Introduction

In purchasing fresh retail meat, the microbiological shelf life, color and general appearance are important features to the consumers. Underlying these features, food safety and product confidence has been increasingly emphasized. Growth of spoilage and pathogenic bacteria is generally reduced by stringent hygienic standards, low storage temperatures and by using modified atmospheres packaging (MAP), the latter by increased levels of carbon dioxide (CO₂) and/or removal of oxygen (O₂). Color of meat is very important at the point of purchase, and establishing and maintaining a bright red and attractive color during retail display is still a challenge. Usually, meat is wrapped in an O₂ permeable film that allows blooming, but results in a poor color stability. An improved, but still limited color stability is achieved by packaging meat in high O₂ atmospheres, with minimum 60 % O₂. High O₂ packaging currently has 10 – 40 % of the retail red meat market share in most countries in Western Europe, but is less common in other parts of the world.

Carbon monoxide (CO) has the ability to form a stable bright red or cherry red color of meat, even in very low concentrations. The color properties of CO were known over 100 years ago, when a patent on a CO₂/CO gas mixture for meat was granted (cited in Church, 1994). In 1985, the Norwegian meat industry started to use a CO mixture with 0.3 – 0.5 % CO/60 – 70 % CO₂/30 – 40 % nitrogen (N₂). This low CO packaging has grown to a current market share of 60 % of the Norwegian retail red meat market. CO in concentrations of 0.5 % or less is temporarily allowed for meat packaging in Norway. However CO is not permitted for this purpose in the USA and European Union (EU) (Cornforth, 1994; European Parliament and Council Directive, 1995).

The aim of this review is to address the different ways of using CO in meat packaging, in particular aspects related to color, microbiological spoilage, pathogenic bacteria, toxicology, workers’ safety and food regulations. In view of the controversial nature of CO, we will try to sort out some myths and facts, and discuss future developments in the use of CO.

Research on CO Packaging

A number of reports have been published on packaging and treatment with atmospheres containing CO. The use of CO in retail meat can be divided into three categories:

- packaging and storage in low CO concentrations, 0.1 – 2.0 %, mainly for color purposes (Table 1)
- packaging and storage in high CO concentrations, 5 – 100 %, both for color and antimicrobiological purposes (Table 1)
- pretreatment with high CO concentrations, 5 – 100 %, followed by vacuum packaging and storage, affecting both color and microbiological status (Table 2)

Color

The main function of CO in MAP of meat is to make a stable, bright red color, as a result of the strong binding of CO to deoxymyoglobin and formation of carboxymyoglobin. The spectrum of carboxymyoglobin is very similar to that of oxy-myoglobin (Tappel, 1957), but carboxymyoglobin is more resistant to oxidation (Lanier et al., 1978). CO in concentrations of one and five percent increased the reduction of metmyoglobin, even in the presence of air (Lanier et al., 1978). Without CO present, meat in CO₂/N₂ atmospheres is vulnerable to discoloration by myoglobin oxidation due to residual O₂, occurring in beef with less than 0.1 % O₂ (Gill & McGinnis, 1995).

Several studies have documented that low concentrations of CO, 0.1 – 2.0 %, improve meat color and color stability.
(Table 1). These reports include meat of beef, pork and poultry. The color improvement by CO seems to be valid if the other gases in the atmospheres are CO₂, N₂, O₂ or air. When increasing the CO concentration to 2%, the color was characterized as "too artificial" by a sensory panel (Renerre & Labadie, 1993). Therefore, concentrations of 0.4 – 1.0% CO can be regarded as sufficient and suitable for color purposes by MAP of meat.

By including 2% CO in air, El-Badawi et al. (1964) managed to stabilize the color of beef for 15 days, compared to five days in air only. Experiments of beef packaged in 0.1 – 10% CO in N₂, showed that concentrations of 0.5% or more stabilized color for more than 30 days (Clark et al., 1976). Studies of beef and pork packaged in a 0.4% CO/60% CO₂/40% N₂ mixture, resulted in a bright red color of the meat until termination of the microbiological shelf life (Sørheim et al., 1999). The color quality and stability of beef steaks was better in the CO mixture than in a 70% O₂/30% N₂ mixture or vacuum at both 4 and 8 °C. Steaks in the high O₂ atmosphere had a bright red color initially, but it deteriorated during storage, more rapidly at 8 than 4 °C. Bone blackening in pork chops was prevented by packaging in a 0.4% CO mixture, probably as a result of CO binding to hemoglobin in the bone marrow (Sørheim et al., 1999). Luño et al. (1998, 2000) recommended to pretreat beef steaks with 5% CO for 24 hours resulting in a high color stability, but not exceeding the microbiological shelf life of the meat. In contrast, pretreatment of beef with CO followed by storage under an O₂ permeable film, did not improve color stability (Clark et al., 1976). Thus, continued storage under anaerobic conditions seems crucial to preserve the bright red carboxymyoglobin.

The penetration of CO and depth of formation of carboxymyoglobin in the meat is dependent on the concentration of CO in the atmosphere, the length of CO exposure, and the structure of the meat (Jayasingh et al., 2001). While 0.5% CO in ground beef penetrated at least 15 mm in one week, CO of the same concentration penetrated 3.5 mm in beef loin steaks after one week and 12 mm after eight weeks.

TABLE 1. Use of carbon monoxide (CO) in modified atmosphere packaging and storage of fresh meat.

<table>
<thead>
<tr>
<th>Gas combinations (%)</th>
<th>CO</th>
<th>CO₂</th>
<th>N₂</th>
<th>O₂</th>
<th>Air</th>
<th>Specie</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>98</td>
<td>50</td>
<td>49</td>
<td>51</td>
<td>49</td>
<td>Beef</td>
<td>El-Badawi et al., 1964</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>51</td>
<td>50</td>
<td>49</td>
<td>51</td>
<td>Beef</td>
<td>Gee &amp; Brown, 1978</td>
</tr>
<tr>
<td>1 &amp; 5</td>
<td>95 &amp; 99</td>
<td>50</td>
<td>51</td>
<td>50</td>
<td>49</td>
<td>Beef</td>
<td>Lanier et al., 1978</td>
</tr>
<tr>
<td>0.1 – 10</td>
<td>90-99.9</td>
<td>50</td>
<td>51</td>
<td>50</td>
<td>49</td>
<td>Beef</td>
<td>Clark et al., 1976</td>
</tr>
<tr>
<td>1</td>
<td>51</td>
<td>18</td>
<td>30</td>
<td>20</td>
<td>9</td>
<td>Beef</td>
<td>Seidemann et al., 1979</td>
</tr>
<tr>
<td>0.1 – 1.0</td>
<td>25.0 – 25.9</td>
<td>49</td>
<td>50</td>
<td>49</td>
<td>49</td>
<td>Beef</td>
<td>Luño et al., 1998; 2000</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>9</td>
<td>70</td>
<td>20</td>
<td>9</td>
<td>Beef</td>
<td>Luño et al., 1998</td>
</tr>
<tr>
<td>2</td>
<td>78</td>
<td></td>
<td></td>
<td>20</td>
<td>9</td>
<td>Beef</td>
<td>Renerre &amp; Labadie, 1993</td>
</tr>
<tr>
<td>0.4</td>
<td>40</td>
<td></td>
<td></td>
<td>60</td>
<td>40</td>
<td>Beef &amp; pork</td>
<td>Sørheim et al., 1999</td>
</tr>
<tr>
<td>0.4</td>
<td>40</td>
<td></td>
<td></td>
<td>60</td>
<td>40</td>
<td>Beef</td>
<td>Nissen et al., 2000; Sørheim et al., 2001</td>
</tr>
<tr>
<td>0.5</td>
<td>49.5</td>
<td></td>
<td>50</td>
<td>50</td>
<td>49.5</td>
<td>Turkey</td>
<td>Fraqueza et al., 2000</td>
</tr>
<tr>
<td>0.5</td>
<td>39.5</td>
<td></td>
<td>50</td>
<td>50</td>
<td>39.5</td>
<td>Beef</td>
<td>Jayasingh et al., 2001</td>
</tr>
<tr>
<td>1 &amp; 10 &amp; 100</td>
<td>69 &amp; 90 &amp; 99</td>
<td></td>
<td>60</td>
<td>60</td>
<td>39.5</td>
<td>Beef &amp; poultry</td>
<td>Tsemakhovich &amp; Shaklai, 2000</td>
</tr>
</tbody>
</table>

TABLE 2. Use of carbon monoxide (CO) for pretreatment of fresh beef.

<table>
<thead>
<tr>
<th>Gas combinations (%)</th>
<th>CO</th>
<th>CO₂</th>
<th>N₂</th>
<th>O₂</th>
<th>Exposure time</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>99</td>
<td>2-16 h</td>
<td></td>
<td>100</td>
<td>3 h</td>
<td>Clark et al., 1976</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>1 h</td>
<td></td>
<td>100</td>
<td>30 min</td>
<td>Rozbeh et al., 1993; Brewer et al., 1994</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>24 h</td>
<td></td>
<td>100</td>
<td>1 h</td>
<td>Jayasingh et al., 2001</td>
<td></td>
</tr>
</tbody>
</table>

a Meat frozen after pretreatment.
Exposure to CO as one of the causes for pink discoloration in cooked, uncurved meat has been studied extensively, in particular for poultry (for reviews see Maga, 1994; Comforth, 1994). Persistent redness in cooked ground beef patties was influenced by CO (Sørheim et al., 2001). Patties containing carboxymyoglobin were cooked to an end point temperature of more than 80 °C while still having traces of pink color and an uncooked appearance. However, the pink color faded rapidly when the patty surfaces were exposed to air.

The color of cooked meat products can also benefit from exposure to CO, as 1 % CO in a N₂ atmosphere stabilized the color of sliced bologna (Aasgaard, 1993). The reason for this color improvement is not known, but CO may bind to partly denatured myoglobin.

**Microbiological Spoilage and Food Safety**

In MAP of meat, the effects of low concentrations of CO on microorganisms seem to be of either no or minor importance. However, Clark et al. (1976) found that by adding 0.5 – 10 % CO to N₂ atmospheres, the shelf life based on odor was extended and the growth of psychrotrophic bacteria was reduced at 0, 5 and 10 °C. In the evaluation of antimicrobiological effects of CO, considerations to other gases in the gas mixtures must be made. In most MAP of meat, bacteriostatic CO₂ in levels of 20 - 100 % is usually present. Low concentrations of 0.4 and 1 % CO did not affect microbiological loads of meat when applied in combination with atmospheres containing CO₂ (Sørheim et al., 1999; Luño et al., 1998). Therefore, in gas mixtures containing high levels of CO₂, the possible antimicrobiological effect of low CO concentrations is likely to be overshadowed by CO₂.

The microbiological benefits of using CO for MAP of meat is two-fold: O₂ can be omitted from the atmosphere, and concentrations of CO₂ can be high, from 60 to near 100 %. The absence of O₂ inhibits the growth of aerobic spoilage bacteria. In storage experiments of beef steaks, ground beef and pork chops in a 0.4 % CO/ 60 % CO₂/ 40 % N₂ gas mixture, the shelf life, as evaluated by off-odor, increased within two to seven days compared to storage in a 70 % O₂/ 30 % CO₂ mixture at 4 and 8 °C (Sørheim et al., 1999). The microflora of the meat in the CO gas mixture was dominated by lactic acid bacteria, and this gas mixture reduced the growth of spoilage flora of *Brochothrix thermosphacta* and pseudomonads. Nissen et al. (2000) studied the growth of pathogens in ground beef stored in 0.4 % CO/ 60 % CO₂/ 40 % N₂ at 4 and 10 °C. The CO mixture reduced the growth of *Yersinia enterocolitica* and *Listeria monocytogenes* compared to a 70 % O₂/ 30 % CO₂ atmosphere and chub packages. *Escherichia coli* O157:H7 was inhibited at 10 °C. However, growth of *Salmonella* spp. was not reduced in meat in the CO mixture at 10 °C, which emphasizes the importance of low storage temperatures for inhibiting these pathogenic bacteria.

Exposure of pure bacterial cultures to high CO concentrations of 5 - 30 % in air inhibited the growth of *E. coli*, *Achromobacter* and *Pseudomonas fluorescens*, but *Pseudomonas aeruginosa* was not affected (Gee and Brown, 1980). In another study, continuous storage of meat in 100 % CO reduced the development of off-odor and bacteria (Tsemakhovich and Shaklai, 2000). Pretreatment of beef steaks with 100 % CO for 30 minutes lowered aerobic plate counts by one log after eight weeks of vacuum storage, compared to no CO pretreatment (Brewer et al., 1994).

**Toxicology**

CO is a colorless, odorless and tasteless gas, which is mainly produced through incomplete combustion of carbon-containing materials. Natural background levels of CO are 0.01 – 0.09 mg/m³ (WHO, 1979). Elevated levels up to 20 mg/m³ are recorded in urban areas. By far the most common cause for elevated CO concentrations in the blood is tobacco smoking. CO binds to the iron atom of hemoglobin in red blood cells, forming carboxyhemoglobin (COHb). The affinity of hemoglobin to CO is approximately 240 times higher than to O₂ (WHO, 1979). The binding of COHb to hemoglobin is reversible, with a half-life of approximately 4.5 hours in individuals who are at rest.

The COHb concentration in blood, often referred to as the COHb percent (COHb %), is a function of the CO concentration in the air, the exposure time, and the level of physical activity of the individual (Coburn et al., 1965). At COHb concentrations of approximately 2.5 %, sensitive individuals suffering from cardiovascular diseases, display changes in cardiac function and report chest pain. However, in healthy adults, no adverse health effects were described at COHb levels below 5 % (Aunan et al., 1992). The average COHb concentration in non-smokers is 1.2 – 1.5 %, compared to 3 – 4 % in smokers. According to a Norwegian expert group, COHb levels greater than 2 % should be avoided to protect the most vulnerable individuals in the population. They recommended that COHb levels should not exceed 1.5 %, taking into consideration endogenous CO formation (Aunan et al., 1992).

In order to prevent a maximum COHb level in the blood of 1.5 % from being exceeded, the CO concentration in the air for a 1-hour period at moderate physical activity should not exceed 24 mg/m³, amounting to a CO intake of 15.1 mg (Sørheim et al., 1997). In comparison, meat that has been treated for 3 days in an atmosphere containing 1 % CO, yielded approximately 0.1 mg of CO per kg meat upon storage and cooking (Watts et al., 1978). Furthermore, the absorption of CO from the gastrointestinal tract into the blood is far less effective, compared with the absorption of the gas from the lungs. Consequently, it is highly improbable that the consumption of one meal of meat packaged in a low CO gas mixture will result in even measurable increases in the blood COHb level.

**Workers’ Safety**

CO as a packaging gas has been evaluated for its potential hazard for workers in meat plants. If pure CO or high concentrations of CO were used for mixing of gases in the plant, they would pose a clear risk. However, the practice of Norwegian
gas suppliers is either to deliver CO as a 1% CO/99% N₂ mixture and then blend this mixture with CO₂ on site, or as a complete 0.3% CO/70% CO₂/30% N₂ mixture. This practice is recognized by the Norwegian health authorities to be safe. Concentrations of CO in working environments should not exceed 29 mg/m³ (Directorate of Labour Inspection, 1996). The concentration of gases, CO included, in working environments is monitored in meat plants on a voluntary basis.

High O₂ atmospheres must be handled carefully because they are explosive gas mixtures. In contrast, low CO gas mixtures are not explosive. Strict safety regulations apply to explosive gas mixtures, increasing the cost of equipment and packaging operations.

Food Regulations

Despite the extensive knowledge and benefits of using CO in meat packaging, CO is presently not allowed for this purpose in the USA and the EU. The EU considers packaging gases as additives, and CO is not on the list of such gases. However, in Norway, CO in concentrations up to 0.5% has temporarily been permitted for MAP of meat. Although Norway is not a member of the EU, the Norwegian food regulations are gradually being adopted to EU regulations due to trade agreements within the European Economic Area. Consequently, the Norwegian meat industry in the summer of 2000 applied to the EU Commission for continued and permanent acceptance of 0.5% CO for meat MAP. The application is now under evaluation in the scientific committee of the EU Commission, and it is supported by meat trade organizations in many European countries.

In the EU and Norway as well, current labeling regulations require packages with meat and other foods in modified atmospheres to be labeled with “Packaged in a protective atmosphere”. The specific gases must not be declared on the packages.

Conclusions and Future Prospects

Main features of low CO for MAP of retail meat:

- no toxic hazard to consumers
- safe to use for meat plant workers
- stable, bright red color
- long microbiological shelf life due to high concentration of CO₂ and absence of O₂
- reduced growth of some pathogenic bacteria
- benefits of case-ready meat (quality control, costs and marketing)
- wider distribution range
- potential for masking spoilage and microorganisms by high color stability.

Although there is a limited industrial experience by using CO, except for Norway, extensive documentation exists on the effects of CO on meat, as demonstrated by this review. An increased interest for research on CO packaging in Norway and other countries has developed over the past decade. The packaging and storage of retail meat in low CO/high CO₂ in Norway has since 1985 been successful in terms of its high market share and positive response from consumers and food stores. The increased color stability combined with extended microbiological shelf life by using low CO/high CO₂ is considerably better than by other packaging methods, like high O₂ atmospheres, enabling a much wider range for distribution.

The main disadvantage of using CO for meat packaging, is the concern about misrepresentation of the condition of the meat and possible masking of the microbiological condition by formation of the stable, bright red carboxymyoglobin color (Kropf, 1980). However, off-odors can still be detected in spoiled meat stored in an atmosphere containing CO. A combination of low levels of CO with a bacteriostatic gas, like CO₂ in high concentrations, which reduce growth of microorganisms, is important to justify approval by regulatory agencies. Recent research on pretreatment with CO followed by vacuum packaging, is promising because of the possibility of better adjusting the color stability of the meat to the time of spoilage (Jayasingh et al., 2001). By adopting CO to meat MAP, the meat industry must label the packages with reliable times for maximum shelf life. However, as with other perishable foods in MAP, such meat has to be handled with strict hygienic standards, low storage temperatures must be kept in a continuous chill chain, and quality control must be implemented in the packaging operations.

References


American Meat Science Association


