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Influence of New Packaging Technologies on the Growth of Microorganisms in Produce

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ABSTRACT

Several new technologies which are intended to extend the shelf life of respiring fruits and vegetables have been or are being developed. This is in response to the increased consumption of fresh fruits and vegetables and the desire to distribute branded products. The most widely studied and adopted technologies are controlled or modified atmosphere packaging. These packaging methods alter the gases surrounding a respiring product in order to slow the normal senescence or decay of the product. Controlled or modified atmosphere packaging can also affect the types and growth rates of microorganisms associated with produce. This may slow the rate of deterioration of the produce but could also provide sufficient time for human pathogens to develop rendering the product unsafe while still edible. This possibility has not been thoroughly researched and so interest in the safety of these technologies exists. Of primary importance is the relationship between the growth rate of pathogenic microorganisms and the rate of decay of the produce. Produce which has spoiled beyond the point where it is edible is of much less risk than produce which remains edible while becoming infectious or toxic. The relationship between the formation of botulinum toxin and "edibility" of extended shelf-life packaged tomatoes is an example of such concern. Often measures of toxin formation are available but not directly compared to the likelihood that a product is acceptable and would be eaten. This paper discusses one such approach.

According to the U.S. Department of Agriculture, Americans consumed 37% more fresh vegetables in 1988 than in 1971 (*31*). Annual per capita fresh fruit consumption increased by 19 lb (ca. 8.6 kg) over the same period. With some exceptions, most fresh fruits and vegetables are sold without packaging and unbranded. There has been a recent interest in producing brand name "fresh" fruit-and/or vegetable-based products which provide both convenience and sufficient shelf life for distribution (*39*). A worldwide market has developed for fresh fruits and vegetables resulting in a need for longer shipping times and increased retail display time. Achieving these goals usually requires that the product have an extended shelf life compared to conventional products.

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A number of technologies have been or are being developed to increase the shelf life of both whole produce and minimally processed produce items including prepared salads, precut vegetables, peeled fruits, and long-life whole vegetables. Low-dose irradiation, low-input thermal processes, additives, controlled/modified atmosphere packaging (C/MAP), and gas absorbers or emitters are being developed and used to fill this need. These technologies have the common objective of extending shelf life by reducing the rate of organoleptic spoilage and nearly always involve sealed containers or packages (*40*).

In modified atmosphere packaging (MAP), the gas or gases surrounding a packaged product are changed (i.e., "modified") to a composition other than that of air (air = ca. <0.1% CO_2, 21% O_2, 78% N_2). The headspace gas composition in sealed packages will change over time as the product respires. Technologies which limit these gas composition changes during storage are termed controlled atmosphere packaging. Both of these technologies can extend shelf life by decreasing the rate of product deterioration.

Extension of produce shelf life with MAP

Kader and coworkers have extensively reviewed the effects of gas atmospheres on the decay or senescence of many fruits and vegetables (*22,23*). Fresh fruits and vegetables are metabolically active long after harvest. Senescence (ripening) and loss of eating quality are a result of intrinsic enzymic processes such as respiration, metabolism (e.g., composition changes), and growth and development which are inherent to plant material. External factors such as physical injury, water loss, storage temperature, and microbial infection also influence deterioration. These factors all combine to influence the rate or speed of deterioration and hence the shelf life.

One primary factor in the rate of decay is the composition of the gas surrounding the product. For example, reducing the O_2 concentration to approximately 2% and increasing CO_2 to 5% results in more than a 10-fold decline in the rate respiration of broccoli florets (Fig. 1; *45*). In addition to slowing respiration, MAP can control some insects, reduce numbers of plant pathogens, and reduce

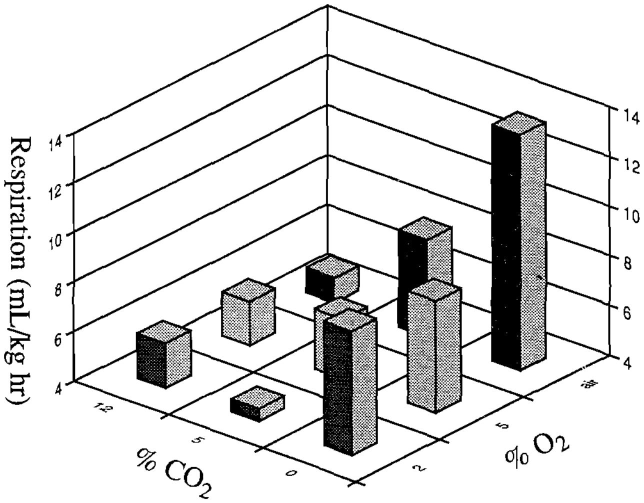

Figure 1. *Effect of different gas mixtures on the respiration of broccoli stored at 0°C for 7 d (adapted from ref. 45).*

sensitivity to ethylene. However, there are limits to the atmospheres used. Too little O_2 or too much CO_2 can cause irregular ripening, increased browning, and the development of off-odors and flavors (*23*). Another disadvantage is that each produce item may have different combinations of gases that reduce deterioration which means that a different packaging system will have to be tailored for each produce item.

Effects of MAP on microorganisms

There are at least five factors affecting the growth of microorganisms on produce and other foods including the numbers and types of microorganisms present, the ability of the food to support microbial growth (i.e., the nutritional composition, release of nutrients due to processing or damage, and/or the presence of antimicrobial additives), the water activity, the temperature, and lastly the gas atmosphere surrounding the product. Besides temperature which has a lower practical limit, the most readily manipulated factor is the gas composition. In addition to the effects on indigenous processes in fresh produce, modified atmospheres (MAs) affect the microorganisms occurring on such products.

The relative importance of microorganisms versus the inherent decay of respiring produce is not clear. For example, Nguyen-The and Prunier (*32*) suggested that a relationship between the deterioration of leafy salads and the growth of *Pseudomonas* spp., particularly *Pseudomonas marginalis*, exists. *Pseudomonas* spp. appear to be the most common organisms on minimally processed vegetables; however, their importance in quality loss is not clear (*16,27*).

MAs may not have the same effect on the microorganisms as on inherent deterioration processes in the produce. Berrang et al. (*4*) compared the growth of naturally occurring microorganisms on asparagus, broccoli, and cauliflower to the time in which the produce became inedible. Storage in reduced O_2 and elevated CO_2 increased the edible shelf life from 14 to 21 d for all products. However, for asparagus and cauliflower there were only small effects on the numbers of microorganisms when compared to controls. For broccoli, there was a larger effect on the general microbial population (Fig. 2). Brackett (*8*) similarly found that packaging method does not necessarily

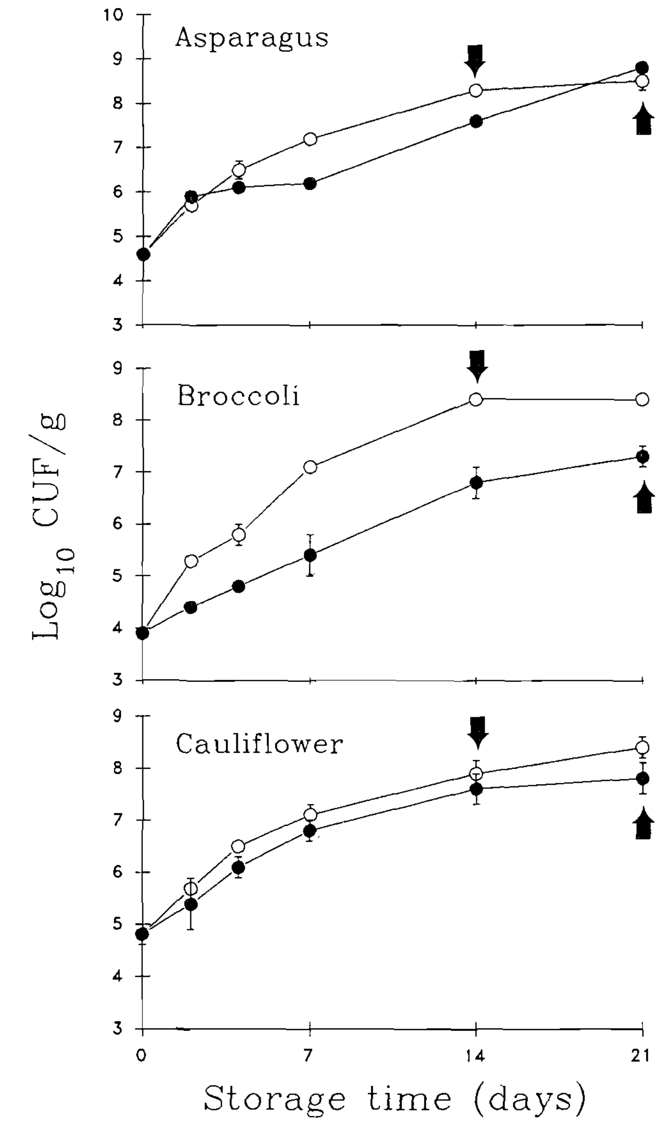

Figure 2. *Growth of aerobic plate count organisms on fresh asparagus, broccoli, and cauliflower stored at 4°C in air (○) or MA (●). The day in which the product was considered inedible is noted with an arrow. Error bar are standard error (adapted from ref. 4).*

affect the microflora of produce in the same manner as the shelf life. The shelf life of packaged peppers was found to increase from 3 to 6 weeks compared to loose peppers. However, the shrink-wrapped green peppers were found to increase in aerobic microorganisms faster than unpackaged peppers even though the packaged product had a longer shelf life. Buick and Damoglou (*10*) also found that MAP significantly increased the shelf life of mixed vegetable salads. Clearly, insufficient research has been conducted on the relationships between microorganisms, inherent enzyme-mediated decay, and gas composition in fresh produce.

Safety of MAP produce

There has been concern that packaging technologies which extend the shelf life of refrigerated produce items might increase risks associated with pathogenic microorganisms. At least four events must occur for an outbreak of foodborne disease:

a. *The food must contain at least one type of pathogenic* microorganism. While there are only a limited number of surveys of the prevalence of pathogens in produce, it must be assumed that pathogens occur with sufficient frequency to cause outbreaks.

b. In most cases, contamination alone is unlikely to be sufficient to cause disease. The food must support pathogen growth and/or toxin production in order to produce sufficient numbers of organisms to be virulent. The ability of fruits and vegetables to support pathogen growth will depend on a number of factors including whether or not the produce is whole or has been cut and prepared.
c. The food processing and/or storage conditions must be insufficient to destroy the pathogens. This is the case with minimally processed fruits and vegetables.
d. Lastly, the food must be consumed. Products which become inedible prior to the development of sufficient pathogens for disease are less likely to be consumed and cause disease than foods which do not have indicators of spoilage but may contain pathogens.

While there is a growing body of literature on the effects of MAP on the shelf life of produce, there is little published work on the effects of MAP on pathogenic microorganisms in raw or minimally processed (e.g., cut and washed) produce. In general, produce has not been considered a major vector for foodborne disease. However, technologies which extend the edible shelf life of raw and minimally processed fruits and vegetables could allow sufficient time for pathogen growth in products which would not, with conventional handling, support sufficient pathogen growth before becoming organoleptically unacceptable. The major objective of C/MAP technologies is to increase the time in which fruits and vegetables remain edible (i.e., reduce spoilage). However, C/MAP could in some cases enhance the growth of pathogenic organisms. The major safety consideration in C/MAP produce is whether or not spoilage (inedibility) occurs before or after pathogenicity.

Beuchat and Brackett (*7*) have recently published a study which points out the importance of the relationship between organoleptic spoilage and pathogenicity. Whole and chopped tomatoes were inoculated with *Listeria monocytogenes* and sampled over time. *L. monocytogenes* grew, albeit slowly, in whole tomatoes and died off slowly in chopped tomatoes held at 21°C. Under normal handling, tomatoes are not likely to support growth. However, a substantial increase in shelf life might give sufficient time for *L. monocytogenes* growth. The authors pointed out that *L. monocytogenes* can remain viable after normal shelf-life expectancy. While tomato products were not good substrates for *Listeria*, technologies which decrease the rate of senescence of whole tomatoes at room temperature might create an increased risk.

L. monocytogenes has been studied in other produce items. *L. monocytogenes* occurs naturally in and as a contaminant of lettuce, cabbage, and other vegetables and is known to occur in soil, vegetation, and agricultural environments (*43*). Given that *L. monocytogenes* can survive and grow under a wide range of conditions, it is not surprising that it has been isolated from raw vegetables. Hofer (*20*) isolated untyped strains of *Listeria* from cabbage and other vegetables. More recently, Steinbruegge et al. (*42*) found that 9% of head lettuce tested positive for *L. monocytogenes*. In a large survey, Heisick et al. (*17*) found several types of vegetables positive for *Listeria* spp. including one of 92 whole-head lettuce samples and two of 92 head lettuce samples. In contrast, Farber et al. (*12*) found no *L. monocytogenes* in whole-head lettuce samples collected in Canada. The author attributed this contrast with previous reports to seasonal differences and/or ability to detect very low levels. Petran et al. (*33*) also did not find any positive head lettuce samples in a smaller survey.

The prevalence of *L. monocytogenes* seems to be higher in prepared (i.e., shredded and mixed) salads of the type which are most likely to be packaged in MAs. Sizmur and Walker (*38*) found four of 60 packaged, prepared shredded lettuce-and cabbage-based mixed salads to contain *L. monocytogenes*. *L. innocua* was isolated from 13 of 60 samples. Bendig and Strangeways (*1*) found three of 16 samples of "english round lettuce" including that used in mixed salads to contain *L. monocytogenes*.

Work by Steinbruegge et al. (*42*), Berrang et al. (*2*), and Beuchat and Brackett (*5*) indicates that *L. monocytogenes* will grow to high numbers in lettuce, cabbage, and other vegetables given sufficient time [interestingly, carrots are inhibitory (*6*)]. Steinbruegge et al. (*42*) found that in most, but not all cases, *L. monocytogenes* grew in lettuce and lettuce juice. *L. monocytogenes* was found to be naturally present in some samples. Berrang et al. (*2*) showed that the composition of the atmosphere in which cut asparagus, broccoli, and cauliflower were stored decreased the rate of deterioration but did not affect the growth of *L. monocytogenes*. The net effect of the MA was to increase the time in which *L. monocytogenes* could grow while the vegetables remained edible. The authors point out that problems not normally encountered in fresh produce could occur if shelf life were extended without inhibition of microbial growth. For example, they found that *L. monocytogenes* was significantly higher in asparagus stored for 21 d under MA compared to 14 d of conventional storage, even though both had similar organoleptic quality.

Similar conclusions can be drawn from the work of Beuchat and Brackett (*5*) who investigated the effect of some processing and storage variables including temperature and MA on *L. monocytogenes* growth in shredded and whole leaf lettuce. Lettuce was inoculated by dipping at a level of ca. 10^4 CFU/g with *L. monocytogenes* and stored at two temperatures for up to 15 d in low barrier bags. At 5°C, significant growth was observed between 8 and 15 d, but at 10°C, differences were seen in 3 d. The MA had no effect on *L. monocytogenes* nor on the shelf life of the lettuce. This was likely due to the use of low barrier bags which would have negated any effect from the MA. Nonetheless, the authors suggested that because MA reduces the rate of organoleptic deterioration the risk of listeriosis may be increased due to the increased time for growth.

While there is no direct proof that *L. monocytogenes* in produce items have been responsible for any outbreaks, there is indirect evidence (*25*). For example, Ho et al. (*19*) after studying a large listeriosis outbreak in Boston concluded that salads made from raw vegetables were most likely responsible. Similarly, cut cabbage was strongly implicated in a Canadian outbreak (*37*). Additional circumstantial evidence (*2*) leads to a similar conclusion. These data have led some to suggest that susceptible populations not consume mixed salads (*1*).

Fewer studies have been undertaken related to the effects of C/MAP on the potential for toxigenesis by *Clostridium botulinum* in packaged produce. Workers at the U.S. Food and Drug Administration found that shredded cabbage that had been packaged in an anaerobic MA would support toxigenesis when incubated at room temperature (*41*). Importantly, these workers concluded that toxigenesis preceded organoleptic spoilage. However, the spoilage criteria used for this observation were not described in detail. This work followed a botulism outbreak in which MA-packaged shredded cabbage was implicated as the source of toxin (*40*).

In other work, packaged fresh mushrooms were implicated as a botulism hazard (*24*). Recently, Malizio and Johnson (*28*) concluded that spoilage preceded toxigenesis in Enoki mushrooms that had been vacuum packaged. Doyle (*11*) has recognized the need for further research into botulism risks in foods packaged in MAs and has recently proposed guidelines for such studies.

The potential for extended shelf-life whole tomatoes to support toxigenesis by *C. botulinum* when packaged in MAs is of interest because of the commercial introduction of extended shelf-life tomatoes. Tomatoes would not be expected to be a *C. botulinum* hazard because their pH is normally below that capable of supporting toxigenesis (*15*); however, stressing could elevate the pH. It is possible that tomatoes which were severely stressed and packaged in anaerobic atmospheres would become toxic. If this were the case, it would be important to know if toxigenesis occurred before or after the time that the tomatoes became inedible. Preliminary experiments were undertaken to determine the relationship between toxin production and shelf life of packaged tomatoes.

While the mouse assay is an accepted and defined criterion for toxigenesis, no such criterion existed for spoilage of whole tomatoes. Prior to the initiating the laboratory work, five "edibility" criterion were developed for whole tomatoes. Meeting any one criterion was sufficient to classify the tomato as inedible:

a. Mold growth covered more than 25%, in aggregate, of the surface area of the tomato.
b. The tomato's surface had one or more ruptures or tears in excess of 3 cm and juice was leaking from the tear.
c. More than 1 ml of juice had leaked from the tomato.
d. At least 50% of the tomato's surface was shriveled or wrinkled.
e. At least 25% of the surface was discolored, indicating internal decay.

After setting these criteria, preliminary inoculation challenge studies were designed. Whole tomatoes were inoculated by injection just under the skin with a mixed culture of 1.1 x 10^4 *C. botulinum* spores in 50 μl of buffer at the stem scar, blossom end, and equator. Tomatoes were then stressed in a manner that might occur in commercial practice. They were slightly overripe, intentionally bruised (dropped without skin rupture), and inoculated under the skin with a composite of *Fusarium* and *Alternaria* mold spores. Tomatoes were then sealed in high- and low- barrier packages and incubated at 13, 21, and 30°C. Tomatoes were assayed for toxin (mouse lethal assay) after 13 or 20 d of storage.

Most tomatoes met one or more criteria for inedibility 2 to 12 d before sampling. Twenty-eight of the 96 tomatoes tested for toxin resulted in toxin-related deaths (4/28 at 13°C; 10/28 at 21°C; 14/28 at 30°C). The initial pH of all 24 randomly tested tomatoes before inoculation was below 4.6. At the time of toxin testing, 25 of 28 had pH values above 4.6, probably as a result of mold growth. Molds can increase pH of their substrate by producing amines. Surprisingly, tomatoes from low-barrier packages were more likely to be toxic than from high-barrier packages. This may be due to the greater mold growth that occurred in the low-barrier packages which resulted in more rapid increase in pH.

If no consideration of spoilage (i.e., organoleptic acceptability) were taken into account, the above results might indicate that packaged tomatoes represent a significant risk of botulism. However, knowing whether or not a nonsterile product can support toxigenesis is insufficient to make a judgment about the level of risk involved. An understanding of the relationship between pathogenicity (e.g., toxin production) and organoleptic spoilage is important if the risk associated with extended shelf-life produce is to be understood (*18*). The above case indicated that tomatoes could, with some degree of spoilage, support toxigenesis by *C. botulinum*. These preliminary results prompted a much larger study designed to determine the relationship between toxin production and organoleptic spoilage. The results of this second expanded study indicated that spoilage preceded toxin formation (*21*). Both studies underscore the importance of objective measurements of spoilage in assessing the risks associated with MAP. The criteria for edibility have recently been expanded (*21*) and are given in Table 1.

A few workers have investigated the potential for growth of pathogens other than *Listeria* and *Clostridia* in packaged produce. Berrang et al. (*3*) found that MA lengthened the shelf life of vegetables but did not affect the growth of *Aeromonas hydrophila*. This may mean that *Aeromonas* has more time to grow while the vegetables remained edible. Satchell et al. (*36*) found that *Shigella sonnei* survived in refrigerated shredded cabbage when packaged aerobically or under MA.

CONCLUSIONS

Extending the shelf life of refrigerated foods might increase microbial risks in packaged produce in at least three ways: First, increasing the time in which food remains acceptable (edible) increases the time in which even slow growing pathogens can develop to significant numbers or produce toxin. This is especially true for pathogens that can

TABLE 1. *Edibility scores for whole tomatoes.*

Rating	9	7	5	3	1
Overall quality	Excellent	Good	Fair	Poor	Inedible
% Edible	100	100	75	50	0
Salability	Salable	Salable	Unsalable	Unsalable	Unsalable
General Description	No signs of decay. No postharvest blemishes.	Minor signs of decay visible to trained eye but of little significance to consumer.	Decay evident but not serious. Decay restricted to areas easily cut away by consumer.	Serious decay. Consumer unlikely to eat any of tomato but could salvage at least 50% if necessary.	Massive decay. Consumer reluctant to touch tomato.
Mold	None	Fine growth. Visible only to trained eye.	Growth readily detected by consumer but restricted to no more than two spots.	Lush growth, but limited to 5% of surface area.	Lush growth covers more than 10% of surface area.
Shrivel	None	Visible only to trained eye.	Readily detected by consumer but restricted to no more than two spots.	Large area (size of half dollar) or several small areas.	Massive shrivel. Dominates view of tomato.
Bruising	None	Visible only to trained eye.	Readily detected by consumer but restricted to shoulder area or other areas easily cut away.	Serious bruising. Evidenced by heavy discoloration (darkening) or water-logged appearance.	Massive bruising.
Internal decay	None	None	Minor. Restricted to no more than two areas near tomato surface--easily cut away.	Serious. Evidenced by heavy discoloration or water-logged appearance.	Massive. Evidenced by tissue rupture and leakage of juice.

grow, albeit slowly, at 3 to 8°C. Secondly, MAP can retard the development of competing spoilage organisms and thereby allow or enhance pathogen growth. Thirdly, packaging respiring produce in MAs could alter the atmosphere such that the growth of pathogens is stimulated. For example, CO_2 has been reported to enhance toxigenesis by *C. botulinum* (*14*).

The ecology of pathogens and nonpathogens in foods is complex, and it is not always possible to predict the effects of changes in atmospheres. For example, Lambert et al. (*26*) recently found that the inclusion of O_2 in sealed packages of pork appeared to enhance *C. botulinum* toxigenesis, perhaps as a result of higher CO_2 production by the aerobic organisms. Similar challenge studies in which toxigenesis is compared to organoleptic spoilage need to be initiated for packaged produce.

Inhibition of spoilage by MAP with concurrent development of a pathogen was demonstrated by Wimpfhiemer et al. (*44*). Spoilage microorganisms in MA-packaged raw chicken were significantly inhibited (up to 60 d) at 4°C, but *L. monocytogenes* grew to levels as high as 10^8 CFU/g.

Industry trade associations (*30*) and regulatory agencies including the U.S. Food and Drug Administration (*35*) have urged caution in the use of MAP and similar these shelf-life extension technologies, Moberg (*29*) has detailed several precautions be taken with these products.

Farber (*13*) has recently reviewed the microbiology of MAP and has concluded that there is a lack of knowledge concerning of the microbial safety of MAP and suggests that further research is needed in several areas including microbial interactions, effects of MAP on psychrotropic pathogens, and effect of package failure and temperature abuse. Farber points out that MAP foods are vulnerable from a safety standpoint because MAs may inhibit aerobic organisms which usually warn consumers of spoilage while the growth of pathogens may be encouraged.

MAP of prepared salads as well as other products is reported by industry sources to be widely practiced (*9,34*). At least two self-modifying systems have been commercially introduced in the last 2-3 years. Hercules' Fresh Pack and Dow Brands' Summerfields® are two examples. The introduction of commercial products gives impetus to further research into the effects of new packaging technologies on microorganisms associated with produce.

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