14

*Modified-Atmosphere Packaging of Produce**

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* This chapter has not been updated from first edition.

14.1 Modified-Atmosphere Packaging—Rationale

Immediately after harvest, the sensorial, nutritional, and organoleptic quality of fresh produce will start to decline as a result of altered plant metabolism and microbial growth. This quality deterioration is the result of produce transpiration, senescence, ripening-associated processes, wound-initiated reactions, and the development of postharvest disorders. In addition, microbial proliferation contributes markedly to postharvest quality loss. The relative importance of individual deterioration processes in determining the end of the shelf life will depend upon specific product characteristics as well as upon external factors. Low temperature and proper hygienic handling of the material are the prime factors that control these processes. In addition, modified-atmosphere packaging (MAP) is a preservation technique that may further minimize the physiological and microbial decay of perishable produce by keeping them in an atmosphere that is different from the normal composition of air [2,26,31,47,62,82].

The MAP of respiring food products such as fresh and minimally processed produce requires a different approach than the MAP of nonrespiring foods. With nonrespiring foods, modified atmospheres (MA) without oxygen are used to minimize oxidative deterioration reactions, such as brown discoloration of meat or rancidity of peanuts, or reduce microbial proliferation, e.g., the growth of molds in cheese and bakery products. High gas barrier films or laminates are used to exclude the exchange of gases (especially O_2) through the package, which would result in a less beneficial in-package gas atmosphere. In contrast, respiring products stay metabolically active after harvest, and this activity is essential for keeping their quality.

Aiming at the extension of the shelf life of respiring products through MAP, a prerequisite for a suitable packaging system will be that the composition of the gas atmosphere allows for a basic level of metabolism, which means that a certain amount of O_2 should be available. The required basic level of metabolism is highly at variance with different commodities (type and maturity) and heavily depends on the storage temperature and the degree of processing (trimming, cutting, slicing, etc.) applied. Owing to the significant respiratory activity of the product, the gas atmosphere inside the package changes during the course of the storage period, and expert knowledge about these changes is necessary to tailor the package design of an individual product to optimize quality shelf life.

The MAP of fresh and minimally processed fruits and vegetables is a preservation system that is nonsterile by design. Fruits and vegetables are characterized by an elaborate microflora, consisting of many different types of bacteria, molds, and yeasts, most of which are involved in the spoilage of the produce but are harmless to the human consumer. Microorganisms that are dangerous to humans (pathogens that are toxic or cause infectious diseases) normally cannot establish a dangerous population density because they have to compete with the spoilage microflora. However, packaging the produce will change the microenvironment perceived by the microorganisms and may well impair this safe balance. Consequently, evaluation of the impact of package design and use in the logistic chain is a mandatory exercise to assure consumer safety.

14.2 Early Research on Modified-Atmosphere Packaging

Packaging techniques based on altered gas conditions have a long history. Ancient Chinese writings report the transport of fruits in sealed clay pots with fresh leaves and grass added. The respiratory activity of the various plant products generated a low-oxygen and high-carbon dioxide atmosphere, which retarded the ripening of the fruit [42,59]. In the beginning of the nineteenth century, Berard [12] demonstrated that fruit placed in closed containers did not ripen. By the end of the nineteenth century, the first patent was granted covering the use of a CO_2/CO mixture to extend the shelf life of meat [31]. Extensive research on the use of altered gas conditions for fruits tailed early in the twentieth century, with the work of Kidd and West [66]. Commercial storage under altered gas conditions was undertaken in England in 1929, when apples were stored in 10% CO_2 and ambient O_2 [64]. Reduced O_2 concentrations and increased CO_2 concentrations also proved to be beneficial for harvested products other than apples. Products with a high potential for a successful commercial application in MAP include apple, banana, broccoli, cabbage, cherry, chicory, and brussels sprouts. The first commercial application of MAP did not take place until 1974, when the technique was used for meat [31]. The use of MA for storage and packaging has increased steadily over the years and contributed strongly to extending the postharvest life and maintaining the quality of fruits and vegetables [60]. In fact, the biggest growth in the use of MA has been for fresh fruits and vegetables, especially for minimally processed salads [31]. The technique of MA is now applied at a range of different sizes, i.e., for bulk storage packages (e.g., red currants), transport packages (e.g., bananas, strawberries), and consumer packages (e.g., apples, broccoli).

14.3 Effects of Modified Gas Atmospheres

The strategy of packaging produce under MA is to slow down the metabolic activity of the product as well as the growth of microorganisms (both spoilage and pathogenic) present by limiting O_2 supply and by application of an elevated level of CO_2. Because the same strategy underlies refrigerated storage, MAP of respiring produce is usually combined with this technique. Many commodities, for instance, avocados, mangoes, papayas, and cucumbers, are very sensitive to low-temperature injury and should not be stored below about 13°C. Commodities like apples, broccoli, and pears are not sensitive to chilling and can be stored near 0°C without ill effect [100].

14.3.1 Reduction of Oxidative Reactions

Plant parts such as seeds, fruits, leaves, or roots continue to live after harvest. The energy that plant cells need to stay alive or to proceed with ripening is generated by aerobic respiratory processes. Respiration involves the consumption of O_2 and the production of CO_2. A reduction of respiration results in a lower energy supply and a reduced rate of changes within the product, like ripening [62]. To extend storage periods, conditions should be created that reduce respiration, for instance, by using low-temperature and low-O_2 concentrations. In general, the reduction of the respiration rate is regarded to be the process that is most strongly affected by altered gas conditions [5,69]. For certain fruits, low O_2 levels inhibit the production and action of the plant hormone ethylene, which results in reduced ripening as well [26]. Because respiration has such an important central position in the overall metabolism of a plant (part), its measurement is often used as a general measure of metabolic rate. Specific metabolic changes, however, may occur without measurable changes in net respiration [64]. Nevertheless, good quantification of the effect of reduced O_2 on respiration rates is essential for MA, as this process helps to generate the modified atmosphere inside MA packages.

With both whole, fresh produce and minimally processed produce, oxidative reactions do not only relate to respiratory activity. In addition, oxygen also has an effect on the activity of certain enzymes present in bruised or wounded tissue. Such enzymes are involved in wound repair reactions and in the defense against intruding microorganisms. Their activity depends on the presence of oxygen and is driven by the metabolic activity of the produce. The most studied enzyme is polyphenol oxidase (PPO), an enzyme that causes browning of plant tissues. In the case of minimally processed product (i.e., chopped, cut, sliced, and peeled), the level of tissue injury is much higher than the whole produce. Consequently, the level of metabolic activity and thus the respiration rate of minimally processed produce is often in orders of magnitude higher than that of the raw material. Also, enzymes such as PPO will be more active and may cause visible browning of cut surfaces. Such responses should be considered and overcome by choosing the correct MAP design. In the case where different types of minimally processed products are included in a MAP, which is often the case in mixed vegetable salads, conflicting levels of O_2 and CO_2 may be optimal for the individual components. A designer's solution for this problem needs to integrate all the different aspects that are important with regard to the quality features of the end product.

14.3.2 Fermentation Reactions

The most optimal MA condition for a product is often considered to be the O_2 concentration, which is as low as possible with regard to product respiration without initiating fermentative reactions [5,26]. Fermentative reactions lead to the production of compounds such as acetaldehyde, ethanol, lactic acid, and ethyl acetate. Alcoholic fermentation is always found in plant tissues exposed to an environment without O_2 [81]. An increased concentration of ethanol and ethyl acetate is often related to quality problems such as off-taste and off-odor [65,67]. A strong correlation was found between ethanol and ethyl acetate concentrations [65]. A relationship between other fermentative metabolites and off-flavors is less clear. With improved detection techniques, compounds like ethanol and acetaldehyde can even be detected at O_2 concentrations higher than those considered to be optimal for packaging of certain produce [80]. It seems that fermentation cannot be avoided completely and that it is not absolutely necessary to be avoided from the point of view of package design. Rather, it is important at what concentration of ethanol (or ethyl acetate) the consumer experiences off-odors or off-flavors. The package design should allow O_2 concentrations to be high enough to avoid an accumulation to that concentration. A complicating factor is that a relatively short period of too low O_2 concentrations can cause irreversible quality damage, because it has been found that strong off-flavors do not disappear once the favorable O_2 levels have been reestablished.

14.3.3 Selective Impact on Microbial Growth

For many minimally processed products, the main factor causing quality loss is not ripening or senescence, but microbial growth. The modified-atmosphere composition has a marked impact on the growth of spoilage microorganisms as well as on pathogens that occasionally occur in minimally processed produce [77]. The very low O_2 (typically 2%–3%) and moderately high CO_2 (5%–20%) levels prevailing inside a package slow down the proliferation of aerobic spoilage microorganisms [20,41,47,55,57–59,82]. The antimicrobial effect of CO_2 on microorganisms has been intensively documented [4,18,34–39,52,76]. However, it has been shown recently that only CO_2 levels well above 20%–50% significantly affect the growth of psychrotrophic pathogens that are relevant to MA-packaged produce [11]. This contradicts the general belief that CO_2 has very pronounced antimicrobial properties. At levels of O_2 and CO_2 that are generally favorable for storage of produce, there is certainly no beneficial effect of CO_2 [11,29].

In *in situ* studies, it was established that the specific conditions of MAP (reduced oxygen, increased carbon dioxide) can lead to marked changes in the epiphytic microflora, especially in chicory endive [10]. Thus, where there may be no direct antimicrobial effect of CO_2, there is an influence on the composition of the microflora and on the competition that pathogens may experience in this ecosystem. A specific safety hazard is that psychrotrophic, facultative aerobic pathogens such as *Listeria monocytogenes* are not suppressed under MA conditions that are optimal for respiring produce [11,15,28,29]. On the contrary, growth may be enhanced in certain cases [3,10], especially because the MA conditions diminish the growth of spoilage microorganisms that would be the competitors of the pathogens.

14.4 Types of Packages

With MAP, the gas composition surrounding the produce inside the package is different from that outside the package. Outside, the gas composition is always close to 78.1 kPa nitrogen, 20.95 kPa oxygen, 0.93 kPa argon, and 0.036 kPa carbon dioxide. Several different types of packages and packaging techniques have been developed to accommodate MA around the produce, and these will be explained in detail below. The modification of the atmosphere generally implies a reduction of O_2 content or an increase of the CO_2 concentration, but in some cases changing the level of carbon monoxide (CO), ethylene, ethanol, or other compounds in the atmosphere can also contribute to shelf-life extension. Modified atmospheres can be created passively by the respiration activity of the product inside the package (product-modified MAP) or actively by introducing the desired gas mixture (gas packing). Other active ways of obtaining MA are the use of gas generators and scrubbers (controlled-atmosphere packaging [CAP]), evacuation of air (hypobaric storage, vacuum packaging), or addition of chemical systems that absorb or generate gases or volatile compounds (active packaging) in packages.

14.4.1 Modified-Atmosphere Packaging

In MAP, the gas composition within the package is not monitored or adjusted. Therefore, the term passive atmosphere packaging (PAM) is sometimes used in this respect. Depending on the oxygen sensitivity and metabolic activity of the product to be packaged, air or a predetermined gas mixture is used to flush packages before closing. The use of ambient air as the packaging gas obviously is most economic, but is an option mainly when the respiration activity under the prevailing storage conditions is high enough to reduce the in-pack O_2 level fast enough to lower levels that do not cause physiological or microbial deterioration. With produce highly sensitive to O_2 (e.g., many minimally processed fruits) or those that have a low level of respiratory activity, flushing with a gas mixture composed of low oxygen and moderately high CO_2 is often used to shorten the time needed to reach the desired in-pack gas composition. After closing the package, the respiration of the product will cause a decrease in the oxygen content and an increase in the carbon dioxide content. These altered gas concentrations, however, cause a decrease in the respiration rate. Finally, an equilibrium concentration inside the package is reached, which is the result of a balance between metabolic rates of the packed product and diffusion characteristics of the package materials. This explains the use of another term, equilibrium-modified atmosphere (EMA) packaging. The package is often designed in such a way that the equilibrium concentrations resemble the optimal gas concentrations found in experiments where products are stored under a range of stable gas conditions.

The course of the atmosphere modification is determined by three interacting processes: respiration of the commodity, gas diffusion through the commodity, and gas permeation through the film. Each of these processes is in turn strongly influenced by several commodity- and environment-generated factors. Respiration of a certain commodity depends, among others, on its physiological stage and temperature, O_2 and CO_2 partial pressures, relative humidity (RH), and ethylene concentration. Gas diffusion is affected by temperature, gas gradient across the limiting barrier, and the commodity's mass, volume, respiration rate, membrane permeability, and gas diffusion path. Some of these variables may vary with the maturity stage of the product or even the degree of illumination. Some variables affecting gas permeation through the film are temperature, gas gradient across the film and film structure, water vapor gradient, thickness, and surface area. A change in product amount, free volume, or any of the variables listed above will affect the EMA and the time in which the steady-state conditions are established. Flushing a package with a premixed gas will influence the time needed to attain the EMA.

Strict temperature control in the distribution chain would be a prerequisite for optimal use of MAP in practice, but in most countries the cooling chain between production, distribution, retail, and the consumer has many uncontrolled links. The changes in the permeabilities of most packaging films to gases in response to changes in temperature are generally lower than changes in product respiration. Most of today's existing plastic films do not have the proper O_2:CO_2 permeability ratio to provide the ideal MA for many commodities at a given temperature. In view of all these variables and knowing that any change within or around the package will alter the dynamic equilibrium between the product and its environment, it is clear that knowledge about the limits of tolerance of a certain commodity is even more important for MAP than it is for CAP.

14.4.2 Controlled-Atmosphere Packaging

In CAP, the altered gas composition inside the package is monitored and maintained at a preset level by means of scrubbers and the inlet of gases. This method closely resembles the practices used in large controlled-atmosphere (CA) storage facilities where produce is stored essentially unpacked in bulk, except that CAP is used for storage or transport of smaller quantities of produce.

Additionally, new areas of attention in CA storage today are ultra-low oxygen (ULO) storage and dynamic CA storage. Obviously these techniques can be used in CAP as well. The ULO storage uses O_2 levels close to the minimum level required for maintenance of plant tissues; lower levels will induce disorders such as browning and tissue necrosis. Using ULO storage at 1°C–2°C with preset levels of 0.5%–1% O_2 and 2%–3% CO_2, for instance, Elstar apples can be stored for almost a whole year without unacceptable quality loss. In the case of dynamic CA storage, sometimes referred to as interactive CA storage, gas levels are not controlled at preset levels but are continuously adapted to the physiological response of the produce [98], for instance, by monitoring fermentation products or cell degradation products. In this way, an optimal match is made between the physiological demand and tolerance of a product and the storage conditions. Although this concept is still in development for CA [87], comparable ideas have been described for packages (see below).

14.4.3 Active Packaging

In some cases, a package cannot be designed in such a way that optimal conditions will be reached passively. "Active packaging" can then provide a solution, by adding materials that absorb or release a specific compound in the gas phase. Compounds that can be absorbed are carbon dioxide, oxygen, water vapor, ethylene, or volatiles that influence taste and aroma. For some leafy vegetables, carbon dioxide levels can induce browning of tissues, while for most fruits increased ethylene levels cause an acceleration of ripening. Even at rather low levels, depending on the type of produce, ethylene can induce senescence and maturation processes that reduce the fresh product quality. Inclusion of ethylene scrubbers like potassium permanganate counteracts the effect of ethylene, although the capacity of such scavengers is finite. In transport packages for grapes, pouches are often added that slowly release sulfur-containing chemicals to reduce fungal growth. Recently, research has been directed to replacing chemicals with compounds retrieved from plant tissues ("green chemicals"). How much of the active compounds needs to be added will depend on a range of interacting factors, such as production rates (carbon dioxide, ethylene), concentrations to be reached, how long the package should be functional, etc. Various possibilities exist, although precise control of O_2 in such packages is not possible [26].

Recently, a number of new "intelligent" concepts have been introduced that involve more than only scrubbing or emitting compounds. These types of packages will only become "active" when a specific prerequisite has been met. Most of these packages focus on prevention of problems associated with anaerobic conditions. In one such system, holes are introduced in the package upon exposure to high temperatures for a certain time; originally, the holes are closed by solid hydrocarbons that have melting points between 10 and 30°C [26]. Because respiration of a product often increases at a faster rate than the diffusion of gases with a rise in temperatures, the hole in the package will prevent the depletion of O_2. Another idea is a sensor for ethanol mounted on a package that informs possible buyers of the history of the package in terms of possible mechanical damage or temperature abuse [26]. Yet another concept, which has seen some use in, for instance, France and the United States, is the "time–temperature indicator" or "time–temperature integrator" (TTI). TTIs used now are in most cases small devices that, attached to the package, will indicate the combined time and temperature history of that product by a gradual change color [92,97]. TTIs integrate the time and temperature by specific enzymatic or chemical reactions that, ideally, have an identical rate constant to the quality or safety feature of the packed product. The consumer can compare the actual color at the time of intended purchase with the indicated sell-by limit color. A TTI is an elegant and user-friendly improvement that informs consumers of the expected shelf life at the point of sale. The concept could well be extended to the home situation.

14.4.4 Vacuum Packaging

Whereas MAP and CAP mostly operate at ambient pressure (101 kPa), storage at reduced atmospheric pressures has been experimented with and, in some cases, has been used for bulk storage (e.g., in the so-called hypobaric storage systems designed by Stanley Burg [23,24] almost a quarter of a century ago. In the Burg system, produce is stored under atmospheric pressure in the range of about 1–10 kPa at refrigerated temperatures. At this low pressure, a constant circulation of fresh air, substantially saturated with water (RH 80%–100%), is maintained. Facilities to constantly scrub CO_2 and ethylene could be included as well. Although the system performed rather well, and shelf lives of different horticultural and floricultural products could be extended 3- to 10-fold, it was technically complex and for this reason was never used as widely as CA or MA storage. Vacuum packaging (VP) may be regarded as a special type of MAP, since part of the normal headspace is removed, leaving an altered initial atmosphere that is not controlled after packaging. VP puts quite a pressure strain on produce and is only suitable when the product is sufficiently durable.

Using a VP system—called a moderate VP system because it operates at 40 kPa—a significant prolongation of quality shelf life at 8°C was obtained with a range of minimally processed fruits and vegetables [48]. In this system, the initial gas composition is that of normal air, but because of the reduced partial gas pressure, the amount of O_2 available at the start of storage is about one-third of the normal amount. As with MAP, the lower O_2 content stabilizes the postharvest product quality by slowing down the metabolism of the produce and the growth of spoilage microorganisms. Compared to refrigeration-only storage, refrigerated storage under moderate vacuum was found to improve microbial quality (e.g., red bell pepper, chicory endive, sliced apple, sliced tomato), sensory quality (e.g., apricot, cucumber), or both (e.g., mung bean sprouts and a mixture of cut vegetables). In some instances, no beneficial effect (mushroom, green bell pepper, and a mixture of cut fruits) or an impeded decrease in sensory quality (strawberries, alfalfa) was noticed. With cut products (vegetables and fruits salad mixes, chicory endive, apple), VP strongly retarded enzymatic browning of the cut surfaces.

14.4.5 Modified-Humidity Packaging

MAP, CAP, and VP all focus on changing the metabolic gases oxygen and carbon dioxide. Modified-humidity packaging (MHP), however, is designed for products where dehydration causes the most important quality losses, and therefore focuses on controlling water vapor levels. When products such as leafy vegetables or bell peppers are not packed, quality losses can be observed very soon (e.g., wilting and shriveling). In most "closed" packages such as MAP, CAP, and VP, the RH is close to saturation due to the water exchange between the product and the headspace. This high humidity increases the probability of condensation and free water accumulating directly on the product, especially when the package is exposed to changing temperatures. Therefore, MHP systems are designed to control not only dehydration but also condensation. The in-pack RH is influenced by the rate of water loss (transpiration) of the product and the transmission rate for water vapor of the package, which are dependent on the prevailing water vapor pressure and temperature of storage. Temperature is one of the most important factors determining the in-pack RH. Weight loss relates more exactly to the vapor pressure deficit than to RH, but at constant temperature weight loss has a linear relationship with relative humidities above 75%–85% [50]. At higher temperatures, the air can contain more water vapor, thereby decreasing the RH value. A package designed to have a high RH at a high temperature will show condensation on the package surface or on the product if the temperature is decreased substantially. To counteract the effect of condensation, films have been developed that are coated with an antifog layer, due to which moisture forms a continuous layer rather than separate droplets on the surface of a film. This allows a clear view of the product and prevents water from forming a pool at the bottom of the package.

For many products, transpiration must be reduced to maintain quality. Products with a large surface area, such as lettuce and endive, are very susceptible to wilting. Bell peppers and tomatoes also benefit from good control of RH [9,88]. Reducing water loss is one of the main aspects related to packaging of minimally processed products, despite the usual emphasis on gas levels [26]. On the other hand, for products such as onions and flower bulbs, humidity should not be too high, as it results in increased sprouting. Like O_2 and CO_2, water vapor levels can be too high or too low, and an optimum level should be reached. For (Israeli) bell peppers, this level was estimated to be 92% RH at 8°C [83]. A lower RH caused too much weight loss, while a higher RH caused decay. Especially for products where water loss is the predominant cause of quality changes (e.g., bell pepper and tomato), MHP can be effectively used to minimize loss of quality. In such cases, the concentrations of oxygen and carbon dioxide in MHP are often close to that of ambient air.

Many commercially available packaging materials that have favorable gas-permeability characteristics for a certain commodity cannot be used because they have a rather low permeability for water vapor. When the in-pack RH is very high (≥95%), a small fluctuation in storage temperature results in condensation, which greatly enhances the proliferation and spread of spoilage microorganisms. Especially for fruits, the high RH conditions cause heavy losses due to microbial decay. Control of the in-package RH may be pursued through the use of packaging materials with high water vapor permeabilities, by inclusion of sachets containing water absorbers like $CaC1_2$, sorbitol, or xylitol in the package ("active packaging") or by use of packaging materials with suitable gas permeabilities onto which such desiccants are coated [6,7,81].

14.5 Important Parameters in Package Design

14.5.1 Product Characteristics

Before a package can be designed, detailed knowledge about the physiological characteristics of the product to be packed and the environmental conditions the package is exposed to after production is essential. Many specific parameters need to be known. Important are not only the optimal O_2, CO_2, and water vapor levels, but also the upper and lower limits of these components beyond which damage can be expected. When low O_2 or high CO_2 is beneficial, it becomes important to quantify the relationship between gas conditions and gas-exchange rates. For good quantification, O_2 uptake and CO_2 production should be measured under a range of O_2 and CO_2 concentrations. Such data sets, however, are still scarce.

Another important product aspect is the influence of light on color changes, for instance, with chicory endive, which changes from the preferred yellow-white to the undesired green color under excess illumination. It is also important to know what mechanical properties of the package should have when delicate products such as berries are to be packaged without any mechanical damage.

14.5.2 Package Characteristics

An important aspect of package design is the selection of the packaging material, and this can be a cumbersome exercise. Exama et al. [40] studied the possible application of 20 different types of polymer films and were still not able to find a suitable match with products with a high respiration rate. Using too-high-barrier package film, O_2 will be fully depleted and fermentation will lead to off-odors and off-flavors. Also, the right combination of low O_2 and high CO_2 is crucial. This highlights two decisive aspects in selecting films: (a) the permeability for O_2 and CO_2 at the temperature to be used and (b) the ratio between O_2 and CO_2 permeability. A serious drawback is that gas permeability specifications given by film manufacturers are usually determined under conditions remote from the high-humidity refrigerated storage conditions of respiring produce. Thus, it is impossible to deduce only from the specifications provided by film manufacturers whether a specific film would provide for an in-package gas atmosphere with tolerable O_2 and CO_2 levels when applied in practice. Thus, the suitability of a film must be tested with the product under the correct practical conditions.

In addition to permeability of the metabolic gases, permeability for water vapor, ethylene, and volatiles can be important. A low permeability for water vapor can increase the risk for condensation. Condensation should always be avoided, since it generates an ideal climate for microbial growth. Also, discoloration of the product can result from condensation.

Currently, polyethylene (PE) and polyvinyl chloride (PVC) films are the most often used polymers. In the past decade, a new type of film was introduced with very small holes (microperforation) as the main pathway for diffusion [95]. The interesting aspect of these films is that diffusion of O_2 and CO_2 through the film is equal. This enables the creation of packages with both low O_2 and high CO_2 concentrations. Such atmospheres are especially suitable for minimally processed products but also for unprocessed products with extremely high respiration rates such as asparagus, broccoli, mushrooms, or mung bean sprouts.

In addition to the selection of the type of film, other important aspects include thickness of the film, the surface area used, the package volume, and for films with microperforation, the number of holes per area. Film thickness, film area, and the number of holes influence the equilibrium gas composition inside the package. Varying package volume and the free volume inside the package influences the rate at which gas concentrations are changing. The final equilibrium concentrations will be equal, but the moment in time at which these concentrations are reached can differ by varying the volume.

14.5.3 Modeling

Since there are so many variables to take into account in package design, a trial-and-error type of approach can lead to numerous attempts to find the best package. The risk is that the best package will not be found. Sufficient control of the many different factors interacting in determining the atmosphere change in a MAP can only be achieved with the help of mathematical modeling [101]. Mathematical models may provide a means to determine and predict important packaging specifications. When optimal (equilibrium) gas conditions are known as well as the respiratory response to various O_2 and CO_2 concentrations, the suitable permeability characteristics of the package can be mathematically deduced. The most frequently used models that relate gas conditions to O_2 uptake and CO_2 production are based on Michaelis–Menten kinetics [5,26,68]. Although an inhibition of CO_2 on respiration is not found for all products, Peppelenbos and van't Leven [79] examined which type of inhibition best described the influence of CO_2. The models of Banks et al. [5] or Peppelenbos et al. [78] can be applied best when not only respiratory CO_2 but also

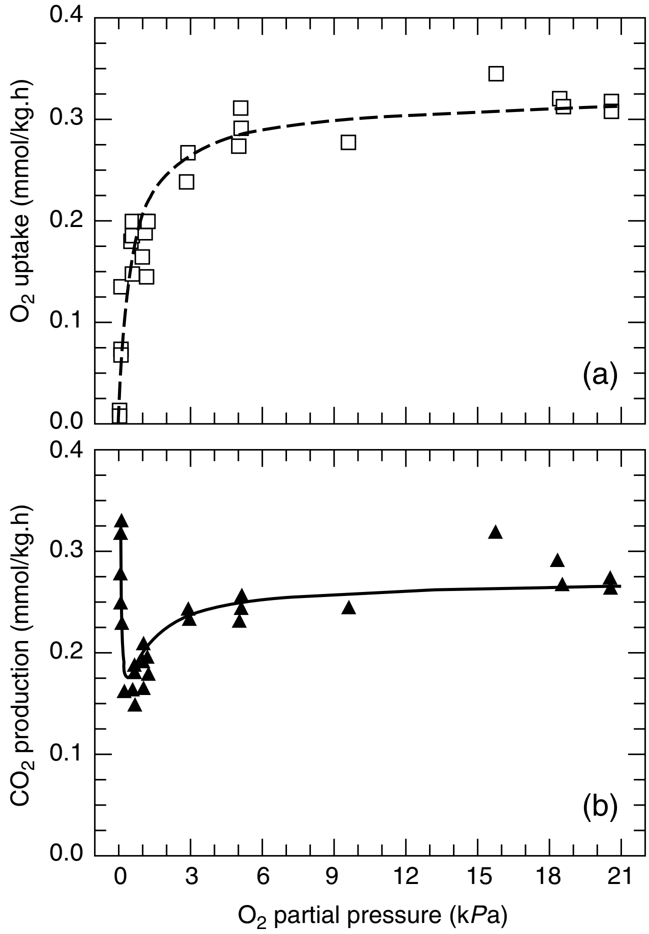

FIGURE 14.1 Gas exchange rates of strawberries (*Fragaria ananassa* cv. *Elsanta*) at 4°C. (a) O_2 uptake rates (□) with O_2 uptake model. (b) CO_2 production rates (▲) with CO_2 production model. (From Peppelenbos et al., *Postharv. Biol. Technol.*, 9:283, 1996.)

fermentative CO_2 production needs to be calculated. An example of gas-exchange modeling is given in Figure 14.1, where at low O_2 concentrations CO_2 production increases due to enhanced fermentation.

The description of gas diffusion through packaging materials is mostly done by applying Fick's law [26]. Although all models mentioned above are static, they can be incorporated into dynamic models to be used for the prediction of changing gas conditions inside a package [56]. Packages can be easily designed with such dynamic models by changing variables such as film type, surface area, and amount of product packed. A very useful extension of simulation models would be the incorporation of expected variance of the achieved equilibrium conditions. Using expected variance, not only can optimal packages be designed, but so can (sub)optimal packages that are also safe. A survey of this variance has already been carried out for broccoli by Talasila et al. [91].

Since temperature in the distribution chain often cannot be strictly controlled, another interesting feature of dynamic modeling is the possibility of simulating products passing through the different links of the distribution chain. Using simulation, for instance, the dynamics of the gas composition inside the package can be evaluated to determine whether gas conditions will remain within the limits of tolerance of the commodity. When necessary, the use of different packaging films can be simulated to obtain the most optimal equilibrium modified-atmosphere condition. The end result of the modeling exercise, however, could be that there is no suitable packaging film available commercially that would be suitable for use. Instead of not packing the product, this information could be used to further improve distribution chains or give suggestions to the packaging industry, defining the requirements for new films in terms of their temperature sensitivity, CO_2:O_2 permeability ratio, etc.

Once proper models have been created and integrated in package design, they should be mandatory in the development of packages that achieve optimal gas conditions at dynamic temperatures encountered in practical situations and of packages that can overcome fluctuations in temperature, which temporarily cause gas conditions to exceed tolerance limits but do not affect product quality.

14.6 Microbial Growth under Modified Atmospheres

14.6.1 Spoilage Microorganisms

Fresh fruits and vegetables normally have an elaborate spoilage microflora, due to intensive contact with various types of microorganisms during growth and postharvest handling. The high acidity of many fruits ($pH < 4.6$) limits spoilage to acid-tolerant molds, yeasts, and lactic acid bacteria. Vegetables generally have a pH around 6.0–7.0 and lack this intrinsic protection. Microbial spoilage of undamaged, healthy products can only be effectuated by microorganisms that are able to penetrate through the skin, which requires the presence of specific enzyme systems. In vegetables, pectinolytic Gram-negative bacteria of the genera *Pseudomonas* and *Enterobacter* are often involved in spoilage. The effect of MAP, and in particular carbon dioxide, on spoilage organisms is distinctly selective, but it is possible to make some broad generalizations. Molds exhibit sensitivity, while yeasts are comparatively resistant. Different species of bacteria, on the other hand, vary greatly in sensitivity. For example, aerobic organisms such as *Pseudomonas, Micrococcus,* and *Bacillus* are inhibited by CO_2, while the *Lactobacillus* species are more resistant. On the other hand, facultative anaerobes such as *Escherichia coli* are comparatively less affected by the level of CO_2 and more by the level of O_2. Most spoilage organisms that pose a quality problem in produce are aerobic, thus a limited supply of O_2 hampers their growth potential. Nitrogen has little inhibitory effect except in displacing oxygen.

Spoilage microorganisms usually pose no safety problem for consumers. The main concern is that applications of MA diminish the competition for oxygen, carbohydrates, other nutrients, and space between spoilage microorganisms and pathogens. This may allow the growth of certain pathogens to hazardous levels, especially during extended shelf life. In addition, the problem of product temperature abuse, either during manufacture, distribution, and retail or by the consumer, must also be considered. In those cases, spoilage microorganisms may be important safety indicators, giving an organoleptic warning signal to consumers that the food product has been mishandled or kept beyond its shelf life and therefore may not be safe to eat. However, when technologies such as MAP are used to extend the product shelf life by suppressing spoilage organisms but not all hazardous pathogens, situations could occur in which the packaged food is organoleptically unspoiled but very unsafe to eat.

14.6.2 Pathogenic Microorganisms

Most fruit products have too low a pH to permit growth of pathogenic bacteria—only figs, peaches, and tomatoes, whose pH is potentially in the range 4.6–4.8, may permit pathogenic growth. In the early days of MAP, attention was focused primarily on anaerobic pathogens, especially proteolytic *Clostridium botulinum,* which produces a deadly toxin but does not grow below 10°C. Since nearly all MAP foods are refrigerated, focus has been mainly on the survival and outgrowth of cold-tolerant pathogens such as *Yersinia enterocolitica* and *L. monocytogenes* that can proliferate under low-oxygen conditions [15,82]. An important factor with respect to microbial safety is whether the MAP food is intended for direct consumption or requires heating before consumption. With MAP foods that are cooked before being eaten, vegetative pathogens should all be killed, provided that the cooking instructions are properly followed. However, the majority of MAP produce is sold as "ready-to-eat." The main potential sources of pathogenic bacteria in fresh and minimally processed produce are the raw material, ingredients, plant workers, as well as the processing equipment and environment. The main pathogens of possible concern to MAP are described below.

14.6.2.1 *Clostridium botulinum*

Because of the potency of their toxin, the potential growth of *Clostridium* sp. in MAP foods has been of especially great concern [90]. The organisms can be present in soils and can thus come into contact with fruits and vegetables easily. *C. botulinum* is not markedly affected by the presence of CO_2, and growth is encouraged by the anaerobic conditions that may exist in MAP. Most strains of *C. botulinum* do not grow at temperatures below 10°C, although nonproteolytic *C. botulinum* types B, E, and F have been recorded as growing and producing toxins at temperatures as low as 3.3°C. Botulism has been linked to coleslaw prepared from MA-packaged, shredded cabbage mixed with coleslaw dressing [63,89]. Shredded cabbage onto which spores of *C. botulinum* types A and B were inoculated and that subsequently was MA-packaged and held at room temperature was found organoleptically acceptable after 6 days, yet type A toxin production was apparent on day 4. A pungent odor was produced and released on opening the bag, after which the cabbage smelt normal. A recent survey by Lilly et al. [72] on the incidence of *C. botulinum* in MAP and VP vegetables involving 1118 packages of a variety of precut produce (including cabbage, pepper, coleslaw, carrot, onion, broccoli, mixed vegetables, stir-fry vegetables, and various salad mixes) found that one package each of shredded cabbage, chopped green pepper, and Italian salad mix contained *C. botulinum*-type spores, while an additional salad mix (main ingredient, escarole) contained both *C. botulinum* type A and type B spores. The overall incidence rate (0.3%–6%) of *C. botulinum* spores thus may be quite low in commercially available precut vegetables.

14.6.2.2 Listeria monocytogenes

The widespread presence of this organism in the environment and its ability to grow at low temperatures make it a pathogen of special concern. A serious outbreak of listeriosis was thought to be derived from cabbage fertilized with manure from infected sheep [86]. In many studies carried out since this outbreak on a variety of produce, it was frequently established that *L. monocytogenes,* when the organism was inoculated onto vegetables, grew as well under MA or CA conditions as in air at 4°C or 15°C [3,13,16,17,27,29,43,45]. By now, growth of this pathogen under MA conditions has been reported for asparagus, broccoli, cauliflower, lettuce, and chicory endive. Studies by Carlin et al. [28] investigated the fate of *L. monocytogenes* in minimally processed foods in the presence of nonpathogens and at temperatures ranging from 3°C to 20°C. It was shown that on unspoiled products *L. monocytogenes* would hardly grow more than 2 log units whatever the storage temperature, but that spoilage of the salad leaves would permit a rapid multiplication. Low storage temperatures reduced the growth of *L. monocytogenes* more than that of the spoilage microflora and are therefore a factor of improving safety. Carbon dioxide concentrations of 10%–20% reduce spoilage development and growth of the spoilage microflora, whereas higher concentrations slightly increased growth of *L. monocytogenes.* On minimally processed green endive, it was found that that high inoculum concentrations overestimated the maximum growth of *L. monocytogenes.* Again, the epiphytic microflora of green endive leaves had a barrier effect against *L. monocytogenes.*

14.6.2.3 Aeromonas hydrophila

This psychrotrophic organism is also widespread in the environment and is mainly waterborne. It has been found in drinking water, fresh and saline water, and sewage water. Cytotoxic strains have been found on seafood, meats, and poultry, as well as on fresh produce, parsley, spinach, celery, and endive. The pathogen grows rapidly at refrigeration temperature [44]. Berrang et al. [14] observed that *Aeromonas* could grow to population densities exceeding 10^6 CFU/g within 2 weeks at 4°C on asparagus, broccoli, and cauliflower and that CA conditions did not markedly affect its growth potential.

14.6.2.4 Yersinia enterocolitica

Animals, specifically swine, are the predominant natural source of *Y. enterocolitica.* However, this cold-tolerant pathogen has also been isolated from raw vegetables. As with the former two pathogens, modified atmospheres optimal for produce do not hamper its growth at refrigeration temperature. With little oxygen (1.5%) present, carbon dioxide levels as high as 50% were found to be required before its growth was significantly reduced [11].

14.6.2.5 Bacillus cereus

This bacterium, a common contaminant of vegetables, does not usually grow below 10°C [53]. However, recent reports have shown that some enterotoxigenic strains can grow at temperatures as low as 4°C and produce toxin at 8°C. *B. cereus* is rather susceptible to the antimicrobial effects of CO_2 [11], and CO_2-rich environments severely reduce the ability of the spores to germinate.

14.6.2.6 *Salmonella* spp.

This organism is most commonly associated with animals and birds and is only present on vegetables through cross-contamination. Nevertheless, two large outbreaks of salmonellosis have been attributed to fresh produce, both involving tomatoes stored at ambient temperatures [54,99]. *Salmonella* species have also been implicated in smaller outbreaks in which raw bean sprouts and different types of melons were the vehicles. Although high levels of CO_2 retard the growth of *Salmonella,* generally the inhibitory effect on the organism is largely dependent on decreased temperature. Most *Salmonella* species are mesophilic bacteria, but many isolates survive well during storage at 5°C [46].

14.6.2.7 *Staphylococcus aureus*

S. aureus does not grow well under chill conditions or in the presence of competing microorganisms. The pathogen has been found on fresh produce and ready-to-eat vegetable salads. It is known to be carried by food handlers. Generally, CO_2 has an adequate inhibitory effect on the growth of *S. aureus* when combined with low-temperature storage.

14.6.2.8 *Escherichia coli*

E. coli is a mesophilic bacteria often used as an indicator of fecal contamination. Enterotoxigenic *E. coli,* the common cause of travelers' diarrhea, is regularly detected on raw vegetables. Strains can grow at temperatures below 10°C, but not usually below 7°C. Some strains reportedly are able to grow and produce toxin at 5°C. Growth of this organism can be inhibited by high levels of CO_2. Enterohemorrhagic *E. coli* O157:H7 is recognized as an important emerging pathogen. Outbreaks of this pathogen have been associated with unpasteurized apple cider and cantaloupe. Also, broccoli is suspected to have carried this type of *E. coli.* MAP had no effect on pathogen growth on shredded lettuce and cucumber in experiments in which storage temperatures were 12°C and higher [1,33].

14.6.2.9 *Campylobacter jejuni*

This organism is still one of the major causes of bacterial enteritis. Poultry and other foods of animal origin are the main sources. The pathogen has been implicated in diseases caused by consumption of fruits and vegetables. Cross-contamination of fresh produce with *C. jejuni* from poultry meats has been suspected. The pathogen has been found to survive sufficiently on sliced watermelon and papaya to be a risk for consumers. Total absence of oxygen was noted, whereas survival, without growth, was enhanced in an atmosphere of 100% N_2. Optimal growth has been documented to occur under atmospheres of reduced oxygen level and high temperatures (42°C–45°C). The minimum growth temperature reportedly is 32°C, so the risk with consumption of refrigerated MAP produce should be minimal.

14.6.2.10 Disinfectant Usage

The use of disinfectants to reduce the microbial load of minimally processed fresh salads is permitted and practiced in many countries around the world. It has been found that after disinfection, the surviving spoilage microorganisms have an increased growth rate and rapidly reach the same level found on nondisinfected products [10,28]. In the case of contamination with *L. monocytogenes* during processing, disinfection of salad leaves would reduce the antagonism from epiphytic bacteria and might increase growth of the foodborne pathogen. Therefore, the major role of disinfectants may be to prevent build-up of contamination in washing water during processing rather than to reduce microbial load of raw salad leaves. In the production of MAP produce, good manufacturing practices should be observed that avoid recontamination of disinfected produce with hazardous pathogens.

14.7 Recommended MA Conditions for Produce

The benefits of CA and MA packaging vary greatly according to the plant product. Storing some products, like apples, under low O_2 conditions can increase the storage period by months. Also some products, like carrots [96], do not respond positively to low O_2 or high CO_2 concentrations. In general, altered gas conditions are regarded as positive only within a certain range of concentrations, the so-called optimum concentrations.

Much research effort has been devoted to the determination of the optimum gas concentrations for individual products [61,75,84]. Table 14.1 gives a recent update on recommended storage conditions for a range of fruits and vegetables. Traditionally, lists of recommended storage conditions have been developed by national research organizations conducting extensive laboratory research [59]. A common experimental procedure is to store products under a range of O_2 and CO_2 concentrations and monitor quality changes. The lower O_2 limit for stored fruits is accomplished empirically by lowering the storage O_2 concentration until intolerable damage occurs. Each commodity and new cultivar requires a large investment in time, equipment, and materials [49,98]. By repeating the trials year after year, it is possible to sense the importance of climatic variation on product behavior [59].

Some caution is needed in the application of optimal concentrations. For the various apple cultivars, for instance, the advised optima differ according to country [75]. Growing conditions like climate and orchard factors influence crop growth and contribute to these differences [21,30,74]. Although this is probably also the case of other plant products, this is never specified.

Another important aspect of optimal values for temperature, O_2, and CO_2 concentrations is that they are often established separately, although interactions between temperature, O_2, and CO_2 concentrations (and probably also humidity) are known. Optimal O_2 concentrations are found to shift to a higher value

TABLE 14.1

Recommended Optimal Storage Conditions

Commodity	**Temperature** (°C)	**RH** (%)	$[O_2]$ %	$[CO_2]$ %	**Potential**
Fruits					
Apple	1–4	90–95	1–3	0–6	A
Avocado	5–13	90	2–5	3–10	B
Banana	12–14	85–95	2–3	8	A
Blackberry	0–2	90–95	5–10	15–20	A
Blueberry	0–2	90–95	2–5	12–20	A
Cherry	0–2	85–90	3–10	10–15	B
Kiwi	0–2	85–90	1–2	3–5	A
Mango	10–15	90	3–7	5–8	B
Melon	8–10	85–90	3–5	5–15	B
Nectarine	0–2	85–90	1–2	3–5	B
Peach	0–2	85–90	1–2	3–5	B
Pear	0–1	90–95	2–3	0–2	A
Persimmon	0–5	90	3–5	5–8	B
Plum	0–2	85–90	1–2	0–5	B
Raspberry	0–2	85–90	5–10	15–20	A
Red currant	0–2	90	5–10	15–20	A
Strawberry	0–2	90	5–10	12–20	A
Sweet corn	0–2	90	2–4	5–10	B
Tomato	1–13	90	3–5	2–4	B
Vegetables					
Artichoke	0–2	90	3–5	0–2	B
Asparagus (green)	0–2	95	10–15	7–12	A
Broccoli	0–1	90–95	2–3	8–12	A
Brussels sprouts	0–1	90–95	2–4	4–6	A
Cabbage	0–1	95	2–3	3–6	A
Celery (stem)	0–2	90–95	3–5	1–4	B
Chicory (witloof)	0–2	90–95	2–3	5–10	A
Leek	0–2	90–95	3–5	3–6	B
Lettuce	0–2	90–95	2–3	2–5	B
Mung bean	0–2	90–95	1–2	1–3	A
Onion	0–2	70–80	1–4	2–5	B
Spinach	0–2	95	21	10–20	B

Note: Value based on the *Proceedings of the 6th and 7th International Controlled Atmosphere Research Conferences* (Ithaca 1993 and Davis 1997). Potential: A, excellent; B, fair. Products with a low potential or no potential are not listed.

Source: Adapted from A. A. Kader, Ed., *Postharvest Technology of Horticultural Crops*, Agriculture and Natural Resources, Publication 3311, University of California (1992), p. 85.

when CO_2 concentrations are increased [7,62,73,93], products are more mature [19,62,71,94], they are kept at a higher temperature [8,65,85,93], or when products sensitive to chilling injury are stored at too low temperatures [73]. For apples, the CO_2 limit at low O_2 concentrations decreases when the temperature is decreased [66]. The effect of RH is often an interacting factor as a cause for, or in the symptom expression of, a disorder. Relative humidity, however, is often not mentioned and frequently not (accurately) measured [87]. The conclusion is that it is very hard to recommend absolute values for optimum O_2 and CO_2 concentrations or O_2 and CO_2 limits for a product without knowledge of other factors. The understanding of actual physiological processes determining the potential of products for MA is developing steadily and will help the further development of optimal and safe packaging techniques.

14.8 Future Outlook

Fruit and vegetable consumers increasingly demand high-quality products. An important quality feature is freshness. No signs of senescence, decay, wilting, or shriveling are accepted. In general, consumers are willing to pay more for products with better quality. Often consumers also have specific expectations as to the ripening stage. For instance, consumers in Northern Europe want firm, not mealy tomatoes (i.e., the fruits should not be too ripe), but also tomatoes with taste and flavor (i.e., the fruits should not be harvested in a very unripe stage). These quality demands result in strict criteria for storage conditions, transport conditions, and shelf conditions. MA packaging is a tool of increasing importance in meeting these criteria.

MA packages are increasingly being used. The total European MAP market handles on average 300 million package units of produce per year with a market value of about \$1 billion [22,32]. In the United States, a market share of about \$8 billion in MAP products has been predicted for the year 2000. Often successful applications are due to good control of the whole distribution chain, from the moment of packing the product until it is displayed on the retail shelf. When film permeabilities or respiration rates are not well characterized, package designers have to resort to empirical studies with MA packages that can be described as “pack and pray” [26]. A thorough understanding of principles and processes will lead to a more rational selection of packaging materials [62]. Nevertheless, even a package that has been well designed in a laboratory may not necessarily perform well in practice, when no information on the actual storage, transport, or shelf-life conditions were considered at the design stage.

Current state-of-the-art MAP systems for minimally processed produce have been optimized mainly for product quality. Safety and cost aspects have not yet been optimized. Also, quality deterioration still occurs, and further improvements can still be achieved through, for example:

- A systematic approach to select appropriate gas conditions in MAP for specific products
- Availability of more data on the interaction between produce and gas composition
- Development of better computer software to aid in the selection of suitable packaging systems (gas compositions plus foils) for use under dynamic conditions (temperature, humidity)
- Combating microbial hazards in MAP systems (i.e., psychrotrophic pathogens) using improved MAP systems or new hurdles to microbial growth
- Use of “smart” films that compensate for temperature fluctuations by changing permeability properties
- Studies on more environment-friendly MAP systems (e.g., simple foils, biodegradable foils)
- Minimizing packaging in MAP systems (including biocoatings as part of the packaging concept)

Table 14.2 lists these and other trends foreseen in MAP. A misconception of MAP is that it can overcome hygienic abuses in the production or handling of a product. MAP is not a panacea for the preservation of food products, but, if used correctly, it slows the natural deterioration of a product. There is no enhancement of product quality but, when starting with a good clean product, the initial fresh state of the product may be prolonged. Strict codes of practice should be enforced to ensure the maximum quality shelf life and safety of MA-packed foods.

With the increasing importance of MA worldwide, the role of temperature and of its control in the successful use of MAP should be considered [25]. MA packages will not be a substitute for adequate

TABLE 14.2

New Developments in Modified Atmosphere Packaging

- Packaging
  - Tailoring gas transmission/selectivity (new plastics, microperforation)
  - Water vapor transmission/selectivity (modified humidity storage)
  - Biodegradability—environment (multimixed-layered plastics fortified with biodegradable mass)
  - Composites (metals/cartons with plastics/liners)
  - New concepts (high oxygen, noble gases, optimization)
  - Chain optimization (controlling basics: chilling, handling, logistics)
- Minimal packaging
  - Integrating functionalities
  - Simpler/less films, better recyclable films
- Active packaging
  - Absorbers (O_2, ethylene, water, off-flavors)
  - Generators (CO_2, antifungals, flavors)
  - Controlled release (antimicrobials, antioxidants)
- Dynamic packaging
  - Temperature dynamic films
  - Humidity dynamic films
- Biopackaging
  - Biodegradable/edible films
  - Custom-made physical properties
- Biocoatings
  - Edible, physical protection (invisible)
  - Functional features (antimicrobials)

temperature management. For all nontropical products only cooling is always better than only MA. But also when only MA can be applied in cases where cooling is not possible, stable temperatures are necessary since no films are yet available that respond to temperature fluctuations of the packed product [40].

Modern consumer demands for convenient and fresh, wholesome produce together with the recent reorganization of the distribution chain will further stimulate the use and the broadening of the application area of MAP. Continued basic physiological and microbiological research on the action of MAs will minimize the risks of loss and safety associated with the use of MAs and will allow for faster MAP optimization using models. New technological developments, especially in the area of more suitable packaging films, will contribute to the success of MA as a preservation technique. The main limiting factors for further expansion of MAP may arise out of environmental concerns. A solution for the huge waste problem, in the long run, may be found in the use of edible and biodegradable films that are able to create a modified atmosphere [51]. The integration of different preservative hurdles, such as refrigeration, MAP, active components, and green chemicals in accordance with the concept of combined processing [70], may not only minimize potential microbial problems but also contribute to optimized product quality. The range of powerful technologies we have at our disposal today will help to ensure a good supply of minimally processed, fresh, safe, and ready-to-eat products.

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