts of beef and pork. Depending on the stability of the metmyoglobin-reducing activity of muscle tissue (Jeong and others 2009), high O_2 retail-ready products can be fabricated successfully from previously VP subprimals. Such integrated systems involve a centralized repacking facility and a group of retail stores in close proximity ($\leq$500 km) where raw material and final product inventory are closely monitored, making a shelf life of $\leq$16 d commercially viable.

Using a high CO_2 MAP environment is an effective method to considerably extend the microbial shelf life of fresh meat (Sørheim and others 1999) and it accommodates longer transportation and retail display periods. In contrast to the normal shelf life in air, atmospheres containing both O_2 and CO_2 are used commercially (80% O_2 + 20% CO_2) to approximately double the shelf life of chilled meat (Buffo and Holley 2005). An atmosphere of CO_2 alone can more than double the shelf life obtained in vacuum packs (Gill and Harrison 1989) by establishment of LAB as the primary spoilage group. The rate of growth of the aerobic spoilage flora, particularly pseudomonads, is decreased by increased concentrations of CO_2 to 20% even in the presence of >1% O_2, but beyond 20% increases in CO_2 cause little further inhibition of pseudomonads in the presence of O_2 (Gill 1988; Gill and Gill 2005). Borch and others (1996) found that very long shelf life may be attained in pure CO_2 and, provided oxygen levels were $\leq$200 rpm and pH was <6, these packages achieved the greatest shelf life of all MAP options at –1.5 °C (Gill and Gill 2005). When stored

Table 4–Important findings in extending shelf life of fresh meats by MAP and other treatments since 2000.

Meat varieties	Conditions	Results	References
Beef	Beef was packaged under normal atmospheric gas composition or CO-MAP (0.4% CO/20% CO_2/79.6% N_2), and irradiated at 0, 0.5, 1.0, 1.5, or 2.0 kGy then held at 4 °C for 28 d.	The color of aerobically packaged beef was greener and less red than CO-MAP packaged beef and red color of CO-MAP packaged samples decreased slightly in some irradiated samples after 14 d of storage. Irradiation at 1.5 or 2.0 kGy reduced total coliform counts below the detection limits.	Ramamoorthi and others (2009)
Lamb	Lamb steaks were packaged with 2 MAP (70%O_2/20%CO_2/10%N_2 and 50%O_2/30% CO_2/20%Ar) using an active film containing a 4% rosemary extract or a 4% oregano extract, or sprayed with rosemary extract and stored under illumination at 1 ± 1 °C for up to 13 d.	Oxidative stability of lamb steaks was enhanced by using a rosemary extract, a rosemary active film, or an oregano active film packaged in MAP and displayed under illumination.	Camo and others (2008)
Beef	Beef pieces were mixed with 5 antimicrobial solutions: 2% and 5% lactic acid, 2% lactic acid + 0.5% sodium ascorbate, 20% potassium lactate, and 20% potassium sorbate, then ground and packaged under MAP (70%O_2/30%CO_2) and stored at 2 ± 0.5 °C for up to 12 d.	The growth of aerobic microorganisms was inhibited by lactic acid alone or in combination with sodium ascorbate, potassium lactate, and potassium sorbate. However, lactic acid also caused significant discoloration. Sodium ascorbate was effective in improving color stability of lactic acid-treated beef.	Friedrich and others (2008)
Beef	Beef samples were injected with potassium lactate to a final lactate concentration of 1.25% or 2.5% and then packaged in vacuum, high-oxygen (80% O_2 /20% CO_2), or 0.4% CO (30% CO_2 /69.6% N_2) MAP and stored at 1 °C in the dark for either 5 or 9 d.	Beef injected with 2.5% lactate had darkened color when packaged in vacuum, high-oxygen, and 0.4% CO-MAP. The redness of beef with 2.5% lactate was improved in high-oxygen MAP. CO-MAP significantly increased color life compared with high-oxygen MAP.	Mancini and others (2008)
Bone-in beef	Bone-in beef steaks were topically treated with either ascorbic acid or sodium erythorbate (0%, 0.05%, 0.1%, 0.5%, 1.0%, or 1.5%) then packaged in high-oxygen MAP (80% O_2 + 20% CO_2) and displayed for 24 h at 1 °C under continuous 1614 lux of fluorescent light.	Ascorbic acid or sodium erythorbate treatments at levels between 0.5% and 1.5% inhibited of vertebrae discoloration and *longissimus lumborum* color oxidization early in display. Sodium erythorbate may be a cost-effective antioxidant substitute for ascorbic acid in high-oxygen packaging.	Mancini and others (2007)
Beef	Five bovine muscles were packaged in MAP (20% O_2/20% CO_2/60% N_2, 20% O_2/20% CO_2/59.6% N_2/0.4% CO, 80% O_2/20% CO_2, 79.6% O_2/20% CO_2/0.4% CO) and displayed under fluorescent lighting of 2150 ± 100 lux at 0.2 ± 3.1 °C and evaluated on days 0, 4, and 7 of retail display.	By increasing oxygen level from 20% to 80%, color stability of all samples was increased, but no effect was observed by inclusion of 0.4% CO with these oxygen levels.	Seyfert and others (2007b)
Beef	Beef steaks were sprayed with *Lactobacillus sakei* CTC 372 and *Lactobacillus* CTC 711 to inoculate the surface with 10^4 to 10^5 *Lactobacillus*/cm^2; then packed in 2 MAP (70%O_2/20%CO_2/10%N_2 and 60%O_2/40% CO_2) and stored at 1 ± 1 °C for up to 28 d.	The growth of spoilage bacteria was inhibited by inoculation of meat with both strains. Formation of metmyoglobin and the development of off-odors were not affected.	Djenane and others (2006)
Beef	Samples were packaged with active films containing rosemary extract under MAP (70%O_2/20% CO_2/10%N_2) and exposed to cool white fluorescent light at 2 ± 1 °C for up to 30 d.	Active film efficiently protected myoglobin against oxidation, thus enhanced the stability of both myoglobin and fresh meat against oxidation processes.	Nerín and others (2006)
Pork	Chops were overwrapped and stored in 0.4% CO/30.0% CO_2/69.6% N_2 (CO-MAP) or 80% O_2/20% CO_2 (HiOx-MAP), placed in the dark at 2 to 4 °C for 1 wk, and then transferred to an open-top retail case, and displayed at 4 °C under fluorescent lights (3013 lux) for 2 d.	Chops packaged in CO-MAP were redder and darker than chops packaged in traditional high-oxygen MAP (HiOx-MAP) packaging. CO-MAP had no effect on flavor or consumer acceptability and only minimal effects on other characteristics.	Wicklund and others (2006)

(*Continued*)

Table 4–(*Continued*)

Meat varieties	Conditions	Results	References
Pork	Pork chops were placed on a polystyrene tray and overwrapped. The overwrap film was punctured to facilitate gas exchange. Then the chops were placed in master packs with either 100% CO_2 or 80%CO_2/19.6%N_2/0.4% CO and stored at 3 °C for up to 8 wk.	Chop color was improved but lipid oxidation was not inhibited with the inclusion of CO. At least 8 wk of spoilage-free refrigerated storage was achieved by CO_2-MAP with or without CO for retail-ready pork in a master-packaging system.	Wilkinson and others (2006)
Lamb	Diets supplemented with various vitamin E concentrations (0, 250, 500, and 1000 mg/kg feed) were fed to lambs for about 37 d. Slices of lamb were packaged under MAP (70% O_2/30% CO_2) and stored at 2 ± 1 °C for 14 and 28 d.	Vitamin E supplementation significantly increased lipid stability. However, neither the microbial load nor the color in meat was significantly affected or improved.	Lauzurica and others (2005)
Pork	Fresh refrigerated pork loins (*longissimus dorsi*) were stored at 4 °C using vacuum (600 mmHg), 100% CO_2, 99% CO_2/1% CO, 100% O_2, and 100% CO followed by vacuum after 1 h of exposure. Packaged loins were stored at 5 ± 0.5 °C for up to 20 d.	The highest consumer acceptance scores were for meat in 99% CO_2/1% CO MAP after 24 h of storage. The shelf life of pork loins in 100% O_2 MAP was the lowest with greater growth of aerobic microorganisms, which induced discoloration after 20 d of storage.	Viana and others (2005)
Pork	Pork loins were packaged in air (AP), MAP (70% O_2/30% CO_2), and VP. All samples were stored at 4 °C and exposed to fluorescent light (600 Lux) for up to 20 d.	High-oxygen (70%) MAP had no benefit with respect to the objective color parameters over AP, and favored lipid oxidation. VP did not notably alter the color but lowered oxidative deterioration of pork.	Cayuela and others (2004)
Beef	Beef was sprayed with carnosine (50 mM), carnitine (50 mM), and L-ascorbic acid (500 ppm) and their combinations. Meats were packaged in MAP (70%O_2/20% CO_2/10% N_2) and stored at 1 ± 1 °C for up to 28 d.	The best antioxidative protection was provided by the combination of carnosine with ascorbic acid. Effective delay of meat oxidation was caused by surface application of carnosine or ascorbic acid alone.	Djenane and others (2004)
Beef steaks and ground beef	Samples were packaged in MAP (0.4%CO/30%CO_2/69.6%N_2) and traditional retail packages. MAP treatments consisted of storage at 1 of 6 temperature/time combinations. Storage at 2 °C and 6 °C for 7, 21, or 35 d for steaks and 7, 14, or 21 d for ground beef. After storage, samples were wrapped in PVC then displayed at 1 °C under fluorescent light of 1614 Lux.	MAPs containing 0.4% CO resulted in typical initial bloomed color at the beginning of sample display. Beef spoilage and microbial unsoundness was not masked by addition of CO to MAP.	Hunt and others (2004)
Beef	Three muscles: semimembranosus (SM), semitendinosus (ST), and biceps femoris (BF) were assigned to 2 packaging types (MAP or polyvinyl chloride overwrap) and displayed for up to 10 d and evaluated by a panel and a Minolta Chroma Meter. (Konika Minolta, Ramsey, NJ, USA).	The select and high choice steaks packaged in MAP showed lower lean color scores and less discoloration at days 7 and 10 than did low choice steaks. In MAP low choice steaks reacted the best, and despite quality grade or packaging, the ST maintained better storage stability.	Behrends and others (2003)
Beef	Beef muscles were sprayed with 2 treatments: rosemary extract (1000 ppm) and L-ascorbic acid (500 ppm) mixture, and n-pentane and then packaged in MAP (70%O_2/20%CO_2/10%N_2) and stored at 1 ± 1 °C with various illumination for up to 25 d.	A 15% inhibition of TBARS formation under any lighting condition was induced by the antioxidant mixture. The rates of metmyoglobin formation and lipid oxidation, as well as microbial growth, were significantly reduced by using the antioxidant mixture of rosemary and vitamin C.	Djenane and others (2003)
Pork	Treatments studied were: aerobic overwrap (high-oxygen-permeable film), vacuum (high-barrier film), MAP (20% CO_2/80% N_2), and MAP-CO (0.5% CO/70% CO_2/29.5% N_2). Packed meats were stored at 0 to 2 °C in lighted display under 6.5 Lux of Deluxe Cool White florescent light.	Both injected and noninjected pork chops achieved a significantly stable, bright-red color over an extended storage period in MAP at low levels of CO (0.5%). When compared with overwrap (aerobic) package, lipid oxidation was also suppressed by CO treatment.	Krause and others (2003)

(*Continued*)

Table 4–(*Continued*)

Meat varieties	Conditions	Results	References
Beef patties	Samples were mixed with 1% borage, 2% borage, 0.02% oregano, 0.1% oregano, 0.05% ascorbic acid, 0.1% rosemary, rosemary + ascorbic acid (0.1% + 0.05%) and packaged under MAP ($70\%O_2/20\%CO_2/10\%N_2$), then stored at 2 ± 1 °C for 24 d.	Lipid oxidation in beef patties was highly inhibited by rosemary extract, oregano extract, and borage meal in MAP. The combination of rosemary and ascorbic acid showed the highest protection against color fading and myoglobin oxidation.	Sánchez-Escalante and others (2003)
Beef	Beef cuts were sprayed with vitamin C (500 ppm), taurine (50 mM), rosemary (1000 ppm), and vitamin E (100 ppm); the 3 latter in combination with 500 ppm of vitamin C, packaged in MAP ($70\%O_2/20\%CO_2/10\%N_2$), and stored at 1 ± 1 °C for 29 d.	An effective delay of oxidative deterioration was observed by surface application of antioxidant combinations prior to MAP. In delaying myoglobin and lipid oxidation, rosemary combination with vitamin C was the most effective.	Djenane and others (2002)
Ground beef	A high-oxygen MAP ($80\%O_2/20\%CO_2$) with an oxygen-impermeable film (control) were used to pack finely and coarsely ground beef chubs separately which were stored at 2 °C up to 10 d.	High-oxygen MAP was effective in maintaining a desirable red color for 10 d of refrigerated storage. However, the TBA value increased greatly in high-oxygen MAP samples compared to controls.	Jayasingh and others (2002)
Beef and pork cuts	Samples were retail VP and stored at 2 °C for 14 to 21 d to minimize loss of metmyoglobin-reducing activity. Groups of 3 retail packs were placed in a master pack and then filled with N_2 and heat-sealed.	For the master packaging, to keep the color of retail-ready meat cuts stable, oxygen scavengers were needed inside retail packages to absorb O_2. The O_2 absorbing capacity in each tray influenced the subsequent retail display life of meat cuts.	Tewari and others (2002)
Beef	Steaks were packaged under MAP ($70\%O_2/20\%CO_2/10\%N_2$) and displayed under various conditions at 1 ± 1 °C for up to 28 d.	A significant delay of meat spoilage was caused by lighting without UV radiation. The retail life of beef steaks was more stable in the absence of UV light.	Djenane and others (2001)
Beef	A diet containing 2000 IU α-tocopheryl acetate/day was fed to steers before slaughter. Beef was minced and supplemented vitamin E, soy oil, Duralox NMC, extracts of rosemary, BHA, and BHT, and then packaged aerobically or under MAP (O_2:CO_2 varied) and stored at 4 °C for 8 d.	Oxidative stability of beef from dietary vitamin E-supplemented muscles was improved and dietary α-tocopheryl acetate-supplemented beef was also significantly improved by adding rosemary extracts or BHA/BHT.	Formanek and others (2001)
Beef	Pretreatment was done with MAP ($5\%CO/60\%CO_2/35\%N_2$) for 24 h or (100% CO) for 1 h, then samples were repackaged under vacuum (VP) and stored at 2 °C.	Exposure to a 24 h 5% CO pretreatment was feasible and safe to allow development of surface redness on beef steaks and to maintain redness for 21 d in VP.	Jayasingh and others (2001)
Turkey, beef, and pork	Samples were individually packaged in either aerobic or vacuum bags, irradiated at 0 or 4.5 kGy, and stored at 4 °C for 7 d.	The packaging effect was more noticeable on the oxidation of cholesterol and lipid than irradiation. Vacuum packaging of raw meats was sufficient to protect cholesterol and fatty acids from oxidation even when irradiated.	Nam and others (2001)
Pork	Pigs were fed diets containing 48 and 170 mg α-tocopherol (α-Toc) acetate/kg feed (VIT-E) before slaughter. Chops were packaged aerobically or in MAP ($80\%O_2/20\%CO_2$) at 4 °C. Patties were made with 1.5% salt, and also stored at 4 °C.	Dietary supplementation of α-Toc delayed lipid oxidation in both salted and unsalted VIT-E pork. However, α-Toc supplementation did not show a significant effect on color of salted or unsalted pork patties, or pork chops.	Phillips and others (2001)
Beef	Beef with natural antioxidants: ascorbic acid (500 ppm), taurine (50 mM), carnosine (50 mM), rosemary (1000 ppm), and their combinations in MAP ($70\%O_2/20\%CO_2/10\%N_2$) was stored at 2 ± 1 °C for 20 d.	Rosemary, either alone or with ascorbic acid, was the most effective oxidation inhibitor of both lipid and myoglobin. Carnosine and carnosine + ascorbic acid were effective lipid oxidation inhibitors.	Sánchez-Escalante and others (2001)
Beef	CO (0.1% to 1%), was used in combination with O_2 (24%), high CO_2 (50%), and N_2 (25 to 25.9%). A reference treatment contained $70\%O_2/20\%CO_2/10\%N_2$ and stored at 1 ± 1 °C.	Bacterial numbers in all atmospheres containing CO were greatly reduced. Shelf life was extended by 5 to 10 d at CO concentrations of 0.5% to 0.75%.	Luño and others (2000)
Ground beef	Ground beef was packed in MAP of high CO_2/low CO mixture ($60\%CO_2/40\%N_2/0.4\%CO$), high O_2 mixture ($70\%O_2/30\%CO_2$) and inoculated with rifampicin-resistant or nalidixic acid/streptomycin-resistant *Y. enterocolitica*, *L.monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp., then stored at 4 and 10 °C for up to 14 d.	In the high CO_2/low CO MAP, growth of *Salmonella* spp. was not inhibited at 10 °C and growth of *Y. enterocolitica* was nearly completely inhibited both at 4 and 10 °C. In both the 2 MAP, growth of *E. coli* O157:H7 at 10 °C was nearly completely inhibited.	Nissen and others (2000)

in 100% CO_2 at 3 °C, *Leuconostoc mesenteroides* subsp. *mesenteroides* predominated in beef loins (Jackson and others 1992), and these organisms plus the lactobacilli and carnobacteria were predictably dominant on long storage of 100% CO_2-packed meats at −1.5 °C. Predominance of slow-growing LAB was responsible for the extended storage life. However, *Enterobacteriaceae* and *Aeromonas* spp. may grow and cause spoilage if pH is >6.0, if initial numbers are high, and if packaging material allows O_2 penetration (Gill and Penney 1985, 1988; Gill and Harrison 1989; McMullen and Stiles 1993). Ordonez and others (1991) found *B. thermosphacta* was the dominant bacterium on refrigerated pork packaged in atmospheres enriched with CO_2 and O_2, but this can be avoided by ensuring that O_2 residuals are not measurable (<200 ppm) or the pH is reduced to ≤5.8 (Gill and Gill 2005).

Use of carbon monoxide (CO) in MAP. Carboxymyoglobin (MbCO) forms when CO irreversibly combines with myoglobin, and it gives a desirable cherry-red color to meat (El-Badawi and others 1964). Concerns have been raised that CO use in MAP may obscure normal spoilage (Pattron 2007; McMillin 2008). In practical use, very low levels of CO (0.4% to 0.5%) can maintain the red color of meat due to the stability of MbCO (Clark and others 1976; Luño and others 1998). Wilkinson and others (2006) indicated that, although CO improves the stable red color of fresh meat, it does not inhibit pathogen growth and at the levels used does not inhibit the spoilage microflora. Therefore, an additional concern arises that CO-MAP may delay the perception of spoilage and result in unnecessary food safety risk under certain conditions. This is an unresolved debate in Europe and Canada where its use is not presently permitted. CO is of little value in promoting color stability in high O_2 MAP because oxymyoglobin predominates, but in low O_2 or vacuum packages 0.5% CO improves color acceptability. It has regulatory approval in the United States and is used there commercially. However, once cooked, meat previously packaged with CO may have some residual pink color (McMillin 2008).

Active packaging

Traditionally, packaging has been used in a passive manner to protect package contents from physical, chemical, and microbiological contamination. More recently, packaging and allied systems have been developed that promote microbial and chemical stability through scavenging or absorbing gases (O_2, CO_2, ethylene), flavors, or moisture. Packaging also serves as platforms for release of CO_2, ethanol, antioxidants, flavors, or preservatives, as well as enzymes to modify package content and even serves to change thermal distribution during heating or cooling (Vermeiren and others 1999; Appendini and Hotchkiss 2002; Cooksey 2005). Some active films have been developed which have features that suggest "intelligence" and allow them to interact with package contents in response to environmental change to improve shelf life (McMillin 2008).

Recently, attention has been directed toward the development of degradable biopolymer films as alternatives to synthetic food packaging films because of waste disposal problems associated with the latter. However, instability at high moisture levels has meant that most edible films are used in conjunction with conventional packaging. Among the former, chitosan and polylactic acid films show promise, particularly chitosan, which can be strongly antimicrobial (Rao and Sachindra 2002; Han and Gennadios 2005; McMillin 2008). A number of these types of films have been shown to reduce lipid oxidation, enhance color retention, and improve the microbial stability of meats (Cutter 2006). Antimicrobial agents with potential for use in conjunction with food packaging materials include: organic acids, acid salts and anhydrides, parabenzoic acids, alcohol, bacteriocins, fatty acids and their esters, chelating agents, enzymes, metals, antioxidants, sterilizing gases, polysaccharides, phenolics, spice and herbal extracts, and probiotics (Quintavalla and Vicini 2002; Cutter 2006).

Nerín and others (2006) applied a plastic film with an embedded rosemary extract (RE) and observed that both myoglobin and lipid oxidation in beef were inhibited, leading to extended display life of meat. Camo and others (2008) investigated the effect of active antioxidant packaging on the extension of lamb display life and observed enhanced oxidative stability of lamb steaks by directly spraying a RE on the meat surface, as well as by packaging meat in films containing rosemary or oregano. They indicated that active films with oregano were the most effective and that these extended fresh odor and color from 8 to 13 d compared to the control.

Application of LAB

LAB have been studied in fresh meat for their ability to inhibit spoilage and pathogenic bacteria. Djenane and others (2006) observed that inoculation of beef with 2 LAB strains (*Lactobacillus sakei* CTC 372 or *Lactobacillus* CTC 711) inhibited the growth of spoilage bacteria. In contrast, Hoyle Parks and others (2012) confirmed that LAB (a mixture of 2 lactobacilli and one strain each of *Pediococcus* and *Lactococcus*) had no effect on the organoleptic shelf life and stability of traditionally or high-oxygen MAP packaged ground beef patties. They concluded that LAB have the potential to be used in ground beef to improve safety without altering spoilage indicators. A number of studies have shown that LAB can inhibit *Escherichia coli* O157:H7 and *Salmonella* spp., but results have not been uniformly successful. Smith and others (2005) observed a reduction of *E. coli* O157:H7 and *Salmonella* by application of LAB in ground beef. After 3 and 5 d of storage at 5 °C, LAB caused a 2 log and 3 log reduction of *E. coli* O157:H7, respectively, compared with the control. In addition, after 5 d, *Salmonella* counts were reduced to nondetectable levels and no adverse effects of LAB on the sensory properties of the ground beef were noted. The authors concluded that foodborne pathogens may be controlled by the addition of LAB to raw ground beef stored at refrigeration temperatures. Hoyle and others (2009) found that direct addition of LAB to ground beef decreased numbers of *E. coli* O157:H7 and *Salmonella* during storage and proposed their use as a processing intervention for control of *E. coli* O157:H7 and *Salmonella* in traditional overwrap (TOP) and MAP without masking microbial spoilage. Ruby and Ingham (2009) found that a *L. sakei* strain (10-EGR-a), the most inhibitory from among 12 ground beef *Lactobacillus* isolates, was unable to inhibit growth of *E. coli* O157:H7 and multidrug-resistant (MDR) *Salmonella* (serovars Newport and Typhimurium) in fresh raw ground beef stored at 5 and 10 °C. The conflicting results of pathogen inhibition by LAB probably result from differences in characteristics of the LAB evaluated.

Meat species

The predominant microorganisms, which occur and multiply on the surface of fresh meats can vary somewhat among meat species and are influenced to the largest extent by the storage environment. The pseudomonads are ubiquitous on most refrigerated meats and are most frequently involved in aerobic spoilage (Jay and others 2003). Nychas and others (1998) found that meat (beef, pork, and lamb) stored under vacuum, or in atmospheres enriched with CO_2, N_2, or O_2 was dominated by LAB and *B.*

thermosphacta rather than pseudomonads. García-López and others (1998) pointed out that on chilled meat, certain species of psychrotrophic *Enterobacteriaceae* normally appear. Since they are able to grow aerobically/anaerobicaly on adipose tissue and on muscle tissue of high pH (>6), these organisms appear to be more prevalent on pork and lamb. In vacuum packages, the shelf life of beef is longer than that of pork and is related to the lower numbers of bacteria initially present on beef rather than to differences in the types of organisms present (Borch and others 1996). The major organisms present in high-pH pork were *Enterobacteriaceae* and *S. putrefaciens* as shown by Whitfield (1998), although these may also be present as dominant organisms on other fresh meats when stored at >9 °C or in packages with low but measurable O_2, respectively. The occurrence of *Enterobacteriaceae* on meats can be reduced by improved handling and storage hygiene.

Effect of organic acids, their salts, and hot water

A large number of studies have shown that the shelf life of fresh meat can also be prolonged by the use of organic acids and their salts as antimicrobial sprays or dips (Castillo and others 2001; Calicioglu and others 2002; Mustapha and others 2002; Stivarius and others 2002; Ahmed and others 2003; Ikeda and others 2003; Sallam and Samejima 2004; Bosilevac and others 2006; González-Fandos and Dominguez 2006; Serdengecti and others 2006); and this was also shown in a review on fresh beef cuts by Jamilah and others (2008). Uyttendaele and others (2001) found that decontamination with 1% and 2% buffered lactic acid (LA), sterile water, or with no treatment (control) resulted in a gradual reduction of *E. coli* O157:H7 during 10 d of storage at 4 °C in the 3 treatments, which indicated that the pathogen was not reduced appreciably by LA. Djenane and others (2003b) investigated the shelf life of beef steaks treated with LA (1.5%) and antioxidants (0.1% RE and 0.05% ascorbic acid) stored under MAP (60% O_2/40% CO_2 or 70% O_2/20% CO_2/10% N_2) and observed that both 40% CO_2 and the LA treatment significantly inhibited growth of LAB, *B. thermosphacta, and Pseudomonas* spp. Jensen and others (2003) observed that lactate/diacetate significantly lowered aerobic plate counts (APC) on pork chops compared to unpumped control chops after a 96-h display. They concluded that the mixture of lactate/diacetate extended shelf life more than the lactate or acetate alone. The combination of organic acid salts seems more efficient than the application of single salt. Huang and others (2005) investigated the retail shelf life of pork dipped in ascorbic acid (500 ppm) or citric acid (250 ppm) and then packaged in several ways. Samples were VP, subjected to air-permeable packaging, MAP (20% CO_2:80% N_2) packaging, as well as with dynamic gas exchange to yield a high O_2 atmosphere (20% CO_2:80% O_2), and they observed reduced psychrotrophic microbial numbers following ascorbic acid dipping. It was found by Naveena and others (2006) that when buffalo steaks were dipped in either a mixture of LA/clove or LA/clove/vitamin C, the display life of the steaks was extended at 4 ± 1 °C. Compared to control and LA-treated buffalo steaks, treatment with LA/clove significantly reduced (1 to 2 log) microbial numbers without influencing meat color and odor after 12 d of display. No difference in microbial numbers was observed between LA/clove and LA/clove/vitamin C-treated samples. Özdemir and others (2006) found that *Salmonella* Typhimurium (ST) and *Listeria monocytogenes* inoculated at 7 log CFU/g on beef were reduced on day 5 of storage at 4 °C to 0.43 log by 1% LA; to 1.78 log by hot water (HW, 82 °C) followed by 2% LA; to 1.69 log by HW; and to 3.84 log by HW followed by 2% LA. Their study suggested that LA and HW treatments could be an additional safety hurdle during production by reducing *S.* Typhimurium and *L. monocytogenes* numbers.

A number of studies (Castillo and others 1998, 1999, 2001; Calicioglu and others 2002; Mustapha and others 2002; Stivarius and others 2002; Ahmed and others 2003; Ikeda and others 2003; Sallam and Samejima 2004; Bosilevac and others 2006; González-Fandos and Dominguez 2006) also showed that total APC, *Enterobacteriaceae*, coliforms, ST, *L. monocytogenes,* and *E. coli* O157:H7 (EC) on beef, sheep, goats, and poultry could be reduced substantially following 2% to 5% LA, 2% polylactic acid, 2% acetic acid, or combinations of 2.5% sodium acetate, 2.5% sodium citrate, and 2.5% sodium lactate, or by 2.5% potassium sorbate, or hot water (82 to 95 °C) spray treatments. Although organic acids were, in general, more effective for reducing EC, coliforms, and APC than hot water, they reduced ground beef redness (Stivarius and others 2002). In commercial practice LA has become the most commonly used organic acid applied prior to evisceration for sanitizing whole carcasses (Bosilevac and others 2006). In a comparison to establish minimum thresholds for interventions, Bosilevac and others (2006) concluded that a hot water wash at 74 °C was better than a 2% LA spray for reduction of EC on beef carcasses. The main concerns with the treatment of organic acids, their salts, and hot water, are the discoloration and off-odor induced by these chemicals.

Fresh Meat Spoilage Resulting from Biochemical Reactions

Meat spoilage is not only caused by extrinsic factors such as microbial activity, but also by intrinsic factors such as lipid oxidation. Lipid oxidation is one of the most common reasons for meat deterioration, which leads to discoloration, drip loss, off-odor, and off-taste, and may even produce potentially toxic compounds (Morrissey and others 1998). Even though many psychrotrophic bacteria produce lipases, the role of bacteria in the lipolytic and oxidative changes that occur in meat is not well established (García-López and others 1998).

Lipid oxidation in meat is a chain reaction where unsaturated fats are oxidized by free radical autoxidation, catalyzed by the products of the reaction. Where the number of double bonds in the fatty acid is high, the rate of oxidation is usually faster. Among the factors consumers use to evaluate meat quality, the color of meat is a primary indicator (Faustman and Cassens 1990; Zerby and others 1999). Changes in meat color are closely related to lipid and pigment oxidation (Buckley and others 1995), although excessive bacterial load may also play a role (Brewer and others 2002; Martínez and others 2006). Meat color changes from an acceptable cherry-red to undesirable brown in beef when oxymyoglobin is oxidized to metmyoglobin (Chan and others 1995). Gill (1996) indicated that steps to slow or prevent the formation of brown metmyoglobin at the muscle surface are most productive in improving color stability. Color change is influenced by the various forms of myoglobin present in response to different oxygen partial pressure at the meat surface, with metmyoglobin in aerobic packages preferentially forming a few millimeters below the meat surface. Meat color also depends upon the distance of oxygen penetration into the meat; the red color perceived is determined by the depth of the oxymyoglobin layer (Belitz and others 2004). Mancini and Hunt (2005) and McMillin (2008) comprehensively reviewed current research in meat color and focused on enzymatic factors that affect myoglobin chemistry, pigment redox stability, and effects of MAP on color stability. Oxidation of myoglobin in high (80%) O_2 + 20% CO_2 packaged fresh meats is the main factor, rather than bacterial activity, which limits shelf life of these

products to ≤16 d.

Effect of muscle source

Muscle color stability is mainly a function of the balance between metmyoglobin reducing activity (MRA) and oxygen consumption rate (OCR). Depending upon the muscle source, different muscles demonstrate different postmortem OCRs and subsequent color stability (Hood 1980; O'Keefe and Hood 1982; Renerre and Labas 1987), which are inversely related (Atkinson and Folett 1973; Lanari and Cassens 1991). Additionally, muscles with greater MRA are generally more color stable than those with less MRA during retail display (Hood 1980; ÓKeefe and Hood 1982; Ledward 1985). Mitochondrial enzymes, especially cytochrome c oxidase, which continue to consume oxygen postmortem control meat OCR and pigment reduction (Tang and others 2005). In meat, consumption of oxygen, otherwise available to bind to myoglobin, results in the formation of deoxymyoglobin, which more rapidly leads to metmyoglobin formation, and these reactions are both accelerated by mitochondrial action (Tang and others 2005).

McKenna and others (2005) investigated the effect of retail display time on the discoloration of 19 bovine muscles. Reducing capacity, the sum of MRA plus resistance to induced metmyoglobin formation (RIMF), and OCR as well as oxidative rancidity were evaluated. They concluded that muscles with high color stability usually had high RIMF, high nitric oxide-reducing ability, high O_2 penetration depth, and possessed low OCRs and oxidative rancidity. It was shown that differences in discoloration between muscles were related to the amount of reducing activity relative to the OCR.

Seyfert and others (2006) investigated muscle MRA, total reducing activity (TRA), and cytochrome c oxidase activity of 5 bovine muscles. Their work confirmed that color stability was different among muscles and that, in terms of explaining the role reducing activity plays in muscle color stability, MRA was more useful than TRA. Seyfert and others (2007b) further confirmed that color stability and reducing activity were different among muscles when packaged in MAP containing 20% or 80% O_2, even with or without 0.4% CO.

Effect of MAP on biochemical reactivity

For red meat packaged under aerobic conditions, lipid oxidation occurs at the same rate as discoloration, but it is faster than microbial growth, which makes it an important factor influencing meat spoilage (Jakobsen and Bertelsen 2000). O'Keefe and Hood (1980–1981) indicated that to prevent discoloration of beef, O_2 levels below 0.1% in the package were required. Meat retains the purple color of reduced myoglobin during storage and "blooms" to a bright red color on re-exposure to air, when O_2 is maintained at a sufficiently low level in a package with CO_2 or N_2.

Jeremiah and others (1992) found that when red meat was packaged under vacuum or CO_2 and stored at chiller temperatures for extended periods, undesirable color changes may be caused by oxidative and/or autolytic reactions. However, pork was resistant to such deterioration under these conditions. Behrends and others (2003) evaluated visual and chemical attributes of muscles and beef steaks from various (United States Department of Agriculture, UDSA) quality grades packaged in high O_2 (80% O_2/20% CO_2) MAP and found that the high O_2 condition increased color stability of the samples. This finding was further confirmed by Seyfert and others (2007b) who indicated that color stability of bovine muscles increased with increasing O_2 level in MAP from 20% to 80% during 7 d of retail display. They also found higher O_2 levels decreased variability among muscles, which increased display case uniformity. Hence, high O_2 MAP is an alternative approach for increasing meat color stability. However, different meat species react differently to high O_2 atmosphere. Cayuela and others (2004) investigated oxidative stability of fat by using vacuum and modified atmospheres with a high O_2 concentration (70%). They found that the latter acted as a pro-oxidation factor for both fatty acids and cholesterol. The use of high O_2 atmosphere provided no significant benefit regarding meat color. It was found that vacuum packaging increased oxidative stability of fresh pork loins, which in turn yielded greater color stability, and thus increased meat shelf life.

The desirable bright red color of fresh meat can be maintained longer when packed in high O_2 MAP than when packed in air and stored at 4 °C (Penney and Bell 1993). High O_2 MAP also prevented growth of anaerobic pathogens (Ogrydziak and Brown 1982). However, the resulting increased lipid oxidation was a disadvantage that caused quality loss in meat during such storage (Renerre and Labadie 1993), especially when antioxidants such as vitamin E were low in the animal diet. It is of interest that anaerobic pathogens have not proven to be problematic in low O_2-packed fresh meats when properly refrigerated (Gill and Gill 2005).

The more rapid oxidation of lipids caused by high package concentrations of O_2, which leads to negative sensory and nutritional qualities of meat, is a major disadvantage associated with this type of MAP. Meat oxidation has been comprehensively reviewed by Kanner (1994), Gray and others (1996), Morrissey and others (1998), Mancini and Hunt (2005), and McMillin (2008).

The generation of toxic compounds such as cholesterol oxidation products (COPs) is another detrimental effect of lipid oxidation. Concern over COPs in foods has arisen since they have biologically adverse effects, particularly regarding the onset of degenerative diseases such as atherosclerosis and cancer (Schroepfer 2000; Osada 2002).

Ledward (1984) established that the conversion of oxymyoglobin to metmyoglobin accelerated meat discoloration. Incorporation of CO in the package atmospheres at low O_2 levels forms MbCO, which stabilizes red color (Clark and others 1976; Silliker and Wolfe 1980; Luño and others 1998). However, Seyfert and others (2007b) found that at O_2 levels in MAP from 20% to 80%, 0.4% CO did not influence color stability, MRA, or O_2 consumption. They attributed this to the greater formation of oxymyoglobin in atmospheres containing 20% to 80% O_2, which dominated or limited the ability of MbCO to form. More consistent color stability of beef steaks resulted from 0.4% CO use (35% CO_2/69.6% N_2) when compared with high-oxygen packaging (80% O_2/20% CO_2) (Mancini and others 2009).

The use of CO for food packaging is not allowed in most countries since CO is toxic. Nevertheless, according to Sørheim and others (1997, 1999), the Norwegian meat industry has been using gas mixtures containing 0.3% to 0.4% CO for over 20 y. The toxicological aspects of CO used in MAP of meat were reviewed by the above-mentioned researchers and they concluded that, with up to about 0.5% of CO, no human toxicity was likely. Lipid oxidation was also suppressed by CO when compared with aerobic overwrap packaging. The U.S. Food and Drug Administration (FDA) has approved the use of CO as a color fixative in retail case-ready meats (USFDA 2004). Cornforth and Hunt (2008) concluded that the major disadvantages of CO-MAP were the negative image of CO held by consumers because of its potential toxicity (when inhaled)

and that meats might look fresh even if bacterial levels were high and the product was spoiled. In view of these concerns about CO, work has continued to investigate the use of CO in fresh meat preservation. Grobbel and others (2008) found that packaging meat in ultra-low O_2 with CO (0.4% CO/35% CO_2/69.6% N_2) MAP was an effective way to maintain red beef color. Mancini and others (2009) observed that the darkening effects of lactate treatment were not counteracted by packaging steaks in 0.4% CO (30% CO_2/69.6% N_2). Important findings in extending the shelf life of fresh meats by MAP alone and by other treatments since 2000 are summarized in Table 4.

Effect of adding antioxidants

Meats contain traces of natural antioxidants such as vitamin E. By acting as hydrogen or electron donors, antioxidants prevent lipid oxidation and interrupt the radical chain reaction by forming nonradical compounds. However, the practical value of naturally occurring antioxidants in meat has been questioned since some of these antioxidants have been shown to produce inconsistent effects (Morrissey and others 1998).

Dietary supplementation with antioxidants in several animal species has been found to be an effective way to inhibit lipid oxidation. Phillips and others (2001) investigated effects of diets containing α-tocopherol on lipid oxidation and found that lipid oxidation was delayed and pork color was not affected. Formanek and others (2001) and Gatellier and others (2001) also observed similar results with beef. Lauzurica and others (2005) and Ripoll and others (2011) found increased lipid and color stability of lamb by diets supplemented with vitamin E. O'Grady and others (2006) investigated the effect of supplementation of beef cattle diets with tea catechins (TC) (1000 mg/d) and RE (1000 mg/d) for 103 d preceding slaughter on the oxidative stability of *longissimus dorsi* (LD) steaks and observed that after aerobic or MAP storage (80% O_2/20% CO_2), for up to 8 d at 4 °C, dietary supplementation with TC and RE did not significantly increase total plasma antioxidant status and lipid stability. However, direct addition of TC (1000 ppm) and RE (1000 ppm) significantly improved the color and lipid stability in LD patties stored under the same conditions. Nieto and others (2010a) investigated the effect of including distilled rosemary leaf (10% and 20%) in the diet of pregnant ewes on the oxidative stability of lamb meat stored in modified atmosphere (70% O_2/30% CO_2) for up to 21 d and observed delayed lipid oxidation. Later Nieto and others (2010b) investigated the effect of diets containing thyme leaves (TL, 3.7% and 7.5%, w/w) under the same conditions and observed that the presence of TL also delayed lipid oxidation, and the effect was more significant at the higher level of TL (7.5%). Luciano and others (2009) conducted an experiment to compare the antioxidative effect of a concentrate-based diet and a fresh herbage-based diet (vetch) on minced raw lamb stored over 14 d in a high-oxygen MAP (80% O_2/20% CO_2), which normally promotes oxidative stress in meat, and were encouraged that lipid oxidation was lower in meat from the herbage-based diet. Therefore, it can be speculated that fresh herbage contains bioactive substances, which protected raw lamb meat from oxidation.

Spraying antioxidants on meat surfaces is another widely used technique to protect meat from lipid oxidation. Djenane and others (2002) have conducted quite a few projects to investigate antioxidants on lipid oxidation. They first examined the antioxidant effects of rosemary, α-tocopherol, and taurine, in combination with vitamin C, on beef steaks and found that oxidative deterioration of the meat was significantly delayed by surface application in combinations. Combinations of either rosemary or taurine with vitamin C significantly extended the shelf life of fresh beef steaks by about 10 d and rosemary was observed to be the most effective treatment in delaying lipid oxidation. Djenane and others (2003a) then observed that the rates of metmyoglobin formation and lipid oxidation were significantly reduced by the combined application of rosemary and vitamin C in the absence of UV light, and the display life was extended from about 10 to 20 d. Djenane and others (2003b) later observed that oxidation of both myoglobin and lipids was significantly delayed by antioxidants (0.1% RE and 0.05% ascorbic acid), which usefully extended the shelf life of beef steaks. In addition, Djenane and others (2004) investigated the antioxidant effects of carnosine and carnitine in fresh beef steaks and found that surface sprays of carnosine (50 mM) or ascorbic acid (500 ppm) alone resulted in a notable delay in onset of oxidative deterioration. The best antioxidant effect against meat deterioration during 28 d storage was achieved by the combination of carnosine with ascorbic acid (50 mM/500 ppm). However, no antioxidant effect by the combination of carnitine and ascorbic acid was observed. Fasseas and others (2007) observed that sage essential oil (3%, w/w) and oregano (3%, w/w) treatment of porcine and bovine ground meat significantly reduced the oxidation of samples after 12 d of storage at 4 °C. The most recent research was conducted by Karabagias and others (2011), who investigated the effect of thyme (TEO) and oregano (OEO) essential oils, as well as MAP, in extending the shelf life of fresh lamb meat and observed that microbial populations were reduced up to 2.8 log CFU/g on day 9 of storage with the most pronounced effect being achieved by the combination of MAP (80% CO_2/20% N_2) plus TEO (0.1%). TBA values varied for all treatments and remained lower than 4 mg MDA/kg throughout storage. Compared to air-packaged samples, the shelf life of lamb meat containing 0.1% TEO, and MAP-packaged samples containing 0.1% TEO, was extended 2 to 3 d and 14 to 15 d at 4 °C, respectively.

Effect of organic acids, their salts, and hot water on color

Treatment with organic acids and their salts has been studied as a means to retard bacterial deterioration and extend distribution time and display life of fresh meat (Bauernfeind and Pinkert 1970). However, it was reported that organic acids caused discoloration and pungent odors in meats (Mountney and O'Malley 1965; Reynolds and Carpenter 1974). Injection is an effective way to include organic acids or their salts in meats to improve the color stability. Injection-enhanced beef color and color stability were also obtained with calcium (2.4% w/v) or potassium lactates (60% w/v) (Lawrence and others 2004; Mancini and others 2005; Kim and others 2006, 2009). Sodium acetate (0.25%) has also been used in injection-enhanced pork to improve color stability (Jensen and others 2003; Livingston and others 2004). Further, a combination of potassium lactate (1.83%)/potassium diacetate (0.17%) has been used to improve the color stability of moisture-enhanced pork (Jensen and others 2003). Injecting a solution containing a combination of potassium lactate (1.5%), sodium chloride (0.3% or 0.6%), sodium tripolyphosphate (0.3%), and sodium acetate (0.1%) into beef rib steak stabilized color life and decreased surface shine (Knock and others 2006). Seyfert and others (2007a) observed improved color stability and metmyoglobin-reducing activity in ground beef following treatment with LA salts and sodium acetate. Additional reduction of surface shininess seemed to result from including sodium acetate (0.1%), which made beef steaks treated with potassium lactate (1.5%) more attractive to consumers because steaks had a better "fresh beef" appearance (Knock

and others 2006). Tumbling meats with organic acids and their salts is another way to enhance their color stability. Exposure of beef pieces by tumbling before grinding with single agents (0.5% cetylpyridinium chloride, 200 ppm chlorine dioxide, 0.5% LA, or 10% trisodium phosphate) or multiple combinations and sequences of antimicrobial agents: (1) 0.5% cetylpyridinium chloride followed by 10% trisodium phosphate; (2) 200 ppm chlorine dioxide followed by 0.5% cetylpyridinium chloride; (3) 200 ppm chlorine dioxide followed by 10% trisodium phosphate; or (4) 2% LA followed by 0.5% cetylpyridinium chloride were found to improve bulk ground beef color, odor, and shelf life (Jimenez-Villarreal and others 2003a,b,c).

Suman and others (2010) found that the color-stabilizing effect of lactate on refrigerated ground beef was dependent on package atmosphere. They incorporated 2.5% potassium lactate into ground beef patties and observed increased surface redness, reduced discoloration, and darkened color in all treatments which included: aerobic packaging in polyvinyl chloride (PVC), high O_2 MAP (HIOX, 80%O_2/20%CO_2), CO MAP (CO MAP, 0.4% CO/19.6% CO_2 /80% N_2), and vacuum. However, lactate did not stabilize the color of ground beef in CO MAP. Further, irrespective of lactate treatment, patties stored in CO MAP showed less discoloration and greater surface redness than in PVC and HIOX.

In addition, color stability of fresh meats can be related to the extent of lipid oxidation. Citric acid has been found to increase lipid oxidation (Cheah and Ledward 1997) due to its pH-decreasing effects. However, Huang and others (2005) observed improved lipid stability by dipping meat in citric or ascorbic acid and then packaging in gas exchange MAP (80% N_2/20% CO_2 followed by 80% O_2/20% CO_2), but the gradually increased O_2 levels led to more lipid oxidation than reported for air-permeable packaging. Ke and others (2009) reported inhibition of lipid oxidation in ground beef acidified with citric acid for 2 d when pH was readjusted with sodium tripolyphosphate or sodium carbonate to values equal to or greater than the untreated raw beef muscle. They suggested that the best acid marination technique for beef would be citric acid since it is effective at both reducing lipid oxidation and improving texture.

Conclusion

The shelf life of packaged fresh meats is primarily influenced by the action of the meat microflora, oxidation of lipids, and myoglobin. Microbial enzymatic and metabolite effects on shelf life are less important in determining the shelf life of high-O_2-packed fresh meats than color deterioration. Efficient chill-chain technology enables cutting, packaging, transportation, and retail marketing of fresh meats under suitable refrigeration. MAP technology through different combinations of O_2, CO_2, N_2, and CO has made it possible to design package environments to satisfy different product and distribution requirements. Some organic acids and their salts can be effective treatments to inhibit microbial spoilage and delay color deterioration. However, European regulations do not recognize meats directly treated with chemicals as being fresh meats. Alternatively, vitamin E and natural antioxidants (clove, oregano, sage, rosemary, and thyme) can be used in livestock diets to delay lipid oxidation in harvested meat. Active packaging holds promise to improve the safety and quality of fresh meats, but commercialization awaits further validation of benefits amidst regulatory uncertainty. Although these technologies can markedly extend shelf life of packaged fresh meats, spoilage caused by microbial growth and biochemical activities is still inevitable during extended storage. There has been considerable progress in the past 10 y to adapt packaging to maximize the safety and quality of meat during fabrication, distribution, and retail display. Further work to expand our understanding will support efforts both preslaughter (animal husbandry, nutrition, feed contamination) and postslaughter (packaging innovation, nanotechnology) to improve meat shelf life and safety.

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