Modified atmosphere packaging of pomegranate arils

Oluwafemi James Caleb

Dissertation presented in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

In the Department of Food Science
Faculty of AgriSciences
Stellenbosch University

Promoter: Prof U.L. Opara

Co-promoter: Prof M. Manley

Co-promoter: Dr P.V. Mahajan

March 2013

Declaration

By submitting this dissertation, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it at any other university for obtaining any qualification.

March 2013

Summary

Modified atmosphere packaging (MAP) is a dynamic process of altering gaseous composition inside a package. It relies on the interaction between the respiration rate (RR) of the produce, and the transfer of gases through the packaging material. These two processes are dependent on numerous factors such as storage temperature, film thickness and surface area, produce weight as well as free headspace within the pack. Therefore, in order to achieve the desired modified atmosphere in a given package, it is important to understand the three basic disciplines of MAP, namely produce physiology, polymer engineering, and converting technology.

In this study the effects of storage conditions and duration on physiological responses i.e. respiration (RR) and transpiration rate (TR) of two pomegranate cultivars 'Acco' and 'Herskawitz', were investigated and mathematical models were developed to predict these physiological responses at given time and storage conditions. The result of this study showed that RR of whole pomegranate fruit was significantly higher than that of fresh arils, and that temperature had a significant impact on the RR of both whole fruit and fresh arils. The influence of time, and the interaction between temperature and time also had significant influences on RR of fresh pomegranate arils. These findings highlight the significance of maintaining optimal cold-storage condition for packaged arils or whole fruit along the supply chain. In addition, mathematical models based on the Arrhenius-type equation and the power function equation coupled with Arrhenius-type equation accurately predicted the effect of temperature and the influence of time and temperature on the RR of fresh pomegranate arils for both cultivars.

Furthermore, the results of experimental and model prediction studies showed that both relative humidity (RH) and storage temperature had significant effects on TR. RH was the variable with the greatest influence on TR, and it was observed that arils were best kept at 5 °C and 96% RH to maintain quality for 8 days. The applicability of the transpiration model developed was validated based on prediction of TR of pomegranate arils under different combinations of storage conditions. The model adequately predicted TR and provides a useful tool towards understanding the rate of water loss in fresh pomegranate arils as affected by storage conditions and duration.

The effect of passive-MAP engineering design parameters as a function of produce weight contained, storage temperature and duration on fresh pomegranate arils was investigated. The result showed that produce weight of aril content, temperature and the interaction between temperature and time had slight but insignificant effects on measured physicochemical quality attributes. However, headspace gas concentration was significantly influenced by produce weight and storage temperature. Oxygen (O_2) composition decline below 2% after day 3 and 5 at 15 and 10 °C, respectively, while samples at 5 °C did not reach below 2% throughout the study. On the other hand, CO_2 levels increased significantly during storage for all packaging conditions. This study showed the importance of a systematic approach to the design of optimal MAP systems. At lowest storage temperature the inability to achieve desired modified atmosphere (MA) required for optimal storage of arils despite the increase in produce weight, suggests that the use of active gas modification (gas flushing with recommended atmosphere) would be necessary. However, the present results show that at higher temperature macro/micro perforations would be required on the polymeric films used in the present study in order to avoid critical levels of O_2 and CO_2.

The influence of passive MAP, storage temperature and duration on volatile composition and evolution of packaged pomegranate arils was investigated. The results showed that changes in aroma compounds were dependent on cultivar differences, storage condition and duration. Using GC-MS analysis of pomegranate juice HS-SPME extracts, a total of 18 and 17 volatiles were detected for 'Herskerwitz' and 'Acco', respectively. Furthermore, flavour life (7 days) was shorter than the postharvest life (10 days) for both cultivars. There was a decrease in volatile composition during the storage period (aldehydes < alcohols < esters) while the concentration (%) and composition of ethyl esters increased with storage time.

These results highlight the need for a more precise definition of flavour shelf life for MA-packaged pomegranate arils and other packaged fresh produce. The importance of maintaining optimal cold storage condition, selection of appropriate packaging materials and a systematic approach to the design and application of MAP systems has also been shown.

Opsomming

Gemodifiseerde atmosfeer-verpakking (GAV) is 'n dinamiese proses waartydens die gassamestelling binne-in 'n verpakking gewysig word. Dit berus op die wisselwerking tussen die respirasietempo (RT) van die produkte en die oordrag van gasse deur die verpakkingsmateriaal. Hierdie twee prosesse is van verskeie faktore soos bergingstemperatuur, dikte van die film en oppervlakte, gewig van die produkte asook vry boruimte binne-in die pakkie afhanklik. Om dus die gewenste gemodifiseerde atmosfeer in 'n gegewe verpakking te verkry, is dit belangrik om die drie fundamentele dissiplines van GAV te begryp, naamlik produkfisiologie, polimeerontwerp, en omsettingstegnologie.

In hierdie studie is die gevolge van bergingstoestande en -duur op fisiologiese reaksie, met ander woorde, respirasie- (RT) en transpirasietempo (TT) van twee geselekteerde granaatkultivars 'Acco' en 'Herskawitz', ondersoek en wiskundige modelle is ontwikkel om ons in staat te stel om hierdie fisiologiese reaksies by gegewe tyd- en bergingstoestande te voorspel. Die resultaat van hierdie studie het aangetoon dat die respirasietempo (RT) van heel granaatvrugte aansienlik hoër was as die RT van vars arils, en temperatuur het beduidende uitwerking op RT van beide heel vrugte en vars arils gehad. Die invloed van tyd, en die wisselwerking tussen temperatuur en tyd het ook 'n beduidende invloed op die RT van vars granaatarils gehad. Hierdie bevinding beklemtoon die belang van die handhawing van optimale koelbewaringstoestande vir verpakte arils of heel vrugte met die aanvoerketting langs. Daarbenewens wiskundige modelle wat gebaseer op die Arrhenius-tipe vergelyking en die magsfunksie-vergelyking gepaard met Arrhenius-tipe vergelyking, die uitwerking van temperatuur en die invloed van tyd en temperatuur op die RT van vars granaatarils vir beide kultivars onderskeidelik voldoende en akkuraat voorspel.

Afgesien die resultate van eksperimentele en modelvoorspellings die studies aangetoon dat beide relatiewe humiditeit (RH) en bergingstemperatuur 'n beduidende uitwerking op TT het. RH was die veranderlike met die grootste invloed op TT, en it was waargeneem dat dit die beste was om arils teen 5 °C en 96% RH te bewaar (8 dae). Die toepaslikheid van die transpirasiemodel wat ontwikkel is, is bevestig op grond van voorspelling van TT van granaatarils onder verskillende kombinasies van bergingstoestande. Die model het TT voldoende voorspel en sou 'n bruikbare instrument wees ten einde die waterverliestempo

in vars granaatarils en ander vars produkte, soos deur bergingstoestande en duur beïnvloed, te begryp.

Die uitwerking van passiewe-GAV ontwerpparameters as 'n funksie van gewig van die produkte, bergingstemperatuur en duur op vars granaatarils is ondersoek. Dit het aan die lig gebring dat gewig van die produkte, temperatuur en die wisselwerking tussen temperatuur en tyd 'n geringe maar onbeduidende uitwerking op gemete fisikochemiese gehalte-eienskappe gehad het. Die gaskonsentrasie in die boruimte is betekenisvol beïnvloed deur gewig van die produkte en bergingstemperatuur. Die O_2-samestelling het tot benede 2% gedaal na 3 en 5 dae by 15 en 10 °C, onderskeidelik, terwyl monsters by 5 °C deur die studie heen nooit laer as 2% was nie. Aan die ander kant, CO_2-vlakke het gedurende berging betekenisvol verhoog wat betref alle verpakkingstoestande. Hierdie studie het die belangrikheid van 'n sistematiese benadering by die ontwerp van 'n optimale GAV-stelsel aangetoon. By die laagste bergingstemperatuur dui die onvermoë om die gewenste gemodifiseerde atmosfeer (GA) wat vir optimale berging van arils benodig word, te verkry – ondanks die toename in die gewig van die produkte – daarop dat die gebruik van aktiewe gasmodifisering (gasspoeling met aanbevole atmosfeer) nodig sou wees. Egter die huidige uitslae aangetoon dat by hoër temperatuur, sou makro/mikroperforasies op die polimeerfilms wat gebruik word in die onderhawige studie egter nodig wees ten einde kritiese vlakke van O_2 en CO_2 te verhoed.

Die invloed van passiewe GAV, bergingstemperatuur en duur op onstabiele samestelling en evolusie van verpakte granaatarils is ondersoek. Die resultate aangetoon dat veranderinge in aromaverbindings afhanklik was van kultivarverskille, bergingstoestande en duur. Met behulp van GC-MS-analise van granaatsap HS-SPME-ekstrakte, het ons 'n totaal van 18 en 17 vlugtige stowwe vir 'Herskawitz' en 'Acco', onderskeidelik bespeur. Verder het ons waargeneem dat die smaakleeftyd (7 dae) korter was as die na-oesleeftyd (10 dae) vir beide kultivar. Daar was 'n afname in vlugtige samestelling (aldehiede < alkohole < esters) terwyl die konsentrasie (%) en samestelling van etielesters het met bergingstyd verhoog.

Hierdie resultate het die aandag gevestig op die behoefte aan 'n meer presiese definisie van geur-raklewe vir GA-verpakte granaatarils en ander verpakte vars produkte. Die belang van die handhawing van die optimale koelbewaringstoestand, seleksie van geskikte verpakkingsmateriaal en 'n sistematiese benadering tot die ontwerp van 'n optimale GAV-stelsel, is ook beskryf.

CONTENTS

Chapter		Page
	Summary	iii
	Opsomming	v
	Acknowledgements	viii
1	Introduction	2
2	Literature review Published as: Caleb, O.J., Opara, U.L. & Witthuhn, C.R. (2012). Modified atmosphere packaging of pomegranate fruit and arils: A Review. *Food and Bioprocess Technology*, **5**(1), 15-30. Published as: Caleb, O.J., Mahajan, P.V., Fahad, A.A., & Opara, U.L. (2012). Modified atmosphere packaging technology of fresh and fresh-cut produce and the microbial consequences - A Review. *Food and Bioprocess Technology,* **6**(2), 303-329.	8
3	Investigating the impact of temperature and relative humidity on the transpiration rate of pomegranate arils Published as: Caleb, O.J., Mahajan, P.V., Fahad, A.A. & Opara, U.L. (2012). Transpiration rate and quality of pomegranate arils as affected by storage conditions. *CyTA – Journal of Food,* DOI 10.1080/19476337.2012.721807	75
4	Evaluating the effect of storage temperature on the respiration rates of pomegranate fruit and arils Published as: Caleb, O.J., Mahajan, P.V., Opara, U.L. & Witthuhn, C.R. (2012). Modelling respiration rate of pomegranate fruit and arils. *Postharvest Biology and Technology,* **64**, 49-54.	102
5	Development of prediction model describing the effect of time and temperature on respiration rate of pomegranate arils Published as: Caleb, O.J., Mahajan, P.V., Opara, U.L. & Witthuhn, C. R. (2012). Modelling the effect of time and temperature on respiration rate of pomegranate arils cv. 'Acco' and 'Herskawitz'. *Journal of Food Science,* **77**(4), E80-E87.	119
6	Evaluation of modified atmosphere packaging design parameters for pomegranate arils Caleb, O.J., Mahajan, P.V., Manley, M. & Opara, U.L. (2013). Evaluation of modified atmosphere packaging engineering design parameters for pomegranate arils. *International Journal of Food Science and Technology*, **(in press)**	140
7	Changes in volatile composition as an indicator of microbial stability and shelf life of modified atmosphere packaged pomegranate arils Caleb, O.J., Opara, U.L., Mahajan, P.V., Manley, M., Mokwena, L. & Tredoux, A.G.J. (2013). Effect of modified atmosphere packaging and storage temperature on volatile composition and postharvest life of pomegranate arils (cv. 'Acco' and 'Herskawitz'). *Postharvest Biology and Technology,* **79**, 54-61.	170
8	General discussion and conclusion	197

Language and style used in this dissertation are in accordance with the requirements of the *International Journal of Food Science and Technology*, as prescribed by the Department of Food Science, Stellenbosch University.

This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Acknowledgements

Unto God almighty for His mercy, grace, and loving kindness, to him I give all praise and adoration, for having favoured and given me strength to pursue this path.

To my promoter Prof U.L. Opara and co-promoter Prof M. Manley, Dr P.V. Mahajan and Prof R.C. Witthuhn (who left for a new appointment at the University of Free State), my sincere thanks and gratitude for your guidance, support, advice, critical reviews and constant encouragement, as well as, giving me the opportunity to undertake this research under your supervision.

To the Department of Food Science and the South African Research Chair in Postharvest Technology, Stellenbosch University, for the privileges and financial support throughout my study. Stellenbosch University Postgraduate International Office Overseas Research Visit (PGIO-ORV) travel grant awarded towards my research trip to University College Cork, Ireland.

Mrs Marie Maree, Nazneen Ebrahim, Daleen du Preez, Petro du Buisson, and Veronique Human for administrative and technical support throughout my research.

Mr Fan Oliver of Houdconstant Pack-house, Porterville, for advice concerning procurement and processing of pomegranate fruit and packaging, as well as Mr Gerrit Nieuwoudt of Colorsfruit for assistance with fruit procurement.

Dr A.G.J. Tredoux (Institute for Wine biotechnology, Department of Viticulture and Oenology) and Mr L. Mokwena (Central Analytical Facilities, Mass Spectrometry Unit), for their assistance and guidance with gas chromatography and mass spectroscopy (GC-MS) analysis.

To my parents, Mr and Deaconess F.O. Caleb, my siblings (Oluwatobi, Folusho, Olumide and Tolulope), and to my love and sustainability manager Ms Maria Huber, for believing in me and most importantly for your prayers, love and support at all times, even when it was difficult and unthinkable to do so. I am grateful for your trust in me.

I am equally indebted to my colleagues (Postharvest Technology Research Lab.), friends and members of RCCG (Desire of Nations, pastor by Dr Funlola Olojede). With the fear that I might leave anyone of you out during my editorial process, I would not list you all by name. But, words cannot express my gratitude enough for your advice, help, encouragement and understanding through the many waters we thread and paths we shared. Thank you.

“Keep hope alive...Keep walking...”

“From the fullness of His grace we have all received one blessing after another”
John 1:16

Chapter 1

Introduction

CHAPTER 1

INTRODUCTION

Almost all the world's pomegranate is cultivated in the northern hemisphere, with India being the world's largest producer of pomegranates with an estimated annual production of about 900,000 tons (INMA, 2008). During 2005/2006, the global export earnings from pomegranate were estimated at US$ 188 million (GOI-UNCTAD DFID, 2007). The harvest dates of pomegranate in the northern hemisphere are between September and November depending on the cultivar (Gil *et al*., 1996a; López-Rubira *et al*., 2005). This opens a window of opportunity when the fruit is not available (due to alternating seasons across the hemisphere), for the southern hemisphere including South Africa to export into the northern hemisphere.

During the last decade, there has been a remarkable increase in the commercial farming of pomegranate fruits globally, due to the potential health benefits of the fruit (Hess-Pierce & Kader, 2003; Holland & Bar Ya'akov, 2008) such as, its high antioxidant, anti-mutagenic, anti-hypertension activities and the ability to reduce liver injury (Viduda-Martos *et al*., 2010). Pomegranate anthocyanins have been demonstrated to scavenge hydroxyl (OH^-) and superoxide (O^-) radicals, preventing lipid peroxidation in rat brain homogenates (Noda *et al*., 2002). Also, the plasma antioxidant status of humans fed pomegranate juice was observed to be higher than those of the control subjects (Seeram *et al*., 2004). This observation suggests that pomegranate polyphenolic compounds are able to elevate the antioxidant capacity of the body. Pomegranate fruit is also known for its anti-inflammatory and anti-atherosclerotic effect activity against osteoarthritis, prostate cancer, heart disease and HIV-1 (Malik *et al*., 2005; Neurath *et al*., 2005; Sumner *et al*., 2005). The edible portion of pomegranate is an excellent dietary source it contains a significant proportion of organic acids, soluble solids, polysaccharides, vitamins, fatty acids and mineral elements of nutritional significance (Ewaida, 1987; Fadavi *et al*., 2006). Furthermore, different varieties of pomegranate fruit have been report to have a high content vitamin C (Dumlu & Gürkan, 2007; Opara *et al*., 2009), significant antimicrobial effects (Opara *et al*., 2009) and various industrial applications this include; their use as dyes, food colourants, inks, tannins for leather and juice (Ergun & Ergun, 2009).

In spite of the numerous health benefits, pomegranate consumption is still limited, due to the difficulties of extracting the arils from the fruit and, the irritation of phenolic metabolites' which stain the hands during preparation of seeds (Gil *et al*., 1996b). Fruit disorder such as sun-burnt husks, splits and cracks, and husk scald on the whole fruit reduces marketability and consumer acceptance (Saxena *et al*., 1987; Defilippi *et al*., 2006; Sadeghi & Akbarpour, 2009). Hence, minimally processed pomegranate fruit (ready-to-eat arils), presents a more appealing produce to consumers than whole fruit (Gil *et al*., 1996a; Gil *et al*., 1996b; Sepúlveda *et al*., 2000; Ergun & Ergun, 2009), and increases the prospect of production and consumption of pomegranate. Therefore, fresh arils could be an excellent way to obtain a commercial profit from unacceptable whole fruit with disorder such as sun-burn husk and cracks.

Modified atmosphere packaging is an active or passive dynamic process of altering gaseous composition inside a packaged. It relies on the interaction between the respiration rate (RR) of the fresh or fresh-cut produce and exchange of gases through the packaging material (Fonseca et al., 2002). Application of MAP for fresh produce slow down physiological processes, delay softening and ripening and a reduced incidence of various physiological disorders and pathogenic infestations (Saltveit, 2003). However, when fruit respiration does not correlate to the permeability properties of packaging film, increase in the concentration of CO_2 will build up resulting in a state of anaerobic respiration and ethanol accumulation in the fruit. This results in the development of off-flavours and decay of fruit while in the package unit (Fonseca *et al*., 2002; Ares *et al*., 2007).

Studies have shown that modified atmosphere packaging (MAP), and controlled atmosphere storage have the ability to delay quality losses and thus extend shelf life of fresh or minimally processed pomegranate arils (Artés *et al*., 2000; Sepúlveda *et al*., 2000; López-Rubira *et al*., 2005). Current research on aroma and flavour of pomegranate fruit concentrated on identification of unique volatiles produced by ripe pomegranate fruit (Calín-Sánchez *et al*., 2011; Melgarejo *et al*., 2011; Mayuoni-Kirshinbanum *et al*., 2012). Calín-Sánchez *et al*. (2011) and Melgarejo *et al*. (2011) suggested that consumer liking of pomegranate juices could be linked with the high levels of monoterpenes. This was corroborated by report of Mayuoni-Kirshinbanum *et al*., (2012), wherein 5 out the 12 detected 'Wonderful' pomegranate aroma-active compounds by the GC-O sniffing panellists were terpens. Thus, this suggests that class of aroma compound and concentration plays a

role among cultivar preference for pomegranate (Melgarejo *et al*., 2011). However, increased interest in minimally processed and fresh-cut pomegranate arils with high nutritional value and improved arils quality has highlighted our limited knowledge of factors that affect flavour development in modified atmosphere packaged pomegranate arils.

However, there is limited information on the quantitative description of physiological response of fresh arils via mathematical modeling, which is essential for the design of MAP. These studies were based on empirical rather than systematic approach as no MAP design was reported. The aim of the current study was to investigate the application of MAP for postharvest storage of pomegranate arils. Highlight the quality changes in physicochemical properties of pomegranate arils during storage. Better understanding of the responses of arils to MAP will extend the shelf or storage life of the fruit. Assist all the role players in the pomegranate value chain including fruit producers, suppliers and processors in selecting packaging materials and storage conditions, in order to maintain physicochemical, sensory and microbial stability of minimally processed pomegranate arils.

References

Ares, G., Lareo, C. & Lema, P. (2007). Modified atmosphere packaging for postharvest storage of mushrooms. A review. *Fresh Produce*, **1**, 32-40.

Artés, F., Villaescusa, R. & Tudela, J.A. (2000). Modified atmosphere packaging of pomegranate. *Journal of Food Science*, **65**, 1112-1116.

Caleb, O.J., Opara, U.L. & Witthuhn, C.R. (2012). Modified atmosphere packaging of pomegranate fruit and arils: a review. *Food and Bioprocess Technology,* **5**, 15-30.

Calín-Sánchez, A., Martínez, J.J., Vázquez-Araú L., Burló, F., Melgarejo, P., & Carbonell-Barrachina, A.A. (2011). Volatile composition and sensory quality of Spanish pomegranates (*Punica granatum* L.). *Journal of Science Food Agriculture*, **91**, 586-992.

Defilippi, B.G., Whitaker, B.D., Hess-Pierce, B.M. & Kader, A.A. (2006). Development and control of scald on Wonderful pomegranate during long-term storage. *Postharvest Biology and Technology*, **41**, 234-243.

Dumlu, M.U. & Gürkan, E. (2007). Elemental and nutritional analysis of Punica granatum from Turkey. *Journal of Medical Food*, **10**, 392-395.

Ergun, M. & Ergun, N. (2009). Maintaining quality of minimally processed pomegranate arils by honey treatments. *British Food Journal*, **111**, 396-406.

Ewaida, E.H. (1987). Nutrient composition of "Taifi" pomegranate (*Punica granatum* L). Fragments and their suitability for the production of jam. *Persian Gulf Science Research Agricultural and Biological Sciences*, **3**, 367-378.

Fadavi, A., Barzegar, M. & Azizi, H. (2006). Determination of fatty acids and total lipid content in oilseed of 25 pomegranate variaties grown in Iran. *Journal of Food Composition and Analysis*, **19**, 676-680.

Fonseca, S.C., Oliveira, F.A.R. & Brecht, J.K. (2002). Modeling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *Journal of Food Engineering,* **52**, 99-119.

Gil, M.I., Artés, F. & Toma-Barberan, F.A. (1996a). Minimal processing and modified atmosphere packaging effects on pigmentation of pomegranate seeds. *Journal of Food Science*, **61**, 161-164.

Gil, M.I., Martínez, J.A. & Artés, F. (1996b). Minimally processed pomegranate seeds. *Lebensmittel-Wissenschaft und Technologie*, **29**, 708-713.

GOI-UNCTAD DFID (2007). Project on strategies and preparedness for trade and globalization in India. Agricultural Finance Corporation Ltd.

Hess-Pierce, B. & Kader, A. (2003). Responses of 'wonderful' pomegranates to controlled atmosphere. *Acta Horticulturae*, **600**, 751-757.

Holland, D. & Bar-Ya'akov, I. (2008). The pomegranate: New interest in an ancient fruit. *Chronica Horticulturae*, **48**, 12-15.

INMA (2008). Iraq – a strategy for pomegranate, Agribusiness Program, USAID.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 174-185.

Malik, A., Afaq, F., Sarfaraz, S., Adhami, V.M., Syed, D.N. & Mukhtar, H. (2005). Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. *Proceedings of the National Academy of Sciences*, **102**, 14813-14818.

Mayuoni-Kirshinbaum, L., Tietel, Z., Porat, R. & Ulrich, D., 2012. Identification of aroma-active compounds in 'wonderful' pomegranate fruit using solvent-assisted flavour

evaporation and headspace solid-phase micro-extraction methods. *European Food Research Technology*, **235**, 277-283.

Melgarejo, P., Sánchez, A.C., Vázquez-Araújo, L., Hernández, F., José-Martínez, J., Legua P. & Carbonell-Barrachina, A.A., 2011. Volatile composition of pomegranates from 9 Spainish cultivars using headspace solid phase microextraction. *Journal of Food Science*, **76**, S114-S120.

Neurath, A.R., Strick, N., Li, Y. & Debnath, A.K. (2005). *Punica granatum* (pomegranate) juice provides an HIV-1 entry inhibitor and candidate topical microbicide. *Annals of New York Academy of Sciences*, **1056**, 311-327.

Noda, Y., Kaneyuki, T., Mori, A. & Packer, L. (2002). Antioxidant activities of pomegranate fruit extract and its anthocyanidins: Delphinidin, cyanidin and pelargonidin. *Journal of Agricultural and Food Chemistry*, **50**, 166-171.

Opara, U.L., Al-Ani, M.R. & Al-Shuaibi, Y.S. (2009). Physico-chemical properties, vitamin C content, and antimicrobial properties of pomegranate fruit (*Punica granatum* L.). *Food and Bioprocess Technology*, **2**, 315-321.

Sadeghi, H. & Akbarpour, V. (2009). Liquid acrylic and polyamide plastic covering affect quality and storability of pomegranate (cv. Malas-e-Saveh). *Journal of Food, Agriculture and Environment*, **7**, 405-407.

Saltveit, M. E. (2003). Is it possible to find an optimal controlled atmosphere? Postharvest *Biology and Technology,* **27**, 3-13.

Saxena, A.K., Manan, J.K. & Berry, S.K. (1987). Pomegranates: Post-harvest technology, chemistry and processing. *Indian Food Packer*, **41**, 43-60.

Seeram, N., Lee, R., Hardy, M. & Heber, D. (2005). Rapid large scale purification of ellagitannins from pomegranate husk, a by-product of the commercial juice industry. *Separation and Purification Technology***, 41,** 49-55.

Sepúlveda, E., Galletti, L., Sáenz, C. & Tapia, M. (2000). Minimal processing of pomegranate var. Wonderful. *CIHEAM-Opitions Mediterraneennes*, **42**, 237-242.

Sumner, M.D., Elliott-Eller, M., Weidner, G., Daubenmier, J.J., Chew, M.H., Marlin, R., Raisin, C.J. & Ornish, D. (2005). Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart disease. *American Journal of Cardiology*, **96**, 810-814.

Viuda-Martos, M., Fernández-López, J. & Pérez-Álvarez, J.A. (2010). Pomegranate and its many functional components as related to human health: A Review. *Comprehensive reviews in Food Science and Food Safety*, **9**, 635-65.

Chapter 2

Literature Review

CHAPTER 2

LITERATURE REVIEW

A. Background

Pomegranate *(Punica granatum* L.*)* belongs to the subclass Rosidae, order Myrtales, which is home to a few other fruits such as the guava *(Psidium sp.)* and feijoa *(Feijoa sp.)*. However, pomegranate is unusual in being one of only two species in its genus, *Punica,* which is the sole genus in the family Punicaceae (ITIS, 2006). Recent molecular studies suggest a taxonomic reconsideration might place the genus *Punica* within the Lythraceae (Graham *et al*., 2005). It is widely considered native to the Mediterranean basin up to northern India. It is capable of adapting to adverse climatic conditions and different soil types (Sepúlveda *et al*., 2000). Pomegranate fruit have an irregular rounded shape with rinds that vary from yellow, green or pink to bright deep red, depending on the stage of ripening and variety (Elyatem & Kader, 1984; Holland *et al*., 2009). However, there are some exceptional cultivars such as the black pomegranate. These cultivars acquire black color very early and remain black until ripening time (Holland *et al*., 2009). Internally, pomegranates have a multi-ovule chambers separated by membranous walls (septum) and a fleshy mesocarp. The chambers are filled with seeds called arils (Fig. 1). The arils are the succulent and edible portion, which develops from the outer epidermal cells of the seed and elongates to a very large extent in a radial direction (Fahan, 1976). Arils vary in size and in hardness depending on the varieties, while some varieties are referred to as seedless but contain soft seeds. The colour of the arils equally varies from white to deep red depending on the variety (Holland *et al*., 2009). And occasionally, a state of metaxenia does occur wherein there are several seeds of different colour within a pomegranate fruit (Levin, 2006).

The physico-chemical properties of pomegranate fruit cultivars grown in different regions have been reported by several researchers (Artés *et al*., 2000; Al-Said *et al*., 2009; Al-Yahyai *et al*., 2009; Opara *et al*., 2009; Zarei *et al*., 2010). The physical properties reported include the fruit weight, whole fruit and aril colour, juice content and juice dry matter content. These and other researchers have also shown that the physic-chemical properties of pomegranate cultivars vary among agro-climatic regions (Al-Said *et al*., 2009; Opara *et al*.,

2009; Zarei *et al*., 2010). Furthermore, several chemical properties and phyto-nutrients such as the vitamin C, total phenolics, total tannins and condensed tannins, total soluble solids and anthocyanins in the peel and arils of various pomegranate cultivar have been reported (Artés *et al*., 2000; Al-Said *et al*., 2009; Opara *et al*., 2009; D'Aquino *et al*., 2010; Zarei *et al*., 2010).

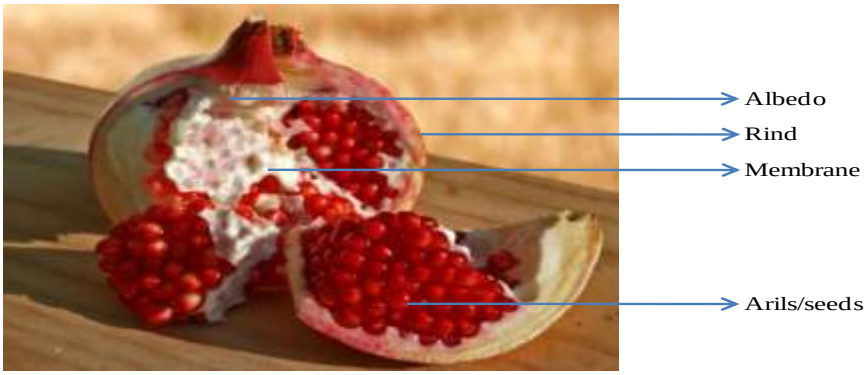


Figure 1 An annotated picture of pomegranate fruit.

Furthermore, current research on aroma and flavour of pomegranate fruit concentrated on identification of unique volatiles produced by ripe pomegranate fruit (Calín-Sánchez *et al*., 2011; Melgarejo *et al*., 2011; Mayuoni-Kirshinbanum *et al*., 2012). Using the headspace solid-phase micro-extraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) Calín-Sánchez *et al*. (2011) and Melgarejo *et al*. (2011) identified 18 and 21 aroma volatiles, respectively, in juices of nine difference Spanish pomegranate cultivars. Mayuoni-Kirshinbanum *et al*. (2012) in their study performed a stir bar sorptive extraction (SBSE), coupled with GC-MS analysis to indentify 23 aroma volatiles in 'Wonderful' pomegranate. The identifications included various classes such as aldehydes, monoterpenes, alcohols, esters, furans and acids, and the most prominent volatiles were ethyl-2-methylbutanoate, hexnal, limonene, *trans*-2-hexenal, *cis*-3-hexenol, *cis*-2-heptenal, β-pinene and β-caryophyllene. Furthermore, Calín-Sánchez *et al*. (2011) and Melgarejo *et al*. (2011) suggested that consumer liking of pomegranate juices could be linked with the high levels of monoterpenes. This was corroborated by report of Mayuoni-Kirshinbanum *et al*., (2012), wherein 5 out the 12 detected 'Wonderful' pomegranate aroma-active compounds by the GC-O sniffing panellists were terpens. Thus, this suggests that class of aroma compound and concentration plays a role among cultivar preference for pomegranate (Melgarejo *et al*., 2011). However, increased interest in minimally processed and fresh-cut pomegranate arils with high nutritional value and improved arils quality has highlighted our limited knowledge of factors that affect flavour development in modified atmosphere packaged pomegranate arils.

B. Pomegranate production in South Africa

The current world pomegranate production is estimated to be about 2.5 million tons, with production dominated by India and Iran. Commercial production of pomegranate fruit in South Africa started less than a decade ago, and presently approximately 1,200 ha of land is under cultivation (Joubert, 2012). South Africa production per hectare contributes less than 1% of total global production (Fig. 2). However, between year 2010 and 2012, the number cartons of pomegranate exported from South Africa has grown from 71,640 to 442,800 (PPECB, 2012). With such an exponential growth in export, pomegranate could become a dominate cash crop within the local and international market for South Africa. Thus, adequate and appropriate post harvest handling and storage condition is required for the sustainability of the young pomegranate production in South Africa.

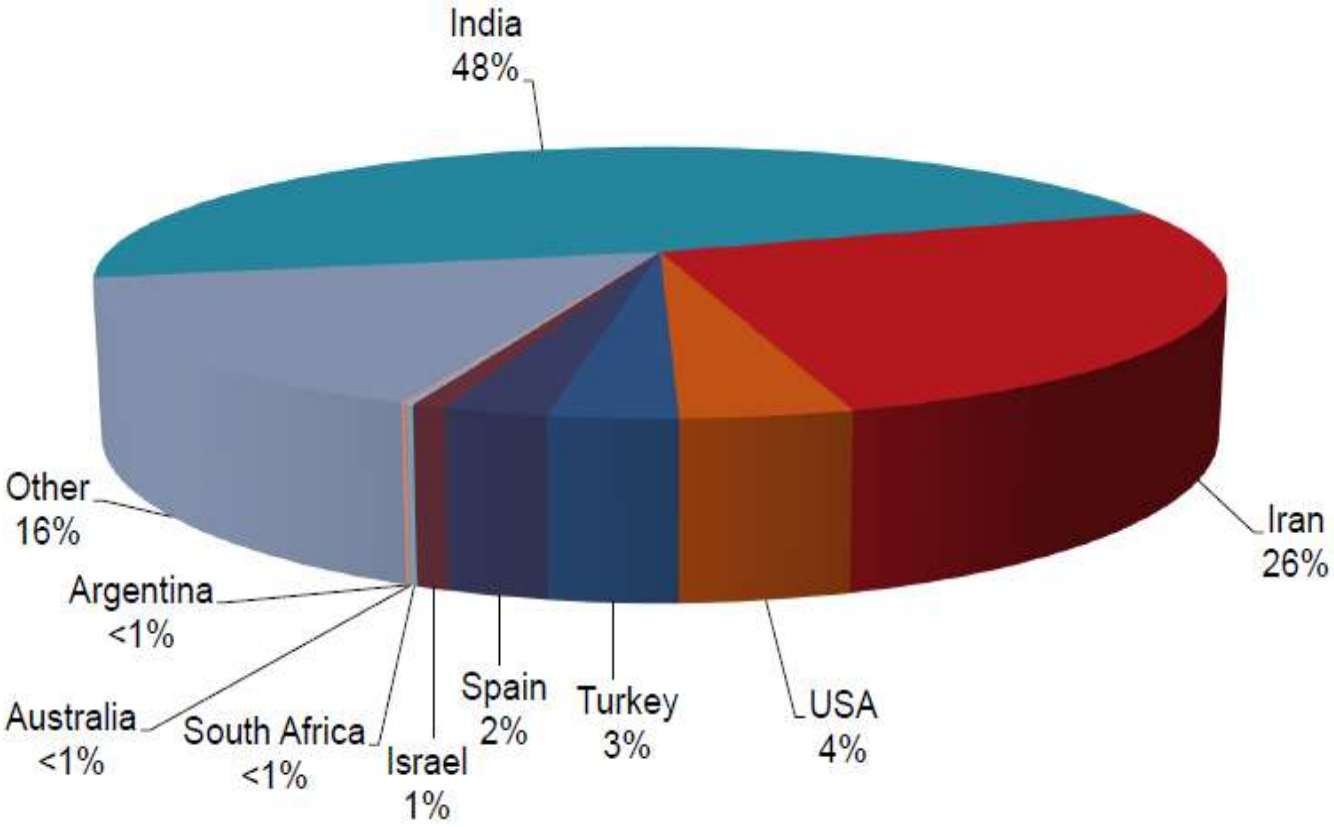


Figure 2 An annotated picture of pomegranate fruit. Source: NAMC, 2011.

C. Deterioration in pomegranate fruit quality

Pomegranate is classified as a non-climacteric fruit, because, maturation and ripening occurs on the plant prior to harvest, fruits harvested before ripening do not continue ripening in storage and are of inferior eating quality (Elyatem & Kader, 1984). Contrary to non-climacteric fruit, the ripening process of climacteric fruit is accompanied by a peak of respiration rate and a concomitant burst of ethylene production (Barry & Giovannoni, 2007). Kader *et al.* (1984) reported that pomegranate fruits had a relatively low respiration rate, which declined with postharvest to a steady rate of 8 mL kg^{-1} hr^{-1} for about 3 months and ethylene production was in trace quantity less than 0.2 µl kg^{-1} hr^{-1}, when stored at 20 °C for 2 weeks. These observed metabolic processes confirms pomegranate as a non-climacteric fruit, being that it exhibits no drastic changes in postharvest physiology and composition. In spite of the non-climacteric nature of the fruit, quantitative and qualitative loss still occur due to postharvest handling processes, resulting in chilling injuries, husk scalding, weight loss and decay (Kader *et al.*, 1984; Ben-Arie & Or, 1986; Artés & Tomás-Barberán 2000; Artés *et al.*, 2000).

Chilling injury

The shelf life of pomegranate fruit based on experimental data with the 'Wonderful' cultivar suggested that fruits quality attributes are best kept or maintained at 5 °C for 8 weeks with relative humidity of above 95 % (Elyatem & Kader, 1984; Kader *et al.*, 1984). However, depending on the cultivar, pomegranate can be stored for 2 to 7 months at temperatures ranging from 0 to 10 °C (Köksal, 1989; Treglazova & Fataliev, 1989; Onur *et al.*, 1992). Pomegranate fruit have been reported to be susceptible to chilling injury if stored longer than one month at temperatures below 5 °C (Elyatem & Kader, 1984; Kader *et al.*, 1984), with symptoms such as skin rotting, etiolating and cracking, browning of the rind, necrotic pitting and internal discolouration and browning of seeds (Elyatem & Kader, 1984; Köksal, 1989; Artés, 1992). High temperature treatment such as water dipping at 45 °C has been reported to reduce incidence of chilling injury and increase the ratio of saturated or unsaturated fatty acids of membrane as well as the concentration of spermidine and putrescine (Mirdehghan *et al.*, 2007a, b). Also intermittent warming of fruits at high

temperature prior to storage has been shown to prevent chilling injury's symptoms and fruit decay (Artés *et al*., 2000).

Husk scald

Husk scald is a superficial browning which is restricted to the husk, with no observable internal changes on the arils or on the white astringent membrane as observed with chilling injury (Ben-Arie & Or, 1986). This physiological disorder is suggested to be due to the oxidation of phenolic compounds on the husk when stored at temperatures above 5 °C. Ben-Arie & Or (1986) observed a correlation between husk scald incidence and the amount of extractable *o*-dihydroxyphenols obtained from the affected husk. In line with Ben-Arie & Or (1986) they observed that, the most effective control of husk scald in 'Wonderful' pomegranates was the storage of late-harvest fruits in 2 % oxygen at 2 °C. However, the treatment resulted in accumulation of ethanol which led to off-flavours in the fruits.

Weight loss

Beside chilling injury another major storage challenge is the effect of weight loss on the pomegranate fruit, which leads to hardening and browning of the rind and arils (Artés *et al*., 2000; Nanda *et al*., 2001; D'Aquino *et al*., 2010). Weight loss is regarded as a major cause of loss in the visual quality for horticultural products, as excessive transpiration can lead to desiccation, shriveling, wilting, reduced firmness and crispness and promotes senescence by lowering the endogenous enzymatic processes or regulators and ageing (Ben-Yehoshua & Rodov, 2003). Nanda *et al*. (2001) reported weight losses of 1.2 - 1.3% in shrink-wrapped 'Ganesh' pomegranates stored at 8 °C for 12 weeks and weight losses of 2.2 - 3.7% for those stored at 15 °C for 10 weeks, in comparison to non-wrapped fruits with weight loss of 20.4 and 30.7% at 8 ° and 15 °C, respectively. In a similar study, by D'Aquino *et al*. (2010) they observed that after 6 weeks of storage at 8 °C unwrapped and untreated control 'Primosole' pomegranate had a weight loss of 5.1%, while polyolephinic film wrapped fruits lost only 0.6%, and weight loss increased up to 12.7% in control as against 3.1 % for wrapped fruits after 12 weeks of cold storage. Artés *et al*. (2000) observed weight losses of 1.15 or 1.34% in unpackaged control 'Mollar de Elche' cultivars exposed to thermal treatment prior to storage at 5 or 2 °C for 12 weeks, compared to weight loss of 0.07 % in thermal treated fruits packaged in standard polypropylene films at both 5 ° and 2 °C for 12 weeks.

Decay

Another limiting factor for long term storage and a major cause of postharvest losses is pomegranate fruit decay, caused by various pathogens such as *Botrytis cinerea*, *Aspergillus niger*, *Penicillum* spp. and *Alternaria* spp. (Roy & Waskar, 1997; Nerya *et al*., 2006; D' Aquino *et al*., 2010). The diseases caused by these pathogens are, grey mould rot by *Botrytis cinerea*; heart rot by *Aspergillus niger* and *Alternaria* spp.; and penicillium rot by *P. expansum* and other *Penicillum* spp. (Roy & Waskar, 1997). *Botrytis cinerea* develops a characteristic grey mycelium on the affected region under a moist condition. The grey mould rot decay usually starts from the calyx, and progresses on to the skin making the skin tough and leathery with a change in skin colour (Ryall & Pentzer, 1974). In heart rot, with *Aspergillus niger* and *Alternaria* spp. infestation the fruits show a slightly abnormal skin colour with a mass of blackened arils within. Often this disease develops while the fruits are on the tree and are usually detected by sorters and removed from the package (Roy & Waskar, 1997). Vyas & Panwar (1976) observed that *Alternaria solani* caused damage to pomegranate fruits during transit and storage. *P. expansum* and other *Penicillum* spp. produce watery areas at the site of infection followed by the development of blue or green spores. Infection usually occurs via skin breaks caused by cracking, insect punctures or mechanical injuries (Sonawane *et al*., 1986).

Treatments with aqueous Topsin-M (0.1%) and Bavistin (0.05 - 0.1%) was reported to inhibit the growth of *Aspergillus niger* (Padule & Keskar, 1988). Also, when pomegranate fruits were treated with fludioxonil (FLU) and stored at 10 °C for 2-5 months, the natural incidence of decay of fruits were shown to be significantly reduced to 0 - 8% (Adaskaveg & Förster, 2003). These reports suggest that one principal factor affecting the quality of pomegranate during postharvest storage is principally the suitability of cultivars to storage conditions and postharvest handling.

D. MAP technology – An overview

MAP is an active or passive dynamic process of altering gaseous composition within a package. It relies on the interaction between the respiration rate of the produce, and the transfer of gases through the packaging material, with no further control exerted over the initial gas composition (Farber *et al*., 2003; Mahajan *et al*., 2007; Caleb *et al*., 2012c). Passive-

MAP can be generated inside a package by relying on the natural process of produce respiration and film permeability to attain the desired gas composition over time (Charles *et al*., 2003; Farber *et al*., 2003). While, active-MAP is a rapid process of gas replacement or displacement, or the use of gas scavengers or absorbers to establish a desired gas mixture within a package (Kader & Watkins, 2000; Charles *et al*., 2003; Farber *et al*., 2003). This involves the addition of active agents into packaged food product, such as O_2, CO_2 and ethylene scavengers (Phillips, 1996; Sandhya, 2010). For example, CO_2 absorbers can prevent a build-up of CO_2 gas to deleterious levels (Kader & Watkins, 2000).

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

The physiological processes of produce (mainly respiration and transpiration) play significant roles in the postharvest quality of MA-packaged fresh and fresh-cut fruit and vegetables. Respiration is a metabolic activity that provides the energy needed for other plant biochemical reactions (Fonseca *et al*., 2002a). Aerobic respiration (referred to as respiration throughout this paper) involves the oxidative breakdown of complex organic compounds such as carbohydrates, lipids, and organic acids into simpler molecules, including CO_2 and water with the release of energy (Fonseca *et al*., 2002a, b). Table 1 summarizes factors that influences fresh or fresh-cut produce respiration rate. Respiration rate can be reduced by decreasing O_2 concentration around the fresh produce. This process induces a decrease in the activity of oxidizing enzymes such as polyphenoloxidase, glycolic acid oxidase and ascorbic acid oxidase (Kader, 1986). Decreasing respiration rate via MA and lowering temperature delays enzymatic degradation of complex substrates and reduces sensitivity to ethylene synthesis (Saltviet, 2003; Tijskens *et al*., 2003), thereby extending the shelf life and

avoiding senescence of the produce. De Santana *et al.* (2011) evaluated the effect MAP on respiration rate and ethylene synthesis during 6 days storage at 1 and 25 °C. They reported that ethylene production was proportional to respiration rate for peaches during ripening at 25 °C. However, lower ethylene synthesis and respiration rate were obtained at lower temperature in MAP treatments. This principle is a critical component to the successful application of MAP. Excessively low O_2 level, below 1% may result in anaerobic respiration leading to tissue deterioration as well as production of off-odours and off-flavours (Lee *et al.*, 1995; Austin *et al.*, 1998; Ares *et al.*, 2007). The influence of CO_2 on respiration rate has not been well clarified as there are varying theories on this, such as the idea that CO_2 being a product of respiration process will cause a feedback inhibition (Fonseca *et al.*, 2002a, b). Another concept considered that elevated CO_2 might affect the Krebs cycle's enzymes and intermediates, while another suggested that CO_2 might inhibit ethylene production instead of having a direct influence on respiration process (Mathooko, 1996; Fonseca *et al.*, 2002a, b). Retarding ethylene synthesis has tremendous benefits for the storage of sensitive horticultural produce. Although, for some non-climacteric produce such as vegetable tissue and citrus ethylene production is under a negative feed-back response, hence reducing ethylene will stimulate its production (Saltveit, 2003).

Table 1 Factors influencing respiration rate quantification

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

*Factors due to produce processing
Source: Fonseca *et al.* (2002a); Kader *et al.* (2002); Monetro-Calderón & Cerdas-Araya (2011)

The other physiological process of significant importance in postharvest quality of fresh and fresh-cut produce is transpiration. Once the fresh produce is detached from the growing plant, they solely depend on internal water content for transpiration resulting in water loss (Mahajan *et al.*, 2008c). The loss of water from fresh produce result in weight loss and shrivelling, leading to unsalable loss during retail marketing and a direct financial loss.

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

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

E. Produce physiology and mathematical predictions

Understanding the multi-complex interactions within various physiological processes towards MAP design requires a suitable model to predict these responses as function of time, temperature, gas composition or RH in the case of transpiration rate. Over the last decade, significant advancements in computing and the use of statistical tools for data fitting

and numerical integration, with more accurate analytical techniques, have enabled a better understanding of the physiological interactions involved on MAP of fresh and fresh-cut produce through the development of predictive models (Charles *et al*., 2003; Mahajan *et al*., 2007). However, there are various limitations to the development of such predictive models. This include time consuming experiments with potentially large experimental errors, and the complex nature of respiration process for the determination of respiration rates of produce for MAP design (Fonseca *et al*., 2002a, b). Other limitations of mathematical models are that, models are based on limited number of experimental observations, and inherent biological variation and the dynamic response of stored fresh or fresh-cut produce to environmental changes is not adequately accounted for. Often these variables are held constants or assumed to be negligible (Saltviet, 2003; Tijskens *et al*., 2003). Therefore, the development of models should incorporate adequate measure of the produce's dynamic response to extrinsic factors such as RH, temperature, light, time and others (Saltviet, 2003; Tijskens *et al*., 2003; Caleb *et al*., 2012b).

Following up on the review by Fonseca *et al*. (2002b), Table 2 presents a summary of articles on respiration rate since 2000, highlighting the produce, experimental approach, experimental conditions, and the types of models developed or applied. Most respiration rate models have been oriented towards either one or two out of the three functions of time, temperature and gas composition. The Michaelis-Menten type equations (uncompetitive, non-competitive, or uncompetitive/competitive) based on CO_2 inhibitory effect (Lee *et a*l., 1991; Peppelenbos & Leven, 1996; Del Nobile *et al*., 2006; Rocculi *et al*., 2006; Bhande *et al*., 2008), and the Arrhenius-type equations, which describe temperature as a function of respiration (Jacxsens *et al*., 2000; Kaur *et al*., 2010; Uchino *et al*., 2004; Torrieri *et al*., 2010), have been widely reported for respiration rate of fresh produce as a function of both temperature and gas composition. A major limitation of respiration rate modeling is of the lack of adequate respiratory data information. Often, data available are either based on O_2 consumption or CO_2 production rates, based on the assumption that the respiratory quotient (RQ) = 1. The downside to this is that if the RQ were to be > 1, the model would underestimate CO_2 production and if RQ < 1, the predictive would underestimate likewise (Fonseca *et al*., 2002b).

Mathematical prediction of transpiration rate for fresh produce is challenging, due to insufficient information on the dynamic interactions between evaporation on the produce surface due to heat released during respiration and the permeability property of the

packaging film (Song *et al*., 2002). Existing models for predicting water loss in fresh produce have been limited in application to cooling process and bulk storage (Sastry & Buffingtin, 1982; Chau & Gaffney, 1985; Gaffney *et al*., 1985), and these models may not be suitable for MAP systems (Song *et al*., 2002). Most models describe moisture loss as a function of the bio-physical and thermo-physical properties such as skin thickness, surface cellular structure and pore-fraction in the skin, thermal diffusivity and geometry of produce. Measuring these properties is time consuming (Song *et al*., 2002). Predicting the rate of water loss is important towards estimating the shelf life of produce, and designing appropriate packaging at optimal storage conditions. To overcome methodological challenges in the measurement and prediction of water loss, the weight loss approach for fresh produce can be adopted (Leonardi *et al*., 1999). This approach was successfully applied by Mahajan *et al*. (2008a).

F. Packaging material

Another critical parameter in the successful use of MAP is the choice of packaging material (Sivalumar & Korsten, 2006). The degree to which modification of the atmosphere takes place in packages is dependent on variables such as film permeability to O_2, CO_2, water vapour, film thickness, package surface area and the free volume inside the package (Mahajan *et al*., 2008b). Gas flux through the package film or film permeability can be mathematically predicted, using permeability equation based on the Fick's diffusion laws for thin and infinite films, where in the gas flux per unit time through the film can be determined (Crank & Park, 1968). Furthermore, Arrhenius equation which describes the temperature sensitivity of film permeability to gases can be coupled with other mathematical models to obtain a more robust and descriptive parameters.

Table 2 Respiration rate models presented in literature from 2002

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

ANN: Artificial neural network; MMC: Michaelis-Menten competitive inhibition; MMUC: Michaelis-Menten uncompetitive inhibition; UCI: Uncompetive inhibition; MMNC: Michaelis-Menten noncompetitive inhibition

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

Table 3 Properties of major packaging material

Packaging material	Properties	
	Advantages	**Disadvantage**
Paper	(i) Strength and rigidity	(i) Opacity
	(ii) Printability	
Tinplate	(i) Corrosion resistance	(i) Higher barrier to gases
	(ii) Excellent barrier to gases, water vapour, light and odour	(i) Tin toxicty
	(iii) Heat-treatable	
	(iv) Ability to seal hermetically; ductility and formability	
Tin-free steel	(i) Corrosion resistance	(i) Higher barrier to gases
	(ii) Excellent barrier to gases, water vapour, light and odour	
	(iii) Heat-treatable	
	(iv) Ability to seal hermetically	
	(v) Ductility and formability	
	(vi) Less expensive compared to tinplate	
Aluminium foil	(i) Negligible permeability to gases, odours and water vapour	(i) Opacity
	(ii) Dimensional stability	(ii) High barrier to gases
	(iii) Grease resistance	
	(iv) Brilliant appearance	
	(v) Dead folding characteristics	
Glass	(i) Formability and rigidity	(i) Higher barrier to gases (ii) Heavy weight adds to transport cost
	(ii) Transparency and UV protection due to colour variation	
	(iii) Impermeable to gases, water vapour and odour	
	(iv) Chemical resistance to all food products	
	(v) Heat stable	
Cellulose film (coated)	(i) Strength	(i) Low permeability barrier
	(ii) Attractive appearance (iii) Low permeability to water vapour, gases, and odours (*coat dependent*)	
	(iv) Grease resistance, printability	
Cellulose acetate	(i) Strength and rigidity	(i) Glossy appearance
	(ii) Dimensional stability, printability	
Ethylene vinyl alcohol (EVOH)	(i) Excellent barrier to gases and odour	(i) Moisture sensitive barrier
	(ii) Effective oxygen barrier material	
Ethylene vinyl acetate (EVA)	(i) Very good adhesive properties	(i) Poor gas barrier
	(ii) Excellent transparency	(ii) Poor moisture barrier
	(iii) Heat-sealability	
Polyethylene	(i) Durability and fexibility	(i) HDPE; Poor clarity
	(ii) Heat-sealability	(ii) LLDPE; heat sensitive
	(iii) Good moisture barrier	
	(iv) Chemical resistance	
	(v) Good low-temperature performance	
	(vi) Permeable to gases	

Table 3 Continued

	Properties	
Packaging material	**Advantages**	**Disadvantages**
Polypropylene	(i) Harder, denser and more transparent than polyethylene	
	(ii) Better response to heat sealing	
	(iii) Excellent grease resistance	
	(iv) Good resistance to chemical	
	(v) Higher gas and water vapour barrier	
Polyesters (PET/PEN)	(i) Excellent durability and mechanical properties	
	(ii) Excellent transparency (iii) Good resistance to heat, mineral oil and chemical degradation	
	(iv) Adequate barrier to gases, water vapour and odours	
Polyvinyl chloride (PVC)	(i) Strong and transparent (ii) Good gas barrier and moderate barrier to water vapour	
	(iii) Excellent resistance to chemicals, greases and oils	
	(iv) Heat-sealability	
Polyvinylidene chloride (PVDC)	(i) Low permeability to gases, water vapour and odours	(i) Low permeability barrier
	(ii) Good resistance to greases and chemicals	
	(iii) Heat-sealability	
	(iv) Useful in hot filling, and low temperature storage	
Polystyrene	(i) High tensile strength	(i) Poor barrier to gas and water vapour
	(ii) Excellent transparency	
Polyamide (nylon-6)	(i) Strong (ii) Moderate oxygen barrier, excellent odour and flavour barrier	(i) Poor water vapour barrier
	(iii) Good chemical resistance	
	(iv) Thermal and mechanical properties similar to PET	
	(v) High temperature performance	

Source: FAD/WFP (1970); Page *et al*. (2003); Marsh & Bugusu (2007); Mangaraj *et al*. (2009)

For example, Koide & Shi (2007) investigated the microbial and physicochemical quality of green peppers stored in a polylactic acid based biodegradable and low-density polyethylene (LDPE) film packaging. Results obtained by the authors showed that physicochemical properties such as weight loss, hardness, colour, ascorbic acid and gas concentrations, and microbial levels did not show significant changes during the storage period. However, the total coliform bacteria increased by 2.3 log CFU g^{-1} in LDPE film and 0.9 and 0.2 log CFU g^{-1} in the perforated LDPE and biodegradable film packaging, respectively. These findings indicated that biodegradable film with higher water vapour permeability would better maintain the quality of green peppers. As no fungal growth was observed in biodegradable

film-packaged green peppers, this was associated to the high water vapour permeability which lowered the relative humidity inside the biodegradable packaging.

Pyla *et al*. (2010) investigated both antimicrobial and antioxidant effect of corn-starch matrix mixed with tannic acid. They found that the matrix exhibited an antimicrobial activity against *Listeria monocytogenes* and *Escherichia coli* O157:H7 and antioxidant effect on soybean oil. Kim *et al*. (2011) reported antimicrobial activity of chitosan biopolymer films (CBFs) with four different viscosities against *L. monocytogenes*, *Samonella typhimurium* and *E. coli* O157:H7. CBFs with 100 mPa s chitosan had an antilisterial effect on 10^4 cfu mL^{-1} inoculation. In a more recent study, Ture *et al*. (2011) investigated the effect of wheat gluten (WG) and methyl cellulose (MC) biopolymers containing natamycin on the growth of *Aspergillus niger* and *Penicillium roquefortii* on the surface of fresh kashar cheese. WG and MC films were found to be effective against *A. niger* with about 2 log reductions in spore count. This information highlights the potential for biodegradable films towards optimal microbiological safety of MA-packaged fresh and fresh-cut produce. As more innovative biodegradable packaging materials emerge within the nanotechnology field (Siracusa *et al*., 2008), it is necessary to conduct research on their microbiological safety to ensure the overall integrity of food.

Another form of biodegradable polymer is edible films, which comprise of a thin layer of edible materials applied to food as surface coating (Mangaraj *et al*., 2009; Campos *et al*., 2011). There are several benefits of using edible films as packaging material, including the ability to minimize microbial growth by lowering the water activity a_w, enzymatic activities and mitigating moisture loss, gas and aroma absorption into food, and improving the mechanical integrity and shelf life of food (Cutter, 2002; Marsh & Bugusu, 2007; Campos *et al*., 2011). As with other MAP technologies, edible films can create a low level of O_2 within package (Odriozola-Serrano *et al*., 2008), which can facilitate the growth of anaerobic pathogens such as *C. botulinum* (Guilbert *et al*., 1996). However, edible films are ideal vehicles for incorporating a wide variety of additives such as antimicrobials, antioxidants, and texture agents to customize the film (Baldwin, 1994; Cutter, 2002; Farber *et al*., 2003; Campos *et al*., 2011).

Table 4 Types of polymeric films and their permeability properties at set conditions

Polymeric film	**Permeance (mol $sec^{-1}m^{-2}Pa^{-1}$ for 25 μm film at 25 °C)**			**WVT(mol $sec^{-1}m^{-2}Pa^{-1}$)**
	Oxygen	Carbon dioxide	Nitrogen	**at 38 °C and 90 % RH**
Ethylene vinyl alcohol (EVOH)	1.87×10^{-14}	-	-	8.01×10^{-5}
Ethylene vinyl acetate (EVA)	5.84×10^{-11}	2.33×10^{-10}	2.29×10^{-11}	2.36×10^{-4}
Polyamide (PA) (Nylon-6)	1.87×10^{-13}	7.94×10^{-13}	6.54×10^{-14}	7.50×10^{-3}
Polyethylene (PE), LD	3.64×10^{-11}	1.96×10^{-10}	1.31×10^{-11}	8.48×10^{-5}
Polyethylene (PE), HD	1.21×10^{-11}	3.55×10^{-11}	3.04×10^{-12}	4.01×10^{-5}
Polypropylene (PP), cast	1.73×10^{-11}	4.67×10^{-11}	3.18×10^{-12}	5.18×10^{-5}
Polypropylene (PP), oriented	9.34×10^{-12}	3.74×10^{-11}	1.87×10^{-12}	2.83×10^{-5}
Polypropylene (PP), oriented, PVDC coated	7.00×10^{-14}	1.98×10^{-13}	4.90×10^{-14}	2.12×10^{-5}
Polystyrene (PS), oriented	2.33×10^{-11}	8.41×10^{-13}	3.74×10^{-12}	5.30×10^{-4}
Polyurethane (Polyester)	5.37×10^{-12}	7.47×10^{-11}	4.20×10^{-12}	2.36×10^{-3}
Rigid, Polyvinyl chloride (PVC)	1.17×10^{-12}	3.39×10^{-12}	4.90×10^{-13}	1.65×10^{-4}
Plasticized, PVC	7.12×10^{-11}	1.11×10^{-10}	2.40×10^{-11}	1.30×10^{-4}
Polyvinylidene chloride (PVDC), coated	5.60×10^{-14}	1.17×10^{-13}	-	-
PVDC-PVC copolymer (Saran)	7.70×10^{-14}	4.67×10^{-13}	1.07×10^{-14}	1.53×10^{-5}

	Oxygen permeance (mol/sec.m^{-2}.Pa at 23°C)	**WVT (mol/sec.m^{-2}.Pa at 23°C and 85 % RH)**
Ethylene vinyl alcohol	4.70×10^{-18}- 4.70×10^{-17}	4.71×10^{-06}- 1.41×10^{-05}
Polyamide (PA)	4.70×10^{-16}- 4.70×10^{-15}	2.36×10^{-06}- 4.71×10^{-05}
Polyethylene (PE)	2.35 - 9.40×10^{-13}	2.36 - 9.43×10^{-06}
Ployethylene terephthalate (PET)	4.71×10^{-15}- 2.35×10^{-14}	2.36 - 9.43×10^{-07}
Ployethylene naphthalate (PEN)	2.35×10^{-15}	3.29878×10^{-06}
Polypropylene (PP)	2.3 - 4.70×10^{-13}	9.43×10^{-07}- 1.89×10^{-06}
Polystyrene (PS)	4.70 - 7.05×10^{-13}	4.71×10^{-06}- 1.89×10^{-05}
Polyvinyl alcohol (PVAL)	9.40×10^{-17}	1.41×10^{-04}
Polyvinyl chloride (PVC)	9.40×10^{-15}- 3.76×10^{-14}	4.71 -9.42×10^{-06}
Polyvinylidene chloride (PVDC)	4.70×10^{-18}- 1.41×10^{-15}	4.71×10^{-07}

Source: Guilbert *et al.* 1996; Phillips, 1996; Chung & Yam, 1999; Park, 1999; Han, 2000; Lange & Wyser, 2003 *Data has been converted to SI unit (Banks et al. 1995).

Several researchers have shown that antimicrobial compounds such as minerals and vitamins, organic acids, bacteriocins, enzymes, proteins and peptides, antibiotics and fungicides could be added to edible films to inhibit microbial growth on a variety of fresh produce (Ayranci & Tunc, 2004; Han *et al*., 2004; Martínez-Romero *et al*., 2006; Tapia *et al*., 2008; Türe *et al*., 2008; Rojas-Graü *et al*., 2009; Corrales *et al*., 2009; Ibarguren *et al*., 2010; Campos *et al*., 2011; Shakeri *et al*., 2011). Basch *et al*. (2012) investigated the antimicrobial effectiveness of nisin and potassium sorbate, incorporate into edible films made with tapioca starch mixed with hydroxypropyl methylcellulose (HPMC). They observed that the combination of both antimicrobial agents was more effective against *L. innocua* and *Zygosaccharomyces bailii*, than their individual incorporation. With growing interest in incorporating nutritional and bioactive compounds into edible films or coatings to improve their functional properties (Campos *et al*., 2011), the concentration of these additives and their potential side effects must be carefully investigated to determine the optimal range of barrier, mechanical and antimicrobial properties.

G. Converting technology and MAP design

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

life of a product, because O_2 and/ or CO_2 levels are out of recommended range, or if the appropriate atmosphere is not rapidly established within the package (Mahajan *et al*., 2007).

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

Table 5 presents a summary of variables involved in MAP design using polymeric films. Once a produce has been selected and its environmental conditions for storage are established, 8 out of these 14 listed variables in Table 6 are fixed. For instance, variables such as the surrounding gas composition and temperature, the product density, the production rate of CO_2, the consumption rate of O_2, and, the gas composition to be attained in the package so that the product shelf life is extended, are all produce- and temperature-specific (Jacxsen *et al*., 1999a; Fonseca *et al*., 2000; Paul & Clarke, 2002; Mahajan *et al*., 2007). These variables must satisfy the design equations (1) and (2) and therefore, the system has 4 design variables, that is, only 4 out of the remaining variables, M, V, A, e, P_{O_2} and P_{CO_2}, can be specified arbitrarily. However, it should be noted that some of these variables are inter-dependent, e.g. once the packaging material is selected both P_{O_2} and P_{CO_2} are fixed and only 2 degrees of freedom remain. Also, some restrictions must be applied as the volume of the package must be large enough to accommodate the required amount of product to be packed, and the area available for gas exchange depends on the type and size of the package (Mahajan *et al*., 2007; Mangaraj *et al*., 2009).

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

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

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

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

$$y_{CO_2}^{out} = y_{CO_2} + \frac{R_{CO_2} eM}{P_{CO_2} A} \quad (4)$$

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

H. Advances in MAP sensing and monitoring

The desire to improve on safety of MAP products and to extend the technology to a broader spectrum of products led to the introduction of 'smart' or 'active' or 'intelligent' packaging system (Sneller, 1986; Labuza, 1989; Summers, 1992; Church, 1994). This advancement is considered as the most significant area of development of MAP technology (Church, 1994). For simplicity the term 'smart' packaging will be used broadly in this paper to designate the packaging systems. 'Smart' packaging can be defined as, an interaction

between the packing system and the product itself which confers intelligence appropriate to function and use of the product with the ability to sense or be sensed and to communicate (Summer, 1992). Due to this interaction there is often a visible change in the properties of the indicator used, such as colour change, which allows the consumer the privilege to monitor the safety and shelf life of the product (Phillips, 1996). 'Smart' packaging of food can divided into active packaging and intelligent packaging (Yam *et al*., 2005; Sandhya, 2010).

Table 5 Components and variables involved in MAP design (adapted from Mahajan *et al*., 2007)

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

Active packaging

Active packaging involves the interaction between the package and food product in order to extend the shelf life of the product (Sandhya, 2010). This involves the addition of active agents into the packaged food product, such as oxygen and carbon dioxide scavengers, carbon dioxide, ethylene and water vapour removals and aroma releasing compounds (Church, 1994; Phillips, 1996; Sandhya, 2010). Two approaches to the development of oxygen scavenging systems have been reported. However, the most successful in commercial application has been the use of sachets and labels that are included in the packaged product. The other approach is the development of oxygen scavenging films with

immobilized oxidizing enzymes (e.g. alcohol oxidase and glucose oxidase) on the inner surface of the packaging film. The cost of this approach makes commercial application unlikely (Church, 1994). Also scavengers with higher absorption speed at low temperatures and those that are microwaveable have been developed (Church, 1993).

Carbon dioxide and ethylene removals have equally been applied with commercial success. Carbon dioxide removals have been used in the packaging of freshly roasted coffee and this approach extended the shelf life more than three times the expected shelf life (Church, 1994). Various ethylene removals based on activated carbon system ('Freshkeep' from Kurarey, Osaka, Japan) or silicon dioxide or potassium permanganate ('Acepack' from Nippon Greener, Tokyo, Japan) or polyethylene film impregnated with mineral ('Peakfresh' from Klerk Plastic Industrie, Noordwijkerhoot, The Netherlands) have been used in the removal of ethylene from packed food product (Church, 1994).

Intelligent packaging

On the other hand, intelligent packaging involves the monitoring of the quality and/ or safety of a food product, while providing an indication or information that can be helpful in the distribution or supply chain (Yam *et al*., 2005; Sandhya, 2010). Examples of such indicators include time-temperature indicators (TTIs), Radio frequency identification RFID tags, gas indicators, sensors, leak detection and edible films (Church, 1994; Yam *et al*., 2005; Sandhya, 2010). TTIs are often small self-adhesive labels attached onto individual consumer packages or shipping containers (Yam *et al*., 2005). They provide a visual indication of temperature history, which gradually changes over time, and the rate of change observed is directly proportional to the increase in temperature (Yam *et al*., 2005; Sandhya, 2010). The observed changes are often in the form of distinct changes in colour intensity or diffusion of a dye (indicator) along a straight path (Yam *et al*., 2005). They can also be used as "freshness indicators" for estimating the shelf life of perishable products (Yam *et al*., 2005). According to Singh (2000), there are basically 3 types of commercially available TTIs this include full history indicator; partial history indicators; and critical temperature indicators. The operating principles and functionality of these indicators have been extensively reviewed in literature (Singh & Wells, 1985; Taoukis *et al*., 1991; Selman, 1995; Taoukis & Labuza, 2003; Smolander *et al*., 2004).

TTIs are more reliable in monitoring the remaining shelf life of perishable produce than expiration dates. Expiration dates assume a specific temperature history, but, variation from this temperature could result in premature spoilage and possible sales of spoiled product. While, TTIs respond directly to temperature and reflect the temperature history of the product (Sandhya, 2010). The setback, however, is that TTIs are sensitive to actinic radiation and must be stored at low temperature prior to usage. These criteria elevate the cost of production and introduce an element of uncertainty based on the reliability of the indicators (Sandhya, 2010). Hence, the need to advance the technology of TTI labels to be more cost efficient with more reliability.

Radio frequency identification (RFID) involves a wireless transfer and collection of data (Sandhya, 2010; Yam *et al*., 2005). A reader emits radio waves which capture data from a RFID tag, and the data is then transferred onto a host computer (with either a local or internet networking) for analysis and interpretations, which guides in decision making (Want, 2004). RFID tags can be classified into 2 types, this include passive tags and active tags (Goodrum & McLaren, 2003; Yam *et al*., 2005). The passive tags are powered by the energy supplied via the reader and have a reading range of about 15 feet, while active tags are battery powered with a broadcasting range of up to 100 feet (Yam *et al*., 2005). The capability of RFID to wirelessly transfer data gives the technology an edge above conventional bar-coding. The ability to wirelessly transfer data enables real time monitoring product and analysis of data, as well as provides a reduction in inventory cost (Sandhya, 2010). Furthermore, RFID tag can be integrated with a TTI or a biosensor to collect time-temperature history and microbiological data (Nambi *et al*., 2003; Want, 2004).

In a modified atmosphere packaged product the respiration of the fresh produce, or gas flux through the packaging film, or gas generation by spoilage microbes, or leakage, may cause a change in gaseous composition within the package. Gas indicators in the form of labels or print on the packaging films can help to monitor the safety and quality of the packed produce (Yam et al., 2005). Oxygen indicators are the most frequently used gas indicator (Krumhar & Karel, 1992; Inoue *et al*., 1994; Ahvenainen *et al*., 1997; Smiddy *et al*., 2002), this is due to the ability of oxygen to cause oxidative colour change and enhance microbial spoilage. Carbon dioxide indicator can be used to detect early spoilage as well as to monitor the levels of carbon dioxide within modified atmosphere packages on transit and within storage facility (Hong & Park, 2000; Neethirajan *et al*., 2009).

I. Microbiological safety of MAP

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

Amanatidou *et al*. (1999) reported that the use of "oxygen shock" or high levels of O_2 was very effective in retarding enzymatic discolouration, anaerobic fermentation process, and both aerobic and anaerobic microbial growth. However, they also observed that high O_2 levels of 80 - 90% stimulated the growth of food-borne pathogenic microbes such as *L. monocytogenes* and *E. coli* were stimulated. The reduction in O_2 levels reduces respiration rate of fruit and vegetables, due to a decrease in the activity of oxidative enzymes such as glycolic acid oxidase, ascorbic acid oxidase and polyphenol oxidase (Kader, 1986). Extremely low level of O_2 may create potential risk for the growth of pathogenic anaerobic microbes such as *Clostridium perfringens*, *C. botulinum* and *L. monocytogenes* (Charles *et al*., 2003; Farber

et al., 2003; Phillips, 1996). Furthermore, at excessively low level of O_2 (< 1%) anaerobic respiration may occur, resulting in tissue deterioration, production of off-flavours and off-odours (Ares *et al*., 2007).

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

Furthermore, other noble gases such as helium, argon and xenon have been reported in successful MAP applications to reduce microbial growth and maintain the quality of fresh produce (Nasar-Abbas *et al*., 2008; Zhang *et al*., 2008; Meng *et al*., 2012), as well as under controlled atmosphere and cold storage conditions (Jamie & Saltveit, 2002; Wu *et al*., 2012a, b). In a recent work, Meng *et al*. (2012) investigated the effect of pressurized argon treatments (2, 4 and 6 MPa) on fresh-cut green peppers placed in polystyrene packages with gas combination of 5 and 8% O_2 and CO_2, respectively and stored at 4 °C and 90% RH for 12 days. Their study showed that pressurized argon treatments were able to maintain the cell integrity of the produce by inhibiting the production of malondialdehyde, as well as the activities of catalase and peroxidase. The treatments were also reported to reduce the proliferation of spoilage microorganisms such as coliforms, yeast and moulds. Yu *et al*. (2009) compared the efficacy of ordinary MAP and argon-MAP on the preservation of cherries stored at ambient temperature. The results showed that the freshness of the cherries was better preserved with argon-MAP, due to the reduced mobility of water molecules.

Inert gases treatment could play a critical role in lowering water activity in fresh and fresh-cut produce, thereby reducing the leaching of organic material from fresh-cuts and movement of microbes into deeper tissues in comparison to other pretreatments (Meng *et al*. 2012). Studies have shown that at specific temperatures and pressures, inert gases can

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

J. Microbiology of packaging

The growth and survival of microorganisms in fresh and fresh-cut fruit and vegetables is significantly influenced by the intrinsic properties of the produce, as well as by extrinsic factors as summarized in Table 6 (Church, 1993; Ahvenainen, 1996; Cutter, 2002). Fruit and vegetables vary in their intrinsic properties. For instance, kernel and pome fruit have a high amount of organic acids which are responsible for their low pH values. In contrast fruit such as melon and avocado have higher pH values, closer to those of vegetables (Willkox *et al*., 1993; Soliva-Fortuny *et al*., 2004; Hounsome *et al*., 2008). The a_w of intermediate-moisture dried fruit ranges from 0.51 to 0.62 for raisins, 0.65 to 0.83 for prunes and figs, and 0.73 to 0.81 for peaches and apricots (Taoukis *et al*., 1988), while, high moisture (HM) dried fruit could retain up to 0.85 a_w (Witthuhn *et al*., 2005). Additionally, due to damage inflicted by mechanical operations on fruit tissue during processing, fresh-cut fruit have a much larger cut surface area resulting in higher a_w in comparison to whole fruits (Gorny *et al*., 2000; Garrett, 2002). Various studies have shown a direct relationship between a_w and the growth

rate of spoilage or pathogenic microorganisms (Wijtzes *et al*., 1993; Samapundo *et al*., 2005; Sağirli *et al*., 2008; Garia *et al*., 2011).

The a_w requirement of various microorganisms varies. Gram-positive non-spore forming bacteria can grow at a_w of between 0.90 - 0.94, while Gram-negative organisms require a minimum a_w of between 0.93 - 0.96. Generally, fungi; yeast and molds have lower a_w requirements ranging from 0.62 to 0.88 in comparison to bacteria (Farkas, 1997; Alzamora *et al*., 2003; Witthuhn *et al*., 2005). A summary of minimum level of water activity for various important microorganisms occurring in foods is has been reported by Lee & Khang, (2004). The predominant microflora of fresh fruit is fungi, due to the low a_w on the surface (Goepfert, 1980). However, processing operations and packaging conditions may transform the microbial ecology of fresh produce (Lanciotti *et al*., 1999; Watson, 2000; Soliva-Fortuny *et al*., 2004). By decreasing or maintaining a low a_w, the lag phase of microbial growth can be extended thereby reducing the microbial growth rate (Farkas, 1997).

Table 6 Intrinsic and extrinsic characteristics influencing the shelf life and microbiological safety of MA-packaged produce

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

Sources: Church, 1993; Cutter, 2002; Ahvenainen, 1996

Furthermore, other intrinsic factors such as storage temperature, pH, nutrient composition and oxidative-reduction potential have a synergistic effect on a_w and can influence microbial growth even at a high a_w value (Wijtzes *et al*., 1993; Samapundo *et al*., 2005; Sağirli *et al*., 2008; Garia *et al*., 2011). For instance, Garcia et al. (2011) reported that

at a constant a_w and low storage temperature of between 25 - 30 °C, a short lag phase was observed for *A. ochraceus*, but, a sharp increase in growth was observed at 37 °C. At a specific temperature, the ability of microbes to grow is restricted as a_w is lowered, while, the availability of nutrients increases the range of a_w over which microorganisms can survive (Jay *et al*., 2005). Furthermore, in packaged produce a_w in conjunction with relative humidity of the storage environment has a critical influence on microbial growth (Jay *et al*., 2005). Caution should be exercised when storing produce with low a_w in environments where relative humidity is high, due to moisture transfer from environment to food (Cutter, 2002). As change in a_w of the produce could affect the microflora associated with the product, resulting in an accelerated rate of decay. In contrast, however, when packaged produce with high a_w are stored in an environment with low relative humidity this could result in moisture loss from the food to the environment (Cutter, 2002).

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

Storage temperature is another extrinsic factor that influences microbial growth in fresh or fresh-cut produce. Microorganisms grow over a wide range of temperatures from as low as -34 °C to highest exceeding 100 °C (Jay *et al*., 2005). Based on temperature requirements, microorganisms can be categorized into three groups, namely: those that grow well at or below 7 °C but optimally between 20 °C and 30 °C are classified as psychrotrophs; the mesophilic group grow well between 20 °C and 45 °C with optimal growth between 30 °C and 40 °C; and, those that grow well at and above 45 °C with optima between 55 °C and 65 °C are classified as thermophiles. Molds are able to grow over the psychrotrophic temperatures. For example species of *Aspergillus*, *Cladosporium* and *Thamnidium* may be found growing on eggs, beef and fruit. Yeasts generally grow optimally within the psychrotrophic and mesophilic temperature ranges but not within thermophilic range (Jay *et al*., 2005). The

possibility of contamination and growth of anaerobic psychrotrophic and some thermophiles foodborne pathogens such as *Aeromonas caviae*, *A. hydrophila*, *Escherichia coli*, *C. perfringens*, *C. botulinum*, *L. monocytogenes*, *Salmonella* spp., is of concern to guarantee the safety of MA-packaged fresh or fresh-cut and/ or minimally processed fruit and vegetables (Philips, 1996; Szabo *et al*., 2000; Farber *et al*., 2003; Soliva-Fortuny *et al*., 2004; Jay *et al*., 2005). Since limited O_2 levels in MAP conditions is proven to inhibit the growth of most aerobic microorganisms (Farber, 1991).

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

The oxidation-reduction potential (ORP) of a substrate refers to the rate at which a substrate gains or losses electrons and is determined by the characteristic pH of the food, its resistance to change in potential (poising capacity), and the oxygen tension of the surrounding atmosphere as well as its access to the product (Jay *et al*., 2005; Kalia & Gupta, 2006). Compounds such as sulphide groups, ascorbic acid and reducing sugars help to

maintain reducing conditions in fruit and vegetables (Jay et *al*., 2005). Aerobic microorganisms such as bacilli, micrococci, actinobacters, and pseudomonas require positive ORP values, while, anaerobes such as clostridia requires a negative ORP or a reduced state for optimal growth, and they cannot lower the ORP of their environment (Jay *et al*., 2005; Kalia & Gupta, 2006). Therefore, the availability of adequate quantities of oxidizing and reducing compounds in food and optimal gas composition within packaged fruit and vegetables is important to militate against microbial activity and growth.

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

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

design. More research is needed on the effects of various atmospheric modifications on the growth and survival of food-associated pathogens on fresh and fresh-cut produce.

K. Regulations on microbiological safety of fresh produce

Microbial quality assurance for MA-packaged fresh and fresh-cut fruit and vegetables is invaluable. Considering the critical points for contamination from farm to fork, this includes postharvest handling, contaminated processing equipment or transportation vehicles, cross-contamination (Farber *et al*., 2003; Oliveira *et al*., 2010), and possibility of abuse of optimal storage conditions (Chua *et al*., 2008; Oliveira *et al*., 2010). Furthermore, modified atmosphere within the package may inhibit the natural microflora on the product, while, growth of pathogens may be enhanced. The ability of MAP to extend product shelf life, pathogens may increase microbial counts above regulated threshold (Farber *et al*., 2003). In Europe and South Africa, food safety criteria for fresh-cut fruits and vegetables are regulated by the amended Commission Regulation EC No. 1441/2007 (OJEU L322/12-29, 7 December 2007) and the Foodstuffs, Cosmetics and Disinfectants (FCD) Act 54 of 1972. These criteria include: absence of *Salmonella* in products placed on the market during their shelf life; and, absence of *L. monocytogenes* in 25 g before the food has left the immediate control of the food processor and < 100 cfu g^{-1} in products placed on the market during their shelf life, among others. In the summary of the commission report, consideration was given to other approaches to the microbiological safety and quality of foods such as the preventive approach based on the principles of Hazard Analysis and Critical Control Points (HACCP) and the development of guides to Good Hygienic Practice (GHP). That will have longer term implications for microbiological standards in EC food hygiene legislation.

Stakeholders in the fresh produce chain have introduced measures to prevent product contamination (FDA/CFSAN, 2001). At the farm level, Good Agricultural Practices (GAPs) and documentation of these practices were introduced. These guidelines help in promoting safe practices, and most retailers encourage the use of these guidelines by demanding results of audits of practices (FDA, 1998a, b). Also, the International Fresh Cut Produce Association (IFPA) published food safety guidelines for fresh-cut food processors. Documents produced include a model HACCP plan, best practice guidelines for activities, a model food allergen plan, as well as a sanitary equipment buying guide and development checklist (James, 2006). The HACCP plan ensures that operations are audited in each area in a pack-house, and risk

assessments are conducted accordingly (James, 2006). Such assessment may also indentify areas where good manufacturing practices (GMPs) are failing and help in improving GMPs. The application of GAPs, GMPs, and HACCP in the fresh fruit and vegetables industry provide the basic framework for safe products for the consumer.

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

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

Additional research is needed to identify suitable surrogates and indicators for fresh and fresh-cut produce. For an extensive research needs in the use of indicators and surrogates, the reader is referred to Busta *et al.* (2003).

L. Predictive microbiology and MA-packaged produce

The risk of food borne disease outbreak involves a series of events, from the possibility of exposure to the microbial pathogen, to the likelihood of infection or intoxication leading to illness and the degree of such illness (Lammerding & McKellar, 2004). MAP of fresh and fresh-cut produce is a complex system with many variables affecting both the probability and the severity of the occurrence of food borne pathogens and diseases. Some of these variables include, gas composition, pre-treatment, properties of packaging films, and storage conditions (Cutter, 2002; Oliveira *et al.*, 2010; Sandhya, 2010; Caleb *et al.*, 2012c). Thus, to manage food safety in MA-packaged produce effectively, a systemic means of understanding these variables is necessary. Often, it is impossible to measure the effects of these factors directly on microbial response in MAP; hence they should be adequately predicted over time by evaluating available data, using mathematical predictions and re-evaluating the critical hazard points.

Over the last decade, predictive microbiology has evolved in its empirical nature ranging from "black box" approaches, such as artificial neural network models, to "grey box" models which include microbial theoretical knowledge in order to describe well characterized microbial response to intrinsic and extrinsic factors (Geeraerd *et al.*, 2004; McMeekin *et al.*, 2008; Fakruddin *et al.*, 2011). In a recent review by McMeekin *et al.* (2002), they described the concepts of predictive microbiology and highlighted on new trends, such as the progressive approximation of the growth or no growth interface and the increased application of probability models. Gompertz and logistic equations have been used extensively by various researchers to fit a variety of microbial growth curves such as: *Penicillium chrysogenum* (Dantigny *et al.*, 2011), *P. expansum* and *Aspergillus niger* (Gougouli *et al.*, 2011), *Yersinia enterocolitica* ATCC 35669 (Chen & Hoover, 2003), *L. monocytogenes* (Chhabra *et al.*, 2002; Corbo *et al.*, 2006), *E. coli* O157:H7 and generic *E. coli* (Kim *et al.*, 2007). Ratkowsky *et al.* (2005) reported a thermodynamically dependent model, describing the effect of temperature on microbial growth rates based on reversible protein denaturation both at low and high temperature. Others includes the Baranyi model (Baranyi

et al., 1993; Baranyi & Roberts, 1994), the Buchanan model (Buchanan & Bagi, 1994; Buchanan *et al*., 1997), the Hills model (Hills & Wright, 1994; Hills & Mackey, 1995), heterogeneous population model (McKeller, 1997), the Fermi model (Peleg 1997; Gastélum *et al*., 2010) and artificial neural networks (ANN) (Jeyamkondan *et al*., 2001). Although, these models have all been used in various studies to describe the behavior of microbes to parameters such as changing temperature, gaseous concentration, pH, and a_w (Farber *et al*., 1996; McKeller, 1997; Jeyamkondan *et al*., 2001; Chen & Hoover, 2003; Braun & Sutherland, 2005; Dantigny *et al*., 2011). More effort should be concerted towards modeling microbial growth 'in situ' in MA-packaged fresh and fresh-cut fruit and vegetables. A recent work although on fish fillet by Speranza *et al*. (2012), reported the use of desirability and polynomial models to predict the inhibition of *Photobacterium phosphoreum*, *Shewanella putrefaciens* and *Pseudomonas fluorescens* in fish fillets using a combination of antimicrobials and MAP technology. They observed that the effectiveness of MAP with a high content of CO_2 combined with antimicrobial solution was consistent with the stability time from the model.

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

M. Influence of MAP on microbial growth and survival on fresh produce

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

MAP has been successfully used to maintain the microbial quality of fresh and fresh-cut fruit and vegetables (Kader & Watkins, 2000; Yahia, 2006), as well as combined application of MAP and antimicrobial agents (Sivakumar *et al*., 2008). However, the effect of MAP on microorganisms can vary depending on the type of produce packaged (Farber *et al*., 2003). For instance, the increase CO_2 and decreased O_2 concentrations used in MAP generally favours the growth of lactic acid bacteria. This can accelerate the spoilage of produce sensitive to lactic acid bacteria such as carrots, chicory leaves, and lettuce (Nguyen-the & Carlin, 1994). Furthermore, oxygen concentrations below 1-2% can create a potential risk for the growth of pathogens such as *C. botulinum* (Charles *et al*., 2003; Farber *et al*., 2003). Therefore, it is necessary to highlight some foodborne pathogens that can be potential health risks due to the vulnerability of MA-packaged produce.

N. MAP of whole pomegranate fruit

Storage conditions of pomegranate fruit varies depending on the cultivar of interest (Kader *et al*., 1984; Salunkle & Desai, 1986; Köksal, 1989; Treglazova & Fataliev, 1989), so do the recommendations for controlled atmosphere storage. Artés *et al*. (1996) recommended a

controlled atmosphere of (5% O_2 + 0-5% CO_2) storage at 5 °C with RH above 95% during the storage of 'Mollar' cultivar, to minimize decay, weight loss and chilling injuries. In contrast, Kader (1997) recommended for the storage of pomegranate a gas composition of (3 to 5% O_2 + 5-10% CO_2) at 5 °C. Studies have shown that the storage of pomegranate at temperatures lower than 5 °C resulted in chilling injuries (Elyatem & Kader, 1984; Kader, 1985; Ben-Arie & Or, 1986; Artés, 1992). However, other researchers have also demonstrated that the optimum storage temperature is cultivar dependent. For instance, Köksal (1989) found that after 4 months storage the lowest weight loss in pomegranate fruit (cv. Gök Bahce) treated with anti-transpirant occurred at 1°C. Furthermore, Onur *et al.* (1992) investigated the effects of storage temperatures (2, 6 and 10 °C) on pomegranate (cv. Hicaz). After 5 months, the authors reported that storage at 2 °C resulted in the least weight loss with slight chilling injury in comparison with other storage temperatures. These studies highlight the importance of cultivar differences in optimizing the storage conditions of pomegranate and other horticultural produce.

Only one publication reported the gas composition on modified atmosphere packaging of whole fruit, other studies focused on the fruit quality attributes obtained with different polymeric films (Caleb *et al.*, 2012c). Artés *et al.* (2000) investigated the effect of different packaging films; unperforated polypropylene (UPP) 25 µm thick, and perforated polypropylene (PPP) 20 µm thick, on the quality attributes, physiological disorders, and decay of sweet 'Mollar de Elche' fruit, during cold storage and shelf life. They observed that at 2 and 5 °C within the sealed UPP bags, traces of C_2H_2 were detected occasionally, while, within perforated bags the atmospheric composition was practically like air at the end of the 12 weeks of storage. At the end of the shelf life, all treatments maintained or increased in pH values, with the exception of PPP treatment at 5 °C which had slightly decreased values. The values of titratable acid (TA) at the end of 12 week storage at 2 or 5 °C were lower than those at harvest except for PPP at 5 °C. Comparing the soluble-solids content (SSC) at harvest with those of the treatments at the end of refrigerated storage, no changes were detected, however, during the refrigerated storage at 2 and 5 °C, the SSC/TA ratio increased significantly in MAP-stored fruits, due to the decrease in TA. Based on their assessment, the best treatment for maintaining red colour of the arils at the end of cold storage was PPP at 5 °C and the lowest physiological disorders were found in MAP treatments at both refrigerated-storage temperatures.

Another study on whole fruit pomegranate "var. Wonderful" harvested from a commercial orchards in the central coastal region of Israel was reported by Porat *et al.* (2009). They recommended the use of either *X*tend® Easy-Tear or regular *X*tend® modified atmosphere or modified humidity bags in 4-5 Kg export cartons. They observed that the *X*tend® film had a higher moisture vapour transmission rates (MVTR) in comparison to conventional polyethylene and polypropylene films, which helps to eliminate excess moisture. *X*tend® packaging reduced weight loss and scald incidence of the fruit and maintains internal quality and taste.

Bayram *et al.* (2009) investigated the storage performance of 07 N 08 Hicaznar cultivar pomegranates, exposed to three different packaging treatment this include, a Strecfilm wrapped (SFW), modified atmosphere packaging and a control with no treatment. The fruits were stored for 6 month at 6 °C and relative humidity greater than 90%. They observed the least weight loss of 3% in the modified atmosphere packaged fruits compared to 24% recorded for the control treatment, and the MAP treatment gave the best visual and quality scores at the end of the storage period.

Nanda *et al.* (2001) studied the effect of shrink film wrapping of two polyolefin films (BDF-2001 and D-955) and skin coating with a sucrose polyester (SPE) Semperfresh™ on the quality and shelf life of soft-seeded 'Ganesh' pomegranates stored at 8, 15 and 25 °C. They observed that the shrink wrapping significantly reduced the respiration rate compared to non-wrapped pomegranates, but, no detectable level of ethylene was found during storage at ambient and low temperature conditions both in wrapped and non-wrapped fruits. Furthermore, shrink wrapping of pomegranates was observed to have a reduction in weight loss during storage at different temperatures in comparison to the other treatments. After 25 days of storage at 25 °C, fruits shrink-wrapped with BDF and D-955 films lost 1.5 and 2.3% of weight respectively, as compared to 14.0 and 7.8% weight lost in non-wrapped and SPE-treated fruits, respectively. Also, the pomegranate fruits remained firm throughout the storage period, in all film-wrapped treatment but it was significantly maintained better when the fruits were wrapped with BDF-2001 film compared to D-955 film at ambient and low temperature storage. Comparing the non-wrapped fruits to the SPE-treated fruits, the SPE-treatment maintained firmness better at all the storage temperatures.

In a recent study on pomegranate "Primosole" cultivar by D' Aquino *et al.* (2010), they investigated the effect of film wrapping and fludioxonil (FLU) application on reducing the

occurrence of husk scald, weight loss and decay of pomegranate fruit. FLU is a synthetic analogue of pyrrolnitrin (Rosslenbroich & Stuebler, 2000), in the class of phenylpyrroles and recently registered for controlling postharvest decay of various horticultural crops including pomegranates in USA (US EPA, 2005). D' Aquino *et al*. observed a reduction in respiration within the first week of storage at 8 °C and 90% RH and no significant difference was detected among treatments after 6 weeks. However, from the 10th week respiration in control treatment was significantly lower than in wrapped fruits. Wrapping retarded weight loss, husk scald and preserved fruit freshness for the whole storage time. Fludioxonil application alone and in combination with wrapping, effectively controlled mold growth with 50-67 % less decay in comparison to control treatments after 12 weeks at 8 °C including one week shelf life.

O. MAP of minimally processed pomegranate arils

Caleb *et al*. (2012c) presented a summary of MAP on arils of various pomegranate cultivars, highlighting the types of packaging adopted, and the modified atmosphere condition attained in the packages. Gil *et al*. (1996a) investigated the influence of different washing solutions, temperatures, and packaging on the anthocyanins content of minimally processed pomegranate "Mollar de Elche" seeds. They found no significant differences in the anthocyanin composition after washing with different solutions. However, unpackaged pomegranate seeds stored for 7 days at 8, 4, and 1 °C, were observed to be shriveling, with almost half of the water originally present in the seeds lost during the unpackaged storage. On the other hand, MAP stored seeds had a minimal water loss compared to unpackaged. During cold storage in modified atmospheres at 1 °C, an increase in anthocyanin content was observed while a decrease was recorded at 8 and 4 °C. Comparing the perforated oriented polypropylene (OPP) and unperforated OPP package bags, stored with arils at 1 °C for 7 days. They observed that the unperforated OPP bags maintained the pigments better compared to perforated OPP bags. However, when the storage condition was extended for additional 4 days at 4 °C to mimic domestic storage, the seeds were better preserved in the perforated films. In a similar study by Gil *et al*. (1996b), the best outcomes in quality and appearance were obtained for pomegranate seeds washed with chlorine (100 mg kg^{-1}) plus antioxidants (5 g L^{-1} ascorbic acid and 5 g L^{-1} citric acid) sealed in OPP film, using an initial atmosphere actively modified to 0 mL L^{-1} CO_2 and 20 mL L^{-1} O_2 and stored for 7 days at 1

°C. Under this condition, the minimally processed seeds maintained good quality without fungal attacks or off-flavour development.

López-Rubira *et al*. (2005) investigated the effect of harvest time, use of different UV-C radiation and passive MAP storage on sensory, chemical and microbial quality as well as on the shelf life of minimally fresh processed arils extracted from "Mollar of Elche" pomegranate. They observed that the rate of respiration of fresh processed arils was higher in the late harvest than in the earlier harvested fruit, with an average respiration rate (RR) of 26.6 ± 1.88 and 14.5 ± 2.48 nmol CO_2 Kg^{-1} s^{-1} respectively. No significant differences were observed between the control and UV-C treated arils and there was no observable interaction between the passive MAP and UV-C treatments. Except that the CO_2 accumulation within aril packages was higher in December harvest than those of October, due to their higher RR. However, microbial counts of minimally fresh processed arils increased throughout the shelf life, with mesophilic counts of control arils processed in October slightly higher than those from December. Their anthocyanin content investigation was in agreement with previous report by Gil *et al*. (1996b). They found no significant change in total anthocyanin content of "Mollar" arils harvested in early October during MAP storage at 1 °C for 7 days. However, their findings suggested that the shelf life of fresh processed arils is at least 10 days, contrary to 7 days reported by Gil *et al*. (1996b) for "Mollar" pomegranate arils harvested in early October and stored at 1 °C under MAP.

García *et al*. (2000) studied the respiratory intensity (RI) of pomegranate "Mollar" seeds and the gas composition inside both a semi-permeable and an impermeable plastic at a storage temperature of 4 °C for 10 days. They observed a RI of 30.8 ± 0.4 (mL CO_2 kg^{-1} h^{-1}) for the pomegranate seeds which was much lower compared to sliced oranges with 57.05 ± 1 (mL CO_2 kg^{-1} h^{-1}) from their study. In the case of modified atmosphere packages the atmosphere within the semi-permeable plastic was inadequate to prolong the shelf life of the minimally processed and refrigerated pomegranates. The high relative humidity within the packages helps reduce weight loss, maintaining the turgency and texture of the pomegranate seeds.

Sepúlveda *et al*. (2000) investigated the influence of various types of antioxidant solutions and three semi-permeable films; two cryovac, based on ethyl vinyl acetate (BE and BB4) and perforated polyethylene film as control on the quality of minimally processed pomegranate 'var. Wonderful' arils from Chile stored at 4 °C ± 0.5 for 14 days. A slight browning of arils

was observed in all treatments, but this was highest in treatments without antioxidants. The weight loss of arils was lower in the arils packaged in BE and BB4 film and was significantly different from the arils in PE packages. After 14 days, all the treatments with BE and BB4 packages showed a very low total count for mesophilic aerobes, which could be attributed to higher concentration of carbon dioxide inside the packages. The use of semi-permeable films allowed successful storage for 14 days at 4 °C ± 0.5, with good physical, chemical, and microbiological quality. Additionally, the decrease in microbial growth was in agreement with Gorny (1997), who observed a decrease in the growth of microorganisms with CO_2 concentrations between 15 and 20%.

Chemical and organoleptic characteristics of minimally processed seeds of pomegranate 'Primosole' were examined after packaging in a 40 µm thick polypropylene film and stored at 5 °C for 10 days by Palma *et al*. (2009). They observed that a passive modified atmosphere was established within the package, with a progressive increase in CO_2 and decrease in O_2 level. Ethylene concentration increased rapidly to the end of storage, the increase in ethylene was associated with wound injuries on the seeds. Furthermore for their study, no significant changes in chemical properties of analyzed juice. However, an increase in titratable acidity was observed in packaged seeds, this increase acidity was attributed to the absorption of CO_2 which lowers pH when dissolved in aqueous phase (Malhotra & Prasad, 1999).

The use of honey treatments has also been explored in preserving the fresh-like quality of arils and to extend their shelf life. Ergun & Ergun (2009) evaluated the efficacy of varying concentration of 10 and 20% honey dip treatment on the quality and shelf life of minimally processed pomegranate arils of 'Hicaznar' stored at 4 °C in loosely closed plastic containers. It was demonstrated that honey treated arils had brilliant aroma throughout the 10 days storage period, compared to arils treated with water. After five days of storage, arils treated with honey solution had a significantly lower rate of softening than control samples. The total aerobic microbial count was lower in honey treated arils compared to the control but the counts increased across all treatment compared to the count immediately after treatment.

Microbial quality criteria are often used to determine the acceptability limit and the shelf life of minimally fresh processed produce and this is used as a minimal standard for processed produce having a limited microbial count and free of pathogenic microorganisms

(Willocx, 1995). Storage of arils under optimal MA have been shown to reduce the risk of *Enterobacteriaceae*, latic acid bactria, mesophilic, psychrotrophic, as well as moulds and yeast counts (Sepúlveda *et al*., 2000; López-Rubira *et al*., 2005). Furthermore, since pomegranate arils are stored at lower temperature, the risk of microbial proliferation is reduced. According to Artés *et al*. (2000), higher levels of decay (mainly due to *Penicillium* spp.) were observed in unpackaged treatments at 5 °C than in those at 2 °C. Similarly, López-Rubira *et al*. (2005) observed a low count of micro-aerophilic lactic acid bacteria after 10 days of aril storage without any trace of fermentative metabolism.

P. Conclusion

Comprehensive review of literature showed that MAP application for pomegranate whole fruit and minimally processed arils is still restricted to certain cultivars either because of the profit margin gained from packaging them or due to limited information on the metabolic properties of the other cultivars. As new cultivars are merging for commercial farming, it is expedient to investigate the postharvest physiology for both the newly introduced cultivars and other unstudied cultivars. It was also shown in this review that different pomegranate cultivars responded differently to MAP. Hence, experimental studies should be carried out separately for each cultivar with a more informative output on the physiological characteristics (e.g. respiration rates of whole fruits or arils) under various conditions, in order to enable the successful application of the available technology. Additionally, challenges such as: the choice of polymeric films, as no single polymeric film can offer all the required properties for MAP, can be overcome buy adequate understanding of the interconnected three disciplines towards an optimal MAP for pomegranate whole fruit and arils.

Furthermore, it has been established that inconsistent or abusive temperature contributes to increased respiration and transpiration rates, which in turn can enhance microbial proliferation and deterioration of MA-packaged fresh or fresh-cuts. This highlights the need for more concerted effort towards the maintenance of strict cold chain along the whole distribution continuum. The integration of multiple intelligent systems coupled with microbiological data should be evaluated towards the optimal success of MAP. The application of novel technologies such as high pressured inert gases, ‘smart’ packaging and pre-packaging treatment for fresh produce offer additional potential to increase produce

shelf-life and microbial safety. This includes the development of optimal inert gas-MAP atmospheric composition for fresh produce; bioactive polymeric films with antimicrobial activity against foodborne pathogens, via immobilization of bacterial cells or antimicrobials on polymers; the incorporation and controlled release of volatile and non-volatile antimicrobial agents into packages; and, the use of biopolymers that are inherently antimicrobial.

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

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

References

Abulhamd, A.T. (2009). Characterization of *Aeromonas hydrophila* isolated from aquatic environments using phenotypic and genotyping methods. *Research Journal of Agriculture and Biological Sciences,* **5**, 923-931.

Adams, M.R. & Moss, M.O. (2000). *Food microbiology,* 2nd ed. Cambridge: Royal Society of Chemistry.

Adaskaveg, J.E. & Förster, H. (2003). Management of gray mold of pomegranate caused by *Botrytis cinerea* using two reduced-risk fungicides, fludioxonil and fenhexamid. *Phytopathology*, **93**, S127-S131.

Ahvenainen, R., Eilamo, M. & Hurme, E. (1997). Detection of improper sealing and quality deterioration of modified-atmosphere-packaged pizza by a colour indicator. *Food Control*, **8**, 177-184.

Ahmed, D.M., Yousef, R.M. & Sarrwy, S.M.A. (2011). Modified atmosphere packaging for maintaining quality and shelf life extension of persimmon fruits. *Asian Journal of Agricultural Science,* **3**, 308-316.

Ahvenainen, J.M. (1996). New approaches in improving the shelf life of minimally processed fruits and vegetables. *Trends in Food Science and Technology,* **7**, 179-186.

Almenar, E., Samsudin, H., Auras, R., Harte, B. & Rubino, M. (2008). Postharvest shelf life extension of blueberries using a biodegradable package. *Food Chemistry,* **110**, 120-127.

Alzamora, S.M., Tapia, M.S., Lopez-Malo, A. & Welti-Chanes, J. (2003). The control of water activity. In: *Food preservation techniques* P. Zeuthen & L. Bøgh-Sørensen (eds.), Pp. 126-153. Cambridge, England: CRC Press.

Amanatidou, A., Smid, E.J., & Gorris, L.G.M. (1999). Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated microorganisms. *Journal of Applied Microbiology,* **86**, 429-438.

Ando, H., Takeya, S., Kawagoe, Y., Makino, Y., Suzuki, T. & Oschita, S. (2009). In situ observation of xenon hydrate formation in onion tissue by using NMR and powder X-ray diffraction measurement. *Cryobiology,* **59**, 405.

Al-Said, F.A., Opara, L.U. & Al-Yahyai, R.A. (2009). Physico-chemical and textural quality attributes of pomegranate cultivars (*Punica granatum* L.) grown in the Sultanate of Oman. *Journal of Food Engineering*, **90**, 129-134.

Al-Yahyai, R., Al-Said, F. & Opara, L. (2009). Fruit growth characteristics of four pomegranate cultivars from northern Oman. *Fruits*, **64**, 1 – 7.

Ares, G., Lareo, C. & Lema, P. (2007). Modified atmosphere packaging for postharvest storage of mushrooms. A review. *Fresh Produce*, **1**, 32-40.

Artés, F. (1992). Factores de calidad y conservación frigorífica de la granada. In : II Jornadas Nacionales del Granado. Univ. Politécnica de Valencia.

Artés, F. & Tomás-Barberán, F.A. (2000). Postharvest technological treatments of pomegranate and preparation of derived products. In: Melgarejo-Moreno et al. (eds.) Production, processing and marketing of pomegranate in the Mediterranean Region, pp 199-204. CIHEAM-Options Mediterraneennes: Série A. Séminaires Méditerranéens no. 42.

Artés, F., Tudela, J.A. & Villaescusa, R. (2000a). Thermal postharvest treatment for improving pomegranate quality and shelf life. *Postharvest Biology and Technology*, **18**, 245-251.

Artés, F., Villaescusa, R. & Tudela, J.A. (2000b). Modified atmosphere packaging of pomegranate. *Journal of Food Science*, **65**, 1112-1116.

Artés, F. Gómez, P., Aguayo, E., Escalona, V. & Artés-Hernández, F. (2009). Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biology and Technology,* **51**, 287-296.

Artin, I., Carter, A.T., Holst, E., Lövenklev M., Mason, D.R., Peck, M.W. & Rådstrom, P. (2008). Effects of carbon dioxide on neurotoxin gene expression in nonproteolytic *Clostridium botulinum* Type E. *Applied and Envirinmental Microbiology,* **74**, 2391-2397.

Artin, I., Mason, D.R., Pin, C., Schelin, J., Peck, M.W., Holst, E., Rådstrom, P. & Carter, T. (2010). *Applied and Environmental Microbiology,* **76**, 1168-1172.

Arvanitoyannis, I., Psomiadou, E., Biliaderis, C.G., Ogawa, H., Kawasaki, N. & Nakayama, A. (1997). Biodegradable films made from low density polyethylene (LDPE), ethylene acrylic acid (EAA), polycaprolactone (PCL) and wheat starch for food packaging applications: Part 3. *Starch*, **49**, 306-322.

Atmar, R. L., Metcalf, T., Neil, F., & Estes, M. (2001). Detection of enteric viruses in oysters by using PCR. *Applied and Environmental Microbiology,* **59**, 631-635.

Ayranci, E. & Tunc, S. (2004). The effect of edible coatings on water and vitamin C loss of apricots (*Armeniaca vulgaris* Lam.) and green peppers (*Capsicum annuum* L.). *Food Chemistry,* **87**, 339-342.

Austin, J.W., Dodds, K.L., Blanchfield, B. & Farber, J.M. (1998). Growth and toxin production by Clostridium botulinum on inoculated fresh-cut packaged vegetables. *Journal of Food Protection*, **61**, 324-328.

Baldwin, E.A. (1994). Edible coatings for fresh fruit and vegetables; past, present, and future. In: *Edible coatings and films to improve food quality.* J. M. Krochta, E. A. Baldwin, M. O. Nisperos-Carriedo (Eds.). Pp. 25-64. Lancaster: Technomic.

Banks, N.H., Cleland, D.J., Cameron, A.C., Beaudry, R.M. & Kader, A.A. (1995). Proposal for a rationalized system of units for postharvest research in gas exchange. *HortScience*, **30**, 1129-1131.

Baranyi, J. & Roberts, T.A. (1994). A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology,* **23**, 277-294.

Baranyi, J., Roberts, T.A. & McClure, P. (1993). A non-autonomous differential equation to model bacterial growth. *Food Microbiology,* **10**, 43-59.

Barbosa, L.N., Carciofi, B.A.M., Dannenhauer, C.É. & Monteiro, A.R. (2011). Influence of temperature on the respiration rate of minimally processed organic carrots (*Daucus carota* L. cv. Brasília). *Ciência e Tecnologia de Alimentos,* **31**, 78-85.

Barry, C.S. & Giovannoni, J.J. (2007). Ethylene and fruit ripening. *Journal of Plant Growth Regulation*, **26**, 143-159.

Basch, C.Y., Jagus, R.J. & Flores, S.K. (2012). Physical and antimicrobial properties of tapioca starch-HPMC edible films incorporate with nisin and/or potassium sorbate. *Food and Bioprocess Technology,* accepted manuscript. DOI: 10.1007/s11947-012-0860-3.

Bayram, E., Dundar, O. & Ozkaya, O. (2009). Effect of different packaging types on storage of Hicaznar pomegranate fruits. *Acta Horticulturae*, **818**, 319-322.

Ben-Arie, R. & Or, E. (1986). The development and control of husk scald on Wonderful pomegranate fruit during storage. *Journal of the American Society for Horticultural Science*, **111**, 395-399.

Ben-Yehoshua, S. & Rodov, V. (2003). Transpiration and water stress. In: *Postharvest physiology and pathology of vegetables*, J.A. Bartz, & J.K. Brecht (eds.). Pp. 111-159. NY, USA: Marcel Dekker Inc.

Berrang, M.E., Brackett, R.E. & Beuchat, L.R. (1989). Growth of *Aeromonas hydrophila* on fresh vegetables stored under a controlled atmosphere. *Applied and Environmental Microbiology,* **55**, 2167-2171.

Bennik, M.H.J., Smid, E.J., Rombouts, F.M. & Gorris, L.G.M. (1995). Growth of psychotrophic foodborne pathogens in a solid surface model system under the influence of carbon dioxide and oxygen. *Food Microbiology,* **12**, 509-519.

Berg, D., Kohn, M., Farley, T. & McFarland, L. (2000). Multistate outbreak of acute gastroenteritis traced to fecal-contaminated oysters harvested in Louisiana. *Journal of Infectious Diseases,* **181**, S381-S386.

Bhande, S.D., Ravindra, M.R. & Goswami, T.K. (2008). Respiration rate of banana fruit under aerobic conditions at different storage temperatures. *Journal of Food Engineering,* **87**, 116-123.

Bidawid, S., Farber, J.M. & Sattar, S. A. (2000). Contamination of foods by food handlers: Experiments on Hepatitis A virus transfer to food and its interruption. *Applied and environmental microbiology,* **66**, 2759-2763.

Bidawid, S., Farber, J.M. & Sattar, S. A. (2001). Survival of hepatitis A virus on modified atmosphere-packaged (MAP) lettuce. *Food Microbiology,* **18**, 95-102.

Bourke, P. & O'Beirne, D. (2004). Effects of packaging type, gas atmosphere and storage temperature on survival and growth of *Listeria* spp. in shredded dry coleslaw and its components. *International Journal of Food Science and Technology,* **39**, 509-523.

Brandenburg, J.S. & Zagory, D. (2009). Modified and controlled atmosphere packaging technology and applications. In: *Modified and controlled atmosphere for the storage, transportation, and packaging of horticultural commodities* E. M. Yahia (ed.). Pp. 73-92. Boca Raton, FL: Taylor & Francis Group, CRC Press:

Braun, P. & Sutherland, J.P. (2005). Predictive modelling of growth and measurement of enzymatic synthesis and activity by a cocktail of selected Enterobacteriaceae and *Aeromonas hydrophila*. *International Journal of Food Microbiology,* **105**, 257-266.

Buchanan, R.L. & Bagi, L.K. (1994). Expansion of response surface models for the growth of *Escherichia coli* O157:H7 to include sodium nitrite as a variable. *International Journal of Food Microbiology,* **23**, 317-332.

Buchanan, R.L., Whiting, R.C. & Damert, W.C. (1997). When is simple good enough: a comparison of the Gompertz, Baranyi, and three phase linear models for fitting bacterial growth curves. *Food Microbiology,* **14**, 313-326.

Busta, F.F., Suslow, T.V., Parish, M.E., Beuchat, L.R., Farber, J.N., Garett, E.H. & Harris L. J. (2003). The use of indicators and surrogate microorganisms for the evaluation of pathogen in fresh and fresh cut produce. *Comprehensive Reviews in Food Science and Food Safety,* **2**, 179-185.

Cahill, M. M. (1990). Virulence factors in motile *Aeromonas* species. A review. *Journal of Applied Bacteriology, 69*, 1-16.

Caleb, O.J., Mahajan, P.V., Opara, U.L. & Witthuhn, C.R. (2012a). Modelling the respiration rate of pomegranate fruit and arils. *Postharvest Biology and Technology,* **64**, 49-54.

Caleb, O.J., Mahajan, P.V., Opara, U.L. & Witthuhn, C.R. (2012b). Modelling the effect of time and temperature on respiration rate of pomegranate arils (cv. "Acco" and "Herskawitz"). *Journal of Food Science,* **77**, E80-E87.

Caleb, O.J., Opara, U.L. & Witthuhn, C.R. (2012c). Modified atmosphere packaging of pomegranate fruit and arils: a review. *Food and Bioprocess Technology,* **5**, 15-30.

Calín-Sánchez, A., Martínez, J.J., Vázquez-Araú L., Burló, F., Melgarejo, P., & Carbonell-Barrachina, A.A. (2011). Volatile composition and sensory quality of Spanish pomegranates (*Punica granatum* L.). *Journal of Science Food Agriculture*, **91**, 586-992.

Cameron, A.C., Beaudry, R.M., Banks, N.H. & Yelanich, M.V. (1994). Modified atmosphere packaging of blueberry fruit: modelling respiration and package oxygen partial pressures as function of temperature. *Journal of American Society for Horticultural Science*, **119**, 534-539.

Campos, C.A., Gerschenson, L.N. & Flores, S.K. (2011). Development of edible films and coatings with antimicrobial activity. *Food and Bioprocess Technology,* **4**, 849-875.

Carey, C.M., Kostrzynska, M. & Thompson, S. (2009). *Escherichia coli* O157:H7 stress and virulence gene expression on Romaine lettuce using comparative real-time PCR. *Journal of Microbiological Methods,* **77**, 235-242.

Carraso, E., Pérez-Rodríguez, F., Valero, A., García-Gimeno, R.M. & Zurera, G. (2008). Growth of *Listeria monocytogenes* on shredded, ready-to-eat iceberg lettuce. *Food Control,* **19**, 487-494.

Cha, D.S. & Chinnan, M.S. (2004). Biopolymer based antimicrobial packaging: A review. *Critical Reviews in Food Science and Nutrition,* **44**, 223-237.

Chau, K. & Gaffney, J.J. (1985). A mathematical model for the transpiration for fruits and vegetables. *ASAE Paper* No. 85-6005.

Charles, F., Sanchez, J. & Gontard, N. (2003). Active modified atmosphere packaging of fresh fruits and vegetables: Modelling with tomatoes and oxygen absorber. *Journal of Food Science*, **68**, 1736-1742.

Chen, H. & Hoover, D.G. (2003). Pressure inactivation kinetics of *Yersinia enterocolitica* ATCC 35669. *International Journal of Food Microbiology,* **87**, 161-171.

Chua, D.K., Goh, K., Saftner, R.A. & Bhagwat, A.A. (2008). Fresh-cut lettuce in modified atmosphere packages stored at improper temperatures support enterohemorrhagic *E. coli* isolates to survive gastric acid challenge. *Journal of Food Science,* **73**, M148-M153.

Church, N. (1994). Developments in modified-atmosphere packaging and related technologies. *Trends in Food Science and Technology*, **5**, 345-352.

Church, P.N. (1993). Meat and meat products. In: *Principles and application of modified atmosphere packaging of food* R. T. Parry (ed.). Pp. 170-187. Glasgow: Blackie.

Corbo, M.R., Del Nobile, M.A. & Sinigaglia, M. (2006). A novel approach for calculating shelf life of minimally processed vegetables. *International Journal of Food Microbiology,* **106**, 69-73.

Corrales, M., Han, J.H. & Tauscher, B. (2009). Antimicrobial properties of grape seed extracts and their effectiveness after incorporation in pea starch films. *International Journal of Food Science and Technology,* **44**, 425-433.

Charles, F., Sanchez, J. & Gontard, N. (2005). Modeling of active modified atmosphere packaging of endives exposed to several postharvest temperatures. *Journal of Food Science,* **70**, E443-E449.

Chhabra, A.T., Carter, W.H., Linton, R.H., & Cousin, M.A. (2002). A predictive model that evaluates the effect of growth conditions on the thermal resistance of *Listeria monocytogenes*. *International Journal of Food Microbiology,* **78**, 235-243.

Chung, D. & Yam, K.L. (1999). Antimicrobial packaging material containing propyl paraben. In: Institute of Food Technologists annual technical meeting program, pp.21 July 1999, Chicago.

Crank, J. & Park, G.S. (1968). Diffusion in polymers. In: Crank et al (eds). Pp 360. London: Academic Press.

Cutter, C.N. (2002). Microbial control by packing: A review. *Critical Reviews in Food Science and Nutrition*, **42**, 151-161.

D' Aquino, S., Palma, A., Schirra, M., Continella, A., Tribulato, E. & La Malfa, S. (2010). Influence of film wrapping and fludioxonil application on quality of pomegranate fruit. *Postharvest Biology and Technology*, **55**, 121-128.

Dantigny, P., Nanguy, S.P., Judet-Correia, D. & Bensoussan, M. (2011). A new model for germination of fungi. *International Journal of Food Microbiology,* **146**, 176-181.

Dash, K.K., Ravindra, M.R. & Goswami, T.K. (2009). Modeling of respiration rate of sapota fruit under aerobic conditions. *Journal of Food Process Engineering,* **32**, 528-543.

Davies, A.R. & Slade, A. (1995). Fate of *Aeromonas* and *Yersinia* on modified atmosphere package (MAP) cod and trout. *Letter of Applied Microbiology,* **21**, 354-358.

Day, B. P. F. (1993). Fruit and vegetables. In: R. T. Parry (Ed.), *Principles and applications of MAP of foods* (pp. 114-133). New York, USA: Blackie Academic and Professional.

De Santana, L.R.R., Benedetti, B.C., Sigrist, J.M.M. & Sato, H.H. (2011). Effect of modified atmosphere packaging on ripening of 'Douradão' peach related to pectolytic enzymes activities and chilling injury symptoms. *Revista Brasileria de Fruticultura*, **33**, 1084-1094.

Del Nobile, M.A., Baiano, A., Benedetto, A. & Massignan, L. (2006). Respiration rate of minimally processed lettuce as affected by packaging. *Journal of Food Engineering,* **74**, 60-69.

Ding, C.–K., Chanchin, K., Ueda, Y., Imahori, Y. & Wang, C.Y. (2002). Modified atmosphere packaging maintains postharvest quality of loquat fruit. *Postharvest Biology and Technology,* **24**, 341-348.

Disalvo, E. A., Lairion, F., Martini, F., Tymczyszyn, E., Frías, M., Almaleck, H. & Gordillo, G. J. (2008). Structural and functional properties of hydration and confined water in membrane interfaces. *Biochimica et Biophysica Acta (BBA) – Biomembranes,* **1778***,* 2655-2670.

Elyatem, S.M. & Kader, A.A. (1984). Post-harvest physiology and storage behaviour of pomegranate fruits. *Scientia Horticulturae*, **24**, 287-298.

Ersan, S., Gunes, G. & Zor, A.O. (2010). Respiration rate of pomegranate arils as affected by O_2 and CO_2, and design of modified atmosphere packaging. *Acta Horticulturae,* **876**, 189-196.

Ergun, M. & Ergun, N. (2009). Maintaining quality of minimally processed pomegranate arils by honey treatments. *British Food Journal*, **111**, 396-406.

FAD/WFP. (1970). In: F. Ashman (Ed.), *Food Storage manual part II. Food and commodities*. Rome: FAO.

Fahan, A. (1976). The seed. In: *Plant anatomy*. Pp 419-430. Jerusalem: Hakkibutz Hameuhad Publication.

Fakruddin, M., Mazumder, R.M. & Mannan, K.S.B. (2011). Predictive microbiology: Modelling microbial response in food. *Ceylon Journal of Science,* **40**, 121-131.

Farber, J.M. (1991). Microbiological aspects of modified atmosphere packaging- a review. *Journal of Food Protection*, **54**, 58-70.

Farber, J.N., Harris, L.J., Parish, M.E., Beuchat, L.R., Suslow, T.V., Gorney, J.R., Garrett, E.H. & Busta, F.F. (2003). Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comprehensive Review in Food Science and Food Safety*, **2**, 142-160.

Farber, J.M., Cai, Y. & Ross, W.H. (1996). Predictive modeling of the growth of *Listeria monocytogenes* in CO_2 environments. *International Journal of Food Microbiology,* **32**, 133-144.

Farkas, J. (1997). Physical methods of food preservation. In: *Food microbiology-fundamentals and frontiers* M. P. Doyle, L. R. Beuchat, & T. J. Montville (eds.). Pp. 497-519. Washington: ASM Press.

Fonseca, S.C., Oliveira, F.A.R. & Brecht, J.K. (2002a). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *Journal of Food Engineering,* **52**, 99-119.

Fonseca, S.C., Oliveira, F.A.R., Frias, J.M., Brecht, J.K. & Chau, K.V. (2002b). Modelling respiration rate of shredded Galega kale for development of modified atmosphere packaging. *Journal of Food Engineering,* **54**, 299-307.

Food and Drug Adminustration (FDA) (1998a). Guide to minimize microbial food safety hazards for fresh fruit and vegetables. Available at http://vm.cfsan.fda.gov/~dms/prodguid.html. Accessed October 2005.

Food and Drug Administration (FDA) (1998b). Guide to minimize microbial food safety considerations for organic produce production: An analysis of Canadian organic production standards compared with US FDA guidelines for microbial food safety. *Food Protection Trends, 24*(4), 246-252.

Food and Drug Administration/ Center for Food Safety and Applied Nutrition (FDA/CFSAN) (2001). Analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. Available at http://www.cfsan.fda.gov/~comm/ift3-7html. Accessed January 2003.

Francis, G.A. & O'Berine, D. (1997). Effects of gas atmosphere, antimicrobial dip and temperature on the fate of *L. innocua* and *L. monocytogenes* on minimally processed lettuce. *International Journal of Food Science and Technology,* **32**, 141-151.

Francis, G. A. & O'Berine, D. (1998). Effects of the indigenous microflora of minimally processed lettuce on the survival and growth of *Listeria innocua*. *International Journal of Food Science and Technology,* **33**, 477-488.

Francis, G. A. & O'Berine, D. (2001). Effects of vegetable type, package atmosphere and storage temperature on growth and survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. *Journal of Industrial Microbiology and Biotechnology,* **27**, 111-116.

Francis, G. A. & O'Berine, D. (2005). Variation among strains of *Listeria monocytogenes*: differences in survival on packaged vegetables and in response to heat and acid conditions. *Food Control,* **16**, 687-694.

Francis, G. A., & Thomas, C. & O'Berine, D. (1999). Review paper: the microbiological safety of minimally processed vegetables. *International Journal of Food Science and Technology,* **34**, 1-22.

Gaffney, J.J., Baird, C.D. & Chau, K.V. (1985). Methods for calculating heat and mass transfer in fruits and vegetables individually and in bulk. *ASHRAE Transaction,* **90**, 333-352.

Garcia, D., Ramos, A.J., Sanchis, V. & Marín, S. (2011). Modelling the effect of temperature and water activity in the growth boundaries of *Aspergillus ochraceus* and *Aspergillus parasiticus*. *Food Microbiology,* **28**, 406-417.

Garcia-Gimeno, R.M., Sanchez-Pozo, M.D., Amaro-Lopez, M.A. & Zurera-Cosano, G. (1996). Behaviour of *Aeromonas hydrophila* in vegetable salads stored under modified atmosphere at 4 and 15 °C. *Food Microbiology,* **13**, 369-374.

Garrett, E. H. (2002). Fresh cut produce: Tracks and trends. In: *Fresh-cut fruit and vegetables: Science, technology, and market.* O. Lamikanra (ed.). Pp 1-10. Boca Raton, Fla. : CRC Press.

Gastélum, G.G., Avila-Sosa, R., López-Malo, A. & Palou, E. (2010). *Listeria innocua* multi-target inactivation by thermo-sonication and vanillin. *Food and Bioprocess Technology,* DOI: 10.1007/s11947-010-0334-4.

García E., Salazar, D.M., Melgarejo, P. & Coret, A. (2000). Determination of the respiration index and of the modified atmosphere inside the packaging of minimally processed products. *CIHEAM-Options Mediterraneennes*, Pp. 247-251.

Gbaruko, B.C., Igwe, J.C., Gbaruko, P.N. & Nwokeoma, R.C. (2007). Gas hydrates and clathrates: flow assurance, environmental and economic perspectives and the Nigerian liquefied natural gas project. *Journal of Petroleum Science and Engineering,* **56**, 192-198.

Geeraerd, A. H., Valdramidis, V. P., Devlieghere, F., Bernaert, H., Debevere, J., & Van Impe, J. F. (2004). Development of a novel approach for secondary modelling in predictive microbiology: incorporation of microbiological knowledge in black box polynomial modelling. *International Journal of Food Microbiology, 91*, 229-244.

Gil, M.I., Artes, F. & Toma-Barberan, F.A. (1996a). Minimal processing and modified atmosphere packaging effects on pigmentation of pomegranate seeds. *Journal of Food Science*, **61**, 161-164.

Gil, M.I., Martínez, J.A. & Artés, F. (1996b). Minimally processed pomegranate seeds. *Lebensmittel-Wissenschaft und Technologie*, **29**, 708-713.

Gleeson, E. & O'Beirne, D. (2005). Effects of process severity on survival and growth of *Escherichia coli* and *Listeria innocua* on minimally processed vegetables. *Food Control,* **16**, 677-685.

Goepfert, J.M. (1980). Vegetables, fruits, nuts and their products. In: *Microbial ecology of food* J. H. Silliker *et al.* (eds.), (pp. 606-642). New York: Academic Press.

Gomes, M.H., Beaudry, R.M., Almeida, D.P.F., & Malcata, F.X. (2010). Modelling respiration of packaged fresh-cut 'Rocha' pear as affected by oxygen concentration and temperature. *Journal of Food Engineering,* **96**, 74-79.

González-Fandos, E., Olarte, C., Giménez, M., Sanz, S. & Simón, A. (2001). Behaviour of *Listeria monocytogenes* in packaged fresh mushrooms (*Agaricus bisporus*). *Journal of Applied Microbiology,* **91**, 795-805.

Goodrum, P.M. & McLaren, M. (2003). A review of smart chip implementation and pilot project at University of Kentucky: A report to the electrical contracting foundation. Lexington, University of Kentucky, Department of Civil Engineering.

Gorny, J. (1997). CA and MA requirements and recommendations for fresh-cut (minimally processed) fruits and vegetables. In: Proceedings of 7th International controlled atmosphere research conference, Vol 5, July 1997, Davis, California, USA.

Gorny, J. R., Cifuentes, R. A., Hess-Pierce, B. H., & Kader A. A. (2000). Quality changes in fresh-cut pear slices as affected by cultivar, ripeness stage, fruit size and storage regime. *Journal of Food Science. 65*, 541-544.

Gougouli, M. & Koutsoumanis, K.P. (2011). Modelling germination of fungal spores at constant and fluctuating temperature conditions. *International Journal of Food Microbiology,* DOI: 10.1016/j.ijfoodmicro.2011.07.030

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B. & Givskov, M. (2002). Food spoilage-interactions between food spoilage bacteria. *International Journal of Food Microbiology,* **78**, 79-97.

Graham, S.A., Hall, J., Sytsma, K. & Shi, S. (2005). Phylogenetic analysis of the Lythraceae based on four gene regions and morphology. *International Journal of Plant Science*, **166**, 995-1017.

Guevara, J.C., Yahia, E.M., Brito de la Fuente, E. & Biserka, S.P. (2003). Effect of elevated concentrations of CO_2 in modified atmosphere packaging on the quality of prickly pear cactus stems (*Opuntia* spp.). *Postharvest Biology and Technology,* **29**, 167-176.

Guilbert, S., Gontard, N. & Gorris, L.G.M. (1996). Prolongation of the shelf life of perishable food products using biodegradable films and coatings. *Food Science and Technology,* **29**, 10-17.

Guillaume, C., Schwab, I., Gastaldi, E. & Gontard, N. (2010). Biobased packaging for improving preservation of fresh common mushrooms (*Agaricus bisporus* L.). *Innovative Food Science and Emerging Technologies,* **11**, 690-696.

Han, J.H. (2000). Antimicrobial food packaging. *Food Technology,* **54**, 56-65.

Han, C., Zhao, Y., Leonard, S.W. & Traber, M.G. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria ananassa*) and raspberries (*Rubus ideaus*). *Postharvest Biology and Technology,* **33**, 67-78.

Hao, Y.Y., Brackett, R.E., Beuchat, L.R. & Doyle, M.P. (1998). Microbiological quality and the ability of proteolytic *Clostridium botulinum* to produce toxin in film-packaged fresh-cut cabbage and lettuce. *Journal of Food Protection,* **61**, 1148-1153.

Harris, L.J., Fraber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H. & Busta, F.F. (2003). Outbreaks associated with fresh produce: Incidence, growth, and survival of

pathogens in fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety,* **2**, 78-141.

Hills, B.P. & Mackey, B.M. (1995). Multi-compartment kinetic models for injury, resuscitation, induced lag and growth in bacterial cell populations. *Food Microbiology,* **12**, 333-346.

Hills, B.P. & Wright, K.M. (1994). A new model for bacterial growth in heterogeneous systems. *Journal of Theoretical Biology,* **168**, 31-41.

Holland, D., Hatib, K. & Bar-Ya'akov, I. (2009). Pomegranate: Botany, horticulture, breeding. *Horticultural Reviews*, **35**, 127-191.

Hong, S.-I. & Park, W.-S. (2000). Use of colour indicators as an active packaging system for evaluating kimchi fermentation. *Journal of Food Engineering*, **46**, 67-72.

Hotchikiss, J.H. (1988). Experimental approaches to determining the safety of food packaged in modified atmospheres. *Food Technology,* **42**, 55-64.

Hounsome, N., Hounsome, B., Tomos, D. & Edwards-Jones, G. (2008). Plant metabolites and nutritional quality of vegetables. *Journal of Food Science,* **73**, R48-R65.

Ibarguren, C., Vivas, L., Bertuzzi, M.A., Apella, M.C. & Audisio, M.C. (2010). Edible films with anti-*Listeria monocytogenes* activity. *International Journal of Food Science and Technology,* **45**, 1443-1449.

Inoue, Y., Hatakeyama, H. & Yoshino, I. (1994). Oxygen indicator. US Patent 5358876 (in United State).

Iqbal, T., Rodrigues, F.A.S., Mahajan, P.V. & Kerry, J.P. (2009a). Mathematical modeling of the influence of temperature and gas composition on the respiration rate of shredded carrots. *Journal of Food Engineering,* **91**, 325-332.

Iqbal, T., Rodrigues, F.A.S., Mahajan, P.V. & Kerry, J.P. (2009b). Mathematical modeling of O_2 consumption and CO_2 production rates of whole mushrooms accounting for the effect of temperature and gas composition. *International Journal of Food Science and Technology,* **44**, 1408-1414.

Iqbal, T., Rodrigues, F.A.S., Mahajan, P.V., & Kerry, J.P. (2009c). Effect of time, temperature, and slicing on respiration rate of mushrooms. *Journal of Food Science,* **74**, E298-E303.

Isobe, S. (2003). Production technology of biodegradable materials using agricultural wastes. *Farming Japan,* **37**, 21-25.

ITIS, 2006. Integrated Taxonomic Information System.

Jacxsens, L., Devlieghere, F. & Debevere, J. (1999a). Validation of a systemic approach to design equilibrium modified atmosphere packages for fresh-cut produce. *Lebensmittel-Wissenschaft und Technologies,* **32**, 425-432.

Jacxsens, L., Devlieghere, F., Falcato, P. & Debevere, J. (1999b). Behaviour of *Listeria monocytogenes* and *Aeromonas* spp. on fresh-cut produce packaged under equilibrium-modified atmosphere. *Journal of Food Protection,* **62**, 1128-1135.

Jacxsens, L., Devlieghere, F., Rudder, T. & Debevere, J. (2000). Designing equilibrium modified atmosphere packages for fresh-cut vegetables subjected to changes in temperature. *Lebensmittel-Wissenschaft und Technologies,* **33**, 178-187.

Jacxsens, L., Devlieghere, F., Van der Steen, C. & Debevere, J. (2001). Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology,* **71**, 197-210.

James, J. (2006). Overview of microbial hazards in fresh fruit and vegetables operations. In: *Microbial hazard identification in fresh fruit and vegetables,* J. James (ed.). Pp. 1-32. John Wiley & Sons, Inc.

Jamie, P. & Saltveit, M. E. (2002). Postharvest changes in broccoli and lettuce during storage in argon, helium, and nitrogen atmospheres containing 2% oxygen. *Postharvest Biology and Technology,* **26**, 113-118.

Janda, J.M. & Duffey, P.S. (1988). Mesophilic aeromonads in human disease: current taxonomy, laboratory identification and infectious disease spectrum. *Reviews of Infectious Diseases,* **10**, 980-997.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005). Modern food microbiology, 7th ed. USA: Springer.

Jeyamkondan, S., Jayas, D.S., & Holley, R.A. (2001). Microbial growth modelling with artifical neural networks. *International Journal of Food Microbiology,* **64**, 343-354.

Jiménez, A., Fabra, M.J., Talens, P. & Chiralt, A. (2012). Edible and biodegradable starch films: A review. *Food and Bioprocess Technology,* accepted manuscript. DOI: 10.1007/s11947-012-0835-4.

Joseph, C.S., Prashanth, K.V.H., Rastogi, N.K., Indiramma, A.R., Reddy, S.Y. & Raghavarao, K.S.M.S. (2011). Optimum blend of chitosan and poly-(ε caprolactone) for fabrication of films for food packaging applications. *Food Bioprocess Technology*, **4**, 1179-1185.

Joubert, R. (2012). Pomegranate power ahead. *Famer's weekly*.

Kader, A.A. (1986). Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. *Food Technology*, **40**, 99-104.

Kader, A.A., Chordas, A. & Elyatem, S. (1984). Response of pomegranates to ethylene treatment and storage temperature. California Agriculture, July-August, 14-15.

Kader, A.A. & Watkins, C.B. (2000). Modified atmosphere packaging- Toward 2000 and beyond. *HortTechnology*, **10**, 483-486.

Kader, A. A. (2002). Postharvest biology and technology: An overview. In: A. A. Kader (Ed.), *Postharvest technology of horticultural crops* (pp. 39-48). University of California, Agriculture and Natural Resources Publication 3311.

Kalia, A., & Gupta, R.P. (2006). Fruit microbiology. In: *Handbook of fruit and fruit processing* Y. H. Hui, M. P., Cano, W., Gusek, , J. W., Sidhu, N. K., Sinha (eds.). Pp. 3-28. Blackwell publishing.

Kaur, P., Rai, D.R. & Paul, S. (2010). Nonlinear estimation of respiratory dynamics of fresh-cut spinach (*Spinacia oleracea*) based on enzyme kinetics. *Journal of Food Process Engineering,* **34**, DOI: 10.1111/j.1745-4530.2009.00508.x

Kirov, S.M. (2001). *Aeromonas* and *Plesiomonas* species. In *Food microbiology: fundamentals and frontiers* 2nd ed M. P. Doyle, L. R. Beuchat, T. J. Montville (eds.). Pp. 301-328, ASM Press.

Kim, K.W., Min, B.J., Kim, Y.–T., Kimmel, R.M., Cooksey, K. & Park, S. I. (2011). Antimicrobial activity against foodborne pathogens of chitosan biopolymer films of different molecular weights. *LWT-Food Science and Technology,* **44**, 565-569.

Kim, S.R., Rhee, M.S., Kim, B.C. & Kim, K.H. (2007). Modelling the inactivation of *Escherichia coli* O157:H7 and generic *Escherichia coli* by supercritical carbon dioxide. *International Journal of Food Microbiology,* **118**, 52-61.

Kirwan, M.J. & Strawbridge, J.W. (2003). Plastics in food packaging. In: *Food Packaging Technology* R. Coles et al (eds.). Pp.174-240. London, UK: Blackwell Publishing, CRC Press.

Koide, S. & Shi, J. (2007). Microbial and quality evaluation of green peppers stored in biodegradable film packaging. *Food Control,* **18**, 1121-1125.

Koopmans, M. & Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology,* **90**, 23-41.

Köksal, A.I. (1989). Research on the storage of pomegranate (cv. Gök Bahce) under different conditions. *Acta Horticulturae*, **258**, 295-302.

Krumhar, K.C. & Karel, M. (1992). Visual indicator system. US Patent No 5096813. Massachusetts Institute of Technology (in United State).

Labuza, T.P. (1989). Active food packaging technologies. In: *Preservation processes and related techniques*. W.E.L. Spiess & H. Schubert (eds.). Pp. 304-311. Engineering and Food, 2, Elsevier Applied Science, London.

Lange, J. & Wyser, Y. (2003). Recent innovation in barrier technologies for plastic packaging-a Review. *Packaging Technology and Science*, **16**, 149-158.

Lakakul, R., Beaudry, R.M. & Hernandez, R.J. (1999). Modeling respiration of apple slices in modified atmosphere packages. *Journal of Food Science*, **64**, 105-110.

Lee, L., Arul, J., Lencki, R. & Castaigne, F. (1995). A review on modified atmosphere packaging and preservation of fresh fruits and vegetables: physiological basis and practical aspects- part 1. *Packaging Technology Science*, **8**, 315-331.

Levin, G.M. (2006). Pomegranate roads: a Soviet botanist's exile from Eden. In: Baer (ed) pp15-183. Forestville, CA: Floreat Press.

Lammerding, A.M., & McKellar, R.C. (2004). Predictive microbiology in quantitative risk assessment. In: R. C. McKellar & X. Lu (Eds.), *Modelling microbial responses in food.* (pp. 263-301). Boca Raton: CRC Press, Taylor & Francis Group.

Lanciotti, R., Corbo, M.R., Gardini, F., Sinigaglia, M. & Guerzoni, M.E. (1999). Effect of hexanal on the shelf life of fresh apple slices. *Journal of Agricultural and Food Chemistry,* **47**, 4769-4776.

Larson, A.E. & Johnson, E.A. (1999). Evaluation of botulinal toxin production in packaged fresh-cut cantaloupe and honeydew melons. *Journal of Food Protection,* **62**, 948-952.

Lees, D. (2000). Viruses and bivalve shellfish. *International Journal of Food Microbiology,* **59**, 81-116.

Lee, D.S., Haggar, P.E., Lee, J. & Yam, K.L. (1991). Model for fresh produce respiration in modified atmospheres based on principles of enzyme kinetics. *Journal of Food Science,* **56**, 1580-1585.

Lee, S.-Y. & Khang, H. (2004). Microbial safety of prickled fruits and vegetables and hurdle technology. *International Journal of Food Safety,* **4**, 21-32.

Leonardi, C., Baille, A. & Guichard, S. (1999). Effect of fruit characteristics and climatic conditions on tomato transpiration in greenhouse. *Journal of Horticultural Science and Biotechnology,* **74**, 748-756.

Li, T. & Zhang, M. (2010). Effects of modified atmosphere package (MAP) with a silicon gum film window and storage temperature on the quality and antioxidant system of stored *Agrocybe chaxingu*. *LWT – Food Science and Technology,* **43**, 1113-1120.

Little, C.L., Monsey, H.A., Nichols, G.L. & de Louvois, J. (1997). The microbiological quality of refrigerated salads and crudities. *Public Health Laboratory Service Microbiology Digest,* **14**, 142-146.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 174-185.

Lund, B. M. & Peck, M. W. (2000). *Clostridium botulinum*. In: *The microbiological safety and quality of food* B. M. Lund, T. C. Baird-Parker, and G. W. Gould (eds.). Pp. 1057-1109. Gaithersburg, MD.

Malhotra, J. & Prasad, D.N. (1999). Role of carbon dioxide in enhancing the microbiological quality of perishable foods; A review. *Microbiology Alims Nutritions*, **17**, 155-168.

Mahajan, P.V., Oliveira, F.A.R., Montanez, J.C. & Frias, J. (2007). Development of user-friendly software for design of modified atmosphere packaging for fresh and fresh-cut produce. *Innovative Food Science and Emerging Technologies*, **8**, 84-92.

Mahajan, P.V., Oliveira, F.A.R. & Macedo, I. (2008a). Effect of temperature and humidity on the transpiration rate of the whole mushrooms. *Journal of Food Engineering,* **84**, 281-288.

Mahajan, P.V., Oliveira, F.A.R., Montanez, J.C. & Iqbal, T. (2008b). Packaging design for fresh produce: an engineering approach. *New Food* **1**, 35-36.

Mahajan, P.V., Rodrigues, F.A.S. & Leflaive, E. (2008c). Analysis of water vapour transmission rate of perforation-mediated modified atmosphere packaging (PM-MAP). *Biosystems Engineering,* **100**, 555-561.

Makino, Y. & Hirata, T. (1997). Modified atmosphere packaging of fresh produce with a biodegradable laminate of chitosan-cellulose and polycaprolactone. *Postharvest Biology and Technology,* **8**, 179-190.

Mangaraj, S., Goswami, T.K. & Mahajan, P.V. (2009). Applications of plastic films for modified atmosphere packaging of fruits and vegetables: A review. *Food Engineering Reviews*, **1**, 133-158.

Marsh, K., & Bugusu, B. (2007). Food packaging-roles, materials, and environmental issues. *Journal of Food Science,* **72**, R39-R55.

Martins, E. A. & Germano, P. M. L. (2008). Microbiological indicators for the assessment of performance in the hazard analysis and critical control points (HACCP) system in meat lasagna production. *Food Control,* **19**, 764-771.

Martínez-Romero, D., Alburquerque, N., Valverde, J.M. *et al.* (2006). Postharvest sweet cherry quality and safety maintenance by aloe vera treatment: a new edible coating. *Postharvest Biology and Technology,* **39**, 93-100.

Mathooko, F.M. (1996). Regulation of respiratory metabolism in fruits and vegetables by carbon dioxide. *Postharvest Biology and Technology,* **9**, 247-264.

Mayuoni-Kirshinbaum, L., Tietel, Z., Porat, R. & Ulrich, D., 2012. Identification of aroma-active compounds in 'wonderful' pomegranate fruit using solvent-assisted flavour

evaporation and headspace solid-phase micro-extraction methods. *European Food Research Technology*, **235**, 277-283.

Mbithi, J.N., Springthorpe, V.S. & Sattar, S.A. (1991). Effect of relative humidity and air temperature on survival of hepatitis A virus on environmental surfaces. *Applied and Environmental Microbiology,* **57**, 1394-1399.

McDonald, K. & Sun, D.-W. (1999). Predictive food microbiology for meat industry: a review. *International Journal of Food Microbiology,* **52**, 1-27.

McMeekin, T.A., Bowman, J., McQuestin, O., Mellefont, L., Ross, T. & Tamplin, M. (2008). The future of predictive microbiology: Strategic research, innovative applications and great expectations. *International Journal of Food Microbiology,* **128**, 2-9.

McKellar, R.C. (1997). A heterogeneous population model for the analysis of bacterial growth kinetics. *International Journal of Food Microbiology,* **36**, 179-186.

McMahon, M.A.S. & Wilson, I.G. (2001). The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables. *International Journal of Food Microbiology,* **70**, 155-162.

Melgarejo, P., Sánchez, A.C., Vázquez-Araújo, L., Hernández, F., José-Martínez, J., Legua P. & Carbonell-Barrachina, A.A., 2011. Volatile composition of pomegranates from 9 Spainish cultivars using headspace solid phase microextraction. *Journal of Food Science*, **76**, S114-S120.

Meng, X., Zhang, M. & Adhikari, B. (2012). Extending shel-life of fresh-cut green peppers using pressurized argon treatment. *Postharvest Biology and Technology,* **71**, 31-20.

Mirdehghan, S.H., Rahemi, M., Castillo, S., Martínez-Romero, D., Serrano, M. & Valero, D. (2007a). Pre-storage application of polyamines by pressure infiltration or immersion improves shelf-life of pomegranate stored at chilling temperature by increasing endogenous polyamine levels. *Postharvest Biology and Technology*, **44**, 26-33.

Mirdehghan, S.H., Rahemi, M., Serrano, M., Guillén, F., Martínez-Romero, D., & Valero, D. (2007b). The application of polyamines by pressure or immersion as a tool to maintain functional properties in stored pomegranate arils. *Journal of Agricultural and Food Chemistry*, **55**, 755-760.

Montero-Calderón, M. & Cerdas-Araya, M.M. (2011). Fruits and vegetables for the fresh-cut processing industry. In: *Advance in fresh-cut fruits and vegetables processing* O. Martín-Belloso, R. Soliva-Fortuny (eds.). Pp. 185-195. Boca Raton: CRC Press, Taylor & Francis Group.

Nambi, S., Nyalamadugu, S., Wentworth, S.M. & Chin, B.A. (2003). Radio frequency identification sensors. In: Proceedings of 7th World Multiconference on Systemic, Cybernetics and informatics, 30 July- 2 August 2003, Dubna, Russia.

Nanda, S., Rao, D.V.S. & Krishnamurthy, S. (2001). Effect of shrink film wrapping and storage temperature on the shelf life and quality of pomegranate fruits cv. Ganesh. *Postharvest Biology and Technology*, **22**, 61-69.

Nasar-Abbas, S.M., Plummer, J.A., Siddique, K.H.M. & White, P. F. (2008). Nitrogen retards and oxygen accelerates colour darkening in faba bean (*Vicia faba* L.) during storage. *Postharvest Biology and Technology,* **47**, 113-118.

National Agricultural Marketing Council (NAMC) (2011). International trade probe No. 31, January 2011.

Nguyen-the, C. & Carlin, F. (1994). The microbiology of minimally processed fresh fruit and vegetables. *Critical Reviews in Food Science and Nutrition,* **34**, 371-401.

Neethirajan, S., Jaya, D.S. & Sadistap, S. (2009). Carbon dioxide sensors for the agric-food industry - A review. *Food and Bioprocess Technology*, **2**, 115-121.

Nerya, O., Gizis, A., Tsvilling, A., Gemarasni, D., Sharabi-Nov, A. & Ben-Arie, R. (2006). Controlled atmosphere storage of pomegranate. *Acta Horticulturae*, **712**, 655-660.

Notermans, S., Gallhoff, G., Zwietering, M.H. & Mead, G. C. (1995). The HACCP concept: specification of criteria using quantitative risk assessment. *Food Microbiology,* **12**, 81-90.

Odriozola-Serrano, I., Soliva-Fortuny, R. & Martín-Belloso, O. (2008). Effect of minimal processing on bioactive compounds and colour attributes of fresh-cut tomatoes. *LWT-Food Science and Technology,* **41**, 217-226.

Oliveira, M., Usall, J., Solsona, C., Alegre, I., Viñas, I. & Abadias, M. (2010). Effect of packaging type and storage temperature on the growth of foodborne pathogens on shredded 'Romaine' lettuce. *Food Microbiology,* **27**, 375-380.

Oms-Oliü, G., Soliva-Fortuny, R. & Martín-Belloso, O. (2008). Modelling changes of headscape gas concentration to describe the respiration of fresh-cut melon under low or superatmospheric oxygen atmospheres. *Journal of Food Engineering,* **85**, 401-409.

Ongeng, D., Ryckeboer, J., Vermeulen, A. & Devlieghere, F. (2007). The effect of micro-architectural structure of cabbage substratum and or background bacterial flora on the growth of *Listeria monocytogenes*. *International Journal of Food Microbiology,* **119**, 291-299.

Onur, C., Pekmezci, M., Tibet, H., Erkan, M., Gözlekci, S. & Tandogan, P. (1992). A research on cold storage of pomegranate cv. Hicaz. 1st National Horticultural Congress of Turkey, 13-16 Oct. 1992, Izmir, Turkey, pp 449-452.

Opara, L.U, Al-Ani, M.R. & Al-Shuaibi, Y.S. (2009). Physico-chemical properties, vitamin C content, and antimicrobial properties of pomegranate fruit (*Punica granatum* L.). *Food and Bioprocess Technology*, **2**, 315-321.

Oshita, S., Seo, Y. & Kawagoe, Y. (2000). Relaxation time of protons in intracellular water of broccoli. In: Proceedings of CIGR international symposium. Kyoto, Japan, pp. 77-82.

Padule, D.N. & Keskar, B.G. (1988). Studies on postharvest treatments for increasing the shelf life of pomegranate fruits. *Maharashtra Journal of Horticulture*, **4**, 73-76.

Palma, A., Schirra, M., D' Aquino, S., La Malfa, S. & Continella, G. (2009). Chemical properties changes in pomegranate seeds packaged in polypropylene trays. *Acta Horticulturae*, **818**, 1-4.

Paul, D.R. & Clarke, R. (2002). Modelling of modified atmosphere packaging based on designs with a membrane and perforations. *Journal of Membrane Science*, **208**, 269-283.

Page, B., Edwards, M. & May, N. (2003). Metal cans. In: *Food packaging technology* R. Coles, D. McDowell, M. J. Kirwan, (eds.). Pp. 121-151. London, UK: Blackwell Publishing, CRC Press.

Park, H.J. (1999). Development of advanced edible coatings for fruits. *Trends in Food Science and Technology,* **10**, 254-260.

Pálmai, M. & Buchanan, R.L. (2002). The effect of *Lactococcus lactis* on the growth characteristic of *Listeria monocytogenes* in alfafa sprout broth. *Acta Alimentaria,* **31**, 379-392.

Pedroso, D.M.N., Iaria, S.T., Cequeiracampos, M.L., Heidtmann, S., Rall, V.L.M., Pimenta, F. & Saad, S.M.I. (1997). Virulence factors in motile *Aeromonas* spp. isolated from vegetables. *Reviews Microbiology,* **28**, 49-54.

Peleg, M. (1997). Modeling microbial populations with the original and modified versions of the continuous and discrete logistic equations. *Critical Reviews in Food Science and Nutrition,* **37**, 471-490.

Peppelenbos, H.W. & Leven, J. (1996). Evaluation of four types of inhibition for modeling the influence of carbon dioxide on oxygen consumption fruits and vegetables. *Postharvest Biology and Technology,* **7**, 27-40.

Perishable Products Export Control Board (PPECB), 2012. Annual report briefing, 2011/ 2012.

Petran, R.L., Sperber, W.H. & Davis, A. R. (1995). *Clostridium botulinum* toxin formation in romaine lettuce and shredded cabbage: effect of storage and packaging conditions. *Journal of Food Protection,* **58**, 624-627.

Phadtare, S. (2004). Recent development in bacterial cold-shock response. *Current Issues on Molecular Biology,* **6**, 125-136.

Phillips, C.A. (1996). Review: Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science and Technology*, **31**, 463-479.

Porat, R., Weiss, B., Fuchs, Y., Sandman, A. & Ward, G. (2009). Modified atmosphere/ modified humidity packaging for preserving pomegranate fruit during prolonged storage and transport. *Acta Horticulturae* **818**, 1-4.

Pyla, R., Kim, T.J., Silva, J.L. & Jung, Y. S. (2010). Enhanced antimicrobial activity of starch-based film impregnated with thermally processed tannic acid, a strong antioxidant. *International Journal of Food Microbiology,* **137**, 154-160.

Rai, D.R. & Singh, R. (2011). Package headspace dynamics for baby corn under modified atmosphere packaging with macroperforations. *Journal of Food Engineering,* DOI: 10.1111/j.1745-4530.2010.00580.x

Rakotonirainy, A.M., Wang, Q. & Padua, G.W. (2001). Evaluation of zein films as modified atmosphere packaging for fresh broccoli. *Journal of Food Science,* **66**, 1108-1111.

Ratkowsky, D.A., Olley, J. & Ross, T. (2005). Unifying temperature effects on the growth rate of bacteria and stability of globular proteins. *Journal of Theoretical Biology,* **233**, 351-362.

Ravindra, M.R. & Goswami, T.K. (2008). Modelling the respiration rate of green mature mango under aerobic conditions. *Biosystems Engineering,* **99**, 239-248.

Rocculi, P., Del Nobile, M.A., Romani, S., Baiano, A. & Dalla Rosa, M. (2006). Use of simple mathematical model to evaluate dipping and MAP effects on aerobic respiration of minimally processed apples. *Journal of Food Engineering,* **76**, 334-340.

Rojas-Graü, M.A., Oms-Oliu, G., Soliva-Fortuny, R. & Martín-Belloso, O. (2009). The use of packaging techniques to maintain freshness in fresh-cut fruits and vegetables: a review. *International Journal of Food Science and Technology, 44*, 875-889.

Ross, T. & McMeekin, T. A. (1994). Predictive microbiology. *International Journal of Food Microbiology,* **23**, 241-264.

Rosslenbroich, H.J. & Stuebler, D. (2000). *Botrytis cinerea*-history of chemical control and novel fungicides for its management. *Crop Protection*, **19**, 557-561.

Roy, S.K. & Wasker, D.P. (1997). Pomegranate. In: *Postharvest physiology and storage of tropical and subtropical fruits,* Mitra S (ed.). Pp 365-374. UK: CAB International.

Ruffine, L., Donval, J.P., Charlou, J.L., Cremière, A. & Zehnder, B. H. (2010). Experimental study of gas hydrate formation and destabilisation using a novel high-pressure apparatus. *Marine and Petroleum Geology,* **27**, 1157-1165.

Ryall, A.L. & Pentzer, W.T. (1974). Handling, transportation and storage of fruits and vegetables. Vol. 2 Fruits and Tree Nuts. Connecticut: AVI Publishing, Westport.

Saltveit, M.E. (2003). Is it possible to find an optimal controlled atmosphere? Postharvest *Biology and Technology,* **27**, 3-13.

Salunke, D.K. & Desai, B.B. (1986). Low temperature storage of pomegranate fruits. In: *Postharvest Biotechnology of fruits*, vol. 2, pp 65-67. Boca Raton, FL: CRC Press.

Samapundo, S., Devlieghere, F., De Meulenaer, B., Geeraerd, A.H., Van Impe, J.F. & Debevere, J. M. (2005). Predictive modeling of the individual and combined effect of water activity and temperature on the radial growth of *Fusarium verticilliodes* and *F. proliferatum* on corn. *International Journal of Food Microbiology,* **105**, 35-52.

Sandhya, (2010). Modified atmosphere packaging of fresh produce: Current status and future needs. *Food Science and Technology*, **43**, 381-392.

Sastry, S.K. & Buffington, D.E. (1982). Transpiration rates of stored perishable commodities: A mathematical model and experiment on tomatoes. *ASHRAE Transactions,* **88**, 159-184.

Sattar, S.A., Tetro, J., Bidawid, S. & Farber, J. (2000). Foodborne spread of hepatitis A: Recent studies on viral survival, transfer and inactivation. *Canadian Journal of Infectious Diseases,* **11**, 159-163.

Sağirli, F., Tağı, Ş., Özkan, M. & Yemiş, O. (2008). Chemical and microbial stability of high moisture dried apricots during storage. *Journal of the Science of Food and Agriculture,* **88**, 858-869.

Selman, J. D. (1995). Time-temperature indicators. In: *Active food packaging* M.L. Rooney (ed.). Pp. 215-237. NY: Blackie Academic & Professional.

Sepúlveda, E., Galletti, L., Sáenz, C. & Tapia, M. (2000) Minimal processing of pomegranate var. Wonderful. *CIHEAM-Opitions Mediterraneennes*, **42**, 237-242.

Shakeri, M., Shahidi, F., Beiraghi-Toosi, S. & Bahrami, A. (2011). Antimicrobial activity of *Zatatria multiflora* Boiss. Essential oil incorporated with whey protein based films on pathogenic and probiotic bacteria. *International Journal of Food Science and Technology,* **46**, 549-554.

Sharma, M., Lakshman, S., Ferguson, S., Ingram, D.T., Luo, Y. & Patel, J. (2011). Effect of modified atmosphere packaging on the persistence and expression of virulence factors of

Escherichia coli O157:H7 on shredded iceberg lettuce. *Journal of Food Protection,* **74**, 718-726.

Singh, A., Dhaduk, B.K. & Ahlawat, T. (2009). Storage of jasmine (*Jasminum sambac*) in passive MAP. In: *Proceedings of 9th International Symposium on Postharvest Quality of Ornamental Plants,* C. -O. Ottosen et al. (eds.), *Acta Horticulturae,* **847**, 321-326.

Singh, R.P. (2000). Scientific principles of shelf-life evaluation. In: *Shelf-life evaluation of food*, D. Man & A. Jones (eds.). Pp. 3-22. Gaithersburg, Md: Aspen Publishers.

Singh, R.P. & Wells, J.H. (1985). Use of time-temperature indicators to monitor quality of frozen hamburger. *Food Technology*, **39**(12), 42-50.

Siracusa, V., Rocculi, P., Romani, S. & Rosa, M.D. (2008). Biodegradable polymers for food packaging : a review. *Trends in Food Science and Technology*, **19**, 634-643.

Sivakumar, D. & Korsten, L. (2006). Influence of modified atmosphere packaging and postharvest treatments on quality retention of litchi cv. Mauritius. *Postharvest Biology* and *Technology*, **41**, 135-142.

Sivakumar, D., Arrebola, E. & Korsten, L. (2008). Postharvest decay control and quality retention in litchi (cv. McLean's red) by combined application of modified atmosphere packaging and antimicrobial agents. *Crop Protection*, **27**, 1208-1214.

Sivertsvik, M., Jeksrud, W.K. & Rosnes, J.T. (2002). A review of modified atmosphere packaging of fish and fishery products – significance of microbial growth, activities and safety. *International Journal of Food Science and Technology,* **37**, 107-127.

Sleator, R.D. & Hill, C. (2001). Bacterial osmoadaptation: The role of osmolytes in bacterial stress and virulence. *FEMS Microbiology Reviews,* **26**, 49-71.

Smiddy, M., Fitzgerald, M., Kerry, J.P., Papkovsky, D.B., O'Sullivan, C.K. & Guilbault, G.G. (2002). Use of oxygen sensors to non-destructively measure the oxygen content in modified atmosphere and vacuum packed beef: impact of oxygen content on lipid oxidation. *Meat Science*, **61**, 285-290.

Smith, R. (2005). Biodegradable polymers for industrial application. Pp. 531. Boca Raton, USA: CRC Press.

Smolander, M., Alakomi, H.-L., Ritvanen, T., Vainionpaa, J. & Ahvenainen, R. (2004). Monitoring of the quality of modified atmosphere packaged broiler chicken cuts stored in different temperature conditions: Time-temperature indicators as quality-indicating tools. *Food Control*, **15**, 217-229.

Sneller, J.A. (1986). Smart films give big lift to controlled atmosphere packaging. *Paper Film Converter*, **12**, 58-59.

Sonawane, C.S., Utikar, P.G. & Shinde, P.A. (1986). Postharvest fungal flora of pomegranate. *Journal of the Agricultural University, Maharashtra*, **11**, 107-110.

Soliva-Fortuny, R.C., Elez-Martínez, P. & Martín-Belloso, O. (2004). Microbiological and biochemical stability of fresh-cut apples preserved by modified atmosphere packaging. *Innovative Food Science and Emerging Technologies,* **5**, 215-224.

Song, Y., Vorsa, N. & Yam, K.L. (2002). Modeling respiration-transpiration in a modified atmosphere packaging system containing blueberry. *Journal of Food Engineering,* **53**, 103-109.

Speranza, B., Bevilacqua, A., Conte, A., Del Nobile, M.A., Sinigaglia, M. & Corbo, M. R. (2012). Use of desirability approach to predict the inhibition of Pseudomonas fluorescens, Shewanella putrefaciens and Photobacterium phosphoreum in fish fillets through natural antimicrobials and modified atmosphere packaging. *Food and Bioprocess Technology*, accepted manuscript. DOI: 10.1007/s11947-012-0889-3.

Srinivasa, P.C., Baskaran, R., Ramesh, M.N., Prashanth, K.V.H. & Tharanthan, R.N. (2002). Storage studies of mango packed using biodegradable chitosan film. *European Food Research and Technology,* **215**, 504-508.

Suparlan, & Itoh, K. (2003). Combined effects of Hot water treatment (HWT) and modified atmosphere packaging (MAP) on quality of tomatoes. *Packaging Technology and Science,* **16**, 171-178.

Szabo, E., Scurrah, K. & Burrows, J. (2000). Survey for psychrotrophic bacterial pathogens in minimally processed lettuce. *Letter of Applied Microbiology,* **30**, 456-460.

Summers, L. (1992). Intelligent packaging. Centre for exploitation of science and technology, London.

Taoukis, P. S., El Meskine, A. & Labuza, T.P. (1988). Moisture transfer and shelf life of packaged foods. In: *Food and packaging interactions.* J. H. Hotchkiss (ed.), *American Chemical Society, Symposium Series* Vol. 365, DOI: 10.1021/6k-1988-0365.ch019.

Taoukis, P.S., Fu, B. & Labuza, T.P. (1991). Time-temperature indicators. *Food Technology*, **45**, 70-82.

Taoukis, P.S. & Labuza, T.P. (2003). Time-temperature indicators (TTIs). In: *Novel food packaging techniques*, R. Ahvenainen (ed.). Pp. 103-126. Cambridge, UK: Woodhead Publishing Limited.

Tapia, M.S., Rojas-Graü, M.A., Carmona, A., Rodriguez, F.J., Soliva-Fortuny, R. & Martín-Belloso, O. (2008). Use of alginate and gellan-based coatings for improving barrier, texture and nutritional properties of fresh-cut papaya. *Food Hydrocolloids,* **22**, 1493-1503.

Távora, L.N., Raghavan, G.S.V. & Orsat, V. (2004). Storage of cranberries in plastic packaging. *Journal of Food Technology,* **2**, 28-34.

Tharanathan, R.N. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in Food Science and Technology,* **14**, 71-78.

Tijskens, L.M.M., Konopacki, P. & Simcic, M. (2003). Biological variance, burden or benefit? *Postharvest Biology and Technology,* **27**, 15-25.

Torrieri, E., Cavella, S. & Masi, P. (2009). Modelling respiration rate of Annurca apple for development of modified atmosphere packaging. *International Journal of Food Science and Technology,* **44**, 890-899.

Torrieri, E., Perone, N., Cavella, C. & Masi, P. (2010). Modelling the respiration rate of minimally processed broccoli (*Brassica rapa* var. *sylvestris*) for modified atmosphere package design. *International Journal of Food Science and Technology,* **45**, 2186-2193.

Treglazova, N.V. & Fataliev, A.T. (1989). The effect of treatment with calcium chloride on pomegranate fruit storage. *Sadovodstva i Vinogradarstvo*, **9**, 25-27.

Türe, H., Eroğlu, E., Soyer, F. & Özen, B. (2008). Antifungal activity of biopolymers containing natamycin and rosemary extract against *Aspergillus niger* and *Penicillium roquefortii*. *International Journal of Food Science and Technology,* **43**, 2026-2032.

Türe, H., Eroğlu, E., Özen, B. & Soyer, F. (2011). Effect of biopolymer containing natamycin against *Aspergillus niger* and *Penicillium roquefortii* on fresh kashar cheese. *International Journal of Food Science and Technology,* **46**, 154-160.

Uchino, T., Nei, D., Wenzhong, H. & Sorour, H. (2004). Development of a mathematical model for dependence of respiration rate of fresh produce on temperature and time. *Postharvest Biology and Technology,* **34**, 285-293.

U.S. Environmental Protection Agency (EPA), (2005). Fludioxonil, pesticide tolerance, vol 70(95). Available at: www.epa.gov/fedrgstr/EPA-PEST/2005/May/Day-18/p9798.htm. Accessed 20 July 2006Valdramidis, V.P., Geeraerd, A.H., Gaze, J.E., Kondjoyan, A., Boyd, A.R., Shaw, H.L. & Van Impe, J. F. (2006). Quantitative description of *Listeria monocytogenes* inactivation kinetics with temperature and water activity as the influencing factors; model prediction and methodological validation on dynamic data. *Journal of Food Engineering,* **76**, 79-88.

Varnam, A.H., & Evan, M.G. (1996). Foodborne pathogens. Lodon: Manson Publishing.

Vescovo, M., Scolari, G., Orsi, C., Sinigaglia, M. & Torriani, S. (1997). Combined effect of *Lactobacillus casei* inoculums, modified atmosphere packaging and storage temperature in

controlling *Aeromonas hydrophila* in ready-to-use vegetables. *International Journal of Food Science and Technology,* **32**, 411-419.

Wang, Z.-W., Duan, H.-W. & Hu, C.-Y. (2009). Modelling the respiration rate of guava (*Psidium guajava* L.) fruit using enzyme kinetics, chemical kinetics and artificial neural network. *European Food Research and Technology,* **229**, 495-503.

Wijtzes, T., McClure, P.J., Zwietering, M.H. & Roberts, T. A. (1993). Modelling bacterial growth of Listeria monocytogenes as a function of water activity, pH and temperature. *International Journal of Food Microbiology*, **18**, 139-149.

Witthuhn, R.C. Engelbrecht, S., Joubert, E. & Britz, T.J. (2005). Microbial content of commercial South African high-moisture dried fruits. *Journal of Applied Microbiology,* **98**, 722-726.

Willkox, F., Mercier, M., Hendrickx, M. & Tobback, P. (1993). Modelling the influence of temperature and carbon dioxide upon the growth of *Pseudomonas fluorescens*. *Food Microbiology,* **10**, 159-173.

Wu, Z.–S., Zhang, M. & Wang, S. (2012a). Effects of high pressure argon treatments on the quality of fresh-cut apples at cold storage. *Food Control,* **23**, 120-127.

Wu, Z.–S., Zhang, M. & Wang, S. (2012b). Effects of high pressure argon and nitrogen treatments on respiration, browning and antioxidant potential of minimally processed pineapples during shelf life. *Journal of Science of Food and Agriculture*, accepted manuscript. DOI: 10.1002/jsfa.5612.

Vyas, N.L. & Panwar, K.S. (1976). A new post-harvest disease of pomegranate in India. *Current Science*, **45**, 76.

Want, R. (2004). RFID: A key to automating everything. *Scientific America*, **290**, 56-65.

Willocx, F. (1995). Evolution of microbial and visual quality of minimally processed foods: a case study on the product life cycle of cut endive. Ph.D. Thesis, Catholic University of Leuven, Belgium.

Yahia, E.M. (2006). Modified and controlled atmosphere for tropical fruits. *Stewart Postharvest Review*, **5**, 1-10.

Yam, K.L., Takhistov, P.T. & Miltz, J. (2005). Intelligent packaging: Concept and applications. *Journal of Food science*, **70**, R1-10.

Yoshioki, S. (2010). A note on transformation between clathrate hydrate structures I and II. *Journal of Molecular Graphics and Modelling,* **29**, 290-294.

Yu, J., Guo, J. & Zhang, L. (2009). Application of argon MAP technology in packaging of fresh cherries. *Package Engineering,* **30**, 4-7.

Zagory, D. (1995). Principles and practice of modified atmosphere packaging of horticultural commodities. In: *Principles of modified atmosphere and sous-vide product packaging* J. M. Farber, K. L. Dodds (Eds.). Pp. 175-204. Lancaster: Technomic Publishing Co.

Zagory, D. & Kader, A. A. (1988). Modified atmosphere packaging of fresh produce. *Food Technology,* **42**, 70-74, 76-77.

Zarei, M., Azizi, M. & Bashiri-Sadr, Z. (2010). Studies on physic-chemical properties and bioactive compounds of six pomegranate cultivars grown in Iran. *Journal of Food Technology*, **8**, 112-117.

Zhan, Z.G. (2005). Study on preservation of asparagus spears and cucumber by pre-treatment with pressurized xenon. Master of Science Dissertation. Southern Yangtze Univeristy, Wuxi, Jiangsu, China.

Zhang, H. & Mittal, G. (2010). Biodegradable protein-based films from plant resources: A review. *Environmental Progress and Sustainable Energy,* **29**, 203-220.

Zhang, M., Zhan, Z.G., Wang, S.J. & Tang, J.M. (2008). Extending the shelf-life of asparagus spears with a compressed mix of argon and xenon gases. *LWT-Food Science and Technology,* **41**, 686-691.

Chapter 3

Investigating the impact of temperature and relative humidity on the transpiration rate of pomegranate arils

CHAPTER 3

Investigating the impact of temperature and relative humidity on the transpiration rate of pomegranate arils

Summary

This study investigated the transpiration rate (TR) of pomegranate (*Punica granatum* L.) arils under various combinations of temperature (5, 10 and 15°C) and relative humidity (RH) (76, 86 and 96%) during storage. TR ranged from 1.14 to 16.75 g kg^{-1} day^{-1} across the various combinations of RH and temperature studied. RH had the most significant impact on TR ($p < 0.05$). TR increased 6 folds when RH was reduced from 96 to 76%, and correlated well with water vapour pressure deficit (WVPD) (R^2 = 96.1%). Aril weight loss increased at higher WVPD. After 8 days of storage, losses in quality attributes of arils were higher with increasing storage temperature and lowering RH. A mathematical model to predict TR as a function of temperature and RH was developed and successfully validated at 8°C. The target water vapour transmission rate of packaging materials for pomegranate arils was found to be 33 to 68 g m^{-2} day^{-1}.

Introduction

Transpiration is one of the critical physiological processes in fresh produce such as fruit and vegetables. Once the produce is detached from the growing plant, it solely depends on its own water content for transpiration (Mahajan *et al*., 2008). The loss of water from fresh produce result in weight loss and shrivelling leading to unsaleable loss during retail marketing and a direct financial loss. Therefore, appropriate packaging and optimal storage conditions are applied to extend the shelf life of both fresh and fresh-cut produce. Fresh

produce large amount of water vapour and without appropriate packaging, water vapour could build-up inside the package, facilitating the growth of microorganisms (Mahajan *et al*., 2008). TR of produce during postharvest handling and storage is influenced by intrinsic factors such as surface-to-volume ratio, surface injuries, morphological and anatomical characteristics, as well as maturity stage (Sastry & Buffington, 1982), and extrinsic factors such as temperature, RH, air movement, and atmospheric pressure (Chourasia *et al*., 2005).

Pomegranate (*Punica granatum* L.) has been well documented for its potential health benefits such as its high antioxidant, anti-mutagenic, anti-hypertension, anti-inflammatory and anti-atherosclerotic activity against osteoarthritis, prostate cancer, heart disease and HIV-1 (Viuda-Martos *et al*., 2010; Ríos-Romero *et al*., 2012). Despite these health benefits, pomegranate consumption is still limited, due to the difficulties of extracting the arils and the inconvenience due to phenolic metabolites which stain the hands during preparation of seeds. Modified atmosphere packaging (MAP) of ready-to-eat arils presents a more appealing product to consumers and increases the prospect of both production and consumption (Caleb *et al*., 2012). However, MAP could lead to water accumulation on the product surface due to water vapour build-up resulting in produce sliminess and enhancement of microbial growth (Song *et al*., 2001; Song *et al*., 2002). It is well known that package gas composition is influenced by respiration rate of the product and the gas permeability of the packaging film. Current MAP design considers the respiration rate of product as the only important parameter for deciding target oxygen (O_2) properties required to achieve equilibrium, mainly O_2 which is suitable for the selected product. However, besides in-package gas composition it is also important to take into consideration the in-package level of humidity for fresh produce, in order to avoid condensation and/or mould and bacterial development in MAP systems (Song *et al*., 2001). Therefore, development of TR model is necessary for estimating the target barrier properties required from the packaging materials. Various types of mathematical models have been proposed for moisture loss, such as physical dynamic models, based on the diffusivity (Ochoa-Martínez *et al*., 2004). The objective of this study was to quantify the water loss of fresh pomegranate arils and develop mathematical model to relate TR to temperature and RH, and to estimate the packaging needs (water vapour transmission rate) of pomegranate arils to achieve RH of 90% inside the package.

Materials and Methods

Plant material and sample processing

Commercially ripened pomegranate fruits (cv. Acco) were procured from Robertson valley farm, Western Cape (33°48′0″S, 19°53′0″E), South Africa and air-freighted in well ventilated boxes to Process and Chemical Engineering Laboratory, University College Cork, Ireland. On arrival, the fruits were immediately stored at 5 °C until the next day when fruit samples were processed in a disinfected cold room at 5 °C, by carefully removing the husks to avoid damaging the arils. Free surface moisture on the arils were gently removed using sterile paper towels after which, the arils were weighed and equilibrated at 5, 10 and 15 °C for 1 h prior to experiment. Physicochemical properties of the pomegranate cv. 'Acco' studied were characterised at the start of experiment and data is presented in Table 1. 100 g of arils were homogenised and filtered using cheesecloth. Juice pH was measured using a digital pH meter (3310 Jenway, pH Meter, UK). Total soluble solids (TSS) were measured by hand refractometer (Atago, Tokyo, Japan). Titratable acidity (TA) expressed as % citric acid was determined potentiometrically by titration to an end point of pH 8.2 using 2 mL of juice diluted with 10 mL distilled water using an autotitrator (Metrohm 785 DMP, Titrino, Switzerland). Hunter colour parameters (*L** (lightness), *a** (redness and greenness), and *b** (yellowness and bluness)) of arils were measured with a colour meter (Minolta Chroma Meter, CR-300, Japan), after calibration against a white tile background. All analyses were presented as mean ± standard error (S.E.) of 10 replicates.

Table 1 Physicochemical properties of pomegranate fruit 'cv. Acco' studied.

Cultivar	**Fruit size (g)**	**CIELAB colour index**			**TA**	**TSS (°Brix)**	**pH**
		L*	***a****	***b****	**(%w/v) citric acid**		
Acco	242.2 ± 2.9	31.3 ± 2.0	15.4 ± 2.2	10.4 ± 0.5	2.1 ± 0.1	17.9 ± 0.2	3.2 ± 0.1

*Values are mean ± S.E.

Experimental setup

The experimental setup consisted of nine air-tight plastic test containers placed within refrigerating incubators with temperature maintained with ± 0.5 ° C of the set temperature of 5, 10 and 15 °C (Fig. 1). RH within the test containers were independently controlled by using saturated salt solutions of sodium chloride, potassium chloride, potassium nitrate giving 76, 86 and 96% RH respectively (Mahajan *et al.*, 2008). The salt solutions were made from reagents of analytical grade, using demineralised water. The solutions were supersaturated in order to ensure that the constant RH was maintained through the experiment, the temperature and RH within the test containers were monitored continually using battery-powered sensor (HMP50, Campbell Scientific Inc., Utah). This setup was found to maintain a constant RH throughout the experimental run. The solutions were poured into the test containers and supports were mounted above the solution level with large aluminium pans to hold the Petri-dishes containing the samples.

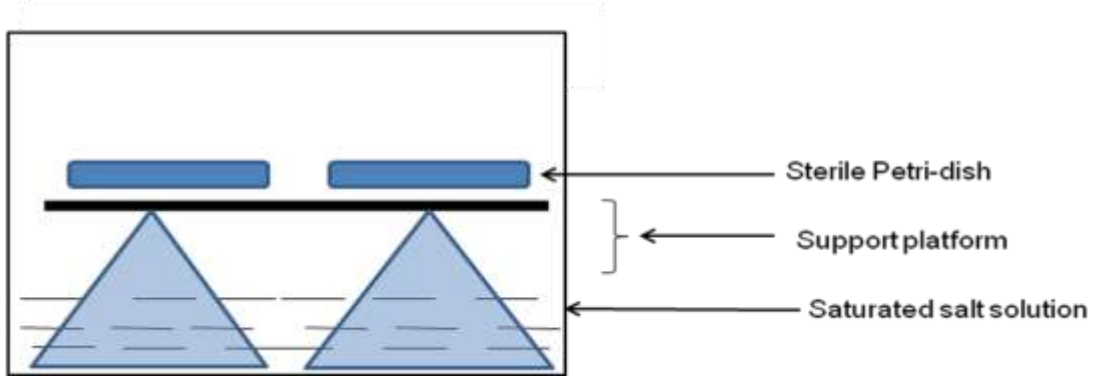


Figure 1 Annotated diagram of the experimental setup for creating RH, with temperature maintained with ± 0.5 ° C of the set temperature of 5, 10 and 15 °C**.**

To evaluate the TR, a weight loss approach was adopted (Leonardi *et al.*, 1999). Approximately 10 g of arils were placed in a petri-dish of known weight and, aril weight loss was measured daily using an electronic balance (Bosch SAE200, GmbH). TR was calculated from the changes in weight over time and expressed as change in aril weight (g) per initial weight of arils (kg) per unit time (day) as shown in the following equation:

$$TR = \frac{M_i - M}{t \times (M_i/1000)} \qquad (1)$$

where, TR is the transpiration rate in g kg^{-1} day^{-1}, M_i is the initial weight of arils in g and M is the weight of arils in g at weighed time t in days.

Two replicates for each storage condition were used for all analyses and the duration as well as measurement interval was the same for all temperature and RH conditions. Experiments were performed according to a full factorial design with two factors temperature and RH at three levels of 5, 10 and 15 °C, and 76, 86 and 96% RH respectively. The total number of runs was 18. An additional set of experiments with all the combinations of 76, 86 and 96% RH was performed at 8 °C in order to validate the mathematical model for TR.

Model building

The flow of water vapour through a fruit is proportional to the difference in water activity (a_w) (RH/100) between the surface of a commodity and the surrounding air. This can be related to Fick's law of diffusion (Ben-Yehoshua, 1987). In this model the RH of fruit internal atmosphere was considered as a first approximation to be 1.0 (or 100% RH). This parameter depends on solute content of the fruit and is slightly less than 1.0 (Mahajan *et al.*, 2008). The term (a_w - a_{wi}) is the difference in concentration of the water vapour across the pomegranate aril membrane for the direction of flow from a_{wi} to a_w. Weight loss due to respiration was considered negligible (*Shirazi & Cameron, 1993*)

$$TR = -K_i \times (a_w - a_{w_i}) \quad (2)$$

where, a_w is the water activity of the container; a_{wi} is the water activity of the arils; K_i is the mass transfer coefficient. At the end of the storage period, the water activity of the arils was measured experimentally (mean a_{wi} = 0.984 ± 0.01) using water activity meter (Aqualab, Pullman, USA) and did not differ among the different storage temperature and RH levels. This showed that a_w of the arils was constant throughout the study period and gave a constant RH gradient across the arils resulting in uniform mass loss. Equation (1) was combined with Equation (2) yielding Equation (3) where TR is transpiration rate

$$TR = \frac{M_i - M}{t \times (M_i/1000)} = K_i \times (a_{w_i} - a_w) \quad (3)$$

Equation (3) was then separated for M which is the weight loss of arils with time as shown in Equation (4).

$$M = M_i - K_i \times \left(a_{w_i} - a_w\right) \times t \times \left(\frac{M_i}{1000}\right) \quad (4)$$

The mass transfer coefficient K_i for each set of experimental conditions was estimated by fitting equation (4) to the experimental data by non-linear regression using Statistica software (Statistica 10.0, Statsoft, USA). Furthermore, temperature term was incorporate in order to estimate the overall effect of temperature on K_i, hence, equation (3) was modified as follows:

$$TR = \frac{M_i - M}{t \times (M_i/1000)} = K_i \times \left(a_{w_i} - a_w\right)(1 - e^{-aT}) \quad (5)$$

Equation (5) was then separated for M in order to fit all the experimental data for weight loss of pomegranate aril (M) with time (t) as shown in Equation (6)

$$M = M_i - \mathrm{K}_i \times \left(a_{w_i} - a_w\right) \times (1 - e^{-aT}) \times t \times \left(\frac{M_i}{1000}\right) \quad (6)$$

Experimental data obtained at all combinations of temperature and RH studied were used to estimate the values of the constants K_i and a. The model equation (6) was fitted by non-linear regression using Statistica software (Statistica 10.0, Statsoft, USA).

Packaging needs

The ability to create a stable pre-determined RH in sealed packages with the potential for reducing decay problems would be valuable for improving the success of MAP systems. This requires proper selection of packaging film material based on water vapour transmission rate (WVTR). Therefore, the TR model developed for arils was used for estimating the target WVTR required to maintain optimal RH inside the package. The package RH is influenced by TR of produce as well as by the water vapour permeability of the packaging film as shown in equation (7). This is a mass balance equation that describes the rate of change of RH or moisture accumulation in the headspace.[7]

$$\frac{dH_i}{dt} = \frac{(\dot{m}_1 - \dot{m}_2)}{W_a} \quad (7)$$

where, $\dot{m}_1$is the rate of water transpiration from produce to headspace (g kg^{-1} day^{-1}); $\dot{m}_2$ is the rate of water permeation from headspace to surrounding (g m^{-2} day^{-1}); W_a is weight of dry air inside the package (g). At equilibrium when the rate of change in humidity is zero,

equation (7) becomes $\dot{m}_1 = \dot{m}_2$. Substituting the TR and permeation rate, equation (7) becomes,

$$K_i \times (a_{w_i} - a_w) \times (1 - e^{-aT}) \times W = WVTR \times A \quad (8)$$

where, *W* is weight of pomegranate arils, kg and *A* is packaging film area, m^2. Re-arranging equation (8),

$$\text{Target WVTR} = \frac{K_i \times (a_{w_i} - a_w) \times (1 - e^{-aT}) \times W}{A} \quad (9)$$

Equation (9) was then used for determining packaging needs for pomegranate arils, which included an estimation of target WVTR required to achieve a stable RH of 90% inside a package containing 200 g of pomegranate arils.

Quality evaluation

Quality evaluation of arils from each of the experimental run was performed on the 8th day of storage. Colour, firmness and decay of arils were determined subjectively using a 1-5 visual rating scale by adapting visual quality descriptors. Firmness was determined based on the resistance of the arils to slightly applied finger pressure and recorded using a 1-5 tactile rating according to method described by Nunes *et al.* (2011) for sliced cucumbers, adapted to pomegranate arils as reported in Table 2. The initial quality evaluation of arils on the visual rating scale for colour and firmness were scored as 5 without any form of decay.

Table 2 Quality scores and descriptors for pomegranate arils

	Scores and description				
Descriptors	**1**	**2**	**3**	**4**	**5**
Colour	Very poor appearance with arils' severely paled-red	Poor appearance, arils is half paled half red	Fair appearance, slightly paled-red arils	Good appearance, completely red	Excellent appearance, completely dark/deep red arils
Firmness	Extremely soft, no resistance to finger pressure	Soft, slightly resistant to finger pressure, with evident loss of turgidity	Minor signs of softness and loss of turgidity slight yield to finger pressure	Firm, slightly loss of turgidity, slight yield to finger pressure	Very firm and turgid, delayed yield to finger pressure
Decay	0 %, no decay	1-25 % of arils decayed	26-50% of arils slight to moderate decay; spots with decay and mycelia growth	51-74 % of arils decayed, moderate to severe decay	76-100 % of arils decayed, severe to extreme decay; the fruit is either partial or completely rotten

Statistical analysis

Pareto analysis was carried out at 95% confidence interval using Statistica software (Statistica 10.0, Statsoft, USA) to assess the effects of RH and temperature, and the interaction between RH and temperature on the TR of pomegranate arils. The experiment was replicated twice for each storage condition.

Result and Discussion

Transpiration rate of pomegranate arils

Weight loss of pomegranate arils during storage at 10°C and 86% RH across the experimental combinations (Fig. 2), constantly decreased with time at all combinations of temperature and RH studied. TR for pomegranate arils as calculated from equation (1) ranged from 1.14 to 16.75 g kg^{-1} day^{-1} across all the combinations of temperature and RH tested. The TR values obtained in this study were lower than those reported by Mahajan *et al.* (2008) for mushroom (TR: 6.5 to 92 g kg^{-1} day^{-1}) at 5, 10 and 15°C with RH 76, 86 and 96%. In contrast, the TR values from this study were higher than that of apple (TR: 0.67 g kg^{-1} day^{-1}) at 10°C, 86% RH (Patel *et al.*, 1988). The high values of TR for mushroom is associated to its lack of a protective skin, therefore, the rate of moisture loss is higher. A higher surface/mass ratio for smaller fruit enhances TR compared to large fruit, this could explain the higher TR in arils compared to apple. TR was found to be higher at 76% RH and 15°C compared to 96% RH and 5°C, (Fig. 3). Furthermore, WVPD was calculated from the temperature and RH conditions used in this study as described by Lichter *et al.* (2011). Weight loss of arils increased with higher WVPD and the effect of WVPD correlated well with TR of arils (R^2 = 96.1%), (Fig. 4). The WVPD in this study ranged from 0.04 to 0.4 kPa and values were highest at low RH of 76%. Our finding corroborates Lichter *et al.* (2011) report. They observed cluster weight-loss increase in grapes with WVPD as well as highest WVPD values at low RH.

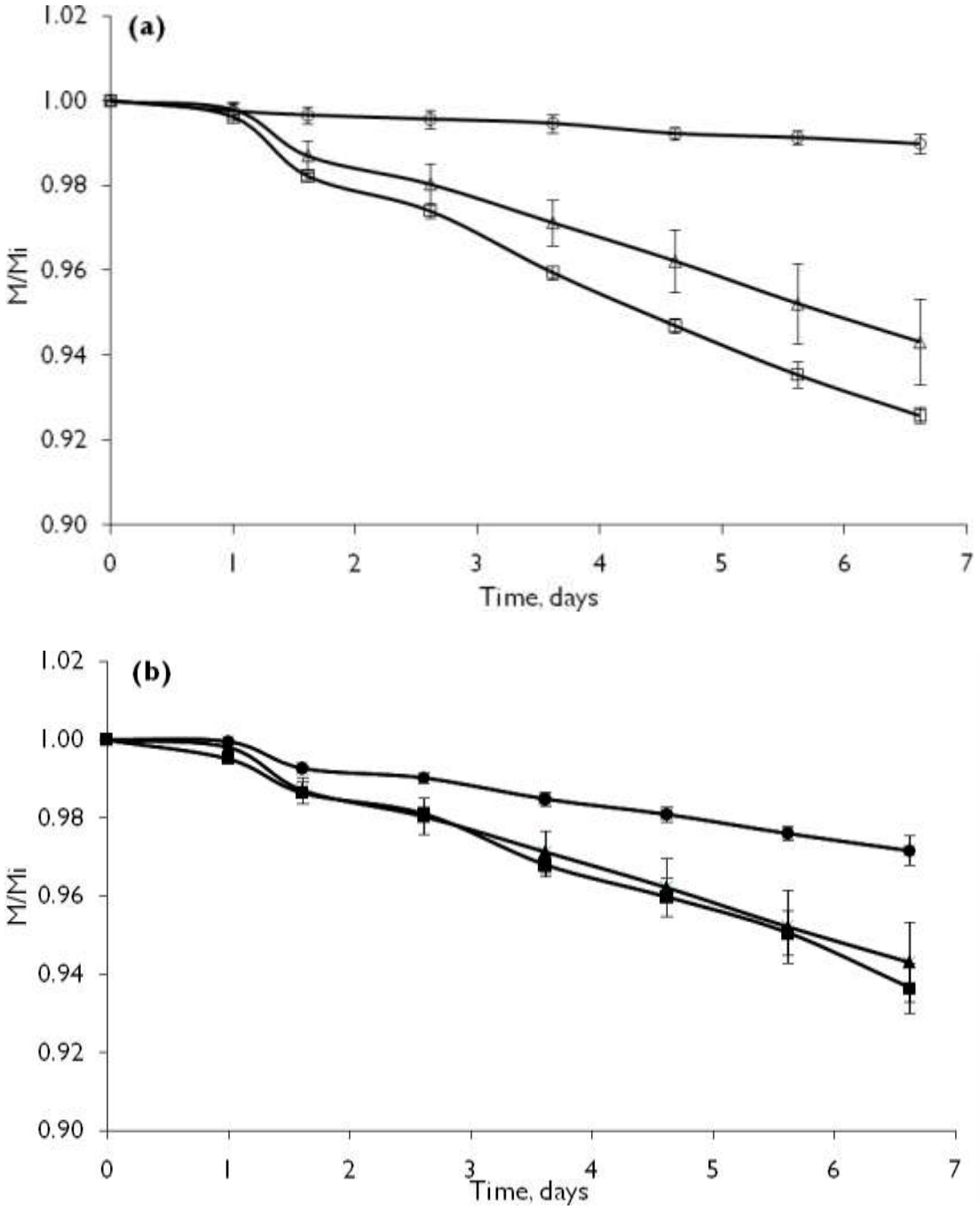


Figure 2 Changes in weight loss of pomegranate arils over time. The values were normalized with respect to the initial weight of pomegranate arils (Mi, g); (a) Effect of RH on weight loss at 10 °C: ○ represents RH at 96 %; Δ represents RH at 86 %; □ represents RH at 76 %. (b) Effect of temperature at 86 % RH: ● represents temperature at 5 °C; ▲ represents temperature at 10 °C; ■ represents temperature at 15 °C. The bars indicate standard error of mean.

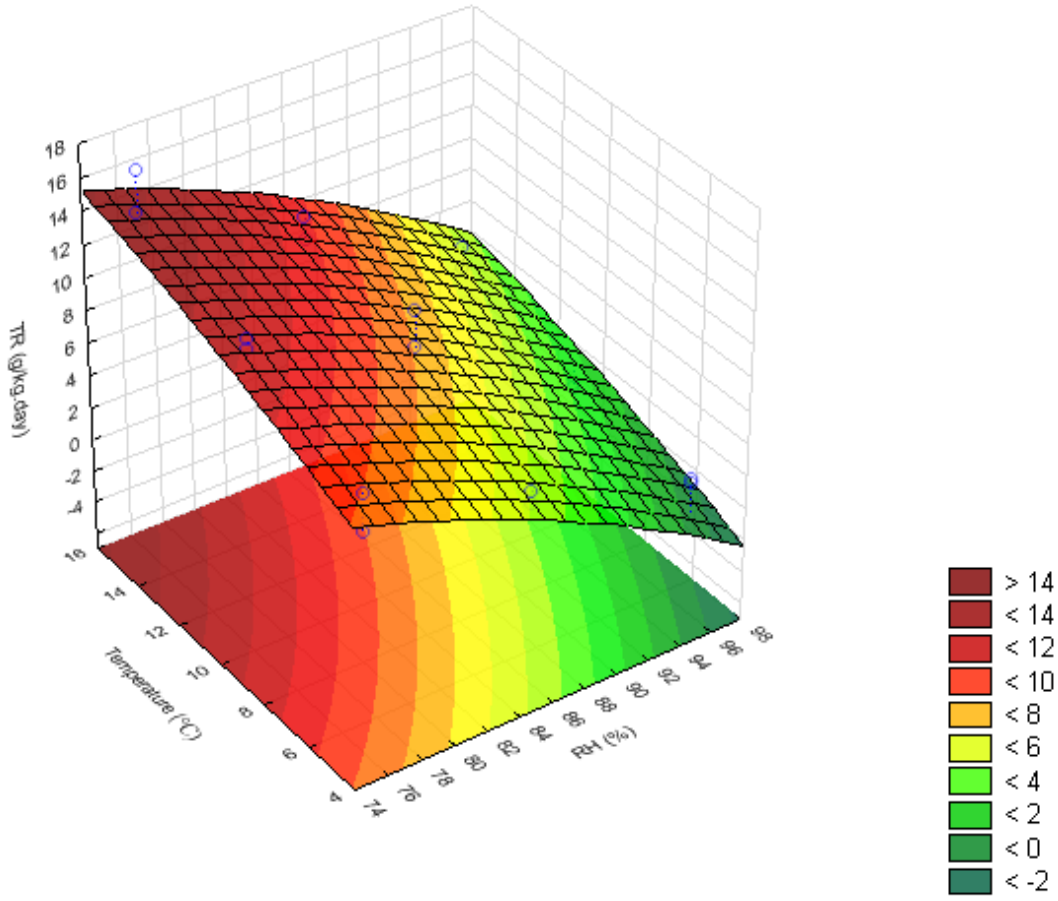


Figure 3 Fitted square surface showing the effect of temperature and RH on transpiration rate (g kg^{-1} day^{-1}).

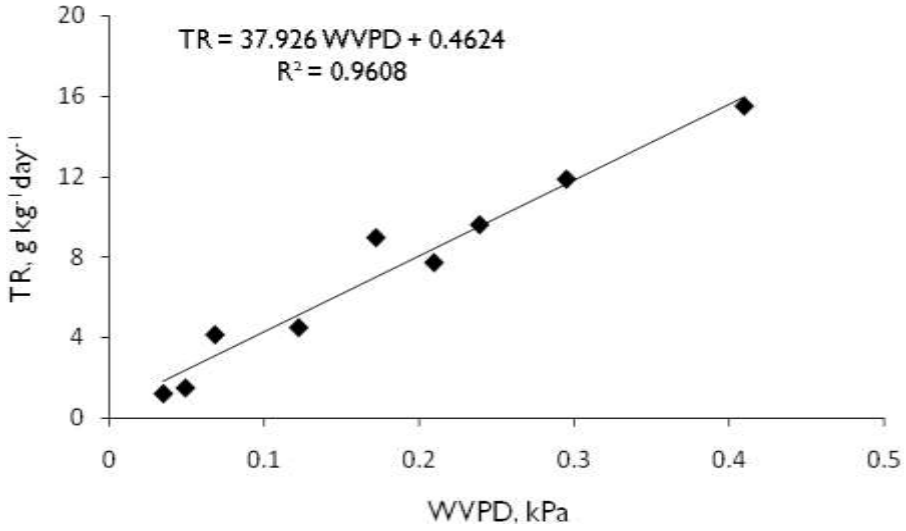


Figure 4 Relationship between transpiration rate (TR) and water vapour pressure deficits for pomegranate arils.

RH was the variable with greatest influence on TR, increasing RH of the container from 76 to 96% decreased TR by 83.5% at 5°C. Temperature as well as the interaction of RH and temperature had significant effect on TR. Ben-Yehoshua (1987) reported that weight loss of 3 to 10% could have an adverse effect on the appearance, salable weight, texture quality of fresh and FC produce. In related report, when RH of storage air for potatoes was decreased below 85% more than 7% water loss was observed (Chourasia *et al*., 2005). Similarly, an increase in water loss during the ripen phase of 'Hass' avocados was observed when the fruits where transferred from RH of 90 to 20%, Burdon *et al*. (2005) reported a 30% increase in moisture loss when RH of stored apples was reduced from 97.5 to 95%. Based on Pareto analysis (Fig. 5), temperature and RH, and their interaction had a significant impact on TR of pomegranate arils ($p < 0.05$).

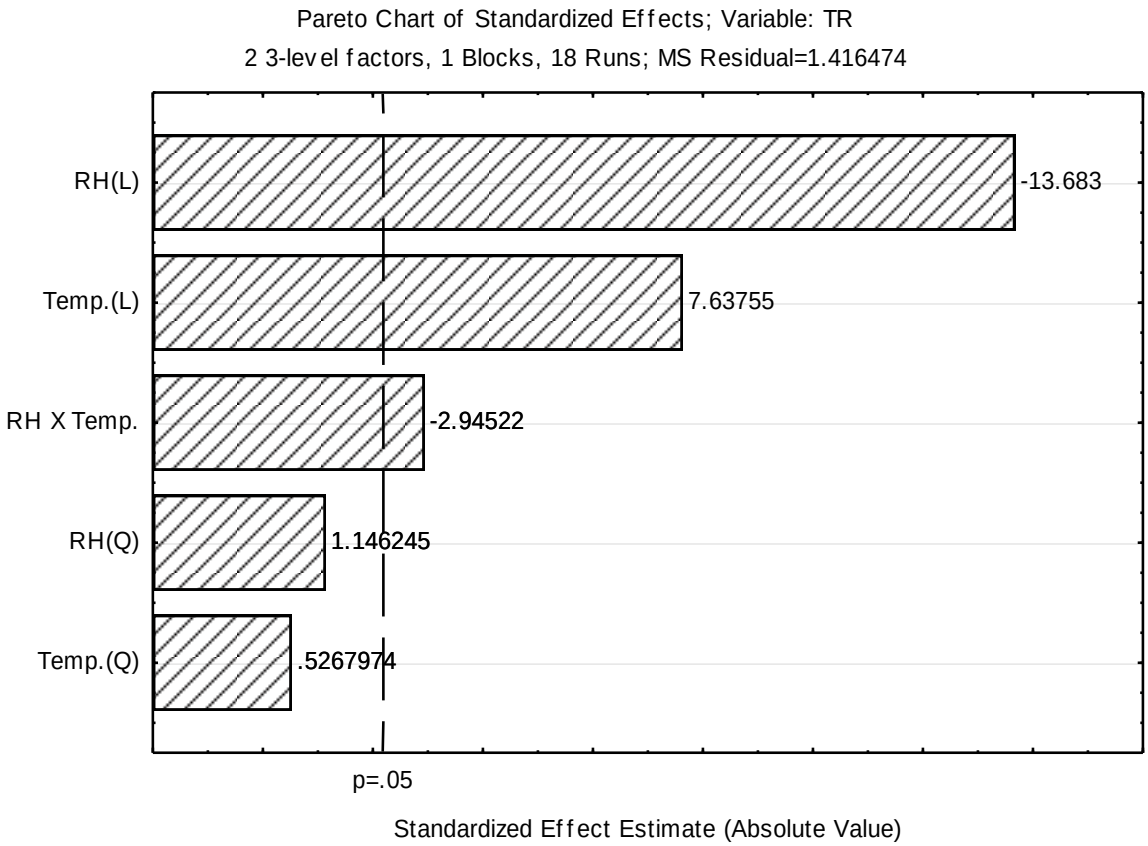


Figure 5 Pareto chart showing the effect of RH and temperature on transpiration rate of pomegranate arils, and the interaction both factors at p = 0.05 indicated with vertical dashed lines. 'L' and 'Q' are linear and quadratic effect of temperature and RH, respectively.

A similar result was reported by Mahajan *et al.* (2008), where the effects of temperature and RH were found to be significant on the TR of whole mushrooms. The effect of RH was more evident than that of temperature in this study and the interactive effects between temperature and RH were also significant. High RH and temperature increases the rate of deterioration, which is detrimental to fresh and fresh-cut fruits. As the RH in most sealed packaged fresh and fresh-cut fruits is close to saturation and fluctuations in temperature during storage and/or on transit may lead to water vapour condensation on both the film surface and produce. Thus supporting the growth of pathogenic and spoilage microorganisms resulting in produce decay (Aharoni *et al.*, 2008).

Quality of pomegranate arils

Texture in terms of firmness and pigment stability (colour) are very important attributes associated with high quality ready-to-eat pomegranate arils and, are directly related to consumer acceptance and commercial value (Gil *et al.*, 1996). After 8 day storage, firmness and colour of arils decreased with increase in storage temperature. The least firm, with the highest percentage of decay being arils stored at 15 °C and 96% RH (Table 3). The effect of increase temperature was more pronounced on visual quality, and this deleterious effect was enhanced at high RH (96%). However, the arils were best kept at 5 °C and optimally with 96% RH. Arils stored at 5 °C and 96% RH had the overall best keeping quality on the 8th day in comparison to those at 76 and 86% RH. Various lengths of shelf life have been reported in literature for pomegranate arils under different storage conditions, but none of these studies reported on storage-RH condition. Palma *et al.* (2009) reported a shelf life of 10 days for pomegranate (cv. Primosole) arils stored in 40 µm thick polypropylene film at 5 °C. They observed no visible symptoms of decay or undesirable defects such as off-flavour and off-taste. López-Rubira *et al.* in their study on minimally fresh processed and UV-C treated pomegranate cv. 'Mollar of Elche' arils. They reported a shelf life of 14 and 10 days for early and late harvested fruit, respectively. An optimal storage RH is essential for successful extension of shelf-life of MAP-packed pomegranate arils to minimise losses during processing. Furthermore, based on these results, the quantification of TR would assist in developing optimal storage conditions and design of MAP for pomegranate arils.

Table 3 Quality attributes (firmness, colour and decay incidence) of pomegranate arils after 8 days under different storage conditions.

Descriptors	5 °C			10 °C			15 °C		
	RH 76%	RH 86%	RH 96%	RH 76%	RH 86%	RH 96%	RH 76%	RH 86%	RH 96%
Colour	5.0a	5.0a	5.0a	4.5a	4.5a	4.5a	2.5b	2.5b	2.0c
Firmness	4.5a	4.5a	5.0a	3.0b	3.0b	2.5b	2.0c	2.0c	1.5b
Decay	1.0a	1.0a	1.0a	1.5b	1.5b	2.5c	2.5c	2.5c	3.5d

*For each column, similar lower case letters are not significantly different at $p < 0.05$.

Model development and validation

The coefficient constant K_i as determined by fitting equation (4) for each set of experimental conditions. K_i was found to increase with temperature (Fig. 6), with R^2 values > 99.5%. The increase in temperature created a higher shear stress or turbulence along the membrane surface of the arils and, this consequently, results in the observed correlation between our experimental temperatures and the coefficient constant K_i. This phenomenon is similar to that reported by Mahajan, Oliveira, & Macedo (2008), for mushrooms and, by Guiné, Henrriques, & Barroca (2012) for the drying of pumpkin. Fitting equation (6) with the experimental data at all combinations, the model described the change in mass adequately as shown with a R^2 value of 94.3%, K_i and a with standard error value of 89.96 (± 6.87) and 0.09 (± 0.01), respectively. A good agreement was obtained between the observed and predicted mass of pomegranate arils (Fig. 7). The values of mass of pomegranate predicted were in close agreement with those experimentally obtained. As expected, both the experimental data and the model prediction showed a decrease in mass with decrease in RH from 96 to 76% as well as with the decrease in temperature from 15 to 5°C. Similarly, a good agreement was obtained between the experimental and predicted TR by the model, (Fig. 8). In order to validate the mathematical model, its predictions of TR at 8 °C with 76, 86 and 96 % RH were compared with experimental data. A good agreement was observed between experimental and predicted TR, at 8 °C. The experimental TR at RH of 76, 86 and 96% were 9.93 (± 0.83), 5.50 (± 0.11) and 1.5 (± 0.59) g kg^{-1} day^{-1} respectively, while the model predicted TR were 10.5, 5.8 and 1.1 g kg^{-1} day^{-1} at the respective RH. Similarly the

predicted TR also decreased with the increase in RH, once more highlighting the influence of RH. These results therefore confirm the predictive ability of the model.

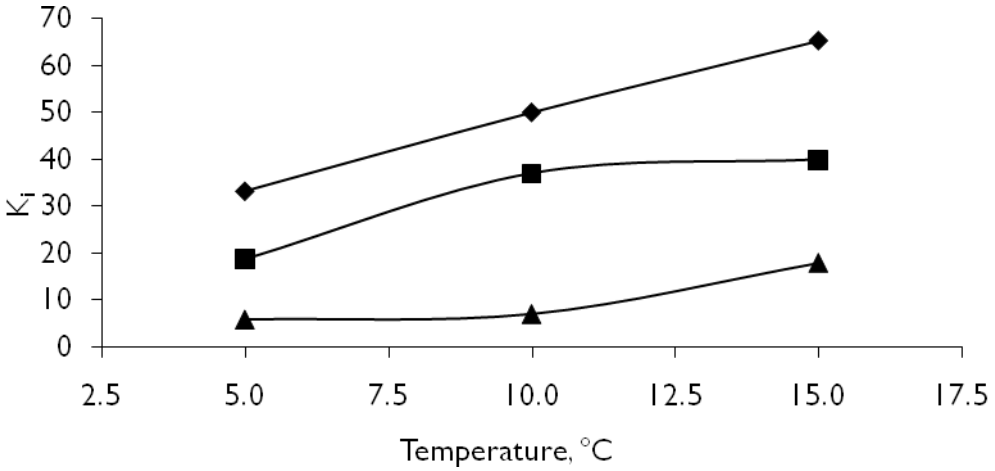


Figure 6 Variation of coefficient K_i of the model Eqn. 4 with temperature and RH. ♦, 76%; ■, 86%; ▲, 96%.

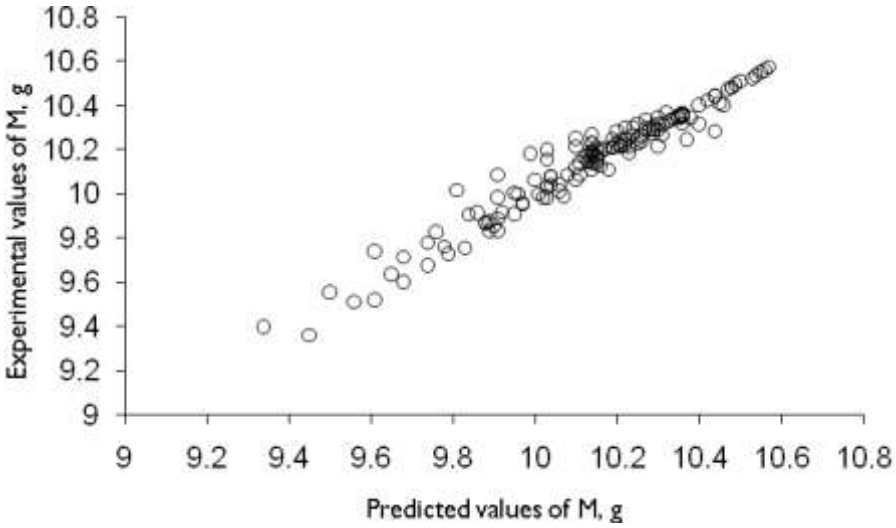


Figure 7 Relationship between experimental and predicted values of pomegranate aril mass.

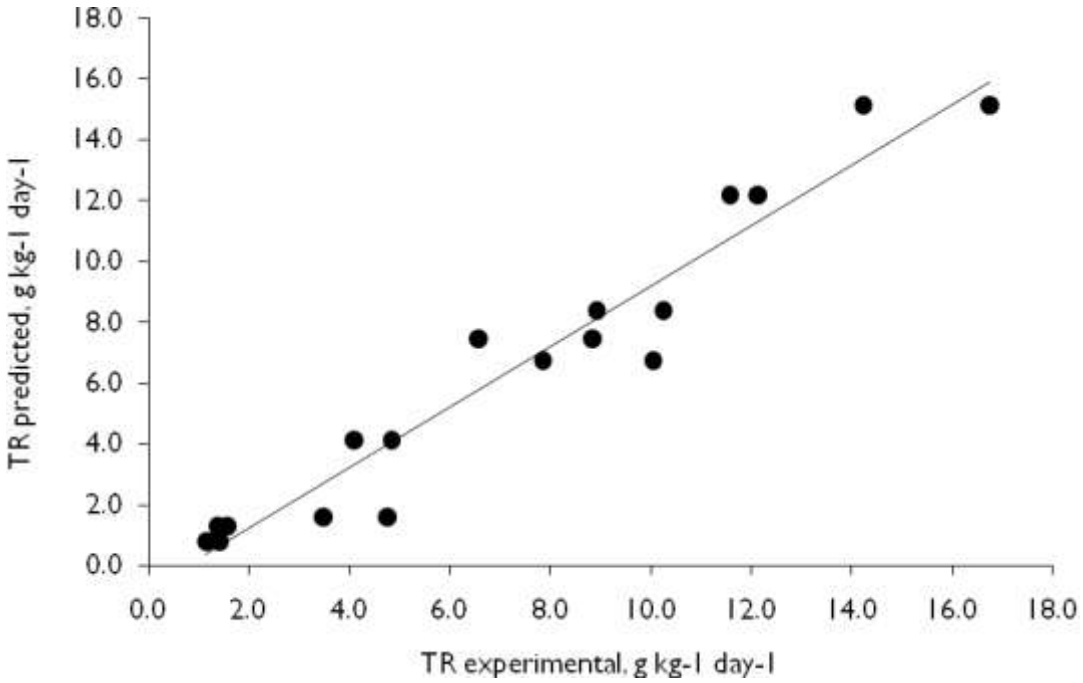


Figure 8 Relationship between experimental and predicted transpiration rate (TR) for pomegranate arils using Eqn. 3.

Packaging needs

One of the ways to reduce weight loss in fresh produce is by appropriate packaging, because this helps to maintain high RH inside the package thereby reducing the effect of WVPD. However, the selection of appropriate packaging material is critical towards achieving the optimum produce quality and to create a stable pre-determined RH in sealed fresh produce packages. The generally recommended level of between 85 to 95% RH for storage of fresh produce represents a compromise to prevent excessive weight loss while providing some control of microbial spoilage (Hardenburg *et al*., 1986). Optimizing the permeability of barriers such as films in order to avoid deterioration in quality of food would be valuable for the success of MAP. From the target WVTR determination using equation (9), it was observed that the target WVTR for maintaining RH of 90% inside package varied from 33 to 68 g m^{-2} day^{-1} for the temperature range of 5 to 15°C, (Fig. 9). Hence, the existing packaging films such as polycarbonate, poly-lactic acid, polyamide and cellulose films would be suitable for packaging pomegranate arils, because they fall within the range of WVTR.

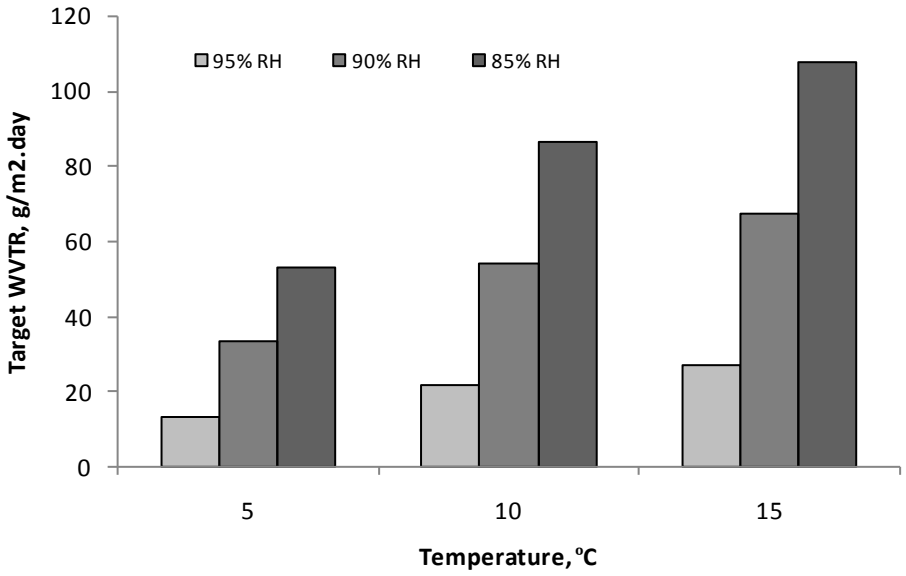


Figure 9 Target WVTR derived with temperature and relative humidity using equation (9).

Conclusions

Weight loss of pomegranate arils increased with higher WVPD, and weight loss was highest at experimental combinations of 15 °C with 76% RH. Additionally, RH was the variable with the greatest influence on TR, and arils were best kept at 5 °C and 96% RH. This highlights the significance of maintaining an optimal produce storage condition. The applicability of the transpiration model developed was verified based on adequate prediction of TR of pomegranate arils during storage at different combinations of temperature and RH. The model would be useful towards understanding the rate of water loss as affected by temperature and RH over time, and thus provides a valuable guide for the storage and designing MAP-system for pomegranate arils. Experimental and model prediction results showed that both RH and temperature had significant effects on TR and quality of stored arils, highlighting the need to maintain optimal storage condition to assure high quality ready-to-eat pomegranate arils with maximum shelf-life.

Nomenclature

a	constant parameter
a_w	water activity of the container (RH/100)
aw_i	water activity of arils
K_i	mass transfer coefficient
M	mass of arils (g)
M_i	initial mass of arils (g)
RH	relative humidity (%)
T	storage temperature (°C)
t	storage time (day)
TR	transpiration rate (g kg^{-1} day^{-1})

References

Aharoni, N., Rodov, V., Fallik, E., Porat, R., Pesis E. & Lurie, S. (2008). Controlling humidity improves efficacy of modified atmosphere packaging of fruits and vegetables. *Acta Horticulturae*, **804**, 121-128.

Ben-Yehoshua, S. (1987). Transpiration, water stress, and gas exchange. In: *Postharvest physiology of vegetables* J. Weichmann (ed.). Pp. 113-170. New York: Marcel Dekker Inc.

Burdon, J., Lallu, N., Haynes, G., Fancis, K., Pidakala, P. & Billing, D. (2005). Mode of action of water loss on fruit quality of 'Hass' avocados. In New Zealand and Australia avocado grower's conference, Tauranga, New Zealand, 20-22 September.

Caleb, O.J, Opara, U.L. & Witthuhn, C. (2012). Modified atmosphere packaging of pomegranate fruit and arils: A review. *Food and Bioprocess Technology*, **5**, 15-30.

Chourasia, M.K., Maji, P., Baskey, A. & Goswami, T.K. (2005). Estimation of moisture loss from the cooling data of potatoes. *Journal of Food Process Engineering*, **28**, 397-416.

Gil, M.I., Artés, F. & Tomás-Barberán, F.A. (1996). Minimal processing and modified atmosphere packaging effects on pigmentation of pomegranate seeds. *Journal of Food Science,* **61**, 161-164.

Guiné, R.P.F., Henrriques, F. & Barroca, M.J. (2012). Mass transfer coefficient for the drying of pumpkin (*Cucurbita moschata*) and dried product quality. *Food and Bioprocess Technology*, **5**, 176-183.

Hardenburg, R.E., Watada, A.E. & Wang, C.Y. (1986). The commercial storage of fruits, vegetables, and florist and nursery stocks. Number 66, USDA-ARS Handbook.

Leonardi, C., Baille, A. & Guichard, S. (1999). Effects of fruit characteristics and climatic conditions on tomato transpiration in greenhouse. *Journal of Horticultural Science and Biotechnology*, **74**, 748-756.

Lichter, A., Kaplunov, T., Zutahy, Y., Daus, A., Alchanatis, V., Ostrovsky, V. & Lurie, S. (2011). Physical and visual properties of grape rachis as affected by water vapour pressure deficit. *Postharvest Biology and Technology*, **59**, 25-33.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 174-185.

Mahajan, P.V., Oliveira, F.A.R., & Macedo, I. (2008). Effect of temperature and humidity on the transpiration rate of the whole mushrooms. *Journal of Food Engineering*, **84**, 281-288.

Nunes, M.C.N., Emond, J-P., Dea, S. & Yagiz, Y. (2011). Distribution centre and retail conditions affect the sensory and compositional quality of bulk and packaged slicing cucumbers. *Postharvest Biology and Technology*, **59**, 280-288.

Ochoa-Martínez, L.A., Gallegos-Infante, J.A., Morales-Castro, J., Medrano-Roldán, H. & Rocha-Guzmán, N.E. (2004). Mathematical model for prediction of moisture content in jalapeño pepper (*Capsicum frutescens*). *Ciencia y Tecnologia Alimentaria*, **4**, 154-157.

Palma, A., Schirra, M., D'Aquino, S., La Malfa, S. & Continella, G. (2009). Chemical properties changes in pomegranate seeds packaged in polypropylene trays. *Acta Horticulturae*, **818**, 323-330.

Patel, P.N., Pai, T.K. & Sastry, S.K. (1988). Effects of temperature, relative humidity and storage time in the transpiration coefficients of selected perishables. *Transactions of the ASHRAE*, **94**, 1563-1587.

Ríos-Romero, E., Tabilo-Munizaga, G., Morales-Castro, J., Reyes, J.E., Pérez-Won, M. & Ochoa-Martinez, L.A. (2012). Effect of high hydrostatic pressure processing on microbial inactivation and physicochemical properties of pomegranate arils. *Ciencia y Tecnologia Alimentaria*, **10**, 152-159.

Sastry, S.K. & Buffington, D.E. (1982). Transpiration rates of stored perishable commodities: A mathematical model and experiments on tomatoes. *Transactions of the ASHRAE*, **88**, 159-184.

Shirazi, A. & Cameron, A.C. (1993). Measuring transpiration of tomato and other detached fruit. *HortScience*, **28**, 1035-1038.

Song, Y., Lee, D.S. & Yam, K.L. (2001). Predicting relative humidity in modified atmosphere packaging system containing blueberry and moisture absorbent. *Journal of Food Processing and Preservation*, **25**, 49-70.

Song, Y., Vorsa, N. & Yam, K.L. (2002). Modelling respiration-transpiration in modified atmosphere packaging system containing blueberry. *Journal of Food Engineering*, **53**, 103-109.

Viuda-Martos, M., Fernández-López, J. & Pérez-Álvarez, J.A. (2010). Pomegranate and its many functional components as related to human health: A Review. *Comprehensive Reviews in Food Science and Food Safety*, **9**, 635-654.

Chapter 4

Evaluating the effect of storage temperature on the respiration rates of pomegranate fruit and arils

CHAPTER 4

EVALUATING THE EFFECT OF STORAGE TEMPERATURE ON THE RESPIRATION RATES OF POMEGRANATE FRUIT AND ARILS

Summary

The design of modified atmosphere packaging (MAP) for fresh and fresh-cut fruits requires adequate prediction of respiration rate (RR). A study was conducted to determine the influences of storage temperature (5, 10 and 15 °C) on RR of whole pomegranate fruit and arils of two pomegranate cultivars (cvs: 'Acco' and 'Herskawitz'). The respiration rates of whole fruit were two to three folds higher, in comparison to those of the fresh arils across all storage temperatures. Temperature had a significant influence on RR ($p < 0.05$). Over the range of storage temperatures studied, RO_2 and RCO_2 increased from 4.53 to 14.67 mL kg^{-1} h^{-1} and 5.67 to 18.53 mL kg^{-1} h^{-1}, respectively, for whole fruit, while RO_2 and RCO_2 of fresh arils ranged from 2.51 to 7.59 mL kg^{-1} h^{-1} and 2.72 to 9.01 mL kg^{-1} h^{-1}, respectively. The cultivar 'Acco' had higher respiration rate (mL kg^{-1} h^{-1} CO_2 production) than 'Herskawitz', especially as the length of storage increased at higher temperature conditions. The respiration quotient (RQ) for the whole fruit of both cultivars ranged from 1.14 to 1.26, while that of fresh arils ranged between 1.06 and 1.62. Experimental evidence showed that the significant influence of higher temperature in increasing RQ of pomegranate arils was more pronounced towards the end of storage period. The effects of temperature on rates of O_2 consumption and CO_2 production of whole fruits and arils was adequately described by an Arrhenius type model. The model was validated for whole fruit stored at 8 °C, and a good agreement was found between experimental and predicted data.

Introduction

Pomegranate is a non-climacteric fruit, with a relatively low respiration rate (RR), which declines during postharvest handling to a steady rate of about 8 mL kg^{-1} h^{-1} for about 3 months when stored between 0-10 °C (Kader *et al*., 1984). Ethylene production rate occurred in trace quantity (less than 0.2 μL kg^{-1} h^{-1}) when 'Wonderful' pomegranate cultivar was stored for 2 weeks at 20 °C (Kader *et al*., 1984). López-Rubira *et al*. (2005) reported an average RR of 14.45 nmol CO_2 kg^{-1} s^{-1} (1.15 mL kg^{-1} h^{-1}) for minimally processed fresh arils (cv. 'Mollar of Elche') stored at 5 °C. Eran *et al*. (2010) reported a minimum RR of 1.5 and 0.52 mL kg^{-1} h^{-1} for RO_2 and RCO_2, respectively, under atmospheric composition of 2% O_2 with 10% CO_2, for (cv. 'Hicaz'). Furthermore, recommended optimum cold storage and controlled atmosphere storage conditions of pomegranate fruit varies depending on cultivar. Artés *et al*. (1998) recommended a controlled atmosphere of (5% O_2+ 0 to 5% CO_2) storage at 5 °C with relative humidity (RH) above 95% for 'Mollar' cultivar to minimize decay, weight loss and chilling injury. In contrast, Kader (1995) recommended a gas composition of 3 to 5% O_2+ 5 to 10% CO_2 at 5 °C for the storage of pomegranates. These studies highlight the importance of understanding the physiological responses of pomegranate cultivars under different storage conditions to assist in developing optimal postharvest handling processes.

Modified atmosphere packaging (MAP) is the dynamic process of altering gaseous composition within a package to extend storage life and optimize fresh produce quality. Desired MAP is achieved through the interaction between two processes: the respiration of the produce and the transfer of gases through the packaging material (Farber *et al*., 2003; Mahajan *et al*., 2007). A quantitative description of RR of fresh produce using mathematical modeling is essential for the design of MAP (Fonseca *et al*., 2002; Mahajan *et al*., 2007). The amount of product, size of packaging material, and perforation density can be adjusted for optimal packaging once RR is known. When fruit respiration does not correlate to the permeability properties of packaging film, increase in the concentration of CO_2 will build up leading to anaerobic respiration and ethanol accumulation inside the fresh produce. This results in the development of off-flavours and decay (Caleb *et al*. 2012). Although there are various studies reported in literature on modeling of RR, of a wide range of fruit such as tomatoes (Charles *et al*., 2003), sliced apple (Lakakul *et al*., 1999), blueberries (Cameron *et al*., 1994), and other fresh produce such as mushroom (Iqbal *et al*., 2009) studies on predictive modeling of the RR of whole pomegranate fruits or fresh arils are lacking.

Therefore, the objectives of this study were: (i) to investigate the effect of temperature on RR of whole pomegranate fruit and fresh arils cvs. 'Acco' and 'Herskawitz' and, (ii) to develop a predictive model relating RR to temperature, thereby, providing valuable information in the design of MAP for pomegranate fruit.

Materials and methods

Produce and sample preparation

Pomegranate (*Punica granatum* L.) fruit sweet-sour cv. 'Acco' and 'Herskawitz' harvested manually during commercial harvest period were obtained from Robertson valley farm, Western Cape (33°48′0″S, 19°53′0″E), South Africa and air-freighted in well ventilated boxes to the Process and Chemical Engineering Laboratory, University College Cork, Ireland. The duration of transportation was about 72 hours. On arrival, the fruits were randomly divided into two groups of 9 fruit each for whole fruit studies and arils studies, respectively, and immediately stored at 5 °C. For arils respiration study, fruit samples were processed in a clean cold room at 5 °C, where the peel (husk) was carefully removed by hand to avoid damaging the arils. Samples of arils (≈ 150 g each sample) and individual fruit were placed inside glass jars of about 428 and 1900 mL, respectively and equilibrated at 5, 10 and 15 °C for at least 1 hr prior to experiment. All experiments were carried out in triplicate.

Experimental setup

The rate of oxygen consumption (RO_2) and carbon dioxide production (RCO_2) of whole fruit and fresh arils were measured using the closed system method (Fonseca *et al*., 2002; Iqbal *et al*., 2009; Torrieri *et al*., 2009). Air-tight glass jars with a lid containing a rubber septum in the middle were used to store samples (one single pomegranate fruit or 150 g of aril) at the different temperatures (5, 10 and 15 °C). To ensure hermetic seal, Vaseline was incorporated into the gap between lid and jar for all the glass jars. The gas composition within the glass jars were monitored over time with an O_2/CO_2 gas analyser with an accuracy of 0.5% (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Gas samples were taken at an hourly time intervals from the jar head space through the rubber septum. An additional set of experiment was performed at 8 °C in order to validate the mathematical

model. RO_2 and RCO_2were determined by fitting experimentally obtained data on y_{O_2} and $y_{CO_2}$with Eqn. 1 and 2, respectively

$$y_{O_2} = y^i_{O_2} - \frac{R_{O_2}.W}{V_f}.(t - t_i) \times 100 \quad (1)$$

$$y_{CO_2} = y^i_{CO_2} + \frac{R_{CO_2}.W}{V_f}.(t - t_i) \times 100 \quad (2)$$

where $y^i_{O_2}$ and y_{O_2} are, respectively, O_2 concentration (%) at the initial time t_i (hours, h) (time zero) and at time t (hr) and $y^i_{CO_2}$ and y_{CO_2} are, respectively, CO_2 concentration (%) at the initial time t_i (h) (or time zero) and at time t (h). RO_2 and RCO_2 are RR in mL kg^{-1} h^{-1} and W is the total weight of the product (kg). V_f is the free volume inside the glass jar (mL), which is the total volume of the glass jar minus volume occupied by the sample. The volume occupied by the fruit was calculated from the mass of fruits over apparent density of pomegranate fruit (0.98 g cm^{-3}). To evaluate the influence of time on respiration rate of pomegranate arils over days, periodic gas samples were taken at hourly interval over a period of 5 hours, after which the glass jars were slightly opened overnight to minimize rapid moisture loss and also to avoid built-up of sub-atmospheric gases. After overnight storage, the jars were closed hermetically, and gas samples were taken. This cycle was repeated over a 5 day storage period and no microbial infestation or decay was observed over this period. The gas samples taken during 5 hour measurement period were used to calculate RO_2 and RCO_2 using Eqn. 1 and 2.

Furthermore, Arrhenius-type equation which describes respiration rate as a function of temperature (Iqbal *et al.*, 2009; Torrieri *et al.*, 2010), for both R_{O2} and R_{CO2} was used in model fitting as presented in Eqn. 3 and 4.

$$R_{O_2} = R_{O_{2,ref}} \times e^{\left[\frac{-E_{a,O_2}}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]} \quad (3)$$

$$R_{CO_2} = R_{CO_{2,ref}} \times e^{\left[\frac{-E_{a,CO_2}}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]} \quad (4)$$

where R_{O2} and R_{CO2} are the respiration rate (mL $kg^{-1}h^{-1}$) at temperature (T, K), $R_{O2,ref}$ and $R_{CO2,ref}$ are respiration rate at reference temperature (T_{ref}, K), R is the universal gas constant (0.008314 kJ $K^{-1}mol^{-1}$), $E_{a,O2}$ and $E_{a,CO2}$ are activation energy (kJ mol^{-1}), T_{ref} is the reference temperature (i.e. average of the temperatures studied = 283 K), and $R_{O2,ref}$, $R_{CO2,ref}$, $E_{a,O2}$ and $E_{a,CO2}$ are model constants. On substituting Eqn. 3 in Eqn. 1 and Eqn. 4 in Eqn. 2 respectively we get:

$$y_{O_2} = y^{i}_{O_2} - R_{O_2,ref} \times e^{\left[\frac{-E_{a,O_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} \frac{W}{V_f}(t - t_i) \times 100 \quad (5)$$

$$y_{CO_2} = y^{i}_{CO_2} + R_{CO_2,ref} \times e^{\left[\frac{-E_{a,CO_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} \frac{W}{V_f}(t - t_i) \times 100 \quad (6)$$

where, $y^{i}_{O_2}$ = 20.6 %, $y^{i}_{CO_2}$ = 0 %, t is the elapsed time (h) during respiration rate measurement, and $R_{O2,\ ref}$, $R_{CO2,\ ref}$, $E_{a,O2}$ and $E_{a,\ CO2}$ were model constants estimated using solver Microsoft Excel (Microsoft Office 2003, USA) and data was further analysed using Statistica software (Statistical 10.0, Statsoft, USA).

Statistical analysis

Pareto analysis (Mahajan *et al*., 2008) was used with two factors (time and temperature) each at three levels of temperatures 5, 10 and 15 °C at 95% confidence interval to assess the effects of time and temperature, and the interaction between time and temperature on the RR data. One-way analysis of variance (ANOVA) at 95% confidence interval was applied to evaluate the effect of time and temperature on respiration rate and respiratory quotient (RQ). All experiments were carried out in triplicate and data were analysed using Statistical software (Statistical 10.0, Statsoft, USA).

Results and Discussion

Modelling the influence of temperature on respiration rate

The model appropriately described the influence of temperature on RR for both whole fruit and fresh arils as shown by the high R^2 (93.0 to 96.8%) obtained for RO_2 and RCO_2. The scatter plot graph in Fig. 1 shows a good relationship between experimental and predicted respiration rate values of pomegranate whole fruit and arils. The distribution of residuals was normal as quantified by Kolgomorov-Smirnov test (d = 0.14) and Lilliefors (p < 0.01) at a significant level of 95% (Fig. 2), this indicates that the trend observed was not biased. Parameter estimates of the models Eqn. 3 and 4 using Eqn. 5 and 6, and relevant statistical data for both RO_2 and RCO_2 of the two cultivars (whole fruit and arils) are presented in Table 1. Furthermore, in order to validate the model developed its predictions of RR for R_{O2}

and R_{CO2} at 8 °C for pomegranate fruits cv. 'Acco' and 'Herskawitz' were compared with the set of experimental data. The experimental RO_2 and RCO_2 of Acco cultivar at 8°C were 7.46 (± 0.71) and 7.81 (± 0.55) mL kg^{-1} h^{-1}, respectively, while the model predicted RO_2 and RCO_2 were 6.63 and 8.83 mL kg^{-1} h^{-1}, respectively. Similarly for the other cultivar Herskawitz the experimental values of RO_2 and RCO_2 at 8 °C were 6.72 (± 0.29) and 8.08 (± 0.74) mL kg^{-1} h^{-1}, respectively, while the model predicted RO_2 and RCO_2 were 6.65 and 9.04 mL kg^{-1} h^{-1}, respectively confirming the predictive ability of the mathematical model.

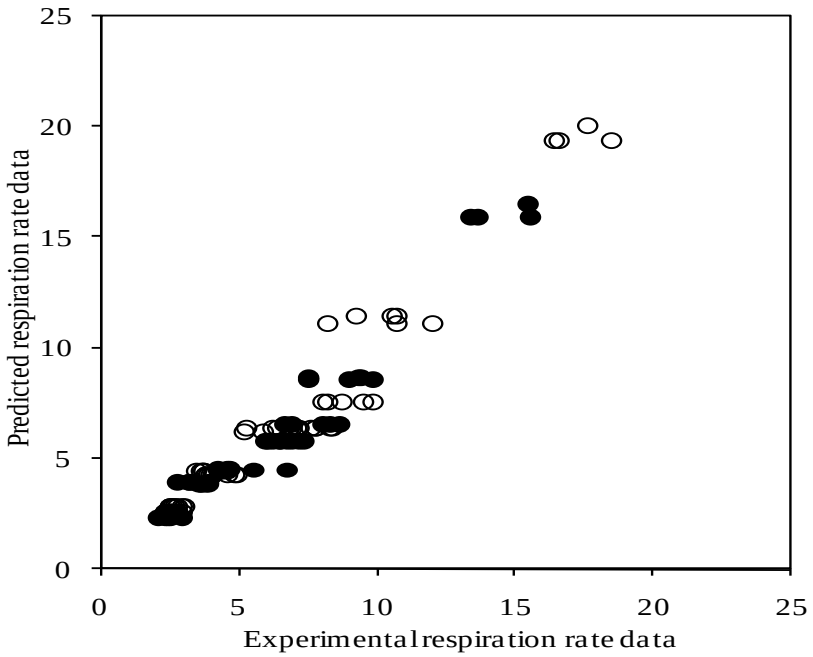


Figure 1 Relationship between experimental and predict respiration rate values of pomegranate whole fruits and arils: Empty circles represent RCO_2, mL kg^{-1} h^{-1}; filled circles represent RO_2, mL kg^{-1} h^{-1}.

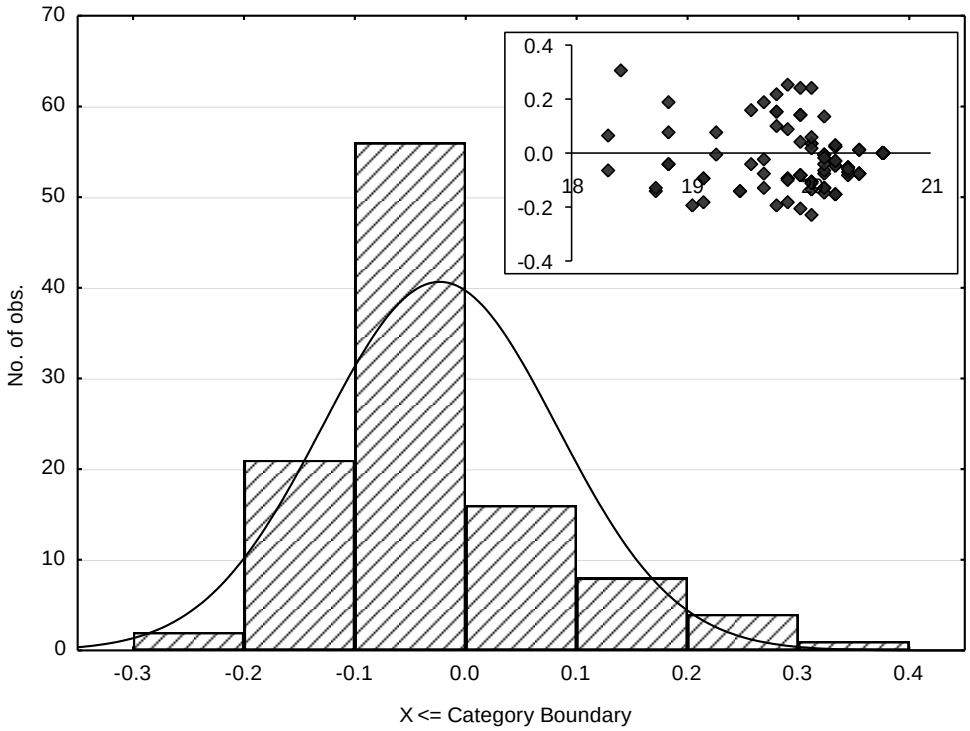


Figure 2 The distribution of residuals obtained from fitting Eqn. 5 and 6. The inside graph shows the pattern of residuals versus O_2 concentrations.

Table 1 Estimated parameters and relevant statistical data of the mathematical model (Eqn. 3 and 4) describing the influence of temperature on respiration rate of pomegranate fruit and arils

Pomegranate	RO_2, ref [mL kg^{-1} h^{-1}]	RCO_2, ref [mL kg^{-1} h^{-1}]	Ea, O_2 [kJ mol^{-1}]	Ea, CO_2 [kJ mol^{-1}]	R^2, O_2 [%]	R^2, CO_2 [%]
Whole fruit						
(cv. Acco)	8.55	11.10	84.18	75.74	96.2	95.8
(cv. Herskawitz)	8.74	11.50	90.64	79.72	94.2	93.0
Fresh arils						
(cv. Acco)	3.90	4.43	69.61	72.13	96.2	96.8
(cv. Herskawitz)	3.84	4.24	54.42	54.36	93.4	93.8

*T_{ref} = Reference temperature, 283 K

Effect of temperature on the respiration rate

The influence of temperature on the O_2 consumption (RO_2) and CO_2 production (RCO_2) of both whole pomegranate fruit and fresh arils for the two cultivars was significant, as shown in fig. 3. RO_2 and RCO_2 were within the range of 4.53 ± 0.23 to 14.67 (± 1.29) mL kg^{-1} h^{-1} and 5.67 (± 0.23) to 18.53 (± 2.84) mL kg^{-1} h^{-1} respectively, for whole fruit, and in the range of 2.51 (± 0.30) to 7.59 (± 0.92) mL kg^{-1} h^{-1} and 2.72 (± 0.17) to 9.01 (± 0.73) mL kg^{-1} h^{-1}, respectively, for fresh arils. Reducing temperature from 15 to 5 °C decreased RO_2 and

RCO_2 by about 68 and 67% for whole fruit and, 67 and 70% for fresh arils, respectively. This significant reduction in fruit respiration rate at lower storage temperature corroborates the finding reported for other types of fresh produce (Tano *et al*., 2007; Nie *et al*., 2005). For instance, Torrieri *et al*. (2010) reported a decrease in RR by 88 and 84% for RO_2 and RCO_2 respectively, when the storage temperature of minimally processed broccoli was reduced from 20 to 3 °C. The slightly lower percentage reduction in respiration rates of both whole fruit and fresh arils found in the present study compared to other types of fresh produce such as broccoli may be attributed to the non-climacteric nature of pomegranate fruit and differences in temperature regimes tested. Furthermore, the whole fruit had a higher RR compared to the arils. This could be associated to the presence of micro-cracks on the cuticle of the whole, which facilitates an increase in metabolic response for the whole fruit. While arils are membrane-bound without micro-cracks and the integrity of each aril is well protected. This implies that the selection of appropriate packaging material is crucial for better keeping of the whole fruit and arils.

The RR of the pomegranate cultivars in this study follows the pattern of other non-climacteric fruit. Wang *et al*. (2009) reported RO_2 and RCO_2 of 6.90 mL kg^{-1} h^{-1} and 6.39 mL kg^{-1} h^{-1}, respectively for guava fruit stored at 10 °C. The authors also observed a significant increase in respiration rate when temperature was increased to 30 °C. Manolopoulou & Papadopoulou (1998) reported RCO_2 range of between 1.0 to 2.5 mL kg^{-1} h^{-1} for four different kiwi cultivars stored at 0 °C. The average RCO_2 for pomegranate whole fruit at 10 °C in this study agrees with that reported by Kader *et al*. (1984), which found an average of 8 mL kg^{-1} h^{-1} for pomegranate cv. 'Wonderful' stored between 0 and 10 °C for about 3 months.

For arils, the values of RR observed in this study were relatively higher than those reported in literature for other pomegranate cultivars. For example, Eran *et al*. (2010) reported a minimum respiration rate (RO_2 and RCO_2) of 1.5 and 0.52 mL kg^{-1} h^{-1} respectively, for pomegranate arils cv. 'Hicaz' stored in 2 % O_2 + 10 % CO_2 at 4 °C. López-Rubira *et al*. (2005) reported RCO_2 of 14.45 (± 2.48) nmol $kg^{-1}s^{-1}$ (1.15 mL kg^{-1} h^{-1}) for pomegranate arils cv. 'Mollar' stored at 5 °C in air condition. Similar R_{CO2} of 14.77 nmol $kg^{-1}s^{-1}$ (1.30 mL kg^{-1} h^{-1}) was reported for fresh arils cv. 'Mollar' stored at 4 °C (Gil *et al*., 1996). The observed difference in RR highlights the possible influences of cultivar, storage condition and growing region, and thus the need for detailed study of commercial cultivars to assist in design and optimization of postharvest handling and processing operations (Caleb *et al*., 2012).

There was no significant difference in respiration rates of the two cultivars ('Acco' and 'Herskawitz') at all experimental temperatures ($p > 0.05$) studied. However, irrespective of cultivar, the RR of whole fruit was significantly higher than those of fresh arils as shown in Fig. 3. The respiration rate of whole fruit was two to three folds higher, in comparison to those of the fresh arils across all experimental temperatures. Contrary to other fresh-cut fruit in which membranes and cells are damaged, resulting in increased tissue metabolic process such as enzymatic browning, increased rate of water loss and respiration rate due to the increased surface area in contact with atmospheric oxygen (Iqbal *et al*., 2009; Torrieri *et al*., 2009), pomegranate arils have a protective membrane which prevents direct tissue or cellular interaction of its succulent portion with atmospheric condition after the husk is carefully removed. Therefore, careful postharvest handling of minimally processed pomegranate arils to avoid membrane cuts and bruises is essential for effective application of MAP (Caleb *et al*., 2012).

Effect of time and temperature on the respiration rate

Changes in respiration rate for pomegranate arils during storage at different temperatures (5, 10 and 15 °C) are summarized in fig. 4. The influence of both time and the interaction between temperature and time on the RO_2 and RCO_2 of fresh arils were significant ($p < 0.05$). These effects were adequately described by the fitted surface plot and Pareto plots which are summarised in fig. 5 and fig. 6, respectively. Fig. 5 shows the change in RO_2 and RCO_2 rate over time and storage temperature for fresh arils. The observed effect of temperature on RR of arils as shown in fig. 3, is similar to those reported by Gil *et al*. (1996), who reported respiration rates of 1.94, 1.30, and 0.53 mL CO_2 kg^{-1} h^{-1} for pomegranate arils cv. 'Mollar' stored at 8, 4, and 1 °C, respectively. However, the difference between the responses of the two cultivars in this study at 15 °C highlights the possible influence of physiological differences between cultivar responses to storage condition (Al-Mughrabi *et al.,* 1995). Furthermore, the spike observed in respiration rate at 15 °C (Fig. 4), suggests the possible influence of ethylene. Devlieghere *et al*. (2003) found a linear relationship when respiration rate at a specific temperature was plotted against the ethylene production rate for different O_2 and CO_2 concentrations for climacteric and non-climacteric fruits.

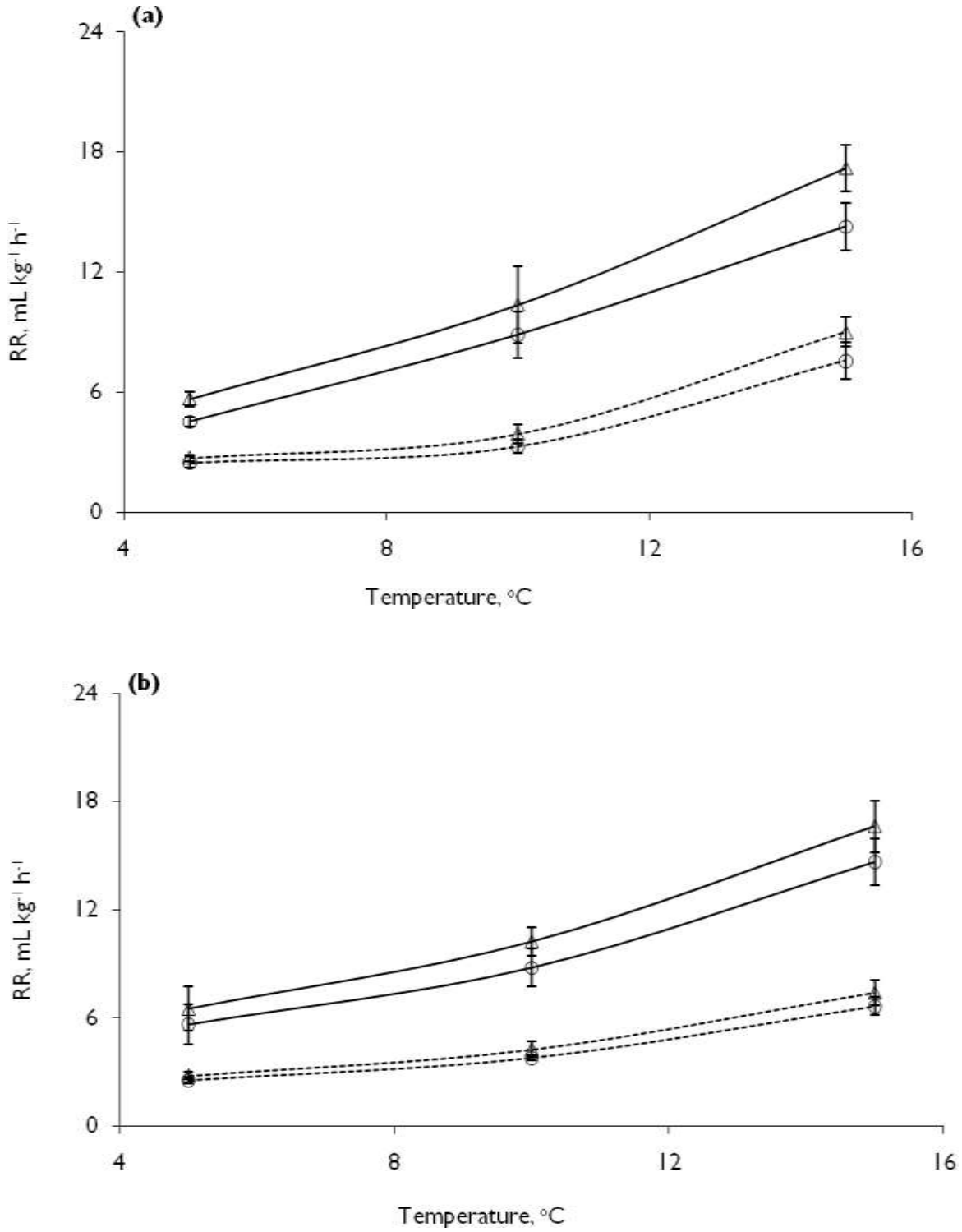


Figure 3 Effect of storage temperature on respiration rate of pomegranate fruit and arils of two cultivars: (a) cv. 'Acco' and (b) cv. 'Herskawitz'. Continuous and dotted lines represent the respiration rate of pomegranate whole fruit and arils, respectively. Circle and triangle represents the O_2 consumption rate and CO_2 production rate, respectively.

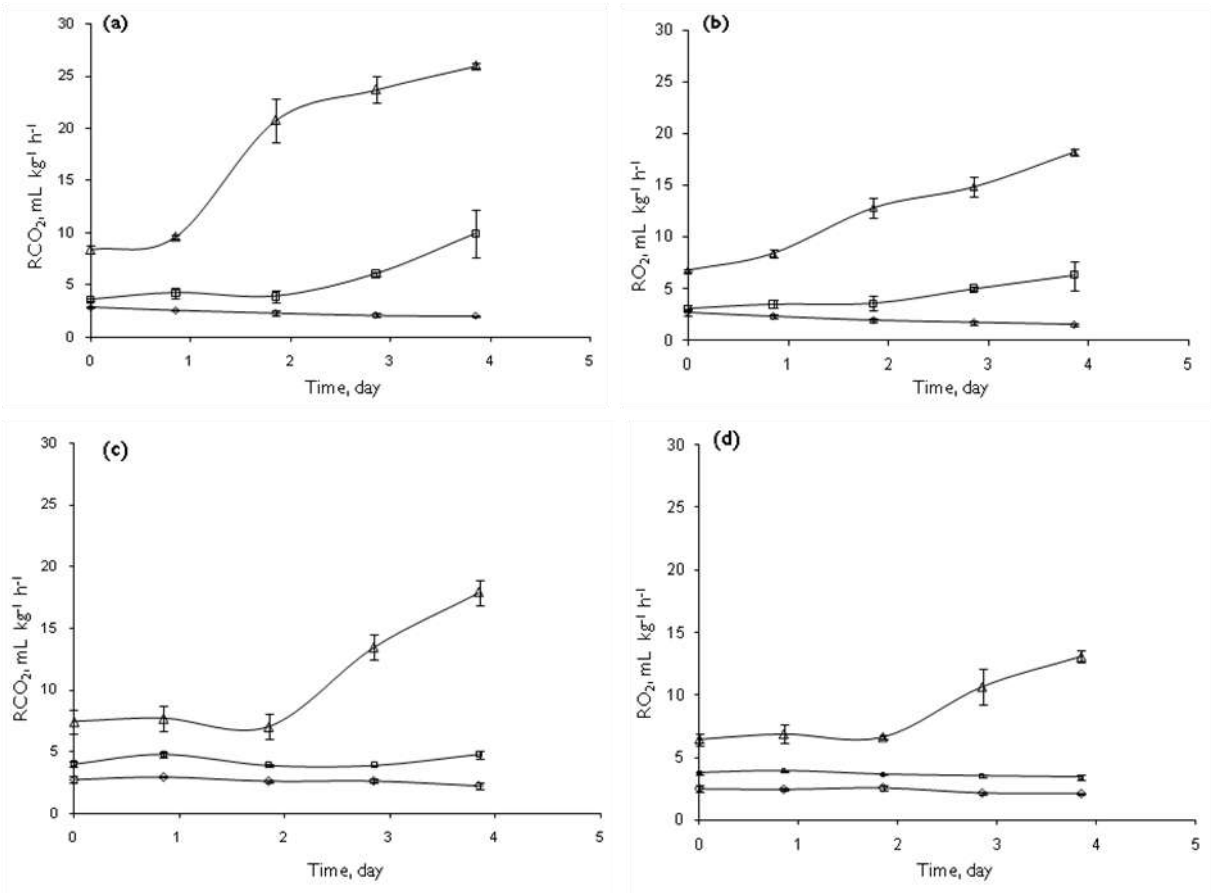


Figure 4 Changes in respiration rate of arils with time at different temperatures: (a) and (b): RCO_2 and RO_2 of arils (cv. 'Acco'); (c) and (d): RCO_2 and RO_2 of arils (cv. 'Herskawitz') with ◊ representing 5 °C, □ for 10 °C and Δ for 15 °C.

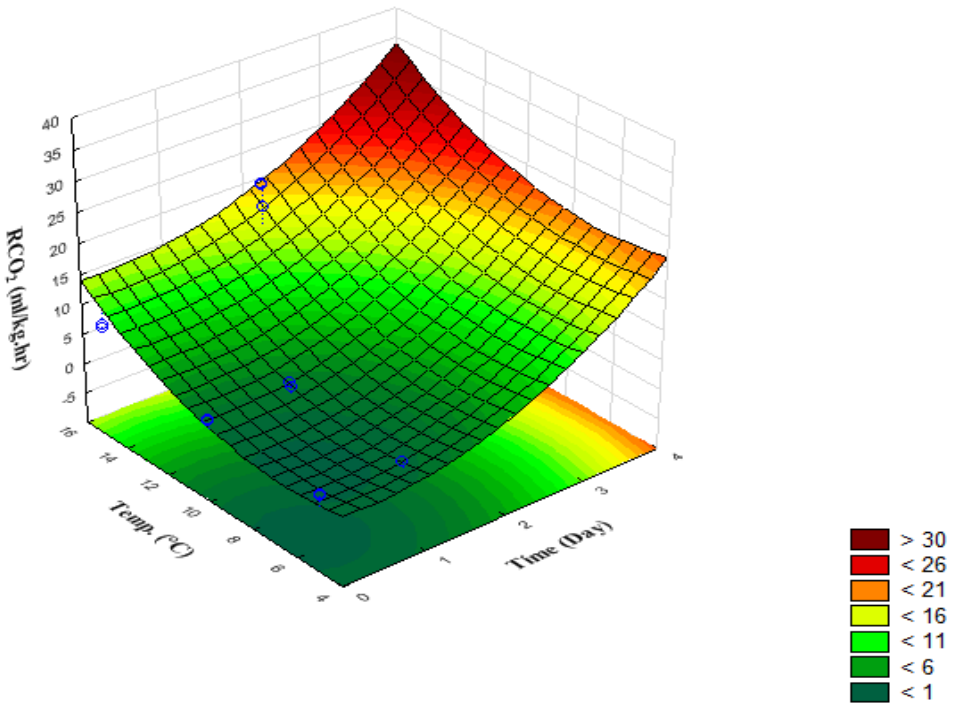


Figure 5 A fitted surface plot showing the effect of temperature and time on respiration rate (RCO_2) for pomegranate arils cv. 'Acco'. *Similar data was obtained for cv. 'Herskawitz' data not shown.

In terms of relevance to MAP design, the pattern of RR of pomegranate arils in relation to storage temperature and time as shown in Fig. 4. Can serve as guiding tool towards other MAP parameters such as package volume to packed arils volume, type of packaging material, barrier properties and temperature sensitivity of packaging material (Fonseca *et al.*, 2002). For instance at 15 °C, if the permeability property of a packaging film does not correlate with the respiration rate observed. This can lead to excessive accumulation of CO_2, resulting in cell membrane damage and physiological injuries to the product (Caleb *et al.*, 2012). Furthermore, at 5 °C storage temperature respiration rate was at its lowest and appeared to be relatively constant over time. Thus, if an inappropriate ratio of package volume to packed arils volume or packaging material is used, it is possible that the gas equilibrium level at steady-state required inside the package for passive-MAP will take a longer time to establish. MAP has been reported to strongly reduce water loss and chilling injuries without incidence of decay in pomegranate fruit (Artés *et al.*, 2000), and to maintain arils pigments (anthocyanins) better in comparison to samples packed without MAP (Gil *et al.*, 1996).

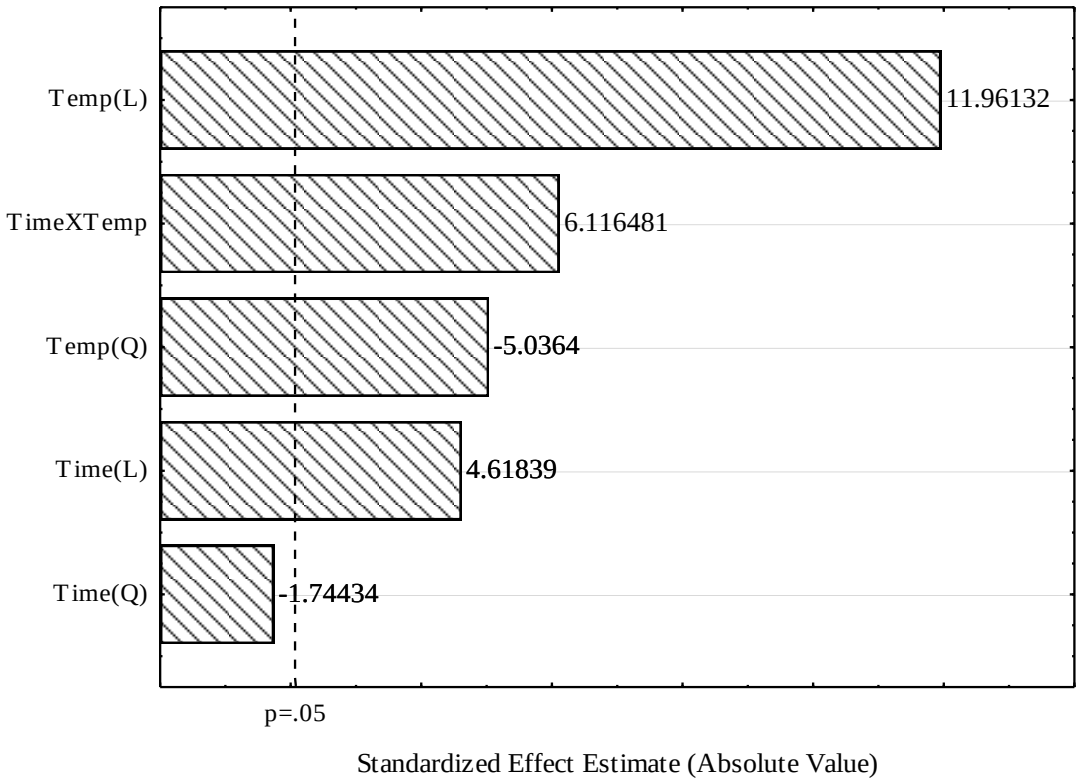


Figure 6 Pareto chart showing the effect of temperature (Temp.) and time on the respiration rate of pomegranate arils; (cv. 'Acco') at 95 % significance level, indicated as a vertical dashed line: 'L' and 'Q' are linear and quadratic effect of temperature and time, respectively. *Similar data was obtained for cv. 'Herskawitz' data not shown.

The RQ for the whole fruit of both pomegranate cv. 'Acco' and 'Herskawitz' ranged from 1.14 (± 0.06) to 1.26 (± 0.13). The RQ for arils cv. 'Acco' ranged between 1.06 (± 0.07) and 1.62 (± 0.04), while, RQ for cv. 'Herskawitz' ranged from 1.01 (± 0.09) to 1.37 (± 0.04). The RQ value of arils estimated by linear regression of RCO_2 vs. RO_2 was 0.98 (± 1.14) (R^2 adj = 98%) at 95% significant level. The values reported in this study, compare favourably with normal RQ limits (0.7 to 1.3) for aerobic respiration (Kader *et al.* 1989), with the exception of pomegranate arils (cv. 'Acco') at 15 °C where a significant increase in RQ was observed. However, there was experimental evidence which suggest the significant ($p < 0.05$) influence of time and temperature on the observed high RQ for pomegranate arils (cv. 'Acco') under aerobic conditions (Fig. 7 and 8). This phenomenon is similar to that reported by Wang *et al.* (2009) for guava fruit. Fig. 4 also confirms the change in respiration pattern with temperature.

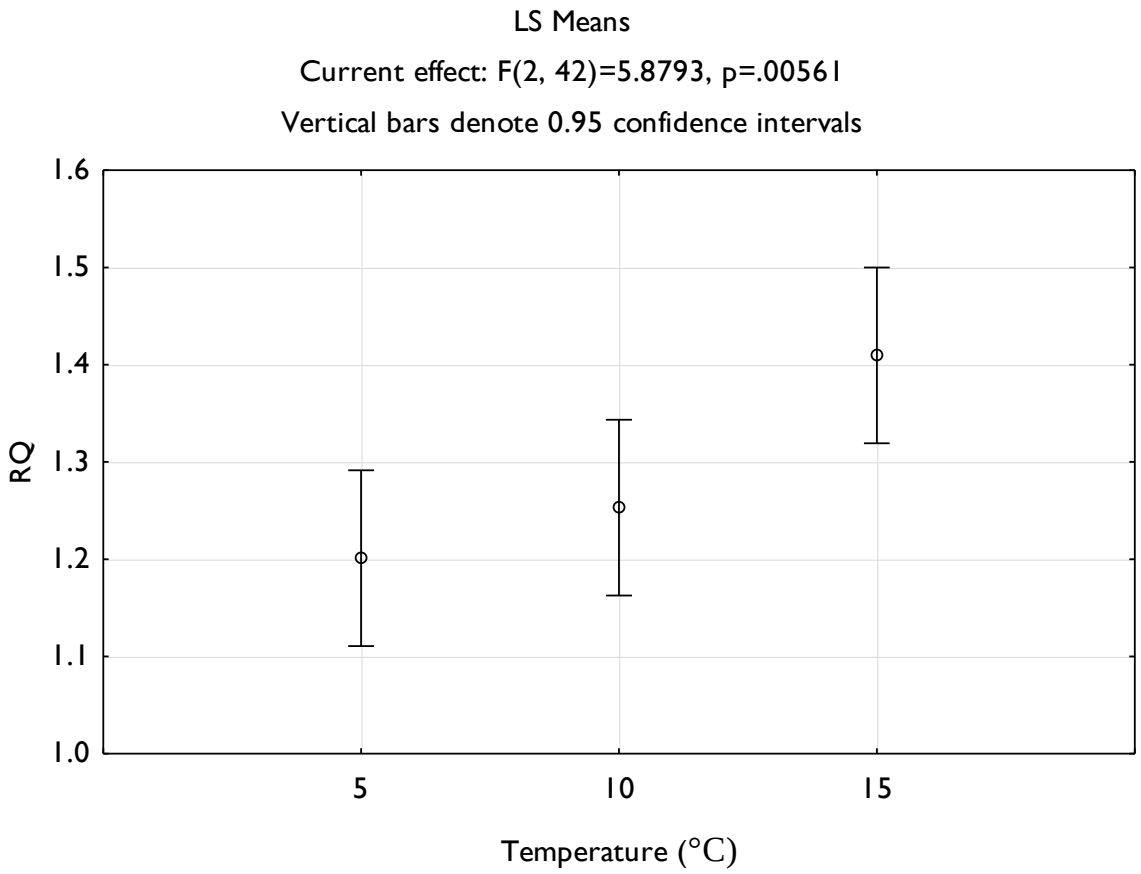


Figure 7 One-way ANOVA analyses showing the influence of temperature on the observed RQ for pomegranate arils (cv. 'Acco').

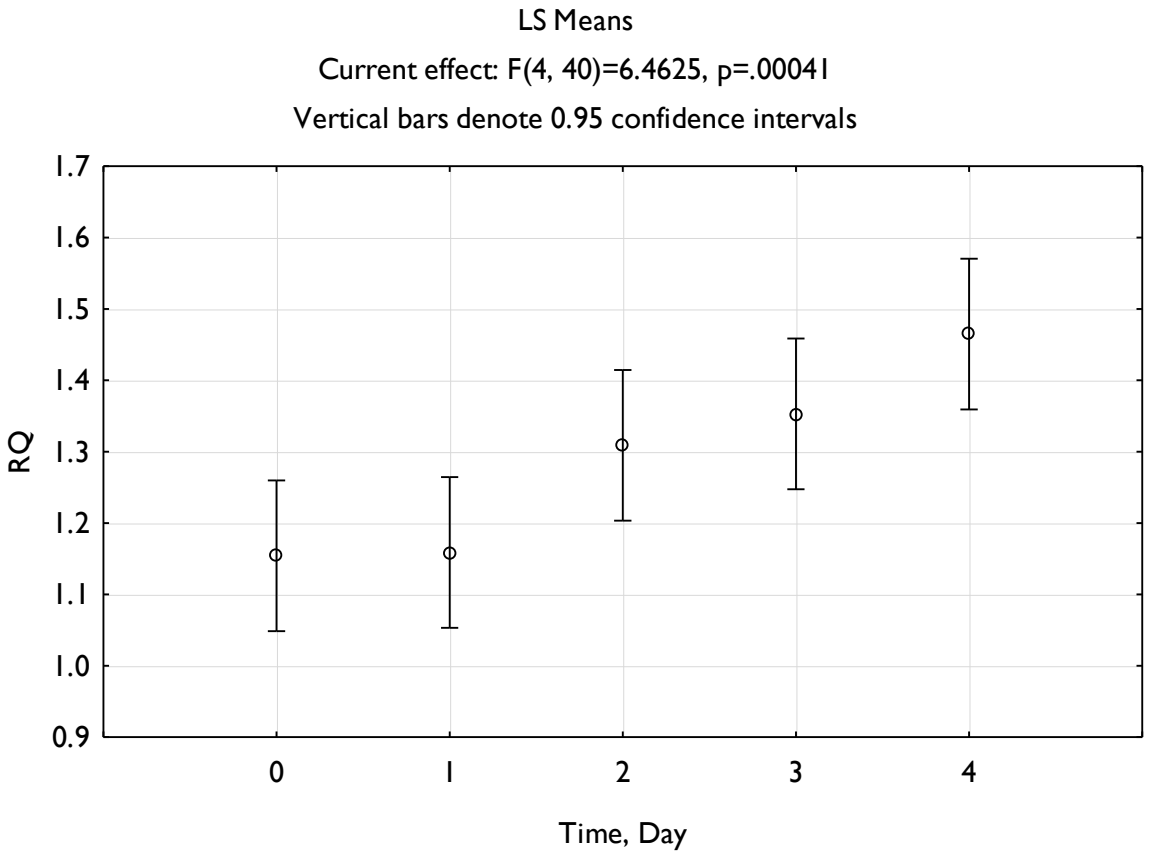


Figure 8 One-way ANOVA analyses showing the influence of time on the observed RQ for pomegranate arils (cv. 'Acco'), similar data was obtained for cv. 'Herskawitz' data not shown.

Conclusions

The respiration rate of pomegranate cv. 'Acco' and 'Herskawitz' whole fruit was significantly higher than the respiration rate of fresh arils. Temperature had a significant impact on the respiration rates of both whole fruit and fresh arils. The influence of time, and the interaction between temperature and time also had a significant influence on the respiration rate of fresh arils. This highlights the importance of maintaining optimal product cold chain, especially when MAP is used for arils. It was found that the RQ of pomegranate arils was dependent on temperature and time, with higher RQ value as storage temperature increased from 5 to 15 °C. An Arrhenius-type equation accurately predicted the effect of temperature on respiration rate of whole fruit and fresh arils. The model would be useful towards MAP-design for postharvest handling of whole pomegranate fruit and freshly processed arils.

References

Al-Mughrabi, M.A., Bacha, M.A. & Abdelrahman, A.O. (1995). Effect of storage temperature and duration on fruit quality of three pomegranate cultivars. *Journal of King Saudi University*, **7**, 239-248.

Artés, F., Marin, J.G. & Martínez, J.A. (1998). Permeability rates of films for modified atmosphere packaging of respiring foods, In: *Food quality* (edited by Nicolaï B.M. *et al.*). Pp. 153–157Leuven: European Commission.

Caleb, O.J., Opara, U.L. & Witthuhn, C. (2012). Modified atmosphere packaging of pomegranate fruit and arils: A review. *Food and Bioprocess Technology*, **5**, 15-30.

Cameron, A.C., Beaudry, R.M., Banks, N.H. & Yelanich, M.V. (1994). Modified atmosphere packaging of blueberry fruit: modelling respiration and package oxygen partial pressures as function of temperature. *Journal of American Society Horticultural Science*, **119**, 534-539.

Charles, F., Sanchez, J. & Gontard, N. (2003). Active modified atmosphere packaging of fresh fruits and vegetables: Modeling with tomatoes and oxygen absorber. *Journal of Food Science*, **68**, 1736-1742.

Devlieghere, F., Jacxsens, L., Tatay, M.S., Debevere, J., Meirlaen, J. & Vanrolleghem, P. (2003). Modelling the relation between ethylene production rate, respiration rate and their influence on climacteric and non-climacteric fruits. *Acta Horticulturae*, **600**, 647-651.

Ersan, S., Gunes, G. & Zor, A.O. (2010). Respiration rate of pomegranate arils as affected by O_2 and CO_2, and design of modified atmosphere packaging. *Acta Horticulturae*, **876**, 189-196.

Farber, J.N., Harris, L.J., Parish, M.E., Beuchat, L.R., Suslow, T.V., Gorney, J.R., Garrett, E.H. & Busta, F.F. (2003). Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, **2**, 142-160.

Fonseca, S.C., Oliveira, F.A.R. & Brecht, J.K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages. *Journal of Food Engineering*, **52**, 99-119.

Gil, M.I., Artes, F. & Toma-Barberan, F.A. (1996). Minimal processing and modified atmosphere packaging effects on pigmentation of pomegranate seeds. *Journal of Food Science*, **61**, 161-164.

Iqbal, T., Rodrigues, F.A.S., Mahajan, P.V. & Kerry, J.P. (2009). Mathematical modelling of O_2 consumption and CO_2 production rates of whole mushrooms accounting for the effect of temperature and gas composition. *International Journal of Food Science and Technology*, **44**, 1408-1414.

Kader, A. A. (1995). Regulation of fruits physiology by controlled and modified atmosphere. *Acta Horticulturae*, **398**, 59-70.

Kader, A.A., Zagory, D. & Kerbel, E.L. (1989). Modified atmosphere packaging of fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, **28**, 1-30.

Kader, A.A., Chordas, A. & Elyatem, S. (1984). Response of pomegranates to ethylene treatment and storage temperature. California Agriculture, July–August. pp. 14-15.

Lakakul, R., Beaudry, R.M. & Hernandez, R.J., (1999). Modeling respiration of apple slices in modified atmosphere packages. *Journal of Food Science*, **64**, 105-110.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 174-185.

Mahajan, P.V., Oliveira, F.A.R. & Macedo, I. (2008). Effect of temperature and humidity on the transpiration rate of the whole mushrooms. *Journal of Food Engineering,* **84**, 281-288.

Mahajan, P.V., Oliveira, F.A.R., Montanez, J.C. & Frias, J. (2007). Development of user-friendly software for design of modified atmosphere packaging for fresh and fresh-cut produce. *Innovative Food Science and Emerging Technology*, **8**, 84-92.

Manolopoulou, H. & Papadopoulou, P. (1998). A study of respiratory and physico-chemical changes of four kiwi fruit cultivars during cool-storage. *Food Chemistry*, **63**, 529-534.

Nie, D., Uchino, T., Sakai, N. & Tanaka, S. (2005). Effect of high temperature on the apparent activation energy of respiration of fresh product. *Postharvest Biology and Technology*, **37**, 277-285.

Tano, K., Oulé, M.K., Doyon, G., Lencki, R.W. & Arul, J. (2007). Comparative evaluation of the effect of storage temperature fluctuation on modified atmosphere packages of selected fruit and vegetables. *Postharvest Biology and Technology*, **46**, 212-221.

Torrieri, E., Cavella, S. & Masi, P. (2009). Modelling the respiration rate of fresh-cut Annurca apples to develop modified atmosphere packaging. *International Journal of Food Science and Technology*, **44**, 890-899.

Torrieri, E., Perone, N., Cavella, S. & Masi, P. (2010). Modelling the respiration rate of minimally processed broccoli (*Brassica rapa* var. *sylvestris*) for modified atmosphere package design. *International Journal of Food Science and Technology*, **45**, 2186-2193.

Wang, Z.-W., Duan, H.-D. & Hu, C.-Y. (2009). Modelling the respiration rate of guava (*Psidium guajava* L.) fruit using enzyme kinetics, chemical kinetics and artificial neural network. *European Food Research and Technology*, **229**, 495-503.

Chapter 5

Development of prediction model describing the effect of time and temperature on respiration rate of pomegranate arils

CHAPTER 5

DEVELOPMENT OF PREDICTION MODEL DESCRIBING THE EFFECT OF TIME AND TEMPERATURE ON RESPIRATION RATE OF POMEGRANATE ARILS

Summary

Understanding the effect of time and temperature on the respiration rate (RR) of fresh-cut produce, towards the design of a suitable modified atmosphere packaging (MAP) system requires an adequate mathematical model for prediction of RR as a function of both time and temperature. This study investigated the effect of temperature (5, 10 and 15 °C) and storage time (1 to 5 days) on the RR (RO_2 and RCO_2) of two pomegranate cultivars (cv. 'Acco' and 'Herskawitz') fresh arils. RO_2 and RCO_2 were 3-4 folds significantly higher with increased temperature from 5 to 15 °C and were within the range of 2.51 to 7.59 mL kg^{-1} h^{-1} and 2.72 to 9.01 mL kg^{-1} h^{-1}, respectively for both cultivars. At 15 °C RCO_2 increased significantly from 8.4 to 25.96 mL kg^{-1} h^{-1} from day 1 to 5, respectively, while at 5 °C RCO_2 changed from 2.9 to 2.05 mL kg^{-1} h^{-1} from day 1 to 5. Temperature had the greatest influence on RR and the interaction of time and temperature also significantly affected RO_2 and RCO_2. The respiratory quotient (RQ) estimated by linear regression was 0.98 at 95% significant level. The dependence of RR on temperature and time was accurately described with a combination of an Arrhenius-type and power equation model for RO_2 and RCO_2 of fresh pomegranate arils.

Introduction

During the last decade, there has been a remarkable increase in the commercial production of pomegranates globally, due to the potential health benefits of the fruit. These benefits have been attributed to the high antioxidant contents and its anti-mutagenic, anti-hypertension, anti-inflammatory and anti-atherosclerotic activities against osteoarthritis, prostate cancer, heart disease and HIV-1 (Viuda-Martos *et al*., 2010). Furthermore, pomegranate fruit is an excellent dietary source rich in organic acids, soluble solids,

anthocyanins, vitamin C, fatty acids and mineral element, and has significant antimicrobial effects (Opara *et al*., 2009; Fawole *et al*., 2012). In spite of these health benefits, pomegranate consumption is still limited, due to the difficulties of extracting the arils and the irritation of phenolic metabolites which stain the hands during preparation (Caleb *et al*., 2012). Furthermore, the presence of external blemish and defects such as sunburnt husks, splits and cracks, and husk scald on the whole fruit reduces marketability and consumer acceptance (Sadeghi & Akbarpour, 2009), even though the arils are of good quality. Hence, modified atmosphere packaged ready-to-eat fresh pomegranate arils presents a more appealing product to consumers and increases the prospect of both production and consumption (Caleb *et al*., 2012; Opara & Al-Ani, 2010).

Modified atmosphere packaging (MAP) is a dynamic process of altering gaseous composition within a package. It relies on the interaction between the RR of the produce, and the transfer of gases through the packaging material, with no further control exerted over the initial gas composition (Farber *et al*., 2003; Mahajan *et al*., 2007). MAP technology extends the shelf-life and maintains quality of fresh-cut produce by lowering the RR and retarding the development of physiological disorders and proliferation of spoilage pathogenic microbes (Artés *et al*., 2000). However, a quantitative description of RR of fresh produce via mathematical modeling is essential for the design of MAP (Fonseca *et al*., 2002; Mahajan *et al*., 2007). When fruit respiration does not correlate to the permeability properties of packaging film, increase in the concentration of CO_2 will build up beyond acceptable levels, leading to anaerobic respiration and ethanol accumulation inside the fresh produce. This results in the development of off-flavours and decay (Caleb *et al*., 2011). Although, some studies have reported data on the RR of arils of selected pomegranate cultivars (Eran *et al*., 2010), there is no predictive model on the RR of fresh pomegranate arils describing the effect of time and temperature. Therefore, the objective of this study was to investigate the effects of time and temperature on respiration rate of fresh arils, and to develop a predictive model relating respiration rate to both time and temperature, thereby providing basic information relevant to the design of MAP for this product.

Materials and methods

Produce and sample preparation

Fully ripe pomegranate (*Punica granatum* L.) fruit sweet-sour cv. 'Acco' and 'Herskawitz' harvested manually during commercial harvest period were procured from Robertson valley farm in the Western Cape (33°48′0″S, 19°53′0″E), South Africa and air-freighted in well ventilated boxes to the Process and Chemical Engineering Laboratory, University College Cork, Ireland. The duration of transportation was about 72 hours. On arrival, fruit were immediately stored at 5 °C until the next day, when they were peeled manually in a clean cold room at 5 °C by carefully removing the arils to avoid damage. Samples of arils were weighed (≈ 150 g each sample), and each sample was placed inside a glass jar of about 428 mL, and equilibrated at the desired storage temperature (5, 10 or 15 °C) for at least 1 hr prior to experiment. The physicochemical properties of the pomegranate cultivars studied were characterised at the start of experiment and data is presented in Table 1. A total of 100 g of arils were homogenised and filtered using cheesecloth. The juice pH was measured using a digital pH meter (3310 Jenway, pH Meter, UK). Titratable acidity (TA) expressed as % citric acid was determined potentiometrically, by titration to an end point of pH 8.2 using 2 mL of juice diluted with 10 mL distilled water using an autotitrator (Metrohm 785 DMP, Titrino, Switzerland). Total soluble solids (TSS) were measured by hand refractometer (Atago, Tokyo, Japan). Hunter colour parameters (*L** (lightness), *a** (redness and greenness), and *b** (yellowness and bluness)) of arils were measured with a colour meter (Minolta Chroma Meter, CR-300, Japan), after calibration against a white tile background (Opara *et al*., 2009; Fawole *et al*., 2012). All analyses were presented as mean ± standard error (S.E.) of 10 replicates.

Table 1 Fruit physicochemical properties of the studied pomegranate cultivars

		CIELAB colour index			TA		
Cultivar(s)	**Fruit size (g)**	***L****	***a****	***b****	**(%w/v) citric acid**	**TSS (°Brix)**	**pH**
'Acco'	242.2 ± 2.9	31.3 ± 1.9	15.43 ± 2.19	10.4 ± 0.5	2.08 ± 0.09	17.8 ± 0.2	3.2 ± 0.06
'Herskawitz'	252.4 ± 8.4	28.9 ± 0.8	22.45 ± 1.49	11.8 ± 0.6	1.82 ± 0.04	16.3 ± 0.2	3.1 ± 0.01

[a]Values are mean ± S. E.

Experimental setup

The rate of oxygen consumption (RO_2) and carbon dioxide production (RCO_2) of the pomegranate fresh arils were measured using the closed system method (Fonseca *et al.*, 2002; Iqbal *et al.*, 2009b; Torrieri *et al.*, 2009). Air-tight glass jars with a lid containing a rubber septum in the middle were used to store aril samples at the different temperatures of 5, 10 and 15 °C. To ensure hermetic seal, Vaseline was incorporated into the gap between lid and the glass jar. Gas composition inside the glass jars were monitored periodically using an O_2/CO_2 gas analyser with an accuracy of 0.5 % (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Gas samples were taken at constant time intervals from the head space through the rubber septum. RO_2 and RCO_2 were determined by fitting experimentally obtained data on y_{O_2} and y_{CO_2} with Eqn. 1 and 2, respectively

$$y_{O_2} = y^i_{O_2} - \frac{R_{O_2}.W}{V_f}.(t - t_i) \times 100 \quad (1)$$

$$y_{CO_2} = y^i_{CO_2} + \frac{R_{CO_2}.W}{V_f}.(t - t_i) \times 100 \quad (2)$$

where:

$y^i_{O_2}$ = O_2 concentration (%) at the initial time t_i (hours, h)

y_{O_2} = O_2 concentration (%) at time t (h)

$y^i_{CO_2}$ = CO_2 concentration (%) at the initial time t_i (h)

y_{CO_2} = CO_2 concentration (%) at time t (h)

RO_2 = respiration rates in mL O_2 kg^{-1} hr^{-1}

RCO_2 = respiration rates in mL CO_2 kg^{-1} hr^{-1}

W = total weight of the product (kg)

V_f = free volume inside the glass jar (ml), which is the total volume ($V_t \approx 428$ ml) of the glass jar minus volume occupied by arils.

The volume occupied by the arils was calculated from the mass of arils over apparent density (0.98g cm^{-3}). Furthermore, in order to characterise the effect of time on respiration rate of the arils, periodic gas samples were taken hourly over a period of 5 hours from the hermetic

sealed jars, after which the glass jars were opened slightly to minimize rapid moisture loss and also to avoid built-up of sub-atmospheric gases. Following overnight storage time the jars were closed hermetically and gas samples were taken. This cycle was repeated over a 5 day storage period and no spoilage was observed over this period. The gas samples taken during 5 hour measurement period were used to calculate RO_2 and RCO_2 using Eqn. 1 and 2.

Statistical analyses

Pareto analysis (Mahajan *et al*., 2008) was used with two factors (time and temperature) each at three levels of temperatures 5, 10 and 15 °C at 95% confidence interval to assess the effects of time and temperature, and the interaction between time and temperature on the respiration rate of pomegranate arils. The experimental data obtained were treated with one-way analysis of variance (ANOVA) at 95% confidence interval to evaluate the effect of time and temperature on RR and RQ. All experiments were carried out in triplicate and analysed using Statistical software (Statistical 10.0, Statsoft, USA).

Results and Discussion

Effect of time and temperature on the respiration rate

The influence of temperature on the RR (O_2 consumption (RO_2) and CO_2 production (RCO_2)) on both cultivars was significant, as shown in Fig.1. RO_2 and RCO_2 values ranged from 2.51 ± 0.30 to 7.59 (± 0.92) mL kg^{-1} h^{-1} and 2.72 (± 0.17) to 9.01 (± 0.73) mL kg^{-1} h^{-1}, respectively. Reducing storage temperature of arils from 15 to 5 °C decreased RO_2 and RCO_2 by about 67 and 70 %, respectively. The significant reduction in RR at lower storage temperature corroborates the finding reported for other types of fresh produce (Cliffe-Byrnes & O'Beirne 2007; Lakakul *et al.,* 1999). Similarly, the effect of reduced temperature was reported by Torrieri *et al.* (2010). They observed a decrease RR by 88 and 84% for RO_2 and RCO_2, respectively, when the storage temperature of minimally processed broccoli was reduced from 20 to 3 °C. However, the slightly lower percentage reduction in RR of fresh arils found in the present study compared to other types of fresh produce such as broccoli

may be attributed to the non-climacteric nature of pomegranate fruit and differences in temperature regimes tested.

Based on Pareto chart (Fig. 2) and fitted surface plot (Fig. 3) show that both storage time and temperature, and their interaction had significant influences on RR of fresh arils ($p < 0.05$). Fig. 2 shows that temperature had the most significant effect in comparison to time. The observed effect of temperature on RR of arils as shown in Fig. 1, is similar to those reported by Gil *et al.* (1996), who reported RR of 1.94, 1.30, and 0.53 mL CO_2 kg^{-1} h^{-1} for pomegranate arils cv. 'Mollar' stored at 8, 4, and 1 °C , respectively. The RR observed in this study was relatively higher than those reported in literature for arils of other pomegranate cultivars. For instance, Eran *et al.* (2010) reported a minimum RO_2 and RCO_2 of 1.5 and 0.52 mL kg^{-1} h^{-1} respectively, for pomegranate arils cv. 'Hicaz' stored in 2 % O_2 + 10 % CO_2 at 4 °C. López-Rubira *et al.* (2005) reported RCO_2 of 14.45 (± 2.48) nmol kg^{-1} s^{-1} (1.15 mL kg^{-1} h^{-1}) for pomegranate arils cv. 'Mollar' stored at 5 °C in air condition. This could be attributed to difference in storage conditions and postharvest handling/ treatment, which was different from this study.

Furthermore, the difference between the responses of the two cultivars in this study at 15 °C highlights the possible influence of physiological differences between cultivar responses to storage conditions (Al-Mughrabi *et al.*, 1995). Furthermore, the observed spike in RR at 15 °C (Fig. 1) could be associated with a stress-induce response due to temperature. Other possible influence could be stress-induce by ethylene synthesis, Oosterhaven & Peppelenbos (2003) reported a linear relationship between RR at a specific temperature and ethylene production rate for different O_2 and CO_2 concentrations for climacteric and non-climacteric fruits.

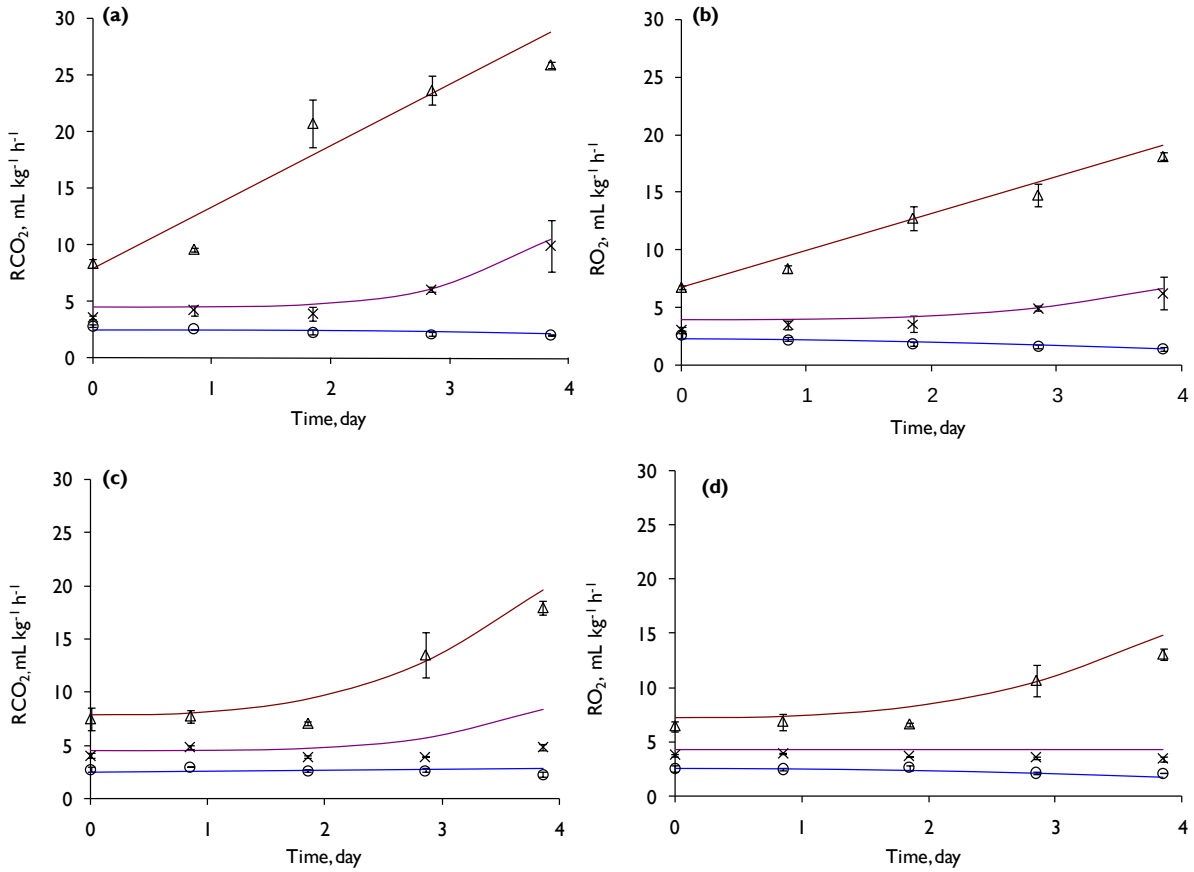


Figure 1 Change of RCO_2 and RO_2 rate as a function of time and temperatures 5, 10 and 15 °C experimental and prediction data (a) and (b) cv. 'Acco', and (c) and (d) cv. 'Herskawitz' respectively: Δ = 15 °C, x = 10 °C, ○ = 5 °C, continuous line represents the predicted values at respective temperatures, the bars represents the standard deviation of the experimental data.

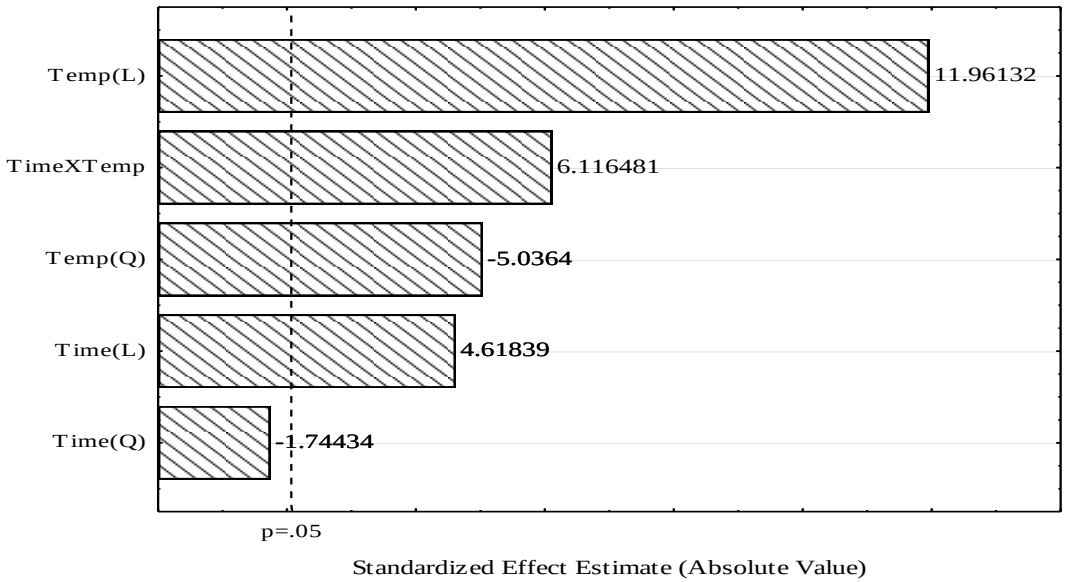


Figure 2 Pareto chart showing the effect of time, temperature (Temp) and their interaction on the respiration rate of pomegranate arils; cv. 'Acco' at 95% significance level, indicated as a vertical dashed line. * Similar data obtained for cv. 'Herskawitz'

RCO_2 increased significantly during storage 15 °C from day 1 to 5 for both cultivars ($p < 0.05$), with values ranging from 9.01 (± 0.73) to 25.9 (± 0.27) mL kg^{-1} h^{-1} for cv. 'Acco', and 7.5 (± 1.04) to 17.9 (± 0.63) mL kg^{-1} h^{-1} for cv. 'Herskawitz'. The significant increase in fruit RR during storage at higher temperature corroborates the finding reported for other types of fresh produce. For instance, Iqbal *et al*., (2009a) reported increased RR of sliced mushroom from 59.5 to 95.2 mL kg^{-1} h^{-1} during 100 h storage at 12 °C. In contrast, the present study showed that during 5 days storage temperature at 5 °C there was a decline in RCO_2 from 2.9 (± 0.09) to 2.1 (±0.06) mL kg^{-1} h^{-1} for cv. 'Acco' and 2.7 (± 0.23) to 2.3 (± 0.27) mL kg^{-1} h^{-1} for cv. 'Herskawitz'. These results are in agreement with findings of Nei *et al*., (2006) who reported a slight decrease in RR of shredded cabbage during storage at 5 °C. Other studies have shown that metabolic and enzymatic processes in fresh produce are retarded at low temperature (Fonseca *et al*., 2002). Hence, it is important to monitor and control storage temperature inside MAP of fresh produce along the supply chain.

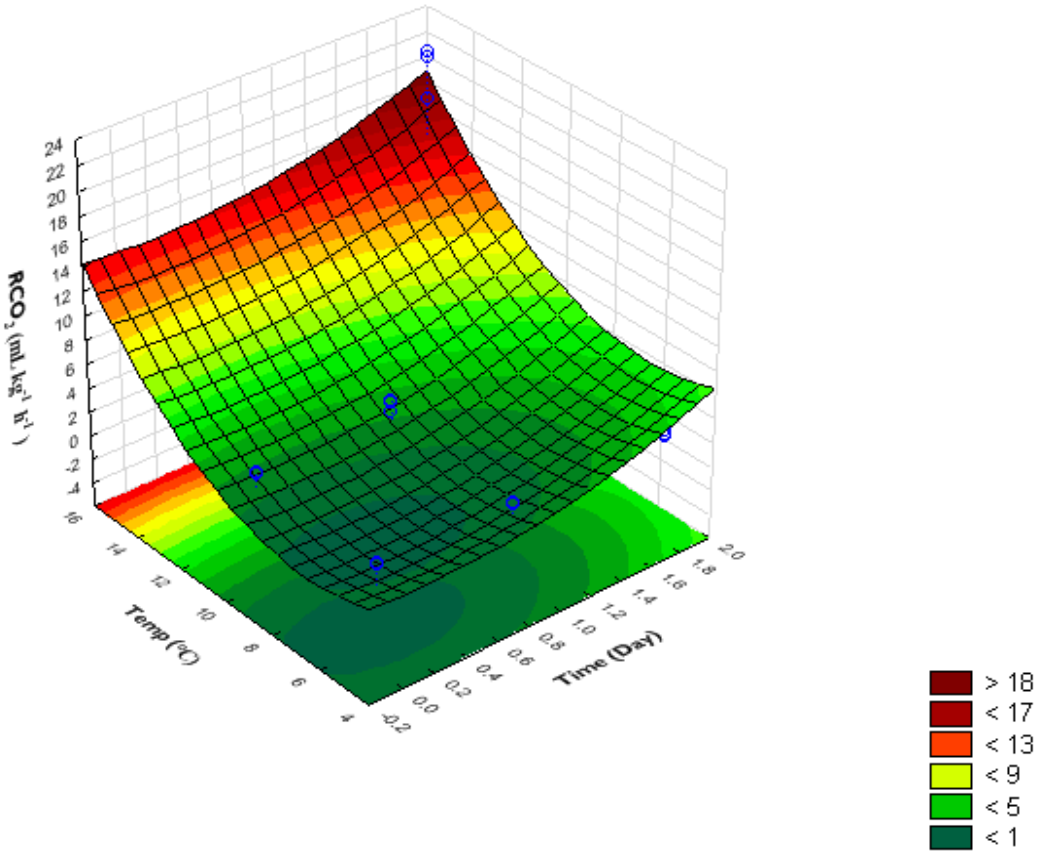
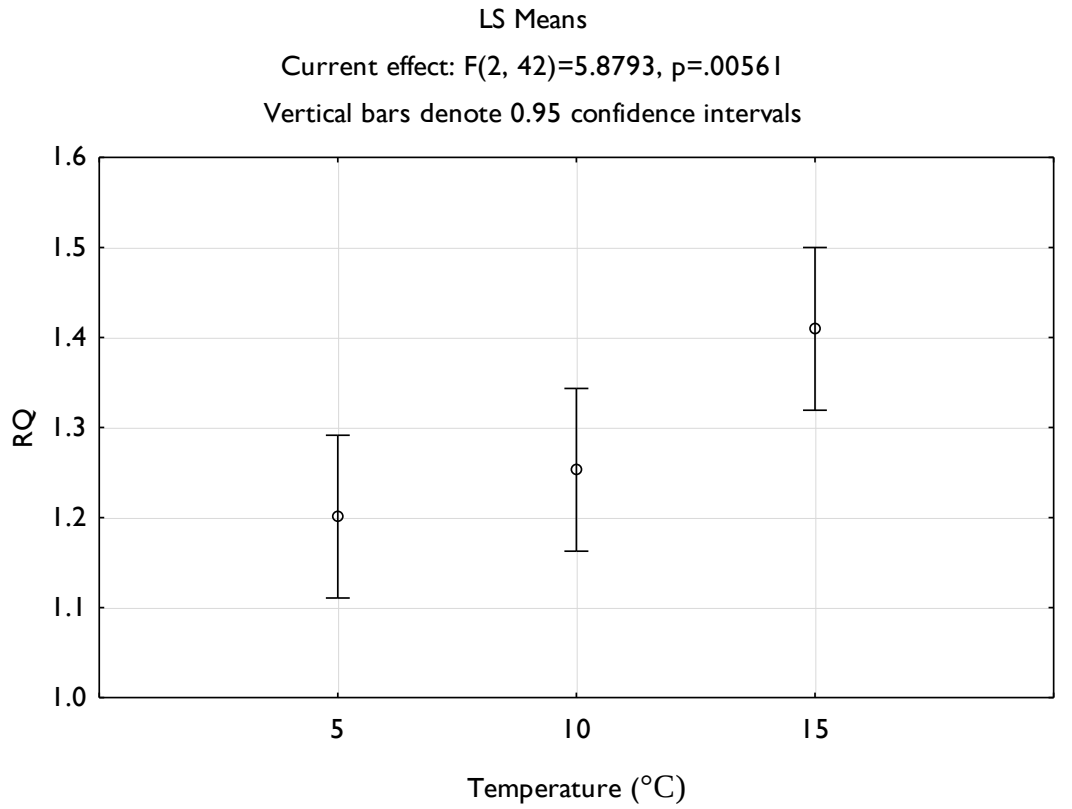


Figure 3 A fitted surface plot showing the effect of temperature and time on respiration rate (RCO_2) for pomegranate arils cv. 'Acco'. * Similar data obtained for cv. 'Herskawitz' data not shown.

The RQ of pomegranate arils ranged between 1.06 (± 0.07) and 1.62 (± 0.04) for cv. 'Acco' and 1.01 (± 0.09) to 1.37 (± 0.04) for cv. 'Herskawitz'. The RQ value of arils estimated by linear regression of RCO_2 vs. RO_2 was 0.98 ± (0.14) (R^2 adj = 98%) at 95% significant level. These values compares favourably with normal RQ limits (0.7 to 1.3) for aerobic respiration (Kader *et al*., 1989), with the exception of pomegranate arils cv. 'Acco' at 15 °C. However, experimental evidence suggests that the significant ($p < 0.05$) influence of time and temperature on the observed high RQ for pomegranate arils (cv. 'Acco') occurred under aerobic conditions (Fig. 4), similar to the findings reported by Wang *et al.* (2009) for guava fruit. Although there was no evidence of anaerobic respiration for the conditions of temperature tested, it is possible that the RQ breakpoint of fresh-cut pomegranate arils (cv. 'Acco') is lower than 15 °C. This breakpoint will be the highest storage temperature that does not induce anaerobic respiration in packaged arils.

Considering variation in temperature during distribution chain, the RR pattern observed in Fig. 1 could serve as a guiding tool for selecting appropriate packaging material for pomegranate arils. For instance at 15 °C, if the permeability property of a selected film does not match with the observed RR, this might lead to excessive accumulation of CO_2 causing physiological injuries and cell membrane damage of product (Caleb *et al*., 2012). Conversely, at 5 °C RR was at its lowest rate and appeared to be relatively constant over time, therefore, it will take longer period to establish an equilibrium gas composition in passive-MAP. Furthermore, the observed RR response highlights the need for strict measures to monitor storage conditions along the supply chain to the retail market using devices such as time temperature indicators (TTIs), radio frequency identification (RFID) tags, and gas indicators (Caleb *et al*., 2012).



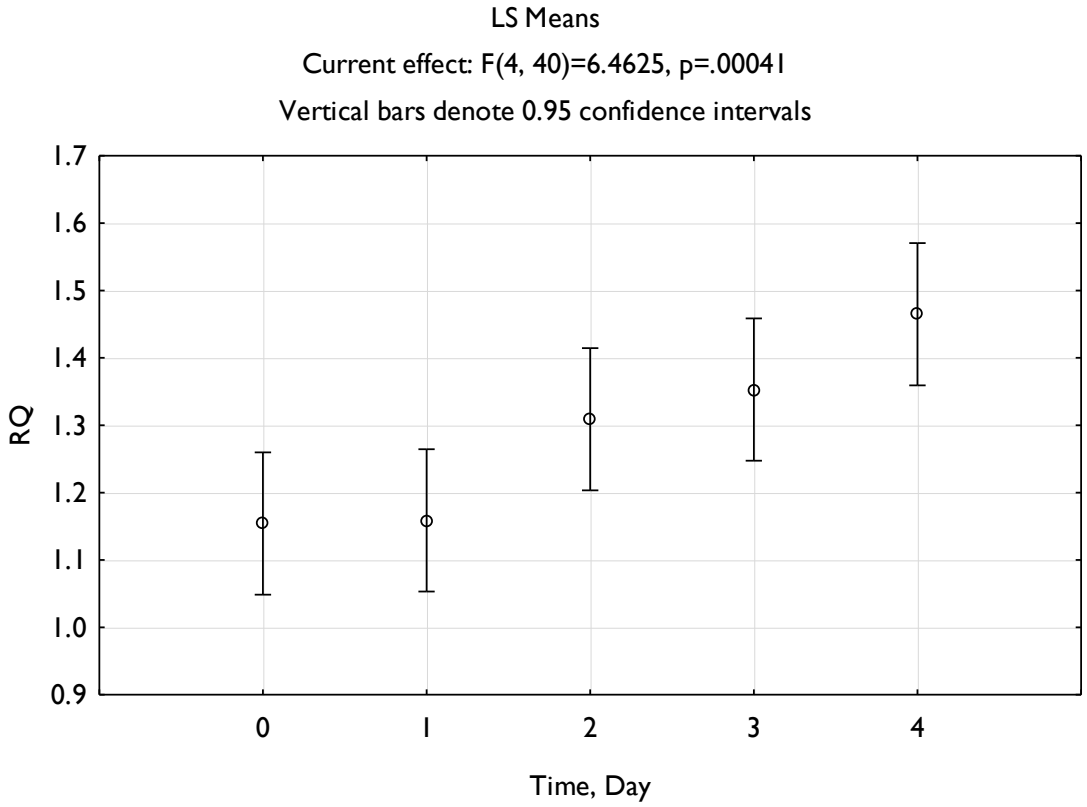


Figure 4 One-way ANOVA analyses showing the influence of temperature and time on the observed RQ for pomegranate arils cv. 'Acco'.

Table 2 Parameter estimates from model (Eqn. 5 and 6) describing the influence of temperature on respiration rate and relevant statistical data

Pomegranate arils	$R^i_{O2,\,ref}$ [mL kg^{-1} h^{-1}]	$R^i_{CO2,\,ref}$ [mL kg^{-1} h^{-1}]	Ea, $_{O2}$ [kJ mol^{-1}]	Ea, $_{CO2}$ [kJ mol^{-1}]	R^2, $_{O2}$ [%]	R^2, $_{CO2}$ [%]
cv. 'Acco'	4.03	4.52	71.25	75.78	94.9	95.4
cv. 'Herskawitz'	4.32	4.46	69.61	77.24	95.4	96.7

Model building

Effect of temperature on respiration rate of pomegranate arils

A simple Arrhenius-type equation which describes temperature as a function of RR (Iqbal *et al.*, 2009b; Torrieri *et al.*, 2010) for both R_{O2} and R_{CO2} was applied in model fitting as presented in Eqn. 3 and 4:

$$R_{O2} = R^{i}_{O2,ref} \times e^{\left[\frac{-E_{a,O2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} \quad (3)$$

$$R_{CO2} = R^{i}_{CO2,ref} \times e^{\left[\frac{-E_{a,CO2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} \quad (4)$$

where R_{O2} and R_{CO2} are RR (mL kg^{-1} h^{-1}) at temperature (T, K), $R^{i}_{O2,ref}$ and $R^{i}_{CO2,ref}$ are initial RR (mL kg^{-1} h^{-1}) at reference temperature (T_{ref}, K), R is the universal gas constant (0.008314 kJ K^{-1} mol^{-1}), $E_{a,O2}$ and $E_{a,CO2}$ are activation energy (kJ mol^{-1}), T is the storage temperature (K), and T_{ref} is the reference temperature (i.e. average of the storage temperatures = 283 K). A secondary model was built by substituting R_{O2} and R_{CO2} in Eqn. 1 and 2, with Eqn. 3 and 4, respectively:

$$y_{O_2} = y^{i}_{O_2} - \left[R^{i}_{O2,ref} \times e^{\left[\frac{-E_{a,O2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]}\right]\frac{W}{V_f}(t-t_i)\times 100 \quad (5)$$

$$y_{CO_2} = y^{i}_{CO_2} + \left[R^{i}_{CO2,ref} \times e^{\left[\frac{-E_{a,CO2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]}\right]\frac{W}{V_f}(t-t_i)\times 100 \quad (6)$$

where, $y^{i}_{O_2}$ = 20.6%, $y^{i}_{CO_2}$ = 0%, t is the elapsed time (hr) during RR measurement, and parameter estimates of $R^{i}_{O2,ref}$, $R^{i}_{CO2,ref}$, $E_{a,O2}$ and $E_{a,\ CO2}$ were estimated using solver on Microsoft Excel (Microsoft Office 2003, USA). Data were further analysed using Statistica software (Statistical 10.0, Statsoft, USA).

The models (Eqn. 5 and 6) appropriately described the influence of temperature on RR for both cultivars as shown by the high R^2 values between 94.9 to 96.7% for R_{O2} and R_{CO2}

(Table 2). The experimental R_{O2} and R_{CO2} data at 5, 10 and 15 °C for both cultivars and the predicted values are presented in fig. 5. The scatter plot in fig. 6 shows a good relationship between experimental and predicted data for the measured CO_2 concentration. The distribution of residuals was normal with the Kolgomorov-Smirnov test of d = 0.13 and Lilliefors of $p < 0.05$ at a significant level of 95% as shown in fig. 7. This observation indicates that the model describing the effect of temperature on respiration in this study was not biased. Table 2 summaries the constant parameters and other relevant statistical data estimated using Eqn. 5 and 6.

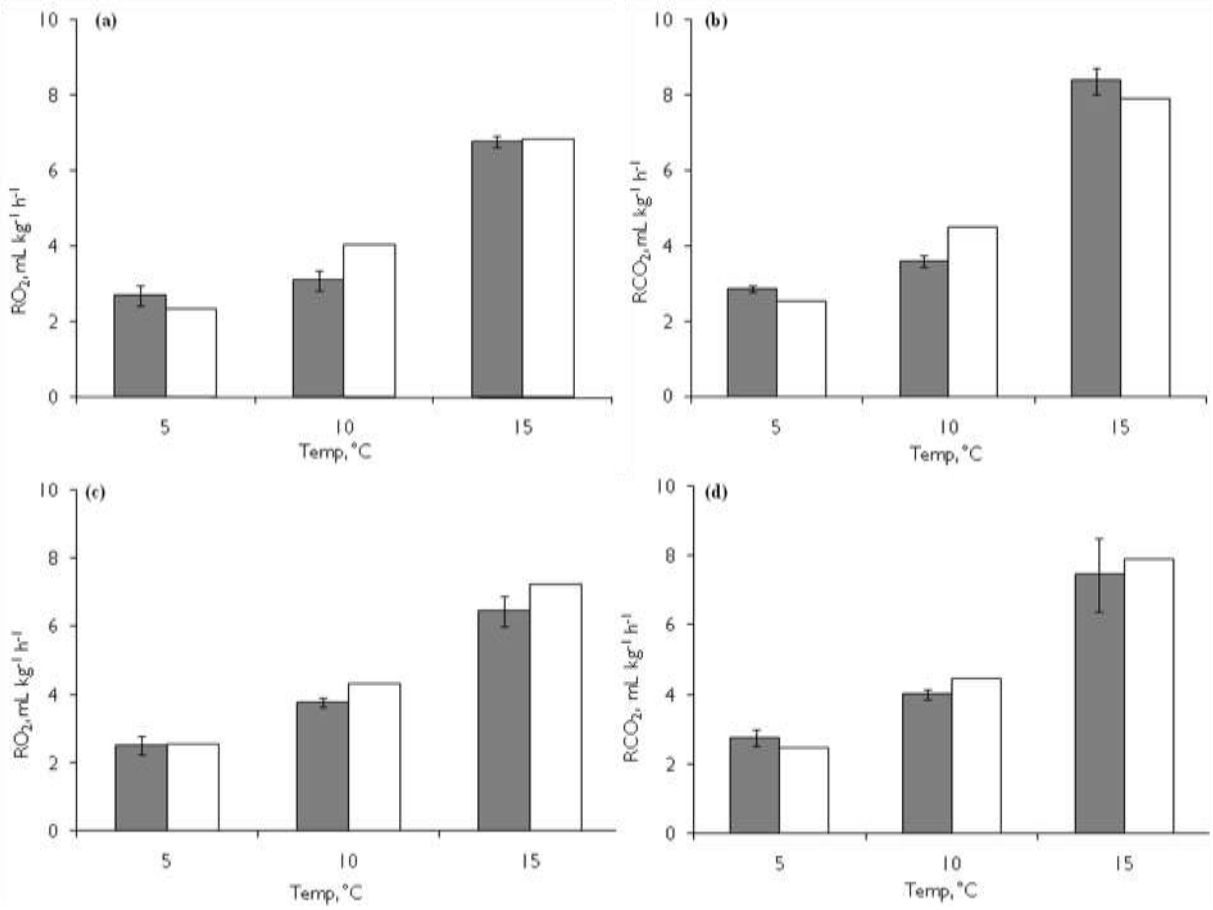


Figure 5 Relationship between experimental respiration rate (R_{O2} and R_{CO2}) and those predicted values at 5, 10 and 15 °C for both cultivars using Arrhenius-type equation (Eq. 3 and 4): (a) and (b) represents R_{O2} and R_{CO2} cv. 'Acco'; and (c) and (d) represents R_{O2} and R_{CO2} cv. 'Herskawitz', respectively; the shaded bars are experimental data and unshaded are predicted values.

Furthermore, the estimated $R^{i}_{O2,ref}$, $R^{i}_{CO2,ref}$ values of 4.03 and 4.52 mL kg^{-1} h^{-1} for cv. 'Acco', and 4.32 and 4.46 mL kg^{-1} h^{-1} for cv. 'Herskawitz', respectively, were close to the experimental R_{O2} and R_{CO2} values of 3.11 (± 0.26) and 3.64 (± 0.14) mL kg^{-1} h^{-1} for cv. 'Acco', and 3.78 (± 0.12) and 4.02 (± 0.15) mL kg^{-1} h^{-1} for cv. 'Herskawitz', respectively. This

outcome implies that at least a single experimental respiration data point is sufficient to predict the RR at other temperatures, thereby, reducing the tedious process of data accumulation towards mathematical prediction. Similarly, the estimate activation energy ($E_{a, O2}$ and $E_{a, CO2}$) values of 69.61 to 71.25 KJ mol^{-1} and 75.78 to 77.24 KJ mol^{-1}, respectively, reported in this study were in accord with the normal range of (29 to 92.9 KJ mol^{-1}) reported in literature for fruits and vegetables exposed to air (Exama *et al.*, 1993).

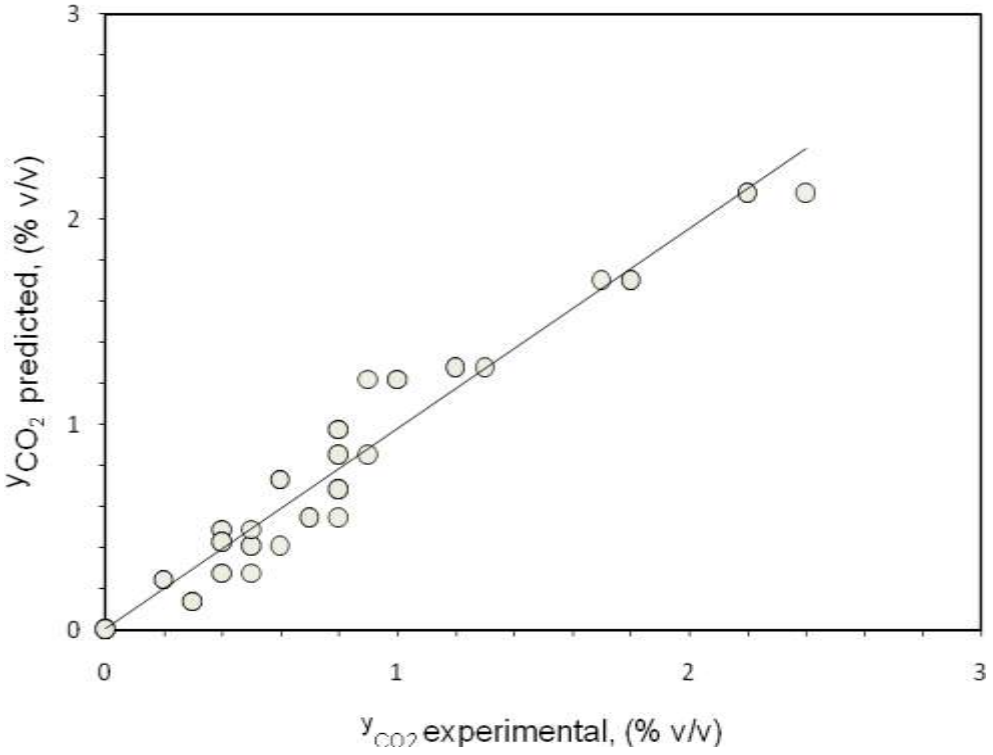


Figure 6 Relationship between CO_2 concentration measured experimentally, and the values predicted using Eqn. 5 and 6 combining all storage conditions.

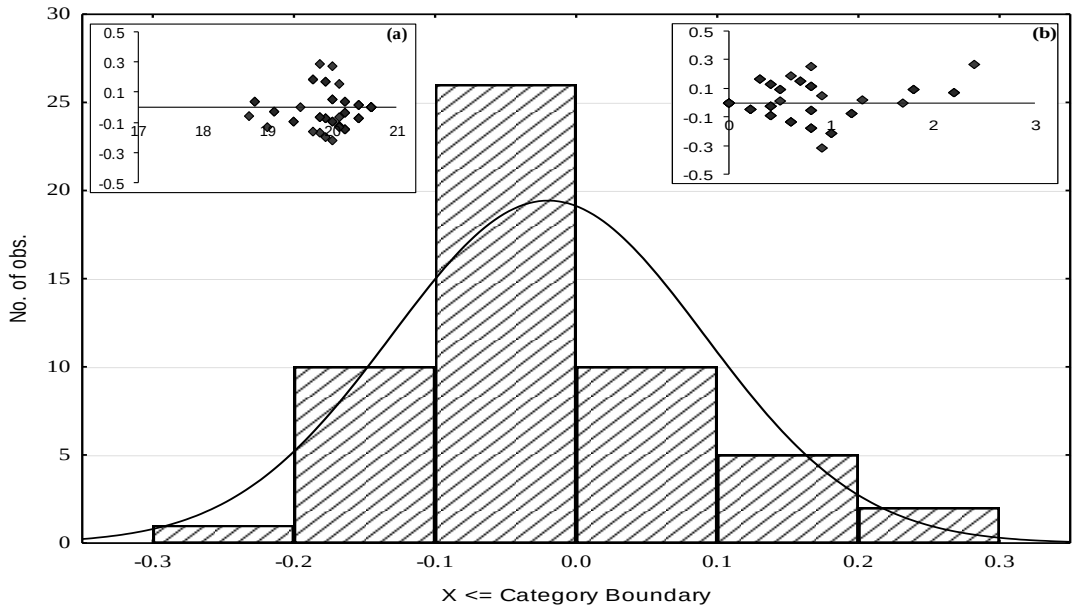


Figure 7 The distribution of residuals obtained from fitting Eqn. 5 and 6 for cv. 'Acco'. The inside graph shows the pattern of residuals of O_2 and CO_2 versus experimental values of O_2 (a) and CO_2 (b) concentrations.

Combined effect of time and temperature on respiration rate of pomegranate arils

The relationship between RR and time as expressed by a power function, which describes the time effect on respiration rate in Eqn. 7 and 8 (Uye & Yashiro, 1988) was combined with Arrhenius-type model, which describes the effect of temperature on respiration rate (Eqn. 3 and 4):

$$R_{O_2} = at^b \tag{7}$$

$$R_{CO_2} = at^b \tag{8}$$

where, t is storage time in days, a and b are the model constants. Combining the above equations 3 to 5, and 7 and 4 to 8, respectively to describe both the influence of temperature and time on the respiration rate of pomegranate arils:

$$R_{O_2} = R^i_{O_2,ref} \times e^{\left[\frac{-E_{a,O_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} + at^b \tag{9}$$

$$R_{CO_2} = R^i_{CO_2,ref} \times e^{\left[\frac{-E_{a,CO_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} + at^b \tag{10}$$

A secondary model was built by substituting R_{O2} and R_{CO2} in Eqn. 1 and 2, with Eqn. 9 and 10, respectively:

$$y_{O_2} = y^i_{O_2} - \left[R^i_{O_2,ref} \times e^{\left[\frac{-E_{a,O_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} + at^b\right]\frac{W}{V_f}(t - t_i) \times 100 \quad (11)$$

$$y_{CO_2} = y^i_{CO_2} + \left[R^i_{CO_2,ref} \times e^{\left[\frac{-E_{a,CO_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} + at^b\right]\frac{W}{V_f}(t - t_i) \times 100 \quad (12)$$

where, parameter estimates of $R^i_{O2,ref}$, $R^i_{CO2,ref}$, $E_{a,O2}$ and $E_{a,\ CO2}$ previously obtained from Eqn. 5 and 6 were incorporated into Eqn. 11 and 12. Model constants a, and b were estimated for each tested temperatures, all data obtained at all combinations of temperature were used to estimate the values of global a, and b for each cultivar, so that RR can be predicted at any temperature. Using solver Microsoft Excel (Microsoft Office 2003, USA), and data was further analysed using Statistica software (Statistical 10.0, Statsoft, USA).

Table 3 summarises the estimates of the constants and relevant statistical data. The model fitted well with the experimental data as shown by the high R^2 of 87.11 to 97.66% obtained across all tested temperatures for both R_{O2} and R_{CO2}. The parameter estimates for global a, and b has R^2 value ranging above 98.4%. Respiration rate for pomegranate arils were observed to change with time at the various storage temperatures of 5, 10 and 15 °C, this was adequately described by the Arrhenius-type equation as summarized with the continuous line fitted into fig. 1. Similarly, Iqbal *et al.* (2009a) reported the increase in RR of sliced and whole mushrooms with storage time and this effect was well described by an Arrhenius-type model. Biological reactions, such as respiration, generally increase 2 to 3-fold for every 10 °C rise in temperature (Fonseca *et al.*, 2002). This was consistent with the observation in this study with about 3-fold increase in RR from 5 to 15 °C, highlighting the significance of temperature. Although fresh and fresh-cut MA-packaged produce are stored at low temperatures, monitoring the effect of time and temperature along the supply-chain on produce should be taken into consideration in order to avoid the abuse of the MAP.

Table 3 Parameter estimates from model (Eqn. 9 and 10) describing the influence of storage time on respiration rate of pomegranates at each temperature tested and relevant statistical data

Pomegranate arils	Estimated parameters	RO_2				RCO_2			
		5 °C	10 °C	15 °C	Global	5 °C	10 °C	15 °C	Global
cv. Acco	a	-0.09	0.03	3.19	0.66	-0.01	0.02	5.43	1.65
	b	1.64	3.22	1.0	1.38	2.97	4.37	1.0	1.16
	R^2 (%)	89.6	92.1	97.7	98.9	88.7	93.3	95.7	98.4
cv. Herskawitz	a	-0.05	-0.02	0.18	0.01	0.09	0.02	0.26	0.04
	b	2.02	1.0	2.77	3.78	1.0	3.95	2.81	3.23
	R^2 (%)	93.8	92.7	94.9	99.5	91.7	87.1	95.9	99.2

Conclusion

Respiration rates were relatively higher for cv. 'Acco' in comparison to cv. 'Herskawitz' across all the experimental temperatures tested. This observation highlights the possible influence of physiological differences between cultivar responses to storage conditions. Temperature had the most significant impact on the RR of arils of both pomegranate cultivars (cv. 'Acco' and 'Herskawitz') and the RR were 3-4 folds significantly higher with increased temperature from 5 to 15 °C. The influence of time, and the interaction between temperature and time also had a significant influence on the RR of fresh arils. This highlights the importance of maintaining optimal cold-storage condition for fresh produce along the supply-chain. The RQ was dependent on both temperature and time as the RQ value increased with rising temperature from 5 to 15 °C towards the end of the storage time. An Arrhenius-type equation accurately predicted the effect of temperature on RR of fresh pomegranate arils. The power function equation combined with Arrhenius-type equation adequately predicted the influence of time and temperature on RR of fresh pomegranate arils for both cultivars. These models would be useful towards the design of appropriate modified atmosphere package for freshly processed pomegranate arils.

References

Al-Mughrabi, M.A., Bacha, M.A. & Abdelrahman, A.O. (1995). Effect of storage temperature and duration on fruit quality of three pomegranate cultivars. *Journal of King Saudi University*, **7**, 239-248.

Artés, F., Villaescusa, R. & Tudela, J.A. (2000). Modified atmosphere packaging of pomegranate. *Journal of Food Science*, **65**, 1112-1116.

Caleb, O.J., Opara, U.L. & Witthuhn, C. (2012). Modified atmosphere packaging of pomegranate fruit and arils: A review. *Food and Bioprocess Technology*, **5**, 15-30.

Cliffe-Byrnes, V. & O'Beirne, D. (2007). Effect of gas atmosphere and temperature on the respiration rate of whole and sliced mushrooms (Agaricus bisporus) implications for film permeability in modified atmosphere packages. *Journal of Food Science*, **72**, E197-E204.

Ersan, S., Gunes, G. & Zor, A.O. (2010). Respiration rate of pomegranate arils as affected by O_2 and CO_2, and design of modified atmosphere packaging. *Acta Horticulturae*, **876**, 189-196.

Exama, J.P., Arul, J., Lencki, R.W., Lee, L.Z. & Toupin, C. (1993). Suitability of plastic films for modified atmosphere packaging of fruits and vegetables. *Journal of Food Science* **58**, 1365-1370.

Farber, J.N., Harris, L.J., Parish, M.E., Beuchat, L.R., Suslow, T.V., Gorney, J.R., Garrett, E.H. & Busta, F.F. (2003). Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, **2**, 142-160.

Fawole, O.A., Opara, U.L. & Theron, K.I. (2011). Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa. *Food and Bioprocess Technology*, **5**, 2934-2940.

Fonseca, S.C., Oliveira, F.A.R. & Brecht, J.K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages. *Journal of Food Engineering*, **52**, 99-119.

Gil, M.I., Artes, F. & Toma-Barberan, F.A. (1996). Minimal processing and modified atmosphere packaging effects on pigmentation of pomegranate seeds. *Journal of Food Science*, **61**, 161-164.

Iqbal, T., Rodrigues, F.A.S., Mahajan, P.V. & Kerry, J.P. (2009a). Effect of time, temperature, and slicing on respiration rate of mushrooms. *Journal of Food Science*, **74**, E298-E303.

Iqbal, T., Rodrigues, F.A.S., Mahajan, P.V. & Kerry, J.P. (2009b). Mathematical modelling of O_2 consumption and CO_2 production rates of whole mushrooms accounting for the effect of temperature and gas composition. *International Journal of Food Science and Technology*, **44**, 1408-1414.

Kader, A.A., Zagory, D. & Kerbel, E.L. (1989). Modified atmosphere packaging of fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, **28**, 1-30.

Lakakul, R., Beaudry, R.M. & Hernandez, R.J., (1999). Modeling respiration of apple slices in modified atmosphere packages. *Journal of Food Science*, **64**, 105-110.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 174-185.

Mahajan, P.V., Oliveira, F.A.R. & Macedo, I. (2008). Effect of temperature and humidity on the transpiration rate of the whole mushrooms. *Journal of Food Engineering,* **84**, 281-288.

Mahajan, P.V., Oliveira, F.A.R., Montanez, J.C. & Frias, J. (2007). Development of user-friendly software for design of modified atmosphere packaging for fresh and fresh-cut produce. *Innovative Food Science and Emerging Technology*, **8**, 84-92.

Nei, D., Uchino, T., Sakai, N. & Tanaka, S. (2006). Prediction of sugar consumption in shredded cabbage using a respiratory model. *Postharvest Biology and Technology*, **41**, 56-61.

Oosterhaven J, Peppelenbos HW, editors. ISHS 2003. Proceedings of the 8th International Conference on Controlled Atmosphere; 2001 July 8-13; Rotterdam, The Netherlands.

Opara, U.L. & Al-Ani, M.R. (2010). Antioxidant components in fresh-cut and whole fruit and vegetables. *British Food Journal*, **112**, 797-810.

Opara, U.L., Al-Ani, M.R. & Al-Shuaibi, Y.S. (2009). Physico-chemical properties, vitamin C content, and antimicrobial properties of pomegranate fruit (*Punica granatum* L *Food and Bioprocess Technology*, **2**, 315-321.

Sadeghi, H. & Akbarpour, V. (2009). Liquid acrylic and polyamide plastic covering affect quality and storability of pomegranate (cv. Malas-e-Saveh). *Journal of Food Agriculture and Environment*, **7**, 405-407.

Torrieri, E., Cavella, S. & Masi, P. (2009). Modelling the respiration rate of fresh-cut Annurca apples to develop modified atmosphere packaging. *International Journal of Food Science and Technology*, **44**, 890-899.

Torrieri, E., Perone, N., Cavella, S. & Masi, P. (2010). Modelling the respiration rate of minimally processed broccoli (*Brassica rapa* var. *sylvestris*) for modified atmosphere package design. *International Journal of Food Science and Technology*, **45**, 2186-2193.

Uye, S. & Yashiro, M. (1988). Respiration rates of planktonic crustaceans from the Inland sea of Japan with special reference to the effects of body weight and temperature. *Journal of Oceanography Society Japan*, **44**, 47-51.

Viuda-Martos, M., Fernández-López, J. & Pérez-Álvarez, J.A. (2010.) Pomegranate and its many functional components as related to human health: A Review. *Comprehensive Reviews in Food Science and Food Safety*, **9**, 635-654.

Wang, Z.-W., Duan, H.-D. & Hu, C.-Y. (2009). Modelling the respiration rate of guava (*Psidium guajava* L.) fruit using enzyme kinetics, chemical kinetics and artificial neural network. *European Food Research and Technology*, **229**, 495-503.

Chapter 6

Evaluation of modified atmosphere packaging design parameters for pomegranate arils

CHAPTER 6

EVALUATION OF MODIFIED ATMOSPHERE PACKAGING DESIGN PARAMETERS FOR POMEGRANATE ARILS

Summary

This study evaluated the effects of passive-MAP engineering design parameters as a function of the amount of product (g), storage temperature (°C) and time (days) on pomegranate arils. Minimally processed pomegranate arils (75, 100 and 125 g) were packed in trays, heat-sealed with polylid film and stored at 5, 10 and 15 °C for 14 days. Packaged products were analysed for various physicochemical quality parameters viz headspace gas composition, weight loss, total soluble solids (TSS), titratable acidity (TA) (citric acid), pH, anthocyanin, aerobic mesophilic bacterial and fungal load (log CFU g^{-1}). At the highest storage temperature and product weight, O_2 concentration continuously decreased, reaching levels below the critical limit (2%) after 4 days, while at 5 °C this lower limit was not reached. CO_2 concentration inside all packages continuously increased over time. Based on the microbial evaluation, the shelf life of packaged 'Acco' and 'Herskawitz' was limited to 10, 7 and 3 days due to fungal growth ≥ 2 log CFU g^{-1} at 5, 10 and 15 °C, respectively. The aerobic mesophilic bacteria count at all storage conditions were in the range of 0.02 - 3.8 log CFU g^{-1}. It was not possible to achieve an equilibrium atmosphere using passive MAP due to very low respiration rate of the arils despite increasing product weight and storing at higher temperature. Storing for 3, 5 and 10 days at 15, 10 and 5 °C, respectively, yielded low O_2 and high CO_2 but storing beyond these times is not recommended in order to avoid anoxia and increased microbial load caused by excessive CO_2 concentration. Using the unsteady state equation this study showed good agreement between simulated results and experimental data (R^2 = 0.98).

Introduction

During the last decade, there has been a remarkable increase in the commercial production of pomegranates globally, due to the potential health benefits of the fruit. These benefits have been attributed to the high antioxidant contents and its anti-mutagenic, anti-hypertension, anti-inflammatory and anti-atherosclerotic activities against osteoarthritis, prostate cancer, heart disease and HIV-1 (Viuda-Martos *et al.,* 2010). Furthermore, pomegranate fruit is an excellent dietary source rich in organic acids, soluble solids, anthocyanins, vitamin C, fatty acids and mineral element, and has significant antimicrobial effects (Opara *et al*., 2009). Minimally processed "ready-to-eat" pomegranate arils have become popular due to convenience, health benefits and high value (Ayhan & Eştürk, 2009).

MAP has been used to extend the shelf life of minimally processed arils (Sepulveda *et al*., 2000; López-Rubira *et al*., 2005; Ayan & Eştürk, 2009; Caleb *et al*., 2012c). Sepulveda *et al*. (2000) observed that minimally processed pomegranate arils cv. 'Wonderful' were storable for 14 days at 4 ± 0.5 °C in semi-permeable films, however, this study was focused on the effect of different types of semi-permeable and antioxidant solutions on arils quality. López-Rubira *et al*. (2005) investigated the shelf life and overall quality of minimally processed pomegranate arils cv. 'Mollar Elche' treated with UV-C and packaged under passive-MAP in polypropylene (PP) baskets sealed with BOPP film and stored at 5 °C. They observed that the shelf life of arils was influenced by the harvested dates (earlier or late harvest). The report obtained on the effect of UV-C radiation on microbial growth was inconclusive, being that microbial count were not systematically reduced. Ayan & Eştürk (2009) studied the effect of various gas compositions in active-MAP on the shelf life and overall quality of minimally processed pomegranate arils stored at 5 °C. The authors observed no significant change in chemical and physical attributes of arils during cold storage, while aerobic mesophilic bacteria were in the range of 2.3 – 4.5 log CFU g^{-1}. However, these studies were based on empirical rather than systematic approach (Caleb *et al*., 2012a) as no MAP design was reported.

Modified atmosphere packaging (MAP) is a dynamic process of altering gaseous composition inside a package. It relies on the interaction between the respiration rate (RR) of the produce, and the transfer of gases through the packaging material, with no further control exerted over the initial gas composition (Fonseca *et al*., 2002; Mahajan *et al*., 2007).

MAP technology extends the shelf-life and maintains quality of fresh-cut produce by lowering the RR and retarding the development of physiological disorders and proliferation of spoilage pathogenic microbes (Caleb *et al*., 2012a). However, a quantitative description of RR of fresh produce via mathematical modelling is essential for the design of MAP (Fonseca *et al*., 2002; Mahajan *et al*., 2007). An inappropriately designed MAP system may be ineffective towards extending the storage life of packaged produce, if the desired or optimal atmosphere is not established rapidly inside the package (Oliveira *et al*., 2012). MAP should be carefully designed taking into account the amount of product, film permeability, and the time to achieve the optimum atmospheric equilibrium at a given temperature in order to maintain product quality (Oliveira *et al*., 2012; Caleb *et al*., 2012b). The objectives of this study were to determine the effect of MAP design parameters on the physicochemical and microbial attributes of mechanically processed pomegranate arils.

Materials and methods

Plant materials and preparation

Sweet-sour pomegranate (*Punica granatum* L.) fruit cvs. 'Acco' and 'Herskawitz' harvested manually during commercial harvest period were obtained from Robertson valley farm, Western Cape (33°48′0″S, 19°53′0″E) in South Africa and immediately stored in the pack-house (Houdoconstant Pack-house, Porterville, South Africa) at 5 °C. Black polypropylene (PP) trays with the dimensions of 15.5 x 11.5 x 3.5 cm^3 and POLYLID polymeric film (55µm with WVTR of 20 - 22 g m^{-2} day^{-1}; CO_2TR of 600 - 700 mL m^{-2} day^{-1}; and OTR 130 - 150 mL m^{-2} day^{-1} at 25 °C, 50% RH and 1 Bar) were provided by Blue Dot Packaging (Cape town, South Africa) and Barkai Polyon Ltd. (Kibbutz Barkai, Israel), respectively. Clear polyethylene terephthalate (PET) clamshell packs was used as control package with 420 µm thick and with the dimensions of 11.5 x 11.5 x 3.5 cm^3. It has a high barrier to water vapour and gas permeability.

Fruit processing and packaging procedures

Fruit were manually sorted to remove mechanically damaged fruit and the outer skins (husk) of healthy whole fruits were washed in sterilized water with 200 μLL^{-1} of sodium hypochlorite (NaOCl) solution. Arils were extracted from fruit using a commercial extraction unit (ArilSystems, Juran Metal Works, Israel). The extracted arils were collected on sterile conveyer belt in order to air dry and manually remove damaged arils. Each cultivar was processed separately and all processing was conducted at temperature below 10 °C. Air dried arils (with no surface moisture) were mixed together to ensure uniformity and weighed into lots of 75, 100 and 125 g (referred to as P-MAP 1, 2 and 3 application, respectively) into PP trays which had been, previously sterilized with ethylene oxide. PP trays were sealed with POLYLID films using a semi-automated heat sealing machine (Food Processing Equipment, South Africa). A label of 7.0 x 3.8 cm^2 area was placed onto each package film, to simulate the labels found in the retails market packages. Our control package was clamshell trays which are frequently used within the fresh-cut industry. At the pack-house packaged products were cooled down to 2 °C and transported in ice-packed cooler boxes fitted with data loggers (Gemini Data Loggers, United Kingdom) to the postharvest research laboratory. On arrival temperature inside the cooler boxes ranged between 3 – 4.5 °C. Packaged samples were stored at 5, 10 and 15 °C and 95 (±2) % RH for 14 d, and sampling was carried out on 0, 3, 7, 10, and 14 d of storage. Two packs were analyzed for each experimental condition on sampling days.

This study involved packaging fresh arils using two different factors namely amount of arils in the pack and storage temperature. A full factorial experimental design was used with 2 factors (amount of pomegranate arils and temperature) and 3 levels each (75, 100 and 125 g and 5, 10 and 15 °C, respectively). The entire experiment was replicated twice with 6 packages analyzed per sampling day.

Headspace gas analysis

Before packages were opened on each sampling day, gas composition inside the packages was determined using a gas analyzer with an accuracy of 0.5% (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Immediately after taking the gas analysis, packages were opened and

used for microbial and physicochemical analyses. Furthermore, additional 18 packages representing 2 replicates for each factor and level were used for gas analysis for the entire duration of the study. These packaging trays were fitted with rubber septum for the daily measurement of headspace gas composition throughout the duration of the study.

Weight loss

Initial and final weight of each packaged arils was measured using an electronic weighing balance (ML3002.E, Mettler Toledo, Switzerland). Weight loss was calculated according to the following equation:

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

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 analyzer (TA-XT Plus, Stable Micro Systems, Surrey, England) with a 35 mm diameter cylindrical probe. Firmness was expressed as maximum compression force (N). A test speed of 1.0 mm s^{-1} and distance of 9.5 mm were used. Average of 10 arils was measured for each experimental condition.

Colour

Aril colour was measured using a colour meter (Minolta Chroma Meter, CR-400, Japan). Before each measurement, the apparatus was calibrated against a white tile background (Illuminants C: Y = 93.6, x = 0.3133, y = 0.3195). Approximately 20 g of arils were placed into a Petri dish and the measurements were taken from 5 different points of the dish. Hunter colour parameters (L^* (lightness), a^* (redness and greenness), and b^* (yellowness and bluness) were measured. All analyses were presented as mean ± standard deviation (S.D.) of 10 replicates.

Titratable acidity, pH, and total soluble solids

Pomegranate arils of each pack were juiced separately using a LiquaFresh juice extractor (Mellerware, South Africa), and the juice was directly used for pH and total soluble solid (TSS) measurement using a pH meter (Crison, Barcelona, Spain) and digital refractometer expressed as °Brix (Atago, Tokyo, Japan), respectively. Titratable acidity (TA) was measured by titration to an end point of pH 8.2 using a Metrohm 862 compact titrosampler (Herisau, Switzerland). All values are presented as mean ± S.D.

Total anthocyanin content

The total juice anthocyanin content was determined by the pH-differential method using 2 buffer systems comprised of potassium chloride (pH 1, 0.025M) and sodium acetate (pH 4.5, 0.4M). One mL of sample juice was mixed with 9 mL of buffer and the absorbance was measured at 520 and 700 nm. Total anthocyanins were calculated as cyaniding-3-glucoside according to the following equation 1:

$$Total\ anthocyanins\ (mg\ 100L^{-1}) = \left[\frac{A \times MW \times DF \times 100}{\varepsilon \times 1}\right] \quad (2)$$

where A = (A520 – A 700) pH 1 – (A520 – A700) pH 4.5; MW (molecular weight) = 449.2 g mol-1 for cyaniding-3-glucoside; DF = dilution factor; 1 = pathlength in cm; ε = 26900 molar extinction coefficient. All analyses were done as 4 replicates (*n* = 4).

Microbial quality

Microbiological stability of samples was screened by total plate count, for aerobic mesophilic bacteria count plate count agar (PCA) was used and for the yeast and mould counts potato dextrose agar (PDA) acidified with 10% tartaric acid. Packages were opened under sterile conditions, and 10 g of each sample was obtained aseptically and homogenized with 90 ml of sterile physiological solution. Further 3-fold dilutions were prepared using 1.0 mL of diluents into 9 .0 mL of PS. In order to enumerate microbial load 1.0 mL of each dilution was pour-plated in triplicate onto appropriate media, PCA for aerobic mesophilic bacteria and PDA

for yeast and molds. Plates for aerobic mesophilic bacteria were incubated at 37 °C for 2 d and at 25 °C for 3 – 5 d for yeast and molds. The results were presented as log CFU g^{-1}.

MAP design

MAP design for fresh produce requires an integrated model incorporating produce respiration rate as a function of temperature and gas composition, amount of product, package geometry and size, package gas transmission rate as a function of temperature, as well as other produce characteristics (Mahajan *et al*., 2007). Based on the assumption that there is no gas stratification inside the package and total pressure is constant, the unsteady-state equations describing behaviour of MAP system during the process of passive modification within a package are given below in Eqs. (3) and (4):

$$V_f \times \frac{\mathrm{d}(y_{O_2})}{\mathrm{d}t} = \frac{P_{O_2}}{e} \times A \times \left(y_{O_2}^{out} - y_{O_2}\right) - R_{O_2} \times M \quad (3)$$

$$V_f \times \frac{\mathrm{d}(y_{CO_2})}{\mathrm{d}t} = \frac{P_{CO_2}}{e} \times A \times \left(y_{CO_2}^{out} - y_{CO_2}\right) + R_{CO_2} \times M \quad (4)$$

where V_f is the headspace (free volume) in the package, y is the gas concentration (in molar fraction), e is the thickness of polymeric film, P is the permeability of the package expressed in volume of gas exchanged per unit time and area, and weight of the product is M; and R is the respiration rate (RR) expressed in volume of gas generated/consumed per unit time; the subscripts O_2 and CO_2 refer to oxygen and carbon dioxide, respectively. In order to gain the understanding of the effect time, so that RR can be predicted at any temperature the global model equations from previous study by Caleb *et al.* (2012b) and shown in Eqn. 5 and 6 was applied. Table 1 summarises the estimates of the constants and relevant statistical data.

$$\mathrm{R}_{O_2} = R_{O_2,ref}^{i} \times e^{\left[\frac{-E_{a,O_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} + \mathrm{at}^{\mathrm{b}} \quad (5)$$

$$\mathrm{R}_{CO_2} = R_{CO_2,ref}^{i} \times e^{\left[\frac{-E_{a,CO_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} + \mathrm{at}^{\mathrm{b}} \quad (6)$$

where, T_{ref} is the Reference temperature, 283.15 K; R_{O2} and R_{CO2} is respiration rate, mL kg^{-1} hr^{-1}; t is storage time, days; R is the universal gas constant (0.008314 kJ K^{-1} mol^{-1}), $E_{a,O2}$ and $E_{a,CO2}$ are activation energy (kJ mol^{-1}), T is the storage temperature (K). Eqn. 3 and 4 were used to predict package O_2 and CO_2 atmosphere during storage period.

Statistical analysis

Pareto analysis was used with two factors (amount of arils and temperature) at three levels of temperatures 5, 10 and 15 °C at 95% confidence interval to assess the effects of amount of arils and temperature, and the interaction between these factors on the gas concentration profile of pomegranate arils. The experimental data obtained were treated with one-way analysis of variance (ANOVA) at 95% confidence interval to evaluate the effect of amount of pomegranate arils, storage time, temperature and their interaction on the quality attributes measured. Least significant difference (LSD) and Tukey Post-hoc tests were performed to identify specific differences in factor levels. All experimental data were analysed using Statistical software (Statistical 10.0, Statsoft, USA).

Table 1 Parameter estimates from model (Eqn. 5 and 6) describing the influence of temperature and time on respiration rate (Caleb *et al.*, 2012b)

Pomegranate	$R^{i}O_{2,ref}$	$R^{i}CO_{2,ref}$	Ea, O_2	Ea, CO_2	R^2, O_2	R^2, CO_2	Global model			
	[mL $kg^{-1}h^{-1}$]		[kJ mol^{-1}]		[%]	[%]	*a*, RO_2	*b*, RO_2	*a*, RCO_2	*b*, RCO_2
Fresh arils										
Acco	4.03	4.52	71.25	75.78	94.9	95.4	0.66	1.38	1.65	1.16
Herskawitz	4.32	4.46	69.61	77.24	95.4	96.7	0.01	3.78	0.04	3.23

Results

Package headspace gas composition

Predicted gas compositions at higher temperature with highest product weight reached anoxia level after 40 and 80 hr for 15 and 10 °C, respectively. Steady state concentrations of 18% O_2 and 5% CO_2 were achieved after day 6 at 5 °C, but this was not enough to create the optimal modified atmosphere of 5% O_2 and 5% CO_2 within the package. While no equilibrium state was observed at 15 °C, it was observed that CO_2 increases rapidly during storage once O_2 drops below critical limit. Furthermore, experimental data was in line with the predicted packaged atmosphere as shown in Fig. 1. For example, in the 125 g (P-MAP 3), package O_2 concentration reached below 2% after day 5 and 3 at 10 and 15 °C, respectively, while samples at 5 °C O_2 was not below 2% throughout the study. On the other hand, CO_2 levels increased significantly during storage for all packaging conditions. Based on Pareto analysis chart, changes in headspace gas composition inside the packs were significantly influenced by the temperature ($p < 0.05$), while amount of product at a given time did not show any significant effect for both cultivars (Fig. 2). Headspace O_2 content significantly decreased over time in packages at the different storage temperature up to day 14, without reaching an equilibrium concentration.

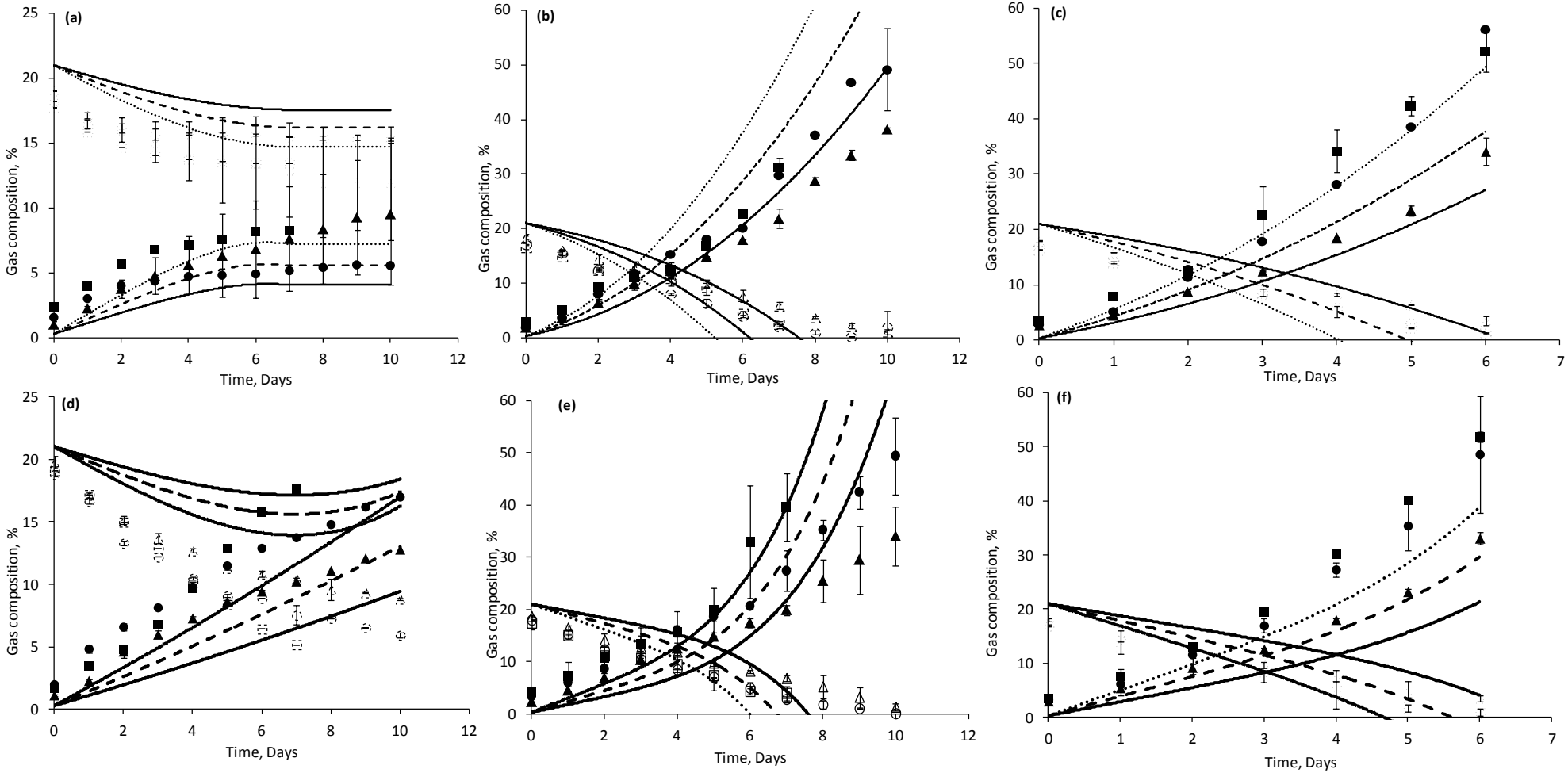


Figure 1 Examples of gas composition profiles for predicted and experimental data at (a) 5 °C; (b) 10 °C; (c) 15 °C for cv. 'Acco' and (d) 5 °C; (e) 10 °C; (f) 15 °C for cv. 'Herskawitz', at the different weight of arils. The amount of arils: ▲, 75 g; •, 100 g; ■, 125 g; shaded and unshaded makers, CO_2 and O_2, respectively.

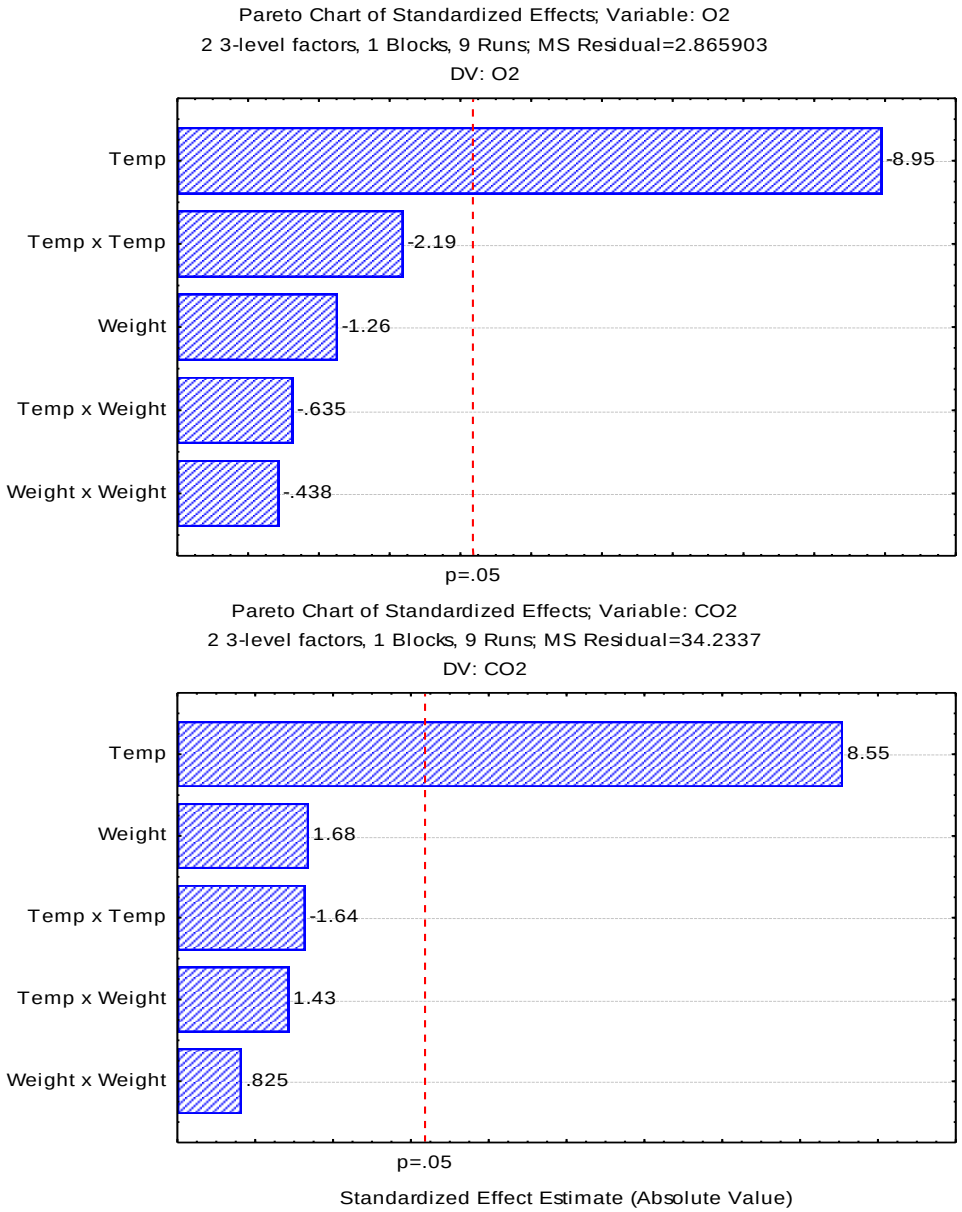


Figure 2 Effect of storage temperature and pomegranate arils' weight cv. 'Acco' on O_2 and CO_2 concentration profiles. *Similar results were observed for cv. 'Herskawitz' figure not shown.

Physical quality

Weight loss did not exceed 0.5 and 0.8% for cv. 'Acco' and 'Herskawitz' at 5 °C; 0.7 and 0.9% at 10 °C; and 2.1 and 1.9% at 15 °C (Fig. 3) in passive-MA packages. While, in the control clamshell packages weight loss did not exceed 0.02, 0.1 and 0.08% at 5, 10, and 15 °C, respectively in both cultivars over the storage period, however, increase in net weight of product occurred throughout the storage period.

The interaction of arils weight, storage temperature and time, had a significant effect on firmness of arils ($p \leq 0.05$), although there was no significant difference between P-MAP applications until day 10. Firmness at day 0 was 76.1 ± 5.1 N and 85.6 ± 8.4 N for cv. 'Acco' and 'Herskawitz', and did not exceed 77.5 ± 7.4 N and 102.4 ± 7.6 N, respectively, at day 14 (Table 2). Firmness of pomegranate arils packaged in control decreased over storage period by about 11%. No significant differences were found in fresh arils under the P-MAP applications throughout the 14 days at 5 °C.

Colour characteristics of pomegranate arils cv. 'Acco' and 'Herskawitz' varied over time with an average L^* values ranging from 39.6 to 26.2 for cv. 'Acco' and 41.6 to 30.0 for cv. 'Herskawitz'. While a^* ranged from 29.3 to 19.3 for cv. 'Acco' and 33.9 to 22.6 for cv. 'Herskawitz', and, b^* ranged from 19.7 to 12.3 for cv. 'Acco' and 19.0 to 12.9 for cv. 'Herskawitz' across all storage conditions (Table 3). A comparison between the two cultivars shows that cv. 'Herskawitz' had better colour stability than 'Acco'. Based on the overall analysis of variance and Tukey Post-hoc test, the effect of P-MAP application and storage time had no significant effect colour parameters L^* (lightness), a^* (redness) and b^* (yellowness) ($p > 0.05$) which are used as indicators of colour stability. Although there were pockets of fluctuations in lightness, redness and yellowness values, these observed changes were not consistent throughout the storage period. However, the interaction of storage temperature (15 °C) and time had a significant effect on colour parameters within a given P-MAP application, but there was no significant difference between the different weights applied in this study.

Table 2 Effect of produce weight, storage temperature and days on pomegranate arils firmness

Cultivar	Treatment (g)	Temp. °C	Firmness (N)				
			Day 0	Day 3	Day 7	Day 10	Day 14
'Acco'							
	P-MAP1	5	76.10 ± 5.1^{A}a	77.22 ± 7.1^{A}a	78.12 ± 11.3^{A}a	73.49 ± 9.79^{A}a	71.40 ± 12.9^{A}a
		10	76.10 ± 5.1^{A}a	70.58 ± 9.2^{A}a	76.90 ± 8.0^{A}a	73.94 ± 7.8^{A}a	75.93 ± 8.1^{A}a
		15	76.10 ± 5.1^{A}a	73.24 ± 12.1 ^{A}a	77.77 ± 12.0^{A}a	82.61 ± 13.1^{A}a	77.25 ± 14.2^{A}a
	P-MAP2	5	76.10 ± 5.1^{A}a	69.62 ± 7.3^{A}a	76.19 ± 9.9^{A}a	74.58 ± 9.7^{A}a	76.72 ± 7.0^{A}a
		10	76.10 ± 5.1^{A}a	67.84 ± 14.5^{AB}a	78.54 ± 12.6^{A}a	72.07 ± 6.9^{AB}a	76.16 ± 10.9^{A}a
		15	76.10 ± 5.1^{A}a	61.84 ± 6.1^{B}b	78.66 ± 6.8^{A}a	69.63 ± 7.4^{B}a	77.50 ± 7.4^{A}a
	P-MAP3	5	76.10 ± 5.1^{A}a	69.90 ± 7.9^{AB}a	76.15 ± 8.6^{A}a	70.40 ± 6.7^{A}a	75.43 ± 8.9^{A}a
		10	76.10 ± 5.1^{A}a	69.25 ± 8.7^{AB}a	72.78 ± 7.0^{A}a	71.42 ± 4.7^{B}b	69.91 ± 11.0^{A}ab
		15	76.10 ± 5.1^{A}a	70.71 ± 12.6^{A}a	75.84 ± 6.0^{A}a	75.06 ± 5.2^{A}a	75.72 ± 7.5^{A}a
	Control	5	76.10 ± 5.1^{A}a	67.84 ± 3.1^{B}b	68.94 ± 8.2^{A}ab	68.63 ± 4.4^{B}b	67.50 ± 2.4^{B}ab
		10	76.10 ± 5.1^{A}a	69.90 ± 7.9^{AB}b	74.90 ± 8.70^{A}a	70.40 ± 6.7^{A}a	Decay visible
		15	76.10 ± 5.1^{A}a	70.25 ± 9.7^{AB}a	69.25 ± 8.8^{A}a	Decay visible	Decay visible
'Herskawitz'							
	P-MAP1	5	85.55 ± 8.4^{A}a	85.51 ± 12.5^{A}a	85.81 ± 11.9^{A}a	73.60 ± 13.8^{AB}ab	93.29 ± 17.7^{A}c
		10	85.55 ± 8.4^{A}a	82.21 ± 18.1^{A}a	93.07 ± 20.6^{A}a	87.41 ± 20.7^{B}ab	91.55 ± 17.2^{A}bc
		15	85.55 ± 8.4^{A}a	79.19 ± 11.4^{A}a	96.35 ± 8.7^{A}ab	98.72 ± 10.2^{B}bc	103.36 ± 7.6^{B}d
	P-MAP2	5	85.55 ± 8.4^{A}a	78.50 ± 11.2^{A}a	90.01 ± 12.1^{A}a	86.56 ± 13.7^{B}a	88.08 ± 9.0^{B}a
		10	85.55 ± 8.4^{A}a	83.52 ± 13.6^{A}a	87.25 ± 18.4^{A}a	89.97 ± 14.5^{B}a	89.57 ± 12.8^{AB}a
		15	85.55 ± 8.4^{A}a	82.56 ± 22.3^{A}a	95.32 ± 10.5^{A}a	98.11 ± 10.9^{B}a	100.24 ± 17.2^{B}a
	P-MAP3	5	85.55 ± 8.4^{A}a	81.91 ± 17.03^{A}a	90.57 ± 14.7^{A}a	87.43 ± 14.9^{B}a	93.60 ± 10.7^{A}a
		10	85.55 ± 8.4^{A}a	81.15 ± 17.1^{A}a	88.19 ± 12.5^{A}a	84.80 ± 15.5^{AB}a	86.31 ± 13.6^{AB}a
		15	85.55 ± 8.4^{A}a	87.94 ± 16.6^{A}a	94.20 ± 15.3^{A}a	98.36 ± 20.0^{B}a	97.32 ± 17.3^{A}a
	Control	5	85.55 ± 8.4^{A}a	74.73 ± 11.8^{A}a	89.40 ± 6.3^{A}ab	74.40 ± 11.6^{B}abc	68.85 ± 13.5^{B}c
		10	85.55 ± 8.4^{A}a	85.52 ± 13.9^{A}ac	88.91 ± 9.4^{A}ab	86.00 ± 6.6^{A}abc	Decay visible
		15	85.55 ± 8.4^{A}a	77.53 ± 21.3^{A}ac	87.3 ± 11.2^{A}ab	Decay visible	Decay visible

* For each column, similar upper case letters in superscript are not significantly different at $p < 0.05$ among the passive MAP treatments. For parameter in rows, similar lower case letters are not significantly different. **P-MAP1: 75 g; P-MAP2: 100 g; P-MAP3: 125 g; control: clamshell package

Table 3 Effect of produce weight, storage temperature and duration on changes in colour parameters of pomegranate arils

Cultivar	Application	Storage time	Temp. ^{o}C	*L**	*a**	*b**	Cultivar	*L**	*a**	*b**
'Acco'		**Day 0**		39.55 ± 5.7^{A}	27.75 ± 6.4^{A}	15.41 ± 2.8^{AB}	**'Herskawitz'**	37.61 ± 6.2^{A}	23.49 ± 5.3^{A}	14.09 ± 1.9^{A}
	P-MAP 1	**Day 3**	5	28.05 ± 9.3^{AB}	26.24 ± 2.7^{A}	13.3 ± 1.8^{A}		36.88 ± 9.9^{A}	30.06 ± 6.3^{A}	14.70 ± 2.1^{A}
			10	33.03 ± 2.4^{A}	25.87 ± 4.4^{A}	14.11 ± 1.3^{A}		38.86 ± 5.7^{A}	29.35 ± 7.0^{A}	16.10 ± 2.7^{A}
			15	37.89 ± 8.3^{A}	27.37 ± 6.8^{A}	19.66 ± 3.9^{AB}		30.85 ± 4.6^{A}	30.88 ± 6.7^{ABC}	15.67 ± 3.3^{A}
		Day 7	5	31.48 ± 6.1^{A}	24.47 ± 5.5^{A}	14.78 ± 3.4^{AB}		30.06 ± 9.6^{A}	25.10 ± 7.2^{A}	14.32 ± 1.3^{A}
			10	32.99 ± 4.8^{A}	21.76 ± 7.4^{A}	16.16 ± 1.1^{AB}		31.63 ± 5.7^{B}	26.29 ± 3.3^{A}	15.47 ± 2.7^{A}
			15	31.82 ± 5.0^{AB}	25.45 ± 7.2^{A}	16.16 ± 1.7^{AB}		31.14 ± 6.1^{AB}	29.26 ± 4.6^{A}	16.61 ± 3.1^{A}
		Day 10	5	32.48 ± 3.9^{A}	20.99 ± 6.2^{A}	14.15 ± 2.1^{AB}		32.84 ± 5.4^{A}	26.52 ± 5.1^{A}	14.58 ± 1.9^{A}
			10	28.99 ± 4.1^{B}	25.09 ± 3.9^{A}	15.2 ± 2.4^{AB}		36.63 ± 5.8^{A}	26.73 ± 7.4^{A}	17.79 ± 1.9^{AB}
			15	30.48 ± 4.9^{AB}	24.95 ± 6.5^{A}	15.66 ± 1.6^{AB}		31.74 ± 5.0^{AB}	28.86 ± 4.5^{AB}	16.81 ± 2.9^{AB}
		Day 14	5	35.32 ± 3.0^{AB}	20.54 ± 5.6^{A}	17.33 ± 0.7^{B}		36.98 ± 8.2^{A}	28.72 ± 2.9^{A}	15.62 ± 1.2^{A}
			10	31.96 ± 4.6^{AB}	21.34 ± 7.5^{A}	14.59 ± 1.2^{A}		41.58 ± 3.9^{ABC}	24.39 ± 10.7^{A}	17.89 ± 2.9^{A}
	P-MAP 2	**Day 3**	5	28.63 ± 4.8^{B}	27.73 ± 6.8^{A}	13.53 ± 3.0^{A}		34.52 ± 4.6^{A}	31.15 ± 3.3^{B}	14.83 ± 1.9^{A}
			10	26.22 ± 4.2^{B}	25.74 ± 2.8^{A}	14.1 ± 2.4^{AB}		34.73 ± 6.2^{A}	27.17 ± 6.4^{A}	13.79 ± 2.7^{A}
			15	30.96 ± 3.6^{A}	22.67 ± 2.7^{A}	14.93 ± 1.7^{AB}		31.94 ± 7.4^{A}	29.83 ± 2.9^{AB}	15.55 ± 1.8^{A}
		Day 7	5	30.63 ± 5.5^{AB}	22.32 ± 3.0^{A}	14.14 ± 1.6^{AB}		33.05 ± 7.4^{A}	25.88 ± 5.0^{A}	13.68 ± 1.9^{A}
			10	36.95 ± 4.5^{AB}	21.93 ± 7.3^{A}	16.78 ± 2.0^{AB}		27.95 ± 8.9^{A}	27.73 ± 2.1^{A}	14.75 ± 2.3^{A}
			15	27.85 ± 3.5^{B}	23.40 ± 3.6^{A}	14.26 ± 3.4^{AB}		33.17 ± 3.8^{A}	29.31 ± 4.7^{A}	16.29 ± 2.2^{AB}
		Day 10	5	29.67 ± 4.9^{AB}	20.75 ± 4.3^{A}	13.46 ± 2.6^{AB}		34.68 ± 4.1^{A}	23.39 ± 3.8^{A}	13.98 ± 0.7^{A}
			10	31.69 ± 3.6^{AB}	22.55 ± 4.4^{A}	15.42 ± 2.5^{AB}		32.16 ± 3.8^{A}	26.56 ± 3.4^{A}	14.53 ± 1.4^{A}
			15	27.44 ± 3.6^{B}	22.79 ± 2.5^{A}	13.26 ± 2.5^{A}		31.84 ± 4.1^{A}	25.97 ± 4.3^{A}	16.29 ± 2.2^{A}
		Day 14	5	31.29 ± 5.2^{A}	19.30 ± 7.0^{AB}	14.60 ± 2.5^{AB}		32.11 ± 7.3^{A}	23.81 ± 8.2^{A}	14.78 ± 2.1^{A}
			10	29.74 ± 6.6 ^{A}	23.61 ± 2.6^{A}	14.67 ± 1.4^{AB}		31.04 ± 7.9^{A}	26.63 ± 3.9^{A}	15.69 ± 1.8^{A}

* For each column, similar upper case letters in superscript are not significantly different at $p < 0.05$.

Table 3(continued)

Cultivar	Application	Storage time	Temp. ^{o}C	*L**	*a**	*b**	Cultivar	*L**	*a**	*b**
'Acco'		**Day 0**		39.55 ± 5.7^{A}	27.75 ± 6.4^{A}	15.41 ± 2.8^{AB}	**'Herskawitz'**	37.61 ± 6.2^{A}	23.49 ± 5.3^{A}	14.09 ± 1.9^{A}
	P-MAP 3	**Day 3**	5	33.61 ± 3.7^{A}	23.79 ± 1.9^{A}	13.78 ± 1.3^{A}		38.64 ± 6.9^{A}	33.95 ± 5.9^{AB}	16.37 ± 3.0^{A}
			10	32.22 ± 1.64^{B}	22.25 ± 8.9^{A}	13.12 ± 2.4^{A}		34.81 ± 4.8^{A}	29.29 ± 6.1^{A}	15.13 ± 2.5^{A}
			15	29.1 ± 2.6^{B}	24.54 ± 4.3^{A}	14.72 ± 1.2^{A}		36.24 ± 10.5^{A}	23.48 ± 3.6^{A}	15.00 ± 2.7^{A}
		Day 7	5	30.96 ± 4.4^{AB}	22.01 ± 2.1^{A}	13.39 ± 1.7^{A}		30.03 ± 7.3^{A}	25.89 ± 4.6^{A}	14.43 ± 2.3^{A}
			10	27.66 ± 1.9^{B}	22.58 ± 5.9^{A}	14.29 ± 1.7^{A}		32.29 ± 6.6^{A}	24.76 ± 5.8^{A}	14.55 ± 1.5^{A}
			15	33.49 ± 6.7^{A}	29.29 ± 5.8^{A}	17.20 ± 2.7^{AB}		31.79 ± 5.4^{A}	26.11 ± 3.6^{A}	15.57 ± 1.8^{A}
		Day 10	5	30.99 ± 5.6^{AB}	19.37 ± 5.8^{AB}	14.02 ± 3.9^{AB}		28.64 ± 3.1^{AB}	22.6 ± 2.8^{B}	12.74 ± 0.9^{B}
			10	25.85 ± 5.5^{B}	19.8 ± 3.7^{AB}	12.28 ± 1.6^{A}		34.62 ± 2.3^{B}	25.66 ± 5.3^{A}	14.72 ± 1.4^{A}
			15	31.65 ± 3.7^{AB}	24.29 ± 3.9^{A}	14. 03 ± 1.7^{A}		30.79 ± 4.6^{A}	25.78 ± 3.4^{A}	14.49 ± 1.6^{A}
		Day 14	5	33.69 ± 8.6^{AB}	19.32 ± 2.3^{AB}	15.72 ± 3.2^{AB}		31.55 ± 3.3^{A}	25.24 ± 6.9^{A}	13.59 ± 1.9^{A}
			10	28.32 ± 5.3^{AB}	21.54 ± 1.9^{A}	14.09 ± 2.1^{A}		34.64 ± 3.4^{A}	25.23 ± 3.6^{A}	16.06 ± 3.8^{A}
	Control	**Day 3**	5	23.61 ± 5.2^{B}	23.19 ± 0.8^{A}	14.58 ± 2.5^{AB}		30.13 ± 7.1^{A}	24.06 ± 3.7^{A}	12.9 ± 1.3^{B}
			10	30.42 ± 4.6^{AB}	25.96 ± 2.9^{A}	14.53 ± 2.6^{AB}		36.27 ± 6.5^{A}	25.55 ± 3.3^{A}	12.99 ± 1.4^{A}
			15	31.76 ± 4.1^{AB}	22.52 ± 1.7^{A}	13.3 ± 1.5^{A}		36.85 ± 4.1^{A}	27.24 ± 4.7^{A}	15.37 ± 1.7^{A}
		Day 7	5	29.42 ± 5.7^{AB}	22.34 ± 1.8^{A}	13.09 ± 1.3^{A}		31.38 ± 4.7^{A}	25.07 ± 5.4^{A}	14.25 ± 1.9^{A}
			10	30.96 ± 6.2^{AB}	20.42 ± 7.0^{A}	13.78 ± 1.9^{A}		35.69 ± 5.6^{A}	23.15 ± 4.9^{A}	15.86 ± 2.7^{A}
			15	Visible decay in package				Visible decay in package		
		Day 10	5	30.99 ± 5.6^{AB}	19.37 ± 5.8^{AB}	15.01 ± 3.0^{AB}		34.59 ± 5.3^{A}	28.40 ± 6.5^{AB}	17.03 ± 2.0^{AB}
			10	32.31 ± 3.6^{AB}	20.94 ± 3.4^{AB}	15.88 ± 2.5^{AB}		37.32 ± 2.9^{AB}	31.28 ± 7.8^{AB}	18.98 ± 1.7^{AB}
			15	Visible decay in package				Visible decay in package		
		Day 14	5	30.01 ± 3.6^{AB}	17.65 ± 1.9^{B}	14.05 ± 2.8^{AB}		36.03 ± 6.2^{B}	27.61 ± 6.3^{A}	17.38 ± 1.9^{AB}
			10	Visible decay in package				Visible decay in package		
			15	Visible decay in package				Visible decay in package		

* For each column, similar upper case letters in superscript are not significantly different at $p < 0.05$; **P-MAP1: 75 g; P-MAP2: 100 g; P-MAP3: 125 g; control: clamshell package.

Changes in pH, total soluble solids and total titratable acidity

Table 4 shows chemical parameters of fresh and modified atmosphere packaged pomegranate arils for both cultivars during storage. A comparison of both cultivars cv. 'Herskawitz' had a significantly higher TA and pH than cv. 'Acco', while cv. 'Acco' had a relatively higher TSS. The TSS/TA was influenced by storage time and temperature. Result shows that there was no significant effect of increased produce weight on TA, pH, and TSS of pomegranate arils throughout the duration of storage compared to control (clamshell package) storage ($p > 0.05$), with some few exceptions. However, the interaction of storage temperature and time had a significant effect on all chemical quality parameters evaluated ($p < 0.05$). There was a significant decrease in TTA on day 3 ($p < 0.05$), afterward stayed relatively unchanged over time for the rest of the storage period but significantly higher than day 3 TTA values.

Similarly, result showed that there was a significant decrease in TSS on day 3 ($p < 0.05$), afterward stayed relatively unchanged over time for the rest of the storage period. At 7 day of storage TSS of cv. 'Acco' was significantly influenced by storage temperature compared to cv. 'Herskawitz'. Furthermore, the changes in pH during storage for cv. 'Acco' increased from 3.80 to 4.12, while, cv. 'Herskawitz' ranged from 3.01 to 3.08. Although changes in pH were negligible, storage temperature and duration was found to have significant effect on the observed changes.

Total anthocyanin content

The amount of arils, storage temperature and duration, as well as the interaction the factors had significant effects on the total anthocyanin content ($p \leq 0.05$). A general trend of decrease in total anthocyanin content was observed as the storage time increased for all treatments (Table 5). Total anthocyanin content was within the range of 21.13 to 13.32 mg C3gE 100 mL^{-1} of pomegranate juice for cv. 'Acco', and 20.42 to 12.32 mg C3gE 100 mL^{-1} for cv. 'Herskawitz'. Pomegranate arils packaged in the control clamshell packages had a significantly lower value in comparison to those packaged under passive MAP at all storage temperatures. P-MAP 3 with the highest amount of arils had a relatively higher total anthocyanin contents than P-MAP 1 and 2 at the end of storage.

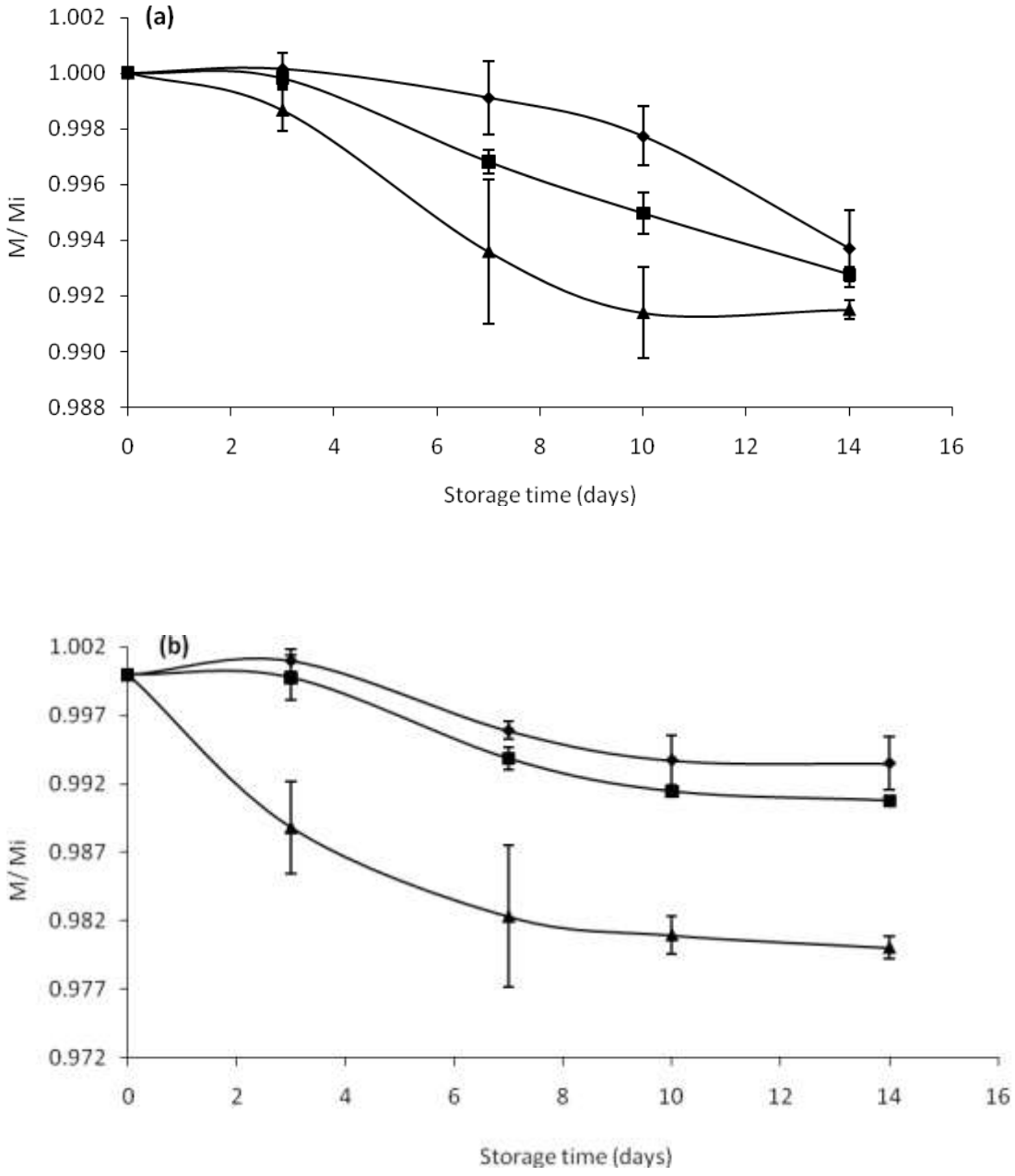


Figure 3 Effect of storage temperature on weight loss of pomegranate arils over time (a) cv. 'Acco' and (b) cv. 'Herskawitz'. The values were normalised with respect to the initial weight of pomegranate arils (Mi, g): ♦, 5 °C; ■, 10 °C; ▲, 15 °C

Table 4 Effect of passive MAP design factors on chemical parameters of two pomegranate cultivars

Cultivar		pH, TSS, TTA, and TSS:TTA												
	Parameter	Treatment	Temp	Day 0	Day 3	Day 7	Day 10	Day 14	Cultivar	Day 0	Day 3	Day 7	Day 10	Day 14
'Acco'	pH	P-MAP 1	5	3.80 ± 0.01^{A}a	3.92 ± 0.18^{A}ab	3.99 ± 0.0^{A}b	4.0 ± 0.06^{A}b	4.12 ± 0.01^{A}c	'Herskawitz'	3.01 ± 0.02^{A}a	3.11 ± 0.01^{A}a	3.02 ± 0.01^{A}a	3.03 ± 0.01^{A}a	3.08 ± 0.01^{A}a
			10	3.80 ± 0.01^{A}	3.96 ± 0.21^{A}a	4.04 ± 0.02^{A}b	3.94 ± 0.06^{A}a	4.06 ± 0.07^{A}a		3.01 ± 0.02^{A}a	3.07 ± 0.06^{A}ab	3.02 ± 0.01^{A}a	3.01 ± 0.01^{A}a	3.07 ± 0.01^{A}b
			15	3.80 ± 0.01^{A}a	3.97 ± 0.19^{A}a	3.63 ± 0.09^{B}b	3.65 ± 0.07^{B}b	Decay visible		3.01 ± 0.02^{A}a	3.09 ± 0.0^{A}a	3.07 ± 0.01^{A}a	3.01 ± 0.02^{A}a	Decay visible
		P-MAP 2	5	3.80 ± 0.01^{A}a	3.95 ± 0.20^{A}a	3.95 ± 0.04^{A}a	3.94 ± 0.07^{A}a	4.02 ± 0.06^{B}b		3.01 ± 0.02^{A}a	3.11 ± 0.01^{A}a	3.03 ± 0.06^{A}a	2.97 ± 0.01^{A}b	3.07 ± 0.01^{A}a
			10	3.80 ± 0.01^{A}a	3.99 ± 0.13^{A}a	3.94 ± 0.04^{A}a	3.97 ± 0.05^{A}ab	4.0 ± 0.01^{B}b		3.01 ± 0.02^{A}a	3.16 ± 0.01^{B}b	3.00 ± 0.03^{A}a	3.0 ± 0.01^{A}a	3.09 ± 0.03^{A}a
			15	3.80 ± 0.01^{A}a	3.77 ± 0.16^{A}a	3.91 ± 0.0^{B}b	3.65 ± 0.07^{B}c	Decay visible		3.01 ± 0.02^{A}a	3.05 ± 0.01^{A}a	3.01 ± 0.03^{A}a	2.95 ± 0.07^{A}a	Decay visible
		P-MAP 3	5	3.80 ± 0.01^{A}a	3.76 ± 0.22^{A}a	3.96 ± 0.06^{A}b	3.97 ± 0.02^{A}b	4.05 ± 0.01^{BC}c		3.01 ± 0.02^{A}a	2.96 ± 0.08^{A}a	2.99 ± 0.01^{A}a	3.01 ± 0.02^{A}a	3.05 ± 0.01^{A}a
			10	3.80 ± 0.01^{A}a	3.86 ± 0.25^{A}a	3.88 ± 0.21^{A}a	3.99 ± 0.07^{A}b	4.01 ± 0.08^{BC}b		3.01 ± 0.02^{A}a	3.01 ± 0.08^{A}a	2.96 ± 0.11^{A}a	3.02 ± 0.04^{A}a	3.06 ± 0.01^{A}a
			15	3.80 ± 0.01^{A}a	3.82 ± 0.03^{A}a	3.84 ± 0.18^{A}a	3.55 ± 0.07^{A}b	Decay visible		3.01 ± 0.02^{A}a	3.22 ± 0.04^{C}b	2.94 ± 0.07^{A}a	2.95 ± 0.07^{A}a	Decay visible
		Control	5	3.80 ± 0.01^{A}a	3.80 ± 0.03^{A}a	4.02 ± 0.18^{A}a	4.02 ± 0.18^{AB}a	4.12 ± 0.0^{A}a		3.01 ± 0.02^{A}a	3.30 ± 0.03^{A}b	3.02 ± 0.18^{A}a	3.02 ± 0.01^{A}a	3.07 ± 0.01^{A}a
			10	3.80 ± 0.01^{A}a	3.82 ± 0.07^{A}a	3.93 ± 0.09^{A}a	4.09 ± 0.11^{B}a	Decay visible		3.01 ± 0.02^{A}a	3.22 ± 0.07^{C}b	3.03 ± 0.19^{A}a	3.02 ± 0.01^{A}a	Decay visible
			15	3.80 ± 0.01^{A}a	3.83 ± 0.09^{A}a	3.96 ± 0.13^{A}a	Decay visible	Decay visible		3.01 ± 0.02^{A}a	3.31 ± 0.09^{C}b	2.96 ± 0.13^{A}a	Decay visible	Decay visible
	TSS	P-MAP 1	5	15.6 ± 0.01^{A}a	14.85 ± 0.92^{A}b	15.45 ± 0.07^{A}a	15.5 ± 0.00^{A}a	15.0 ± 0.14^{A}a		15.03 ± 0.01^{A}a	13.80 ± 0.14^{A}b	14.60 ± 0.57^{A}a	15.05 ± 0.21^{AB}a	15.20 ± 0.71^{A}a
			10	15.6 ± 0.01^{A}a	14.80 ± 0.85^{A}a	14.35 ± 0.21^{B}a	15.2 ± 0.42^{A}b	14.45 ± 0.21^{B}a		15.03 ± 0.01^{A}a	13.70 ± 0.14^{A}b	13.90 ± 0.85^{A}b	14.00 ± 0.85^{A}b	14.20 ± 0.28^{A}b
			15	15.6 ± 0.01^{A}a	14.90 ± 0.42^{A}a	14.15 ± 0.07^{B}b	14.75 ± 0.07^{A}a	Decay visible		15.03 ± 0.01^{A}a	13.85 ± 0.07^{A}b	13.75 ± 0.21^{A}b	13.93 ± 0.12^{A}b	Decay visible
		P-MAP 2	5	15.6 ± 0.01^{A}a	15.20 ± 0.71^{AB}a	14.80 ± 0.14^{C}b	15.4 ± 0.42^{A}a	15.05 ± 0.07^{C}c		15.03 ± 0.01^{A}a	14.35 ± 0.07^{B}b	14.70 ± 0.28^{A}b	15.30 ±0.14^{B}c	15.10 ± 0.28^{A}abc
			10	15.6 ± 0.01^{A}a	15.70 ± 0.0^{B}a	14.45 ± 0.07^{A}b	14.8 ± 0.57^{A}b	14.45 ± 0.07^{B}b		15.03 ± 0.01^{A}a	14.05 ± 0.21^{B}b	14.35 ± 0.64^{A}bc	14.75 ± 0.07^{A}bc	14.55 ± 0.21^{A}bc
			15	15.6 ± 0.01^{A}a	14.50 ± 1.56^{A}b	13.75 ± 0.78^{D}c	14.65 ± 0.07^{A}b	Decay visible		15.03 ± 0.01^{A}a	14.40 ± 0.0^{B}b	13.90 ± 0.28^{A}c	13.81 ± 0.59^{A}bc	Decay visible
		P-MAP 3	5	15.6 ± 0.01^{A}a	14.85 ± 0.21^{A}b	15.0 ± 0.14^{C}c	15.45 ± 0.21^{A}a	15.0 ± 0.0^{A}d		15.03 ± 0.01^{A}a	14.80 ± 0.0^{C}b	14.80 ± 0.28^{A}b	15.40 ± 0.14^{B}c	15.15 ± 0.07^{A}c
			10	15.6 ± 0.01^{A}a	13.40 ± 1.70^{A}b	14.7 ± 0.42^{A}c	14.95 ± 0.21^{A}c	14.45 ± 0.35^{B}c		15.03 ± 0.01^{A}a	14.70 ± 0.14^{C}b	14.40 ± 0.14^{A}b	14.65 ± 0.07^{A}b	14.60 ± 0.14^{A}b
			15	15.6 ± 0.01^{A}a	14.70 ± 0.14^{A}b	13.7 ± 0.57^{D}c	14.65 ± 0.07^{A}b	Decay visible		15.03 ± 0.01^{A}a	14.50 ± 0.14^{B}b	13.50 ± 0.85^{A}c	14.42 ± 0.59^{A}bc	Decay visible
		Control	5	15.6 ± 0.01^{A}a	15.03 ± 0.01^{C}b	15.50 ± 0.35^{A}a	15.05 ± 0.07^{A}c	14.95 ± 0.07^{AC}d		15.03 ± 0.01^{A}a	14.03 ± 0.01^{B}b	14.50 ± 0.35^{A}c	15.05 ± 0.07^{A}a	14.75 ± 0.07^{A}c
			10	15.6 ± 0.01^{A}a	13.97 ± 0.26^{D}b	13.85 ± 1.06^{BCD}b	14.8 ± 0.26^{A}bc	Decay visible		15.03 ± 0.01^{A}a	13.87 ± 0.46^{AB}b	13.85 ± 1.06^{A}b	14.80± 0.26^{A}b	Decay visible
			15	15.6 ± 0.01^{A}a	14.90 ± 0.14^{A}b	13.70 ± 0.85^{D}c	Decay visible	Decay visible		15.03 ± 0.01^{A}a	14.20 ± 0.14^{AB}b	13.70 ± 0.85^{A}b	Decay visible	Decay visible

Table 4 (continued)

Cultivar	Parameter	pH, TSS, TTA, and TSS:TTA												
		Treatment	Temp	Day 0	Day 3	Day 7	Day 10	Day 14	Cultivar	Day 0	Day 3	Day 7	Day 10	Day 14
'Acco'	TA	P-MAP 1	5	0.37 ± 0.01^{A}a	0.34 ± 0.03^{AB}a	0.38 ± 0.01^{A}a	0.38 ± 0.02^{A}a	0.38 ± 0.01^{A}a	'Herskawitz'	1.73 ± 0.01^{A}a	1.66 ± 0.01^{A}b	1.78 ± 0.08^{AB}a	1.84 ± 0.11^{A}ac	1.87 ± 0.03^{A}c
			10	0.37 ± 0.01^{A}a	0.33 ± 0.01^{AB}b	0.39 ± 0.01^{A}ac	0.42 ± 0.02^{A}c	0.41 ± 0.02^{A}c		1.73 ± 0.01^{A}a	1.65 ± 0.04^{A}b	1.77 ± 0.06^{AB}ab	1.79 ± 0.04^{A}ab	1.95 ± 0.04^{A}c
			15	0.37 ± 0.01^{A}a	0.35 ± 0.01^{AB}a	0.45 ± 0.0^{B}b	0.57 ± 0.01^{B}c	Decay visible		1.73 ± 0.01^{A}a	1.79 ± 0.11^{A}a	1.70 ± 0.01^{A}b	1.95 ± 0.07^{A}c	Decay visible
		P-MAP 2	5	0.37 ± 0.01^{A}a	0.36 ± 0.02^{AB}a	0.35 ± 0.01^{C}a	0.39 ± 0.02^{AC}a	0.39 ± 0.01^{A}a		1.73 ± 0.01^{A}a	1.75 ± 0.03^{A}a	1.82 ± 0.06^{B}a	1.91 ± 0.11^{A}b	1.83 ± 0.06^{A}a
			10	0.37 ± 0.01^{A}a	0.36 ± 0.01^{AB}a	0.43 ± 0.0^{D}b	0.52 ± 0.01^{C}c	0.46 ± 0.02^{B}b		1.73 ± 0.01^{A}a	1.70 ± 0.01^{A}b	1.83 ± 0.07^{B}c	2.00 ± 0.08^{A}c	1.87 ± 0.18^{A}c
			15	0.37 ± 0.01^{A}a	0.36 ± 0.05^{AB}a	0.52 ± 0.09^{BDE}b	0.58 ± 0.01^{BD}b	Decay visible		1.73 ± 0.01^{A}a	1.66 ± 0.06^{A}a	1.83 ± 0.06^{B}b	1.98 ± 0.07^{A}c	Decay visible
		P-MAP 3	5	0.37 ± 0.01^{A}a	0.35 ± 0.0^{AB}b	0.38 ± 0.01^{A}a	0.41 ± 0.05^{A}ab	0.40 ± 0.01^{A}a		1.73 ± 0.01^{A}a	1.72 ± 0.03^{A}a	1.87 ± 0.06^{B}b	1.89 ± 0.12^{A}b	1.83 ± 0.03^{A}b
			10	0.37 ± 0.01^{A}a	0.33 ± 0.02^{AB}b	0.42 ± 0.04^{ABD}a	0.50 ± 0.07^{AC}a	0.47 ± 0.06^{A}a		1.73 ± 0.01^{A}a	1.79 ± 0.04^{A}b	1.79 ± 0.01^{AB}b	2.00 ± 0.16^{A}c	1.96 ± 0.11^{A}c
			15	0.37 ± 0.01^{A}a	0.43 ± 0.08^{A}a	0.54 ± 0.01^{E}b	0.60 ± 0.02^{D}c	Decay visible		1.73 ± 0.01^{A}a	1.74 ± 0.03^{A}a	1.85 ± 0.02^{A}b	2.08 ± 0.01^{B}c	Decay visible
		Control	5	0.37 ± 0.01^{A}a	0.32 ± 0.02^{B}b	0.41 ± 0.09^{ABC}abc	0.43 ± 0.03^{A}c	0.36 ± 0.06^{A}ac		1.73 ± 0.01^{A}a	1.50 ± 0.11^{B}b	1.65 ± 0.03^{C}c	1.86 ± 0.11^{A}d	1.77 ± 0.08^{A}ad
			10	0.37 ± 0.01^{A}a	0.34 ± 0.04^{AC}a	0.38 ± 0.10^{ABCD}a	0.50 ± 0.05^{C}b	Decay visible		1.73 ± 0.01^{A}a	1.56 ± 0.15^{AB}b	1.90 ± 0.37^{ABC}abc	1.88 ± 0.01^{A}c	Decay visible
			15	0.37 ± 0.01^{A}a	0.31 ± 0.01^{BC}b	0.34 ± 0.03^{A}a	Decay visible	Decay visible		1.73 ± 0.01^{A}a	1.53 ± 0.23^{AB}a	1.90 ± 0.38^{ABC}a	Decay visible	Decay visible
	TSS:TA	P-MAP 1	5	42.16	43.68	40.66	40.79	39.47		8.69	8.31	8.20	8.18	8.13
			10	42.16	44.85	36.79	36.19	35.24		8.69	8.30	7.85	7.82	7.28
			15	42.16	42.57	31.44	25.88	Decay visible		8.69	7.74	8.09	7.14	Decay visible
		P-MAP 2	5	42.16	42.22	42.29	39.49	38.59		8.69	8.20	8.08	8.01	8.25
			10	42.16	43.61	33.60	28.46	31.41		8.69	8.26	7.84	7.38	7.78
			15	42.16	40.28	26.44	25.26	Decay visible		8.69	8.67	7.60	6.97	Decay visible
		P-MAP 3	5	42.16	42.43	39.47	37.68	37.50		8.69	8.60	7.91	8.15	8.28
			10	42.16	40.61	35.00	29.90	30.74		8.69	8.21	8.04	7.33	7.45
			15	42.16	34.19	25.37	24.42	Decay visible		8.69	8.33	7.30	6.93	Decay visible
		Control	5	42.16	46.97	37.80	501.67	41.53		8.69	9.35	8.79	8.09	8.33
			10	42.16	41.09	36.45	29.60	Decay visible		8.69	8.89	7.29	7.87	Decay visible
			15	42.16	48.06	40.00	Decay visible	Decay visible		8.69	9.28	7.21	Decay visible	Decay visible

* For each column, similar upper case letters in superscript are not significantly different at $p < 0.05$ among the passive MAP treatments. For parameter in rows, similar lower case letters are not significantly different. **P-MAP1: 75 g; P-MAP2: 100 g; P-MAP3: 125 g; control: clamshell package

Table 5 Effect of passive MAP design factors on total anthocyanin content in pomegranate arils

Cultivar	Total anthocyanin content (mg C_3gE 100 mL^{-1})												
	Treatment	Temp	Day 0	Day 3	Day 7	Day 10	Day 14	Cultivar	Day 0	Day 3	Day 7	Day 10	Day 14
'Acco'	P-MAP 1	5	21.13 ± 0.45^{A}a	19.74 ± 4.11^{A}ab	12.62 ± 1.83^{A}b	13.32 ± 0.58^{AB}b	13.80 ± 1.58^{A}c	'Herskawitz'	20.42 ± 0.68^{A}a	18.05 ± 0.91^{A}b	14.39 ± 2.09^{A}c	17.75 ± 0.56^{A}bd	16.06 ± 0.87^{A}cd
		10	21.13 ± 0.45^{A}a	18.44 ± 2.04^{A}b	12.10 ± 0.40^{A}c	15.12 ± 1.78^{BC}b	13.38 ± 1.70^{A}c		20.42 ± 0.68^{A}a	17.85 ± 0.96^{AB}b	14.56 ± 0.13^{A}c	15.06 ± 0.57^{B}d	14.45 ± 0.14^{B}c
		15	21.13 ± 0.45^{A}a	13.60 ± 0.81^{B}b	11.51 ± 0.09^{A}c	11.65 ± 0.07^{D}c	Decay visible		20.42 ± 0.68^{A}a	15.80 ± 1.13^{B}b	15.09 ± 1.97^{AB}b	14.01 ± 0.02^{C}c	Decay visible
	P-MAP 2	5	21.13 ± 0.45^{A}a	20.79 ± 3.26^{A}a	14.82 ± 0.22^{B}b	15.00 ± 0.22^{C}b	13.01 ± 0.19^{A}c		20.42 ± 0.68^{A}a	19.32 ± 0.27^{A}b	17.43 ± 0.53^{B}c	17.43 ± 0.53^{A}c	18.22 ± 1.24^{C}bc
		10	21.13 ± 0.45^{A}a	18.57 ± 1.03^{A}b	17.95 ± 2.61^{BC}b	17.95 ± 0.05^{E}b	14.26 ± 0.55^{A}c		20.42 ± 0.68^{A}a	17.48 ± 2.59^{AB}ab	18.33 ± 0.87^{B}ab	18.33 ± 0.07^{A}b	18.04 ± 1.31^{C}b
		15	21.13 ± 0.45^{A}a	19.46 ± 3.11^{A}ab	18.38 ± 1.28^{C}b	13.65 ± 0.07^{A}c	Decay visible		20.42 ± 0.68^{A}a	12.99 ± 1.57^{C}b	18.88 ± 0.50^{B}c	13.88 ± 0.50^{C}bd	Decay visible
	P-MAP 3	5	21.13 ± 0.45^{A}a	17.70 ± 1.33^{A}b	15.89 ± 2.43^{B}bc	15.89 ± 0.42^{C}c	16.32 ± 0.64^{B}bc		20.42 ± 0.68^{A}a	13.42 ± 3.30^{BC}b	16.96 ± 0.39^{B}bc	16.96 ± 0.02^{A}c	17.06 ± 0.12^{AC}c
		10	21.13 ± 0.45^{A}a	16.88 ± 1.54^{A}b	15.83 ± 0.30^{B}b	15.69 ± 0.30^{C}b	14.02 ± 0.0^{A}c		20.42 ± 0.68^{A}a	17.17 ± 1.19^{A}b	18.95 ± 0.45^{B}b	17.90 ± 0.45^{A}b	17.06 ± 1.01^{A}b
		15	21.13 ± 0.45^{A}a	20.42 ± 2.05^{A}ab	19.24 ± 0.53^{C}b	13.55 ± 0.17^{A}c	Decay visible		20.42 ± 0.68^{A}a	14.74 ± 5.97^{ABC}ac	17.06 ± 0.91^{B}b	14.95 ± 0.07^{B}c	Decay visible
	Control	5	21.13 ± 0.45^{A}a	16.95 ± 0.22^{A}b	13.90 ± 1.80^{AB}c	14.04 ± 0.29^{A}b	13.32 ± 0.25^{D}c		20.42 ± 0.68^{A}a	17.65 ±1.22^{A}b	13.99 ± 0.81^{AD}c	17.04 ± 0.09^{A}b	13.32 ± 0.25^{D}c
		10	21.13 ± 0.45^{A}a	13.67 ± 0.62^{B}b	12.18 ± 0.20^{A}c	11.12 ± 0.11^{D}b	Decay visible		20.42 ± 0.68^{A}a	11.67 ± 0.62^{C}b	13.18 ± 0.28^{D}c	13.02 ± 0.11^{D}c	Decay visible
		15	21.13 ± 0.45^{A}a	13.55 ± 0.19^{B}b	10.16 ± 0.41^{E}c	Decay visible	Decay visible		20.42 ± 0.68^{A}a	15.55 ± 0.09^{B}b	10.46 ± 2.01^{E}c	Decay visible	Decay visible

* For each column, similar upper case letters in superscript are not significantly different at $p < 0.05$ among the passive MAP treatments. For parameter in rows, similar lower case letters are not significantly different. **P-MAP1: 75 g; P-MAP2: 100 g; P-MAP3: 125 g; control: clamshell package

Microbial quality

Temperature and time had significant effects on microbial growth ($p < 0.05$), while the amount of arils used in this study had no significant influence on microbial load. The aerobic mesophilic bacterial and fungal growth remained below detection limit for all passive MAP applications until day 10 and 7 of storage at 5 °C, respectively, for both cultivars. In contrast, microbial growth were observed in the control (clamshell package) treatment from day 3 increasing to over 3.5 log CFU g^{-1} at 5 °C and decay was visible after day 10 and 14 in samples stored at 15 and 10 °C, respectively. We observed a significant difference between the microbial stability of the two cultivars in this study, cv. 'Herskawitz' were more microbiologically stable compared to cv. 'Acco'. Yeast and mould count were higher than bacterial count at all storage conditions. Yeast and mould count were in the range of 0.36 to 2.17 log CFU g^{-1} for 'Herskawitz' and 1.76 to 2.59 log CFU g^{-1} for 'Acco' after 14 days of storage at 5 °C. While, the aerobic mesophilic bacterial count were in the range of 1.10 and 1.73 for 'Herskawitz' and 1.76 to 2.41 log CFU g^{-1} for 'Acco' after 14 days of storage at 5 °C (Fig. 4).

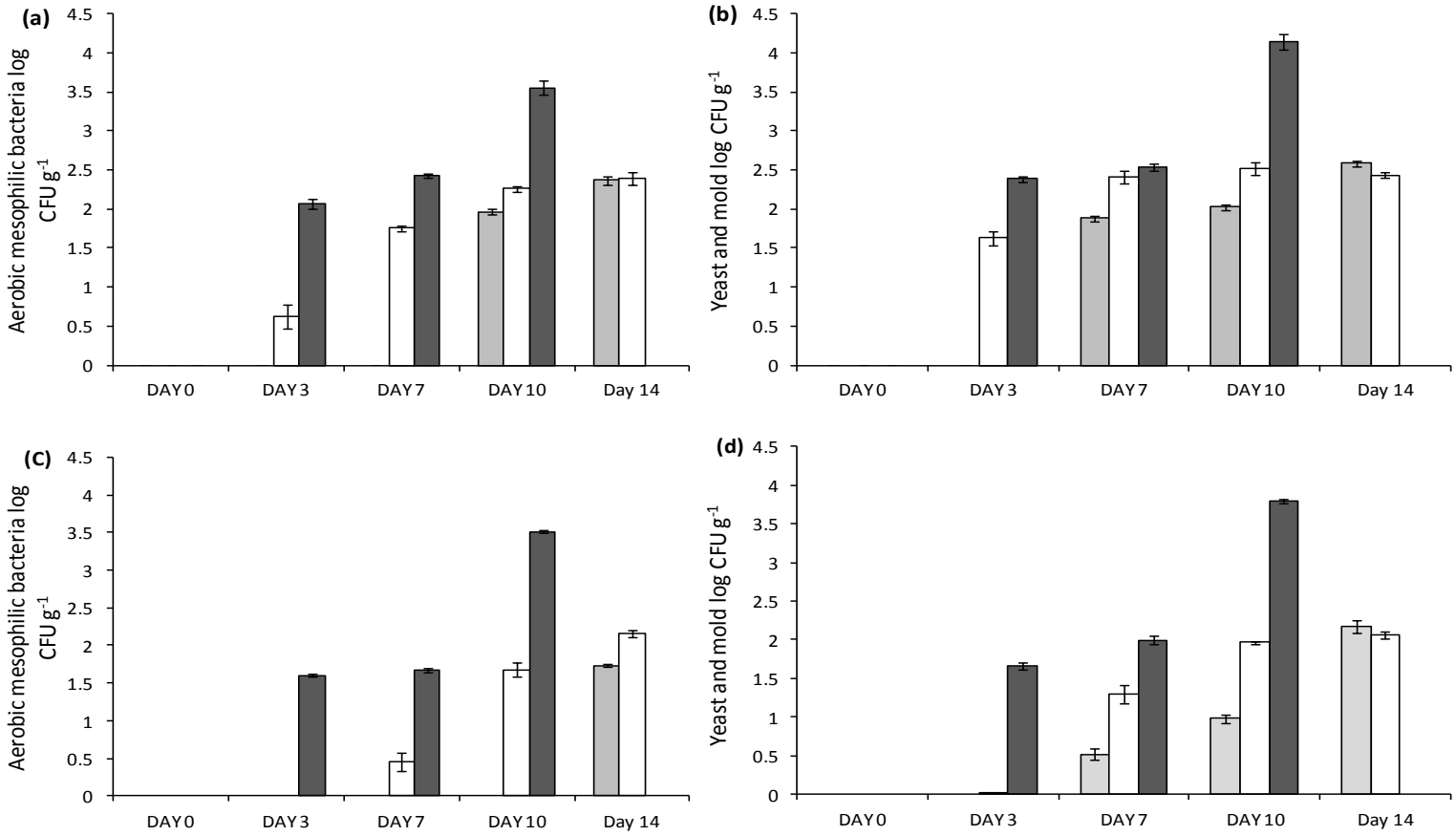


Figure 4 Effect of storage temperature on growth of aerobic mesophilic bacteria and yeast and mould in fresh-cut pomegranate arils from P-MAP treatment, (a, b) cv. 'Acco' and (c, d) cv. 'Herskawitz'. Dark shaded bars, 15 °C; Unshaded bard, 10 °C; Gray shaded bars, 5 °C

Discussions

Package headspace gas composition and optimal MAP design

It is important to integrate and validate mathematical modelling for MAP design of fresh produce with experimental data (Sousa-Gallagher & Mahajan, *in press*). The present study showed good agreement between simulated results and experimental data (R^2 = 0.98) (Figure 1). Slow changes in headspace gas composition could be explained by the very low respiration rate of pomegranate arils at the set temperatures (Caleb *et al*., 2012b). At lowest temperature the inability to achieve an equilibrium MAP (eMAP) required for optimal storage of arils, suggests that the use of active gas modification (gas flushing with recommended atmosphere) may be necessary. However, if the product is stored for longer duration and at higher temperature, micro/macro perforations would be required on polymeric film in order to avoid critical levels of O_2 and CO_2. According to Soliva-Fortuny *et al.* (2004) a decrease in O_2 level below fermentative threshold limit could induce anaerobic respiration, which results in the production of off-flavours and –odours. The increase in CO_2 caused off-flavour development as perceived after day 10, when fresh packages were opened. This observation suggests that rapid increase in CO_2 can indicate the end of product shelf life, and also highlights the need for a polymeric film with higher permeability for CO_2 in order to avoid accumulation inside the package. Additionally, our result identifies the important of engineering design of a MAP system, which takes into consideration amount of product; produce respiration rate and permeability of the packaging polymeric film as affected by temperature; and headspace gas composition.

Furthermore, based on the Pareto chart analysis of investigated parameters at a given time, only temperature had a significant impact on the headspace gas composition. This highlights the importance of maintaining an optimal cold chain and retail shelf temperature. Our experimental and predicted data identifies the significance of time effect on respiration rate of fresh or fresh-cut produce. In order to achieve a desired eMAP time must be taken into consideration. This corroborates the study by Caleb *et al.* (2012b), which reported that time and temperature play a crucial role in the respiration rate of pomegranate arils.

Physical quality attributes

Storage temperature and duration had significant effects on the weight of packaged arils. Weight loss increased with increase in temperature from 5 to 15 °C and was observed at day 7. Gil *et al*. (1996) reported a weight loss of 0.52, 0.56 and 0.70% for chlorine-treated pomegranate arils cv. 'Mollar Elche' stored at 1, 4 and 8 °C, respectively, while weight loss of 0.68, 0.84 and 0.72% for those treated with chlorine plus antioxidant at 1, 4 and 8 °C stored for 7 days. Singh *et al.* (2009) reported a minimal loss in weight for jasmine buds packaged using polypropylene film under passive MAP compared to non-MAP stored buds at 2 °C. MAP was able to minimize weight loss by retarding respiration and transpiration rate. Furthermore, the use of polymeric films in MAP serves as mechanical barrier to the movement of water vapour and this helps to maintain a high level of RH within the package, and reduce produce weight loss (Suparlan & Itoh, 2003). However, an excessively high level of RH within the package can result in moisture condensation on produce, thereby creating a favourable condition for the growth pathogenic and spoilage microorganisms (Távora *et al*., 2004; Aharoni *et al*., 2008). This corroborates the observation in the control experiment with clamshell packages, with increase in weight due moisture condensation.

The decrease in firmness of arils packed in clamshell trays could be attributed to the accumulation of water vapour inside the packages, which softens the membrane of the arils. Furthermore, the absence of significant changes in firmness of fresh arils over time at 5 °C under passive MAP indicates the fruit structure is well kept at this storage condition in combination with MAP. This observation in similar to report by Ayan & Eştürk (2009), they observed slight or no significant change in firmness for pomegranate cv. 'Hicaznar' arils in stored in passive-MAP or active-MAP until 15 d at 5 °C. Furthermore, the non-uniform flesh characteristics of arils contribute to the large variability among individual aril's mechanical attribute. And the relatively minimal changes observed in pomegranate textural attribute at 5 °C highlight the need to identify other quality and non-destructive parameters could adequately measure real-time changes in packaged fresh-cuts.

The non-significant and fluctuating effects of the amount of arils, temperature and time on colour parameters agrees with data reported for other pomegranate arils cultivars that were minimally processed and stored under MAP conditions (Gil *et al*., 1996; Sepúlveda *et al*., 2000; Ayhan & Eştürk, 2009). For instance, Gil *et al*. (1996) reported a relatively small

change in *L** parameter for cv. 'Mollar' arils packed in OPP bags stored at 8, 4 and 1 °C for 7 d; Sepúlveda *et al.* (2000) observed no colour change in minimally processed pomegranate arils cv. 'Wonderful' stored at 4 °C ± 0.5 in semi-permeable films for 14 d. Ayhan & Eştürk (2009) reported that MAP application or storage time had no significant effect on redness *a** and yellowness of *b**, but observed small fluctuations throughout the 18 d of storage at 5 °C.

Changes in pH, total soluble solids, total titratable acidity

The non significant effect of passive MAP applications chemical attributes such as pH, TTS and TTA corroborates previous study reported by Artés *et al.* (2000) and Ayan & Eştürk (2009). Artés *et al.* (2000) reported, that at the end of shelf life, all MAP treatment maintained or had an increase in pH of arils, except for samples stored in perforated PP at 5 °C, which had lower pH values. Ayan & Eştürk (2009) also observed little changes in chemical quality of minimally processed pomegranate arils stored under modified atmosphere condition. The variability of pH, TSS, and TTA values found in the studies could be explained by several factors such as cultivar differences and the relative solubility effect of CO_2 in water molecules surrounding the freshly packed pomegranate arils.

Total anthocyanin content

The observed decrease in total anthocyanin reported in our study is in agreement with previous studies on the effect of MAP on pomegranate arils (Artés *et al.*, 2000; Ayhan & Eştürk, 2009). Artés *et al.* (2000) reported a general decrease in total anthocyanin content for all treatments of modified atmosphere packaged pomegranate fruit cv. 'Mollar' stored at 5 °C for 12 wk. Ayhan & Eştürk (2009) also reported that the total anthocyanin content of pomegranate arils of cv. 'Hicaznar' were significantly influence by MAP application, storage time, and the interaction of both MAP application × storage time. The total content of anthocyanin reported in this study was lower than that reported by Ayhan & Eştürk (2009) for pomegranate cv. 'Hicaznar' (31.13 to 26.53 mg C3gE 100 mL^{-1}). This is probably due to differences in cultivar and agro climatic regions.

Microbial quality

The highest yeast and mould count in all passive MAP applications was less than 5 log CFU g^{-1} which is the maximum limit for yeast and mould in raw and fresh-cut fruit allowed by the South African legislation (FCD Act 54 1979). However, after day 10, 7, and 3, fermentative headspace gas resulting in off-odours was noticed in P-MAP samples at 5, 10 and 15 °C, respectively. Compared to the control (clamshell) package in this study, passive MAP prolonged the shelf life of fresh pomegranate arils for 10 days at 5 °C. Higher counts of yeast and mould and a shorter lag phase reported in this study, is explained based on the fact that yeast and mould are capable of growing at lower pH in comparison to aerobic mesophilic bacteria (Suárez-Jacobo *et al*., 2010; Varela-Santos *et al*., 2012). Our result is in agreement with report by Varela-Santos et al. (2012). They observed that aerobic mesophilic bacteria count were lower than yeast and mould counts in untreated pomegranate juice of cv. 'Wonderful'. Furthermore, based on the aerobic mesophilic bacteria counts, they suggested that shelf life of unpressurized pomegranate juice was about 13 days; while on the basis of the moulds and yeasts shelf life was around 8 days. Furthermore, the lower microbial count observed for 'Herskawitz' in comparison with 'Acco' may be attributed to differences in chemical characteristics such as organic acids and pH of the two pomegranate cultivars. For instance, cv. 'Herskawitz' has higher TTA and pH than 'Acco', this can influence microbial growth. Soliva-Fortuny & Martin-Belloso (2003) reported that fruit physicochemical properties such as pH and TTA have an important effect on microbial shelf life of fresh-cut fruit.

Conclusion

Headspace gas concentration was significantly influenced by produce weight and storage temperature. Physicochemical quality attributes evaluated in this study were not significantly affected by passive MAP application. Passive MA-packaged pomegranate arils had a better keeping quality and longer shelf life in comparison to those packed in clamshell trays. The influence of storage temperature, time and their interaction on evaluated parameters shows the significance of maintaining optimal cold-storage condition for fresh and fresh-cut products along the supply chain. Based on the aerobic mesophilic bacteria counts, the shelf

life of passive MAP fresh pomegranate arils extends beyond day 14 at 5 °C, whereas, on the basis of the yeasts and moulds the shelf of product was around 10 days at 5 °C. The relatively small changes observed in physicochemical attributes at optimal temperature 5 °C in this study highlight the need to identify other non-invasive indictors that could indicate the changes occurring inside MA-packaged fresh arils during storage and shelf life. Further studied are recommended to evaluate the impact of produce weight and temperature on active MAP performance of arils.

References

Aharoni, N., Rodov, V., Fallik, E., Porat, R., Pesis, E. & Lurie, S. (2008). Controlling humidity improves efficacy of modified atmosphere packaging of fruit and vegetables. In: S. Kanlayanarat *et al*. (Eds.), Proceedings of EURASIA Symposium on Quality Management in Postharvest Systems, *Acta Horticulturae,* **804**, 189-196.

Artés, F., Villaescusa R. & Tudela, J.A. (2000). Modified atmosphere packaging of pomegranate. *Journal of Food Science*, **65**, 1112-1116.

Ayhan, Z. & Eştürk, O. (2009). Overall quality and shelf life of minimally processed and modified atmosphere packaged "ready-to-eat" pomegranate arils. *Journal Food Science*, **74**, C399-C405.

Caleb, O.J., Mahajan, P.V., Fahad, A.A. & Opara, U.L. (2012a). Modified atmosphere packaging technology of fresh and fresh-cut produce and the microbial consequences - A Review. *Food and Bioprocess Technology,* DOI 10.1007/s11947-012-0932-4.

Caleb, O.J., Mahajan, P.V., Opara, U.L. & Witthuhn, C.R. (2012b). Modelling the effect of time and temperature on respiration rate of pomegranate arils (cv. 'Acco' and 'Herskawitz'). *Journal of Food Science,* **77**, E80-E87.

Caleb, O.J., Opara, U.L. & Witthuhn, C.R. (2012c). Modified Atmosphere Packaging of Pomegranate Fruit and Arils: A Review. *Food and Bioprocess Technology,* **5**, 15-30.

Fonseca, S.C., Oliveira, F.A.R., & Brecht, J.K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *Journal of Food Engineering*, **52**, 99-119.

Gil, M.I., Martínez, J.A. & Artés, F. (1996). Minimally processed pomegranate seeds. *Lebensmittel Wissenschaft und Technologie*, **29**, 708-713

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 174-185.

Mahajan, P.V., Oliveira, F.A.R., Montanez, J.C. & Frias, J. (2007). Development of user-friendly software for design of modified atmosphere packaging for fresh and fresh-cut produce. *Innovative Food Science and Emerging Technologies*, **8**, 84-92.

Oliveira, F., Sousa-Gallagher, M.J., Mahajan, P.V. & Teixeira, J.A. (2012). Evaluation of MAP engineering design parameters on quality of fresh-sliced mushrooms. *Journal of Food Engineering*, **108**, 507-514.

Opara, U.L., Al-Ani, M.R. & Al-Shuaibi, Y.S. (2009). Physicochemical properties, vitamin C content, and antimicrobial properties of pomegranate fruit (*Punica granatum* L.). *Food Bioprocess Technology*, **2**, 315-321.

Sepúlveda, E., Galletti, L., Sáenz, C. & Tapia, M. (2000). Minimal processing of pomegranate var. Wonderful. *CIHEAM-Opitions Mediterraneennes*, **42**, 237-242.

Soliva-Fortuny, R.C., Elez-Martínez, P. & Martín-Belloso, O. (2004). Microbiological and biochemical stability of fresh-cut apples preserved by modified atmosphere packaging. *Innovative Food Science and Emerging Technologies,* **5**, 215-224.

Soliva-Fortuny, R.C. & Martín-Belloso, O. (2003). New advances in extending shelf-life of fresh-cut fruits: a review. *Trends in Food Science and Technology*, **14**, 341-353.

Sousa-Gallagher, M.J. & Mahajan, P.V. (in press). Integrative mathematical modeling for MAP design of fresh-produce: Theoretical analysis and experimental validation. *Food Control (accepted in press)*.

Singh, A., Dhaduk, B. K. & Ahlawat, T. (2009). Storage of jasmine (*Jasminum sambac*) in passive MAP. In: C. -O. Ottosen *et al.* (Eds.), Proceedings of 9th International Symposium on Postharvest Quality of Ornamental Plants, *Acta Horticulturae,* **847**, 321-326.

Suparlan, & Itoh, K. (2003). Combined effects of Hot water treatment (HWT) and modified atmosphere packaging (MAP) on quality of tomatoes. *Packaging Technology and Science,* **16**, 171-178.

Suárez-Jacobo, Á., Gervilla, R., Guamis, B., Roig-Sagués, A.X. & Saldo, J. (2010). Effect of UHPH on indigenous microbiota of apple juice: A preliminary study of microbial shelf-life. *International Journal of Food Microbiology*, **136**, 261-267.

Távora, L.N., Raghavan, G.S.V. & Orsat, V. (2004). Storage of cranberries in plastic packaging. *Journal of Food Technology,* **2**, 28-34.

Varela-Santos, E., Ochoa-Martinez, A., Tabilo-Munizaga, G., Reyes, J.E., Pérez-Won, M., Briones-Labarca, V. & Morales-Castro, J. (2012). Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. *Innovative Food Science and Emerging Technologies*, **13**, 13-22.

Viuda-Martos, M., Fernández-López, J. & Pérez-Álvarez, J.A. (2010). Pomegranate and its many functional components as related to human health: A Review. *Comprehensive reviews in Food Science and Food Safety*, **9**, 635-65.

Chapter 7

Changes in volatile composition as an indicator of microbial stability and shelf life of modified atmosphere packaged pomegranate arils

CHAPTER 7

CHANGES IN VOLATILE COMPOSITION AS AN INDICATOR OF MICROBIAL STABILITY AND SHELF LIFE OF MODIFIED ATMOSPHERE PACKAGED POMEGRANATE ARILS

Summary

This study investigated the effects of passive modified atmosphere packaging (MAP), storage temperature (5, 10 and 15 °C) and duration of 14 days on the postharvest quality attributes, compositional change in flavour attributes and microbiological quality of minimally processed pomegranate arils (*Punica granatum* L.), cvs 'Acco' and 'Herskawitz'. Volatile compounds were extracted via headspace solid phase micro-extraction (HS-SPME) and analysed by gas chromatography-mass spectrometry (GC-MS). Storage conditions and duration had significant effects ($p < 0.05$) on measured postharvest quality attributes and the composition of volatile compounds. A total of 17 and 18 volatiles were tentatively detected and identified in the headspace of pomegranate juices of 'Acco' and 'Herskawitz', respectively. Based on the physicochemical attributes and microbial evaluation, the postharvest life of MA-packaged 'Acco' and 'Herskawitz' was limited to 10 days due to fungal growth ≥ 2 log CFU g^{-1} at 5 °C. However, the concentration (%) and compositional changes in volatile compounds indicated that the flavour/aroma life (7 days) was shorter than the postharvest shelf-life (10 days) for both cultivars.

Introduction

Assessment of postharvest shelf life of fresh-cut or minimally processed packaged fruit and vegetables is often based on changes/stability in physical attributes such as colour, firmness, juiciness, absence of decay, and chemical attributes such as total soluble sugars (TSS), pH and titratable acidity (TA). These attributes reflects visual acceptance and physicochemical properties associated with produce quality; however, they neglect the significance of flavour or aroma quality (Pelayo *et al*., 2003; Kader, 2008). The development of desired

characteristic flavour in packaged fresh-cuts plays a crucial role in consumer preference and this influences future decision to purchase the produce. Furthermore, identification of characteristic aroma during storage life of packaged fresh-cuts can serve as an indicator product shelf-life. Thus, the understanding of volatile development should be incorporated into the postharvest life concept for fresh-cut fruit and vegetables.

During the last decade, there has been an increased global production and consumption pomegranate, due to its health benefits and enriched bioactive phytochemicals (Viduda-Martos *et al*., 2010). Current research on aroma and flavour of pomegranates have focused on the identification of unique volatiles produced by ripe fruit (Calín-Sánchez *et al*., 2011; Melgarejo *et al*., 2011; Mayuoni-Kirshinbanum *et al*., 2012). Using the headspace solid-phase micro-extraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS), Calín-Sánchez *et al*. (2011) and Melgarejo *et al*. (2011) identified 18 and 21 aroma volatiles, respectively, in juices of nine different Spanish pomegranate cultivars. Mayuoni-Kirshinbanum *et al*. (2012) in their study performed a stir bar sorptive extraction (SBSE), coupled with GC-MS analysis to indentify 23 aroma volatiles in 'Wonderful' pomegranate. The identifications included various classes such as aldehydes, monoterpenes, alcohols, esters, furans and acids, and the most prominent volatiles were ethyl-2-methylbutanoate, hexnal, limonene, *trans*-2-hexenal, *cis*-3-hexenol, *cis*-2-heptenal, *β*-pinene and *β*-caryophyllene. Furthermore, Calín-Sánchez *et al*. (2011) and Melgarejo *et al*. (2011) suggested that consumer liking of pomegranate juices could be linked to high levels of monoterpenes. This observation was corroborated by report of Mayuoni-Kirshinbanum *et al*. (2012), where 5 out the 12 detected 'Wonderful' pomegranate aroma-active compounds by the GC-O sniffing panellists were terpens, which suggests that this class of aroma compounds and concentration plays a role among cultivar preference for pomegranate (Melgarejo *et al*., 2011). However, increased interest in minimally processed and fresh-cut pomegranate arils due to its high nutritional value and improved arils quality has highlighted the limited knowledge of factors that affect flavour development in modified atmosphere packaged pomegranate arils.

Modified atmosphere packaging (MAP) is a dynamic process of altering gaseous composition inside a package. It relies on the interaction between the respiration rate (RR) of the produce, and the transfer of gases through the packaging material, with no further control exerted over the initial gas composition (Caleb *et al*., 2012). MAP has been reported to extend the shelf life of minimally processed arils (Sepulveda *et al*., 2000; López-Rubira *et*

al., 2005; Ayan & Eştürk, 2009). Sepulveda *et al*. (2000) observed that minimally processed pomegranate arils cv. 'Wonderful' were storable for 14 days at 4 °C ± 0.5 in semi-permeable films. This study was focused on the effect of different types of semi-permeable and antioxidant solutions on arils quality. López-Rubira *et al*. (2005) investigated the shelf life and overall quality of minimally processed pomegranate arils cv. 'Mollar Elche' treated with UV-C and packaged under passive-MAP in polypropylene (PP) baskets sealed with BOPP film and stored at 5 °C. They observed that the shelf lives of arils were influenced by the harvested dates (earlier or late harvest). The report obtained on the effect of UV-C radiation on microbial growth was inconclusive, being that microbial count were not systematically reduced. Ayan & Eştürk (2009) studied the effect of various gas compositions in active-MAP on the shelf life and overall quality of minimally processed pomegranate arils stored at 5 °C. They observed no significant change in physicochemical attributes of arils during cold storage, while, aerobic mesophilic bacteria were in the range of 2.30 – 4.51 log CFU g^{-1}. However, none of these studies provided information on the development of flavour in MA-packaged pomegranate arils.

In this study we investigated the postharvest shelf life based on physicochemical properties, microbial stability and on changes in concentration and composition of volatile compounds of two pomegranate cultivars during storage under passive MAP at 5, 10 and 15 °C. Our goal was to evaluate the potential of using changes in volatile composition as indicators of shelf life.

Materials and methods

Plant materials and preparation

Sweet-sour pomegranate (*Punica granatum* L.) fruit cvs. 'Acco' and 'Herskawitz' harvested manually during commercial harvest period were obtained from Robertson valley farm, Western Cape (33°48′0″S, 19°53′0″E) in South Africa and immediately stored in the pack-house, at the Houdoconstant Pack-house (Porterville, South Africa) at 5 °C. Black polypropylene (PP) trays with the dimensions of 15.5 x 11.5 x 3.5 cm^3 and POLYLID polymeric film (55µm with WVTR of 20 - 22 g m^{-2} day^{-1}; CO_2TR of 600 - 700 mL m^{-2} day^{-1};

and OTR 130 - 150 mL m^{-2} day^{-1} at 25 °C, 50% RH and 1 Bar) were provided by Blue Dot Packaging (Cape town, South Africa) and Barkai Polyon Ltd. (Kibbutz Barkai, Israel), respectively.

Fruit processing and packaging procedures

Fruit were manually sorted to remove those with blemish after which the outer skins (husk) of healthy whole fruit were washed in sterilized water with 200 μLL^{-1} of sodium hypochlorite (NaOCl) solution. Fruit husk were mechanically processed for aril extraction using a commercial pomegranate aril extraction unit (ArilSystems, Juran Metal Works, Israel). The extracted arils were collected on sterile conveyer belt in order to air dry and manually remove damaged arils. Each cultivar was processed separately and all processing was conducted at temperature below 10 °C. Arils were mixed to ensure uniformity and portions of 125 g arils were weighed into polypropylene (PP) trays which had been previously sterilized with ethylene oxide. PP trays were sealed with POLYLID films using a semi-automated heat sealing machine (Food Processing Equipment, South Africa). A label of 7.0 x 3.8 cm^2 area was placed onto each package film to simulate the labels found in the retails market packages. At the pack-house, packaged products were cooled down to 2 °C and transported in ice-packed cooler boxes fitted with data loggers (Gemini Data Loggers, United Kingdom) to the postharvest research laboratory. On arrival temperature inside the cooler boxes ranged between 3 – 4.5 °C. Packaged samples were stored at 5, 10 and 15 °C and 95 ± 2% RH for 14 d, and sampling was carried out on 0, 3, 7, 10, and 14 d of storage. Two packs were analyzed for each experimental condition on each sampling day. A full factorial experimental design was used and were replicated six times (n = 6).

Headspace gas analysis

Before packages were opened on sampling days, the gas composition inside the packages was determined using a gas analyzer with an accuracy of 0.5% (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Immediately after taking the gas analysis, packages were opened and used for microbial, physicochemical and volatile analyses.

Weight loss

Initial and final weight of each packaged arils was measured using an electronic weighing balance (ML3002.E, Mettler Toledo, Switzerland). Weight loss was calculated according to the following equation:

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

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 arils was measured using texture analyzer (TA-XT Plus, Stable Micro Systems, Surrey, England), with a 35 mm diameter cylindrical probe. Firmness was expressed as maximum compression force (N). A test speed of 1.0 mm s-1 and distance of 9.5 mm were used. An average of 10 arils was measured individually for each experimental condition.

Titratable acidity, pH, total soluble solids and total anthocyanin content

Arils (125 g) for each pack were juiced separately using a LiquaFresh juice extractor (Mellerware, South Africa) and the juice was directly used for pH and total soluble solid (TSS) measurement using a pH meter (Crison, Barcelona, Spain) and digital refractometer expressed as °Brix (Atago, Tokyo, Japan), respectively. Titratable acidity (TA) was measured by titration to an end point of pH 8.2 using a Metrohm 862 compact titrosampler (Herisau, Switzerland). Total anthocyanin content was determined by the pH-differential method, using 2 buffer systems, namely potassium chloride (pH 1, 0.025M) and sodium acetate (pH 4.5, 0.4M). One ml of sample juice was mixed with 9 mL of buffer and the absorbance was measured at 520 and 700 nm. Total anthocyanins were calculated as cyaniding-3-glucoside according to the following equation:

$$Total\ anthocyanins\ (mg\ 100L^{-1}) = \left[\frac{A \times MW \times DF \times 100}{\varepsilon \times 1}\right] \quad (2)$$

where *A* = (A520 – A 700) pH 1 – (A520 – A700) pH 4.5; *MW* (molecular weight) = 449.2 g mol-1 for cyaniding-3-glucoside; *DF* = dilution factor; l = pathlength in cm; ε = 26900 molar extinction coefficient. All analyses were done as 4 replicates (*n* = 4) and values are presented as mean ± S.D.

Microbial quality

Microbiological stability of samples was screened by total plate count. For aerobic mesophilic bacteria count, plate count agar (PCA) was used and for the yeast and mould count, potato dextrose agar (PDA) acidified with 10% tartaric acid was used. Packages were opened under sterile conditions and 10 g of each sample was obtained aseptically and homogenized with 90 mL of sterile physiological solution (PS). Furthermore, 3-fold dilutions were prepared using 1.0 mL of diluents into 9 .0 ml of PS. In order to enumerate microbial load, 1.0 ml of each dilution was pour-plated in triplicate onto PCA plates for aerobic mesophilic bacteria and PDA for yeast and moulds. Plates for aerobic mesophilic bacteria were incubated at 37 °C for 2 d and at 25 °C for 3 – 5 d for yeast and moulds. The results were presented as log CFU g^{-1}.

Extraction procedure of volatile compounds and chromatographic analyses

Approximately 5 mL of aliquots of pomegranate juice were taken from the total samples thawed overnight at refrigerating temperature and was placed in 20 mL SPME vials. These aliquots were mixed with equal amounts of 30% NaCl, to inhibit enzymatic degradation and facilitate the evolution of volatiles into the headspace. The aroma volatiles were trapped and extracted from the vial headspaces an SPME method described before by Melgarejo *et al.* (2011) and Mayuoni-Kirshinbanum *et al.* (2012). The vials were allowed to equilibrate for 10 min at 50 °C in the CTC autosampler incubator and after this equilibration time, a 50/30 μm three phase fiber coated with divinylbenzene/-carboxen/-polydimethylsiloxane (needle size 23 ga, StableFlex, 57298-U Supelco, Sigma-Aldrich) was exposed to the headspace for 20 min at 50 °C. After extraction, desorption of the volatile compounds from the fibre coating was carried out in the injection port of the gas chromatography-mass spectrometry (GC-

MS) during 2 min in splitless mode and then 8 min in split mode to clean fibre. The temperature of the injection was maintained at 250 °C.

Separation of the volatile compounds was performed on a gas chromatograph using Agilent 6890 N (Agilent, Palo Alto, CA), coupled with an Agilent mass spectrometer detector Agilent 5975 MS (Agilent, Palo Alto, CA). The GC-MS system was equipped with an Rtx®-5Sil MS, with a 95% polydimethyl siloxane/ 5% polydiphenyl siloxane stationary phase and the dimensions were 30 m length; 0.25 mm inner diameter; and 0.5 µm film thickness. Analyses were carried out using helium as carrier gas with a flow of 1.2 mL min^{-1}. The injector temperature was maintained at 250 °C. The oven temperature was as follows: 40 °C for 2 min; and then ramped up to 250 °C at 5 °C min^{-1} and held for 5 min. The MSD was operated in full scan mode and the ion source and quadropole were maintained at 240 °C and 150 °C, respectively. The transfer line temperature was maintained at 280 °C. Where authentic standards were available, compounds were tentatively identified by comparison of retention times (RI); Kovats retention indices (KI); and, by comparison with mass spectral libraries (NIST, version 2.0). For quantification, the calculated relative abundances were used.

The formula to obtain experimental Kovats indexes is described in the following equation:

$$I = 100n + 100z\frac{(\log tRA - \log tRn)}{(\log tRN - \log tRn)} \quad (3)$$

where *I* is the Kovats index, *A* is the unknown compound, *n* is the number of carbon atoms in the smaller n-alkane, *N* is the number of carbon atoms in the lager n-alkane, *z* is the difference in the carbon atoms in the smaller and larger n-alkanes and *tR* is the retention time.

Statistical analysis

The experimental data obtained were treated with one-way analysis of variance (ANOVA) at 95% confidence interval to evaluate the effect of amount of pomegranate arils inside the MAP, storage time, temperature and their interaction on the quality attributes. Least significant difference (LSD) and Tukey Post-hoc tests were performed to identify specific

differences in factor levels. All experimental data were analysed using Statistical software (Statistical 10.0, Statsoft, USA).

Results and discussion

Package headspace gas composition

Headspace O_2 content significantly decreased over time inside packages at the different storage temperature up to day 14, without reaching an equilibrium concentration (Fig. 1a). Oxygen composition went below 2% in packages stored at 10 and 15 °C on day 7 and 4, respectively, while samples at 5 °C did not reach below 2% throughout the study. According to Soliva-Fortuny *et al*. (2004), a decrease in O_2 level below fermentative threshold limit could induce anaerobic respiration, which results in the production of off-flavours and –odours. On the other hand, CO_2 levels increased significantly during storage for all packaging conditions; however, the increase was highest at 15 °C (Fig. 1b). At the end of the storage, O_2 and CO_2 concentration reached approximately 4.8 and 27.8%, respectively, at 5 °C. This observation suggests that rapid increase in CO_2 can indicate the end of product shelf life, and also highlights the need for a polymeric film with higher permeability for CO_2 in order to avoid accumulation inside the package.

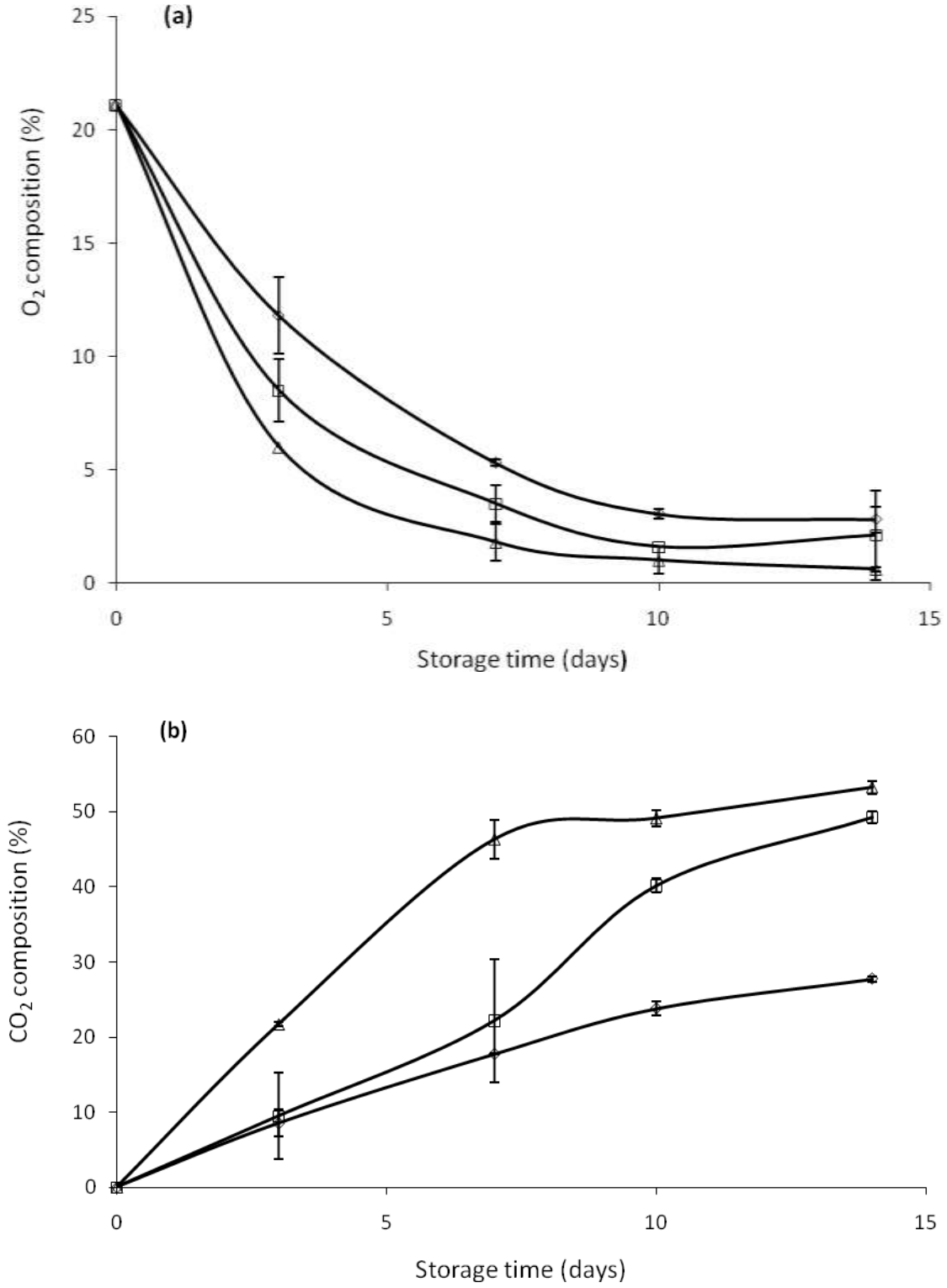


Figure 1. Effect of storage temperature and time on the percentage gas composition inside MA-packaged cv. 'Acco' pomegranate arils: (◊) 5 °C, (□) 10 °C, (Δ) 15 °C. *Data similar for cv. 'Herskawitz'.

Changes in physical attributes

Weight loss did not exceed 0.53 and 0.79% at 5 °C; 0.70 and 0.91% at 10 °C; and 2.14 and 1.94% at 15 °C for 'Acco' and 'Herskawitz', respectively. Gil *et al.* (1996) reported a weight loss of 0.52, 0.56 and 0.70% for chlorine-treated pomegranate arils cv. 'Mollar Elche' stored at 1, 4 and 8 °C, respectively, while weight loss of 0.68, 0.84 and 0.72% for those treated with chlorine plus antioxidant at 1, 4 and 8 °C stored for 7 days. Furthermore, the use of polymeric films in MAP serves as mechanical barrier to the movement of water vapour and this helps to maintain a high level of RH within the package, and reduce produce weight loss (Suparlan & Itoh, 2003).

Firmness at day 0 was 76.10 ± 5.1 N and 85.55 ± 8.4 N for cv. 'Acco' and 'Herskawitz', and did not exceed 77.50 ± 7.4 N and 102.36 ± 7.6 N, respectively, at day 14 (Table 1). Storage temperature, time, and their interaction had no significant effect on firmness of arils as storage progressed ($p \leq 0.05$). This observation in similar to report by Ayan & Eştürk (2009), they observed slight or no significant change in firmness for pomegranate cv. 'Hicaznar' arils in stored in passive-MAP or active-MAP until day 15 at 5 °C.

With regards to colour characteristics of pomegranate arils, the average *L** values measured ranged from 39.55 to 26.22 for 'Acco' and 41.58 to 30.03 for cv. 'Herskawitz', while *a** ranged from 29.29 to 19.30 for 'Acco' and 33.95 to 22.60 for cv. 'Herskawitz', and, *b** ranged from 19.66 to 12.28 for 'Acco' and 18.98 to 12.90 for cv. 'Herskawitz' across all storage conditions. Comparison of the two cultivars showed that 'Herskawitz' had better colour stability than 'Acco', based on the overall analysis of variance (ANOVA) and Tukey Post-hoc test. Storage time had no significant effect colour parameters *L** (lightness), *a** (redness) and *b** (yellowness) ($p > 0.05$) which are commonly used in industry as indicators of colour stability (Table 2). The non-significant or fluctuating effects of passive MAP and temperature on colour parameters agrees with data reported for other minimally processed pomegranate arils cultivars and stored under MAP conditions (Gil *et al.*, 1996; Sepúlveda *et al.*, 2000; Ayhan & Eştürk, 2009). Gil *et al.* (1996) reported a relatively small change in *L** parameter for cv. 'Mollar' arils packed in oriented polypropylene (OPP) bags stored at 8, 4 and 1 °C for 7 day. Sepúlveda *et al.* (2000) observed no colour change in minimally processed pomegranate arils 'Wonderful' stored at 4 °C ± 0.5 in semi-permeable films for 14 day. Ayhan & Eştürk (2009) reported that MAP application or storage time had no

significant effect on redness *a** and yellowness of *b**, but observed small fluctuations throughout the 18 day of storage at 5 °C.

Table 1 Effect of passive MAP, storage temperature and duration on pomegranate arils firmness.

Cultivar	Temp. °C	Firmness (N)				
		Day 0	Day 3	Day 7	Day 10	Day 14
Acco						
	5	76.10 ± 5.1^{A}a	69.90 ± 7.9^{AB}a	76.15 ± 8.6^{A}a	70.40 ± 6.7^{A}a	75.43 ± 8.9^{A}a
	10	76.10 ± 5.1^{A}a	69.25 ± 8.7^{AB}a	72.78 ± 7.0^{A}a	71.42 ± 4.7^{B}b	69.91 ± 11.0^{A}ab
	15	76.10 ± 5.1^{A}a	70.71 ± 12.6^{A}a	75.84 ± 6.0^{A}a	75.06 ± 5.2^{A}a	75.72 ± 7.5^{A}a
Herskawitz						
	5	85.55 ± 8.4^{A}a	81.91 ± 17.03^{A}a	90.57 ± 14.7^{A}a	87.43 ± 14.9^{B}a	93.60 ± 10.7^{A}a
	10	85.55 ± 8.4^{A}a	81.15 ± 17.1^{A}a	88.19 ± 12.5^{A}a	84.80 ± 15.5^{AB}a	86.31 ± 13.6^{A}a
	15	85.55 ± 8.4^{A}a	87.94 ± 16.6^{A}a	94.20 ± 15.3^{A}a	98.36 ± 20.0^{B}a	97.32 ± 17.3^{A}a

* For each column, similar upper case letters in superscript are not significantly different at $p < 0.05$. For parameter in rows, similar lower case letters are not significantly different.

Table 2 Effect of packaging, storage temperature and duration on pomegranate arils' CIE*L*a*b** colour indices.

Cultivar	Storage time	Temp. ^{o}C	*L**	*a**	*b**	Cultivar	*L**	*a**	*b**
'Acco'	**Day 0**		39.55 ± 5.7^{A}	27.75 ± 6.4^{A}	15.41 ± 2.8^{A}	**'Herskawitz'**	37.61 ± 6.2^{A}	23.49 ± 5.3^{AB}	14.09 ± 1.9^{A}
	Day 3	5	33.61 ± 3.7^{AB}	23.79 ± 1.9^{A}	13.78 ± 1.3^{A}		38.64 ± 6.9^{A}	33.95 ± 5.9^{A}	16.37 ± 3.0^{A}
		10	32.22 ± 1.6^{B}	22.25 ± 8.9^{A}	13.12 ± 2.4^{A}		34.81 ± 4.8^{A}	29.29 ± 6.1^{AB}	15.13 ± 2.5^{A}
		15	29.1 ± 2.6^{B}	24.54 ± 4.3^{A}	14.72 ± 1.2^{A}		36.24 ± 10.5^{A}	23.48 ± 3.6^{B}	15.00 ± 2.7^{A}
	Day 7	5	30.96 ± 4.4^{AB}	22.01 ± 2.1^{A}	13.39 ± 1.7^{A}		30.03 ± 7.3^{A}	25.89 ± 4.6^{AB}	14.43 ± 2.3^{A}
		10	27.66 ± 1.9^{B}	22.58 ± 5.9^{A}	14.29 ± 1.7^{A}		32.29 ± 6.6^{A}	24.76 ± 5.8^{AB}	14.55 ± 1.5^{A}
		15	33.49 ± 6.7^{AB}	29.29 ± 5.8^{A}	17.20 ± 2.7^{A}		31.79 ± 5.4^{A}	26.11 ± 3.6^{AB}	15.57 ± 1.8^{A}
	Day 10	5	30.99 ± 5.6^{AB}	19.37 ± 5.8^{A}	14.02 ± 3.9^{A}		28.64 ± 3.1^{A}	22.6 ± 2.8^{B}	12.74 ± 0.9^{A}
		10	25.85 ± 5.5^{B}	19.8 ± 3.7^{A}	12.28 ± 1.6^{A}		34.62 ± 2.3^{A}	25.66 ± 5.3^{AB}	14.72 ± 1.4^{A}
		15	31.65 ± 3.7^{AB}	24.29 ± 3.9^{A}	14. 03 ± 1.7^{A}		30.79 ± 4.6^{A}	25.78 ± 3.4^{AB}	14.49 ± 1.6^{A}
	Day 14	5	33.69 ± 8.6^{AB}	19.32 ± 2.3^{A}	15.72 ± 3.2^{A}		31.55 ± 3.3^{A}	25.24 ± 6.9^{AB}	13.59 ± 1.9^{A}
		10	28.32 ± 5.3^{B}	21.54 ± 1.9^{A}	14.09 ± 2.1^{A}		34.64 ± 3.4^{A}	25.23 ± 3.6^{AB}	16.06 ± 3.8^{A}

* For each column, similar upper case letters in superscript are not significantly different at $p < 0.05$.

Changes in pH, total soluble solids, total titratable acidity and total anthocyanin content

Table 3 shows the chemical parameters of fresh and modified atmosphere packaged pomegranate arils for both cultivars during storage. A comparison of both cultivars showed that cv. 'Herskawitz' had a significantly higher TA and pH than 'Acco', while 'Acco' had a relatively higher TSS. The TSS/TA ratio was influenced by storage duration and temperature. Overall, cv. 'Herskawitz' was more stable compared to 'Acco' based the observed changes in physico-chemical attributes. The interaction of storage temperature and time had a significant effect on all chemical quality parameters evaluated ($p < 0.05$). There was a significant decrease in TA day 3 ($p < 0.05$), afterward stayed relatively unchanged over time for the rest of the storage period but significantly higher than day 3. Decrease observed in acidity reported on day 3, could be related initial arils' response and metabolic activities during storage. Our findings are in agreement with other reports on the effect of packaging on the stability of chemical attributes of pomegranate arils (Artés *et al*., 2000; Ayan & Eştürk, 2009). Artés *et al*. (2000) reported in their study, that at the end of the shelf life all MAP treatment maintained or had an increase in pH values, except for samples stored in perforated PP at 5 °C, which had lower pH values. Ayan & Eştürk (2009) also observed little changes in chemical quality of minimally processed pomegranate arils stored under modified atmosphere condition. The variability of pH, TSS, and TA values found in the studies could be explained by several factors such as cultivar differences and the relative solubility effect of CO_2 in water molecules surrounding the freshly packed pomegranate arils.

There was a significant effect of storage temperature and duration, as well as their interaction on the total anthocyanin content ($p \leq 0.05$). A general trend of decrease in total anthocyanin content was observed as the storage time increased for all treatments (Table 3). Total anthocyanin content was within the range from 21.13 to 13.32 mg C3gE 100 mL^{-1} of pomegranate juice for 'Acco', and 20.42 mg C3gE 100 mL^{-1} to 12.32 mg C3gE 100mL^{-1} for 'Herskawitz'. Ayhan & Eştürk (2009) also reported that the total anthocyanin content of pomegranate arils of cv. 'Hicaznar' were significantly influence by packaging, storage time, and their interaction. The total content of anthocyanin reported in this study was lower than that reported by Ayhan & Eştürk (2009) for pomegranate cv. 'Hicaznar' (31.13 to 26.53 mg C3gE 100 mL^{-1}). This is probably due to differences in cultivar and agro climatic regions.

The increase reported in the studies could be explained by several factors such as cultivar differences and the relative solubility effect of CO_2 in water molecules surrounding the freshly packed pomegranate arils.

Table 3 Effect of passive MAP design factors on chemical parameters of two pomegranate cultivars.

Cultivar		pH, TSS, TA, and TSS:TA										
	Parameter	Temp	Day 0	Day 3	Day 7	Day 10	Day 14	Cultivar	Day 0	Day 3	Day 7	Day 10	Day 14
'Acco'	pH	5	3.80 ± 0.01^{A}a	3.76 ± 0.22^{A}a	3.96 ± 0.06^{A}b	3.97 ± 0.02^{A}b	4.05 ± 0.01^{BC}c	'Herskawitz'	3.01 ± 0.02^{A}a	2.96 ± 0.08^{A}a	2.99 ± 0.01^{A}a	3.01 ± 0.02^{A}a	3.05 ± 0.01^{A}a
		10	3.80 ± 0.01^{A}a	3.86 ± 0.25^{A}a	3.88 ± 0.21^{A}a	3.99 ± 0.07^{A}b	4.01 ± 0.08^{BC}b		3.01 ± 0.02^{A}a	3.01 ± 0.08^{A}a	2.96 ± 0.11^{A}a	3.02 ± 0.04^{A}a	3.06 ± 0.01^{A}a
		15	3.80 ± 0.01^{A}a	3.82 ± 0.03^{A}a	3.84 ± 0.18^{A}a	3.55 ± 0.07^{A}b	Decay visible		3.01 ± 0.02^{A}a	3.22 ± 0.04^{C}b	2.94 ± 0.07^{A}a	2.95 ± 0.07^{A}a	Decay visible
	TSS	5	15.6 ± 0.01^{A}a	14.85 ± 0.21^{A}b	15.0 ± 0.14^{C}c	15.45 ± 0.21^{A}a	15.0 ± 0.0^{A}d		15.03 ± 0.01^{A}a	14.80 ± 0.0^{C}b	14.80 ± 0.28^{A}b	15.40 ± 0.14^{B}c	15.15 ± 0.07^{A}c
		10	15.6 ± 0.01^{A}a	13.40 ± 1.70^{A}b	14.7 ± 0.42^{A}c	14.95 ± 0.21^{A}c	14.45 ± 0.35^{B}c		15.03 ± 0.01^{A}a	14.70 ± 0.14^{C}b	14.40 ± 0.14^{A}b	14.65 ± 0.07^{A}b	14.60 ± 0.14^{A}b
		15	15.6 ± 0.01^{A}a	14.70 ± 0.14^{A}b	13.7 ± 0.57^{D}c	14.65 ± 0.07^{A}b	Decay visible		15.03 ± 0.01^{A}a	14.50 ± 0.14^{B}b	13.50 ± 0.85^{A}c	14.42 ± 0.59^{A}bc	Decay visible
	TA	5	0.37 ± 0.01^{A}a	0.35 ± 0.0^{AB}b	0.38 ± 0.01^{A}a	0.41 ± 0.05^{A}ab	0.40 ± 0.01^{A}a		1.73 ± 0.01^{A}a	1.72 ± 0.03^{A}a	1.87 ± 0.06^{B}b	1.89 ± 0.12^{A}b	1.83 ± 0.03^{A}b
		10	0.37 ± 0.01^{A}a	0.33 ± 0.02^{AB}b	0.42 ± 0.04^{ABD}a	0.50 ± 0.07^{AC}a	0.47 ± 0.06^{A}a		1.73 ± 0.01^{A}a	1.79 ± 0.04^{A}b	1.79 ± 0.01^{AB}b	2.00 ± 0.16^{A}c	1.96 ± 0.11^{A}c
		15	0.37 ± 0.01^{A}a	0.43 ± 0.08^{A}a	0.54 ± 0.01^{E}b	0.60 ± 0.02^{D}c	Decay visible		1.73 ± 0.01^{A}a	1.74 ± 0.03^{A}a	1.85 ± 0.02^{A}b	2.08 ± 0.01^{B}c	Decay visible
	TSS:TA	5	42.16	42.43	39.47	37.68	37.50		8.69	8.60	7.91	8.15	8.28
		10	42.16	40.61	35.00	29.90	30.74		8.69	8.21	8.04	7.33	7.45
		15	42.16	34.19	25.37	24.42	Decay visible		8.69	8.33	7.30	6.93	Decay visible

Cultivar	Total anthocyanin content (mg C_3gE 100 mL^{-1})										
	Temp	Day 0	Day 3	Day 7	Day 10	Day 14	Cultivar	Day 0	Day 3	Day 7	Day 10	Day 14
'Acco'							'Herskawitz'					
	5	21.13 ± 0.45^{A}a	17.70 ± 1.33^{A}b	15.89 ± 2.43^{B}bc	15.89 ± 0.42^{C}c	16.32 ± 0.64^{B}bc		20.42 ± 0.68^{A}a	13.42 ± 3.30^{BC}b	16.96 ± 0.39^{B}bc	16.96 ± 0.02^{A}c	17.06 ± 0.12^{AC}c
	10	21.13 ± 0.45^{A}a	16.88 ± 1.54^{A}b	15.83 ± 0.30^{B}b	15.69 ± 0.30^{C}b	14.02 ± 0.0^{A}c		20.42 ± 0.68^{A}a	17.17 ± 1.19^{A}b	18.95 ± 0.45^{B}b	17.90 ± 0.45^{A}b	17.06 ± 1.01^{A}b
	15	21.13 ± 0.45^{A}a	20.42 ± 2.05^{A}ab	19.24 ± 0.53^{C}b	13.55 ± 0.17^{A}c	Decay visible		20.42 ± 0.68^{A}a	14.74 ± 5.97^{ABC}ac	17.06 ± 0.91^{B}b	14.95 ± 0.07^{B}c	Decay visible

*For each column, similar upper case letters in superscript are not significantly different at $p < 0.05$ among the passive MAP treatments. For parameter in rows, similar lower case letters are not significantly different. TSS, Total soluble solids; TA; Total titratable acidity

Microbial quality

Storage conditions and duration had significant effect on microbial growth ($p < 0.05$). The aerobic mesophilic bacterial and fungal growth remained below detection limit until day 10 and 7 of storage at 5 °C, respectively, for both cultivars. We observed a significant difference between the microbial stability of the two cultivars in this study, which showed that 'Herskawitz' arils were more microbiologically stable compared to 'Acco'. Yeast and mould count were higher than bacterial count at all storage conditions. Yeast and mould count were in the range of 0.36 - 2.17 log CFU g^{-1} for 'Herskawitz' and 1.76 - 2.59 log CFU g^{-1} for 'Acco' after 14 days of storage at 5 °C. While, the aerobic mesophilic bacterial count were in the range of 1.10 and 1.73 for 'Herskawitz' and 1.76 to 2.41 log CFU g^{-1} for 'Acco' after 14 days of storage at 5 °C (Fig. 2). Although, the highest yeast and mould count in all passive MAP applications was fewer than 5 log CFU g^{-1}, which was established as the maximum limit for yeast and mould in raw and fresh-cut fruit by the South African legislation (FCD Act 54 1979). However, at day 10, 7, and 3, fermentative headspace gas resulting in off-odours was observed at 5, 10 and 15 °C, respectively.

The higher levels of yeast and mould count with a shorter lag phase found in this study may be attributed to the fact that yeast and mould are capable of growing at lower pH in comparison to aerobic mesophilic bacteria (Suárez-Jacobo *et al.*, 2010; Varela-Santos *et al.*, 2012). These findings agree with the report by Varela-Santos *et al.* (2012), who observed that aerobic mesophilic bacteria count were lower than yeast and mould counts in untreated pomegranate juice of 'Wonderful'. Furthermore, the lower microbial count observed for 'Herskawitz' in comparison with 'Acco' may be attributed to differences in chemical characteristics of the two pomegranate cultivars. For instance, cv. 'Herskawitz' has higher TA and pH than 'Acco', this can influence microbial growth. Soliva-Fortuny & Martin-Belloso (2003) reported that fruit physicochemical properties such as pH and TA have an important effect on microbial shelf life of fresh-cut fruit.

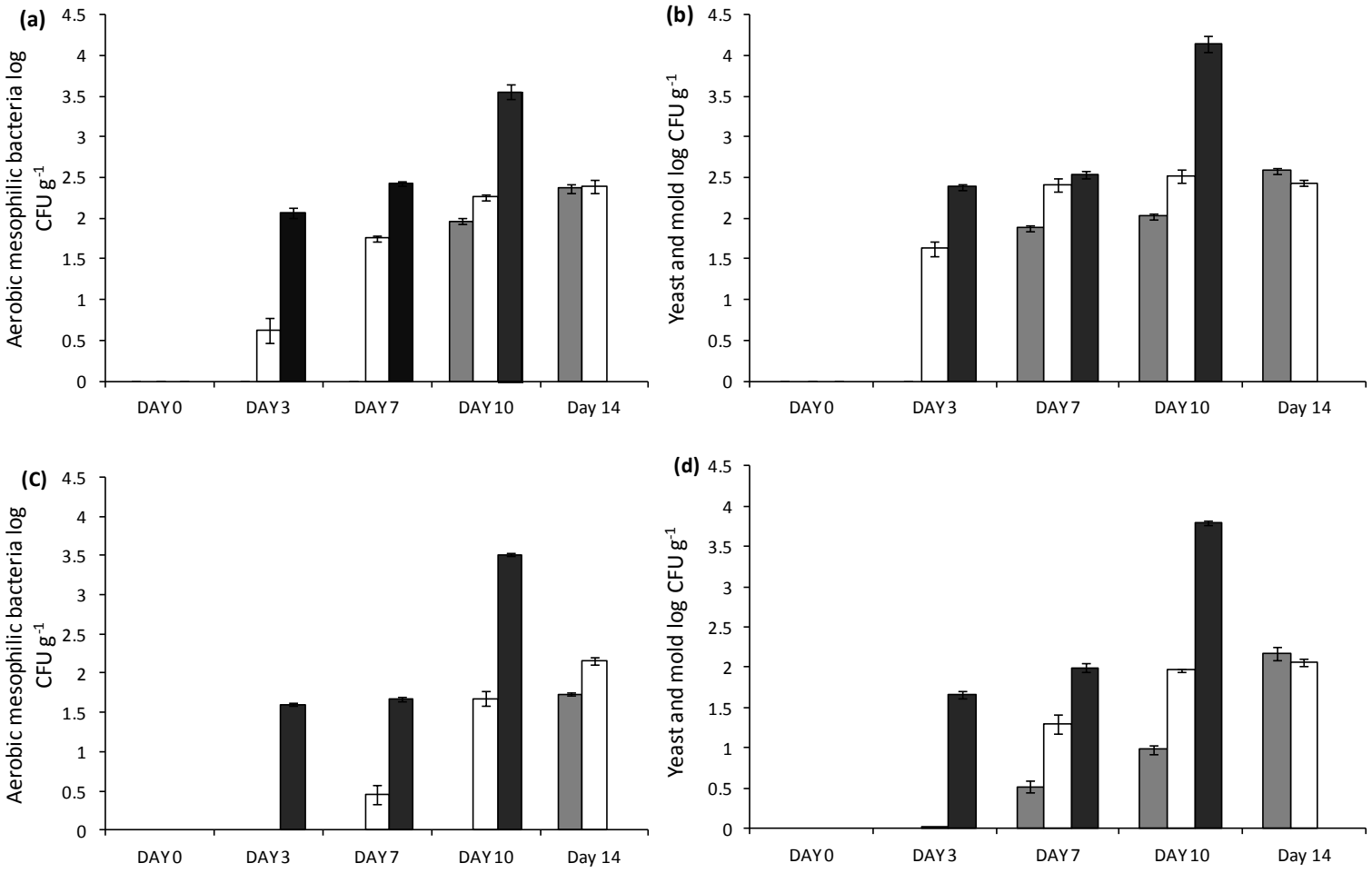


Figure 2. Effect of storage temperature on growth of aerobic mesophilic bacteria and yeast and mould in MA-packaged pomegranate arils, (a, b) cv. 'Acco' and (c, d) cv. 'Herskawitz'. *Dark shaded bars, 15 °C; Unshaded bard, 10 °C; Gray shaded bars, 5 °C. Mean separation by LSD, $p < 0.05$.

Volatile composition and evolution

Using GC-MS analysis of pomegranate juice HS-SPME extract, a total of 18 and 17 volatiles were detected for 'Herskawitz' and 'Acco', respectively. In general, temperature and storage duration had significant effects on the evolution of volatiles ($p > 0.05$). The cultivars differed quantitatively in aroma compounds; however, they exhibited the same volatile profiles which were categorized into primary and secondary volatiles based on their evolution. Primary volatiles were identified in day 0 (fresh samples), while secondary volatiles evolved over the storage period at different temperatures. The most abundant volatiles in both cultivars were *trans*-3-hexen-1-ol, 1-hexanol, 3-methyl-1-butanol acetate, hexyl acetate and 2-octanone (only in 'Acco'). Several of the other volatiles identified in both cultivars were present in very low concentration (%) for example limonene, benezeacetaldehyde, α-terpineol, and 2-nonanone (Table 4). The volatile compounds found in pomegranate juices can be grouped in 7 chemical classes: (a) monoterpenes: limonene; (b) monoterpenoids: L-terpinen-4-ol, α-terpineol; (c) aldehydes: benzenacetaldehyde; (d) ketones: 2-octanone, 2-nonanone and 2-undecanone; (e) alcohols: *trans*-3-hexen-1-ol, 1-hexanol, 2-phenylethanol and 2-nonanol; (f) esters: 3-methyl-1-butanol acetate, cis-3-hexenyl acetate, hexyl acetate, 2-phenylethyl acetate, octanoic acid-, decanoic acid- and dodecanoic acid-ethyl ester; and (g) sesquiterpenes: trans-α-bergamotene.

The concentration of ketones decreased over time during the storage. Concentration of aldehydes, alcohols and esters decreased in the following order during the storage period: aldehydes < alcohols < ester. It is well known that most types of fruit have the ability to metabolize aldehydes into alcohols, and then into their corresponding esters during ripening as well as storage (Dixon & Hewett, 2000; Pelayo *et al*., 2003; Vazquez-Cruz *et al*., 2012). For instance, Pelayo *et al.* (2003) reported a decrease in the level of aldehydes and alcohols at the end of postharvest life of CO_2-stored 'Aromas' and 'Diamante' strawberries with a notable increase in the concentration of ethyl esters. This was also the finding in this study, concentration and composition of ethyl esters increased with the storage period. For example, 2-phenylethyl acetate, octanoic acid ethyl ester and decanoic acid ethyl ester were detected from day 3 and in all storage conditions and duration, with 2-phenylethyl acetate exhibiting the highest concentration among all esters. A higher level of ethyl esters was observed in 'Acco' in comparison to 'Herskawitz' after 10 days of storage. Enhanced

synthesis of ethyl esters requires a fermentation process in order to supply high amounts of alcohol precursor.

According to Purvis (1997), fermentative metabolism can be enhanced in fruit via various stress factors such as extrinsic (temperature, hypoxic conditions), intrinsic (ripening, senescence) and biotic (microbial growth) factors. In this study we observed that increased temperature and microbial growth were correlated with the evolution of ethyl esters after day 3 of storage. Microorganisms have been reported to produce high levels of ethyl esters and alcohols on fresh food produce (Longo & Sanromán, 2006; Deetae *et al*., 2007). In addition, enhanced production of ethyl esters exhibits an increased activity of alcohol acyltransferase enzyme (AAT), which promotes the last stage in biosynthesis of esters (Zhu *et al*., 2008). Therefore, the ability to maintain the original volatile profile during MAP storage depends on maintaining strict optimal cold chain, processing hygiene to reduce microbial load and the ability of pomegranate cultivars to maintain a reduced rate of fermentative metabolism. 'Acco' pomegranate arils did not maintain a low rate of this metabolic pathway compared to 'Herskawitz'. This observation highlights the need for precise definition of stored produce flavour life (Pelayo *et al*., 2003), and offers the possibility to use volatile evolution in MA-packaged fresh/ fresh-cuts as an indicator for intelligent packaging.

Furthermore, the esters chemical group had the highest percentage composition and representation among the isolated, identified and quantified groups of volatiles which evolved as secondary volatiles. The finding in this study is in contrast with others in literature by Calín-Sánchez *et al*. (2011), Melgarejo *et al*. (2011) and Mayuoni-Kirshinbaum *et al*. (2012). For instance, Calín-Sánchez *et al*. (2011) and Melgarejo *et al*. (2011) identified 18 and 21 aroma volatiles, respectively, in juices of nine different Spanish pomegranate cultivars. The most abundant of these volatiles were limonene, hexanal, cis-3-hexenol and trans-2-hexenal. Mayuoni-Kirshinbaum *et al*. (2012) reported that majority of aroma compounds in 'Wonderful' pomegranate were terpenes and aldehydes. These differences could be associated to cultivar, influence of agro-climatic regions on the fruit as well as the extraction methods used and retention time reported. However, the observation in this study is principally due to the influence of minimal processing, storage condition and duration on fresh pomegranate arils, which.

Flavour life can be defined as the maximum period of storage in which fruit maintain a similar flavour profile to that detected in freshly harvested fruit (Pelayo *et al*., 2003). However, the described changes in aroma compounds during MAP storage created new profiles that could influence flavour perception. Thus, flavour life could be described based on the compounds with the highest concentration/odour threshold that contributes to the global odour of a given food (Alonso *et al*., 2009). Based on the correlations found between the increasing levels of ethyl esters, changes in volatile composition and microbial growth during MAP storage, both cultivars exhibited a shorter flavour life (7 days) than postharvest life (10 days). Therefore, changes in headspace volatile composition could serve as an indicator of microbial stability and postharvest shelf life for MA-packaged pomegranate arils.

Table 4 Aromatic compounds found in the headspace of MA-packaged pomegranate juice using GC-MS analysis of HS-SPME extracts.

Cultivar	Common volatiles	RT* mins	Kovats indexes		Day 0	Day 3			Day 7			Day 10		Sensory descriptor†
	Relative peak area (%)**		Lit.[a]	Exp.[a]		5°C	10°C	15°C	5°C	10°C	15°C	5°C	10°C	
Acco	*trans*-3-Hexen-1-ol	**8.3**	**814**	**804**	12.7 ± 1.8a	_	6.4 ± 2.2b	_	4.6 ± 0.5b	2.4 ± 0.7c	_	2.2 ± 0.2c	0.2 ± 0.1d	Plant, fruity, aromatic
	1-Hexanol	**8.6**	**831**	**836**	16.6 ± 2.6a	10.7 ± 0.3b	18.2 ± 5.8a	_	11.5 ± 1.3b	5.1 ± 2.1c	_	2.5 ± 0.05d	0.5 ± 0.3e	Mint, grass
	3-Methyl-1-butanol acetate	**8.8**	**850**	**858**	12.2 ± 1.0a	25.5 ± 2.5b	25.7± 6.5bcd	20.2±2.2bc	16.1 ± 2.5d	20.3 ± 3.1bc	11.5 ± 2.2a	20.2 ± 5.9bc	33.2 ± 4.5d	Fruity, sweet-like, banana
	2-Octanone	**12.4**	**977**	**975**	16.5 ± 2.5a	14.07	5.0 ± 4.3b	_	1.0 ± 0.3c	2.7 ± 1.0d	_	1.3 ± 0.2c	0.2 ± 0.04e	
	cis-3-Hexenyl Acetate	**12.9**	**986**	**991**	3.1 ± 0.02a	2.22	0.85	1.17	0.8 ± 0.4b	1.0 ± 0.02b	1.07	2.4 ± 0.6a	1.4 ± 0.1b	Fresh, leafy, green,
	Acetic acid, hexyl ester	**13.1**	**997**	**998**	9.5 ± 0.3a	10.7 ± 1.0a	2.4 ± 1.4b	2.48	2.6 ± 1.1b	2.4 ± 0.2b	0.88	2.8 ± 0.2c	2.4 ± 0.9c	Apple, cherry, floral, pear
	Limonene	**13.7**	**1020**	**1017**	3.4 ± 0.27a	1.6 ± 0.2b	2.2 ± 0.7a	0.10	1.3 ± 0.1b	0.8 ± 0.5d	_	0.6 ± 0.03d	0.3 ± 0.01e	Mild, citrus, sweet, orange
	Benzeneacetaldehyde	**14.2**	**1036**	**1033**	2.3 ± 0.1a	0.75	_	0.4 ± 0.01e	0.9	1.3 ± 0.3b	0.51	0.5 ± 0.02c	0.98 ± 0.1b	Honey, sweet, flowery
	2-Nonanone	**15.6**	**1069**	**1079**	4.3 ± 0.5a	3.07	7.7 ± 1.0b	3.05	0.6 ± 0.2c	9.9 ± 1.0d	8.74	2.2 ± 0.9d	4.9 ± 0.1a	Cheesy, green, fruity, dairy
	2-Phenylethanol	**16.3**	**1081**	**1104**	0	8.2	2.95		_	6.6 ± 0.2a	7.3 ± 0.2bc	6.7 ± 1.4ac	5.0 ± 1.8a	Flowery, roses
	α-Terpineol	**18.8**	**1169**	**1186**	3.3 ± 1.2a	1.3 ± 0.1b	_	0.73	0.8 ± 0.1c	0.9 ± 0.1c	_	1.4 ± 0.2b	_	Lilac
	Secondary volatiles													
	2-Nonanol	**15.9**	**1087**	**1091**	0.00	2.18	_	1.8 ± 0.4a	1.3 ± 0.7a	1.9 ± 0.2a	5.3	0.57	2.0 ± 0.04a	cucumber
	Octanoic acid, ethyl ester	**18.8**	**1183**	**1184**	0.00	1.18	1.37	1.4	1.4 ± 0.02a	0.36	6.53	1.1 ± 0.1b	2.2 ± 0.5c	Fruity, fresh, sweet-like
	2-Phenylethyl acetate	**20.5**	**1224**	**1241**	0.3 ± 0.02a	1.9 ± 0.4b	1.36	5.5 ± 0.2c	2.54	10.8 ± 0.3d	17.2 ± 1.1e	27.7 ± 0.1f	27.2 ± 0.2f	Flowery, cooked apple
	2-Undecanone	**21.5**	**1274**	**1275**	0.00	1.65	_	1.24	_	0.9 ± 0.1a	2.72	0.31	0.9 ± 0.1a	
	Decanoic acid, ethyl ester	**24.2**	**1383**	**1364**	0.00	1.49	0.74	1.7 ± 0.2a	_	0.25	3.31	1.9 ± 0.3a	1.5 ± 0.5a	Rancid
	Dodecanoic acid, ethy ester	**29.2**	**1581**	**1526**	0.00	0.5 ± 0.02a	_	0.6	_	_	0.9± 0.1b	0.16	0.3 ± 0.1c	Dry, metallic

** Peak areas are means of two GC-MS runs; *RT = retention time; [a] Lit = literature (MS software, NIST version 2.0), Exp. = experimental. Similar lower case letters in rows are not significantly different ($p < 0.05$). †Melgarejo et al. 2011; SAFC (2008)

Table 4 continues

Cultivar	Common volatiles / Relative peak area (%)**	RT* mins	Kovats indexes Lit.[a]	Kovats indexes Exp.[a]	Day 0	Day 3 5°C	Day 3 10°C	Day 3 15°C	Day 7 5°C	Day 7 10°C	Day 7 15°C	Day 10 5°C	Day 10 10°C	Sensory descriptor†
Herskawitz	*trans*-3-Hexen-1-ol	**8.3**	**814**	**804**	10.1±0.02a	9.7 ± 0.4a	2.2± 0.3b	1.8±0.1c	3.3 ± 1.0b	1.0±0.8bc	0.3 ± 0.05d	0.5 ± 0.02e	0.2 ± 0.1f	Plant, fruity,
	1-Hexanol	**8.6**	**831**	**836**	11.9± 0.2a	12.3±1.9a	6.6 ± 1.6b	2.8 ± 0.4c	8.1 ± 0.6b	2.5 ± 1.0c	0.7 ± 0.3d	0.9 ± 0.2d	0.9 ± 0.3d	Mint, grass
	3-Methyl-1-butanol acetate	**8.8**	**850**	**858**	14.0± 0.5a	31.6±14.9bc	36.9 ± 3.0c	35.3±1.7c	31.7± 12.7c	38.2 ± 4.6c	21.9 ± 0.6b	33.6±13.2bc	35.2 ± 2.5c	Fruity, sweet-like
	cis-3-Hexenyl Acetate	**12.9**	**986**	**991**	2.9± 0.1a	2.1 ± 0.7ac	2.3 ± 0.5a	2.7 ± 1.0a	1.7± 0.7ac	3.1 ± 0.8a	1.4 ± 0.05b	2.1 ± 0.2c	3.0 ± 0.3a	Fresh, leafy, green,
	Acetic acid, hexyl ester	**13.1**	**997**	**998**	17.0	14.4 ± 2.1b	11.5 ± 0.6c	8.4 ± 2.6c	5.1 ± 2.9d	10.4 ± 0.1c	2.0 ± 0.4e	9.2 ± 0.7c	7.8 ± 0.9c	Apple, cherry, floral
	Limonene	**13.7**	**1020**	**1017**	2.1± 0.6a	3.3 ± 1.2a	1.8± 0.5abc	0.9± 0.7b	2.6 ± 0.9a	1.1 ± 0.1c	0.4 ± 0.02d	0.4 ± 0.3d	0.5 ± 0.04d	Mild, citrus, lemon
	Benzeneacetaldehyde	**14.2**	**1036**	**1033**	1.4± 0.02a	1.3 ± 0.05a	2.0 ± 0.24b	1.3±0.1a	1.2± 0.5ab	2.2 ± 0.01c	0.9± 0.7abd	0.8± 0.6abd	1.4 ± 0.4ad	Honey, flowery
	2-Nonanone	**15.6**	**1069**	**1079**	2.9±0.04ac	2.4 ± 0.6ac	5.1 ± 0.1b	2.9±0.2ac	4.7 ± 0.3b	2.4 ± 1.0cd	1.7 ± 0.03d	0.6 ± 0.3e	1.0 ± 0.4e	Cheesy, fruity, dairy
	2-Phenylethanol	**16.3**	**1081**	**1104**	0.7± 0.1a	5.5±5.1abcd	4.7 ± 3.2bd	6.7±3.3bd	1.9 ± 0.2c	2.8 ± 0.1b	7.0 ± 0.05d	7.8 ± 2.5d	4.6 ± 0.6d	Flowery, roses
	L-Terpinen-4-ol	**18.4**	**1137**	**1172**	0.9±0.05	_	_	_	_	_	_	_	_	Must, turpentine,
	α-Terpineol	**18.8**	**1169**	**1186**	2.8± 0.1a	1.0 ± 0.3b	0.5 ± 0.01c	0.9± 0.1b	1.4±0.02d	_	2.9 ± 0.1e	1.4 ± 0.1d	_	Lilac
	trans-α-Bergamotene	**25.3**	**1434**	**1445**	0.6±0.02a	0.4 ± 0.1b	0.71	0.99	1.20	1.08	0.6 ± 0.2ab	0.78	0.4 ± 0.02c	Woody, terpene-like
	Secondary volatiles													
	Ethyl hexanoate	**12.7**	**984**	**984**	0.00	0.2±0.01a	0.6 ± 0.2b	0.5 ± 0.04c	_	0.4 ± 0.1d	0.9 ± 0.4bc	0.5 ± 0.2bcd	0.49	Fruity, candy
	2-Nonanol	**15.9**	**1087**	**1091**	0.00	1.6± 0.2a	1.8 ± 0.01a	0.7 ± 0.01b	1.3±0.7ab	1.4 ± 0.8ab	8.5 ± 0.3c	0.77	0.55	cucumber
	Octanoic acid, ethyl ester	**18.8**	**1183**	**1184**	0.00	1.1± 0.3a	0.5	1.1 ± 0.02a	1.4±0.02c	2.6 ± 0.2d	1.6 ± 0.2c	2.7±1.7abcde	3.6 ± 0.3e	Fruity, fresh
	2-Phenylethyl acetate	**20.5**	**1224**	**1241**	0.00	0.00	2.9 ± 0.2a	5.78	1.0± 0.2b	4.4 ± 0.02c	10.8 ± 1.8d	24.4 ± 18.4d	11.9± 1.3d	Flowery
	Decanoic acid, ethyl ester	**24.2**	**1383**	**1365**	0.00	1.14	0.68	0.57	_	1.2 ± 0.4a	1.7 ± 0.3b	1.9 ± 0.7b	3.1 ± 0.2c	Rancid
	Dodecanoic acid, ethyl ester	**29.2**	**1581**	**1585**	0.00	0.25	_	_	_	0.42	0.82	0.3 ± 0.04a	0.6 ± 0.1b	Dry, metallic

** Peak areas are means of two GC-MS runs; *RT = retention time; [a] Lit = literature (MS software, NIST version 2.0), Exp. = experimental. Similar lower case letters in rows are not significantly different ($p < 0.05$). †Melgarejo et al. 2011; SAFC (2008)

Conclusion

Changes in quality attributes and aroma compounds were dependent on cultivar differences, and storage condition and duration. At 5 °C storage condition MA-packaged pomegranate arils of cv. 'Acco' and 'Herskawitz', were best kept in comparison samples at 10 and 15 °C. It was evident by the extension of the postharvest life based on physicochemical properties and the inhibition of microbial growth at the lowest storage temperature in the two cultivars. This shows importance of maintaining optimal cold chain in postharvest handling of fresh/ fresh-cut produce. However, flavour life was shorter than the postharvest life and was significantly influenced by storage temperature. Additional sensory evaluations would be needed to confirm this observation. Flavour life can be a selected parameter as a good indicator to determine the quality of minimally processed fresh produce. Although optimum flavour life is difficult to establish, due to cultivar differences, a more precise definition of flavour shelf life is required for MA-packaged pomegranate arils. This could be achieved by considering recommended levels of flavour components in order to ensure acceptable flavour. Further research is warranted in this area, especially given the importance of flavour in consumer perception of quality and purchase of pomegranate arils and other fresh produce.

References

Alonso, A., Vázquez-Araújo, L., García-Martínez, S., Ruiz, J.J. & Carbonell-Barrachina, A.A. (2009). Volatile compounds of traditional and virus-resistant breeding lines of Muchamiel tomatoes. *European Food Research and Technology*, **230**, 315-323.

Artés, F., Villaescusa R. & Tudela, J.A. (2000). Modified atmosphere packaging of pomegranate. *Journal of Food Science*, **65**, 1112-1116.

Ayhan, Z. & Eştürk, O. (2009). Overall quality and shelf life of minimally processed and modified atmosphere packaged "ready-to-eat" pomegranate arils. *Journal of Food Science*, **74**, C399-C405.

Caleb, O.J., Opara, U.L. & Witthuhn, C.R. (2012). Modified atmosphere packaging of pomegranate fruit and arils: a review. *Food and Bioprocess Technology*, **5**, 15-30.

Calín-Sánchez, A., Martínez, J.J., Vázquez-Araú L., Burló, F., Melgarejo, P. & Carbonell-Barrachina, A.A. (2011). Volatile composition and sensory quality of Spanish pomegranates (*Punica granatum* L.). *Journal of the Science Food Agriculture*, **91**, 586-992.

Deetae, P., Bonnarme, P., Spinnler, H.E. & Helinck, S. (2007). Production of volatile aroma compounds by bacterial strains isolated from different surface-ripened French cheeses. *Applied Microbiology and Biotechnology*, **76**, 1161-1171.

Dixon, J. & Hewett, E.W. (2000). Factors affecting apple aroma/flavour volatile concentration: a review. *New Zealand Journal of Crop andHorticultural Science*, **28**, 155-173.

Gil, M.I., Martínez, J.A. & Artés, F. (1996). Minimally processed pomegranate seeds. *Lebensmittel Wissenschaft und Technologie*, **29**, 708-713.

Kader, A.A. (2008). Perspective flavour quality of fruits and vegetables. *Journal of the Science Food Agriculture,* **88**, 1863-1868.

Longo, M.A. & Sanromán, M.A. (2006). Production of food aroma compounds: microbial and enzymatic methodologies. *Food and Bioprocess Technology*, **44**, 335-353.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology Technology*, **37**, 174-185.

Mayuoni-Kirshinbaum, L., Tietel, Z., Porat, R. & Ulrich, D. (2012). Identification of aroma-active compounds in 'wonderful' pomegranate fruit using solvent-assisted flavour evaporation and headspace solid-phase micro-extraction methods. *European Food Research and Technology,* **235**, 277-283.

Melgarejo, P., Sánchez, A.C., Vázquez-Araújo, L., Hernández, F., José-Martínez, J., Legua P. & Carbonell-Barrachina, A.A. (2011). Volatile composition of pomegranates from 9 Spainish cultivars using headspace solid phase microextraction. *Journal of Food Science*, **76**, S114-S120.

Pelayo, C., Ebeler, S.E. & Kader, A.A. (2003). Postharvest and flavour quality of three strawberry cultivars kept at 5 °C in air or air + 20 kPa CO_2. *Postharvest Biology Technology*, **27**, 171-183.

Purvis, A.C. (1997). The role of adaptative enzymes in carbohydrate oxidation by stressed and senescing plant tissues. *HortScience*, **32**, 1165-1168.

SAFC. 2008. Flavours and fragrances. European ed. Catalogue 2007-2008. Madrid, Spain: SAFC Specialities.

Sepúlveda, E., Galletti, L., Sáenz, C. & Tapia, M. (2000). Minimal processing of pomegranate var. Wonderful. *CIHEAM- Options Mediterraneennes*, **42**, 237-242.

Soliva-Fortuny, R.C., Elez-Martínez, P. & Martín-Belloso, O. (2004). Microbiological and biochemical stability of fresh-cut apples preserved by modified atmosphere packaging. *Innovative Food Science and Emerging Technologies*, **5**, 215-224.

Soliva-Fortuny, R.C. & Martín-Belloso, O. (2003). New advances in extending shelf-life of fresh-cut fruits: a review. *Trends in Food Science and Technology*, **14**, 341-353.

South African legislation 1979. Foodstuffd, Cosmetics and Disinfectant (FCD) Act 54.

Suparlan & Itoh, K. (2003). Combined effects of Hot water treatment (HWT) and modified atmosphere packaging (MAP) on quality of tomatoes. *Packaging Technology and Science*, **16**, 171-178.

Suárez-Jacobo, Á., Gervilla, R., Guamis, B. & Roig-Sagués, A.X., Saldo, J. (2010). Effect of UHPH on indigenous microbiota of apple juice: A preliminary study of microbial shelf-life. *International Journal of Food Microbiology*, **136**, 261-267.

Varela-Santos, E., Ochoa-Martinez, A., Tabilo-Munizaga, G., Reyes, J.E., Pérez-Won, M., Briones-Labarca, V. & Morales-Castro, J. (2012). Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. *Innovative Food Science and Emerging Technologies*, **13**, 13-22.

Vazquez-Cruz, M.A., Jimenez-Gracia, S.N., Torres-Pacheco, I., Guzman-Maldonado, S.H., Guevara-Gonzalez, R.G. & Miranda-Lopez, R. (2012). Effect of maturity stage and storage on flavour compounds and sensory description of berrycactus (*Myrtillocactus geometrizans*). *Journal of Food Science*, **77**, C366-373.

Viuda-Martos, M., Fernández-López, J. & Pérez-Álvarez, J.A. (2010). Pomegranate and its many functional components as related to human health: a review. *Comprehensive Reviews in Food Science and Food Safety*, **9**, 635-65.

Zhu, Y., Rudell, D.R. & Mattheis, J.P. (2008). Characterization of cultivar differences in alcohol acyltransferases and 1-aminocyclopropane-1-carboxylate synthase gene

expression and volatile ester emission during apple fruit maturation and ripening. *Postharvest Biology Technology*, **49**, 330-339.

Chapter 8

General Discussion and Conclusion

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

According to Brandenburg & Zagory (2009), in order to achieve the desired modified atmosphere in a given package, it is expedient to understand the three basic disciplines underpinning MAP, namely produce physiology (such as the extrinsic and intrinsic factors affecting produce respiration rate), polymer engineering (which identifies the choice of specific polymer's physical, chemical, and gas transmission rate properties), and converting technology (which entails the fabrication of raw polymers, films, adhesives, inks and additives into packages of desired format monolayer or multi to complex layers, with or without perforation). The aim of the current study was to investigate the application of MAP for postharvest handling of pomegranate arils. Better understanding of the responses of arils to passive-MAP will assist fruit processors in selecting packaging materials and storage conditions, in order to optimize physicochemical, sensory and microbial stability of minimally processed pomegranate arils. Active-MAP was not considered in this study due to the principal need to understand the basic response of pomegranate arils without complex tools, and to show an affordable, flexible and adaptable technique for the 'young' pomegranate agro-economic community in South Africa. In addition to lowering postharvest losses of pomegranate fruit, this is one of the critical challenges in developing countries.

The effects of storage conditions and duration on physiological responses (i.e. respiration and transpiration rate (TR)) of pomegranates cvs. 'Acco' and 'Herskawitz' were investigated and mathematical models were developed to predict these physiological responses at given time and storage condition. The result of this study showed that the respiration rate (RR) of whole pomegranate fruit was significantly higher than the RR of fresh arils, and temperature had a significant impact on the RR of both whole fruit and fresh arils. Generally, biological reactions such as respiration increase 2 to 3-fold for every 10 °C rise in temperature (Fonseca *et al*., 2002). This was in line with the observation in this study with about 3-fold increase in RR when storage temperature increased from 5 to 15 °C. The influence of time, and the interaction between temperature and time also had a significant effect on the RR of

fresh pomegranate arils. This finding highlights the significance of maintaining optimal cold chain conditions for packaged arils or whole fruit. Additionally, the mathematical models based on Arrhenius-type equation and the power function equation coupled with Arrhenius-type equation accurately predicted the effect of temperature and the influence of time and temperature on the RR of fresh pomegranate arils for both cultivars, respectively.

Furthermore, the experimental and model prediction results showed that both relative humidity (RH) and storage temperature had significant effects on transpiration (TR). RH was the variable with the greatest influence on TR, and the result showed that arils were best kept at 5 °C and 96% RH. Previous studies have shown close relationships between storage condition (temperature and RH) and transpiration rate of fresh produce (Mahajan *et al*., 2008), which in turn plays a significant role in determining the optimal storage conditions to maintain quality. The use of polymeric films in MAP serves as mechanical barrier to the movement of water vapour and this helps to maintain a high level of RH within the package and reduces produce weight loss (Suparlan & Itoh, 2003). However, an excessively high level of RH within the package can result in moisture condensation on produce, thereby creating a favourable condition for the growth of pathogenic and spoilage microorganisms (Aharoni *et al*., 2003; Távora *et al*., 2004). Thus, the selection of appropriate packaging material is critical in order to create a stable RH in sealed fresh produce packages. The applicability of the transpiration model developed in this study was validated based on prediction of TR of pomegranate arils under different storage conditions. The model adequately predicted TR and could be a useful tool towards understanding the rate of water loss in fresh pomegranate arils and other fresh produce, as affected by storage conditions and duration.

Evaluation of the effect of passive-MAP engineering design parameters as a function of produce weight, storage temperature and duration on fresh pomegranate arils revealed that produce weight, increase in temperature and the interaction between temperature and time had a slight or no significant effect on measured physicochemical quality attributes such as firmness, colour, pH, total soluble sugars (TSS), and total titratable acidity (TTA). This result is consistent with previous studies in the literature on the effects of MAP on pomegranate arils (Artés *et al*. 2000; Sepúlveda *et al*., 2000; Ayhan & Eştürk, 2009). The relatively small changes observed in this study on the physicochemical attributes of arils at 5 °C over the storage period highlights the need to identify other non-invasive or intelligent indictors that could adequately monitor the changes occurring inside MA-packaged fresh pomegranate arils during storage, transit and shelf life.

The headspace gas concentration inside MAP was significantly influenced by produce weight and storage temperature, Oxygen (O_2) concentration went below 2% after day 5 and day 3 at 10 and 15 °C, respectively, while the O_2 concentration inside MAP stored at 5 °C did not reduce below 2% throughout the study. On the other hand, CO_2 levels increased significantly during storage for all packaging conditions. The rate at which headspace gas composition changed in this study could be explained by the very low respiration rate of pomegranate arils at the set temperatures reported by Caleb *et al.* (2012), in comparison to fruit such as kiwifruit and guava (Manolopoulou & Papadopoulos 1998; Wang *et al.*, 2009). This study showed the importance of a systematic approach in designing optimal MAP system and the need to understand the three basic disciplines underpinning MAP, namely produce physiology, polymer engineering, and converting technology.

Furthermore, the simulation model and evaluation of the effect of selected design parameters on the physiological response of pomegranate arils shows that, at low storage temperature the inability to achieve an equilibrium MAP (eMAP) required for optimal storage of arils despite the increase in produce weight, suggest that the use of active gas modification (gas flushing with recommended atmosphere) may be necessary in modified atmosphere packaging of pomegranate arils. Result of simulation study using unsteady-state models showed that the recommended atmosphere to maintain aril quality storage at 5 °C was: 5% O_2 + 5% CO_2. However, if the product is stored for longer duration and at higher temperature, and packaged with polymeric film with similar permeability properties to the one used in this study, macro/micro perforation of the film would be required to avoid critical levels of O_2 and CO_2. This requires the use of full factorial experiments to select the appropriate number of perforations for the desired storage temperature, and the recommended atmospheric composition has to be validated experimentally.

Temperature and time had a significant influence on total aerobic mesophilic bacterial and yeast and mould growth. However, passive Map arils were best kept at 5 °C with a shelf life of 10 days. This highlights the importance of optimum maintaining optimum cold storage practice and good agricultural and manufacturing practices (GAP and GMP). During minimal processing, contamination with food borne pathogens can become a problem for the industry. Thus, the integration of Hazard Analysis and Critical Control Points-based programs, GAP and GMP is essential in order to ensure food quality and safety. These include accurate auditing of each area in the pack-house and conducting risk assessments of potential points.

With regards to aroma and flavour composition and sensory descriptors for packaged pomegranate arils, the influence of passive MAP, storage temperature and duration on the volatile composition and evolution were investigated. The results showed that changes in aroma compounds were dependent on cultivar, storage condition and duration. Using GC-MS analysis of pomegranate juice HS-SPME extract, a total of 18 and 17 volatiles were detected for 'Herskawitz' and 'Acco', respectively. The importance of maintaining optimal cold chain in postharvest handling of fresh/ fresh-cuts was evident due to the observed extension in postharvest life and an extended lag phase of microbial growth in the two cultivars at lowest temperature. Furthermore, the flavour life was shorter than the postharvest life, based on the reported accumulation of ethyl esters and the decrease in primary volatiles while physicochemical parameters remained relatively unchanged. The change in volatile composition had a correlation to microbial growth. There was a decrease in volatile composition in the following order aldehydes < alcohols < ester during the storage period, and the concentration (%) and composition of ethyl esters increased with storage time. This was consistent with literature evidence (Pelayo *et al.,* 2003) which reported a decrease in the level of aldehydes and alcohols at the end of postharvest life of CO_2-stored 'Aromas' and 'Diamante' strawberries with a notable increase in the concentration of ethyl esters. Additional sensory evaluations would be required to confirm this observation with focus on lowest temperature; however, the present findings highlight the potential towards an innovative food packaging solution for modified atmosphere packaging of pomegranate arils. These include (a) nanoencapsulation of natural antimicrobials on biopolymers with a controlled release mechanism which controls microbial growth phase in packaged arils (Donsi *et al*., 2011), and (b) the addition of nanosensor on polymeric film to detect changes in volatile composition or in the evolution of secondary volatiles such as the ethyl esters' group based on pH (Xu *et al*., 2011). For example, in their concise review, Brody *et al*. (2008) reported the development of a NanoBioluminescence detection spray containing a luminescent protein that is engineered to bind to the surface of microbes such as *E. coli* and *Salmonella*. When bound, it emits a visible glow that varies in intensity based on the microbial load. This principle can be explored via nanoencapsulation of bioluminescence of desired volatile precursors on polymeric films.

This study showed that storage conditions and duration play a crucial role on physiological responses (i.e. RR and TR) of whole pomegranate fruit and arils. The

experimental and model prediction results showed show a good agreement with the produce physiological response. Passive-MA packaged arils were best kept at 5 °C with longer shelf life (10 days) compared to the clamshell packages (< 7 days). Produce weight, increase in temperature and the interaction between temperature and time had a slight or no significant effect on measured physicochemical quality. However, the interaction of produce weight and temperature has a significant influence on the in-package headspace gas composition. Pomegranate arils cv. 'Herskawitz' had a lower microbial load and better flavour stability compared to 'Acco'. Thus the choice of cultivar for MAP is critical to the success of the technology for pomegranate arils. Furthermore, this study showed that the flavour life of packaged arils was shorter than the postharvest life. This identified the need for a more precise definition of flavour shelf life for MA-packaged pomegranate arils and other packaged fresh produce. Although this is difficult to establish, due in part to cultivar differences, it could be achieved by considering recommended levels of flavour components in order to ensure acceptable flavour. Currently there is no research data available on the flavour or volatile composition for MA-packaged pomegranate arils.

Reference

Aharoni, N., Rodov, V., Fallik, E., Porat, R., Pesis, E. & Lurie, S. (2008). Controlling humidity improves efficacy of modified atmosphere packaging of fruit and vegetables. In: S. Kanlayanarat *et al*. (Eds.), Proceedings of EURASIA Symposium on Quality Management in Postharvest Systems, *Acta Horticulturae,* **804**, 189-196.

Artés, F., Villaescusa R. & Tudela, J.A. (2000). Modified atmosphere packaging of pomegranate. *Journal of Food Science*, **65**, 1112-1116.

Ayhan, Z. & Eştürk, O. (2009). Overall quality and shelf life of minimally processed and modified atmosphere packaged "ready-to-eat" pomegranate arils. *Journal Food Science*, **74**, C399-C405.

Brandenburg, J.S., & Zagory, D. (2009). Modified and controlled atmosphere packaging technology and applications. In: E. M. Yahia (Ed.), *Modified and controlled atmosphere for the storage, transportation, and packaging of horticultural commodities* (pp. 73-92). Boca Raton, FL: Taylor & Francis Group, CRC Press.

Brody, A.L., Bugusu, B., Han, J.H., Sand, C.K. & McHugh, T.H. (2008). Innovative food packaging solutions. *Journal of Food Science*, **73**, R107-R116.

Caleb, O.J., Mahajan, P.V., Opara, U.L. & Witthuhn, C.R. (2012). Modelling the effect of time and temperature on respiration rate of pomegranate arils (cv. 'Acco' and 'Herskawitz'). *Journal of Food Science,* **77**, E80-E87.

Donsi, F., Annunziata, M., Sessa, M. & Ferrari, G. (2011). Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *LWT – Food Science and Technology*, **44**, 1908-1914.

Fonseca, S.C., Oliveira, F.A.R. & Brecht, J.K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *Journal of Food Engineering*, **52**, 99-119.

Mahajan, P.V., Rodrigues, F.A.S., & Leflaive, E. (2008). Analysis of water vapour transmission rate of perforation-mediated modified atmosphere packaging (PM-MAP). *Biosystems Engineering,* **100**, 555-561.

Manolopoulou, H. & Papadopoulou, P. (1998). A study of respiratory and physicochemical changes of four kiwi fruit cultivars during cool-storage. *Food Chemistry*, **63**, 529-534.

Pelayo, C., Ebeler, S.E., Kader, A.A., 2003. Postharvest and flavour quality of three strawberry cultivars kept at 5 C in air or air + 20 kPa CO2. *Postharvest Biology and Technology*, **27**, 171-183.

Sepúlveda, E., Galletti, L., Sáenz, C. & Tapia, M. (2000). Minimal processing of pomegranate var. Wonderful. *CIHEAM-Opitions Mediterraneennes*, **42**, 237-242.

Suparlan, & Itoh, K. (2003). Combined effects of Hot water treatment (HWT) and modified atmosphere packaging (MAP) on quality of tomatoes. *Packaging Technology and Science,* **16**, 171-178.

Távora, L.N., Raghavan, G.S.V. & Orsat, V. (2004). Storage of cranberries in plastic packaging. *Journal of Food Technology,* **2**, 28-34.

Wang, Z.-W., Duan, H.-D. & Hu, C.-Y. (2009). Modelling the respiration rate of guava (*Psidium guajava* L.) fruit using enzyme kinetics, chemical kinetics and artificial neural network. *European Food and Research Technology*, *229*, 495-503.

Xu, J., Sun, L., Li, J., Liang, J., Zhang, H. & Yang, W. (2011). FITC and $Ru(phen)_3^{2+}$ co-doped silica particles as visualized ratiometric pH indicator. *Nanoscale Research Letters*, **6**, 561-568.