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REVIEW PAPER

Modified Atmosphere Packaging Technology of Fresh and Fresh-cut Produce and the Microbial Consequences—A Review

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Abstract Modified atmosphere packaging (MAP) technology offers the possibility to retard the respiration rate and extend the shelf life of fresh produce, and is increasingly used globally as value adding in the fresh and fresh-cut food industry. However, the outbreaks of foodborne diseases and emergence of resistant foodborne pathogens in MAP have heightened public interest on the effects of MAP technology on the survival and growth of pathogenic organisms. This paper critically reviews the effects of MAP on the microbiological safety of fresh or fresh-cut produce, including the role of innovative tools such as the use of pressurised inert/noble gases, predictive microbiology and intelligent packaging in the advancement of MAP safety. The integration of Hazard Analysis and Critical Control Points-based programs to ensure fresh food quality and microbial safety in packaging technology is highlighted.

Keywords MAP · Foodborne pathogen · Predictive microbiology · Noble gases · HACCP

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Introduction

Global drive for healthier diet, changes in consumer life style and the advancement of retail marketing have led to a remarkable increase in the demand for fresh, healthy and convenient food produce. Fruit and vegetables are often associated with high nutritional quality and freedom from synthetic additives used to preserve and enhance characteristics such as flavour and colour in industrially manufactured foods (Bruhn 2000; Rico et al. 2007; Gialamas et al. 2010). Fresh and fresh-cut fruit and vegetables continue all metabolic processes, and are susceptible to quality deterioration and microbial infestation due to increase in enzymatic activities, transpiration and respiration (Mahajan et al. 2008c; Caleb et al. 2012c). Modified atmosphere packaging (MAP) technology offers the possibility to extend the shelf life of fresh produce, by retarding produce respiration rate, and delaying enzymatic degradation of complex substrates (Kader 1986). Cost-effective MAP design depends on many other factors such as the product weight, temperature and properties of the packaging material (e.g. film thickness, permeability, perforation density and surface area; Charles et al. 2003; Sandhya 2010; Caleb et al. 2012a, c). Successful applications of MAP in fresh and fresh-cut fruit and vegetables has been extensively reported in the literature (Farber et al. 2003; Sandhya 2010)

Concerns have been expressed on the influence of MAP on the survival and growth of pathogenic microorganisms which may render fresh and fresh-cut produce unsafe while still edible (Jay 1992; Philips 1996; Farber et al. 2003). Additionally, MAP may significantly inhibit spoilage organisms or eradicate desirable produce microflora due to the non-selective antimicrobial effect of carbon dioxide (CO_2; Farber et al. 2003). As the interaction between natural microflora and food pathogens may play a significant role in product safety. Studies have shown that optimal gas composition, as well as the presence and competitive effect of background microflora have inhibitory effect on foodborne pathogens (Bourke and O'Beirne 2004).

Furthermore, the antimicrobial effect of CO_2 has been reported to have differential effects on bacterial and viral population density, and on toxin gene expression of some foodborne pathogen (Francis et al. 1999; Bidawid et al. 2001; Guevara et al. 2003; Artin et al. 2008, 2010). These findings highlight the need for implementing an effective and robust hazard analysis and critical control points (HACCP) programs in fresh produce packaging facilities and along the cold chain. More research on the microbial interactions of background microflora and foodborne pathogens under various MAP storage conditions is recommended (Farber et al. 2003). There is a need for better understanding of the effects of different gas atmospheres on the survival, growth and expression of toxin gene of potential foodborne pathogens on fresh and fresh-cut fruit and vegetables.

Table 1, summarises several recent reviews which examined various specific aspect of MAP technology for various food products including the basic principles of MAP, the effect on produce quality, microbial contamination and safety, and MAP of specific fresh food products such as pomegranate, mushroom and fish. None of these reviews extensively covered the role of predictive microbiology, regulations and role of HACCP and other programs towards achieving optimal benefits of MAP. This paper presents a critical review on the current status of MAP technology for fresh and fresh-cut fruit and vegetables, with emphasises on the microbiological safety of MAP application, influence of MAP conditions on genetic expression in microorganisms and integration of the advances in MAP technology into existing regulations.

MAP Technology—An Overview

MAP is an active or passive dynamic process of altering gaseous composition within a package. It relies on the interaction between the respiration rate of the produce, and the transfer of gases through the packaging material, with no further control exerted over the initial gas composition (Farber et al. 2003; Mahajan et al. 2007; Caleb et al. 2012c). Passive MAP can be generated inside a package by relying on the natural process of produce respiration and film permeability to attain the desired gas composition over time (Charles et al. 2003; Farber et al. 2003). While, active MAP is a rapid process of gas replacement or displacement, or the use of gas scavengers or absorbers to establish a desired gas mixture within a package (Kader and Watkins 2000; Charles et al. 2003; Farber et al. 2003). This involves the addition of active agents into packaged food product, such as O_2, CO_2 and ethylene scavengers (Philips 1996; Sandhya 2010). For example, CO_2 absorbers can prevent a build-up of CO_2 gas to deleterious levels (Kader and Watkins 2000).

Both produce respiration rate and film permeability are dependent on extrinsic factors such as temperature. Therefore, the purpose of applying MAP is to maintain a desirable atmosphere within a specific temperature range. If the temperature changes by more than a few degrees, the package atmosphere will also change and may become inappropriate or even injurious to the product (Zagory 1995). Therefore, in order to achieve the desired modified atmosphere in a given package, it is expedient to understand the three basic disciplines underpinning MAP (Brandenburg and Zagory 2009), namely produce physiology (such as the extrinsic and intrinsic factors affecting produce respiration rate), polymer engineering (which identifies the choice of specific polymer's physical, chemical, and gas transmission rate properties), and converting technology (which entails the fabrication of raw polymers, films, adhesives, inks and additives into packages of desired format monolayer or multi to complex layers, with or without perforation). Effective and resource-efficient MAP design exists at the intersections of these three disciplines, creating innovative packaging solution that is driven by consumer demand and balanced with environmental sustainability.

The physiological processes of produce (mainly respiration and transpiration) play significant roles in the postharvest quality of MA-packaged fresh and fresh-cut fruit and vegetables. Respiration is a metabolic activity that provides the energy needed for other plant biochemical reactions. Aerobic respiration (referred to as respiration throughout this paper) involves the oxidative breakdown of complex organic compounds such as carbohydrates, lipids, and organic acids into simpler molecules, including CO_2 and water with the release of energy (Fonseca et al. 2002a, b). Table 2 gives a summary of factors that influences fresh or fresh-cut produce respiration rate. Respiration rate can be reduced by decreasing O_2 concentration around the fresh produce. This process induces a decrease in the activity of oxidising enzymes such as polyphenoloxidase, glycolic acid oxidase and ascorbic acid oxidase (Kader 1986). Decreasing respiration rate via MA and lowering temperature delays enzymatic degradation of complex substrates and reduces sensitivity to ethylene synthesis (Saltveit 2003; Tijskens et al. 2003), thereby extending the shelf life and avoiding senescence of the produce. De Santana et al. (2011) evaluated the effect MAP on respiration rate and ethylene synthesis during 6-day storage at 1 and 25 °C. They reported that ethylene production was proportional to respiration rate for peaches during ripening at 25 °C. However, lower ethylene synthesis and respiration rate were obtained at lower temperature in MAP treatments. This principle is a critical component to the successful application of MAP. Excessively low O_2 level, below 1 % may result in anaerobic respiration leading to tissue deterioration as well as production of off-odours and off-flavours (Lee et al. 1995; Austin et al.

Table 1 Selected reviews on microbial safety and related topics on MAP of various food products

References	Scope of the review	Product(s) of focus
Buchanan (1993)	Predictive food microbiology[a]	
Church (1994)	New developments in MAP	Fresh products[c]
Notermans et al. (1995)	Specification of criteria towards application of HACCP[a, b]	
Philips (1996)	Evaluted the effect of MAP on microbiological quality and safety	Meat, fish, fruit and vegetables[b, c]
Francis et al. (1999)	Microbial safety of minimally processed vegetables: processing, packaging and storage	Vegetable[a–c]
McDonald and Sun (1999)	Predictive food microbiology	Meat industry[a]
Kader and Watkins (2000)	Research need and future prospect of MAP	Fresh and fresh-cut fruit and vegetables[b,c]
Cutter (2002)	Microbial control by packaging[a, b]	
Jayas and Jeyamkondan (2002)	Modified atmosphere storage	Meat, grains, fruit and vegetables[b,c]
Sivertsvik et al. (2002)	Significance of MAP on microbial growth, activities and safety of fish and fishery products	Fish[a,c]
Farber et al. (2003)	Food safety of CA and MA-packaging technology and commerically available applications	Fresh and fresh-cut fruit and vegetables[a–c]
Harris et al. (2003)	Incidence, growth and survival of pathogens in fresh and fresh-cut produce	Fresh and fresh-cut fruit and vegetables[a–c]
Soliva-Fortuny and Martín-Belloso (2003)	Advances in extending the microbiological, physico-chemical and sensory quality of fresh-cuts	Fresh-cut fruits[b,c]
Lee and Khang (2004)	Hurdle technologies and microbial safe of pickled fruit and vegetables	Pickled fruit and vegetables[a–c]
Koopmans and Duizer (2004)	Foodborne viruses	Food products[a, b]
O'Beirne (2007)	Microbial safety of fresh cuts	Fresh-cut vegetables[b]
Rico et al. (2007)	Methods of extending the shelf-life of fresh-cut produce	Fresh-cut fruit and vegetables[a–c]
Dainelli et al. (2008)	Legal aspects and safety concerns of packaged foods[a, b]	
Rojas-Graü et al. (2009)	Advances in the use of innovative MAP	Fresh-cut fruit and vegetables[a–c]
Varzakas and Arvanitoyannis (2008)	Comparison of ISO2200 to HACCP for processing ready-to-eat vegetables	Vegetable[a, b]
Artés et al. (2009)	Sanitation techniques for fresh cuts	Fruit and vegetables[a–c]
Oms-Oliü et al. (2009)	Overview on recent developments in the use of MAP	Fresh-cut fruits and vegetables[b,c]
Mastromatteo et al. (2010)	Overview on the use of natural compounds combined with MAP	Dairy, meat, fish, fruit and vegetables[b, c]
Caleb et al. (2012c)	Overview on MAP of pomegranate fruit and arils	Pomegranate[b, c]
Luber et al. (2011)	Recommendations for improving prevention and control of *L. monocytogenes*	Food products[a]
Neethirajan and jayas (2011)	Overview on current and future applications of nanotechnology	Food products[a–c]

[a] Review do not contain information on role of predictive microbiology

[b] Review do not contain information on HACCP, good hygenic practice, GMP etc.

[c] Review do not contain information on systemic MAP—i.e. modelling respiration and package permeability

1998; Ares et al. 2007). The influence of CO_2 on respiration rate has not been well clarified as there are varying theories on this, such as the idea that CO_2 being a product of respiration process will cause a feedback inhibition (Fonseca et al. 2002a, b). Another concept considered that elevated CO_2 might affect the Krebs cycle's enzymes and intermediates, while another suggested that CO_2 might inhibit ethylene production instead of having a direct influence on respiration process (Mathooko 1996; Fonseca et al. 2002a, b). Retarding ethylene synthesis has tremendous benefits for the storage of sensitive horticultural produce. Although, for some non-climacteric produce such as vegetable tissue and citrus, ethylene production is under a negative feed-back response, hence reducing ethylene will stimulate its production (Saltveit 2003).

The other physiological process of significant importance in postharvest quality of fresh and fresh-cut produce is transpiration. Once the fresh produce is detached from the growing plant, they solely depend on internal water content for transpiration resulting in water loss (Mahajan et al. 2008c). The loss of water from fresh produce result in weight loss and shrivelling, leading to unsalable loss during retail marketing and a direct financial loss. Transpiration rate of produce during postharvest handling and storage is influenced by produce factors such as surface-to-volume ratio, surface injuries, morphological and anatomical

Table 2 Factors influencing respiration rate quantification

Intrinsic factors	Extrinsic factors
Produce cultivar	Temperature
Growing season	Level of oxygen
Farming system	Level of carbon dioxide
Growing region	Storage time
Produce maturity level	
Pre-treatment processes	
Type of cuts[a]	
Size of cuts[a]	
Type of cutting blade[a]	

Fonseca et al. (2002a, b); Kader et al. (1989); Montero-Calderón and Cerdas-Araya (2011)

[a] Factors due to produce processing

characteristics, as well as maturity stage and environmental factors including, temperature, relative humidity (RH), air movement and atmospheric pressure (Kader 2002; Mahajan et al. 2008c). Studies have shown that there is a close relationship between temperature and relative humidity on transpiration rate (Mahajan et al. 2008c), which plays a significant role in determining the optimal storage conditions of fresh and fresh-cut produce. At a given RH, the increase in transpiration rate is directly proportional to the increase in temperature (Kader 2002; Mahajan et al. 2008c).

Furthermore, the use of polymeric films in MAP serves as mechanical barrier to the movement of water vapour and this helps to maintain a high level of RH within the package, and reduce produce weight loss (Suparlan and Itoh 2003). However, an excessively high level of RH within the package can result in moisture condensation on produce, thereby creating a favourable condition for the growth pathogenic and spoilage microorganisms (Zagory and Kader 1988; Aharoni et al. 2008; Távora et al. 2004). Ding et al. (2002) reported a minimal water loss of 0.9–1.5 % in modified atmosphere-packaged loquat fruit, in comparison to perforated polyethylene packaged fruit which had 8.9 % water loss after storage for 60 days at 5 °C. It was also observed in their study that MAP significantly maintained loquat organic acid levels and fruit quality. Suparlan and Itoh (2003) investigated the combined effect of hot water treatment and MAP on the quality of tomatoes. MAP was found to reduce the weight loss of tomatoes to about 41 % compared to the unpacked samples during a 2-week storage period at 10 °C. Singh et al. (2009) reported a minimal physiological loss in weight and a higher shelf life for jasmine buds packaged using polypropylene film under passive MAP compared to non-MAP stored buds at 2 °C. These finding shows that lowering temperature and applying other technology such as MAP to decrease the rate of physiological process has a beneficial effect on preservation of fresh produce.

Produce Physiology and Mathematical Predictions

Understanding the multi-complex interactions within various physiological processes towards MAP design requires a suitable model to predict these responses as function of time, temperature, gas composition or RH in the case of transpiration rate. Over the last decade, significant advancements in computing and the use of statistical tools for data fitting and numerical integration, with more accurate analytical techniques, have enabled a better understanding of the physiological interactions involved on MAP of fresh and fresh-cut produce through the development of predictive models (Charles et al. 2003; Mahajan et al. 2007). However, there are various limitations to the development of such predictive models. This include time-consuming experiments with potentially large experimental errors, and the complex nature of respiration process for the determination of respiration rates of produce for MAP design (Fonseca et al. 2002a, b). Other limitations of mathematical models are that, models are based on limited number of experimental observations, and inherent biological variation and the dynamic response of stored fresh or fresh-cut produce to environmental changes is not adequately accounted for. Often, these variables are held constants or assumed to be negligible (Saltveit 2003; Tijskens et al. 2003). Therefore, the development of models should incorporate adequate measure of the produce's dynamic response to extrinsic factors such as RH, temperature, light, time and others (Saltveit 2003; Tijskens et al. 2003; Caleb et al. 2012b).

Table 3 presents a summary of articles on respiration rate since 2000, highlighting the produce, experimental approach, experimental conditions and the types of models developed or applied. Most respiration rate models have been oriented towards either one or two out of the three functions of time, temperature and gas composition. The Michaelis–Menten type equations (uncompetitive, non-competitive, or uncompetitive/competitive) based on CO_2 inhibitory effect (Lee et al. 1991; Peppelenbos and Leven 1996; Del Nobile et al. 2006; Rocculi et al. 2006; Bhande et al. 2008), and the Arrhenius-type equations, which describe temperature as a function of respiration (Jacxsens et al. 2000; Kaur et al. 2010; Uchino et al. 2004; Torrieri et al. 2010), have been widely reported for respiration rate of fresh produce as a function of both temperature and gas composition. A major limitation of respiration rate modelling is of the lack of adequate respiratory data information. Often, data available are either based on O_2 consumption or CO_2 production rates, based on the assumption that the respiratory quotient (RQ)=1. The downside to this is that if the RQ were to be >1, the model would underestimate CO_2 production and if RQ<1, the predictive would underestimate likewise (Fonseca et al. 2002a, b).

Mathematical prediction of transpiration rate for fresh produce is challenging, due to insufficient information on

Table 3 Respiration rate models presented in literature from 2002

Produce	Experimental approach	Storage T (°C)	Model	Reference
Shredded Galega Kale	Close system; gas chromatography (Gow-Mac series 580)	1, 5, 10, 15 and 20	MMUC	Fonseca et al. (2002a, b)
Blueberry	Close system; gas chromatography (Hewlett Packard 5890A)	15 and 25	Regression equation	Song et al. (2002)
Tomatoes	Close system; gas chromatography (Micro GC, CP2003)	20	MMNC	Charles et al. (2003)
Fresh endives	Close system; gas chromatograhy (Micro GC, CP2003)	5, 8 and 20	MMNC	Charles et al. (2005)
Minimally processed lettuce	MA packaged; gas chamber (M.K.S. Baratron 221A)	5	MM	Del Nobile et al. (2006)
Sliced golden delicious apple	Active MA packaged; gas anlyser (PBI Dansensor)	4	MM	Rocculi et al. (2006)
Banana	Closed system; gas analyser (PBI Dansensor)	10–30	Regression equation and UCI	Bhande et al. (2008)
Fresh-cut melons	Active MA packaged; gas anlyser (Micro-GC Chrompack)	4	Weibull model and logistic	Oms-Oliü et al. (2008)
Green mature mango	Closed system; gas chromatograph (Nucon AIMIL 5765)	5, 10, 15, 20, 25 and 30	MMUC	Ravindra and Goswami (2008)
Sapota	Closed system; gas analyser (PBI Dansensor)	0, 5,10, 15, 20, 25 and 30	Regression equation and UCI	Dash et al. (2009)
Fresh-cut 'Annurea' apple	Modified closed system; gas analyser (PBI Dansensor)	5, 10, 15 and 20	MMUC and Arrhenius-type	Torrieri et al. (2009)
Shredded carrots	Closed system; gas analyser (PBI Dansensor)	0, 4, 8, 12, 16 and 20	MMUC and Arrhenius-type	Iqbal et al. (2009a)
Whole mushroom	Closed system; gas analyser (PBI Dansensor)	0, 4, 8, 12, 16 and 20		Iqbal et al. (2009b)
Whole and sliced mushroom	Closed system; gas analyser (PBI Dansensor)	0, 4, 8, 12, 16 and 20		Iqbal et al. (2009c)
Guava	Closed system; gas analyser (PAC CHECK, Model 325, MOCON)	5, 10, 15, 20, 25 and 30	MM; Arrhenius-type and ANN	Wang et al. (2009)
Pomgranate arils	Closed system; gas analyser (PBI Dansensor)	4	MMUC; MMC; MMNC; MMUC & MMC	Ersan et al. (2010)
Fresh-cut 'Rocha' pear	Permeable system; gas analyser	0, 5, 10 and 15	MM and non-competitive inhibition	Gomes et al. (2010)
Fresh-cut spinach	Closed system; gas analyser (Quantek Instrument)	10 to 15	Arrhenius-type	Kaur et al. (2010)
Minimally processed broccoli	Modified close system; gas analyser (PBI Dansensor)	3, 5, 7, 10, 15 and 20	MMC	Torrieri et al. (2010)
Minimally processed organic carrots	MA packaged; gas chromatograph (Model 35)	1, 5 and 10	MMUC; MMC; MMNC and Arrhenius-type	Barbosa et al. (2011)
Litchi	Close system; gas chromatograph (Model 100 Knaur, Germany)	0, 5, 10, 15, 20, 25 and 30	MMUC and enzyme kinetic model	Mangaraj and Goswami (2011)
Baby corn	Close system; gas analyser (Model 902 D Dualtrak, Quantek)	5, 10 and 15	Fourth order Runge-Kutta method	Rai and Singh (2011)
Pomgranate fruit and arils	Closed system; gas analyser (PBI Dansensor)	5, 10 and 15	Arrhenius-type	Caleb et al. (2012a)

ANN Artificial neural network, *MMC* Michaelis–Menten competitive inhibition, *MMUC* Michaelis–Menten uncompetitive inhibition, *UCI* uncompetive inhibition, *MMNC* Michaelis–Menten noncompetitive inhibition

the dynamic interactions between evaporation on the produce surface due to heat released during respiration and the permeability property of the packaging film (Song et al. 2002). Existing models for predicting water loss in fresh produce have been limited in application to cooling process and bulk storage (Sastry and Buffington 1982; Chau and Gaffney 1985; Gaffney et al. 1985), and these models may not be suitable for MAP systems (Song et al. 2002). Most models describe moisture loss as a function of the biophysical and thermo-physical properties such as skin thickness, surface cellular structure and pore-fraction in the skin, thermal diffusivity and geometry of produce. Measuring these properties is time consuming (Song et al. 2002). Predicting the rate of water loss is important towards estimating the shelf life of fresh and fresh-cut produce, and designing appropriate packaging at optimal storage

conditions. Concerted research effort should be made to develop simple but comprehensive models for predicting of water loss in fresh and fresh-cut fruit and vegetables. In order to overcome the methodological challenges in the measurement and prediction of water loss, the weight loss approach for fresh produce can be adopted (Leonardi et al. 1999). This approach was successfully applied by Mahajan et al. (2008a).

Packaging Material

Another critical parameter in the success of MAP is the choice of packaging material. The degree to which modification of the atmosphere takes place in packages is dependent on variables such as film permeability to O_2, CO_2, water vapour, film thickness, package surface area and the free volume inside the package (Beaudry 1999; Cameron et al. 1994; Mahajan et al. 2008b). Gas flux through the package film or film permeability can be mathematically predicted, using permeability equation based on the Fick's diffusion laws for thin and infinite films, where in the gas flux per unit time through the film can be determined (Crank and Park 1968). Furthermore, Arrhenius equation which describes the temperature sensitivity of film permeability to gases can be coupled with other mathematical models to obtain a more robust and descriptive parameters. In order to predict the package oxygen partial pressure as a function of product mass, film surface area and thickness, and temperature (Cameron et al. 1994; Lakakul et al. 1999).

Tables 4 and 5 presents the properties of various packaging materials and the permeability of some commonly used polymeric films at set conditions. Although petroleum-based polymeric materials are mostly used in packaging of fresh produce, with their advantages summarised in Table 4. These materials are not biodegradable and burning them leads to environmental pollution, which poses a global ecological challenge and detrimental to human health (Isobe 2003; Kirwan and Strawbridge 2003; Tharanathan 2003; Siracusa et al. 2008; Zhang and Mittal 2010). Hence, the growing paradigm shift due to environmental awareness by consumers towards packaging films which are biodegradable, and processes which are user- and eco-friendly (Tharanathan 2003). Raw materials used to make biodegradable films can be classified into three groups, namely extracts derived from agricultural raw materials (e.g. protein, lipids, and starch), by-products from microorganisms (e.g. polyhydroxyalcanoates and poly-3-hydroxy-butyrate), and synthesis from bio-derived monomers (e.g. polylactic acid; Cha and Chinnan 2004; Smith 2005; Siracusa et al. 2008; Joseph et al. 2011; Jiménez et al. 2012). Other source of biodegradable films include a matrix of synthetic and natural polymers, for example, the properties of a mixture of wheat starch, ethylene acrylic acid and low-density polyethylene (LDPE) were investigated by Arvanitoyannis et al. (1997). Several studies have compared the properties of biodegradable films and their effect on the quality of fresh produce (Makino and Hirata 1997; Rakotonirainy et al. 2001; Srinivasa et al. 2002; Del Nobile et al. 2006; Almenar et al. 2008; Siracusa et al. 2008; Guillaume et al. 2010). However, little information is available on the effectiveness of various biodegradable film packaging on microbial safety of packaged produce during storage (Koide and Shi 2007).

For example, Koide and Shi (2007) investigated the microbial and physicochemical quality of green peppers stored in a polylactic acid based biodegradable and LDPE film packaging. Results obtained by the authors showed that physicochemical properties such as weight loss, hardness, colour, ascorbic acid and gas concentrations, and microbial levels did not show significant changes during the storage period. However, the total coliform bacteria increased by 2.3 log CFU/g in LDPE film and 0.9 and 0.2 log CFU/g in the perforated LDPE and biodegradable film packaging, respectively. These findings indicated that biodegradable film with higher water vapour permeability would better maintain the quality of green peppers. As no fungal growth was observed in biodegradable film-packaged green peppers, this was associated to the high water vapour permeability which lowered the relative humidity inside the biodegradable packaging. Pyla et al. (2010) investigated both antimicrobial and antioxidant effect of corn starch matrix mixed with tannic acid. They found that the matrix exhibited an antimicrobial activity against *Listeria monocytogenes* and *Escherichia coli* O157:H7 and antioxidant effect on soybean oil. Kim et al. (2011) reported antimicrobial activity of chitosan biopolymer films (CBFs) with four different viscosities against *L. monocytogenes*, *Samonella typhimurium* and *Escherichia coli* O157:H7. CBFs with 100 mPa s chitosan had an antilisterial effect on 10^4 cfu/ml inoculation. In a more recent study, Türe et al. (2011) investigated the effect of wheat gluten (WG) and methyl cellulose (MC) biopolymers containing natamycin on the growth of *Aspergillus niger* and *Penicillium roquefortii* on the surface of fresh kashar cheese. WG and MC films were found to be effective against *A. niger* with about 2-log reductions in spore count. This information highlights the potential for biodegradable films towards optimal microbiological safety of MA-packaged fresh and fresh-cut produce. As more innovative biodegradable packaging materials emerge within the nanotechnology field (Siracusa et al. 2008), it is necessary to conduct research on their microbiological safety to ensure the overall integrity of food.

Another form of biodegradable polymer is edible films, which comprise of a thin layer of edible materials applied to food as surface coating (Mangaraj et al. 2009; Campos et al. 2011). There are several benefits of using edible films as

Table 4 Properties of major packaging materials

Packaging material	Properties	
	Advantages	Disadvantage
Paper	Strength and rigidity Printability	Opacity
Tinplate	Corrosion resistance	Higher barrier to gases
	Excellent barrier to gases, water vapour, light and odour	Tin toxicty
	Heat-treatable Ability to seal hermatically	
	Ductility and formability	
Tin-free steel	Corrosion resistance Excellent barrier to gases, water vapour, light and odour	Higher barrier to gases
	Heat-treatable	
	Ability to seal hermatically	
	Ductility and formability	
	Less expensive compared to tinplate	
Aluminium foil	Negligible permeability to gases, odours and water vapour	Opacity
	Dimensional stability	High barrier to gases
	Grease resistance Brilliant appearance	
	Dead folding characteristics	
Glass	Formability and rigidity	Higher barrier to gases
	Transparency and UV protection due to colour variation	Heavy weight adds to transport cost
	Impermeable to gases, water vapour and odour Chemical resistance to all food products	
	Heat stable	
Cellulose film (coated)	Strength Attractive appearance	Low permeability barrier
	Low permeability to water vapour, gases, and odours (*coat dependent*)	
	Grease resistance	
	Printability	
Cellulose acetate	Strength and rigidity Dimensional stability	Glossy appearance
	Printability	
Ethylene vinyl alcohl (EVOH)	Excellent barrier to gases and odour Effective oxygen barrier material	Moisture sensitive barrier
Ethylene vinyl acetate (EVA)	Very good adhesive properties	Poor gas barrier
	Excellent transparency Heat sealability	Poor moisture barrier
Polyethylene	Durability and fexibility	HDPE; poor clarity
	Heat sealability	LLDPE; heat sensitive
	Good moisture barrier Chemical resistance	
	Good low-temperature performance	
	Permeable to gases	
Polypropylene	Harder, denser and more transparent than polyethylene Better response to heat sealing	
	Excellent grease resisitance	
	Good resistance to chemical	
	Higher gas and water vapour barrier compared to polyethylene	
Polyesters (PET/PEN)	Excellent durability and mechanical properties Excellent transparency	
	Good resistance to heat, mineral oil and chemical degradation	

Table 4 (continued)

Packaging material	Properties	
	Advantages	Disadvantage
	Adequate barrier to gases, water vapour and odours	
Polyvinyl chloride (PVC)	Strong and transparent Good gas barrier and moderate barrier to water vapour	
	Excellent resistance to chemicals, greases and oils	
	Heat sealability	
Polyvinylidene chloride (PVDC)	Low permeability/high barrier to gases, water vapour and copolymer odurs Good resistance to greases and chemicals	Low permeability barrier/high gas barrier
	Heat sealability	
	Usefull in hot filling, retorting and low temperature storage	
Polystyrene	Hight tensile strength Excellent transparency	Poor barrier to gas and water vapour
Polyamide (nylon-6)	Strong Moderate oxygen barrier, and excellent odour and flavour barrier	Poor water vapour barrier
	Good chemical resistance	
	Thermal and mechanical properties similar to PET	
	High temperature performance	

FAD/WFP (1970), Page et al. (2003), Marsh and Bugusu (2007), Mangaraj et al. (2009)

packaging material, including the ability to minimise microbial growth by lowering the water activity (a_w), enzymatic activities and mitigating moisture loss, gas and aroma absorption into food, and improving the mechanical integrity and shelf life of food (Cutter 2002; Marsh and Bugusu 2007; Campos et al. 2011). As with other MAP technologies, edible films can create a low level of O_2 within package (Odriozola-Serrano et al. 2008), which can facilitate the growth of anaerobic pathogens such as *Clostridium botulinum* (Guilbert et al. 1996). However, edible films are ideal vehicles for incorporating a wide variety of additives such as antimicrobials, antioxidants and texture agents to customise the film (Baldwin 1994; Cutter 2002; Farber et al. 2003; Campos et al. 2011). Several researchers have shown that antimicrobial compounds such as minerals and vitamins, organic acids, bacteriocins, enzymes, proteins and peptides, antibiotics and fungicides could be added to edible films to inhibit microbial growth on a variety of fresh produce (Ayranci and Tunc 2004; Han et al. 2004; Martínez-Romero et al. 2006; Tapia et al. 2008; Türe et al. 2008; Rojas-Graü et al. 2009; Corrales et al. 2009; Ibarguren et al. 2010; Campos et al. 2011; Shakeri et al. 2011). Basch et al. (2012) investigated the antimicrobial effectiveness of nisin and potassium sorbate, incorporate into edible films made with tapioca starch mixed with hydroxypropyl methylcellulose. They observed that the combination of both antimicrobial agents was more effective against *Listeria innocua* and *Zygosaccharomyces bailii*, than their individual incorporation. With growing interest in incorporating nutritional and bioactive compounds into edible films or coatings to improve their functional properties (Campos et al. 2011), the concentration of these additives and their potential side effects must be carefully investigated to determine the optimal range of barrier, mechanical and antimicrobial properties.

Converting Technology and MAP Design

The combination of various packaging material results in the development of a wide variety of MAP formats, ranging from the very simple monolayer side weld bags to complex multilayer coextruded, metalized, laminated-reverse-printable, thermoformed multilayer tray with peelable lids and nanocomposites polymers with or without micro perforations (Farber et al. 2003; Brandenburg and Zagory 2009; Lange and Wyser 2003; Mangaraj et al. 2009; Marsh and Bugusu 2007). The objective of MAP design is to define conditions that will create the atmosphere best suited for the extended storage of a given produce, while minimising the equilibrium time required in achieving this atmosphere (Mahajan et al. 2007). This includes the determination of intrinsic properties of the produce, i.e. respiration rate, optimum O_2 and CO_2 gas concentrations, and film permeability characteristics. Determining optimum package permeability characteristic involves the selection of suitable films for a given produce, including its area and thickness, filling weight, equilibrium time and the equilibrium gas composition at isothermal and non-isothermal conditions (Mahajan et al. 2007; Mangaraj et al. 2009). Poorly

Table 5 Types of polymeric films and their permeability properties at set conditions

Polymeric film	Permeance (mol s^{-1} $m^{-2}Pa^{-1}$) for 25 μm film at 25 °C			WVT (mol s^{-1} $m^{-2}Pa^{-1}$) at 38 °C and 90 % RH
	Oxygen	Carbon dioxide	Nitrogen	
Ethylene vinyl alcohol (EVOH)	1.87×10^{-14}	–	–	8.01×10^{-5}
Ethylene vinyl acetate (EVA)	5.84×10^{-11}	2.33×10^{-10}	2.29×10^{-11}	2.36×10^{-4}
Polyamide (PA) (Nylon-6)	1.87×10^{-13}	7.94×10^{-13}	6.54×10^{-14}	7.50×10^{-3}
Polyethylene (PE), LD	3.64×10^{-11}	1.96×10^{-10}	1.31×10^{-11}	8.48×10^{-5}
Polyethylene (PE), HD	1.21×10^{-11}	3.55×10^{-11}	3.04×10^{-12}	4.01×10^{-5}
Polypropylene (PP), cast	1.73×10^{-11}	4.67×10^{-11}	3.18×10^{-12}	5.18×10^{-5}
Polypropylene (PP), oriented	9.34×10^{-12}	3.74×10^{-11}	1.87×10^{-12}	2.83×10^{-5}
Polypropylene (PP), oriented, PVDC coated	7.00×10^{-14}	1.98×10^{-13}	4.90×10^{-14}	2.12×10^{-5}
Polystyrene (PS), oriented	2.33×10^{-11}	8.41×10^{-13}	3.74×10^{-12}	5.30×10^{-4}
Polyurethane (Polyester)	5.37×10^{-12}	7.47×10^{-11}	4.20×10^{-12}	2.36×10^{-3}
Rigid, Polyvinyl chloride (PVC)	1.17×10^{-12}	3.39×10^{-12}	4.90×10^{-13}	1.65×10^{-4}
Plasticized, PVC	7.12×10^{-11}	1.11×10^{-10}	2.40×10^{-11}	1.30×10^{-4}
Polyvinylidene chloride (PVDC), coated	5.60×10^{-14}	1.17×10^{-13}	–	–
PVDC-PVC copolymer (Saran)	7.70×10^{-14}	4.67×10^{-13}	1.07×10^{-14}	1.53×10^{-5}
	Oxygen permeance (mol s^{-1} $m^{-2}Pa^{-1}$ at 23 °C)		WVT (mol s^{-1} $m^{-2}Pa^{-1}$ at 23 °C and 85 % RH)	
Ethylene vinyl alcohol	4.70×10^{-18}–4.70×10^{-17}		4.71×10^{-06}–1.41×10^{-05}	
Polyamide (PA)	4.70×10^{-16}–4.70×10^{-15}		2.36×10^{-06}–4.71×10^{-05}	
Polyethylene (PE)	2.35–9.40×10^{-13}		2.36–9.43×10^{-06}	
Ploye thylene terephthalate (PET)	4.71×10^{-15}–2.35×10^{-14}		2.36–9.43×10^{-07}	
Ploye thylene naphthalate (PEN)	2.35×10^{-15}		3.29878×10^{-06}	
Polypropylene (PP)	2.3–4.70×10^{-13}		9.43×10^{-07}–1.89×10^{-06}	
Polystyrene (PS)	4.70–7.05×10^{-13}		4.71×10^{-06}–1.89×10^{-05}	
Polyvinyl alcohol (PVAL)	9.40×10^{-17}		1.41×10^{-04}	
Polyvinyl chloride (PVC)	9.40×10^{-15}–3.76×10^{-14}		4.71–9.42×10^{-06}	
Polyvinylidene chloride (PVDC)	4.70×10^{-18}–1.41×10^{-15}		4.71×10^{-07}	

Day (1993), Greengrass (1993), Guilbert et al. (1996), Philips (1996), Chung and Yam (1999), Park (1999), Han (2000), Lange and Wyser (2003)
Data reported by authors in different units were converted to SI unit following Banks et al. (1995)

designed MAP systems may be in-effective or even shorten the storage life of a product, because O_2 and/or CO_2 levels are out of recommended range, or if the appropriate atmosphere is not rapidly established within the package (Mahajan et al. 2007).

The development of MAP in industry has been mainly empirical with 'trial and error' or 'pack and pray' approach, which is time consuming with financial and safety consequences. This results in most commercial fresh produce packages often deviating from the optimal MAP (Mahajan et al. 2008b; Mangaraj et al. 2009). To overcome this setback, a systemic approach in designing optimal equilibrium modified atmosphere packaging (EMAP) for fresh produce was developed by Mahajan et al. (2007). The software contains a database on respiration rate of various fruits and vegetables, optimum temperature, optimum range of O_2 and CO_2 levels and gas permeability properties of commonly used packaging films, including micro-perforated films. The Pack-in-MAP software is accessible online (www.packinmap.com). It enables the user to define the type of product and the system then selects the optimum temperature, the O_2 and CO_2 concentrations, and calculates the respiration rate for the product. Furthermore, it identifies the best possible packaging material and/or amount of product required to achieve optimal packaging conditions.

Table 6 presents a summary of variables involved in MAP design using polymeric films. Once a produce has been selected and its environmental conditions for storage are established, eight out of these 14 listed variables in Table 6 are fixed. For instance, variables such as the surrounding gas composition and temperature, the product density, the production rate of CO_2, the consumption rate of O_2, and, the gas composition to be attained in the package so that the product shelf life is extended, are all produce- and temperature-specific (Jacxsen et al. 1999a; Fonseca et al. 2002a; Paul and Clarke 2002; Mahajan et al.

Table 6 Components and variables involved in MAP design (adapted from Mahajan et al. (2007))

MAP components	Variables	Designation
Produce-related	Produce mass	M
	Produce density	ρ
	Respiration rate	RO_2, RCO_2
	Desired gas composition	yO_2^{eq}, yCO_2^{eq}
Environment-related	Gas composition	yO_2^{out}, yCO_2^{out}
	Temperature	T
Package-related	Volume	V
	Thickness of the film	E
	Available film surface area for gas flux	A
	Gas permeability	PO_2, PCO_2
Macro-perforated films	Number of perforations	N_H
	Radius of perforations	R_H
Tube-mediated perforation	Number of tubes	N_p
	Length of tubes	L_p
	Diameter of tubes	D
	Porosity of the tube packing	ε

2007). These variables must satisfy the design Eqs. (1) and (2) and therefore, the system has four design variables, that is, only four out of the remaining variables, M, V, A, e, P_{O2} and P_{CO2}, can be specified arbitrarily. However, it should be noted that some of these variables are inter-dependent, e.g. once the packaging material is selected both P_{O2} and P_{CO2} are fixed and only 2° of freedom remain. Also, some restrictions must be applied as the volume of the package must be large enough to accommodate the required amount of product to be packed, and the area available for gas exchange depends on the type and size of the package (Mahajan et al. 2007; Mangaraj et al. 2009).

$$V_f \frac{d(y_{O_2})}{dt} = \frac{P_{O_2}}{e} A\left(y_{O_2}^{out} - y_{O_2}\right) - R_{O_2} M \tag{1}$$

$$V_f \frac{d(y_{CO_2})}{dt} = \frac{P_{CO_2}}{e} A\left(y_{CO_2}^{out} - y_{CO_2}\right) + R_{CO_2} M \tag{2}$$

where V_f is the headspace (free volume) in the package, y is the gas concentration (in molar fraction), e is the thickness of polymeric film, P is the permeability of the package expressed in volume of gas exchanged in volume of gas generated/consumed per unit time and weight of the product is M; the subscripts O_2 and CO_2 refer to oxygen and carbon dioxide, respectively. The limitation of these models, however, is that they are only useful for describing the unsteady-state behaviour of MAP system during the process of passive modification within a package (Mahajan et al. 2007). At steady-state the accumulation term is zero. Thus, in order to adequately describe the dynamic equilibrium behaviour of MAP system, where the rate of evolution of CO_2 equals the rate of efflux of CO_2 through the package and the O_2 consumption rate equals the influx rate of O_2 into the package, Eqs. (3) and (4) below is applicable. With Eqs. (3) and (4), it is equally important to keep track of design variables involved (Mahajan et al. 2007).

$$y_{O_2}^{out} = y_{O_2} + \frac{R_{O_2} eM}{P_{O_2} A} \tag{3}$$

$$y_{CO_2}^{out} = y_{CO_2} - \frac{R_{CO_2} eM}{P_{CO_2} A} \tag{4}$$

In the case of long storage of produce, the dynamic equilibrium behaviour is more important in comparison to the unsteady state behaviour.

Microbiological Safety of MAP

It is important to differentiate between the categories of MA-packaged fresh produce. This includes those with or without minimal pre-treatment such as antimicrobial solution, ozone, super-atmospheric oxygen, or artificial ultraviolet light (UV-C) prior to packaging (Artés et al. 2009), which are eaten without heat treatment immediately prior to consumption such as 'ready-to-eat'. Or, those produce with or without any minimal pre-treatment prior to packaging, which is subsequently cooked or heat treated prior to consumption (Philips 1996; Sivertsvik et al. 2002). The safety concern for pathogenic microbial contamination is minimal in later since they are subsequently cooked, and vegetative cells of pathogens are killed in this process (Hotchikiss 1988). However, for ready-to-eat product, the microbial load as well as infestation of pathogenic microorganisms during postharvest handling, processing and distribution is of critical importance. The safety and stability of MA-packaged produce depends on its natural microflora, which is produce-dependent and the storage conditions (Philips 1996; Farber et al. 2003). The success and microbiological safety of MA-packaged produce is anchored on controlled low temperature storage, and produce intrinsic and extrinsic characteristics, as summarised in Table 6. Therefore, maintaining the quality of fresh and fresh-cut produce during postharvest processing, distribution and storage is mainly by retarding of growth spoilage microorganisms at an optimal storage condition (Philips 1996). Oliveira et al. (2010) reported a significant increase in non-pathogenic strain of *E. coli* O157:H7 (NCTC 12900), *Salmonella choleraesuis* BAA-709 (ATCC) and *L. monocytogenes* inoculated onto MA-packaged shredded 'Romaine' lettuce stored at 25 °C compared to those stored at 5 °C.

Similarly, they observed a decrease in *E. coli* O157:H7 and *S. choleraesuis* on MA-packaged shredded lettuce stored at 5 °C.

Amanatidou et al. (1999) reported that the use of 'oxygen shock' or high levels of O_2 was very effective in retarding enzymatic discolouration, anaerobic fermentation process, and both aerobic and anaerobic microbial growth. However, they also observed that high O_2 levels of 80–90 % stimulated the growth of foodborne pathogenic microbes such as *L. monocytogenes* and *E. coli* were stimulated. The reduction in O_2 levels reduces respiration rate of fruit and vegetables, due to a decrease in the activity of oxidative enzymes such as glycolic acid oxidase, ascorbic acid oxidase and polyphenol oxidase (Kader 1986). Extremely low level of O_2 may create potential risk for the growth of pathogenic anaerobic microbes such as *Clostridium perfringens*, *C. botulinum* and *L. monocytogenes* (Charles et al. 2003; Farber et al. 2003; Philips 1996). Furthermore, at excessively low level of O_2 (<1 %), anaerobic respiration may occur, resulting in tissue deterioration, production of off-flavours and off-odours (Ares et al. 2007).

Nitrogen (N_2) is an inert, odourless, tasteless and colourless gas, which is used as a filler gas in MAP gas mixture to balance the volume decrease due to CO_2 absorption into produce tissue and to prevent package collapse (Sandhya 2010; Philips 1996). In MA-packaged products such as fresh meat packed with high concentration of CO_2, package collapse could occur due to the solubility of CO_2 in meat tissue (Philips 1996). For example, Ahmed et al. (2011) reported the use of 100 % N_2 gas in MAP to maintain the quality and shelf life of persimmon fruit stored at 0 °C and 85–90 % RH for 90 days. They observed that the fruit quality parameters such as firmness, colour and chemical properties were maintained and the shelf life of the fruit was extended at optimum storage conditions. Additionally, N_2 is used to displace O_2, thereby, helps to retard oxidative processes as well as the growth of aerobic spoilage microorganisms (Farber et al. 2003).

Furthermore, other noble gases such as helium, argon and xenon have been reported in successful MAP applications to reduce microbial growth and maintain the quality of fresh produce (Nasar-Abbas et al. 2008; Zhang et al. 2008; Meng et al. 2012), as well as under controlled atmosphere and cold storage conditions (Jamie and Saltveit 2002; Wu et al 2012a, b). In a recent work, Meng et al. (2012) investigated the effect of pressurised argon treatments (2, 4 and 6 MPa) on fresh-cut green peppers placed in polystyrene packages with gas combination of 5 and 8 % O2 and CO2, respectively, and stored at 4 °C and 90 % RH for 12 days. Their study showed that pressurised argon treatments were able to maintain the cell integrity of the produce by inhibiting the production of malondialdehyde, as well as the activities of catalase and peroxidase. The treatments were also reported to reduce the proliferation of spoilage microorganisms such as coliforms, yeast and moulds. Yu et al. (2009) compared the efficacy of ordinary MAP and argon MAP on the preservation of cherries stored at ambient temperature. The results showed that the freshness of the cherries was better preserved with argon MAP, due to the reduced mobility of water molecules.

Inert gases treatment could play a critical role in lowering water activity in fresh and fresh-cut produce, thereby reducing the leaching of organic material from fresh-cuts and movement of microbes into deeper tissues in comparison to other pretreatments (Meng et al. 2012). Studies have shown that at specific temperatures and pressures, inert gases can form ice-like crystals called clathrate hydrates, in which molecules are trapped within cage-like structure of water molecules and stabilised by bonding via van der Waals forces (Gbaruko et al. 2007; Disalvo et al. 2008; Ruffine et al. 2010). The mobility of water is restricted by the formation of calthrate hydrates (Yoshioki 2010). Previous studies have reported the formation of calthrate hydrates in certain fruit and vegetables (Zhan 2005; Zhang et al. 2008; Ando et al. 2009). Zhan (2005) investigated the effect of a mixture of argon and xenon at a pressure range of 0.4–1.1 MPa on cucumber samples. The study found that the formation of calthrate hydrates had occurred and that the activity of intracellular water was restrained due to this formation. Similarly, Oshita et al. (2000) observed a reduced mobility of intracellular water in broccoli under xeon gas with a partial pressure of 0.45 MPa at 298 K, and the visual quality of broccoli was well preserved. All these finding highlights the potential inherent in the use of inert gases to maintain both microbial safety and keeping quality of fresh produce. The clathrate hydrates phenomenon could be used to maintain the microbiological quality of MA-packed products, by maintaining desired a_w level. Hence, more research on the role of inert gases in the optimization of MAP for fresh and fresh-cut produce should be investigated.

Microbiology of Packaging

The growth and survival of microorganisms in fresh and fresh-cut fruit and vegetables is significantly influenced by the intrinsic properties of the produce, as well as by extrinsic factors as summarised in Table 7 (Church 1993; Ahvenainen 1996; Cutter 2002). Fruit and vegetables vary in their intrinsic properties. For instance, kernel and pome fruit have a high amount of organic acids which are responsible for their low pH values. In contrast, fruit such as melon and avocado have higher pH values, closer to those of vegetables (Willkox et al. 1993; Soliva-Fortuny et al. 2004; Hounsome et al. 2008). The a_w of intermediate-moisture dried fruit ranges 0.51–0.62 for raisins, 0.65–0.83 for prunes and figs, and 0.73–0.81 for peaches and apricots (Taoukis et al. 1988), while, high moisture (HM) dried fruit could retain up to 0.85 a_w (Witthuhn et al. 2005). Additionally, due to damage inflicted by mechanical

Table 7 Intrinsic and extrinsic characteristics influencing the shelf life and microbiological safety of MA-packaged produce

Intrinsic properties	Extrinsic properties
Water activity (a_w)	Storage temperature at all stages
pH	Storage relative humidity
Nutrient composition	Time interval before packaging
Oxidation-reduction potential	Initial and final gas composition
Presence of natural antimicrobial compounds	Gas purity
Microbial flora	Headspace to product ratio
Natural flora present	Barrier properties of packaging film(s)
Microbial succession	MAP design
Growth rate	HACCP procedures: hygienic produce processing
Presence of spores	Finished product
Concentration and type of preservatives used	

Church (1993), Cutter (2002), Ahvenainen (1996)

operations on fruit tissue during processing, fresh-cut fruit have a much larger cut surface area resulting in higher a_w in comparison to whole fruits (Gorny et al. 2000; Garrett 2002). Various studies have shown a direct relationship between a_w and the growth rate of spoilage or pathogenic microorganisms (Wijtzes et al. 1993; Samapundo et al. 2005; Sağirli et al. 2008; Garcia et al. 2011).

The a_w requirement of various microorganisms varies. Gram-positive non-spore-forming bacteria can grow at a_w of between 0.90 and 0.94, while Gram-negative organisms require a minimum a_w of between 0.93 and 0.96. Generally, fungi, yeast and moulds have lower a_w requirements ranging from 0.62 to 0.88 in comparison to bacteria (Farkas 1997; Alzamora et al. 2003; Witthuhn et al. 2005). A summary of minimum level of water activity for various important microorganisms occurring in foods is has been reported by Lee and Khang (2004). The predominant microflora of fresh fruit is fungi, due to the low a_w on the surface (Goepfert 1980). However, processing operations and packaging conditions may transform the microbial ecology of fresh produce (Lanciotti et al. 1999; Soliva-Fortuny et al. 2004). By decreasing or maintaining a low a_w, the lag phase of microbial growth can be extended thereby reducing the microbial growth rate (Farkas 1997). Furthermore, other intrinsic factors such as storage temperature, pH, nutrient composition and oxidative reduction potential have a synergistic effect on a_w and can influence microbial growth even at a high a_w value (Wijtzes et al. 1993; Samapundo et al. 2005; Sağirli et al. 2008; Garcia et al. 2011). For example, Garcia et al. (2011) reported that at a constant a_w and low storage temperature of between 25 and 30 °C, a short lag phase was observed for *Aspergillus ochraceus*, but, a sharp increase in growth was observed at 37 °C. At a specific temperature, the ability of microbes to grow is restricted as a_w is lowered, while, the availability of nutrients increases the range of a_w over which microorganisms can survive (Jay et al. 2005). Furthermore, packaged produce a_w in conjunction with relative humidity of the storage environment has a critical influence on microbial growth (Jay et al. 2005). Caution should be exercised when storing produce with low a_w in environments where relative humidity is high, due to moisture transfer from environment to food (Cutter 2002). As change in a_w of the produce could affect the microflora associated with the product, resulting in an accelerated rate of decay. In contrast, however, when packaged produce with high a_w are stored in an environment with low relative humidity this could result in moisture loss from the food to the environment (Cutter 2002).

Additionally, the osomoregulatory capability in response to low a_w differs for bacteria and fungi. The strategy adopted by microorganisms to protect against osmotic stress involves the accumulation of compatible solutes, such as the maintenance of high potassium chloride (KCl) in the cytoplasm of halophiles, and/or the increase in compatible solutes via their uptake from environment or de novo synthesis (Jay et al. 2005). The compatible solutes have no net charge nor do they adhere to or react with intracellular macromolecules (Sleator and Hill 2001). The three most common compatible solutes in most bacteria are glycine betaine, carnitine and proline, while fungi have been reported to accumulate polyhydric alcohols to a concentration commensurate with their extracellular a_w (Pitt 1975; Edgley and Brown 1978; Reed et al. 1987; Ko et al. 1994; Jay et al. 2005).

Storage temperature is another extrinsic factor that influences microbial growth in fresh or fresh-cut produce. Microorganisms grow over a wide range of temperatures from as low as −34 °C to highest exceeding 100 °C (Jay et al. 2005). Based on temperature requirements, microorganisms can be categorised into three groups, namely: those that grow well at or below 7 °C but optimally between 20 °C and 30 °C are classified as psychrotrophs; the mesophilic group grow well between 20 and 45 °C with optimal growth between 30 and 40 °C; and, those that grow well at and above 45 °C with optima between 55 and 65 °C are classified as thermophiles (Eddy 1960; Morita 1975; Jay 1987). Moulds are able to grow over the psychrotrophic temperatures. For example species of *Aspergillus*, *Cladosporium* and *Thamnidium* may be found growing on eggs, beef and fruit. Yeasts generally grow optimally within the psychrotrophic and mesophilic temperature ranges but not within thermophilic range (Jay et al. 2005). The possibility of contamination and growth of anaerobic psychrotrophic and some thermophiles foodborne pathogens such as *Aeromonas caviae*, *Aeromonas hydrophila*, *E. coli*, *C. perfringens*, *C. botulinum*, *L. monocytogenes*, *Salmonella* spp., is of concern to guarantee

the safety of MA-packaged fresh or fresh-cut and/or minimally processed fruit and vegetables (Philips 1996; Szabo et al. 2000; Farber et al. 2003; Soliva-Fortuny et al. 2004; Jay et al. 2005) since limited O_2 levels in MAP conditions is proven to inhibit the growth of most aerobic microorganisms (Farber 1991).

The influence of temperature on microbial growth in MA-packaged fresh or fresh-cut fruit and vegetables has been well documented (Debevere 1996; Jacxsens et al. 1999b, 2002; Valdramidis et al. 2006; Oliveira et al. 2010). However, there is still limited information regarding the influence of temperature on microbial gene expression in MA-packaged fresh and fresh-cut fruit and vegetables (Chua et al. 2008; Li and Zhang 2010; Sharma et al. 2011). Recent studies have shown that although psychrotrophic microbes grow slower under refrigerated conditions, they also express different genes and are physiologically different from mesophilic microorganisms (Phadtare 2004). Change in temperature has also been reported to influence gene expression and synthesis of other proteins such as toxin (Chua et al. 2008; Carey et al. 2009; Li and Zhang 2010; Sharma et al. 2011). On leafy green 'Romaine' lettuce inoculated with *E. coli* O157:H7 strain expressing both stx_1 and stx_2 stored at 4 °C, Carey et al. (2009) observed an up-regulation in stx_2 and intimin (*eae*) gene expression after 9 days of storage under atmospheric conditions. In a study investigating the effect of MAP and storage temperature, on the persistence and expression of virulence factors of *E. coli* O157:H7 on shredded Iceberg lettuce, Sharma et al. (2011) observed a significantly greater expression of *eae*, *iha*, stx_2, *ehxa*, and *rfhE* genes on day 10 at 15 °C in MA packages subjected to near-ambient atmospheric conditions with micro-perforations. Similarly, Chua et al. (2008) reported that enterohemorrhagic *E. coli* isolates with defective *rpoS* genes, which were inoculated into MA-packaged fresh-cut lettuce were able to induce acid resistance over the 8-day storage at 15 °C. No acid resistance was induced for MAP-stored lettuce kept at 5–10 °C.

The oxidation–reduction potential (ORP) of a substrate refers to the rate at which a substrate gains or losses electrons and is determined by the characteristic pH of the food, its resistance to change in potential (poising capacity), and the oxygen tension of the surrounding atmosphere as well as its access to the product (Jay et al. 2005; Kalia and Gupta 2006). Compounds such as sulphide groups, ascorbic acid and reducing sugars help to maintain reducing conditions in fruit and vegetables (Jay et al. 2005). Aerobic microorganisms such as bacilli, micrococci, actinobacters and pseudomonas require positive ORP values, while, anaerobes such as clostridia requires a negative ORP or a reduced state for optimal growth, and they cannot lower the ORP of their environment (Jay et al. 2005; Kalia and Gupta 2006). Therefore, the availability of adequate quantities of oxidising and reducing compounds in food and optimal gas composition within packaged fruit and vegetables is important to militate against microbial activity and growth.

CO_2 is the only gas used in MAP that confers a significant level of antimicrobial influence on the product. Farber (1991) suggested various theories to explain the antimicrobial influence of carbon dioxide on MAP product this include direct inhibition of enzyme systems or decrease in rate of enzyme reactions; alteration of cell membrane function including uptake and absorption of nutrient; gas penetration of bacterial membranes leading to decrease in intracellular pH; direct changes in the physical and chemical properties of proteins. Growth of microorganism is retarded at high concentration of CO_2 in various products, due to an increased lag phase and generation time during the logarithmic phase of microbial growth (Philips 1996; Guevara et al. 2003; Soliva-Fortuny et al. 2004; Oliveira et al. 2010). Guevara et al. (2003) reported on the effect of elevated concentrations of CO_2 on MA-packaged prickly pear cactus stems stored at 5 °C. They found that semi-active MAP with 20 kPa CO_2 significantly influenced microbial population after 15–20 days of storage, in comparison to semi-active MAP with 40 kPa CO_2 and semi-active MAP with 80 kPa CO_2. Semi-active MAP with 20 kPa CO_2 decreased the microbial counts for total aerobic mesophiles, moulds and yeast, but, observed a slight increase in the total anaerobic mesophilic bacteria.

The inhibitory effect of CO_2 is not universal and this is dependent on the microbial flora present and the produce characteristics. For instance, while aerobic bacteria such as the pseudomonads are inhibited by moderate to high levels of CO_2, microbes such as lactic acid bacteria and yeasts can be stimulated at such levels of CO_2 (Amanatidou et al. 1999; Guevara et al. 2003; Soliva-Fortuny et al. 2004; Oliveira et al. 2010). Furthermore, food-associated pathogens such as *C. perfringens*, *C. botulinum* and *L. monocytogenes* are minimally affected by CO_2 levels below 50 % (Philips 1996; Charles et al. 2003; Farber et al. 2003). Therefore, the use of CO_2 is most effective on produce where the spoilage microorganisms consist mainly of aerobic, psychrotropic Gram-negative bacteria. Better understanding of the background microflora for each MA-packaged produce is essential towards a successful MAP design. More research is needed on the effects of various atmospheric modifications on the growth and survival of food-associated pathogens on fresh and fresh-cut produce.

Regulations on Microbiological Safety of Fresh Produce

Microbial quality assurance for MA-packaged fresh and fresh-cut fruit and vegetables is invaluable. Considering the critical points for contamination from farm to fork, this includes postharvest handling, contaminated processing equipment or transportation vehicles, cross-contamination

(Farber et al. 2003; Oliveira et al. 2010), and possibility of abuse of optimal storage conditions (Chua et al. 2008; Oliveira et al. 2010). Furthermore, modified atmosphere within the package may inhibit the natural microflora on the product, while, growth of pathogens may be enhanced. The ability of MAP to extend product shelf life, pathogens may increase microbial counts above regulated threshold (Farber et al. 2003). In Europe, food safety criteria for fresh-cut fruits and vegetables are regulated by the Commission Regulation EC No. 2073/2005 (OJEU L338/1-26, 22 December 2005), which was amended by EC No. 1441/2007 (OJEU L322/12-29, 7 December 2007). These criteria include: absence of *Salmonella* in products placed on the market during their shelf life; and, absence of *L. monocytogenes* in 25 g before the food has left the immediate control of the food processor and <100 cfu/g in products placed on the market during their shelf life. In the summary of the commission report, consideration was given to other approaches to the microbiological safety and quality of foods such as the preventive approach based on the principles of HACCP and the development of guides to good hygienic practice. That will have longer-term implications for microbiological standards in EC food hygiene legislation.

Stakeholders in the fresh produce chain have introduced measures to prevent product contamination (FDA/CFSAN 2001). At the farm level, good agricultural practices (GAPs) and documentation of these practices were introduced. These guidelines help in promoting safe practices, and most retailers encourage the use of these guidelines by demanding results of audits of practices (FDA 1998a, b). Also, the International Fresh Cut Produce Association published food safety guidelines for fresh-cut food processors. Documents produced include a model HACCP plan, best practice guidelines for activities, a model food allergen plan, as well as a sanitary equipment buying guide and development checklist (James 2006). The HACCP plan ensures that operations are audited in each area in a pack-house, and risk assessments are conducted accordingly (James 2006). Such assessment may also indentify areas where good manufacturing practices (GMPs) are failing and help in improving GMPs. The application of GAPs, GMPs and HACCP in the fresh fruit and vegetables industry provide the basic framework for safe products for the consumer.

The integration of HACCP into the fresh and fresh-cut fruit and vegetables and the pack-house should be more comprehensive with regulations towards optimising MAP, and HACCP implementation can be standardised and improved by incorporating MAP technology. For example, the monitoring and control of gas and water vapour permeability, package integrity, accuracy of gas mixtures, headspace gaseous composition, storage temperature, humidity and microbial activity (Ooraikul 1991). The HACCP plan should include selection of appropriate packaging material for produce storage and distribution; identification of potential microbiological risk factors in the product design; identification of ways to reduce packaged product risks by adopting microbiological barriers such as low pH and a_w, competitive microflora, thermal processing, preservatives and modified atmosphere; and consumer awareness and education programme on the proper handling and storage of packaged foods (Cutter 2002). Examples of such indictors used within the food industry include time–temperature indicators, radio frequency identification tags, gas indicators, and leak detectors (Church 1994; Yam et al. 2005; Sandhya 2010).

Furthermore, the success of HACCP is centred largely on adequate efforts to establish GAPs and GMPs thereby hazard analysis can be limited to few critical control points (CCPs) by which the safety of food product is ensured (Notermans et al. 1995; James 2006). Although complete elimination of a hazard is impossible for foods, an acceptable level must be defined (Notermans et al. 1995; James 2006). Tools such as quantitative risk assessment, surrogates and indicator microorganisms can be used in assessing the safety of fresh fruit and vegetables, and to measure the effectiveness of control points (Notermans et al. 1995; Busta et al. 2003; James 2006). For example, Martins and Germano (2008) investigated the validation of control measures in order to establish performance indicators of HACCP system in the manufacturing process of Lasagna Bolognese, using total mesophile and faecal coliform counts as microbial indicators (MIs). They reported non-significant change in the MI count on lasagna meat after storage. Their finding shows that if the HACCP system allowed them to meet both the company and Brazilian government regulations. The use of indicators and surrogates can serve as scientific basis to obtain quantitative information to support the development and validation of fresh produce decontamination and packaging processes. Additional research is needed to identify suitable surrogates and indicators for fresh and fresh-cut produce. For an extensive research needs in the use of indicators and surrogates, the reader is referred to Busta et al. (2003).

Predictive Microbiology and MA-Packaged Produce

The risk of foodborne disease outbreak involves a series of events, from the possibility of exposure to the microbial pathogen, to the likelihood of infection or intoxication leading to illness and the degree of such illness (Lammerding and McKellar 2004). Modified atmosphere packaging of fresh and fresh-cut produce is a complex system with many variables affecting both the probability and the severity of the occurrence of foodborne pathogens and diseases. Some of these variables include gas composition, pre-treatment, properties of packaging films and storage conditions (Cutter 2002; Oliveira et al. 2010; Sandhya 2010; Caleb et al.

2012c). Thus, to manage food safety in MAP-packaged produce effectively, a systemic means of understanding these variables is necessary. Often, it is impossible to measure the effects of these factors directly on microbial response in MAP; hence, they should be adequately predicted over time by evaluating available data, using mathematical predictions and re-evaluating the critical hazard points.

Over the last decade, predictive microbiology has evolved in its empirical nature ranging from 'black box' approaches, such as artificial neural network models, to 'grey box' models which include microbial theoretical knowledge in order to describe well-characterised microbial response to intrinsic and extrinsic factors (Geeraerd et al. 2004; McMeekin et al. 2008; Fakruddin et al. 2011). In a recent review by McMeekin et al. (2002), they described the concepts of predictive microbiology and highlighted on new trends, such as the progressive approximation of the growth or no growth interface and the increased application of probability models. Gompertz and logistic equations have been used extensively by various researchers to fit a variety of microbial growth curves such as: *Penicillium chrysogenum* (Dantigny et al. 2011), *Penicillium expansum* and *A. niger* (Gougouli and Koutsoumanis 2011), *Yersinia enterocolitica* ATCC 35669 (Chen and Hoover 2003), *L. monocytogenes* (Chhabra et al. 2002; Corbo et al. 2006), *E. coli* O157:H7 and generic *E. coli* (Kim et al. 2007). Ratkowsky et al. (2005) reported a thermodynamically dependent model, describing the effect of temperature on microbial growth rates based on reversible protein denaturation both at low and high temperature. Others includes the Baranyi model (Baranyi et al. 1993; Baranyi and Roberts 1994), the Buchanan model (Buchanan and Bagi 1994; Buchanan et al. 1997), the Hills model (Hills and Wright 1994; Hills and Mackey 1995), heterogeneous population model (McKellar 1997), the Fermi model (Peleg 1997; Gastélum et al. 2010) and artificial neural networks (Jeyamkondan et al. 2001). Although, these models have all been used in various studies to describe the behaviour of microbes to parameters such as changing temperature, gaseous concentration, pH and a_w (Farber et al. 1996; McKellar 1997; Jeyamkondan et al. 2001; Chen and Hoover 2003; Braun and Sutherland 2005; Dantigny et al. 2011). More effort should be concerted towards modelling microbial growth 'in situ' in MA-packaged fresh and fresh-cut fruit and vegetables. A recent work although on fish fillet by Speranza et al. (2012), reported the use of desirability and polynomial models to predict the inhibition of *Photobacterium phosphoreum*, *Shewanella putrefaciens* and *Pseudomonas fluorescens* in fish fillets using a combination of antimicrobials and MAP technology. They observed that the effectiveness of MAP with a high content of CO_2 combined with antimicrobial solution was consistent with the stability time from the model.

Furthermore, methods which define the physiological state/stage of foodborne pathogens under various storage conditions should be developed (Fakruddin et al. 2011) in order to provide real-time reporting for MA-packaged produce. Additionally, models which take into consideration possible interactions between microbial flora present in products should be explored (Ross and McMeekin 1994; Gram et al. 2002). Especially for fresh and fresh-cut produce where natural microflora could be influenced by handling and processing, as well as microbial succession due to change in gas composition. Mathematical predictions which combine enzymatic and microbial growth kinetics data would be very beneficial to the fresh produce industry and HACCP, providing valuable quantitative information of microbial growth kinetics. The combination of predictive microbiology will aid in optimising processing conditions, identifying critical control points and establishing corrective actions towards optimal safety and security of MA-packaged fresh and fresh-cut produce (Notermans et al. 1995; McDonald and Sun 1999).

Influence of MAP on Microbial Growth and Survival on Produce

The commonly encountered microflora of fruit and vegetables such as *Pseudomonas* spp., *Erwinia herbicola*, *Flavobacterium*, *Xanthomonas*, *Enterobacter agglomerans*, *Lactobacillus* spp., *Leuconostoc mesenteroides*, moulds and yeasts are largely associated with spoilage of fresh produce (Farber et al. 2003). The microflora population found on fruit and vegetables is dependent on the type of produce and storage conditions. Nonetheless, the safety of fresh and fresh-cut produce is mostly related to the maintenance of the cold chain. Low temperatures have been reported to retard the growth of foodborne pathogens such as *Salmonella*, *Shigella*, *E. coli* O157:H7 (Leverentz et al. 2001; Oliveira et al. 2010; Sharma et al. 2011). Exceptions to this are other psychrotrophic foodborne pathogens including *L. monocytogenes*, *Y. enterocolitica*, *C. botulinum* and *A. hydrophilia*, which studies have shown to multiply on the surface of shredded 'Romaine' lettuce, cut melons, chopped parsley, wounded apple tissue and chopped tomatoes stored at low temperatures (Harris et al. 2003; Oliveira et al. 2010).

MAP has been successfully used to maintain the quality of fresh and fresh-cut fruit and vegetables (Kader and Watkins 2000; Yahia 2006; Mangaraj et al. 2009; Sandhya 2010). However, the effect of MAP on microorganisms can vary depending on the type of produce packaged (Farber et al. 2003). For instance, the increase CO_2 and decreased O_2 concentrations used in MAP generally favours the growth of lactic acid bacteria. This can accelerate the spoilage of produce sensitive to lactic acid bacteria such as carrots, chicory leaves, and lettuce (Nguyen-the and Carlin 1994). Furthermore, oxygen concentrations below 1–2 % can create a potential risk for the growth of pathogens such as *C. botulinum* (Charles et al. 2003; Farber et al. 2003). Therefore, it is necessary to highlight some foodborne pathogens that can

be potential health risks due to the vulnerability of MA-packaged produce.

L. monocytogenes

Concerns about the possible *L. monocytogenes* contamination in MAP produce have been raised, due to its facultative anaerobic and psychrotrophic nature (Francis and O'Berine 1997, 1998). However, published data on the effect of modified atmosphere on the survival and growth of *L. monocytogenes* on refrigerated fresh-cut produce are conflicting. For instance, Oliveira et al. (2010) observed that the passive MAP conditions in their study did not influence the growth of *L. monocytogenes* on shredded 'Romaine' lettuce at 5 °C. While, Carraso et al. (2008) reported an inhibitory effect of active MAP system on the growth of *L. monocytogenes* on shredded ready-to-eat 'iceberg' lettuce stored at 5 °C. Rodriguez et al. (2000) reported that MAP conditions at 8 °C, did not affect the growth of *L. monocytogenes* on trimmed fresh green asparagus. Similarly, Gleeson and O'Beirne (2005) and Francis and O'Berine (1997) reported that low levels of O_2 in MA-packaged vegetables may increase the growth of *L. monocytogenes*. These discrepancies over the behaviour of *L. monocytogenes* at low temperatures may be due to differences in fresh produce investigated, different mixture and concentration of gases used to create a modified atmosphere, the microflora competitors on produce, *L. monocytogenes* strain variation and the properties of the packaging film (Nguyen-the and Carlin 1994; Varnam and Evans 1996; Rodriguez et al. 2000; Francis and O'Berine 2001, 2005).

Studies conducted by Rodriguez et al. (2000) on the growth of *L. monocytogenes* in packaged fresh green asparagus, clearly demonstrated the importance of refrigeration. As *L. monocytogenes* population decreased at 2 and 4 °C in all packages, while, at higher temperatures 8, 12 and 20 °C the growth rate of *L. monocytogenes* was accelerated. Also, Carraso et al. (2008) reported that the growth rate of *L. monocytogenes* on MA-packaged shredded 'iceberg' lettuce was slower at 5 °C in comparison to 13 °C with a lag phase of about 5.6 days. Furthermore, Jacxens et al. (1999b) reported a decline in *L. monocytogenes* on MA-packaged carrots and Brussels sprout stored at 7 °C. However, they observed in the same study, that severe storage temperature abuse at 25 °C for 1–2 days followed by storage at 4 or 10 °C for packaged Caesar salad and coleslaw mix supported the growth of *L. monocytogenes*. The growth of *L. monocytogenes* at abusive temperature highlights the safety risk of this microorganism with respect to certain MAP produce, and reiterates the importance of HACCP, GMPs and GAPs for postharvest handling and processing of agricultural produce.

Additionally, research on the effect of interaction between the indigenous/background microflora and pathogens such as *L. monocytogenes* on MAP produce has been limited. In recent studies on microbial interactions, Leverentz et al. (2006) investigated the antagonistic effect of 17 natural microflora of fresh-cut apples on the growth of *L. monocytogenes*. They observed that *Gluconobacter asaii* (T1-D1), *Candida* sp. (T4-E4), *Discosphaerina fagi* (STI-C9), and *Metscnikowia pulcherrima* (T1-E2) proved effective in preventing the growth or survival of *L. monocytogenes* on fresh-cut apple tissue. Pálmai and Buchanan (2002) used a 'sprout juice' as a model system to examine the effect of *Lactococcus lactis* on the growth of *L. monocytogenes* in alfalfa broth. Their observation suggested an inhibitory effect of *L. lactis* on population density of *L. monocytogenes*, but this inhibitory effect was decreased at 10 °C. In contrast, Ongeng et al. (2007) reported that the interaction of *L. monocytogenes* and background microflora on fresh-cut cabbage had no effect on growth and survival of *L. monocytogenes*, except, at higher population densities of approximately 8 log CFU/g. At which in the case of fresh-cut vegetables, spoilage will have occurred.

Francis and O'Berine (1998) used a surface model agar system to investigate the effects of modified atmospheric conditions on *L. monocytogenes* and background microflora such as *Leuconostoc citreum*, *P. fluorescens*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *E. agglomerans*. Their findings suggested that MAP conditions of 3 % O_2 and 5–20 % CO_2 might increase the growth rate of *L. monocytogenes*. However, they observed that at increased CO_2 levels, the growth of lactic acid bacteria increased and inhibited the growth of *L. monocytogenes*. This was associated to the possible production of anti-listerial agents. Also, Bourke and O'Beirne (2004) investigated the effects of packaging type, atmospheric composition and storage temperature on survival and growth of *Listeria* spp. in shredded dry coleslaw and its components. They observed a decline in *Listeria* spp. in packaging film type at 3 °C between days 10 and 12 of storage, while growth remained at initial inoculation levels at 8 °C. Their reports suggested that the inhibition of *Listeria* spp. could be due to the optimal gas composition between days 10 and 12, as well as, the presence and competitive effect of background microflora. They also reported differences in the survival characteristics between the different *Listeria* strains. González-Fandos et al. (2001) reported that *L. monocytogenes* population increased between 1 and 2 log units within the first 2 days at 4 °C, and remained relatively stable from days 3–8 on MA-packaged mushrooms. But, they further observed that the growth within the first 2 days correspond to the lag phase of competitors (*Pseudomonas* spp.) in this study. Although their results highlighted the sanitary risk of MA-packaged mushrooms due to the initial increase of 2 log CFUs of *L. monocytogenes* observed, however, it also suggested the possible inhibitory effect of *Pseudomonas* spp. on the growth of *L. monocytogenes* from day 3. These reports suggest the need for more research towards a better

understanding of the influence of different atmospheres, storage temperatures, background microflora and their interactions on the survival and growth of *L. monocytogenes* on MA-packaged fresh and fresh-cut produce.

C. botulinum

Spores of *C. botulinum* are commonly found in agricultural soils and on the surfaces of fruit and vegetables. *C. botulinum* is classified into non-proteolytic and proteolytic group. Proteolytic *C. botulinum* growth is inhibited under storage conditions of below 12 °C, pH of 4.6, a_w of 0.95 and NaCl concentrations above 10 %. While, non-proteolytic *C. botulinum* can grow at a minimum of 3 °C, a_w above 0.97, pH above 5.0 and NaCl concentration above 4 % (Zagory 1995; Lund and Peck 2000). The risk for *C. botulinum* to grow and produce toxin due to abusive temperature under anaerobic conditions, further heightens the concern about the use of MAP with respect to fresh and fresh-cut fruit and vegetables (Farber et al. 2003; James 2006). In a study investigating the effect of CO_2 concentration on neurotoxin gene expression in non-proteolytic *C. botulinum* type E, Artin et al. (2008) reported that CO_2 concentration had a significant effect on neurotoxin gene expression and neurotoxin formation. They observed that the expression of *cntE* mRNA and the formation of extracellular neurotoxin were twofold higher with a headspace CO_2 concentration of 70 % (*v*/*v*) in comparison to headspace of 10 % (*v*/*v*). Their findings shed a cautionary light on the potential risk of botulism associated with use of MAP for ready to eat produce. However, Artin et al. (2010) reported a marked contrast on the effect of CO_2 (10–70 %, *v*/*v*) for proteolytic *C. botulinum* type A1 ATCC 3502. CO_2 concentration had little effect on the expression of *cntE* mRNA and the formation of extracellular neurotoxin. At all CO_2 concentrations, the relative expression of neurotoxin cluster genes peaked in the transition between exponential and stationary phases. These reports confirmed that gene expression differs between proteolytic and non-proteolytic *C. botulinum*, and that the expression of *C. botulinum* type A1 ATCC 3502 neurotoxin cluster genes was dependent on growth phase rather than CO_2 concentration.

Furthermore, Petran et al. (1995) investigated the effect of storage temperature and packaging conditions (vented and non-vented flexible pouches) on *C. botulinum* toxin formation in 'Romaine' lettuce and shredded cabbage. They observed no toxin formation in either of the packages at storage temperatures of 4.4 and 12.7 °C. However, in the non-vented pouches of 'Romaine' lettuce stored at 21 °C, growth of inoculate *C. botulinum* spores and toxin production were reported after 14 days. Also, lettuce samples packaged in vented pouches at 21 °C became toxic after 21 days. In the case of shredded cabbage, toxin formation was detected in the non-vented packages stored at 21 °C after 7 days. And all toxin-positive samples were judged to be inedible prior to toxin detection. Hao et al. (1998) reported similar observation for shredded carrots and green beans packaged under four different films with varying oxygen transmission rates. Larson and Johnson (1999) confirmed the same results, when they investigated the incidence of *C. botulinum* toxin production on inoculated honeydew and cantaloupe. The findings of Petran et al. (1995) indicated that the formation of anaerobic microenvironments within the pouches is favourable for the growth of *C. botulinum*. This stresses the importance of correlating the permeability properties of the packaging films with the respiration rate of the produce, and taking into consideration the influence of extrinsic factors such temperature on both processes.

Aeromonas spp.

Aeromonas spp. is ubiquitous in an environment where fresh or salt water and chlorinated drinking water are sources for the contamination of food product (McMahon and Wilson 2001; Harris et al. 2003; Abulhamd 2009). *Aeromonas* spp. isolated from vegetables has been reported to represent a potential risk to human health (Pedroso et al. 1997), due to their presumptive pathogenicity and the ability to grow at refrigerating temperatures (Janda and Duffey 1988; Cahill 1990; Davies and Slade 1995). According to Adams and Moss (2000) and Kirov (2001), *Aeromonas* spp. especially *A. hydrophila* currently has the status of a foodborne pathogen of emerging importance. Similar to *L. monocytogenes*, studies have shown that *A. hydrophila* can grow at CO_2 levels of up to 50 % and low O_2 levels of about 1.5 % (Francis et al. 1999). McMahon and Wilson (2001) examined 86 organic vegetables for the presences of foodborne pathogens. They isolated *Aeromonas* spp. from 31 % of the total number of samples examined and 41 % from the ready-to-eat group of organic vegetables that were minimally processed by washing. Additionally, a survey conducted by the UK Public Health Laboratory Service (PHLS) and non-PHLS laboratories on 2,552 retail samples of refrigerated salads and crudités, found *A. hydrophila* to be present in 54 % (Little et al. 1997). This figure indicated that *A. hydrophila* do contaminate fresh-cut produce.

Several studies have also reported the ability of *A. hydrophila* to grow on vegetables stored under MAP at low temperatures. Berrang et al. (1989) investigated the effect of gas atmospheres on the growth and survival of *A. hydrophila* on fresh broccoli, asparagus and cauliflower. They observed that *A. hydrophila* reached high populations on all the vegetables during storage at 4 and 15 °C, and the growth of *A. hydrophila* was not significantly affected by gas atmosphere. However, the authors noted that the shelf life of the three vegetables studied was prolonged by modified atmosphere and were

judged as acceptable for consumption. Furthermore, Bennik et al. (1995) using a solid surface model system studied the effect of gas atmosphere on the growth of *A. hydrophila*. Their study revealed that, although the maximum specific growth rate of *A. hydrophila* decreased with increasing CO_2 concentrations, the maximum population densities were not affected by CO_2 concentration of up to 50 %. The growth pattern of *A. hydrophila* remained the same at O_2 concentration of 1.5 and 21 %. A study to evaluate the effect of high oxygen MAP on microbial growth and sensorial qualities of fresh-cut produce was conducted by Jacxsens et al. (2001). They observed in the in-vitro studies at 4 °C, that the growth of *A. caviae* (HGA) was retarded under high O_2 level of 70, 80 and 95 %. And no significant growth after 34 days found during storage under 95 % O_2 at 4 °C. On the overall achievable shelf life of the fresh-cut mushrooms, celeriac and chicory endives at 4 °C. They concluded that the high O_2 atmosphere of 95 % had a beneficial effect on the colour retention of the three produce. The other sensorial qualities were also not significantly affected by high O_2 level when compared to those stored in low O_2 EMAP.

Furthermore, various studies have proposed the use of microbial interaction combined with MAP and low storage temperatures to reduce the survival and/or growth of *Aeromonas* spp. Vescovo et al. (1997) investigated the combined effect of *Lactobacillus casei* inoculums, MAP and chilled temperature on the growth and survival of *A. hydrophila* in ready-to-use vegetables. Their findings suggested that *L. casei* inoculums size was the main factor that influenced the kinetic parameters of *A. hydrophila*, while CO_2 concentration in the package extended the lag phase of *A. hydrophila*. Similarly, Garcia-Gimeno et al. (1996) demonstrated that an increase in lactic acid bacteria combined with high levels of CO_2 of about 33 %, decreased pH and retarded the population density of *Aeromonas* spp. on vegetable salads. Therefore, more research needs to be done to examine the influence of background microflora competition, different composition of gas atmospheres and storage temperatures on the survival and growth of *Aeromonas* spp. on MAP fresh-cut produce.

Enteric Viruses

Foodborne illness has been documented for several groups of enteric viruses (Berg et al. 2000; Koopmans and Duizer 2004). However, recent studies have shown that the norovirus and hepatitis A virus (HAV) are the most important human viral foodborne pathogens with regards to the number of outbreaks (Sattar et al. 2000; Koopmans and Duizer 2004). Other relevant foodborne viruses are those capable of infecting the cells lining the intestinal tract and are dispersed by shedding into the stool or through emesis are summary in Table 8. Foodborne viral infections differs from bacterial infections in that only a few particles are needed to produce illness, a high number of viral particles (~10^{11} particles per gram of stool) are shed from infected persons and foodborne viruses are relatively stable outside the host and are acid-resistant (Koopmans and Duizer 2004).

Recent studies have shown that HAV can survive on nonporous environmental surfaces over a wide range of temperatures and relative humidity. Mbithi et al. (1991) investigated the influence of temperatures 5, 20 and 35 °C, as well as RH range of 25–95 % on the half-lives of HAV (HM-175). They observed that at refrigeration temperature of 5 °C, HAV had a half-life of more than 4 days, irrespective of RH. At 20 °C with low RH, its half-life was almost 7.8 days, but, did not survive well when held at storage conditions of 35 °C with RH of about 95 %. Bidawid et al. (2001) studied the survival of HAV on MA-packaged lettuce stored at room temperature and 4 °C. Their findings indicated that the survival of HAV at 4 °C was not influenced by MAP, and the virus survival on lettuce was slightly improved in the presence of high CO_2 levels for packaged samples stored at room temperature. However, this observation was attributed to the inhibition of spoilage-inducing enzymes in lettuce, which may have reduced their toxic effect on the virus. Furthermore, Bidawid et al. (2000) investigated the transfer of HAV from hands to foods. This was carried out by bringing experimentally HAV-contaminated finger pads of adult in contact with clean pieces of lettuce for approximately 10 s at a pressure of 0.2–0.4 kg/cm^2. They observed that touching the lettuce with the contaminated finger pads resulted in transfer of 9.2±0.9 % of the infectious virus. However, when finger pads were treated with topical agents or alcohol before the lettuce was touched, the amount of viral transfer to lettuce was reduces from 9.2 % to between 0.3 and 0.6 % depending on the topical agent used, which was about 30-fold reduction in viral transfer. These findings reinforce the importance of GMP and GAP for postharvest handling and processing of agricultural produce, before their packaging in MAP.

Most of the current food hygiene guidelines have been optimised for the prevention of bacterial infections and may be partially effective against viruses (Koopmans and Duizer 2004). This can be attributed to the complexity of the most common foodborne viruses, which grow poorly or not at all in cell culture (Lees 2000; Atmar et al. 2001), thereby making the study of the inactivation of these pathogens impossible (Koopmans and Duizer 2004). This highlights the need for a concerted effort towards the development of rapid, simple and reproducible techniques for the detection of foodborne viral particles. In terms of MAP, more information is needed on viral survival on different fresh and fresh-cut produce at various gas atmosphere and storage conditions.

Table 8 Enteric viruses, mode of transmission and associated illness or infection

Infection(s)	Virus	Possible mode of transmission
Gastroenteritis	Norovirus	Commonly by food or water
	Enteric adenovirus (types 40/41)	Occasionally by food or water
	Rotavirus (group A–C)	
	Sapovirus	
	Astrovirus	
	Coronavirus	
	Aichivirus	
Hepatitis	Hepatitis A virus	Commonly by food or water
	Hepatitis E virus	Water
Others	Enterovirus	Occasionally by food or water

Koopmans and Duizer (2004)

Overview of Outbreaks

Over the past decade, the frequency of reported outbreaks of foodborne illnesses has increased. There could be a number of possible reasons for this observed increase, including increased consumption of fresh-cut produce and advanced epidemiological surveillance programs (Beuchat 2002). Fresh produce-related outbreaks accounted for about 6 % of reported foodborne outbreaks in the 1990s in comparison to only 0.7 % in the 1970s (Sivapalasingam et al. 2004). Between 1996 and 2006, more than 20 foodborne illness were traced to fresh and fresh-cut leafy green vegetables, and the Center for Disease Control and Prevention has identified leafy green vegetables as one of the most important vector of foodborne illness outbreaks caused by bacterial pathogens including *E. coli* O157:H7 (Herman et al. 2008; Lynch et al. 2009). Additionally, an increasing volume of data supports and suggests that salad vegetables, such as cabbage, lettuce, celery, onions, cucumber, leeks, watercress, among others can have a high incidence of *L. monocytogenes*, while some of these produce have been implicated in outbreak of foodborne listeriosis (Beuchat 2002; Thunberg et al. 2002; Leverentz et al. 2003). The growth of potential foodborne pathogens is higher on fresh-cut produce compared to whole produce with protective peel or rind, due to the availability of nutrients and moisture on the cut surface (Ngsuyen-the and Carlin 1994; Francis et al. 1999).

Last year's outbreak of *E. coli* O104:H4, which was linked to sprouts as the most likely vehicle of infection resulted in a total of 3,816 cases (including 54 deaths) in Germany (Buchholz et al. 2011; Frank et al. 2011). Isaacs et al. (2005) reported an international outbreak of salmonellosis associated with fresh almonds contaminated with a rare type of *Salmonella enteritidis* phage. Sivapalasingam et al. (2004) presented food categories involved in foodborne outbreaks these data included fresh fruit and vegetables as well as fresh cuts. With the increase in the number of people traversing international boundaries daily and the globalisation of food production, marketing and distribution, the risk of importing and exporting the foodborne pathogens is more substantial. Hence, the application of GAPs, GMPs, with a robust HACCP in the fresh or fresh-cut fruit and vegetables industry towards MAP technology will provide safe products for the consumer.

Conclusions

Comprehensive review of the literature showed that modified atmosphere packaging technology plays a major role in the preservation of the quality of a wide range of fresh produce. However, it also highlighted the concerns of potential foodborne pathogen which may be stimulated with moderate CO_2 levels within fresh food packages. Under storage conditions which are predisposed to increase in temperature, increase in temperature around temperature-sensitive packaging may result in anoxic state. So far, only limited information is available on permeability properties of films at different storage temperatures. For optimal MAP performance, the mechanical properties of the polymeric film must also be balanced with its flexibility and peelability for the convenience of consumers. Mathematical modelling and prediction offers considerable benefits towards attaining optimal water vapour transmission and gas permeability rates for various packaging materials.

Furthermore, it has been established that inconsistent or abusive temperature contributes to increased produce respiration and transpiration rates, which in turn can enhance microbial proliferation and deterioration of MA-packaged fresh or fresh cuts. This highlights the need for more concerted effort towards the maintenance of strict cold chain along the whole distribution continuum. The integration of multiple intelligent systems coupled with microbiological data should be evaluated towards the optimal success of MAP.

The application of novel technologies such as high pressured inert gases, 'smart' packaging and pre-packaging treatment for fresh produce offer additional potential to increase produce shelf-life and microbial safety. This includes the development of optimal inert gas MAP atmospheric composition for fresh produce; bioactive polymeric films with antimicrobial activity against foodborne pathogens, via immobilisation of bacterial cells or antimicrobials on polymers; the incorporation and controlled release of volatile and non-volatile antimicrobial agents into packages; and, the use of biopolymers that are inherently antimicrobial.

It was also shown in this review that the interaction of background microflora with foodborne pathogens in various MAP conditions could retard the growth of potential foodborne pathogens. Different microbial species, as well as different species strains showed different phenotypic and genetic expressions to gas atmospheres. Therefore, the investigation of individual potential foodborne pathogen should be conducted independently over a wide range of MAP and storage conditions, as well as, their interactions with background microflora specific for each MA-packaged produce. Similarly, the behaviour and survival of enteric viral and bacterial foodborne pathogens on MA-packaged produce should be studied extensively and this information may play an integral role in assuring product safety and successful application of MAP.

Given the increasing importance of MAP in the postharvest handling and marketing of fresh and fresh-cut produce, and the critical need to assure produce safety, collaboration among research institutions in critical areas like predictive microbiology, with the industry and regulatory agencies will be a key to the success of quality and safety assurance systems, especially for maintain the microbiological safety of MA-packaged fresh and fresh-cut produce.

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