Journal of Food Protection, Vol. 54, No. 1, Pages 58-70 (January 1991)

Microbiological Aspects of Modified-Atmosphere Packaging Technology - A Review[1]

J. M. FARBER

Microbiology Research Division, Bureau of Microbial Hazards, Food Directorate, Health and Welfare Canada, Ottawa, Ontario K1A 0L2

(Received for publication April 25, 1990)

ABSTRACT

Modified-atmosphere packaged (MAP) foods have become increasingly more common in North America, as food manufacturers have attempted to meet consumer demands for fresh, refrigerated foods with extended shelf life. Although much information exists in the general area of MAP technology, research on the microbiological safety of these foods is still lacking. The great vulnerability of MAP foods from a safety standpoint is that with many modified atmospheres containing moderate to high levels of carbon dioxide, the aerobic spoilage organisms which usually warn consumers of spoilage are inhibited, while the growth of pathogens may be allowed or even stimulated. In the past, the major concerns have been the anaerobic pathogens, especially the psychrotrophic, nonproteolytic clostridia. However, because of the emergence of psychrotrophic pathogens such as *Listeria monocytogenes, Aeromonas hydrophila,* and *Yersinia enterocolitica,* new safety issues have been raised. This stems mainly from the fact that the extended shelf life of many MAP products may allow extra time for these pathogens to reach dangerously high levels in a food. This review focuses on the effects of MAP on the growth and survival of foodborne pathogens. Considered are the major psychrotrophic pathogens, the mesophiles such as the salmonellae and staphylococci, as well as the microaerophilic *Campylobacter jejuni*. The use of MAP in various food commodities such as beef, chicken, fish, and sandwiches is also discussed. Examples of various foods currently being packaged under MAP in North America are given, along with the specific atmospheres employed for the various food groups. Major safety concerns that still need to be addressed include the potential for growth and toxin production of *Clostridium botulinum* type E in MAP fish products, the growth of *L. monocytogenes* and *A. hydrophila* under modified atmospheres in various food commodities, and the enhanced survival of anaerobic spores and *C. jejuni* under certain gas atmospheres. Additional research with MAP foods is needed to ensure the microbiological safety of the numerous MAP products that will be available to the consumer in the next decade and beyond.

Modified-atmosphere packaging (MAP), although relatively new in North America, is a well established technology, dating back to the 1930's when fresh beef was shipped from Australia and New Zealand stored under carbon dioxide. A big turning point in MAP technology came in 1981 when Marks and Spencer in the United Kingdom introduced a wide range of fresh meat products packaged under a modified atmosphere (MA). The impetus behind these products came mainly from an increased consumer demand for fresh and chilled products, although other factors, such as a consumer desire for preservative-free products, the growth of centralized packaging and portion control, and a decline in the growth of canned and frozen foods, certainly played a role. There are numerous advantages of using MAP technology, but it must be noted that there are basic problems which also must be considered (Table 1).

Although much information exists in the area of MAP technology as related to packaging films, machinery, etc., research into the microbiological safety of MAP foods has lagged far behind. One of the major problem areas as far as the safety of MAP foods is concerned, is that of refrigerated ready-to-eat foods with extended shelf life. Given the long storage life of these products, it is felt that pathogenic microorganisms which can grow at refrigeration temperatures may be a particular problem. Another major area of concern is the growth and potential toxin production of *Clostridium botulinum* type E in MAP fresh fish.

TABLE 1. *Potential advantages and disadvantages of MAP.*

I. Advantages
• Potential shelf-life increases of 50 to 400% (*53.*)
• Reduced economic loss.
• Products can be distributed over longer distances and with fewer deliveries, leading to decreased distribution costs.
• Provides a high quality product.
• Easier separation of slices (e.g. vacuum-packaged vs. MAP bacon.)
II. Disadvantages
• Visible added cost.
• Temperature control necessary.
• Different gas formulations needed for each product type.
• Special equipment and training required.

This review will briefly discuss the major effects of MA, mainly carbon dioxide (CO_2), on the survival and growth of food pathogenic organisms as well as the use of MAP in

[1]*Based on a paper presented at a workshop on "Modified Atmosphere Packaging Technology", Technical University of Nova Scotia, Halifax, N.S., November 14-15, 1989.*

various nonrespiring food commodities such as beef, chicken, and fish.

A. *Gases used in MAP*

A general, all inclusive definition of a food stored in a MAP environment is one in which a food product is stored in something other than air. This would include, among other things, vacuum-packaging, gas-flushing, naturally-respiring products that use special permeable films and controlled-atmosphere packaging (CAP). In CAP, product is continually exposed to a constant mixture of gases, while in gas-flushing or gas-packaging, the particular gas mixture desired is flushed only once at the time of packaging into an evacuated or nonevacuated environment surrounding the food. For the purposes of this review, we will be mainly concerned with gas-flushed products.

Oxygen, nitrogen, and carbon dioxide are the three main gases used commercially, although trace gases such as carbon monoxide, nitrous oxide, and sulphur dioxide are mentioned as possible gases for MAP of foods.

Oxygen (O_2) will generally stimulate the growth of aerobic bacteria and can inhibit the growth of strictly anaerobic bacteria, although there is a very wide variation in the sensitivity of anaerobes to oxygen. One of the major functions of O_2 in MAP meats is to maintain myoglobin in its oxygenated form, oxymyloglobin. This is the form responsible for the bright red color which most consumers associate with fresh red meat.

Nitrogen (N_2) is an inert tasteless gas which displays little or no antimicrobial activity on its own. Because of its low solubility in water, the presence of N_2 in a MAP food can prevent pack collapse that can occur when high concentrations of CO_2 are used. In addition, N_2, by displacing O_2 in the pack, can delay oxidative rancidity and also inhibit the growth of aerobic microorganisms.

Carbon dioxide (CO_2) is both water and lipid soluble and is mainly responsible for the bacteriostatic effect seen on microorganisms grown in MA environments. The effects of CO_2 on microorganisms have been known for many years. Pasteur and Joubert in 1877 *(89)* observed that *Bacillus anthracis* can be killed by CO_2. Not only does CO_2 have biostatic activity, but it is known to have an inhibitory effect on product respiration. Although the specific way in which CO_2 exerts its bacteriostatic effect is unknown, the overall effect on microorganisms is an extension of the lag phase of growth and a decrease in growth rate during the logarithmic phase. For practical purposes, in most foods, gaseous CO_2 applied to a biological tissue would exist in the liquid phase of the tissue primarily as dissolved CO_2 gas and carbonic acid (about 2%) *(21)*. At pH $\geq$6.0, carbonic acid will dissociate to form bicarbonate and hydrogen ions, the latter of which likely causes the small pH drop (<0.1 pH unit) often observed in muscle tissue packaged in a CO_2 atmosphere *(21)*. This minimal pH decrease would not cause any significant biostatic activity.

There have been many theories regarding the way in which CO_2 exerts its influence on a bacterial cell. These can be summarized as follows *(21,22)*:

a) alteration of cell membrane function including effects on nutrient uptake and absorption; b) direct inhibition of enzymes or decreases in the rate of enzyme reactions; c) penetration of bacterial membranes, leading to intracellular pH changes; and d) direct changes to the physico-chemical properties of proteins.

The inhibitory effects of CO_2 on microorganisms in a culture medium or food system are dependent on many factors. These include the partial pressure of CO_2, CO_2 concentration, volume of headspace gas, temperature, acidity, water activity, the type of microorganism, the microbial growth phase, and the growth medium used. For maximum antimicrobial effect, the storage temperature of a MAP product should be kept as low as possible, because the solubility of CO_2 decreases dramatically with increasing temperature *(21)*. Thus, improper temperature control will usually eliminate the beneficial effects of elevated CO_2.

B. *Experimental protocols*

There are four main experimental protocols which have been used to study the microbiological safety of MAP food products *(53)*. Storage parameters are crucial in these types of studies, and they should always be conducted under well defined conditions of storage.

I. Inoculation studies

A particular food is inoculated with a pathogen, and the survival and/or growth of the organism in a MAP food is followed with time.

II. Organoleptic spoilage and toxigenicity

A food product is usually inoculated with *C. botulinum* and the earliest time at which toxin can be detected and at which organoleptic spoilage occurs is then determined. The central question here is whether detectable spoilage precedes or follows toxigenesis.

III. Predictive modeling

In these types of experiments, mathematical models are used to generate regression equations, which may enable the investigator to predict the probability of microbial growth and/or toxin production in a certain food product. The role of spoilage is generally ignored in these experiments. Drawbacks include the inability to extrapolate from a liquid culture to a food system and also the inability to extrapolate beyond the parameters used in the experiment.

IV. Relative spoilage and pathogenicity

The concept of the "safety index" ratio has been developed by Hintlian and Hotchkiss *(57)*. A food product is inoculated both with a pathogenic and a food spoilage microorganism and growth in a MA environment is followed with time. The ratio of the log of spoilage organisms to the log of pathogenic organisms is used as a measure of the relative safety of the test conditions. Although this ratio does not give information about the absolute safety of the test conditions, it does address the issues of both spoilage and pathogenicity in a quantitative manner *(53)*.

MAP - Safety issues. The great vulnerability of MAP foods from a safety standpoint is that a particular MA may inhibit organisms that might warn consumers of spoilage while either allowing or promoting the growth of pathogens.

Other MAP safety issues which have not been properly addressed include product safety under conditions of temperature abuse, gas-flush system failure, and loss of packaging integrity.

In the past, anaerobic pathogens such as the nonproteolytic psychrotrophic strains of *C. botulinum* have been the major safety concern associated with MAP foods. While the growth of aerobes may be inhibited under a certain MA, these nonproteolytic, anaerobic toxin producers may be stimulated and also would not usually cause any detectable organoleptic deterioration. Thus, the food may appear to be unspoiled long after the expiration date, but may contain toxins.

However, new concerns have also been raised with some of the newly recognized psychrotrophic pathogens such as *L. monocytogenes* which may be able to grow well in refrigerated MAP products *(113)*.

C. *Effect of MAP on growth and survival of foodborne pathogens*

I. Psychrotrophic pathogens

1. Clostridium botulinum (nonproteolytic). C. botulinum is a gram-positive, spore-forming, anaerobic rod. Strains can be grouped into seven different types A to G, each type producing a serologically distinct neurotoxin. The most common types involved in human illness are A, B, and E. A few ng of toxin is sufficient to cause disease in humans. The biological properties of *C. botulinum* proteolytic group I and nonproteolytic group II are shown in Table 2. It is evident that there are major differences between these groups, with the most important ones being temperature ranges for growth and spore heat resistance *(51)*.

For foodborne botulism to occur, basically four conditions must exist: the presence of the organism *C. botulinum*, inadequate processing, a food that is capable of supporting toxin formation, and the food to be ingested. Although both the incidence and levels of *C. botulinum* spores in raw and semipreserved meats in North America are very low, the incidence of *C. botulinum* spores in fish has been as high as 23.5% in some studies *(51)*. Furthermore, the seriousness of the disease warrants protection of the consumer against such potentially hazardous situations.

In addition to the redox potential, headspace composition, pH, water activity, and preservatives, both temperature and substrate can greatly influence the ability of nonproteolytic *C. botulinum* to produce toxin. At temperatures of 5.6, 4.4, or 3.3°C, the time for *C. botulinum* (nonproteolytic B) to produce detectable toxin was 27, 33, and 129 d, respectively *(102)*. Kautter et al. *(62)* found that *C. botulinum* type E could produce toxin in nitrogen-packed hamburger sandwiches stored at 12°C but not those stored at 8°C. Thus, the importance of keeping food storage temperatures as low as possible is quite evident.

As far as substrate influence is concerned, *C. botulinum* type E has been found to produce toxin in synthetic laboratory broth at 4 and 8°C but not in crabmeat under the same experimental conditions *(102)*. In addition, nitrogen-packed hamburger sandwiches were capable of supporting growth of and toxin production by *C. botulinum* type E at 12°C, whereas turkey or sausage sandwiches were not (*62*). The effect of MAP on growth and toxin production by *C. botulinum* in fish products will be discussed in a later section.

2. Yersinia enterocolitica. Yersinia enterocolitica is a facultatively anaerobic psychrotrophic, gram-negative rod which belongs in the family *Enterobacteriaceae*. Swine appears to be the principal reservoir of virulent strains. Transmission of the organism to humans occurs mainly via ingestion of contaminated foods and water. Many of the outbreaks linked to foods have involved dairy products *(19)*. The minimum infective dose for humans is unknown. There are many complications, including arthritis which can occur after the initial *Yersinia* infection. The symptoms of arthritis can begin 1-2 weeks after the onset of gastrointestinal (G-I) symptoms and usually persist for 1-4 months *(19)*.

Zee et al. *(115)* examined the effect of different MA on the growth of *Y. enterocolitica* in trypticase soy broth at 25°C. While 10% CO_2 (remainder argon) appeared to be stimulatory to the growth of *Yersinia* as compared to growth in air, 40% CO_2 increased the lag phase and 100% CO_2 both increased the lag phase and decreased the growth rate during the logarithmic period. Studies with *Y. enterocolitica* have shown that, compared to the percentage growth in air, growth at 2, 6, and 20°C (for 23 d) under an atmosphere of 100% CO_2 was reduced by 100, 98, and 43%, respectively *(25)*. However, Gill and Reichel *(45)* found *Y. enterocolitica* to be capable of growing on high-pH beef packaged under 100% CO_2 at both 5 and 10°C, even though *L. monocytogenes* and *A. hydrophila* could only grow at the latter temperature under the same conditions. This CO_2-packaged beef stored at 0, 2, or -2°C was incapable of supporting the growth of *Y. enterocolitica*. Other studies have shown *Y. enterocolitica* to be incapable of growing in minced beef at 4°C in a MA of 20% CO_2 and 80% O_2. However at 15°C, growth of *Y. enterocolitica* was equal to that of the air control, while growth at 10°C was only slightly delayed *(63)*. Interestingly, the presence of a large competitive background flora was capable of inhibiting the growth of Y. *enterocolitica* at all temperatures tested (1, 4, 10, and 15°C)

TABLE 2. *Characteristics of* C. botulinum, *Groups I and II.*

Properties	Group I	Group II
Proteolytic	+	-
Association with		
Human outbreaks	+	+
Infant botulism	+	-
Toxin types[a]	A,B,F	B,E,F
Inhibitory pH	4.6	5.0
Inhibitory NaCl concentration	10%	5%
Minimal a_w	0.94	0.97
Temperature range for growth and toxin production	10-48°C	3.3-45°C
D_{100} of spores	25 min	<0.1 min

Adapted from Hauschild *(51)*.

[a]Where association can be made, illness due to *C. botulinum* type A is mainly associated with home-preserved vegetables, nonproteolytic type B with home-cured meats, and type E with fish products *(51)*.

to the extent that there was little or no additional effect of CO_2 on the microorganism *(63)*.

3. *Listeria monocytogenes*. *L. monocytogenes* is an organism which has caused great concern to both food industry and regulatory agencies since the early 1980's. It is a gram-positive, rod-shaped motile organism which causes disease mainly in pregnant women and their fetuses, newborns, the elderly, and immunocompromised people. The concern surrounding the presence of this organism in foods stems from the following. (a) It is a psychrotrophic pathogen and thus can grow at chill temperatures in products such as the "new generation" of foods including cook-chill MAP products with extended shelf lives. (b) It is very widespread in the environment. (c) It is a very hardy organism and appears to be more heat tolerant than many other vegetative microbes. (d) The disease it causes (listeriosis) is associated with a high mortality rate which averages 30% in food outbreak situations *(34)*.

There has been very little research on the effects of MA on the growth and/or survival of *L. monocytogenes* in foods. Berrang et al. *(11)* examined the behavior of *L. monocytogenes* inoculated onto vegetables, which were then stored under controlled-atmosphere (CA) conditions. *L. monocytogenes* grew as well on vegetables stored under CA-storage as those stored in air, either at 4 or 15°C. Enumeration of total *Listeria* at the end of the shelf life for CA-stored asparagus (21 d; 4°C) demonstrated that significantly higher levels of *Listeria* were present than in air-stored samples (shelf life 14 d). However, it should be noted that even with the most restrictive atmospheres used in this study (broccoli - 11% O_2, 10% CO_2, 79% N_2), one would not generally expect biostatic activity towards the growth and/or survival of *L. monocytogenes*. Gill and Reichel *(45)* found *L. monocytogenes* incapable of multiplying on high-pH beef packaged under 100% CO_2 at temperatures of 5°C or less. However, the organism did grow in CO_2-packed meat stored at 10°C, as well as on vacuum-packed meat stored at 0, 2, 5, and 10°C. Studies done following the survival and/or growth of *L. monocytogenes* on minced raw chicken have also shown that the organism does not survive well in a anaerobic MA (75% CO_2, 25% N_2). However, if a little O_2 (5%) is added, *L. monocytogenes* can grow, even at 4°C *(112)*. Of particular concern was the observation that, although *L. monocytogenes* could grow in the aerobic MA at 4°C, the aerobic background microflora was substantially inhibited. Thus, in this case, the spoilage organisms which normally grow and give an indication to the consumer that a food may be unsafe to eat, would be inhibited and a potentially unsafe product would appear organoleptically acceptable to the consumer.

4. *Aeromonas hydrophila*. This psychrotrophic, motile, gram-negative rod is a member of the family *Vibrionaceae*. It is very widespread in the environment, especially in fresh water and brackish water, and appears to be part of the normal intestinal flora of healthy fish *(91)*. Although the organism has been isolated from a wide variety of foods *(17, 38)* and is capable of growing in these foods at low temperatures *(17,84)*, the link between food and human illness has not been definitively established *(2,76)*.

Not much work related to growth of this organism under MA has been published. Enfors et al. *(31)* found that, although *A. hydrophila* was present in pork stored under nitrogen for 10 d at 4°C at levels of about 10^6 organisms/cm^2, it was not present in CO_2-stored pork. Similarly, *A. hydrophila* was unable to grow well on high-pH beef packaged under CO_2 at temperatures of 5°C or less, although it did grow well at 10°C *(45)*.

Other studies have examined the ability of *A. hydrophila* to grow in surimi-type products (cooked mince, low salt, and salt-added surimi) stored under 36% CO_2, 13% O_2, remainder N_2 *(59)*. After 8 d of air storage at 4°C (trial 1), the population of *A. hydrophila* had increased by 6.4, 6.3, and 2.4 logs, respectively, as compared to $\log_{10}$ increases of 3.1, 4.8, and -0.3, respectively, for MA-stored product. Experiments performed at 13°C also showed that, compared to air storage, the MA environment did not stimulate the growth of *A. hydrophila*. In general, *A. hydrophila* was found to compete well with a *Pseudomonas fragi* strain that was co-inoculated on both mince and low-salt surimi stored either under air or MA. The authors concluded that MA storage of surimi-based products could not be used to significantly inhibit *Aeromonas*, and that potentially significant numbers of the organism may be present, even when the food appears organoleptically acceptable *(59)*. The growth of *A. hydrophila* at 4 or 15°C on CA-stored fresh vegetables was also found not to be significantly affected, as compared to growth in air, even though the CA extended the shelf life of the vegetables *(10)*. The health concerns about vegetables stored for extended periods at 4°C under CA are valid, since the extra length of time that the vegetables remain acceptable for consumption allows psychrotrophic pathogens such as *Listeria* or *Aeromonas* additional time to grow to perhaps significantly higher levels.

II. Pathogens capable of growing between 5-12°C

1. *Salmonella* spp. Silliker and Wolfe *(98)* inoculated ground beef with six *Salmonella* strains and then stored the meat under different gas atmospheres at either 10 or 20°C. After 10 d at 10°C, the number of *Salmonella* in samples stored in air had increased by more than 3 logs, while in MA-stored samples (60% CO_2, 25% O_2, 15% N_2) the number had only increased by approximately 0.5 logs. At 20°C, however, *Salmonella* grew almost equally as well in air or in MA-stored samples. Luiten et al. *(71)* observed that *S. typhimurium* was unable to grow on beef steaks stored at 10°C in an atmosphere containing 60% CO_2, 40% O_2, although there was no die-off of the organism during the 9-d storage period. This is in contrast to air-stored beef samples in which an approximate 3-log increase in the numbers of *Salmonella* was observed. The growth of *S. typhimurium* inoculated into a meat culture, or trypticase soy broth (TSB) culture, and stored at 2, 7, or 13°C was also found to be inhibited in an 80% CO_2 (remainder air) environment relative to air *(6)*. However, at both 7° and especially 13°C, the organism did multiply. Hintlian and Hotchkiss *(56)* also found *S. typhimurium* inhibited by MA environments. In this case roast beef was used as substrate, and a MA containing 75% CO_2 with various concentrations of oxygen, balance N_2, was used as the gas atmosphere. Generally, however, the MA's used were less effective in

inhibiting *Salmonella* than other pathogens such as *Staphylococcus aureus* or *Clostridium perfringens*. In addition, storage of meat under air or MA at 4°C did not appear to affect the ability of *Salmonella* to recover and grow when the meat was subsequently transferred to 12.8°C for 7 d.

Other studies have also demonstrated partial inhibition of either *S. typhimurium* in TSB *(25)* or *S. enteritidis* on chicken by 100% CO_2 *(49)*.

There has been one report of growth stimulation of *S. enteritidis* in a 20 or 60% CO_2 atmosphere as compared to air. Whether this is a strain specific effect, due to the fact that trypticase soy agar (TSA) plates stored in a plexiglass chamber were used as substrates, or is a real phenomenon is unclear at present. Although gases were allowed to flow continuously in these experiments, no actual measurements of gas composition were taken within the chambers in which the plates were stored *(27)*.

2. *Clostridium perfringens*. Carbon dioxide at atmospheric pressure has been found to stimulate the germination of *C. perfringens* spores relative to nitrogen *(28)*. Germination of *C. perfringens* spores at 37°C was either slightly stimulated, unaffected, or stopped at 4, 10, and 25 atm of CO_2, respectively. Parekh and Solberg *(85)* found no significant difference in the growth rate between eight strains of *C. perfringens* growing in fluid thioglycollate broth whether in a 100% CO_2 or 100% N_2 atmosphere. However, these experiments were conducted at 43°C, a temperature at which CO_2 might not have much biostatic activity because of its decreased solubility at high temperatures.

In experiments done at 12.8°C in which cooked roast beef was co-inoculated with *C. perfringens* and *P. fragi*, it was demonstrated that a combination of low temperature and MA (75% CO_2, remainder O_2) could prevent the outgrowth of *C. perfringens (57)*. However, upon increasing the storage temperature to 26.7°C, the MA had no effect on *Clostridium* and the organism was able to grow well. Studies done at 4°C in which cooked roast beef was co-inoculated with *P. fragi, S. typhimurium, S. aureus,* and *C. perfringens* and then stored either in air or 75% CO_2, 10% O_2, 15% N_2 for 42 d, showed that *C. perfringens* was unable to grow either in air or the MA. Interestingly, when samples were transferred at various times during the 42-d sampling period to 12.8°C for a final 7 d, no organisms could be recovered after 21 d, and for samples stored less than 21 d, recovery of *C. perfringens* was always greater in air-stored samples. In general, it was noted that the inclusion of 10% O_2 in the MA inhibited *C. perfringens*, as compared to those meat samples stored under CO_2 and N_2 *(57)*.

3. *Bacillus cereus*. Most studies to date indicate that *B. cereus* is very sensitive to the antimicrobial effects of CO_2. The ability of *B. cereus* spores to germinate in the presence of 100% CO_2 was reduced by 70-90% as compared to germination under 100% N_2 *(28)*. Molin *(75)* found that, of 11 organisms tested, *B. cereus* was the most sensitive to inhibition by 100% CO_2 in that growth at 25°C was inhibited by 83 and 67%, as compared to aerobic or anaerobic (5% CO_2, 95% N_2) growth, respectively. Other investigators have also documented the CO_2 sensitivity of *B. cereus (26,30)*.

4. *Staphylococcus aureus*. Ground beef was inoculated with four strains of *S. aureus* and stored at 10 and 20°C under different gas atmospheres *(98)*. In air-stored samples *S. aureus* counts decreased slightly at 10°C and increased by approximately 2 logs at 20°C. In samples stored in 100% CO_2 at 10°C, *S. aureus* counts decreased by greater than 1.5 logs, while at 20°C, counts increased similar to air-stored samples. Growth of *S. aureus* in broth culture was decreased by 72% in 100% CO_2 as compared to growth in air *(75)*.

Growth of *S. aureus* in TSB or meat culture at 2, 7, or 13°C was inhibited in a MA (80% CO_2, remainder air) compared to an air control *(5)*. Inhibition under the MA was much greater at 2°C than at 7 or 13°C, again emphasizing the importance of storing foods at as low a temperature as possible. This is not only because it is well known that lowering the growth temperature can dramatically increase the bacterial lag phase *(83)* but, as stated before, because of the increased solubility of CO_2 at lower temperatures. The influence of substrate on the growth of *S. aureus* in a MA was also observed in this study. At both 7 and 13°C, *S. aureus* growing in TSB in 80% CO_2 (remainder air) reached levels greater than 10^6/ml after 120 h, while levels never reached 10^6/ml in the meat culture in the MA environment. It is usually necessary to reach a level of 10^6 orgs/g of food before detectable enterotoxin is observed *(9)*.

Experiments using cooked roast beef as substrate at 12.8°C demonstrated that a MA of 75% CO_2 (O_2 0-25%, remainder N_2) was quite inhibitory to the growth of *S. aureus* as compared to air *(57)*. The different levels of O_2 had little influence on the inhibition. Interestingly, similar work done at 26.7°C suggested that inhibition was due more to the amount of O_2 present, than to the CO_2 content *(57)*.

III. Pathogens capable of growing between 31-45°C

Campylobacter jejuni. *C. jejuni* is a motile, gram-negative, slender, curved bacterium in the family *Spirillaceae*. It is microaerophilic, growing best in an atmosphere containing 5% oxygen, and at 42-43°C. It is a very fragile organism and a poor competitor. Only within the last 10 years has the organism become recognized as one of the most common causes of acute gastrointestinal infection in humans. Many studies have identified foods as the most significant cause of *Campylobacter* enteritis, with raw milk and undercooked poultry most often involved in outbreak situations. Other vehicles implicated in causing *Campylobacter* enteritis include uncooked or poorly cooked meat, raw clams, and water *(106)*.

Hanninen et al. *(50)* inoculated fresh beef with three strains of *C. jejuni* and then followed growth and/or survival of the organism at three different temperatures (37, 20, and 4°C) and under different MAs. At 37°C, strains generally grew best in an atmosphere of 10% CO_2, 5% O_2, 85% N_2, which is usually optimal for microaerophiles, and poorest under an atmosphere of 20% CO_2, 80% N_2. However, survival of the organism at 4°C was similar in all packaging treatments. Survival of *C. jejuni* in ground beef at 4°C, was highest in a 100% N_2 atmosphere, followed by, in decreasing order of survival, (a) 10% CO_2, 5% O_2, 85% N_2; (b) 80% CO_2, 20% N_2; and (c) vacuum.

Phebus et al. *(90)* found that *C. jejuni* inoculated onto turkey roll stored at 4 and 21°C survived best at 4°C and at the higher concentrations of CO_2 tested (40-100% CO_2).

TABLE 3. *Relative effect of CO_2 on the growth of foodborne microorganisms*[a].

Organism	CO_2 effect				
	Growth[b] unaffected	Growth[b] inhibited	Growth[b] weakly inhibited	Growth[b] stimulated	References
Gram-negative					
Acinetobacter		+			*(46)*
Aeromonas hydrophila		+	+		*(59,75)*
Alteromonas spp.		+	+		*(46,103,114)*
Campylobacter jejuni					
survival	+			+	*(50,90,105)*
growth		+	+		
Enterobacter spp.	+	+			*(1,47,115)*
Escherichia coli		+	+		*(25,26,75,77,115)*
Moraxella spp.		+			*(36)*
Proteus spp.		+			*(115)*
Pseudomonas spp.		+	+		*(6,25,29,30,56,57)*
Salmonella spp.		+	+	+[c]	*(6,25,27,56,57,71,98)*
Serratia spp.	+	+			*(1,115)*
Vibrio spp.		+			*(36)*
Yersinia enterocolitica		+			*(25,46,115)*
Gram-positives					
Bacillus spp.		+	+		*(25,26,28,29,30,75)*
Brochothrix thermosphacta		+	+		*(13,25,75)*
Clostridium botulinum	+		+[d]	+[e]	*(23,28,37)*
Clostridium PA3679		+			*(98)*
C. sporogenes-survival		+		+	*(6,22,115)*
C. perfringens		+		+[e]	*(56,57)*
Enterococci		+	+		*(75,98)*
Corynebacterium spp.		+			*(1,36)*
Lactobacillus spp.	+		+[f]	+	*(13,25,115)*
Leuconostoc spp.		+			*(115)*
Listeria monocytogenes	+[g]	+			*(113)*
Staphylococcus aureus		+	+		*(6,49,56,70,75,98)*
Streptococcus spp. *(besides enterocci)*	+				*(29)*
Molds		+[h]	+		*(25,56,77,100,101)*
Yeasts	+		+		*(25,60,61,72,77)*

[a]Degree of inhibition increases as temperature decreases.
[b]Growth and/or survival considered relative to growth and/or survival in air.
[c]Only 1 report of growth stimulation.
[d]At atmospheric pressure 100% CO_2 can delay toxin production when compared with an atmosphere of 100% N_2.
[e]Spore germination enhanced at 1 atmosphere CO_2.
[f]Some of the lactobacilli especially the homofermenters have been reported to be inhibited by 100% CO_2; see *(75)*.
[g]Provided at least 5% O_2 included.
[h]Mainly due to oxygen exclusion.

Although samples stored under 100% CO_2 gave the best recovery of *C. jejuni*, an atmosphere of 100% N_2 also enhanced recovery of the organism, as compared to samples stored in air.

The relative effects of CO_2 on other foodborne pathogens as well as spoilage microorganisms are listed in Table 3.

D. *The use of MAP for various food commodities*

I. Beef

Early workers in the field *(18)* demonstrated that CO_2 or combinations of CO_2 with O_2, could be effective in extending the shelf life of meats. At an early stage it was also recognized that the closer the temperature to 0°C, the higher the CO_2 concentration, and the lower the numbers of bacteria at the time CO_2 was applied, the longer the shelf-life extension would be.

Generally, when dealing with MAP of meats there are four main areas to be concerned with; namely, the control of bacterial pathogens and spoilage microorganisms, the maintenance of meat color, the control of weight loss, and the development of meat tenderness.

1. *Gases used in MAP of meats.* For fresh meats in which red color maintenance is important, 60-85% O_2 is generally used so that the formation of metmyoglobin, which gives a brown color to the meat, will be retarded for an extended period of time.

CO_2 is generally used in concentrations ranging from

15-40%, as higher concentrations can cause bleaching or discoloration of the meat surface due to the denaturation of meat proteins. Optimal inhibition of meat spoilage bacteria is achieved using concentrations of 40-60% CO_2 *(14)*, although some studies indicate that 20-30% CO_2, or even 10% CO_2 may be sufficient to retard bacterial growth *(14,95)*. The variability in the reported percentages of CO_2 required for optimal inhibition, relates to the fact that although most research is reported in terms of % gas, the important parameter is probably the amount of gas available. Excess CO_2 above the volume which will dissolve in meat is necessary for optimal antimicrobial effectiveness, with a rough figure being 1 to 1.5 liters of gas per kilogram of meat *(24,44)*.

2. *Effect of CO_2 on meat spoilage bacteria. Pseudomonas, Moraxella,* and *Acinetobacter* species, the main spoilage bacteria of aerobically stored chilled fresh meats, are generally inhibited by concentrations of 20% CO_2 or greater, while gram-positive microorganisms such as *Lactobacillus* spp. and *Brochothrix thermosphacta* are usually resistant to inhibition by CO_2. Thus, a shift from an initial gram-negative aerobic spoilage flora to a predominantly gram-positive facultatively anaerobic microflora dominated by *Lactobacillus* spp., usually occurs in muscle foods during MA storage. This is very beneficial in the sense that the by-products of the metabolism of the lactobacilli are usually produced very slowly and are inoffensive compared to the typical spoilage odors produced by the pseudomonads. Generally, under an anaerobic MA, the lactobacilli will usually outcompete the background microflora *(79)*. Organisms which appear to be a problem in high pH (pH >6.0), dark, firm, dry (DFD) meats are *Alteromonas putrefaciens, Enterobacter liquefaciens*, and *Y. enterocolitica (43)*, which would all be most likely inhibited by a typical MA environment used for fresh meats (see Table 3). Examples of the types of meats which have had their shelf life extended by storage in a MA environment are shown in Table 4. Normally, MAP meats stored at 4°C, have a shelf life 2 to 3 times greater than air-packaged products *(101)*.

TABLE 4. *MAP - Meat products.*

Product	Temp.	Atmosphere	Shelf life (d)	Reference
Beef	4°C	20% CO_2, 80% O_2	>15	(97)
Pork loins	1°C	50% CO_2 25% O_2, 25% N_2	Between 14 and 20	(99)
Pork	4°C	100% CO_2	>24	(31)
Lamb chops	-1°C	20% CO_2, 80% N_2 (low O_2)	6 wk	(80)
		(No O_2)	8 wk	
Frankfurter sausages	4°C	100% CO_2	>210	(12)
DFD beef Frankfurters	4°C	100% CO_2	?	(32)
	4.4°C	5-96%	?	(82)
Cooked beef	12.8 or 26.7°C	75% CO_2 in 0-25% O_2 (balance N_2)	?	(57)
Venison loins	-1°C	100% CO_2	Ca. 12 wk	(96)

II. Chicken

As for most other MAP foods, the shelf life obtained with gas-packed poultry will depend on the storage temperature, the types and initial numbers of bacteria, the initial CO_2 concentration in the container, how good a barrier the packaging film is to CO_2, and the amount of headspace gas in the container *(54)*.

One of the earliest studies which examined the microbiology of MAP chicken was done by Ogilvy and Ayres *(81)*. Cut-up chicken portions were stored at 0-10°C under both MAP and CAP type systems. CAP proved to be the most effective in extending shelf life when using CO_2 concentrations varying from 5-25%. Although the shelf life of the chicken increased linearly with increasing CO_2 concentration up to 25%, at this concentration the meat became discolored. Many other early studies also observed an extended shelf life for chicken packaged in MA, as compared to air storage (see Table 5).

TABLE 5. *Examples of early studies on MAP poultry.*

Product	Temp.	Gas atmosphere	Shelf life (d) Air	MAP	Reference
Chicken	1.5°C	50 and 80% CO_2	14	≥23	(41)
Chicken carcasses	1.1°C	CO_2[a] (two levels)	10	22-27	(94)
Whole carcasses	2°C	20 or 65% CO_2		18 (maximum shelf life)	(3,4)
Chicken pieces	0-10°C	5-25% CO_2	ca 9-17[b]	35-37[b]	(81)
Chicken carcass	1°C	10 and 20% CO_2 (CA system)	?	?	(110)

[a]3.61 x 10^{-4} and 7.22 x 10^{-4} m^3/kg.
[b]For freshly dressed broilers at 4.4°C.

More recently, Baker et al. *(5)* stored samples of ground chicken tissue at 2°C for up to 28 d under MAs ranging from 20-100% CO_2 (remainder air). The effectiveness of any level of CO_2 depended on the storage time. After 7 d, 20% CO_2 was as effective as higher CO_2 loads in inhibiting bacteria, while after 28 d, 80% CO_2 was needed to keep the total microbial load below 10^8/g. After 28 d in CO_2-treated samples, *Lactobacillus* spp. predominated, with pseudomonads making up only 1-10% of the final population. The basic conclusions from this study were that the optimum concentration of CO_2 depended in part on the desired shelf-life extension, and that for a shelf-life of approximately 28 d, it is necessary to apply a CO_2 concentration of around 60-80%. In a follow-up study done with chicken quarters, similar results were obtained. A shift from a gram-negative to a gram-positive bacterial population resulted upon application of 60-80% CO_2, with the shelf-life of the product extended to at least 35 d at 2°C, compared to 12-14 d for air-stored samples *(55)*. In more practical terms, poultry processors who use CO_2 for gas packing are reportedly achieving a 17-18 d shelf life *(52)*. The general consensus is that (a) the shelf life of fresh poultry can be extended by using MAP, providing the MA contains greater than 20% CO_2, and (b) the optimum CO_2 concentration to use depends

on the desired shelf-life extension.

In Canada, the organisms most often associated with foodborne disease involving poultry include (in order of importance) *Salmonella* spp., *S. aureus, C. perfringens,* and then *B. cereus* and *Campylobacter (108).* Concerning the safety of MAP chicken, the possible problem organisms would be *C. jejuni*, which may be able to survive better in a MAP product, and *L. monocytogenes* and *A. hydrophila*, which may, because of the extended storage lives of the MAP products, have additional time to grow to potentially high numbers. Although *C. perfringens* may be able to survive better in some MA as compared to air, it would not be able to grow at the chill temperat... products. Thus, it would not be mu... d in a MAP product unless the product is t... sed, because high numbers of the organism m... to cause illness *(64).*

III. Fish

As for most other foods, there are three majo... by which fish can be stored in MAP environments.

1. *Large van.* Looking for more efficient means t... fresh salmon from remote fishing villages to U.S. a... foreign markets, Alaskan fisheries started shipping dressed salmon from Anchorage to Seattle by sea van for distribution fresh in the Northwest and California. Salmon, which were usually less than 36 h old, were stored in 20 to 40 foot vans containing a CO_2-enriched MA. The vans were filled with salmon in cartons, the atmosphere injected, and the van then sealed with polyethylene sheeting. The MA sea van w... found to compete economically not only with air shipm... but with frozen production as well *(109).*

2. *Master pack.* In this type of packaging ... fish product is first overwrapped with PVC film ... s then placed in a large partial barrier film, master ... bag that has usually been flushed with CO_2. Prior ... ail display, the consumer package is taken out of t... ster package, and, once removed, has a shelf life ... ximately 3 to 4 d *(16).*

3. *Individual consu...* of the potential hazards of MAP fish, there is currently little MAP fish being sold in the United States at the retail level. In Western Europe and Canada, some MAP is being applied at the retail level.

Seafoods unlike other muscle foods (see from...

enhance growth of C. botulinum. ... C. botulinum type E is a natural...

...which are eaten without further cooking, which would include products such as cooked peeled shrimp and smoked salmon. The health risks associated with eating MAP smoked fish products may be high because the general contamination of fish with botulinal spores is high, the spores of *C. botulinum* can survive smoking, there are fewer competing microorganisms, smoked-fish products are handled more than fresh fish, and they are often eaten without prior cooking. However, because of the built-in safety factors (such as salt in combination with smoking) in some of the MAP fish products, if any problems do arise in these products, these would probably be a result of insufficient salting, gross temperature abuse, or heavy initial contamination with botulinal spores *(51).*

Research on the effects of CO_2 on fish packaged in a MA (100% CO_2) began in the 1930's and demonstrated that cod, whiting, and ha... 100% CO_2 at 0°C had a shelf-life extens... 100% compared to air controls at the same tem... e *(20).*

4. *Shelf-life exten... MAP fish.* There have been numerous studies sh... that the shelf life of MAP fish is substantially incre... as compared to air controls at the same temperatu... any different types of fish, storage temperatures, ... A's have been used (Table 6). Generally ... CO_2-MAP ... es for consistently improved stability of ... h fish ... higher initial CO_2 levels resulting in in... ed ... life extensions. However, this holds true only ... chilled product is hygienically handled from time ... ting and is held at proper temperatures. MAP

... P - Fish products.

Prod...		Atmosphere	Shelf life (d) Air	MAP	Reference
Cod f...		48 to 100% CO_2 ... ainder O_2 or N_2	-	-	(*104*)
Trout, golden croa...		00% CO_2	-	-	(*7*)
Cooked cray fish	4	CO_2 : ... ir	14	≥21	(*111*)
Trout	1.7°C	:	12	ca. 20	(*8*)
Spotted shrimp	0-2°C		2-16		(*67*)
Swordfish		40 and 70% CO_2 remainder O_2 or N_2			(*66*)
Brown shrimp	4°C	100% CO_2	4-5	>14	(*65*
		66% CO_2:	"	ca. 13.5	
		...		...	
		...		...	
		...		...	
		20% air ...			
crab		100% CO ...			(*48*)
bluefish croaker	10°C				
Rock cod	4°C	80% CO_2 : 20% air	7	≥21	(*74*)
Prawns	4°C	100% CO_2	6-7	8-9	(*88*)

on the desired shelf-life extension.

In Canada, the organisms most often associated with foodborne disease involving poultry include (in order of importance) *Salmonella* spp., *S. aureus, C. perfringens,* and then *B. cereus* and *Campylobacter (108).* Concerning the safety of MAP chicken, the possible problem organisms would be *C. jejuni,* which may be able to survive better in a MAP product, and *L. monocytogenes* and *A. hydrophila,* which may, because of the extended storage lives of the MAP products, have additional time to grow to potentially high numbers. Although *C. perfringens* may be able to survive better in some MA as compared to air, it would not be able to grow at the chill temperatures commonly used for MAP products. Thus, it would not be much of a health hazard in a MAP product unless the product is temperature abused, because high numbers of the organism must be ingested to cause illness *(64).*

III. Fish

As for most other foods, there are three major modes by which fish can be stored in MAP environments.

1. *Large van.* Looking for more efficient means to get fresh salmon from remote fishing villages to U.S. and foreign markets, Alaskan fisheries started shipping dressed salmon from Anchorage to Seattle by sea van for distribution fresh in the Northwest and California. Salmon, which were usually less than 36 h old, were stored in 20 to 40 foot vans containing a CO_2-enriched MA. The vans were filled with salmon in cartons, the atmosphere injected, and the van then sealed with polyethylene sheeting. The MA sea van was found to compete economically not only with air shipments, but with frozen production as well *(109).*

2. *Master pack.* In this type of packaging, the fish product is first overwrapped with PVC film and is then placed in a large partial barrier film, master pouch bag that has usually been flushed with CO_2. Prior to retail display, the consumer package is taken out of the master package, and, once removed, has a shelf life of approximately 3 to 4 d *(16).*

3. *Individual consumer package option.* Because of the potential hazards of MAP fish, there is currently little MAP fish being sold in the United States at the retail level. In Western Europe and Canada, some MAP is being applied at the retail level.

Seafoods, unlike other muscle foods, are very susceptible to both microbiological and chemical deterioration. The major spoilage organisms found on spoiled fish include *Pseudomonas, Moraxella, Acinetobacter, Flavobacterium,* and *Cytophaga* species. From a microbiological safety standpoint, the organisms of greatest concern when dealing with MAP fish products are the nonproteolytic *C. botulinum* type E. The restricted growth of the normal fish spoilage bacteria by MAP may enhance growth of *C. botulinum.* As mentioned previously, *C. botulinum* type E is a natural seafood contaminant which can grow at temperatures as low as 3.3°C. One of the major safety concerns is with fish products which are eaten without further cooking, which would include products such as cooked peeled shrimp and smoked salmon. The health risks associated with eating MAP smoked fish products may be high because the general contamination of fish with botulinal spores is high, the spores of *C. botulinum* can survive smoking, there are fewer competing microorganisms, smoked-fish products are handled more than fresh fish, and they are often eaten without prior cooking. However, because of the built-in safety factors (such as salt in combination with smoking) in some of the MAP fish products, if any problems do arise in these products, these would probably be a result of insufficient salting, gross temperature abuse, or heavy initial contamination with botulinal spores *(51).*

Research on the effects of CO_2 on fish packaged in a MA (100% CO_2) began in the 1930's and demonstrated that cod, whiting, and haddock packaged under 100% CO_2 at 0°C had a shelf-life extension of 50-100% compared to air controls at the same temperature *(20).*

4. *Shelf-life extension of MAP fish.* There have been numerous studies showing that the shelf life of MAP fish is substantially increased, as compared to air controls at the same temperature. Many different types of fish, storage temperatures, and MA's have been used (Table 6). Generally CO_2-MAP provides for consistently improved stability of fresh fish, with higher initial CO_2 levels resulting in increased shelf-life extensions. However, this holds true only if a quality chilled product is hygienically handled from time of harvesting and is held at proper temperatures. MAP

TABLE 6. *MAP - Fish products.*

Product	Temp.	Atmosphere	Shelf life (d) Air	MAP	Reference
Cod fillets	2°C	48 to 100% CO_2 - remainder O_2 or N_2	-	-	(*104*)
Trout, golden croaker	4°C	100% CO_2	-	-	(*7*)
Cooked cray fish	4°C	80% CO_2 : 20% air	14	≥21	(*111*)
Trout	1.7°C	80% CO_2 : 20% N_2	12	ca. 20	(*8*)
Spotted shrimp	0-2°C	50% CO_2 : 50% air	12-16		(*67*)
		100% CO_2			
Swordfish	3.5°C	100% CO_2 plus 40 and 70% CO_2 remainder O_2 or N_2	-	-	(*66*)
Brown shrimp	4°C	100% CO_2	4-5	>14	(*65*
		66% CO_2: 34% O_2	"	ca. 13.5	
		38% CO_2 : 62% O_2	"	ca. 11.5	
		65% CO_2 : 35% N_2	"	ca. 11.5	
		35% CO_2 : 65% N_2	"	ca. 8.5	
Rockfish fillets	1.7°C	80% CO_2 : 20% air	≤6	≥13	(*86*)
Dungeness crab	1.7°C	80% CO_2 : 20% air	ca 14	≥25	(*87*)
Perch seatrout, bluefish croaker	1.1 or 10°C	100% CO_2	-	-	(*48*)
Rock cod	4°C	80% CO_2 : 20% air	7	≥21	(*74*)
Prawns	4°C	100% CO_2	6-7	8-9	(*88*)

cannot be used to salvage inferior quality fish products. Some investigators have also looked at the possibility of combining MAP with preservatives in the form of ice *(35)* or dips *(73,93)*.

5. *Safety of MAP fish products*. The safety of MAP fish products has been extensively examined and/or reviewed by many authors *(15,16,33,39,40,42,53,58,69,92,103,107,112)*. As discussed previously, the major safety concern is growth and toxin formation by the nonproteolytic clostridia which can grow at low temperatures. Although controversy still exists as to whether toxin production can or cannot occur before organoleptic spoilage at room temperature or above *(53)*, there is no doubt that under certain conditions of temperature abuse, or even at 4°C under an anaerobic MA, botulinum toxin can be detected well before any evidence of organoleptic deterioration *(92)*. Because the proper refrigeration of foods throughout the distribution chain cannot be ensured, most experts feel, that at this time, consumer-sized packages of MAP fish should not be available for sale at the retail level. The National Academy of Sciences in the U.S. has stated that "fishery products which are vacuum or controlled-atmosphere packaged should not be held at refrigerated temperatures" *(78)*.

Another concern is that, although *C. sporogenes* cannot grow in 80% CO_2 (remainder air) at 2, 7, or 13°C, survival of the organism appears to be much greater in the MA, as compared to an air control *(6)*. *C. sporogenes* is often used in challenge studies or other lab experiments to mimic the biological characteristics of the proteolytic clostridia because it does not produce toxin *(102)*.

The only effective way to assure the safety of refrigerated vacuum-packaged or MAP fish products would be to either (a) keep the product below 3°C at all times, (b) heat the product sufficiently to destroy spores of all strains, or (c) heat the product sufficiently to inactivate the nonproteolytic spores and then keep the product well below 10°C. The latter two points may be effective from a theoretical standpoint, but in practice it may be difficult in a fish-processing environment to avoid post-processing contamination with spores of *C. botulinum*.

IV. Other MAP foods

Other foods which are currently being packaged under MAP in North America are listed in Table 7, and examples of MAP sandwiches (a strong growth area in Canada) and the type of atmosphere in which they are being stored are given in Table 8. In addition, examples of the atmospheric conditions under which a wide variety of other foods have been stored are demonstrated (Table 9).

E. *Summary*

The success of MAP depends on many factors including good initial product quality, good hygiene from slaughter on, correct packaging material selection, the appropriate gas mix for the product (determined by consultation and by in-house or contract research), reliable packaging equipment, and maintenance of controlled temperatures. It is important to realize that storage under controlled or modified atmospheres will not improve the quality of the product, it will only delay the rate of spoilage. As well, CA or MAP is not a substitute for good sanitation, nor a replacement for strict temperature control.

TABLE 7. *Examples of food currently being packaged under MAP in North America.*

A. Fresh Raw Meats	
e.g.	sliced bacon
	steak
	beef hearts
	pork kidneys
	ox tails
B. Cooked Meats	
e.g.	hamburgers
	beef jerky
	sausage rolls
	sliced meats
	cretons (head cheese)
	wieners
C. Poultry	
e.g.	whole carcasses
	nuggets
	chicken parts
	peeled hard cooked eggs
D. Fish (Canada only)	
E. Cheese	
F. Prepared salads (mainly at restaurant level)	
G. Pasta	
H. Various types of sandwiches (see Table 8)	

One generally wants to avoid high hazard, abusive type conditions where spoilage organisms are low in numbers and pathogenic bacteria high. Obviously the ideal situation is one in which lactic acid bacteria predominate at the onset, and then continue to multiply throughout the storage period, keeping the background microflora, including any pathogenic microorganisms which may be present, at very low levels. In the future, the addition of lactic acid starter cultures may prove to be very beneficial in certain MAP products. This is in keeping with the hurdle/barrier concept *(68)* in which additional barriers or hurdles, such as acidity, water activity, temperature, competitive flora, redox potential, and preservatives may be built into a particular food (MAP) product. These hurdles (including modified atmosphere) could interact either directly or synergistically to secure microbial stability of a particular food product *(68)*. The development of such safe food systems, rather than safe products, would hopefully prevent the development of any potential health hazard.

Information on the microbiological safety of MAP products appears to be lacking regarding (a) the effects of MAP on the growth of psychrotrophic pathogens, (b) interactive effects on microorganisms, (c) effect of failures in packaging systems, and (d) storage conditions and possible consequences of temperature abuse by the consumer. Although there appears to be great potential for abuse with

TABLE 8. *Examples of various MAP sandwiches currently distributed in Canada.*

Product	Gas mixture	Expected shelf life (d)
Head cheese	80 : 20 CO_2 : N_2	30
Meat submarine sandwich	50 : 50 CO_2 : N_2	30
Submarine sandwich	30 : 70 CO_2 : N_2	30
Large sandwich	20 : 80 CO_2 : N_2	30
Meat submarine	5 : 95 CO_2 : N_2	30
Pepperoni, ham, cheese sandwich	100% N_2	30
Cretons	90 : 10 CO_2 : N_2	50
Cretons	15 : 85 CO_2 : N_2	42
Cretons	75 : 25 CO_2 : N_2	60
Creton sandwich	5 : 95 CO_2 : N_2	30
Turkey Breast sandwich	30 : 70 CO_2 : N_2	30
Turkey sandwich	5 : 95 CO_2 N_2	21
Cheeseburger	CO_2 : N_2 20 : 80	30
Pizza sub	100% CO_2	17
	20 : 80 CO_2 : N_2	30
	50 : 50 CO_2 : air	30
Mixed meat sub	100% CO_2	21
Ham'n cheese sandwich	50 : 50 CO_2 : N_2	35
	100% CO_2	30
Beef Jerky (Chinese style)	100% N_2	30
European cocktail wieners	100% N_2 or N_2/CO_2	42
Deviled egg sandwich	30 : 70 CO_2 : N_2	30

TABLE 9. *Examples of gas mixtures - MAP products.*

Product	% CO_2	% O_2	% N_2
Fresh meat	30	30	40
	15-40	60-85	-
Cured meat	20-50	0	50-80
Sliced cooked roast beef	75	10	15
Eggs	20	0	80
	0	0	100
Poultry	25-30	0	70-75
	60-75	5-10	≥20
	100	0	0
	20-40	60-80	0
Pork	20	80	0
Processed meats	0	0	100
Fish (white)	40	30	30
Fish (oily)	40	0	60
	60	0	40
Cheese (hard)	0-70		30-100
Cheese	0	0	100
Cheese; grated/sliced	30	0	70
Sandwiches	20-100	0-10	0-100
Pasta	0	0	100
	70-80	0	20-30
Bakery	20-70	0	20-80
	0	0	100
	100	0	0

MAP foods, there have been no reported illnesses of foodborne disease linked to the consumption of these products. This may be due to several factors. For one, products of superior initial quality are generally used. This would ensure that the product initially contains low bacteria counts. Starting out with a low bacterial load results in a longer bacterial lag phase and a lower final cell density. In addition, the presence of pathogens is minimal, and the lactic acid bacteria dominate more easily. Secondly, temperature control is stressed both at the manufacturing and retail level. As stated previously, keeping product at temperatures close to 1°C results in a maximum extension of the lag phase and a long generation time, as well as ensuring optimum CO_2 solubility.

Although most pathogens would not be able to survive and/or grow any better in a MA as compared to air storage, and, in most cases, would fare worse in a properly refrigerated product, there are several products and/or organisms of concern. As previously discussed, it appears that some of the clostridia and *Campylobacter* species may be able to survive better in a MA as compared to an air atmosphere. However, only the nonproteolytic clostridia would grow at 4°C. It is because the nonproteolytic clostridia are psychrotrophic that much concern has been raised about he health hazards of MAP or vacuum-packed fish held at refrigeration temperatures. In addition, *L. monocytogenes, Y. enterocolitica,* and *A. hydrophila* appear capable of growing at chill temperatures in certain MAs. Therefore, MA products with extended refrigerated shelf lives could be a problem.

In North America, we will be witnessing great advances in the production of MAP products. Only by taking a proactive stance and doing the necessary research on microbiological safety, will government agencies be able to ensure the safety of the wide variety of new MAP products that will be on our grocery shelves in the 1990's.

ACKNOWLEDGMENTS

The author would like to thank Dr. J. Hotchkiss for his extremely helpful comments and critical review of this manuscript.

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