PERFORATION-MEDIATED MODIFIED ATMOSPHERE PACKAGING (PM-MAP) AND SHELF-LIFE OF POMEGRANATE FRUIT ARILS (cv. ACCO)

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

Perforation-mediated modified atmosphere packaging (PM-MAP) offers the possibility of optimising polymeric films in order to compensate for barrier limitations of conventional modified atmosphere packaging (MAP). The aim of this study was to investigate the effects of PM-MAP and storage duration on the physico-chemical quality attributes, microbial quality, phytochemicals (anthocyanins, phenolics and ascorbic acid) and antioxidant activities of arils from fresh minimally processed pomegranate (cv. Acco). The effects of number of perforations (0, 3, 6 and 9; Ø = 0.8 mm) and storage temperature (5, 10 and 15 ºC) on water vapour transmission rate (WVTR, g/m^2.day) of synthetic 'Polylid' and biodegradable (Nature flex^{TM}) polymeric films were investigated. The results showed that non-perforated biodegradable film had higher WVTR at all storage temperatures, and irrespective of film type, increasing the number of perforations (from P-3 to P-9) had higher impact on WVTR than increasing storage temperature (from 5 to 15 ºC).

Furthermore, this study investigated the effects of PM-MAP on the physico-chemical properties, phytochemicals components and antioxidant activities of fresh minimally processed arils. Arils (100 g) were packaged in polypropylene trays (10.6 x 15.1 cm^2) and heat-sealed with a polymeric film POLYLID®. Perforations (0, 3, 6 and 9; Ø = 0.8 mm) were made on the top of the film and all samples were stored at 5 ± 1 ºC and 95 ± 2% relative humidity for 14 days. Samples were analysed at intervals of 3, 6, 9, 12 and 15 days. Microbial analysis included tests for *Escherichia coli*, aerobic mesophilic bacteria, yeast and moulds at days 0, 6, 10 and 14.

The results showed that headspace gas composition was significantly influenced by the number of perforations, which helped balance the decrease in O_2 with corresponding increase in CO_2 levels, thus preventing anoxic conditions. Total soluble solids, titratable acidity and firmness of arils were slightly reduced by PM-MAP compared to clamshell trays. Colour attributes was generally maintained across all treatments and throughout the storage duration. The highest counts of aerobic mesophilic bacteria (5.5 log CFU/g), yeast and moulds (5.3 log CFU/g) were observed in P-0 and P-9 packages, respectively. Overall, P-3 and P-6 better maintained the physico-chemical properties and microbial quality of arils. Total phenolics and anthocyanin contents were higher in arils packaged in PM-MAP while ascorbic acid was slightly reduced. Antioxidant activities tested against FRAP and DPPH radical-scavenging activity increased across all types of MAP over storage duration. However, antioxidant activities were significantly higher in pomegranate arils packaged in

PM-MAP due to O_2-promoted biosynthesis of phenolics and anthocyanins which constitute the antioxidant properties.

Overall, the results reported in this study showed that the use of PM-MAP in cold chain could be suitable for the preservation of physico-chemical quality, phytochemical contents and antioxidant properties of arils packaged in passive PM-MAP compared to clamshell and non-perforated packages during postharvest handling and storage. Perforating MAP films showed potential in preventing the incidence of in-package moisture condensation which is a common problem during postharvest handling and storage of fresh produce packaged inside non-perforated MAP. The results also showed the importance of keeping PM-MAP packs in closed refrigerated shelves to avoid cross contamination or ingress of foodborne pathogens.

OPSOMMING

Perforasie-bemiddelde gewysigde-verpakking (PM-MAP) maak dit moontlik om polimeer films te optimiseer en om sodoende te kompenseer vir die versperring beperkings van die konvensioneel-gewysigde atmosfeer verpakking (MAP). Die doelwit is om die effek van PM-MAP en die duur van stoor op die fisioko-chemiese gehalte kenmerke, mikrobiale gehalte, fitochemikale (antisianien, fenolies en askorbiensuur) en antioksidant aktiwiteite van granaatarils van vars, minimaal geprosesseerde granate, te ondersoek (cv. Acco). Die effek van die aantal perforasies (0, 3, 6 en 9; Ø =0.8 mm) en stoortemperatuur (5, 10 en 15 ºC) op die waterdamp transmissie koers (WVTR, g/m^2.day) van sintetiese 'Polylid' en biodegradeerbare (Nature flex^{TM}) polymeriese films is ondersoek. Die resultate het bewys dat nie-perforeerde biodegradeerbare film by alle stoortemperature 'n hoër WVTR het, en dat by alle tipes film, 'n verhoogde aantal perforasies (van P-3 tot P-9) 'n hoër impak op WVTR het as 'n verhoogde stoortemperatuur (van 5 tot 15 ºC).

Verder is die effek van PM-MAP op die fisiko-chemiese kenmerke, fitochemikale komponente en antioksidant aktiwiteite van vars, minimaal-geprosesseerde granaatarils bestudeer. Die granaatarils (100 g) is verpak in in polipropilien (PP) platkissies (10.6 x 15.1 cm^2) en verseël met polimeer film, POLYLID®. Perforasies (0, 3, 6 en 9; Ø =0.8 mm) is aan die bo-end van die film aangebring en alle voorbeelde is vir 14 dae teen 5 ± 1 ºC en 95 ± 2 % relatiewe humiditeit. Die voorbeelde is met tussenposes van 3, 6, 9, 12 en 15 dae ontleed. Die ontleding het toetse vir *Escherichia coli*, aerobiese mesofiliese bakterië, suurdeeg en skimmel op tussernposes van 0, 6, 10 en 14 dae ingesluit.

Die resultate bewys dat die komposisie van die gas beïnvloed word deur die aantal perforasies. Dit help om die vermindering in O_2 met 'n ooreenkomstige toename in CO_2 vlakke te balanseer en om dus toestande wat deur 'n gebrek aan suurstof veroorsaak is, te verhoed. Die totaal van oplosbare vaste stowwe, titreerbare suurgehalte en fermheid van die granaatarils is deur die PM-MAP verminder veral as dit vergelyk word met "clamshell trays". Die kleur kenmerke het oor die algemeen dieselfde gebly by al die behandelings en dwarsdeur die stoortydperk. Die hoogste aantal aerobiese mesofiliese bakterië (5.5 log CFU/g), gis en skimmel (5.3 log CFU/g) is in die P-0 en P-9 verpakkings onderskeidelik opgemerk. Oor die algemeen is P-3 en P-6 beter in staat om die fisioko-chemiese kenmerke en mikrobiale gehalte van die granaatarils te behou. Die totaal van die fenoliese and antosianiin inhoud was hoër in granaatarils wat verpak is in PM-MAP maar die askorbiensuur was effens laer. Antioksdant aktiwiteite is getoets teen FRAP en DPPH aktiwiteite het by al die tipes van MAP tydens stoor vermeerder. Antioksidant aktiwiteite was egter heelwat hoër

in granate wat in PM-MAP verpak is. Dit is die gevolg van die biosintese van fenolies en antosianins wat deur O_2 in die hand gewerk word en wat die basis van antioksidant kenmerke vorm.

Oor die algemeen toon die resultate van hierdie studie dat die gebruik van PM-MAP in die koue ketting geskik is vir die behoud van fisieko-chemiese gehalte, fitochemiese inhoud en antioksidant kenmerke van granaatarils wat in passiewe MAP verpak is, veral as dit vergelyk word met die vrugte wat in ‘clamshell’ en nie-geperforeerde verpakking tydens hantering na die oes en tydens stoor verpak is. Die perforeer van MAP films kan die voorkoms van die kondensasie in die verpakking verminder. Hierdie kondensasie is ’n algemene probleem tydens die hantering en stoor van vars vrugte wat in MAP sonder gaatjies verpak is. Die resultate toon ook hoe belangrik dit is om die PM-MAP verpakking in toe, verkoelde rakke te hou en om sodoende kruis-kontaminasie asook kontaminasie deur kieme wat in vrugte teenwoordig is, te voorkom.

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TABLE OF CONTENTS

This thesis is a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable. Language and styles used in this thesis are in accordance with the requirements of the International Journal of Food Science and Technology.

CHAPTER ONE: General Introduction

Pomegranate (*Punica granatum* L.) fruits are nearly round in shape with a top crown of a prominent calyx and a hard, leathery skin (pericarp) and a spongy mesocarp (Holland *et al*., 2009; Da Silva *et al*., 2013). The edible portion, called aril sacs, contains bright-red pulp with individual seeds enclosed in membranous compartments made of papery tissue divided into several chambers (Fig. 1.1). The colour of arils and skin (pericarp) depends on cultivar and ripening stage of the fruit. The aril colour ranges from yellow, deep red to nearly colourless (Stover & Mercure, 2007; Ozgen *et al*., 2008; Da Silva *et al*., 2013), while that of pericarp ranges from yellow, green or pink, overlain with pink to deep red or indigo to fully red, pink or deep purple (Stover & Mercure, 2007; Holland *et al*., 2009). Some cultivars such as black pomegranates exceptionally maintain black colour of skin throughout the entire stages of fruit development up to the ripening stage (Holland *et al*., 2009).

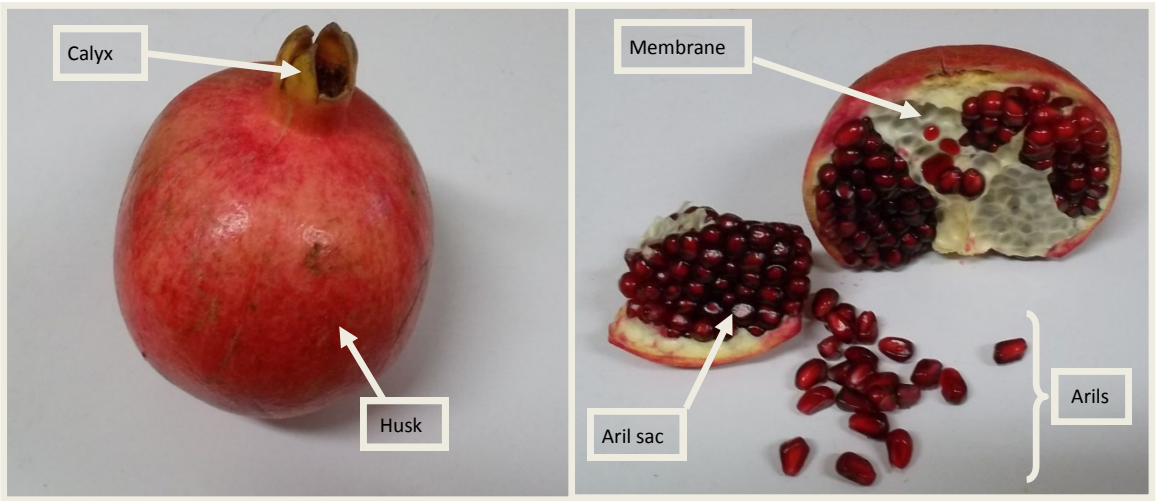

Figure 1.1 Pomegranate whole fruit (cv. Acco) and its interior structure and loose arils

Global pomegranate cultivation is concentrated in the northern hemisphere (Citrogold, 2011; Pomona, 2013). Currently, commercial orchards of pomegranate trees are grown in many geographical regions, spanning from the Mediterranean basin in the northern hemisphere to southern hemisphere, including India, Iran, Egypt, China, Israel, Tunisia, Syria, Lebanon, Turkey, Greece, Cyprus, Italy, France, Spain, Portugal, USA and Oman. Other countries include; Australia, Peru, Chile, Argentina and South Africa (Stover & Mercure, 2007; Holland & Bar-Ya'akov, 2008; Opara *et al*., 2009; Fawole *et al*., 2013). South Africa has emerged as a new commercial producer, competing with a few countries in the southern hemisphere such as Chile and Argentina (Fawole *et al*., 2013). Due to differences in geographical

locations, harvest season for pomegranate alternates across two producing regions, southern and northern hemispheres. This seasonal variation coupled with limited storage potential of pomegranate fruit creates a window of opportunity for enhanced production and export to the northern market (Citrogold, 2011; Fawole *et al*., 2013). To date, South Africa, has a total pomegranate planted area of just over 1000 ha, thus joining other countries in the southern hemisphere including Peru, Chile and Argentina to contribute more than 4% of the total global commercial production (Pomona, 2013).

Global production and consumption of pomegranate have gained momentum in recent years. This is attributed to the high content of health-promoting phytonutrients and high antioxidant capacity of pomegranate fruit, with nutritional and medicinal benefits (Hassan *et al*., 2012; Fawole *et al*., 2013). Knowledge of health promoting benefits of pomegranate fruit had been long standing since ancient times, and is deeply embedded in various native cultures (Holland & Bar-Ya'akov, 2008; Holland *et al*., 2009; Hassan *et al.,* 2012). Pomegranate fruit has been reported to be effective in the treatment of numerous diseases such as cancer (skin, breast, prostate, and colon), coronary heart diseases, inflammation, diabetes, cardiac disorders, hypoxia, ischemia, aging, brain disorders and HIV/AIDS (Malik *et al*., 2005; Mir *et al.*, 2012).

In spite of the reported health benefits, the consumption of pomegranate has been limited compared to other types of fruit due to the difficulty of extracting the arils caused by hardness of the rind and staining of fingers during peeling as a result of high content of polyphenols (Defilippi *et al*., 2006; Caleb *et al*., 2012a). Furthermore, despite their excellent internal quality, the marketing and consumption of fresh pomegranates has been limited due to high incidence of preharvest defects such as sunburn and cracking, and postharvest disorders such as bruising and chilling injury, respectively. The presence of these defects and disorders result in downgrading of fruit quality and incidence of losses during postharvest handling operations (Lopez-Rubira *et al*., 2005; Defilippi *et al*., 2006).

Thus, minimal processing of pomegranate arils offers convenience to consumers as it preserves both sensory and nutritional quality attributes of the produce using simple and cost-effective operations such as peeling, slicing or cutting of the fresh produce without much change in natural fresh-like properties (Siddiqui *et al*., 2011; Caleb *et al*., 2012b). However, the risk of quality deterioration of fresh minimally processed produce remains a major challenge in the food industry due to physiological ageing, biochemical changes and microbial spoilage (Fonseca *et al*., 2002; Ragaert *et al*., 2007; Sandhya, 2010).

The application of modified atmosphere packaging (MAP) technology combined with optimum cold chain maintenance has been reported to extend shelf-life of minimally

processed fresh and fresh-cut produce (Riad & Brecht, 2002; Montanez *et al*., 2005; Caleb *et al*., 2012a,b). Modified atmosphere packaging is a dynamic process of altering the gaseous composition inside a package via the interaction between the natural process of produce respiration and permeation of gas through the packaging film (Mahajan *et al*., 2008; Caleb *et al*., 2012b). Generated atmospheres in MAP extend shelf-life of fresh produce by supressing metabolic activities such as respiration and ethylene biosynthesis. In turn, this slows down physiological and biological changes of produce such as senescence, softening, decay and the rate of changes in texture, colour, flavour and nutritional quality attributes (Mangaraj *et al*., 2011; Siddiqui *et al*., 2011; Mahajan *et al*., 2014)

However, preservation of fresh-cut and minimally processed fruits and vegetables using MAP technology still poses challenges to food processors. The inability to single out a film with desirable properties for an optimum MAP use is a critical problem (Mahajan *et al*., 2008). Commercial polymeric films used in MAP of various fresh produce have several critical limitations that are based on both film structure and permeability to gases and water vapour (Oliveira *et al*., 1998; Mangaraj *et al*., 2009). Most polymeric films are characterized by high barriers to water vapour, such that small changes in temperature may lead to moisture condensation inside the package (Oliveira *et al*., 1998; Montanez *et al*., 2005). Consequently, moist condition increases susceptibility to microbial growth inside the package and enhances decay of packaged produce (Montanez *et al*., 2005; Sandhya, 2010). Furthermore, such films are characterised by difficulties in generating desired and safe in-package modified atmosphere resulting in risk of quality deterioration of produce due to high carbon dioxide (CO_2) to oxygen (O_2) gas permeability ratio (β) (Gonzalez *et al*., 2008; Mahajan *et al*., 2008; Mangaraj *et al*., 2009). For instance, high levels of CO_2 in the headspace of MAP containing fresh produce could create suitable environment for growth of foodborne pathogens such as *Listeria monocytogenes* and *Escherichia coli*, while very low levels of O_2 favours growth of *Clostridium botulinum* and potential risk of fermentative reactions (Farber *et al*., 2003; Ragaert *et al*., 2007; Sandhya, 2010). As a result there is a possibility for edible packaged produce to be rendered unsafe due to remarkably high load of harmful microorganisms (Farber *et al*., 2003; Caleb *et al*., 2013). Quality deterioration and reduction of shelf-life of fresh and processed food due to poor packaging has been identified as major cause of high incidence of postharvest losses (Opara & Mditshwa, 2013; Mahajan *et al*., 2014).

Perforation-mediated modified atmosphere packaging (PM-MAP) offers the possibility to prevent condensation inside the package and generate desired in-pack MA in terms of safe ratios of CO_2 to O_2 concentration suitable for storage of fresh produce (Riad & Brecht, 2002; Montanez *et al*., 2005; Mahajan *et al*., 2008; Montanez *et al*., 2008). Perforation-mediated

modified atmosphere packaging technology relies on the use of a single to multiple perforations or tubes (50-200 µm perforation diameter (Ø) referred to as micro-perforations or Ø >200 µm as macro-perforations) (Lange, 2000; Montanez *et al*., 2005; Gonzalez *et al*., 2008; Gonzalez-Buesa *et al.,* 2012). Perforations enhance the permeability rate of relevant gases (O_2 and CO_2) and water vapour of the film, thereby generating modified atmospheres suitable for highly respiring produce such as fresh minimally processed produce (Montanez *et al*., 2005; Gonzalez *et al*., 2008; Montanez *et al*., 2010). This benefit makes PM-MAP a cost-effective packaging technology with a potential to reduce postharvest losses of minimally processed fresh produce (Sanz *et al*., 2002; Montanez *et al*., 2005; Mahajan *et al*., 2008). Successful application of PM-MAP has been reported in cherry tomatoes (Briassoulis *et al*., 2013), Mandarin segments (Del-Valle *et al*., 2009), strawberry (Almenar *et al*., 2007; Kartel *et al*., 2012), shredded carrots (Montanez *et al*., 2005), sliced onions (Lee and Renault, 1998) and sweet corn (Riad & Brecht, 2002).

Earlier studies reporting postharvest storage of fresh produce have shown that different types of fresh produce respond differently to same MAP conditions (Oliveira *et al*., 1998; Ščetar *et al*., 2010). It is therefore essential to conduct research on suitability of PM-MAP for each specific produce (Oliveira *et al*., 1998; Mahajan *et al*., 2008). Although PM-MAP has been successfully applied on various types of fresh produce, to the best of our knowledge, no work has been reported on the physiological responses and changes in quality of pomegranate arils under PM-MAP. Hence, the overall aim of this research was to investigate the use of perforation-mediated modified atmosphere packaging on extending shelf-life and reduce losses of fresh processed pomegranate fruit arils (cv. Acco).

The research aim was accomplished by achieving the following specific objectives;

a. Evaluating the effects of number of perforations and temperature on water vapour transmission rate (WVTR) of selected commercial packaging films.

b. Investigating the effects of PM-MAP and storage duration on physico-chemical properties and microbial quality of fresh minimally processed pomegranate arils.

c. Investigating the effects of PM-MAP and storage duration on phytochemical and antioxidant properties of minimally processed pomegranate arils.

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CHAPTER TWO: Literature review on perforation-mediated modified atmosphere packaging (PM-MAP) of fresh and minimally processed produce

A REVIEW ON PERFORATION-MEDIATED MODIFIED ATMOSPHERE PACKAGING (PM-MAP) OF FRESH AND MINIMALLY PROCESSED PRODUCE

Introduction

Fruit and vegetables are rich sources of micronutrients, fibers, vitamins and remarkable contents of phytochemicals such as anthocyanins, carotenoids, polyphenols and flavonoids which have been reported to possess antioxidant properties. This makes them essential components of the daily human diet (Allende *et al*., 2006; Rico *et al*., 2007). Consumption of fresh fruits and vegetables is associated with a number of nutritional and health benefits, and is highly recommended as health diets to fight against sedentary life style and degenerative diseases such as cancer, cardiovascular diseases and ageing (Allende *et al*., 2006; Rico *et al*., 2007; Ramos *et al*., 2013). In recent decades, there has been rapid expansion of fresh and minimally processed produce industry to multiple digit growth (Allende *et al*., 2004; Montanez *et al*., 2010; Siddiqui *et al*., 2011). This has been attributed to changes in consumer life style and increase in consciousness to health diets leading to high demand for healthy, fresh-like and ready-to-eat fruits and vegetables (Rico *et al*., 2007; Caleb *et al*., 2013a; Ramos *et al*., 2013).

A major challenge facing production and marketing of fresh minimally processed produce is the rapid quality deterioration and reduced shelf-life (Allende *et al*., 2004; Siddiqui *et al*., 2011; Caleb *et al*., 2013a; Ramos *et al*., 2013). Life processes of fresh fruits and vegetables and fresh-cuts continue after harvest due to active metabolic activities including respiration, maturation and ripening which continue in cells or plant parts until senescence and death (Jacxsens *et al*., 2000; Irtwange, 2006; Sandhya, 2010). These biological (internal) causes of deterioration lead into undesirable changes in harvested food which are characterized by changes in color, texture, flavour, and nutritive value (Kader, 2005). Additionally, rapid quality deterioration and reduced shelf-life may also result from physiological disorders and presence of mechanical injuries, which present major quality challenges for the marketing of fresh minimally processed produce (Siddiqui *et al*., 2011). Overall, the management of these quality challenges may result in reductions in availability, edibility, quality as well as wholesomeness, contributing to the incidence of postharvest food losses and subsequent financial losses (Fallik & Aharoni, 2004; Kader, 2005; Irtwange, 2006; Mahajan *et al*., 2014).

High levels of postharvest losses coupled with increasing global market demand for fresh fruits and vegetables press the need for appropriate postharvest technologies to reduce quality loss and extend shelf-life of whole fresh and minimally processed produce (Kader, 2005; Montanez *et al*., 2010; Opara & Mditshwa, 2013). As one of the most promising

postharvest technologies to reduce fresh food losses, researchers have examined various aspects of modified atmosphere packaging (MAP) for various types of fresh produce. A good number of published reviews have addressed advancement in the use of MAP and its potential to preserve quality and extend shelf-life of fresh and minimally processed produce (Rai *et al*., 2002; Oms-Oliu *et al*., 2009; Rojas-Grau *et al*., 2009; Sandhya, 2010; Caleb *et al*., 2013a). Others have examined the influence of MAP on growth of resistant foodborne pathogens and subsequent outbreaks of foodborne diseases (Farber *et al*., 2003; Caleb *et al*., 2013a). In this review, the basic principles of MAP and parameters affecting the performance of MAP are discussed. This is followed by a detailed discussion of perforation-mediated modified atmosphere packaging (PM-MAP), including the principles, functions and applications to fresh and minimally processed produce.

Overview of postharvest technologies applied to reduce losses and extend shelf-life of fresh horticultural produce

The quality of fresh produce cannot be improved after harvest; nevertheless it remains possible to slow down the rate of undesirable changes and maintain the quality for a longer time (Kim *et al*., 2010). Postharvest technologies refer to various techniques applied to reduce losses, extend quality and shelf-life of fresh and minimally processed produce. In this regard, various postharvest technologies to preserve quality and extend shelf-life during distribution and short-term storage of fresh and minimally processed produce have been reviewed (Soliva-Fortuny *et al*., 2003; Barry-Ryan *et al*., 2007; Siddiqui *et al*., 2011; Ramos *et al*., 2013; Mahajan *et al*., 2014). The use of chemical-based treatments such as washing with sanitizers, antioxidant treatments and ozonised water are among the postharvest preservation methods that have been successfully applied in the fresh fruit and vegetable industries (Francis *et al*., 1999; Garcia *et al*., 2002; 2003; Beltran *et al*., 2005). Furthermore, physical treatments such as application of heat (such as blanching, heat-shock and hot water dips) have been used to delay physiological deterioration of fresh produce such as pomegranate arils (Maghoumi *et al*., 2012) and citrus (Hong *et al*., 2014). Other physical methods include irradiation which is based on exposing food to different sources of radiant energy and ultraviolet light reportedly used as antimicrobial treatments (Fallik, 2004; Lopez Rubira *et al*., 2005; Tahir *et al*., 2009; Maghoumi *et al*., 2012; Hong *et al*., 2014).

The application of a wide range of edible and antimicrobial coatings represents another group of important postharvest treatment technologies which have received considerable attention over the years (Arzu *et al*., 2004; Bourtoom, 2008; Campos *et al*., 2011; Dhall, 2013). Edible coatings incorporate thin layers of edible materials applied on food produce or

at the interfaces between different layers of food components (Bourtoom, 2008; Falguera *et al*., 2011). Such coatings serve an important role as protection against microbial and antioxidant activity, physical damage and prevention of moisture loss (Bourtoom, 2008; Falguera *et al*., 2011; Dhall, 2013). Smart or intelligent packaging (IP) is another interesting innovation that has gained interest in the horticultural food industry which is able to track produce, sense the external and internal environment of the package and communicate any changes to consumer or food manufacturer, thus monitoring the quality and safety status of produce (Yam *et al*., 2005; Caleb *et al*., 2013a). Intelligent packaging is also commonly referred to as 'interactive packaging' due to its ability to give information about produce quality along the chain, during transport and storage (Yam *et al*., 2005; Sandhya, 2010). Active packaging is another useful technology which is characterised by the use of absorbers and emitters (or releasing systems) of active ingredients (such as O_2, CO_2), ethylene scavengers/ emitters and moisture absorbers in the package (Rodriguez-Aguilera & Oliveira, 2009). Active ingredients in an active package modify the atmosphere surrounding produce inside the package, thereby extending produce shelf-life (Vermeiren *et al*., 2003). However, the practical application and widespread use of active and intelligent packaging systems is limited mainly due regulatory issues (e.g. application of antimicrobial packaging systems) and technical limitations such as high cost associated with these technologies (Yam *et al*., 2005; Realini & Marcos, 2014).

Increasing consumer awareness about health benefits and safety of food has driven the fresh produce industry to minimise the use of chemicals that have hitherto been commonly applied as sanitizing and preservative agents (Meyer *et al*., 2002; Ramos *et al*., 2013). Apart from the health concerns, it has been reported that use of chemical sanitizers and washings cannot guarantee the microbial quality of produce without compromising sensory quality (Rico *et al*., 2007). As the result, most of the inorganic chemical treatments and washing sanitizers such as chlorine-based chemicals have recently faced critical challenges to gain widespread acceptance in the fresh produce industry (Meyer *et al*., 2002; Rico *et al*., 2007). Most recently, the combination of different preservation techniques (hurdle technology) as a preservation strategy has successfully been applied in controlling microbial growth and reduction of quality losses (Allende *et al*., 2006; Rico *et al*., 2007). Hurdle technology relies on various combinations of control of temperature, acidity, redox potential, water activity, and use of preservatives and modified atmospheres to delay quality deteriorations. However, the selection of hurdles should be tailor-made to achieve the desired control of quality attributes of a specific produce (Allende *et al*., 2006). Accordingly, combining appropriately selected hurdles lowers the intensity at which each individual preservation techniques can be applied

while achieving a collective preservation role to minimise loss of quality and/ or suppress microbial growth (Allende *et al.,* 2006; Ramos *et al*., 2013).

Modified atmosphere packaging (MAP)

Overview of MAP

Despite the existence of a wide range of various postharvest technologies and applications in the horticultural industry, modified atmospheres packaging (MAP) and controlled atmosphere (CA) storage represent some of the most widely commonly applied to extend the shelf-life and maintain quality of fresh and minimally processed produce (Sandhya, 2010; Caleb *et al*., 2012; Mahajan *et al*., 2014). Studies have shown that the application of MAP or CA, in combination with appropriate temperature control, remains a common practice for maintaining quality, reducing incidences of decay and extending shelf-life of fresh and minimally processed produce (Mahajan *et al*., 2014). Controlled atmosphere storage (CAS) relies on continuous monitoring and control of the storage atmosphere to maintain desirable and stable storage conditions such as gas composition, temperature and humidity, usually in containers larger than retail-sized packages (Kader, 2005; McMillin, 2008). On the other hand, MAP is mostly useful on retail-sized smaller quantities of produce. Additionally, the controlled conditions in CA are maintained throughout storage while in MAP the atmosphere may only be modified initially (Mahajan *et al*., 2014). Despite the huge potentials for both systems in maintaining produce quality in the horticultural industry, MAP is generally more economical and more preferred, as it is designed to provide the optimal atmosphere for packaged produce only (Mahajan *et al*., 2014). On the other hand, CA storage is limited to entire storage facility in relatively large-scale systems, usually requires extended storage period and thus more capital intensive (Irtwange, 2006; Sandhya, 2010; Mahajan *et al*., 2014).

Modified atmosphere packaging relies on the alteration of the atmosphere inside the package, which under passive MAP, is achieved by the natural interplay between produce respiration and the permeation of gases through packaging film to achieve in-package gas equilibrium (Irtwange, 2006; Mahajan *et al*., 2007; 2008). As a result of generation of an atmosphere low in O_2 and higher in CO_2, MAP affects produce respiration rate (RR), ethylene production and action, decay and physiological changes caused by oxidation of plant tissues (Sivakumar & Korsten, 2006; Mahajan *et al*., 2007). The modified atmosphere (MA) in the package influences quality preservation of packaged produce by retaining

moisture and reducing the risk of decay through reduced incidence of physiological disorders and infestation of pathogenic microorganisms (Mir & Beaudry, 2004; Caleb *et al*., 2012).

Internal atmosphere of the package in MAP can be modified either naturally (passive MAP) or artificially (active MAP). In passive MAP, respiring produce modifies the atmosphere naturally by consuming oxygen (O_2), while elevated carbon dioxide (CO_2) is continuously depleted via permeation through the packaging film until equilibrium concentrations of the two gases is reached (Fonseca *et al*., 2000; Mahajan *et al*., 2008; Montanez *et al*., 2009). Traditionally, passive MAP takes longer time (7-14 days) to attain the desired gas composition and equilibrium modified atmosphere (EMA), which in turn is a function of produce, RR, weight, storage temperature and properties of packaging film (gas exchange area, film thickness and whether it is perforated or not) (Del Valle *et al*., 2009; Sandhya, 2010; Caleb *et al*., 2013a). Early generation of correct EMA can have beneficial effects on quality and shelf-life of packaged produce which is attributed to lower RR, which in turn slows down the rate of senescence and produce deterioration, thereby extending produce shelf-life (Rojas-Grau *et al*., 2009; Caleb *et al*., 2012). As most commercial polymeric films are suited to promote low levels of O_2 and high levels of CO_2 in the package headspace (Ščetar *et al*., 2010), EMA is usually not reached at right time, and this increases the risk of reaching of anoxic conditions due to high levels of CO_2 above the fermentation threshold (Mahajan *et al*., 2007; Lucera *et al*., 2010). These conditions are usually associated with formation of acetaldehyde compounds and the incidence of off-odour and off-flavour (Oms-Oliu *et al*., 2008; Briassoulis *et al*., 2013).

In active MAP, the in-package gas composition is modified actively by replacing normal air (mainly 78% N_2, 21% O_2 and 0.035% CO_2) with a desired mixture of gases (Charles *et al*., 2003; Irtwange, 2006; Caleb *et al*., 2012). Active modification of atmosphere in MAP rapidly establishes the desired EMA (Charles *et al*. 2003; Mir & Beaudry, 2004; Ščetar *et al*., 2010). However, in spite of the initial introduction of gas mixture in the actively MA package headspace, maintenance of final EMA ultimately depends on film permeability and produce respiratory characteristics (Mahajan *et al*., 2014). Used in combination with MAP, the inclusion of adsorbent substances such as CO_2, O_2 and ethylene absorbers has been reported to offer beneficial effects of delaying produce metabolic activities in active MAP (Charles *et al*., 2003; Siddiqui *et al*., 2011; Caleb *at al*., 2012a). Regulation of ethylene produced in MAP by use of ethylene scavengers restricts ripening process while CO_2 absorbers have been reported to prevent build-up of CO_2 beyond a level that is harmful for safe storage of produce (Sandhya, 2010; Caleb *et al*., 2013a). Furthermore, oxygen absorbers are useful for controlling the rapid build-up of O_2 partial pressure in MAP headspace (Charles *et al.,* 2003). Researchers have reported successful incorporation of

release substances such as antimicrobial agents, antioxidants and flavour compounds in active MAP, which offer additional benefits of extending produce shelf-life (Rodriguez-Lafuente *et al*., 2010).

In summary, MAP has been proven as a useful tool for delaying quality deterioration, and extending shelf-life of fresh fruits and vegetables throughout the distribution system (Riad & Brecht, 2002; Montanez *et al*., 2005; Sivakumar & Korsten, 2006; Mahajan *et al*., 2008). Apart from serving a general purpose of protecting the produce from deleterious external environmental conditions, the use of MAP in fresh postharvest handling reduces also exposure to both pathogens and potential contaminants (Mangaraj *et al*., 2009). MAP is relatively a low cost postharvest technology, and offering a number of advantages over other preservation technologies including the ease of application and low maintenance cost (Mangaraj *et al*., 2012). The ability to preserve quality of fresh produce without use of synthetic chemicals has greatly enhanced the public acceptance of MAP (Lopez-Rubira *et al*., 2005; Sivakumar & Korsten, 2006; Mangaraj *et al*., 2009). Furthermore, MAP system is capable of maintaining beneficial EMA conditions in the package along the postharvest chain as well as during retail display (Riad & Brecht, 2002). Reported commercial benefits associated with applying MAP to extend shelf-life of produce include improved product value and reduction of wastage in manufacturing and retail display (Caner *et al*., 2008).

Principles of MAP

The atmosphere inside modified atmosphere packages is continuously changing depending on the permeability of film used. High permeability favours excess supply of O_2 into the package, resulting in a roughly normal produce RR. On the other hand, if the permeability is too low, O_2 level will fall below critical limits in favour of anaerobic respiration, leading to fermentation process and subsequent production of undesirable metabolites (Oms-Oliu *et al*., 2008; Del-Valle *et al*., 2009). Equilibrium modified atmosphere packaging (EMAP) is determined by the rates of O_2 consumption and CO_2 evolution, and is usually achieved when transmission rate of gases through the film matches the RR of the packaged fresh produce (Caner *et al*., 2008; Jacxsens *et al*., 2000). Therefore, the optimization of EMAP is the only means to prolong the shelf-life of fresh packaged produce (Del-Valle *et al*., 2009; Mistriotis *et al*., 2011). This implies that rapid generation and maintenance of the desired EMA within the desirable range will guarantee the optimum exploitation of MAP benefits.

The most critical and general requirement for any MAP to function properly is the use of packaging material with desirable permeability properties (Mahajan *et al*., 2007; Caner *et al*.,

2008; Mistriotis *et al*., 2011). Whether the in-package atmosphere is modified by gas flushing to achieve desired EMA or passively, the importance of film permeability remains decisive. Despite the quick generation of desirable EMA by pulling the vacuum and replace with desired mixture of gases, the primary goal of gas flushing remains to reduce the initial O_2 level within the package (Ščetar *et al*., 2010). Whether EMA is achieved passively depending of produce RR and film permeability or actively by gas flushing, the generated EMA becomes effective and beneficial if it matches the optimum requirement for the packaged produce (Lee & Renault, 1998; Jacxsens *et al*., 2000).

The combined effects of low O_2 and high CO_2 concentrations generates aerobic conditions which potentially reduce RR, ethylene production, retard enzymatic reactions and delay aerobic spoilage microorganisms (Oms-Oliu *et al*., 2008; Sandhya, 2010; Caleb *et al*., 2012). High level of CO_2 in MAP of fresh produce is associated with bacteriostatic and fungistatic effects which retard the growth of most moulds and aerobic bacteria in packaged produce (Farber *et al*., 2003; Hutton, 2003). However, exposure of fresh produce to extremely high CO_2 levels may cause physiological damage due to anaerobic fermentative reactions (Caner & Aday, 2009). On the other hand, extremely low concentration of O_2 that favours anaerobic conditions endangers the quality and safety of packaged produce due to high risk of growth of pathogenic microorganisms such as *Clostridium botulinum* (Charles *et al*., 2003; Caleb *et al*., 2013a; Oliveira *et al*., 2012a). Therefore, the physiological conditions and nutritional quality of fresh packaged produce are affected by the gaseous composition inside the package. Table 2.1, summarizes a list of physiological responses of fresh produce resulting from the possible gas composition inside modified atmosphere packaging.

Table 2.1 Physiological responses of fruits and vegetables driven by reduced levels of O_2 and/or elevated CO_2

Physiological response	Possible effects due to			
	Reduced O_2		Elevated CO_2	
Respiration rate	>1%	None	<15 - 20%	None
	<1%	Increase	>15 - 20%	Increase
Development of off - flavours	<1%	Increase	>15 - 20%	Increase
Ethylene action	None		None	
Chlorophyll degradation	None		None	
Fungal growth	<1 %	None	Slight/None	
Carotenoid biosynthesis	None		None	
Anthocyanin development	None		None	
Loss of vitamin C	None		None	
Enzymatic browning	Near 0%	None	None	

Source: Brandenburg & Zagory (2009).

Design of modified atmosphere packaging

Designing suitable MAP systems requires critical analysis of appropriate factors and necessary conditions for creating an optimum and timely EMAP to prolong the postharvest life of the fresh produce to be packaged (Charles *et al*., 2003; Mahajan *et al*., 2007). Improper design of MAP endangers quality, safety and storage life of packaged produce (Mahajan *et al*., 2007; Oliveira *et al*., 2012a). Properties of the packaging material and the characteristics of the environment surrounding the produce are key extrinsic factors of important design considerations. Design approach of any MAP system includes the selection of suitable film with the required permeability to match the requirement of a given produce (Oliveira *et al*., 2012a), including parameters such as film area and thickness, filling weight, time required to reach equilibrium as well as the equilibrium gas composition at fluctuating temperature conditions (Mahajan *et al*., 2007; Del-Valle *et al*., 2009; Oliveira *et al*., 2012b). Matching film transmission rate of relevant gases (O_2 and CO_2) with the RR of produce is the only means to suitably generate safe MA to extend postharvest life of produce in MAP.

Produce RR and optimum gas concentration represent another set of extrinsic factors to consider in designing an effective MAP (Oliveira *et al*., 1998; Mahajan *et al*., 2007). Adequate knowledge of polymer properties such as specific physical, chemical and gas transmission rates (GTR), O_2 transmission rate (O_2TR) and CO_2 transmission rate (CO_2TR), is very important in MAP design. Different whole fresh or minimally processed produce have different specific packaging requirements. Therefore, matching the GTR properties of polymeric film to specific produce MAP requirement becomes paramount in MAP design (Ščetar *et al*., 2010).

Brandenburg and Zagory (2009) recommended the use of combined approach of combining knowledge of produce physiology with polymer engineering and the science of converting technology in designing an effective MAP system. Knowledge of produce physiology provides good understanding of the physiological properties and requirements of perishable produce. On the other hand, polymer engineering provides adequate understanding of the desired features and suitability of polymeric film for MAP use, while converting technology combines raw polymer materials with other relevant materials to create the desired package which has added beneficial features other than just film permeability. The intersection of these three science disciplines results into an effective MAP design (Brandenburg & Zagory, 2009).

Temperature fluctuation (below or above optima) is a common phenomenon affecting the permeability of MAP film during storage, transport and distribution chain (Mahajan *et al*., 2007; Caner *et al*., 2008). A change in film permeability disturbs the generated equilibrium

MAP, leading to poor performance. Elevated temperatures accelerate produce RR with less corresponding increase in film permeability to gases, and thus affecting the internal composition of gases and establishment of EMA (Sanz *et al*., 1999; Jacxsens *et al*., 2000; Caner *et al*., 2008). As a result of difficulties in generating EMA in the package, excessive accumulation of CO_2 and/ or depletion of O_2 at higher temperatures are common occurrences which contribute to produce deterioration (Mahajan *et al*., 2007; Caner *et al*., 2008).

In view of aforementioned factors affecting effective MAP design, earlier research using common polymeric films have shown that these films are suitable for packing produce with low or moderate RR such as leek, apples, melons, apricots and onion (Kadel *et al*., 1989; Exama *et al*., 1993; Cameroon *et al*., 1994). In addition to the effects of produce RR, film suitability for MAP application is also influenced by the desired handling temperature and atmospheric condition (Del Nobile *et al*., 2007). The interaction of these factors with corresponding permeability of the packaging film determines atmosphere modification in MAP (Mahajan *et al*., 2007). Thus, systematic design that involves modelling and simulation to establish parameters for MAP of produce should be a preliminary step towards effective MAP design (Montanez *et al*., 2010; Mangaraj *et al*., 2012; Pandey & Goswami, 2012).

Application of polymeric films in MAP

Preservation of quality and safety of fresh produce packaged in MAP requires selection of the most appropriate packaging materials (Mangaraj *et al*., 2009; Caleb *et al*, 2013a). Precise selection of these materials depends on factors such as type of package intended (i.e. flexible pouch, rigid or semi-rigid lidded tray), gas permeability properties, gas ratio inside the package and water vapour transmission rate (WVTR) of the material (Mangaraj *et al*., 2009; Kirwan *et al*., 2011). Other requirements that affect the choice of polymeric film include sealing reliability (the ability to seal to itself or to other material by heating), machinability, strength, clarity, durability, resistance to chemical degradation, non-toxicity, chemical inertness, printability, and commercial suitability (Lange, 2000; Mangaraj *et al*., 2009; Kirwan *et al*., 2011). As summarised in Table 2.2, a combination of several factors determine the suitability and choice of polymeric films for MAP applications.

Advancements in polymer processing technology have enabled the combination of various packaging materials and polymers in MAP, in turn leading to the availability of a wide range of MAP formats for fresh and minimally processed produce (Farber *et al*. 2003; Brandenburg & Zagory, 2009; Mangaraj *et al*., 2009). The application of co-extrusion, lamination, coating

technologies or their combinations has led to the development of different MAP formats in the market, ranging from flexible bags, pouches, pillow packs and top webs in sealed tray systems to rigid and semi-rigid structures for base trays, dishes, cups and tubs (Silva *et al*., 1999; Mangaraj *et al*., 2009; Mullan & McDowell, 2011).

Despite the foregoing advantages, the application of polymeric films in MAP has limitations including: (i) unpredictable changes in film permeability characteristics when stretched or punctured; (ii) relatively high barriers to water vapour, causing condensation inside packages, especially under fluctuating temperature conditions which promote the development of optimal conditions for microbial growth; (iii) non-uniformity in permeation characteristics of films that may cause gas stratification; (iv) higher permeability to CO_2 than O_2 of most films, which is unfavourable for high respiring products such as strawberries, grapes, citrus, mushrooms, broccoli and asparagus, and (v) high ratio of CO_2 to O_2 permeability coefficients which is not suitable for products requiring high CO_2 and low O_2 concentrations due to increased risk of anaerobiosis (Oliveira *et al*., 1998; Fonseca *et al*., 2000). Due to these limitations, it is known that polymeric films may generate in-package atmospheric conditions outside the ideal optimal requirements for most fresh produce (Lee & Renault, 1998; Mahajan *et al*., 2007; 2008). For these reasons, their application of MAP for preserving whole and minimally processed produce is quite restricted.

Table 2.2 A summary of benefits and drawbacks of polymeric films commonly used in fresh food packaging

Polymeric film	MAP use suitability	
	Benefit properties	Drawbacks
Polyvinylidene chloride (PVdC)	Good heat sealabilty that provide peelable feature of MAP. Excellent gas, dour and water barrier properties. Good resistance to oil, grease and organic solvents. Excellent heat sealabilty (able to seal to itself and to other materials)	High barrier to water vapour and gases limit is use for MAP of high respiring produce.
Linear Low Density Polyethylene (LLDPE)	Good sealing quality, and therefore its application on the sealing face allows a peelable seal to be made. Used as a sealant layer on base trays and lidding films.	
Polyvinyl alcohol (PVOH)	Good barrier properties against water vapour. Can be copolymerised with ethylene to produce ethyl vinyl alcohol with improved water vapour permeability.	
Bi-axially Oriented Polypropylene (BOPP)	Rigid and hard plastic material. Being bi-axially oriented, it has improved tensile strength and hence useful as a base tray. Good barrier to water vapour and gases.	Higher barrier to water vapour limits its suitability to MAP of some fresh produce.
Polyamide	Good barrier to gas, flavour, odour loss. High resistance to stress cracking and puncture. High water vapour permeability.	Not suitable for MAP of high respiring produce. Tends to absorb moisture from their environment.
Ethyl Vinyl Alcohol (EVOH)	Excellent barrier to oxygen thus used as a gas barrier layer in MAP applications of low-moisture foods. Resistant to the absorption and permeation of oil, fat and sensitive aromas and flavours. Good processing properties(machinability)	Less sensitive to the presence of moisture. Not suitable for MAP of high respiring produce.
Polystyrene (PS)	Stiff and brittle material with high gas permeability. Foamed PS used as structural layer for preformed MAP base tray applications	Cannot be used alone in MAP application due to high gas permeability, unless combined with EVOH

Table 2.2 Summary continued

Polymeric film	MAP use suitability	
	Benefit properties	Drawbacks
Polyvinyl chloride (PVC)	Has low softening temperature, good processing properties, thus suitable material for producing thermoformed packaging structures. Excellent oil and grease resistance. Common structural material in MAP thermoformed base trays.	Unplasticised PVC has moderate gas and water vapour barrier properties, thus not suitable as film for MAP of high respiring produce
Ethylene Vinyl acetate (EVA	Excellent heat-sealing properties. Useful as heat seal layer in some MAP applications.	
High Density Polyethylene (HDPE)	Tough and stiff material. Commonly used for rigid and semi-rigid structures.	

Source: Blakistone, 1999; Kirwan *et al*., 2011; Mullan & McDowell, 2011.

The manufacture of suitable packaging films with a wide range of physical properties, such as good permeability to gases and water vapour (WV), heat sealability, anti-fogging properties, puncture resistance can be obtained by combining several types of polymeric films through processes like lamination and co-extrusion or blending of several polymers during extrusion process (Scetar *et al*., 2010; Mangaraj *et al*., 2009). However, these processes rely on high technology machines adding to high cost of obtaining films of suitable permeability for MAP use (Mangaraj *et al*., 2009). This necessitates the need for alternative technologies to optimise film permeability to meet the MAP requirements of medium and high respiring fresh produce.

Transmission of gases and water vapour through MAP film

Modified atmosphere is generated via a dynamic process balanced by produce respiration and gas permeation through the packaging film (Mir & Beaudry, 2004; Mahajan *et al*., 2008). Exchange of gas between packaging film and surrounding atmosphere is driven by the partial pressure gradient across MAP film (Mullan & McDowell, 2011). The rate at which O_2 is consumed and CO_2 is produced by respiring fresh produce inside MAP depends on the concentrations of O_2 and CO_2 at a given temperature (Mangaraj *et al*., 2012). In this regard, barrier properties of packaging films in relation to permeant molecules (O_2, CO_2 and water

vapour) plays a significant role in MAP of fresh whole and minimally processed produce. These gases permeate in and outside the package across the film thereby influencing changes in quality and shelf-life of produce. Most importantly, CO_2 is particularly an important gas in MAP as it reduces the RR of packaged produce while suppressing the growth of spoilage microorganisms (Curtzwiler *et al*., 2008; Siracusa *et al*., 2008).

Mass transfer is an important physical phenomenon that influences the movement of gases and water vapour through MAP films. During mass transfer, the sorption of permeant molecules into barrier surface occurs by diffusional molecular exchange and desorption on the opposite surface (Hu *et al*., 2001; Rodriguez-Aguilera & Oliveira, 2009). In conventional, non-perforated polymeric films, flow of gas across a barrier film increases with increasing concentration gradient between the package headspace and surrounding environment (Mir & Beaudry, 2004; Mullan & McDowell, 2011). This gradient creates the driving force for gas diffusion through the polymeric film produced by the reduced O_2 and elevated CO_2 resulting from the actively respiring produce (Mir & Beaudry, 2004). Equilibrium levels of O_2 and CO_2 are finally achieved in the package when the rates of O_2 uptake and CO_2 production by the packaged produce are equal to that permeating through the film, a situation favoured by steady-state (constant) RR (Mir & Beaudry, 2004; Caner *et al*., 2008)

Barrier properties of a packaging material to gases (CO_2, O_2) or water vapour is measured by the transmission rate, which is defined as the quantity of gas or water vapour passing across a film of known area over a given time. The permeability of most common plastic polymers is affected by temperature of the surrounding, and therefore, transmission rate values are often reported for specific temperature ranges (Siracusa *et al*., 2008; Mullan & McDowell, 2011). However, the effect of temperature on permeability of perforated film is minimal (Mir & Beaudry, 2004). The permeability coefficient of a packaging polymer which relates film thickness and driving force is a useful parameter that permits the comparison of barrier properties of different packaging films (Mullan & McDowell, 2011).

The diffusion rates of CO_2 and O_2 through perforated and non-perforated polymeric films differ significantly (Mir & Beaudry, 2004). For non-perforated film, diffusion rate of CO_2 is between 2 to 8 times faster than O_2 (Mir & Beaudry, 2004; Šcetar *et al*., 2010). This gives a wide range of permeability ratios of CO_2TR to O_2TR (referred to as β), which are always higher than the recommended optima values for most produce (Al-ati & Hotchkiss, 2003; Mahajan *et al*., 2007; Šcetar *et al*., 2011). Due to unequal permeability rate between O_2 and CO_2 of most of commercially available films, their β values lie within a range of 2.2-8.7 (Mahajan *et al*., 2007). Such low O_2 and /high CO_2 permeability is undesirable for suitable

application in the MAP of most minimally processed produce that require the selected film to match their high respiration rates (RRs) (Mahajan *et al*., 2007).

In perforated films, the scenario is quite different such that CO_2 diffuses 0.77 faster than O_2, thus resulting in more or less equal generation of gradient of gases (Gonzalez, Ferrer, Oria & Salvador, 2008; Mir & Beaudry, 2004). This implies that for any given level of O_2, perforated MAP will generate a considerably higher O_2 and lower CO_2 regimes suitable for a wide range of produce (Mir & Beaudry, 2004). Modified atmosphere packaging application of polymeric films whose β values range from 4 - 6 is associated with high risk of generating an equilibrium atmosphere low in CO_2 which may be suitable for a few commodities tolerating lower CO_2 levels such as banana, grapes, mango and apples (Mahajan *et al*., 2006; 2008). For such fresh produce with low and medium rates of respiration, a small list of polymers has been reported to generate suitable in-package modified atmosphere (Kader & Watkins, 2000; Rennie & Tavoularis, 2009).

Modified atmosphere packaging of pomegranate whole fruit and arils – an update

Several studies have reported successful use of MAP technology to achieve different postharvest objectives during storage, transport and on-shelves display for both fresh whole and minimally processed pomegranate (Gil *et al*., 1996a; Artes & Tomas-Barberan, 2000; Lopez-Rubira *et al*., 2005; Ersan *et al*., 2010; Maghoumi *et al*., 2012; Caleb *et al*., 2013c). Caleb *et al.* (2012) reported a comprehensive review on modified atmosphere packaging of pomegranate fruit and arils. The review in this section provides an update and highlights some important aspects which enhance the effectiveness of MAP application that were not elaborated by the authors.

Gil *et al*. (1996a) investigated the combined effects of MAP and different washing treatments with chlorine and antioxidants (ascorbic and citric acids) on quality retention and shelf-life of 'Mollar' pomegranate arils. To identify the best storage condition, different temperatures (1, 4 and 8 ºC) were studied. The authors reported that chlorine wash followed by antioxidant solution dip was effective in controlling oxidation of arils. Furthermore, pomegranate arils were best kept at 1 ºC inside MAP with polypropylene film. The initial level of modified atmosphere of oxygen (20 mL/L) and carbon dioxide (0 mL/L) achieved inside the packages was credited for the lower incidence of total physical losses and improved visual appearance of the pomegranate arils.

In a subsequent similar study, Gil *et al*. (1996b) evaluated the combined impacts of packaging types, washing treatment and storage temperature on pigment stability of 'Mollar'

pomegranate arils. Under 1 ºC storage, both perforated polypropylene bags (normal air) and non-perforated polypropylene (passive MAP) packaging maintained stability of anthocyanin concentration of arils for up to 7 days. Based on these results, it was recommended that either packaging types could be used for successful storage of minimally processed arils in combination with washing with chlorine. Furthermore, Sepulveda *et al*. (2000) studied the quality of minimally processed 'Wonderful' pomegranate arils packaged in different types of semi-permeable Cryovac ethyl vinyl acetate-based MAP films. The study also evaluated the use of ascorbic acid and citric acid solutions mixture as antioxidant solution on quality of arils. It was observed that irrespective of antioxidant dip, both types of films successfully maintained the physical and microbiological quality of arils at 4 ºC for 14 days under passively modified atmosphere. Similarly, Ayhan & Esturk (2009) studied the shelf-life of fresh 'Hicaznar' pomegranate arils under commercial conditions using MAP with bioriented polypropylene (BOPP) film heat sealed on polypropylene tray. The authors reported that storing arils in MAP at 5 ºC coupled with pre-treatment with chlorine sanitizer maintained quality and freshness of arils for 18 days.

Ersan *et al*. (2010) investigated the effects of different atmospheric conditions on the RR of fresh 'Hicaznar' pomegranate arils. The evaluation of suitability of common packaging materials (low density polyethylene (LDPE) and PP) against the required material properties for MAP of fresh pomegranate arils and generation of EMAP was the scope of the study. Target levels of O_2 and CO_2 to slow down RR of fresh arils were 10 or 20% CO_2 and 2% O_2 at 4 ºC. The authors concluded that the studied packaging materials could not achieve desirable gas composition due to their limited permeability.

In their study of the shelf-life and quality preservation of minimally processed fresh 'Mollar of Elche' pomegranate arils, Lopez-Rubira *et al*. (2005) assessed the effects of a passive generated MAP with BOPP heat-sealed film, chlorine treatment and UV-C radiation. The study also examined the effects of harvest date and found that the quality of arils was maintained at 5 ºC storage for 10 and 14 days under MAP storage for late and early harvested fruit, respectively. However, the authors reported that unclear results of the effect of UV-C radiation on the microbial growth of pomegranate arils were obtained. Similar study on effects of passive MAP, storage temperature (5, 10 and 15 ºC) and duration of storage on the quality of minimally processed pomegranate arils 'Acco' and 'Herskawitz' was reported by Caleb *at al*. (2013c). Fungal growth observed on arils both cultivars limited the shelf-life to 10 days at 5 ºC. The study also reported that other physico-chemical quality attributes such as pH, total soluble solids, titratable acidity and total anthocyanin content were best maintained at 5 ºC. Cultivar differences were found to be the dominant factor affecting changes in aril quality attributes and aroma compounds.

The effect of passive MAP, storage temperature (5, 10 and 15 ºC) and storage duration on the compositional change in flavour attributes of pomegranate arils cvs. 'Acco' and 'Herskawitz' was also reported by Caleb *et al*. (2013c). The authors revealed that production of volatiles for both fruit arils cultivars under passive storage was significantly affected by storage temperature and duration while quantitative differences were found among cultivars. The concentration and compositional changes in volatiles for both cultivars showed that the 'flavour-life' did not exceed 7 days as compared to overall postharvest shelf-life (10 days) based on appearance and other physico-chemical quality attributes. Similarly, Mayuoni-Kirshinbaum *et al*. (2013) investigated the effect of MAP on the changes in sensory quality and aroma volatile composition of 'wonderful' pomegranate whole fruit. The results of descriptive flavour analyses showed that flavour life of pomegranate fruit decreased after 16 to 20 weeks of cold (7 ºC) storage under commercial Xtend modified atmosphere bags. However, the external visual quality of pomegranate fruit was extended up to 4-5 months after harvest, which was 4 weeks longer than 'flavour-life'.

Perforation-mediated modified atmosphere packaging (PM-MAP)

Importance of PM-MAP

Permeability properties of most polymeric films commonly used in MAP represents an important limitation of MAP, especially for highly respiring produce such as mushrooms, citrus, asparagus, strawberries, grapes and broccoli. Low permeability to O_2 than CO_2 is the characteristic of most films (Mangaraj *et al*., 2009; Mahajan *et al*., 2008; Sandhya, 2010), and consequently, the levels of atmosphere attained using traditional MAP are rarely sufficient to ensure longer shelf-life and achieve high quality produce during storage. Due to anaerobiosis, development of undesirable off-odours under low O_2 and elevated CO_2 atmospheres are common occurrences that severely modify volatiles profile of packaged produce (Oms-Oliu *et al*., 2007; Rojas-Grau *et al*., 2009; Caleb *et al*., 2013a). Alternatively, the use of perforations in perforation-mediated modified atmosphere packaging (MP-MAP) has been proposed as a technique to overcome these limitations (Rodriguez-Aguilera & Oliveira, 2009; Oliveira *et al*., 2012a).

Perforations in MAP are used to achieve higher transmission rates of gases and water vapour through commonly used polymeric films (Del-Valle *et al*., 2004; Gonzalez *et al*., 2008). The technique involves use of single or multiple perforations on polymeric films to allow for regulation of gas and water vapour exchange rates in packaged fresh produce (Riad & Brecht, 2002; Montanez *et al*., 2005; Mahajan *et al*., 2008). Several authors have

analyzed the impacts of perforations on gas and water vapour exchange rates as being beneficial in generating safe and desirable modified atmosphere within packages of fresh produce (Gonzalez *et al*., 2008; Pandey & Goswami, 2012).

Principles and functions of PM-MAP

In PM-MAP system, micro-perforations (50-200 µm perforation diameter) or macro-perforations (perforation diameter > 200 µm) can be made by laser, flame, or mechanical technologies (Lange, 2000; Gonzalez *et al*., 2008; Gonzalez-Buesa *et al*., 2012). The use of perforations fosters rapid and sufficient build-up of adequate CO_2 and O_2 levels to establish a safe EMAP (Gonzalez *et al*., 2008; Kartal *et al*., 2012). Micro-perforations are developed to improve film permeability to O_2, CO_2 and water vapour above that of the film alone (Mir & Beaudry, 2004; González-Buesa *et al*., 2012) due to the fact the exchange of gases through PM-MAP takes additional route of perforations (Lange, 2000; Pandey & Goswami, 2012). Perforation-mediated MAP potentially reduces the risk of anaerobiosis and microbial growth associated with moisture condensation due to fluctuating temperatures (Lee & Renault, 1998; Silva *et al*., 1999; Fonseca *et al*., 2000). The exchange of O_2 and CO_2 through perforations on the packaging film facilitates achievement of the desired EMAP.

Perforation-mediated MAP is a useful technique to achieve safe modification of internal atmosphere of package for safe storage and quality retention of horticultural produce in comparison with conventional non-perforated MAP system (Riad & Brecht, 2002; Montanez *et al*., 2005). High and medium respiratory products (such as cherries, strawberries blueberries, sweet corn, spinach and mushroom) require relatively high concentration of CO_2 and low O_2, and the use of perforated packaging system provides an alternative means to equilibrate the in-package gas composition, in which a reduced O_2 and relatively higher CO_2 is achieved. Perforation helps to maintain higher relative humidity (RH) inside the package (Pandey & Goswami, 2012). In addition, besides improving gas and moisture transfer, perforating an air-tight package serves other crucial functions in MAP. For instance, the use of perforations has been reported to shorten cooling time and prevent condensation of water vapour inside the package. Furthermore, perforation can be used to achieve safe and desired atmospheres inside package through the effects on altering film permeability as well as a means to attain pressure equilibrium inside the package (Oliveira *et al*., 1998; Fonseca *et al*., 2000).

Design of PM-MAP

Design process of PM-MAP is complex because each produce has its specific and often unique packaging requirements different from the others (Jacxsens *et al*., 2000; Mahajan *et al*., 2007). As a result, a number of variables need to be optimised simultaneously so as to meet the target MAP for specific produce (Rodriguez-Aguilera & Oliveira, 2009). Designing process needs to consider the gaseous composition requirement (O_2 consumption and CO_2 production rates) of specific produce, the mass transfer coefficients for the gas exchange through the packaging material and the response of these MAP parameters to change in environmental factors such as storage temperature (Mahajan *et al*., 2007; Rodriguez-Aguilera & Oliveira, 2009).

Design of PM-MAP system must take into account: the number and dimensions of the perforations as the major factors controlling the exchange rate of relevant gases through the perforations (Montanez *et al*., 2010; Kartel *et al*., 2012). The effect of changes in external environment (temperature and atmospheric pressure) on the rate of mass transport through perforations is another critical factor (Montanez *et al*., 2010). Therefore, optimal PM-MAP design is also dependent on knowledge of these variables and their correlation with the mass transfer coefficients of packaging films (Fonseca *et al*., 2002; Montanez *et al*., 2010) as summarized in Table 2.3.

Furthermore, MAP design using perforated polymeric films involves prediction of gas permeability of the film by modelling the exchange of gases through perforated area (Pandey & Goswami, 2012). Mathematical models simulating various conditions of MAP are useful tools in design and validation of correct MAP (Montanez *et al.,* 2005; Mahajan *et al*., 2007). Modelling allows pre-determination of key MAP determinant factors such as exchange rates of gases, water vapour and changes in gas composition in MA package prior to MAP design (Kader & Watkins, 2000). Modelling of produce respiration and exchange of permeant (gas and water vapour) or mass transfer through film and perforations is based on different physical laws. Graham's law of effusion, Fick's law and Stephan-Maxwell law of diffusion and/ or a combination of more than one physical law have been used to predict permeation of gas and water vapour through non-perforated and perforated packaging systems (with micro-perforations) (Kader & Watkins, 2000; Gonzalez *et al*., 2008). However, in some cases, the combination of diffusion and sorption laws (such as Henry's law for sorption) has proven not to be adequate in describing the mass transport process alone. In this case, Knudsen diffusion and effusion and/ or hydrodynamic flow laws (such as Poiseuille's) have been applied to describe permeation of gas through perforated films (Zinderighi, 2001; Del-Valle *et al*., 2003; Gonzalez-Buesa *et al*., 2009).

Table 2.3 Factors and related variables influencing the design of PM-MAP

Factors	Variables	Designation
Extrinsic factors: package-related	Film thickness	E
	Film surface area for gases exchange	A
	Volume of the package	V
	Number of micro perforations	n
	Diameter of micro perforation	D
	Film permeability	PO_2, PCO_2
surrounding-related	Gas composition	yO_2^{in}, yCO_2^{out}
	Temperature	T
	Atmospheric pressure	P
Intrinsic factors: produce-related	Produce mass	M
	Produce density	ρ
	Respiration rate	RO_2, RCO_2
	Desired equilibrium gases composition	yO_2^{eq}, yCO_2^{eq}

Adopted from Mahajan *et al*., 2007.

Various mathematical models have been developed to describe gas exchange of perforation-mediated packaging system and prediction of O_2 and CO_2 mass transport coefficients, some of which are summarised in Table 2.4. Gas exchange through micro-perforated films takes either of the two major assumptions: (i) some models are developed based on perforations as the major route of gas transport with the final gas transfer rate being the additive term of permeation through perforation and diffusion across the film (Mir & Beaudry, 2004; Montanez *et al*., 2010); or (ii) other models takes into account the transfer of gas through perforations, while assuming the film as impermeable (Del-Valle *et al*., 2009; Montanez *et al*., 2010). In cases where modelling of mass transport of gas through micro-perforated packaged produce is assumed to take place through multi-component including the commodity, headspace, perforations and/or permeable film, the Maxwell-Stefan equation has been used to determine diffusive flux of gases. This model was reported to be more appropriate for describing relationships between the fluxes and concentration gradients of gases in multicomponent systems (Chung *et al*., 2003; Rennie & Tavoularis, 2009; Gonzalez-Buesa *et al*., 2012).

Most of the models for predicting gas exchange rates depend merely on the product RR and film permeability (also known as a steady-state or constant RR condition), normally simulated at a single temperature and very often at low RH (Kadar & Watkins, 2000). Perhaps, possible large experimental errors, time consuming experiments for determination of RR for MAP and the complexity nature of the process are main limitations of many predictive models (Fonseca *et al*., 2002).

However, during MAP design, the dynamism caused by temperature fluctuation during storage and distribution coupled with changing humidity conditions and consequent responses of film permeability to these changes need to be considered. The need for considering such dynamic processes in the modelling becomes crucial to avoid the risk of exposing the product to undesirable gas and humidity composition atmosphere (Kader & Watkins, 2000; Mahajan *et al*., 2007).

Table 2.4 Summary of reported models for predicting exchange of gases and water vapour through perforated films

Basis of the model	Mathematical equation	Number of perforations(n)	Film thickness (l)	Reference
Fick's law	$J = -D(c_W)\frac{\partial c_W}{\partial x}$		28 µm	Del Nobile et al., 2002
Stephan-Maxwell's law	$-\frac{P\partial C_1}{RT\partial l} = \varphi_{pi}\sum_{\substack{j=1 \\ j\neq i}}^{n}\frac{\varphi_{pi}C_j - \varphi_p Y_i}{D_{ji}}$	1 – 5	0.00284 ≤ n ≤0.102 cm	Paul & Clarke, 2002
Fick's law (Modified)	$M_1 = \frac{D_1 A\Delta c}{l_1 + \varepsilon}$			Chung *et al*., 2003
Knudsen's law	$J_{k,A} = D_{k,A}\frac{\partial c_A}{\partial X}$	3 – 6	0.2 mm	Del-Valle *et al*., 2003
Fick's law	$WTR_z = -D\left[\frac{M_W A P_T}{RTpAlm}\right](pW_1 - pW_2)$	1, 3, 6, 12, 18 and 24 (Holes per 38.5 cm^2).	0.2, 0.5, 1.75 mm	Dirim *et al*., 2004
Fick's law	$\frac{dO_2}{dt} = AkO_2\left(pO_2^{in} - pO_2^{out}\right)$	0.13 m^2 Diffusion area	35 µm	Ozdemir *et al*., 2005
Fick's law	$\frac{dV(t)}{dt} = n_p D_i\left(P_i^{out} - P_i^{in}\right)$	1 hole	0.012, 0.025 mm	Techavises & Hikida, 2008
Fick's law	$J_{fi} = -\frac{P}{RT}\frac{Q_i A\left(P_i^{in} - P_i^{out}\right)}{L}$	0 – 14	40 µm	Gonzalez-Buesa *et al*., 2009
Fick's law	$J_{fi} = \frac{A_f P_{fi}\left(C_{i,out} - C_{i,in}\right)}{L_f}$	1 hole	45 µm	Gonzalez *et al*., 2008; Pandey & Goswami, 2012

Designing an optimal atmosphere capable of reducing the risk of water vapour condensation in the package and thus maintain produce weight loss to the minimum has been a major challenge of modified atmosphere and humidity packaging (Mahajan *et al*., 2014). Water uptake from fresh produce as water vapour also known as transpiration is the common physiological process of significant importance as it substantially influence postharvest quality of fresh and minimally processed produce (Mahajan *et al*., 2008; Caleb *et al*., 2013b). During storage, transpirational water molecules evaporated from the produce need to escape outside the package. However, commercial polymeric films commonly used in MAP have lower water vapour permeability relative to transpiration rate of packaged fresh produce (Dirim *et al*., 2004; Mistriotis *et al*., 2011; Mahajan *et al*., 2014). Therefore, design of PM-MAP needs to take into consideration the important factors affecting transpiration rate of packaged fresh produce. Environmental factors such as temperature, RH, air movement and atmospheric pressure influence the rate of transpiration of fresh horticultural produce (Kader, 2002; Mahajan *et al*., 2008; Xanthopoulos *et al*., 2012; Caleb *et al*., 2013b).

Exchange of water vapour through MAP film is crucial due to its potential role in regulating in-package humidity, which in turn influences produce physiological responses and quality (Dirim *et al*., 2004; Techavises & Hikida, 2007). Proper regulation of RH in MAP system should depend on produce requirement, focusing the desirable level of humidity required to maintain the quality of packaged produce. A desirable humidity is required to maintain produce firmness. As high humidity favours the growth of microorganisms such as yeast and moulds, in order to reduce incidences of microbial spoilage of packaged produce, it remains necessary to maintain desirable in-package humidity (Techavises & Hikida, 2007; Pandey & Goswami, 2012). Furthermore, regulation of in-package humidity is necessary to avoid incidence of excessive weight loss (Mistriotis *et al*., 2011). High RH can encourage moisture condensation on the produce, creating conditions suitable for microbial growth. On the other hand, low RH increases produce transpiration that results in substantial water loss and desiccation leading to loss produce quality (Song *et al*., 2002; Techavises & Hikida, 2007).

Permeability properties of packaging film with high WVTR such as hydrophilic polymers influence the effect of perforations to water vapour permeability (Mistriotis *et al*., 2011). As a result, excess moisture due to transpiration diffuses across the film surface while O_2 and CO_2 permeate through perforations (Mistriotis *et al*., 2011; Briassoulis et *al*., 2012; Mistriotis & Briassoulis, 2012). Perforation-mediated modified atmosphere packaging system takes control of both respiration and transpiration process through adjustment of gases and water vapour to generate desirable EMAP for a specific horticultural produce (Briassoulis et *al*., 2012). Design of the EMAP system to regulate water vapour condensation should not only consider the respiration but also the transpiration rate of packaged produce (Xanthopoulos

et al., 2012; Caleb *et al*., 2013b). The regulation of these two processes is the key to efficient EMAP of fresh produce (Song *et al*., 2002; Mistriotis & Briassoulis, 2012).

Application of PM-MAP in fresh and minimally processed produce

Extensive review of literature summarized in Table 2.6 shows that PM-MAP has been applied more extensively on whole fresh fruit and vegetables and less to fresh minimally processed produce. This includes fresh produce such as strawberries (Sanz *et al*., 1999; 2000; Almenar *et al*., 2007), broccoli (Fernandez-Leon *et al*., 2012), Loquat fruit (Amoros *et al*., 2007), sweet corn (Riad & Brecht, 2002), sliced mushrooms (Simon *et al*., 2005; Oliveira *et al*., 2012a), mandarin (Del-Valle *et al*., 2009) and many other high and medium respiring produce. However, researchers have recommended the need for systematic approach in order to obtain a successful application of PM-MAP (Mahajan *et al*., 2007; Montanez *et al*., 2010a). Scientific research and validation of PM-MAP technique is therefore needed for each specific produce in order to develop commercially applicable solutions for industry.

Almenar *at al*. (2007) evaluated the effects of micro-perforations on generation of EMAP suitable for safe storage of wild strawberries under 10 ºC. The authors reported that the use of poly (ethylene terephthalate) (PET)/polypropylene film with one and three perforations (average diameter of 100 µm) heat-sealed on plastic cups (125 mL capacity) retained the quality of strawberries through the generation of adequate equilibrium concentrations of gases (4-13% CO_2 and 5-18% O_2). The authors concluded that 6 days shelf-life was achieved for strawberries packaged in MAP with three perforations while maintaining berry quality with little or no incidence of fungal decay and off flavours. Similarly, De Reuck *et al*. (2010) reported that quality of litchi (cv. McLean's Red) was maintained for up to 21 days under 2 ºC packaged in perforated MAP with 10 perforations (0.6 mm diameter). However, the authors did not describe the gas exchange area that was perforated. The EMAP of 17% O_2 and ~5% CO_2 was attained within 6 days of storage and successfully maintained quality attributes of Litchi including acceptable pericarp color, total soluble solids (TSS), titratable acidity (TA) and TSS to TA ratio thereby preventing the loss of taste and flavour.

The quality of fresh-cut 'Gala' apple slices stored in micro-perforated film was evaluated by Cliff *et al*. (2010). The use of micro-perforated MAP (2-100 µm diameter perforations per package of 14 cm x 14 cm) maintained both sensorial and physico-chemical quality attributes of apple slices such as volatile compounds, soluble solids concentration, titratable acidity, colour and relative juice loss. Enhancement of quality attributes of fresh-cut 'Gala'

apple slices was attributed to the established MAP composition (14 kPa O_2 and 7 kPa CO_2 partial pressures) and lower in-package of ethylene concentration.

Table 2.5 Selected PM-MAP of whole and fresh-cut produce reported from 1999-2013

Produce	Type of PM – MAP		Gas Level	Storage Temp.	Optimum Shelf-life	Reference
	No of holes/tubes	Perforated area (A) /Diameter(D)				
Strawberries	2 or 4 holes	1.57 mm^2 or 3.14 mm^2	18.7% CO_2, 5% O_2 or 13.3% CO_2, 7.6% O_2,	20 ºC	4 d	Sanz *et al*., 1999
Mango fruit		75 × 75 cm perforated area	~5% CO_2, ~15% O_2	12 ºC	3 weeks	Pesis *et al*., 2000
Sweet corn		0.001% of 0.75×0.75 m^2	5 kPa CO_2	2 ºC	2 weeks	Rodov *et al*., 2000
Charentais melons		0.00025% of 0.56 m^2 size	13-14 kPa CO_2, O_2	7 ºC	12 d	Rodov *et al*., 2002
Sweet corn		4 mm D.	15, 20 or 25% CO_2	1 ºC	10 d	Riad & Brecht, 2002
Strawberries	3 tubes	1.57, 3.14, 4.71mm^2	13.6-14.9% O_2, 7.2 -8.8% CO_2	2 ºC	10 d	Sanz *et al*., 2002
Strawberries	2, 4, or 6 holes	1.57 mm^2 or 3.14 mm^2	18.7% CO_2, 5% O_2 or 13.3% CO_2, 7.6% O_2,	20 ºC	4 d	Sanz *et al*., 1999
Sweet cherry		35 µm perforation D	3 - 4% CO_2	4 ºC	8 d	Alique *et al*., 2003
Citrus fruit		0.002% perforated area	2-3% CO_2, 17-18% O_2	6 ºC	5 weeks	Porat *et al*., 2004
Sliced mushroom		0.102 m^2 perforated area	2.5% CO_2, 10 - 20% O_2	4 ± 1 ºC	13 d	Simon *et al*., 2005
Bananas	4 and 10 cm diffusion channels	50.29 cm^2 diffusion area	3% O_2 and 3.5% CO_2	15 ºC	42 d	Stewart *et al*., 2005
Litchi cv. Mauritius		0.00939% of 720 cm^2	17.0% O_2, 6.0% CO_2	2 ºC	34 d	Sivakumar & Korsten, 2006

Table 2.5 Selected PM-MAP Continued

Produce	Type of PM - MAP		Gas Level	Storage Temp.	Optimum Shelf-life	Reference
	No of holes/tubes	Perforated area (A) /Diameter(D)				
Wild strawberries	1 and 2 holes	0.0785 m^2 perforated area	10% CO_2 , 10% O_2	10 ºC	6 d	Almenar *et al*., 2007
Loquat fruit		(20×30) cm^2 bag	2-4 kPa O_2, 16-18 kPa CO_2	2 ºC	14 d	Amoros *et al*., 2008
Litchi cv. McLean's Red	10 holes	0.6 mm D	~17% O_2, ~5% CO_2,	2 ºC	21 d	De Reuck *et al*., 2009
Mandarin		~150 µm D	19.8% O_2, 1.2% CO_2	3 ºC	21 d	Del-Ville *et al*. 2009
Fresh-cut apple		2 -100 µm D in 196 cm^2	14 kPa O_2, 7 kPa CO_2	5 ºC	21 d	Cliff *et al*., 2010
Mango	80 -100 holes	~50 -70 µm D	9 kPa O_2, 17 kPa CO_2	12 ºC	30 d	Boonruang *et al*., 2011
Fresh sliced mushroom	2 holes	0.33 mm D	3.6% O_2, 11.5% CO_2	10 ºC	3 d	Oliveira *et al*., 2012a
Broccoli		625 cm^2 A	10 % O_2, 5% CO_2	5 ºC	12 d	Fernandez-Leon *et al*., 2012
Strawberries	7 and 9 holes	90 µm D	5 kPa O_2, 15 kPa CO_2	4 ºC	Beyond 14 d	Kartal *et al*., 2012
Cherry tomatoes	5 holes	200 µm D	4.0 ± 0.1 CO_2 kPa	20 ºC	60 d	Briassoulis *et al*., 2013
Peaches	100 holes	200 µm D	3.3 ± 0.01 CO_2 kPa	20 ºC	12 d	Briassoulis *et al*., 2013

However, besides the wide application of PM-MAP in the fresh horticultural food industry, there is a growing concern over the potential risk to permeation of moisture, volatile organic compounds and ingress of microorganisms through perforations, especially during wet or moist handling conditions (Chung *et al*., 2003; Del-Valle *et al*., 2004; Dirim *et al*., 2004). The study conducted by Del-Valle *et al*. (2004) reported a significant permeation of volatile organic compounds through a porous package which resulted in loss of odour and rapid deterioration of organoleptic properties of packaged produce. Furthermore, microbial contamination through perforations might be a hurdle towards the successful application of PM-MAP technology. This highlights the need for more research to evaluate the extent and severity of microbial safety of this postharvest technology.

Successful use of MAP to maintain quality and extend shelf-life of fresh produce must be accompanied by appropriate storage temperature, use of good quality of produce with minimal physiological damage and the application of appropriate treatments to reduce microbial spoilage (Krasnova *et al*., 2012). Various measures can be taken to reduce deterioration of fresh produce, including good agricultural and processing practices (such as harvesting produce at optimum maturity stage and minimising mechanical injuries), proper sanitation procedures, adherence to HACCP principles as well as the application of the optimal postharvest treatment (Artés *et al*., 2009; Weerakkody *et al*., 2010; Caleb *et al*., 2013a; Mahajan *et al*., 2014). This would help to minimize quality deterioration and the risk of microbial contamination in perforated modified atmosphere packages (Montanez *et al*., 2010b; Boonruang *et al*., 2011; Oliveira *et al*., 2012b).

Summary and future prospects

This review has explored the systematic approaches to MAP design, highlighting important factors to consider in MAP design, as well as examples of successful use of MAP technology to delay quality deterioration and prolonging shelf-life of fresh fruit (including pomegranate fruit) and vegetables. Furthermore, specific applications of PM-MAP to a number of fresh whole and minimally processed produce were highlighted. Overall, the review showed that PM-MAP offer many benefits over conventional MAP through the ability to rapidly establish desirable in-package gas composition for safe storage and quality retention of horticultural produce with medium to high respiration rates. Based on specificity of packaging requirement for each produce, the need for better understanding of the critical factors influencing PM-MAP design was emphasised. Similarly, factors affecting the performance of PM-MAP system such as produce RR, film permeability and changes in external

environment conditions (temperature and RH) were identified as important factors to consider in the design process.

Despite the fact that PM-MAP has been proven as a potential postharvest tool to preserve quality and extend shelf-life of various horticultural produce, two major concerns need to be addressed in order to gain consumer confidence. First is the concern about overall produce safety and quality of PM-MAP packaged fresh produce during postharvest handling and storage. The risk of permeation of moisture, loss of volatile organic compounds and ingress of microorganisms through perforations of PM-MAP during wet or moist handling situations has been spotted. Therefore, future prospects must focus on investigating the effect of perforation on flavour life and microbiological quality of fresh packaged produce. Second, there is a need for more research and validation on the suitability of PM-MAP for storage and quality retention of minimally processed pomegranate fruit arils before its ultimate application. Although PM-MAP has been successfully applied on various types of fresh produce, to the best of our knowledge, no work has reported the application of this technology in pomegranates. Hence, the research reported in this thesis focuses on the application of PM-MAP to preserve the postharvest quality and shelf-life of fresh minimally processed pomegranates (cv. Acco) grown in South Africa.

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CHAPTER THREE: Effects of number of perforations and storage temperature on water vapour transmission rate of synthetic polymeric and biodegradable films for applications in food packaging

EFFECTS OF NUMBER OF PERFORATIONS AND STORAGE TEMPERATURE ON WATER VAPOUR TRANSMISSION RATE OF SYNTHETIC POLYMERIC AND BIODEGRADABLE FILMS FOR APPLICATIONS IN FOOD PACKAGING

Abstract

Most of the commercial films used in fresh food packaging are characterized by high water vapour barrier, which often results in condensation inside the package. Under favourable storage conditions, the accumulation of water vapour inside package containing whole fresh or minimally processed produce results in accelerated microbial growth and deterioration. This study investigated the effects of number of perforations (0, 3, 6 and 9) and storage temperature (5, 10 and 15 ºC) on the water vapour transmission rate (WVTR) of one biodegradable and one synthetic polymeric film using the dry cup technique based on American society for testing and materials methods. Perforations were made using a 0.8 mm diameter needle and changes in weight of cups were monitored daily for 30 days. Number of perforation had greater influence on WVTR than temperature for both types of polymeric film. WVTR increased with increase in number of perforations and temperature. At 5 ºC and 0 perforations, WVTR was significantly higher for biodegradable (1.35 ± 0.05 g/m^2.day) than synthetic polymeric film (0.95 ± 0.13 g/m^2.day). However, there was an increase in permeability with the increase in number of perforations for both synthetic polymeric and biodegradable films. The effects of number of perforations and storage temperatures on WVTR were adequately described by a combination Arrhenius-exponential model. The model developed was validated for all perforations at 8 ºC, and a good agreement was observed between experimental and predicted data (R^2 = 93.8%). This study showed that perforation technique could be used to optimize the water vapour permeability properties of polymeric films for packaging of fresh produce and to minimize the incidence of vapour condensation.

Introduction

During postharvest storage and handling, the shelf-life of packaged food product is determined by a number of complex interactions between produce related-parameters and those associated with the external environment (Morillon *et al*., 2000; Siracusa, 2012). The interaction between packaged foods and the external environment result in subsequent modifications in chemical, physical, enzymatic or microbiological quality attributes of packaged produce which in turn affect shelf-life (Morillon *et al*., 2000). Thus, developing

optimum packaging and storage duration is essential in maintaining postharvest life of whole fresh and minimally processed fruit and vegetables.

Plastic polymers are increasingly used in packaging industry for both food and non-food applications (Mangaraj *et al*., 2009; Kirwan *et al*., 2011; Siracusa, 2012). The reasons for their success and rapidly increasing application, especially in food packaging industry, includes their versatility which makes them easily produced as flexible films or containers of various sizes and shapes (Mangaraj *et al*., 2009; Del-Valle *et al*., 2003; Kirwan *et al*., 2011; Siracusa, 2012). In addition, thermosetting or thermoplastic properties of plastic provide heat sealing, transparency, colour, heat resistance and barrier properties, which are remarkably suitable for the application of advanced packaging technologies such as modified atmosphere packaging (Morillon *et al*., 2000; Caner *et al*., 2008; Mangaraj *et al*., 2009; Kirwan *et al*., 2011).

Packaging plays a crucial role of reducing water loss of fresh produce by maintaining desirable humidity in the headspace atmosphere. However, excessive in-pack humidity may stimulate food spoilage, such as development of spoilage microorganisms and decay (Techavises & Hikida., 2008; Mistriotis *et al*., 2011). Nevertheless, depending on the rate of water permeation through the package, water is still lost owing to the difference in humidity between the internal and external atmospheres (Almenar *et al*., 2007). Therefore, a certain degree of film permeability is crucial to maintain gas and moisture composition within the package (Del Nobile *et al*., 2002; Del-Valle *et al*., 2004; Kirwan *et al*., 2011).

Modifying the permeability of the packaging film through perforation technique helps to optimize permeability of the packaging film and generates in-package equilibrium modified atmosphere (EMAP) to meet specific produce requirement (Del Valle *at al*., 2004; Caner *et al*., 2008; Briassoulis *et al*., 2013). Perforation modifies the permeability of packaging films by increasing rate of gases (O_2 and CO_2) exchange and water vapour transfer which in turn reduces the risk of spoilage and guarantee longer shelf-life of fresh produce (Dirim *et al*., 2004; Techavises & Hikida, 2008; Mistriotis *et al*., 2011; Briassoulis *et al*., 2013). The use of perforation enables various films to suit packaging applications of specific produce while ensuring the good preservation of produce (Pandey & Goswami, 2012). For instance, Dirim *et al*. (2004) investigated the effect of perforations (0.2, 0.5 and 1.75 mm diameters) on the water vapour transmission rate (WVTR) of synthetic low density polyethylene (LDPE) film. The authors reported a good correlation between WVTR (mg/h) and perforated area of investigated films at various temperature (10, 20, 30 ºC) and relative humidity (RH) (25, 30, 77 and 79%) conditions. Similarly, the work reported by Mastromatteo *et al*. (2012) showed different number of micro perforations (2, 7, 40 and 100 micro-holes per 50 cm^2 of package)

of varying diameters had a positive impact on water vapour transmission rate of polypropylene based films at 23 ºC and 90% RH.

As perforation technology becomes a more useful alternative to optimise the permeability of commercially available films, studies to investigate its effect in relation to other physical parameters is paramount. Therefore, the aim of this study was to investigate the water vapour transmission rate of two commercially obtained polymeric films commonly used in the fresh food industry. The specific objective was to investigate the effects of number of perforations and storage temperature on water vapour transmission rate (WVTR).

Materials and methods

The dry cup technique (ASTM, 2005) method E96-95 was used with slight modification to determine WVTR gravimetrically. Aluminium test cups (Diameter: 5.6 cm and depth: 1.5 cm) with open top-screw lid (Comar International, Cape Town, South Africa) were filled with 8.0 ± 0.5 g of anhydrous calcium chloride salt ($CaCl_2$). Samples of two polymeric films, synthetic POLYLID® 107 polyethylene (thickness 55 µm; WVTR, 4.2 g/m^2.day at 38 ºC, 90% relative humidity and 1 Bar) (Barkai Polyon Ltd, Kibbutz Barkai, Israel) and biodegradable 'Natureflex™', (thickness 55 µm; WVTR, 14 g/m^2.day at 38 ºC, 90% relative humidity and 1 Bar) (Innovia films Ltd, Wigton, Cumbria, UK), were used. Each film was placed on top of test cups, tightly closed exposing film surface area of 25 cm^2. Each cup was sealed by an O-ring rubber and grease to ensure airtight and moisture proof condition. Perforations 0 (control), 3, 6 and 9 (P-0, P-3, P-6 and P-9, respectively) were made using a 0.8 mm diameter sharp needle by manually piercing the film surface of each test cup at equi-distant positions (table 3.1). All the test cups were preconditioned at room temperature (20 ± 2 ºC) for 24 hours prior to making the holes. The set up was replicated in triplicate making a total of 36 cups for each studied film and stored at 5, 10 and 15 ºC and 90 ± 3% RH. All test cups were stored in an environmental test chamber (Sanyo Electric Co, Osaka, Japan) with controlled constant air movement. A control experiment without perforation was conducted for both polymeric films across all the temperatures. An additional experimental set-up at 8 ºC was included for model validation.

Weight gain of each test cup was measured every 24 h over a period of 30 days. The WVTR (g/m^2.day) of films was calculated on basis of mass gain in water by $CaCl_2$ salt in the test cup over time, using the following equation:

$$WVTR = \frac{W_t - W_i}{\Delta t} \times \frac{1}{\Delta p} \qquad (1)$$

where W_i is the initial weight of test cup (g); W_t is the weight of test cup at time and t (in days); and Δp is the differential water vapour pressure (kPa) across the test cup (Δp = 1; given the assumption that, the internal cup pressure and external atmospheric pressure was the same.

Table 3.1 Perforated area of sample films per test cup mouth area used in the experiment for analysis of water vapour transmission rate

Number of perforations	Perforation area (cm^2)	% perforated area (per 25 cm^2 of a test cup)
0	0	0
3	0.015	0.06
6	0.030	0.12
9	0.045	0.18

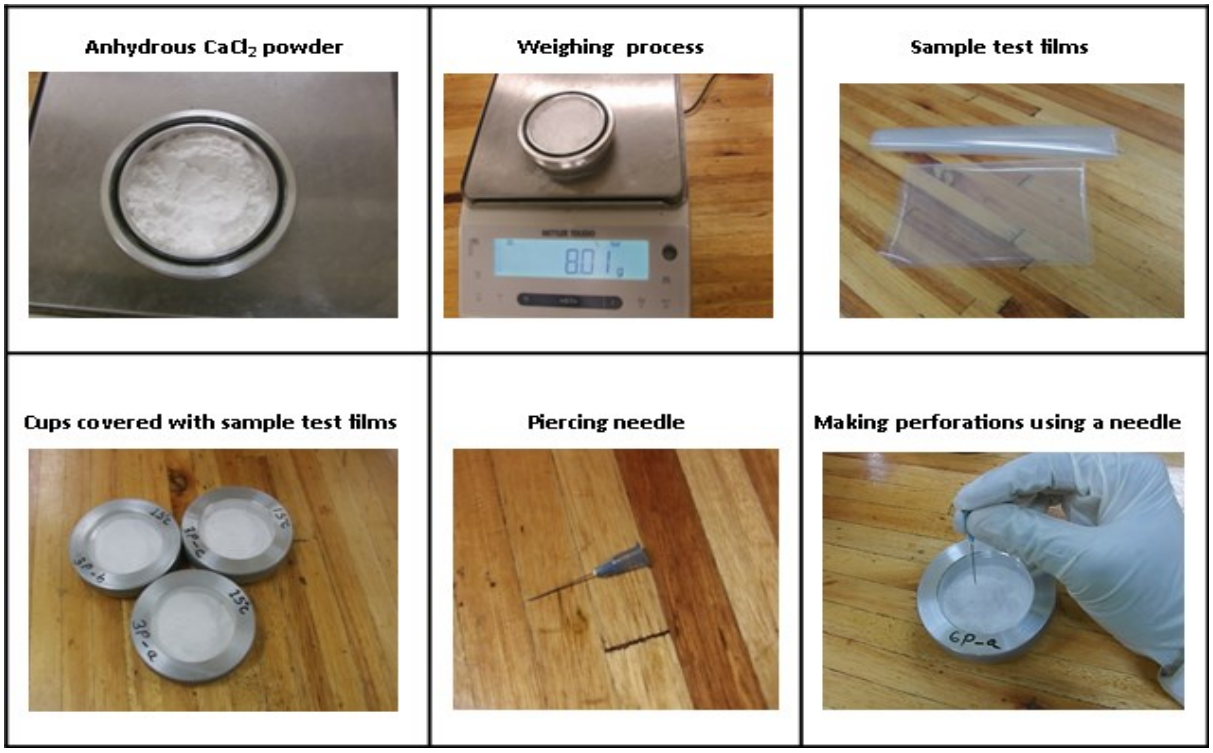

Figure 3.1 Pictorial presentation of experimental set up for the study of water vapour transmission rate of studied films, polylid and biodegradable.

Results and discussion

Comparison of biodegradable and synthetic polymeric film

For non-perforated samples (control), WVTR was significantly higher (p <0.05) in biodegradable films compared to synthetic polylid films, across all treatments (Figure 3.2). At all experimental temperatures, WVTR of biodegradable film was higher than the synthetic polylid by two-fold. For instance, at 10 ºC, the WVTR of polylid film was 0.75 g/m^2.day compared to 1.6 g/m^2.day for biodegradable film. These results support previous findings in the literature reported by Macedo *et al*. (2013), who reported that a biodegradable 'Natureflex N913' film had higher WVTR than a synthetic biaxial oriented polypropylene (BOPP) film by two-fold at different sets of experimental temperatures (10, 30, 40 ºC) and relative humidity (RH) (32.5 ± 0.5, 75.5 ± 0.5 and 92.5 ± 3.5%). Furthermore, in similar study reported by Koide and Shi (2007), the WVTR of polylactic acid (PLA) bio-based film was 100 times higher than that of the low density polyethylene (LDPE) film at the same temperature condition (10 ºC). The remarkably higher permeability values obtained in their study might be due to high hydrophilic nature of PLA film that influences water vapour permeability (Mistriotis *et al*., 2011). The observed increase in WVTR of biodegradable film could be attributed to the influence of hydrophilic character of biodegradable film due to the presence of cellulose materials (Yang & Paulson, 2000; Lucera *et al*., 2010; Mistriotis *et al*., 2011). Starch chains in biodegradable films are not sufficiently packed together such that they leave micro-pores that enable transmission of water molecules through the film (Arvanitoyannis *et al*., 1998). In contrast, synthetic polymeric films are semi-crystalline in structure and hydrophobic in nature (Arvanitoyannis *et al*., 1998), and thus there is no interaction between permeating water molecule and the hydrophobic film. This means that permeation of water vapour through non-perforated polylid film was influenced by water vapour pressure difference of both sides of the film (Morillon *et al*., 2000).

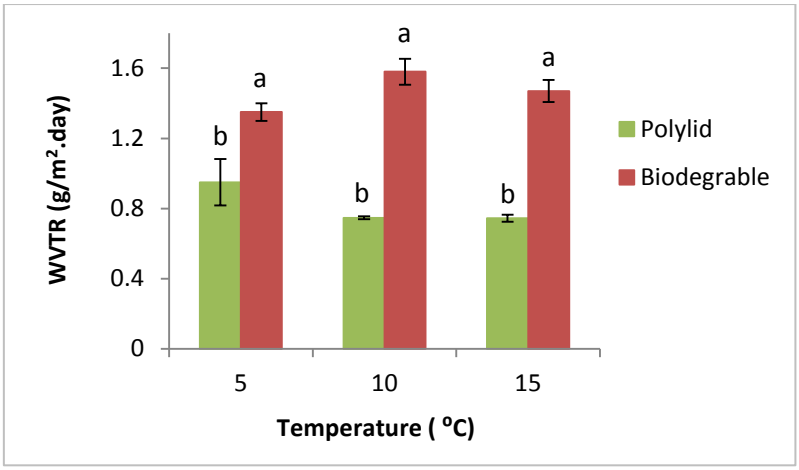

Figure 3.2 WVTR of non-perorated (P-0) films, polylid and biodegradable at 5, 10 and 15 ºC experimental temperature conditions. Error bars indicate a 95% confidence interval. Bars with the different letters are significantly different (p <0.05).

Nevertheless, the difference in WVTR between biodegradable and polylid was reduced from 49% in non-perforated (P-0) to 17%, 19% and 10% in P-3, P-6 and P-9, respectively due to the effect of perforations (Table 3.2). For instance, at 5 ºC, water vapour transmission rates (WVTRs) of non-perforated polylid and biodegradable films were 0.95 ± 0.1 and 1.35 ± 0.05 g/m^2.day, respectively. While with P-3 perforations polylid and biodegradable films were 5.2 ± 01 and 6.1 ± 0.1 g/m^2.day, respectively. The difference between the WVTR of non-perforated and perforated films could be attributed to the effect of perforation (Mistriotis *et al*., 2011), which enhanced the water vapour permeation for both polylid and biodegradable films. This is because perforations provide additional routes for the flow of water vapour through holes (Mistriotis *et al*., 2011; Mastromatteo *et al*., 2012). This provided additive effect to water vapour permeation for both polylid and biodegradable film.

Table 3.2 Water vapour transmission rate of biodegradable and synthetic polylid sample films at different storage temperatures. All results presented as mean (± SE)

Sample film	Temperature (ºC)	Water vapour transmission rate (g/m^2.day)			
		P-0	P-3	P-6	P-9
Biodegradable Nature flex	5	1.4±0.05^{j}	6.1±0.08^{h}	10.5±0.20^{f}	16.1±0.08^{e}
	10	1.6±0.24^{j}	8.5±0.15^{g}	17.2±0.02^{d}	21.4±0.12^{b}
	15	1.5±0.06^{j}	10.5±0.09^{f}	20.1±0.04^{c}	26.1±0.45^{a}
Synthetic polylid	5	0.9±0.13^{k}	5.2±0.13^{i}	9.9±0.21^{fg}	15.2±0.51^{e}
	10	0.7±0.01^{k}	6.5±0.42^{h}	13.3±0.28^{i}	19.3±0.32^{c}
	15	0.7±0.02^{k}	8.9±0.00^{g}	15.6±0.33^{e}	22.5±0.46^{b}

Mean values with different letters are significantly different (p <0.05), according to Duncan's multiple range test.

Effect of perforations and temperature on WVTR of films

Increasing the number of perforations had a higher impact on measured WVTR (g/m^2.day) than increasing storage temperature for both polylid and biodegradable films as shown in Pareto chart (Figure 3.3). For instance, increasing the number perforations from P-3 to P-9 increased the WVTR of polylid by 164.5, 151.8 and 147.7 % at 5, 10 and 15 ºC, respectively. An increase in storage temperature from 5 to 15 ºC increased the WVTR by 63, 103.7 and 62.3 % for P-3, P-6 and P-9, respectively. Additionally, at 95% confidence level as shown by line crossing over the bars that represent standardised effects (Figure 3.3), both temperature and perforation and their interaction had significant effects on WVTR. Our results corroborated previous findings by Dirim *et al*. (2004) who observed an increase in WVTR of low density polyethylene (LDPE) film (38.5 cm^2 surface area) with increase in number of perforations of 0.2 mm diameter from 2.04 mg/h (1 perforation) to 3.84 mg/h (24 perforations). Similar findings were reported by Mastromatteo *et al*. (2012) who investigated the effects of different number of micro-perforations (2, 7, 40, 100) and diameters (50, 70, 90, 110 µm) on water vapour permeability of polypropylene based films. The authors revealed that water vapour permeability of films increased with increase both in diameter and number of perforations per unit area. As perforations offer a much lower resistance to the movement of water vapour compared to the bulky polymeric matrix, increase in number of perforations per unit area increases rate of permeation of water vapour (Mastromatteo *et al*., 2012).

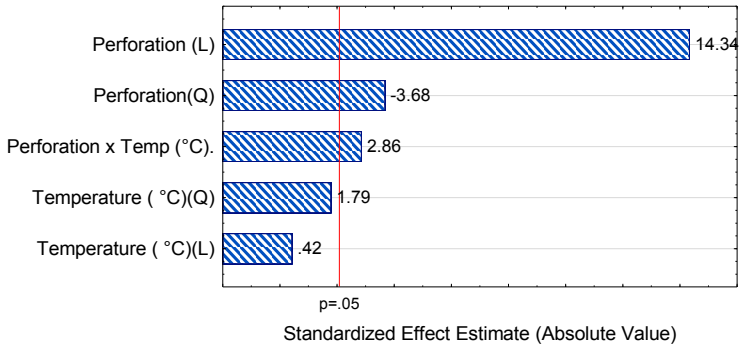

Figure 3.3(a) Standardised Pareto chart showing the effect of temperature, perforation and their interaction on WVTR (g/m^2.day) of polylid film.

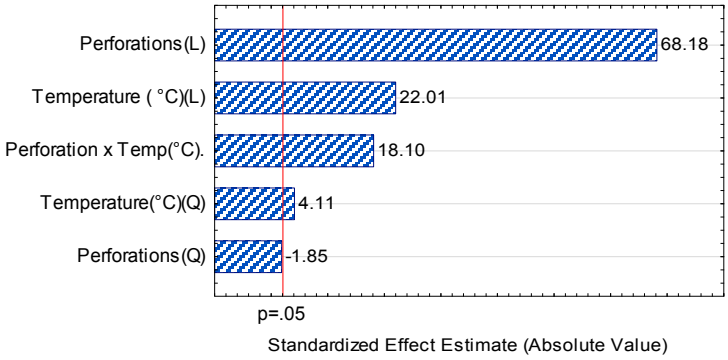

Figure 3.3(b) Standardised Pareto chart showing the effect of temperature, perforation and their interaction on WVTR (g/m^2.day) of biodegradable film.

Furthermore, the effects of temperature were much pronounced at higher number of perforations (P-6 and P-9) for both polylid and biodegradable films stored at 10 ºC and 15 ºC (Figure 3.4). The effects of temperature on water vapour permeability was also investigated by Techavises and Hikida (2008) who reported an increase in WVTR of LDPE film from 96.5 to 105.1 g/day kPa when the storage temperature increased from 5 to 15 ºC for 5 mm diameter perforations. However, the authors observed higher permeability values in comparison to this study, probably due to the use of the macro perforation tubes of larger diameter (0.05 mm) in their study. Similarly, Mahajan *et al*. (2008) also reported an increase in WVTR from 0.045, 0.086 and 0.152 to 0.049, 0.124 and 0.19 g/day kPa for 9, 13 and 17 mm perforation diameters, respectively. Furthermore, the interaction effect of number of perforations x storage temperature on WVTR was significant (p <0.05) for both synthetic polylid and biodegradable film samples. Contrary to these results, Mahajan *et al*. (2008) reported a negative interaction effect between perforation tube diameter x tube lengths, and tube lengths x storage temperature while the interaction of tubes diameter x storage temperature was not significant (p >0.05).

The biodegradable film investigated in the present study generally showed a relatively higher response to changes in storage temperature as evidenced by higher permeability values compared to polylid (Figure 3.4). These results are comparable to those reported by Macedo *et al*. (2013) who found that temperature (10, 20 and 30 ºC) had higher effects on WVTR of three types of biodegradable Innovia (NatureFlex^{TM}) films than BOPP film. The authors also found that the effects of temperature were more pronounced at higher humidity conditions (75.5 ± 0.5 and 92.5 ± 3.5% RH). Similar observation was reported by Lucera *et al*. (2010) who reported that the WVTR of bio-polymeric film (COEX) with the exposed film surface area of 50 cm^2 was about two-folds higher than polypropylene based (OPP) at 23 ºC and 85% RH. It has been established that changes in temperature affect the diffusion and

solubility coefficient of permeating molecules (Morillon *et al*., 2000; Bertuzzi *et al*., 2007). As a result, increase in temperature causes a slight decrease in solubility coefficient and subsequent increase in diffusion coefficient resulting from enhanced movement of polymer segments and increased energy level of permeant molecules (Bertuzzi *et al*., 2007). These mechanisms explain the prospects for an increase in temperature to promote permeation of water vapour across MAP films.

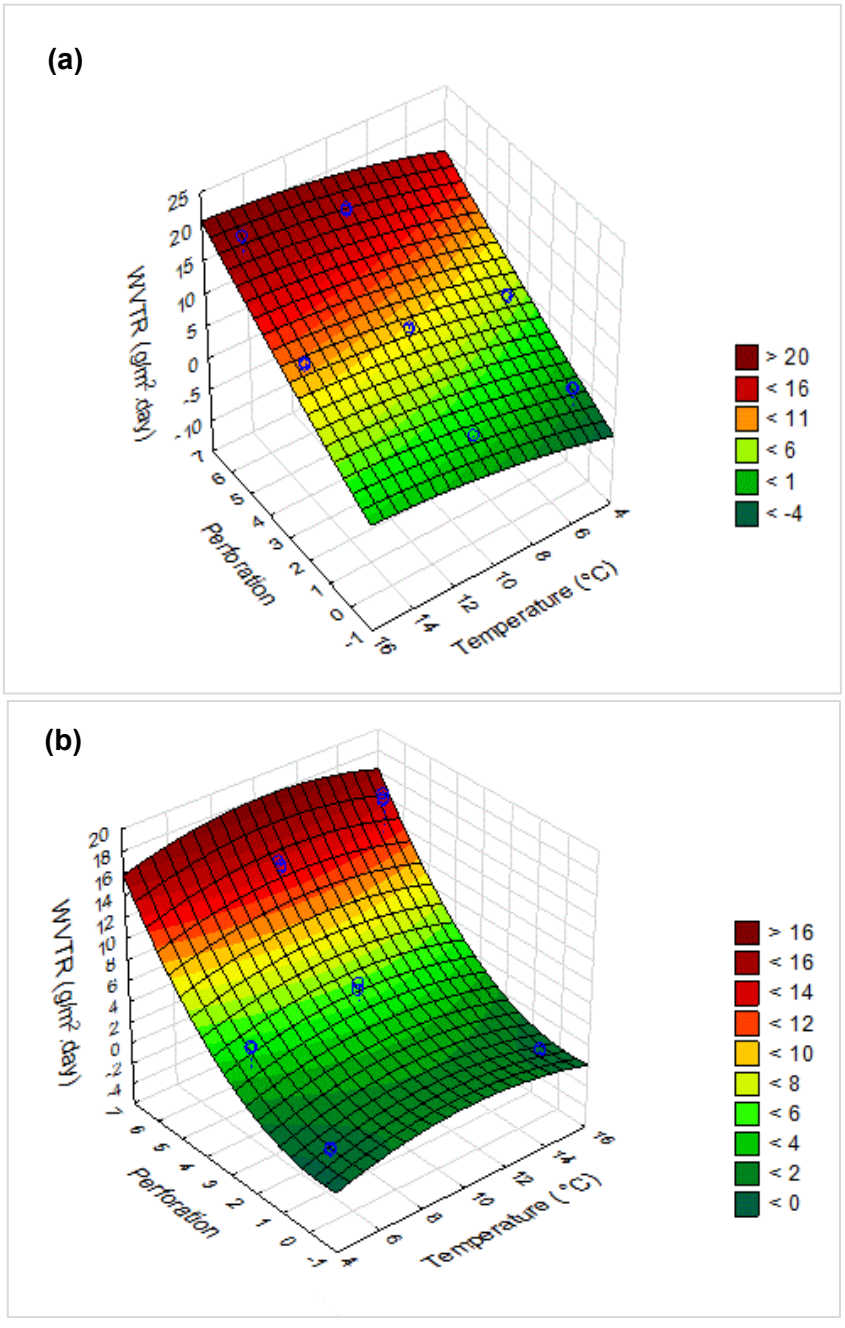

Figure 3.4 The surface fitted plots displaying the effects of number of perforations and storage temperature on WVTR (g/m^2.day) for biodegradable (a), and polylid film (b) at different storage temperature conditions.

Model building

An exponential equation describing the effect of number of perforations on WVTR was used to fit the experimental data as describe in Eq. 2:

$$WVTR = a \times e^{b \times N_i} \quad (2)$$

where *a,* and *b* were estimated parameters, N_i is the number of perforations. The model was found to fit the experimental data with R^2 = 99.8% (Figure 3.5) and it adequately described the effects of number of perforations on WVTR (g/m^2.day).

Furthermore, an Arrhenius-type equation which describes change in storage temperature as a function of WVTR (Mahajan *et al*., 2008) was applied in model fitting as presented in Eq. 3

$$WVTR = \left[1 \times \frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad (3)$$

where *WVTR* is the water vapour transmission rate (g/m^2.day), at temperature (*T*, K), *T* is the storage temperature (K), *R* is the universal gas constant (0.008314 kJ/ K.mol), E_a is the estimated activation energy of water vapour transmission (kJ/mol), and T_{ref} is the reference temperature (i.e. average of the experimental storage temperatures = 283 K).

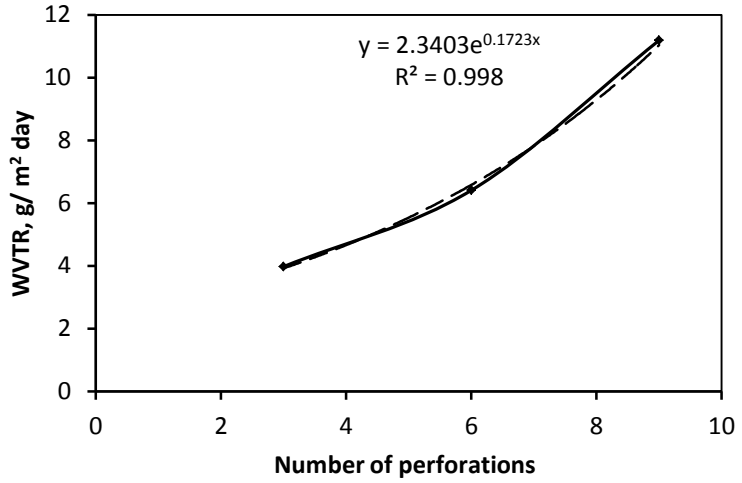

Figure 3.5 Water vapour transmission rate (WVTR) as the function of number of perforations.

In order to understand the combined effects of temperature and number of perforations, Eq. 2 and 3 were combined to build a secondary model Eq. 4:

$$WVTR = \left[1 \times \frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \times a \times e^{b \times N_i} \quad (4)$$

Parameter estimate E_a and model constants *a* and *b* were estimated using the global equation (4). Data were analysed using solver analysis in Statistica software (Statistica, 11.0, Statsoft, USA). The estimated model parameters and other statistical data are shown in Table 3.3. The global model adequately predicted (R^2 = 93.9%) the combined effects of number of perforations and storage temperature on the WVTR for both biodegradable and polymeric films as shown in Figure 3.6.

Table 3.3 Parameters of the mathematical model Eq. (4) describing the effect of temperature and number of perforations on the water vapour transmission rate of perforated films

E_a (kJ mol^{-1})	a	b	R^2 (%)
64.88	3.07	0.14	93.88

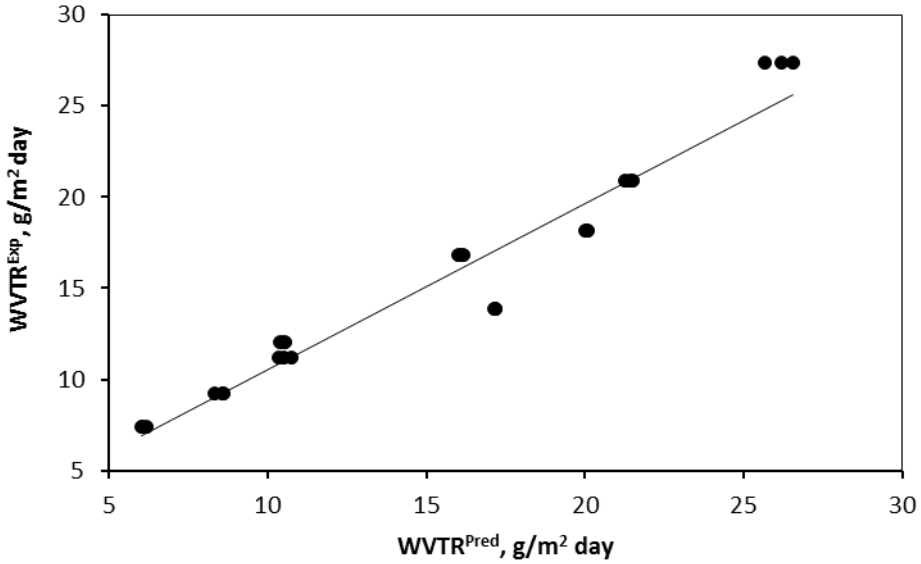

Figure 3.6 Cluster graph describing the relationship between calculate experimental and predicted changes in WVTR in test cup during storage.

The model parameters *a* and *b* for WVTR shown in Table 3.3 differ from the values 0.0077 and 1.68, respectively, reported by Mahajan *et al*. (2008). These differences could be attributed to variations in storage conditions as well as differences in perforation methods. The work done by Mahajan *et al*. (2008) used tubes of different diameters (9, 13, 17 mm) and lengths (10, 20, 30 mm) which were inserted into the lid of the test cup to make the desired perforations. In their study to analyse the effect of external turbulence on the gas

exchange rate through small perforations, Montanez *et al*. (2010) reported that air circulation patterns created by the refrigeration system could have an impact on the permeability rate of CO_2 and O_2. This could be attributed to the use of test cups that were not enclosed in air-tight containers.

Furthermore, the estimated E_a value in the current study (Table 3.3) was higher than those reported by Mahajan *et al*. (2007) and Bedane *et al*. (2012). This difference in activation energy could be attributed to the type of packaging material and perforation techniques used as well as the number and size of perforation. The current study shows an increase in permeability with increase in temperature and perforation, according to the Arrhenius-exponential type of dependency (Figure 3.7). This indicated positive E_a and is consistent with the diffusion dominated processes for water permeation through the samples (Bedane *et al*., 2012). The higher the E_a, the more temperature-dependent of the film property is the permeation process (Koo *et al*., 2005). That means, for polymeric films whose permeation processes are temperature-dependent, their diffusion and permeation rates increase with increase in temperature (Bertuzzi *et al*., 2007; Bedane *et al*., 2012). Thus, underestimation or overestimation of E_a during modelling process could lead to incorrect prediction of the rate of water vapour permeability through packaging film.

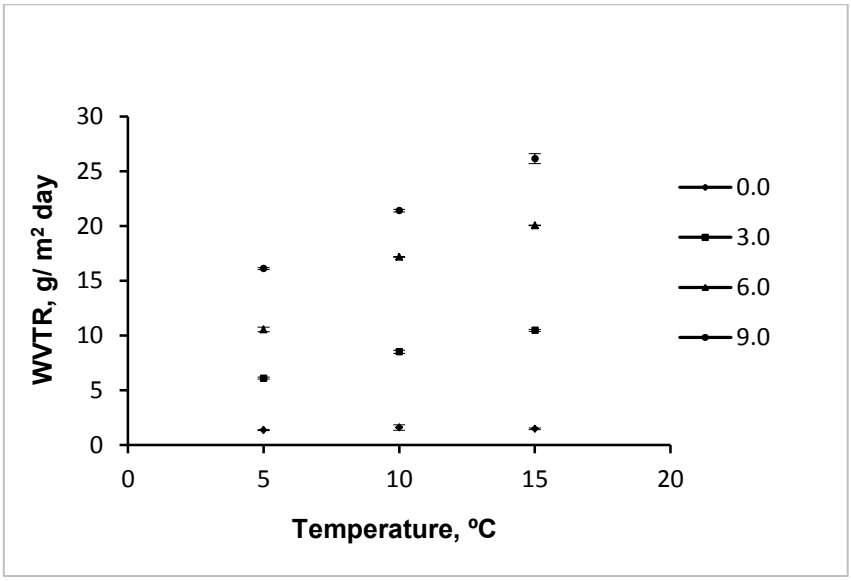

Figure 3.7 Effect of temperature on water vapour transmission rate (WVTR) for P-0, P-3, P-6 and P-9 perforations of polylid film samples.

Conclusion

This study has revealed that water vapour permeabilities of non-perforated polylid and biodegradable sample films were significantly different, such that the WVTR of biodegradable film was two-fold higher than that of polylid at all storage conditions. Based on these results, biodegradable starch-based polymers such as Natureflex^{TM} may be preferred due to their good permeability to water vapour. However, with the use perforations, WVTR rate of polylid film was reduced from 49% in non-perforated (P-0) to 17%, 19% and 10% in perforated films with 3, 6 and 9 perforations, respectively.

Perforation and temperature had a positive effect on water vapour permeability of both studied films, such that WVTR of polylid and biodegradable films increased with increased number of perforations and storage temperatures. The better water vapour permeability results obtained with perforation highlight the potential of this technology for optimising permeability of synthetic polymeric films such as polylid that are predominantly used in the food industry. Furthermore, application of the global model developed to predict WVTR of perforated films gave good correlation between the experimental data and predicted values (R^2 = 93.8%), indicating the potential practical relevance for in the design of perforation-mediated MAP. Overall, findings reported in this study show that the application of perforation to optimise film permeability to water vapour presents a low-cost alternative to high cost biodegradable films in the fresh produce industry.

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CHAPTER FOUR: Perforation-mediated modified atmosphere packaging (PM-MAP) and storage duration affect physico-chemical properties and microbial quality of fresh minimally processed 'Acco' pomegranate arils

PERFORATION-MEDIATED MODIFIED ATMOSPHERE PACKAGING (PM-MAP) AND STORAGE DURATION AFFECT PHYSICO-CHEMICAL PROPERTIES AND MICROBIAL QUALITY OF FRESH MINIMALLY PROCESSED 'ACCO' POMEGRANATE ARILS

Abstract

This study investigated the effects of the number of perforations (P: 0, 3, 6 and 9 per 160.1 cm^2) and storage duration on the physico-chemical quality attributes and microbiological quality of fresh minimally processed pomegranate fruit arils (*Punica granatum* L., cv. Acco) Pomegranate arils (100 g) were packaged in polypropylene trays and heat sealed with polymeric film of known permeability property. Perforations were made on the film using a 0.8 mm diameter sterile needle and packaged arils were stored at 5 ºC for 14 days. Arils were analysed for physico-chemical quality attributes at 3 d intervals over 14 days. Microbial analyses for aerobic mesophilic bacteria, yeast and moulds were made on 0, 7 and 14 d, and the presence of *Escherichia coli* was tested before and at the end of storage. Results showed that headspace gas composition was significantly influenced by number of perforation. Highest CO_2 accumulation was observed in non-perforated MAP, while O_2 composition increased with an increase in the number of perforations in PM-MAP. The measured physico-chemical quality attributes were influenced by the PM-MAP and storage duration. The lowest of pH 3.6 ± 0.2 was measured for arils packaged in P-3 after 14 days. PM-MAP packaged arils showed higher percentage loss in weight. The use of PM-MAP maintained overall aril colour, although chroma (*C**) and hue angle (*h°*) values decreased slightly across all treatments during storage. Total soluble solid (TSS) decreased across all treatments but higher decrease in TSS observed in PM-MAP from 15.4 ± 0.02 to 13.9 ± 0.2, 13.5 ± 0.4 and 13.1 ± 0.4 ºBrix for P-3, P-6 and P-9 PM-MAP packaged arils, respectively. Titratable acidity (TA) decreased from 1.1 ± 0.01 mg CA/100 mL to 0.7 ± 0.02 and 0.4 ± 0.05 mg CA/100 mL in P-0 and MP-MAP packaged arils, respectively after 14 d storage as the result of increased metabolic activities. Among perforated packages, aril firmness was highest in in P-3 MAP (6.2 ± 0.3N). The highest counts of aerobic mesophilic bacteria (5.5 log CFU/g) and yeast and moulds (5.3 log CFU/g) were observed in P-0 and P-9 PM-MAP. No *E. coli* were detected before and after storage. Overall, P-3 and P-6 MP-MAPs better maintained quality attributes than in P-0 and P-9. Thus, MAP with the appropriate number of perforations (in this study 3 or 6 perforations per 160.1 cm^2) could be a useful tool to avoid excessive CO_2 accumulation or anoxic state, thereby maintaining microbial and overall quality of minimally processed pomegranate arils.

Introduction

Pomegranate (*Punica granatum* L.) fruit consumption has increased in time due in part to its unique sensory and nutritional properties coupled with medicinal benefits attributed to high content of phytonutrients and antioxidant properties (Opara *et al.,* 2008; Hassan *et al*., 2012). Minimally processed ready-to-eat pomegranate arils have high economic importance due to their convenience, healthiness and their desirable sensory characteristics as compared to whole produce, which poses difficulties in extracting the arils (Defilippi *et al*., 2006; Artes *et al*., 2007; Caleb *et al*., 2012). Nevertheless, the shelf-life of pomegranate arils is shorter than that of the whole fruit. While the later can be stored for 3-4 months at temperatures below 10 ºC (Artés *et al*., 2000; Nanda *et al.*, 2001; Ghafir *et al*., 2010; Fawole & Opara, 2013b), pomegranate arils can last for a period of 1-2 weeks when stored under 5 ºC (López-Rubira *et al*., 2005; Ayhan & Estürk, 2009; Caleb *et al*., 2013). Fresh-cuts or minimally processed produce are highly susceptible to microbial growth (via the cut surfaces and juice exudates), enzymatic disorders and physiological response, which limit the shelf-life (Farber *et al*., 2003; Ragaert *et al*., 2007).

Modified atmosphere packaging (MAP) is a postharvest tool used to preserve quality of various fresh whole and minimally processed fruits and vegetables (Sepúlveda *et al*., 2000; López-Rubira *et al*., 2005; Ayhan & Estürk, 2009; Caleb *et al*., 2012). Modified atmosphere packaging relies on the dynamic process of alteration of gaseous composition inside a package, determined by permeability of packaging film and produce respiration (Mahajan *et al*., 2007). However, barrier properties to gases (O_2 and CO_2) and water vapour limit MAP applicability of many commercial polymeric films (Mangaraj *et al*., 2009). Conventional (passive MAP) that uses high barrier polymeric films are characterized by the generation of unsuitable in-package gas composition, condensation of water vapour and subsequent microbial growth causing loss of quality and impaired shelf-life (Lucera *et al*., 2010; Mistriotis *et al*., 2011).

Hence, perforation-mediated modified atmosphere packaging (PM-MAP) offers the possibility of optimising polymeric films in order to compensate for barrier limitations. Studies have reported successful use of PM-MAP on quality preservation and extension of shelf-life of various fresh whole and minimally processed fruit and vegetables such as mandarin (Del-Valle *et al*., 2009), fresh-cut apple slices (Cliff *et al*., 2010), Litchti (De Reuck *et al*., 2009), strawberries (Almenar *at al*., 2007; Kartel *et al*., 2012), Fresh sliced mushroom (Oliveira *et al*., 2012) and broccoli (Fernández-León *et al*., 2013). To reduce losses, maintain quality and prolong shelf-life of minimally processed pomegranate arils, the need for researching appropriate packaging technology is paramount. The purpose of this study was to

investigate the effects of PM-MAP technology on the postharvest quality attributes and microbial safety of minimally processed pomegranate fruit (cv. Acco).

Materials and methods

Plant material and sample preparation

Pomegranate fruit (cv. Acco*)* grown in a commercial orchard located in Porterville, Wellington area (33°38'S 18°59'E), of the Western Cape, in South Africa, were harvested manually at commercial maturity based on external colour of the fruit peel, total soluble solids (TSS) (15.4 ºBrix), titratable acidity (TA) (1.1 mg CA/100 mL) and TSS:TA ratio (13.6). Fruits were sorted manually to get rid of damaged ones, and healthy fruits were washed in sodium hypochlorite (NaOCl) solution (200 mg/L). Sterilized fruit were aseptically hand processed by carefully removing arils (without crushing) under cool temperature (6 ºC). Approximately 100 g of fresh arils were packaged in polypropylene (PP) trays (10.6 x 15.1 cm^2) and heat sealed using a semi-automated machine (Food Packaging Equipments, Cape Town, South Africa) with a polymeric film POLYLID® 107 polyethylene (thickness 55 µm; carbon dioxide transmission rate, 600-700 mL/m^2.day; oxygen transmission rate, 130-150 mL/ m^2.day and water vapour transmission rate, 4.2 g/m^2.day at 38 ºC, 90% relative humidity and 1 Bar) (Barkai Polyon Ltd, Kibbutz Barkai, Israel). Heat sealed films were manually perforated using sterilized needle (0.8 mm diameter) with 3, 6 and 9 number of perforations to obtain P-3, P-6 and P-9 on film surface of 160.1 cm^2. Non-perforated film (P-0) was used as a control while clamshell tray was included to simulate traditional packaging of fresh pomegranate arils in the South African market. Labels of 7.0 x 3.8 cm^3 area were placed onto each package film to simulate retail conditions. At the processing facility, packaged products were cooled down to 2 ºC, and packed in sterile cooler boxes with dry ice to maintain low temperature during transportation from the processing facility to the Postharvest Technology Research Laboratory at Stellenbosch University. Boxes were fitted with data loggers (Gemini Data Loggers, West Sussex, UK) to monitor the cold chain. On arrival, the packaged samples were stored at 5 ºC and 95 ± 2% relative humidity (RH) for 14 days. A baseline analysis to investigate the microbial and physico-chemical properties of pomegranate fruit samples was conducted on fresh arils prior to storage. Sampling for further physico-chemical analyses was taken at 3 d intervals during 14 d of storage while microbial analysis included tests for *Escherichia coli*, aerobic mesophilic bacteria, yeast and moulds at days 0, 6, 10 and 14. Three packages were analysed for each experimental condition on each day.

Changes in gas composition during storage

Before opening the package on each sampling day, gas (CO_2 and O_2) composition inside the packages was determined using a gas analyser with an accuracy of 0.5% (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Levels of CO_2 and O_2 were presented in percentage (%). Immediately after gas analysis, each package was reweighed before the physico-chemical properties of aril were analysed.

Analysis of physico-chemical properties

Weight loss

Weight of each pack of arils was measured using an electronic weighing balance (ML3002.E, Mettler Toledo, Switzerland) with an accuracy of ± 0.01g. Using the initial weight of sample recorded prior to storage, weight loss (*WL*) was obtained using the following equation:

$$WL = \frac{W_o - W_f}{W_o} \times 100 \qquad (1)$$

where, *WL* is the weight loss (%), W_0 is the initial weight (g) and W_f is the final weight (g) prior to package analysis.

Texture

Firmness of individual arils was measured using texture analyser (TA-XT Plus, Stable Micro Systems, England, UK) by compression using a 35 mm diameter cylindrical probe, set at compression strain of 60% and 9.5 mm distance to rupture the aril membrane. The probe was set at a speed of 1.0 and 10.0 mm/s for test and post-test, respectively. Averages of 15-20 arils were tested for each treatment. Firmness was expressed as maximum compression force in Newton (N).

Colour

Colour of arils was determined on basis of CIE *L*a*b** colour system by Commission International del' Eclairage (CIE) measured using a digital colour meter (Minolta Chroma Meter, CR-400, Japan). Calibration of the colour meter was performed against a white tile background (Illuminants C: Y = 89.53, x = 0.3247, y = 0.3198) prior to each measurement.

Arils were spread to cover a petri dish and colour measurements were taken from 5 different points of the dish. Colour parameters, lightness (*L**), redness/greenness (*a**) and yellowness/ blueness (*b**) were measured and means of all measurements were determined for each package type. Using software Exponent v.4 (Stable MicroSystem Ltd., Godalming, UK), colour measurement data were interpreted and the results presented as the mean (±S.E) of the number of measurement taken. Chroma (*C**) values, which indicate the quantitative attribute of colour intensity and hue angle (*h*°) which is considered as the qualitative attribute of colour of samples were calculated using equations (2) and (3), respectively (Mangaraj *et al*., 2011; Pathare & Opara, 2013).

$$C^* = \sqrt{a^{*2} + b^{*2}} \qquad (2)$$

$$h^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right) \qquad (3)$$

Titratable acidity, pH, and total soluble solids

Pomegranate juice (PJ) was prepared from arils of each pack separately using LiquaFresh juice extractor (Mellerware, South Africa). PJ pH and total soluble solid (TSS) were measured using a pH meter (Crison, Barcelona, Spain) and digital refractometer expressed as ºBrix (Atago, Tokyo, Japan), respectively. Titratable acidity (TA) of aqueous diluted PJ was determined potentiometrically by titration with 0.1N NaOH (Merck) to an end point of pH 8.2 using a Metrohm 862 compact auto titrosampler (Herisau, Switzerland). Titratable acidity was expressed as milligrams of citric acid (CA) per a hundred millilitres of crude PJ (mg CA/100 mL).

Analysis of microbial quality

Microbiological stability of pomegranate arils was analysed according methods described by Caleb *et al.* (2013), for aerobic mesophilic bacteria and yeast and moulds. Plate count agar (PCA) was used for aerobic mesophilic bacteria, while yeast and mould counts was characterised using potato dextrose agar (PDA) acidified with 10% tartaric acid. For indicator microorganisms *Escherichia coli* were analysed using tryptone bile x-glucuronide (TBX) agar. Packages for this purpose were opened under sterile conditions, and 10 g of pomegranate arils from each sample was obtained aseptically and homogenized with 100 mL of physiological sterile solution (PSS) (8.5 g/L). The three-fold dilutions were prepared using 1.0 mL of homogenised sample into 9.0 mL of PSS. To enumerate microbial load of samples, 1.0 mL of each dilution was pour-plated in triplicate onto appropriate prepared

media, PCA, PDA and TBX agars for aerobic mesophilic bacteria yeast and mould counts and *E. coli*, respectively. Plates for aerobic mesophilic bacteria and *E.coli* were incubated for 2 days at 37 ºC and 35 ºC, respectively, while yeast and moulds plates were incubated at 26 ºC for 5 days. After incubation, colonies grown on plates were counted (only between 30-300 colonies), and results presented as log of colony-forming units per gram (log CFU/ g) of sample arils.

Statistical analysis

All experimental were data obtained subjected to factorial analysis of variance (ANOVA) at 95% confidence interval using Statistical software (Statistica 12.0, Statsoft, USA). Main effects (number of perforations and storage duration) and interaction effects (number of perforations x storage duration) were assessed using Pareto analysis at 95% confidence interval. Post-hoc test (Duncan's Multiple Range Test) was used to test for statistical significance such that observed differences at p <0.05 were considered significant.

Results and discussion

Weight loss

Generally, weight loss of packaged arils increased progressively over storage time. Slight percentage increase in weight was observed for arils packaged in P-0 MAP after day 3 and in clamshell trays up to day 9 (Figure 4.2). Increase in weight in these packages could be the result of in-package water vapour condensation from evaporating aril surface moisture as shown in Figure 4.1. Similar results was reported by Caleb *et al*. (2013), who observed an initial increase in weight of MAP packaged pomegranate arils (cvs. Acco and Herskawitz) stored at 5 and 10 ºC. At the end of storage, changes in weight was higher in perforated packages, where 1.9, 4.4 and 6.2% weight loss were recorded in P-3, P-6 and P-9 PM-MAP packaged arils, respectively. However, previous findings reported by Caleb *et al*. (2013) revealed 0.53% weight loss of fresh MAP packaged arils (cv. Acco) after 14 d of storage at 5 ºC. Progressive decrease in weight of arils packaged in PM-MAP (P-3, P-6 and P-9) could be attributed to moisture loss in arils through perforations. As reported by Lucera *et al*. (2010), high water vapour permeability enhanced by perforations of MAP film enhance water uptake from packaged produce by evaporation, resulting into weight loss. Bhatia *et al*. (2013) observed higher physiological loss in weight of minimally processed 'Mridula' pomegranate arils packaged in low density polyethylene (LDPE) and KPA bags of higher water vapour permeability over those in polypropylene bags.

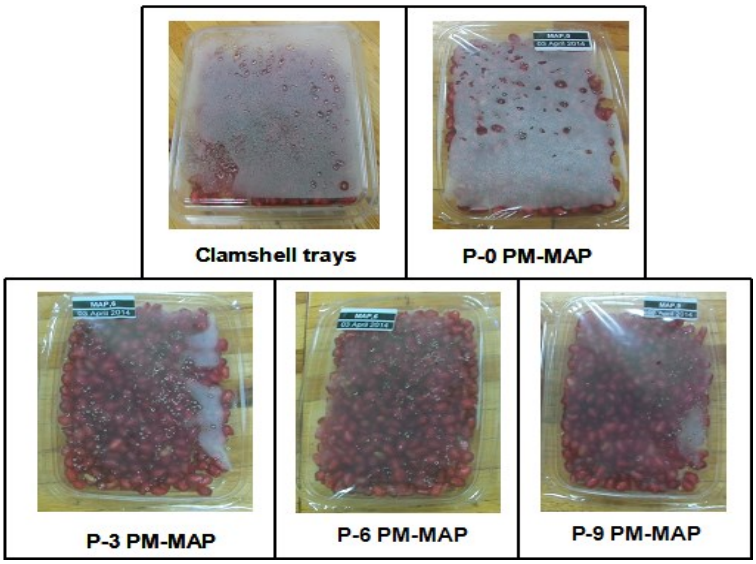

Figure 4.1 Water vapour (moisture) on surface of pomegranate arils packages at day 9 during cold storage (5 ºC). All respective packages had similar trend of moisture condensation at the end of storage.

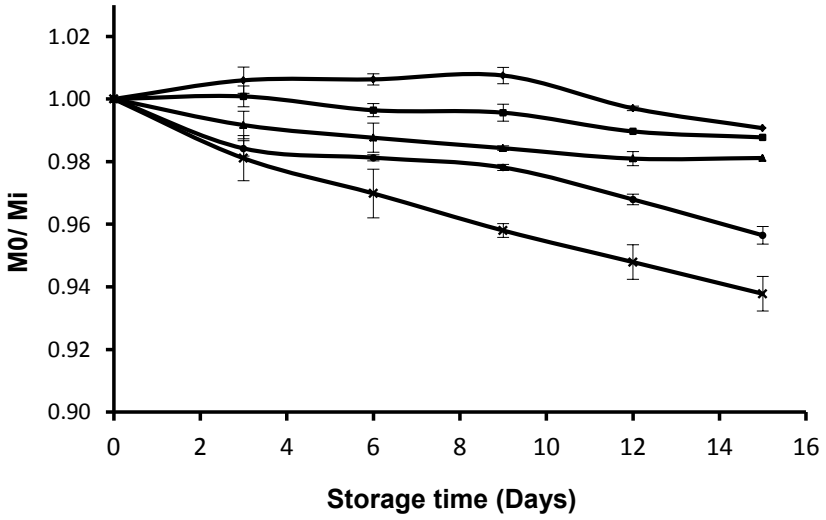

Figure 4.2 Changes in weight of PM-MAP-packaged pomegranate arils cv. 'Acco' over storage duration (Normalised values with respect to the initial weight of pomegranate arils, Mi in g); ♦ = Clamshell trays; ■ = P-0; ▲=P-3; ● = P-6; x = P-9. Error bars indicate a 95% confidence interval.

Gas compositions within packages

During cold storage, gas composition changed significantly in all packages (p <0.05) as shown in Table 4.1. In non-perforated (P-0) packages, O_2 composition decreased progressively to critical level (1.3%) by day 15. This level of O_2 is detrimental to produce as it favours fermentative anaerobic reactions leading to off odours and off flavours (Almenar *et*

al., 2007; De Reuck *et al*., 2009). Similar results were reported by Almenar *et al*. (2007) who observed critical level of O_2 (>5%) in strawberries packaged in MAP cups without micro-perforations after 6 days of storage. Clamshell trays had the highest level of O_2 (19.2%) while perforated packages P-3, P-6 and P-9 PM-MAP maintained higher level of O_2 of 17.6%, 18.6% and 18.4%, respectively, at the end of storage. Poor hermetic seal of clamshell trays enhanced non-resistant flow of O_2 in trays while perforations in PM-MAP influenced permeation and accumulation of O_2. Changes in O_2 in PM-MAP were consistent with previous report by Almenar *et al*. (2007) who observed increase in O_2 concentration (5-18%) in 125 mL-capacity MAP cups of strawberries with one and three perforations (~100 µm diameter). On the contrary, the composition of CO_2 increased in all packages after 3 days of storage, with P-0 packages having the highest CO_2 accumulation and 9.6% and 34.0 % on day 3 and 15, respectively. Clamshell had the lowest level of CO_2 which stabilised throughout the storage period. This could be attributed to the known poor hermetic seal of clamshell trays (Caleb *et al*., 2013). The level of CO_2 in perforated packages decreased significantly at the end of 14 days in storage with increase in number of perforations, from 1.3% for P-3 to 0.6% and 0.7% for P-6 and P-9, respectively. The lack of difference in CO_2 composition between P-6 and P-9 packages could be due to erroneous variation in size of perforations that may lead to considerable small deviation of in-package atmosphere achieved. Perforations enhanced the permeability of the polymeric film and prevented the accumulation of CO_2 in PM-MAP. De Reuck *et al*. (2009) revealed that increase in number of perforations from 4 to 10 increased headspace O_2 and reduced CO_2 concentration (~17% O_2 and ~5% CO_2) for PM-MAP of litchi (cv. Mauritius). However, the authors did not describe the size of exchange area through the MAP film. Similar observations were reported on MAP of semi-permeable films of minimally processed 'Wonderful' pomegranate (Sepulveda *et al*., 2000).

Table 4.1 Changes in package headspace gas composition during postharvest cold storage (5 ºC) of fresh pomegranate arils. All results presented as mean values (± SE)

Gas Composition (%)	PM-MAP Type	Storage duration (Days)					
		0	3	6	9	12	15
O_2	Clamshell	19.6±0.10^{a}	19.1±0.03^{bcd}	19.1±0.05^{bcd}	19.0±0.03^{cde}	19.0±0.11^{cde}	19.2± 0.05^{abc}
	P-0	19.5±0.02^{ab}	8.7±0.10^{j}	5.9±0.10^{k}	2.7±0.10^{i}	1.7±0.10^{m}	1.3±0.30^{m}
	P-3	19.5±0.05^{a}	18.7±0.10^{d-g}	18.4±0.2^{gh}	18.1±0.10^{h}	17.5±0.41^{i}	17.6±0.20^{i}
	P-6	19.6±0.01^{a}	18.8±0.01^{c-g}	18.5±0.10^{fgh}	18.5±0.20^{gh}	18.6±0.03^{d-g}	18.6±0.10^{efg}
	P-9	19.5±0.10^{a}	19±0.10^{cde}	18.7±0.10^{c-g}	19±0.03^{c-f}	18.6±0.10^{d-g}	18.4±0.30^{gh}
CO_2	Clamshell	0.04±0.00^{h}	0.1±0.03^{h}	0.2±0.03^{h}	0.2±0.03^{h}	0.3±0.14^{h}	0.1±0.10^{h}
	P-0	0.05±0.00^{h}	9.6±0.14^{e}	13.7±0.20^{d}	21.1±1.00^{c}	27.9±0.90^{b}	34.0±0.20^{a}
	P-3	0.05±0.01^{h}	0.5±0.03^{gh}	0.9±0.10^{gh}	1.3±0.03^{fg}	1.96±0.70^{f}	1.3±0.10^{fg}
	P-6	0.04±0.00^{h}	0.5±0.1^{gh}	0.6±0.05^{gh}	0.6±0.02^{gh}	0.7±0.03^{gh}	0.6±0.10^{gh}
	P-9	0.04±0.01^{h}	0.2±0.03^{h}	0.5±0.03^{gh}	0.6±0.01^{gh}	0.8±0.20^{gh}	0.7±0.05^{gh}

According to Duncan's multiple range test, means presented in the same column with different letters are significantly different (p <0.05). Means presented in the row with different superscript numbers are significantly different (p <0.05).

Colour

The colour of pomegranate arils and juice is an important quality attribute perceived by consumers (Pathare & Opara, 2013; Fawole & Opara, 2013a). Aril colour was 10.3 ± 0.43, 8.4 ± 0.47 and 3.8 ± 0.11 for *L*, a** and *b**, respectively, at day 0 (Table 4.2). However, these values are much lower than those reported by Caleb *et al*. (2013) for the same cultivar (Acco) while the *L** and *b** values are close to those reported by Ahyan and Esturk (2009) for freshly harvested 'Hicaznar' pomegranate arils. Increase in lightness (*L**), redness (*a**) and yellowness (*b**) of packaged arils was slight but significant (p <0.05) across all treatments up to day 3 of storage. The observed changes in colour could be due to initial response of packaged arils to metabolic activities. After day 3, the changes in colour across all treatments were not significant (p >0.05) until end of storage. Colour changes of pomegranate arils observed in the present study was in agreement with those reported by Ahyan and Esturk (2009) who reported that MAP gas composition had no significant effect on the *a** and *b** values of 'Hicaznar' pomegranate arils after 18 d of storage. Similarly, Caleb *et al*. (2013) found passive MAP had no significant effect on colour parameters (*L*, a** and *b**) used as indicators of colour stability of 'Acco' pomegranate arils after 14 d of storage. However, from day 12 there was a slight but significant increase of *a** from 9.8 ± 0.13 to 10.5 ± 0.91 in P-6 and *b** from 3.7 ± 0.20 to 4.3 ± 0.24 and 3.6 ± 0.23 to 4.3 ± 0.31 in P-6 and P-9 packaged arils, respectively. Similar trend in colour attributes (*a** and *b**) was observed in fresh 'Mridula' pomegranate arils packaged in MAP of semi-permeable low density polyethylene (LDPE) and KPA-Cryovac based-films during cold storage (5 ± 2 ºC) (Bhatia *et al*., 2013).

Chroma (*C**) values and hue angle (h^{o}) changed significantly (p <0.05) across all packaging treatments over storage duration. Arils packaged in of P-0 had an exceptional increase in *C** from 9.8 ± 0.43 on day 0 to 10.4 ± 0.17 after 14 d, in agreement with the effects observed by Almenar *et al*. (2007) in fresh strawberry fruit packaged in non-perforated high-CO_2 atmospheres of MAP using cups. Slight significant decrease in *C** was observed in arils packaged in clamshell trays and PM-MAP end of storage (from 9.8 ± 0.43 to 8.4 ± 0.33, 9.7 ± 0.10, 9.4 ± 0.15 and 9.6 ± 0.21 for clamshell, P-3, P-9 and P-9, respectively. Almenar *et al*. (2007) observed lower chroma values in strawberry fruits stored in high-permeable MAP films with 7 micro-perforations (50 µm diameter) and flexible semi-permeable PVC bags. Accordingly, decrease in *C** indicates loss in red colour and is related to high permeability of package (Almenar *et al*., 2007; De Reuck *et al*., 2009). However, changes in *C** observed in this current study were too small to affect the red colour of pomegranate arils.

Hue angle (*h°*) declined slightly across all packages from 25.5 ± 0.24 on day 0 to 25.1±0.33, 24.6 ± 0.31, 23.2 ± 0.51, 22.2 ± 0.14 and 24.52 ± 0.3 for clamshell, P-0, P-3, P-6 and P-9 packaged arils, respectively at the end of storage. These results are in agreement with De Reuck *et al*. (2009) who reported decrease in *C** and *h*° angle during 21 days of cold storage (2 ºC), in both perforated (with 10 and 4 perforations of 0.6 mm diameter), and non-perforated MAP packaged fresh litchi (cvs. Mauritius and McLean's Red). Authors related decline in *h*° values to high CO_2 injury in non-perforated MAP, but no clear reasons were given for decrease in *h*° observed in perforated packages.

Table 4.2 Effects of PM-MAP on colour attributes of fresh minimally processed pomegranate arils during cold (5 ± 1 ºC) storage. Results presented as mean values (± SE)

Colour attributes	PM-MAP Type	Storage duration (Days)					
		0	3	6	9	12	15
*L**	Clamshell	10.3±0.43^{cd}	13.2±1.22^{abc}	13.6±0.41^{abc}	12.7±1.01^{abc}	13.5±0.09^{abc}	13.7±1.07^{abc}
	P-0	10.3±0.43^{cd}	11.5±1.51^{bc}	13±0.51^{abc}	11.6±0.50^{abc}	12.7±0.29^{abc}	13.6±0.25^{abc}
	P-3	10.3±0.43^{cd}	13±0.73^{abc}	13.2±0.64^{abc}	13.5±0.63^{abc}	12.5±0.59^{abc}	12.4±0.18^{abc}
	P-6	10.3±0.43^{cd}	13.6±0.44^{abc}	13.2±0.63^{c}	13.6±13^{abc}	12.9±0.18^{abc}	13.5±0.45^{abc}
	P-9	10.3±0.43^{cd}	13.4±0.43^{abc}	12.5±0.74^{abc}	13.9±0.82^{abc}	12.2±0.74^{abc}	12.9±0.19^{abc}
*a**	Clamshell	8.4 ±0.47^{ac}	9.7±1.11^{abc}	9.1±0.62^{abc}	8.2±0.52^{abc}	7.9±0.89^{bc}	7.5±1.02^{bc}
	P-0	8.4±0.47^{ac}	8±1.91^{abc}	8.6±0.41^{abc}	8.5±0.40^{abc}	8.5±0.04^{abc}	8.2±1.00^{abc}
	P-3	8.4±0.47^{ac}	9.3±0.61^{abc}	9.7±0.51^{abc}	9.6±0.55^{abc}	8.5±0.78^{abc}	8.8±1.04^{abc}
	P-6	8.4±0.47^{ac}	8.8±0.33^{abc}	9.1±0.65^{abc}	9.7±0.70^{abc}	9.8±0.13^{ab}	10.5±0.91^{a}
	P-9	8.4±0.47^{ac}	8.9±0.52^{abc}	8.6±0.40^{abc}	9.2±0.08^{abc}	9.2±0.06^{abc}	10.2±1.03^{a}
*b**	Clamshell	3.8 ±0.11^{bc}	4±0.42^{ab}	3.8±0.31^{ab}	3.9±0.12^{ab}	4.2±0.53^{ab}	3.6±0.32^{ab}
	P-0	3.8±0.11^{bc}	3.8±0.48^{ab}	3.9±0.23^{ab}	3.7±0.32^{ab}	3.9±0.73^{ab}	3.7±0.33^{ab}
	P-3	3.8±0.11^{bc}	3.5±0.16^{ab}	4.2±0.12^{ab}	4.1±0.52^{ab}	3.6±0.14^{ab}	3.6±0.43^{ab}
	P-6	3.8±0.11^{bc}	4±0.34^{ab}	3.7±0.72^{ab}	4.3±0.32^{ab}	3.7±0.20^{ab}	4.3±0.24^{a}
	P-9	3.8±0.11^{bc}	4.1±0.20^{ab}	4.1±0.13^{ab}	3.8±0.33^{ab}	3.6±0.23^{ab}	4.3±0.31^{a}
*C**	Clamshell	9.8±0.43^{ae}	9.3±0.12^{cde}	9.2±0.16^{fgh}	8.9±0.15^{c-h}	8.7±0.52^{bc}	8.4±0.33^{e-h}
	P-0	9.8±0.43^{ae}	9.2±0.40^{c-f}	9.2±0.31^{c-f}	9±0.34^{d-h}	9.9±0.51^{ab}	10.4±0.17^{a}
	P-3	9.8±0.43^{ae}	9.8±0.22^{h}	9.6±0.33^{bc}	9.5±0.11^{bcd}	9.6±0.17^{bc}	9.7±0.10^{gh}
	P-6	9.8±0.43^{ae}	9.7±0.22^{gh}	9.4±0.43^{cde}	9.4±0.12^{cde}	9.4±0.42^{cde}	9.4±0.15^{cde}
	P-9	9.8±0.43^{ae}	9.3±0.20^{fgh}	9.3±0.25^{fgh}	9.5±0.43^{bcd}	9.6±0.72^{bcd}	9.6±0.21^{bcd}
h°	Clamshell	25.5±0.24^{ac}	25.2±0.40^{abc}	25.2±0.30^{abc}	25.2±0.46^{abc}	25.2±0.92^{abc}	25.1±0.33^{a-d}
	P-0	25.5±0.24^{ac}	25.5±0.30^{abc}	24.6±0.44^{bcd}	24.4±0.14^{bcd}	24.4±0.04^{c-f}	24.6±0.31^{bcd}
	P-3	25.5±0.24^{ac}	25.1±0.64^{a}	24.9±0.51^{a-d}	23.7±0.32^{d-g}	23.3±0.44^{e-h}	23.2±0.51^{d-g}
	P-6	25.5±0.24^{ac}	24.4±0.55^{cde}	24.4±0.45^{abc}	23.2±0.54^{fgh}	23.1±0.20^{fgh}	22.2±0.14^{h}
	P-9	25.5±0.24^{ac}	25.2±0.33^{abc}	25.2±0.3^{abc}	24.8±0.5^{bcd}	24.5±0.40^{cd}	24.5±0.32^{abc}

All means presented in the same column with different letters are significantly different (p <0.05) among PM-MAP treatments. Means presented in the same row with different letters are significantly different (p <0.05) among storage duration, according to Duncan's multiple range test.

Total soluble solids (TSS), titratable acidity (TA), Brix-acid (TSS:TA) ratio and pH

The results in Table 4.3 show that number of perforations in PM-MAP and storage duration had significant effects on TSS of 'Acco' pomegranate juice (p <0.05). It was observed that at the end of storage, clamshell trays and P-0 had slightly higher TSS value (14.7 ± 0.14 and 14.3 ± 0.13 ºBrix, respectively) than PM-MAP. Perforated MAP (P-3, P-6 and P-9) resulted in gradual but progressive decrease in TSS until the end of the 14 days of storage duration. The decrease in TSS could be attributed to increased metabolic activities of pomegranate arils during storage such as the conversion of soluble sugars into other organic acids such as citric, malic, oxalic and succinic accelerated by high concentration of O_2 in these packages (Bhatia *et al*., 2013). Similar trend of decrease in TSS of pomegranate arils packaged in MAP of high permeable LDPE and KPA Cryovac films was reported by Bhatia *et al*. (2013). However, contrary to these results, other studies showed that there was increase in TSS of fresh processed 'Wonderful' pomegranate arils packaged in MAP of perforated oriented polypropylene (POPP) film (with 33 perforations of 2 mm per dm^2 in 9 x 12 cm^2 area) (Gil *et al*., 1996). Similarly, Sepulveda *et al*. (2000) observed similar trend in TSS of 'Molar' pomegranate arils packaged in semi-permeable Cryovac ethyl vinyl acetate-based MAP film. Increase in TSS was related to loss of water due to high dehydration observed in in these packages (Gil *et al*., 1996; Sepulveda *et al*., 2000).

Furthermore, prior to storage, the concentration of titratable acidity of pomegranate juice was 1.1 ± 0.01 mg CA/100 mL followed by significant changes (p <0.05) over storage duration. After 3 days of storage, arils packaged in non-perforated (P-0) MAP had the lowest decrease in TA (0.7 ± 0.01 mg CA/100 mL) which remained unchanged for the rest of the storage duration. These results are in agreement with the effects observed by Caleb *et al*. (2013) in fresh 'Acco' pomegranate arils packaged in MAP of non-perforated biaxial oriented-PP film. Accordingly, the observed decrease in TA was attributed to initial response and metabolic activities of the packaged arils during storage. Titratable acidity of arils packaged in clamshell trays, P-3, P-6 and P-9 decreased to 0.4 ± 0.01, 0.5 ± 0.05, 0.4 ± 0.01 and 0.4 ± 0.03 mg CA/100 mL, respectively. The decrease in TA of pomegranate arils in perforated packages could be attributed to the increase in metabolic activities due to high O_2 concentrations observed in PM-MAP, in which citric acid was used as substrate. These results were supported by Amoros *et al*. (2007) who reported that micro-perforated polyethylene MAP bags reduced malic acid content of loquat fruit (cv. Algerie) by ~30% after 6 weeks of cold storage (6 ºC). According to Amoros *et al*. (2007), metabolic activities such as respiration resulted from MAP composition of higher O_2 (16-18%) and lower CO_2 (2-4%) caused the decrease in malic acid. Similar effects were also observed by Almenar *et al*. (2007) who reported a decrease in TA of strawberry fruit packaged in MAP cups (125 mL-

capacity) using micro-perforated film (7 micro-perforations of 50 µm diameter) during storage.

The Brix:acid (TSS:TA) ratio determines the taste and flavour quality of most fruit species including pomegranate arils at harvest and during postharvest handling (Arendse, 2014). The changes in Brix:acid ratio during storage was dependent on changes in both TSS and TA contents in pomegranate arils during storage. As shown in table 4.3, Brix-acid ratio of arils increased significantly (p <0.05) by more than 100% reaching highest values of 36.9 ± 0.04, 27.9 ± 0.06, 29.8 ± 1.95 and 32.8 ± 1.14 at the end of storage, in clamshell, P-3, P-6 and P-9 packaged arils, respectively. A fluctuation in the ratio in the TSS:TA ratio of arils in P-0 after day 3 was observed. The Brix:acid ratios measured in clamshell and PM-MAP packaged arils at the end of storage were close to the range (37.48 - 55.48) reported by Fawole (2013), which was descriptive for 'sweet-sour' taste of 'Acco', 'Arakta', 'Bhagwa', 'Ganesh', 'Herskawitz' and 'Ruby' pomegranate fruit arils.

Generally, the pH values of aril juice varied across all treatments throughout the storage duration (Table 4.3). The observed changes in pH showed a fluctuating trend of decrease and increase which was statistically significant (p <0.05). After 3 days of storage, the pH values decreased slightly from initial value of 3.6 ± 0.01, across all packages. Thereafter, the pH values of arils packaged in P-0 MAP increased significantly with storage duration to 4.2 ± 0.02, and then decreased to 3.7 ± 0.01 by the end of day 14 of storage. The highest mean pH values were 3.9 ± 0.01 and 3.7 ± 0.01 for clamshell and P-0 MAP, respectively. The fluctuating trend in pH was observed in the rest of packages. This fluctuation in pH of arils can be explained by the differences in CO_2 accumulated in the PM-MAP during storage. Over the period of 14 days of storage the CO_2 composition inside PM-MAP packages increased from 0.05 ± 0.01% to 1.3 ± 0.10% in P-3, 0.04 ± 0.00% to 0.6 ± 0.10% in P-6 and 0.04 ± 0.00% to 0.7 ± 0.05%, although there were some pockets of fluctuation in between. Similar trend was observed in 'Hicaznar' pomegranate arils during cold storage under passive MAP (Ahyan & Esturk, 2009). Caleb *et al*. (2013) also found slight but statistically significant changes in pH of passively MAP stored 'Acco' and 'Herskawitz' pomegranate arils.

Firmness of arils

In this study, firmness of arils decreased with increase in the number of perforations over the duration of storage (Table 4.3). The interaction of number of perforations on package and storage duration had a significant effect on the firmness of arils (p <0.05). Aril firmness was

best maintained under P-3 (6.2 ± 0.33 N), while the highest loss in firmness were observed in P-9 (3.8 ± 0.22 N) after 14 days of storage. Decrease in firmness of arils packaged in PM-MAP may be attributed to loss in moisture influenced by perforation. Our results corroborated with previous findings reported by Ayhan and Esturk (2009), who found that variation in the firmness of 'Hicaznar' pomegranate arils stored under passive MAP of PP film was related to water loss through the packages during 18 days of storage at 5 ºC. Similarly, Bhatia *et al*. (2013) reported that after 14 days of cold storage (5 ± 2 ºC) of 'Mridula' pomegranate arils packaged under passive MAP, highest loss in firmness was observed in arils packaged in LDPE and Cryovac based-laminate film which had highest water vapour permeability (5.6 and 15 cm^3/m^2.day, respectively) among the studied packages. According to Gimenez *et al*. (2003), high O_2 concentration may also contribute to loss of texture in arils packaged in PM-MAP. However, this is in contrast to observations reported by Caleb *et al*. (2013), where pomegranate (cvs. Acco and Herskawitz) arils packaged in clamshell trays had higher rate of firmness loss compared with those packaged in passive MAP during 14 days of cold storage at 5 ºC.

Table 4.3 Changes in physico-chemical properties of fresh processed pomegranate arils during cold storage (5 ± 1 ºC) under PM-MAP. Results presented as mean values (± SE)

Physico-chemical properties	PM-MAP Type	Storage duration (Days)					
		0	3	6	9	12	15
pH	Clamshell	3.6±0.01^{gh}	3.4±0.1^{jk}	3.9±0.10^{cd}	3.9±0.03^{bc}	4±0.02^{b}	3.9±0.01^{bc}
	P-0	3.6±0.01^{gh}	3.5±0.02^{hij}	3.8±0.01^{ef}	3.4±0.01^{jk}	4.2±0.02^{a}	3.7±0.01^{ijk}
	P-3	3.6±0.01^{gh}	3.3±0.03^{k}	3.8±0.01^{ef}	3.4±0.03^{jk}	4.0±0.02^{b}	3.6±0.10^{gh}
	P-6	3.6±0.01^{gh}	3.2±0.09^{i}	3.9±0.02^{bc}	3.6±0.05^{gh}	4.0±0.14^{b}	3.6±0.02^{ghi}
	P-9	3.6±0.01^{gh}	3.4±0.01^{jk}	3.8±0.03^{fg}	3.9±0.02^{bc}	3.8±0.11^{cde}	3.5±0.02^{jk}
TA (mg CA/100 mL)	Clamshell	1.1±0.01^{ac}	0.6±0.04^{abc}	0.6±0.03^{abc}	0.5±0.01^{a-d}	0.5±0.01^{a-d}	0.4±0.01^{e-i}
	P-0	1.1±0.01^{ac}	0.7±0.01^{ab}	0.7±0.03^{ab}	0.7±0.01^{ab}	0. 7±0.05^{ab}	0.7±0.02^{ab}
	P-3	1.1±0.01^{ac}	0.7±0.03^{ab}	0.7±0.03^{ab}	0.6±0.01^{abc}	0.5±0.2^{1a-d}	0.5±0.05^{a-d}
	P-6	1.1±0.01^{ac}	0.6±0.02^{abc}	0.6±0.01^{abc}	0.5±0.02^{a-d}	0.4±0.02^{ghi}	0.4±0.01^{f-i}
	P-9	1.1±0.01^{ac}	0.6 ±0.01^{abc}	0.5±0.01^{a-d}	0.4±0.01^{f-i}	0.4±0.01^{f-i}	0.4±0.03^{f-i}
TSS (ºBrix)	Clamshell	15.4±0.02^{abc}	15.6±0.05^{ab}	16±0.05^{a}	15 ±0.05^{bce}	14.7±0.5^{def}	14.7±0.14^{def}
	P-0	15.4±0.02^{abc}	15.4±0.42^{abc}	15±0.20^{b-e}	14.6±0.20^{def}	14.1±0.03^{f}	14.3±0.13^{df}
	P-3	15.4±0.02^{abc}	15.5±0.30^{abc}	14.1±0.22^{fg}	14.3±0.14^{def}	14.0±0.05^{f}	13.9±0.22^{g}
	P-6	15.4±0.02^{abc}	15.0±0.21^{b-e}	14.8±0.33^{c-f}	14.4±0.10^{def}	13.9±0.33^{g}	13.5±0.14^{gh}
	P-9	15.4±0.02^{abc}	14.8 ±0.12^{c-f}	14.6±0.15^{def}	14.3±0.03^{def}	13.8 ±0.05^{g}	13.1±0.04^{i}
Brix-acid ratio (TSS:TA)	Clamshell	13.6±0.41^{i}	26.1±0.16^{def}	27.5±0.37^{cde}	29.7±0.41^{c}	29.4±0.68^{c}	36.9±0.04^{a}
	P-0	13.6±0.41^{i}	22.3±0.33^{gh}	20.7±1.05^{h}	20.8±0.17^{h}	19.9±0.41^{h}	20.5±0.23^{h}
	P-3	13.6±0.41^{i}	22.6±0.39^{gh}	21.1±0.65^{h}	23.9±1.08^{fg}	27.9±0.05^{cd}	27.9±0.06^{cd}
	P-6	13.6±0.41^{i}	25.0±0.47^{efg}	26.2±1.57^{def}	28.9±0.06^{cd}	32.4±2.31^{b}	29.8±1.95^{c}
	P-9	13.6±0.41^{i}	24.1±0.61^{fg}	27.7±1.45^{cde}	35.8±0.08^{a}	34.6±0.08^{b}	32.8±1.14^{b}
Firmness (compression/N)	Clamshell	7.8±0.03^{a}	7.8±0.18^{ab}	7.2±0.44^{a-e}	7±0.11^{b-g}	6.6±0.32^{e-j}	5.4±0.20^{m}
	P-0	7.8±0.03^{a}	6.7±0.21^{e-j}	7.8±0.22^{a}	6.8±0.10^{d-i}	6.3±0.31^{g-l}	5.6±0.12^{lm}
	P-3	7.8±0.03^{a}	6.6±0.31^{e-j}	7.1±0.20^{a-f}	6.9±0.32^{d-i}	6.3±0.14^{f-k}	6.2±0.33^{h-l}
	P-6	7.8±0.03^{a}	6.8±0.22^{d-i}	7.6±0.34^{a-d}	7±0.23^{c-h}	6±0.12^{j-m}	5.8±0.13^{klm}
	P-9	7.8±0.03^{a}	6.9±0.13^{d-h}	6.8±0.25^{def}	6.3±0.24^{d-g}	6.1±0.44^{i-m}	3.8±0.22^{n}

Means presented in the same column with different superscript letters are significantly different (p <0.05) among PM-MAP treatments. Means presented in the same row with different letters are significantly different (p <0.05) among storage duration, according to Duncan's multiple range test.

Microbial quality

Microbial counts on minimally processed pomegranate arils increased significantly with storage duration (Figure 4.3). Both storage duration and packaging type significantly influenced the growth of all analysed microorganisms (p <0.05). On day 0, initial aerobic mesophilic bacteria and yeast and mould counts on fresh arils were below 1 log CFU/g. There was no *E. coli* detected in all packages after processing and packaging of arils and at the end of storage. Lowest aerobic mesophilic bacteria count was observed in P-0 (4.1 ± 0.02 log CFU /g), and highest counts in P-9 PM-MAP packaged arils (5.5 ± 0.01 log CFU/g). In contrast, lowest yeast and moulds counts were observed in P-6 MAP (5 ± 0.03), while the highest counts was in P-0 MAP (5.3 ± 0.01 log CFU/g) at the end of storage. Higher counts of yeasts and moulds count found in this study collaborates with the report by Caleb *et al*. (2013) who observed that aerobic mesophilic bacteria counts were lower than yeast and moulds in minimally processed fresh 'Herskawitz' and 'Acco' pomegranate arils under passive MAP stored at 5 ºC. This may be attributed low pH values of packaged arils, which favours the growth of yeast and moulds in comparison with aerobic mesophilic bacteria (Caleb *et al*., 2013). Similarly, the higher yeast and mould counts observed in P-0 MAP packaged arils in comparison to P-9 PM-MAP could be attributed to higher level of CO_2 recorded in P-0 MAP samples. Studies have suggested that higher yeast and moulds counts in packaged produce can be attributed to increase in or accumulation of CO_2 inside packages (Farber *et al*., 2003; Lopez-Rubira *et al*., 2005).

After 14 days of storage, aerobic mesophilic bacteria counts were below the maximum (7 log CFU/g) acceptable counts for raw and fresh-cut fruits imposed by South African regulation (FCD, Act 54 1979). On the other hand, yeast and mould counts in P-3 and P-6 PM-MAP packaged arils did not exceed maximum acceptable limit for raw and fresh-cut fruits (5 log CFU/g) (FCD, Act 54 1979) at the end of storage. In general, higher microbial counts were found in this study in comparison to previous findings on MAP of fresh pomegranate arils (Lopez-Rubira *et al*., 2005; Ahyan & Estürk, 2009; Caleb *et al*., 2013). This variation may be attributed in part to the presence of perforations on MAP which provide points of entry for microorganisms during storage. The trend of progressive increase in aerobic mesophilic counts with perforations of packages may be a basis of this explanation. Also possible difference in cultivars investigated, maturity of arils and storage conditions. *E. coli* is a hygienic criterion that indicates level of fecal contamination during the manufacturing processes (Abadias, 2008). The absence of *E. coli* in the investigated packaged arils shows that preparation of samples were performed in a highly hygienic facility and the presence of perforation on packaged fresh could be microbiological safe.

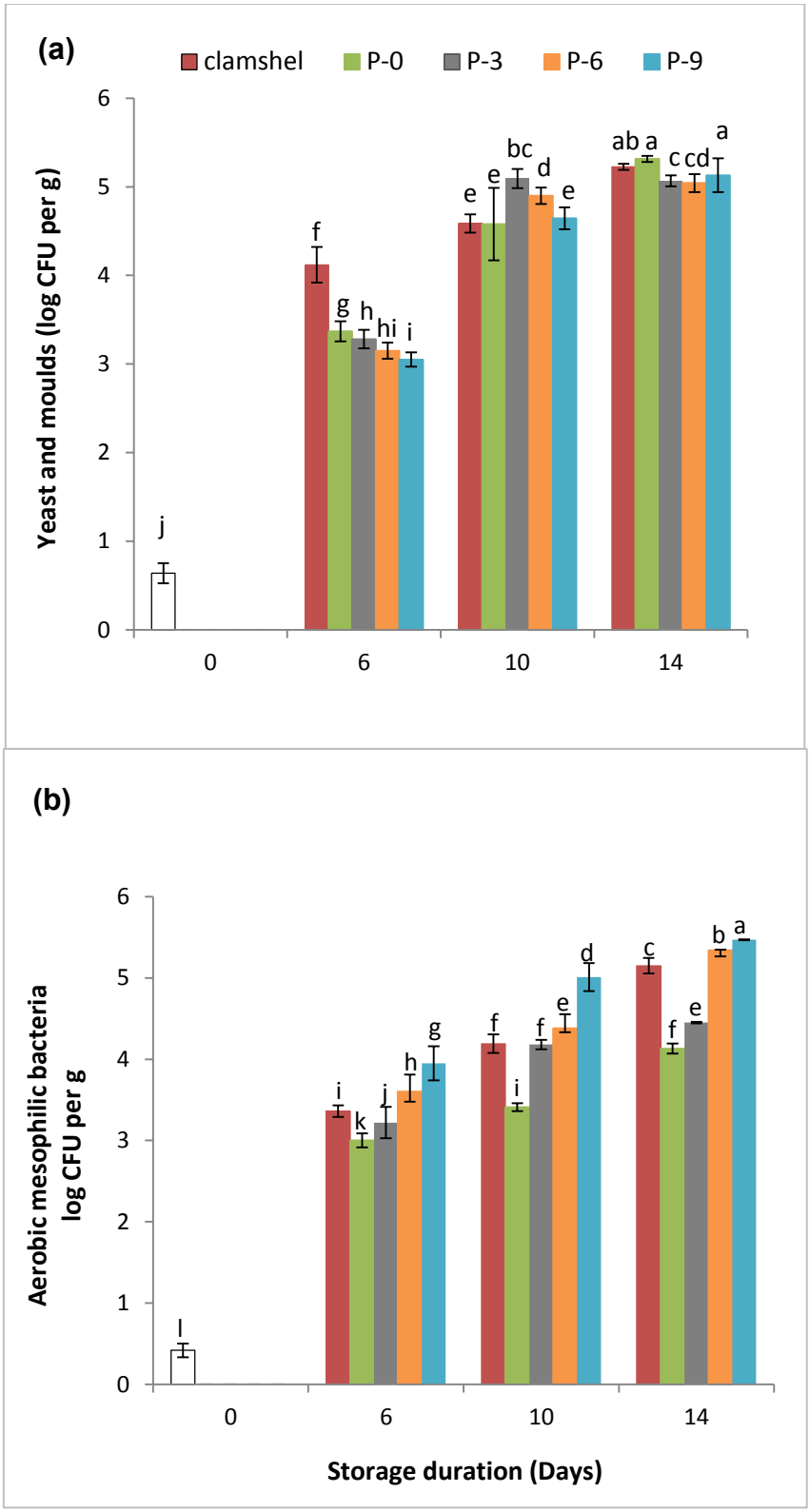

Figure 4.3 Effect of PM-MAP on growth of yeast and moulds (a), and aerobic mesophilic bacteria (b) in fresh processed pomegranate arils cv. 'Acco' during storage at 5 ºC. Error bars indicate a 95% confidence interval. Bars with the different letters are significantly different (p <0.05).

Conclusion

Perforations on modified atmosphere packaging prevented build-up of CO_2 in the headspace atmosphere during 14 days of cold the storage. Modified atmospheres rich in O_2 and poor in CO_2 prevented anaerobic fermentative reactions in favour of aerobic respiration. In this study, water vapour condensation was observed in clamshell trays and non-perforated (P-0) MAP due to poor permeability. As such, arils in these packages had poor visual quality and signs of decay. The results highlight the industrial relevance of using perforation as a feasible low-cost approach to optimise film permeability and guarantee longer shelf-life of fresh produce.

Perforated packages showed a potential to prevent the growth of aerobic mesophilic bacteria, yeast and moulds for 14 days of storage. Yeast and moulds did not exceed maximum acceptable limit in industry (5 log CFU/g) in P-3 and P-6 MAPs while no of growth of *E.coli* was detected throughout the 2-week storage period. These results suggest that the use of perforations (P-3 or P-6) did not limit microbial quality of fresh pomegranate arils. However, good processing practices, optimal cold chain and contamination free environment should be ensured during commercial postharvest practices, handling and storage. Due to the impact of microbial load on detectable changes in sensory properties of fresh produce, It is proposed that future research should include the evaluation of sensory quality (in combination with microbial analysis) to determine consumer acceptability of fresh minimally processed pomegranate arils packaged in perforated MAP.

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CHAPTER FIVE: Phytochemical and antioxidant properties of minimally processed pomegranate arils (cv. Acco) as affected by perforation-mediated modified atmosphere packaging (PM-MAP) and storage duration

PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF MINIMALLY PROCESSED POMEGRANATE ARILS (CV. ACCO) AS AFFECTED BY PERFORATION-MEDIATED MODIFIED ATMOSPHERE PACKAGING (PM-MAP) AND STORAGE DURATION

Abstract

The effects of perforation-mediated modified atmosphere packaging (PM-MAP) on the phytochemicals and antioxidant properties of fresh processed pomegranate arils (cv. Acco) kept at 5 ºC for 15 d were investigated. About 100 g of fresh ready-to-eat arils were packaged in polypropylene trays and heat sealed with a polymeric film of known permeability. Perforations (P) were made on the film using a 0.8 mm diameter sterile needle (P-3, P-6 and P-9) with non-perforated P-0 and clamshell trays as control. Packaged arils were stored at 5 ºC for 14 days and analysis were done on day 3, 6, 9, 12 and 15. Total anthocyanins increased slightly across all treatments, P-6 had the highest content (49.2 ± 0.71 mg C3gE/100 mL). Total phenolic content was highest (253.6 ± 5.13 mg GAE/100 mL) in P-0, while P-3, P-6 and P-9 had approximately 215.8 ± 1.22, 224.5 ± 1.28 and 227.8 ± 2.12 mg GAE/100 mL, respectively. Ascorbic acid decreased significantly across all treatments (p <0.05), compared to the initial values (15.3 ± 0.13 mg AA/100 mL) prior to storage. A threefold increase in antioxidant capacity was observed at the end of storage when tested against ferric ion reducing antioxidant power **(**FRAP) initial value (43.52 ± 0.73 µM TE/mL) in PM-MAP by end of 14 d storage. The DPPH assay showed an initial increase in antioxidant activities for arils in PM-MAP followed by stabilization until end of storage. Perforation-mediated MAP better maintained phytochemical and antioxidant properties of passive MAP packaged arils compared to clamshell and non-perforated packages during cold storage.

Introduction

Postharvest shelf-life of minimally processed fruits and vegetables has been traditionally defined by a number of physico-chemical quality attributes such as colour, firmness, juiciness, absence of decay, pH, titratable acidity, and total soluble sugars (Ayala-Zavala *et al*., 2004; Caleb *et al*., 2013a). However, phytonutrients and bioactive components such as anthocyanins, phenolic compounds, and antioxidant capacity are increasingly becoming important quality determining attribute of fresh produce (Ayala-Zavala *et al*., 2004; Lutz *et al*., 2011). The role postharvest storage technology is to manipulate metabolism of fresh produce during storage for the purpose of extending produce shelf-life (Kalt *et al*., 1999).

However, changes in metabolic activities during postharvest storage may affect the content of phytonutrient and their constitutive antioxidant activity during this period (Shiri *et al*., 2011). In this regard, it is essential to evaluate changes phytochemical and antioxidant attributes during storage of minimally processed produce.

Pomegranate (*Punica granatum* L.) fruit is characterized by high content of phytochemicals and antioxidants with preventive and therapeutic effects for various degenerative diseases such as inflammatory diseases, diabetes, cardiac disorders, Alzheimer's disease, brain disorders and different types of cancer (Malik *et al*., 2005; Seeram *et al*., 2005; Pala & Toklucu, 2011; Fawole *et al*., 2012a). Diverse group of phytochemicals such as flavonoids and polyphenols are linked to the potent antioxidant capacity of pomegranate fruit and juice (Sareem *et al*., 2008; Fawole *et al*., 2012a). The role of food antioxidants in the prevention of certain diseases through various mechanisms such as blocking the generation of free radical chain reactions and neutralizing free radicals which release electrons had been epidemiologically proved (Ayala-Zavala *et al*., 2004; Garcia-Alonso *et al*., 2004; Stanner et *al*., 2004).

Earlier findings by Lopez-Rubira *et al*. (2005) showed non-significant changes in total anthocyanin content of pomegranate arils (cv. Mollar of Elche) during postharvest storage under passive MAP. After 13 d of cold storage, total anthocyanin content was in the range of 178.63 and 197.35 µg/mL for late and early harvest respectively. Similarly, no significant changes observed in the antioxidant activities of arils after 13 days of storage under same conditions, changing from 8.37 to 6.6 mM/mM AAE and 7.63 to 7.27 mM/mM AAE for arils of early and late harvested pomegranate fruit respectively. Gil *et al*. (1996) revealed non-significant change in total anthocyanin content of pomegranate arils cv. 'Mollar of Elche' after 7 d of MAP storage at 1 ºC. However, studies to investigate the stability of phytochemicals and antioxidant capacity during processing, packaging and storage of pomegranate arils are still limited. The objective of this study was to investigate the effects of perforation-mediated modified atmosphere packaging (PM-MAP) and storage duration on the stability of phytochemicals and antioxidant properties of fresh minimally processed pomegranate fruit arils (cv. Acco).

Materials and methods

Plant material and sample preparation

Pomegranate fruit (*Punica granatum* L., cv. Acco) were obtained during commercial harvest from a commercial orchard located in Porterville (33°38'S 18°59'E), Western Cape, South

Africa. Fruits were sorted manually to get rid of damaged ones, healthy fruits were washed in sodium hypochlorite (NaOCl) solution (200 mg/L). Sterilized whole fruit were aseptically hand processed to carefully remove arils (without crushing) under cool temperature (6 ºC). Approximately 100 g of fresh arils were packaged in polypropylene (PP) trays (10.6 x 15.1 cm^2) and heat sealed using a semi-automated machine (Food Packaging Equipments, South Africa) with a polymeric film POLYLID® 107 polyethylene (thickness 55 µm; carbon dioxide (CO_2) transmission rate; 600-700 mL/m^2.day; oxygen (O_2) transmission rate; 130-150 mL/ m^2.day and WVTR 4.2 g/m^2.day at 38 ºC, 90% relative humidity (RH) and 1 Bar) (Barkai Polyon Ltd, Kibbutz Barkai, Israel). Heat seals films were manually perforated using sterilized needle (0.8 mm diameter) with 3, 6 and 9 numbers of perforations to obtain P-3, P-6 and P-9. Non-perforated film (P-0) was used as a control while clamshell tray was included to simulate traditional packaging of fresh pomegranate arils in South Africa market. Labels of 7.0 x 3.8 cm^3 area were placed onto each package film to simulate retail conditions. At the processing facility packaged products were cooled down to 2 ºC, and packed in sterile cooler boxes with dry ice to maintain low temperature during transportation from the processing facility to the postharvest technology research laboratory. Boxes were fitted with data loggers (Gemini Data Loggers, West Sussex, UK) to monitor the cold chain. On arrival, the packaged samples were stored at 5 ºC and 95 ± 2% RH for 14 days. A baseline analysis to determine the phytochemical and antioxidant properties of pomegranate fruit samples was conducted on fresh arils prior to storage. Sampling for further analyses were taken every 3 days interval on day 3, 6, 9, 12 and 15. Three packages were analysed for each experimental condition on sampling days.

Change in headspace gas compositions

Analysis of headspace gas composition (CO_2 and O_2) inside the packages was performed on each sampling day prior to opening the package at the end of 15 d storage period. Using a gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark) with an accuracy of 0.5% (Checkmate 3, PBI Dansensor, Ringstead, Denmark), CO_2 and O_2 composition were measured and presented in percentage (%). After every gas analysis, each package was reweighed, opened and aril samples used for determination of phytochemicals and antioxidant properties.

Preparation of fruit juice extracts

Pomegranate juice (PJ) was extracted from arils of each pack separately, using LiquaFresh juice extractor (Mellerware, South Africa). From each package, approximately 50 mL of PJ sample was obtained. From each sample, about 1 mL of PJ sample was diluted with 50% aqueous methanol in a centrifuge tube and then sonicated in cold water for 12 min. In order to prevent interference of particulates when measuring the absorbance of the extract, the mixture was thereafter centrifuged at 4000 rpm for 20 min and the supernatant was carefully transferred into clean tubes without disturbing the sediments. The extract obtained was used in chemical analysis of total anthocyanin content, total phenolics and antioxidant capacity of PJ samples. All analyses were conducted in triplicates.

Analysis of antioxidant properties

Total phenolic content

Total phenolic content for each treatment was determined according to the Folin–Ciocalteu (Folin C) method described by Makker (2007). Approximately 50 µL of diluted aqueous methanolic PJ extracts in the test tube was mixed with 450 µL of 50% methanol. 500 µL of the Folin C reagent was added to the methanolic PJ extract mixture. After 2 min, 2.5 mL of sodium carbonate solution was added to the mixture of methanolic PJ extract and methanol and Folin C. The tubes containing solution mixture were vortexed, and then incubated in dark chamber for 40 min at room temperature (15 ºC). The absorbance of the solution mixture was measured at 725 nm using an UV-visible spectrophotometer (Thermo Fisher Scientific, Madson, USA). The results was presented as the mean of duplicate analyses and expressed as milligrams of gallic acid equivalent per a hundred millilitre of crude PJ (mg GAE/100 mL).

DPPH radical-scavenging activity

The antioxidant activity of PJ on DPPH was determined calorimetrically. The DPPH assay is based on scavenging of the 2, 2-diphenil-1-picrylhydrazil (DPPH) radical from the antioxidants of PJ, producing a spectrophotometric loss in absorbance at 515 nm (Perez-Jimenez & Saura-Calixto, 2006). The DPPH assay (Sigma Chemical Co.) was carried out according to the method reported by Fawole *et al*. (2012b). 15 µL of methanolic extract of PJ sample was diluted with 735 µL 100% methanol in test tubes. Exactly 750 µL of 0.1 mM methanolic DPPH reagent solution was added to the mixture of PJ extract and methanol and

this was incubated at room temperature in a dark chamber for 30 min. After incubation, absorbance of the mixture was measured at 517 nm using a UV–vis spectrophotometer (Thermo Fisher Scientific, Madson, USA). The absorbance was compared with the standard curve of varying concentration of ascorbic acid from 0.0 - 2.0 mM, with the linear equation, $y = -0.46x + 0.56$ and R^2 = 0.98. Free-radical scavenging capacity of PJ based on DPPH reaction was expressed as millimoles of ascorbic acid equivalent per a hundred millilitres of crude PJ (mM AAE/100 mL).

Ferric ion reducing antioxidant power (FRAP)

The antioxidant activity of PJ was determined using calorimetric method with a FRAP assay as described by Fawole *et al*. (2012b). FRAP assay is based on the ferric ion reducing ability such that, in the presence of antioxidants the ferric-tripyridyl-s-triazine (Fe^{3+}-TPTZ) complex is reduced to its ferrous coloured complex under acidic medium, causing an increase in absorbance at 515 nm (Perez-Jimenez & Saura-Calixto, 2006). FRAP was freshly prepared by mixing 50 mL of 300 mM acetate buffer, 5 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 5 mL of 20 mM ferric chloride. Prior to its use, the fresh mixture was incubated in a water bath at 37 ºC for about 15 min to stabilise contents of the mixture. Exactly, 150 µL of diluted aqueous methanolic PJ extracts was transferred into clean test tubes followed by addition of 2850 µL of FRAP solution in triplicates. The mixture was vortexed and incubated in a dark chamber for 30 min before measuring the absorbance at 515 nm in a UV-vis spectrophotometer (Thermo Fisher Scientific, Madson, USA). The results for ferric ion reducing antioxidant power of PJ were obtained through extrapolation of trolox (100-1000 µM) standard curve using a linear equation, $y = 1.75x + 0.15$, and R^2 = 0.98. Antioxidant activity of PJ was expressed as micromoles of trolox equivalent per millilitre of crude PJ (µM TE/mL).

Total anthocyanin content

The anthocyanin content of PJ samples was determined by the pH-differential method using 2 buffer systems comprised of potassium chloride (pH 1, 0.025 M) and sodium acetate (pH 4.5, 0.4 M) as described by Fawole *et al*. (2012b). Pomegranate juice (1 mL) was mixed with 9 mL of buffer and the absorbance was measured at 520 and 700 nm. Total anthocyanin content was calculated and expressed as milligram of cyanidin-3-glucoside equivalent per a hundred millilitres of crude PJ (mg C3g E/100 mL) using the following equation;

$$Total\ anthocyanins\ (mg\ 100L^{-1}) = \left[\frac{A \times MW \times DF \times 100}{\varepsilon \times 1}\right] \quad (1)$$

where, A = (A520 – A700) pH 1 – (A520 – A700) pH 4.5; MW (molecular weight) = 449.2 g/ mole for cyaniding-3-glucoside; DF is the dilution factor; 1 = pathlength in cm; molar extinction coefficient (ε) = 26900.

Ascorbic acid

Ascorbic acid content of PJ was determined calorimetrically, based on method reported by Barros *et al*. (2007). Pomegranate juice was diluted with 1% metaphosphoric acid (MPA) (1 mL of PJ to 9 mL of 1% MPA) at room temperature (15 ºC). Diluted sample was vertexed for 30 sec and sonicated in ice for about 5 min. The sample (diluted) was then centrifuged at 40000 rpm for 20 min at 4 ºC. The supernatant was carefully transferred into a clean tube without disturbing the sediments at the bottom of centrifuge tubes. Approximately, 1 mL of the supernatant was mixed with 9 mL of 2, 6-dichlorophenolindophenol (dye), shaken to mix the content thoroughly and incubated for 20 min in a dark chamber. After incubation, the absorbance of samples was measured at 515 nm against a blank. Ascorbic acid content of each sample was calculated on the basis of the calibration curve of standard L-ascorbic acid (0.01 - 0.1 mg/mL), using linear equation, $y = -49.21 + 0.56$, and R^2 = 0.99. Results for ascorbic acid content of sample PJ was expressed as milligram of ascorbic acid per a hundred millilitres of crude PJ (mg AA/100 mL).

Statistical analysis

Factorial analysis of variance (ANOVA) at 95% confidence interval using statistical software (Statistical 12.0, Statsoft, USA) was used to analyse the effects of treatments and duration on phytochemicals and antioxidant capacity of pomegranate arils. Main and interaction effects of the number of perforations and storage duration were described using Pareto analysis at 95% confidence interval. Statistical significance of treatments was tested using Post-hoc test (Duncan's Multiple Range Test) and observed differences at p <0.05 were considered significant.

Results and discussion

Headspace O_2 and CO_2 compositions

The headspace gas composition changed significantly in all packages of arils during cold storage (p <0.05). The levels of O_2 composition decreased progressively to critical level (1.3%) in non-perforated (P-0) packages, while clamshell trays had the highest level of O_2 (19.2%) at the end of storage. Changes in O_2 composition in P-0 MAP were consistent with previous report by Almenar *et al*. (2007) who found that O_2 composition in the headspace atmosphere of strawberries packaged in unperforated (P-0) MAP ranged between 1-2% during 6 days storage. Perforations in P-3, P-6 and P-9 MA packages helped to maintain higher level of O_2 to 17.6%, 18.6 % and 18.4%, respectively at the end of storage. Pandey and Goswami (2012) revealed that increase in number of perforations increased headspace O_2, while reducing CO_2 build-up. Almenar *et al*. (2007) observed that O_2 and CO_2 compositions in PM-MAP packaged strawberries with 1, 3 and 7 perforations (100 μm diameter) ranged between 5-18% and 4-14%, respectively. Similar observations were reported on MAP of semi-permeable films of minimally processed 'Wonderful' pomegranate arils (Sepulveda *et al*., 2000) and passive PM-MAP of Litchi with ten and four (0.6 mm diameter) perforations (De Reuck *et al*., 2009).

On the other hand, the composition of CO_2 increased in all packages after 3 days of storage with P-0 packages having the highest CO_2 accumulation from 9.6 % to 34.0 % on days 3 to 15, respectively. Clamshell had the lowest level of CO_2 which stabilised throughout the storage period. According to Caleb *et al*. (2013b) lowest level of CO_2 in clamshell trays could be attributed to the known poor hermetic seal of clamshell trays. The level of CO_2 in perforated packages varied significantly, with P-3, P-6 and P-9 reaching 1.3%, 0.6 % and 0.7 % respectively at the end of storage. These results showed that perforations increased the permeability of packaging films and prevented the accumulation of CO_2 (Almenar *et al*., 2007; Pandey & Goswami, 2012).

Total anthocyanin content

Total anthocyanin content of arils increased during storage across all treatments (Table 5.1). Package type had a significant impact on the increase in total anthocyanin (p <0.05). Arils packaged in clamshell trays and P-0 MAP had a slight increase in anthocyanin content towards the end of storage. Highest anthocyanin content (49.2 ± 0.71 mg C3gE/100 mL)

was observed in arils stored under P-6 PM-MAP at day 9. The observed increase in the concentration of anthocyanin in pomegranate juice could be attributed the biosynthesis of anthocyanins during storage of pomegranate arils and changes resulting from anthocyanin extraction process after storage of pomegranate arils (Gil *et al*., 1996). In addition, the increase in anthocyanin concentration of juice could also be due to water loss in arils packaged under PM-MAP. The results from this study corroborated those reported by Gil *et al*. (1996). The authors observed an increase in anthocyanin concentration for pomegranate arils cv. 'Mollar of Elche' stored in non-perforated passive MAP and control macro-perforated oriented polypropylene (POPP) film bags (33 holes of 2 mm per dm^2) at 1 ºC. However, our results contrast the findings of previous studies on MAP of pomegranate arils. For example, no changes in anthocyanin content were reported in UV-C treated and MAP packaged pomegranate arils cv. 'Mollar of Elche' stored at 5 ºC after 13 days (Lopez-Rubira *et al*., 2005). Furthermore, a decrease in anthocyanin concentration was reported for pomegranate arils cv. 'Hicaznar' packaged under active MAP with varying gas compositions over storage time, although high content was observed in high O_2 MAP (Ahyan & Esturk, 2009). Caleb *et al*. (2013b) reported a decrease in total anthocyanins of pomegranate arils cv. 'Acco' and 'Herskawitz' packaged under passive MAP across all storage temperatures (5, 10 and 15 ºC) investigated. The anthocyanin concentration reported in this study for PM-MAP packaged arils was about two fold higher than that reported by Caleb *et al*. (2013b) for 'Acco' cultivar (21.1 to 13.3 mg C3gE/100 mL). This could be attributed to stress factors such as accumulation of CO_2 and depletion of O_2 under passive MAP, which may have influenced biosynthesis of anthocyanins content during storage.

Total phenolic content

The effects of number of perforations and storage duration were significant (p <0.05) on the change in phenolics content as shown in Table 5.1. A continuous increase in total phenolics of pomegranate arils was observed across all packages; from an initial value of 157.7 ± 0.84 mg GAE/100 mL to 222.7 ± 1.11, 253.6 ± 5.13 and 227.8 ± 2.12 mg GAE/100 mL in clamshell, P-0, and P-9, respectively. In P-3 and P-6 PM-MAP a fluctuation in phenolic content was observed. Metabolic processes, such as respiration, ethylene production and enzyme activity such as oxidation phenolic compounds by polyphenol oxidase are suggested factors for the decrease in phenolic content of arils during storage (Shiri *et al*., 2011). In addition, changes in physico-chemical properties such as titratable acidity and total soluble solids could influence the total phenolic content of pomegranate arils during storage (Ahyan & Esturk, 2009). In this regard, Kalt *et al*. (1999) and Peano *et al*. (2013) attributed

the increase in phenolics, including anthocyanin during MAP storage of raspberry fruits to the biosynthesis of new phenolics which involves the use of carbon skeletons obtained from organic acids and total solids through interconversion reactions.

Ascorbic acid

Results in Table 5.1 show that after 14 days of storage, ascorbic acid concentration of packaged arils decreased across all treatments from 15.3 ± 0.13 mg AA/100 mL to 14.6 ± 0.12, 12.4 ± 0.25, 11.3 ± 0.06 mg AA/100 mL in P-3, P-6 and P-9, respectively. Clamshell packaged arils had the highest ascorbic acid concentration (15.6 ± 0.25 mg AA/100 mL), while fluctuation in ascorbic acid level was observed in P-0 until last day of storage. A general trend of slight decrease in ascorbic acid concentration with an increase in permeability of PM-MAP was observed. This may be attributed to high O_2 and low CO_2 concentration in P-3, P-6 and P-9 packages that may have caused oxidation of ascorbic acid into dehydroascorbic acid (DHA) leading to subsequent decrease in ascorbic acid (Mahajan *et al*., 2014). However, high CO_2 concentration stimulates degradation of ascorbic acid in fresh-cut due to its high stimulating effects on the oxidation of ascorbic acid and/or inhibition of DHA reduction to ascorbic acid compared to the equivalent O_2 concentration (Agar *et al*., 1999, Lee & Kader, 2000). Regeneration of ascorbic acid to close to initial concentration for arils packaged in clamshell and P-0 MA package towards the end of storage can be explained by the oxidation of DHA to ascorbic acid. During this reversible process, decrease in ascorbic acid concentration correlates with corresponding increase in DHA and vice versa (Gil *et al*., 2006). Previous findings have reported the stability of ascorbic acid content of fruits during cold storage. Del Caro *et al*. (2004) observed significant decrease in ascorbic acid towards end of 14 days of cold (4 ºC) storage of minimally processed citrus segments under MAP. Similarly, Agar *et al*.(1999) reported that MAP of higher O_2 (21%) and low CO_2 (5%) decreased vitamin C content of fresh-cut kiwi fruit slices by 14% during cold storage (0 ºC). Furthermore, ascorbic acid content of fresh-cut and whole strawberries fruit slightly increased during storage, followed by slight decrease in fresh-cut slices after 9 days of storage (Gil *et al*., 2006).

Table 5.1 Effects of PM-MAP and storage duration on phytochemical properties of fresh processed pomegranate fruit arils under cold storage (5 ± 1 ºC). All results presented as mean values (± SE)

Phytochemicals	PM-MAP Type	Storage duration (Days)					
		0	3	6	9	12	15
Total phenolics (mg GAE/100mL)	Clamshell	157.7±0.84^{ij}	214.6±0.191^{i}	213.9±1.17^{hi}	219.03±3.18^{e-i}	220.1±0.33^{d-i}	222.7±1.1^{d-h}
	P-0	157.7±0.84^{ij}	234.5±0.26^{bc}	240.2±4.11^{b}	241.2±0.26^{b}	241±0.44^{b}	253.5±5.13^{a}
	P-3	157.7±0.84^{ij}	237.6±0.52^{b}	227.1±2.32^{cde}	223.2±3.45^{d-g}	214.6±1.67^{hi}	215.8±1.22^{ghi}
	P-6	157.7±0.84^{ij}	228.3±1.91^{cd}	226.2±2.15^{de}	224.5±0.37^{def}	216.4±1.90^{f-i}	224.5±1.28^{def}
	P-9	157.7±0.84^{ij}	220 ±1.03^{e-h}	222.5±3.1^{d-h}	228.1±2.34^{de}	224.4±2.73^{def}	227.8±2.12^{cd}
Total anthocyanin (mg C3gE/100mL)	Clamshell	41.1±0.41^{def}	43.4±0.90^{cde}	44.4±0.43^{cde}	45.6±1.23^{b-e}	46±1.05^{a-d}	42.6±1.05^{de}
	P-0	41.1±0.41^{def}	45.9±0.83^{a-e}	43.3±1.51^{cde}	43.6±1.22^{cde}	46.8±1.02^{abc}	46.1±1.23^{a-d}
	P-3	41.1±0.41^{def}	42.3±0.73^{e}	44.9±1.41^{cde}	45.4±0.33^{b-e}	45.3±1.44^{b-e}	43.6±2.02^{cde}
	P-6	41.1±0.41^{def}	44.4±0.94^{cde}	45.8±1.22^{b-e}	48.5±1.14^{ab}	49.2±0.71^{a}	45.8±0.19^{b-e}
	P-9	41.1±0.41^{def}	46.7±0.85^{abc}	45.5±0.03^{b-e}	44.6±1.01^{cde}	44.8±0.81^{cde}	44.8±1.11^{cde}
Ascorbic acid (mg AA/100mL)	Clamshell	15.3±0.13^{de}	13.7±0.25^{ef}	16.4±0.23^{a}	16.4±0.13^{a}	15.3±0.14^{bc}	15.6±0.25^{b}
	P-0	15.3±0.13^{de}	12.7±0.21^{ij}	15.3±0.12^{bc}	15.5±0.14^{b}	14.6±0.15^{d}	14.9±0.05^{cd}
	P-3	15.3±0.13^{de}	11.6±0.12^{kl}	13.2±0.09^{gh}	14.6±0.12^{d}	14.1±0.22^{e}	14.6 ±0.12^{e}
	P-6	15.3±0.13^{de}	11.8±0.12^{k}	13.0±0.22^{hi}	13.9±0.11^{e}	12.8±0.15^{hi}	12.4±0.25^{j}
	P-9	15.3±0.13^{de}	11.2±0.16^{l}	12.7±0.05^{ij}	13.4±0.05^{fg}	12.6±0.04^{ij}	11.3±0.06^{l}

All means presented in the same column with different letters are significantly different (p <0.05). All means presented in the same row with different letters are significantly different (p <0.05) according to Duncan's multiple range test.

Antioxidant activity

The potential antioxidant activity of pomegranate juice of arils was evaluated using DDPH and FRAP assays. Modified atmosphere packaging and storage duration had a significant impact on the antioxidant activity of pomegranate arils juice (p <0.05) for the two assays investigated. FRAP assay showed a progressive increase (up to three fold) in the antioxidant activity of arils juice extract (Table 5.2). The TPTZ ferric ions were reduced by the antioxidants of PJ into ferric-tripyridyltriazine complex. At the end of storage, P-3 and P-9 PM-MAP had highest values (171.1 ± 0.05 and 167 ± 1.62 µM TE/mL, respectively).

The results from DPPH assay followed similar trend as observed with the FRAP as depicted in Table 5.2. Although the interaction of package type and storage duration had no significant effect on the radical scavenging activity by PJ antioxidants, the effect of packaging type and storage duration was significant (p <0.05). After 3 days of storage, antioxidant activity of pomegranate arils juice increased across all treatments from an initial value of 173.9 ± 0.71 mM AAE/100 mL. Antioxidant activity of arils packaged in P-3, P-6 and P-9 increased to the highest (183.9 ± 2.13, 186.5 ± 2.43 and 186.1 ± 2.08 mM AAE/100 mL, respectively) at the end of 14 days of storage. These results do not corroborate with previous findings reported on the antioxidant capacity of fresh-cut table grape, which decreased towards end of storage life. It was suggested that decrease in antioxidant capacity with prolonged storage may be due to the O_2-promoted oxidation of the constitutive phenolic compounds and ascorbic acid (Shiri *et al*., 2011). Similarly, Ahyan & Esturk (2009) reported that the total antioxidant capacity based DPPH assay of pomegranate arils cv. 'Hicaznar' packaged under passive MAP increased initially during storage and then decreased towards the end storage. Increase in phenolic contents and total anthocyanin have been identified as a primary course for increased antioxidant activity in pomegranate arils (Scalzo *et al*., 2004; Kulkarni & Aradhya, 2005; Peano *et al*., 2013). Fluctuation in antioxidant activity was observed from arils packaged in clamshell. Furthermore, P-0 MA packaged arils maintained a relatively constant antioxidant throughout the storage period. This is consistent with the findings reported by Lopez-Rubira *et al*. (2005), in which antioxidant activity of pomegranate arils cv. 'Mollar of Elche' stored under passive MAP did not significantly change after 13 days at 5 ºC. Overall, the results reveal a difference in antioxidant activity of PJ samples, independent of all treatments between the assays used. This may be attributed to the chemical nature of biological system of samples (pomegranate arils) and multiplicity of antioxidant systems present that result into complex interactions in the food matrix (Lutz *et al.,* 2011). Due to this complexity, there is no single universal assay that can accurately reflect all antioxidants in *vivo*. Therefore, it is essential to perform multiple assays for better estimate of the antioxidant activity of samples (Sareem *et al*., 2008; Lutz *et al*., 2011).

Table 5.2 Effects of PM-MAP and storage duration on antioxidant properties of fresh processed pomegranate fruit arils under cold storage (5 ± 1 ºC). All results presented as mean values (± SE)

Antioxidant activity	PM-MAP Type	Storage duration (Days)					
		0	3	6	9	12	15
FRAP (TE µM/ml)	Clamshell	43.5±0.73^{ac}	50.2±0.55^{p}	91.7±0.09^{m}	117.2±0.25^{k}	141.9±0.64^{ef}	155.1±1.42^{cd}
	P-0	43.5±0.73^{ac}	53.2±2.32^{p}	96.3±1.13^{m}	122.2±2.03^{j}	143.8±1.14^{ef}	153.4±1.91^{d}
	P-3	43.5±0.73^{ac}	61.6±1.23^{o}	90.7±3.11^{m}	128±1.14g^{h}	140.7±1.04^{f}	171.1±0.05^{a}
	P-6	43.5±0.73^{ac}	58.8±1.04^{o}	96.5±2.10^{m}	129.7±0.73^{g}	145.7±1.21^{e}	160.2±1.52^{b}
	P-9	43.5±0.73^{ac}	69.5±2.22^{o}	102.1±2.11^{i}	124.5±1.64^{hi}	158.4±1.37^{bc}	167±1.62^{a}
DPPH (mM AAE100/mL)	Clamshell	173.9±0.71^{bcd}	182.6±1.50^{a-d}	178.3±1.14^{cde}	176.5±0.32^{de}	175.9±4.33^{e}	175.2±0.61^{e}
	P-0	173.9±0.71^{bcd}	182.4±2.22^{a-d}	182.8±1.15^{a-d}	182.3±2.03^{a-d}	183.1±2.03^{a-d}	183.4±2.64^{a-d}
	P-3	173.9±0.71^{bcd}	179.1±2.51^{a-e}	179.8±4.17^{a-e}	182.1±0.27^{a-d}	183.4±1.07^{a-d}	183.9±2.13^{a-d}
	P-6	173.9±0.71^{bcd}	181.2±1.44^{a-e}	184.9±3.13^{abc}	185.5±0.27^{abc}	186.4±0.52^{a}	186.5±2.43^{a}
	P-9	173.9±0.71^{bcd}	183.5±2.02^{a-d}	185.1±1.15^{abc}	185.5±1.12^{abc}	186.6±0.62^{a}	186.1±2.08^{ab}

All means presented in the same column with different letters are significantly different (p <0.05). All means presented in the same row with different letters are significantly different (p <0.05) according to Duncan's multiple range test.

Conclusion

The use of perforations in PM-MAP enhanced permeability of O_2 gas and water vapour and inhibited build-up of CO_2 gas. These conditions contributed to a significant increase in the anthocyanin concentration and total phenolics content of arils throughout the storage period. However, there was a slight decrease in concentration of ascorbic acid in PM-MAP probably due to oxidative reversible conversion into dehydroascorbic acid (DHA). This highlights the need for assessment of both ascorbic acid and DHA in order to avoid underestimation of ascorbic acid content.

The trend of progressive increase in antioxidant activity was revealed with both FRAP and DPPH assays showing packaging type as main significant factor. Pomegranate arils packaged in PM-MAP had the highest antioxidant activity until end of 14 days of storage compared to those in P-0 and clamshell trays. However, irrespective of all treatments, different results were obtained using two selected antioxidant determination assays. This shows the relevance of performing multiple assays to obtain better estimate of the antioxidant activity of analysed samples. Overall, the application of cold storage (5 ºC) and packaging system that enables enhanced exchange of gases (O_2, CO_2) and water vapour between packaged produce and the external environment could be suitable for better preservation of phytochemical components and antioxidant properties of fresh 'ready-to-eat' pomegranate arils.

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CHAPTER SIX: General discussion and conclusion

GENERAL DISCUSSION AND CONCLUSION

General Introduction

Global production and consumption of pomegranate fruit has gained momentum in recent years due to the reported high contents of health-promoting phyto-nutrients and high antioxidant capacity, with numerous nutritional and medicinal benefits (Hassan *et al*., 2012; Fawole *et al*., 2013). Studies have shown that bioactive components of pomegranates have preventive and therapeutic effects for various degenerative diseases (Pala & Toklucu, 2011). In spite of these benefits, the consumption of pomegranates has been limited due to the difficulty of peeling the rind in addition to the staining of fingers (Defilippi *et al*., 2006; Caleb *et al*., 2012a). Thus, minimal processing into ready-to-eat arils offers convenience to consumers (Caleb *et al*., 2012b). However, the risk of quality deterioration of fresh minimally processed produce is a major challenge due to rapid physiological ageing and microbial spoilage (Fonseca *et al*., 2002; Ragaert *et al*., 2007). Modified atmosphere packaging (MAP) technology combined with optimum storage conditions have been used to maintain fresh and desirable qualities of fresh minimally processed produce. However, most commercial packaging films are characterised by high barrier properties to gas and water vapour. This results in the generation of anoxic conditions and moisture condensation inside the packages, which subsequently accelerates microbial growth (Farber *et al*., 2003; Mangaraj *et al*., 2009). Hence, this study was carried out to optimise permeability of two selected packaging films by analysing the effect number of perforations on water vapour transmission rate at different storage temperatures. Furthermore, the study evaluated the effect of perforation-mediated modified atmosphere packaging (PM-MAP) on the physico-chemical properties and shelf-life of fresh processed pomegranate fruit arils (cv. Acco). The study went further to investigate the influence carbon dioxide (CO_2) and oxygen (O_2) concentrations generated in PM-MAP on phytochemicals and antioxidant properties of fresh minimally processed pomegranate fruit arils cv. ‘Acco’.

Chapter two: Literature review on PM-MAP of fresh and minimally processed produce

Modified atmosphere packaging technology combined with optimum storage conditions has shown promise in extending shelf-life of fresh and minimally processed produce (Caleb *et al*., 2012b; Mahajan *et al*., 2014). On these bases, the principles, functions and role of MAP and PM-MAP on postharvest quality and extension of shelf-life of fresh and processed produce were reviewed in this chapter. A critical discussion of principles and functions of

perforations in PM-MAP and current application on fresh and minimally processed produce was explored. This review has showed the importance of applying a systematic modelling approach in the design of MAP and PM-MAP to establish pertinent relationships among important parameters such as film permeability and produce RR. The benefits of matching film permeability to produce metabolic rates was undisclosed. The review highlighted the need for research on suitability of PM-MAP before ultimate commercialization for each specific produce. The concern about overall produce safety and quality of PM-MA packaged fresh produce due to the risk of permeation of moisture, loss of volatile organic compounds and ingress of microorganisms through perforations of perforation-mediated MAP was also discussed.

Research chapter three

This study in this chapter evaluated the use of perforations as a simple and cost efficient technique to optimise WVTR of polymeric films for food packaging applications. The effect of number of perforation (3, 6 and 9) and storage temperature (5, 10 and 15 ºC) on WVTR (g/m^2.day) of synthetic polymeric 'polylid' and biodegradable 'Nature flex' films was investigated. For non-perforated films, biodegradable had higher WVTR than synthetic polymeric film. These results were in consistent with previous reported findings (Koide & Shi, 2007; Macedo *et al*., 2013). Number of perforations, storage temperature and their interaction had significant effects on WVTR of studied films, although the effect of temperature was more pronounced in biodegradable sample films. Increase in number of perforations increased WVTR of both films by close to three-fold as compared to temperature. Furthermore, mathematical model to predict water vapour transmission rate films as a function of number of perforations and storage temperature was successfully developed by combining the exponential and Arrhenius type equations. At a temperature of 8 ºC, the global model was found to fit very well (R^2 = 93.9%) to the experimental data.

The results of WVTR of perforated polylid have shown the potential of perforation technology in optimisation of water vapour permeability of synthetic polymeric films commonly used for packaging of fresh produce. Apparently, most of the biodegradable starch-based polymers such as Natureflex™ may be preferred in MAP applications due to their better permeability to water vapour as compared to conventional polymeric films (Koide & Shi, 2007; Mistriotis *et al*., 2011). However, in light of the results found in the present study, the use of perforations seems to have a potential to significantly improve water vapour permeability of synthetic polymeric such as polylid films that are predominantly used by food companies. Thus, application of perforation technique can provide a wide spectrum of commonly used

polymeric films with better permeability results (Dirim *et al*., 2004; Mahajan *et al*., 2008). This could replace the need for 'ready-made' laminate films of desirable permeability properties whose cost remain higher than the petro-based synthetic polymeric films. A good fit observed between the experimental data and predicted values (R^2 = 93.9%) using global model is of industrial relevance due to its potential in validation of commercial instruments specifically designed for perforation of food packaging films.

Research chapter four

The application of high barrier polymeric films in MAP of fresh produce is associated with generation of unsuitable in-package gas composition, moisture condensation and subsequent microbial growth, causing loss of quality and limited shelf-life. Perforation-mediated modified atmosphere packaging offers the possibility of optimising polymeric films in order to compensate for barrier limitations. The effect of PM-MAP and storage temperature (5 ºC) on extension of shelf-life of fresh pomegranate arils was investigated and reported in this chapter. Physico-chemical properties and microbial quality of aseptically hand processed pomegranate arils packaged in PM-MAP with perforations (P) (0, 3, 6 and 9) and stored at 5 ^{0}C were investigated at interval of 3, 6, 9, 12 and 15 days. Weight loss was found significant in PM-MAP (P-3, P-6 and P-9) packaged arils and increased over storage duration, in consistent with other reports (Lucera *et al*., 2010; Bhatia *et al.,* 2013). Previous reports have related water vapour permeability of packaging material to weight loss of packaged produce due to enhanced water uptake from packaged produce by evaporation (Lucera *et al*., 2010; Krasnova *et al*., 2012; Bhatia *et al*., 2013). On the other hand, the initial slight increase in weight of clamshell and P-0 packaged arils could be attributed to incidences of moisture condensation observed in the packages, which in turn may also have influenced microbial growth (Caleb *et al*., 2013). These results highlight the need for modelling of water vapour permeability of packaging film with perforations during the process of PM-MAP design.

In-package gas composition during storage was affected by the presence of perforations, which helped balance decrease in O_2 with corresponding increase in CO_2 levels, thus preventing anoxic conditions (De Reuck *et al*., 2009; Pandey & Goswami, 2012). Highest concentration of CO_2 was measured in P-0 packages which increased progressively with storage duration from 9.6 % to 34.0 % on day 3 to 15, respectively. Higher O_2 and low CO_2 concentrations achieved in PM-MAP influenced changes of both physico-chemical and microbial quality of packaged arils. Based on the gas composition results obtained in this study, it could be established that different number of perforations can achieve different in-

package gas concentrations. This knowledge in combination with the understanding of fruit physiology could help in designing of tailor-made PM-MAP packages suitable for extending shelf-life of specific fresh produce.

Furthermore, in this study we observed a significant decrease in total soluble solids (TSS) and titratable acidity (TA) of packaged arils in P-3, P-6 and P-9 PM-MAP. This was attributed to possible increase in respiratory activities due to high levels of O_2 in the PM-MAP as reported by Bhatia *et al*. (2013). There were changes in pH attributed to changes in CO_2 levels across all packages, with the highest pH (4.2 ± 0.02) measured in P-0 due to accumulation of CO_2. The increase in pH was in consistent with previous reports on MA-packaged pomegranate arils (Ahyan & Esturk, 2009; Caleb *et al*., 2013). Number of perforations in PM-MAP affected moisture loss significantly. Loss in moisture influenced decrease in firmness, as previously reported by several authors (Ayhan & Esturk, 2009; Bhatia *et al*., 2013). However, P-3 packaged arils had better firmness after 14 days of storage compared to P-6 and P-9. Overall, variation of pH, TSS, TA and texture observed of this study in comparison with previous reported results in literature could be explained in part by differences in cultivar, agro climatic regions and postharvest pre-treatment techniques (Caleb *et al*., 2013), and to a certain extent by differences in MAP gas compositions achieved during storage.

Packaging and storage duration had significant affect *(p* < 0.05) on microbial growth, while *E. coli* was below detection level across all treatment. The lowest (4.1 log CFU/g) and highest (5.5 log CFU/g) aerobic mesophilic bacteria counts were observed in P-0 and P-9 PM-MAP, respectively. Nonetheless, the counts did not exceed maximum acceptable limit (7 log CFU/g) at the end of 14 d storage. Yeast and moulds counts were below acceptable limit (5 log CFU/g) in PM-MAP and higher in P-0 MAP. Higher microbial counts were found in this study in comparison to previous similar studies of conventional (high barrier) MAP (Lopez-Rubira *et al*., 2005; Ahyan & Estürk, 2009; Caleb *et al*., 2013). In part, this could be the result of cross-contamination of packaged arils by various sources of spoilage microorganisms in the storage room. The current results revealed the importance of keeping PM-MAP packages in contamination free environment such as closed refrigerated shelves or displays to avoid cross contamination or ingress of foodborne pathogens.

Overall, the results in this chapter have revealed that high O_2 and low CO_2 atmospheres in PM-MAP inhibited the development of anoxic conditions. Despite the slight decrease TSS and TA of fresh arils, P-3 and P-6 packaged arils better maintained these quality attributes. Higher aerobic mesophilic bacteria reported in this study could be attributed to the high O_2 level available to the perforated packages which enhanced their growth. Thus, the study has

shown the need for good processing practices, optimal cold chain and sanitation all along the postharvest handling chain of fresh minimally processed produce. Future research should consider the evaluation volatile compounds and microbial analysis for better evaluation of shelf-life of fresh pomegranate arils. Also, the role of PM-MAP on the development of characteristic flavour quality of pomegranate arils should be investigated.

Research chapter five

Due to the effect of metabolic activities on various phytonutrients and their constitutive antioxidant activities, evaluation of changes in the content and activity of bioactive compounds during postharvest storage of fresh minimally processed produce is very essential. The effect of PM-MAP on the stability of phytochemicals and antioxidant properties of fresh processed pomegranate fruit arils was discussed in chapter 5. Package type had a significant effect on changes in the total anthocyanin, total phenolics and ascorbic acid concentration (p <0.05). Arils packaged in PM-MAP had highest content of anthocyanin. Total anthocyanins content of arils packaged in PM-MAP increased slightly for arils packaged in P-3 (43.6 ± 2.03 mg C3g E/100 mL), P-6 (45.8 ± 0.9 mg C3g E/100 mL) and P-9 (44.8 ± 1.1 mg C3g E/100 mL), respectively at the end of storage. This increase could be related to higher O_2 levels and moisture loss observed in PM-MAP during storage of pomegranate arils that lead to biosynthesis and concentration of anthocyanins in pomegranate juice, respectively. These results were in agreement with previous findings reported by (Gil *et al*., 1996). However, previous findings reported a significant decrease in anthocyanin of various pomegranate arils cultivars during postharvest storage (Lopez-Rubira *et al*., 2005; Ahyan & Esturk, 2009; Caleb *et al*., 2013). This could be due to low O_2 and relatively high CO_2 achieved level with traditional MAP (that use high barrier film).

The highest content of total phenolics content was measured in P-0 MAP. However, arils in PM-MAP showed an increase in phenolics with substantial fluctuation towards the end of storage. Metabolic activities and subsequent changes in TSS and TA during storage could influence changes in the total phenolics content through new biosynthesis and interconversion reactions (Ahyan & Esturk, 2009; Peano *et al*., 2013). Ascorbic acid was negatively affected by gas compositions in PM-MAP. Ascorbic acid concentration was maintained in P-0 and clamshell trays, although there was a slight decrease towards end of storage in these packages. Lower concentrations of ascorbic acid in PM-MAP could be attributed to the oxidation of ascorbic acid into dehydroascorbic acid (DHA) due to high O_2 and low CO_2 levels in P-3, P-6 and P-9 packages (Lee & Kader, 2000).

The antioxidant activities evaluated using FRAP assay showed a three-fold increase across all PM-MAP from initial value of 43.52 µM TE/mL after 14 days of storage. The DPPH assay showed an initial increase in antioxidant activities for arils in PM-MAP followed by stabilization until end of storage. Contrary to our results, previous findings in literature reported decrease in antioxidant activity that could have been attributed to O_2-promoted oxidation of the constitutive phenolic compounds and ascorbic acid (Ahyan & Esturk, 2009; Shiri *et al*., 2011). In P-0 MAP, antioxidant activity of arils was maintained, in agreement with results reported on MAP of arils cv. 'Mollar of Elche' by Lopez-Rubira *et al*. (2005).

High O_2 and low CO_2 compositions generated in PM-MAP better maintained the bioactive components of fresh pomegranate arils during storage. The decrease in ascorbic acid (AA) concentration in PM-MAP packaged arils that could have been attributed to reversible oxidation of AA to DHA enlighten the need for evaluating the two interconversion products to avoid underestimation of ascorbic acid content. Additionally, this study has shown the importance of using multiple assays in evaluation of antioxidant activities for better estimate. Overall, this study suggests that high O_2 and relatively low CO_2 achieved by PM-MAP were suitable for the stability of bioactive compounds over traditional low O_2 MAP. Therefore, perforation-mediated modified atmosphere packaging showed a high potential in maintaining the phytochemical and antioxidant properties of fresh pomegranate arils.

Recommendations and future prospects

This current study has shown that perforation-mediated modified atmosphere packaging could be suitable for extending the shelf-life of fresh pomegranate arils as it offered many benefits over conventional or high barrier MAP. The use of perforations in PM-MAP enabled rapid establishment of desirable in-package gas composition which prevented fermentative reactions of packaged arils, maintained the concentration of bioactive compounds at the cost of slight decrease in physico-chemical quality attributes.

Based on physico-chemical and microbial quality measured in this study, arils packaged in packages with fewer numbers of perforations (P-3 and P-6) had better quality and longer shelf-life. These results have enlightened the significance of the combination of number and size of perforations used in PM-MAP. However, this combination could influence not only the gas compositions but also the degree of ingress of microorganisms, undesirable odour and dusts from the surrounding storage environment while enhancing egress of volatile organic compounds (VOCs) from the package. This highlights on the need for future study on the impact of perforations (PM-MAP) on change in characteristic VOCs in packaged

fresh/minimally processed produce. Additionally, studies have shown possible correlation between VOCs and microbial growth in packaged products. Hence, a better understanding of changes in VOCs under PM-MAP could ensure consumer confidence towards PM-MAP packaged produce. Therefore, future prospects must focus on investigating the effect of perforations on flavour life and microbial quality of fresh packaged arils. Furthermore, polymeric film permeability can be optimized via numerous micro-perforation using laser perforation technologies instead of the use of manual perforation. Hence, future research need is required towards establishing the desired number of micro-perforation to maintain quality and extend shelf-life of fresh pomegranate arils.

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