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Recent Advances in Controlled and Modified Atmosphere of Fresh Produce

Postharvest technologies to reduce food waste and maintain fresh produce quality

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World trade has transformed food retailing and driven the development of technology for the transportation and storage of horticultural products, providing year-round supply of fruit and vegetables. Horticultural produce is highly perishable, as fruit and vegetables continue their metabolic processes that lead to ripening and senescence after harvest, making them ultimately unmarketable. Advanced postharvest technologies are essential for reducing food waste while maintaining high standards of safety and quality. Together with cold storage, controlled atmosphere (CA) and modified atmosphere packaging (MAP) have been applied to alter the produce's internal and external environment, decreasing its metabolic activity and extending shelf-life. Both CA and MAP have benefitted from technological innovation. Respiratory quotient control has improved the management of conventional and recently developed CA systems; gas scavengers have made MAP more efficient; and the inclusion of natural additives has enhanced food safety across the supply chain. This paper critically reviews the application of new postharvest techniques to manipulate gaseous environments and highlights areas that require further study.

Introduction

Over the past decades, the nature of food retailing has been transformed by worldwide trade. The development of infrastructure, facilities and technology across the supply chain, together with the liberalisation of the global economy, have driven consumers' expectations for year-round availability of fresh fruit and vegetables (1). Maintaining freshness requires the efficient transport and storage of highly perishable horticultural produce. After harvest, fruit and vegetables maintain their physiological systems and continue with their metabolic activity. Respiration and transpiration lead to the consumption of substrates, such as sugars and organic acids, and the loss of water, which accompanies ripening and senescence, eventually making the produce non-marketable. Food waste is a global problem that has increased in the last ten years.

In developed countries, access to advanced postharvest technology is essential for reducing loss and waste while maintaining food safety and quality. Historically, cold storage such as cellars, basements, caves and ice houses have been used to preserve fresh produce (2). The technology has

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advanced since the recognition of microbial food spoilage in the 18th century. Fruit and vegetables must be cooled to remove heat: before processing, transporting and storing (3). Nowadays, refrigeration units are more cost-effective, sustainable and consume less energy. They can be used as centralised systems that operate at a wide range of temperatures and respond quickly to changes in working temperature. Reducing storage temperature decreases enzymatic activity, respiratory and metabolic processes, and hence can extend shelf-life. Yet, current market requirements are more demanding, having longer postharvest periods where high quality and food safety standards must be maintained. Because of this, other techniques such as CA storage and MAP are used to enhance and augment cold storage. These, either actively or passively, alter the atmosphere composition surrounding and within the produce in order to influence cellular metabolism, causing a reduction in catabolism in climacteric fruit and vegetables (4), and an inhibition of enzymatic reactions (5). Each commodity has its own optimal CA and MAP conditions which, together with controls on storage duration, relative humidity and ethylene concentration, may influence shelf-life and flavour-life. An important feature of the technologies is that they are innocuous and can be applied to organic fruit and vegetables. CA and MAP techniques have been evolving because of the development of new technology and improved knowledge of fresh produce physiology. This review outlines the most recent approaches in CA and MAP techniques highlighting their advantages, disadvantages and main applications.

Controlled Atmosphere Storage

CA technology is one of the most successful techniques developed by the postharvest industry in the 20th century. However, *ca*. 100 BC the Romans already stored grain in sealed underground pits (6, 7). Jacques Etienne Berard observed in the early 1800s in France that fruit did not ripen in a low oxygen atmosphere (8). In 1927, Kidd and West found that a reduction in respiration rate in apples was correlated to an extension of storage life (9). Since this time, postharvest scientists have progressively studied the effect of different atmospheres on most horticultural produce to obtain optimal concentrations of gases (10–13). The application of conventional CA generally consists of increasing carbon dioxide levels and decreasing the oxygen concentration. It has been shown that CA alters the atmosphere surrounding the product and thus the internal gas composition, reducing the fruit or vegetable metabolic activity and delaying senescence. Some controversy exists around the use of CA. This is because the consumer may think that CA storage confers a counterfeit freshness to the produce they buy. The reality is that CA extends the seasonal availability of produce, maintains the physicochemical and functional quality and can reduce the cost to the consumer. On top of these advantages, the reduction of storage disorders such as chilling injury (14–16) help reduce food waste, which lowers economic, social and environmental impact (17). Furthermore, its potential as an alternative to using postharvest chemicals is a subject of high interest (18, 19).

The effectiveness of CA depends on: cultivar, climacteric nature, storage temperature, selected concentration of gases, stage of maturity, commodity quality at harvest and pre-storage treatments. If the conditions are optimal for the chosen crop, senescence will be delayed by: reducing respiration rate and substrate oxidation, delaying ripening of climacteric fruit and reducing the rate of ethylene production (20). Also, CA reduces the pathogen respiration rate, and can maintain natural disease resistance. In summary, CA prolongs storage life. However, inappropriate CA store management can provoke the development of off-odours, off-flavours and physiological disorders. To obtain the best results, it is essential to have a deep knowledge about the produce physiology and adapt the technology to each scenario. It is generally accepted that applying CA as soon as possible is the best option to maximise efficacy. Yet this causes a dramatic change in the surrounding environment that can elicit abiotic stress in the product (21). Recent studies propose CA scheduling as a means to better adapt to produce metabolism. Chope *et al*. (22) reported that delaying the start of CA on onions for three weeks was as effective at controlling sprout growth using continuous CA. Alamar *et al*. (13) applied different CA timings on strawberry, finding that the application of CA for 2.5 days mid-way through storage at 5ºC (2.5 days; 15 kPa CO_2 + 5 kPa O_2 after 2 days in air) increased shelf-life by 3 days. Likewise, it is recommended to use low-temperature conditions during pre-storage. The equipment and the methods used are under constant development. However, the following key components should be installed for an efficient CA facility: gas tight stores or cabins, a refrigeration system, gas control instrumentation and robust

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monitoring systems (for example oxygen, carbon dioxide, ethylene, temperature and humidity sensors).

The optimal gas concentrations should be adapted to each commodity. Preferably, fruit and vegetables must be stored under low oxygen concentrations, close to the anaerobic compensation point (ACP); taking into account that oxygen levels above the ACP quickly increase respiration rate, and when below, fermentation will adversely affect fruit metabolism (23). In the 1990s, it was demonstrated that fruit like apples can be stored at oxygen levels as low as 0.5% (24–26). If the storage is carried out below 2.5 kPa of oxygen, it is considered ultra-low oxygen (ULO) storage. Although applying ULO is more expensive than conventional CA methods, its use has resulted in better firmness and quality retention (27).

Another option is reducing the initial oxygen concentration with the objective of conditioning the fruit to resist further abiotic stresses. This technique is known as initial low oxygen stress (ILOS) and it has been found to be effective against superficial scald, avoiding the use of chemical treatments (28). CA and ULO storage are static systems, which means that the atmosphere is set to an optimal level and does not vary according to the product response (29). This has several disadvantages: the lowest optimal oxygen content must be adjusted for each produce and condition (such as cultivar and seasonal variation) and it is difficult to access fruit within a container without disturbing the atmosphere, which gives no access to real-time information (30).

The CA technique has evolved with the development of more accurate control systems, to dynamic controlled atmosphere (DCA storage). DCA storage aims for the lowest possible oxygen level, as per ULO, but adapts the gas concentrations dynamically on the basis of the changing physiological response of the produce (31). If the system detects low-oxygen stress, it increases the oxygen level until the commodity response is back to the optimal threshold (23). This method is attractive because it uses existing CA technology that is improved by controlling parameters in near real-time, extending the produce storage life longer than traditional CA. It can also reduce the impact of storage disorders such as superficial scald in apples and pears. Until recently, superficial scald was prevented by using the postharvest antioxidant diphenylamine (DPA) or ethoxyquin (only for pears), but their use is no longer permitted within the European Union (32).

In order to achieve an accurate gas control, CA rooms are continuously monitored to detect the aforementioned stress. Ethanol production (dynamic control system (DCS)), chlorophyll fluorescence (DCA-CF), and the assessment of the respiratory quotient (RQ) are the main parameters measured. DCS uses ethanol, the final product of fermentation, as the stress signal for anaerobic conditions. It is determined in the headspace of a sample box placed in the storage room with sensors such as a quartz crystal microbalance (33, 34). The main issue with this method is that most of the ethanol produced during fermentation remains in the cells, making its detection difficult (23).

DCA-CF is another non-destructive method for measuring the primary processes of photosynthesis such as light absorption, excitation energy transfer and the photochemical reaction in photosystem II (35). These processes are affected by factors such as light intensity, temperature, humidity and gas composition. In this sense, changes in CF measurements are indicators to stress, such that CF can detect cellular injury in advance of symptom development (36). It has been successfully used to perceive low oxygen stress in CA environments for storage of apple, avocado, pear and kiwifruit (30, 36, 37). The limitations of this system are: that sensors can only measure a small portion of an individual fruit, extrapolating the results; they cannot repeatedly measure at the same point; the sensors are still expensive; they need to be calibrated; and peaks in CF can also be caused by other kind of stress, for example abiotic stress (drought, chilling injury). The most popular system for DCA-CF is based on fluorescence interactive response monitor (FIRM) sensors, which detect fluoresced light (Isolcell, s.P.a., Italy).

An alternative to these methods is the RQ measurement of stored produce, which can be used as a stress signal to adapt gas levels in the storage facility (23). RQ is the ratio of the carbon dioxide production rate to the oxygen consumption rate of the stored fruit or vegetable (**Figure 1**). The RQ will remain under one in aerobic conditions and increase exponentially over unity if oxygen concentration approaches zero, caused by a shift from aerobic respiration to fermentation, which implies low oxygen stress (5, 38). In this case, the limitation when applied to DCA systems is the leakage of the storage facility, which introduces noise to the results. A new automatic DCA control system based on online real-time RQ measurements has been recently developed that is

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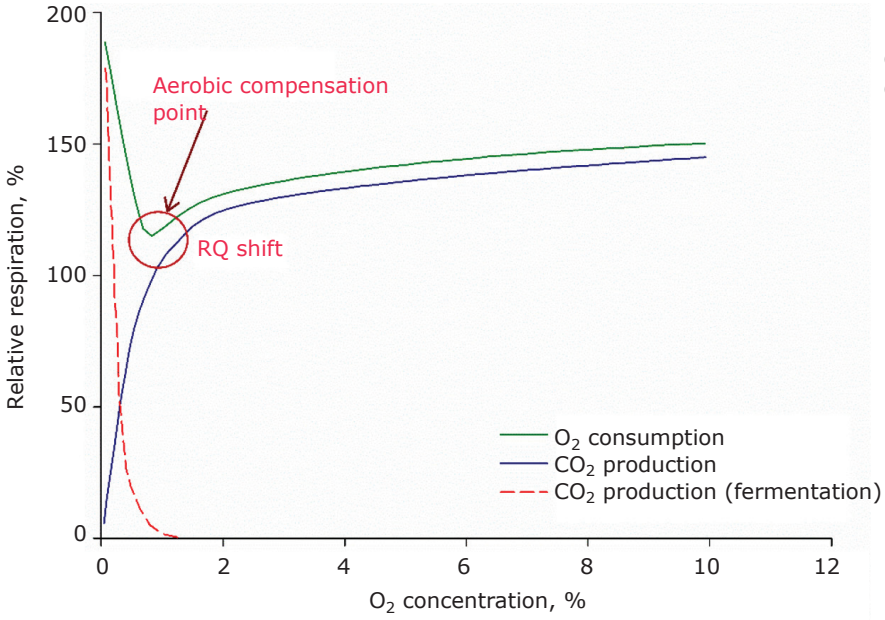

Fig. 1. Effect of oxygen concentration on oxygen consumption and carbon dioxide production in fresh produce

integrated into the control system of the CA facility (39). This enables the CA system to adjust the gas concentrations immediately according to RQ readings, avoiding the mentioned noise as it takes into account the leakage in a predictive model (23).

This technology can be applied in individual sample containers that are representative of the conditions of the storage facility. An example of this option is the LabPod (Storage Control Inc, USA), a hermetically water-sealed container with a stainless steel base and a transparent plastic cover (**Figure 2**). Each pod has oxygen, carbon dioxide and temperature sensors with digital communication to a central operating panel. In it, RQ is periodically and automatically measured and used to set the gas concentrations in the storage room. It is recommended for products that are kept in long-term storage, such as apples, kiwifruit and pear, as, at this moment, it requires a capital investment and is expensive to operate (40). Novel biosensors and photonics are now being developed to better understand physiologically-targeted CA interventions to control ripening. They will also allow real-time phenotyping, which offers new insight into fruit and vegetable quality and safety aspects (41).

Fig. 2. LabPod (Storage Control Inc, USA) storing Gala apples

Apart from the factors already mentioned, the action of ethylene (C_2H_4) has to be carefully considered. Ethylene is a natural plant hormone which works at trace levels stimulating or regulating fruit ripening (especially in climacteric fruit) (42). CA storage implies the increase of carbon dioxide and the reduction of oxygen. Low oxygen and/or elevated carbon dioxide concentrations inhibit the ethylene production rate by suppressing 1-aminocyclopropane-1-carboxylic acid synthase transcripts, the key enzyme in the synthesis pathway of ethylene (43). Another effective option to inhibit ethylene is the application of 1-methylcyclopropene (1-MCP) (**Figure 3**). 1-MCP is a gaseous cyclic olefin which binds irreversibly to ethylene receptors avoiding ethylene-dependent responses (44–46). 1-MCP is very efficient because its affinity for the receptor is around ten

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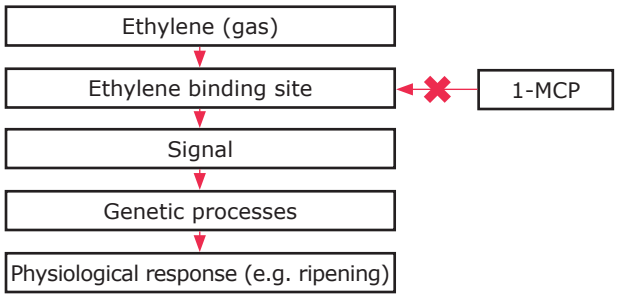

Fig. 3. Diagram showing the sequence of ethylene action and 1-MCP interaction in one of the possible sites

times greater than that of ethylene (44). Some recent studies show that the effects of 1-MCP are comparable to CA in maintaining fresh produce quality (47). However, DCA is the solution that can allow optimal results during postharvest storage (48).

Research has been focused on inhibiting ethylene action in the last decade. However, scrubbing technologies are also available and their efficiency has been widely proven. These techniques include high-temperature catalytic degradation, oxidation of ethylene through potassium permanganate ($KMnO_4$)-based mechanisms, activated carbon and impregnated zeolite (42). The most commercially used technique to remove ethylene is simple ventilation but it is not compatible with environments which require sealing such as CA or some MAP solutions and ethylene adsorption materials.

Modified Atmosphere Packaging

Packaging should be designed according to the marketing and distribution needs of the product. It should do the following: protect the product from mechanical damage, avoid moisture loss and modify the internal atmosphere to prolong shelf-life. Physical injuries (vibration and compression bruises or abrasion damage) can be reduced by proper package design which acts as a shock-absorber. Packages must also allow the product to reach the optimal storage temperature quickly.

MAP is a technology that alters the atmosphere within the package according to the interaction between the product respiration rate and the transfer of gases through the package (49). Diffusion through the package depends on the film characteristics (permeability, area and thickness) and the temperature of the surrounding environment (50). When the packaging technology is adapted to the produce respiration rate, an equilibrium modified atmosphere (EMA) can be established in the package, leading to a reduction in the respiration rate and metabolic processes, and with it, increased product shelf-life. The most used gases in MAP are oxygen, carbon dioxide and nitrogen. As previously mentioned, whilst oxygen is consumed during storage life, carbon dioxide is generated through respiration. This process, as well as the interchange with the surrounding environment, will help to achieve EMA.

Packaging systems delay senescence by decreasing respiration rate, metabolic activity and microbial growth (51). There are two types of MAP based on gaseous transmission rates: passive and active. The former uses the natural permeability and thickness of the packaging film to establish the desired atmosphere for the product as a result of its respiration (52). Despite the promise of MAP, it is not yet used ubiquitously in the food industry (53) for the following reasons: the cost of the technology packaging machinery and materials, the analytical equipment necessary to ensure the correct gas mixture, and the fact that some benefits of MAP are lost once the package is opened or where there are leaks. The most common polymers used are polyamide (PA), polypropylene (PP, oriented or not), polyethylene (PE), low density polyethylene (LDPE), linear low density polyethylene (LLDPE), polystyrene (PS), polyester (PES), polyethylene terephthalate (PET), ethylene vinyl alcohol (EVOH) and polyvinylchloride (PVC) (54, 55).

The technique has been successfully applied to whole and fresh-cut products such as artichokes (56), lettuce (57) and strawberry (58). In order for the produce to create the optimal atmosphere the packaging material must be permeable. These packaging films can be microperforated to enable gas interchange between the inside and the outside of the packaging. Xtend® packaging (Johnson Matthey, UK) helps equilibrate the packed produce atmosphere within the optimal range of oxygen and carbon dioxide for a specific fruit or vegetable. It is also able to retain humidity within the package, reducing weight loss during storage. Another example is PerfoTec® (PerfoTec BV, The Netherlands). The film permeability for a particular product is determined and the PerfoTec® laser system carries out the required microperforations.

New structural polymers are now available to improve packaging materials towards bio-based and bio-degradable, non-petroleum sustainable

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packaging materials such as polylactic acid (PLA; for example, NATIVIA®, UAE) made from corn or other starch or sugar sources (59), polylactide aliphatic copolymer (CPLA) (60) and polymers derived from high proportions of recycled plastics (61). At this point, these new materials have limitations in terms of their cost and technical performance. MAP is highly dependent on respiration and temperature. To overcome this, membranes like BreatheWay® contain thermoresponsive crystalline polymers that allow high gas transmission rates at high temperatures (62).

One of the current challenges of MAP is the control of transpiration rate (TR) in fresh produce storage (63). TR is related to the mass transfer process from the stored product to the surrounding atmosphere (64) and is affected by fresh produce factors such as maturity stage, and environmental factors such as water vapour pressure deficit gradient (65). Water loss after harvest leads to weight loss and quality reduction of the produce while an accumulation of water at the product surface will help the growth of spoilage microorganisms (63). Nowadays macroperforations are used to diminish the impact of this problem, yet their presence precludes the creation of a modified atmosphere.

Microbial growth within the package is one of the challenges for MAP. Nanotechnology can enhance packaging functionality by adding antimicrobial, structural and barrier properties (66). This technology can also improve mechanical properties of films and reduce oxygen transmission rates (61). Other gases have also enriched MAP: helium, argon, xenon and nitrous oxide (N_2O). They are also reported to reduce microbial growth and maintain quality (67–69), but are yet to be widely used commercially.

Active MAP is based on the alteration of gases within the package to achieve the ideal gas equilibrium earlier than passive MAP. The techniques used include flushing pre-set gas mixtures into the package; introducing gas scavengers, such as oxygen and carbon dioxide scavengers, moisture absorbers, and ethylene scrubbers; and inserting gas emitters, such as carbon dioxide emitters (70). In the case of flushing gas mixtures, it is proven that high initial concentrations of oxygen (above 70 kPa) have an antimicrobial effect on aerobic and anaerobic microorganisms (71–73). This is also effective for helping inhibit enzymatic browning (74, 75) and avoiding loss of firmness (74, 76). However, operating in high oxygen environments carries the risk of fire. High carbon dioxide concentrations inhibit several enzymes of the Krebs' cycle (77), slowing down ripening processes and decay. However, their efficacy will depend on cultivar, maturity stage and storage conditions. With respect to active inserts, oxygen scavengers are traditionally based on a metal powder (generally iron, ferrous carbonate or metallic platinum), ascorbic acid and enzymes (glucose oxidase and alcohol oxidase).

Active inserts are defined according to their scavenging reaction (such as enzyme mediated oxidation and oxidation speed), and their scavenging capacity (millilitres of oxygen removed). They can lower oxygen concentrations within the sealed packs, slowing deterioration caused by oxidation (78). The use of sulfites, such as potassium sulfite, and natural antioxidants, including tocopherols, lecithin, organic acids and plant extracts, are currently being explored (79) to reduce the oxidation of the fresh produce and delay denaturation of proteins (80). Currently they are applied to breads, nuts, candies and confectioneries, coffee and tea and processed, smoked and cured meats, among others, to improve storage conditions. Generally these scavengers are designed for oxygen removal from sealed food packaging and not semi-permeable fresh produce EMA packaging. More research is needed as existing oxygen scavenger formats are typically cumbersome and not appropriate for fresh produce storage conditions.

Carbon dioxide scavengers (chemical absorbers such as calcium hydroxide, sodium carbonate, calcium oxide; physical absorbers such as zeolite and activated carbon) can similarly delay senescence and reduce browning and mould decay (81). This is particularly interesting for climacteric products, which produce high concentrations of carbon dioxide affecting their organoleptic characteristics.

Another option is the removal of ethylene from the package. Ethylene scrubbers, such as potassium permanganate pellets (Ryan Co, USA) and clay mineral coated strips (It's Fresh, UK) (42), can slow down senescence and reduce decay by neutralising the effect of the plant hormone. Carbon dioxide emitters increase carbon dioxide concentration within the package, helping achieve the optimal gas mixture for each product (70).

A recent trend, known as smart or intelligent packaging, is to fit packaging with sensors able to monitor quality, microbiological growth or temperature along the supply chain (82, 83). Intelligent packaging components include radio frequency identification sensors (84),

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time-temperature and ripeness indicators (for example, ripeSense®, New Zealand), and biosensors (85). Also, carbon dioxide and oxygen gas sensors are being developed for real-time monitoring of produce quality (86). Some low cost intelligent packaging options are available to provide visual information on freshness: fluorescent dyes or molybdenum ions (87, 88). These can inform not only about food quality, but also food safety (89).

It is possible to create physical barriers on the fruit surface which provide protection against moisture loss and can help control oxygen and carbon dioxide concentrations, in a similar way to MAP as they are able to change the internal atmosphere of the produce. This technique is known as edible coating (90). The ideal edible coating should be able to extend storage life without causing anaerobiosis and reduce decay and water loss (90), acting also as antimicrobial agents. The development of this technique began with the application of wax coatings on fruit using dipping methods. The material used to formulate them has to be generally regarded as safe (GRAS) and has evolved with time. According to Arvanitoyannis and Gorris (91), the edible coating must: be water resistant and cover the product completely when applied, reduce water vapour permeability, generate the optimal atmosphere, improve the produce appearance, melt over 40ºC without decomposition, dry with high efficiency performance, have low viscosity, be easily emulsifiable, be economical, be translucent and not interfere with produce quality. The composition of edible coatings has advanced to be based on natural compounds. Some of the latest examples are: *Aloe vera* gel (92), alginate-based edible coatings (93), shellac (94) or silk fibroin (95).

At a commercial level, AgriCoat NatureSeal Ltd, UK, provides a sucrose ester based edible coating for whole fruit, mainly melon (Semperfresh®, UK) and fresh-cut produce (NatureSeal®, UK), which are sulfite-free (GRAS) and delay ripening effects. Edible coatings are able to extend the shelf-life of perishable products, maintain initial appearance, including colour and gloss, and delay decay. The correct formulation should not affect flavour or appearance. To maintain safety within the packaging an application of solutions such as natural antimicrobial like cinnamon or vanillin (93), and essential oils (96) can be used within the edible coating or on their own. Films can also be coated with inhibitors such as titanium dioxide (TiO_2), which is able to inactivate pathogens like *Escherichia coli* (97, 98). These packaging options are required to respond to consumers' demand for ready to eat fruit and vegetables. The fresh-cut industry has to face not only physiological issues that lead to ripening and senescence of fresh produce, but also the likely microbial growth caused by the exposure of tissues to the environment. Mechanical wounding, due to minimal processing, damages cells making it easier for pathogens to contaminate the produce and for enzymes to catalyse non-desirable processes such as browning. Hence, the application of the correct gas mixture environment, edible coatings and natural antimicrobials are critical in this case (99).

Other postharvest technologies can complement MAP. In order to control microbial growth, non-ionising, germicidal and artificial ultraviolet C (UV-C) light (100) can be applied. Some studies show an enhancement of bioactive compounds when this technique is used (100, 101). There are no residues left in the fruit or vegetable after UV-C treatment, which is an advantage in meeting new consumer requirements. A promising technique to improve food safety is cold plasma technology (NSW Department of Primary Industries, Australia). It is created by applying an electric current to normal air or a gas to generate reactive gaseous species with antimicrobial activity. It involves no chemicals, and therefore, no residues.

Future Prospects

The growing demand to decrease postharvest use of chemicals and the need for more sustainable technologies has led to the development of improved CA and MAP storage methods. More research is needed to understand the dynamic physiological responses of fresh produce to CA and MAP in order to determine the optimal conditions for each cultivar and scenario. Critically, a better understanding of how flavour can be extended is required; one of the most repeated consumers' complaints is the lack of fresh fruit and vegetable flavour. With respect to MAP, the structure and functionality of film polymers should be improved, and new sustainable materials developed and deployed. Moreover, a reduction of cost will make these technologies accessible to a wider number of companies within the sector, improving the adoption of MAP and reducing food waste.

Research should focus on optimising gas concentrations by selection of appropriately permeable packaging materials, and on improving their interaction with active materials such as scavengers, emitters, and nanoparticles. In this case, a modelling approach taking into account

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different materials, gas compositions and temperatures will enhance results in the short term. Studies on consumer response to active materials and the information presented by intelligent packaging is needed. Finally, these advances will drive the development of microbiologically safe products, with high functional and sensory quality.

Concluding Remarks

In conclusion, CA storage and MAP serve as important tools to maintain fruit and vegetable quality along the supply chain, reducing food waste and extending fresh produce availability all year round. They need however to be adapted to the new requirements of consumers, being innocuous and applicable to 'residue free' produce. Thanks to recent technological developments, it is possible to create storage environments that adjust their settings to the physiological response of the commodity, further extending postharvest life while maintaining quality. The advances in CA and MAP will drive the development of more sustainable materials and more efficient gas control, which are essential instruments for postharvest management. However, these technologies have the following main limitations: imprecise monitoring of fruit and vegetable response, high energy requirements, high cost of materials and reduced retention of initial quality (such as flavour-life). These problems can be overcome through physiologically-targeted CA and MAP.

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