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Opinion of the Scientific Committee on Food on the use of carbon monoxide as component of packaging gases in modified atmosphere packaging for fresh meat

(adopted on 13 December 2001)

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Terms of Reference

The Committee was asked to evaluate the safety of carbon monoxide as a packaging gas for meat in a mixture with carbon dioxide and nitrogen.

Background

The Norwegian Meat Co-operative and the Norwegian Independent Meat Association, representing the Norwegian meat industry, have applied for the use of a gas mixture containing 60%-70% carbon dioxide (CO_2), 30%-40% nitrogen (N_2) and <0.5% carbon monoxide (CO) as components of the packaging gas in modified atmosphere packaging (MAP) for fresh red meat (mainly beef, pork, lamb but also horse, goat, reindeer, game etc.).

The high level of CO_2 inhibits growth of many pathogenic and non-pathogenic micro-organisms thus giving an extended microbiological shelf life. The presence of CO provides a stable cherry red colour of the meat.
This MAP is used in Norway to prolong shelf life for displaying and selling fresh retail meat in all parts of the country (Norwegian Food Control Authority, 2001).
In interpreting the Terms of Reference, the Committee considered both microbial and toxicological aspects of safety.

Technological aspects

The CO used in the MAP has a degree of purity higher than 99.3%, the major impurities being oxygen and argon, hydrogen and nitrogen, carbon dioxide, water and total hydrocarbons. The gas is supplied as pre-mixtures in gas containers to prevent potential hazards from occupational exposure to CO. The proposed gas mixture for fresh meat packaging is as follows: 60%-70% CO_2, 30%-40% N_2, and 0.3%-0.5% CO (high CO_2/low CO).

Two pigments, myoglobin and haemoglobin, contribute principally to the colour of fresh meat. Myoglobin represents about 80%-90% of the pigment in meat, while haemoglobin, catalase and cytochromes contribute the remainder. The ultimate colour depends on the oxidation state of the Fe-atom in the non-protein haem ring and the compound bound to the iron at the free binding site. The relatively stable oxygenated oxymyoglobin with the structure {globin-tetrapyrrole ring-$Fe^{+2}(O_2)$} is bright red, myoglobin, with the structure {globin-tetrapyrrole ring-$Fe^{+2}(H_2O)$} inside the reduced atmosphere of the muscle cell, is purple, the oxidised metmyoglobin with the structure {globin-tetrapyrrole ring-$Fe^{+3}(OH)$}, is brown.

CO ligates to the free binding site on the Fe-atom of the haem to form the cherry red carboxymyoglobin with the structure {globin-tetrapyrrole ring-$Fe^{+2}(CO)$}.
High partial pressure of oxygen favours the formation of oxymyoglobin for use in the metabolism of the muscle cell while low partial pressure of oxygen favours the formation of myoglobin and metmyoglobin. Carboxymyoglobin is more resistant to oxidation than oxymyoglobin because of the stronger binding of CO to the Fe-binding site on the myogobin molecule.

Shelf life aspects

Either vacuum packing or MAP improves shelf life of fresh meat. End-product characteristics affecting the shelf life depend among others on type of product, initial contamination, atmosphere, storage temperature, packaging material and design (Church and Parsons, 1995). The major spoilage flora of fresh meat consists of aerobic *Pseudomonas* spp. This flora is inhibited by the anaerobic conditions in vacuum or non-oxygen MAP systems or due to CO_2 concentrations exceeding 10-20% in oxygen MAP (Gill and Molin, 1991; White and Roberts, 1992). The shift in ecology from a gram-negative aerobic spoilage flora to a facultative gram-positive flora of *Brochotrix thermosphacta* and especially *Lactobacillus* will exhibit a certain competition towards pathogens that might be associated with fresh meat.

A disadvantage with vacuum packing or anaerobic MAP for fresh meat is the change in meat colour. A mixture of CO_2 and oxygen is usually used in MAP to avoid this change. CO_2 is used for its inhibitory effect on the gram-negative spoilage flora and oxygen as a stabiliser of the red colour. The shelf life during this storage is not as predominant compared to anaerobic storage in CO_2 (Dainty and Mackey, 1992).

Studies were carried out to evaluate the effect of high carbon dioxide/low CO MAP on the shelf life of fresh meat and meat products under MAP conditions (Sørheim et al., 1997; Sørheim et al., 1999; Nissen et al., 1999).

In comparative studies the shelf lives of fresh meat products packaged in high CO_2/low CO gas mixtures were evaluated against products packaged in alternative MAP gas mixtures having following composition: 70% O_2/30% CO_2 and 60% CO_2/40% N_2 with an O_2 absorber. The products were stored in the dark at 4^0C or 8^0C for up to 21 days.

Meat in the high CO_2/low CO mixtures had a stable bright red colour. The shelf lives at 4^0C in this gas mixture were, 11 days for ground beef, 14 days for beef loin steaks and 21 days for pork chops. After these storage times off-odours developed. The samples stored under high O_2 showed initially a bright red colour of the meat, but the colour was unstable and off-odours developed rapidly after shorter storage periods of 8, 10 and 14 days respectively. Increasing the storage temperature up to 8^0C reduced the shelf life, under both MAP conditions, to nearly half that at 4^0C.

The observed off-odours were probably caused by the growth of *Brochothrix thermosphacta*. Indeed, at chill temperatures above 1^0C, *B. thermosphacta* often causes spoilage of meat stored in high O_2 atmospheres (Dainty and Mackey, 1992). High concentrations of CO_2, removal of O_2 and low temperatures inhibit the growth of *B. thermosphacta* (Gill, 1996; Nissen et al., 1996). Meat in high O_2 is often spoiled by *Pseudomonas* spp., but the growth of pseudomonads is retarded under anaerobic conditions (Dainty and Mackey, 1992; Gill, 1996). A shift in the metabolism of lactic acid bacteria under anaerobic conditions can also produce off-odours (Nissen et al., 1996). In the studies that were considered here, the number of coliforms or *Escherichia coli* did not exceed 10^3 colony forming units (cfu)/g in any samples and thus were probably not involved in off-odour production.

Microbiological aspects

The inclusion of CO in MAP is controversial because the stable cherry-colour can last beyond the microbial shelf life of the meat and thus mask spoilage (Kropf, 1980). The extended shelf life obtained by MAP may, therefore, under certain conditions imply increased risk of growth of pathogens (Silliker and Wolfe, 1980; Hintlian and Hotchkiss, 1986; Farber, 1991; Lamberts et al., 1991).

Meat packed in high CO_2/low CO acquires a stable colour and the shelf life at 4^0C, based on odour, is significantly longer than in the other gas mixtures. At this temperature *Yersinia enterocolitica* and *Listeria monocytogenes* are considered to be the most serious pathogens in meat. At abuse temperatures (>8^0C) *E. coli* 0157:H7 and *Salmonella* spp. also may grow and increase the health risk of the consumer. This issue was discussed by the Scientific Committee Food (SCF Reports, 1997).

In studies submitted by the petitioner (Sørheim et al., 1997; Sørheim et al., 1999; Nissen et al., 1999; Nissen et al., 2000) growth of the pathogens *Y. enterocolitica*, *L. monocytogenes*, *E. coli* 0157:H7 and strains of *Salmonella* was compared in ground beef packed in high CO_2/low CO, in a high O_2 mixture (70% O_2/30% CO_2) and in chub packs. Ground beef was chosen because it is considered as a risk product since pathogens may be mixed into the product and not be properly heated before being eaten.

Ground beef was inoculated with rifampicin- or nalidixic acid/streptomycin-resistant strains (to aid their recovery) at a final concentration of 10^2 – 10^3 bacteria/g. Packs were stored at 4^0C or 10^0C for up to 14 days.

At 4^0C shelf life measured on the basis of colour stability and a low background flora, was prolonged for the high CO_2/low CO MAP compared with the other packs. At 10^0C the shelf life was below 8 days for all packs.

The growth of *Y. enterocolitica* was totally inhibited at both 4^0C and 10^0C in the high CO_2/low CO mixture, while the bacterial numbers in the samples packed in the high O_2 mixture increased from about 5.10^2 bacteria/g at day 0, to about 10^4 on day 5 at 4^0C and to 10^5 by day 5 at 10^0C.

L. monocytogenes showed very little growth at 4^0C in all treatments. At 10^0C it grew from about 5.10^3 bacteria/g to about 10^4 by day 5 in the high CO_2/low CO mixture, while the numbers in the high O_2 and the chub packs were about 10 times higher.

E. coli 0157:H7 does not grow at 4^0C but even at 10^0C in ground beef it was almost totally inhibited in both high CO_2/low CO mixture and in the high O_2 mixture. In the chub packs growth was much higher reaching 10^5 bacteria/g on day 5.

Salmonella spp. also do not grow at 4^0C but at 10^0C the pathogens *S. typhimurium*, *S. dublin*, *S. enteritidis* and *S. enterica* 61:k:1,5,(7) grew better by days 5 and 7 in the high CO_2/low CO MAP than the high O_2 MAP (Nissen et al., 1999; Sørheim et al., 1999).

The data presented in the studies show that the prolonged shelf life at 4^0C did not increase growth of *Y. enterocolitica* and *L. monocytogenes* in ground beef stored in the high CO_2/low CO mixture. However, the observed growth of strains of *Salmonella* at the abuse temperature of 10ºC in this mixture and in the chub packs does stress the importance of temperature control during storage.

History of use

The MAP packaging of fresh meat using high CO2/low CO mixtures has been in use in Norway since the mid eighties. Currently 50-60% of retail meat and up to 85% of ground beef is packaged under such conditions (Norwegian Food Control Authority, 2001).

The Norwegian Food Control Authority has not registered outbreaks or a higher frequency of sporadic cases of food-borne diseases linked to such products during this time (Norwegian Food Control Authority, 2001).

Toxicological aspects

CO is present in the normal atmosphere, mainly through the incomplete combustion of carbon-containing materials, the oxidation of methane in the troposphere and from the decay of chlorophyll. The natural background levels of CO are 0.01–0.9 mg/m^3. In urban areas, 8-h mean concentrations of CO[a] are generally <20 mg/m^3. However, maximum 8-h concentrations of up to 60 mg/m^3 have been reported (WHO, 1979).

CO is eliminated from the atmosphere through oxidation to CO_2 by hydroxyl radicals in the upper layers of the atmosphere and through oxidation by soil bacteria.

CO is formed in the human body through oxidation of the carbon of the methylene bridge in the tetrapyrrole ring in haemoglobin or myoglobin. (Marquardt & Schäfer, 1994). By far the most common cause of elevated CO concentrations in the blood is tobacco smoking (WHO, 1987).

The toxic action of CO is due to the blockage of the oxygen-carrying function of haemoglobin through the formation of carboxyhaemoglobin (HbCO) instead of oxyhaemoglobin (HbO_2). The binding of CO to haemoglobin is reversible, with a half-life of ~4.5 h in individuals at rest.

A small amount of CO is formed naturally in the human body from the breakdown of haemoproteins. Such production leads to a HbCO concentration of ~0.5% of total haemoglobin. The average HbCO concentration in non-smokers is 1.2-1.5%. In smokers the concentration is in the range from ~3 - 4% (Aunan, 1992).

Concentrations above 2% have been observed to have adverse effects ranging from reduced attention to anoxia and death at concentrations of 30%-50% or more of the total haemoglobin (Marquardt & Schäfer, 1994). Concentrations of HbCO below 2% of the total haemoglobin do not have any measurable adverse effects in humans. It is therefore accepted that, to protect the most vulnerable section of the population, the level of HbCO should not exceed 1.5% (Coburn et al., 1965).

The mean normal air inhalation of an adult in 24 hr is ~15 m3 (or ~0.625 m3/hr). In order to prevent a maximum HbCO concentration level in the blood of 1.5% being exceeded, the CO concentration in air for a 1 hr period of moderate physical activity should not exceed 24 mgCO/m3 (Aunan, 1992)

Exposure aspects

Very little information exists in the literature on the exposure to CO following consumption of meat that has been treated with CO gas. The exposure of beef to an atmosphere containing 1% CO for 3 days resulted in ~30% saturation of the meat myoglobin (Watts et al., 1978).

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[a] mean CO concentrations measured for each possible 8 hr interval during a 24 hr period, then averaged

In the absence of carbon monoxide, CO is lost, from previously CO-treated meat during storage, with a half-life of ~3 days. When further cooked at 195S only 0.1 mg CO/kg meat remains. This amounts to a loss on cooking of ~85% (Watts et al., 1978).

Fresh packaged meat packaged stored in high CO2/lowCO MAP could contain, next to endogenous CO, an additional 0.7 mg CO/kg meat. This is a worst case estimate as it does not take into account losses on cooking which may be up to 85% of the CO bound to carboxymyoglobin and carboxyhaemoglobin present in the packaged meat.

An assumed consumption of 250 g fresh meat/24 hrs, could therefore release 0.18 mg CO (equivalent to 0.018 % HbCO) on digestion in the gut. Assuming 100% transfer of CO from the gut to the blood and complete transformation to HbCO, only a negligible amount of HbCO would be added to the 0.5% HbCO, resulting from endogenous CO production, and the 0.7%-1.0% HbCO formed from inhalation of urban air by a non-smoker.

These figures are deduced from the observation that an intake of 15.1 mg CO/hr through inspired air leads to the formation of 1.5% HbCO (Sørheim et al., 1997).

Exposure through inhalation of headspace gas on opening a package of meat with a MAP containing 0.3%-0.5% CO would equally contribute insignificantly to the HbCO in the blood when compared to the other sources of inhalation of CO.

Conclusion

Meat packaged in MAP containing a high concentration of CO_2 and 0.3%-0.5% CO remains microbiologically stable for 11-21 days when stored at a maximum temperature of 4°C.
High CO_2/low CO gas mixtures inhibit the growth of *L. monocytogenes, Y. enterocolitica* and *E. coli* O157:H7 during storage at 4°C, however some strains of *Salmonella* will grow at 10°C. Thus, close control of temperature throughout packaging, distribution, retailing and storage by the consumer of MAP products is essential.

The use of meats packaged in MAP containing 0.3%-0.5% CO contributes in negligible amounts to the overall exposure to CO and the HbCO concentration in humans.

The Committee therefore concluded that there is no health concern associated with the use of 0.3%-0.5% CO in a gas mixture with CO_2 and N_2 as a modified atmosphere packaging gas for fresh meat provided the temperature during storage and transport does not exceed 4°C. However the Committee wishes to point out that, should products be stored under inappropriate conditions, the presence of CO may mask visual evidence of spoilage.

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