POSTHARVEST PHYSIOLOGY AND EFFECTS OF MODIFIED ATMOSPHERE PACKAGING AND ANTI-BROWNING TREATMENT ON QUALITY OF POMEGRANATE ARILS AND ARIL-SAC (CV. BHAGWA)

WILHELMINA VULIKENI AINDONGO

Thesis presented in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN FOOD SCIENCE

Department of Food Science

Faculty of AgriSciences

Stellenbosch University

Supervisor: Prof Umezuruike Linus Opara

Co-supervisors: Prof Marena Manley

Dr Oluwafemi Caleb

April 2014

DECLARATION

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

Signature: ……

Date: April 2014

ABSTRACT

Knowledge of postharvest quality attributes of minimally processed packaged fruit is essential in order to establish the optimum shelf life period. The aim of this study was to investigate the effects of Passive-modified atmosphere packaging (MAP) on the quality of minimally processed pomegranate (cv. Bhagwa) arils and aril-sacs. These began by understanding the physiological processes i.e. respiration and transpiration rates of the whole fruit, arils and aril-sacs. The respiration rates (RR) of whole fruit, aril-sacs and arils were studied at 5, 10, 15 and 22°C, and comparisons were made among these fruit fractions. A high RR was observed in aril-sacs compared to whole fruit and arils across all storage temperatures. A 74.5% decrease in RR was observed when storage temperature was reduced from 22°C to 5°C. A significant increase in RR occurred from day 3 of storage across all fruit fractions and storage temperatures. The transpiration rates (TR) of arils and aril-sacs were studied at storage conditions of 5, 10 and 15°C and 76, 86 and 96% relative humidity (RH), and was found to increase with increase in temperature and decrease in relative humidity, with lowest TR occurring in fruit fractions stored at 5°C and 96% RH showing lower TR. Arils had high TR compared to aril-sacs, and this may be related to high surface area to volume ratio of exposed arils.

The effects of modified atmosphere packaging and application of anti-browning agents on quality of arils and aril-sacs stored at 5°C were studied. Compared to clamshell packaging, Passive-MAP using POLYLID® 107 polyethylene (PE) polymeric film showed greater positive effects in maintaining the quality and extends the shelf life of the arils and aril-sacs. Furthermore, the anti-browning agents used controlled browning on the cut-surfaces of the peel of the aril-sacs and reduced microbial growth in both arils and aril-sacs. When the effects of MAP and anti-browning were combined, aril-sacs stored better than arils. These treatments extended the shelf life of aril-sacs to 12 days while arils lasted up to 9 days.

The water vapour transmission rate (WVTR) of pomegranate fruit membrane was evaluated at cold storage (5°C, 90% RH) and room condition (18.7°C, 70% RH). A high WVTR occurred in membranes stored at room condition, compared to those stored at cold storage. Further studies are warranted to improve our understanding of the biophysical properties of pomegranate membranes in relation to possible exchange of water vapour and gases between the aril-sacs.

In summary, the use of MAP in combination with anti-browning agents showed a high potential in maintaining the quality of pomegranate arils and aril-sacs and consequently increase their shelf-life.

OPSOMMING

Kennis van naoes- gehalte-eienskappe van minimaal geprosesseerde verpakte vrugte is essensieel ten einde optimum rakleeftyd te bepaal. Die doel van hierdie studie was om die gevolge van passiewe gemodifiseerde atmosfeerverpakking (GAV) op die gehalte van arils en arilsakkies van minimaal geprosesseerde granaat (kv. Bhagwa) te ondersoek. 'n Aanvang is gemaak deur die fisiologiese prosesse, m.a.w. respirasie- en transpirasietempo's van die hele vrugte, arils en arilsakkies, te begryp. Die respirasietempo's (RT) van hele vrugte, arilsakkies en arils is by 5, 10, 15 en 22°C bestudeer, en vergelykings is getref tussen hierdie vrugdele. 'n Hoë RT is waargeneem by arilsakkies in vergelyking met hele vrugte en arils oor alle bergingstemperature heen. 'n Afname van 74.5% RT is waargeneem toe bergingstemperatuur van 22°C na 5°C verminder is. 'n Beduidende toename in RT het van dag 3 van berging af oor alle vrugdele en bergingstemperature heen voorgekom. Die transpirasietempo's (TR) van arils en arilsakkies is by bergingstoestande van 5, 10 en 15°C en 76, 86 en 96% relatiewe humiditeit (RH) bestudeer, en daar is bevind dat dit verhoog met 'n toename in temperatuur en 'n afname in relatiewe humiditeit, met die laagste TR wat voorkom by vrugdele geberg by 5°C en 96% RH wat dus laer TR toon. Arils het hoë TR gehad in vergelyking met arilsakkies, en dit kan verband hou met die verhouding van hoë oppervlakarea tot volume blootgestelde arils.

Die gevolge van gemodifiseerde atmosfeerverpakking en aanwending van middels vir die voorkoming van verbruining op gehalte van arils en arilsakkies geberg teen 5°C is bestudeer. In vergelyking met verpakking in toeknipbakkies (*clamshell packaging*), het passiewe GAV waarby POLYLID® 107 poliëtileen- (PE) polimeriese film gebruik is, groter positiewe gevolge by die behoud van gehalte getoon, en die rakleeftyd van die arils en arilsakkies is verleng. Daarbenewens het die middels vir die voorkoming van verbruining beheerde verbruining op die sny-oppervlakke van die skil van die arilsakkies gebruik en mikrobiese groei in beide arils en arilsakkies verminder. Toe die gevolge van GAV en die voorkoming van verbruining gekombineer is, het arilsakkies beter as arils geberg. Hierdie behandelings het die rakleeftyd van arilsakkies tot 12 dae verleng terwyl arils tot 9 dae gehou het.

Die waterdamptransmissiespoed (WDTS) van granaatvrugtemembraan is geëvalueer by koel berging (5°C, 90% RH) en kamertoestande (18.7°C, 70% RH). 'n Hoë WDTS het voorgekom by membrane wat by kamertoestande geberg is in vergelyking met dié wat in koelbewaring geberg is. Verdere studies is geregverdig vir verbetering van ons begrip van die biofisiese eienskappe van granaatmembrane in verhouding met moontlike uitruiling van waterdamp en atmosfere tussen die arilsakkies.

ACKNOWLEDGEMENTS

Glory to the Almighty God for giving me strength, power and ‘’taking the wheel’’ during my studies.

I would like to extend my genuine gratitude to the German Academic Exchange Service (DAAD) In-country scholarship, the Namibian Government Scholarship Training Program (NGSTP) for financial assistance to take up the MSc studies.

Special thanks go to my supervisor Prof. Umezuruike Linus Opara, co-supervisors Prof. Marena Manley and Dr Oluwafemi Caleb, for their guidance, encouragement, and technical advice and for giving me the opportunity to work under their supervision. I am humble for that.

To Dr Mahajan Pramod of Montpellier University, France, for his guidance and assistance on the mathematical modelling area.

To Prof. Martin Kidd, for his assistance and guidance on statistical analysis of the research data.

Office of the South African Research Chair in Postharvest Technology, Stellenbosch University: Ms Marie Maree, Ms Nazneen Ebrahim, fellow postgraduate students for your kind support and technical assistance. I am grateful for being part of this great team.

Food Science Department, Stellenbosch University: Ms Petro Du Buisson, Ms Veronique Human, Prof. Trevor Britz and Ms Daleen for their assistance, especially during my microbiology experiments.

I am indebted to Mr Fan Olivier of Houdocostant Pack-house, Porterville, for his guidance during the processing and packaging of pomegranate products used this study.

Credit goes to my dad, mom, sisters, brother and the adorable nieces and nephews for their prayers, love, care, support and inspirations during my studies and entire life.

I am grateful for the love and support from my friends who were patient with me, thank you for creating a home for me away from home. God bless you all.

I thank the South African Research Chair in (SARChI) Postharvest Technology, Stellenbosch University, for the privileges and financial support throughout my study.

To my Family

TABLE OF CONTENTS

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

Chapter 1: Introduction

CHAPTER 1

INTRODUCTION

Consumer demand for fresh, convenient, healthy, safe and nutritious food has contributed to the recent dramatic increase in chilled fresh-cut produce in the market (James & Ngarmsak, 2010). For the food industry to meet these demands, creative product development, use of new processing and innovative food packaging technologies are needed to maintain product quality and safety as well as assure convenience to the consumer. Various fruits have been processed into fresh-cut products, including apple, banana, mango, pineapple, watermelon and papaya (Gonzalez-Aguilar *et al.,* 2000; Arias *et al.,* 2007; Liu *et al*., 2007). Additionally, pomegranate (*Punica granatum* L.) is one of the fruit which is minimally processed into fresh arils (Ayhan & Esturk, 2009; Martinezo-Romero *et al.,* 2013). There has been a rapid increase in pomegranate consumption and this is attributed to its reported potential health benefits such as anti-helminthic, antioxidant and anti-hypertension activities (Gil *et al*., 2000; Viuda-Martos *et al.,* 2010).

Pomegranate was commonly used in ancient times as traditional medicine for removing parasites, as an anti-helminthic, to treat ulcers, microbial infections and respiratory pathologies (Viuda-Martos *et al*., 2010). Apart from its medicinal use, the seeds were regarded as an agent of revival by the Babylonians. Persians believed the seeds gave them hiddenness on the battle fields, while for the Chinese it symbolises immortality and longevity (Aviram *et al*., 2000). In modern society, the edible part of the pomegranate fruit is consumed as fresh arils or processed into fresh juice, canned as pastes and jam (Opara *et al.*, 2009; Ersan *et al.,* 2010; Viuda-Martos *et al*., 2010; Martinezo-Romero *et al.,* 2013).

Pomegranate is reported to have originated from the Mediterranean region (Fadavi *et al.,* 2006) and commercial production is now widely distributed globally, with the highest production being in India, USA, Spain and Turkey (Citrogold, 2012). Other countries where cultivation and research on pomegranate are done include Malaysia, Saudi Arabia, Egypt, Sultanate of Oman, China and South Africa (Fadavi *et al.*, 2006; Opara *et al.*, 2009; Anon., 2012). The harvesting period of pomegranate in the northern hemisphere is between September and November (Lopez-Rubira *et al.,* 2005), which offers a window of opportunity for countries in the southern hemisphere such as South Africa to supply in the counter season. The harvesting season for pomegranate in South Africa is between March and May, and the most commonly grown cultivars are Bhagwa, Arakta, Wonderful and Ruby (Citrogold, 2012). Although commercial pomegranate production in South Africa is at an early stage of development, farmers need to look broader on value addition to these fruit and taking advantage of the window of opportunity mentioned above.

Pomegranate fruit has a unique structure that is made up of compartments which contain arils. These compartments are separated by a membrane, which aid as a shield to the arils against water loss (Figure 1.1). Mature and ripe arils are red in colour owing to the high content of anthocyanins and flavonoids and contain about 10% total sugar (mainly fructose and glucose), 1.5% pectin and organic acids such as citric, ascorbic and malic acids (Viuda-Martos *et al.*, 2010). Pomegranate seeds are rich in lipids, which are mainly polyunsaturated fatty acids such as linoleic acid. However, there are difficulties involved when opening the fruit to remove the arils hence causing irritation from the phenolic metabolites which consequently tint the hands (Gil *et al*., 1996b). Therefore, processing of the pomegranate fruit into minimally processed aril-sac will ease the removal of arils and subsequently the consumption.

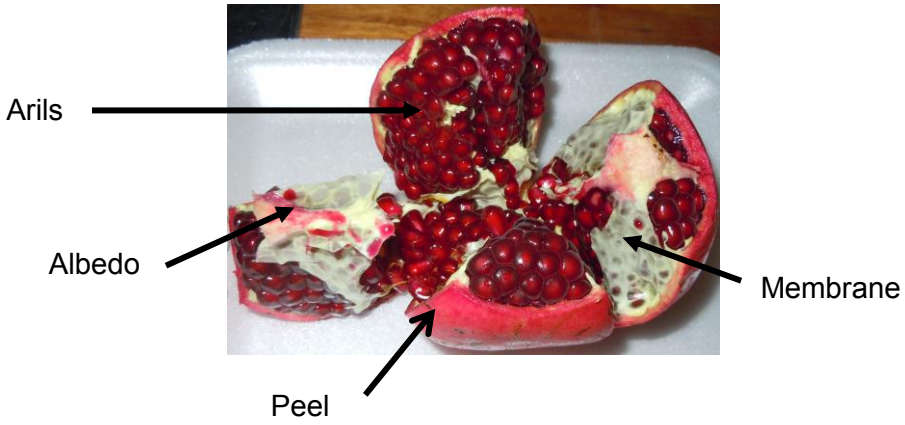

Figure 1.1 Parts of the pomegranate fruit

Minimally processed fresh produce deteriorate rapidly during storage and processing such as peeling and cutting that cause damage to the tissues (Garcia & Barret, 2002; Toivonen & DeEll, 2002). Consequently, the damage leads to the onset of enzymatic browning reactions and accelerated respiration rate and water loss (Toivonen & Deell, 2002; Liu *et al*, 2007; Mahajan *et al.,* 2008). Hence, this damage make them susceptible to microbial growth, degradation in colour, flavour and texture properties (Chonhenchob *et al*., 2006; Liu *et al*., 2007). Therefore, proper handling, hygiene, low temperature storage as well as the use of food graded anti-browning solutions offer the possibility to reduce degradation of food quality.

In order to extend the shelf-life of minimally processed fresh produce, biological processes such as enzymatic browning reactions, respiration and transpiration rates should be controlled. Various enzyme/browning inhibitors have been reported on a range of minimally processed produce. These include ascorbic acid and 4-hexylresorcinol (4-HR) to control polyphenoloxidase (PPO) activity in pears (Arias *et al.,* 2007), potassium sorbate, 4-HR and D-isoascorbic acid on fresh-cut mangoes (Gonzalez-Aguilar *et al.,* 2000), ascorbic acid on apple slices (El-shimi, 1993) and a combination of citric and ascorbic acids on fresh-cut pineapple (Montero-Calderon *et al.,*

2008). Furthermore, these pre-treatment conditions can be combined with food packaging which is a crucial step in the processing chain as it preserves and protects the food as well as aid in transportation and distribution (Robertson, 2006). In recent years, the use of low temperature in combination with enzyme/browning inhibitors and/or modified atmosphere packaging (MAP) has been found to be effective in extending the shelf life of minimally processed fruits (Artes *et al.,* 2000; Gonzalez-Aguilar *et al.*, 2000; Lopez-Rubira *et al.,* 2005; Arias *et al*., 2007; Liu *et al*, 2007)

Pomegranate is a non-climacteric fruit with low respiration rate (RR) which decreases through the postharvest period, reaching a stable state of about 4 mL CO_2 $kg^{-1}h^{-1}$ after 2 months when stored at 5°C under controlled atmosphere (CA) conditions (5% O_2, 0-5% CO_2 and >95%RH) (Artes *et al.,* 1996). Modified atmosphere packaging (MAP) has been used in the food processing and packaging industry for many years to extend and maintain product quality (Floros & Matsos, 2005). MAP involves the alteration of the gases inside the package as a result of direct injection of a mixture known gases (active MAP) or due to the interaction between the package contents and the air in the package and consequently causing the package atmosphere to change over time (passive MAP) (Floros & Matsos, 2005; Robertson, 2006). Preferred headspace condition can be achieved from the relationships between the produce respiration and the permeation of gases through the packaging film (Farber *et al.*, 2003; Mahajan *et al.*, 2008). Thus, the respiration and transpiration rate of the produce are needed to determine the favourable gas composition, relative humidity (RH), as well as suitable packaging film for the MAP (Song *et al*., 2002). MAP has beneficial effects in terms of colour and water retention, delaying microbial growth, and reducing metabolic processes such as respiration and browning (Artes *et al.,* 2009; Ayhan & Esturk, 2009). MAP application has been reported to increase the shelf-life and maintain the quality of pomegranate fruit and arils (Artes, *et al*., 1996; Caleb *et al*., 2012), as well as other fresh food like mushroom (Ares *et al*., 2007) and fresh-cut pineapples (Liu *et al*., 2007).

Recent studies have reported the effects of extrinsic factors such as gas composition, temperature, time and RH on the physiological responses of pomegranate arils and whole fruit packed under modified atmosphere (Artes *et al.,* 1996; Caleb *et al*., 2012). However, there is limited information on the physiological responses and quality attributes of minimally processed pomegranate aril-sacs (arils attached to its peel). The hypothesis of this study is that aril-sacs keep longer as compared to loose arils when packed under modified atmosphere. This raises the following question: does pomegranate stored as aril-sac have better keeping quality compared to arils when stored under modified atmosphere packaging? To answer this research question requires new insights into the respiration and transpiration rates of the fruit fraction. Furthermore, understanding the role of the internal fruit membrane structure on produce physiological responses such as water vapour transmission rate (WVTR) can also aid in answering the research question.

Therefore, the aim of this study was to investigate the effects of passive-MAP on pomegranate arils and aril-sac (cv. Bhagwa*)*. This was achieved by accomplishing the following specific objectives:

- Evaluate the respiration rates of the whole fruit, aril-sac and arils at selected storage temperatures;
- Determine the effects of storage conditions (temperature, %RH) on the transpiration rate (weight loss) of aril-sac and arils;
- Investigate the influence of modified atmosphere packaging and anti-browning treatment on quality attributes of aril-sac and arils; and
- Assess the water vapour transmission rate (WVTR) of pomegranate membranes.

References

Anonymous (2012). Pomegranate nurseries in South Africa. [Internet document]. URL http://www.safruitfarms.com. 26/07/2012.

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

Arias, E., Gonzalez, J., Oria, R. & Lopez-Buesa, P. (2007). Ascorbic acid and 4-hexylresorcinol effects on pear PPO and PPO catalysed browning reaction. *Journal of Food Science*, **72**(8), 422-429.

Artes F., Gines Marin J. & Martinez J.A. (1996). Controlled atmosphere packaging of pomegranate. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung*, **203**, 33-37.

Artes F., Villaescusa, R. & Tudela J.A. (2000). Modified atmosphere packaging of pomegranate. *Journal of Food Science*, **65**(7), 1112-1116.

Aviram M., Dornfeld L., Rosenblat M., Volkova N., Kaplan M., Coleman R., Hayek T., Presser D. & Fuhrman B. (2000). Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in human and in atherosclerotic apolipoprotein E-deficient mice. *American Journal of Clinical Nutrition* **71**, 1062-1076.

Ayhan, Z. & Esturk, O. (2009). Overall quality and shelf life of minimally processed and modified atmosphere packaged ‘’ready-to-eat’’ pomegranate arils. *Journal of Food Science*, **74** (5), 399-405.

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

Chonhenchob, V., Chantarasomboon, Y. & Singh, S.P. (2006). Quality changes of treated fresh-cut tropical fruits in rigid modified atmosphere packaging containers. *Packaging Technology and Science*, **20**, 27-37.

Citrogold (2012). Producing pomegranates in South Africa [Internet document]. URL http://www.citrogold.co.za/definitions.html. 26/07/2012.

El-shimi, N.M. (1993). Control of enzymatic browning in apple slices by using ascorbic acid under different conditions. *Plant Foods for Human Nutrition*, **43,** 71-76.

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.

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

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

Floros, J.D. & Matsos, K.I. (2005). Introduction to modified atmosphere packaging. In: *Innovations in Food Packaging*. Pp. 159-171. London, UK: Elsevier Ltd.

Garcia, E. & Barret, D.M. (2002). Preservative treatments for fresh-cut fruits and vegetables. In: *Fresh-cut Fruits and Vegetables: Science, Technology and Market*. Pp. 267-303., Boca Raton, Florida, USA: CRC press.

Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., Holcroft, D. M., &Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry*, **48**(10), 4581–4589.

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

Gonzalez-Aguilar, G.A., Wang, C.Y. & Buta, J.G. (2000). Maintaining quality of fresh-cut mangoes using anti-browning agents and modified atmosphere packaging. *Journal of Agricultural Food Chemistry*, **48**, 4204-4208.

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

James, J.B. & Ngarmsak, T. (2010). Processing of fresh-cut tropical fruits and vegetables: A technical guide. Food and Agriculture Organisation of the United Nations, Bangkok.

Liu, C., Hsu, C. & Hsu, M. (2007). Improving the quality of fresh-cut pineapples with ascorbic acid/sucrose pre-treatment and modified atmosphere packaging. *Packaging Technology and Science*, **20**, 337-343.

Lopez-Rubira, V. Conesa, A., Allende, A. & Artes, 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., Oliviera, F.A.R. & Macedo, I. (2008). Effect of temperature and relative humidity on the transpiration rate of the whole mushrooms. *Journal of Food Engineering*, **84**, 281-288.

Martínez-Romero, D., Castillo, S., Guillén, F., Díaz-Mula, H.M., Zapata, P.J., Daniel Valero, D. & Serrano, M. (2013). *Aloe vera* gel coating maintains quality and safety of ready-to-eat pomegranate arils. *Postharvest Biology and Technology,* **86,** 107–112.

Montero-Calderon M., Rojas-Grau, M.A. & Martin-Belloso, O. (2008). Effect of packaging conditions on quality and shelf life of fresh-cut pineapple (*Ananas comosus*). *Postharvest Biology and Technology*, **50,** 182-189.

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

Robertson, G.L. (2006). Modified atmosphere packaging. In: *Food Packaging, Principles and Practice.* 2nd Ed. Pp. 313-330. USA: CRC press, Taylor & Francis Group.

Song, Y., Vorsa, N. & Yam, K.L. (2002). Modelling respiration-transpiration in a modified atmosphere packaging system containing blueberry. *Journal of Food Engineering*, **53** (2) 103-109.

Toivonen, P.M.A. & DeEll, J.R. (2002). Physiology of fresh-cut fruits and vegetables. In: *Fresh-cut Fruits and Vegetables*. Pp. 91-123. USA: CRC Press.

Viuda-Martos, M., Fernandez-Lopez, J. & Perez-Alvarez, 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 2: Literature review

Table of contents

CHAPTER 2

LITERATURE REVIEW ON POSTHARVEST PHYSIOLOGY AND MODIFIED ATMOSPHERE PACKAGING OF POMEGRANATE

1. Introduction

Pomegranate (*Punica granatum* L.) belongs to Lythraceae and it is commonly consumed as fresh fruit (arils) or made into juice. It is high in antioxidant and antibacterial properties, hence used for therapeutic purposes (Viuda-Martos *et al*., 2010). Pomegranate is classified as a non-climacteric fruit, due to its relatively low respiration rate (RR), which steadily declines during postharvest storage.

Respiration and transpiration are the major physiological processes in fresh or fresh-cut fruits, including pomegranate and they are influenced by factors such as the type and maturity stage of the fruit, temperature, relative humidity and processing (Fonseca *et al.,* 2002; Garcia & Barrett, 2005). These physiological processes continue during postharvest handling and storage and their rates impact on the quality, and subsequently shelf life (Fonseca *et al.*, 2002). Hence, an understanding of the rate at which this processes occur helps postharvest technologists and food processors in making decision on what technology to develop or use, to extend produce shelf life and maintain wholesomeness. Like most fruits, the quality of pomegranate declines with time, more especially when minimally processed.

Optimum cold storage during postharvest used in combination with other techniques such as modified atmosphere packaging (MAP) has been reported to maintain the quality of fresh or minimally processed fruit and vegetables (Ersan *et al.,* 2010; Ayhan & Esturk, 2009; Porat *et al.,* 2009; Artes *et al*., 2000). The application of MAP on minimally processed fruits such as pomegranate, mangoes and apricot has been widely reported to maintain eating quality and extend shelf life (Gonzalez-Aguilar *et al*., 2000; Pretel *et al.*, 2000; Lopez-Rubira, 2000; Artes *et al.,* 2000; Caleb *et al.,* 2013). Ayhan and Esturk (2009) reported that the use of MAP on pomegranate arils maintained the physicochemical quality and extend the shelf life to about 18 days.

Several treatments, which includes the use of natural products has been found to help extend the shelf lives of fresh-cut fruits by effectively reducing or delay browning in these products (Gonzalez-Aguilar *et al.,* 2000; Lopez-Rubira *et al*., 2005; Arias *et al*., 2007; Liu *et al*., 2007; Montero-Calderon *et al.,* 2008; Martinez-Romero *et al*., 2013; Nabigol & Asghari, 2013). Moreover, studies on pre-treatment of pomegranate arils have been done and they include the use of aloe vera gel (Martinez-Romero *et al*., 2013; Nabigol & Asghari, 2013), UV-C radiation treatment

(Lopez-Rubira *et al*., 2005), the use of citric acid (Ayhan & Esturk, 2009) and treatment with honey (Ergun & Ergun, 2009).

This review presents detailed information on the economically important physiology and nutritional attributes of pomegranate, with a brief overview of the principles, techniques, and importance of MAP in postharvest management. Furthermore, the effects of MAP and use of anti-browning agents on physiology and quality of pomegranate fruit and arils will be highlighted.

2. Pomegranates: Origin and production

Pomegranates are reported to have originated from the Mediterranean region (Fadavi *et al.,* 2006) and commercial production is now globally distributed, with the highest production being in India, Iran, USA, Spain and Turkey (Citrogold, 2012). Other countries where pomegranate is cultivated includes Malaysia, Saudi Arabia, Egypt, Sultanate of Oman, China and South Africa (Anon., 2012; Fadavi *et al.*, 2006; Opara *et al.*, 2009; Fawole *et al*., 2013).

Pomegranate shrubs grow well in arid and semi-arid climates where summers are hot with little or no rainfall and winters are cool (Anon., 2012). Pomegranate shrubs can withstand drought conditions but may need supplemental irrigation to give the best yield (Citrogold, 2012). The harvesting usually start in the third year after planting, where about 50-60 fruits are allowed to mature, equating to 10-15 kg of fruits per tree (Citrogold, 2012). The fruits are categorised as early, mid-season and late according to their harvest date. The early cultivars usually reach their maturity in February, while the late cultivars mature in April. The closing of the calyx at the end of the fruit and when the skin indents slightly are usually the indicators of ripeness.

A summary of global production of pomegranate is presented in Table 2.1. Almost all of the world's pomegranate production is in the Northern Hemisphere; hence there is an opportunity for the countries in the Southern Hemisphere to supply fruit to these markets during off-season, since pomegranate shelf life is limited to about 4-5 months (Anonymous, 2012). Citrogold (2012) has categorised cultivar Bhagwa, Ruby and Arakta, under ''commercially recommended'' group. The production and marketing of these cultivars are known and the farmers can plant the cultivar with success, with little or no risk. This cultivar has been reported to be successfully grown in Western Cape, South Africa. In South Africa, the commercial production of pomegranate is still at an early stage (Citrogold, 2012). Provinces where pomegranate production is carried out include Western Cape, Northern Cape, Limpopo, North West and Mpumalanga (Wohlfarter *et al*., 2010). The area of land, under which pomegranate is cultivated commercially in South Africa is estimated to be about 1,200 hectares (Gedders, 2013). The harvested pomegranate fruits are usually exported to areas such as Northern Europe, Asia, Mediterranean, United Kingdom, Northern Africa, Canada

and Indian Ocean Island, to mention a few Perishable Products Export Control Board (PPECB) (2012). The export volume is reportedly to have increased since year 2010 from 788 pallets to 2,460 pallets in 2012 (PPECB, 2012).

Care is usually taken when harvesting to avoid bruising. For commercial purposes, the grading is carried out based on the weight and physical qualities such as free cracks, cuts, sun scalding and decay (Anon., 2012). Other quality indices include the evaluation of the skin colour and smoothness, the flavour, which depend on the sugar-to-acid ratio and the soluble solids contents above 17% with tannins below 0.25%. In South Africa, fruits for exports are packed in a single layer in 3.5 to 5 kg boxes. According to Citrogold (2012) the pomegranate is currently ranked 18th in terms of fruit consumed annually in the world.

Table 2.1 Pomegranate production in major selected countries (Citrogold, 2012)

Country	**Quantity (tons)**	**Main varieties**
China	160,000	Chonghuang, Chongfen, Baiyue, Dame, Dasuan
USA	100,000	Wonderful, Early Foothill
Turkey	75,000	Hicaz Fellahyemez
Iran	65,000	Malas, Saveh, Sara
Spain	60,000	Mollar de Elche, Tendral
India	20,000	Bhagwa, Arakta, Ruby
Israel	20,000	Wonderful
South Africa	5,700	Arakta, Ruby, Bhagwa, Acco, Herskawitz, Wonderful

2.1. Fruit structure

Pomegranate fruit is round, with leathery skin or rind, normally red, yellow or yellow-red, with yellow to light-yellow membrane and albedo (Figure 2.1) (Ozgen *et al*., 2008). The fruit contains many seeds each enclosed by juicy, sweet-acidic, red, pink or whitish, translucent, pulp or aril (Figure 2.2) (Ozgen *et al*., 2008). With specific reference to Bhagwa cultivar, fruit colour is deep red and the rind is thinner than other cultivars. It has large dark red arils, with soft seeds and is sweet with no aftertaste. Whole fruit mass ranges between 229 and 350 grams (Anon., 2012).

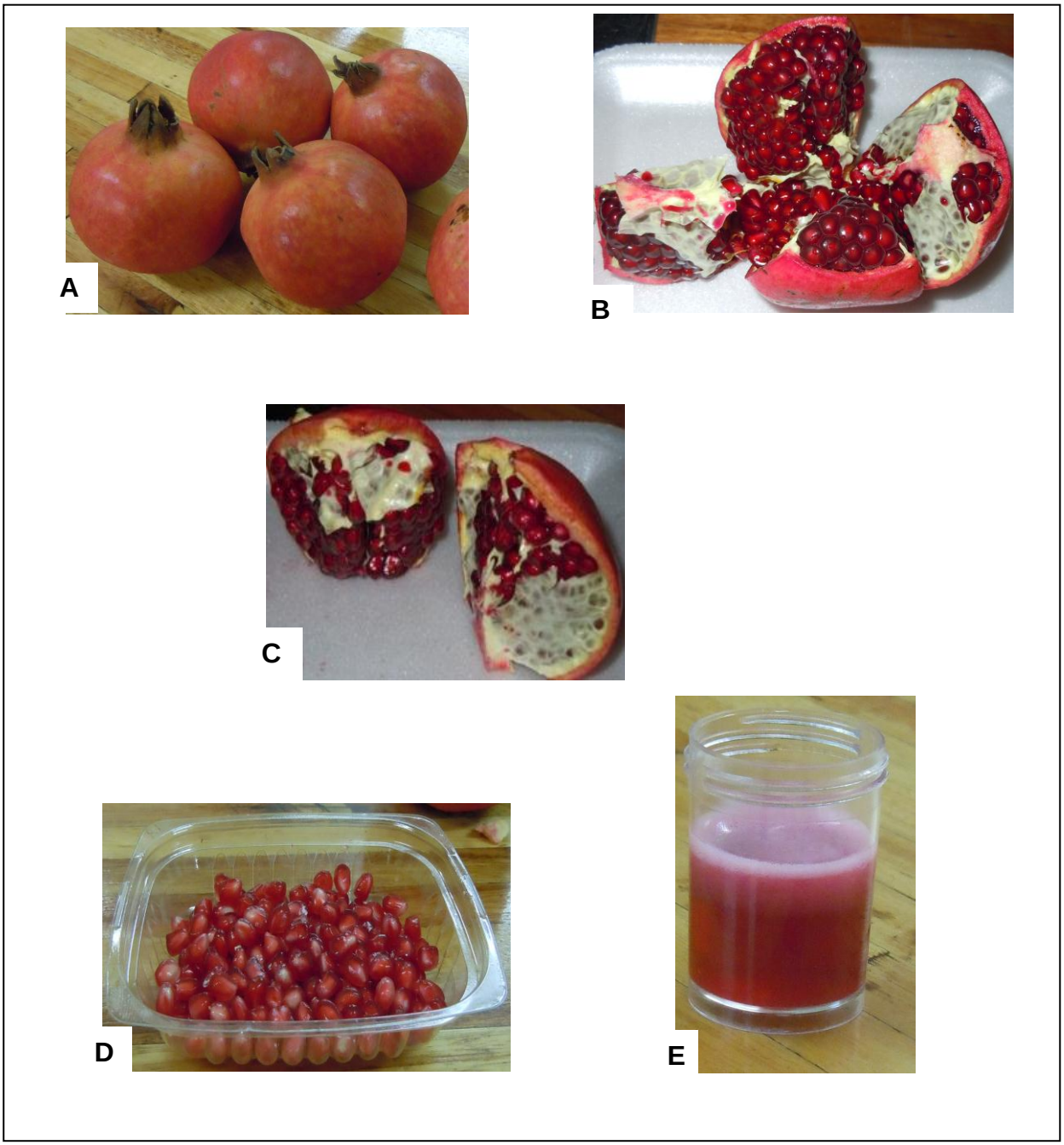

Figure 2.1 Different parts of the pomegranate fruit (A); B: section of pomegranate fruit; C: aril-sacs; D: arils; E: juice

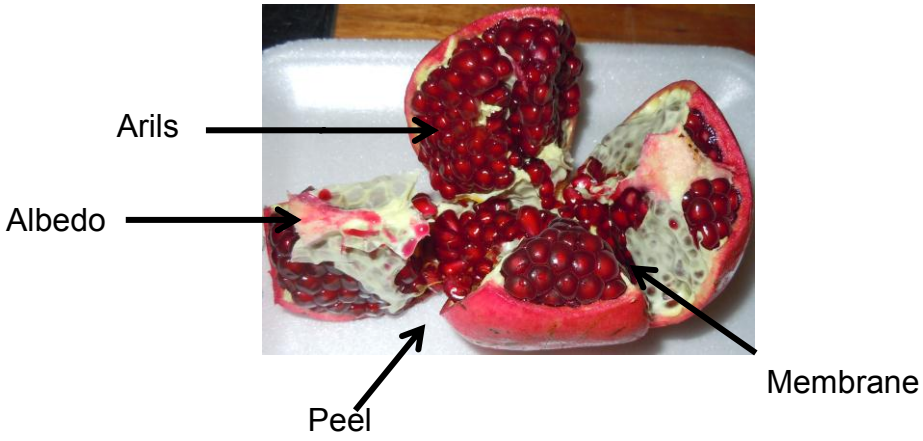

Figure 2.2 Parts of the pomegranate fruit

3. Economic importance of pomegranate

Pomegranate was commonly used in ancient times as traditional medicine, for removing parasites, as an anti-helminthic, to treat ulcers, microbial infections and respiratory pathologies (Viuda-Martos *et al*., 2010). Apart from its medicinal use, the seeds were regarded as an agent of revival by the Babylonians. Persians believed the seeds gave them hiddenness on the battle fields, whereas for the Chinese it meant immortality and longevity (Aviram *et al*., 2000). In modern society, edible part of pomegranate fruit is consumed as fresh arils or processed into fresh juice, canned as pastes and jam. The fruit is also used as a flavour and colour enhancer in the beverage industry (Opara *et al.*, 2009). Principal functions and medicinal effects of pomegranate fruit includes improved oral health, anti-diabetic properties, improved skin health, improved cardio-vascular health, antimicrobial, anti-tumoral, anti-inflammatory (Ozgen *et al*., 2008).

Pomegranate fruit is a good source of vitamins (vitamin C, A, B, thiamine, niacin etc.) minerals and dietary fibre, hence playing a good role in human nutrition (MRC, 2010; Simson & Straus, 2010; Kader, 2002). In addition, they contain various compounds that may help reduce the risk of heart diseases, cancer and other health conditions. These compounds include flavonoids (anthocyanins and phenolics), isoflavones and other phytochemicals (Simson & Straus, 2010; Kader, 2002).

Valuable compounds are found in different parts of the pomegranate fruit. Depending on various factors such as growing region, cultivar, maturity, storage conditions, cultivation practice and climate, the chemical composition of fruit may differ (Viuda-Martos *et al.,* 2010). The peel contributes about 50% of total fruit weight (Li *et al.*, 2006) and is reported to be a vital source of

bioactive compounds such as ellagitannins (ETs), phenolic, flavonoids, and pro-anthocyanidin compounds, minerals and complex polysaccharides (Li *et al.*, 2006; Fawole *et al*., 2012). Generally, edible part of the fruit is made up of 40% arils, whereby arils contain about 85% water, 10% total sugars, mainly glucose and fructose and 1.5% pectin, organic acids (ascorbic acid, citric acid and malic acid) and bioactive compounds such as phenolics, and flavonoid (Aviram *et al*., 2010; Tezcan *et al*., 2009). Seeds are a good source of total lipids and seed oil content contributes about 12-20% of total seed weight (Aviram *et al.,* 2010; Fadavi *et al.,* 2006). Pomegranate seed oil has high content of polyunsaturated (n-3) fatty acids such as linoleic, linolenic, other lipids such as oleic acid, punic acid and stearic acid (Fadavi et al., 2006; Ozgul-Yucel, 2005).

Opara *et al*. (2009) reported a variation in vitamin C content among five cultivars of pomegranate (Indian white, Indian red (Ruby), Indian red (Bhagwa)), from Oman and Egypt. The vitamin C content of these cultivars ranged from 52.8 - 72.0 mg 100 g^{-1} of fresh weight for arils and 76.8 - 118.4 mg 100 g^{-1} fresh weight for peels with cv. Bhagwa having higher content in peels than others. According to the Medical Research Council (MRC) (2010), raw peeled pomegranate has higher content of potassium (259 mg 100 g^{-1}) compared to other minerals (Table 2.2).

Table 2.2 Nutritional content of pomegranate arils (MRC, 2010)

Typical Nutritional Information (Information refers to raw peeled pomegranate)	Per 100g	**Per 87 g single serving**
Moisture (g)	81.0	70.5
Energy (kJ)	321.0	279.3
Total Protein (g)	1.0	0.9
Total Fat (g)	0.3	0.3
Available Carbohydrate (g)	16.6	14.4
Of which total sugars	16.6	14.4
Glucose (g)	9.0	7.8
Fructose (g)	7.3	6.4
Sucrose (g)	0.3	0.3
Total dietary fibre (g)	0.6	0.5
Insoluble dietary fibre (g)	0.5	0.4
Soluble dietary fibre (g)	0.1	0.1
Ash (g)	0.6	0.5
Organic acids		
Malic acid (mg)	399.0	347.1
Citric acid (mg)	1357.0	1180.6
Vitamins and Bioactive Components		
Vitamin A (µg retinol equivalents)	6.0	5.2
Niacin (mg)	0.3	0.3
Folate (µg)	6.0	5.2
Panthothenic acid (mg)	0.6	0.5
Biotin (µg)	3.8	3.3
Vitamin C (mg)	6.0	5.2
Vitamin E (mg)	0.6	0.5
Total carotenoids (µg)	33.0	28.7
Of which β-carotene (µg)	26.0	22.6
Minerals		
Calcium (mg)	3.0	2.6
Iron (mg)	0.3	0.3
Magnesium (mg)	3.0	2.6
Phosphorous (mg)	8.0	7.0
Potassium (mg)	259.0	225.3
Sodium (mg)	3.0	2.6
Manganese (µg)	197.0	171.4

4. Quality parameters

4.1. Visual quality

Consumers' perception of fresh produce quality is initially influenced by the visual attributes such as size, colour and absence of decay and blemishes (Kader, 2002; Pathare *et al.*, 2012). Some postharvest defects found on fruits may be physical, physiological, morphological or pathological (Kader, 2002). Furthermore, temperature related disorders such as freezing, chilling injuries, sunburn or sunscalds affects the quality of whole fruits and fresh-cut products, hence good postharvest management is required to control them.

Colour

Colour is an important visual quality attribute in the food and bioprocess industries (Pathare *et al*., 2012). It represents the first contact between the product and the consumer (buyer), therefore it has a direct influence on the buying action (Hess-pierce & Kader, 2003; Mena *et al*., 2011). Pathare *et al*., (2012), reported that the food colour is influenced by the chemical, biochemical, physical changes and microbial which occur during growth maturation, postharvest handling and processing. Colour and acid content are generally used as maturity indices for separation of harvested pomegranate fruit into required grades as well as in the harvest management (Kader & Barret 2005).

The skin colour of pomegranate fruits varies widely among cultivar as well as the geographical area, according to the study done on cultivars such as Indian red (Bhagwa), Indian red (Ruby), Indian white, cultivars obtained from Egypt and Oman (Opara *et al*., 2009). A high *L** of 69.73 was reported on cultivar found in Oman, and Indian red (Bhagwa) showed a lower *L** of 42.44. The *a** value, which indicates the redness was high in Indian red (Ruby) of 39.16 and lower in Oman cultivar, which was -3.83 (Opara *et al*., 2009). The cv. 'Mollar' has been reported to have a yellow/pink skin, cv. 'Arakta' has very deep scarlet colour skin, and cv. 'Bhagwa' has deep red skin and cv. 'Ruby has a pillar-box red colour (Citrogold, 2012). Artés *et al.* (1996) reported that the colour intensity of the juice increased with storage under controlled atmosphere. Furthermore, the colour of pomegranate arils cv. Acco) was reported to be about *L**: 39.55, *a**: 27.75 and *b**: 15.41, after commercial harvest (Caleb *et al.,* 2013). Various studies have looked at the effects of different storage conditions as well as the treatments on the colour of arils. The arils colour does not vary much with storage period or condition such as MAP (Ayhan & Esturk, 2009).

Texture

Texture quality factor includes the firmness, juiciness and toughness of the products. The texture is not only vital in eating and processing quality, but on the shipping ability too (Kader, 2002). When

shipping soft fruits, they are likely to suffer from physical injuries, leading to postharvest losses. Hence it is crucial to maintain the right texture of the fruits, depending on the end-use purpose. The texture of arils was reported to be in the range of 76.10 to 85.55 N (Caleb *et al*., 2013).

Firmness

Firmness of pomegranate arils have been reported to decrease throughout the storage period, however the use of pre-treatments such as Aloe vera helped delay the softening of arils in comparison to those that are not treated (Martinezo-Romero *et al*., 2013). The reduction in firmness throughout storage has been reported in various products such as fresh-cut mangoes, fresh-cut radishes and non-treated arils (Gonzalez-Aguilar *et al*., 2000; Gonzalez-Aguilar *et al*., 2001). The texture of arils can be affected by water loss as well as microbial growth which lead to decay (Gonzalez-Aguilar *et al*., 2000; Martinezo-Romero *et al*., 2013). Such developments can be controlled by storing the products at lower temperature (Gonzalez-Aguilar *et al*., 2000; Martinezo-Romero *et al*., 2013).

4.2. Chemical attributes

Total soluble solids titratable acidity and pH

Apart from the physical quality attributes, the total soluble solids (TSS), titratable acidity (TA) and pH are important factors when describing the quality of the fruit product, as they contribute to its flavour (Kader 2009). Total soluble solids are referred to total soluble sugar content available in the fruits. They are reported to increase with fruit maturity as starch is converted to carbohydrates (Hardy & Sanderson, 2010). Total soluble solids is determined by measuring the refractive index of the beam of light that get refracted as it pass through the juice and expressed as °Brix. Total soluble solid content is reported to range between 14.07-15.10 °Brix for cv. Arakta, Bhagwa and ruby (Fawole *et al.*, 2011). Various studies have found that the TSS of pomegranate arils decreases with storage period (Lopez-Rubira *et al*., 2005; Caleb *et al*., 2013; Martinezo-Romero *et al*., 2013).

Titratable acidity is the measure of total acidity as opposed to pH which measures the concentration of the hydrogen ion present in the juice (Hardy & Sanderson, 2010). Titratable acidity in pomegranate is mostly reported as grams of citric acid (CA) per 100 mL of juice. Fawole *et al*, (2011) reported the TA of pomegranate arils to range between 0.22 and 0.32 g CA 100 ml^{-1} of pomegranate juice. The pH of pomegranate juice is reported to be in the range of 3.32 and 3.64 (Fawole *et al*, 2011). The ratio of TSS/TA is used as the maturity index at harvest as well as during processing (Kader, 2009). A lower TSS/TA ratio is usually found in immature fruits, due to low sugar and high acid levels, hence making fruit to taste sour. As the fruit ripen and reach a maturity

stage, the TSS increases while the acids decrease resulting in a high TSS/TA ratio which causes the fruit to have a sweet taste (Hardy & Sanderson, 2010). The TSS and TA generally depends on the cultivar, the maturity stage and the position on the tree before harvest, whereby fruits under the canopy usually have a lower TSS as compared to those outside the canopy (Hardy & Sanderson, 2010).

4.3. Bioactive compounds

Natural polyphenols can be simple molecules such as phenolic acids, flavonoids, phenylpropanoids or highly polymerised compounds which include lignins, tannins and melanins (Viuda-Martos *et al.,* 2010). Phenolics are secondary metabolites responsible for the functional properties or benefits in many fruits including pomegranate (Haminiuk *et al.,* 2012). Thus there has been a growing interest in these compounds which may be related to their antioxidant potential and the relationship between their consumption and prevention of some diseases (Haminiuk *et al.,* 2012). Phenolic acids present in pomegranate juice can be divided into 2 groups: (1) hydroxybenzoic acids, mainly gallic acid and ellagic acid (EA) and (2) hydroxycinnamic acids, principally caffeic acid, chlorogenic, and *p*- coumaric acids (Viuda-Martos *et al.* 2010).

Pomegranate fruit extracts (PFE), are rich sources of two polyphenolic compounds: anthocyanins and hydrolysable tannin (Gil *et al*., 2000). Anthocyanins such as delphinidin, cyaniding and pelargonidin) are responsible for the red and purple colours of the fruit and juice (Gil *et al*., 2000; Afaq *et al*., 2005). These compounds forms the most important group of flavonoids found in arils (Afaq *et al*., 2005). Other compound are the hydrolysable tannins which include punicalin, pedunculagin, punicalagin, gellagic and ellagic acid esters of glucose, which contributes to the 92% of the antioxidant activity of the whole fruit (Gil *et al.*, 2000). Anthocyanins content for some of the pomegranate cultivars found in South Africa, which are Arakta, Bhagwa and Ruby, were reported as 16.53, 26.93 and 18.14 mg C_3gE 100 mL^{-1} of pomegranate juice, respectively. Apart from anthocyanins, pomegranate peel is rich in hydrolysable tannins, and these are polyphenols that gives the fruit the astringency attribute, when available in high content (Viuda-Martos *et al.,* 2010).

5. Physiological behaviour of pomegranate

5.1. Respiration

Respiration is defined as the metabolic process from which plants receive their energy for biochemical processes. This involves the use of oxygen (O_2) to break down complex organic reserves into simpler molecules whereby energy is released in addition to the production of carbon

dioxide (CO_2) and water (Fonseca *et al.*, 2002). The respiration quotient (RQ) is the ratio of CO_2 produced to O_2 used up during this metabolic process, it is defined by the type of substrate used (Saltveit, 2012). The RQ of the fruit is generally equal to 1.0 when carbohydrates are used as substrates, while values lower than 1.0 indicate lipid as a substrate and the RQ more than 1.0 indicates acid as the metabolic substrate used. The occurrence of anaerobic respiration is normally indicated by the RQ which is greater than 1.0 (Saltveit, 2012).

Respiration rate (RR) is reported to differ among various fruits and the stage of maturity (Gonzalez-Aguilar *et al*., 2000; Saltveit, 2012). In case of non-climacteric fruits, respiration rate is always higher at the early stage of maturity, and will decreases as produces reach their maximum maturity, however with climacteric fruits, it reaches its maximum at the maximum maturity stage and decline at the post-climacteric. Hence, this may be due to the availability of organic molecules since the sugar content also decreases (Saltveit, 2012). Respiration rate is influenced by an array of internal and external factors (Caleb *et al.*, 2013). Internal factors include the type and maturity stage of the fruit, whilst external factors include temperature, relative humidity and processing (Fonseca *et al.,* 2002; Garcia & Barrett, 2005; Caleb *et al.*, 2013). The extent to which fresh produce metabolic processes occurs have an impact on the product quality attributes, such as colour, texture and microbial growth, hence leading to the reduction in shelf life (Fonseca *et al.,* 2002; Saltveit , 2012).

Even though the general aim of processing fruit is to prolong their shelf life as observed in processing methods such as drying, canning and freezing, minimally processed fruits become more susceptible to deterioration (Garcia & Barret, 2005). When preparing fruits into fresh-cut products, the wounding of the tissues lead to various physical and physiological stresses on the produce (Saltveit 2012). Garcia and Barret (2005) further mentioned that responses such as increase in RR and ethylene product have a negative effect on the produce's quality and consequently its shelf life. An increase in RR can be related to the increase in cellular metabolism due to direct tissue contact with oxygen, which speeds up the rate of deterioration (Fonseca *et al.*, 2002). During processing of fresh-cut fruits, the damage imposed on the protective epidermis or skin of the fruit causes the gas diffusion to change which consequently cause an increase in water loss and stimulate microbial contamination (Garcia & Barret, 2005).

Respiration rate of fresh produce can either be expressed as the rate of O_2 consumption and/or the rate of CO_2 production. Various studies reported on the RR of pomegranate. Artes *et al.* (1996), reported that pomegranate followed the non-climacteric pattern of fruits since it showed a low RR which decreases through postharvest period reaching a stable state of about 4 mL CO_2 $kg^{-1}h^{-1}$ for nearly 2 months when stored at 5°C, under controlled atmosphere condition (5% O_2, 0-5% CO_2 and RH above 95%). Furthermore, Caleb *et al.* (2012) reported the RR of whole fruit cv

''Acco'' and ''Herskawitz'' to be in the ranges of 4.53 to 14.67 mL O_2 $kg^{-1}h^{-1}$ and 5.67 to 18.53 mL CO_2 $kg^{-1}h^{-1}$, respectively, when stored at temperatures between 5 and 15°C. Thus, this difference in RR of pomegranate whole fruit explains the effects of storage temperature on the metabolism of the fruit. Lopez-Rubira (2005), reported a minimum respiration rate of arils (cv. 'Mollar') of about 1.15 mL CO_2 $kg^{-1}h^{-1}$ when stored at 5°C, while Ersan *et al.,* (2010), reported 0.52 mL CO_2 $kg^{-1}h^{-1}$ (cv. 'Hicaz') under a storage condition of 2% O_2 with 10% CO_2 at 4°C. Furthermore, results from the study conducted by Caleb *et al*. (2012), indicated the arils (cv. 'Acco' and 'Herskawitz') respiration rate of about 2.72 CO_2 ml $kg^{-1}h^{-1}$, when stored at 5°C under ambient air condition. The respiration rate of the arils differs, depending on the storage condition as well as on the cultivar.

5.2. Transpiration/water loss

Transpiration is the process by which fresh fruits and vegetables lose moisture. Fresh produce transpiration process involves various stages which are the movement of moisture through the skin of the produce, the evaporation of moisture from the produce surface and the mass transfer moisture to the surroundings (Becker & Fricke, 2013). As opposed to the attached fruit where water lost during transpiration is replaced from the flow of sap, the detached fruit relies on its water content. Hence, the fruit become dependent only on its own food reserves and water content, in order to survive (Mahajan *et al.,* 2008). This water loss consequently leads to shrinkage and wilting of the fruits and soon its quality deteriorates, notably when it lost 5% or 10% of its fresh weight (FAO, 2012), impaired flavour can also be experienced (Becker & Fricke 2013). Water loss in fresh produce results in the loss of salable weight, appearance, nutritional quality and texture quality that includes softening, as well as loss of crispness and juiciness (Kader and Barret, 2005).

Transpiration rate process is influenced by various factors through different mechanisms. Factors such as temperature, surface area, relative humidity (RH), air movement and respiration rate (Ben-Yehoshua & Rodov, 2002; Kader and Barret, 2005; Mahajan *et al*., 2008) were reported to have effects on water loss of fresh produce. Food and Agricultural Organization (FAO) (2012) reported that the moisture content in the air determines the rate of water loss, such that a lower moisture content in the air cause an increase in transpiration rate of the fruit. It was further reported that the rate at which water is lost is dependent on the difference between vapour pressure inside the fruit and that in the air; hence a moist environment is needed to keep water loss in fruits minimal.

The rate of air movement plays a role too in water loss whereby a higher water loss is incurred whenever the surrounding air of the fruit moves faster. Though it is needed in removing the heat of respiration, it needs to be kept as low as possible (Becker & Fricke 2013). Furthermore, the rate of water loss depends on the type of produce, i.e. leafy vegetables have a higher rate of water loss

compared to other vegetables or fruits which have thicker skins (Raghavan *et al.*, 2005). Above all, the ratio of the surface area to volume plays a critical role on the TR. The rate of water loss is directly proportional to the surface area and volume ratio (Kader & Barret, 2005). Other factors that influence water loss includes the surface injuries, maturity stage or produce related factors (Kader, 2002).

Lower temperature and high relative humidity has been reported to play a major role in maintaining the produce quality by reducing its rate of water loss (Mahajan *et al*., 2008, Caleb *et al*., 2013b). However, these storage conditions are produce-dependent, with the optimal RH ranging between 85 and 96% and temperatures between 4 and 7°C, for various produces such as eggplant, mizuna, and fig fruits (Hung *et al.*, 2011), pomegranate arils (Caleb *et al*., 2013b), and mushrooms (Mahajan et al., 2008). Caleb *et al*. (2013b) reported that the weight loss of pomegranate arils cv. 'Acco' was notably higher at 15°C and 76% RH, while at 5°C and 96% RH, the quality of arils were best kept.

Similarly, Mahajan *et al*. (2008) reported an increase in the RH from 76% to 96% decreased the TR of mushrooms by 87% at 4°C, while a decrease in temperature from 16 to 4°C reduced TR by 61% at 96% RH. The transpiration rate of pomegranate arils in the study by Caleb *et al*., (2013b), was found to be in the range of 1.14 to 16.75 g kg^{-1} d^{-1} across the combination of the temperature 5, 10 and 15°C and RH of 76, 86 and 96%. Moreover, Mahajan *et al*. (2008) reported on the TR of mushroom to range between 6.5 to 96 g kg^{-1} d^{-1}, when stored under the same storage condition used by the above mentioned researchers. Thus it is crucial to predict/measure the rate of water loss, as this helps to estimate the shelf life of the fresh or minimally processed produces as well as designing the suitable packaging at optimal storage conditions (Caleb *et al.,* 2012).

6. Browning control

Apart from the respiration and water-loss that occur in fresh-cut products, the most common physical change that affects the fresh-cut products quality and shelf life is the surface browning. Browning of the surface is induced by the physical injuries such as cutting or peeling during processing or as a result of improper handling of fruits during harvest or storage (Garcia & Barrett, 2005). Thus, this unit operations cause the fruits epidermis cells to rupture and release their contents which includes enzymes that then comes in contacts with the substrates (Garcia & Barrett, 2005). The polyphenol oxidase (PPO), which is the group of enzymes that occur in higher quantity in fruits such as mangoes, apples, banana, leads to the discolouration of the surface which is referred to enzymatic browning.

Physical and chemical methods can be used to control enzymatic browning and in most cases they are used in combination. Physical methods include storage at lower temperature, the use of modified atmosphere packaging, reduction of oxygen and the use of edible coatings (Garcia & Barret, 2005, Arias *et al*., 2007; Liu *et al*., 2007), and treatment by the use of high pressure or gamma irradiation (Garcia & Barret, 2002). Additionally chemical methods used involve the use of treatments that can inhibit the enzyme polyphenol oxidase by removing its substrates which are oxygen and phenolics (Gonzalez-Aguilar *et al.,* 2000; Garcia & Barret 2005; Arias *et al*., 2007; Liu *et al*., 2007; Maghoumi *et al*., 2013). Traditionally, heat application is used on the fresh-cut products through cooking or by blanching. During blanching, the PPO is expected to be inactivated by heat. However, care needs to be taken when exposing such product to heat as to avoid the interruption on the respiration of the produce and avoid disruption of tissues. Various anti-browning agents are used based on their mode of actions. Anti-browning agents are used to directly prevent the PPO activities e.g. 4-hexyresercinol, other reacts with the outcomes of the PPO e.g. ascorbic acid, and other agents provide a medium which is not enough for the browning reaction to take place e.g. sodium erythorbate (Gonzalez-Aguilar *et al.,* 2000; Garcia & Barret, 2002).

Generally, these chemicals or agents used to control browning are used as solutions, mostly as formulation containing more than one compound (Gonzalez-Aguilar *et al.,* 2000). These agents are categorised in groups such as: acidulants, reducing agents, chelating, complexing agents and enzyme inhibitors. The most common agents include the acidulants, reducing agent and enzyme inhibitors. Acidulants lower the pH of the product, since the optimum PPO activity of most fruits and vegetables range between pH 6.0 and 6.5, while a lower activity can be encountered below pH 4.5 (Whitaker, 1994). Mallic and citric acids are normally used as acidulants. The reducing agents control the enzymatic browning by reducing the colourless O-quinone which is the product of PPO reaction, back to the O-diphenols (Garcia & Barret, 2002). Ascorbic acid is the reducing actions and it further lowers the pH of the product (Whitaker, 1994). The 4-hexylresorcinol chemical is one of the anti-browning agents which successfully prevent discolouration on shrimps, hence used as an enzyme inhibitor (Garcia & Barret, 2002). Several studies have been done on the use of anti-browning agents in fresh-cut products such as mangoes, pears, apples and pineapples as summarized in Table 2.3 (Gonzalez-Aguilar *et al.,* 2000; Arias *et al.,* 2007; Liu *et al*., 2007). From these studies, one can conclude that the browning control is effectively achieved through a combination of various anti-browning agents with different mode of actions. These agents include citric acid, potassium sorbate, calcium chloride, sodium erythorbate, to mention a few (El-shimi, 1993; Sapers *et al.*, 1994; Gonzalez-Aguilar *et al.,* 2000; Arias *et al.,* 2007; Liu *et al*., 2007; Montero-Calderon *et al*., 2008).

The combination of 4-hexylresorcinol (0.001 M), potassium sorbate (0.05 M) and D-isoascorbic acid (0.5M) was reported to reduce discolouration and microbial growth in fresh-cut mangoes and no change in sensory characteristics was perceived (Gonzalez-Aguilar *et al.,* 2000). Moreover, Montero-Calderon *et al*. (2008) found a combination of 1% citric acid and 1% ascorbic acid solution to have anti-browning effects on pineapple fresh-cuts when dipped in this solution for 2 minutes. Apart from the anti-browning effects of the above mentioned agents, anti-microbial effects are also reported to be observed as a result of such agents (Gonzalez-Aguilar *et al.,* 2000; Montero-Calderon *et al*., 2008).

Table 2.3 Some reported anti-browning agents used in minimally processing of fruits and vegetables

Product	Anti-browning agent	References
Apple	Ascorbic acid	El shimi (1993)
Mushroom	Sodium erythorbate, Cysteine, EDTA (disodium salt)	Sapers *et al.* (1994)
Mangoes	4-hexylresorcinol, potassium sorbate, isoascorbic acid	Gonzalez-Aguilar *et al.,* (2000)
Radish	4-hexylresorcinol, potassium sorbate, N-acetylcysteine isoascorbic acid	Gonzalez-Aguilar *et al.,* (2001)
Pineapple	Ascorbic acid, sucrose	Liu *et al.* (2007)

7. Principles of modified atmosphere packaging (MAP)

Food packing is important as it prevents the loss of quality in products and provides the protection against the environmental contaminants (Al-Ati & Hotchkiss, 2002). One of the most commonly used packaging techniques is modified atmosphere packaging (MAP). Robertson (2006) defined MAP as an enclosure of food in a package where the atmosphere inside is modified or changed to provide an optimum atmosphere for increasing the shelf life and preserve the food quality. The modification or alteration of the atmosphere inside the package depends on the respiration of the produce packed and the permeability of the packaging film to gases and water vapour, which can be influenced by temperature and relative humidity (Caleb *et al*., 2012c).

Floros and Matsos (2005) highlighted the objectives of MAP to extend the shelf life of food products and prevent or reduce any undesirable changes in the safety, sensory and overall quality attributes and nutritive values of foods. MAP is used to delay the deterioration of the foods that are not sterile and their enzymatic activities are still active (Robertson, 2006; Gorny, 2003). This is done since it reduces the respiration rates of stored/packed products (Raghavan *et al*., 2005).

The above mentioned objectives are thus based on three principles: MAP reduces undesirable physiological, physical, chemical and biochemical changes; control/inhibit the microbial proliferation/growth and prevent contamination of food products (Robertson, 2006) and consequently extend their shelf lives. The nature of the environment in which food is stored plays a vital role on its shelf life. Raghavan *et al.,* (2005) highlighted the three main descriptors of the storage environment, and these are; temperature, relative humidity (RH) and gas composition of the storage environment.

Apart from baked products, MAP is reportedly to be used in combination with cold/chill temperatures (Artes *et al* 2007; Caleb *et al*., 2012c; Farber *et al*, 2003). Ayhan (2010) reported that the success of MAP depend on the low temperature storage and chain management especially for minimally processed and fresh fruits. Chill temperatures are normally taken as -1 to 7°C such that temperatures close to or higher than the freezing point of the fresh produce dealt with (Robertson, 2006). Therefore, by combining the chill temperatures with modification of gas atmosphere, their preservative effects can be enhanced. Since some deteriorative reactions involves aerobic respiration where food or micro-organism consumes O_2 and produces CO_2 and water, the reduction of oxygen and an increase in carbon dioxide can slow down aerobic respiration and reduce/inhibit microbial growth, respectively.

7.1. Gases used in MAP

According to Brandenburg & Zagory (2009), most packaging strategies for extending the shelf life of food products involve the use of atmospheric gases in proportions that are different from those found in air. Three main gases used in MAP are Nitrogen (N_2), Oxygen (O_2) and Carbon dioxide (CO_2) (Sandhya, 2010; Robertson, 2006; Floros & Matsos, 2005; Mullan, 2002). At sea level, the atmosphere air composition is approximately 78.1% N_2, 20.9% O_2 and 0.03% CO_2 (Floros & Matsos, 2005). The choice and amount of gas used depends mainly on its properties and the food product being packed (Floros & Matsos, 2005; Mullan, 2002). These gases are usually used singly or in combination to extend the shelf life with optimal organoleptic properties of food (Anon., 2013b; Sandhya, 2010).

Nitrogen

Nitrogen is described as an inert odourless and tasteless gas (Floros & Matsos, 2005; Robertson, 2006). The low solubility of N_2 in water (0.018 g kg^{-1} at 100kPa, 20°C) (Sandhya, 2010) makes it favourable for use in MAP as it is used to replace O_2 and balance the volume decrease due to the dissolution of CO_2, hence used as a filler gas to prevent package collapse (Sandhya, 2010; Robertson, 2006; Floros & Matsos, 2005; Mullan, 2002).

Oxygen

Oxygen gas is colourless, odourless and highly reactive, with solubility in water of 0.040 g kg^{-1} at 100kPa, 20°C (Sandhya, 2010). In general, O_2 inhibit the growth of anaerobic micro-organisms, but promote the growth of aerobic micro-organisms (Anon., 2013b; Floros & Matsos, 2005; Mullan, 2002). Additionally, it is responsible for various unfavourable reactions in food which includes oxidation and rancidity in fatty food, browning reactions, fast ripening and senescence in fruits and vegetables, pigment oxidation, stalling in bread and microbial spoilage (Anon., 2013a; Sandhya, 2010; Mullan, 2002). Therefore, due to its negative effects on food preservation, it is generally avoided or kept low in MAP of many food products. For MAP, the recommended oxygen level should be between 2-5%, where by any quantity below this may lead to anaerobic respiration of the produces which leads to undesirable characteristics on the product (Lopez-Rubira *et al*., 2005; Simson & Straus, 2010) However, in some products its presence in smaller quantities is essential (Floros & Matsos, 2005).

Carbon dioxide

Carbon dioxide gas is colourless and has a slight pungent odour when in high concentration. It has a water solubility of 1.57 g kg^{-1} at 100kPa, 20°C (Sandhya, 2010). Carbon dioxide has a bacteriostatic effect and it can slow down the respiration rate of many products (Robertson, 2006; Floros & Matsos, 2005). The solubility of CO_2 increase with a reduction in temperature. This dissolution into the product hence results in package collapse (Sandhya, 2010; Robertson, 2006; Mullan, 2002). However, pack collapse is favourable, for example in MAP of flow wrapped cheese for retail sale (Mullan, 2002). Thus the antimicrobial activity of CO_2 is prominent at temperatures below 10°C compared to temperatures above 15°C (Mullan, 2002).

7.2. Gas mixture

Gas mixture to be used in MAP depends on the type of food being packed as well as the spoilage organisms that are likely to be encountered (Robertson, 2006) . When spoilage by microorganism is likely to be experienced, the CO_2 level in the mix should be high but limited to the level where negative effects such as package collapse does not occur (Robertson, 2006; Mullan, 2002). Therefore, a typical gas composition of this condition is 40-60% N_2, and 30-60% CO_2, whereas, for

oxygen sensitive food products, where oxidative rancidity or anaerobic microbial spoilage is likely to occur a 100% N_2, or N_2/CO_2 mixture is used (Robertson, 2006). A gas mixture too high in CO_2 or too low in O_2 levels should be avoided, when dealing with products with high respiration rates (Simson & Straus, 2010; Roberson, 2006; Mullan, 2002). MAP is usually designed to maintain 2-5% of O_2 and 10-12% of CO_2 in a package of fresh-cut fruits and vegetables (Simson & Straus, 2010). Gorny (2001) have compiled various gas composition mixture mainly O_2 and CO_2 (Table 2.4) for fresh cut fruits.

Table 2.4 Modified atmosphere recommendations for Fresh-cut fruits (From Gorny, 2003)

		Atmosphere		
Fresh-Cut Product	Temperature (°C)	$\%O_2$	$\%CO_2$	Efficacy
Apple, Sliced	0-5	<1	4-12	Moderate
Cantaloupe, Cubed	0-5	3-5	6-15	Good
Grapefruit, Slices	0-5	14-21	7-10	Moderate
Honeydew, Cubed	0-5	2	10	Good
Kiwifruit, Sliced	0-5	2-4	5-10	Good
Mango Cubes	0-5	2-4	10	Good
Orange, Sliced	0-5	14-21	7-10	Moderate
Peach, Sliced	0	1-2	5-12	Poor
Pear, Sliced	0-5	0.5	<10	Poor
Persimmon, Sliced	0-5	2	12	Poor
Pomegranate, Arils	0-5	-	15-20	Good
Strawberry, Sliced	0-5	1-2	5-10	Good
Watermelon Cubes	0-5	3-5	10	Good

Tissue softening in fruits such as kiwi, apple, banana etc., were reported to be lowered through MAP, thus helps reduce the ethylene effects (Brandenburg & Zagory 2009). Oxidative browning in fresh-cut salads is reduced by using a low oxygen MAP. Thus, the breakdown of chlorophyll pigments in fruits such as cucumber, spinach, lettuce can be reduced through the use of MAP (Brandenburg & Zagory, 2009). A low CO_2 level was reportedly used in strawberry shipping to help slow down the growth of certain spoilage bacteria and moulds.

Table 2.5 Physiological effects of reduced O_2 and elevated CO_2 on fruits and vegetables (From Kader *et al*., 1988)

	General Effects of	
Cause of Deterioration	Reduced O_2	Elevated CO_2
Respiration rate	(>1%) − (<1%) +	(<15%–20%) − (>15%–20%) +
Ethylene action	−	−
Chlorophyll degradation	−	−
Anthocyanin development	−	−
Carotenoid biosynthesis	−	−
Enzymatic browning	− (Near 0%)	−
Off-flavours	+ (<1%)	+ (>15%–20%)
Vitamin C loss	−	−
Fungal growth	− (<1%)	− (>10–15%)
Bacterial growth	− or 0	− or 0

7.3. MAP techniques

The gas inside the packages can be obtained by two main techniques, based on the quantity of product packaged, the cost entailed as well as the availability of the equipments needed to achieve these. These techniques are referred to as active and passive (Fig 2.3).

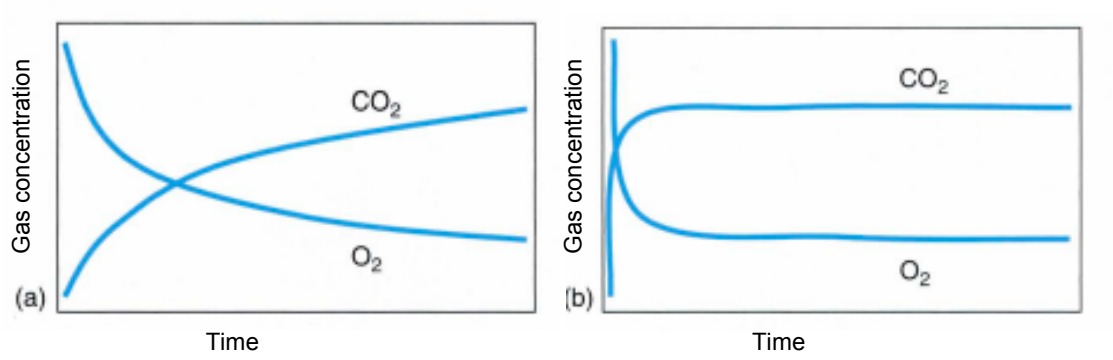

Figure 2.3 Graphic illustration of (a) passive and (b) active atmosphere modification. Adapted from Floros & Matsos (2005)

Passive MAP

In this approach, the required gas composition in the package (which is high in CO_2 and low in O_2) which may require a week or longer before an equilibrium is reached (Farber *et al.,* 2003, Caleb *et al* 2012c). The composition of the atmosphere that develops under passive modification evolves as a result of various factors, these includes the respiration rate of the produce or metabolism of microorganism associated with the food, the film permeability to gases and water vapour (which varies depending on the storage temperature and the nature/type of the film) (Robertson, 2006).

Active MAP

Active MAP is a rapid technique that involves displacing the air with a desired mixture of gases or use of gas scavengers/absorbers (Blakistone, 1999, Farber 2003). Agents such as O_2, CO_2 and ethylene scavengers are used as active agents in this technique, by being incorporated in the packaging film or within the packaging container to modify the headspace atmosphere and consequently extend the product shelf life (Blakistone, 1999; Ayhan, 2010; Kader & Watkins, 2000). Although active MAP may translate into high cost due to the additional use of the active agents, the desired atmosphere is established rapidly, unlike in passive where the equilibrium in gas composition is reached after a number of days (Kader & Watkins, 2000).

On both techniques, ideally, a film used for MAP should have the gas permeability where enough O_2 can enter the package to prevent anoxic conditions and anaerobic respiration, whilst excess CO_2 can be able to escape from the package as to avoid undesirable flavour changes in produce quality (Blakistone, 1999; Kader & Watkins, 2000; Brandenburg & Zagory, 2009).

7.4. Advantages and disadvantages of MAP

When MAP is applied appropriately, it can supplement refrigerated storage of some products which can contribute to the reduction in postharvest losses (Simson & Straus, 2010). Various authors have reported the many benefits that MAP offer. It is emphasised that MAP offers many benefits to consumers and food producers (Floros & Matsos, 2005). MAP extends the shelf life of products (Artes *et al*., 2000; Ares *et al*., 2007; Caleb *et al*., 2013) and this can be achieved as it reduces the senescence that is associated with the biochemical changes, such as ethylene production, softening, compositional changes in fresh produce and respiration rate (Simson & Straus, 2010).

Furthermore, at the levels of O_2 and CO_2 below 8% and 1%, respectively, the sensitivity of produces to ethylene action is reduced, thus leading to shelf life extension. A ‘’natural’’ and ‘’healthy’’ product can be achieved as it reduces and sometimes eliminates the need for chemical preservatives (Floros & Matsos, 2005). MAP have good benefits on the logistics, whereby products can be packed centrally and distribution lost is reduced leading to fewer deliveries to be done over long distances and require less storage space/time as well as labour for the retailers (Floros & Matsos, 2005). Moreover, the promotion of longer shelf life allows wider distribution of food to remote destinations and help increase the product markets (Floros & Matsos, 2005).

Different gas formulation required in MAP increases the need of expensive equipment and packaging materials, and a need for special training of production staffs, thus all this cost will be paid by customers through pricing (FSN, 2013; Floros & Matsos, 2005). Moreover, the need of temperature control and product safety is required (Floros & Matsos, 2005). Hence, potential safety risk may be encountered, since people may not keep their food at prescribed temperature ranges (FNS, 2013). Other disadvantage of MAP is that, it losses all its benefits when consumers open the package (Floros & Matsos, 2005).

7.5. Effects of MAP on respiration and transpiration rates

Modified atmosphere packaging aims to minimise the respiration rates of the products, as well as overpowering the ethylene production, hence hasten the onset of senescence in fruits and vegetables (Floros & Matsos, 2005). Furthermore, MAP helps maintain a high relative humidity in the package and reduce water loss (Robertson, 2006). As the produce respire, it consumes the O_2 inside the package and an approximate equal (based on the respiratory quotient) amount of CO_2 is produced. Thus the reduction in O_2 and increase in CO_2 concentrations leads to the creation of a gradient between the package atmosphere and the extreme condition, to leave the package (Blakistone, 1999).

Initially, RR of the produce is much higher than the permeation rates of CO_2 and O_2 through the package, i.e. respiration flux is higher than package gas exchange (Lee *et al*., 1996). Various studies have evaluated the effects of different MAP conditions on the internal headspace gas compositions, which translate into respiration rate (Lopez-Rubira *et al*., 2005; Caleb *et al*., 2013; Martinezo-Romero et al., 2013). Ayhan and Esturk, (2009) reported a lower respiration rate of pomegranate arils when stored under low oxygen (5% O_2 + 10% CO_2 + 85% N_2) MAP, in comparison to MAP where air was the dominant condition. Generally, the concentration of oxygen under MAP decreases with storage period while CO_2 increases. However, Caleb *et al*., (2013), reported the weight loss on pomegranate arils packed under MAP (polylid (PE) polymeric film) to be high compared to those that were packed in clamshell packages. The weight loss was about, 0.7% under passive MAP and 0.02% in clamshell packages, bot stored at 5 °C, for cv. Acco, respectively.

7.6. Effects of MAP on quality and shelf life

Generally, the nutrient content of fruits and vegetables is influenced by factors such a genetic, maturity, agronomic factors and postharvest handling methods (Ahvenainen, 2003). Thus storing fresh fruits within optimum levels of low O_2 and/or increased CO_2 conditions reduces their physiological processes such as ethylene production and respiration rate, and these help reduce the onset of oxidative reactions. Oxidant constituents of fruits are special interest when considering the effects of MAP (Floros and Matsos, 2005). Few changes in texture and less noticeable discolouration on mushroom when stored under modified atmosphere at 4°C have been reported (Ares *et al*., 2007). Furthermore, the anthocyanins content of pomegranate arils were maintained well under MAP at 5°C, compared to the control which was normal air storage condition (Lopez-Rubira *et al*., (2005); Ayhan & Esturk (2009). Storing produces at low oxygen atmosphere helps control the growth of aerobic bacteria that are mainly responsible for spoilage, hence extending the shelf life of the produce (Kader, 2002). The application of MAP has also been reported to increase the shelf life of fresh cut pineapple (Liu *et al*., 2007; Mushroom (Ares *et al*., 2007), pomegranate fruit and arils (Caleb *et al*., 2013; Artes, *et al*., 1996), through the reduction of produce respiration rates and water loss.

7.7. Effect of MAP on safety

The required safety of food products can be achieved with the help of the MAP application, especially when the CO_2 concentration is maintained at the suitable level (Floros & Matsos, 2005). At the level of about 10% or more, the antimicrobial effect of CO_2 is prominent. Therefore, the use of CO_2 levels that are about 20% helps control the growth of aerobes including pseudomonas, acetobacter, yeast and mould (Floros & Matsos, 2005; Ayhan & Esturk, 2009). However, the

growth of anaerobes such as Clostridium botulinum may be induced at a very high CO_2 concentration (Blakistone, 1999). Blakistone (1999) highlighted that proper film permeability, a health resident micro flora and good refrigeration storage temperatures can work well together to help maintain the safety of fresh-cut products.

MAP helps reduce the incidences of decay which may be associated with microbial growth. The application of MAP alone or in combination with other treatments such as the use of 4-HR, potassium sorbate, N-acetylcysteine, aloe vera or UV-C radiation doses, have been found to have a good influence on the produces safety, through the reduction of microbial growth such as Fungi (mould and yeast) and bacteria (Gonzalez-Aguilar *et al*., 2001; Lopez-Rubira *et al*., 2005; Martinez-Romero *et al*., 2013). The yeast count and lactic acid bacteria on pomegranate arils have been reported to be maintained below the recommended level (5 log cfu g^{-1}) when arils were packed under passive MAP for a period of 12 days (Lopez-Rubira *et al*., 2005).

8. Overview of MAP application and film permeability

Modified atmosphere packaging (MAP) has been reported to extend the shelf life of minimally processed arils (Artes, *et al*., 1996; Ayhan & Esturk, 2009; Caleb *et al*., 2013). Various gas compositions are used, when active MAP is applied, with the semipermeable films and various temperature range and duration as indicated in table 2.6. In order to achieve the target modified atmosphere, plastic films used needs to be permeable to gases (Brandenburg & Zagory, 2009; Thompson, 2010). The permeability of the films is influenced by factors such as the storage temperature, chemical composition of the film, i.e. the type of material from which the film is made from, relative humidity, thickness of the film and the accumulation, concentration and gradient of the gases (Brandenburg & Zagory, 2009; Thompson, 2010). Apart from plastic films, barrier films are used mostly on low-respiring produces, and they include the use of O_2 scavenging technology, high permeability films, low permeability films, CO_2 scavengers and emitters, gas indicators, time-temperature indicators, to mention a few (Thompson, 2010). Table 2.7 lists the type of different polymers used in MAP and their characteristics. For proper selection of the best fitting polymer film, the understanding of the respiration rate of the produce to be packaged as well as its O_2 and CO_2 transmission rates needs to be established (Al-Ati & Hotchkiss, 2002; Kader, 2002; Brandenburg & Zagory, 2009). The gas and water vapour transmission rate of the polymer film depends on the gas transmission rate of each film used to create the final polymer film, through co-extrusion or applying adhesive between the layers, respectively. The film permeability is described by Equation 1 (Al-Ati & Hotchkiss, 2002; Thompson, 2010).

$$P = \frac{\mathrm{J\,x}}{A\,(\mathrm{p_1 - p_2})} \qquad (1)$$

Where;

J= volumetric rate of gas flow through the film at steady state,

x: thickness of film

A: area of permeable surface

p_1: gas partial pressure on side 1 of the film; and

p_2: gas partial pressure on side 2 of the film ($p_1 > p_2$).

Equation 1 shows that gas flow across the film increases with an increase in surface area and concentration gradient. However, as the film resistance to gas diffusion increases the gas flow across the film decreases (Brandenburg & Zagory, 2009).

Table 2.6 MAP application on pomegranate fruit and arils

Fruit fraction	Shelf life	Temperature and Relative humidity (RH)	Packaging condition	References
Arils	7 days	1°C	Perforated OPP film (passive MAP)	Gil *et al*. (1996)
Whole fruit	12 weeks	5°C	Perforated PP film (passive MAP)	Artes *et al.* (2000)
Whole fruit	3-4 months	6°C	Xtend® Easy-Tear bags (passive MAP)	Porat *et al*. (2009)
Arils	18 days	5°C; 75% RH	BOPP film (under air, nitrogen and oxygen atmospheres)	Ayhan & Esturk (2009)
Arils	10 days	5°C; 95 ± 2% RH	Polylid (PE) polymeric film (passive MAP)	Caleb *et al*. (2013)

PP: Polypropylene; BOPP: Bi-axially oriented polypropylene; OPP: Oriented polypropylene; PE: Polyethylene

Table 2.7 Common polymers used in MAP and their characteristics (Brandenburg & Zagory, 2009)

Polymer	Abbreviation	Characteristic	Typical OTR (cc=100 in.2= mil=atm=day)	Application Most Commonly Used
Low-density polyethylene	LDPE	General-purpose polymer	450–500	Both
Linear low-density polyethylene	LLDPE	Increased stiffness	480–500	Both
Linear medium-density polyethylene	LMDPE	Increased stiffness, lower OTR, decreased clarity	300–350	Both
High-density polyethylene	HDPE	Relatively stiff, opaque	150	Rigid
Ultralow-density polyethylene	ULDPE	High OTR, decreased stiffness	900	Flexible
Plastomer metallocenes	—	Very high OTR, soft	1100	Flexible
Amorphous polyethyleneterapthalate	APET	Clear, rigid	5	Rigid
Polyvinyl chloride Clear,	PVC	rigid	10	Rigid
Polypropylene	PP	Decreased clarity	300	Both
Ethylene vinyl acetate	EVA	Sealability	600–900	Flexible
Polystyrene	PS	Stiffness	350	Rigid

9. Summary and future research prospects

Increasing consumer demand for pomegranate has spurred the need for improved postharvest handling and preservation techniques. Pomegranate is mainly processed into juice or loose arils as fresh-cut produce. Although pomegranate arils are packed into several sacs (compartments) that are separated by membranes, little is known about the water vapour and gas transmission properties of the membrane and the possibility of marketing the fruit as fresh-cut aril-sacs Fresh-cut products are susceptible to deterioration due to the acceleration of physiological processes (respiration and transpiration rates) as well as enzymatic browning. Thus the use of treatments

such as anti-browning agents and natural products in combination with MAP, have been demonstrated to maintain the products quality and consequently extend their shelf life. To achieve the desired effects of MAP, knowledge of the respiration and transpiration rates of the produce is important. Furthermore, the type of film and storage condition used should fit the physiological characteristics of the product to be packaged. Various studies reported the application of active-MAP on pomegranate fruit and its arils, however little is known on the passive-MAP application on pomegranate arils. Furthermore, the processing of pomegranate into aril-sacs has not been explored as well as the application of MAP this fruit fraction. The use of anti-browning agent have been extensively studied on fresh cut fruits such as mango, apples and pears, but there is limited information on the use of anti-browning agents such as 4-hexyresercinol, and potassium sorbate on pomegranate. Hence application of the above mentioned anti-browning agents will help us control browning occurrence in aril-sacs.

10. References

Afaq, F., Saleem, M., Krueger, C.G., Reed J.D., Mukhtar H. (2005). Anthocyanin and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-kappa B pathways and inhibits skin tumorigenesis in CD-1 mice. *International Journal of Cancer,* **113**, 423–33.

Ahvenainen, R. (2003). *Novel Food Packaging Techniques*, Pp. 215-216. USA: Woodhead Publishing Ltd.

Al-Ati, T. & Hotchkiss, J.H. (2002). Application of Packaging and Modified Atmosphere to Fresh-cut Fruits and Vegetables. In: *Fresh-cut fruits and vegetables: science, technology and market* (edited by O. Lamikanra). Pp. 311-344. Washington, D.C. USA: CRC Press.

Anonymous (2012). Pomegranate Nurseries in South Africa. [Internet document].URL http://www.safruitfarms.com. 26/07/2012.

Anonymous (2013a). Modified atmosphere packaging in the food industry. [Internet document]. URL http://www.public.web-il.net/tmi-barak/files/LMMappe_UK.pdf. 25/07/2013.

Anonymous (2013b). Modified atmosphere packaging. Food Science Network. [Internet document]. URL https://www.uoguelph.ca/foodsafetynetwork/modified-atmospheric-packaging-map. 03/08/2013.

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

Arias, E., Gonzalez, J., Oria, R. & Lopez-Buesa, P. (2007). Ascorbic acid and 4-hexylresorcinol effects on pear PPO and PPO catalysed browning reaction. *Journal of Food Science*, **72**(8), 422-429.

Artes F., Villaescusa, R. & Tudela J.A. (2000). Modified atmosphere packaging of pomegranate. *Journal of Food Science*, **65** (7), 1112-1116.

Artes, F., Marin, J.G. & Martinez, J.A. (1996). Controlled atmosphere storage of pomegranate. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung*, **203**, 33-37.

Aviram M., Dornfeld L., Rosenblat M., Volkova N., Kaplan M., Coleman R., Hayek T., Presser D. & Fuhrman B. (2000). Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in human and in atherosclerotic apolipoprotein E-deficient mice. *American Journal of Clinical Nutrition,* **71**, 1062-1076.

Ayhan, Z. & Esturk, O. (2009). Overall quality and shelf life of minimally processed and modified atmosphere packaged ''ready-to-eat'' pomegranate arils. *Journal of Food Science*, **74** (5), 399-405.

Becker, B. R. & Fricke, B. A. (2013). Transpiration and respiration of fruits and vegetables. http://b.web.umkc.edu/beckerb/publications/chapters/trans_resp.pdf (03 April 2013).

Ben-Yehoshua, S. & Rodov, V. (2002). Transpiration and water stress. In: *Postharvest Physiology and Pathology of Vegetables*, 2nd ed (edited by J.A. Bartz & J.K. Brecht). Pp. 119-160. New York, US: Marcel Dekker, Inc.

Blakistone, B.A. (1999). *Principles and Applications of Modified Atmosphere Packaging of Foods*, 2nd Ed. Pp. 4-5. Maryland: Aspen Publishers, Inc.

Brandenburg, J.S. & Zagory, D. (2009). Modified and controlled atmosphere packaging technology and applications. In: *Modified and controlled atmospheres for the storage, transportation, and packaging of horticultural commodities* (edited by: E.M. Yahia). Pp. 73-92. London: CRC Press.

Caleb, O.J., Mahajan, P.V., Al-Said, F.A. & Opara, U.L. (2012c). Modified atmosphere packaging technology of fresh and fresh-cut produce and the microbial consequences: a review. *Food Bioprocess Technology*, **6**: 303-329.

Caleb, O.J., Mahajan, P.V., Manley, M. & Opara, U.L (2013). Evaluation of parameters affecting modified atmosphere packaging engineering design for pomegranate arils. *International Journal of Food Science and Technology*, doi:10.1111/ijfs.12220.

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

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

Chonhenchob, V., Chantarasomboon, Y. & Singh, S.P. (2007). Quality changes of treated fresh-cut tropical fruits in rigid modified atmosphere packaging containers. *Packaging Technology and Science*, **20**, 27-37.

Chung, C., Huang, T., Yu, C., Shen, F. & Chen, H. (2011). Bactericidal effects of fresh-cut vegetables and fruits after subsequent washing with chlorine dioxide. In: *Proceedings of the International Conference on Food Engineering and Biotechnology*, *IPCBEE*, **9**, 107-112. Singapore.

Citrogold (2012). Producing pomegranates in South Africa [Internet document]. URL http://www.citrogold.co.za/Producing%20Pomegranates%20in%20South%20Africa%20Citrogold%202011.pdf. 26/07/2012.

El-shimi, N.M. (1993). Control of enzymatic browning in apple slices by using ascorbic acid under different conditions. *Plant Foods for Human Nutrition,* **43**, 71-76.

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.

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

Farber, J.N., Harris, L.J., Parish, M.E., Beuchat, L.R., Suslow, T.V., Gorney, J.R., Garret, E.H. & Busta, F.F., (2003). Microbial safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety Journal,* **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 Bioprocess Technology*, DOI 10.1007/s11947-011-0533-7.

Floros, J.D. & Matsos, K.I. (2005). Introduction to modified atmosphere packaging. In: *Innovations in Food Packaging*. Pp. 159-171. London, UK: Elsevier Ltd.

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.

Garcia, E. & Barret, D.M. (2002). Preservative treatments for fresh-cut fruits and vegetables. In: *Fresh-Cut Fruits and Vegetables: Science, Technology and Market*. Pp. 267-303. Boca Raton, Florida, USA: CRC press.

Garcia, E. & Barrett, D.M. (2005). Fresh Cut Fruits. In: *Processing Fruits: Science and Technology*. 2nd Ed. Pp. 53-72. Boca Raton, Florida, USA: CRC press.

Gedders, B. (2013). South African pomegranate production on the increase. FreshPlaza, March 14, 2013.

Gil, M.I., Artes, F. & Toma-Barberan, F.A. (1996). Minimally processing and modified atmosphere packaging effects on pigmentation of pomegranate seeds. *Journal of Food science*, **61(1),** 161-164.

Gil, M.L., Holcroft, D.M., Kader, A.A. (1997). Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *Journal of Agricultural and Food Chemistry*, **45**, 1662-1667.

Gonzalez-Aguilar, G.A., Wang, C.Y. & Buta, J.G. (2000). Maintaining quality of fresh-cut mangoes using anti-browning agents and modified atmosphere packaging. *Journal of Agricultural Food Chemistry*, **48**, 4204-4208.

Gorny, J.R. (2003). A summary of CA and MA requirements and recommendations for fresh-cut (minimally processed) fruits and vegetables. In: *Proceedings of the 8th International Controlled Atmosphere research conference*. *Acta Horticulturae*, ISHS, Pp. 609-614.

Haminiuk, C. W. I., Maciel, G. M., Plata-Oviedo, M. S. V., & Peralta, R. M. (2012). Phenolic compounds in fruits - an overview. *International Journal of Food Science & Technology*, **47**(10), 2023–2044.

Hardy, S. & Sanderson, G. (2010). Citrus maturity testing. Prime facts. [Internet document]. URL http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0020/320294/Citrus-maturity-testing.pdf. 16/09/2013.

Hess-pierce, B., & Kader, A. A. (2003). Responses of “Wonderful” pomegranates to controlled atmospheres. In: *Proceedings of the 8th International Controlled Atmosphere Conference*. *Acta Horticulturae*, **600**, 751-757.

Hu, Y., Topolkaraev, V., Hiltner, A. & Baer, E. (2001). Measurement of water vapour transmission rate in highly permeable films. *Journal of Applied Polymer Science*, **81,** 1624-1633.

Jalikop, S.H., Venugopala, R. & Kumar, R. (2010). Association of fruit traits and aril browning in pomegranate. *Euphytica, **174**,* 137-141.

Kader, A.A. & Barret, D.M. (2005). Classification, composition of fruits and postharvest maintenance of quality. In: *Processing Fruits: Science and Technology*. 2nd Ed. Pp. 3-22. Boca Raton, Florida, USA: CRC press,

Kader, A.A. (2002). Fresh-cut fruit and vegetable products. In: *Fresh-cut fruits and vegetables: science, technology and market* (edited by O. Lamikanra). Pp. 21-30. Washington, D.C. USA: CRC Press.

Kader, A.A. (2009). Fruit maturity, ripening and quality relationships. In: *Proceedings of the International Symposium on Effect of Pre- and Post-Harvest Factors on Storage of Fruits*. *Acta Horticulturae,* **485***, 203-208.*

Kader, A.A., Zagory, D. & Kerbel, E.L. (1988). Modified atmosphere packaging of fruits and vegetables. *Critical review of food science and nutrition.* **28** (1) 1-30

Kalt, W., Forney C.F., Martin, A. & Prior, R.L. (1999). Antioxidant capacity, vitamin C, phenolic and anthocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry,* **47**, 4638-4644.

Leet, L., Arult, J., Lencki, R. & Castaignet, F. (1996). A review on modified atmosphere packaging and preservation of fresh fruits and vegetables: physiological basis and practical aspects-part II. *Journal of Packaging and Science*, **9,** 1-17.

Leonardi, C., Baille, A. & Guichard, S. (2000). Predicting transpiration of shaded and non-shaded tomato fruits under greenhouse environments. *Scientia Horticulturae*, **84**, 297-307.

Li Y., Guo C., Yang J., Wei J., Xu J., Cheng S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry,* **96**(2), 254–60.

Liu, C., Hsu, C. & Hsu, M. (2007). Improving the quality of fresh-cut pineapples with ascorbic acid/sucrose pre-treatment and modified atmosphere packaging. *Packaging Technology and Science*, **20**, 337-343.

Longtin, R. (2003). The pomegranate: nature's power fruit? *Journal of the National Cancer Institute,* **95**, 346-348.

Lopez-Rubira, V., Conesa, A., Allende, A. & Artes, 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.

Maghoumi, M., Gómez, P. A., Mostofi, Y., Zamani, Z., Artés-Hernández, F., & Artés, F. (2013). Combined effect of heat treatment, UV-C and superatmospheric oxygen packing on phenolics and browning related enzymes of fresh-cut pomegranate arils. *LWT - Food Science and Technology*, **54**(2), 389–396.

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

Marin, F.R., Martinez, M., Uribesalgo, T., Castillo, S. & Frutos, M.J. (2001). Changes in nutraceutical composition of lemon juices according to different industrial extraction systems. *Journal of Food Chemistry,* **78**, 319-324.

Medical Research Council (MRC) (2010). Fruit. In: *Condensed Food Composition Tables for South Africa* (edited by Wolmarans, P., Danster, N., Dalton, A. Rossouw, K. & Schonfeldt, H.) 1st ed. Pp. 3-15. Cape Town: Medical Research Council (As cited by O'Grady, 2012).

Meerts, I.A.T.M., Verspeek-Rip, C.M., Buskens, C.A.F., Keizer, H.G., Bassaganya-Riera, J., Jouni, Z.E., Van Huygevoort, A.H.B.M., van Otterdijk, F.M. & van de Waart, E.J. (2009). Toxicological evaluation of pomegranate seed oil. *Food and Chemical Toxicology*, **47**(6), 1086-1092.

Mena, P., García-Viguera, C., Navarro-Rico, J., Moreno, D. A, Bartual, J., Saura, D., & Martí, N. (2011). Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L.) cultivars grown in Spain. *Journal of The Science of Food And Agriculture*, ***91***(10), 1893-906.

Mullan, W.M.A. (2002). Science and technology of modified atmosphere packaging. [Internet document]. URL http://www.dairyscience.info/packaging-/117-modified-atmosphere-packaging.html. 25/07/2013.

Nanda, S., Sudhakar Rao, D., & Krishnamurthy, S. (2001). Effects of shrink film wrapping and storage temperature on the shelf life and quality of pomegranate fruits cv. Ganesh. *Postharvest Biology and Technology*, **22**(1), 61–69.

O'Grady, L. (2012). Effects of Postharvest handling on nutritional quality of pomegranate (*Punica granatum*) arils. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

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

Ozgen, M. , Durgaç, C., Serçe, S. & Kaya, C. (2008). Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. *Journal of food chemistry,* **111**(3) 703-706.

Ozgul-Yucel, S. (2005). Determination of conjugated linolenic acid content of selected oil seeds grown in Turkey. *Journal of the American Oil Chemist's Society,* **82** (12), 893-897.

Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2013). Colour measurement and analysis in fresh and processed foods: a review. *Food and Bioprocess Technology*, **6**:36–60.

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

Porat, R., Weiss, B., Fuchs, Y., Kosto, I., Sandman, A., Ward, G., & Agar, T. (2009). Modified atmosphere / modified humidity packaging for preserving pomegranate fruit during prolonged storage and transport. In: *Proceedings of the International Symposium on Pomegranate*. *Acta Horticulturae*, **818**, 299-304.

Pretel, M. T., Souty, M., & Romojaro, F. (2000). Use of passive and active modified atmosphere packaging to prolong the postharvest life of three varieties of apricot (*Prunus armeniaca*, L.). *European Food Research and Technology*, ***211***(3), 191-198.

Raghavan, G.S.V., Vigneault, C., Gariepy, Y., Markarian, N.R. & Alvo, P. (2005). Refrigerated and controlled/modified atmosphere storage. In: *Processing Fruits: Science and Technology*. 2nd Ed. Pp. 23-52. Boca Raton, Florida, USA: CRC press.

Robertson, G.L. (2006). Modified atmosphere packaging. In: *Food Packaging, Principles and Practice.* 2nd Ed. Pp. 313-330. USA: CRC press, Taylor & Francis Group.

Rojas-grau, M.A., Grasa-guillem, R. & Martın-Belloso, O. (2007). Quality changes in fresh-cut fuji apple as affected by ripeness stage, anti-browning agents, and storage atmosphere. *Journal of Food Science*, **72** (1), 36-43.

Salveit, M.E. (2012). Respiratory Metabolism. [Internet document] URL www.ba.ars.usda.gov/hb66/019respiration.pdf. 07/09/2012.

Sapers, G.M., Miller, R.L., Miller, F.C., Cooke, P.H. & Choi, S. (1994). Enzymatic browning control in minimally processed mushrooms. *Journal of Food Science*, **59**(5), 1042-1047.

Simson, S.P. & Straus, M.C. (2010). *Post-Harvest Technology of Horticultural Crops.* Pp. 124-125. Narayan Niwas, Delhi: Oxford Book Company.

Song, Y., Vorsa, N. & Yam, K.L. (2002). Modelling respiration-transpiration in a modified atmosphere packaging system containing blueberry. *Journal of Food Engineering*, **53** (2), 103-109.

Tezcan, F, Gultekin-Ozguven, M., Diken T., Ozcelik, B. & Erim, F.B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Journal of Food Chemistry,* **115**, (3), 873-877.

Thompson, A.K. (2010). *Controlled atmosphere storage of fruits and vegetables*. 2nd Ed. Pp. 81-113. United Kingdom. Meredith Carroll.

Toivonen, P.M.A. & DeEll, J.R. (2002). Physiology of fresh-cut fruits and vegetables. In: *Fresh-cut Fruits and Vegetables*. Pp. 91-123. USA: CRC Press.

Viuda-Martos, M., Fernandez-Lopez, J. & Perez-Alvarez, 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.

Watada, A.E., Ko, N.P. & Minnot, D.A. (1996). Factors affecting quality of fresh-cut horticultural products. *Journal of Postharvest Biology and Technology*, **9**, 115-125.

Wohlfarter, M., Giliomee, J.H. & Venter, E. (2010). A Survey of the Arthropod Pests Associated with Commercial Pomegranates, *Punica granatum* (Lythraceae), in South Africa. *African Entomology* **18**, 192-199.

Yahia, E.M. & Gonzalez-Aguilar, G. (1998). Use of passive and semi-active atmospheres to prolong the postharvest life of avocado fruit. *Lebensm Wiss Technol*., **31**, 602-606.

Chapter 3: Respiration rate of pomegranate fruit fractions at different temperatures

CHAPTER 3
RESPIRATION RATES OF POMEGRANATE FRUIT FRACTIONS AT DIFFERENT TEMPERATURES

Abstract

Temperature is one of the factors that affect fruit respiration rate (RR). Thus modelling its effects on the RR is crucial in designing a modified atmosphere packaging (MAP) system. This study investigated the effect of storage temperature (5, 10, 15 and 22°C) on the RR of the pomegranate (*Punica granatum* L.) cv. 'Bhagwa' whole fruit, aril-sac and arils and a mathematical model on RR as influenced by temperature was developed. Temperature had a significant effect on RR across all experimental samples. Aril-sac had the highest RR at all storage temperatures in comparison to the whole fruit and arils. Overall, carbon dioxide production (RCO_2) and oxygen consumption (RO_2) of aril-sac were in the range of 2.95 to 27.66 mL kg^{-1} h^{-1} and 5.49 to 48.44 mL kg^{-1} h^{-1}, respectively, while whole fruit ranged between 2.66 to 22.97 mL kg^{-1} h^{-1} and 3.71 to 33.3 mL kg^{-1} h^{-1}, respectively, and arils were between 1.96 to 18.64 mL kg^{-1} h^{-1} and 3.19 to 28.91 mL kg^{-1} h^{-1}, respectively. Reducing storage temperature from 22°C to 5°C resulted in a reduction in RR of about 74.5% across all the samples. The effect of temperature on RR of whole fruit, aril-sac and arils were adequately predicted by an Arrhenius type equation (R^2 > 97.1%). The model validated RR for samples stored at 22°C and a good correlation was found between experimental and predicted data.

Introduction

Pomegranate fruit (*Punica granatum* L.) is a non-climacteric fruit, with relatively low respiration rate (RR), and produces trace amounts of ethylene (Crisosto *et al*., 2012). Traditionally, pomegranate has been used as anti-helminthic, anti-atherosclerotic, anti-osteoarthritis, and against heart disease and prostate cancer (Viuda-Martos, Fernandez-Lopez, & Perez-Alvarez, 2010). It is rich in phenolic compounds, anti-oxidants, organic acids, anthocyanin's, fatty acids and vitamin C (Opara *et al.* 2009). In recent years, the consumption and processing of pomegranate into products like fresh arils, jam and colourants has increased (Ersan *et al.,* 2010). In spite of these numerous benefits, the difficulties of peeling this fruit and the colour stains that one gets when peeling them makes it inconvenient for consumption (Gil, Martinez & Artés, 1996). Minimally processing of pomegranate into arils or aril-sac can bring about the convenience for food services and consumers (Ersan *et al.,* 2010). Even though minimal processing of this fruit may bring convenience, its quality may be reduced as a result of an increase in respiration rate.

Respiration rate is influenced by a range of internal and external factors (Caleb *et al.*, 2013). Internal factors include the type and maturity stage of the fruit, whilst external factors include

temperature, relative humidity and processing (Fonseca *et al.,* 2002; Garcia & Barrett (2005). The extent to which fresh produce's metabolic processes occurs will have an impact on the product quality attributes, such as colour, texture and microbial growth; hence leading to the reduction in shelf life. Various studies have reported that respiration rate of fresh produce can be retarded by the modification of external factors such as storage temperature and gas composition in the package, and consequently extend their shelf life (Nanda *et al.,* 2001; Ersan *et al*., 2010). Modified atmosphere packaging (MAP) is defined as a dynamic process of altering in-package gas composition (Caleb *et al.,* 2012). It offers the benefits of lowering RR and maintaining the quality of whole or minimally processed fruit and vegetables (Gil *et al*., 1996b; Artes *et al.,* 2000; Arias *et al.,* 2007; Liu *et al*., 2007; Ayhan & Esturk, 2009; Ersan *et al.,* 2010). In order for these benefits of MAP to be achieved, a quantitative description of RR of fresh produce is critical for the selection of appropriate MAP packaging material and optimum storage conditions.

Previous studies have reported on the respiration rate of pomegranate whole fruit and arils, respectively (Lopez-Rubira, 2005; Ersan *et al.,* 2010; Caleb, *et al.,* 2012). However, there is limited information on the RR of pomegranate fractions; whole fruit, aril-sac (arils still attached to the peel and partly covered with the membrane) and arils in literature. Hence, the main objective of this study was to evaluate effects of different storage conditions on RR of pomegranate (cv. 'Bhagwa') whole fruit, aril-sac and arils and to develop a mathematical model relating RR as a function of temperature.

Materials and methods

Source of materials and preparation

Pomegranate fruit (*Punica granatum L.*) cv. 'Bhagwa' (sweet cultivar) were obtained at their commercial harvest conditions from a farm situated in the Wellington area of the Western Cape, (33°38'S 18°59'E) South Africa. The fruit was transported in an air-conditioned and ventilated vehicle to the Postharvest Research Laboratory at Stellenbosch University, South Africa. It was thereafter stored in a cold room at about 5 ± 0.5°C and 95% RH until used for the experiments. Fruits with no physical defects were selected for processing. Each fruit was washed and disinfected using 70% ethanol, and carefully cut in cross-sections. Arils were manually removed carefully to avoid mechanical damage. Processing of fruit was done in a disinfected cold room below 8°C to avoid physiological stress. For the aril-sacs, fruit were carefully cut into three or four sections to obtain an intact aril-sac (arils still attached to the peel and albedo). Whole fruit, arils, and aril-sac were weighed into sample sizes of 230-245 g, 150 g, and 48-75 g, respectively. The samples of whole fruit were placed in 3,100 mL, while arils and aril-sac were placed into 1,100 mL air-tight glass jars, respectively. To ensure that the jars were completely sealed, petroleum jelly

was used around the lid and jar rims. The samples were equilibrated to 5, 10, 15 and 22°C for an hour, prior to closing the jars.

Experimental setup

A closed system method was used to measure the rate of oxygen consumption and carbon dioxide production of the whole fruit, arils and aril-sac (Caleb, *et al.,* 2012). Headspace gas samples were taken every hour for the duration of five hours over a period of five days, with a gas analyser with an accuracy of 0.5% (Checkmate 3, PBI Dansensor, Ringstead, Denmark). To monitor the effect of time on the samples' respiration rates, the jars were left open slightly overnight after every day of measurement and closed first thing the next day, for five days. This was done to avoid excess gas accumulation. The oxygen consumption (RO_2) and carbon dioxide production (RCO_2) rates were calculated from the experimental data using equation 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^j_{O2} and y_{O2} are O_2 concentration (%) at the initial time t_i (hours, h) (time zero) and at time t (h); and y^j_{CO2} and y_{CO2} are CO_2 concentration (%) at the initial time t_i (h) (or time zero) and at time t (h). RO_2 and RCO_2 are the 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), i.e. difference of total volume of glass jar and volume filled up by the test sample (Caleb, *et al.,* 2012). The volume filled up by the fruit was calculated from the mass of the fruit over apparent density of pomegranate fruit (0.98 g cm^{-3}).

Additionally, an Arrhenius-type model as reported by Torrieri *et al.* (2010) was used in model fitting to quantify the effects of temperature on the respiration rate (RCO_2 and RO_2) of the whole fruit, arils and aril-sac, as represented in equation 3:

$$\boldsymbol{R_{CO_2} = R^i_{CO_{2,ref}} \times e^{\left[\frac{-E_{a,CO_2}}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]}} \quad (3)$$

where R_{CO2} is the respiration rate (mL Kg^{-1} h^{-1}) at temperature (T, K); R^i_{CO2ref} is the RR at reference temperature (T_{ref}, K); $E_{a,CO2}$ is the activation energy (kJ mol^{-1}); R is the universal gas constant (0.008314 kJ K^{-1} mol^{-1}); and T_{ref} is the reference temperature (i.e. the mean of all the storage temperatures used in the study i.e. 10°C plus 273.15 = 283.15 K). The model was validated for RCO_2 with separate data obtained at 10°C for pomegranate whole fruit, aril-sacs and arils, and the strength of the model was tested at higher temperature of 22°C.

Statistical analysis

All experiments for each treatment were run in triplicate. Mixed Model Analysis of Variance (ANOVA) of repeated measures was conducted on all data using Statistica software (STATISTICA 11.0, StatSoft, USA) to determine the effect of temperature and time. Significant differences between fruit fractions and storage temperatures were established at *p-value* 0.05.

Results and discussion

Effects of temperature and time on respiration rate

Temperature had a significant effect ($p < 0.05$) on RR of the whole fruit, aril-sac and arils during storage (Fig.3.1). Reducing the storage temperature from 22°C to 5°C shows a reduction in RCO_2 and RO_2 of about 74.5 and 88.8% on average, respectively across all the samples. Similar effects were reported by Caleb *et al* (2012) for pomegranate cv. 'Acco' and 'Herskawitz', when the storage temperature was lowered from 15 to 5°C. They observed a decrease in RR by 70 and 67% for RCO_2 and RO_2 of pomegranate arils and 67 and 68% of RCO_2 and RO_2 for whole fruits, respectively. The higher percentage reduction on the average RR in the current study can be attributed to the higher RR of the aril-sac, which can be related to the physical stress induced on the peel during sample preparation, as well as the storage temperature used.

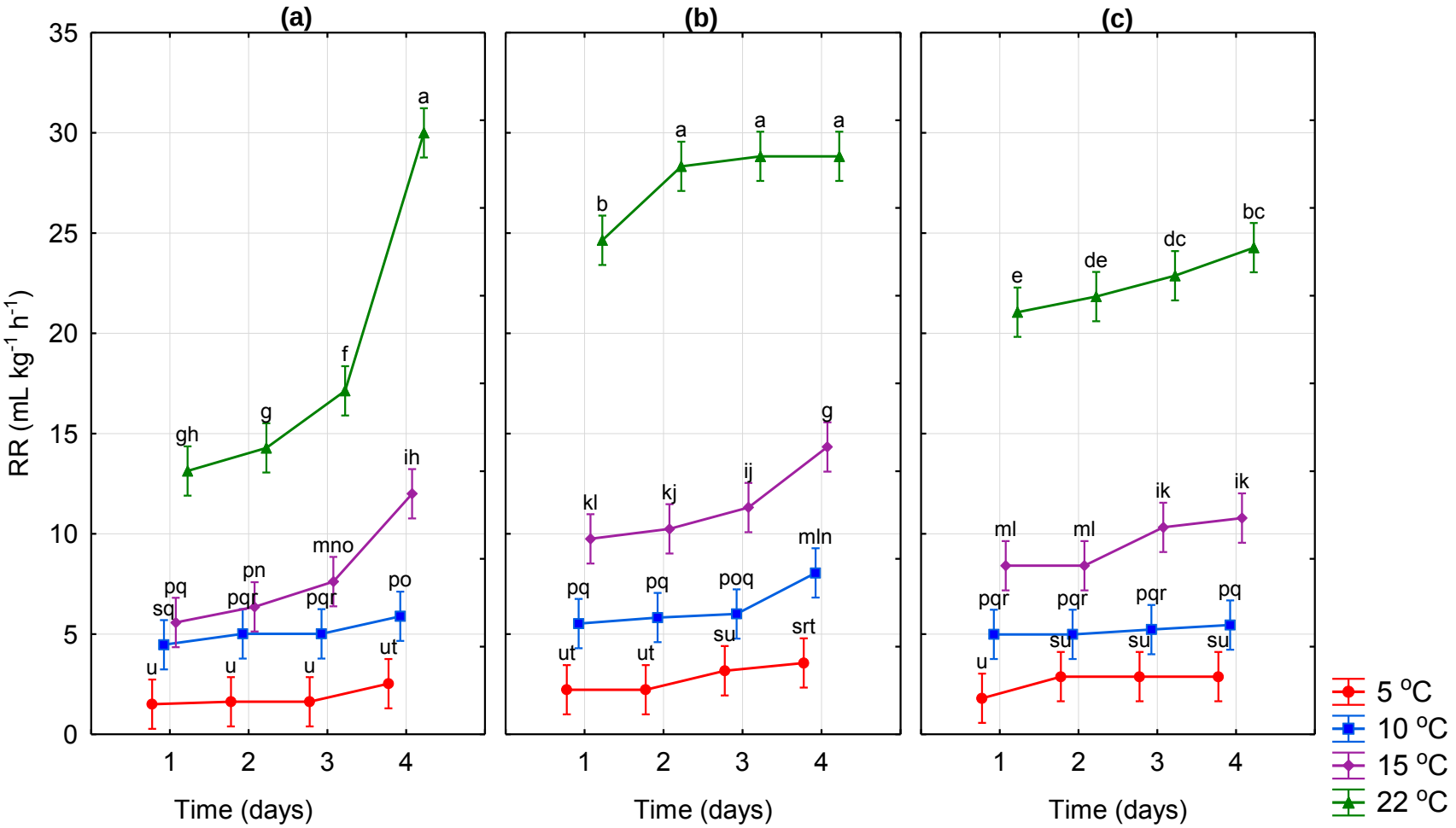

Figure 3.1 Effect of storage temperature (5, 10, 15 and 22°C) on RR of pomegranate (cv. Bhagwa) arils (a); aril-sacs (b) and whole fruit (c).Data points with similar letters are not significantly different according to Fisher LSD test (p=0.05), n=3.

Aril-sac had the highest RR at all the respective storage temperatures (Fig. 3.1 (b)). The lowest RR of 1.96 mL CO_2 kg^{-1} h^{-1} and 3.19 mL O_2 kg^{-1} h^{-1} (arils); 2.66 mL CO_2 kg^{-1} h^{-1} and 3.71 mL O_2 kg^{-1} h^{-1} (whole fruit); and 2.95 mL CO_2 kg^{-1} h^{-1} and 5.49 mL O_2 kg^{-1} h^{-1} (aril-sac) were observed at 5°C. Similar effects at lower storage temperature were reported by Maghoumi *et al.* (2012) on the respiration rate of pomegranate arils (cv. 'Mollar de Elche') which were found to be about 1.58 mL CO_2 kg^{-1} h^{-1}. These lower RRs were attributed to the effects of pre-treatments on the arils before packing, such as hot water and Ultraviolet-C light treatment, and high oxygen inside the packages. Caleb *et al.* (2012) reported the RR of arils to range between 2.51-7.59 mL O_2 kg^{-1} h^{-1} and 2.72-9.01 mL CO_2 kg^{-1} h^{-1}, while the RR of whole fruits ranged between 4.53-14.67 mL O_2 kg^{-1} h^{-1}, and 5.67-18.53 mL CO_2 kg^{-1} h^{-1} when stored between 5 and 15°C (Table 3.1). Therefore, the different responses of these products to changes highlight the possible influence of cultivar differences and storage temperature. The effects of storage time on the RR of the fruit fractions were observed (Fig. 3.1). From these observations, the RR of all the fruit fractions increases with storage period, reaching the maximum levels of 30.00 mL kg^{-1} h^{-1} for arils, 28.83 mL kg^{-1} h^{-1} for aril-sac and 24.27 mL g^{-1} h^{-1} for whole fruit.

Additionally, the observed difference in RR of whole fruit, aril-sac and arils can be associated with the effect of minimal processing. Garcia and Barret (2005) reported the RR of whole peach to be about 0.0081 mL CO_2 kg^{-1} h^{-1} compared to that of sliced peach which is about 0.01 mL CO_2 kg^{-1} h^{-1}, when stored at 5°C. In addition, they have indicated that at 10°C, the RR of sliced peaches was 0.0186 mL CO_2 kg^{-1} h^{-1}, still higher than that of whole peaches which was 0.015 mL CO_2 kg^{-1} h^{-1}. They emphasized that the RR increased with storage temperature as well as the degree of processing or injury played a significant role in the observed RR response. Similarly, Chonhenchob *et al.* (2007) found that unit operations such as peeling and slicing can increase the RR of fresh-cut mangoes, pineapples and melons. Zagory (1999) hypothesised that the increase in respiration rate of fresh-cut produce can be due to an increase in the surface area exposed, which induces a high diffusion rate of oxygen into the internal cells of the fruit and consequently increasing the metabolic processes of these injured cells.

Table 3.1 Comparison of pomegranate fruit and arils respiration rate from various sources and this study.

Fruit fraction	Cultivar	Respiration rate (mL CO_2 $kg^{-1} \cdot h^{-1}$)	Storage temperature	References
Arils	Bhagwa	1.96	5°C	This study
Arils	Mollar	1.15	5°C	Lopez-Rubira (2005)
Arils	Hicaz	0.52	4°C	Ersan *et al.* (2010)
Arils	Acco and Herskawitz	2.72	5°C	Caleb *et al.* (2012)
Whole fruit	Bhagwa	2.66	5°C	This study
Whole fruit	Wonderful	4.00	5°C	Artes *et al.* (1996)

Based on Pareto analysis (Fig. 3.2a, b & c) storage temperature had the most significant influence on RR for pomegranate whole fruit and its fractions. The interaction of storage temperature and duration had an influence on the RR of only the arils in this study (Fig. 3.2c). These findings corroborate with the study of Caleb *et al. (2012).* The observed difference in the response of whole fruit, aril-sac and arils, implies that at a given time only temperature plays a critical role on RR of whole fruit and aril-sac (Fig. 3.2a, b & c). This highlights the importance of maintaining optimum processing and storage temperature during postharvest handling of pomegranate fruit.

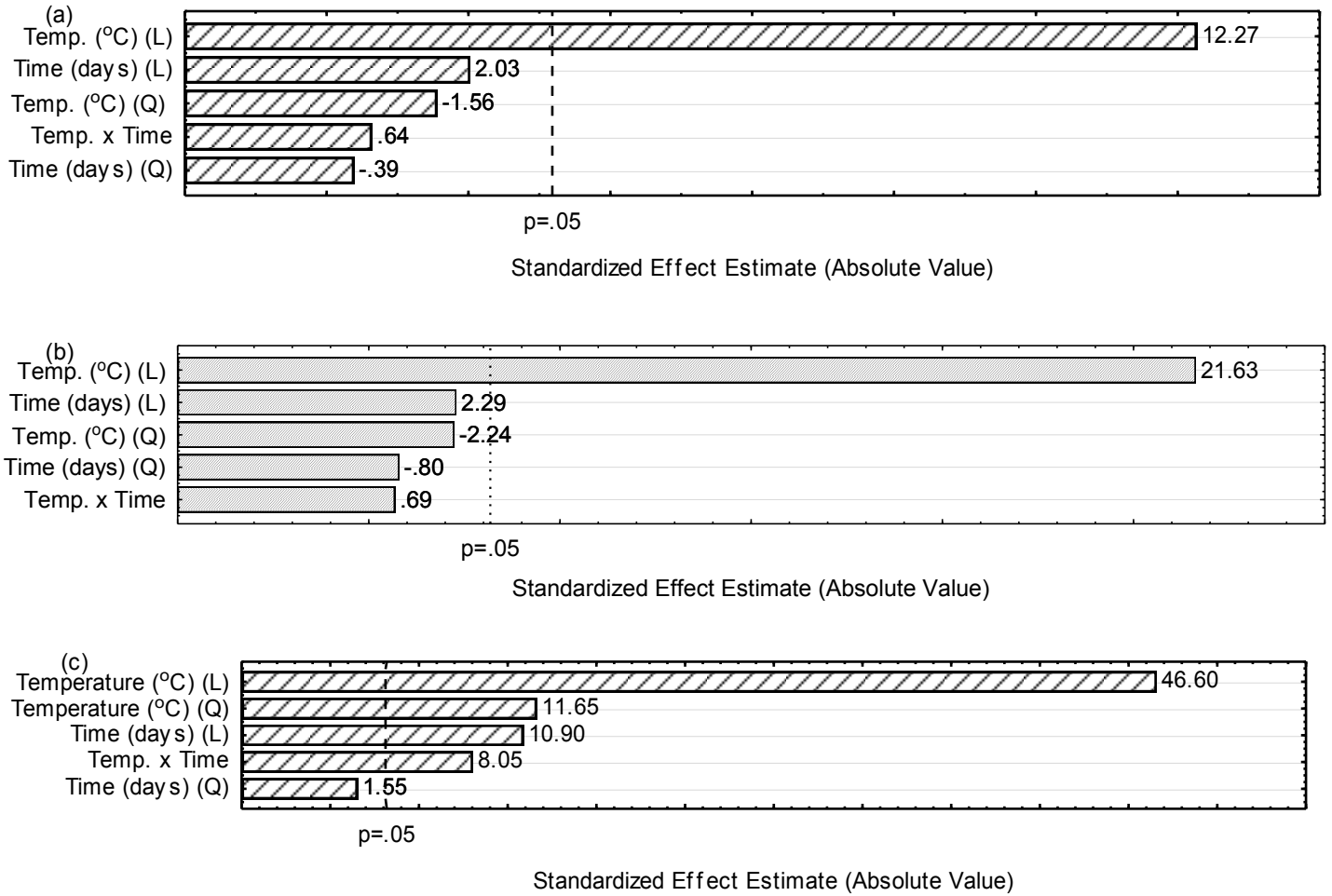

Figure 3.2 Pareto chart showing the effects of storage temperature, time and their interactions on the RR of whole fruit (a), aril-sac (b) and arils (c), at the significant level of p = 0.05 as indicated by the vertical line crossing the bars. Letter 'L' and 'Q' signify linear and quadratic effects.

Model validation

The Arrhenius-type model adequately described the effect of storage temperature on RCO_2 of the whole fruit, aril-sac and arils with R^2 above 97.1%. The scatter plot shows a good relationship between the experimental and predicted RR (Fig.3.3). Estimated parameters and relevant statistical data are presented in Table 3.2.

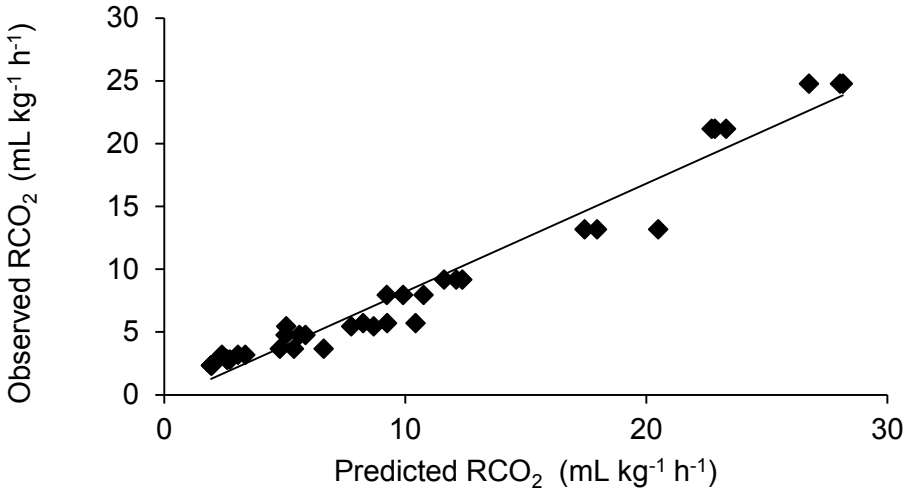

Figure 3.3 Relationship between experimental and predicted respiration rate values (mL kg^{-1} h^{-1}) of the pomegranate whole fruit, aril-sac and arils for all the experimental data.

Table 3.2 Parameters of the mathematical model (Equation 2) and relevant statistical data

Pomegranate fraction	$R^{i}CO_{2,\,ref}$ (mL kg^{-1} h^{-1})	E_a, CO_2 (kJ mol^{-1})	R^2 (%)
Whole fruit	4.73	70.07	98.81
Aril-sac	5.43	70.97	97.13
Arils	3.66	59.93	97.13

The model was successfully validated for RCO_2 with separate data obtained at 10°C for pomegranate whole fruit, aril-sacs and arils, and the strength of the model was tested at the higher temperature of 22°C. The experimental RCO_2 for cv. 'Bhagwa' compared favourably with the predicted data, even at the higher temperature of 22°C (Fig. 3.4). The accuracy of estimated value

of R^{i}_{CO2ref} with measured value at reference temperature (10°C) implies that experimental data obtained for RR at a single temperature is adequate to predict the RR at various temperatures, hence avoiding the accumulation of data that may not be useful. The activation energy (E_a) value ranged between 59.93 and 70.97 kJ mol^{-1} and corresponded with that reported by Caleb *et al*. (2012). It also fits in the range of 29 to 92.9 kJ mol^{-1} that Exama *et al. (1993)* reported for various fruits and vegetables stored under atmospheric condition.

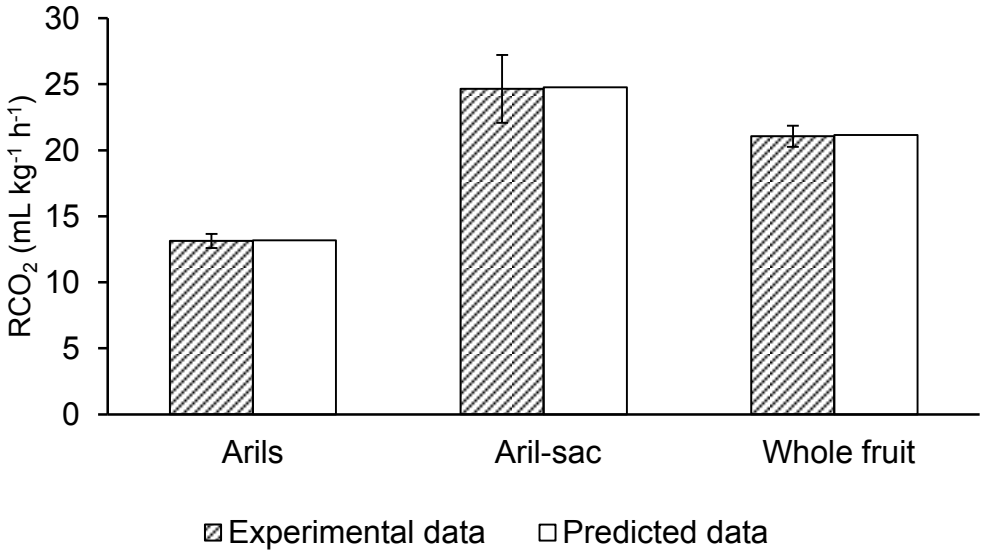

Figure 3.4 Relationship between respiration rates (RCO_2) obtained experimentally and predicted by Eqn. 3 at 22°C, for all the fruit fractions ($p > 0.05$).

Conclusion

Temperature had the most significant influence as compared to storage time on the RR of the pomegranate whole fruit, aril-sac and arils cv. '*Bhagwa*'. While the interaction of temperature and time was significant on the RR of the arils only. The study showed that the RR of pomegranate fruit and its fractions can be reduced significantly by storing them at lower temperature (5°C). Aril-sac had the highest RR in comparison to that of arils and whole fruit. Minimally processing of pomegranate fruit into aril-sac induces high rates of respiration. This provides useful guide towards MAP design, postharvest handling and processing of pomegranate fruits. The Arrhenius model used in this study was found to be useful in predicting the respiration rates of the pomegranate whole fruit and minimally processed fractions.

References

Arias, E., Gonzalez, J., Oria, R. & Lopez-Buesa, P. (2007). Ascorbic acid and 4-hexylresorcinol effects on pear PPO and PPO catalyzed browning reaction. *Journal of Food Science*, **72**(8), 422-429.

Artes, F., Gines Marin, J. & Martinez, J.A. (1996). Controlled atmosphere packaging of pomegranate. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung*, **203**, 33-37.

Ayhan, Z. & Esturk, O. (2009). Overall quality and shelf life of minimally processed and modified atmosphere packaged ''ready-to-eat'' pomegranate arils. *Journal of Food Science*, **74** (5), 399-405.

Caleb, O.J., Mahajan, P.V., Al-Said, F.A.J. & Opara, U.L. (2013). Modified atmosphere packaging technology of fresh and fresh-cut produce and the microbial consequences: a review. *Food Bioprocess Technology,* **6**, 303-329

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

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

Chonhenchob, V., Chantarasomboon, Y. & Singh, S.P. (2006). Quality changes of treated fresh-cut tropical fruits in rigid modified atmosphere packaging containers. *Packaging Technology and Science*, **20**, 27-37.

Crisosto, H.C., Mitcham, E.J. & Kader, A.A. (2012). Pomegranate: recommendations for maintaining postharvest quality. [Internet document] URL www.postharvest.ucdavis.edu/PFfruits/Pomegranate/ . 28/08/2012.

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, A., 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**,1365–70.

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.

Garcia, E. & Barrett, D.M. (2005). Fresh Cut Fruits. In: *Processing Fruits: Science and Technology*. 2nd ed. Pp. 53-72. Boca Raton, Florida, USA: CRC press.

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

Liu, C., Hsu, C. & Hsu, M. (2007). Improving the quality of fresh-cut pineapples with ascorbic acid/sucrose pre-treatment and modified atmosphere packaging. *Packaging Technology and Science*, **20**, 337-343.

Lopez-Rubira, V., Conesa, A., Allende, A. & Artes, 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.

Maghoumi, M., Gomez, P.A., Artes-Hernandez, F., Mostofi, Y., Zamani, Z. & Artes, F. (2012). Hot water, UV-C and super atmospheric oxygen packaging as hurdle techniques for maintaining overall quality of fresh-cut pomegranate arils. *Journal of the Science of Food and Agriculture,* DOI: 10.1002/jsfa.5868.

Nanda, S., Sudhakar Rao, D., & Krishnamurthy, S. (2001). Effects of shrink film wrapping and storage temperature on the shelf life and quality of pomegranate fruits cv. Ganesh. *Postharvest Biology and Technology*, **22**(1), 61–69.

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

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**, 2186–93.

Viuda-Martos, M., Fernandez-Lopez, J. & Perez-Alvarez, 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.

Zagory, D. (1999). Controlled and modified atmosphere. General aspects of film technology and selection. In: *5th Annual Workshop on Fresh-cut Products: Maintaining Quality and Safety*. Pp. 1-3. (Section 7a). University of California, Davis.

Chapter 4: Effects of temperature and relative humidity on transpiration rate of pomegranate aril-sacs and arils

CHAPTER 4

EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON TRANSPIRATION RATE OF POMEGRANATE ARIL-SACS AND ARILS

Abstract

The objective of this study was to investigate the effects of temperature (5, 10, 15 and 22°C) and relative humidity (RH) (76, 86 and 96%) on the transpiration rate (TR) of the pomegranate (*Punica granatum* L.) cv. 'Bhagwa' fruit fractions, namely arils and aril-sac. Results showed that both temperature and RH had significant effects on the transpiration rate of fruit fractions. Transpiration rate increased with an increase in temperature and decrease in RH, with the fruit fraction stored at 5°C and 96% RH showing the lowest TR in comparison to other storage conditions. Arils showed higher TR than aril-sac under all storage conditions. The TR of the arils at 96% RH was in the range of 1.42-15.23 g kg^{-1} d^{-1} while for the aril-sac was 0.63-9.95 g kg^{-1} d^{-1}, respectively. In brief, the higher TR of fruit arils may be attributed to the larger surface area as compared to the aril-sac whereby some of the arils are covered with the membrane, albedo and peel. Mathematical model was applied and the model adequately predicted the TR for arils and aril-sacs stored at 22°C and RH 76, 86 and 96%, with a good correlation found between experimental and predicted data. Additionally, results showed that the WVTR of pomegranate membrane was significantly ($p < 0.05$) affected by the storage conditions applied and but did not change significantly during storage. Membrane WVTR was 40.6% higher when stored at room condition than in cold storage. In comparison with literature evidence, these results showed that pomegranate aril-sac membranes have lower WVTR than the polymeric films widely used in modified atmosphere packaging (MAP) of arils.

Introduction

During postharvest handling and storage, fresh fruits and vegetables continue to lose water through the process of transpiration. This happens when there is a gradient of water vapour pressure between the produce skins and the surrounding air (Ben-Yehoshua & Rodov, 2002). Transpiration process has a negative effect on the commercial and physiological properties of the produce. These effects include loss of firmness, shrivelling, wilting and all components associated with freshness, thus leading to reduction of produce quality and make it unsuitable for processing into fresh-cut products (Baldwin & Bai, 2001). Factors that influence the produce price such as texture, appearance, and weight of the fruits, are greatly affected as a result of water loss. Moreover, fresh-cut/minimally processed produce are reported to have a higher water loss (Ayaha-

Zavala *et al.,* 2008). This can be due to skin damage and increase of surface area to volume ratio, as a result of the cutting process and subsequently juice leakage (Ben-Yehoshua & Rodov, 2002).

Transpiration rate (TR) of produce is generally influenced by factors such as temperature, surface area, relative humidity (RH), air movement and respiration rate (Mahajan *et al.,* 2008; Ben-Yehoshua & Rodov, 2002). Lower temperature and high relative humidity has been reported to play a major role in maintaining the produce quality by reducing its rate of water loss (Mahajan *et al.,* 2008, Caleb *et al.,* 2013a). However, these storage conditions are produce dependent, with the optimal RH ranging between 85-96% and temperatures between 4-7°C, for various produces such as eggplant, mizuna, fig fruits (Hung *et al.,* 2011), pomegranate arils (Caleb *et al.,* 2013b), and mushrooms (Mahajan *et al.,* 2008). Caleb *et al.* (2013b) reported that the weight loss of pomegranate arils cv. 'Acco' was notably higher at 15°C and 76% RH, while at 5°C and 96% RH, the quality of arils were best kept. Similarly, Mahajan *et al.* (2008) reported an increase in the RH from 76% to 96%, decreased the TR of mushrooms by 87% at 4°C, while a decrease in temperature from 16 to 4°C caused a reduction in TR by 61% at 96% RH, respectively.

Packaging materials serve as a mechanical barrier and help control of in-package RH; however, water vapour accumulates in the package as the produce transpires. The accumulation of water droplets may create a conducive environment for microbial growth, which leads to sliminess of produce and decay (Song *et al.,* 2001; Caleb *et al.,* 2013a). These usually occur when the water vapour transmission rate of the packaging film used does not correlate with the physiological processes of the produce (Caleb *et al.,* 2013a). Hence, understanding the effect of storage on transpiration rate of the fruit helps the food and postharvest technologists to make informed decisions on the packaging technologies to use.

Furthermore, Water vapour transmission rate (WVTR) is relevant to the package performance as it is used as a standard measurement to compare the film/membrane abilities to resist moisture transmission (Hu *et al*., 2001; Maixner, 2002; Alyanak, 2004; Siracusa, 2012). Packages with high WVTR barrier properties help to maintain the products moisture constant throughout storage, i.e. dry products are prevented from gaining moisture and wet products are prevented from losing moisture, as this affects their quality (Mahajan *et al*., 2007; Anonymous, 2013). Permeability of the film is influenced by factors such as the storage temperature, type of material used to make up the film, relative humidity (RH), film thickness and concentration and gradient of the gases (Mahajan *et al*., 2007; Brandenburg & Zagory, 2009; Thompson, 2010).

Some of the films used in the food industry include bi-axially oriented polyethylene (BOPP), low density polyethylene (LDPE) and polyethylene (PE) (Artes *et al.*, 2000; Ayhan & Esturk, 2009; Brandenburg & Zagory, 2009; Siracusa, 2012), and their WVTR properties have been extensively

reported (Ayhan & Esturk, 2009; Caleb *et al*., 2013). The structure of the pomegranate fruit has prompted the need to explore the role or properties of the membrane that separates the aril sacs inside the fruit. The membrane as shown in Figure 4.1 is the yellowish tissue that is attached to the inner side of the peel and the albedo, hence forming the aril-sacs. There may be possibilities that the membrane aid in moisture and gas exchange between the aril-sacs. There is limited information in literature on the barrier properties of pomegranate membrane.

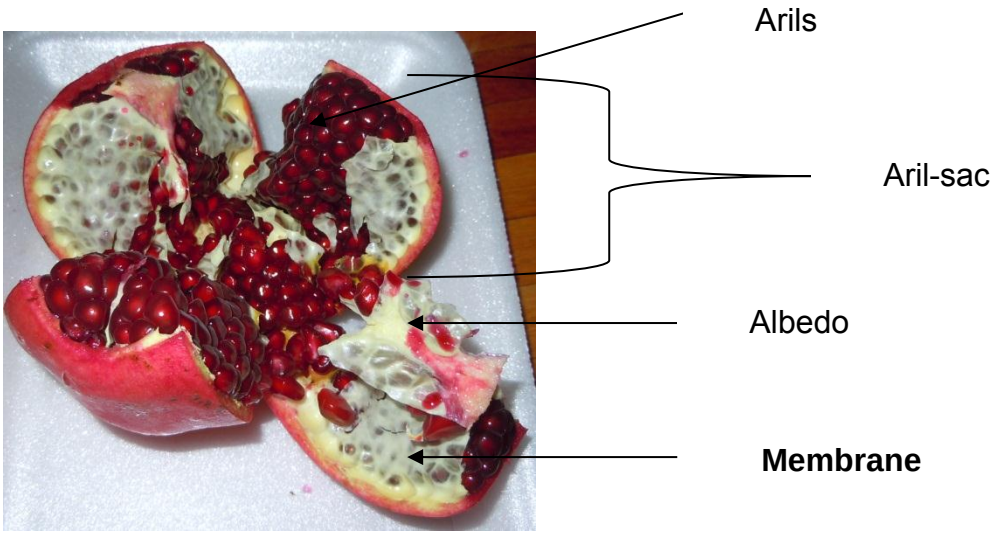

Figure 4.1 Cross section of pomegranate fruit

Pomegranate (*Punica granatum* L.) fruit is rich micro- and macro-nutrients, and has excellent health benefits which are well documented in literature such as high anti-oxidant, anti-anti-atherosclerotic, anti-mutagenic and anti-hypertension (Viuda-Martos *et al.,* 2010; Fawole & Opara, 2012). However, despite its potential health benefits, difficulties in peeling and arils extraction have limited their consumption (Opara *et al.,* 2009). Thus, minimally processed pomegranate fruit such as arils and aril-sac (arils still attached to the peel and partly covered with the membrane) will offer a more appealing product to consumers. Study by Caleb *et al*., (2013b) focused on transpiration rate pomegranate arils, but no comparative study has been done on the effect of storage conditions on transpiration rate of pomegranate arils and aril-sac. The objective of this study was to investigate the effects of storage temperature (5, 10, 15 and 22°C) and RH (76, 86 and 96%) on the transpiration rate of the pomegranate cv. 'Bhagwa' fruit fractions, namely arils and aril-sac. Furthermore, based on the hypothesis that the membrane plays a role on the moisture retention of the arils, a study was done to measure the water vapour transmission rate of the membrane.

Materials and methods

Plant material and preparation

Pomegranate fruits (*Punica granatum* L.) cv. 'Bhagwa' (sweet cultivar) were obtained at their commercial harvest conditions from a farm situated in the Wellington area of the Western Cape, (33°38'S 18°59'E) South Africa. The fruit was transported in an air-conditioned and ventilated vehicle to the Postharvest Research Laboratory at Stellenbosch University, South Africa. It was thereafter stored in a cold room at 5 ± 0.5°C and 95% RH, until used for the experiments. Fruits with no physical defects were selected for processing. Each fruit was surface sterilised using 70% ethanol. To obtain arils, each fruit was cut in cross-sections and arils were manually removed carefully as to avoid mechanical damage. For the aril-sacs, the fruits were carefully cut into four or three sections to obtain an intact aril-sac (arils attached to the peel and albedo). All processing was done in a disinfected cold room with a temperature below 8°C, in order to avoid physiological stress on the fruit.

Measurements of transpiration rate

The experimental setup consisted of test containers filled with salt solutions and kept at each experimental temperature. Supersaturated salt solutions from analytical grade of sodium chloride (NaCl), potassium chloride (KCl) and potassium nitrate (KNO_3) (Merck (Pty) Ltd., Modderfontein, South Africa) were made using de-ionised water in order to obtain 76, 86 and 96% RH, respectively. Storage temperatures used were 5, 10, 15 and 22°C, respectively. Arils of about 12 g and aril-sac weighing about 45 g were placed in sterilised Petri dishes which were placed in the respective containers with supersaturated solutions. Temperature and RH data loggers (Tiny tag view 2, TV-4500, UK) were placed inside the test containers to monitor the temperature and RH.

To calculate the TR of arils and aril-sacs, a weight loss method as reported by Mahajan *et al.* (2008) was used. TR was expressed as weight loss (g kg^{-1} d^{-1}) as described in equation 1:

$$\mathbf{TR} = \frac{\boldsymbol{Mi - M}}{\boldsymbol{t} \times \left(\frac{\boldsymbol{Mi}}{\mathbf{1000}}\right)} \qquad \textbf{(1)}$$

Where;

M_i =initial mass of samples (g)

M = mass of sample (g) at weighed time t in days

t = time (days)

Three replicates for each treatment and fruit fraction were stored at each temperature regime. Weight measurements were taken daily for a period of 5 days, using an analytical balance (Mettler Toledo, ML104/01, Switzerland).

Model building

According to Ben-Yehoshua and Rodov (2002), the flow of water vapour through a produce happens when there is a gradient of water vapour pressure between the produce skins and the surrounding air. Based on Fick's law of diffusion which states that the rate of transfer of vapour through a sheet of tissue is proportional to the tissue area and the pressure difference between the two sides and inversely proportional to the sheet thickness (Ben-Yehoshua & Rodov, 2002). In this model, the RH of the fruit internal atmosphere was estimated to be around 100%, which is proportional to the solute content inside the fruit and slightly below 0.1 (Mahajan *et al.,* 2008). TR was calculated using equation 2 (Caleb *et al.,* 2013):

$$\boldsymbol{TR} = \mathbf{K_i} \times \left(\boldsymbol{a_{w_i}} - \boldsymbol{a_w}\right) \quad \textbf{(2)}$$

Where a_w is the water activity of the container; a_{wi} is the water activity of the arils and aril-sacs; K_i is the mass transfer coefficient. Furthermore, to predict TR of arils and aril-sacs as a function of temperature and RH, the temperature term was incorporated to estimate K_i in equation 2 yielding equation 3.

$$\boldsymbol{TR} = \mathbf{K_i} \times \left(\boldsymbol{a_{w_i}} - \boldsymbol{a_w}\right) \times \left(\mathbf{1} - \boldsymbol{e^{-aT}}\right) \quad \textbf{(3)}$$

Where, T is temperature in °C, and experimental data obtained at all combinations of temperature and RH were used to estimate the constant parameter a and K_i . The model Eqn. 3 was fitted by non-linear regression using Solver tool on Microsoft Excel (version 2010.). In order to validate the accuracy and robustness of our model, additional experiments was set up at 10 and 22°C for arils and aril-sac.

Measurements of WVTR

Sweet pomegranate fruit (*Punica granatum* L.) cv. Bhagwa harvested manually during commercial harvest period was used in this study. Fruit were obtained from Houdocostant farm in Porterville, Western Cape, South Africa and transported in a ventilated vehicle to the Postharvest Research Laboratory, Stellenbosch University. Upon arrival fruits were put in a cold storage (7 ± 0.5°C, 85 ± 1.0% RH) before use. Aluminium foil (heavy duty) and *Rapid-Epoxy* glue (10 minute Epoxy Adhesive, Alcolin) were purchased from a retail outlet.

Experimental setup

A wet cup method, as described by Hu *et al*. (2001) was followed to measure the water vapour transmission rate of the membrane. This method is a modification of the technique described in ASTM E 96-95. The experimental setup was done in a way to measure the WVTR by monitoring the weight loss of the test cup over time. This was done by covering a Petri dish (≈ 9.65 g) with aluminium foil, whereby a hole of known diameter was made at the centre of the aluminium foil. This hole was then covered carefully with a membrane which is obtained from the pomegranate fruit cross-section. In order to ensure hermetic seal, *Rapid-Epoxy* glue was used to seal the membrane on the foil to the Petri dish (Fig. 4.2). Before the foil was sealed on the Petri dish, 30 mL of distilled water was added to the Petri dish. The epoxy was allowed to dry for about 30 minutes before the test cups were placed at their respective temperature.

Test samples were stored in an environment chamber (5°C, 90% RH) or at room condition (18.7°C, 70% RH). Four replicates per storage condition were used. Petri dishes with the foil mask that does not have pomegranate membrane glued on foil were used as control at each storage condition to help monitor the efficiency of the aluminium foil as a barrier property. A Vernier caliper (Absolute Digimatic, model CD-6''CX, Mitutoyo Corp., Kawasaki, Japan) was used to measure the area of circular hole/windows covered by the membrane. An analytical balance (Mettler Toledo, ML 104/01, Switzerland) was used to take the weight of the petri dish. Weight measurements were taken daily for a period of five days. Four samples under each storage condition were measured.

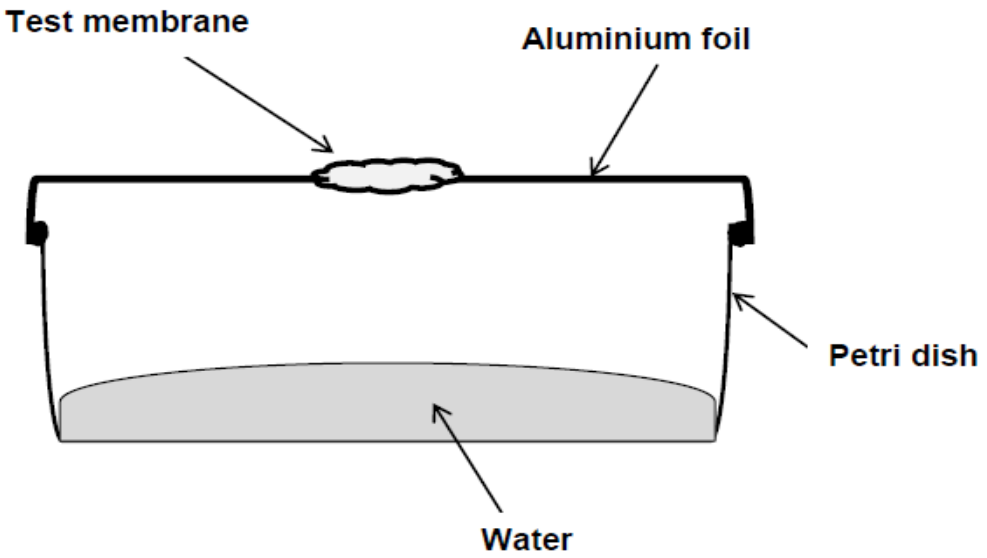

Figure 4.2 Schematic of the wet cup test for water vapour determination

WVTR calculations

A weight loss approach was adopted and WVTR was calculated based on the changes in weight of water in the petri dish over time and expressed as change in water weight (g) per area of membrane (m^2) per unit time (day) as shown in equation 1:

$$WVTR = \frac{M_i - M}{T \times A} \quad (1)$$

where M_i is the initial known weight of water (g), M is the weight observed at time (T) in days and A is the area of membrane (m^2).

Statistical analysis

Experiments were performed according to the full factorial design. Three replicates of each test product were done per treatment. All experimental data were analysed using Statistica software, Version 11.0 (Statistica, Statsoft, USA). The effects of temperature, relative humidity and their interaction on the transpiration rate were analysed by a three-way factorial Analysis of Variance (ANOVA) and Pareto analysis of effects, respectively. Significant differences were established at $p \leq 0.05$.

Results and discussion

Effects of temperature and RH on transpiration rate

The influence of temperature and RH on TR of the arils and aril-sac were significant ($p \leq 0.05$) (Fig. 4.3). The TR, which is expressed as weight loss in g kg^{-1} d^{-1}, increased with an increase in temperature and a decrease in RH. Significant differences ($p \leq 0.05$) were observed among the TR of the arils and aril-sac under some of the storage conditions. The TR of arils and aril-sac ranged from 1.42 to 15.23 g kg^{-1} d^{-1} and 0.63 to 9.95 g kg^{-1} d^{-1}, respectively, across all storage conditions. Lowest TRs was observed at storage conditions of 5°C, 96% RH, while the highest TRs was at 22°C and 76% RH for both fruit fractions.

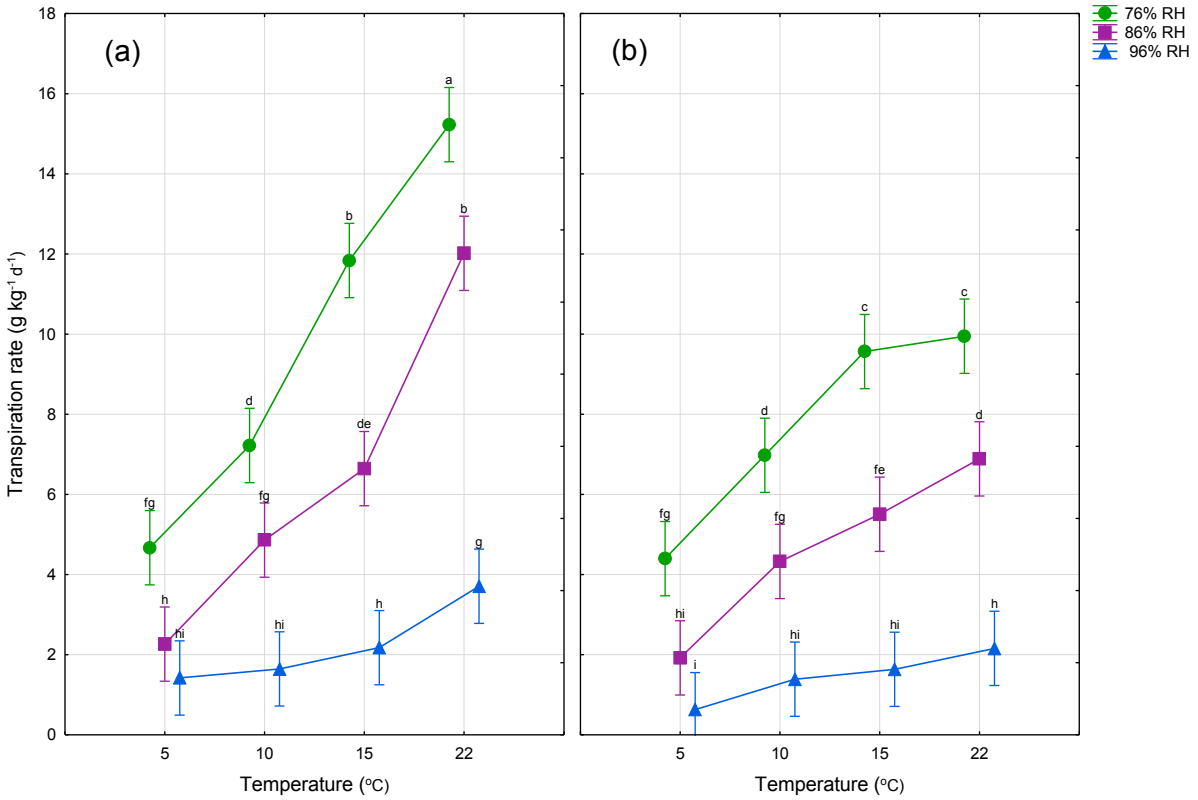

Figure 4.3 Effects of temperature and relative humidity (RH) on transpiration rate (TR) of arils (a) and aril-sac (b); error bars depicts the mean standard deviation. Different superscript letters indicate statistically significant differences ($p < 0.05$) between data points.

Similarly, the lowest weight loss was observed in aril-sac at 5°C and 96%, with TR value of 0.63 g kg^{-1} d^{-1}, which is 55.63% lower than that of the arils which was 1.42 g kg^{-1} d^{-1} under the same storage condition. This trend was also observed in pomegranate arils and mushrooms by Caleb *et al.* (2012) and Mahajan *et al.* (2008), respectively. Caleb *et al.* (2013b) reported a decrease in water loss of arils stored at 5°C and 96% RH. In addition, Mahajan *et al.* (2008) reported a reduction of water loss in mushrooms when stored under the temperature of 4°C and 96% RH. The TR of arils and aril-sac obtained in this study is in the range with that reported by Caleb *et al.* (2013b), and lower than the TR of mushroom reported by Mahajan *et al.* (2008).

Thus, a higher water loss in arils can be related to the large surface area exposed as compared to the aril-sac whereby most of the arils are covered partly by the peel and membrane. The effects of RH on the TR of the arils and aril-sac can be observed on the trend that the TR followed between the RH conditions (Fig. 4.4a). For example, increasing the RH of storage containers from 76% to 96% sees a reduction in TR by 68% and 86% of arils and aril-sac, respectively, at 5°C. Temperature effect was also observed, as indicated (Fig.4.4b). For example, a decrease in temperature from 15°C to 5°C showed a 35% and 62% decrease in the TR of arils

and aril-sac, respectively, under RH of 96%. Caleb *et al.* (2013b) also reported that an increase in the relative humidity from 76-96% decreased the arils TR by 83.5% at 5°C, whilst a decrease by 68.9% was observed when the storage temperature was reduced from 15 to 5°C, respectively.

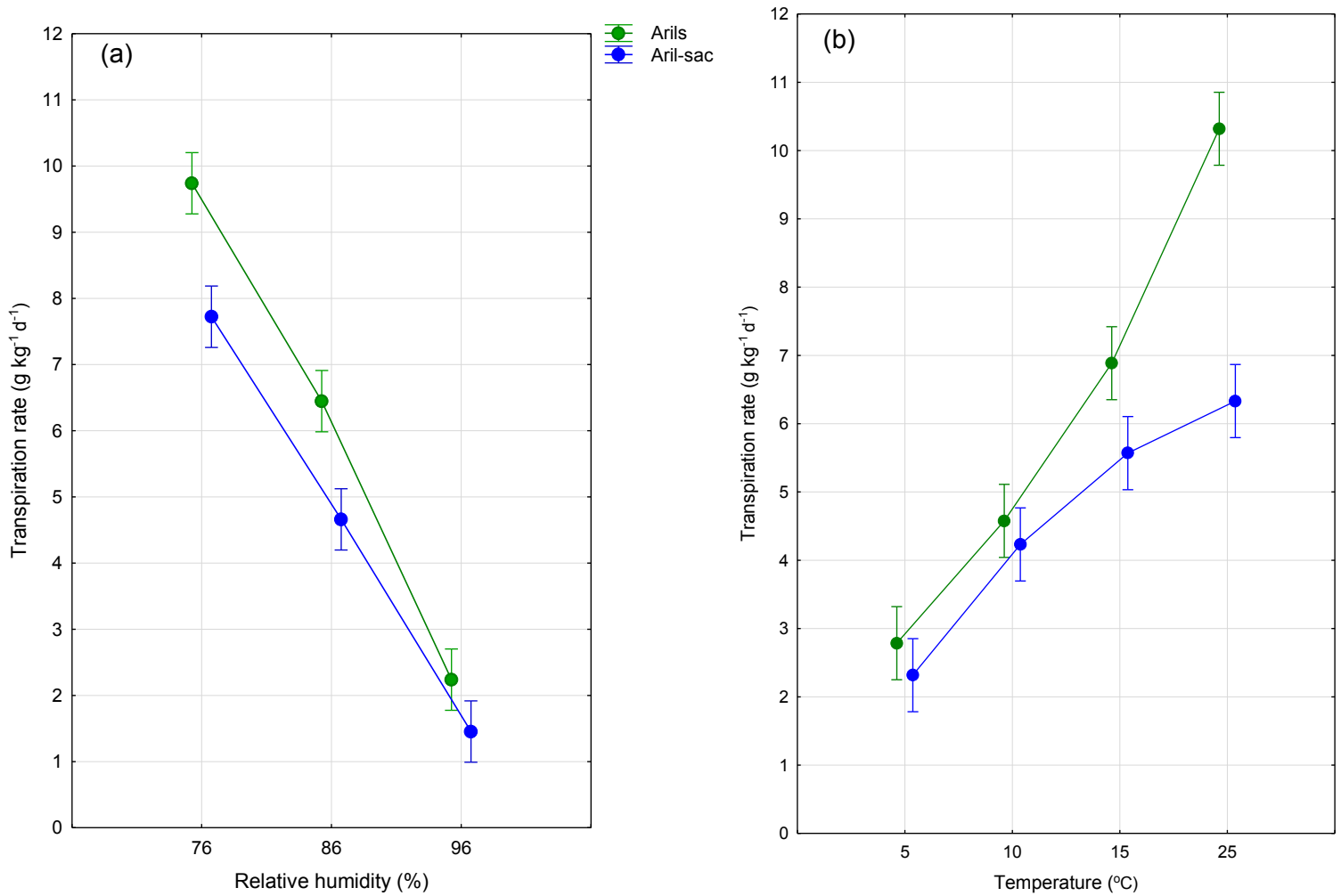

Figure 4.4 One-way ANOVA analyses depicting the effects of RH (a), and temperature (b) on the TR of arils and aril-sac. Error bars depicts the mean standard deviation.

Additionally, this observation was supported by Pareto analysis (Fig. 4.5), which indicates which factor have a higher effect on the TR. Evidently, the effect of RH on TR was more prominent in comparison to that of the temperature. Although, higher in-packaged humidity reduces water loss from fresh produce, excessively high or saturated RH could result in accelerated deterioration of product quality and enhance microbial growth (Ayaha-Zavala *et al.,* 2008). Therefore, determining the suitable relative humidity and storage temperature helps in designing the packaging system of the arils and aril-sac, especially the modified atmosphere packaging technique. Effects of storage period on the weight loss of the arils and aril-sac were also studied, as shown in Fig. 4.6. It has been observed that the weight loss of these fruit fraction increased with storage time, across all the treatment combinations. Similar effects were observed by Caleb *et al.* (2013b) on pomegranate arils, when stored under the same storage condition used in this study.

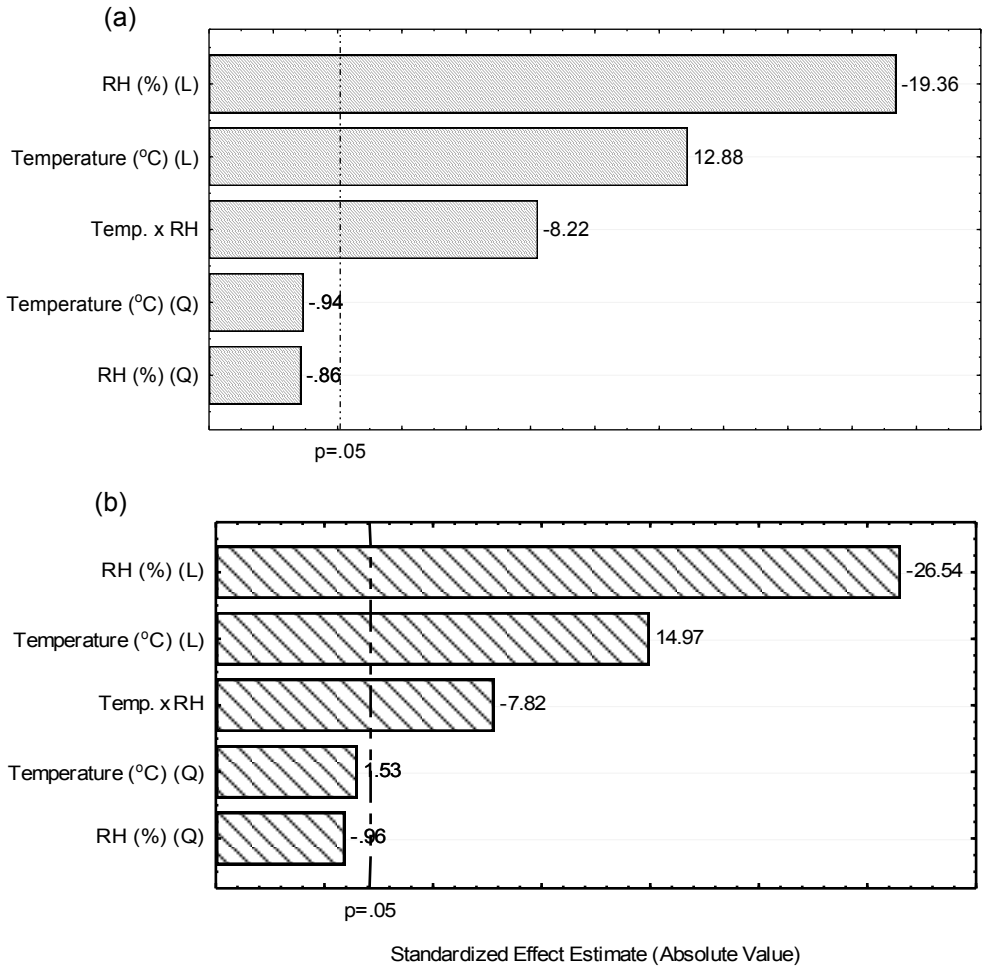

Figure 4.5 Pareto chart of standard effect estimate (absolute values) of relative humidity, temperature and their interactions on the TR of arils (a) and aril-sac (b), with *p*-value of 0.05, shown as dashed lines.

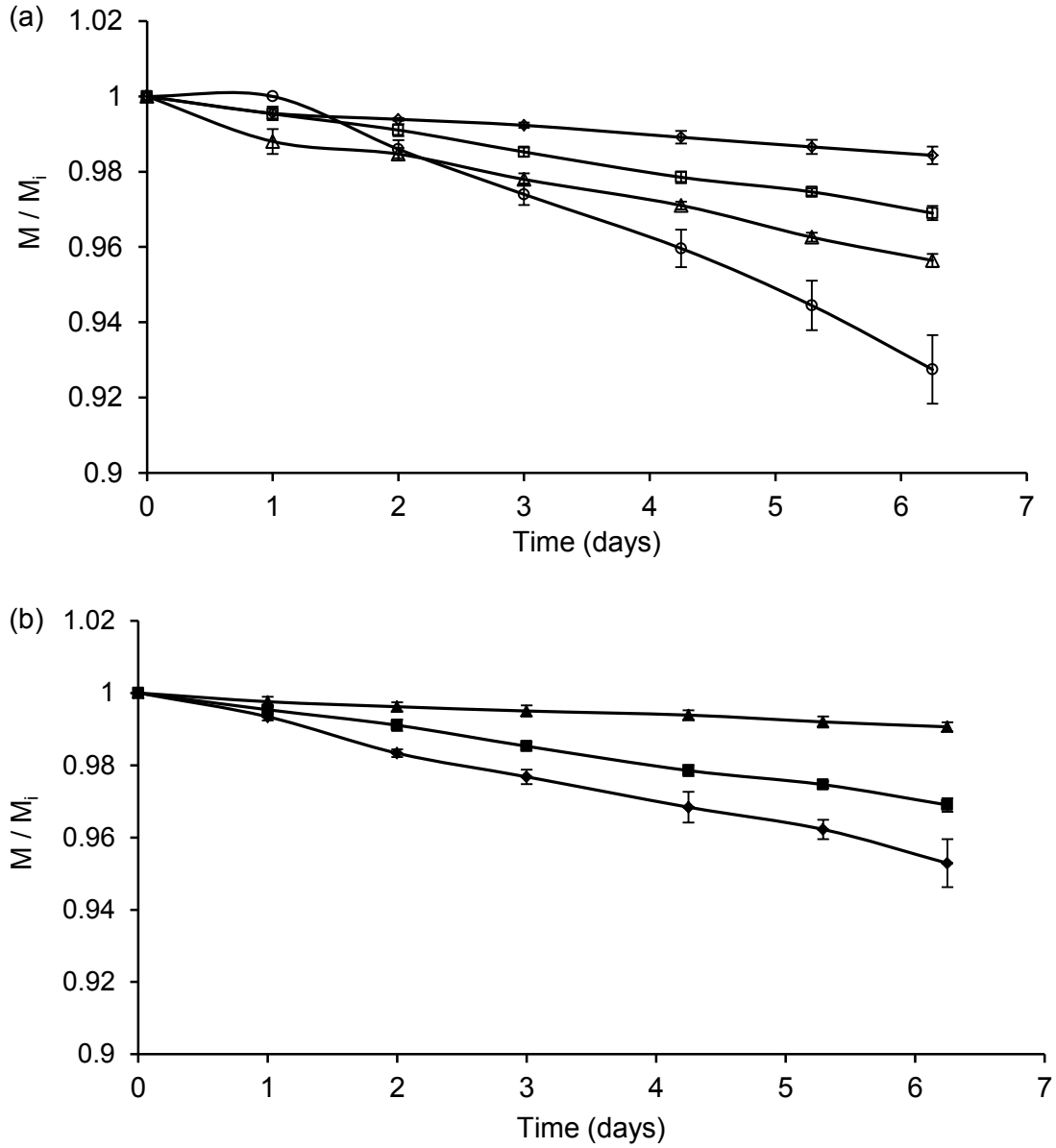

Figure 4.6 Changes in the weight of pomegranate arils over storage time. Values were normalized with respect to the initial weight of the arils (M_i, g): (a) effect of temperature on weight loss at 86% RH (◊, 5°C; □, 10°C; ∆, 15°C; ○, 22°C); and (b) effect of RH on weight loss at 10°C (▲, 96%; ■, 86%; ♦, 76%). Error bars depicts the mean standard deviation.

Model validation

The TR of the arils and aril-sac as influenced by the storage temperature and RH were adequately predicted at 22°C. A good correlation was found between the experimental data and the predicted

value, as depicted in Fig. 4.7. As in experimental data, the predicted value also showed a decrease in water loss with an increase in relative humidity from 76 to 96% and a decrease in storage temperature from 15 to 5°C. The efficiency of the model in predicting the TR of the fruit fractions was well demonstrated by a high R^2 value of 83.59%. The results obtained from the prediction follow the same trend as that of the prediction results reported by Caleb *et al.* (2013b) and Mahajan *et al.* (2008). The estimated parameters used in the prediction model are presented in Table 4.2.

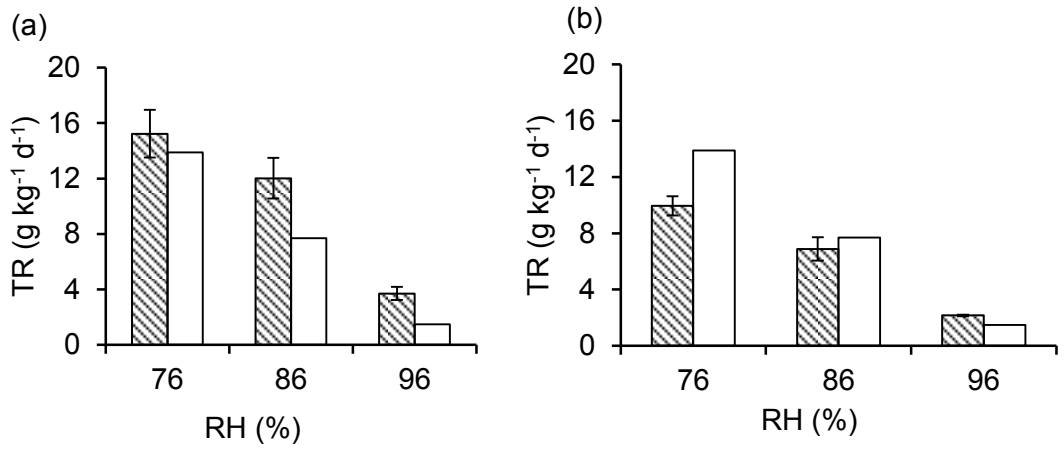

Figure 4.7 Relationship between experimental and predicted TR of arils (a) and aril-sacs (b) model validation at 22°C. Patterned filled bars, represents experimental data; Unshaded bars, represents predicted data.

Table 4.2 Parameters of the mathematical model (Eq. (2)) and relevant statistical data

Pomegranate fraction	**K_i**	***a***	**R^2 (%)**
Aril-sac & arils	84.10	0.05	83.59

WVTR findings

Resulted obtained showed that storage condition had significant ($p < 0.05$) effects on WVTR of pomegranate membrane; however, storage time effects were insignificant (Fig. 4.8). A significantly ($p < 0.05$) lower WVTR (40.6% lower) was observed in membranes stored in cold storage (5°C, 90% RH) compared to those stored at room condition (18.7°C, 70% RH) (Fig. 4.9). The higher WVTR of membranes stored at higher temperature condition (18.7°C, 70% RH) can be related to

the high temperature and lower relative humidity which cause the membrane to dry up fast and leads to the deformation in structure of the membrane, hence allowing water vapour to permeate through it. Hu *et al*. (2001) investigated the effects of lower RH on WVTR of Celgard films (3-mil thickness) at 37.4°C and 14% RH and 37.5°C and 18% RH using a wet cup method. A high WVTR was reported to be high at lower RH of 14% in comparison to 18% RH. Thus from these results, one can relate the membrane role of creating a barrier by reducing the rate at which moisture escape from the arils tissues. Hence cold storage of the pomegranate especially when processed into aril-sacs is crucial.

Several researchers have reported the WVTR of films used in MAP of fresh produce. For instance, BOPP films were reported to have WVTR of 6.5 g m^{-2} d^{-1} at 38°C and 75% (Ayhan & Esturk, 2009) while polylid PE had 20-22 g m^{-2} d^{-1} at 25°C and 50% RH and 1 bar (Caleb *et al*., 2013). Comparing the WVTR of the membrane to those of the polylid PE film and BOPP film, the membrane showed a low WVTR than these films, although the storage conditions were different. The WVTR of the membrane at cold storage (2.05 ± 0.43) was 89.8% and 73.1% lower than that of the polylid PE and BOPP films, respectively. Similarly, the WVTR of the membrane stored at room condition (3.45 ± 0.67) was 82.8% and 46.9% lower than that of the polylid and BOPP films, respectively.

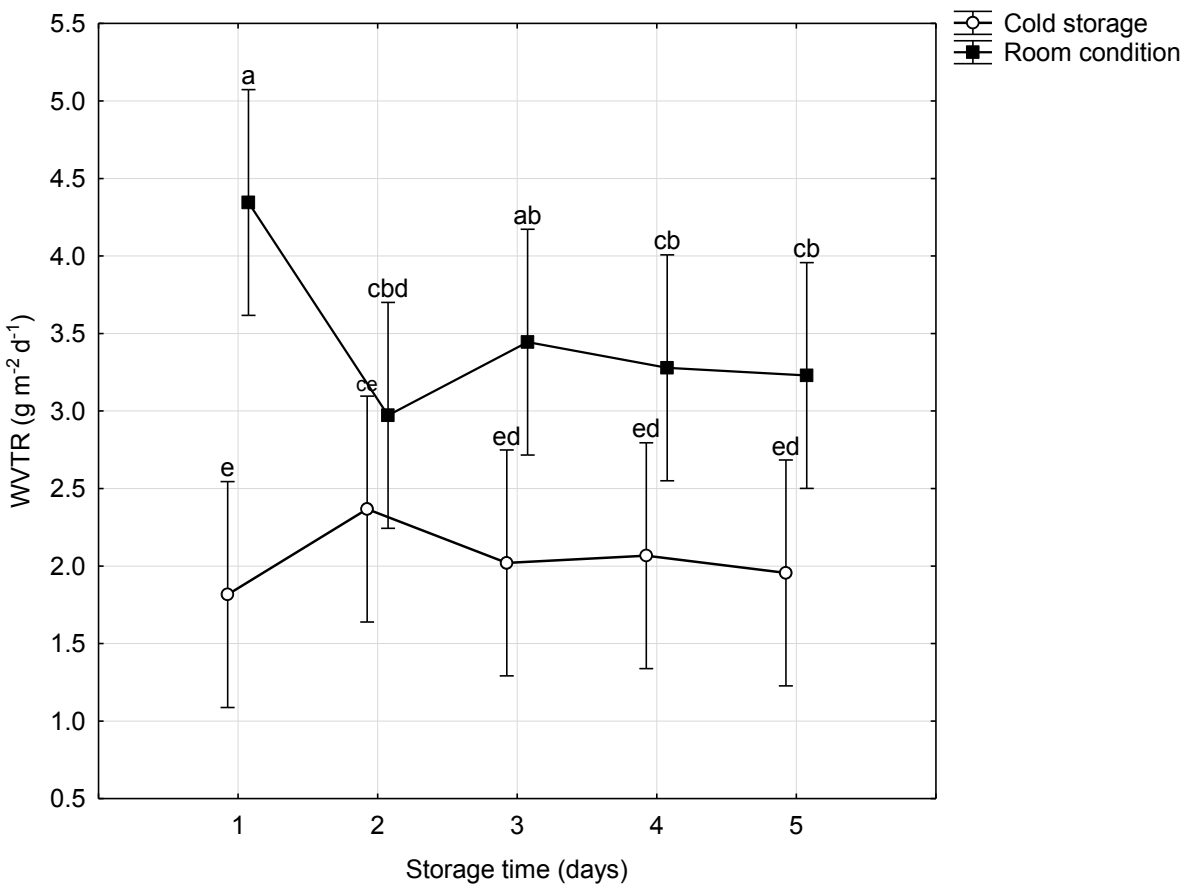

Figure 4.8 WVTR of pomegranate membrane measured at cold storage (5°C, 90% RH) and room temperature (18.7°C, 70% RH) over a period of 5 days. Vertical bars denote 0.95 confidence intervals.

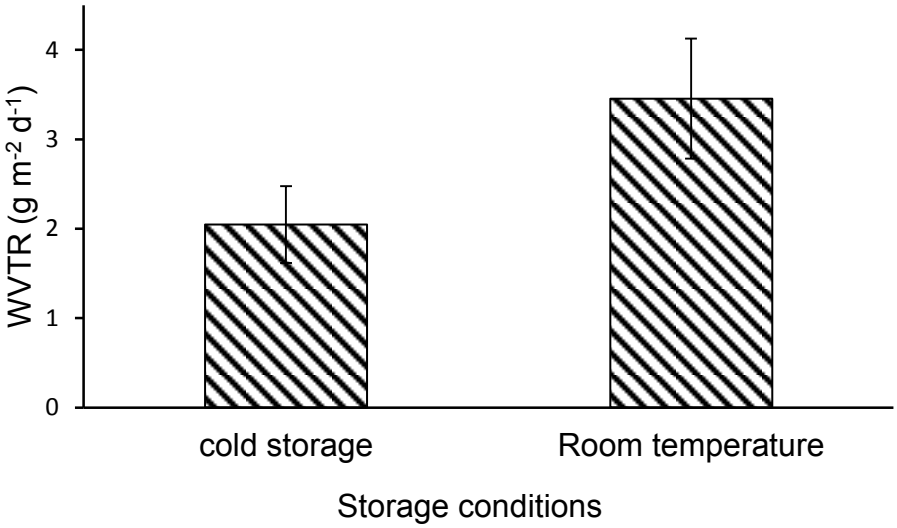

Figure 4.9 Average WVTR of membrane at cold storage (5°C, 90% RH) and room temperature (18.7°C, 70% RH) over 5 days duration. Vertical bars denote error bars.

Conclusion

Both temperature and RH have significant effects on the water loss of pomegranate arils and aril-sac. The fruit fractions had the lowest TR at 5°C and 96% RH. Storing pomegranate arils and aril-sacs at the lower temperature and higher RH helps reduce water loss. Relative humidity had the most significant influence on the TR of the arils and aril-sac, whereby an increase in RH from 76% to 96% decreased the TR by 68% and 86% of arils and aril-sac, respectively, at 5°C. Moreover, temperature and interaction between RH and temperature also had a significant effect on the transpiration rate of the arils and aril-sac. Hence this stresses the importance of keeping an optimum storage condition of fresh-cut fruits. Arils had a higher TR in comparison to aril-sac. The transpiration model used in this study adequately predicted the TRs of both arils and aril-sac, and the model can be used to gain a good understanding of the effects of RH and temperature on the TR of minimally processed produces. The influence of temperature and RH highlighted the critical role of optimal postharvest handling and storage conditions, in order to preserve the keeping quality of fresh or fresh-cut pomegranate fruit.

The wet cup method provided an approach effective to measure the WVTR of pomegranate membrane. Though the study gave us an insight on the barrier properties of the membrane, more research should be done to explore the role and structure of the membrane. Therefore, one can recommend the exploration of the membrane structure i.e. the porosity by using electron microscope to gain an understanding of membrane porosity in relation to water vapour permeability. Studies using Bhagwa pomegranate cultivar showed the WVTR of membranes were higher at room temperature than cold storage temperature.

References

Alyanak, D. (2004). Water vapour permeable membranes. MSc in Biotechnology and Bioengineering Program. Izmir Institute of Technology, Turkey.

Anonymous (2013). Water vapour transmission rate. Polyprint. [Internet document]. URL www.polyprint.com/flexographic.wvtr.htm.19/09/2013.

Ayala-Zavala, J.F., Del-Toro-Sanchez, L., Alvarez-Parrilla, E. & Gonzalez-Aguilar, G.A. (2008). High relative humidity in-package of fresh-cut fruits and vegetables: advantages or disadvantages considering microbiological problems and antimicrobial delivering systems. *Journal of Food Science*, **73** (4), 41-47.

Ayhan, Z. & Esturk, O. (2009). Overall quality and shelf life of minimally processed and modified atmosphere packaged ''ready-to-eat'' pomegranate arils. *Journal of Food Science*, **74** (5), 399-405.

Baldwin, E.A. & Bai, J. (2001). Physiology of fresh-cut fruits and vegetables. In: *Advances in Fresh-Cut Fruits and Vegetables Processing*. Pp. 87-95. Boca Raton, USA: CRC Press.

Ben-Yehoshua, S. & Rodov, V. (2002). Transpiration and water stress. In: *Postharvest Physiology and Pathology of Vegetables*, 2nd ed. (edited by J.A. Bartz & J.K. Brecht). Pp. 119-160. New York, USA: Marcel Dekker, Inc.

Brandenburg, J.S. & Zagory, D. (2009). Modified and controlled atmosphere packaging technology and applications. In: *Modified and controlled atmospheres for the storage, transportation, and packaging of horticultural commodities* (edited by: E.M. Yahia). Pp. 73-92. London: CRC Press.

Caleb, O.J., Mahajan, P.V., Al-Said, F. A. & Opara, U.L. (2013a). Modified atmosphere packaging technology of fresh and fresh-cut produce and the microbial consequences. *Food and Bioprocess Technology*, **6**(2), 303-329.

Caleb, O.J., Mahajan, P.V., Al-Said, F. A., Opara, U.L. (2013b). Transpiration rate and quality of pomegranate arils as affected by storage conditions. *CyTA – Journal of Food*, doi:10.1080/19476337.2012.721807.

Fawole, O.A. & Opara, U.L. (2012). Composition of trace and major minerals in different parts of pomegranate (*Punica granatum*) fruit cultivars. *British Food Journal*, **114**, 1518-1532.

Hu, Y., Topolkaraev, V., Hiltner, A. & Baer, E. (2001). Measurement of water vapour transmission rate in highly permeable films. *Journal of Applied Polymer Science*, **81**, 1624-1633.

Hung, D. V., Tong, S., Tanaka, F., Yasunaga, E., Hamanaka, D., Hiruma, N. & Uchino, T. (2011). Controlling the weight loss of fresh produce during postharvest storage under a nano-size mist environment. *Journal of Food Engineering*, **106** (4), 325-330.

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

Maixner, R. (2002). Testing water vapour transmission rates for flexible barrier materials including metalized structures. In: Proceedings of the TAPPI 2002 Polymers Laminations Adhesives Coatings Extrusion (PLACE) Conference, Boston.

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

Siracusa, V. (2012). Food packaging permeability behaviour: A report. *Review article*. *International Journal of Polymer Science*, **2012**, 1-11.

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.

Thompson, A.K. (2010). *Controlled atmosphere storage of fruits and vegetables*. 2nd Ed. Pp. 81-113. United Kingdom. Meredith Carroll.

Viuda-Martos, M., Fernandez-Lopez, J., Perez-Alvarez, 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 5: Maintaining quality of pomegranate arils and aril-sacs using anti-browning agents and modified atmosphere packaging (cv. Bhagwa)

CHAPTER 5

MAINTAINING QUALITY OF POMEGRANATE ARIL-SACS AND ARILS USING ANTI-BROWNING AGENTS AND MODIFIED ATMOSPHERE PACKAGING (CV. BHAGWA)

Abstract

Assessment of postharvest quality attributes of minimally processed packaged fruits and vegetables is essential in order to establish the produce shelf-life. The objective of this study was to evaluate the effects of anti-browning agents combined with passive-modified atmosphere packaging (MAP) on the physicochemical, sensory and microbial quality of pomegranate arils and aril-sac. Pomegranate (cv. Bhagwa) arils and aril-sacs were treated with food-grade anti-browning agents (4-hexylresersinol (0.001 M), potassium sorbate (0.05 M) and ascorbic acid (0.5 M)) and packed in polypropylene (PP) trays heat-sealed with polylid film, and control samples were packed in clamshell polyethylene terephthalate (PET) trays. Samples were stored at 5°C for a period of 12 days, and sampling was done at 3-day intervals. The results showed that anti-browning treatments had significant effects on produce quality attributes, especially yeast and mould growth, browning index and overall acceptability. Microbial growth was low in aril-sac treated with anti-browning agents. The overall yeast and mould counts were in the range of 1.52 - 4.97 log CFU g^{-1} for arils and 1.83 - 4.29 log CFU g^{-1} for aril-sac after 12 days of storage across all treatments. Positive correlations were observed between the pH, microbial load and fermentation, i.e. alcohol taste of arils perceived by sensory assessors. The headspace gas composition was significantly ($p < 0.05$) influenced by storage time, fruit fraction, anti-browning treatment, type of packaging and their interactions. Steady state gas compositions within the recommended range for modified atmosphere packaging (MAP) (2-5% O_2 and 5-10% CO_2) were observed in arils that were non-treated (MAP-AN), aril-sac non-treated (MAP-ASN) and aril-sac treated (MAP-AST). The overall quality of pomegranate arils and aril-sacs were best maintained by the combination of anti-browning treatment and passive-MAP. Aril-sacs treated with anti-browning agents and packaged under passive-MAP had the highest physicochemical quality attributes, lowest microbial growth and high acceptability. Under the postharvest treatments applied, pomegranate aril-sacs had longer shelf life (12 days) than arils (9 days) due to the development of off-odour and undesirable changes in other quality attributes investigated.

Introduction

Pomegranate fruit follows a non-climacteric ripening pattern (Gil *et al*., 2000). It is a good source of antioxidants, vitamin A, B and C and has been used as an anti-hypertension and anti-helminthic (Gil *et al.*, 2000; Viuda-Martos *et al*., 2000; Martinez-Romero *et al*., 2013). In spite of these beneficial properties, consumption of pomegranate fruit is not widespread mainly due to the difficulties involved in extracting the arils (Gil *et al*., 1996). This limitation promotes the need to process the fruit into minimally processed ready-to-eat arils (Artes *et al*., 1996; Gil *et al*., 1996). Given the sensitivity of the whole fruit to sunburn, cracking and high water loss, processing the externally damaged fruit into ready-to-eat arils will help farmers obtain a profit from fruits that are otherwise unacceptable due to the presence of these external rind or skin disorders (Artes *et al*., 2000; Lopez-Rubira *et al*., 2005).

During processing of fruit into fresh-cut products, tissues are wounded and this leads to an increase in physiological responses such as respiration, water loss, ethylene production and enzymatic browning. These physiological responses have detrimental effects on product shelf life and may lead to postharvest losses (Garcia & Barret, 2005; Saltveit, 2012). Fresh-cut fruits and vegetables may be packed under active modified atmosphere (MA) with desirable gas composition based on produce respiration rate and stored under cold temperature to slow undesirable quality changes and increase shelf life (Gonzalez-Aguilar *et al.,* 2000; Lopez-Rubira *et al.,* 2005; Thompson, 2010; Kader, 2002). Passive modification of the atmosphere (passive-MAP), created by the packed product, has been reported to reduce decay and other undesirable changes (Gonzalez-Aguilar *et al.,* 2000; Lopez-Rubira *et al.,* 2005; Montero-Calderon *et al.,* 2008; Caleb *et al.,* 2013). Both active and passive modified atmosphere packaging (MAP) technology offer the possibility to maintain produce quality and extend the shelf life by slowing down physiological processed, microbial spoilage and the development of physiological disorders (Liu *et al.,* 2007; Ayhan & Esturk, 2009; Caleb *et al.,* 2013).

Various studies have been done to maintain the quality and extend the shelf life of minimally processed pomegranate arils using MAP and other pre-treatments such as aloe vera, UV-C radiation and honey (Ayhan & Esturk, 2009; Caleb *et al.,* 2013; Martinez-Romero *et al*., 2013; Nabigol & Asghari, 2013). Shelf life of pomegranate arils depends on the pre-treatment applied, the type of packaging material used and storage conditions. Caleb *et al*. (2013) reported the shelf life of the arils to be limited to 10 days due to fungal growth, and 7 days when the flavour and aroma were considered. In their study, arils were packed in polypropylene trays covered with a polylid film and samples were stored at 5, 10 and 15°C. Similarly, the shelf life of 10 days was reported by Lopez-Rubira *et al*. (2005) when UV-C radiation was applied to arils before MA-packaging at 5°C.

A shelf life of 18 days was reported for arils pre-treated with citric acid and packed in polypropylene trays covered with Biaxially oriented polypropylene (BOPP) film and stored at 5°C (Ayhan & Esturk, 2009). Ergun & Ergun (2009) reported a shelf life of 9 days after arils were dipped in 20% honey, packed in plastic containers and stored at 4°C.

Several treatments, including the use of natural food grade products have been found to be effective in extending the shelf life of fresh-cut fruits by reducing browning and delaying onset of microbial spoilage (Gonzalez-Aguilar *et al.,* 2000; Lopez-Rubira *et al*., 2005; Arias *et al*., 2007; Liu *et al*., 2007; Montero-Calderon *et al.,* 2008; Martinez-Romero *et al*., 2013; Nabigol & Asghari, 2013). Anti-browning agents and other derivatives such as ascorbic acid, isoascorbic acid, sucrose, potassium sorbate, 4-Hexyresercinol have been used on fresh-cut fruit such as pear, pineapples and mangoes (Gonzalez-Aguilar *et al.,* 2000; Arias *et al*., 2007; Liu *et al*., 2007). These anti-browning agents can either be used singly or in combination with other packaging techniques such as MAP (Gonzalez-Aguilar *et al.,* 2000; Liu *et al*., 2007). The objective of this study was to evaluate the effects of anti-browning pre-treatments and MAP on the gas composition, physicochemical, sensory and microbiological quality and shelf life of pomegranate arils and aril-sac.

Materials and methods

Plant and packaging materials

Sweet pomegranate fruit (*Punica granatum* L.) cv. Bhagwa, harvested manually during commercial harvest period, was used in this study. Fruit were obtained from the Houdocostant farm Porterville, Western Cape, South Africa and stored in the pack-house (Houdoconstant Pack-house, Porterville, South Africa) at 5°C prior to processing. Two types of packaging materials were used: (a) clear polyethylene terephthalate (PET) trays with dimensions of 15.4 × 12.85 × 3.6 cm, which was used as control, and (b) black polypropylene (PP) trays with dimensions of 15.5 × 11.5 × 4.5 cm (Blue Dot Packaging, Cape Town, South Africa) (Fig. 5.1). POLYLID® 107 polyethylene (PE) polymeric film (thickness 55 µm; WVTR 20-22 g m^{-2} day^{-1}; CO_2 TR 600-700 mL m^{-2} day^{-1} and O_2TR 130-150 mL m^{-2} day^{-1} at 25°C, 50% RH and 1 Bar) (Barkai Polyon Ltd. Kibbutz Barkai, Israel) was used to seal the trays. For anti-browning treatment, pure food grade salts of 4-hexylresersinol, potassium sorbate and ascorbic acid (Sigma-Aldrich Co., Steinheim, Germany) were used in the following concentrations: 4-hexylresersinol (0.001 M), potassium sorbate (0.05 M) and ascorbic acid (0.5 M). Sterile distilled water was used to prepare the solutions (Gonzalez-Aguilar *et al.,* 2000).

Sample preparation

Sample preparation was done according to Good Manufacturing Practices (GMP) used in the processing facility. Pomegranate fruit were manually sorted to remove mechanically damaged fruit and those with physiological disorder such as cracks and splits. Selected wholesome fruit were washed in sterilised water containing 200 mg L^{-1} of sodium hypochlorite (NaOCl). To obtain aril-sacs, fruit were carefully cut in four cross-sections in order to obtain intact aril-sacs (i.e. arils still attached to the peel). Care was taken not to damage the arils. Arils were extracted from the whole fruit using a commercial extraction machine (ArilSystem, Juran Metal Works, Israel). Extracted arils were collected on a conveyer belt in order to air-dry the samples and manually remove damaged arils. Air-dried arils were carefully mixed to ensure uniformity of arils. All processing operations were conducted below 8°C. Extracted arils and processed aril-sac were divided equally into two groups. The first group from each fruit fraction were dipped for 2 min in each anti-browning solution using a strainer and then drained by placing the strainer containing the samples over a sterile paper towel. The second group were not treated with any of the anti-browning solutions.

Approximately 200 g of arils were weighed into each package previously sanitised with 70% ethanol, and about four to five pieces of aril-sacs were packed in each tray depending on the size of the individual aril-sac, ranging between 149 and 180 g for each package. PP trays were heat sealed with POLYLID film using a semi-automated heat sealing machine (Food Processing Equipment, Cape Town, South Africa) and the PET trays were closed tightly with the lids. With the combination of passive-MAP and anti-browning treatment, the following eight treatments were obtained: control arils non-treated (C-AN); control arils treated (C-AT); MAP arils non-treated (MAP-AN); MAP arils treated (MAP-AT); control aril-sac non-treated (C-ASN); control aril-sac treated (C-AST); MAP aril-sac non-treated (MAP-ASN) and MAP aril-sac treated (MAP-AST).

To mimic retailer packaging practices, a label (7.0 x 3.8 cm) was placed on the right corner of the film or PET tray lid. The information contained on the label included: date of production, anti-browning status (i.e. treated or non-treated) and cultivar name (*Bhagwa*). After all the packaging procedures were done, packages were cooled down to 2°C and then packed in iced-cooler boxes and transported to the Postharvest Technology Research Laboratory, Stellenbosch University. Data loggers (Tiny tag view, TV-4500, UK) were placed in the cooler boxes to monitor the temperature throughout the transportation. Upon arrival at the Postharvest Technology at Stellenbosch University, the samples were stored in a cold room at 5 ± 0.5°C and 90% relative humidity. Three trays from each treatment were randomly taken for analysis every sampling day (0, 3, 6, 9 and 12). Additional sixteen packages representing two replicates for each treatment were

used for gas analysis for the entire duration of the study. The PP trays were fitted with a septum to aid the headspace gas measurement process.

Figure 5.1 Packaging types used in the study containing arils or aril-sacs with A) clamshell (PET) trays used as control and B) PP trays with a polylid film, used to create the passive MA condition.

Headspace gas analysis

Headspace gas compositions of oxygen (O_2) and carbon dioxide (CO_2) were taken from each package before the packages were opened on sampling days. A gas analyser with an accuracy of 0.5% (Checkmate 3, PBI Dansensor, Ringstead, Denmark) was used. In-package gas composition was expressed as percentage (%) of O_2 and CO_2. After gas measurements, the packages were opened and used for physicochemical, sensory and microbial analyses.

Weight loss

The weight (± 0.01 g) of each package of arils or aril-sac were taken before storage and at each sampling day using an analytical balance (Mettler Toledo, ML104/01, Switzerland). Weight loss was expressed in percentage using equation (1):

$$\text{Weight loss} = \left(\frac{W_0 - W_f}{W_0}\right) \times 100 \qquad (1)$$

where W_o is the initial weight in grams and W_f is the final weight (g).

Colour

Minolta Chroma meter CR-400 (Minolta Corp. Osaka, Japan) was used to measure the colour of arils and peel in CIELAB (*L**, *a**, *b**) coordinates where *L** represents the lightness, *a**: red (+)/green (-) and *b*:* yellow (+)/blue (-). Before measurements were taken, the equipment was calibrated on a white background (Illuminants C: Y = 83.44, x = 0.3051, y = 0.3202). During colour measurements, approximately 30 grams of arils was placed in a Petri dish and measured on a non-reflective white background. Measurements were taken at five points of the sample in the Petri dish. Arils from the aril-sacs were removed manually prior to colour measurements. For aril-sacs, the browning index of the cut surface was determined from the sensorial scores from zero to five (0 = absence of browning, 5 = high incidence of browning).

Texture

A texture profile analyser (TA-XT Plus, Stable Micro Systems, UK) with a 35 mm diameter cylindrical aluminium probe was used to measure the firmness of the arils, expressed as maximum compression force (N). A test speed of 1.0 mm/sec and distance of 8.5 mm were used in the measurements. Arils on the aril-sac were removed manually from peel prior measurement, and care was taken to ensure that are not damaged. Each aril was tested individually and an average of 15 arils was tested for each treatment.

Titratable acidity, pH and total soluble solids

Arils were juiced using the LiquaFresh juice extractor (Mellerware, South Africa). A Methrohm 862 Compact titrosampler (Herisau, Switzerland) was used to measure the titratable acidity (TA) to the titration end point of pH 8.2. A digital refractometer (Atago, Tokyo, Japan) and pH meter (Crison, Barcelona, Spain) were used to measure the total soluble solids (TSS; °Brix) and pH, respectively. All measurements were done in triplicate for each treatment.

Total phenolics

Total phenolics were determined by following the Folin-Ciocalteu (Folin-C) colourimetric method with modifications as reported by Fawole *et al.* (2012). The crude juice was diluted with 10 mL of 50% methanol and quantified using Folin-Ciocalteu reagent. Total phenolics was evaluated by a spectrophotometer at 750 nm and expressed as gallic acid equivalents (GAEs) per 100 mL crude juice. Measurements were in triplicate and reported as mean ± SD.

Anthocyanins

A pH differential method was followed to determine the total anthocyanins contents or in the juice as reported by Fawole *et al.* (2012). A pH 1 buffer (potassium chloride 0.025 M) and pH 4.5 buffer (sodium acetate 0.4 M) were used to dilute the juice. A UV-Vis spectrophotometer was used to evaluate the absorbance of the mixtures at the two absorbance of 520 nm and 700 nm, respectively. The concentration of anthocyanin pigments was calculated using equation (2) and expressed as cyanidin-3-glucoside equivalents (mg of C_3gE 100 mL^{-1}):

$$\textit{Anthocyanin pigment } (C_3gE\ 100\ mL^{-1}) = \frac{A \times MW \times DF \times 100}{\varepsilon \times 1} \qquad (2)$$

where Absorbance (A) = ($A_{520\ nm}$- A_{700nm}) pH1.0 - (A_{520nm}-A_{700nm}) pH4.5; DF= Dilution factor; MW (molecular weight) = 449.2 g mol^{-1} for cyanidin-3-glucoside (cyd-3-glu); 1= path length in cm and ε = 26 900 molar extinction coefficient n L × mol^{-1} × cm^{-1}, for cyanidin-3-glucoside. Measurements were done in triplicate for each treatment.

Microbial quality

Microbial quality was studied to evaluate the effects of the different packaging types and anti-browning treatment via total plate count method. Potato dextrose agar (PDA) acidified with 10% tartaric acid was used for yeast and mould count. Packages were opened aseptically, and approximately 10 g was taken from each treatment and homogenised in 90 mL of physiological solution (PS). Threefold dilution was made by adding 1 mL of each diluent into 9 mL of PS and 1 mL from each dilution was pour-plated on the PDA. The analyses were done in triplicate. Plates were incubated at 25°C and counted after three to five days of incubation. The results were reported as log CFU g^{-1}. In order ensure that the microbial analysis was free of contaminations, sterile PDA plates were opened for 5 min in the work area before and after the dilutions were plated.

Sensory evaluation

Sensory analyses were carried out to evaluate the effects of the packaging types and anti-browning treatment on the aril-sac and arils. Sensory attributes that were assessed on the samples include odour, colour, texture, taste, flavour, aroma and overall acceptability. A non-trained panel consisting of 10 individuals who are familiar and regular consumers of pomegranate fruit were used.

Statistical analysis

A full factorial experiment design with three treatment levels: packaging type (MAP and clamshell); fruit fraction (aril-sacs and arils), anti-browning treatment (treated and non-treated) and three replications per treatment combination were used in this study. Analysis of variance (ANOVA) using Statistica software (Statistica 11.0, Statsoft Inc., Tulsa, OK, USA) was applied to each treatment combination. Interactions between various factors were analysed. Fisher least significant difference (LSD) post hoc testing was used to further investigate differences when the F-test *p*-values were significant. A *p*-value of 0.05 was used to determine the level of significance. To investigate possible relationships between physicochemical and sensory variables, Pearson's correlations were calculated.

Results and discussion

Gas compositions

Figure 5.2 (A-H) summarises the headspace gas composition (O_2 and CO_2) of all the treatment combinations. Storage time, package type, and the interaction between fruit fraction × package had significant effects ($p < 0.05$) on both O_2 and CO_2 compositions in the headspace. Furthermore, the anti-browning treatment, the interaction of fruit fraction × package × time had significant effects ($p < 0.05$) on O_2 concentration; however, only the anti-browning treatment and interaction of fruit fraction × package × anti-browning had a significant effect on the CO_2 level in comparison to the many factors highlighted above that influenced the O_2 level. Similar patterns in the increase of CO_2 and decrease in O_2 levels were observed among the clamshell packages C-ASN, C-AT and C-AST (Fig. 5.2 B, C & D), except for C-AN (Fig. 5.2 A), where a noticeable increase in CO_2 was observed, reaching 6.17% and O_2 decreasing to 11.50% in comparison to other clamshell packages. An equilibrium was achieved in MAP-ASN and MAP-AST at after day 9(Fig.5.2 F & H). Though the CO_2 level of the three treatment combinations mentioned above was within the recommended level of 5-10%, only the MAP-AN had O_2 level within the recommended 2-5% level. At the end of storage (day 12), MAP-ASN had a significantly ($p < 0.05$) higher CO_2 of 18.53% and

lower O_2 of 2.27% than all the other treatments. These observations can be related to the difference in respiration rates of arils and aril-sacs reported in Chapter 3, where aril-sacs showed a high respiration rate (RR) compared to arils when stored at 5°C. Based on Pearson's correlation test, it was found that the O_2 composition had a negative correlation (-0.20) with the alcoholic taste perceived by the sensory assessors, while CO_2 showed a weak positive correlation (0.14).

The changes observed in the in-package atmosphere in this study are consistent with similar reports for pomegranate arils, cultivars Arakta and Bhagwa when stored at 4°C in PET packages (O'Grady, 2012), and cv. Acco and Herskawitz stored at 5°C packed in PP trays with POLYLID film (Caleb *et al.*, 2013). Equilibrium gas composition was also reached at around day 9 of storage for fresh-cut mangoes treated with anti-browning treatment combination of 4-hexylresorcinol, potassium sorbate and D-isoascorbic acid at different concentrations (González-Aguilar *et al.,* 2000). At the end of storage day 12, only MAP-AN shows a gas composition that was within the MAP recommended range of 2 - 5% O_2 and 5 - 10% CO_2 for packages of fresh-cut fruits and vegetables (Lopez-Rubira *et al*., 2005; Simson & Straus, 2010).

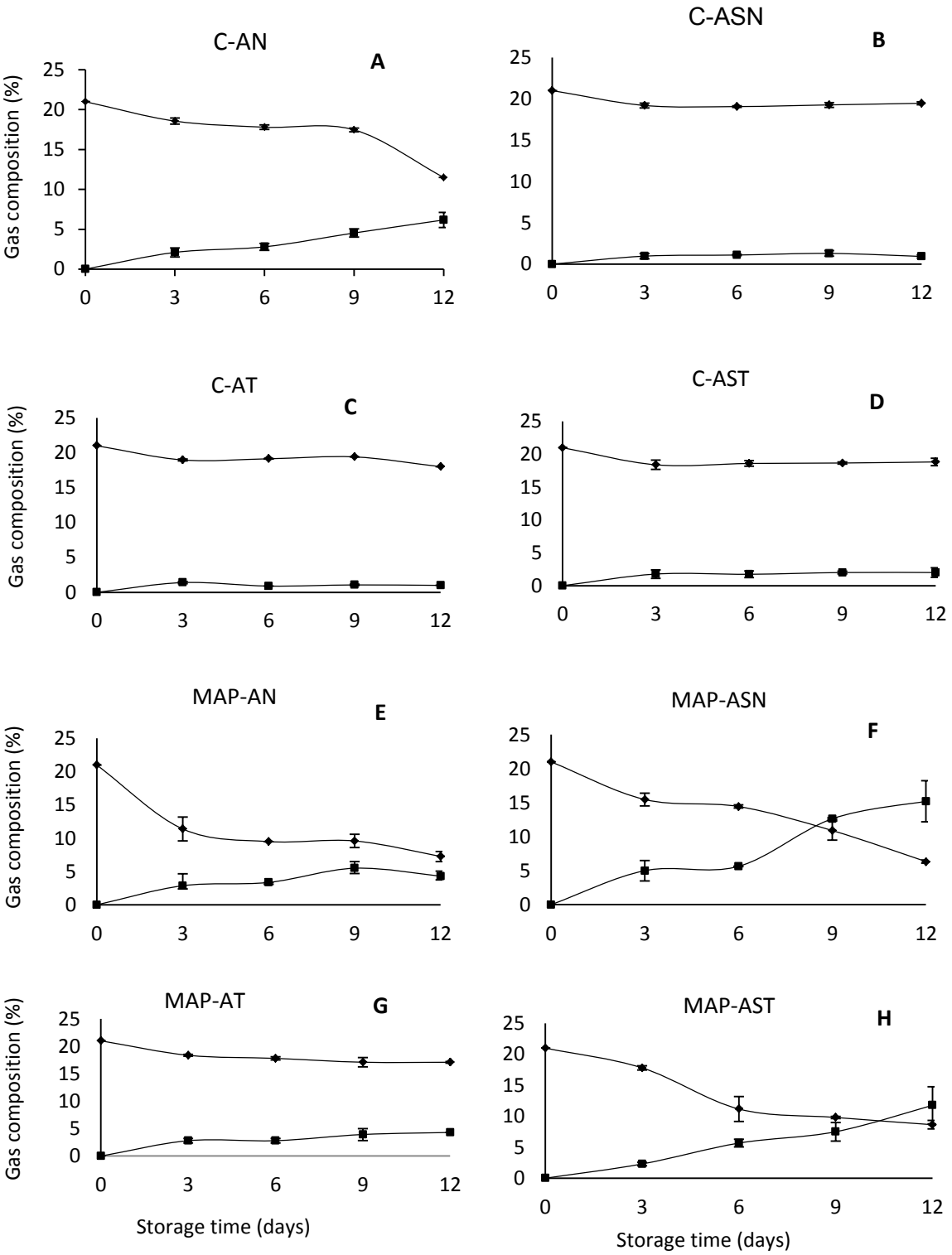

Figure 0.2 In-package atmosphere of O_2 (♦) and CO_2 (■) for arils (A, C, E, G) and aril-sac (B,D,F,H), for all treatments. Vertical bars depicts standard error.

C-AN: control arils non-treated; C-AT: control arils treated; MAP-AN: MAP arils non-treated; MAP-AT: MAP arils treated; C-ASN: control aril-sacs non-treated; C-AST: control aril-sacs treated; MAP-ASN: MAP aril-sacs non-treated; MAP-AST: MAP aril-sacs treated

Total soluble solids, titratable acidity, and pH

Fruit fraction, anti-browning, storage time and the interaction of fruit fraction × anti-browning had significant effects ($p < 0.05$) on the TSS of the arils and aril-sacs as shown in Fig. 5.3 and 5.4. However, packaging did not have a significant effect on TSS. On day 0, TSS content was 16.20 °Brix, and a significant ($p < 0.05$) decrease was observed at day 12, with value ranging between 13.87 - 15.27 °Brix across all treatments. Treated and non-treated aril-sacs showed significantly higher TSS values in comparison to arils. The TSS was best maintained after day 12 in the C-AST and C-AT samples. In C-AST, a 5.74% decrease was noted in comparison to the 14.38% decrease in C-AN sample. Ayhan and Esturk (2009) and Martinez-Romero *et al.* (2013) also reported slight changes in the TSS of pomegranate arils treated with 1% citric acid and packed under MAP and those treated with *Aloe vera*, respectively. Pomegranate flavour, perceived by sensory assessors had positive correlations (0.16) with the TSS. However, a negative correlation (-0.23) between the TSS and alcoholic taste was observed. These results are in agreement with the general process of fermentation, whereby sugars are depleted or converted to acids.

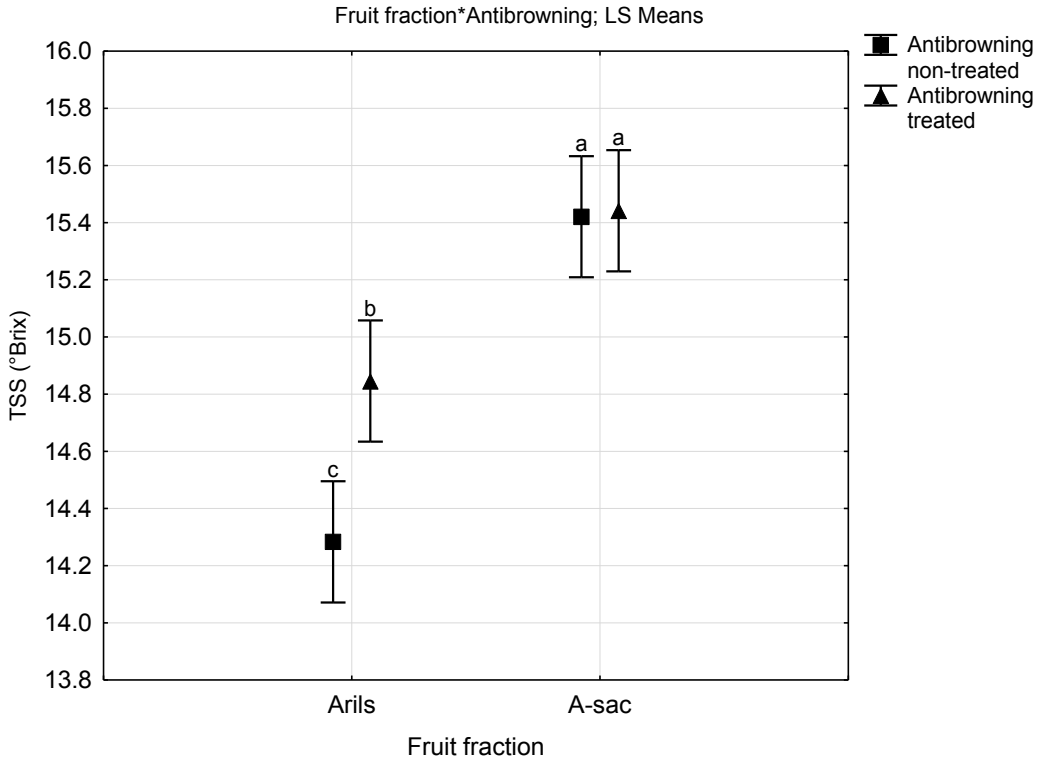

Figure 5.3 Effects of fruit fraction x anti-browning interaction on the TSS content of pomegranate arils and aril-sacs during storage at 5°C.

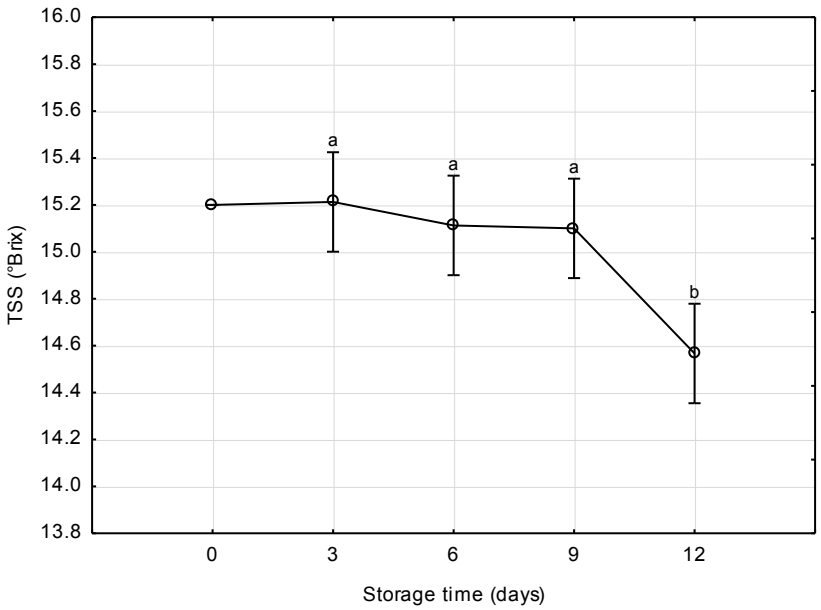

Figure 5.4 Effects of storage time on the TSS content of pomegranate arils and aril-sacs during storage at 5°C.

Titratable acidity (TA) decreased with storage period, whereby lower values were observed at day 12, though this decrease was not significant ($p > 0.05$). However, all the main factors and their interactions had no significant influence on the TA. At day 0, the TA was at 0.47 g CA 100 mL^{-1} of juice and this decreased to the range of 0.34 to 0.40 g CA 100 mL^{-1} juice for arils and 0.36 to 0.42 g CA 100 mL^{-1} juice for aril-sacs, respectively at day 12, across all treatment combinations. A lower TA was observed in arils in comparison to aril-sacs. The MAP-AST best maintained the TA content (0.42 g CA 100 mL^{-1}) in comparison to other treatments. Similarly, reduction in TA was reported by Ayhan and Esturk (2009) and Artes *et al*. (2000) for MA-packaged pomegranate arils cv. Hicaznar and Mollar de Elche. In contrast, Martinez-Romero *et al.* (2013) reported an increase in acidity for cv. Mollar de Elche. They emphasised that this could be related to the organic acid treatments (citric and ascorbic) that were applied to the arils before storage. Thus, variations in TA reported in these studies could be related to the cultivar used, packaging system used as well as on the pre-treatments applied.

The pH level was significantly influenced by anti-browning treatment, fruit fraction, time as well as the interactions of fruit fraction × time and fruit fraction × package × time ($p < 0.05$). Significantly high pH values were observed in arils packed under both MAP and clamshell packages, in comparison to the aril-sacs, with lower pH values at day 12 (Fig 5.5). Among the treatments with aril-sac, MAP-ASN and MAP-AST had high pH values of 2.64 and 2.60, respectively, while for arils, higher pH were observed in C-AT and C-AN with values of 3.36 and 3.43, respectively.

Overall, pH was high in arils samples and lower in aril-sacs. These results follow similar trends reported on pomegranate arils cultivars 'Acco' and 'Herskawitz' packed under passive-MAP, without any pre-treatment (Caleb *et al.,* 2013), and those treated with *Aloe vera* and packaged in plastic containers (Martinez-Romero *et al.,* 2013). Based on the Pearson correlation test done, the alcoholic taste perceived during sensory evaluation had a negative correlation (-0.37) with the pH.

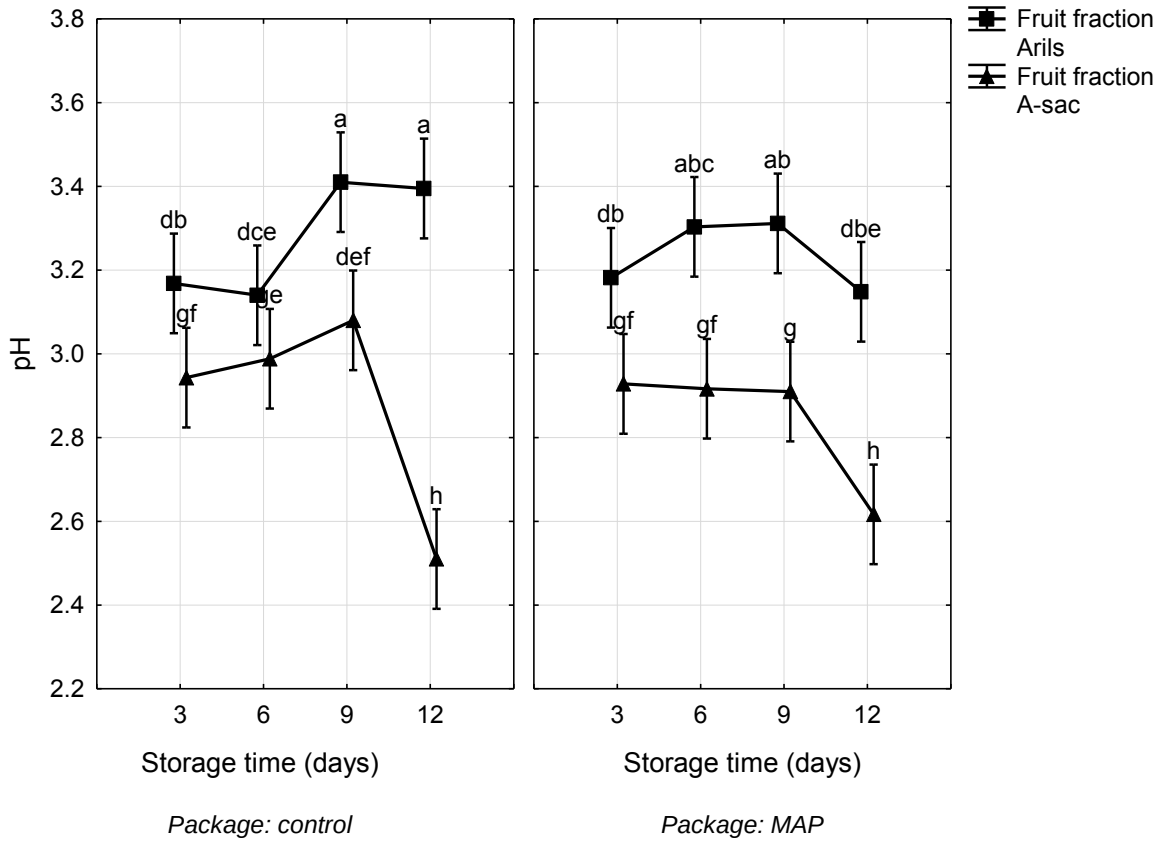

Figure 5.5 Effects of fruit fraction × package × time interactions on the pH of pomegranate arils and aril-sacs during storage at 5°C.

Physical quality

Arils colour

Colour is an important attribute influencing consumer willingness to buy, especially if the product is packed and cannot be touched or smelled. Changes in colour of both arils and cut surface of peels of aril-sacs are shown in Tables 5.2 and 5.3, respectively. From the overall analysis, none of the factors had a significant effect on the redness (a*) which depicts colour stability as well as on the lightness (*L**) values, of the arils. However, the interaction of fruit fraction × time had significant effects ($p < 0.05$) on the yellowness (*b**) values of the pomegranate arils (Fig.5.6). Colour characteristics of the treatment combinations at day 0 were: 15.78 ± 2.60 for lightness (*L**); 22.91 ±

0.34 for redness (*a**) and yellowness (*b**) was 11.41 ± 0.17 and at day 12 they ranged between 12.99 and 27.25 (*L**); 12.02 and 25.38 (*a**) and 5.65 – 13.76 (*b**), across all the treatment combinations. At day 12, a higher *L** was observed in MAP-AT while C-AST showed a lower *L** value. Similarly, a high a* value (25.38 ± 1.89) was observed in MAP-AT and lowest (12.02 ± 0.61), in MAP-ASN. The higher value of b* (13.76 ± 1.42) was observed in MAP-AT and lowest in MAP-ASN.

In comparison to the initial colour values, the MAP-AT treatment combination maintained the colour of the arils. Generally, anthocyanins pigments are responsible for the colour of the pomegranate arils (Afaq *et al.,* 2005) and from the present study, no factor had significant effects on the anthocyanins, which supports the colour measurement results of the *L** and *a**, which are generally main colour indicators in pomegranate arils. Caleb *et al*. (2013), reported that the effects of passive-MAP and storage time was not significant on the *L**, *a** and *b** values of the pomegranate arils when stored at 5°C. However, Ayhan and Esturk (2000), reported that lightness (*L**) values of pomegranate arils was significantly affected by the interaction of MAP application × storage duration when stored at 5°C for 18 days.

Peel colour

No factor had significant effects on the *L** values of peel cut surface, however, the interaction of package × anti-browning had significant effects on *a** and *b** values ($p < 0.05$). A high yellowness (*b**) value was observed on MAP products, especially the MA-AST in comparison to the clamshell packages (Fig. 5.7). At day 12, the colour values of the peels were in the range of 42.34 to 47.41 (*L**); 29.97 to 34.44 (*a**) and 26.43 to 31.29 (*b**) (Table 5.3). Passive MA-packaged aril-sacs non-treated maintained a relatively higher *L** compared to treated aril-sacs under passive MA, however, no significant difference was observed. These results thus explain the effects of the anti-browning treatment on the peel by maintaining a high lightness value on the peels.

Browning index

Browning index was observed to increase with storage period, and high scores (> 3) were observed in C-ASN and MAP-ASN (Fig 5.8). Treatment C-AST and MAP-AST showed lower browning index, thus indicating the effects of anti-browning agents used on controlling browning. The anti-browning effects of similar agents were also reported on fresh-cut mangoes treated with 4-Hexylresercinol, potassium sorbate and D-Isoascorbic acid at various combinations (Gonzalez-Aguilar *et al*., 2000). The same author further reported that the use of MAP alone was not effective in controlling browning; hence the effects were improved up on the use of anti-browning agents.

Table 5.2 Effects of packaging and anti-browning treatment combinations on the colour of pomegranate arils and those obtained from aril-sacs, at 12th day of storage at 5°C.

	L*	***a****	***b****
Day 0	15.78 ± 1.28^{cd}	22.91 ± 0.34^{a}	11.41 ± 0.17^{a}
Treatments[1]		**After 12 days at 5°C**	
C-AN	18.67 ± 2.29^{cb}	20.27 ± 1.10^{b}	11.41 ± 1.96^{a}
C-AT	17.84 ± 2.27^{cd}	21.83 ± 2.21^{ab}	10.81 ± 1.31^{a}
MAP-AN	23.34 ± 4.72^{ab}	22.44 ± 0.62^{ab}	11.61 ± 1.03^{a}
MAP-AT	27.25 ± 5.24^{a}	25.38 ± 1.89^{c}	13.76 ± 1.42^{b}
C-ASN	15.51 ± 1.69^{cd}	17.96 ± 2.68^{d}	8.34 ± 1.55^{c}
C-AST	12.99±1.98^{d}	17.39 ± 0.44^{d}	8.33 ± 0.23^{c}
MAP-ASN	15.12 ± 3.11^{cd}	12.02 ± 0.61^{e}	5.65 ± 0.24^{d}
MAP-AST	13.77 ± 3.08^{cd}	17.82 ± 0.68^{d}	8.36 ± 0.54^{c}

Means with same letter within columns are not significantly different according to Fisher LSD test (p = 0.05).

[1]C-AN: control arils non-treated; C-AT: control arils treated; MAP-AN: MAP arils non-treated; MAP-AT: MAP arils treated; C-ASN: control aril-sacs non-treated; C-AST: control aril-sacs treated; MAP-ASN: MAP aril-sacs non-treated; MAP-AST: MAP aril-sacs treated

Table 5.3 Effects of packaging and anti-browning treatment combinations on the peel colour of pomegranate aril-sacs at the 12th day of storage at 5°C.

	L*	***a****	***b****
Day 0	46.29 ± 3.78[b]	38.03 ± 6.24[a]	32.14 ± 3.46[a]
Treatments[1]	**Storage at 5°C after 12 days**		
C-ASN	42.34 ± 2.63[b]	34.44 ± 5.01[a]	26.43 ± 2.30[a]
C-AST	42.61 ± 1.42[b]	32.37 ± 2.95[a]	26.61 ± 3.65[a]
MAP-ASN	47.41 ± 2.60[a]	29.97 ± 1.48[a]	26.93 ± 2.10[a]
MAP-AST	45.12 ± 0.80[ab]	33.14 ± 0.30[a]	31.29 ± 0.22[b]

Same letter within columns are not significantly different according to Fisher LSD test (p = 0.05).

[1] C-ASN: control aril-sacs non-treated; C-AST: control aril-sacs treated; MAP-ASN: MAP aril-sacs non-treated; MAP-AST: MAP aril-sacs treated

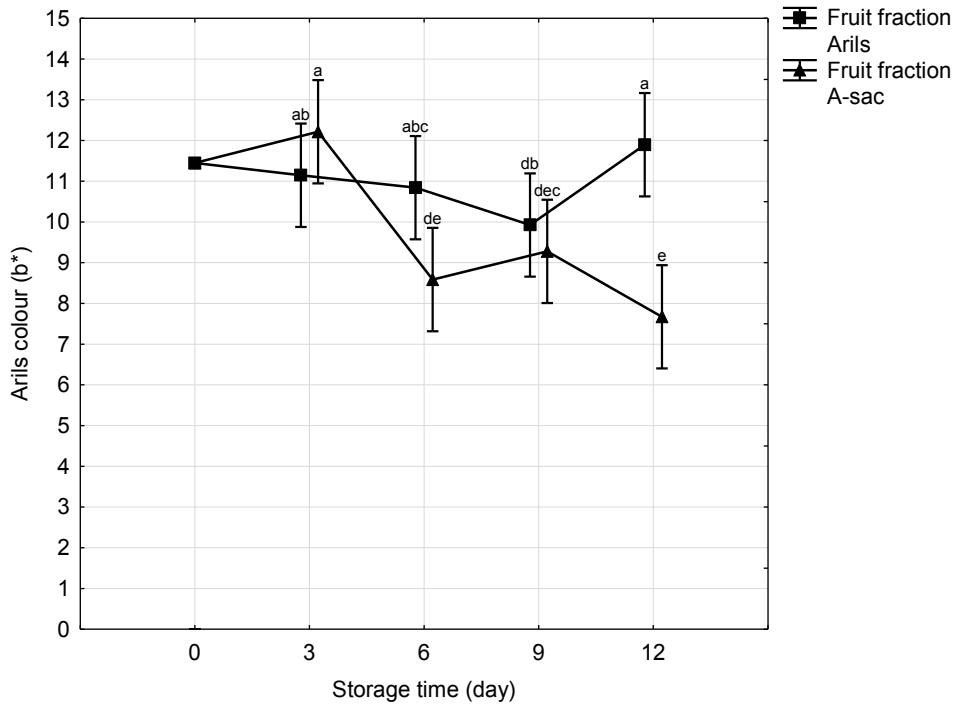

Figure 5.6 Effects of storage time on the colour (*b**) of pomegranate arils and those obtained from aril-sacs during storage at 5°C.

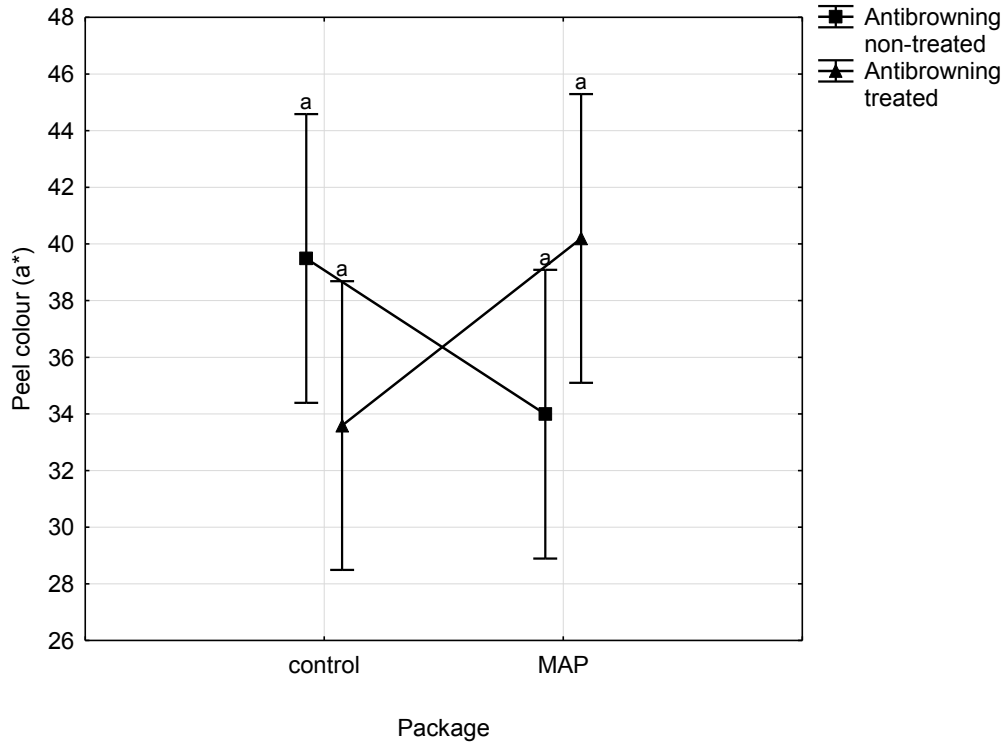

Figure 5.7 Effects of fruit fraction on the peel colour (*a**) of pomegranate aril-sacs during storage at 5°C.

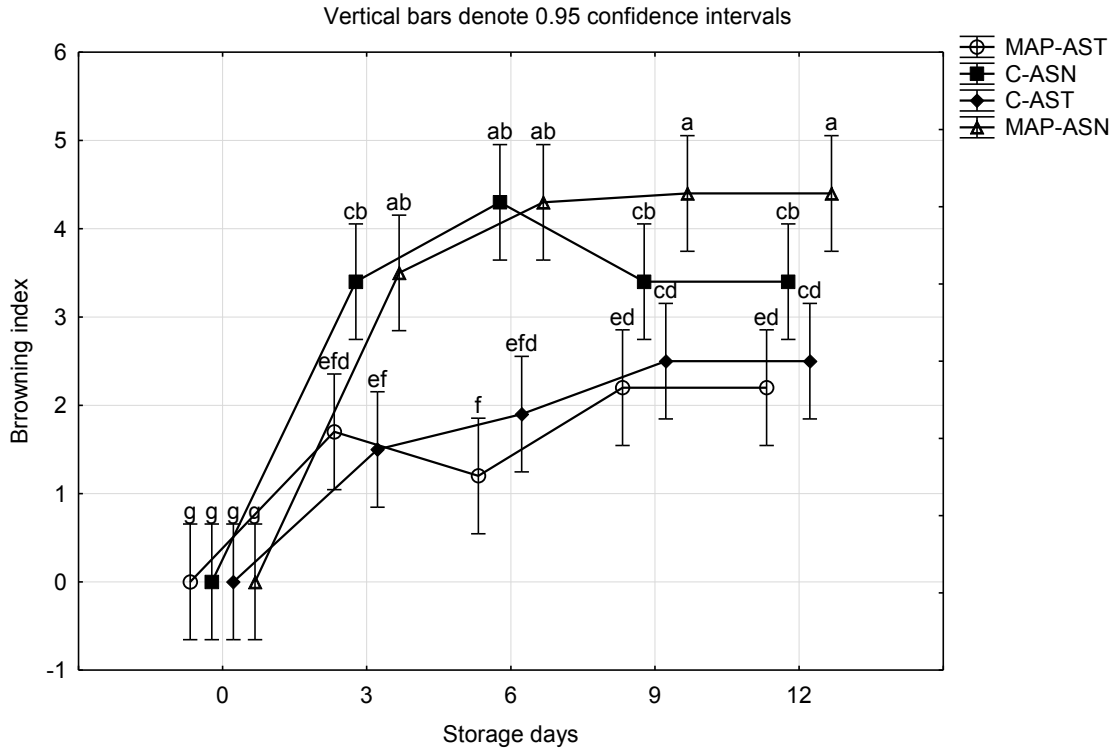

Figure 5.8 Browning index of the cut surface of aril-sacs under different treatment combinations stored at 5°C

Firmness

Firmness varied among treatment combinations and across storage time. Table 5.4 shows the firmness of the products at day 6 and day 12, respectively. In the overall analysis of variance packaging, time and anti-browning had no significant effect on the firmness of the test samples ($p > 0.05$). However, fruit fractions (arils and aril-sacs) had a significant influence on the firmness of arils. At day 12, a higher firmness was observed in MAP-AT (66.71 ± 2.09 N) and the lowest in C-ASN and C-AST. These results were lower than those reported by Caleb *et al*. (2013) for cv. Herskawitz and Acco of 102.4 ± 7.6 N and 77.5 ± 7.4 N, respectively, when after stored under passive-MAP for 14 days.

Table 5.4 Effects of packaging and anti-browning treatments on the firmness (N) of pomegranate arils and those obtained from aril-sacs, at the 6th and 12th day of storage at 5°C.

Treatments	**Day 6**	**Day 12**
	55.93±3.05^{h} (**Day 0**)	
C-AN	55.43±1.98^{cd}	60.53±3.03^{cb}
C-AT	49.51±3.99^{ef}	58.90±2.04^{cb}
MAP-AN	56.31±1.16^{cd}	62.09±2.70^{ab}
MAP-AT	51.62±2.54^{ed}	66.71±2.09^{a}
C-ASN	47.75±2.43^{eg}	47.71±0.55^{eg}
C-AST	49.59±4.06^{ef}	47.81±6.64^{eg}
MAP-ASN	45.97±2.44^{gf}	60.01±3.77^{cb}
MAP-AST	43.25±2.03^{g}	48.44±3.77^{ef}

Means with same letter across each column and row are not significantly different according to Fisher LSD test (p = 0.05).

Weight loss

High weight loss was observed in aril-sacs (0.12%) in comparison to arils (0.09%) though insignificant ($p > 0.05$). Weight loss fluctuated throughout the storage period (Figs. 5.9 & 5.10). For example at days 6 and 9, higher weight loss was observed in MAP-aril-sac non-treated in comparison to MAP-arils non-treated. At the end of storage (day 12), a lower weight loss was observed in C-AST of aril-sacs and C-AN of arils, while the highest weight loss was found in MAP-ASN, MAP-AST, MAP-AT and C-ASN. High values observed on aril-sacs can be related to the moisture loss from the bruised peels through cut surfaces as a result of processing. However, these results contradict those reported in Chapter 4, whereby aril-sacs had lower weight loss compared to arils. All the factors showed insignificant effects ($p > 0.05$) on the weight loss. However, Caleb *et al* (2013) reported that there were no significant difference between the weight loss of cv. Acco and Herskawitz under passive MAP. The weight loss observed in this study was within the range reported for arils by Caleb *et al*. (2013) which was below 0.8% when stored at 5°C under passive-MAP. Hardness perceived by sensory assessors had a positive correlation (0.31) with the weight loss measured.

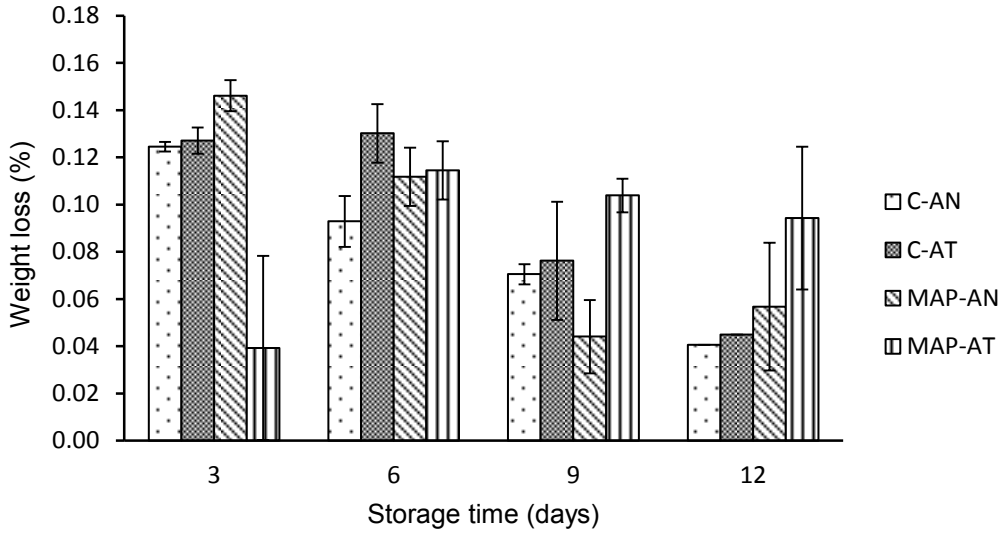

Figure 5.9 Effects of packaging and anti-browning treatment combinations on the weight loss of arils stored at 5°C, over time.

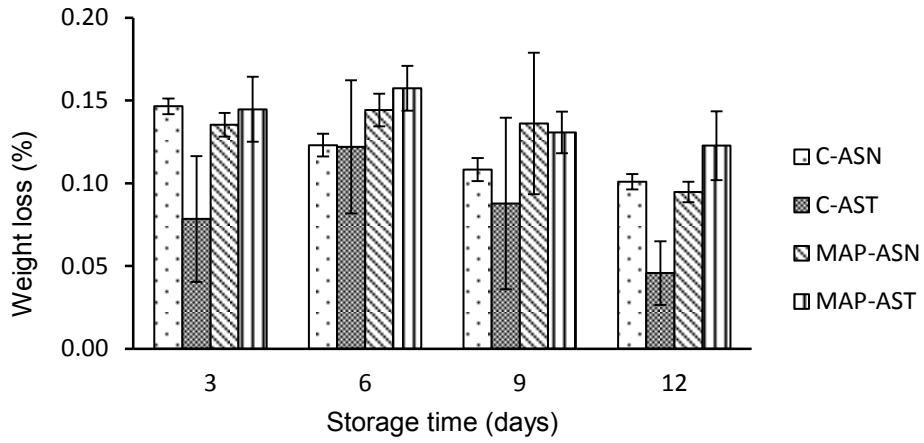

Figure 5.10 Effects of packaging and anti-browning treatment combinations on the weight loss of aril-sacs stored at 5°C, over time.

Anthocyanins and phenolics

Anthocyanin pigments are responsible for the colour of arils and they have been reported to increase during storage (Martinez-Romero *et al*., 2013). Figure 5.11 shows the concentration of total anthocyanins after 12 days of storage. None of the factors had a significant ($p > 0.05$) effect on the anthocyanins content of the fruit fractions. The content of anthocyanins varies with storage time and treatment combinations, however, an increase was observed with reference to the initial level of 12.80 ± 0.51 mg C_3gE 100 mL^{-1}. At day 12, C-AST and MAP-ASN showed a significantly high anthocyanin contents compared to the other treatment combinations. Total anthocyanins concentration found in this study (20.87 to 23.13 mg C_3gE 100 mL^{-1}) were 17.1% lower than that (27.92 mg C_3gE 100 mL^{-1}) reported for MA-packaged arils cv. Hicaznar (Ayhan & Esturk, 2009).

With respect to total phenolics, the concentration of pomegranate juice on day 0 was 2280.91 mg GAE 100ml^{-1} and decreased to a range of 1890.70 to 1321.32 mg GAE 100ml^{-1} by day 12 across all treatment conditions, as depicted in Figure 5.12. However, only the fruit fraction factor had significant effects on the phenolics content. Fruit fraction had significant ($p < 0.05$) effects on the phenolics, whereby a high concentration of phenolic content was found in aril-sacs as compared to arils, there was no observed significant differences among the eight treatments. Among the aril-sacs treatments, the highest phenolic content at day 12 was observed in MAP-ASN and C-AST, while in arils C-AT had the highest content. Phenolic contents of about 1504.32 mg GAE 100 mL^{-1} of pomegranate juice was reported by Ayhan and Esturk (2009), 12% higher than that of the phenolic content observed in this study. Thus this variation can be related to cultivar difference.

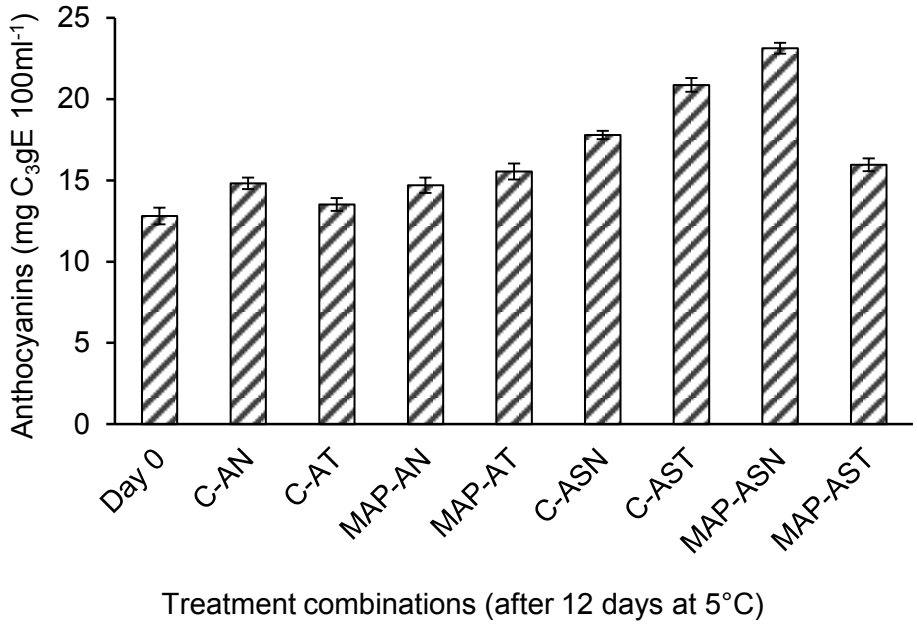

Figure 5.11 Effects of packaging and anti-browning treatment combinations on the anthocyanins content of arils and those obtained from aril-sacs, at the 12th day of storage at 5°C.

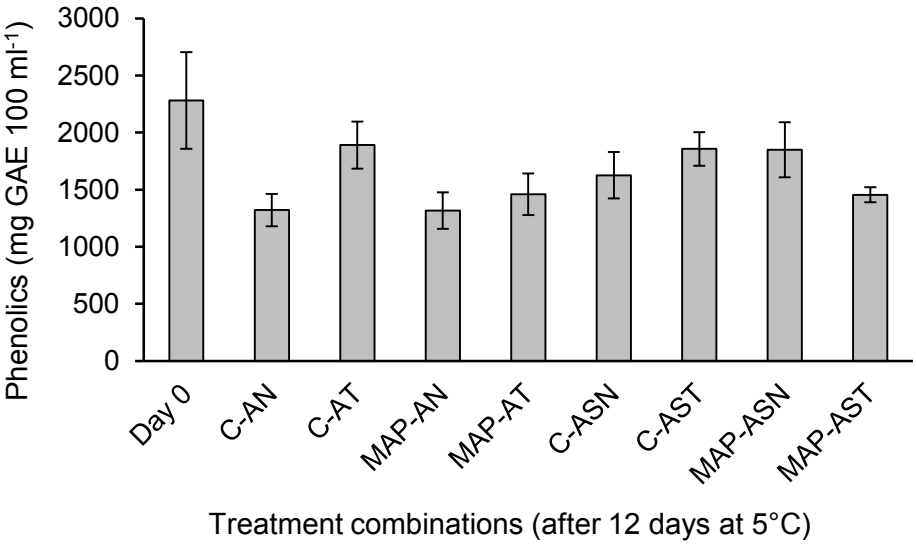

Figure 5.12 Effects of packaging and anti-browning treatment combinations on the phenolics content of arils and those obtained from aril-sacs, at the 12th day of storage at 5°C.

Microbial quality

The fruit fraction × anti-browning × time interaction had significant effects ($p < 0.05$) on the yeast and mould growth of the packaged products. However, packaging alone did not have significant effects on microbial growth. Pre-treatments applied on arils and aril-sacs were observed to be effective in extending the lag phase of microbial growth in comparison to non-treatment. Yeast and mould growth were observed on day 3 and 6 for non-treated and treated arils, respectively, while growth was only observed on day 12 on aril-sacs for both the control and MA-packaged samples (Fig 5.13).

Yeast and mould count for both treated and non-treated were in the range of 1.52 to 4.97 log CFU g^{-1} for arils and 1.83 to 4.29 log CFU g^{-1} for aril-sac after 12 days of storage. Highest microbial load were observed in clamshell packaged non-treated arils, while treated aril-sacs had the lowest microbial load. However, the yeast and mould counts observed in this study are below the maximum limit of 5 log CFU g^{-1} for yeast and mould in raw and fresh-cut fruit allowed by the South African legislation (FCD Act 54 1979). The pre-treatments applied in this study were able to delay the onset of spoilage and the development of off-odour. Furthermore, the yeast and mould growth increase can be related to the high CO_2 observed at day 12 as reported under the gas composition. The microbial load observed were within the range reported by Caleb *et al.* (2013) for cv. Herskawitz (0.36 - 3.8 log CFU g^{-1}) and Acco (1.76 - 4.25 log CFU g^{-1}) after 14 days of storage. Mould and yeast growth below 1 log CFU g^{-1} was reported in arils treated with 100% Aloe vera + 0.5% citric acid + 0.5% ascorbic acid1.0% acid, after 12 days of storage (Martinezo-Romero *et al.,* 2013). The observed differences between these reports could be attributed to applied treatment and cultivar differences. The physicochemical properties of pomegranate fruit such as pH and TA, as well as cultivar have a significant impact on the microbial shelf life (Caleb *et al*., 2013). A positive correlation (0.48) was observed between the mould growth and the alcoholic taste (fermentation) perceived by the sensory assessors. Negative correlation (-0.35, $p < 0.01$) as observed between the overall acceptability and the microbial growth detected.

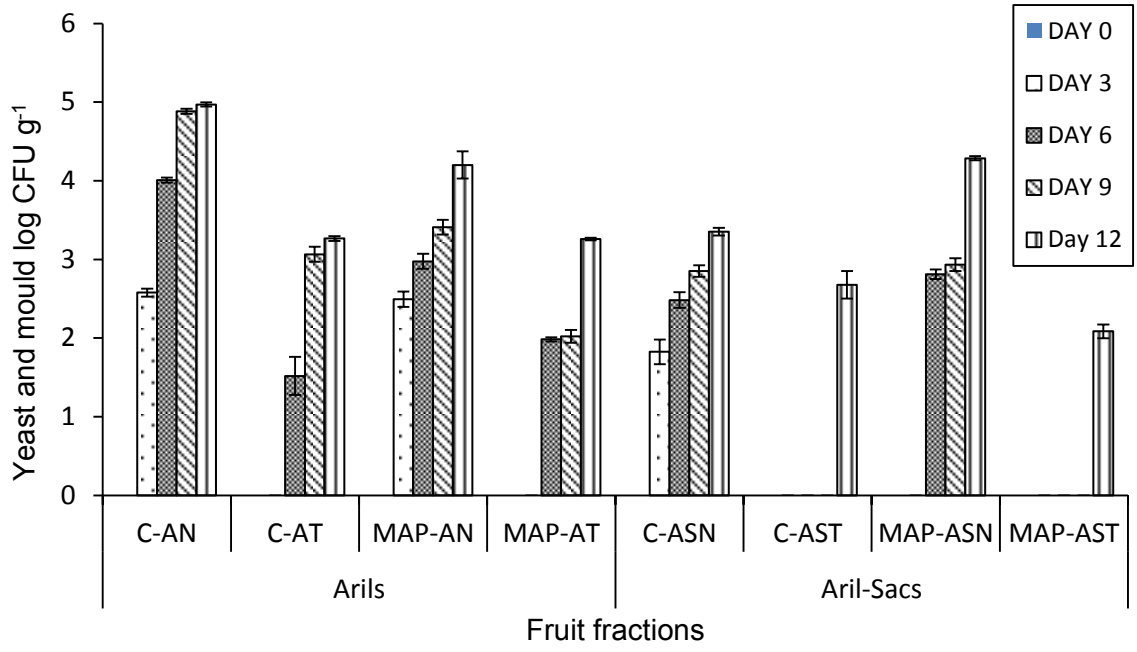

Figure 5.13 Yeast and moulds growth during storage of MAP and anti-browning treated arils and aril-sacs. Vertical bars denote standard deviation of the mean of three replicates.

Sensory evaluation

The radar chart (Fig. 5.14) shows the sensorial scores of the different treatment combinations at day 12 of storage. Aril colour (loose and those obtained from the aril-sacs) was significantly (p < 0.05) influenced by the type of fruit fraction, with arils obtained from aril-sacs having high colour score and overall acceptability in comparison to loose arils. The alcoholic taste which is associated with fermentation was significantly influenced by factors such as fruit fraction, anti-browning and time. The alcoholic taste scores were significantly (p < 0.05) high in arils and generally increased with storage period. Significantly (p < 0.05) high alcoholic taste score was perceived in non-treated arils (Fig 5.15). By day 12, treated arils packed under MA had a highest alcoholic taste while lowest scores were found in all the aril-sacs treatments. A positive correlation between the alcoholic taste perceived and the microbial growth tested was observed (0.48). Fruit fraction, anti-browning, time and fruit fraction × time interaction had significant effects on the overall acceptability of the products (p < 0.05). A significantly high score on overall acceptability was observed in samples treated with anti-browning agents, compared to the non-treated (p < 0.05). On day 12 of storage, all the packed aril-sacs (C-ASN, C-AST, MAP-ASN and MAP-AST) showed a high acceptability level, though no significant differences observed among them. The 12 days of acceptable shelf life of aril-sacs under passive MAP (MAP-AST & MAP-ASN) obtained in the present study is longer than the shelf life of 10 days reported by Caleb *et al.* (2013) for loose arils

of cv. Acco and Herskawitz. However the shelf life of treated arils (MAP-AT) of 6 days is four days shorter than for passive-MAP arils reported by Caleb *et al*. (2013), as they had high scores of alcoholic taste (fermentation), which may be attributed to the high mould and yeast growth, hence reducing their acceptability.

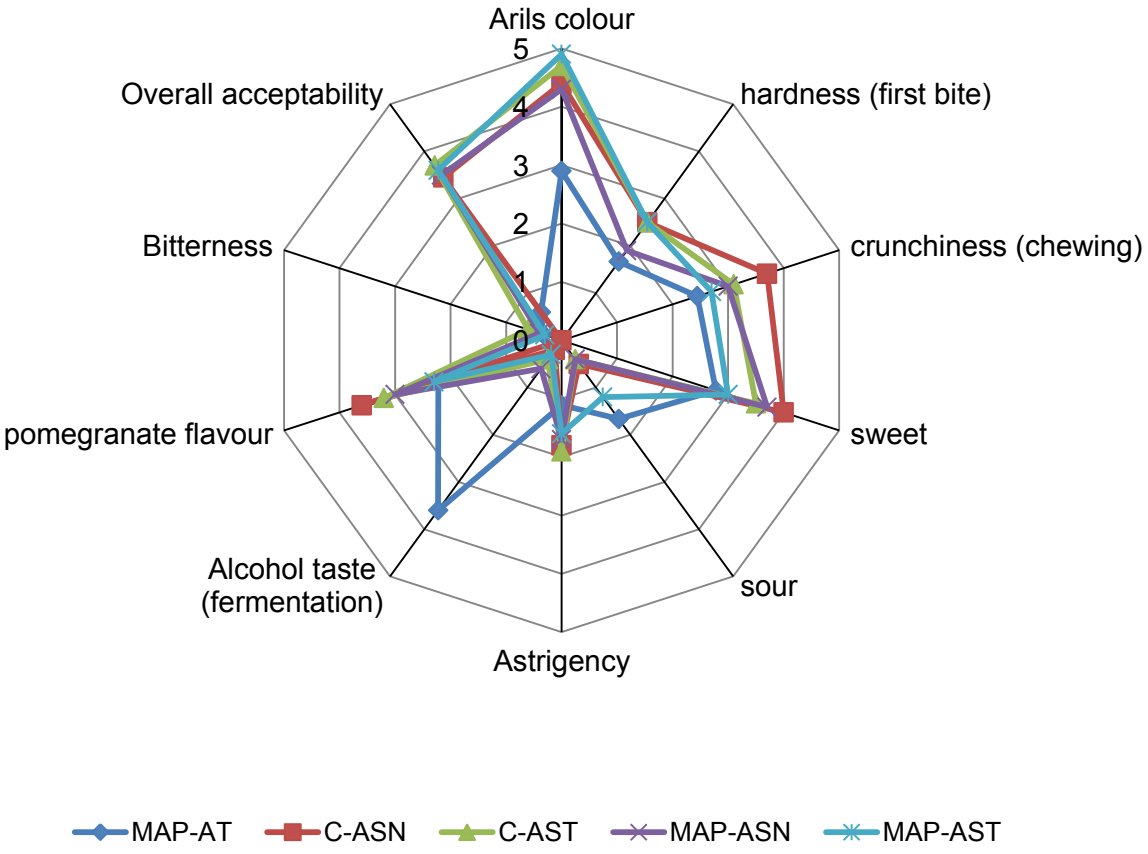

Figure 5.14 Sensorial attributes of the treatment combinations after 12 days of storage at 5°C. Data are the mean of evaluation performed by 10 assessors.

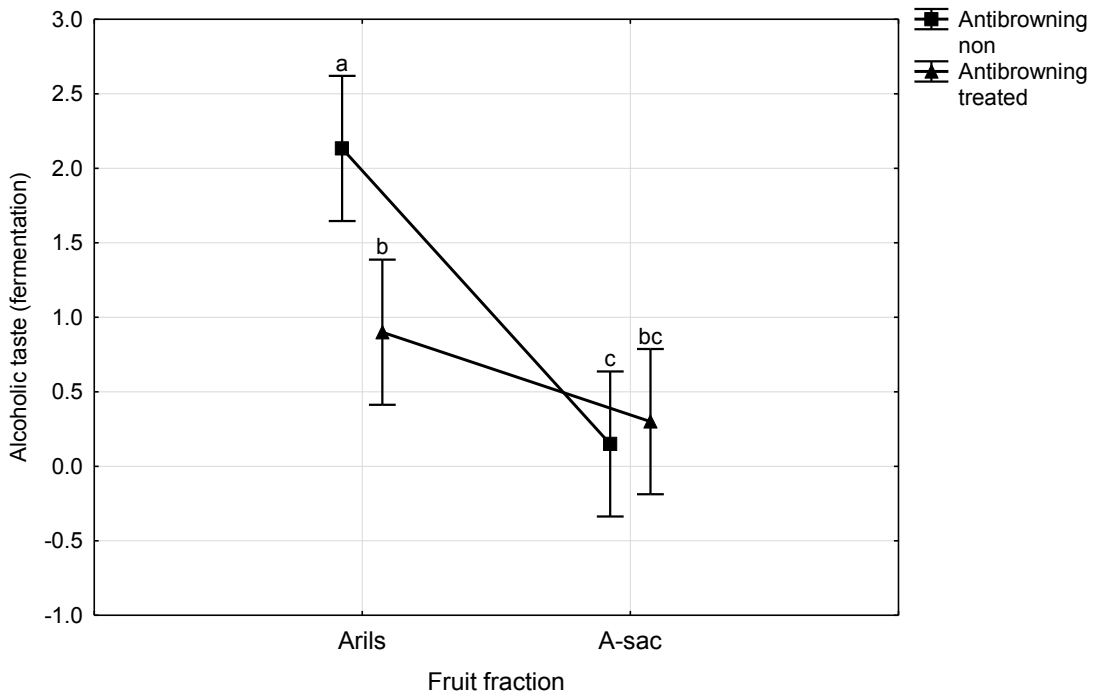

Figure 5.15 Effects of fruit fraction × anti-browning interaction on the alcoholic taste (fermentation) of arils and those obtained from aril-sacs, as perceived by sensory assessors.

Conclusion

The headspace gas composition of O_2 and CO_2 were significantly influenced by the storage time, fruit fraction, anti-browning treatment, type of packages and their interactions with some exceptions. Loose arils that were not treated (MAP-AN) showed a steady state within the recommended level of 2-5% O_2 and 5-10% CO_2 level hence occurrence of undesirable flavour which may occur due to anoxic conditions was prevented. Slight but significant changes were observed in physicochemical quality properties during storage at 5°C. Positive correlation between pH, microbial growth and fermentation (alcoholic taste) gives a clear indication of the product shelf life thus influenced the overall acceptability of the products. Quality attributes and overall acceptability was positive in aril-sacs as compared to arils. Anti-browning treatment of products had a significant influence on the quality attributes, whereby aril-sacs treated with anti-browning agents were best kept in comparison to other samples throughout the storage duration. Passive-MA packaging gave a better performance in comparison to the control packages. MA Packaged arils non-treated (MAP-AN) created gas close to recommended steady states and prevented excessive accumulation of CO_2. This treatment prolonged the shelf life for 12 days compared to clamshell packed arils that only lasted up to day 9 of storage.

Considering quality factors such as microbial growth, physicochemical attributes (colour TA and TSS), alcoholic taste and overall acceptability, the aril-sacs treated with anti-browning agent

and stored under passive MAP (MAP-AST) provided the best option for cold chain handling of minimally processed pomegranates. For the arils, the MA Packaged treated arils (MAP-AT) is the best by default as it is the only arils treatment that reached day 12 of storage, with no decay observed, though the overall acceptability was very low, below the score of 3. Overall, the MAP-AST can be considered to be the best among all the eight treatments, with the shelf life of about 12 days. For the MAP-AT, the shelf life can be around 6 days of storage, as its acceptability level fall below the score of 3 during the sensory evaluation and its alcoholic taste perceived was higher (above the score of 3) than all the treatments. Therefore, it can be concluded that the MAP-AST could be used to extend the shelf life and maintain the quality of processed pomegranate aril-sacs.

References

Arias, E., Gonzalez, J., Oria, R. & Lopez-Buesa, P. (2007). Ascorbic acid and 4-hexylresorcinol effects on pear PPO and PPO catalysed browning reaction. *Journal of Food Science*, **72**(8), 422-429.

Artes F., Villaescusa, R. & Tudela J.A. (2000). Modified atmosphere packaging of pomegranate. *Journal of Food Science*, **65** (7), 1112-1116.

Artes, F., Marin, J.G. & Martinez, J.A. (1996). Controlled atmosphere storage of pomegranate. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung*, **203**, 33-37.

Ayhan, Z. & Esturk, O. (2009). Overall quality and shelf life of minimally processed and modified atmosphere packaged ‘’ready-to-eat’’ pomegranate arils. *Journal of Food Science*, **74** (5), 399-405.

Caleb, O.J., Mahajan, P.V., Manley, M. & Opara, U.L. (2013). Evaluation of parameters affecting modified atmosphere packaging engineering design for pomegranate arils. *International Journal of Food Science and Technology*, doi:10.1111/ijfs.12220.

Ergun, M. & Ergun, N. (2009). Maintaining quality of minimally processed pomegranate arils by honey treatments. *British Food Journal*, **111**(4), 396-406.

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 Bioprocess Technology*, DOI 10.1007/s11947-011-0533-7.

Garcia, E. & Barrett, D.M. (2005). Fresh Cut Fruits. In: *Processing Fruits: Science and Technology*. 2nd Ed. Pp. 53-72. Boca Raton, Florida, USA: CRC press.

Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Anti-oxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry*, **48**(10), 4581–4589.

Gil, M.I., Artes, F. & Toma-Barberan, F.A. (1996). Minimally processing and modified atmosphere packaging effects on pigmentation of pomegranate seeds. *Journal of Food science*, **61(1),** 161-164.

Gonzalez-Aguilar, G.A., Wang, C.Y. & Buta, J.G. (2000). Maintaining quality of fresh-cut mangoes using anti-browning agents and modified atmosphere packaging. *Journal of Agricultural Food Chemistry*, **48**, 4204-4208.

Kader, A.A. (2002). Fresh-cut fruit and vegetable products. In: *Fresh-cut fruits and vegetables: science, technology and market* (edited by O. Lamikanra). Pp. 21-30. Washington, D.C. USA: CRC Press.

Liu, C., Hsu, C. & Hsu, M. (2007). Improving the quality of fresh-cut pineapples with ascorbic acid/sucrose pre-treatment and modified atmosphere packaging. *Packaging Technology and Science*, **20**, 337-343.

Lopez-Rubira, V., Conesa, A., Allende, A. & Artes, 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.

Martínez-Romero, D., Castillo, S., Guillén, F., Díaz-Mula, H. M., Zapata, P. J., Valero, D. & Serrano, M. (2013). Aloe vera gel coating maintains quality and safety of ready-to-eat pomegranate arils. *Postharvest Biology and Technology*, **86**, 107–112.

Montero-Calderon, M., Rojas-Grau, M.A. & Martin-Belloso, O. (2008). Effect of packaging conditions on quality and shelf life of fresh-cut pineapple (*Ananas comosus*). *Postharvest Biology and Technology*, **50**, 182-189.

Nabigol, A. & Asghari, A. (2013). Antifungal activity of aloe vera gel on quality of minimally processed pomegranate arils. *International Journal of Agronomy and Plant Production*, ***4*** (4), 833–838.

O'Grady, L. (2012). Effects of Postharvest handling on nutritional quality of pomegranate (*Punica granatum*) arils. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Salveit, M.E. (2012). Respiratory Metabolism. [Internet document] URL www.ba.ars.usda.gov/hb66/019respiration.pdf. 07/09/2012.

Simson, S.P. & Straus, M.C. (2010). *Post-Harvest Technology of Horticultural Crops.* Pp. 124-125. Narayan Niwas, Delhi: Oxford Book Company.

Thompson, A.K. (2010). *Controlled atmosphere storage of fruits and vegetables*. 2nd Ed. Pp. 81-113. United Kingdom. Meredith Carroll.

Viuda-Martos, M., Fernandez-Lopez, J. & Perez-Alvarez, 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 6: General discussion and conclusion

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

The consumption of pomegranate fruit has been influenced by its functional properties and health promoting benefits (Gil *et al.*, 2000). The fruit can either be packaged as whole fruit or processed into fresh arils, jams, flavourant and other high value products (Lopez-Rubira *et al*., 2005; Opara *et al*., 2009; Martinez-Romero *et al*., 2013). Manual processing limits the consumption of the fruit due to the difficulty of peeling and staining of fingers. Hence, processing of whole fruit into arils create a potential to increase consumption. The physiological processes (i.e. respiration and transpiration rates) of the arils are enhanced due to minimal processing (Garcia & Barret, 2005; Liu *et al.,* 2007; Ayhan & Esturk, 2009; Caleb *et al.,* 2012). This study was carried out to evaluate the effects of passive MAP on the quality of pomegranate cv. Bhagwa arils and aril-sacs. This was done by understanding the effects of storage conditions (temperature and relative humidity (RH)) on the physiological processes (transpiration and respiration rates) of pomegranate whole fruit, arils and aril-sacs. The study went further to assess the influence of the use of anti-browning treatment in combination with passive-MAP on the quality of arils and aril-sacs. Furthermore, the WVTR of the pomegranate membrane was measured. Active-MAP (i.e. gas flushing/mixing) was not considered in this study in order to provide data on the basic response of the produce.

Effects of storage conditions on the physiological processes (TR and RR) of pomegranate arils and aril-sacs were studied and mathematical models were applied in order to predict and validate the observed physiological responses under different storage conditions. Results obtained from this study agreed with those reported in literature on the RR of whole fruit and arils (Lopez-Rubira *et al*. 2005; Ersan *et al.* 2010; Caleb *et al.* 2012; Maghoumi *et al.* 2012). Storage temperature, time and their interaction had significant effects on RR. A high RR was observed in aril-sac as compared to the whole fruit and arils across all storage temperature. A significant increase in RR was encountered as from day 3 of storage across all fruit fractions and storage temperature. A 74.5% decrease in RR was observed when storage temperature is reduced from 22°C to 5°C. Additionally, the efficiency of the model in predicting the RR of the fruit fractions was well demonstrated by a high R^2 value of 97.1%, using the Arrhenius type equation at 22°C.

Effects of temperature and RH on the TR of the arils and aril-sacs were studied. The TR was found to increase with an increase in temperature and decrease in RH, with the fruits stored at 5°C and 96% RH, showing a lower TR. Between the two fruit fractions, the arils had a high TR compared to the aril-sacs. This can be related to the large surface area of loose arils in comparison to aril-sacs, which are partly covered with the peel and the arils are stuck together. The model used in this study adequately predicted the TRs of arils and aril-sacs. Similar trend on the effects of temperature and RH on TRs of arils and mushroom were reported by Caleb *et al*. (2013) and

Mahajan *et al*. (2008), respectively. Furthermore, the WVTR of the membrane studied was found to be high art room temperature conditions (18.7°C, 70% RH) than at cold storage temperature (5°C, 90% RH). A wet cup method was effective in determining the WVTR of the pomegranate membrane.

Once the understanding on the physiological behaviour of arils and aril-sacs was established. Further study was taken on the packaging of these fruit fractions. The effects of passive-MAP combined with anti-browning agents on the physicochemical, sensory, microbial growth and shelf-life of the arils and aril-sacs were investigated. Storage time, fruit fraction, anti-browning, package and their interactions, had impacts on the headspace gas composition of CO_2 and O_2. The MAP-AN showed a steady state at day 9 which was within the recommended level of 2 - 5% O_2 and 5 - 10% CO_2 (Lopez-Rubira *et al*., 2005; Simon & Straus, 2010), thus preventing the anoxic conditions inside the package. The headspace gas composition in this study follows the same trend reported for pomegranate arils cv. Arakta and Bhagwa when stored at 4°C in PET packages (O'Grady, 2012) and cv. Acco and Herskawitz packed in PP trays with polylid films stored at 5°C (Caleb *et al*., 2013). However, Gonzalez-Aguilar *et al*. (2000) reported that an equilibrium gas composition was reached at day 9 of minimally processed mangoes treated with anti-browning combinations (4-HR. potassium sorbate and D-isoascorbic acid) at different combination levels.

Slight significant ($p < 0.05$) changes were observed in physicochemical quality attributes during storage at 5°C between both fruit fraction and packages. Anti-browning treatment had significant effects on the quality attributes, more especially the yeast and mould growth, browning index and overall acceptability. Lowest yeast and mould count was observed in anti-browning treated aril-sacs. Positive correlations were observed between pH, microbial growth and fermentation (alcoholic taste), giving a clear indication of the product shelf life, thus perceived in the overall acceptability of the products. Non-treated samples received lower acceptability scores. Thus, these results highlight the importance of the choice of packaging, anti-browning treatment as well as storage time on the quality of arils and aril-sacs.

This study showed the importance of optimum storage conditions, passive-MAP and the use of anti-browning have an impact on the quality and shelf life of arils and aril-sacs. The RR of pomegranate fruit and its fractions can be reduced significantly by storing them at lower temperature as low as 5°C. Furthermore, storing arils and aril-sacs at lower temperature such as 5°C and a relative humidity above 90% help reduce their transpiration rates (water loss) as well as the WVTR of the membrane. The arils and aril-sacs quality was best kept when packaged under passive-MAP, compared to clamshell trays. Treating arils and aril-sacs with anti-browning agents help reduce the growth of yeast and mould. Thus a high overall acceptability was perceived in aril-sacs at day 12 while for arils the best acceptability level was only perceived until day 9. In summary, the research question was answered, since the study has found that aril-sacs are kept

longer than arils under modified atmosphere packaging. Therefore, one would recommend analysis of the volatile organic compounds present in the headspace of the package at the end or the storage period. Moreover, research should be done to explore the role and structure of the membrane, such that exploring its porosity by using electron microscope.

References

Ayhan, Z. & Esturk, O. (2009). Overall quality and shelf life of minimally processed and modified atmosphere packaged ''ready-to-eat'' pomegranate arils. *Journal of Food Science*, **74** (5), 399-405.

Caleb, O.J., Mahajan, P.V., Manley, M. & Opara, U.L (2013). Evaluation of parameters affecting modified atmosphere packaging engineering design for pomegranate arils. *International Journal of Food Science and Technology*, doi:10.1111/ijfs.12220.

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

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.

Garcia, E. & Barrett, D.M. (2005). Fresh Cut Fruits. In: *Processing Fruits: Science and Technology*. 2nd Ed. Pp. 53-72. Boca Raton, Florida, USA: CRC press.

Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Anti-oxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry*, **48**(10), 4581–4589.

Gonzalez-Aguilar, G.A., Wang, C.Y. & Buta, J.G. (2000). Maintaining quality of fresh-cut mangoes using anti-browning agents and modified atmosphere packaging. *Journal of Agricultural Food Chemistry*, **48**, 4204-4208.

Liu, C., Hsu, C. & Hsu, M. (2007). Improving the quality of fresh-cut pineapples with ascorbic acid/sucrose pre-treatment and modified atmosphere packaging. *Packaging Technology and Science*, **20**, 337-343.

Lopez-Rubira, V., Conesa, A., Allende, A. & Artes, 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.

Maghoumi, M., Gómez, P. A., Mostofi, Y., Zamani, Z., Artés-Hernández, F., & Artés, F. (2013). Combined effect of heat treatment, UV-C and super atmospheric oxygen packing on

phenolics and browning related enzymes of fresh-cut pomegranate arils. *LWT - Food Science and Technology*, **54**(2), 389–396.

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.

Martínez-Romero, D., Castillo, S., Guillén, F., Díaz-Mula, H. M., Zapata, P. J., Valero, D. & Serrano, M. (2013). Aloe vera gel coating maintains quality and safety of ready-to-eat pomegranate arils. *Postharvest Biology and Technology*, **86**, 107–112.

O'Grady, L. (2012). Effects of Postharvest handling on nutritional quality of pomegranate (*Punica granatum*) arils. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

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

Simson, S.P. & Straus, M.C. (2010). *Post-Harvest Technology of Horticultural Crops.* Pp. 124-125. Narayan Niwas, Delhi: Oxford Book Company.