

reported that the growth of *C. perfringens* was retarded by carbon dioxide.

In conclusion, the earlier presumed hazards for growth and toxin formation by clostridia in refrigerated meats and fish packed in modified atmospheres seem exaggerated. Most products are judged inedible well before any toxin of *C. botulinum* can be found in the products. This general conclusion was also drawn by others.^{82,107}

10.8.2 Psychrotrophs with Pathogenic Potential

In the safety discussion concerning clostridia, it is important to remember that most clostridia are unable to grow at chill temperatures. Those able to do so, such as psychrotrophic strains of *Clostridium botulinum*, grow only poorly under these conditions, ie, they are unable to grow below 3°C.¹³¹ However, there are also psychrotrophs with considerable pathogenic potential that can reach high numbers in refrigerated foods, for example, *Listeria monocytogenes*, *Aeromonas* species,^{98,118,145} and *Yersinia enterocolitica*.^{84,88,94} There might also be other species of the family enterobacteriaceae to consider, for example, *Citrobacter freundii* and *Enterobacter cloacae*.⁹⁷ Another potential threat, in refrigerated fish, might be *Vibrio* species.⁹⁸

The Gram-negative bacteria occasionally occur as a significant part of the spoilage flora of refrigerated fresh meat (*Aeromonas* and enterobacteriaceae) and fish (*Aeromonas*, enterobacteriaceae, and *Vibrio*) stored in air or in modified atmospheres. Certain environmental parameters are beneficial for their development. For example, a high pH is beneficial for *Aeromonas*¹²⁴ and *Yersinia enterocolitica*¹³⁴ and a somewhat higher refrigeration temperature would be beneficial for the enterobacteriaceae.^{8,33} Furthermore, a modified atmosphere with a relatively low partial pressure of carbon dioxide can favor the development of enterobacteriaceae and *Aeromonas*^{33,66} (Tables 10-1 and 10-2). The best protection against *Aeromonas* and enterobacteriaceae is offered by the combination of low storage temperature and a modified atmosphere that provides a high partial pressure of carbon dioxide.³³

10.8.3 *Listeria monocytogenes*

Listeria monocytogenes is not likely to compete successfully in refrigerated raw meat and fish.^{47,66} Theoretically, a hazard may arise in modified atmosphere packs after extended storage periods, at moderate partial pressures of carbon dioxide, and in the presence of oxygen.^{101,139,157} Another important parameter is the pH, ie, a pH below 5.0 will decrease

substantially the likelihood for growth of *L. monocytogenes*.^{47,69}

However, the problem with *L. monocytogenes* is more connected to products that are consumed directly without heat treatment and in products where the normal spoilage flora has been inhibited.⁴⁷ The modified atmosphere might eventually increase the hazards due to extended storage times. In general, a high partial pressure of carbon dioxide in combination with a low storage temperature will decrease the chances for growth of *L. monocytogenes*.^{47,66} For example, the growth in cottage cheese is stopped by packaging in elevated concentrations of carbon dioxide.^{19,48} Furthermore, the chances of *L. monocytogenes* growth decreases if the domination of lactobacilli in the product is strong.⁴⁷

The possibility of growth of *L. monocytogenes* is relatively higher in, for example, some soft cheeses and in some vegetables,^{47,121} and may be higher in preprepared salads packed in modified atmosphere with only a low concentration of carbon dioxide. Vegetables are seldom stored in atmospheres exceeding 10% (v/v) carbon dioxide. In the case of cheese, the chances of growth of *L. monocytogenes* can be decreased by packaging in modified atmospheres containing a high partial pressure of carbon dioxide.^{19,48}

10.9 CONCLUDING REMARKS

Carbon dioxide is the most powerful gas component in modified atmosphere packaging to inhibit microbial growth. Growth inhibition increases with increase in the partial pressure of carbon dioxide and low temperature reinforces the growth-inhibitory effect.

The composition of the spoilage flora of refrigerated meat is controlled by the gas atmosphere according to the following principles:

1. *Pseudomonas*, which dominates the spoilage of meat stored in air, is retarded at a partial pressure of carbon dioxide of about 10 kPa (10% [v/v] in the gas phase at atmospheric pressure).^{54,109}
2. *Brochothrix*, enterobacteriaceae, and to some degree also *Aeromonas*, are the first to take over in atmospheres where *Pseudomonas* has been inhibited due to an increased carbon dioxide concentration^{26,46,112} (Table 10-1).
3. *Brochothrix* is slightly more resistant to carbon dioxide than members of the enterobacteriaceae, but has a much slower growth rate under anaerobic conditions and is, because of this, outnumbered by enterobacteriaceae in an anaerobic environment. On the other hand, in the presence of oxygen *B. thermosphacta* will dominate due to its higher resistance to carbon dioxide.^{23,26,46,112}

4. The growth of both enterobacteriaceae and *Brochothrix* is inhibited by partial pressures of carbon dioxide close to atmospheric pressure. The bacterial flora will instead be dominated by lactobacilli, primarily of the genus *Lactobacillus* but also substantial numbers of *Leuconostoc* and *Carnobacterium* can occur.^{12,23,137}

Meat in modified atmosphere should preferably be stored at temperatures below 4°C.

One obstacle with modified atmosphere packaging of fresh fish is the growth of the relatively carbon dioxide-resistant *Shewanella putrefaciens*. The distribution of fresh fish, from the microbiological point of view, preferably ought to be performed at 0°C or below, and in a gas atmosphere of pure or nearly pure carbon dioxide. However, there can be considerations other than the microbiological ones, eg, the appearance of the product and the package, and the loss of water from the product. If the carbon dioxide is supplemented with another gas for reasons other than microbiological ones, it should be with oxygen. Nitrogen should be avoided in connection with fresh fish.

The partial pressure of carbon dioxide in modified atmosphere packaging of processed meat is less critical than for fresh meat. The growth of Gram-negative bacteria is retarded by factors such as NaCl and decreased water activity. The assignment of the modified atmosphere is to maintain a dry surface and keep out the oxygen; a suitable atmosphere is 20% to 40% carbon dioxide in 60% to 80% nitrogen. To ensure the absence of oxygen, an oxygen absorber can be included in the package.

The use of modified atmosphere packaging is increasing, and the packing technique and the control of the atmosphere within the package will most probably further improve in the future. Besides the effect on eating quality, it is equally important to consider the safety aspects of extended storage times. The longer the storage time, the greater the potential microbiological hazards. Sophisticated packaging systems for susceptible refrigerated foods must be combined with advanced systems for microbial control, founded on solid knowledge of microbial ecology of foods.

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