MODELLING AND OPTIMIZATION OF ACTIVE MODIFIED ATMOSPHERE PACKAGING FOR POMEGRANATE ARILS

by

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Declaration

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ABSTRACT

Active modified atmosphere packaging (active-MAP) is a well-proven postharvest technology used to preserve the quality and extend the storage and shelf life of fresh fruit under optimally designed conditions. Successful active-MAP design can be achieved by the mathematical integration of produce physiological characteristics, packaging material properties, and equilibrium gas mixture suitable for the product. The mechanisms by which active-MAP influences fruit quality involve physiological and enzymatic reactions that can be accelerated or reduced depending on the environmental conditions during storage. Therefore, understanding the experimental design and fundamental physiological processes occurring during storage condition are important in the development of an optimal produce-specific active-MAP.

Low O_2 limit for pomegranate arils was identified at 5 and 10 °C and the responses were monitored using real time respiration rate (RR), respiratory quotient (RQ), emission of volatile organic compounds (VOCs) and microbial growth. The results showed that pomegranate arils could tolerate down to 2.18% O_2 during storage at 5 °C and 2.28% O_2 at 10 °C. These findings highlighted the importance of selecting appropriate MAP materials with desired permeability to alleviate rapid depletion of O_2 and excessive accumulation of CO_2 at 10 °C inside the package.

The impact of active-MA on quality attributes of ‘Wonderful’ pomegranate arils were investigated at cold (5 ℃, 95 ± 2% RH) and ambient storage (20 ℃, 65 ± 2% RH) conditions. Low O_2 (5-10%) atmospheres significantly maintained antioxidant properties of arils, whereas significantly lower aerobic mesophilic bacteria, yeast and mould counts were found at super-atmospheric O_2 (70%). Storing pomegranate arils under ambient condition resulted in quality deterioration and short shelf life.

The effects of low O_2 and super-atmospheric O_2 on RR of ‘Wonderful’ pomegranate arils were analysed at 5 °C. Michaelis-Menten (MM) enzyme kinetic models were applied to determine the inhibition effects of CO_2 concentration on O_2 consumption rate. The results showed that both storage atmosphere and temperature had significant effects on aril RR. The MM competitive inhibition model best described the effect of CO_2 on O_2 consumption rate at low O_2 and super-atmospheric O_2 ($R^2 > 99\%$). The findings showed that super-atmospheric O_2 had no effect to retard the metabolic process.

A simplex lattice mixture design (SLMD) approach was applied to optimize gas composition for storing arils, and effects of temperature on the optimum gas was studied. A special cubical model were developed for the responses (RR, RQ, ethylene production rate and microbial quality) and the coefficients of model parameter estimates ($\beta_1, \beta_2, \beta_3, \beta_{12}, \beta_{13}, \beta_{23}$ and β_{123}) and ternary contour plots were characterised. The predicted optimium gas mixture (O_2:CO_2) was 2% O_2:18% CO_2); and at this atmosphere, the minimum values of RR were 0.26

mL O_2 kg^{-1} h^{-1} and 0.78 mL CO_2 kg^{-1} h^{-1}, while ethylene production rate was below the detection limit. Under these conditions, the growth of aerobic mesophilic bacteria (3.9 log CFU mL^{-1}), yeast (3.8 log CFU mL^{-1}) and mould (2.3 log CFU mL^{-1}) were quantified. Increasing the storage temperature by 10 °C resulted in a threefold increase in aril RR.

Using the same SLMD approach, the optimum gas composition required to maintain individual quality attributes was predicted. Variation in optimal gas mixture for individual quality attributes of arils were observed. According to the model parameter estimates, the optimum gas composition (O_2:CO_2) was established (6-7% O_2:7-8% CO_2) for individual sugars, organic acids, antioxidants and colour attributes. The optimum gas composition to maintain aril hardness and volatile compounds (monoterpene and ketones) was 2% O_2 and 18% CO_2. On the other hand, the optimum atmosphere for aldehydes was 2% O_2 and 2% CO_2.

An integrated designing approach was applied to configure a packaging system for arils capable of modifying atmosphere and in-package relative humidity by designing modified atmosphere humidity package (MAHP) system. Cellulose based NatureFlex® (NF) film, bi-axial oriented polypropylene (BOPP) (PF) film and combinations of the two films were used. The 100% NF package created the lowest in-package RH (60-66%) and the highest reduction of O_2, which resulted in arils dryness during storage. The 100% BOPP film resulted in saturated RH and in-package water vapour condensation. The optimized package design using 66% PF and 33% NF films best maintained the overall quality of pomegranate arils.

This study demonstrated the potential of SLMD as an innovative tool to optimize the gas composition and improve packaging design for effective cold storage of minimally-processed fresh produce such as pomegranate arils. The results obtained also provide new information on the optimum condition required to maintain specific quality parameters for the commercialization of active-MA for packaging and marketing of pomegranate arils.

OPSOMMING

Aktiewe gemodifiseerde atmosfeerverpakking (aktiewe-MAP) is 'n beproefde na-oes tegnologie wat gebruik word om die kwaliteit van vars vrugte in stand te hou, en die berging en rakleeftyd onder optimaal ontwerpte toestande te verleng. Suksesvolle aktiewe-MAP ontwerp kan bereik word deur wiskundige integrasie van fisiologiese eienskappe, verpakkingsmateriaal eienskappe en ewewig gasmengsel wat geskik is vir die produk. Die meganismes waardeur aktiewe-MAP die vrugkwaliteit beïnvloed behels fisiologiese en ensimatiese reaksies wat versnel of verminder kan word, afhangende van die omgewingstoestande tydens berging. Daarom is die begrip van eksperimentele ontwerp en fundamentele fisiologiese prosesse wat tydens bergingstoestande plaasvind, belangrik in die ontwikkeling van 'n optimale produkspesifieke aktiewe-MAP.

‘n Lae O_2-limiet van 5 en 10 °C is vir granaat-arils geïdentifiseer en die respons is gemonitor deur gebruik te maak van waretyd respirasie tempo (RR), respirasie kwosiënt (RQ), emissie van vlugtige organiese samestellings (VOCs) en mikrobiese groei. Die resultate het getoon dat granaat arils tot 2.18% O_2 kan verdra tydens berging by 5 °C en 2.28% O_2 by 10 °C. Hierdie bevindinge beklemtoon die belangrikheid om die gepaste MAP materiaal met die gewenste deurlaatbaarheid te kies, om by 10 °C vinnige uitputting van O_2 en oormatige ophoping van CO_2 binne die verpakking te verlig.

Die impak van aktiewe-MA op kwaliteitseienskappe van ‘Wonderful’ granate is ondersoek teen koue (5 °C, 95 ± 2% RH) en omringende (20 °C, 65 ± 2% RH) bergingstoestande. Lae atmosferiese O_2 (5-10%) het die antioksidante eienskappe van arils aansienlik gehandhaaf, terwyl aansienlike laer vlakke van aërobiese mesofiele bakterieë, gis en swam tellings by super-atmosferiese O_2 (70%) gevind is. Granate wat onder omgewingstoestande geberg is, het gelei tot 'n afname in die kwaliteitsgehalte en 'n kort rakleeftyd.

Die effekte van lae O_2 en super atmosferiese O_2 op RR van 'Wonderful' granate is by 5 °C geanaliseer. Michaelis-Menten (MM) ensiem-kinetiese modelle is toegepas om die inhibisie-effekte van CO_2-konsentrasie op O_2-verbruikerstempo te bepaal. Die resultate het getoon dat beide bergingsatmosfeer en -temperatuur beduidende effekte op aril RR gehad het. Die MM-mededingende inhibisie model het die effek van CO_2 op O_2-verbruikerstempo by lae O_2 en super-atmosferiese O_2 ($R^2 > 99\%$) die beste beskryf. Die bevindinge het getoon dat super-atmosferiese O_2 geen effek gehad het om die metaboliese proses te vertraag nie.

'n Simpleks rooster mengsel ontwerp (SLMD) benadering is aangewend om die gas samestelling vir die berging van arils te optimaliseer, en die effekte van temperatuur op die optimale gas samestelling te ondersoek. 'n Spesiale kubiese model is ontwikkel vir die veranderlikes (RR, RQ, etileenproduksietempo en mikrobiese gehalte) en die koëffisiënte van modelparameterskattings ($\beta1$, $\beta2$, $\beta3$, $\beta12$, $\beta13$, $\beta23$ en $\beta123$) en ternêre kontoerplotte is gekenmerk. Die voorspelde optimale gasmengsel (O_2:CO_2) was 2% O_2: 18% CO_2; En by hierdie atmosfeer was die minimum RR waardes 0.26 ml O_2 kg^{-1} h^{-1} en 0.78 ml CO_2 kg^{-1} h^{-1}, terwyl die etileenproduksietempo 0,0 μL kg^{-1} h^{-1} was. Onder hierdie toestande is die groei van aërobiese mesofiele bakterieë (3.9 log CFU mL^{-1}), gis (3.8 log CFU mL^{-1}) en swamme (2.3 log CFU mL^{-1}) gekwantifiseer. Die 10 °C verhoging in bergingstemperatuur het gevolglik die RR van arils drievoudig laat toeneem.

Die vereiste optimale gassamestelling om individuele kwaliteitseienskappe te handhaaf, is met dieselfde SLMD-benadering voorspel. Die optimale gasmengsel vir individuele kwaliteitskenmerke van arils het gevarieer. Volgens die skattings van die modelparameters is die optimale gassamestelling (O_2: CO_2) vasgestel (6-7% O_2: 7-8% CO_2) vir individuele suikers, organiese sure, antioksidante en kleur-eienskappe. Terwyl die optimale gassamestelling om aril hardheid en vlugtige verbindings (VOCs) soos monoterpeen en ketone te onderhou, 2% O_2 en 18% CO_2 was. Aan die ander kant was die optimale atmosfeer vir aldehiede 2% O_2 en 2% CO_2.

'n Geïntegreerde ontwerpbenadering is toegepas om 'n verpakkingstelsel op te stel vir arils waarvan die binne-verpakking relatiewe humiditeit (MAHP) en gas samestelling verander kan word. Sellulose-gebaseerde NatureFlex® (NF) film, bi-aksiale georiënteerde polipropileen (BOPP) (PF) film en kombinasies van die twee films is gebruik. Die 100% NF-verpakking het die laagste binne-verpakking RH (60-66%) en die hoogste verlaging van O_2 geskep, wat die arils uitgedroog het tydens die bergingsperiode. Die 100% BOPP film het gelei na versadigde RH en binne-verpakking waterdamp kondensasie. Die geoptimaliseerde verpakkingsontwerp wat 66% PF en 33% NF-films gebruik, het die algehele gehalte van granaat arils die beste behou.

Hierdie studie het die potensiaal van SLMD as 'n innoverende instrument getoon om die gassamestelling te optimaliseer en die verpakkingsontwerp te verbeter, vir effektiewe verkoeling van minimaal verwerkte vars produkte soos granate. Hierdie resultate verskaf ook nuwe inligting oor die vereiste optimale toestand om spesifieke gehalte-parameters te handhaaf vir die kommersialisering van aktiewe MA vir verpakking en bemarking van granate.

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NOMENCLATURE

Variable	Meaning
a	Transfer rate constant coefficient
a*	Redness/greenness
A_{ic}	Peak area of the identified volatile compound
A_{itc}	Peak area of the internal standard
A_{p}	Area of the packaging material
ATP	Adenosine triphosphate
a_w	Water activity of package headspace
a_{wi}	Water activity of the arils
b*	Yellowness/blueness
BOPP	Bi-axial oriented polypropylene
BOP	Bi-axial oriented polyester
C*	Chroma
C_{itc}	Final concentration of internal standard
C_{p}	Specific heat
CFU	Colony forming unit
D	Diffusion coefficient
DCP	Dichlorophenolindophenol
DF	Dilution factor
E_{a}	Activation energy
F	Flow rate of gases and water vapour
FCDA	Foodstuffs, Cosmetics and Disinfectant Act
FCF	Fresh cut fruit
J	Diffusion flux
K_{m}	Michaelis-Menten constant
K_{i}	Inhibition constant for CO_2
K_m	Mass transfer coefficient
K_{ta}	Transpiration coefficient on mass bases
L	Thickness of the packaging film
LOL	Low O_2 limit
L*	Lightness
NF	NatureFlex film
M	Mass of the fresh produce
M_{w}	Molecular weight
MAP	Modified atmosphere packaging
MAHP	Modified atmosphere and humidity packaging
MMU	Michaelis-Menten uncompetitive
MM	Michaelis-Menten
MMC	Michaelis-Menten competitive
MMN	Michaelis-Menten non competitive
MPA	Metaphosphoric acid
MTT	Magness-Taylor test
$\dot{M}_1$	Water transpiration from produce to headspace
$\dot{M}_2$	Rate of water permeation from headspace to surrounding

Variable	Meaning
n	Number of moles per gas
N	Newton
OTR	Oxygen transmission rate
p	Partial pressure of gas
P_s	water vapour pressure at the evaporating surface
P	Permeability of the packaging material
$p\infty$	ambient water vapour pressure
pO_2	Permeability of O_2
pCO_2	Permeability of carbon dioxide
PPO	Polyphenoloxidase
P_{atm}	Atmospheric pressure
PCA	Plate count agar
PF	PropaFilm
R	Universal gas constant
RA	Relative abundances
RBCA	Rosebengal chloramphenicol agar
RH	Relative humidity
RQ	Respiratory quotient
RR	Respiration rate
RO_2	Respiration rate due to O_2 consumption
RCO_2	Respiration rate due to CO_2 production
R_{ref}	Respiration rate at reference temperature
SHS	Static headspace sampling
$T_{P}gas$	Tissue permance
t	Time
T	Temperature
TCA	Tricarboxylic acid
T_p	Temperature on the product surface
TR_m	Transpiration rate per unit area
T_r	Transpiration rate of the commodity per mass
TPC	Total phenolic concentration
TR	Transpiration rate
T_{ref}	Reference temperature
T_s	Temperature of gas around the product
V_f	Free volume inside the package
V	volume of the package
VPD	Vapour pressure deficit
V_{max}	Maximum respiration rate due to O_2 consumption
WVTR	Water vapour transmission rate
Y	Concentration
YO_2	Concentration of oxygen
YCO_2	Concentration of carbon dioxide
W	Weight of the fruit
W_a	Weight of dry air inside the package

Variable	Meaning
WL	Mass loss
W_0	Initial mass
ϱ	Density
$h°$	Hue angle
ε	Molar extinction coefficient
β	Simplex lattice model parameter coefficient
Δc	Concentration difference

Subscripts

f	Final	CO_2	Carbon dioxide
i	Initial	O_2	Oxygen
ref	Reference	N_2	Nitrogen
Atm	In the surrounding atmosphere		
Pkg	In the gas		

Note

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

List of published and submitted articles, and conference presentations from this thesis

Belay, Z.A., Caleb, O.J. & Opara, U.L. (2016). Modelling approaches for designing and evaluating the performance of modified atmosphere packaging (MAP) systems for fresh produce: A review. *Food Packaging and Shelf Life*, **10**, 1-15. https://doi.org/10.1016/j.fpsl.2016.08.001

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Belay, Z.A., Caleb, O.J., Pramod, P.V. & Opara, U.L. Pomegranate arils (cv. Wonderful) tolerance to low O_2 stress during active modified atmosphere storage: based on real time respiration rate. *Acta Horticulturae (in press).*

CHAPTER 1

INTRODUCTION

Global market for modified atmosphere packaging (MAP) is projected to reach US$13.78 billion by 2020 and growing at a compound annual growth rate (CAGR) of 4.3% during the forecast period from 2015 to 2020 (Research & Markets, 2016). In 2015, this market was led by demand for convenience food, with a market share of 24.9%. According to this report, the market was estimated dominated by North America (32.2%), followed by Europe. The Asia-Pacific region and the rest of the world markets were projected to grow at the highest CAGR owing to the rapid growth in the demand for MAP in this region. The growing demand for convenience and ready-to-eat food in the emerging economies is also contributing in driving this market.

Modified atmosphere packaging technology relies on changing the atmosphere composition surrounding the product (Sousa-Gallagher *et al*., 2013), thereby influencing the biochemical, enzymatic, and microbial actions to maintain the quality of produce (Artés *et al*., 2000). Active-MAP consists essentially of gas flushing, gas scavenging or emitting (O_2, CO_2 and ethylene) to quickly establish equilibrium condition within the package and to avoid the high concentration of unsuitable gases (Charles *et al*., 2003). This enables packed fresh and minimally-processed fruit and vegetables to maintain visual, textural, and nutritional appeal for a longer period (López-Rubira *et al*., 2005; Oms-Oliu *et al*., 2008). It allows a fresh state of produce without chemical treatments used in competitive food preservation technique. The atmosphere inside a MAP system depends on product physiological characteristics (respiration and transpiration), environmental condition (temperature and relative humidity (RH)) and packaging material properties (thickness, perforation, permeability to water vapour and gases) (Caleb *et al*., 2012a; Song *et al*., 2002; Techavises & Hikida, 2008; Torrieri *et al*., 2010). Therefore, MAP is capable of maintaining the quality of fresh produce when the above-mentioned characteristics are at optimum condition (Rodriguez-Aguilera & Oliveira 2009; Sandhya, 2010; Sousa-Gallagher *et al*., 2013).

One of the most important parameters which affect the benefits of MAP during storage is the gas composition of the atmosphere. The use of low O_2 and enriched or high CO_2 concentration during storage has been extensively reported in literature to maintain quality, reduce respiration rate (RR) and ethylene production rate, delay enzymatic reactions and consequently extend the shelf life of many types of fruit (Beaudry, 2000; Oms-Oliu *et al*., 2008; Fonseca *et al.,* 2005; Giuggioli *et al*., 2015). However, under sub-optimum conditions, O_2 concentration could decline below critical limits where the fresh produce cannot tolerate, which then could induce anaerobic respiration and further lead to the synthesis of fermentative metabolites and development of off-odour (Chunyang *et al*., 2010; Giuggioli *et al*., 2015;

Saenmuang *et al*., 2012). Similarly, exposure to excessive CO_2 concentration could induce anaerobiosis, tissue injury and disrupt enzyme systems such as lipoxygenase pathway (Giuggioli *et al.*, 2015).

Recently, the application of super-atmospheric O_2 ($\geq$ 70 %) has introduced as a novel MAP technique. Super-atmospheric O_2 is an effective alternative to low O_2 atmosphere for inhibiting microbial growth and enzymatic deterioration as well as maintaining produce quality (Jacxsens *et al*., 2001; Allende *et al*., 2007). However, the beneficial effects of super-atmospheric O_2 depend on the physiological response of the specific produce (Jacxsens *et al*., 2001; Maghoumi *et al*., 2014).

Furthermore, selection of packaging films with suitable barrier properties is of crucial importance to maintain the desired gas composition inside the package and to ensure an extend shelf life of produce (Martínez-Romero *et al*., 2013). Maintenance of optimum RH during storage is also essential, which has been demonstrated to affect condensation inside the package, weight loss of produce and microbial growth (Giuggioli *et al*., 2015; Silveira *et al*., 2014). Therefore, to overcome the lack of RH control in MAP technique, a good understanding of the factors that affect the MAP system is required. This will help to optimize the storage condition (Chunyang *et al*., 2010; Conesa *et al*., 2007).

In order to optimize MAP for a specific produce such as fruit, the concentration of gases and its effects on the overall quality have to be quantified. Often, this task is carried out by a series of randomly targeted gas concentrations in a selected packaging material and environmental condition. This process involves lengthy trial and error experimentation. However, to achieve optimal solution, it has been recommended that modelling RR and optimizing gas concentration for effective MAP application should involve a systematic mixture design approach (Pintado & Malcata, 2000; Saenmuang *et al*., 2012). Mixture design methodology is very useful to predict the response of any combination of components by investigating the functions of an individual factor and component interaction (Cornell, 2011).

Mixture design experiments represent a special case in response surface methodologies using mathematical and statistical techniques (Cornell, 2011; Myers *et al*., 2016). The experimental designs largely follow from Scheffé's simplex lattice and simplex centroid designs (Scheffé, 1958). In mixture experiments, the measured response is assumed to be dependent only on the relative proportions of the ingredients or components in the mixture and not on the summation of the mixture. The main distinction between mixture experiments and independent variable experiments is that with the former, the input variables or components are non-negative proportionate amounts of the mixture, and if expressed as fractions of the mixture, they must sum to one (Scheffé, 1958). This approach is beneficial for selecting experimental design points and solving the problem of searching the optimal proportion of the components (Azevedo *et al*., 2011; Hron & Macak, 2015; Yilmaz *et al*., 2015). Therefore, this study aimed to optimize active-MAP for 'Wonderful' pomegranate fruit arils using simplex lattice mixture design approach.

Pomegranate fruit (*Punica granatum.* L) is known as 'super-fruit' due to its nutritional value and health benefits such as anti-inflammatory and anti-atherosclerotic effects against osteoarthritis, prostate cancer and heart disease (Caleb *et al*., 2012b; Opara *et al*., 2009; O'Grady *et al*., 2014). Cultivation of the fruit has expanded to several contents (Africa, Asia, Europe, USA and South America), with India, Iran, China, Turkey, United States, Spain, South Africa, Peru, Chile and Argentina as the major countries in terms of production and role players in the international fruit trade. Globally, 'Wonderful' is the most widely grown and consumed pomegranate cultivar (Holland *et al*., 2009). South Africa's production of pomegranates amounted to approximately 7,337 tons in 2016, with 69% destined for exports and the remainder for the local market (5.4%) and processing (25.5%). 'Wonderful' is also the major cultivar grown in South Africa, accounting for over 70% and 85%, respectively, of total export and local market of pomegranates (Hortgro, 2017). The edible portion (arils) of 'Wonderful' pomegranate fruit comprises approximately 50% of the whole fruit (Arendse *et al*., 2016).

Pomegranate arils packed in MAP is one of the main commercial ready-to-eat products, which offers convenience for consumers in pomegranate fruit consumption. Several studies have demonstrated that minimally-processed pomegranate arils is a rich source of bioactive compounds and phytochemicals, which provide potential health promoting benefit, as well as fresh characteristics and convenience for the consumer (Aindongo *et al*., 2014; O'Grady *et al*., 2014). However, maintaining postharvest quality and microbial safety of pomegranate arils is a critical challenge (Munhuweyi *et al*., 2017a; O'Grady *et al*., 2014). The limiting factors affecting the overall quality and shelf life of minimally-processed pomegranate arils include microbial growth, loss of nutritional and physicochemical attributes caused by active metabolic processes related to enzyme activity, enhanced RR or oxidation of phenolic compounds (Munhuweyi *et al*., 2017b; Ersan *et al*., 2010; Ghasemnezhad *et al*., 2011; Maghoumi *et al*., 2014).

Various studies have reported different approaches to determine suitable atmospheric and environmental conditions of MAP for pomegranate arils (Ayhan & Eştürk, 2009; Banda *et al*., 2015; Caleb *et al*., 2012a; Ersan *et al*., 2010; López-Rubira *et al*., 2005). The results reported in these studies demonstrated that the application of MAP systems was effective to maintain quality and extended shelf life of pomegranate arils. However, the studies all emphasized the need for optimization of MAP system for pomegranate arils to fully benefit from its application. Therefore, the aim of this study was to design and optimize MAP system for 'Wonderful' pomegranate arils using the simplex lattice mixture design approach. Special emphasis was placed on the physiological response and quality changes during exposure to different modified atmospheres, packaging configuration and storage temperature. To accomplish the aim of this study, the following objectives have been structured into research chapters:

- Asses the role of mathematical models in the design of MAP systems, and effects of active-MAP on the quality of fresh and fresh-cut fruit based on literature review;
- Determine the physicochemical changes of pomegranate arils during low O_2 atmosphere storage and identify the critical low O_2 limit;
- Investigate the effects of low and super-atmospheric O_2 and storage temperatures on physiological response and quality pomegranate arils;
- Study the applicability of Michaelis-Menten mathematical model to describe the inhibition effect of CO_2 to low and super-atmospheric O_2 during respiration of pomegranate arils;
- Apply simplex lattice mixture design to establish the optimum gas concentration based on the physiological response of pomegranate arils and investigate the effect of temperature on maintaining the optimum gas composition;
- Investigate the effects of active modified atmosphere mixture design on quality attributes of pomegranate arils;
- Propose improved design of packaging material configuration for pomegranate arils by matching the selected optimum gas concentration and avoiding water vapour condensation.

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Declaration by the candidate:

With regard to Chapter 2 (pp 10-57); the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Literature search and writing of chapter	75

The following co-authors have contributed to Chapter 2 (pp 9-55):

Name	E-mail address	Nature of contribution	Extent of Contribution (%)
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Declaration by co-authors:

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 2 (pp 10-57)
2. no other authors contributed to Chapter 2 (pp 10-57) besides those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 2 (pp 10-557) of this dissertation.

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Declaration with signature in possession of candidate and supervisor.

CHAPTER 2

LITERATURE REVIEW

MODELLING APPROACHES FOR DESIGNING AND EVALUATING THE PERFORMANCE OF MODIFIED ATMOSPHERE PACKAGING (MAP) SYSTEMS AND IMPACTS OF ACTIVE-MAP ON QUALITY OF FRESH AND FRESH-CUT FRUIT

ABSTRACT

Fresh and minimally-processed produce continue physiological and metabolic processes after harvest, hence they are susceptible to quality deterioration and reduced shelf life. Modified atmosphere packaging (MAP) is well-proven postharvest technology for preserving natural quality of fresh and minimally-processed produce and extending the storage life under optimum MAP design and storage conditions. Successful MAP design is achieved by the mathematical integration of dynamic produce physiological characteristics, properties of packaging material, coupled with optimum equilibrium atmospheric conditions for the given product. This review examined the various mathematical approaches applicable for postharvest handling and storage of fruit and vegetables. This included the interaction between fresh produce physiological process, packaging and storage conditions, and their influences on produce shelf life and quality. Different mathematical modelling approaches are presented, factors affecting modelling performance are discussed and examples of model applications are highlighted to demonstrate relevance to specific types of produce. This review found that the mechanisms of active-MAP influence fruit quality (physicochemical, bio-active compounds, and microbial safety) due to the physiological or enzymatic reactions that can be accelerated or declined. The review showed that active-MAP has benefits compared to the passive-MAP, therefore, the initial gas modification can be considered as a better approach to maintaining the quality of fresh and fresh-cut fruit without any residual gas effects. However, this benefit is only achieved at the optimum atmosphere and temperature. Future prospects for modelling MAP are highlighted, including the importance of an integrated modelling approach for improved understanding of package performance for fresh and minimally-processed produce under MAP.

Key words: Low O_2, Super-atmospheric O_2; Bioactive compounds; Respiratory energy; Transpiration rate; Film permeability

Introduction

Modified atmosphere packaging (MAP) relies on changing the surrounding gas composition by the interplay between film permeability to gases (O_2 and CO_2) and respiration rate (RR) of produce inside the package (Mahajan & Mezdad, 2013; Reinas *et al*., 2016). Various researchers have reported that MAP system is capable of maintaining the quality of fresh produce if the optimum gas compositions and permeability of the film are designed properly (Rodriguez-Aguilera & Oliveira 2009; Sandhya, 2010). Designing MAP systems is a complex task, which involves the need to understand the dynamic interaction and integrated knowledge of product characteristics, packaging material, and atmospheric conditions for the given product (Sousa-Gallagher & Mahajan, 2013). Good MAP design should also enable accurate prediction of these important parameters such as gas concentration, respiration rate (RR) and film permeability (Li *et al*., 2010) and prediction is possible with the help of mathematical models (Sousa-Gallagher *et al*., 2013). Various studies reported that mathematical models are useful to specify and relate input parameters such as RR, transpiration rate (TR), as well as gas permeability to film thickness, storage temperature, time, and product weight (Fonseca *et al*., 2002a; Guevara *et al*., 2006; González-Buesa *et al*., 2009). Summary of parameters considered in MAP design and modelling is presented in Table 1.

Active modified atmosphere packaging (active-MAP) systems accelerate the establishment of optimum gas within the packaged product by initial displacement of atmosphere for desired and suitable gas composition (Charles *et al*., 2003; Tomás-Callejas, 2011; Costa *et al*., 2011; Ye *et al*., 2012). Initial displacement of gas has the favourable effect to reduce the respiratory activity and prolonging the shelf life of fresh produce (Oms-Oliu *et al*., 2008a; Mangaraj *et al*., 2009; Gomes *et al.,* 2010; Costa *et al*., 2011). Hence, responses differ for each fresh produce under MAP conditions, it is necessary to quantify the effect of the applied atmosphere for an individual product (Jacxsens *et al*., 2001). Removal of oxygen reduces RR, inhibits rate of oxidation, which directly slows the rate of degradation of valuable sensory quality and colour components and result subsistence delayed of proliferation of aerobic microorganisms (Fante *et al*., 2014; Li & Zahng 2015; Rosa *et al*., 2007; Teixeira *et al*., 2016; Van der Steen *et al*., 2002). However, O_2 concentration could decline below the concentration required to sustain aerobic respiration, then fermentation and, potentially, off-flavour could occur (Beaudry, 2000; Soliva-Fortuny *et al*., 2003; Fonseca *et al*., 2005). On the other hand, super-atmospheric O_2 atmosphere has been particularly effective to decrease enzymatic discoloration and preventing anaerobic respiration, inhibiting microbial growth and effective at reducing decay of fresh produce (Chunyang *et al*., 2010). However, the elevated O_2 could enhance the production of reactive oxygen species (ROS), thus damage the cytoplasm and inhibit various metabolic activities led to deterioration of produce quality (Limbo *et al*., 2007), but sensitivity to O_2 toxicity, which is the ability to defend against oxidative stress in plants varies among species (Ayala-Zavala, 2007; Yang *et al*., 2008).

Therefore, this chapter presented a critical review of the literature on designing MAP for fruit and vegetable, with a brief overview of mathematical modelling approaches for MAP. Mathematical models that take into account the physiological response of fresh produce and film permeability during MAP design were discussed. The effects of active gas modification approach on the quality attributes of different fresh and fresh-cut fruit (FCF) were presented.

Rationale for mathematical modelling of modified atmosphere packaging – an overview

Fresh fruit and vegetables keep respiring after harvest, during which they consume O_2 and produce CO_2. Fresh fruit and vegetables RR can be slowed down or accelerated depending on factors associated with the environment, packaging material and product characteristics (Al-Ati & Hotchkiss, 2003). The dynamics of atmosphere generation inside MAP depends on product physiological characteristics (respiration and transpiration), environmental condition (temperature and relative humidity (RH)) and properties of the packaging material (thickness, perforation, permeability to water vapour and gases). Various studies have reported modelling approaches for describing and predicting these interactions using mathematical equations for successful MAP design (Mahajan *et al*., 2008; Gomes *et al*., 2010). Most of the model applications aimed to extend shelf life and maintain the quality of packaged produce (Oms-Oliu *et al*., 2008a). Others assessed the effects of the design parameters on physicochemical and microbial attributes of produce (Caleb *et al*., 2013a), while some studies focused on models used to test different package configurations to achieve optimum postharvest storage conditions (Barrios *et al*., 2014a). These studies have used experimental data considering the effects of the product characteristics, environmental factors and packaging material properties to predict both physiological responses of produce and properties of packaging material in order to identify the optimum packaging conditions. This indicates that a successful MAP design requires integration of these essential parameters.

Integrative mathematical modelling MAP of fresh produce is a highly interdisciplinary research. This involves a combination of bioprocess engineering (produce physiology), packaging material science (Mahajan *et al*., 2008; Sousa-Gallagher & Mahajan, 2013) and design data to predict the impacts of various factors on food quality/safety, and simulate packaging design alternatives (Gomes *et al*., 2010; Kwon *et al*., 2013). To develop an overall MAP model, sub-models are required which account for the influence of MAP on the physiology of packaged product beyond gas exchange (Iqbal *et al*., 2005). Most common type of models used include Michaelis–Menten (MM) enzymatic kinetics equations (which describes RR of fresh produce as a function of gas concentration) (Rennie & Tavoularis 2009; Ersan *et al*., 2010); Arrhenius-type equations, to investigate the effect of temperature on RR of fresh produce (Caleb *et al*., 2012).

Furthermore, MM type equations have been extensively reported in the literature as giving good predictions of RR for different types of fresh produce. Gomes *et al*. (2010) developed a model to describe RO_2 of fresh-cut 'Rocha' pear as a function of O_2 concentration (YO_2) with the possible effect of CO_2. The study further incorporated the effect of temperature by Arrhenius equation and the probability of fit was assessed by the root mean square error (RMSE). The results showed that RR as a function of YO_2 and temperature can be accurately predicted by MM combined with the Arrhenius-type equation. Similar studies were reported for various fresh produce such as for blueberry (Song *et al*., 2002), tomato (Charles *et al*., 2003), shredded carrots (Iqbal *et al*., 2009) and minimally-processed broccoli (Torrieri *et al*., 2010).

On the other hand, various studies used the data from RR and temperature modelling to predict the diffusion process of gases through the packaging material. Models to estimate the transient gas exchange in a passive MAP were developed for prickly pear cactus (*Opuntia spp*.) stems by Guevara-Arauza *et al*. (2006). These authors considered the effects of temperature (T) and, RH on film permeability (P), RR and tissue permeance (TP_{gas}). In this study, diffusion process through the tissue and film, and the effects of respiration were combined in a descriptive model. Validation result showed that the model adequately predicted the parameters, which can facilitate the packaging design. Mahajan *et al*. (2008) has reported an integrated model describing the effects of different combinations of perforation, film type and thickness and validated for the permeation response of the perforation holes that accounted for simultaneous convective transport through the hole. Similarly, integrated models were reported by Techavises and Hikida (2008) to investigate the effects of temperature on gas and water vapour transmission rates for perforation films, and by Lakakul *et al*. (1999) to predict the effects of temperature, film thickness, and fruit mass on package O_2.

In addition, Kwon *et al*. (2013) investigated the diffusion phenomenon and developed empirical models to estimate the atmosphere in the perforated film for King Oyster mushroom. The study considered the effects of gas diffusion and convection through perforations, as well as produce RR and gas permeation through packaging film. This study found that the diffusion model satisfactorily estimated the gas atmospheres. Other studies have modelled gas permeability of MAP films using diffusion models based on Fick's law (Xanthopoulos *et al*., 2012). These studies highlight the importance of incorporating different modelling approaches for the successful design of MAP. The rest of this review will focus on approaches for modelling packaging materials used in MAP and the physiological responses as well as the impact of the active modified atmosphere on the quality fresh produce.

Modelling respiration rate of fresh produce in modified atmosphere packaging

Measuring and modelling of RR of fresh produce are essential for the engineering design of MAP (Iqbal *et al*., 2005). Changes in gas concentration due to fruit respiration can be expressed as production of CO_2 (R_{CO2}) and consumption of O_2 (R_{O2}) (Gomes *et al*., 2010). Thus in MAP, the O_2 partial pressure is typically reduced while that of CO_2 is increased. The purpose of this is to lower the RR and slow down the metabolic pathways that negatively affect the quality of the stored product (Costa *et al*., 2011; Sen *et al*., 2012). Measuring RR of fresh produce under different gas compositions and temperature is required for the development of mathematical models and for defining the package requirement (Yam & Lee, 1995).

Various methods have been reported in literature for measuring RR, the most common methods include a closed or static system, flowing or flushed system, and permeable system (Fonseca *et al*., 2002b). As can be seen from the equations presented in Table 2, in closed system method, a respiring sample is closed in an airtight container with known volume (V_f), of a packaging and weight of a product (M_p), initial oxygen and carbon dioxide concentrations ($Y_{O2i,f}$ and $Y_{O2i,f}$) and time (t_f-t_i). In the flow through system, the produce with a known weight (M) used in a gas of know concentrations at a constant rate over the sampling time in a barrier container. The RR is determined by the differences in the inflow and outflow gases ($Y_{O2in,\,out}$ and $Y_{O2in,\,out}$). Permeable systems use a packaging film of known gas transmission rate and size filled with produce. For RR measurement under permeable packaging films, the thickness (L) and permeability of O_2 and CO_2 (P_{O2} and P_{CO2}) of the film have also used, in addition to the other parameters considered in closed and permeable system, to compute the respiration rate of produce in terms of O_2 consumption and CO_2 production (R_{O2} and R_{CO2}). However, limitations exist in all of these methods. For instance, in flow through systems, the variation in flow rate can affect the measurement of the difference in gas concentration and it is also not effective for low respiring produce. In the closed system, the accumulation of high CO_2 can affect the RR, but in the permeable system the parameters that should be included to determine the RR makes it very complex to calculate. Fonseca *et al*. (2002b) extensively reviewed various RR measuring and modelling approaches applicable for fresh produce. The ratio of CO_2 produced to O_2 consumed by the produce is designated as respiratory quotient (RQ) (Iqbal *et al*., 2009). Typical values of RQ are usually in the range from 0.7 to 1.3 depending on the substrate (Fonseca *et al*., 2002a). The value of RQ > 1.3 implies the presence of anaerobic respiration and the substrate in the metabolic process would be acidic. Moreover, increase in RQ (Cameron *et al*., 1994) or higher CO_2 production (Conesa *et al*., 2007) can be associated with the loss of fresh produce quality.

Accounting for all factors that affect RR is rather complex (Kaur *et al*., 2010). Hence, models developed have considered the parameters that could be studied experimentally such as gas composition, temperature, and time and often based on the combination of one or two of these parameters (Fonseca *et al.,* 2002a). Most models have been developed by either considering the RR as a function of gas concentration or time elapsed (Salvador *et al*., 2002; Torrieri *et al*., 2010). Other studies suitably modified model parameters for temperature dependency by linear interpolation or using an Arrhenius relationship (Caleb *et al*., 2012). In addition to this, modelling RR gives an advanced insight into the respiratory kinetics of the packaging and storage system and also helps to predict the RQ. However, in order to benefit from MAP application, the RR data should be integrated with the permeability of the packaging material. This involves selecting the ratio of CO_2 to O_2 gas permeability (selectivity) of the polymeric film. Film selectivity and product respiration can determine the relationship between the concentrations of O_2 and CO_2 that evolve with in a package RQ (Cliffe-Byrnes & O´ Beirne, 2007). The selectivity requirement depends on the ratio of CO_2 production to O_2 consumption RQ of the product and the target optimal compositions of both O_2 and CO_2. Therefore, only when packaging selectivity matches the product requirement can an optimal atmosphere of both O_2 and CO_2 be achieved (Al-Ati & Hotchkiss, 2003; Ravindra & Goswami. 2008).

Enzyme kinetics based models

Based on the fact that respiratory mechanism is governed by enzymatic reactions, many researchers have used MM enzyme kinetics model to describe RR of fresh produce (Fonseca *et al*., 2002a; Kaur *et al.,* 2010). The MM enzyme kinetics model provides a simple description, based on the assumption that the diffusion and solubility of O_2 and CO_2 in plant tissue regulate reactions catalysed by enzymes (Salvador *et al*., 2002; Sen *et al*., 2012). The constant in this equation used to account for any resistance to gas diffusion or solubility encountered in the skin or flesh of the product (Cliffe-Byrnes & O'Beirne, 2007). The effect of O_2 in the respiration process, which is expressed as the ratio of O_2 concentration and O_2 consumption, can be described by equation 1:

$$R = (V_{max} * Yo_2)/(\mathrm{K_m} + Yo_2) \tag{1}$$

where R is respiration rate (mL kg^{-1} h^{-1}), V_{max} is maximum respiration rate due to O_2 consumption and K_m is MM constant.

However, in some products, both O_2 and CO_2 have an influence on quality; therefore, according to a detailed explanation by Fonseca *et al*. (2002b), the overall influence of CO_2 was included in the MM equation as CO_2 acts as an inhibitor during the respiration process. This influence is used to characterise the models as competitive, uncompetitive, and non-competitive inhibition models. Competitive inhibition (Equation 2) occurs when both the inhibitor (CO_2) and the substrate compete for the same active site of the enzyme. Uncompetitive inhibition (Equation 3) occurs when the inhibitor reacts with the substrate enzyme complex but in non-competitive (Equation 4) models inhibition occurs when the inhibitor reacts both with the enzyme and with the enzyme substrate complex. Therefore, the maximum RR is lower in high CO_2 concentrations, and much influenced by high CO_2 concentration for competitive and uncompetitive inhibition. Therefore, for the respiration process, these inhibition mechanisms can be described by the following equations.

$$Ro_2, Rco_2 = \frac{(V_{max} \times Y_{O_2})}{K_m * \left(1 + \frac{Y_{CO_2}}{K_i}\right) + Y_{O_2}} \tag{2}$$

$$Ro_2, Rco_2 = \frac{(V_{max} \times Y_{O_2})}{K_m + Y_{O_2} \times \left(1 + \frac{Y_{O_2}}{K_i}\right)} \tag{3}$$

$$Ro_2, Rco_2 = \frac{(V_{max} \times Y_{O_2})}{(K_m + Y_{O_2}) * \left(1 + \frac{Y_{CO_2}}{K_i}\right)} \tag{4}$$

where Ki is the carbon dioxide inhibition constant.

As shown in Table 3, MM enzyme kinetics modelling approach has been applied by several studies to describe the relationship between respiration and gas concentration including the effect of CO_2 on the RR. Gomes *et al.* (2010) characterised the respiration of fresh-cut 'Rocha' pear as a function of O_2 concentration and temperature, with accurate prediction using MM kinetics model. Iqbal *et al.* (2005) used MM uncompetitive model to assess the influence of gas composition on the respiration rate of shredded carrots. Moreover, temperature has a major effect on the rate of metabolic processes that will inevitably lead to the product deterioration therefore; the effect of temperature on the headspace gas composition on oxygen consumption and carbon dioxide production has been accounted through the enzyme kinetics respiration rate model by integrating Arrhenius equation and produce a secondary model equation 5:

$$RR = \frac{V_{max} * Yo_2}{K_m + Yo_2} \times exp\left[-\frac{E}{R} * \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \tag{5}$$

where RR is the respiration rate, E is the activation energy and T is the temperature, R_c is the universal gas constant, T_{ref} is reference temperature.

As shown in Table 4, several studies have applied the integrated MM type equation and Arrhenius relation in order to get the best fit of the model. An integrated Arrhenius and MM competitive global model were developed by Torrieri *et al*. (2010) to investigate the effects of gas composition and temperature on the RR of minimally-processed broccoli. The study showed a good agreement between experimental and predicted values, providing that the MM competitive model with Arrhenius equation accurately predicted the influence of gas composition and temperature. The influence of temperature on the enzyme kinetics has reported by Fonseca *et al*. (2002a) where the MM model constant increased exponentially with temperature and further the RR decreased with time.

Furthermore, MM model parameters (V_{max} and K_m) have been reported to be useful in designing effective packaging systems (Hong & Kim, 2001). V_{max} is the maximum RR due to O_2 consumption, while K_m is the MM constant. Higher K_i value indicates that CO_2 minimally influence the RR (Gomes *et al*., 2010). These parameters are economical and can easily be related to temperature compared to exponential model (Cameron *et al*., 1994). The model parameters can be verified by comparing predicted and experimental values (Song *et al*., 2002). Most of these studies further stated that mathematical model developed considering temperature can form an integral part of selecting suitable packaging film, the size of the film, the number of perforation, and the amount of product, minimizing experimental trial needed to conclude the packaging type and optimize the shelf life of fresh produce.

Modelling transpiration rate for fresh produce

Transpiration is one of the critical physiological processes in fresh produce (Caleb *et al*., 2013b). Transpiration rate (TR) is related to the mass transfer process whereby water moves from the surface of the plant origin or from the stored commodity into the surrounding air. Water loss after harvest reduces the weight and quality of fresh produce during postharvest storage (Caleb *et al*., 2013b; Sousa-Gallagher *et al*., 2013). Conversely, accumulation of water at the product surface during MAP favours the growth of spoilage microorganisms (Caleb *et al*., 2013a; Mahajan *et al*., 2008). Transpiration rate during postharvest handling storage is influenced by fresh product factors (morphological characteristics, surface to volume ratio, maturity stage, injuries) and environmental factors (water vapour pressure difference (WVPD), air velocity, storage temperature, RH and RR) (Mahajan *et al*., 2008; Sousa-Gallagher *et al*., 2013). Bio-physical properties of the skin, respiration heat generation, and vapour as variables also affect the TR (Sousa-Gallagher *et al*., 2013). TR increase with increasing storage temperature (Xanthopoulos *et al*., 2014), and with decreasing RH from 100 to 75% (Tano *et al*., 2005). Similarly, Aindongo *et al*. (2014) reported that low temperature and high RH reduced water loss of pomegranate arils. The authors further reported 68% reduction in TR of arils when the RH increased from 76% to 96%. A similar study reported increasing weight loss with decreasing RH from 96 to 76% (Caleb *et al.*, 2013b), while (Mahajan *et al.*, 2008) found higher weight loss of mushrooms at 76% RH and 16 °C compared to 96% RH and 4 °C.

As presented in equation 6, TR can be expressed by the difference in weight loss of a produce through time (Sousa-Gallagher *et al.*, 2013). Mathematical models for the prediction of TR in fresh and fresh-cut products are limited in the literature (Caleb *et al.*, 2013b; Xanthopoulos *et al.*, 2014). This is due to the complex interaction between moisture evaporation on the product surface as result of WVPD and moisture released as result of product metabolic activity (Xanthopoulos *et al.,* 2014). The driving force for TR is the vapour pressure difference between the fruit and the surrounding (Ngcobo *et al.*, 2012). Most models describe the moisture transfer through the skin as a function of the bio and thermo-physical properties such as surface cellular structure, skin thickness, pore fraction in the skin, geometry and thermal diffusivity of produce, which are not easily measured or determined (Song *et al.*, 2002).

$$TR_m = \frac{M_i \; - \; M_t}{t \; \times \; (\; M_{\;i} / \; 100)} \tag{6}$$

Where TR_m is the transpiration rate per unit area of initial mass of the product in g kg^{-1} h^{-1}, M_i is the initial weight (g), M_t is the weight of the product (g) at time t (h),

The simplest type of model applied to TR is the linear model (Equation 7), where steady state conditions prevail and the commodity is assumed to be in thermal equilibrium with the environment (Ngcobo *et al.*, 2012).

$$T_r = k_{ta}(p_s - p\infty) \tag{7}$$

where T is the transpiration rate of the commodity per mass (p) of the product (g kg^{-1} d^{-1}), K_{ta} is transpiration coefficient of commodity on mass basis (g kg^{-1} d^{-1} Pa^{-1}), ps is water vapour pressure at the evaporating surface (Pa) and $p\infty$ is ambient water vapour pressure (Pa).
Mathematical models have been developed describing TR as a function of temperature and RH for mushrooms using Fick´s law of diffusion (Mahajan *et al.*, 2008) and for strawberries by fitting the experimental data (Sousa-Gallagher *et al.*, 2013). In these studies, the models found to be useful for understanding the water loss with temperature, RH, time and determining the target water vapour barrier properties required for maintaining optimal RH inside the package. Sousa-Gallagher *et al.* (2013) further reported the importance of considering both gas and water vapour transpiration rate during packaging design for decreasing TR. In addition, a

mathematical model was developed to investigate the effect of temperature and RH on the TR and successfully validated as a function of temperature for pomegranate arils (Caleb *et al*., 2013b). The study reported that the model would be useful towards understanding the rate of water loss as affected by the design parameters and further mentions the model would provide a valuable guide for storage and design of MAP system for pomegranate arils.

On the other hand, the importance of developing TR model for estimating barrier properties required for the packaging materials was emphasised by Xanthopoulos *et al*. (2014). The study further developed semi empirical and analytical model to correlate the mass loss of grape tomatoes with the storage condition (temperature and time) and duration. Both models provided a satisfactory fit to the experimental data.

Modelling permeability of packaging materials

The barrier properties of packaging film in relation to gases and water vapour play a significant role in MAP of fresh whole or minimally-processed produce (Guevara-Arauza *et al.,* 2006; Hussein *et al*., 2015). Gas and water vapour exchanges in a packaging depend on the combination of the rate of respiration and transpiration by the produce and the permeation of permeants (gas or water vapour) in the packaging film (Techavises & Hikida, 2008). This phenomenon can be influenced by film type and film thickness (Fonseca *et al*., 2000); environmental conditions such as RH and temperature (Mahajan *et al*., 2008; Opara *et al*., 2015) and by the product respiration rate itself (Rodriguez-Aguilera & Oliveria, 2009). Anaerobic respiration and condensation can occur if the permeability of the packaging material is not high enough to balance the respiration rate with permeability (Kwon *et al*., 2013). Therefore, studies have been applied mathematical models to predict the optimal O_2 and CO_2 permeation rates (optimal CO_2/O_2 permselectivitiy) and water vapour (Dash *et al*., 2012; Opara *et al*., 2015) for MAP design.

Permeation process through polymeric films consists of three steps: (i) absorption of gas molecules on one side of the film (which indicates how much gas a polymeric film can take up); (ii) diffusion through the film (the mobility of the permeant molecules in the film); and (iii) desorption of the gas molecules or water vapour on the other side of the film (Mangaraj *et al*., 2009). This process is described by diffusion model, using both Henry and Fick's laws to obtain the expression that relates the permeation rate with the area and thickness of the film as shown in Figure 1 (Siracusa, 2012). Fick's First Law in equation 8 describes permeate (gas or vapour) flux, indicated by J. The permeability coefficient is, therefore the product of a solubility coefficient (the thermodynamic parameter) and diffusion parameter (the kinetic parameter) (Rodriguez-Aguilera & Oliveira, 2009). Fick's law that relates the diffusive flux to the concentration field describes the permeability coefficient. Knowledge of this property of the film is very important to determine the atmospheric composition inside the package (Li *et al.*, 2010).

$$J = -D * \Delta c \qquad (8)$$

where J is the diffusion flux (expressed in mol cm^{-2} s^{-1}), for one-dimensional diffusion through a polymer membrane and in stationary condition. J can be written as $-D * \Delta c/l$, where J is diffusion flux (expressed in mol cm^{-2} s^{-1}), D is the diffusion coefficient or diffusivity (expressed in cm^{2} s^{-1}), Δc is the concentration difference (expressed in mol cm^{-3}) across the packaging film and L is the position, the dimension of which is length expressed in (cm).

Mathematical models of film permeability in MAP

Mathematical models are needed to predict the permeability of packaging material to gases (O_2 and CO_2) or water vapour as affected by factors such temperature, RH and water vapour, film thickness and perforation, and the interaction between product respiration, package permeability and environment (Mahajan *et al.*, 2008; Dash *et al.*, 2012; Opara *et al.*, 2015). Gas exchange is commonly used to describe the permeation process of a gas through a plastic film via the solution-diffusion model (Mc-Gonigle *et al.*, 2001) or by the classic permeability equation based on the first law of Fick's diffusion for thin and infinite films (Guevara-Arauza *et al.*, 2006). This relationship can be implemented to model steady state mass transfer where there is an assumed linear relationship between diffusion flux and concentration gradient between the two sides of the film (Montanez *et al.*, 2010). Generally, the Fick's law diffusion process is considered for gas transport in the polymer (Mangaraj *et al.*, 2009). Since the diffusion is in solid media, other studies have reported the use of Henry's law describing the transfer of gases through the film as a function of permeability coefficient and partial pressure of gases (Zhu *et al.*, 2002).

The water vapour exchange in the MAP system affects in-package relative humidity (RH). RH plays an important role in the physiological responses of packaged produce and influences product quality (Li *et al.*, 2010; Techavises & Hikida, 2008). High humidity condition prevails in the packages, causing moisture condensation, microbial growth and decay of the product if the package is not optimally designed (Hussein *et al.*, 2015). Water vapour exchange in the MAP involves a mass balance of a given packaging system in terms of water vapour inside and outside the package (Sousa-Gallagher *et al.*, 2013). Permeability models depend on the type of packaging system, and different systems may require different combinations of fundamental, semi-empirical and empirical models. The most common type of packaging is a simple polymeric film that exhibits a given permeability to the gases (Rodriguez-Aguilera & Oliveira, 2009). As shown in Table 5, various studies developed gas permeability models by incorporating parameters, *i.e* gas concentration, gas exchange inside the package due to respiration process, the gas permeated through the packaging film, volume of packaging material and molar flux (N) of the fresh produce (Guevara-Arauza *et al*., 2006; Salvador *et al*., 2002; Zhu *et al*., 2002). Other researchers have developed water vapour (WVTR) permeability models using transpiration rate data (Li *et al*., 2010). Several mathematical models describing O_2 and CO_2 exchanges in MAP have been developed (Salvador *et al*., 2002; Techavises & Hikida, 2008). Few researchers have developed a model that includes atmospheric gas and water vapour exchanges in MAP with edible films (Techavises & Hikida, 2008), while most studies investigated the role of perforations (Del-Valle *et al*., 2003; Techavises & Hikida, 2008; Li *et al*., 2010).

A simple mathematical model was applied to estimate the optimal conditions for MAP of 'Burlat' cherries (Salvador *et al*., 2002). The model described gas exchange in flexible packages, taking into account fruit RR and transmission rate of gases through the film. Recently, De-Bonis *et al*. (2015) used a computational method by incorporating all the phenomenological variations such as mass and heat transfer notations with respiration, microbial growth kinetics and variation of moisture content. The researchers reported a method to verify existing MAP configurations or to design new ones. These studies showed that the major important parameters for modelling permeability of packaging material for MAP can be associated with the initial gas concentration coupled with the respiration rate, temperature RH and packaging characterises. Overall, modelling approaches that integrated Fick's law of diffusion with Arrhenius equations in design of MAP, provided more robust solutions applicable to wider application.

Factors affecting film permeability modelling

Achieving the optimal film permeability for MAP depends on numerous factors, including packaging type, film thickness and surface area, a number of diffusion sites, and rubbery or glassy state of the polymeric film (Al-Ati & Hotchkiss, 2003; Rodriguez-Aguilera & Oliveira, 2009; Xanthopoulos *et al*., 2012). As presented in Table 6, various studies reported different gas or water vapour permeability ($WVTR$) models by considering the effect of environmental conditions (temperature and RH) and packaging material (perforation, perforation tube diameter and thickness). Techavises and Hikida (2008) observed that film thickness was not a dominant factor on effective permeability of macro perforations in thin films, whereas Fonseca *et al*. (2000) reported that the increase in film thickness decreases the effective permeability to O_2 and CO_2. However, these thicknesses were not as small as the film thicknesses used commercially for fruit and vegetable (Techavises & Hikida, 2008). The results from the permeability model developed by Al-Ati and Hotchkiss (2003) aimed to estimate the effect of changing packaging type, dimension, product weight and free volume and O_2 and CO_2 concentration showed that changing the free volume and reducing the film thickness affects the time required to reach steady state.

In most cases, permeability is constant for a polymer permeant system at a given temperature and it is common to characterise the effect of temperature by Arrhenius relationship (Yam & Lee, 1995; Mangaraj *et al*., 2009; Li *et al*., 2010; Opara *et al*., 2015). Salvador *et al*. (2002) developed a mathematical model using Fick´s law of diffusion for predicting the gas and water vapour exchange in MAP without incorporating the effect of temperature and perforation for 'Burlat' cherries in MAP. The validation results showed that model prediction was influenced by the initial gas concentration, were model failed to predict CO_2 concentration when the final experimental concentration reached 20% for initially flushed packaged with 20% O_2 and 10% CO_2. Nevertheless, in the case of cherries packed under air, the predicted values agreed with the experimental data.

In addition, a similar observation was reported by Guevara-Arauza *et al*. (2006) were O_2 concentration was underestimated and CO_2 concentration was overestimated for 20% CO_2 by the permeability models developed using Fick´s law integrated with Arrhenius equations for prickly pear cactus. However, the O_2 concentration was overestimated and the CO_2 concentration was suitably described when prickly pear cactus was packed in the air. On the other hand, Techavises and Hikida (2008) proposed an effective permeability model and reported that a good prediction of gas concentration and RH was obtained when compared to experimental results for MAP of kiyomi fruit. These studies further demonstrated that temperature exerted a significant positive effect on gas transmission rate through polymeric film. However, Techavises and Hikida (2008) observed that the type of gas inside the packaging film by itself did not affect the permeability. For perforated films, increase in temperature could increase the transmission rate of gas and water, whereas increase film thickness could decrease the transmission rate (Li *et al*., 2010).

Generally, due to the complexity physiological and metabolic processes of the fresh produce, most of the approaches presented in literature rely on empirical models. However, the application of numerical models in MAP design and performance evaluation offers new opportunities for improved understanding and prediction of the interaction between product characteristics, packaging performance and environmental conditions. The importance of the interaction of fresh produces shelf life and quality to physiological process, packaging and environment conditions. Optimum MAP requirements differ from one fresh produce to another. All fruit and vegetables require specific and optimized gas concentration, film permeability, temperature and RH for optimal MAP performance. Therefore, research is needed to optimize gas concentration taking into consideration the permselectivity of the packaging materials. Further research on the influence of active MAP modelling with the different gas composition at various storage temperatures is required for the emerging horticultural crops. Research effort should be focused towards the development of an integrated mathematical model which includes product characteristics as well as packaging material property including the mass and heat transfer phenomena for successful MAP design.

Effect of initial gas modification on physical quality attributes

Modifying an atmosphere is a simple and inexpensive way of postharvest treatment (Gomes *et al*. 2010). Fresh and fresh-cut fruit (FCF) produce response to modified O_2 concentration has been characterized by the level of primary metabolite (RR) and secondary metabolite (fermentative metabolites and volatile compounds) (Beaudry, 2000). As can be seen in Table 7, the effect of storage atmospheres varied for different fresh and FCF with in fruit type, gas concentration, and tolerance limit of the fruit to the exposed atmosphere, storage temperature, and packaging material. This is evident that sensitivity to O_2 toxicity varies among species and developmental stage of the tissue (Ayala-Zavala *et al*., 2007). Blokhina *et al*. (2003) stated that during low O_2 atmosphere storage condition, the anoxia induced metabolic change could be an avoidable depending on the tolerance limit of the fresh produce. Under abiotic stress condition, the resultant impairment has been characterized as decline in adenosine triphosphate (ATP) level, pyruvate, dehaydrolase activity and cytoplasmic pH which leads to physicochemical changes (Kader & Saltveit, 2003). On the other hand, the most acceptable explanation for O_2 toxicity under elevated O_2 concentration is the formation of superoxide radicals, which can destruct some function of cell metabolism, where the cells shift to the alternate oxidase respiratory pathway (Wszelaki *et al*., 2000). According to Kader and Ben-Yehoshua (2000), the effect of super-atmospheric O_2 varies between the whole and cut fruit. However, exposure to high O_2 concentration may stimulate or have no effect depending on the commodity, storage time and temperature (Kader and Ben-Yehoshua, 2000). In order to investigate the change in the quality of a product during MAP, it has been recommended to consider the compatibility of the gas permeability properties of a packaging film with the storage atmospheres (Sousa-Gallagher & Mahajan, 2013; Tomás-Callejas *et al*., 2011). If film permeability is low relative to RR, a rapid accumulation of CO_2 and depletion of O_2 could results (Mangaraj *et al*., 2009). On the other hand, if the product is packed in a relatively permeable film, little atmosphere modification occurs (Mangaraj *et al*., 2009; Sandhya *et al*., 2010). Apart from the storage conditions, processing causes many physiological changes for FCF, such as an increase in RR and physical damage such as disruption of cells; this influence product quality and shelf life (Cliffe-Byrnes *et al*., 2001).

Weight loss

Fresh fruit is highly susceptible to weight loss, which adversely affects the quality (appearance, texture, flavour, and nutritional value) (Manolopoulou & Varzakas, 2013). Weight loss (mostly due to water loss) is dependent on the physiological and morphological characteristics of individual fruit (Giuggioli *et al*., 2015; Nunes & Emond, 2007). Substantial water loss from fruit could lead to a significant loss of fresh weight, resulting in economic loss (if the commodity is sold by weight), and further accelerates senescence, increase pathogen invasion, and increased susceptibility to chilling injury (Guevara *et al*., 2006). Reducing the respiratory activity is one of the favourable effect of initial displacement of gases during MAP (Gomes *et al*., 2010), which directly reduce transpiration rate and weight loss (Zenoozian, 2011). Low weight loss at reduced RR could be associated with the consecutive decline in TR, due to low respiratory heat and low water vapour pressure deficit (WVPD) that affects the rate of water evaporated from the product (Bovi *et al.,* 2016). Furthermore, MAP can also reduce weight loss by preventing dehydration and by maintaining the appearance of fresh produces (Barrios *et al*., 2014, Manolopoulou & Varzakas, 2013).

The advantage of active MAP has been reported by various studies, where Brackmann *et al*. (2013) found low RR and low mass loss for 'Fuyu' persimons fruit stored under (1.0% O_2 + 0.0% CO_2). Ochoa-Velasco and Guerrero-Beltran (2014) studied the effect of MAP for prickly pear. The study reported that prickly pear stored in MAP (active and passive) at 4 and 10 °C did not lose significant weight compared to unpacked pear which lost 26% of its initial weight at 10 °C. The study further associated the lowest weight loss of prickly pear with the packaging material (polyethylene) used for the study, which has high moisture barrier property. According to Giuggioli *et al*. (2015), the weight losses of raspberries under MAP were ranged from 0.0 to 0.2%, which has shown the advantage of MAP treatments to maintain a good state of hydration of the fruit. on the other hand, the highest mass loss of grape packed under active MAPs (5% O_2 + 3% CO_2 + 92% N_2; 10% O_2 + 3% CO_2 + 87% N_2 and 15% O_2 + 3% CO_2 + 82% N_2) was reported by Costa *et al.* (2011) compared to grape sealed under normal atmospheric conditions at 5 °C. The study associated the result with the evaporation of moisture from package during the initial vacuolization that was applied prior to injecting the gas in the package and absence of humidity in the gas mixture. This is a good indication of the advantage of using humidified air to control in-package RH.

The rate of weight loss varies widely among different fruit types, even when stored under the same environmental conditions (temperature and RH). Therefore, controlling the in-package RH and storage temperature are other factors responsible to control weight loss or water vapour accumulation inside the package (Mangaraj *et al.*, 2009). Low RH can increase TR, RR and ultimately lead to an unmarketable product, whereas high in-package RH associated with condensation (Bovi, 2016). Moisture loss contributes to rapid decline in quality of most fresh and FCF, and in general, a loss of 3 to 10% may render a wide range of horticultural crops unaccepted for sale (Nunes & Emond, 2007). In addition to the above factors, differences in weight loss among genotypes could be attributed to differences in form and structure, such that produce with protective membranes have low water loss (Kays, 1991). In fresh and FCF, it has been reported that quality deteriorates as weight loss increased and this is exacerbated by high metabolic rates (Giuggioli *et al.*, 2015). The first symptom of water loss was perceived as a loss of firmness, consecutively changes in colour followed and changes in tissue firmness (Nunes & Emond, 2007) and loss of water also could contribute to increasing total soluble solid concentration (Li & Zhang, 2015). Therefore, to minimize the challenge of controlling weight loss during MAP system, it is better to start from controlling the RR and TR of the produce. This can be achieved by the integrated approach of selecting desired gas composition and permeability as well as controlling the in-package RH.

Colour

Changes in fruit colour are commonly associated with the breakdown of cellular chloroplasts and chromoplasts and change in natural pigments (chlorophylls, anthocyanins, carotenoids, flavonoids) (Hui, 2006). Almost all of these pigments can be affected by processing and storage conditions (Barrett *et al.*, 2010). Colour is the main perception for consumer acceptability and it can be influenced by many factors, such as maturity, genotype and cultivar (Pathare *et al.*, 2013; Giuggioli *et al.*, 2015). Furthermore, change in colour is the potential indicator of change in shelf life and maturity of fresh and FCF (Fagundes *et al.*, 2015). Various studies reported the beneficial effect of active MAP on the significant retention of colour such as, for 'Hicaznar' pomegranate (Ayhan & Eştürk, 2009), for 'Wonderful' pomegranate (Belay *et al.*, 2017) for banana (Kudachikar *et al.*, 2011), fresh-cut papaya (waghmare & Annapure, 2013), 'Eva' apple (Fante *et al.*, 2014), and strawberry (Giuggioli *et al.*, 2015).

Colour change related to the effect of an enzymatic browning under high O_2 MAP has been associated with the substrate inhibition of enzyme polyphenol oxidase (PPO) (Limbo *et al.*, 2007). Molinu *et al.* (2016) also linked the increase in hue angle (*h*°), which implicates a little colour change from reddish-orange to red-magenta with polyphenol oxidase (PPO) activity during storage for 'Sanguinello Comune' blood orange. During MAP, the type of packaging material significantly affected the evolution of PPO activity of fresh-cut apples, whereas, those packed in plastic bags with low O_2 permeability exhibited steadier inhibition (Soliva-Fortuny *et al*., 2001). In this study, storage atmosphere also significantly affected the PPO depilation, where apple cubes packed in MAP were efficiently preserved from browning when O_2 is initially absent.

In most literature, the advantages of low or super-atmospheric O_2 concentrations have been reported for maintaining the different colour attributes. Higher (L*) and hue angle (h°) values of the cut surface of pears were best maintained under low O_2 (2.5%) and passive atmosphere, on the contrary, a significant decrease in (lightness) L* and h° values under super-atmospheric O_2 was observed (Oms-Oliu *et al*., 2008a). Comparable L* and h° values were found among all super-atmospheric O_2 (40, 60, 80 or 100% O_2) condition in Chinese bayberry and strawberry compared to air storage (Yang *et al*., 2008). The significant effect of super-atmospheric O_2, maintaining the vivid colour, Chroma (C*) and L* of strawberries compared to air storage were reported by Ayala-Zavala *et al*. (2007). Similarly, L* of fresh-cut kiwifruit maintained during active MAP (5% O_2, 5% CO_2 and 90% N_2O) was reported by Rocculi *et al*. (2004). However, there is still a limitation of MAP to maintain the required colour of the other fruit, due to enzymatic browning of such as fresh-cut pears was reported by (Oms-Oliu *et al*., 2008b), carotenoids deceased in orange (Alasalvar *et al*., 2005) and colour loss of litchi (Sivakumar & Korsten, 2006).

Ayhan & Eştürk (2009) reported that gas composition (low and super-atmospheric O_2) did not have a significant effect on the colour parameters for 'Hicraznnar' pomegranate arils stored at 5 °C for 9 days. Similarly, no significant effect of active MAP was reported by Maghoumi *et al*. (2014) on C* value during storage of 'Malese-Saveh' pomegranate arils. However, loss of colour may not be exclusively attributed to chemical reaction, but rather to a summation of many appearance defects, some of which may result from excessive loss of water (Nunes & Emond, 2007). This indicates that the loss of colour during MAP can be maintained by monitoring and controlling both enzymatic and non-enzymatic activities. The enzymatic activities can be controlled by reducing the O_2 concentration, and loss of colour related to water loss can be controlled by appropriately selecting packaging film, which can regulate the in-package RH.

Texture

Texture profile is an important physical attribute that has been associated with quality and storage life of fruit (Ali *et al*., 2004; Toivonen *et al*., 2008). Texture profile is used to determine produce freshness and it can be affected by traits such as cellular organelles and biochemical constituents, water content and cell wall composition (Aday *et al*., 2011; Waghmare &

Annapure, 2013). Thus, any external factor affecting these traits can modify texture and can lead to changes in final quality (Barrett *et al*., 2010). The effect of MAP on maintaining of fruit firmness is usually related to the control of weight loss, where in most studies samples with highest weight loss have shown a higher reduction in texture. MA can influence the textural quality and water content of fruit and vegetables by reducing the RR (Angós *et al*., 2008; Oms-Oliu *et al*., 2008a). Postharvest softening of fruit structure occurs due to the biochemical processes that involve the direct suppression of activities of enzymes (pectin esterase and polygalacturonase enzymes) leading to deterioration in the cell structure, cell wall composition and intracellular materials (Fagundes *et al*., 2015). According to Ali *et al*. (2004), MAP can suppress the activity of the major cell wall hydrolysing enzymes.

Under active-MAP, the potential effect of high and super-atmospheric O_2 concentrations towards maintaining or improving the texture properties of fresh produce has been widely reported than the low O_2 atmosphere. The texture of fresh-cut pears has best maintained by using super-atmospheric O_2 atmosphere, but loss of texture has observed at low O_2 atmosphere (2.5%) (Oms-Oliu *et al*., 2008). This study associated the loss of texture with excessive low O_2 atmosphere and accumulation of CO_2 in the package. However, in this study, it has reported that the progress decrease of texture did not dramatically affect the general appearance of fresh cut pears. Belay *et al*. (2017) reported a similar effect of super-atmospheric O_2 for 'Wonderful' pomegranate arils. The study reported by Allende *et al*. (2007) showed that strawberries treated with super-atmospheric O_2 and CO_2 enriched atmospheres were firmer than air stored fruit, both studies associated this with the accumulation of CO_2 in the package, but not directly related to the effect of super-atmospheric O_2. Similarly, Pérez and Sanz (2001) reported that firmness of strawberries was maintained by storing under super-atmospheric O_2 and enriched CO_2 (80% O_2, 20% CO_2 and 90% O_2, 10% CO_2) at 8 °C. This study further speculated that the mechanism of super-atmospheric O_2 induced firmness retention due to maintenance of constant activity of cell wall hydrolysing enzymes in high O_2 condition.

According to Harker *et al*. (2000), the enhancement of texture during super-atmospheric O_2 condition could be due to the effect of high CO_2 accumulation, where the study detail explained that cell-to-cell adhesion could be increased by 60% at CO_2 enriched atmosphere due to the change in the pH of the apoplast, with the subsequent precipitation of soluble pectin. However, according to Villanueva *et al*. (2005) loss of texture is more associated with the secondary cell wall components (*i.e.* lignin and cellulose) rather than the primary cell wall component (pectic substances); where the study further explained changes in pectic substances is less influenced by CO_2 concentration. On the contrary, Huyskens-Keil and Herppich (2013) reported that high CO_2 concentration reduces texture by inhibiting the synthesis of all cell wall components. Similarly, low firmness of strawberries treated with super-atmospheric O_2 (95%) was observed than those stored at low O_2 (3%) (Van der Steen *et al*., 2002). The author associated this phenomenon with the accumulation of CO_2 in the package. However, CO_2 mediated effects on texture are highly dependent on species, physiological age at the time of exposure, CO_2 concentration and duration of exposure (Huyskens-Keil & Herppich, 2013). On the other hand, Soliva-Fortuny *et al*. (2003) reported higher firmness retention for fresh-cut pear slices in the absence of O_2 during MAP; the study associated this with the oxidative damage causes a reduction in membrane integrity, which is eliminated during this storage. The study reported by Ayhan and Eştürk (2009) found that active-MAP application did not have significant affect ($p \geq 0.05$) the firmness of 'Hicaznar' pomegranate arils until 9 days cold storage at 5 °C

Various studies have reported the combination of chemical treatments, such as calcium chloride or of calcium lactate with MAP to maintain firmness of fresh-cut fruit (Rico *et al*., 2007; Soliva-Fortuny *et al*., 2003; Toivonen *et al*., 2008). Calcium application improved cell-to-cell adhesion, and thus mechanical strength, by forming ionic bridges between dimethyl esterified pectin molecule to produce cross-linked polymer network or either by retarding senescent changes (Toivonen *et al*., 2008). Abbasi *et al*. (2009) recommended the use of edible coating on fresh produce to reduce the availability of O_2 for respiration, and which in turn was considered to be responsible for reducing pectin-esterase and polygalacturonase enzymes activities, and hence retention of fruit firmness. Therefore, it can be suggested that various combinations of MAP, with or without chemical treatment, could be advantageous; however, further study is necessary to understand the combined effects of modified atmsophere and other postharvest treatments on individual fruit.

Organic substrates and bio-active compounds

Organic acids and total soluble solids

As the main substrates of respiratory metabolism, sugars and acids are depleted, thereby causing corresponding changes in total soluble solid (TSS) and titratable acidity (TA) during fruit storage (Zheng *et al*., 2008). According to Conesa *et al*. (2007), a slight decrease of the total organic acid concentration was unavoidable during MA storage as a general rule; this is consistent with their use as respiratory substrates. The acidity of the fruit is determined by the concentrations of the organic acids such as citric acid, malic acid and tartaric acid (Kader 2008). Abbasi *et al*. (2009) reported a decrease in malic and citric acid in mango, where the study associated this with conversion into sugars and further utilization in the metabolic process of the fruit. High CO_2 concentration can highly influence malic and citric acid concentrations by changing the pH (Holcroft & Kader, 1999). This study explained that malic and citric acid could decrease when CO_2 hydration and the production of HCO_3^- and H^+ may reduce intercellular pH. Similarly, negative correlation between TA and pH has reported by Abbasi *et al*. (2009). However, the response to CO_2 atmospheres can be very different in fruit that has a large, acidic vacuole than in fruit with a higher pH, or leafy tissues that do not accumulate high concentration of organic acids. Therefore, the decrease of malic acid could be due fruit ripeness (Pérez and Sanz, 2001).

Various studies reported the decrease in TA value during MAP storage. Ayhan and Eştürk (2009) reported that TA of 'Hicaznar' pomegranate arils decreased under MA conditions (passive and active). A similar result was observed for 'Mollar de Elche' pomegranate arils stored under UV-C treatment and super-atmospheric O_2 condition (Maghoumi *et al*., 2013). Both studies associated the decrease of TA with the metabolic activity during storage, in the fact that organic acids were used as a substrate for respiratory activity and an indicator for the degree of ripeness. Similarly, Sanz *et al*. (2000) found significantly lower TA concentration in strawberry fruit exposed to super-atmospheric O_2 (90% O_2 +10% CO_2) than fruit held in air after 9 days of storage at 8 °C. Pérez and Sanz (2001) found significantly lower TA value of strawberries under super-atmospheric O_2 (80% O_2, 20% CO_2 and 90% O_2, 10% CO_2). On the other hand, Zheng *et al*. (2008) reported that TA values under high O_2 and air treated fruit in berries (Chinese bayberry, strawberry and blueberry fruit) were not significantly different. It is possible to highlight from these studies that, the higher the metabolic rate due to super-atmospheric O_2 could accelerate the consumption of the acid, however the variation of the results for the same type of fruit could be cultivar dependent or factors related with fruit cultivars.

During storage, TSS of produce could decline, increase or remain unchanged depending on the type of fruit. For instance, climacteric fruit continue the ripening process after harvest (which can result in major changes in sugar content). This is also further associated with high RR of produce during storage. For instance, it was reported that the TSS value of 'Hicaznar' pomegranate arils remained unchanged under passive, low and super-atmospheric O_2 (Ayhan & Eştürk, 2009). Similarly, Maghoumi *et al*. (2013) detected no change in TSS of 'Mollar de Elche' pomegranate arils under super-atmospheric and UV-C treatment. Ayala-Zavala *et al*. (2007) reported that TSS of strawberry fruit decreased due to higher RR stored under high O_2 atmospheres, while strawberry kept under air storage showed a continuous increase in the TSS concentration until day 12, then decreased at the end of the storage period. The different change patterns of TA and TSS in different fruit and studies seem to be associated with the different effects of elevated O_2 on commodity RR (Zheng *et al*., 2008). Highest RR and a parallel TSS decrease in berries exposed to super-atmospheric O_2 (90 and 100% O_2 with 15% CO_2) for 14 days were reported by Wszelaki and Mitcham (2000).

In addition to storage atmosphere, RH could affect TSS. Storing fruit at lower RH (< 85%) could increase evapotranspiration, which can lead to higher TSS concentration in the tissue (Beckles, 2012). Abbasi *et al*. (2009) reported higher TSS produce stored under limited O_2 concentration due to the inhibitory effect on RR. The study further stated that the decrease in TSS was caused by a decline in the amount of carbohydrates and pectin, partial hydrolysis of protein and decomposition of glycosides into sub-unit during respiration. Therefore, according to the studies reported above, the effect of active MAP on TA and TSS values varies in different fruit type and storage conditions, therefore monitored by controlling RR and TR could be vital to maintain both TA and TSS.

Bioactive compounds

The concentration of bioactive compounds in fruit can be influenced by different postharvest treatments (Molinu *et al*., 2016) as well as preharvest factors (Mphahlele *et al*., 2014). The activities of antioxidants, such as ascorbic acid, anthocyanins, phenolic and various types of secondary metabolites, mostly composed of total phenolic compounds such as flavones, and flavonols have been linked to function as reactive O_2 species (ROS) scavengers (Yang *et al*., 2008). It has been reported that fruit stored under elevated O_2 concentration exhibited good antioxidant capacity but the decline could happen during prolonged storage possibly due to O_2 promoted oxidation of the main antioxidants including anthocyanin and other phenolic compounds (Zheng *et al*., 2003). The effect of MA on antioxidant activity varies in literature (Table 1). Ayala-Zavala *et al*. (2007) stated that the effect of O_2 concentrations on the properties; such as phenolic and antioxidant capacity could vary depending on the product, O_2 concentration, storage period and temperature. Furthermore, such diversification partly arises from the response specificity of a particular plant species for different stress conditions.

Total anthocyanin and phenolic concentration

The reported impacts of active MA on anthocyanin concentration varies in literature. Ayhan and Eştürk (2009) reported that active MAP significantly maintained anthocyanin concentration of 'Hicaznar' pomegranate stored at 5 °C for 18 days. Anthocyanin concentration of strawberry and blueberry fruit stored under 60% O_2 atmosphere showed a 1.5-folds increase (Zheng *et al*., 2003). Similarly, Molinu *et al*. (2016) reported a dramatic enhancement of anthocyanin concentration for blood orange under super-atmospheric O_2, resulting three times higher than the fruit stored at normal air. These studies associated the increase of anthocyanin concentration with the physiological response to oxidative stress during storage. On the contrary, anthocyanin concentration of ready-to-eat shredded orange was decreased under super-atmospheric O_2 (95%) storage (Alasavar *et al*., 2005). The study indicated that, the initial concentration of 5.1 ± 0.2 mg 100 g^{-1} reduced to 4.8 ± 0.2 mg 100 g^{-1}, 4.6 ± 0.2 mg 100 $g^{-1,}$ and 4.9 ± 0.2 mg 100 g^{-1} on day 13 under air, 95% and 90% O_2 atmospheres, respectively. Banda *et al*. (2015) also reported lowest anthocyanin concentration under a high O_2 atmosphere (30% O_2 and 10% CO_2) for 'Wonderful' pomegranate arils. The decline of anthocyanin under super-atmospheric O_2 atmosphere could be due to the ascorbic acid oxidation (Oms-Oliu *et al*., 2008a; Maghoumi *et al*., 2014), delay of fruit ripening process (Allende *et al*., 2007) or could be due damage during the fruit peeling (Ghasemnezhad *et al*., 2011).

Similar to anthocyanin, the effect of active MAP on the phenolic concentration of fresh fruit and vegetables varies widely in literature. Oms-Oliu *et al*. (2008b) reported higher production of phenolic compounds for ´Piel de Sapo´ melon under low O_2 atmosphere (2.5% O_2 and 7% CO_2) compared to those, which kept at normal air, 30 and 70% O_2 atmospheres. on the contrary, the lower phenolic concentration of strawberries stored under low O_2 concentration (20 to 40%) and higher phenolic concentration when the O_2 concentration increases to 60, 80 and 100% was observed by Ayala-Zavala *et al*. (2007). Similarly, Zheng *et al*. (2008) reported that high O_2 could induce the accumulation of phenolic compounds during the initial treatment period, but it also promotes the oxidation of these compounds after prolonged storage. Yang *et al*. (2008) found out an increase in phenolic concentration of Chinese bayberry under super-atmospheric O_2 concentration (80%) at 5 °C. Similarly, Maghoumi *et al*. (2013) reported increase in phenolic concentration of 'Malese-Saveh' pomegranate arils exposed to high O_2 compared to samples stored under air, while marked reduction of the phenolic compounds in strawberry was observed under super-atmospheric O_2 (Allende *et al*., 2007).

Furthermore, the reduction of phenolic concentration might be due to cell structure breakdown as part of senescence during storage and oxidation of phenolic compounds with polyphenol oxidase (PPO) and (POD) activities (Ghasemnezhad *et al*., 2011), which results in the synthesis and accumulation of phenolic compounds. However, the effect of super-atmospheric O_2 concentration on the total phenolic may vary depending on the commodity, genotypes, oxygen concentration, storage time and temperature (Zheng *et al*., 2008; Ghasemnezhad *et al*., 2011). Furthermore, studies associated the increase in phenolic concentration with increase in the activity of the phenylpropanoid pathway under a stressful condition such as the low or high concentration of O_2 (Ayala-Zavala *et al*., 2007). According to Oms-Oliu *et al*. (2008a), the accumulation of phenolic compounds is a physiological response to stress or injuries, wounding may stimulate phenylalamine ammonia lyase (PAL) activity during minimally-processing with the consequent further production of the phenolic compounds. From the above studies, a clear observation could be drawn that the effect of low or high O_2 atmosphere on phenolic concentration may vary depending on the commodity and O_2 concentration.

Ascorbic acid

Ascorbic acid is one of the most sensitive phytochemical components when fruit undergoes adverse postharvest handling and storage conditions (Li & Zhang 2015). Several studies reported that MAP application preserves ascorbic acid concentration more than another method (Fernández-León *et al*., 2013; Ye *et al*., 2012; Manolopoulou *et al*., 2013). Ascorbic acid oxidation is greatly favoured by the presence of O_2 and thus, a marked decrease was observed in fresh cut melon stored at 4 °C under 10% O_2 + 7% CO_2, air, 30 and 70% O_2, and the highest depletion was detected in fresh cut melon packed under > 30% O_2 atmosphere (Oms-Oliu *et al*., 2008b). Similarly, Siliveira *et al*. (2014) observed higher ascorbic acid concentration in watercress leaves under lower O_2 and CO_2 atmosphere. The study further stated that the concentration of O_2 and CO_2 has effect on ascorbic acid oxidation. High CO_2 at injurious concentrations for the commodity may reduce AA by increasing ethylene production and thus, the activity of ascorbate peroxidase (Lee & Kader, 2000). On the other hand, a marked decrease of ascorbic acid also observed for samples under a low O_2 atmosphere (2.5% O_2 + 7% CO_2) from the beginning of storage despite the restriction in O_2 concentration. The study associated this result with the presence of oxidative stress, in turn an increase in peroxidase activity and ascorbic acid oxidation.

Tomás-Callejas *et al*. (2011) reported minimum ascorbic acid losses under active MAP treatments than passive MAP. This study further stated that ascorbic acid losses were between 8 and 15% for active MAP and 50% for passive MAP. However, Oms-Oliu *et al*. (2008b) reported marked decrease of ascorbic acid under MAP treatment. The study by Allende *et al*. (2007) reported higher retention of ascorbic acid for strawberries under super-atmospheric O_2 and enriched CO_2 (80% O_2 + 10% O_2 + 10% CO_2). Yang *et al*. (2008) reported a reduction of ascorbic acid concentration for Chinese bayberry stored under super-atmospheric O_2 concentrations (80 and 100%) and normal air packaging at 5 °C. However, no significant difference was found in the loss of ascorbic acid between the air and super-atmospheric O_2 treated fruit. In addition to the effect of storage atmosphere, fruit nature by itself has an effect on ascorbic acid concentration during storage, where for climacteric fruit; the increase of ascorbic acid during storage time could be associated with the postharvest dehydration and ripening. Therefore, the higher ascorbic acid found in the advanced stages of ripening might be related to increased level of glucose, which is a precursor of AA (Ornelas-Paz *et al*., 2012). Therefore, in order to control reduction of ascorbic acid during storage under MAP, emphasis should be given to both the storage atmosphere and the type of the fruit.

Volatile organic compounds

The increase in the synthesis and formation of volatiles organic compounds (VOCs) can be explained by an acceleration of metabolism as a response to storage atmosphere, which could lead to stress and disrupt enzyme systems (Amaro *et al*., 2012; Giuggioli *et al*., 2015). Prolonged storage can cause adverse effects, such as accumulation of ethanol, acetaldehyde and aerobic respiration when O_2 concentration decreased below 2% (Soliva-Fortuny *et al*., 2003; Fonseca, 2005; Oms-Oliu *et al*., 2008). This leads to undesirable fermentation, causes off-flavour and odours (Van der Steen *et al*., 2002). Low O_2 concentration (< 2%) and high concentration of CO_2 (> 20%) can initiate the development of hazardous anaerobic conditions (Jacxsens *et al*., 2001) membrane damage (Li *et al*., 2014) or further it could cause cell injury and senescence (Li & Zhang, 2014). However, acute low O_2 injury cannot be expressed until the tissue is re-aerated, and a consequent uncontrolled O_2 burst occurs resulting lipid peroxidation, protein denaturation and membrane injury (Toivonen & Hodges 2011).

Low O_2 and high CO_2 concentrations are responsible for the accumulation of alcohol, which leads to the production of ethyl esters and the reduction of other esters (Giuggioli *et al*., 2015). Furthermore, the increase of VOCs at low O_2 could be associated with exceeding the range of tolerance induced anaerobic metabolism, which can stimulate the production of fermentative compounds (Zhang *et al*., 2013a). Amanatidou *et al*. (2000) also reported that ethanol and acetaldehyde production increased for processed carrots stored at 1% O_2 and 10% CO_2. The study further observed a higher rate of acetate ester production under a low O_2 atmosphere (5%). This phenomenon has discussed by Blokhina *et al*. (2003), where the low O_2 condition reach anoxic metabolism, it leads to acidification of the cytoplasm and decreased pH which attributed to inhibition of lactate dehydrogenase and activation of pyruvate decarboxylase by the acidic pH leads to accumulation of acetaldehyde. On the other hand, Amaro *et al*. (2012) reported the significant inhibition of ethylene biosynthesis when the concentration of O_2 reduced below 8%, In contrast, ethanol concentration, were very low under low O_2 (1% and 3% O_2) atmospheres.

Kader and Ben-Yehoshua (2000) reported that the elevated O_2 atmosphere could affect the synthesis and accumulation of some VOCs associated with respiratory metabolism. According to Oms-Oliu *et al*. (2008a), acetaldehyde concentration began to accumulate in packages stored under super-atmospheric O_2 (70%) and low O_2 (2.5%) atmospheres after 1 week of storage with a continuous increase throughout the storage time. It was reported that accumulation of acetaldehyde, ethanol and ethyl esters are the first indicator of fermentative metabolism and among these, the most harmful is acetaldehyde, which can rapidly convert into ethanol by the enzyme alcohol dehydrogenase (ADH) (Thewes *et al*., 2015).

Similarly, Ayala-Zavala *et al*. (2007) also reported the effect of the super-atmospheric O_2 atmosphere on the synthesis and accumulation of aroma compounds. The study reported that the higher the O_2 concentration the lower the emission of aroma compounds, where the high O_2 atmosphere has limited the production of methyl methanoate, ethyl butanoate, butyl acetate, ethyl hexanoate and hexyl acetate. Strawberries stored at pure O_2 atmosphere showed a clear difference from those stored in 20% CO_2 combined with 2-20% of O_2 (Berna *et al*., 2007). In this study, it has reported that the presence of O_2 without CO_2 suppressed the production of ethyl acetate which is one of the most important off-flavour volatiles after four days of storage. On the other hand, Teixeira *et al*. (2016) reported relative concentration of Limonene for guava stored under super-atmospheric O_2 concentration (100%).

Microbial growth under active atmosphere

The total microbial load on fruit can be modified by the initial O_2 and CO_2 concentration (Masson *et al*., 2002). A combination of a low O_2 (2–6%) and an elevated CO_2 (7–15%) is often used to extend the shelf life of fresh-cut produce by inhibiting fast-growing aerobes (Sandhya, 2010). Low growth rates of aerobic psychrophilic bacteria were observed in cut pears stored under MAP with an O_2 concentration below 2.5% than under passive MAP conditions (Oms-Oliu *et al*., 2008b). However, in most studies in literature, higher reduction of microbial growth by the effect of super-atmospheric O_2 is profound. Super-atmospheric O_2 has a potential effect of inhibiting the growth of naturally occurring spoilage microorganisms (Jacxsens *et al*., 2001; Oms-Oliu *et al*., 2008a). According to Jacxsens *et al*. (2001) effective inhibition of microbial growth can be achieved by the combined effect of super-atmospheric O_2 (70%) with high concentrations of CO_2 (10-20%) rather than using the individual gases alone.

The growth of yeast and aerobic bacteria contaminating pineapple cubes was retarded markedly by high O_2 and CO_2 concentrations (50% O_2 + 50% CO_2) (Zhang *et al*., 2013b). MAP with 95% O_2 concentration suppressed the growth of *L. monocytogenes* for fresh-cut celery (González-Buesa *et al*., 2014). Studies associated the significant microbial growth inhibition effect of super-atmospheric O_2 with high CO_2 concentration. High CO_2 concentration is generally effective to control the growth of a most aerobic microorganism, especially bacteria and moulds (Al-Ati & Hotchkiss, 2003). High O_2 concentration combined with high CO_2 concentrations (10–20%) was more effective in microbial control than the individual gases alone (Amanatidou *et al*., 2000). Thus, the application of super-atmospheric O_2 in a low O_2 permeable film allows the accumulation of CO_2 and, enhances its inhibitory effect on the growth of microorganisms. The effect of CO_2 could be explained by the formation of carbonic acid due to relative solubility of CO_2 in water, which has been suggested to inhibit microbial growth (Zhang *et al*., 2013b). The solubility of CO_2 enables penetration of bacterial membrane, which leads to intercellular pH changes, or formation of carbonic acid that has a bacteriostatic effect (Banda *et al.*, 2015; Paul & Clarke, 2002).

Banda *et al.* (2015) reported that yeast and mould counts did not significantly ($P \geq 0.05$) differ across the MAP treatments (low and high O_2) for 'Wonderful' pomegranate arils packed in the low barrier BOP film. In addition, Gomes *et al.* (2012) reported that the kinetic rate of microbial growth on the fresh-cut slices was not affected by O_2 concentration. However, different organisms vary greatly in their sensitivity to O_2 concentration and some of them could have developed strategies, such as the induction of other enzymes that decompose ROS, to avoid lethal damage (Kader & Ben-Yehoshua, 2000).

Conclusions

This review highlighted the state-of-the-art knowledge on approaches applied by researchers to design MAP systems, including the performance of packaging materials, physiological responses of fresh produce and the complexity of the mechanism by which active MAP affects produce quality attributes. The review showed the availability of appreciable amount of research data on the importance of proper design of MAP systems. Due to the complexity physiological and metabolic processes of the fresh produce, most of the approaches presented in literature rely on empirical models. However, the application of numerical models in MAP design and performance evaluation offers new opportunities for improved understanding and prediction of the interaction between product characteristics, packaging performance and environmental conditions. Limited studies reported on integrated modelling approach for MAP design of different fruit and vegetables. Integrated MAP models have the advantage of being relatively complete in describing the dynamic processes in a MAP system. Therefore, the modelling approach for MAP must balance between the product characteristics and its surrounding conditions. Therefore, research is needed to optimize gas concentration by considering the packaging atmosphere and permselectivity of the packaging materials. Further research on the influence of active MAP modelling with the different gas composition at various storage temperatures is required for the emerging horticultural crops.

Furthermore, the compiled information in this review shows that the major factors influencing the performance of active MAP include the storage atmosphere and environmental condition, type of fruit, and packaging material. The mechanisms by which storage atmosphere affected fruit quality were mostly associated with the action of enzymatic oxidation, reactive O_2 species (ROS), pH and water content. Most of the literature reported that low O_2 atmosphere, alone or in combination with elevated CO_2 is successful to extend the shelf life by reducing decay, organic acid metabolism, delay softening, and retain colour. On the other hand, the effect of super-atmospheric O_2 atmosphere has widely reported to inhibit microbial growth and maintain fruit firmness but adversely affected fruit colour, reduction in organic acids. However, a contradiction exists in the literature regarding the benefit of active MAPs; this indicates the importance of research for individual fruit type at different storage conditions. Furthermore, the review found out that, the potential use of biodegradable packaging films on active MAP application is limited.

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Table 1. Summary of parameters considered in MAP modelling and design.

Produce physiology
Respiratory oxygen consumption and carbon dioxide production as a function of fruit internal O_2 and CO_2 concentration and fruit temperature
Cumulative O_2 consumption
Carbon loss through respiration

Gas Transport
Exchange of gases between the fruit internal atmosphere and the packaging atmosphere
Permeation of O_2, N_2 and CO_2 through the packaging film
Diffusion and flow of O_2, N_2 and CO_2 across the packaging film

Moisture Transport
Evaporation of moisture from the fruit surface
Sorption of moisture from the product
Condensation of moisture on the surface of the fruit and on the internal surface of the packaging film
Permeation of water vapour through the packaging film
Diffusion and flow of water vapour through the packaging film

Heat Transfer
Respiratory heat generated
Evaporative heat loss from the fruit
Convection heat transfer at the fruit and the packaging film surface
Conduction heat transfer through the packaging material

Source: (Merts, 1996)

Table 2. Methods for measuring and quantifying respiration rate of fresh produce.

Type of system	**Basic equation**	**References**
Closed system	$Ro_2 = \frac{(Yo_{2ti} - Yo_{2\,tf}) \times V_f}{100 \times M \times (t_f - t_i)}$ $Rco_2 = \frac{(Yco_{2tf} - Yco_{2\,ti}) \times V_f}{100 \times M \times (t_f - t_i)}$	Iqbal *et al.* (2009); Song *et al.* (2002); Ravindra and Goswami (2008); Mangaraj and Goswami (2011)
Flow through system	$Ro_2 = \frac{(Yo_{2in} - Yo_{2\,out}) \times F}{100 \times M}$ $Rco_2 = \frac{(Yco_{2\,out} - Yco_{2\,in}) \times F}{100 \times M}$	Cliffe-Byrnes and Beirne (2007); Finnegan *et al.* (2013)
Permeable system	$Ro_2 = \frac{(Yo_2 \times A)}{100 \times l \times M} \times (Po_{2i} - Po_{2f})$ $Rco_2 = \frac{(Yco_2 \times A)}{100 \times l \times M} \times (Pco_{2f} - Pco_{2i})$	Gomes *et al.* (2010)

Table 3. Examples of the application of experimental methods and model equations to estimate the respiration rate of selected types of fresh produce under MAP.

Produce	**Experimental method**	**Model**	**Model formulas**	**Fitting**	**References**
Romaine lettuce	CS	MM, Quadratic model Linear model	$Ro_2 = (\%O_2 \times 10)\,{}^{V}/_{W_t}$ $Rco_{2_2} = b + m_1Yco_2 + m_2Yo_2 + m_3Yco_2Yo_2 + m_4{Yco_2}^2 + m_5{Yo_2}^2$ $Ro_2 = b + m_1Yco_2 + m_2Yo_2$		Segall and Scanlon (1996)
Blueberry	CS	MM	$Ro_2 = \frac{V_{max} \times Yo_{2i}}{Km + (1 + Yco_{2i}/K_i)Yo_{2i}}$ $Rco_2 = \frac{V_{max} \times Yo_{2i}}{Km + (1 + Yco_{2i_i}/K_i)Yo_2}$	Good	Song *et al.* (2002)
Prickly pear cactus cladodes	CS	Arrhenius- And linear Model	$\ln(RR) = \left(-0.1985 + 50.96 \times \left(\frac{1}{273.15 + T}\right)\right) \times RH + 67.64 - 18430 \times \left(\frac{1}{273.15 + T}\right)$	NA	Guevara *et al.* (2006)
Tomato	PF	MMN	$Ro_2 = \frac{V_{max} \times Yo_2}{(Km + Yo_2) \cdot \left(1 + \frac{Yco_2}{Ki}\right)}$	Fit	Charles *et al.* (2003)
Shredded carrots	CS	MMU	$Ro_2 = \frac{V_{max} \times Yo_2}{Km + Yo_2 \times \left(1 + \frac{Yco_2}{K_i}\right)} \times e^{\left[\frac{E_a}{R_c} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]}$	NA	Iqbal *et al.* (2009)
Fresh-cut pear	PF	MMU	$Ro_2 = \frac{V_{max} \times Yo_2}{(Km + Yo_2) \times (1 + Yco_2/Ki)}$	NA	Gomes *et al.* (2010)
Pomegranate Arils	CS	MMU	$Ro_2 = \frac{3.1 \times O_2}{O_2 + 3.8 \times \left(1 + \frac{CO_2}{115.2}\right)}$	Good	Ersan *et al.*, (2010)
Minimally processed broccoli	CS	MMC	$Ro_2 = \frac{V_{max} \times Yo_2}{Km \cdot \left(1 + \frac{Yco_2}{K_i}\right) + Yo_2} \times e^{\left[\frac{E_a}{R} \times \left(\frac{1}{T} - \frac{1}{T_o}\right)\right]}$	Good	Torrieri *et al.* (2010)

RO_2 , O_2had been presented as KO_2, and Yo_2, co_2 has been presented as$[o_2]$, $[co_2]by$ Segall & $Scanlon$ (1996) and Yo_2, co_2 has been presented as $[gas]$, by Song *et al.* (2002), CS (closed system), PS (permeability system), FS (flow through system)

Table 3. (continued)

Produce	**Experimental method**	**Model**	**Model formulas**	**Fitting**	**References**
Applicable for fruit and vegetables	CS	MMC and MMU	$Ro_2 = \frac{e^{\left[\frac{E_a}{R_c}\times\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]}[O_2]}{Km(1+[CO_2]/K_i)+[O_2](1+[CO_2]/K_i)} RRM_{O2}$	NA	Rennie and Tavoularis (2009)
		MMC and MMU	$Ro_2 = \frac{e^{\left[\frac{E_a}{R_c}\times\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]}[Yo_2]}{Km(1+[Yco_2]/K_i)+[Yo_2](1+[Yco_2]/K_i)} RRM_{O2}$ $Rco_2 = \frac{e^{\left[\frac{E_{aCO2}}{R_c}\times\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]}[Yo_2]}{Km(1+[Yco_2]/K_i)+[Yo_2](1+[Yco_2]/K_i)} RRM_{CO2}$	NA	
Pomegranate fruit and arils	CS	Arrhenius-type equation	$YO_2 = Y_{O2} - R_{O2.ref} \times e^{\left[\frac{(-E_aO_2)}{R\left(\left(1/T\times 1/T_{ref}\right)\right)}\right]} \frac{W}{V_f}(t-t_i)\times 100$ $YCO_2 = Y_{CO2} + R_{CO2.ref} \times e^{\left[\frac{(-E_aCO_2)}{R\left(\left(1/T\times 1/T_{ref}\right)\right)}\right]} \frac{W}{V_f}(t-t_i)\times 100$	Good	Caleb *et al.* (2012a)
Pomegranate fractions	CS	Arrhenius	$Rco_2 = R_{CO2.ref} \times e^{\left[\frac{E_{aco2}}{R}\times\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]}$	Good	Aindongo *et al.* (2014)
Strawberries	PS	MM and Arrhenius	$Ro_2 = \frac{V_{max} Yo_2}{Km + Yco_2}$ $Ro_2 = RQ \cdot R_{O_2}$ $vm_{o_2} = A\, exp\left(\frac{-Ea}{RT}\right)$	Good	Barrios *et al.* (2014a)
Fresh-cut pineapple	FS	Exponential decay	$\mathrm{CO_2^{out}} = \frac{\left[\mathrm{R_{co2}^{eq}} + \left(\mathrm{R_{co2}^{i}} - \mathrm{R_{co2}^{eq}}\right)\mathrm{e}^{-\alpha t}\right]\cdot \mathrm{W} \times 100}{\mathrm{V_F} \times 60} + \mathrm{CO_2^{in}}$	NA	Finnegan *et al.* (2013)
		Multiple linear regression	$Ro_2 = \frac{V_{mmax}[Yo_2]}{Km + (1+[Yco_2]/K_i)[Yo_2]}$ $Rco_2 = \frac{V_{max}[Yo_2]}{Km + (1+[Yco_2]/K_i)[Yo_2]}$	NA	Lu *et al.*, (2013)

CS (closed system), PS (permeability system), FS (flow through system)

Table 4. Parameters considered in the development of mathematical models for respiration rate of fresh produce**.**

Type of Model	**Parameters**	**Produce**	**References**
MMU	Temp and GC	Shredded galega kale	Fonseca *et al.* (2002a)
MMU	GC, RH and Temp	Blueberry	Song *et al.* (2002)
MM & Arrhenius	Temp and GC	Whole & sliced mushrooms	Cliffe-Byrnes and Beirne (2007)
MMU	Temp, GC and time	Green mango	Ravindra & Goswami (2008)
MMU	Temp and GC	Shredded carrots	Iqbal *et al.* (2005)
Arrhenius	Temp and GC	Whole mushrooms	Iqbal *et al.* (2009)
MMN & Arrhenius	OC and Temp	Pear	Gomes *et al.* (2010)
MMC & Arrhenius	Temp and GC	Minimally processed broccoli	Torrieri *et al.* (2010)
MMU & Arrhenius	Temp and GC	Guava	Mangaraj and Goswami (2011)
Arrhenius	Storage Temp and time	Pomegranate fruit and arils	Caleb *et al.* (2012)
Exponential decay	Temp and GC	Fc pineapple	Finnegan *et al.* (2013)
Arrhenius	Time and Temp	Fc produce	Waghmare *et al.* (2013)
Arrhenius	Temp	Pomegranate fractions	Aindongo *et al.* (2014)

GC & OC are gas and oxygen concentration respectively, Temp is temperature, RH is relative humidity and Fc is fresh cut

MM: Michaelis–Menten; MMU: Michaelis–Menten uncompetitive; MMC: Michaelis–Menten competitive; MMN: Michaelis–Menten non-competitive

Table 5. Selected methodologies applied to model gas and water vapour permeability in MAP of various types of fresh produce.

Method	Model equations	Produce	References
Gas permeability models	$J_i = P_i \frac{A_P}{L}(p_{i.1} - p_{i.2})$	Burlat cherries	Salvador *et al.* (2002)
	$J_{CO_2} = \frac{P_{CO_2}}{L} A_P\left(pCO_2^{in} - pCO_2^{out}\right)$	Cut rutabaga	Zhu *et al.* (2002)
	$\frac{\partial[\mathrm{Yo_2, co}]_{pkg}}{\partial_t} = \frac{P^{gas}}{dV_{pkg}}\left([\mathrm{Yo_2, co}]_{atm} - [\mathrm{Yo_2, co}]_{pkg}\right)$	Prickly pear cactus	Guevara-Arauza *et al.* (2006)
	$\ln \frac{\mathrm{Yo_2, co_2}{}_{i,0}^{in} - \mathrm{Yo_2, co_2}{}_{\mathrm{i}\,i,e}^{out}}{\mathrm{Yo_2, co_2}{}_{i,0}^{in} - \mathrm{Yo_2, co_2}{}_{i,e}^{out}} = D_i \frac{P_T t}{V_b}$	Kiyomi fruit.	Techavises and Hikida (2008)
	$\ln\left(\frac{\mathrm{Yo_2, co_{2.t} - Yo_2, co_{2.i}}}{\mathrm{Y} - Y_{\mathrm{out,i}}}\right) = \mathrm{P_i} \frac{P_T t^{\Delta X}}{\mathrm{V}}$	Fresh Produce	Li *et al.* (2010)
	$\frac{\partial c_{O_2}}{\partial t} + \nabla.\left(-D_{O_{2\_air}} \nabla \mathrm{c}_{O_2}\right) = 0$ $\frac{\partial c_{CO_2}}{\partial t} + \nabla.\left(-D_{CO_{2\_air}} \nabla \mathrm{c}_{CO_2}\right) = 0$	Strawberries	Barrios *et al.* (2014b)
Water vapour permeability models	$(mNA)_{W_2} = -D\left[\frac{m_W A_P P_T}{RT}\right]\left(P_{W_1} - P_{W_2}\right)$	Fresh fruit and vegetable	Dirim *et al.* (2004)
	$(mNA)_{W_2} = \frac{d(p_1 - p_2)}{d_2}$		
	$\mathrm{F_p}{}^{H2O} = +\mathrm{F}_{\mathrm{K}_{\mathrm{VPD}}^{\mathrm{gas}}}\left(\mathrm{F}_{\mathrm{P}_O^{H2O}} + \mathrm{F}_{\mathrm{P}_{ref}^{H20}}\, exp^{\alpha VPD}\right)$		
	$WVTR = \frac{M_t - M_o}{\Delta t}.\frac{1}{A_P}$	Fresh Produce	Li *et al.* (2010)
	$P_{O_2CO_2} = P_{ref}{}^{e\left(-E_a/R\left[(1/T)-\left(1/T_{ref}\right)\right]\right)}$	Strawberries	Sousa-Gallagher *et al.* (2013)

$[gas]$ is O_2 and CO_2: Subscript; *i* O_2; CO_2 & N_2 gases, P_T is total pressure inside the package; M is molecular weight, N is molar flux

Table 6. Approaches for modelling permeability properties of packaging materials used in MAP of fresh produce.

Parameters	**Model**	**Produce**	**References**
Perforation tube diameter & Temp	Gas permeability	Fruit and vegetables	Fonseca *et al*. (2000)
Perforation hole	Gas permeability	Fresh fruit and vegetable	Paul and Clarke (2002)
Perforation, Temp & RH	Gas permeability	Mandarin segments and strawberries	Del-Valle *et al.* (2003)
Temp & RH	Gas permeability modelling	Prickly pear cactus cladodes	Guevara-Arauza *et al.* (2006)
Perforation tube, diameter, length, & Temp	WVTR	Mushrooms	Mahajan *et al.* (2008)
Size & number of micro perforation	Gas permeability	Fresh-cut fruit and vegetables	González-Buesa *et al.* (2009)
Perforation diameter, film thickness & Temp	Gas and WVTR	Fruit produce	Li *et al.* (2010)
Amount of produce and perforation tube dimension	Gas permeability	Fresh products	Montanez *et al.* (2010)
Perforation	Gas permeability	Strawberries	Xanthopoulos *et al.* (2012)

Temp, temperature; RH, relative humidity; WVTR, Water vapour transmission rate

Table 7. Recent publication on effect of MAP on quality, shelf life and individual gas concentration for fruit.

Fresh produce	**MAP types**	**MAP conditions**	**Effects on quality**	**References**
Strawberries	Super-atmospheric O_2 and CO_2 enriched atmospheres	80 and 10% O_2 Treatment with UV-C and ozone	Super-atmospheric O_2 and CO_2 enriched atmosphere, reduced total phenolic content The use of UV-C light and O_3 significantly reduced total phenolic content	Allende *et al.* (2007)
Strawberries	Air, high and Super-atmospheric O_2	20, 40, 60, 80 and 100% O_2	High and super-atmospheric O_2 atmospheres maintained high antioxidant capacity, total phenolic, less decay and long shelf life, whereas lower concentration of volatile compounds than air	Ayala-Zavala *et al*. (2007)
Strawberries	Low, Air, high and Super- atmospheric O_2	5, 20,50, 60, 80 and 100% O_2	Super-atmospheric O_2 without CO_2 suppressed the production of ethyl acetate. Samples stored in the absence of CO_2 were characterized by methyl hexanoate, which increases during ripening. Off-flavour at air atmosphere	Berna *et al*. (2007)
Fresh cut melon	Low and Super-atmospheric O_2	2,5 and 70% O_2	Anaerobic fermentation prevented by 70% O_2, whereas, low O_2 (2.5%) triggered fermentative pathway. Super-atmospheric O_2 maintained colour and firmness better than low O_2 or air	Oms-Oliu *et al*. (2008a)
Fresh cut melon	Low, Air, high and Super-atmospheric O_2	2.5, 10, 21 and 70% O_2	Vitamin C and phenolic maintained at low O_2, low O_2 (2.5%) induced peroxide activity, which is directly related to the change in vitamin C Super-atmospheric O_2 reduced wounding stress and deteriorative changes related to high peroxide activity in tissue	Oms-Oliu *et al*. (2008b)
Chinese bayberry fruit	Super-atmospheric O_2	80 and 100% O_2,	Decay caused by fungus was not suppressed by the atmosphere. Both MAPs' maintained higher total soluble solid (TSS), reduced ethylene production and lower pH, ascorbic acid was not significantly affected	Yang *et al*. (2008)
Plum		18 and 10.3% CO_2	Titratable acidity lower, Pulp hardness deceased	Peano *et al.* (2009)

Table 7. *continue*

Fresh produce	**MAP types**	**MAP conditions**	**Effects on quality**	**References**
Ready to eat table grape	Super-atmospheric O_2	5, 10, 15 % O_2 and 3% CO_2	Acceptable sensory quality from 3-50 days Bacterial count was below the detection limit (10^2 log CFU mL^{-1})	Costa *et al*. (2011)
Fresh-cut papaya	Low O_2	$CaCl_2$ (1% w/v), citric acid (2% w/v), 5% O_2 and 10% CO_2	Better firmness, lightness decreased, decreases in sensory scores Shelf life 25 days	Waghmare and Annapure (2013)
Guava	Air and low O_2	5% O_2 and 1, 5, 10, 15 and 20% CO_2	The increased in CO_2 concentration negatively affected the fruit quality by accelerating changes in colour (h) and firmness CO_2 injury observed at 5% O_2 with 15 and 20% CO_2 with increase in pH value and soluble pectin content, inversely related to firmness	Teixeira *et al*. (2016)
Prickly pear	Air and low O_2	Air, 6.25% O_2 + 0.1% CO_2	Delay in weight loss and total colour change No significant difference has shown on phenolic concentration across the atmospheres	Ochoa-Velasco and Guerrero-Beltrán (2016)
Blood orange		Air 76% O_2	Super-atmospheric O_2 atmosphere increased anthocyanin concentration three times higher than those stored under air Increase in phenolic concentration under super-atmospheric O_2 atmosphere Decrease of sugars (glucose, fructose and sucrose)	Molinu *et al*. (2016)

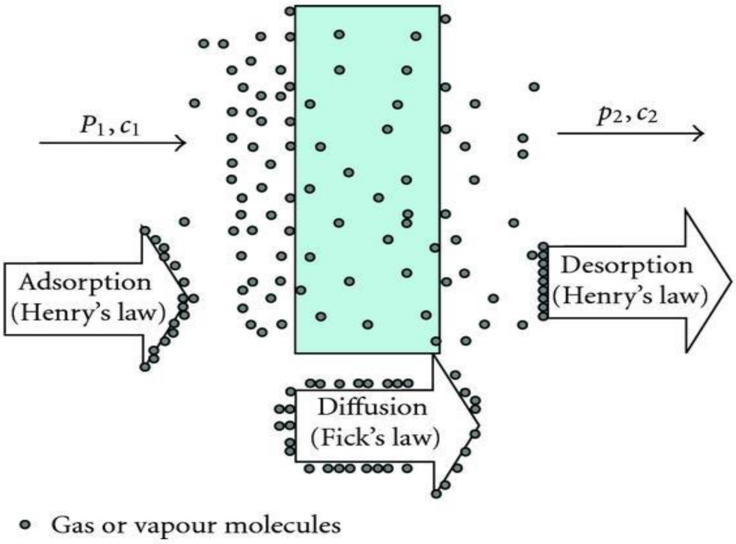

Figure 1. Permeation process for gas or water vapour through the Polymeric film (adapted from Siracusa, 2012). P_1 and C_1 are permeant pressure and permeant concentration respectively in the film at high concentration side; P_2 and C_2 are permeant pressure and permeant concentration in the film at low concentration side.

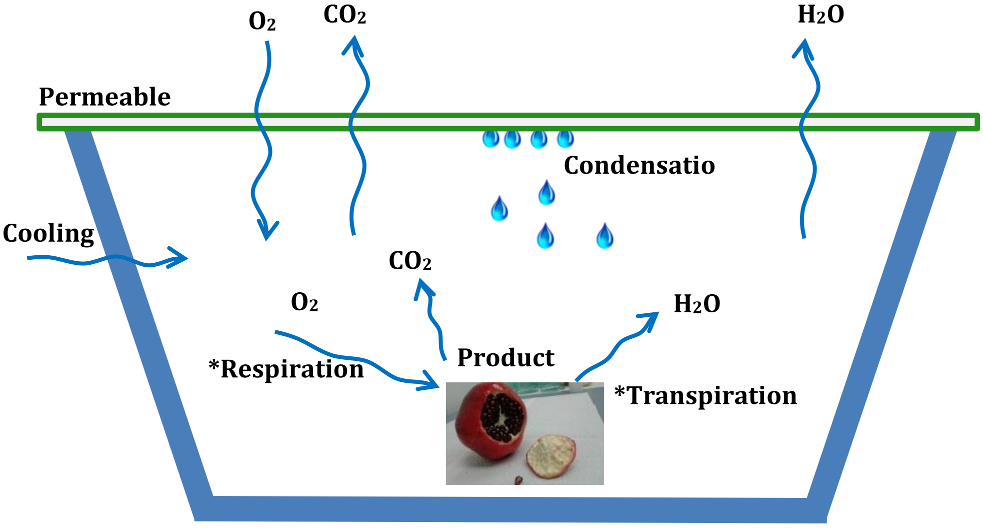

Figure 2. Overall process in modified atmosphere packaged fresh product. *Continuous phenomena within and around packaged product.

Declaration by the candidate:

With regard to Chapter 3 (pp 59-79); the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data collection and analysis, and writing of chapter	75

The following co-authors have contributed to Chapter 3 (pp 59-79):

Name	E-mail address	Nature of contribution	Extent of contribution (%)
Prof U.L. Opara	opara@sun.ac.za	Research input, editorial suggestion, experimental design, and proof reading	10
Dr O.J. Caleb	CalebO@arc.agric.za	Research input, editorial suggestion and proof reading	10
Dr P.V. Mahajan	PMahajan@atb-potsdam.de	Research input, editorial suggestion and proof reading	5

Signature of candidate: Z.A. Belay

Date: 03/11/2017

Declaration by co-authors:

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 3 (pp 59-79)
2. no other authors contributed to Chapter 3 (pp 59-79) besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 3 (pp 59-79) of this dissertation.

Signature	Institutional affiliation	Date
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Declaration with signature in possession of candidate and supervisor.

CHAPTER 3

RESPONSE OF 'WONDERFUL' POMEGRANATE ARILS TO LOW O_2 STRESS UNDER ACTIVE MODIFIED ATMOSPHERE

ABSTRACT

Successful characterization of the relationship between respiration rate (RR) and low O_2 limit (LOL) is critical for optimizing modified atmosphere packaging (MAP). It is well documented that low O_2 atmosphere reduces the RR of fresh produce, but could also lead to abiotic stress due to the accumulation of glycolysis end products. Therefore, this study investigated the response of pomegranate arils exposed to low O_2 atmosphere (2% O_2 + 18% CO_2 + 80% NO_2) and identified the low O_2 limit at 5 °C and 10 °C. The study aim was achieved by using a real time RR and respiration quotient (RQ) data, microbial growth and by identifying changes in volatile organic compounds (VOCs) profile (based on shift from aerobic to anaerobic respiration). The headspace gas evolution was significantly affected by the storage temperature, however minimum change was observed at 5 °C. RQ value was (1.0) at 5 °C and a sharp increase around 3.0 was observed at 10 °C. Response of the arils to low O_2 stress involves making alterations to the metabolic composition, especially those involved in anaerobiosis such as the accumulation of ethanol, acetaldehyde, and hydrazine, methyl-complex as observed in this study. Furthermore, the increase in yeast growth was observed as the O_2 concentration declines. The low O_2 tolerance limit of 2.1 and 2.3% was observed at 5 °C and 10 °C, respectively.

Keywords: Respiration rate; Respiratory quotient; Volatiles; Off-odour; Microbiology

Introduction

Active modified atmosphere packaging (active-MAP) systems accelerate the establishment of optimum gas within the packaged product by initial displacement of atmosphere for desired and suitable gas composition (Ye *et al*., 2012). Delaying physiological deterioration of fresh fruit under low O_2 atmosphere storage is commonly lined to the slowing down of respiration rate (RR). It is well documented that low O_2 concentration in modified atmosphere slows down physiological responses and maintains quality of pomegranate arils during postharvest storage (Banda *et al*., 2015; Ersan *et al*., 2009; López-Rubira *et al*., 2005). Ersan *et al*. (2009) showed that storage of 'Hicaz' pomegranate arils under low O_2 atmosphere (2% O_2 and 10% CO_2) at 4 °C resulted in the minimum RR of 1.5 mL kg^{-1} h^{-1} and 0.5 mL kg^{-1} h^{-1} for O_2 and CO_2, respectively. Similarly, the advantage of low O_2 (1-3% O_2 and 5-10% CO_2) at 5 °C for 'Mollar de Elche' pomegranate arils was reported by López-Rubira *et al*. (2005). In general, lower metabolic rates are known to provide longer postharvest life and quality of fresh produce (Weber *et al*., 2011). However, at low O_2 storage; successful characterization of the relationship between RR and low O_2 limit (LOL) is critical for optimizing modified atmosphere packaging (MAP) for fruit (Yearsley *et al*., 1996). Low O_2 limit is the consequence of the interplay of a diversity of physiological and biochemical processes (Yearsley *et al*., 1996). This may change with storage temperature, physiological age of the produce, and the duration of exposure to hypoxic atmospheres. Therefore, LOL should be used cautiously for optimization of storage atmosphere.

Under low O_2 condition, metabolism can switch from aerobic to anaerobic respiration (fermentation), which enables the continuation of glycolysis and substrate-level phosphorylation to produce energy in the form of ATP to insure survival. This process can lead to the production of off-flavour (Ampofo-Asiama *et al*., 2014). Therefore, this critical O_2 limit should be avoided during MAP in order to maintain fruit quality (Kader, 2002). According to Beaudry *et al*. (1992), the O_2 concentration at which tissue fermentation is induced may be taken as the lowest O_2 limit. Similarly, identification of the optimum atmosphere requires integration of information on the susceptibility of the fruit to physiological disorders and the effect of the atmosphere on the aroma production and other sensory attributes (Dadzie *et al*., 1996). The response of fruit to stress under low O_2 involves making alterations to metabolic composition, especially those involved in anaerobiosis, alcohol dehydrogenase and pyruvate dehydrogenase complex (Ampofo-Asiama *et al*., 2014).

The optimum storage atmosphere occurs just above the LOL at which aerobic respiration rate is at the lowest, without the development of anaerobic metabolism (Yearsley *et al*., 1996). Anaerobic respiration can be detected by the upswing in the RQ associated with the synthesis of acetaldehyde, ethanol and CO_2 (Beaudry *et al*., 1992). Identification of fermentative volatiles emitted due to anaerobic respiration to understand the response of horticultural commodity to low O_2 and other abiotic perturbations has gained tremendous attention in the last decade (Ampofo-Asiama *et al*., 2014).

Tolerance to low O_2 is product dependent, and tolerance to various exposure times is characteristic for each commodity (Bender *et al*., 2000). However, no study has been done to determine LOL for pomegranate arils under active MAP system. Therefore, the aim of this study was to investigate the effect of low O_2 storage and temperature on RR, RQ, a shift in volatile organic compounds (VOCs) and microbial growth. The goal of this study is to estimate the tolerance of 'Wonderful' pomegranate arils to low O_2 atmosphere and to identify the low O_2 limit.

Materials and methods

Fresh produce and sample preparation

Pomegranate fruit cv. 'Wonderful' were obtained at commercial maturity with characteristic deep-red skin and arils with mature kernel (Arendse *et al*., 2016; Crisosto *et al*., 2001), from Sonlia Pack House, Wellington, Western Cape (33°38′23″S, 19°00′40″E), South Africa. The fruit were air-freighted in well-ventilated boxes to Freshness Laboratory, Department of Horticultural Engineering, Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Germany. On arrival, the fruit were stored in a cold storage chamber at 5 °C until the fruit samples processed to extract the arils in the cold room at 5 °C. Damaged fruit were removed and the outer skin of selected healthy fruit was surface disinfected using 70% ethanol prior to processing (Belay *et al*., 2017). Arils were extracted manually by carefully removing the husk. Extracted arils were collected in a tray and mixed to assure uniformity.

Respiration rate set up and measurement

Arils (350 g) were transferred into 4000 mL glass cylinder, designed to achieve a completely hermetic seal. For continuous headspace gas monitoring, three glass jars at each temperature were set aside for this purpose and quality analysis was done at the end of storage. For quality attributes analysis a total of 12 jars were prepared for two storage temperatures for multiple triplicate sampling and three jars were pulled out for each sampling day. Each glass chamber lid was fitted with three valves (inlet, outlet and a gas sampling port). A rubber ring was fixed between the cylinder and the lid seal to prevent air leakage. A plastic tube was attached to the inlet valve, which was inserted down to the bottom of the jar to ensure uniform flushing of the gas mixture. Each storage chambers were flushed with humidified mixed gas (2% O_2 + 18% CO_2 + 80% N_2) until equilibrium archived. Thereafter, the glass chambers were closed. The gas concentrations inside the jars was measured every two minutes non-invasively and continuously using O_2 and CO_2 sensors for 9 days at 5 and 10 °C according to the method described by Luca *et al*. (2016). Calibration of the O_2 and CO_2 sensors was performed prior to each measurement. The O_2 sensor (PreSens, Precision Sensing GmbH, Germany) consisted of an electrochemical cell which measured the O_2 concentration in the range of 0-100% with a resolution of 0.01%. The CO_2 sensor (Vaisala GmbH, Bonn, Germany) consisted of an infrared port which measured the CO_2 concentration in the 0-25% range with an accuracy of 0.2%. The sensors were connected to a Squirrel data logger (Grant Instruments Ltd., Shepreth, United Kingdom) for automatic transfer of the measured voltage signals to a computer. From these data, the O_2 and CO_2 concentrations were calculated and the respiration rates were expressed in mL O_2 consumed (RO_2) and mL CO_2 produced (RCO_2) per kg^{-1} h^{-1} (mL kg^{-1} h^{-1}) using equation 1 & 2. From the RR data, the respiratory quotient (RQ) was calculated as RCO_2/RO_2, and the LOL was designated as the O_2 concentration at which RQ > 1. To evaluate the effects of low O_2 stress on the emission of volatile organic compounds and microbial quality, arils were analysed on days 0, 3, 6, and 9 of storage.

$$R_{O_2} = \frac{\left(O_{2_i} - O_{2_f}\right)}{(\Delta t)} \frac{V_f}{W} \tag{1}$$

$$R_{CO_2} = \frac{\left(CO_{2_f} - CO_{2_i}\right)}{(\Delta t)} \frac{V_f}{W} \tag{2}$$

where $R_{CO_2}$and $R_{O_2}$was respiration rates expressed in mL kg^{-1} h^{-1}, O_{2_i} and CO_{2_i}= is the concentration of O_2 and CO_2 (%) in the jar at beginning of the experiment respectively, $O_{2_f}$and CO_{2_f}= concentration of O_2 and CO_2 (%) at time t, respectively, Δt is change in time, W is fresh mass of arils, and V_f is free volume.

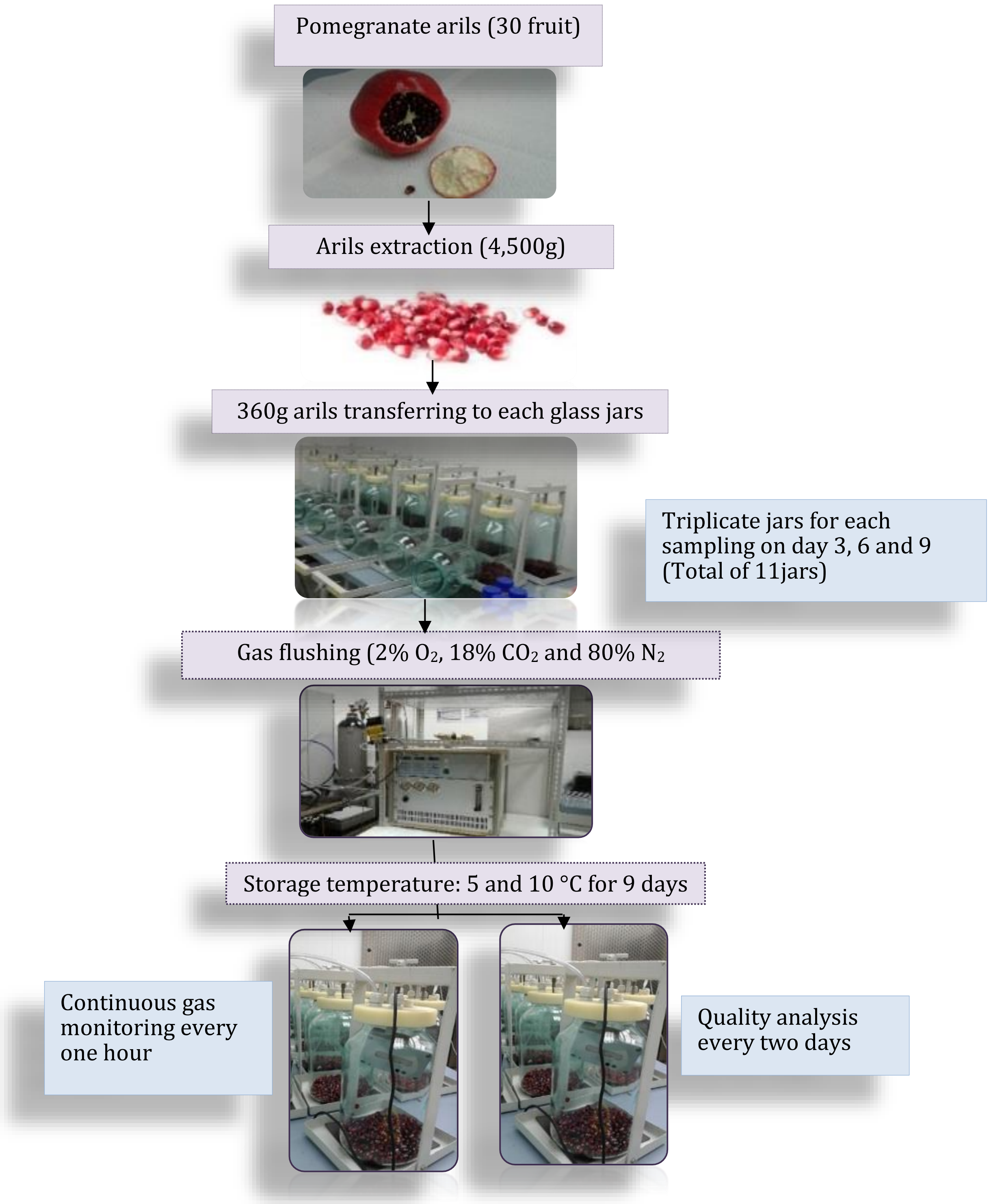

Figure 1. Flow diagram of experimental design for determining the effect of low O_2 atmosphere and storage temperature on the physiological response and quality of pomegranate arils.

Volatile organic compounds

Volatile organic compounds (VOCs) were extracted by static headspace sampling (SHS) method described by Caleb *et al*. (2016). Pomegranate arils from each package were homogenized into puree and 7 g was placed in 20 mL glass vial with 100 µL of 3-octanol (0.1 g L^{-1}) as internal standard. The vials were tightly capped and allowed to equilibrate at 80 °C for 10 min in the headspace auto-sampler incubator. Gas sample (1 mL) was automatically withdrawn from the headspace of each vial (HS-20 automated-sampler, Shimadzu Europa GmbH, Duisburg, Germany). Sampling condition for HS-20 auto-sampler was maintained as follows: the oven, sampling line and transfer line temperature was 80 and 150 °C, respectively; pressurizing time of 30 s at 76%. To increase the sensitivity of the SHS method on the GC-MS, the vial shaking level of 3, load and injection time of 1 min each and split ratio (1:10) at constant pressurizing time (30 s) with single injection parameters were used. Gas samples were transferred from HS-20 sampler into the GCMS-QP2010 (Shimadzu Europa GmbH, Duis-burg, Germany) for separation of volatiles. Due to the volatility, nonpolar character and reactivity of volatile sulphur compound a mid-polar 1.4 mm film thickness Zebron™ capillary column, with 30 m length and 0.25 mm inner diameter was used (ZB-624, Phenomenex, Aschaffenburg, Germany). Analyses were carried out using helium as carrier gas with a constant flow rate of 0.03 mL s^{-1}. The GC temperature was held at 50 °C for 3 min, then ramped up to 170 °C at 0.17 °C s^{-1} and held at this temperature for 3.5 min in total run time of 15 min. The mass selective detector (MSD) in this study was operated in full scan mode and mass spectra in the 35-500 m/z range were recorded. The ion source and interface temperature were maintained at 200 and 230 °C, respectively.

Individual volatile compounds were tentatively identified by their retention time (RT) Kovats retention index using n-alkane group, which was compared to those of the National Institute for Standards and Technology mass spectral libraries (NIST v. 08 and 08s, Gaithersbug, MD, USA). Only compounds with the correlation coefficient (R^2) above 90% were considered. Semi-quantification of the identified compounds was estimated using equation 3:

$$RA = \frac{A_{ic}}{A_{itc}} C_{itc} \tag{3}$$

where RA is the relative abundances of the identified compound (g L^{-1}), A_{ic} is the peak area of the identified compound, A_{its} is the peak area of the internal standard, C_{its} is the concentration (0.1 g L^{-1}) of internal standard in the sample.

Microbial analysis

Microbial quality of pomegranate arils was studied according to the method described by Caleb *et al*. (2016) using total plate count method. The total aerobic mesophilic bacterial count was determined using plate count agar (PCA), while yeast and mould counts were determined using Rose Bengal chloramphenicol agar (RBCA). Sample (10 g) of pomegranate arils was taken for each treatment and transferred into 90 mL peptone buffered water. Samples were homogenized for 2 min at speed 4 strokes s^{-1} in a lab blender (BagMixer1400CC1, Interscience, France) and thereafter threefold serial dilution was made by adding 30 µL of each diluent into 270 µL of PS Rotilabo1-microtest plates (96er U-profile, Carl Roth GmbH & Co KG, Germany) and 100 µL from each dilution was pour-plated on respective growth media. All analyses were done in duplicate for each package treatment. PCA plates for aerobic mesophilic bacterial were incubated at 30 °C for 72 h, and RBCA plate for yeast and mould, respectively, were incubated at 25 °C for 5 days. After incubation, colonies (between 30 and 300 colonies) on each plate were counted and the results were expressed as log colony forming unit per weight (log CFU mL^{-1}).

Statistical analysis

The data from measured attributes of pomegranate arils at 5 and 10 °C stored for 9 days were subjected to analysis of variance (ANOVA). Duncan Multi range test was also used to determine the difference between mean combination values. Results were presented as mean value of triplicate and data were analysed using Statistica software (Statistica 13.0, Statsoft, USA).

Results and discussion

Change in headspace gas evolution and its effect on respiration rate

The results obtained showed that the concentration of O_2 decreased and CO_2 increased continuously during storage (Fig 1). The storage temperatures and duration ($p < 0.05$) significantly affected changes in headspace gas concentration for pomegranate arils. At 5 °C storage, the initial gas compositions of 2.3% O_2 reduced to 2.16% O_2 and 17.8% CO_2 increased to 18% at the end of the storage (9 days). Arils stored at 10 °C showed slight changes at the end of storage compared to the initial concentration to 2.06% O_2 and 19.02% CO_2 concentrations. Furthermore, the results have shown that both O_2 and CO_2 concentrations did not exceed the optimum atmosphere recommended for pomegranate arils storage by López-Rubira *et al.* (2005). Similarly, the efficiency of low temperature (5 °C) to maintain the headspace gas composition for 'Hicaznar' pomegranate arils was reported by Ayhan and Eştürk (2009). Additionally, the significant increase in headspace gas concentration during MAP storage of pomegranate arils due to increase in temperature has been reported (Caleb *et al*., 2013; Ersan *et al*., 2009).

Carbon dioxide production was also depended on temperature and O_2 concentration, where CO_2 production declined with decreasing temperature and in general with decreasing O_2 concentration. Storage temperature had significant effects on RR (Fig 2), with RCO_2 of 0.26 and 1.22 mL kg^{-1} h^{-1} at 5 and 10 °C, respectively at the end of storage. Similar effects of temperature on the RR of minimally-processed fresh produce have been reported in literature (Beaudry, 2000; Caleb *et al*., 2013, Aindongo *et al*., 2014). The increase in temperature greatly increases RR; therefore, higher external O_2 concentration is required to maintain aerobic RR (Ke *et al*., 1991). Hence, a small change in O_2 concentration at low partial pressure of O_2 has a greater effect on RO_2 than a small change at higher partial pressure (Dadzie *et al*., 1996). This implies that reduction in RR per unit area of O_2 decreased is much higher at low O_2 concentration (Dadzie *et al*., 1996). Furthermore, the storage duration also had a significant effect ($p \leq 0.05$) on CO_2 production rate of pomegranate arils at 10 °C, where the significant change was observed at day 3.

The depression in RR also directly affected the RQ, the mean asymptotic RQ of pomegranate arils was marginally above 1.0 at 5 °C with a sharp increase at the end of storage. A similar trend was observed for arils at 10 °C, except that the RQ (3.0) was higher at the end of storage. A gradual increase in RQ was observed as O_2 concentration decreased to about 2.2%, at 5 and 10 °C. The increased RQ could be link to the marked reduction in O_2 concentration or increased CO_2 production rate exceeds O_2 consumption rate (Beaudry, 2000). The low O_2 limit was noted when the O_2 concentration dropped to 2.19 and 2.28 at 5 °C and 10 °C, respectively. Wright *et al*. (2010) reported different low O_2 limit for apples at varying storage temperature. The study has shown that the LOL may differ among fruit of the same species, harvested from different growing regions or at different dates, and due to storage temperature. Therefore, low O_2 atmosphere should be studied before it is applied to a product (Weber *et al*., 2011). Furthermore, the correlation of temperature to LOL could be due to the solubility of gases more at cooler than warmer temperatures. Hence, it is possible that decline in O_2 may be a contributing factor to higher limits at higher temperatures. In addition, it could be associated with changing enzymatic activities and O_2 demand of the fruit with temperature change (Wright *et al*., 2010).

Volatile organic compounds

Pomegranate arils stored at different storage temperatures initially emitted similar VOCs, with differences in relative abundance concentrations but a shift in VOC profiles was observed at the end of storage. Volatile evolution is associated with a sequence of the metabolic events and biochemical pathways due to a change in respiration via ATP biosynthesis (Bangerth *et al*., 2012; Song & Bangerth, 2003). Jacxsens *et al*. (2001) explained that due to the respiratory nature of the fruit, under hazardous anaerobic conditions (< 2% O_2 and > 20% CO_2) and high temperature storage undesirable fermentation reaction could occur. Furthermore, Bangerth *et al*. (2012) and Weber *et al*. (2011) reported the increase in accumulation of volatile compounds due to higher RR. Lower volatile emission was observed with the decrease in RR of "Royal Gala" apples stored at 0.5% O_2 storage (Both *et al*., 2014). Pomegranate arils RR was significantly lower at 5 °C in comparison to 10 °C, which implied that reduction in RR could have an effect on the synthesis of VOCs.

In the current study, a total 27 VOCs were tentatively identified as presented in Table 1. The VOCs identified include esters (5), alcohols (4), aldehydes (7), ketones (4), terpenes (1), sulphur compounds (1), alkane derivatives (4) and monoterpenoids (1). Previous studies identified various VOCs for pomegranate arils; Calín-Sánchez *et al.* (2010) identified 18 compounds including monoterpenes, aldehydes, alcohols, monoterpenoids and linear hydrocarbons. The study found out that the most abundant compounds in freshly harvested Spanish cultivars (sweet, sweet-sour and sour) pomegranate were *trans*-2-hexenal, 3-carene, α-terpinene and α-terpineol. Melgarejo *et al*. (2011) identified 21 VOCs grouped under aldehydes, monoterpenes, and alcohols from the juice of nine Spanish cultivars. Caleb *et al*. (2013) reported 17 and 18 VOCs for 'Acco' and 'Herskawitz' pomegranate arils, respectively under MAP. On another study, Caleb *et al*. (2015) reported 41 VOCs (12 primary and 28 secondary) for 'Bhagwa' pomegranate arils stored under MAP. The relative abundance of VOCs could be varied among fruit cultivars (Caleb *et al*., 2013; Fawole and Opara, 2013), fruit growing location (Mphahlele *et al*., 2015), and packaging gas composition and storage atmosphere (Luca *et al*., 2016).

Aldehydes production

Aldehyde VOCs were the abundant volatiles emitted in the current study at different concentration in both storage temperatures. From all aldehydes VOCs, acetaldehyde had the highest concentration throughout the storage. The storage temperature had significant effect ($p \leq 0.05$) on the emission of acetaldehyde after day 3 of storage. The increase in glycolysis end products was evident in the acetaldehyde concentration emitted from pomegranate arils at higher storage temperature. This changed from initial concentration of 0.13% to 0.17% at 10 °C by the end of the storage. The marked increase at 10 °C could be associated with the higher reduction of O_2 concentration compared to 5 °C, which could be associated with metabolic stress response process. Similar observation was reported for apples due to increase in temperature (Yearsley *et al*., 1996).

In addition, a correlation was observed between acetaldehyde emission and reduction in O_2 concentration. The role of acetaldehyde could be important in post-hypoxic injuries, as ethanol which is less toxic than acetaldehyde is rapidly oxidized (Chervin *et al*., 1996). However, rather than a continuous increase in acetaldehyde concentration during storage at 10 °C, the evolution of aldehyde volatiles such as 2-methyl-propanal, furfural, heptanal and decanal were observed. Caleb *et al*. (2013) reported the decrease in aldehyde content of 'Acco' and 'Herskawitz' pomegranate arils. Similarly, Belay *et al*. (2017) also reported the decrease in aldehyde content for 'Wonderful' pomegranate arils. Furthermore, the decline of aldehydes was consistent with the increase in ethanol (as a respiratory metabolite) in the glycolysis cycle, wherein decarboxylation produces acetaldehyde that can be reduced to ethanol (Allwood *et al*., 2014; Both *et al*., 2014).

Alcohols and esters production

Ethanol was the most abundant alcohol detected in pomegranate arils stored at 5 and 10 °C. Ethanol production was observed after 3 days of storage, and production rates increased from day 6 to 9. The concentration of ethanol was significantly higher ($p \leq 0.05$) at 10 °C compared to 5 °C. At the end of the storage (day 9), ethanol concentration was 0.63 ± 0.09 and 1.2 ± 0.18 at 5 and 10 °C, respectively. In addition, 3-methyl-1-butanol was emitted only in pomegranate arils stored at 10 °C at the end of storage. The concentration of these two alcohols notably increased with the decline in O_2 concentration. The elevated ethanol concentration in pomegranate arils during storage described a possible shift in energy metabolism from respiration to fermentation. This is supported by a comparison of the ethanol concentration at 5 and 10 °C as shown in Table 2, which corresponded to a reduction in O_2 concentration. Furthermore, this was evident that the immediate increase in RQ was also observed at O_2 concentration reached at 2.18% and 2.28% as can be seen in Figure 3. However, there were no visible injury symptoms or off-flavour have observed.

On the other hand, alcohol VOCs such as Isopropyl alcohol, (3-methyl-oxiran-2-yl)-methanol and 2-methyl-1-propanol were emitted intermittently. It has been proposed in previous studies that low O_2 concentration could lead to a decrease in the biosynthesis of fatty acids, the precursors of both alcohols and straight-chain esters (White *et al*., 2016). The cumulative reduction of alcohols was well correlated with higher production of ester compounds since alcohol is the precursor of ester (Song & Bangerth, 2003). Esters are important for characteristics aroma, fruity or floral flavour (Both *et al*., 2014). Thus, it is likely that the lowest O_2 concentration used for this study may have inhibited the synthesis of important substrates for the further production of esters at 5 °C. On the other hand, esters such as acetic acid, hydrazide (0.016 ± 0.01), ethyl acetate (0.061 ± 0.01), and hexanoic acid and ethyl ester (0.002 ± 0.00) were emitted and detected in pomegranate arils stored at 10 °C.

In the present study, the concentration of ethyl ester decreased during storage. Caleb *et al*. (2013) reported the initial increase in concentration and gradual decomposition of ethyl esters for pomegranate arils. Both *et al*. (2014) suggested that the reduction of esters was related to the negative effect of storage atmosphere in the development of aroma in apples. Ke *et al*. (1994) stated that when fruit are exposed to hypoxia, the concentration of ethyl esters increases, since the excess of ethanol synthesised is ready available to react with acyl-CoAs. Therefore, the decrease in ethyl ester in the current study could indicate that the concentration of ethanol available at both storage temperatures was not high.

Microbial growth

Aerobic mesophilic bacterial growth increased gradually until the end of storage and reached a value of 4.5 log CFU mL^{-1} and 6.58 log CFU mL^{-1} at 5 °C and 10 °C, respectively (Fig 4a). Storage temperature had a significant impact ($p \leq 0.05$) on bacteria growth, as the growth was observed higher at 10 °C in comparison to 5 °C. However, aerobic mesophilic bacterial count at the different temperatures did not exceed the maximum growth limit (7 log CFU mL^{-1}) recommended for fresh fruit and vegetable by the South African legislation (FCD Act 54 1979). For pomegranate arils stored at 5 °C, the yeast growth was below the detection limit until day 6, therefore, the reading was considered as < 1 log CFU mL^{-1}. However, the yeast count (5.4 log CFU mL^{-1}) for arils stored at 10 °C exceeded the maximum limit growth at the end of storage according with South African legislation. On the other hand, the mould growth increased only by 6% for arils stored at 5 °C at the end of storage.

Previous studies have reported the potential effect of low O_2 atmosphere and cold storage to retard microbial growth of fresh or minimally-processed fruit. For instance, Pan *et al*. (2015) observed low O_2 (4%) and enriched CO_2 (5%) markedly reduced the growth of yeast and mould on fresh-cut pineapple at 10 °C. For pomegranate arils cv. 'wonderful' stored under low O_2 (5%) a higher aerobic mesophilic bacteria count was reported Banda *et al*. (2015) at 5

°C in comparison to this current study. The effects of low (5%) and super-atmospheric O_2 (70%) was investigated by Ayhan and Eştürk (2009). The authors showed that the lowest growth of aerobic mesophilic bacteria (2.30 log CFU mL^{-1}) was found at super-atmospheric O_2 after 18 days, but at low O_2 atmosphere the growth was higher (3.36 log CFU mL^{-1}). Oms-Oliu *et al*. (2008) found that yeast and mould growth on fresh-cut melon stored under low O_2 and enriched CO_2 (2.5% O_2 + 7% CO_2) atmosphere were inhibited until the third week of storage. The significant effect of storage temperatures (5 °C and 10 °C) on the aerobic mesophilic bacteria, yeast and mould growth on 'Acco' and 'Herskawitz' pomegranate arils during MAP were reported by Caleb *et al*. (2013). The authors recorded higher yeast and mould growth than aerobic mesophilic bacteria. The effect of low O_2 and moderate CO_2 tend to be mutually offsetting so that any benefit that low O_2 might provide for any decay control is likely to be contracted by adding a tolerable CO_2 concentration (Burg, 2004).

In the current study, the shelf life of pomegranate arils was limited to 9 days at 10 °C due to yeast and mould growth. The occurrence of anaerobic respiration was correlated to the microbial growth with the reduction of O_2 concentration. The highest yeast and mould growth at 10 °C could be an indication that there was a slightly acidic environment due to fermentative condition (Ampofo-Asiama *et al*., 2014; Ke *et al*., 1991). Yeast and mould grow relatively better in acidic media than bacteria (Smelt, 1998). This could provide an insight for selecting appropriate packaging film with desired gas permeability to avoid anaerobic respiration.

Conclusion

This study showed that, storage condition (temperature and RH) significantly affected the increase in respiratory quotient (RQ) of pomegranate arils and this increase was well correlated with the decline in O_2 concentration. Different low O_2 limits were obtained based on the immediate increase of RQ when O_2 concentration reduced at 5 and 10 °C. Similarly, the emission and relative concentration of volatiles were found to be storage temperature dependent. Accumulation of ethanol was consistent with the decline in O_2 concentration, immediate increase of RQ and yeast growth during storage. This stress response indicated the induction of fermentative metabolisms. Based on these criteria, pomegranate arils can tolerate up to 2.18% of O_2 concentration at 5 °C and 2.28% of O_2 concentration at 10 °C. This study showed the potential of low O_2 atmosphere to maintain the quality of pomegranate arils at 5 and 10 °C. However, selecting appropriate packaging material with the desired permeability to alleviate higher reduction of O_2 at 10 °C is of critical importance. Therefore, further studies are necessary to design appropriate packaging.

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Table 1. Group of volatile compounds according to their functional groups, tentatively identified in pomegranate arils stored at low O_2 concentration at different temperature for 9 days.

Functional groups	VOCs	(RT)	Kovats index
Aldehyde	Acetaldehyde[a]	1.96-1.98	480
	2-methyl-Propanal[ad]	4.476	552
	Heptanal[ad]	12.23	880
	Nonanal[b]	15.86-15.88	1079
	Decanal[ac]	17.483	1185
	Furfural[a]	11.41-11.45	835
	2-Furancarboxaldehyde-5-(hydroxymethyl)-[a]	19.46-19.50	1267
Keton	Acetone[a]	3.32-3.35	806
	Methylthio-2-propanone[bc]	3.38-3.39	863
	3-Octanone[a]	13.79-13.83	991
	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one[ad]	17.48	1269
Alcohol	Ethanol[b]	2.86-2.92	463
	Isopropyl Alcohol[ad]	3.58-3.75	495
	2-methyl-1-Propanol[ad]	6.56	594
	3-methyl-1-Butanol[a]	9.01	744
Ester	Acetic acid, hydrazide[a]	3.73-3.763	527
	Ethyl Acetate[b]	5.55-5.60	628
	Sulfurous acid, cyclohexylmethyl heptyl ester[bc]	20.10-20.38	2100
	Hydrazine, methyl-[a]	2.89-2.93	594
	Hexanoic acid, ethyl ester[bd]	13.69	982
	Decane[a]	13.19-13.23	1015
Alkane derivatives	Trans-1,2-bis-(1-methylethenyl)cyclobutane[b]	14.14-14.19	1720
	Undecane[b]	14.962-19.471	1100
	Methane[b]	3.72-3.75	1012
	Dimethyl sulfide[bd]	3.36	505
Sulphur compounds	D-Limonene[bc]	14.155	1056
Terpenes	α-terpinol[ad]	5.586-5.587	1400
Monoterpenoids			

RT, retention time

[a] Primary volatile compounds, [b] secondary volatile compounds,

[c] volatile compounds only found at 5 °C and [d] volatile compounds only found at 10 °C

Table 2. Effect of low O_2 stress on the change in volatile concentration and composition for pomegranate arils stored at 5 °C and 10 °C for 9 days.

Volatiles	Day 1	Day 3		Day 6		Day 9	
		5 °C	10 °C	5 °C	10 °C	5 °C	10 °C
Acetaldehyde	0.128 ± 0.01^{a}	0.082 ± 0.02^{a}	0.078 ± 0.04^{a}	0.048 ± 0.02^{b}	0.099 ± 0.01^{a}	0.084 ± 0.00^{b}	0.167 ± 0.10^{a}
Ethanol	nd	Nd	nd	0.469 ± 0.04^{b}	0.915 ± 0.12^{a}	0.628 ± 0.09^{b}	1.179 ± 0.18^{a}
Hydrazomethane	1.706 ± 0.09^{a}	0.982 ± 0.10^{a}	0.978 ± 0.64^{a}	nd	nd	nd	0.488 ± 0.49^{a}
Acetone	0.439 ± 0.01^{a}	Nd	0.275 ± 0.15^{a}	0.044 ± 0.04^{a}	0.116 ± 0.12^{a}	0.198 ± 0.08^{a}	nd
Isopropyl alcohol	0.011 ± 0.00^{a}	Nd	0.005 ± 0.00^{a}	nd	nd	nd	nd
methylthio-2-propanone	nd	0.004 ± 0.00^{a}	nd	0.001 ± 0.00^{a}	nd	nd	0.002 ± 0.00^{a}
Methane	nd	Nd	nd	0.001 ± 0.00^{a}	nd	nd	nd
Dimethyl sulphide	nd	Nd	nd	nd	0.001 ± 0.00^{a}	nd	nd
Acetic acid, hydrazide	nd	0.006 ± 0.02^{a}	nd	0.002 ± 0.00^{b}	0.013 ± 0.01^{a}	0.003 ± 0.02^{b}	0.016 ± 0.01^{a}
2-methyl-propanal	nd	Nd	0.007 ± 0.00^{a}	nd	nd	nd	nd
Ethyl Acetate	nd	0.007 ± 0.00^{a}	nd	0.207 ± 0.20^{b}	0.415 ± 0.41^{a}	nd	0.061 ± 0.01^{a}
α-terpinol	1.743 ± 0.09^{a}	Nd	1.175 ± 0.65^{a}	nd	nd	nd	nd
2-methyl-1-propanol	nd	Nd	0.008 ± 0.00^{a}	nd	nd	nd	nd
Pentanoic acid, 4-oxo-, methyl ester	nd	Nd	nd	nd	nd	0.616 ± 0.09^{a}	nd
3-methyl-1-butanol	0.002 ± 0.00^{a}	Nd	0.001 ± 0.00^{a}	nd	nd	nd	0.002 ± 0.00^{a}
Furfural	nd	0.012 ± 0.00^{a}	0.010 ± 0.00^{b}	0.002 ± 0.00^{a}	nd	nd	nd
Heptanal	0.002 ± 0.00^{a}	Nd	0.002 ± 0.00^{a}	nd	nd	nd	nd
Decane	0.005 ± 0.00^{a}	0.001 ± 0.00^{b}	0.003 ± 0.00^{a}	0.002 ± 0.00^{a}	nd	nd	0.002 ± 0.00^{a}
Hexanoic acid, ethyl ester	nd	Nd		nd	nd	nd	0.002 ± 0.00^{a}
Undecane	nd	0.008 ± 0.00^{a}	nd	nd	nd	0.003 ± 0.00^{a}	0.001 ± 0.00^{b}
3-Octanone	0.001 ± 0.00^{a}	0.003 ± 0.00^{a}	0.001 ± 0.00^{b}	nd	0.001 ± 0.00^{a}	nd	0.001 ± 0.00^{a}
D-Limonene	nd	Nd	nd	nd	nd	0.001 ± 0.00^{a}	nd
Trans-1,2-bis-(1-methylethenyl)cyclobutane	nd	0.003 ± 0.00^{a}	nd	0.002 ± 0.00^{b}	0.001 ± 0.00^{a}	nd	0.001 ± 0.00^{a}
Nonanal	nd	0.001 ± 0.00^{a}	nd	0.001 ± 0.00^{a}	0.001 ± 0.00^{a}	0.002 ± 0.00^{a}	nd
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	0.011 ± 0.00^{a}	0.001 ± 0.00^{b}	0.007 ± 0.00^{a}	nd	nd	nd	nd
Decanal	nd	Nd		0.001 ± 0.00^{a}		nd	nd
2-Furancarboxaldehyde-5-(hydroxymethyl)-	0.228 ± 0.00^{a}	0.057 ± 0.01^{b}	0.158 ± 0.07^{a}	0.015 ± 0.01^{a}	0.003 ± 0.00^{b}	0.006 ± 0.00^{a}	0.004 ± 0.00^{b}
Sulfurous acid, cyclohexylmethyl heptyl ester	nd	0.010 ± 0.01^{a}	nd	nd	nd	nd	nd

Mean values (n = 3) in the same column along the rows with different lower case superscript are significantly different based on Duncan test at 95 % confidence interval. nd, not detected.

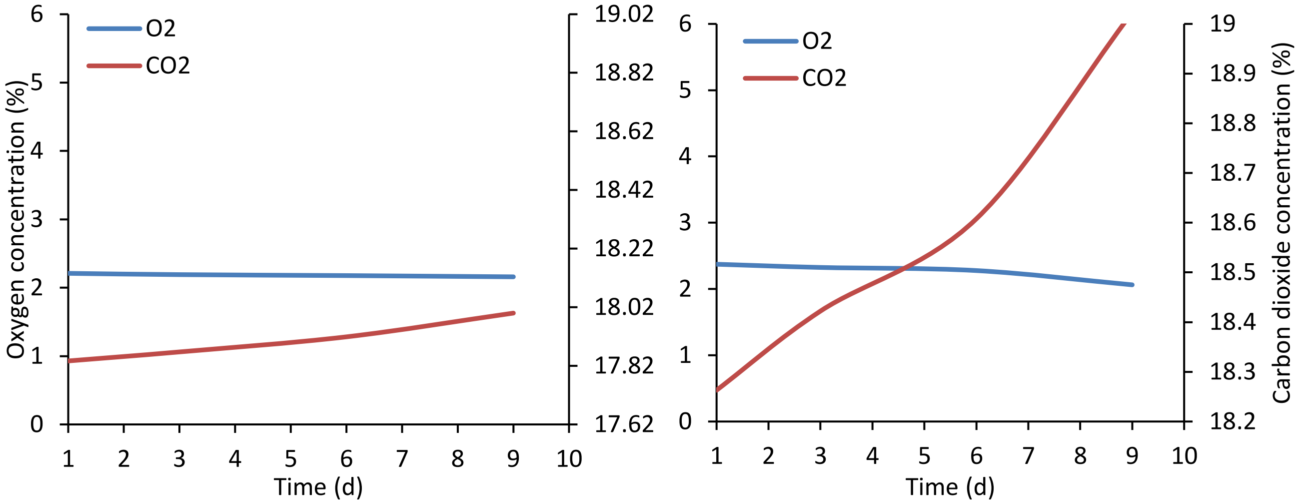

Figure 1. Change in the headspace gas concentration of Pomegranate arils stored at low O_2 atmospheres at and different temperature (5 and 10 °C)

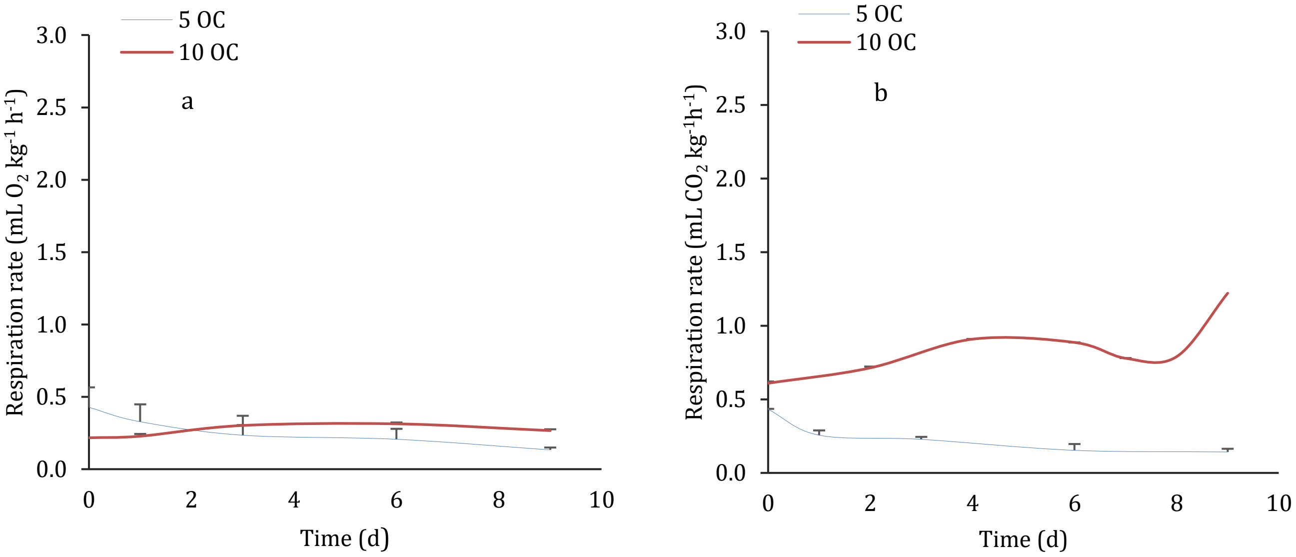

Figure 2. Effect of low O_2 atmospheres on the respiration rate (a) RO_2 and (b) RCO_2 of pomegranate arils during storage at 5 and 10 °C

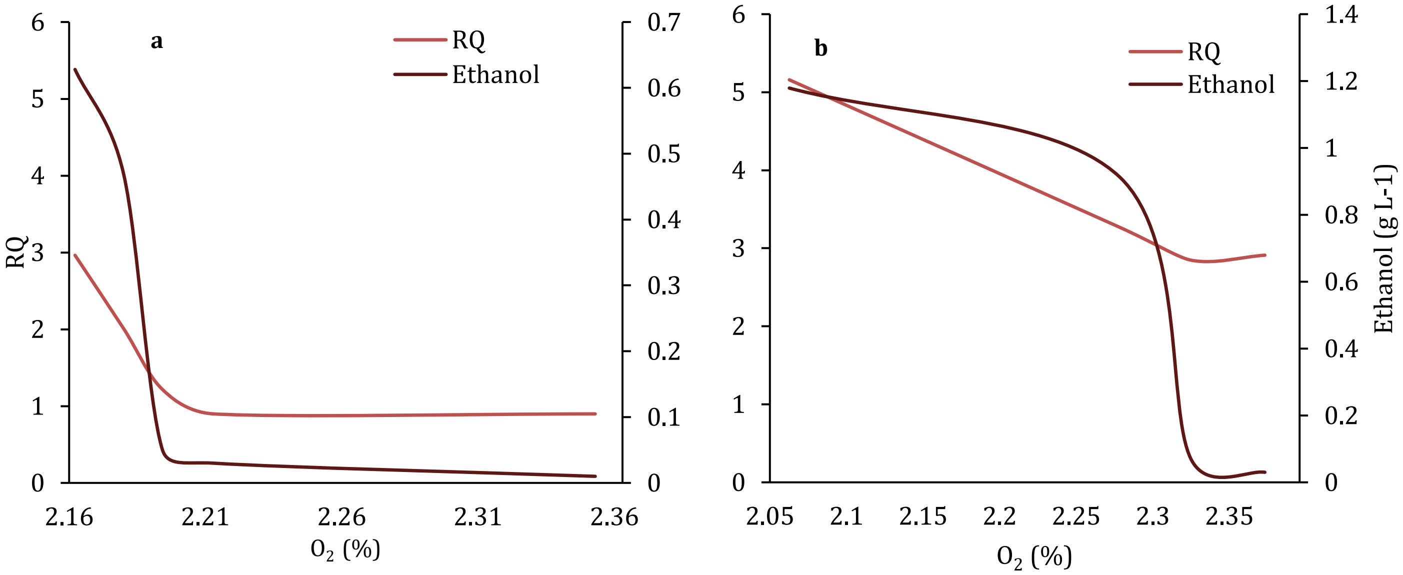

Figure 3. Emission of ethanol and increase respiration quotient (RQ)of pomegranate arils response to low O_2 stress during storage at 5 °C (a) and 10 °C (b)

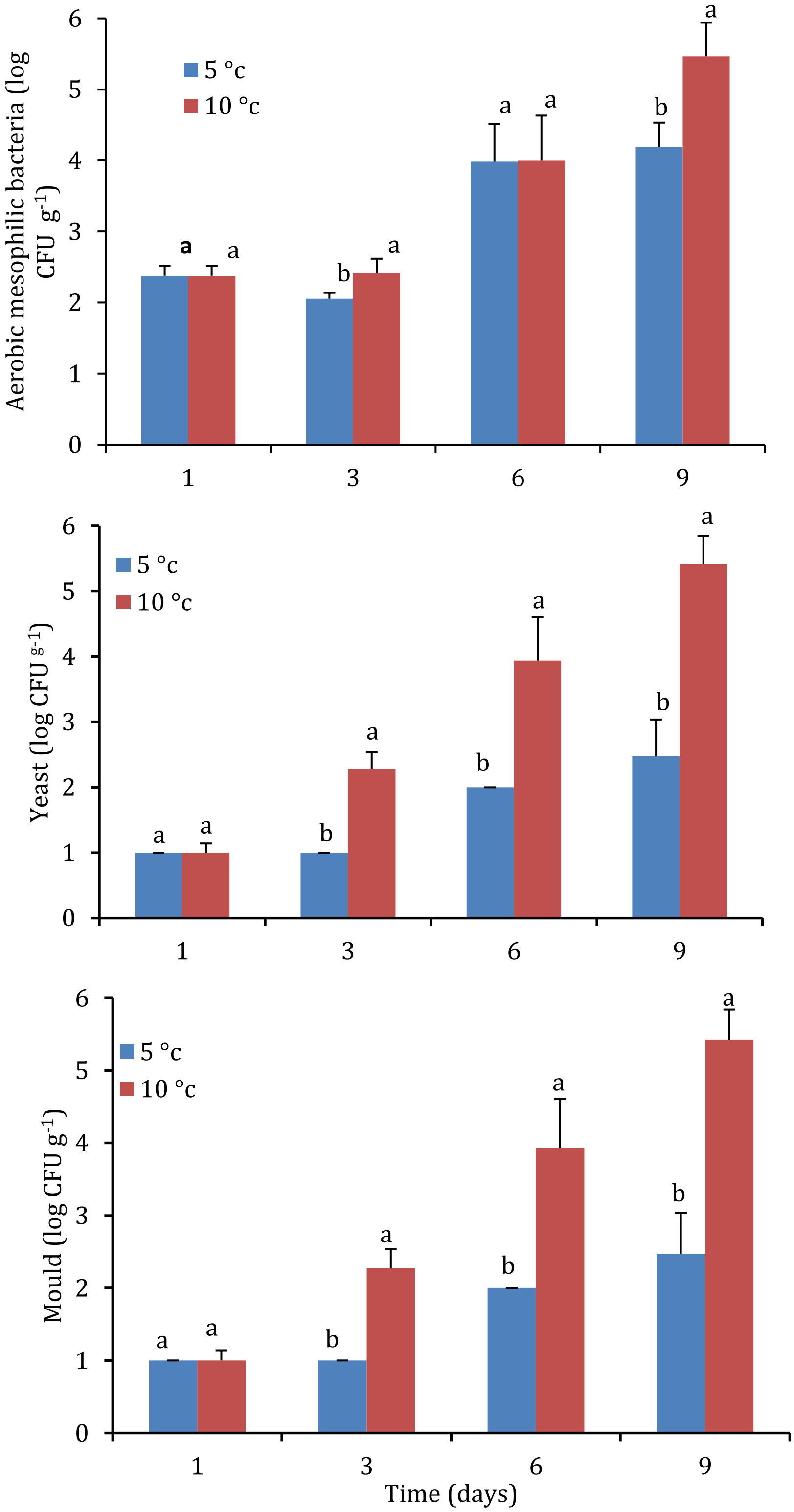

Figure 4. Response of microbial growth on pomegranate arils to low O_2 atmosphere at 5 °C (blue fill) and 10 °C (red bars) stored for 9 days, where (a) is aerobic mesophilic bacteria, (b) is yeast and (c) is mould counts. Error bars represent standard deviation (SD) of mean values (n = 6), and bar with similar letters are not significantly different at 95% confident interval ($p < 0.05$) according to Duncan's multiple range test.

Declaration by the candidate:

With regard to Chapter 4 (pp 81-105); the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data collection and analysis, and writing of chapter	75

The following co-authors have contributed to Chapter 4 (pp 81-105):

Name	E-mail address	Nature of contribution	Extent of contribution (%)
Prof U.L. Opara	opara@sun.ac.za	Research input, editorial suggestion and proof reading	15
Dr O.J. Caleb	CalebO@arc.agric.za	Research input, data analysis, editorial suggestion and proof reading	10

Signature of candidate: Z.A. Belay

Date: 03/11/2017

Declaration by co-authors:

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 4 (pp 81-105)
2. no other authors contributed to Chapter 4 (pp 81-105) besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 4 (pp 81-105) of this dissertation.

Signature	Institutional affiliation	Date
Prof U.L. Opara	Department of Horticultural science, Stellenbosch University	03/11/2017
Dr O.J. Caleb	Postharvest and Agro-processing Technologies, Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Stellenbosch, South Africa	03/11/2017

Declaration with signature in possession of candidate and supervisor.

CHAPTER 4

IMPACTS OF LOW AND SUPER-ATMOSPHERIC OXYGEN CONCENTRATIONS ON QUALITY ATTRIBUTES, PHYTONUTRIENT CONTENT AND VOLATILE COMPOUNDS OF MINIMALLY-PROCESSED ‘WONDERFUL’ POMEGRANATE ARILS

ABSTRACT

The impact of active modified atmosphere (MA) storage under low oxygen (MA-1; 5% O_2 + 10% CO_2 + 85% N_2 and MA-2; 10% O_2 + 5% CO_2 + 85% N_2), super-atmospheric oxygen O_2 (MA-3; 70% O_2 + 10% CO_2 + 85% N_2) and air (MA-4; 21% O_2 + 0.03% CO_2 + 78% N_2), on the, phytonutrient, volatile organic compounds (VOCs) and microbiological safety of minimally-processed ‘Wonderful’ pomegranate arils stored at 5 ℃ for 12 days was studied. The effect of temperature fluctuation at ambient conditions (20 ℃) on the physical quality and microbiology safety was further evaluated. Low O_2 (MA-1) atmosphere significantly maintained phytonutrient content of arils at cold (5 ℃) storage whereas, the highest compositions of VOCs and significantly lower aerobic mesophilic bacteria, yeast and mould counts were found at super-atmospheric O_2 (MA-3) at both cold (5 ℃) and ambient storage (20 ℃). Gas modification significantly influences the texture and colour properties of pomegranate arils at both cold (5 ℃) and ambient storage (20 ℃). Based on the observed microbial count and other quality attributes, the shelf life of ‘Wonderful’ pomegranate arils was limited to 12 days for all MA conditions at 5 ℃ and 6 days for low O_2 (MA-1 and MA-2) and air (MA-4) at ambient condition (20 ℃).

Key words: Cold storage; Active modified atmosphere; Microbiology

Introduction

Several studies have demonstrated that minimally-processed pomegranate arils is a rich source of bioactive compounds and phytochemicals, which provide potential health promoting benefit, as well as fresh characteristics and convenience for the consumer (Aindongo *et al*., 2014; O’Grady *et al*., 2014). However, maintaining postharvest quality and microbial safety of pomegranate arils is a critical challenge (O’Grady *et al*., 2014). The limiting factors affecting the overall quality and shelf life of minimally-processed pomegranate arils include microbial growth, loss of nutritional and physicochemical attributes caused by active metabolic processes related to enzyme activity, enhanced respiration rate or oxidation of phenolic compounds (Ersan *et al*., 2010; Ghasemnezhad *et al*., 2011; Maghoumi *et al*., 2013)

Creating and maintaining a desired atmosphere have been shown to provide benefits of quality preservation of fresh produce (Jo *et al*., 2014). One of the important goals in atmosphere modification was to generate sufficiently low O_2 conditions to influence the metabolic process and reduce respiration rate, oxidative stress, tissue senescence and ethylene synthesis (Beaudry, 1999). Thereby, maintaining postharvest quality and extending the shelf life of fresh produce (Artés *et al*., 2000). Various studies have been investigated on the effect of low O_2 atmosphere on quality attributes of different cultivars of pomegranate arils such as 'Primosole' (Aquino *et al*., 2010), 'Mollar de Elche' (Artés *et al*., 2000), 'Acco' and 'Herskawitz' (Caleb *et al*., 2013), and 'Wonderful' (Banda *et al*., 2015). It was established from these studies that low O_2 atmosphere has potential for reducing chilling injury, decay and weight loss, inhibit fungal growth and retard postharvest ripening. However, the O_2 concentration in most of the studies decreased to a lower limit, which could induce anaerobic respiration and further lead to the synthesis of fermentative metabolites and off-odour (Saenmuang *et al*., 2012).

Recently, the use of super-atmospheric O_2 (≥ 70 %) has been shown as an effective alternative to the low O_2 atmosphere, for inhibition of microbial growth and enzymatic deterioration as well as maintaining quality (Jacxsens *et al*., 2001; Allende *et al*., 2004). For pomegranate arils, studies reported on the application of super-atmospheric O_2 were focused on selected quality attributes and shelf life (Ayhan & Eştürk, 2009; Maghoumi *et al*., 2014; Banda *et al*., 2015). Among the studies that have been performed on pomegranate arils, none have evaluated the effect of super-atmospheric O_2 on the change in volatile organic compounds (VOCs), phytonutrient and microbial safety for 'Wonderful' pomegranate arils. Furthermore, there is limited information on the impact of super-atmospheric O_2 and temperature fluctuation on the quality attributes and microbial stability of pomegranate arils. Thus, the objective of this study was to investigate the effect of modified atmosphere (MA) storage and the impact of temperature fluctuation on the quality attributes, change in VOCs and microbial stability of pomegranate arils at 5 °C and ambient storage (20 °C) for 12 days.

Materials and methods

Material preparation

Pomegranate fruit (cv. Wonderful) were obtained at commercially ripe stage with characteristic deep-red skin and deep red arils with the mature kernel (Mphahlele *et al*., 2016), from Sonlia Pack House, Wellington, Western Cape, South Africa (33°38′23″S, 19°00′40″E). The fruit was transported in an air-conditioned and ventilated vehicle to the Postharvest Research Laboratory at Stellenbosch University and stored in MAP bags at 5 °C and 95% RH for four months prior to processing. This was done to simulate long-term shipping duration from southern hemisphere production region to the northern hemisphere market and vice-versa. The damaged fruit was removed and the outer skin of healthy whole fruit was surface disinfected using 70% ethanol prior to aril extraction (Aindongo *et al*., 2014). Arils were extracted manually by carefully removing the husk. Extracted arils were collected in a tray and mixed to assure uniformity. Arils (350 g) were transferred to 3000 mL air-tight glass jars which were designed to achieve a completely hermetic seal. Four types of modified atmosphere (MA) conditions were randomly selected based on extensive literature study, which comprises a wide range of gases to identify if the arils could response differently. The range of atmospheres includes: i) low O_2 (MA-1; 5% O_2 + 10% CO_2 + 85% N_2) and (MA-2; 10% O_2 + 5% CO_2 + 85% N_2), ii) super-atmospheric oxygen (MA-3; 70% O_2 + 10% CO_2 + 20% N_2), and iii) air (MA-4; 21% O_2 + 0% CO_2 + 78% N_2) as control.

A total of 48 glass jars were prepared in multiple triplicate for each gas mixture for a specific sampling days. Three jars per treatment were opened and used on each sampling day at regular intervals of 0, 3, 6, 9, and 12 days of storage at 5 °C. To evaluate the effect of temperature fluctuation; 150 g of arils from the opened jars were transferred from cold storage (5 °C) on each sampling day and kept at ambient temperature (20 °C) for additional 2 days. Only physical and microbiology quality of arils were evaluated at 20 °C. For analysis of microbial quality and physical properties (firmness and colour) arils were directly used after sampling. Pomegranate juice was extracted by using a cheesecloth and the samples obtained were used to analyse chemical properties in triplicate at room temperature.

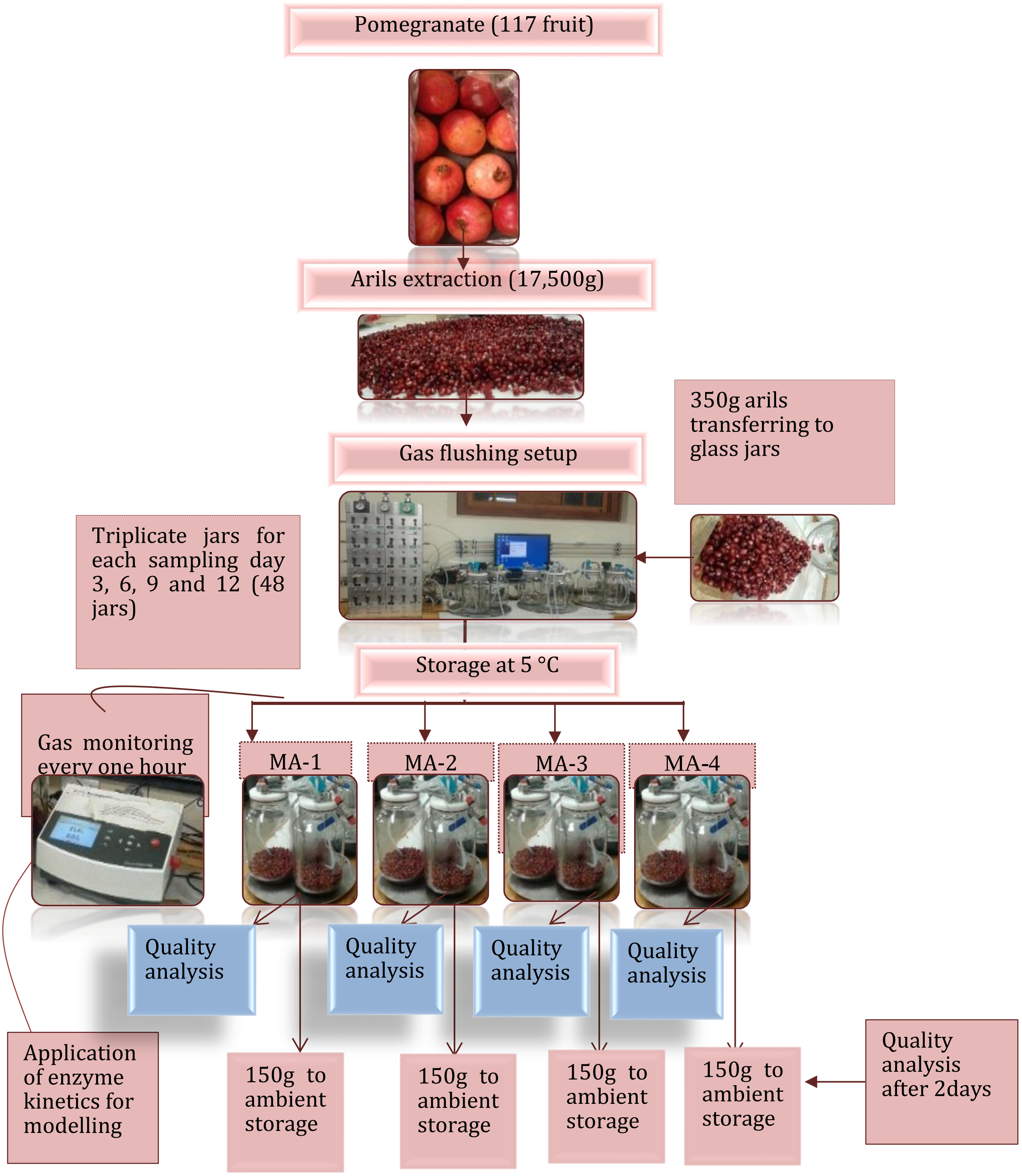

Figure 1. Flow diagram of experimental design for determining the effect of low and super-atmospheric O_2 atmosphere and storage temperature on the physiological response and quality of pomegranate arils

Physical properties

Firmness

Firmness of individual arils was measured using texture analyser (TA-XT Plus, Stable Micro Systems, Surrey, UK) with a 35 mm diameter cylindrical probe. Magness Taylor test (MTT) which is an empirical hardness indicator test was performed according to (Szychowski *et al*., 2015). Measurements were recorded by modifying the input parameters of the 35 mm diameter cylindrical probe to the penetration rate of 0.3 mm^{-1} for 5 s after contacting the surface of pomegranate arils, and results were expressed as N. A total of 20 arils were measured per treatment.

Colour

Pomegranate arils colour was measured using a colour metre (CR-400 Minolta Chroma Meter, Minolta Corp, Osaka, and Japan) according to the method presented by Caleb *et al*. (2013). Colour parameters of (L^*, a^*, b^*) were measured and results were expressed as the CIELAB colour space unit. Colour parameter Chroma (C^*) which describes the length of the colour vector in the plane formed by a^* and b^*, and the hue angle ($h°$) that determines the position of the vector were calculated. Mean of 15 measurements were calculated for each treatment.

Ascorbic acid

Ascorbic acid was determined spectrophotometrically against a standard curve using 2, 6-dichlorophenolindophenol (DCP) dye and metaphosphoric acid (MPA) as described by O'Grady *et al*. (2014). The combination of blue coloured DCP dye and colourless MPA resulted in a pink coloured solution, which was decolourised or reduced in the presence of ascorbic acid. Ascorbic acid of unknown concentrations in pomegranate juice samples was quantified using a standard curve of known concentrations from a stock solution (1 g L^{-1}) L-ascorbic acid. Both the stock solution and the juice samples were diluted with a DCP-MPA solution and absorbance was measured at 515 nm wavelength. Pomegranate juice samples were thawed at room temperature, diluted with MPA, vortexed (Model nr. G560E, Scientific Industries, USA) and sonicated (Ultrasonic Cleaner DC400H, MRC Ltd., Holon, Israel) for 3 min in cold water to extract the ascorbic acid present in the juice. The extract was centrifuged at 12857 g at 4 °C to obtain a clear homogenous solution, diluted with DCP dye and kept in a dark cabinet for 10 min. To correct for the natural pink colour of pomegranate juice, another set of centrifuged extract samples were taken and diluted with distilled water instead of MPA. The absorbance of the samples (MPA and water diluted extracts) and a standard curve was read at 510 nm wavelength. Ascorbic acid values were extrapolated from a standard curve with $R^2 > 0.90$. Ascorbic acid concentration was expressed as mg ascorbic acid per unit volume pomegranate juice (mg L^{-1}).

Total phenolic content

Total phenolic concentration (TPC) was measured using the Folin-Ciocalteu (Folin-C) method as described by Mphahlele *et al*. (2014). The mixture was vortexed and absorbance read at 725 nm using a UV-vis spectrophotometer (Thermo Scientific Technologies, Madison, Wisconsin). Gallic acid standard curve (0.02 to 0.10 g L^{-1}) was used and TPC was expressed as mass Gallic acid equivalent (GAE) per unit volume of pomegranate juice (mg L^{-1}).

Total monomeric anthocyanins

Total anthocyanin content was determined by the pH-differential method described by Banda *et al*. (2015) using 2 buffer systems: potassium chloride buffer for pH, 1.0 (0.025 M) and sodium acetate buffer for pH, 4.5 (0.4 M). The sample diluted with corresponding buffer and absorbance was measured at 520 and 700 nm using a Helis Omega UV–vis spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA). Duplicate readings were done for the triplicate arils juice samples total anthocyanins were calculated as cyanidin-3-glucoside according to the following equation:

$$Total\ anocyanins\ (mg\ L^{-1}) = \frac{A \times MW \times DF \times 1000}{\varepsilon} \times L \qquad (1)$$

where A is (A_{520}-A_{700}) pH1-(A_{520}-A_{700}) pH4.5; MW is molecular weight of cyanidin-3-glucoside (449.2 g mol^{-1}, DF is dilution factor; ε is molar extinction coefficient (26900) and L is path length in cm. All analysis was carried out in triplicate and results were expressed as mass cyanidin-3-glucoside equivalent per volume of pomegranate arils juice (mg L^{-1}).

Volatile organic compounds

For extraction of volatile compounds and chromatographic analysis, approximately 5 mL of aliquots of pomegranate juice were taken from samples thawed overnight at refrigerating temperature and were placed in 20 mL SPME vials. These aliquots were mixed with 2 g of NaCl to inhibit enzymatic degradation and facilitate the movement of volatiles into the headspace. The aroma volatiles were trapped and extracted from the vial headspaces via SPME method described by Caleb *et al*. (2013). The vials were allowed to equilibrate for 10 min at 50 °C in the CTC auto sampler incubator and after this equilibration time, a 50/30 mm divinylbenzene/-carboxen/-polydimethylsiloxane coated fibre was exposed to the headspace for 20 min at 50 °C. After extraction, desorption of the volatile compounds from the fibre coating was carried out in the injection port of the gas chromatography-mass spectrometry (GC–MS) during 2 min in splitless mode and then 8 min in split mode to clean fibre. The temperature of the injection was maintained at 250 °C.

Separation of the volatile compounds was performed on a gas chromatograph using Agilent 6890N (Agilent, Palo Alto, CA), coupled with an Agilent mass spectrometer detector Agilent 5975 MS (Agilent, Palo Alto, CA). The GC–MS system was equipped with an Rtx1-5Sil MS, with a 95% polydimethylsiloxane/5% polydiphenylsiloxane stationary phase and the dimensions were 30 m length; 0.25 mm inner diameter; and 0.5 mm film thickness. Analyses were carried out using helium as a carrier gas with a flow of 1.2 mL min^{-1}. The injector temperature was maintained at 250 °C. The oven temperature was as follows: 40 °C for 2 min; and then ramped up to 250 °C at 5 °C min^{-1} and held for 5 min. The MSD was operated in full scan mode and the ion source and quadropole were maintained at 240 °C and 150 °C, respectively. The transfer line temperature was maintained at 280 °C. Where authentic standards were available, compounds were tentatively identified by comparison of retention index (RI). Quantification was performed based on the relative abundances (%) of peak area using integration data.

Microbial quality

Microbiological quality of the arils samples were analysed for aerobic mesophilic bacteria, yeasts and moulds by pour-plate method, using plate count agar (PCA) and potato dextrose agar (PDA). To enumerate microbial load ten grams of each sample were taken aseptically and homogenize with 90 mL of sterile physiological solution. From each dilution, 1 mL of was pour-plate in triplicate on to PCA and PDA for aerobic mesophilic bacteria and yeast and moulds respectively. Plates were incubated at 37 °C for 2 days for aerobic mesophilic bacteria, and at 25 °C for 5 days for yeast and moulds. After incubation, plates with 15-300 colonies were counted. The results presented as log CFU mL^{-1} (Caleb *et al*., 2013).

Statistical analysis

Data were analysed using STATISTICA (version 10, StatSoft Inc. Tulsa, USA). The effect of gas concentration, storage duration and their interaction on the measured quality attributes were analysed by Two-way ANOVA. Post-Hoc Test (LSD Fishers' test) was used to confirm significant differences observed ($p \leq 0.05$), and descriptive tests were used to show a correlation between experimental data sets.

Result and discussion

Colour

Data obtained showed that all MA conditions, storage time and their interaction had a significant effect ($p \leq 0.05$) on the colour intensity Chroma (C*) and hue angle (h°) of pomegranate arils stored at 5 °C and ambient condition, respectively (Table 1). Both C* and h° values increased at day 3 in all MA conditions followed by a subsequent decrease at day 9. This could be due to the variation of the change in storage atmosphere due to time. Highest *C** (30.14 ± 7.1) and a slight increase in hue (*h*°, 36.51 ± 8.6) were observed for low O_2 atmosphere (MA-1) at the end of storage day 12. On the other hand, super-atmospheric O_2 (MA-3) maintained the initial values of *C** (14.91 ± 2.9) and *h*° (30.21 ± 5.3) throughout the storage compared to samples stored under MA-1, MA-2 and MA-4. The lower hue angle and higher Chroma value indicate red colour and corresponding to the high level of anthocyanin concentration (Pérez & Sanz, 2001). However, in the current study, the anthocyanin concentration of arils under super-atmospheric O_2 condition (MA-3) was low. This could be explained by the presence of high CO_2, which can decrease the anthocyanins of internal tissue content but does not affect the anthocyanin of the external tissue. Ayala-Zavala *et al.* (2004) reported that changes in anthocyanins in external and internal tissues might not be necessarily the same in response to the different treatment. However, besides anthocyanins other antioxidant compounds, such as phenolic (hydroxycinnamic acid), which exist in the arils juice and also contribute to the colour (Tzulker *et al.*, 2007). Martínez-Romero *et al.* (2013) observed that hue angle reduction for 'Mollar de Elche' pomegranate arils treated with Aloe vera gel, citric and ascorbic acid. On the other hand, hue angle increase in control non-treated arils.

Both *C** and *h*° values at ambient condition decreased for all modified atmosphere conditions and the atmosphere modification showed a significant effects ($p \leq 0.05$) on the colour on the *C** than *h*°. The result reported by Artés *et al.* (2000) for 'Mollar de Elche' pomegranate arils indicated a slight decrease of hue angle during shelf life period. In contrast, Ayhan and Eştürk (2009) found no significant effect of gas composition (low and super-atmospheric O_2) on the colour parameters for 'Hicraznnar' pomegranate arils stored at 5 ℃ for 9 days. The difference of these results can be associated with the different in cultivar, harvesting season, geographical location of the fruit and processing and packaging conditions.

The study reported by Palma *et al*. (2015) for 'Primosole' pomegranate fruit and arils at cold storage (5 °C) showed that the tendency of a slight decreasing of *C** and increasing of $h°$ reflect a loss of colour intensity occurring during storage of a whole fruit and ready to eat arils. However, in the current study, no significant differences ($p \leq 0.05$) on the $h°$ were observed among all treatments at the end of storage (day 12) for cold storage (5 °C).

Texture

Texture of pomegranate arils stored under all MA conditions was not significantly ($p \geq 0.05$) affected by gas atmosphere modification for both cold (5 ℃) and ambient (20 °C) storage (Table 1). This result is consistent with other studies in literature. Ayhan & Eştürk (2009) reported no significant differences between active and passive MAP applications until day 9 of storage for 'Hicaznar' pomegranate arils stored at 5 ℃ for 18 days. Caleb *et al*. (2013) also reported that storage temperature, time and their interaction had no significant effect on firmness for 'Acco' and 'Herskawitz' pomegranate arils stored under passive MA at 5 °C until 10 days.

Arils stored under super-atmospheric O_2 (MA-3) showed an increase of hardness values than low O_2 (MA-1) treatment. Similarly, Ayhan and Eştürk (2009) recorded the highest hardness of arils stored under super-atmospheric O_2. Furthermore, the hardness values reported in the current study was relatively higher compared to values reported by Szychowski *et al*. (2015) for 'Wonderful' pomegranate arils. The authors reported hardness values of 5.73 N using Magness-Taylor test (MTT). The variation of hardness values could be due to a different method of texture analyser (TA) measurement, the impact of postharvest treatment or effect of growing location of fruit type. On the other hand, at ambient (20 °C) storage hardness of pomegranate arils decreased across all treatment, while the occurrence of decay was observed after 12 + 2 day. In contrast, arils hardness increased from 8.71 to 8.93 N under super-atmospheric O_2 (MA-3) condition. This study is in line with Bessemans *et al*. (2016) where firmness decreased during ambient condition due to increased respiration rate up on removal from the storage containers. Overall, for texture profile of pomegranate arils, there was no significant effect of gas concentration, storage time and their interaction on this parameter.

Ascorbic acid and phenolic concentration

Initial ascorbic acid concentration (3035.4 ± 8.3 mg L^{-1}) of pomegranate arils decreased gradually across all MA conditions during the storage (Table 2). Gas composition and storage time and the interaction of both has a significant ($p \leq 0.05$) effect on the ascorbic acid concentration of pomegranate arils stored at 5 °C for 12 days. Ascorbic acid oxidation was greatly favoured by the presence of oxygen and thus, a marked decrease in ascorbic acid concentration was observed in samples stored under air (MA-4) and super-atmospheric (MA-3) conditions. The highest (four-fold) depletion of ascorbic acid was observed in pomegranate arils stored under air (MA-4) (Table 2). Maghoumi *et al*. (2014) also reported a reduction of vitamin C concentration of 'Malese-Saveh' pomegranate arils stored at 4 °C for 14 days in super-atmospheric O_2 (MA-3). Several studies have reported on the oxidation of ascorbic acid due to high O_2 (Oms-Oliu *et al*., 2009; Li & Zhang, 2015) or under anaerobic environment (Brar *et al*., 2012) for fresh cut produce. In the current study, the gradual decrease of ascorbic acid concentration was observed for arils stored at MA-3 atmosphere despite the high concentration of CO_2. High CO_2 level could lead the oxidation of ascorbic acid by ascorbate peroxide or inhibition of dehydroascorbic acid (DHH) reduction to ascorbic acid or by ethylene production. Overall, arils under low O_2 storage (MA-1 and MA-2) showed better retention of ascorbic acid concentration compared to MA-3 and MA-4 storage (Table 2).

Storage MA condition, duration and their interaction had a significant effect ($p \leq 0.05$) effect on the on the total phenolic concentration of pomegranate arils ($p < 0.05$) as shown in Table 2. The observed increase in total phenolic under low O_2 storage (MA-1 and MA-2) in this study could be associated with an increase in the activity of the phenylpropanoid pathway under a stressful condition such as low concentration of O_2. This results in the synthesis and accumulation of phenolic compounds (Ayala-Zavala *et al*., 2004; Brar *et al*., 2012). Selcuk and Erkan (2014) found that the phenolic concentration of 'Hicraznnar' pomegranate fruit packed in ZOEpac bag at 6 °C plus 3 days at 20 °C storage increased with storage time. Maghoumi *et al*. (2013) reported an increase in the phenolic concentration of 'Malese-Saveh' pomegranate arils exposed to high O_2 compared to samples stored under air. However, the effect of high O_2 concentration on the total phenolic may vary depending on the commodity, genotypes, oxygen concentration, storage time and temperature (Zheng *et al*., 2007; Ghasemnezhad *et al*., 2011).

The total phenolic concentration of pomegranate arils stored under MA-3 and MA-4 slightly declined, from initial 18.7 ± 6.3 mg L^{-1} to 12.5 ± 3.8 and 13.9 ± 2.8 mg L^{-1}, respectively. However, no significant difference was found at the end of storage ($p \geq 0.05$). The decreasing in phenolic concentration might be due to cell structure breakdown as part of senescence during storage and oxidation of phenolic compounds with polyphenol oxidase (PPO) and (POD) activities (Ghasemnezhad *et al*., 2011). However, in this study, no browning of arils was observed for all low, super-atmospheric oxygen or air atmospheric conditions. The study reported by Kulkarni and Aradhya (2005) on 'Ganesh' pomegranate fruit identified phenolic compounds as substrates for enzymatic browning, and the authors suggested that reduction in phenolic concentration may reduce the incidence of enzyme browning.

Total monomeric anthocyanins

The result in the current study showed that with progressing storage duration, the concentration of total anthocyanins declined slightly throughout the storage period in all MA treatments. This is in agreement with findings by Artés *et al*. (2000), Caleb *et al*. (2013) and Palma *et al*. (2015) who reported that anthocyanin contents of pomegranate arils decreased with increasing storage time. Anthocyanin degradation may be due damage of pomegranate arils during the fruit peeling or oxidation process (Ghasemnezhad *et al*., 2011). The results further showed that there was no significant effect of atmosphere modification, storage time and their interaction on the anthocyanins concentration of pomegranate arils ($p > 0.05$) except arils stored at super-atmospheric O_2 (MA-3) at day 12 (Table 2). This result agreed with those reported by López-Rubira *et al*. (2005) where no significant change in total anthocyanin concentration in early harvested pomegranate arils was observed. Similarly, Gil *et al*. (1996) also observed no significant difference in total anthocyanin concentration of 'Mollar' pomegranate arils.

Super-atmospheric O_2 (MA-3) had the lowest anthocyanin concentration (0.03 ± 0.0 mg L^{-1}) at the end of storage, which was about a two-fold decrease from the initial concentration of 0.07 mg L^{-1}. Banda *et al*. (2015) reported a similar trend where, the lowest anthocyanin concentration observed at high O_2 atmosphere (30% O_2 and 10% CO_2). This may be due to the ascorbic acid oxidation on the presence of super-atmospheric O_2 (Oms-Oliu *et al*., 2009; Maghoumi *et al*., 2014).

Volatiles

A total of 24 volatile organic compounds (VOCs) were identified for 'Wonderful' pomegranate under the different MA conditions during 12 days of storage as shown in Table 3 and 4. Identified volatiles were characterised as primary and secondary groups based on the time of detection. Most abundant chemical groups of VOCs found during storage were alcohol (27%), followed by esters (19%), monotepenes and ketones (15%), seseqitpenoids (12%), aldehydes (8%) and monoterpenoids (4%). Aldehydes were detected in fresh samples on day 0 and decreased below the detection level after day 3 of storage. This observation was in agreement with the previous report by Caleb *et al*. (2013). The authors showed that aldehydes concentration decreased over time for pomegranate arils 'Acco' and 'Herskawitz' during storage at 5 °C. The decline in aldehydes may be related to oxidation of unsaturated fatty acids when cells are disrupted (Róth *et al.*, 2007), as well as metabolism of aldehyde to alcohol and ester (Caleb *et al.*, 2013). Additionally, low aldehydes VOCs in this study could be related to the low microorganism level in MA-3 throughout the storage. As explained by Feng *et al.* (2015) aldehyde is mainly induced by carbonyl compounds due to microbial activity.

Highest VOCs composition was found in arils stored under super-atmospheric O_2 (MA-3) while the lowest composition was found in arils stored at air (MA-4) at the end of storage day 12. The increase in the synthesis and formation of volatiles can be explained by an acceleration of metabolism as a response to wounding (Amaro *et al.*, 2012), or due to a high CO_2 concentration which leads to disrupt enzyme systems, such as the lipoxygenase pathway Giuggioli *et al.* (2015). These findings clearly show the effect of CO_2 on the production of alcohol compounds, with a progressive increase in alcohol production as CO_2 level increased under super-atmospheric O_2. Furthermore, the results indicated that the production of volatile compounds during storage was directly proportional to the CO_2 production and inversely proportional to O_2 consumption.

For the five volatile chemical groups identified in this study, a total relative abundance of alcohols across all treatments increased from the initial 27% on day 0 to 42.3% at the end of storage. As summarized in Table 4 the significant ($p \leq 0.05$) effect of atmosphere modification and storage duration varies between the identified VOCs. The highest significance difference was observed for alcohol and ester VOCs identified for all MA conditions. At the end of storage day 12, samples stored under low O_2 (MA-1) had the highest composition of alcohols (33.3%) of the total VOCs, followed by super-atmospheric O_2 (MA-3), while the lowest (13.3%) was for air (MA-4) at 5 °C.

Giuggioli *et al.* (2015) reported the effect of low O_2 and high CO_2 concentrations on the accumulation of alcohol, which leads to the production of esters. Hence, for MA-1 treated arils, the decrease in headspace O_2 and high CO_2 concentration could result in an anaerobic condition that may stimulate the production of VOCs (Zhang *et al.*, 2013). As observed from the result the highest quantity of alcohols compounds produces at the end of storage day 12. As can be seen from Figure 1 the production of alcohol and esters varies according to the different modified atmosphere conditions. The highest relative abundance of alcohol compounds found in MA-1 could be associated with the highest yeast count observed at the end of storage day 12, which is characterised by fermentation of carbohydrate to produce CO_2, esters and alcohol. The other reason for this could be the decrease of O_2 concentration close to 2% for MA-1. This could change the aerobic respiration to anaerobic fermentation, whereas for MA-3 the reason could be due to the presence of high concentration of CO_2. Meanwhile, the ester compounds identified in this experiment such as 1-butanol-3- methyl acetate were only detected under super atmosphere O_2 (MA-3) at the end of storage. 3-methyl-1-butanol acetate has been reported to have effective antimicrobial inhibitor potential (Mitchell *et al.*, 2010). Therefore, the antimicrobial inhibition effect of 3-methyl-1-butanol acetate could be related to the low microbial load of arils stored at super-atmospheric O_2 (MA-3), which was found in this study. Furthermore, at the end of storage, all MA treatments maintained the emission of β-pinene, and α-terpineol volatile compounds while limonene was found only in samples under low (MA-1) and super-atmospheric oxygen (MA-3) conditions. Caleb *et al.* (2015) reported that β-pinene and α-terpineol and limonene were the major VOCs and flavour descriptors in pomegranate arils. Overall, MA conditions inhibited the synthesis and/or enhanced the accumulation of some volatile compounds, which can be the primary cause of off flavour development such as acetaldehyde, ethanol and ethyl acetate.

Microbial quality

Microbial count of minimally-processed pomegranate arils at different gas composition and temperature was investigated. The results showed that the interaction of MA types and storage time had significant ($p \leq 0.05$) effect on aerobic mesophilic bacteria and yeast and mould count of pomegranate arils stored at 5 ℃ and ambient (20 ℃) conditions. Furthermore, the maximum microbial count during storage of pomegranate arils was affected by the different gas concentrations as shown in Figure 2. The growth of yeast and mould were two-folds lower under MA-3 (2.5 log CFU mL^{-1}) than MA-1 (4.9 log CFU mL^{-1}) at the end of storage at 5 ℃. Furthermore, pomegranate arils stored under MA-3 condition had the lowest aerobic mesophilic bacteria counts, while the highest count was observed for arils kept at normal air condition (MA-4) (5.04 log CFU mL^{-1}). Similarly, Banda *et al.* (2015) and Ayhan and Eştürk (2009) reported that high O_2 atmosphere maintained significantly lower aerobic mesophilic bacterial count throughout the storage time. However, in the current study, total aerobic bacteria and fungi counts were lower than the maximum acceptable limit of 7 log CFU mL^{-1} and 5 log CFU mL^{-1}, respectively for fresh cuts according to South African legislation (FCDA, 1979). However, at ambient (20 °C) condition, the yeast and mould limit of 5 log CFU mL^{-1} was exceeded for arils stored under MA-2 and MA-1 on day 6 and 9, respectively, and the acceptable limit for aerobic mesophilic bacteria count was exceeded after 9 days for all MA conditions except MA-3.

The experimental finding that super-atmospheric O_2 condition is capable of reducing the microbial count could be attributed to the reactive oxygen species (ROS, O_2^-,H_2O_2, OH) generated at high O_2 partial pressure (Zhang *et al*., 2013). The reactive oxygen species damage vital cell components and thereby reducing cell viability when oxidative stress overwhelm cellular protection systems (Oms-Oliu *et al*., 2008). The combined effect of super-atmospheric O_2 (70%) with a high level of CO_2 (10 to 20%), for effective microbial inhibition, was noticed rather than using the individual gases alone (Jacxsens *et al*., 2001). Furthermore, the accumulation of CO_2 and its antimicrobial inhibition effect could also be associated with the lower microbial count. The solubility of CO_2 enables penetration of bacterial membrane, which leads to intercellular pH changes, or formation of carbonic acid, which has a bacteriostatic effect (Paul & Clarke, 2002; Zhang *et al*., 2013; Banda *et al*., 2015).

Conclusions

Storing 'Wonderful' pomegranates arils under super-atmospheric O_2 (MA-3) significantly maintained the lowest microbial counts, better colour and texture properties compared to other MA conditions at both 5 °C. In addition, super-atmospheric O_2 condition promoted the production of highest composition and amount of volatile compounds (VOCs) responsible for flavour profile and antimicrobial effects identified in pomegranate arils. On the other hand, cold storage of arils under low O_2 (MA-1) atmosphere better maintained the antioxidant properties such as phenolic, anthocyanins and ascorbic acid contents. Maintaining or enhancing the different quality attributes of pomegranate arils varied through the different modified atmospheres. Therefore, further study would be necessary to optimize gas concentration for modified atmosphere packaging and storage of pomegranate arils under low or super-atmospheric O_2 at cold storage. Given that the pomegranate fruit investigated in this study were processed after long-term cold storage (simulating shipping duration), further studies on the impact of various pre-processing periods coupled with MA on physiological response and quality attributes of pomegranate arils are required.

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Table 1. Effect of different modified atmosphere conditions on physical quality of pomegranate arils stored at 5 °C for 12 days and additional 2 days at ambient (20 °C).

Parameters	**Cold storage** ^{a}					**Cold storage + 2 d at ambient (20 °C)**				
	Storage time	**MA-1**	**MA-2**	**MA-3**	**MA-4**		**MA-1**	**MA-2**	**MA-3**	**MA-4**
Chroma (C^*)	Day 0	$14.91 \pm 2.9_{b}^{A}$	$14.91 \pm 2.9_{b}^{A}$	$14.91 \pm 2.9_{a}^{A}$	$14.91 \pm 2.9_{c}^{A}$	Day-0+2	$23.09 \pm 5.1_{a}^{AB}$	$24.80 \pm 8.9_{ab}^{AB}$	$19.42 \pm 2.0_{ab}^{B}$	$24.86 \pm 1.1_{a}^{A}$
	Day 3	$26.42 \pm 5.1_{a}^{A}$	$21.46 \pm 9.8_{b}^{A}$	$19.42 \pm 2.0_{a}^{A}$	$33.02 \pm 5.2_{ab}^{A}$	Day-3+2	$23.00 \pm 3.2_{a}^{A}$	$15.93 \pm 1.7_{b}^{B}$	$20.84 \pm 2.4_{a}^{A}$	$15.81 \pm 2.9_{b}^{B}$
	Day 6	$29.82 \pm 4.8_{a}^{A}$	$17.78 \pm 2.6_{b}^{B}$	$18.65 \pm 2.9_{a}^{B}$	$16.83 \pm 2.8_{bc}^{B}$	Day-6+2	$13.13 \pm 5.9_{b}^{AB}$	$19.82 \pm 1.5_{a}^{A}$	$18.38 \pm 2.7_{a}^{AB}$	$17.88 \pm 0.8_{b}^{B}$
	Day 9	$25.26 \pm 1.8_{a}^{A}$	$17.99 \pm 1.0_{b}^{B}$	$18.14 \pm 1.2_{a}^{B}$	$18.42 \pm 2.4_{bc}^{B}$	Day-9+2	$15.20 \pm 2.1_{b}^{A}$	$15.66 \pm 2.4_{b}^{A}$	$17.17 \pm 2.2_{a}^{A}$	$13.93 \pm 4.2_{b}^{A}$
	Day 12	$30.14 \pm 7.1_{a}^{AB}$	$37.79 \pm 1.5_{a}^{A}$	$14.91 \pm 2.9_{a}^{C}$	$28.78 \pm 6.5_{a}^{B}$	Day-12+2	DO	DO	DO	DO
Hue ($h°$)	Day 0	$30.19 \pm 5.7_{b}^{A}$	$30.19 \pm 5.7_{bc}^{A}$	$30.19 \pm 5.7_{abc}^{A}$	$30.19 \pm 5.7_{ab}^{A}$	Day-0+2	$39.61 \pm 7.4_{a}^{A}$	$36.09 \pm 4.0_{a}^{A}$	$29.44 \pm 4.0_{ab}^{A}$	$25.16 \pm 7.6_{a}^{A}$
	Day 3	$36.27 \pm 12.8_{a}^{A}$	$40.06 \pm 3.0_{a}^{A}$	$33.68 \pm 8.4_{ab}^{AB}$	$25.48 \pm 2.5_{b}^{B}$	Day-3+2	$26.96 \pm 4.7_{b}^{A}$	$29.43 \pm 1.8_{b}^{A}$	$28.31 \pm 2.0_{a}^{A}$	$29.98 \pm 4.8_{a}^{A}$
	Day 6	$30.63 \pm 10.0_{ab}^{A}$	$25.90 \pm 2.4_{c}^{A}$	$24.50 \pm 0.1_{c}^{A}$	$26.83 \pm 1.9_{b}^{A}$	Day-6+2	$27.02 \pm 7.9_{ab}^{AB}$	$27.03 \pm 2.0_{bc}^{AB}$	$24.44 \pm 1.2_{b}^{B}$	$28.48 \pm 2.0_{a}^{A}$
	Day 9	$46.06 \pm 5.2_{a}^{A}$	$24.77 \pm 0.2_{c}^{B}$	$25.53 \pm 2.6_{c}^{B}$	$25.36 \pm 1.4_{b}^{B}$	Day-9+2	$27.67 \pm 5.2_{ab}^{A}$	$25.77 \pm 0.9_{c}^{A}$	$26.25 \pm 1.5_{a}^{A}$	$25.48 \pm 1.2_{a}^{A}$
	Day 12	$36.51 \pm 8.6_{ab}^{A}$	$33.32 \pm 1.5_{b}^{A}$	$30.21 \pm 5.3_{b}^{A}$	$32.96 \pm 4.5_{a}^{A}$	Day-12+2	DO	DO	DO	DO
Texture (N)	Day 0	$7.98 \pm 1.9_{a}^{A}$	$7.98 \pm 1.9_{aA}$	$7.98 \pm 1.9_{a}^{A}$	$7.98 \pm 1.9_{a}^{A}$	Day-0+2	$8.83 \pm 1.7_{a}^{A}$	$8.26 \pm 1.7_{ab}^{A}$	$8.95 \pm 2.1_{a}^{A}$	$8.87 \pm 2.6_{ab}^{A}$
	Day 3	$7.91 \pm 1.5_{a}^{A}$	$7.87 \pm 1.5_{aA}$	$8.62 \pm 1.4_{a}^{A}$	$8.71 \pm 1.6_{a}^{A}$	Day-3+2	$7.78 \pm 1.9_{a}^{A}$	$9.53 \pm 0.5_{a}^{A}$	$8.78 \pm 0.6_{a}^{A}$	$8.65 \pm 0.7_{a}^{A}$
	Day 6	$9.68 \pm 1.6_{a}^{A}$	$8.69 \pm 0.8_{aA}$	$7.85 \pm 1.3_{a}^{A}$	$7.85 \pm 1.6_{a}^{A}$	Day-6+2	$8.99 \pm 1.4_{a}^{A}$	$8.12 \pm 0.9_{ab}^{A}$	$7.90 \pm 1.2_{a}^{A}$	$8.04 \pm 1.1_{ab}^{A}$
	Day 9	$8.77 \pm 1.1_{a}^{A}$	$8.86 \pm 1.6_{aA}$	$8.83 \pm 0.6_{a}^{A}$	$7.72 \pm 1.0_{a}^{A}$	Day-9+2	$7.71 \pm 1.0_{a}^{AB}$	$7.22 \pm 1.6_{b}^{AB}$	$8.93 \pm 0.7_{a}^{A}$	$6.91 \pm 0.6_{b}^{B}$
	Day 12	$7.91 \pm 1.5_{a}^{A}$	$7.87 \pm 1.5_{aA}$	$8.62 \pm 1.4_{a}^{A}$	$8.71 \pm 1.6_{a}^{A}$	Day-12+2	Day-9+2	DO	DO	DO

Mean values standard deviation in the same column with different lowercase subscript letters are significantly different ($p \leq 0.05$) along storage duration, and mean values in the same row with different superscript upper case letters are significantly different ($p \leq 0.05$) among treatments.

DO: Decay observed. ^{a} Storage at 5 °C.

Table 2. Effect of different modified atmosphere conditions on the phytonutrient property of pomegranate arils stored at 5 °C.

Parameters	**Storage time**	**MA-1**	**MA-2**	**MA-3**	**MA-4**
Ascorbic acid (mg L^{-1})	Day 0	$3035.4 \pm 8.3_{a}^{A}$	$3035.4 \pm 8.3_{a}^{A}$	$3035.4 \pm 8.3_{a}^{A}$	$3035.4 \pm 8.3_{a}^{A}$
	Day 3	$2645.7 \pm 76.5_{b}^{A}$	$1868.5 \pm 37.6_{b}^{B}$	$928.8 \pm 255.8_{b}^{C}$	$617.7 \pm 71.3_{c}^{D}$
	Day 6	$2647.9 \pm 78.9_{b}^{A}$	$1943.5 \pm 2.5_{b}^{B}$	$1132.5 \pm 422.2_{b}^{C}$	$681.0 \pm 45.3_{c}^{c}$
	Day 9	$2649.4 \pm 75.0_{b}^{A}$	$2059.7 \pm 15.9_{b}^{B}$	$1200.1 \pm 427.8_{b}^{C}$	$769.2 \pm 78.9_{c}^{c}$
	Day 12	$2786.9 \pm 15.4_{b}^{A}$	$2167.1 \pm 18.4_{b}^{B}$	$1339.1 \pm 413.1_{b}^{C}$	$883.9 \pm 11.1_{b}^{D}$
Phenolic (mg L^{-1})	Day 0	$18.71 \pm 6.3_{ab}^{A}$	$18.71 \pm 6.3_{ab}^{A}$	$18.71 \pm 6.3_{a}^{A}$	$18.71 \pm 6.3_{a}^{A}$
	Day 3	$14.76 \pm 1.1_{b}^{B}$	$22.77 \pm 0.4_{a}^{A}$	$13.28 \pm 1.7_{a}^{B}$	$15.31 \pm 1.3_{a}^{B}$
	Day 6	$16.29 \pm 4.8_{b}^{A}$	$22.52 \pm 2.8_{a}^{A}$	$18.16 \pm 6.9_{a}^{A}$	$15.09 \pm 4.3_{a}^{A}$
	Day 9	$23.97 \pm 2.0_{a}^{A}$	$22.09 \pm 2.8_{a}^{A}$	$11.41 \pm 2.3_{a}^{B}$	$15.04 \pm 2.1_{a}^{B}$
	Day 12	$27.19 \pm 3.5_{a}^{A}$	$17.06 \pm 1.9_{b}^{B}$	$12.51 \pm 3.8_{a}^{B}$	$13.99 \pm 2.8_{a}^{B}$
Anthocyanins (mg L^{-1})	Day 0	$0.07 \pm 0.0_{a}^{A}$	$0.07 \pm 0.0_{a}^{A}$	$0.07 \pm 0.0_{a}^{A}$	$0.07 \pm 0.0_{a}^{A}$
	Day 3	$0.06 \pm 0.0_{a}^{A}$	$0.07 \pm 0.0_{a}^{A}$	$0.07 \pm 0.0_{a}^{A}$	$0.07 \pm 0.0_{a}^{A}$
	Day 6	$0.06 \pm 0.0_{a}^{A}$	$0.07 \pm 0.0_{a}^{A}$	$0.05 \pm 0.0_{a}^{A}$	$0.05 \pm 0.0_{a}^{A}$
	Day 9	$0.06 \pm 0.0_{a}^{A}$	$0.06 \pm 0.0_{a}^{A}$	$0.06 \pm 0.0_{a}^{A}$	$0.04 \pm 0.0_{b}^{A}$
	Day 12	$0.06 \pm 0.0_{a}^{A}$	$0.04 \pm 0.0_{b}^{A}$	$0.03 \pm 0.0_{b}^{A}$	$0.05 \pm 0.0_{a}^{A}$

Mean values standard deviation in the same column with different lowercase subscript letters are significantly different ($p \leq 0.05$) along storage duration, and mean values in the same row with different superscript uppercase letters are significantly different ($p \leq 0.05$) among treatments.

Table 3. Group of volatile compounds according to their molecular class, identified in pomegranate arils stored in different gas composition at 5 °C for 12 days**.**

Group	Volatile compounds	RT	RI (Cal[d])	RI (Lib)
Aldehyde	Hexanal[a]	4.8	778	778
	Decanal[a]	10.7	1001,51	1000
Monotepenes	β.-Pinene[a]	4.9	782,67	945
	β.-Myrcene[a]	5.8	816,62	1020
	β.-Phellandrene[a]	6.5	840,84	1059
	Limonene[a]	6.6	846,48	1056
Monoterpenoids	α-Terpineol[b]	13.2	1092,56	1300
Sesquiterpenes	trans-.α.-Bergamotene [a]	11.8	1041,01	1522
	(trans)-β-Farnesene [b]	12.8	1092,56	1391
	(cis)-β-Farnesene[b]	13.1	1089,18	1536
Ester	1-Butanol, 3-methyl-, acetate [c]	5.3	796,67	1042
	Ethyl hexanoate [a]	7.0	861,16	809
	Acetic acid, hexyl ester[b]	7.6	883,36	997
	2,2,4-trimethyl-1,3-pentanediol 1-isobutyrate[b]	15.2	1164,43	1365
keton	2-Heptanone[b]	6.3	832,56	845
	Isopentyl methyl ketone[b]	6.3	832,94	862
	3-Octanone[a]	7.4	874,33	
	2-Octanone[a]	7.8	891,26	1009
Alcohols	1-Hexanol[a]	8.8	928,51	880
	3-Hexen-1-ol, (cis)[b]	9.2	943,94	859
	3-Ethyl-3-octanol[a]	10.1	978,55	1107
	2-Ethyl-1-hexanol[a]	10.6	995,48	1019
	1-Octanol[b]	11.5	1029,73	1053
	1-Nonanol[b]	12.7	1076,01	1155

[a] Primary detected volatiles

[b] secondary detected volatiles

[c] volatile detected only at super-atmospheric O_2

[d] Calculated RI

RT: retention time (minute); RI: Retention index

Table 4. Effect of modified atmosphere condition on the change in volatile concentration and composition for pomegranate arils stored at 5 °C for 12 days.

Volatile compounds			**DAY 3**				**DAY 6**			
	RT	**DAY 0**	**MA-1**	**MA-2**	**MA-3**	**MA-4**	**MA-1**	**MA-2**	**MA-3**	**MA-4**
Hexanal	4.8	0.4 ± 0.1^{f}	0.9 ± 0.1^{e}	–	0.9 ± 0.6^{e}	0.1 ± 0.0^{g}	0.2 ± 0.0^{g}	–	–	–
β -Pinene	4.9	0.1 ± 0.0^{g}	0.1 ± 0.0^{g}	0.1 ± 0.0^{g}	–	0.1 ± 0.0^{g}	0.1 ± 0.0^{g}	–	–	0.2 ± 0.2^{g}
1-Butanol, 3-methyl-, acetate	5.3	–	–	–	–		–	–	–	–
beta.-Myrcene	5.8	0.1 ± 0.0^{g}	0.1 ± 0.0^{g}	–	–	0.2 ± 0.0^{g}	–	0.2 ± 0.0^{g}	–	–
2-Heptanone	6.3	–	–	–	–	–	–	–	–	–
2-Hexanone, 5-methyl-	6.3	1.2 ± 0.1^{e}	–	–	–	–	–	–	–	–
β -Phellandrene	6.5	–	0.1 ± 0.0^{g}				–	–	–	–
Limonene	6.6	1.3 ± 0.1^{e}	–	1.3 ± 0.1^{e}	2.3 ± 0.3^{e}	1.4 ± 0.0^{e}	1.8 ± 2.1^{e}	–	0.3 ± 0.0^{g}	–
Hexanoic acid, ethyl ester	7.0	0.1 ± 0.0^{g}	–	–	–	0.3 ± 0.1^{g}	0.1 ± 0.0^{g}	–	0.1 ± 0.0^{g}	–
3-Octanone	7.4	15.9 ± 0.6^{b}	16.2 ± 0.8^{b}	12.9 ± 3.5^{b}	15.1 ± 1.8^{b}	19.1 ± 2.6^{b}	15.1 ± 2.3^{b}	18.7 ± 4.5^{b}	13.2 ± 0.3^{b}	7.9 ± 10.7^{bc}
Acetic acid, hexyl ester	7.6	–	–	–	–	–	–	–	–	–
2-Octanone	7.8	7.0 ± 0.0^{c}	0.6 ± 0.7	–	–	0.4 ± 0.2	6.7 ± 0.2^{c}	1.9 ± 1.9^{b}	10.9 ± 3.6^{bc}	2.0 ± 1.1^{e}
1-Hexanol	8.8	0.8 ± 0.0^{f}	0.5 ± 0.1^{ab}	–	1.3 ± 0.1^{e}	1.4 ± 0.1^{e}	–	–	–	–
3-Hexen-1-ol, (cis)-	9.2	–	–	–	0.2 ± 0.1^{g}	0.2 ± 0.0^{g}	–	–	–	–
3-Ethyl-3-octanol-	10.1	0.6 ± 0.0	–	–	–	–	–	–	–	–
2-Ethyl-1-hexanol	10.6	4.5 ± 0. 5^{d}	4.7 ± 0.0^{d}	5.2 ± 2.1^{cd}	–	7.2 ± 1.4^{c}	4.7 ± 0.0^{d}	6.3 ± 1.2^{c}	3.8 ± 0.3^{d}	2.9 ± 3.9^{cdf}
Decanal	10.7	0.4 ± 0.0^{f}	0.4 ± 0.0^{f}	–	0.4 ± 0.0^{f}	–	–	–	–	–
1-Octanol	11.5	–	–	0.6 ± 0.0^{f}	0.8 ± 0.4^{e}	0.8 ± 0.1^{f}	–	–	1.5 ± 0.4^{e}	
Trans-.alpha.-Bergamotene	11.8	0.3 ± 0.3^{fg}	0.3 ± 0.1^{g}	0.4 ± 0.1^{g}	0.3 ± 0.0^{g}	1.2 ± 1.9^{eg}	0.9 ± 0.7^{e}	1.0 ± 1.1^{eg}	0.8 ± 0.9eg	0.4 ± 0.3^{fg}
1-Nonanol	12.7	–	0.6 ± 0.1^{f}	2.2 ± 0.3^{d}	1.0 ± 0.6^{e}	–	–	–	2.2±1.9^{de}	2.9±1.9^{de}
(Trans)-β-Farnesene	12.8	0.4 ± 0.1^{f}	0.7 ± 0.0^{f}	–	0.3 ± 0.0^{g}	0.6 ± 0.1^{f}	0.6 ± 0.0^{f}	0.5 ± 0.3^{f}	–	–
(Cis)-β-Farnesene	13.1	0.1 ± 0.0^{g}	0.2 ± 0.0^{g}	–	–	–	–	–	0.3 ± 0.0^{g}	–
α-Terpineol	13.2	–	–	–	–	–	0.6 ± 0.1^{f}	0.6 ± 0.1^{f}	0.6 ± 0.1^{f}	–
2,2,4-trimethyl-1,3-pentanediol 1-isobutyrate	15.1	0.1 ± 0.0^{g}	0.3 ± 0.0^{g}	0.2 ± 0.0^{g}	0.2 ± 0.0^{g}	0.3 ± 0.0^{g}	–	0.7 ± 0.6^{f}–	0.7 ± 0.1^{f}	0.6 ± 0.1^{f}

Means (*n* = 3) ± standard deviation with different letters across each row are significantly different by Duncan's multiple range test (p ≤ 0.05).

– Represents volatile organic compounds were below the detection limit of the GC–MS.

Table 4, *Continue*

Volatile compounds	**DAY 9**				**DAY 12**			
	MA-1	**MA-2**	**MA-3**	**MA-4**	**MA-1**	**MA-2**	**MA-3**	**MA-4**
Hexanal	0.2 ± 0.0^{g}	_	_	_	0.2 ± 0.0^{g}	0.8 ± 0.3^{e}		0.7 ± 0.3^{ef}
β-Pinene	0.1 ± 0.0^{g}	0.1 ± 0.0^{g}	0.1 ± 0.0^{g}	_	0.1 ± 0.0^{g}	0.2 ± 0.0^{g}	0.1 ± 0.0^{g}	
1-Butanol, 3-methyl-, acetate	_	_	_	_	_	_	0.3 ± 0.1^{g}	
β-Myrcene	_	0.1 ± 0.0^{g}	0.1 ± 0.0^{g}	0.3 ± 0.0^{g}	_	_	0.1 ± 0.0^{g}	0.2 ± 0.0^{g}
2-Heptanone	_	_	_	1.4 ± 0.1^{e}	_	_	_	_
2-Hexanone, 5-methyl-	_	_	_	_	_	_	_	_
β-Phellandrene	_	1.6 ± 1.8^{e}	_	0.9 ± 0.9^{e}	_	_	0.2 ± 0.1	0.2 ± 0.2^{g}
Limonene	1.5 ± 0.1^{e}	_	0.5 ± 0.1^{f}	1.1 ± 0.9^{e}	1.4 ± 0.0^{e}	_	0.3 ± 0.0	_
Hexanoic acid, ethyl ester	_	_	_	_	0.1 ± 0.0^{a}	_	1.6 ± 0.2^{e}	_
3-Octanone	13.6 ± 0.8^{b}	_	_	0.1 ± 0.0^{b}	15.0 ± 0.2^{b}	15.0 ± 0.2^{b}	0.2 ± 0.1^{acd}	14.6 ± 0.8^{b}
Acetic acid, hexyl ester	_	0.6 ± 0.1f	0.7 ± 0.1^{f}	0.6 ± 0.1^{f}	_	_	_	_
2-Octanone	10.6 ± 0.1^{c}	19.0 ± 5.8^{bc}	57.9 ± 2.9^{abd}	15.9 ± 1.5^{b}	1.6 ± 0.6^{e}	_	15.7 ± 1.1^{b}	_
1-Hexanol	_	_	0.6 ± 0.2f	_	2.9 ± 0.0^{e}	_	0.9 ± 0.4^{e}	_
3-Hexen-1-ol, (cis)-	_	0.5 ± 0.1^{e}	_	_	0.3 ± 0.0	0.2 ± 0.1^{g}	0.6 ± 0.1f	_
-Ethyl-3-octanol-	_	_	1.9 ± 0.4^{e}	0.7 ± 0.1^{f}	0.8 ± 0.1^{e}	0.7 ± 0.2^{e}	1.4 ± 0.3^{e}	0.4 ± 0.2^{eg}
2-Ethyl-1-hexanol	_	_	0.8 ± 0.1^{f}	0.7 ± 0.1^{f}	5.7 ± 0.3d	_	4.8 ± 0.5d	_
Decanal	4.7 ± 0.2^{d}	6.2 ± 2.7^{c}	5.8 ± 0.4^{d}	_	_	0.4 ± 0.1^{a}	5.5 ± 0.6^{d}	_
1-Octanol	0.3 ± 0.0^{g}	_	0.4 ± 0.4^{ef}	_	_	_	1.0 ± 0.2^{e}	_
Trans-. α--Bergamotene	_	1.6 ± 1.8^{ef}	_	0.9 ± 0.9^{eg}	_	0.6 ± 0.2^{f}	1.3 ± 1.6^{ef}	_
1-Nonanol	0.4 ± 0.0^{f}	-	_	_	0.3 ± 0.0^{g}	_	0.8 ± 0.2^{e}	_
(Trans)-β-Farnesene	_	0.5 ± 0.1^{f}	_	0.3 ± 0.1^{g}	_	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.1^{g}
(Cis)-β-Farnesene	_	0.5 ± 0.1^{f}	0.9 ± 0.8^{e}	_	_	_	2.6 ± 0.1^{e}	_
α-Terpineol	_	0.6 ± 0.1^{ef}	0.7 ± 0.1^{e}	0.6 ± 0.1^{f}	0.8 ± 0.0^{e}	0.8 ± 0.2^{ef}	1.0 ± 0.2^{e}	0.7 ± 0.0^{f}
2,2,4-trimethyl-1,3-pentanediol 1-isobutyrate	0.7 ± 0.0^{e}	0.5 ± 0.6^{f}	0.9 ± 0.8^{e}	_	0.3 ± 0.0^{g}	_	0.6 ± 0.0^{f}	0.3 ± 0.2

Means (*n* = 3) ± standard deviation with different letters across each row are significantly different by Duncan's multiple range test ($p \leq 0.05$).
– Represents volatile organic compounds were below the detection limit of the GC–MS.

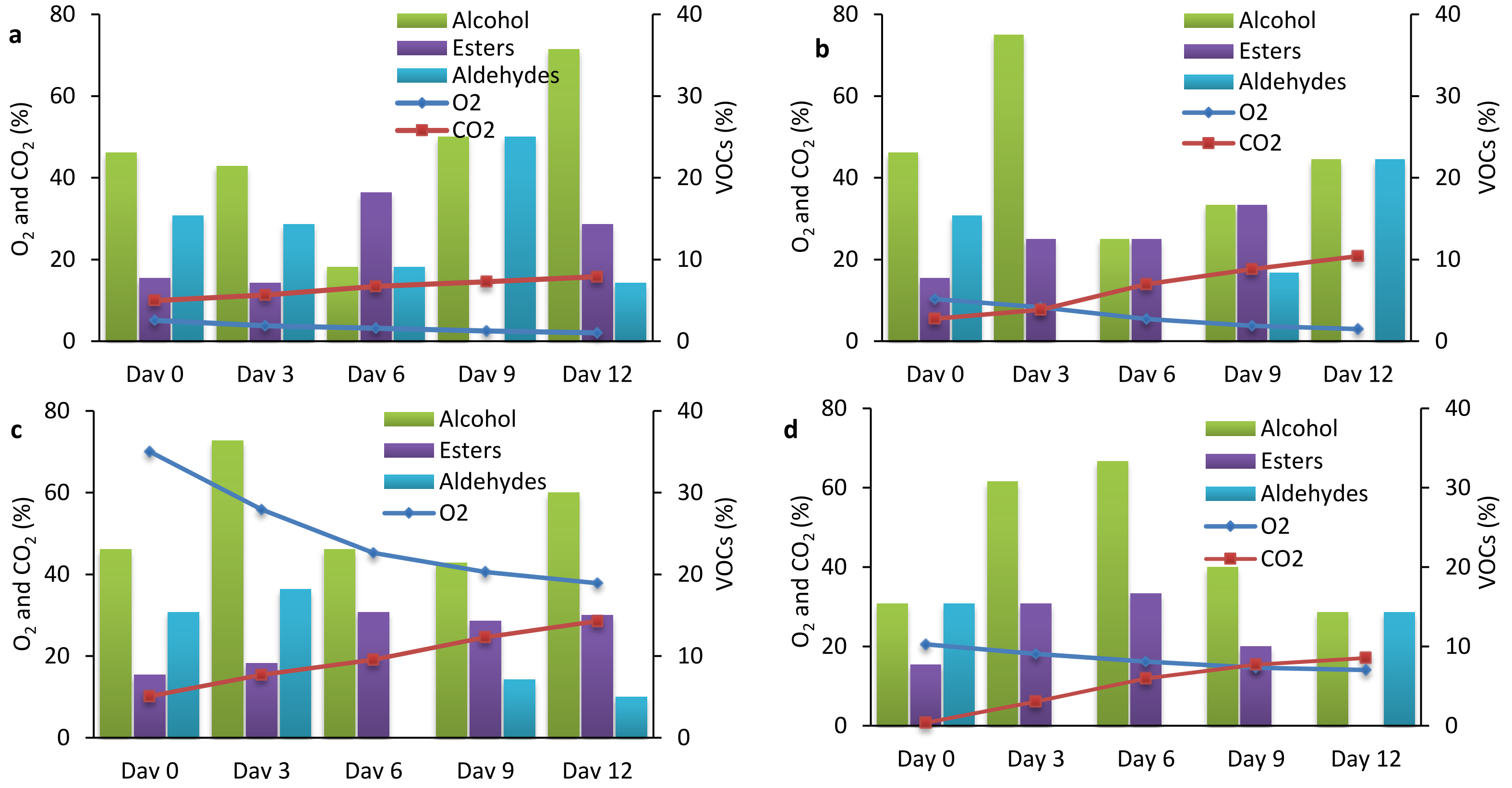

Figure 1. Summary of cumulative change in relative abundance (%) of alcohols, esters and aldehydes at different modified atmospheres for pomegranate 'wonderful' arils stored at 5 °C for 12 days; (a) = MAa-1: (5% O_2 + 10% CO_2 + 85% N_2), (b) = MA-2: (10% O_2 + 5% CO_2 + 85% N_2), (c) = MA-3: (70% O_2 + 10% CO_2 + 20% N_2), and (d) = MA-4: (air, 21% O + 0% CO_2 + 78% N_2) as control. Alcohol: green shaded bars; esters: purple shaded bars; aldehydes: blue filled bars; ◆: O_2 and ■ CO_2 (%). Vertical bars represent the standard deviation (SD) of mean values (n = 9); means with different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

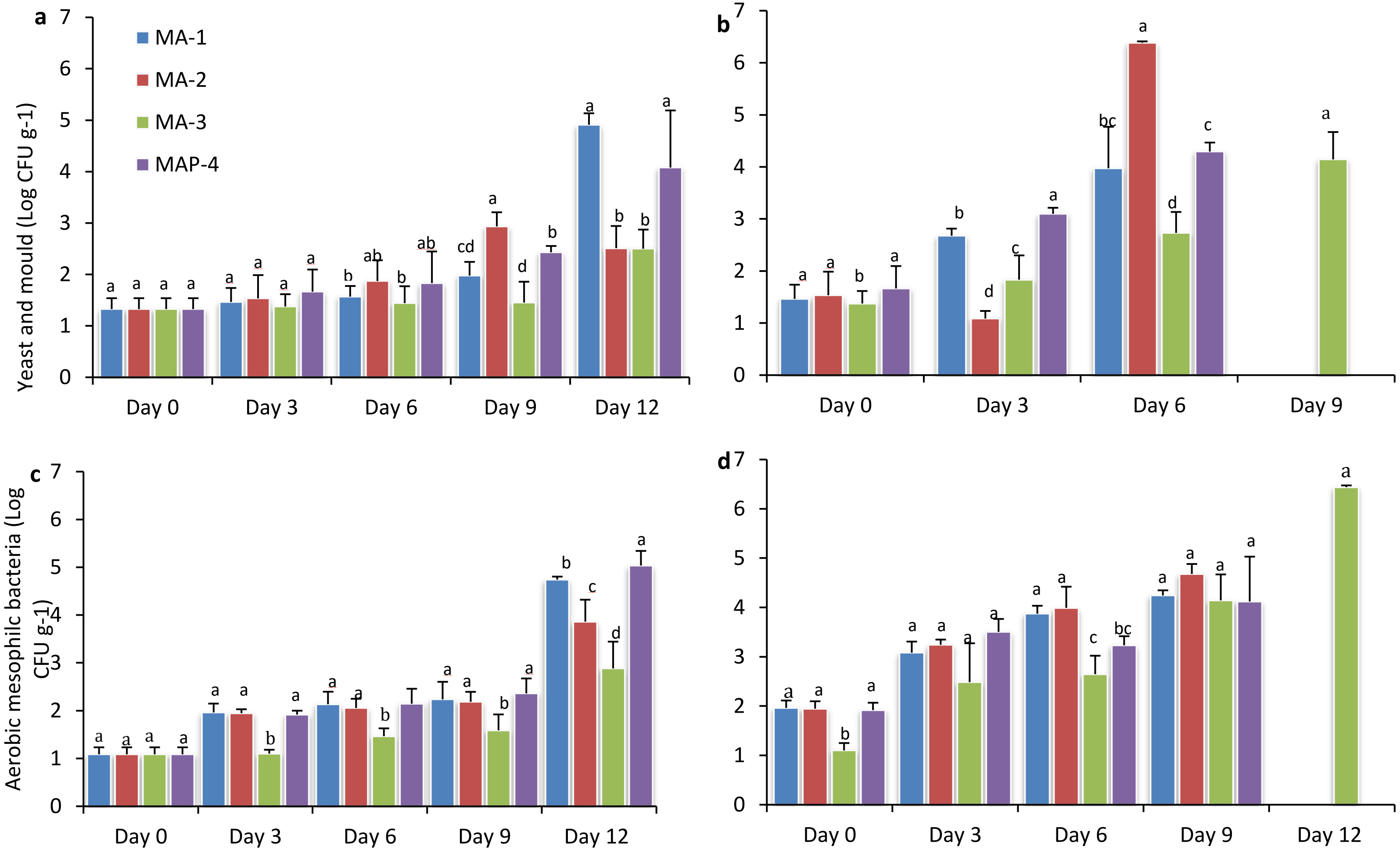

Figure 2. Effect of gas composition on yeast and mould counts of 'Wonderful' pomegranate arils stored at 5 °C (a) and ambient (b) conditions, respectively, and aerobic mesophilic bacteria counts for samples stored at 5 °C (c) and ambient (d) storage conditions, respectively for 12 d. MA-1: (5% O_2 + 10% CO_2 + 85% N_2), MA-2: (10% O_2 + 5% CO_2 + 85% N_2), MA-3: (70% O_2 + 10% CO_2 + 20% N_2), and MA-4: (air, 21% O_2 + 0% CO_2 + 78% N_2) as control. Vertical bars represent the standard deviation (SD) of mean values (n = 9); means with different letters are significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

Declaration by the candidate:

With regard to Chapter 5 (pp 107-126); the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data analysis and writing of chapter	75

The following co-authors have contributed to Chapter 5 (pp 107-126):

Name	E-mail address	Nature of contribution	Extent of contribution (%)
Prof U.L. Opara	opara@sun.ac.za	Research input, editorial suggestion and proof reading	10
Dr O.J. Caleb	CalebO@arc.agric.za	Research input, assistance with modelling, editorial suggestion and proof reading	15

Signature of candidate: Z.A. Belay

Date: 03/11/2017

Declaration by co-authors:

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 5 (pp 107-126)
2. no other authors contributed to Chapter 5 (pp 107-126) besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 5 (pp 107-126) of this dissertation.

Signature	Institutional affiliation	Date
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Declaration with signature in possession of candidate and supervisor.

CHAPTER 5

ENZYME KINETICS MODELLING TO EVALUATE THE IMPACT OF HIGH CO$_2$ AND SUPER-ATMOSPHERIC O$_2$ CONCENTRATIONS ON RESPIRATION RATE OF POMEGRANATE ARILS.

ABSTRACT

Super-atmospheric O$_2$ has been shown to affect the respiration rate (RR), but no model describing its effect on RR of 'Wonderful' pomegranate arils has been reported. This study investigated the effects of four different gas compositions (5% O$_2$, 10% CO$_2$ & 85% N$_2$; 10% O$_2$, 5% CO$_2$ & 85% N$_2$; 70% O$_2$, 10% CO$_2$ & 20% N$_2$; and air) on RR of pomegranate arils stored at 5 °C. Michaelis–Menten (MM) enzyme kinetics models were used to investigate the effect of CO$_2$ inhibition on O$_2$ consumption rate. Respiratory quotient (RQ) was used to determine the fermentation threshold. Gas composition significantly affected the RR. The O$_2$ consumption rate increased from 0.87 to 2.81 mL kg^{-1} h^{-1}, with increase in O$_2$ concentration from 5% to 70%. All enzyme kinetics model parameters adequately described the influence of gas concentration on aril RR with correlation coefficient (R^2_{adj} = 81-91%).

Key words: Modified atmosphere; Postharvest physiology; Michaelis-Menten

Introduction

After harvest, fresh produce are susceptible to increased physiological stress and deterioration in quality due to accelerated respiration rate (RR) (Aindongo *et al*., 2014; Caleb *et al*., 2012a). This process consumes O$_2$ and produced CO$_2$ in a series of enzymatic reaction (Iqbal *et al*., 2009). Thus, higher RR could indicate a faster rate of physiological ageing, senescence and deterioration of fresh produce (Cliffe-Byrnes & O'Beirne, 2007). Improved postharvest handling methods which apply modified atmosphere (MA) around the produce can be used to reduce RR and slow down metabolic processes, thereby maintaining quality and extending shelf life (Belay *et al*., 2016; Caleb *et al*., 2012a; Jing-jun *et al*., 2012).

Studies have reported different approaches to determine suitable gas concentration for reducing the RR of pomegranate arils. Ersan *et al*. (2009) investigated the effect of O$_2$ (2, 10 and 21%) and CO$_2$ (0, 10 and 20%) concentration on the RR of 'Hicaznar' pomegranate arils at 5 °C. Similarly, Banda *et al*. (2015) studied the effect of O$_2$ (5, 21 and 30%) and CO$_2$ (0, 10 and 40%) on the RR of 'Wonderful' pomegranate arils. These studies showed that low O$_2$ atmosphere significantly reduced the RR. In addition, López-Rubira *et al*. (2005) recommended

2-4% O_2 for maintaining qualities of ‘Mollar de Elche’ pomegranate arils. Overall, the results reported in these studies demonstrated that the application of MA systems was effective in maintaining quality and extended shelf life of pomegranate arils. However, low O_2 concentration can cause the build-up of anaerobic atmospheres which then leads to fermentation. Recent studies have introduced the benefits of super-atmospheric O_2 (> 70%) to overcome the limitation of the low O_2 atmosphere on postharvest physiology and quality maintenance of fresh produce (Belay *et al*., 2017; Maghoumi *et al*., 2013; Molinu *et al*., 2016).

In addition, predicting respiratory kinetics of different fruit and vegetables has been based on consideration of the RR as a function of gas composition using enzyme kinetics model (Iqbal *et al*., 2009; Torrieri *et al*., 2010; Belay *et al*., 2016). For pomegranate fruit, Caleb *et al*. (2012b) investigated the effect storage temperature (5, 10 and 15 °C) and time on RR of ‘Acco’ and ‘Herskawitz’ pomegranate arils by using Arrhenius type model. Similarly, Aindongo *et al*. (2014) studied the effect of temperature (5, 10, 15 and 22 °C) on RR of different ‘Bhagwa’ pomegranate fractions by using Arrhenius equation. These studies reported that temperature had a significant effect on RR of pomegranate aril and Arrhenius type model adequately predicted this effect regarding of different temperature and cultivar used.

On the other hand, the effects of gas composition (2, 10, 21% O_2 and 0, 10, 20% CO_2) on the RR of ‘Hicaz’ pomegranate arils was studied by Ersan *et al*. (2009) using MM model. The study showed that MAs affected the RR of pomegranate arils and this relationship was best expressed using the MM competitive inhibition model. However, their study did not report on the application of super-atmospheric O_2 condition and its effects on the physiological response of ‘Wonderful’ pomegranate arils. In addition, pomegranate fruit under-go a long-term shipping of from southern hemisphere (producers) to northern hemisphere (market) and vice-versa, prior to processing into arils (Belay *et al*., 2017). There is a limited report on the impacts of this long supply chain on the physiology of the fruit. Therefore, the aim of this study was to investigate the effects of low and super-atmospheric O_2 combined with low and high CO_2 concentrations on the RR of ‘Wonderful’ pomegranate arils after long-term storage. The goal of this study is to provide valuable information on the respiratory kinetics of ‘Wonderful’ pomegranate arils, which would guide the role players along the value chain to better design and optimize the postharvest handling and packaging of arils.

Materials and methods

Plant materials and sample preparation

Pomegranate fruit (cv. Wonderful) were obtained at the commercially ripened stage with characteristic deep-red skin and arils with the mature kernel (Mphahlele *et al.*, 2016), from Sonlia Pack House, Wellington, Western Cape (33°38′23″S, 19°00′40″E), South Africa. Fruit were transported in an air-conditioned and ventilated vehicle to the Postharvest Research Laboratory at Stellenbosch University and stored in a cold room (about 5 °C and 95% relative humidity (RH)) for 4 months until the experiment started. The storage was done to simulate pre-shipping storage plus long-term shipping duration from southern hemisphere production region to the northern hemisphere market and vice-versa. Damaged fruit were removed and the outer skin of selected healthy fruit was surface disinfected using 70% ethanol (Aindongo *et al.*, 2014). Arils were extracted manually by carefully removing the husk. Extracted arils were collected in a tray and mixed together to create a homogenous batch of all the fruit. Arils (350 g) were transferred to 3000 mL air-tight glass jars, which were designed to achieve a completely hermetic seal. The glass jars were prepared in triplicate for each gas mixture and stored at 5 °C. Measurement was performed using four gas mixtures as shown in Table 1.

Experimental setup

Rate of O_2 consumption (R_{O_2}) and CO_2 evolution (R_{CO_2}) of the arils were measured using a closed system as described by (Iqbal *et al.*, 2009, Mahajan & Goswami, 2001; Techavuthiporn & Boonyaritthongchai, 2016). Airtight jars of size 3000 mL made up of glass fitted with lid were used. Each jar lid had three valves (inlet, outlet and gas sampling ports). A rubber ring was fixed between the bottle and the lid seal from air leakage. A plastic tube was attached to the inlet valve, which was inserted down to the bottom of the jar to ensure uniform flushing of gas mixture. Before sealing, jars were flushed with humidified air and the selected gas composition, until equilibrium archived. Three jars were flushed for each of the four MAs, resulting in 12 jars. The headspace gas (about 100 µL) was sampled at hourly intervals for the duration of 5 h at 5 °C through the silicon septum provided in the jar lid. The headspace gases were analysed for O_2 and CO_2 concentrations using O_2/CO_2 gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark). This measurement cycle was repeated over a period of 12 days, based on experimental design reported by Ayhan and Eştürk (2009). R_{O2} and R_{CO2} were calculated using equation 1 & 2:

$$R_{O_2} = \left(\frac{Y_{O_{2i}} - Y_{O_{2t}}}{t - t_i}\right) * \frac{V_f}{W} * \frac{1}{RT} \qquad (1)$$

$$R_{CO_2} = \left(\frac{Y_{CO_{2f}} - Y_{CO_{2i}}}{t - t_i}\right) * \frac{V_f}{W} * \frac{1}{RT} \tag{2}$$

where Y_{O2i} and Y_{O2t} are, respectively, O_2 concentration (%) at the initial time t_i (hours, h) and at time t (h) and Y_{CO2i} and Y_{CO2t} are, respectively, CO_2 concentration (%) at the initial time ti(h) and at time t (h). R_{O2} and R_{CO2} are RR in mL kg^{-1}h^{-1}, T is temperature (Kelvin), R is universal gas constant (0.008314 kJ K^{-1} mol^{-1}), W is the total weight of the product (kg), and V_f is the free volume ($V_f = V - \frac{W}{\rho}$), where V is the volume of the jar (m^3), W is the weight of the minimally-processed arils (kg) and p the apparent density of the arils (0.98 g cm^{-1}). The ratio of CO_2 produced to O_2 consumption is further expressed by the respiration quotient as follows:

$$RQ = \frac{R_{CO_2}}{R_{O_2}} \tag{3}$$

Development of mathematical model of aril respiration rate

Respiration rate was described by enzyme kinetics approach as a function of one limiting enzymatic reaction in which the substrate is O_2 by using a simple MM model as described by equation 4.

$$R_{O_2} = \frac{R_{CO_2} \times Y_{O_2}}{R_{O_2} + Y_{O_2}} \tag{4}$$

In order to account for the possible inhibitory effect of CO_2, R_{O2} was described by a MM type model for change in gas composition under MA storage (Guillard *et al.*, 2012; Paul & Clarke, 2002). MM competitive inhibition (Equation 5), where both the inhibitor (CO_2) and the substrate (O_2) compete for the same active site; uncompetitive (Equation 6), where CO_2 reacts with the substrate-enzyme complex; and non-competitive (Equation 7), when CO_2 reacts both with the enzyme and within the enzyme-substrate complex, models were fitted to the data obtained experimentally to account for the possible effect of Y_{CO_2} on O_2 consumption rate (Gomes *et al.*, 2010). According to Fonseca *et al.* (2002), during competitive inhibition the maximum RR is lower in high CO_2 concentration, however, for uncompetitive inhibition, the maximum RR is not much influenced by high CO_2; on the other hand, for non-competitive, the maximum RR lies between competitive and uncompetitive inhibitions. The models variables were analysed and compared to select the best fit of the experimental data by using equation 5 to 7:

$$R_{O_2} = \frac{V_{max} \times Y_{O_2}}{K_m \times \left(1 + {}^{Y_{CO_2}}\!/_{K_i}\right) + Y_{O_2}} \tag{5}$$

$$R_{O_2} = \frac{V_{max} \times Y_{O_2}}{K_m + \left(1 + {}^{Y_{CO_2}}\!/_{K_i}\right) \times Y_{O_2}} \tag{6}$$

$$R_{O_2} = \frac{V_{max} \times Y_{O_2}}{\left(K_m + Y_{O_2}\right) \times \left(1 + {}^{Y_{CO_2}}\!/_{K_i}\right)} \tag{7}$$

where V_{max} is the maximal value of respiration rate for O_2 consumption R_{O2} (mL $kg^{-1}h^{-1}$), Y_{O2} and Y_{CO2} are the concentration of O_2 and CO_2 inside the glass jar respectively in %, K_m is MM constant for O_2 consumption, and K_i is inhibition constant for CO_2 evolution. Estimation of model MM parameters and constants, based on the experimental data was performed by non-linear regression based on the Levenverg-Marguardt method using STATISTICA software (vr. 13 StatSoft Inc., Tulsa, USA). The model estimates obtained at each modified atmosphere conditions were used to describe the dependency of the model parameters on gas composition. The accuracy of the models estimation was given by standard error value.

Statistical analysis

The results were presented as mean and standard deviation of three measurements. The data were analysed using STATISTICA software (vr. 13 StatSoft Inc., Tulsa, USA). Two-way ANOVA was used to investigate the effects of atmosphere modification and storage duration on RR of pomegranate arils. Fishers' least significant difference (LSD) test, a Post-Hoc analysis, was used to determine significant differences among the means of response variables ($p \leq 0.05$).

Results and discussion

Change in headspace gas composition

The concentration of O_2 and CO_2 changed significantly ($p \leq 0.05$) with respect to the different initial gas concentrations and storage duration as shown in Figure 1. The decrease in O_2 concentration showed a similar trend. The initial O_2 concentration of 5% decreased to a concentration of 2.04% for low O_2 (MA-1) and from 10% to 2.96% for MA-2 atmospheres at day 12. Similarly, O_2 concentration decreased from 21% to 14.06% for air storage and from 70.05% of O_2 decreased to 37.83% under super-atmospheric O_2. Meanwhile, the headspace CO_2 concentration increased in all MA storage from the first day of storage and it reached the recommended range of < 10-20% CO_2 for pomegranate arils except under MA-3 storage treatment where the CO_2 concentration was > 20% on day 6. Furthermore, CO_2 increased from 9.3% to 15.7% under low O_2 atmosphere and from 0.03% to 17.07% under MA-4 at day 12. However, the increase in CO_2 was highest (28.56%) for arils stored under super-atmospheric O_2 condition at day 12 compared with the initial concentration (10.15%).

The increase in O_2 consumption and CO_2 production corresponding to different initial gas concentrations were observed. This phenomenon was reflected in the gradual linear reduction of slope for O_2 concentration while coefficients of determination (R^2) for fitted linear models ranged from 0.98 to 0.99 for all MA conditions. The linearity of the model could be attributed to the effect of increasing production of CO_2 and decreasing O_2 concentration over time. The linear changes in O_2 concentration in this study were in agreement with the pattern of a linear decline under aerobic conditions, whereas non-linearity can be indicative of anaerobic RR or suppression of respiratory activity due to limited O_2 concentration (Guevara *et al*., 2006). The result showed significant ($p \leq 0.05$) effects of storage MA condition and duration on headspace O_2 consumption and CO_2 evolution for arils. Similarly, Ayhan and Eştürk (2009) reported high CO_2 concentration (35%) for O_2 enriched atmosphere at 18 days of storage. Maghoumi *et al.* (2014) also reported accumulation of CO_2 concentration under high O_2 atmosphere. This study further reported that the presence of high O_2 concentration and accumulation of CO_2 resulted in to enhance the production of reactive O_2 species that could cause some respiratory stress, increase in RR, induced more CO_2 and depletion of antioxidants. Therefore, the increase in RR at MA-3 in the current study could be associated with the reason which shown the highest accumulation of CO_2.

Influence of initial gas composition on respiration rate

The potential effect of initial gas concentration on RR of pomegranate arils stored at different modified atmospheres was analysed. The results indicated that gas concentration had significant ($p \leq 0.05$) effect on RR of pomegranate arils during cold storage. However, the interaction of time and MA types did not significantly affect the RR of pomegranate arils for all MA conditions applied. Based on the calculated RR values obtained from O_2 consumption and CO_2 production, it was observed that RR decreased over storage duration across all MA conditions. Comparing the RR of pomegranate arils at different atmospheres; RO_2 decreased from 3.14 mL kg^{-1} h^{-1} to 0.87 mL kg^{-1} h^{-1} at low O_2 atmosphere for MA-1 and RO_2 decreased from 4.11 mL kg^{-1} h^{-1} to 0.94 mL kg^{-1} h^{-1} for MA-2 and from 7.25 mL kg^{-1} h^{-1} to 2.81 mL kg^{-1} h^{-1} at super-atmospheric O_2 atmosphere. For arils at ambient atmosphere, RO_2 decreased from 5.16 mL kg^{-1} h^{-1} to 1.95 mL kg^{-1} h^{-1} (Fig. 2). Generally, the RR appeared to slow down as time progressed for all MA storage conditions. Furthermore, the effect of O_2 concentration on RR of pomegranate arils clearly showed when the RR decreases with the decreased in O_2 concentration. Similarly, RO_2 pomegranate arils increased from 0.875 mL kg^{-1} h^{-1} to 2.81 ± 0.37 mL kg^{-1} h^{-1} by increasing the O_2 concentration from 5 to 70% at day 12. Lowest CO_2 production rate (RO_2) was 1.15 mL kg^{-1} h^{-1} under MA-1 atmosphere compared to MA-2, MA-3 and MA-4. For the other modified atmosphere conditions (MA-2 and MA-4), which had 10 and 21% initial O_2 concentration, respectively, RO_2 of arils ranging from 0.94 mL kg^{-1} h^{-1} for MA-2 and 1. 95 mL kg^{-1} h^{-1} for MA-4 was observed at the end of storage time (day 12).

In addition, reducing the O_2 concentration from 70 to 5% decreased the R_{CO2} at the same magnitude as R_{O2}. This finding is supported by the fact that RR decreases with decreasing availability of O_2 through the reduction of overall metabolic activity (Oms-Oliu *et al*., 2008). The reduction in RR response due to low O_2 could be as a result of a decrease in activity of oxidative enzymes such polyhenoloxidase, ascorbic acid oxidase and glycolic acid oxidase (Conesa *et al*., 2007). MA-2 enhanced CO_2 production rate after 5 days of storage in comparison to MA-1 and MA-4 atmospheres. Furthermore, the rapid depletion of O_2 and accumulation of CO_2 can be related to the low barrier properties of the glass jars used for the experiment. Similarly, low RR of pomegranate arils 'Hicaz' stored at 4 °C under a combination of low O_2 (2%) and high CO_2 (10%) was reported by Ersan *et al.* (2009). The authors reported that the minimum R_{O2} of 1.5 mL kg^{-1} h^{-1} and R_{CO2} of 0.52 mL kg^{-1} h^{-1} of pomegranate arils was obtained at low O_2 (2%). Aindongo *et al.* (2014) reported R_{O_2} and R_{CO_2} of 'Bhagwa' pomegranate arils ranges from 1.9-18.6 mL kg^{-1} h^{-1} and 3.2-28.9 mL kg^{-1} h^{-1}, respectively, stored at 5 to 22 °C. Similarly, Caleb *et al.* (2012c) observed R_{O_2} of 2.5 to 7.6 mL kg^{-1} h^{-1} and R_{CO_2} ranges from 2.7 to 9.0 mL kg^{-1} h^{-1} for 'Acco' and 'Herskawitz' pomegranate arils stored at 5 to 15 °C. In addition, Banda *et al.* (2015) investigated the effects of different combination of atmospheres (5% O_2 + 10% CO_2 + 85% N_2; 30% O_2 + 10% CO_2 + 60% N_2; 100% N_2; and air) on post-storage RR of 'Wonderful' pomegranate arils. The authors reported that R_{CO_2} significantly changed over time and the highest RR was observed for pomegranate arils stored under passive MA in high barrier films, while arils packed under 100% N_2 maintained the lowest RR (0.58 ± 0.12 mL kg^{-1} h^{-1}) at day 12.

The increase in RR (from 1.77 to 2.30 mL kg^{-1} h^{-1}) of 'Malese-Saveh' pomegranate arils under super-atmospheric (90%) O_2 condition has been reported (Maghoumi *et al*., 2013).

Furthermore, increase in RR under super-atmospheric O_2 concentration was reported by Jacxsens *et al*. (2002) for mushroom, grated celeriac and shredded chicory endives. Allende *et al*. (2004) reported a similar observation for baby spinach leaves under super-atmospheric O_2 (> 70%). The authors observed highest R_{O_2} and R_{CO_2} under super-atmospheric O_2 MA condition. The increase in RR was associated with the production of reactive O_2 species and respiratory stress due to the presence of high O_2 concentration (Jacxsens *et al*., 2002). However, the effect of super-atmospheric O_2 depends on the commodity, maturity, ripeness stage, O_2 and CO_2 concentration, time and storage temperature (Kader & Ben-Yehoshua, 2000). In addition, comparison of the RRs of pomegranate arils observed in this study with other related studies reported in literature, showed that the RR of pomegranate arils were not affected by the pre-processing storage duration. Overall, the data suggested that low O_2 atmosphere would be important to lower the respiration rate of pomegranate arils at 5 °C compared to super-atmospheric O_2 and passive atmosphere.

Respiration quotient (RQ)

The average ratio of CO_2 produced and O_2 consumed (RQ) value of pomegranate arils stored at all MA conditions was within the range of acceptable limit (0.7-1.3) for aerobic respiration of fruit and vegetables (Kader *et al*., 1989). In contrast, arils under super-atmospheric O_2 (MA-3) condition after 10 days of storage had the highest RQ of 1.54. The lowest RQ was observed for Pomegranate arils stored under MA-4 treatment. The results further showed that the RQ for all MA conditions increased slightly at the end of 7 days' storage. Based on the assumption that the value of RQ for fresh produce is equal to 1 when the metabolic substrates oxidized during respiration are carbohydrates (Castro-Giraldez *et al*., 2013; Fonseca *et al*., 2002), it can be suggested that the fermentation threshold for stored pomegranate arils was not reached across all MA conditions. On the other hand, a slight increase in RQ > 1 suggests that the oxidized substrates were organic acidic (Fonseca *et al*., 2002). Furthermore, Conesa *et al*. (2007) associated the higher value of RQ with the production of high CO_2 for fresh cut bell peppers. Similar to the current study, RQ values range from 1.14 to 1.26 for minimally-processed 'Acco' and 'Herskawitz' pomegranate arils were reported by Caleb *et al*. (2012b) and RQ values of 0.9-1.24 for citric acid treated and untreated 'Wonderful' pomegranate arils by Banda *et al*. (2014) at passive atmosphere.

The results showed that gas concentration had no influence on the RQ of pomegranate arils as shown in Table 2. However, for the low O_2 (MA-1) atmosphere storage, RQ increased (38%) from 0.95 to 1.33 when the O_2 concentration declined below 2.81% on day 12. The low O_2 limit is the O_2 concentration that causes the immediate increase (50%) in the RQ as stated by Beaudry (1999) and Hong and Kim (2001) and therefore, the lowest O_2 limit of 'Wonderful' pomegranate arils at low O_2 storage can be estimated to be less than 2.8%. This further showed the influence of gas concentration on RQ and the slight but non-significant ($p \geq 0.05$) change in the respiratory process.

Modelling the influence of gas composition on respiration rate

MM model with different types of inhibition was tested using the experimental data. The predictions from the MM models as well as the inhibition models (competitive, un-competitive and non-competitive) were compared by using the model parameter estimates as presented in Table 3. The MM model parameters for each gas concentration ($V_{max}o_2$ and $K_m o_2$) and the CO_2 inhibition constant ($K_i co_2$) were estimated individually from the experimental data obtained at each gas concentration and their MA dependence subsequently studied using Eq. 5, 6 and 7. All the four types of MM enzyme kinetics models were capable of describing the O_2 consumption rate as a function of MA (MA-2, MA-3 and MA-4 treatments) with observed percentage variance (R^2_{adj} = 81–91%). On the other hand, lower percentage variance of $R^2_{adj} >$ 70% were found for (MA-1) which had the lowest initial O_2 concentration (2%), except for competitive inhibition model. This could be due to the presence of very low O_2, which can influence effectiveness of MM model, since the model is effective when the reaction is in MM type (Hertog *et al*., 1998).

Both the maximum O_2 consumption rate and the MM model constant ($V_{max}o_2$and $K_m o_2$) varied between the different modified atmospheres. Highest $V_{max}o_2$ was observed in MA-3 and lowest was for MA-1. This can be inferred that both parameters were influenced by gas composition. The increase in V_{max} with increase in O_2 concentrations suggested that V_{max} was dependent on gas composition. The gas concentration dependency of R_{O_2} increased substantially when O_2 concentration increased from low to super-atmospheric O_2 (Fig. 3). In addition, the highest value of $K_m o_2$, which represent the O_2 concentration at which half the maximum RR is reached (assuming no inhibition by CO_2) was observed under super-atmospheric O_2 (MA-3). Given that all the arils were harvested at commercial maturity and processed under the same condition, the observed variation in the $K_m o_2$ values might be attributed to the differences in the tissue resistance to diffusion commonly associated with storage modified atmosphere (Gomes *et al*., 2010). Furthermore, this observation could suggest that the internal O_2 concentration in the arils increased proportional to the external O_2 concentration applied (Hertog *et al*., 1998).

Comparing simple and inhibition models for each atmosphere, there was no clear difference in effect of CO_2 on O_2 consumption rate for all MAs. However, according to the $K_i co_2$values, which showed the extent to which RR can be inhibited by CO_2 in Table 3, the simultaneous existence of competitive, un-competitive, and non-competitive inhibition were observed depending on the initial gas concentration. Comparing all models relatively higher $K_i co_2$ value was observed in MA-1 during competitive inhibition model, which implies inhibition by CO_2 has not occurred, and a slight un-competitive and non-competitive inhibition was observed. This indicated that a model that combined un-competitive and non-competitive inhibition could better explain the CO_2 effect on the RR (Guillard *et al*., 2012). On the contrary, the RR of pomegranate arils for the other atmospheres was competitive inhibition, with the lowest $K_i co_2$. However, the influence of CO_2 on the metabolic rate could be due to its effect on changing the pH than its direct influence on enzymatic reaction (Torrieri *et al*., 2010).

The $V_{max} o_2$ values obtained from the current experiment were similar to those reported by Ersan *et al*. (2009) for 'Hicaz' pomegranate arils at 5 °C. At low $O_{2,}$ $V_{max} o_2$ of 3.6 mL kg^{-1} h^{-1} was comparable to 3.1 mL kg^{-1} h^{-1} reported by Ersan *et al*. (2009). In contrast, the $K_m o_2$ and K_m values were different. The authors found relatively higher $K_m o_2$ ranging from 3.8 to 5.1 for 'Hicaz' pomegranate arils; however, the values in the current experiment were within the range of 0.29 to 2.67 for 'Wonderful' under low O_2 atmosphere. This variation in $K_m o_2$ could be associated with the different model fitting methods used. Ersan *et al*. (2009) linearized the data before fitting, while for the current study a non-linear regression model has used, since linearization is equivalent to changing the weight given to the data in the estimation procedure and it should be avoided (Fonseca *et al*., 2002).

Comparing the accuracy of the parameter estimates by using the estimated SEs for all models, small SEs values of all $V_{max} o_2$ and $K_m o_2$ were observed. Low SE values (lower value than the sample mean) for the parameter estimates can be considered as well defied (Hertog *et al*., 2007). Thus, it can be concluded that the models accurately predicted the influence of modified atmosphere on the RR of 'Wonderful' pomegranate arils. On the other hand, the accuracy for the competitive and non-competitive inhibition model for MA-1 and for non-competitive inhibition for MA-3 could be less defined by the increase in SE for K_m. However, the higher SE could also explain the coexistence of both competitive and uncompetitive inhibition of RR by CO_2 (Hertog *et al*., 2007). Furthermore, the model parameters were statistically significant ($p \leq 0.05$) and the correlation coefficient of determination was (R^2 = 0.85-0.97%) for all atmospheres. This showed a good fit of the model to the experimental data. Therefore, these results demonstrated that the MM enzyme kinetics models can be used to describe the dependency of RR of pomegranate arils on the modified storage atmospheres.

Since the results for MA-2, MA-3, and MA-4 showed a competitive inhibition RR and a slight combination of competitive and un-competitive inhibition for MA-1, a non-linear regression was applied to the whole set of experimental data using competitive model. The model adequately predicted the O_2 consumption rate (R^2 = 0.94%) as shown in Figure 4, with a normal distribution of the residual values. Based on this result, it can be suggested that competitive inhibition can be able to describe the effect of CO_2 to O_2 consumption rate at low and super-atmospheric O_2 condition.

Conclusions

This study showed that RR of 'Wonderful' pomegranate arils was significantly affected by initial gas concentrations during storage. Low O_2 MA conditions reduced the RR of arils. In contrast, the use of super-atmospheric O_2 concentration (70%) induced physiological stress, and consequently slightly increased the RR of arils. Based on the respiratory kinetics of pomegranate arils, this study showed that super-atmospheric O_2 is of little or no benefit in slowing down of the metabolic process of pomegranate arils. The MM enzyme kinetics model (using competitive inhibition type equation) adequately described the effect of CO_2 on O_2 consumption rate at low O_2 (5%), super-atmospheric O_2 (70%) and enriched CO_2 concentrations. The model parameter estimates obtained provide a guiding step for the management of MA system to avoid unfavourable conditions for 'Wonderful' pomegranate arils.

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Table 1. Experimental gas combinations investigated.

Modified atmospheres	**Name**	**Gas composition**
Low oxygen	MA-1	5% O_2 + 10% CO_2 +85% N_2
	MA-2	10% O_2 + 5% CO_2 + 85% N_2
Super-atmospheric oxygen	MA-3	70% O_2 + 10% CO_2 + 20% N_2
Passive atmosphere	MA-4	21% O_2 + 0.03% CO_2 + 78% N_2

Table 2. Effects of modified atmosphere on respiratory quotient (RQ) of pomegranate arils stored at 5 °C for 12 days.

	Modified atmosphere conditions*			
No. of days	**MA-1**	**MA-2**	**MA-3**	**MA-4**
0	1.02 ± 0.3	1.10 ± 0.2	1.12 ± 0.6	1.20 ± 0.2
1	1.00 ± 0.3	1.07 ± 0.2	1.15 ± 0.4	1.17 ± 0.2
2	1.22 ± 0.4	1.00 ± 0.4	1.14 ± 0.2	1.20 ± 0.3
3	1.01 ± 0.3	1.02 ± 0.4	1.07 ± 0.3	1.19 ± 0.4
4	1.05 ± 0.2	0.96 ± 0.4	1.19 ± 0.1	1.22 ± 0.4
5	0.95 ± 0.1	1.07 ± 0.1	1.25 ± 0.7	1.24 ± 0.4
6	0.95 ± 0.0**	1.06 ± 0.1**	1.07 ± 0.2**	1.16 ± 0.2**
7	1.15 ± 0.2	1.19 ± 0.6	1.22 ± 0.4	1.19 ± 0.2
8	1.17 ± 0.2	1.30 ± 0.1	1.23 ± 0.2	1.20 ± 0.3
9	1.31 ± 1.0	1.19 ± 0.2	1.21 ± 0.3	1.44 ± 0.4
10	1.27 ± 0.7	1.09 ± 0.4	1.13 ± 0.1	1.52 ± 0.8
11	1.27 ± 1.1	1.05 ± 0.9	1.36 ± 0.3	0.98 ± 0.2
12	1.33 ± 0.8	1.27 ± 0.2	1.54 ± 0.4	1.12 ± 0.1

Values are means ± standard deviation (n = 3). Different lower case superscript letters across the rows and upper case subscript letters along the columns are significantly different ($p \leq 0.05$). *MA compositions are presented in Table 1, ** significantly different.

Table 3. Model parameter estimates obtained from non-linear regression analysis of enzyme kinetics model for O_2 consumption rate in a closed system under various initial O_2 and CO_2 concentrations.

Models	**Storage atmospheres**	R^2_{adj}	**V_{max}, mL kg^{-1} h^{-1}**	**SE**	**K_m, % O_2**	**SE**	**K_i, % CO_2**	**SE**
Simple Michaelis-Menten	MA-1	0.67	3.67	0.29	0.94	0.39	-	-
	MA-2	0.86	6.49	0.98	5.52	1.93	-	-
	MA-3	0.91	19.83	7.32	140.59	73.68	-	-
	MA-4	0.81	8.31	0.01	1.07	0.54	-	-
Competitive Inhibition	MA-1	0.71	3.67	2.67	0.94	0.23	6.83	547.06
	MA-2	0.89	4.62	1.95	5.00	5.59	0.11	31.4
	MA-3	0.91	10.69	15.12	31.23	179.57	24.93	183.3
	MA-4	0.81	8.86	1.15	109.60	0.69	18..69	64.89
Uncompetitive Inhibition	MA-1	0.68	3.64	0.31	0.85	0.00	-*	-*
	MA-2	0.86	6.50	1.03	5.50	2.23	58.53	0.00
	MA-3	0.91	20.00	8.45	141.00	85.09	-*	-*
	MA-4	0.81	8.45	0.18	-*	1.90	-*	-*
Non-competitive Inhibition	MA-1	0.68	3.64	0.45	1.31	3.02	132.9	1087
	MA-2	0.86	6.51	4.00	5.56	12.00	79.60	1157
	MA-3	0.91	20.40	52.3	147.61	644.7	207..9	1396.2
	MA-4	0.81	8.02	1.59	305.7	1.69	57.93	6.00

V_{max} is highest respiration rate due to O_2 consumption, K_m is Michaelis-Menten constant, K_i is CO_2 inhibition constant, SE is standard error, R^2_{adj} *is* percentage variance accounted for, "-*" were very high value.

MA compositions are presented in Table 1.

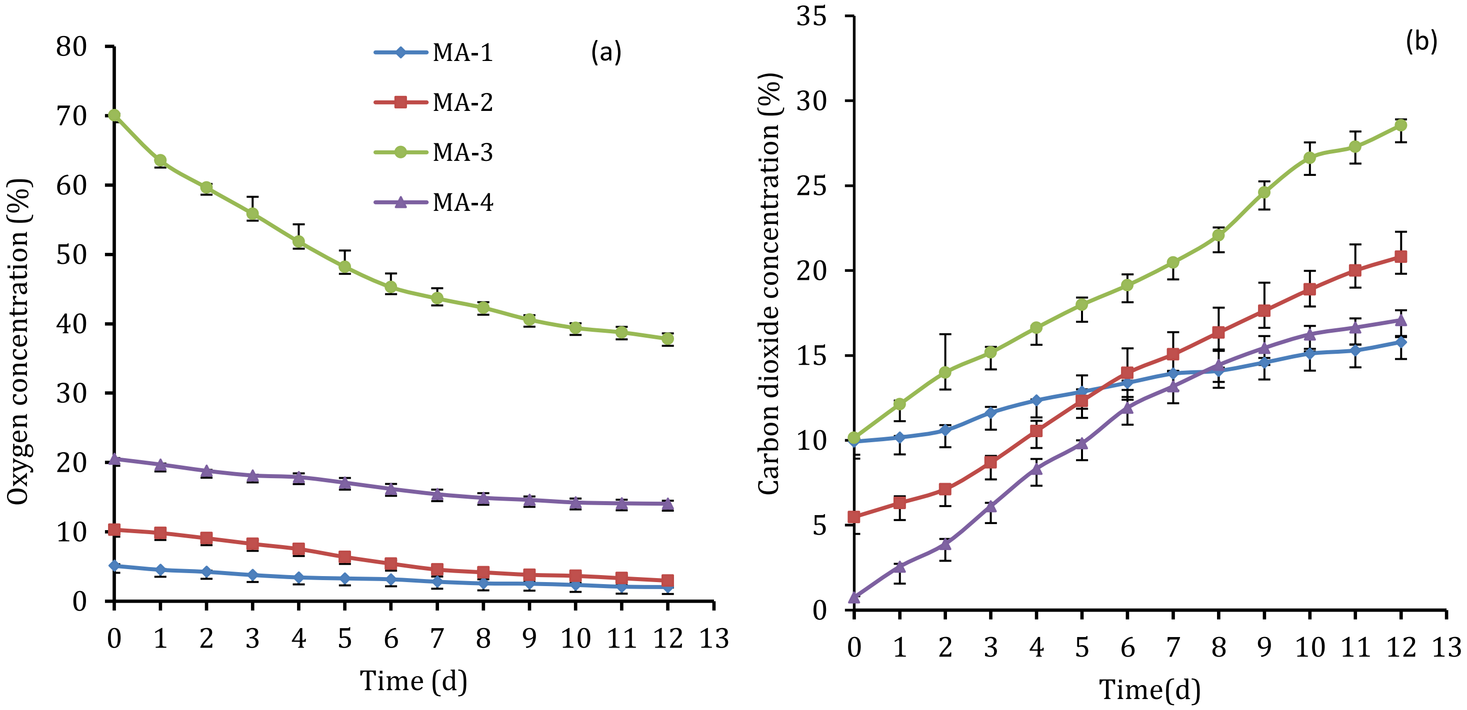

Figure 1. Effect of initial gas atmosphere on the change in headspace gas concentration of pomegranate arils stored at 5 °C for 12 days. Error bar represents standard deviation of mean values (n = 3) at 95% confident interval. (a) O_2 consumption and (b) is CO_2 production. MA-1: (5 % O_2, 10%, CO_2 & 85% N_2); MA-2: (10% O_2, 5% CO_2 & 85% N_2), MA-3: (70% O_2, 10% CO_2 & 20% N_2); and MA-4 (air).

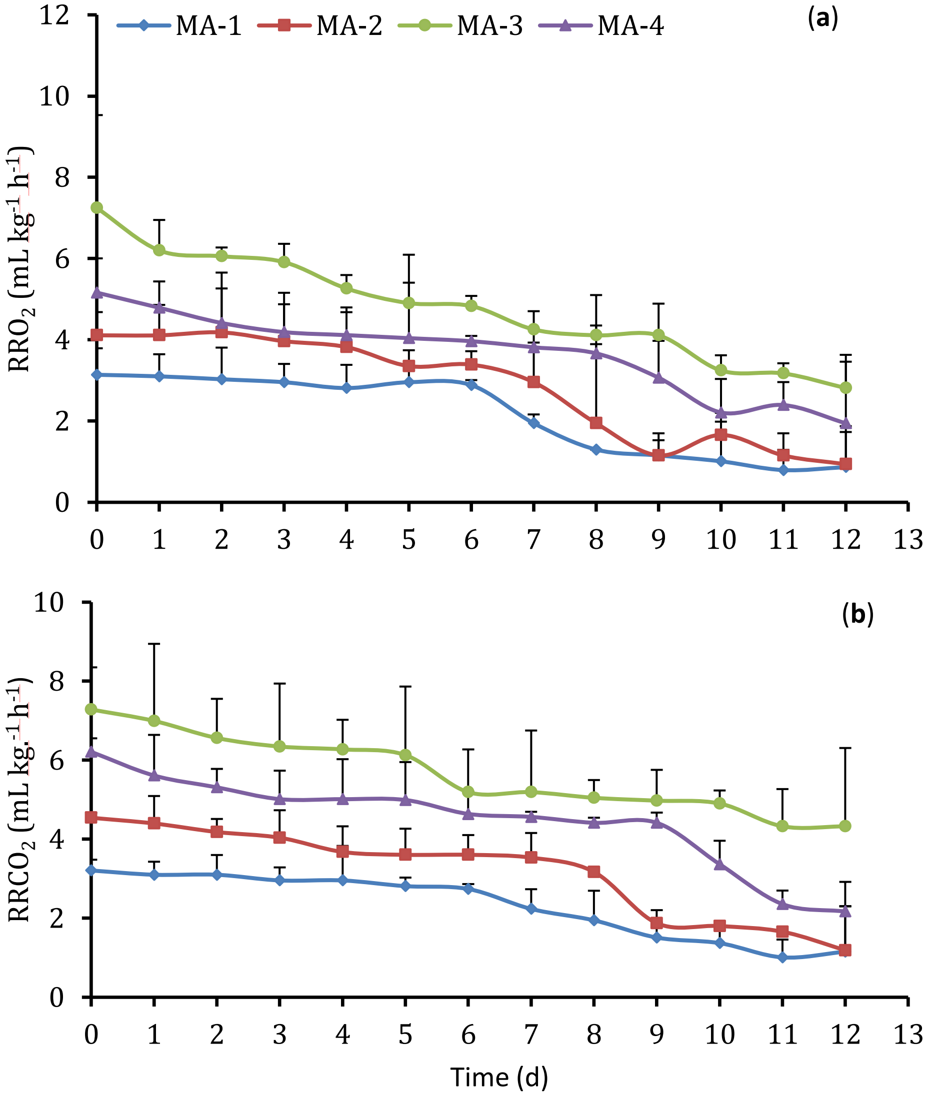

Figure 2. Effect of gas concentration on the calculated respiration rate (RR) due to oxygen consumption (a) and due to carbon dioxide production (b) of minimally-processed pomegranate arils stored at 5 °C for 12 days. Error bar represents standard deviation of mean values (n = 3) at 95% confident interval. MA-1: (5% O_2, 10%, CO_2 & 85% N_2); MA-2: (10% O_2, 5% CO_2 & 85% N_2), MA-3: (70% O_2, 10% CO_2 & 20% N_2); and MA-4 (air).

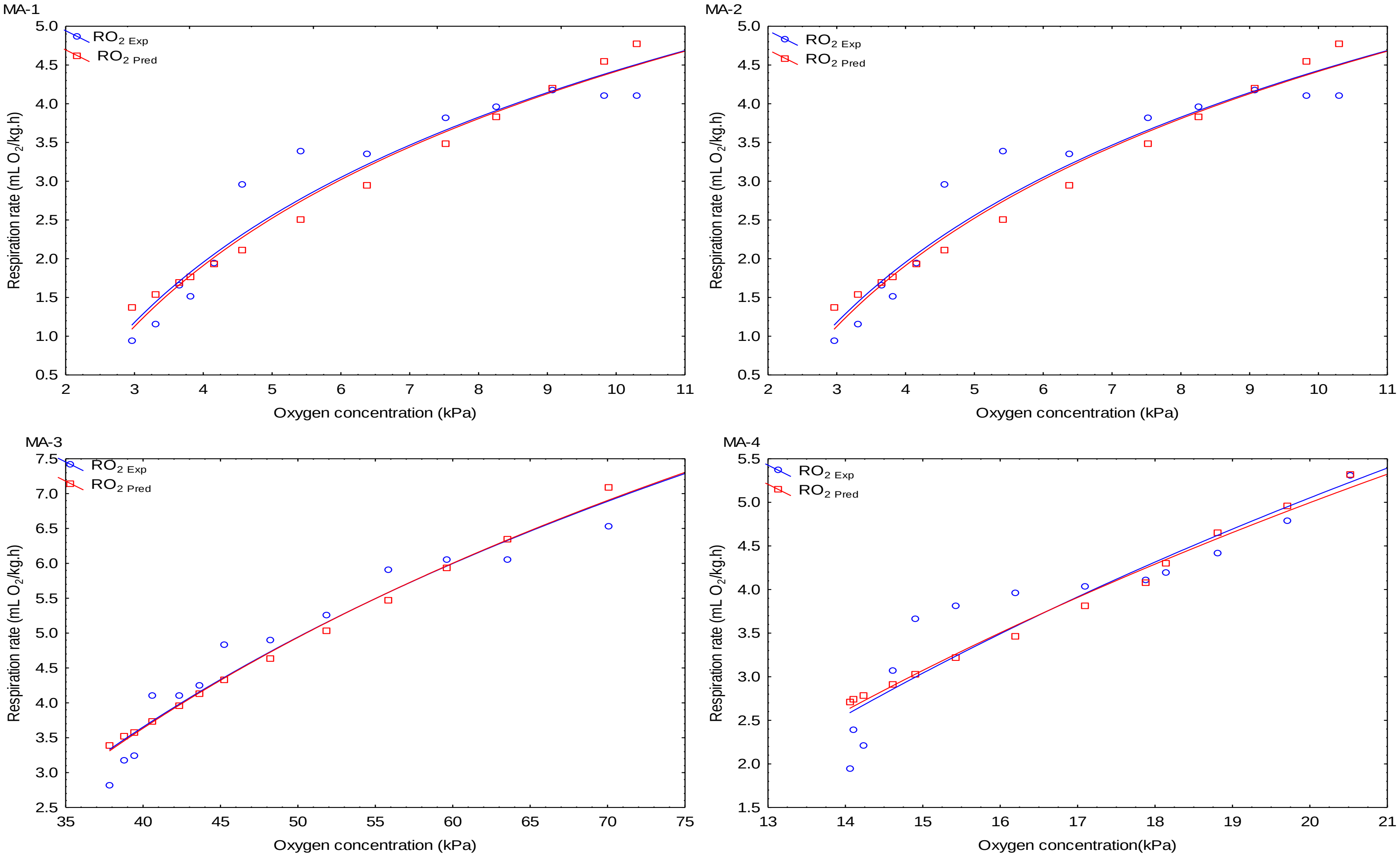

Figure 3. Correlation of headspace O_2 concentration (%) on the O_2 consumption rate (mL kg^{-1} h^{-1}) of minimally-processed pomegranate arils stored at 5 °C for 12 days in a closed system. MA-1: (5% O_2, 10%, CO_2 & 85% N_2); MA-2: (10% O_2, 5% CO_2 & 85% N_2), MA-3: (70% O_2, 10% CO_2 & 20% N_2); and MA-4 (air).

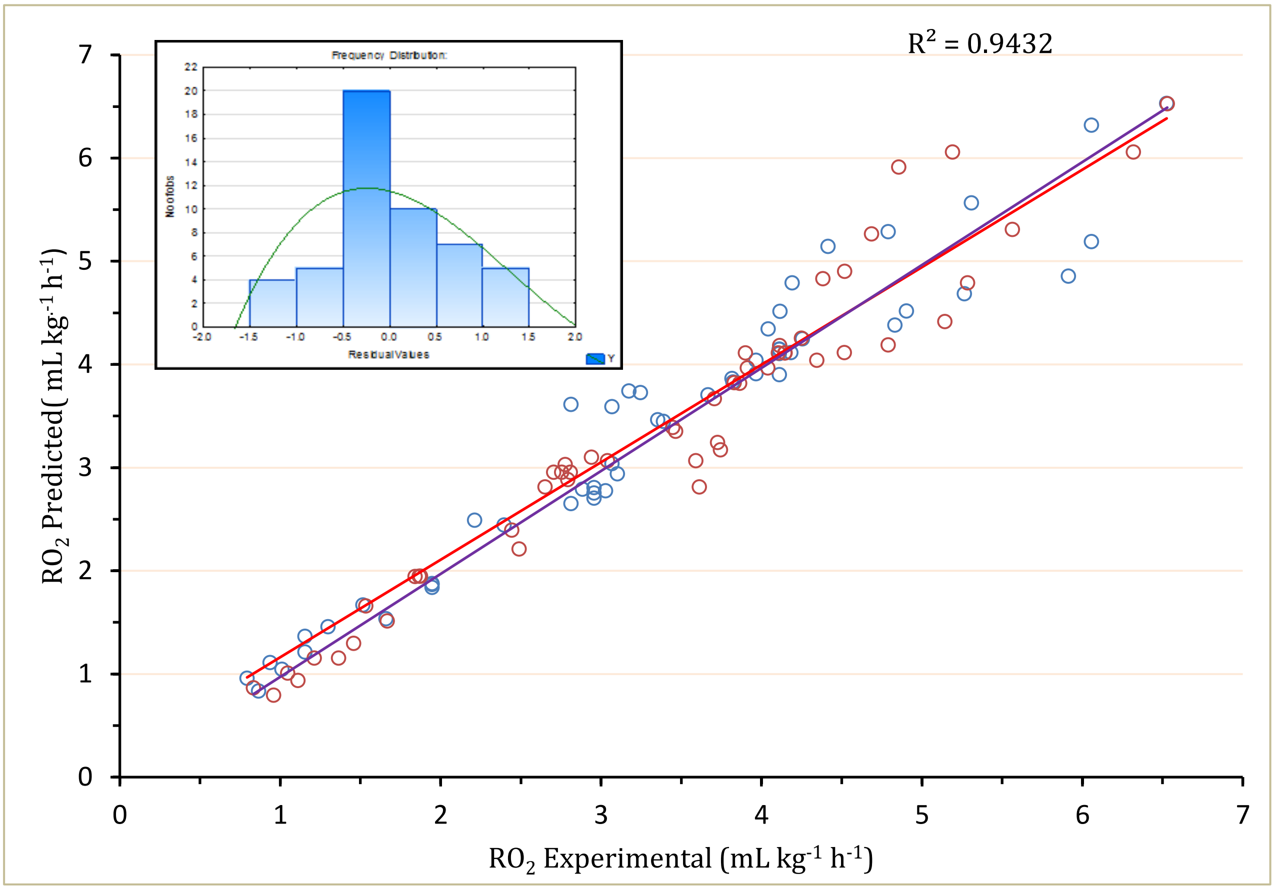

Figure 4. Correlation between experimental data and predicted RO_2 (mL kg^{-1} h^{-1}) using Michaelis-Menten competitive inhibition model. Small plot inserted shows the frequency distribution of the residuals.

Declaration by the candidate:

With regard to Chapter 6 (pp 128-157); the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data collection, data analysis and writing of chapter	75

The following co-authors have contributed to Chapter 6 (pp 128-157):

Name	e-mail address	Nature of contribution	Extent of contribution (%)
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Dr O.J. Caleb	CalebO@arc.agric.za	Research input, editorial suggestion and proof reading	5
Dr P.V. Mahajan	PMahajan@atb-potsdam.de	Research input, assistance with optimization, editorial suggestion and proof reading	10

Signature of candidate: Z.A. Belay

Date: 03/11/2017

Declaration by co-authors:

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 6 (pp 128-157)
2. no other authors contributed to Chapter 6 (pp 128-157) besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 6 (pp 128-157) of this dissertation.

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Declaration with signature in possession of candidate and supervisor.

CHAPTER 6

A SIMPLEX LATTICE MIXTURE DESIGN TO OPTIMIZE ACTIVE MODIFIED ATMOSPHERE CONDITION FOR STORING ‘WONDERFUL’ POMEGRANATE ARILS: PART I – DETERMINING OPTIMUM GAS COMPOSITION AND ASSESSING THE EFFECTS OF STORAGE TEMPERATURE ON PHYSIOLOGICAL RESPONSES

ABSTRACT

Understanding the experimental design and principles guiding gas mixture are important in the development of an optimal produce-specific modified atmosphere (MA) system. Exposure to gas combination below or above the tolerance limit of fresh produce may result in subsequent undesirable changes in product quality and shortened shelf life. Therefore, this study investigated the design of an optimal gas composition for storage of pomegranate arils using the simplex lattice mixture design approach with seven gas combinations at 10 °C. The impact of initial gas composition was also determined by evaluating the physiological response and microbial quality (aerobic mesophilic bacteria, yeast and mould). The correlation of simplex lattice design experimental data to the predicted response parameters were explained effectively by Scheffé’s special cubic model ($R^2 > 94\%$). Parameter estimates showed a significant ($p \leq 0.05$) synergetic effect of single model components for all the response variables. The use of high CO_2 concentration (> 10%) decreased ethylene production rate and microbial load while reducing O_2 to acceptable level reduced respiration rate (RR). Based on the results obtained, the optimal gas composition was established at 4.67% O_2, 12.67% CO_2 and 82.67% N_2. Under this optimal atmosphere condition, increasing in storage temperature by 10 °C resulted in a threefold increase in RR. These results demonstrate that optimum storage temperature also plays a critical role is the success of MA systems.

Key words: Mixture design; Storage; Modelling; Respiration rate; Ethylene production; Microbiology

Introduction

Pomegranate fruit is highly valued mainly due to its strong antimutagenic, anticancer, anti-inflammatory and antioxidant potential (Fawole & Opara, 2013a; Opara *et al.*, 2009; Mphahlele & Opara, 2014a,b). However, consumption of pomegranate is not very widespread mainly due to the difficulty of extracting the arils (Al-Said *et al.*, 2009; López-Rubira *et al.*, 2005; Allende & Artés, 2005). Furthermore, the fruit is very sensitive to sunburn, cracking, cuts or bruises in the husk, as well as chilling injury (Fawole & Opara, 2013b). Therefore, minimally processing of pomegranate fruit to obtain pomegranate arils with intact sensory and nutritional properties is one of the major approaches to facilitate the fruit consumption (Ayhan & Eştürk, 2009; Caleb *et al.*, 2012a). However, maintaining the quality and shelf life of pomegranate arils has been a major challenge due to rapid deterioration commencing once the fruit is peeled (O'Grady *et al.*, 2014).

Modified atmosphere (MA) is a technique used to prolong the shelf life of fresh or minimally-processed produce, based on its ability to decrease the overall metabolic activity of plant tissues (Caleb *et al.*, 2012a; Caleb *et al.*, 2013; Fonseca *et al.*, 2005). Modified atmosphere packaging utilizing low O_2 and enriched or high CO_2 partial pressures during storage have been extensively reported on to maintain quality, reduce respiration rate (RR) and ethylene production rate, delay enzymatic reactions and consequently extend the shelf life of many types of fresh fruit and vegetables (Oms-Oliu *et al.*, 2008; Fonseca *et al.,* 2005; Giuggioli *et al.*, 2015). The beneficial effects of MAP are obtained only if the appropriate levels of O_2 and CO_2 exist inside the package, which depends on the RR of the fruit and the barrier property of the packaging material (Sousa-Gallagher *et al.*, 2013). When a specific atmosphere for a commodity based on its physiological tolerance to O_2 and CO_2, are not within the range it can lead to the risk of undesirable effects such as loss in sensory quality (Beaudry, 2000; Fonseca *et al.*, 2005). Exposure to O_2 below the tolerance limit of a specific commodity may result in the subsequent production of undesirable metabolites and anaerobic fermentation (Chunyang *et al.*, 2010). On the other hand, exposure to excessive CO_2 could induce anaerobiosis, tissue injury and disrupt enzyme systems, such as the lipoxygenase pathway (Giuggioli *et al.*, 2015). Therefore, at the present time, the key to successful low O_2 with enriched or high CO_2 atmosphere packaging is to establish an optimal storage condition depending on the metabolism of the specific fruit (Conesa *et al.*, 2007; Chunyang *et al.*, 2010).

Considerable research over the last decade have studied the possibility of obtaining an optimum gas composition in modified atmosphere for pomegranate arils by changing packaging atmosphere using a trial and error approach. López-Rubira *et al.* (2005) suggested that 1-3% of O_2 and 5-10% of CO_2 resulted in the best quality 'Mollar Elche' pomegranate arils stored at 5 °C with average RR of 1.15 mL CO_2 kg^{-1} h^{-1}. Ersan *et al.* (2009) selected 2% O_2 and 10 or 20% CO_2 as an optimum gas mixture for 'Hicaz' pomegranate arils based on minimum aerobic RR. This study further observed a RR of 1.5 mL kg^{-1} h^{-1} and 0.52 mL kg^{-1} h^{-1} due to O_2 consumption (R_{O_2}) and CO_2 production (R_{CO_2}), respectively. Longer shelf life of 'Hicaznar' pomegranate arils at high O_2 (70% O_2 + 10% CO_2 + 20% N_2) compared to low O_2 (5% O_2 + 10% CO_2 + 85% N_2) atmosphere was reported by Ayhan and Eştürk (2009). Martínez-Romero *et al.* (2013) recommended an optimal CO_2 concentration range of 5-15% to maintain quality and safety of ready-to-eat pomegranate arils coated with Aloe Vera gel. These variations in gas composition clearly indicate the need for a systematic approach where the RR of pomegranate arils is measured at different selected atmospheres to determine the optimum modified atmosphere condition for packaging design (Ersan *et al*., 2009). Therefore, as new cultivars emerge from crop improvement program there is the need for continuous investigation on the optimal atmosphere for each cultivar in order to guide the role players along the pomegranate value chain. To optimize the gases in modified atmosphere, the mixture design approach has been recommended rather than using a trial and error approach (Pintado & Malcata, 2000). However, no study has used a mixture design approach to optimize active modified atmosphere conditions for pomegranate arils yet.

In addition to the optimum gas composition, storage temperature has been extensively studied and shown to play an important role in the physiological responses of pomegranate arils (Caleb *et al*., 2012a). Temperature fluctuation during the distribution chain is an avoidable challenge (Tietel *et al*., 2012), which has a significant detrimental impact on produce quality and shelf life. To benefit from modified atmosphere condition, efficient temperature control during distribution and marketing remains important in addition to maintaining the optimum gas concentration inside the package (Van de Velde & Hendrickx, 2001; Caleb *et al*., 2012b). Therefore, the objectives of this study were to determine the optimal gas combination for storage of pomegranate 'Wonderful' pomegranate arils and to quantify the effect of temperature on the respiration rate of arils stored under optimal gas atmosphere. The simplex lattice mixture approach, Cornell (2011) was used to design the experiments and to optimize the proportion of gases for modified atmosphere. In chapter 6, the optimum gas composition to maintain individual physicochemical quality attributes of arils and volatile organic compounds will be presented.

Materials and methods

Simplex lattice mixture design

The simplex lattice design was applied to optimize the gas concentration during modified atmosphere storage for pomegranate arils as described by Mahajan *et al*. (2014). In this study, three gas components (CO_2, O_2 and N_2) were evaluated by changing their concentrations simultaneously and keeping the total concentration constant. According to Cornell (2011), the starting point of mixture theory is that the sum of the proportion of the mixture components is one and these proportions must be non-negative. The simplex lattice design for a three component system can be represented by an equilateral triangle in a two-dimensional space, with all possible combinations (Fig. 1).

In order to optimize the gas concentration, the partial pressure of O_2, CO_2 and N_2 were chosen as independent factors. For this study, a simplex lattice design resulted in 7 experimental design points: three points at the vertex of the triangle (0, 0, 1), (0, 1, 0) and (1, 0, 0) represent a pure gas mixture where the individual component is considered to be a mixture by itself; another three gas mixtures were represented by the three augmented points (⅔, ⅙, ⅙), (⅙, ⅔, ⅙) and (⅙, ⅙, ⅔) located at the midpoints of the 3 edges of the triangle; and one centroid mixture (⅓, ⅓, ⅓) at the centre. The three gas mixture varied from 0 to 1 in a given mixture according to the design (Table 1).

Fresh produce and sample preparation

Pomegranate fruit cv. 'Wonderful' were obtained at the commercially ripened stage with characteristic deep-red skin and arils with the mature kernel (Arendse *et al*., 2016; Crisosto *et al*., 2001), from Sonlia Pack House, Wellington, Western Cape (33°38′23″S, 19°00′40″E), South Africa. Fruit were air-freighted in well-ventilated boxes to the Horticultural Engineering Department Laboratory, Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Germany. On arrival, fruit were stored in a cold storage chamber at 5 °C until processing to extract the arils in the cold room at 5 °C. Damaged fruit were removed and the outer skin of selected healthy fruit was surface disinfected using 70% ethanol prior to processing (Belay *et al*., 2017). Arils were extracted manually by carefully removing the husk. Extracted arils were collected in a tray and mixed to assure uniformity. Table 1 outlines the different gas combinations used to store arils.

Storage chambers

Arils (350 g) were transferred to 4000 mL airtight glass jars, designed to achieve a completely hermetic seal, and stored at 10 °C. Each jar lid had three valves (inlet, outlet and a gas sampling port). A rubber ring was fixed between the bottle and the lid seal to prevent air leakage. A plastic tube was attached to the inlet valve, which was inserted down to the bottom of the jar to ensure uniform flushing of the gas mixture. Each storage chamber was flushed with humidified air containing the desired gas mixtures (Table 1) until equilibrium was achieved. The changes in O_2 and CO_2 concentration inside the headspace of each jar were monitored over a storage period of 8 days using a gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark).

Determination of respiration rate

The RR of pomegranate arils based on CO_2 production rate (R_{CO_2}) was determined according to the method described by Caleb *et al.* (2016a), using a non-invasive and continuous monitoring of carbon dioxide (CO_2) in a closed system. Respiration measurement was conducted in duplicate for each gas composition, and a total of 14 cuvettes were used. Each cuvette was made from an acrylic material and equipped with a non-dispersive infrared CO_2 sensor (Vaisala GmbH, Bonn, Germany) with a measuring capacity range of 0.1 x 10^{-3} to 5 g L^{-1}. Pomegranate arils (150 g) in a tray were placed inside the acrylic containers of the respirometer setup. Each container was hermetically sealed with O-rings between the lid and the container. Data were acquired for a total of 7 h at an interval of every 2 min and respiration rate was calculated using equation 1:

$$R_{CO_2} = \left(Y_{CO2\,t_f} - Y_{CO2\,t_i}/\Delta t\right) V_f/W \qquad (1)$$

Where $Y_{CO2\,t_f}$ and $Y_{CO2\,t_i}$ are CO_2 concentration (%) at time t_f(h) and time t_i(h), respectively. R_{CO_2} is RR due to CO_2 production in mL kg^{-1} hr^{-1}, V_f is free volume of the containers and W is the total weight of the product (kg).

Measuring ethylene production

Ethylene production rate was determined according to Mahajan *et al*. (2014) and Mditshwa *et al*. (2017). For analysis, 100 µL of gas was withdrawn with an airtight syringe through the septum in the lid and manually injected into a gas chromatograph (GC-17A, Shimadzu, Kyoto, Japan), fitted with a Porapak® Q column, and flame ionization detector. Identification of ethylene was done by injecting a gas standard (Mikrolab, Højbjerg, Denmark) and comparing its retention time with that of the unknown peak in the chromatograph. Quantification of ethylene production rate was done by running a calibration curve for ethylene production rate and the values were expressed in µL kg^{-1} h^{-1}.

Microbial analysis

Microbial quality of pomegranate arils was studied according to the method described previously by Caleb *et al*. (2016a) and Banda *et al*. (2015a) using total plate count method. The total aerobic mesophilic bacterial count was determined using plate count agar (PCA), while yeast and mould counts were determined using rosebengal chloramphenicol agar (RBCA). Sample (10 g) of pomegranate arils was taken for each treatment and transferred into 90 mL peptone buffered water. Each sample was homogenized for 2 min at speed 4 strokes/s in a lab blender (BagMixer1400CC1, Interscience, France) and thereafter, threefold serial dilution was made by adding 30 µL of each diluent into 270 µL of PS Rotilabo1-microtest plates (96er U-profile, Carl Roth GmbH & Co KG, Germany) and 100 µL of each dilution was pour-plated on respective growth media. All analyses were done in duplicate for each package (n = 7, per treatment). PCA plates for aerobic mesophilic bacteria were incubated at 30 °C for 72 h, and RBCA plate for yeast and mould, respectively, were incubated at 25 °C for 5 days. After incubation, colonies (between 30 and 300 colonies) on each plate were counted, and the results were expressed as log colony forming unit per weight (log CFU mL^{-1}).

Mathematical modelling

The results from the statistical analysis of simplex lattice design batches were used for model analysis following Scheffé's special cubical model for three response variables. For modelling the response variables (respiration rate, ethylene production rate and microbial quality) seven gas mixtures were used according to the method described by Mahajan *et al*. (2014). The cubical polynomial model consists of single, binary and ternary terms, while the linear model consists only of single terms that were used to fit the experimental data. The computational work was performed using STATISTICA (version 13, DOE StatSoft Inc.Tulsa, USA). The ternary contour graphical presentations of the models including estimating the constant of all models was done by fitting the experimental data to a linear model using equation 2 and special cubical polynomial model using equation 3 by nonlinear regression:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \tag{2}$$

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \tag{3}$$

Where Y represents the response variables, β is a coefficient of model parameter estimate for linear and non-linear terms, X_1 represents O_2, X_2 is CO_2, and X_3 is N_2, subscripts 1, 2, and 3 to O_2, CO_2, and N_2 component, respectively. To accommodate a polynomial equation to represent the surface, design points were spread over the simplex factor space, an arrangement also known as lattice (Van de Velde & Hendrickx, 2001). The resulting three linear equations can, in turn, be algebraically solved with respect toX_1, X_2, and X_3. The main effects (X_1, X_2 and X_3)

represent the average result of changing 1 factor at a time from low to high values (Mandlik *et al*., 2012) while the interaction term (X_1X_2, X_2X_3 *and* $X_1X_2X_3$) show how the response changes when two or more factors are simultaneously changed. The sign of the coefficients of the effect estimated (β_{11}, β_{22} *or* β_{33}) gives immediate indication whether the locus is a maximum (-) or a minimum (+) (Pintado & Malcata, 2000). The model fitting was performed using the coded variables and the goodness of fit was determined by the coefficient of determination (R^2).

Effect of temperature on optimised gas concentration

In order to assess the effect of temperature on the RR of pomegranate arils, the second set of experiments were conducted at three different temperatures (5, 10 and 15 °C) using the optimum gas atmosphere by the method described by Luca *et al*. (2016) with some modification. For this experimental batch, 350 g of arils was transferred to a 4000 mL jar and flushed with the optimum gas identified (4.67% O_2 + 12.67% CO_2 + 82.67% N_2). The concentration of O_2 inside the jar was continuously measured using the non-invasive O_2 sensor spots (PreSens-Precision Sensing GmbH, Germany), which were mounted to the inner side of the jar before the jar was closed. The O_2 concentration was measured through the glass wall of the jar by monitoring the fluorescence signals of the spots connected with fibre optic O_2 transmitter (Fibox 3 LCD trace), which connected to the computer for data transfer. The CO_2 sensor consists of infrared optics with the measuring capacity range of 0-25 ± 0.2% CO_2. CO_2 sensors were connected to a data logger (ALMEMO® 5690-2 Ahlborn Mess-und Regelungstechnik GmbH, Germany) for automatic transfer of the measured voltage signal to a computer. On both sensors, the O_2 and CO_2 concentrations were measured every minute for 60 h. From this data the real time respiration rate (R_{CO_2}, R_{O_2}) of pomegranate arils were calculated.

Results and discussion

Headspace gas composition

The headspace atmosphere changed significantly ($p \leq 0.05$) throughout the storage duration (Fig. 2). A common trend in CO_2 accumulation and O_2 consumption was observed. Highest reduction of headspace O_2 below the minimum limit (2%) (Artés, Gomez, & Artés-Hernández, 2006) was observed for two modified atmospheres (MA-3 and MA-2), which was reduced from approximately 2% to 1.4% for both atmospheres. Therefore, these results indicated that arils stored initially at a low O_2 concentration (2%) were susceptible to the risk of anaerobic respiration. On the other hand, the minimum reduction was observed for MAP-4 (4.67% O_2 + 12.67% CO_2), where the O_2 concentration reduced from 4.75% to 3.81% at the end of storage. This suggested the MA-4 sustained aerobic respiration while it has low initial O_2 concentration. Consistent with previous studies, the concentration of CO_2 increased throughout the storage duration for all modified atmosphere conditions (Banda *et al*., 2015a; Belay *et al.,* 2017). However, relatively higher CO_2 concentration (21.77% CO_2) was obtained for arils stored under high CO_2 concentration (MA-2). This could be attributed to either the highest initial CO_2 concentration (18%) or to the highest reduction in O_2 (1.3%) at the end of storage. Furthermore, CO_2 production rate did not slow down as much as O_2 consumption for all modified atmospheres except for MA-3, MA-4 and MA-5. This suggested that the respiratory quotient (RQ) of the tissue was not equal to one. It was likely that CO_2 was being liberated not only by aerobic respiration but also by anaerobic respiration when the O_2 concentration decreased below the tolerance level of the tissue (Segall & Scanlon, 1996). For the remaining MA treatments, the concentration of CO_2 was within the range of 9.75-15.23%, with the exception of MA-3 and MA-2, where it was 7.4 and 21.77%, respectively. However, as can be seen in Figure 2 the highest increase in CO_2 compared to the initial concentration was observed in MA-1, where the concentration increased from 2.17 to 9.75% at day 8. According to Conesa *et al*. (2007), the physiological activity of fresh cut fruit generates an equilibrium atmosphere within packages with low O_2 and moderately high CO_2 levels, which in turn produces dependent.

Effect of modified atmosphere on respiratory quotient

The RQ of the different gas combinations is summarized in Table 2. The RQ is defined as the ratio of CO_2 produced and O_2 consumed, which is normally assumed to be equal to 1 if the oxidized metabolic substrate is carbohydrate, and higher than 1 if the substrate is acidic (Caleb *et al*., 2016b). Under low O_2 atmosphere conditions, the amount of O_2 consumption was lower than the rate of CO_2 production, resulting in RQ much higher than 1. Figure 3 illustrates the immediate increase of RQ when O_2 concentration decreased below 5%. Thereafter, significantly higher values of RQ (8.64 ± 0.42 and 6.98 ± 0.15) were observed for the lowest O_2 atmosphere (2%; MA-3) and for highest CO_2 atmosphere (18%; MA-2), respectively. This observation of high RQ values could indicate a shift in the respiration (aerobic to anaerobic) or fermentation process. However, for the atmosphere with higher O_2 concentration (12% MA-6), the RQ was in the range of 1.13 to 1.32. The increase in RQ is generally indicative of fermentative reaction (Conesa *et al*., 2007; Oms-Oliu *et al*., 2008). This observation is consistent with previous reports which showed increased RQ of fresh-cut bell peppers in the presence of high concentration of CO_2 (Conesa *et al*., 2007) and increase in RQ of wild rocket when the O_2 decreased to 0.6% (Luca *et al*., 2016).

The contour plot (Fig. 4a) also shows that the RQ tended to increase towards lower O_2 and higher CO_2 atmospheres. All single gas components were significantly affected by the RQ values, including the binary gas interactions of O_2-N_2 and CO_2-N_2. A range RQ values for pomegranate arils have been reported in the literature. At passive atmosphere, Caleb *et al*. (2012) observed RQ value of 0.98 ± 1.14 for 'Acco' pomegranate arils, while Banda *et al*. (2015b) reported RQ values of (0.9 to 1.24) for 'Wonderful' pomegranate arils treated and non-treated with citric acid. In the current study, the O_2 concentration (2.95%) at which an increase in RQ was noted could be considered the low O_2 limit (LOL) of 'Wonderful' pomegranate arils. Similarly, Belay *et al*. (2017) reported that O_2 concentration of ≤ 2.8% as a lower limit for 'Wonderful' pomegranate arils under active MAP. However, the LOL may vary among fruit of the same species, region, year of production, and storage temperature; therefore, low O_2 atmosphere should be studied before it applied to a product (Weber *et al*., 2011).

Effect of modified atmosphere on respiration rate

The RR of arils based on CO_2 production rate (R_{CO_2}) varied from 5.18 mL kg^{-1} h^{-1} to 26.57 mL kg^{-1} h^{-1}, where the highest R_{CO_2} was observed under MA-2 (2% O_2 + 18% CO_2 + 90% N_2). The contour plot (Fig. 4b) illustrated that R_{CO_2} of pomegranate arils increased towards the highest CO_2 and O_2 atmospheres (MA-2, MA-6 and MA-1), and decreased under lowest O_2 and CO_2 atmospheres (MA-3). The decline in RR can be associated with absence of enough respiration substrate (Chunyang *et al.*, 2010). Furthermore, the reduction in RR under low O_2 MA could be attributed to a decrease in the activity of oxidative enzymes such as polyphenol oxidase, ascorbic acid oxidase and glycolic acid oxidase (Kader & Ben-Yehoshua, 2000). This is clearly shown under MA-1 and MA-3, where CO_2 concentrations were both 2% and O_2 concentration was 18 and 2%, respectively, while the R_{CO_2} of arils under MA-1 was 1.5 times higher than under MA-3. Higher RR could be due to increased physiological stress of arils stored under high CO_2 concentration (Sandhya, 2010), presence of the tissue damage (Iqbal *et al.,* 2008) or microbial growth (Silveria *et al*., 2014).

However, in the current study the presence of higher initial concentration of CO_2 led to increasing in carbon dioxide production rate (R_{CO_2}). Guevara *et al*. (2006) suggested that high CO_2 concentration could either decrease or increase RR depending on its impact on ethylene biology. RR can also increase when the O_2 concentration approaches zero if there is excessive tissues damage (Conesa *et al*., 2007). Furthermore, the interaction of O_2 and CO_2 at elevated CO_2 concentration makes plant material more sensitive to low concentration of O_2 such that the fermentation threshold occurs at higher O_2 partial pressures (Kader & Ben-Yehoshua, 2000).

The observed R_{CO_2} values in the present study are in agreement with findings by Banda *et al*. (2015b), where R_{CO_2} reached 28.3 mL kg^{-1} h^{-1} for pomegranate 'Wonderful' arils, under passive atmosphere. RR of 'Bhagwa' pomegranate arils ranging from 1.9 to 18.6 mL kg^{-1} h^{-1} and 3.2to 28.9 mL kg^{-1} h^{-1} for R_{O_2} and R_{CO_2} respectively, were reported by Aindongo *et al*. (2014) when produce were stored under temperature ranging from 5 to 22 °C. Caleb *et al*. (2012) also found R_{O_2}values between 2.5-7.6 mL kg^{-1} h^{-1} and R_{CO_2}values between 2.7-9.0 mL kg^{-1} h^{-1} for 'Acco' and 'Herskawitz' pomegranate arils. On the other hand, low R_{O_2} of 1.5 mL $kg^{-1}h^{-1}$ and R_{CO_2} of 0.52 mL kg^{-1} h^{-1} for 'Hicaz' pomegranate arils stored under a combination of low O_2 (2%) and high CO_2 (10%) at 4 °C were reported by Ersan *et al.* (2009). The differences in RR found in the present study and current literature could be due to various factors such as, differences in the initial concentration of gases, the type of RR measuring method and fruit cultivar.

Effect of modified atmosphere on ethylene production

Ethylene production rate decreased when CO_2 concentration increased from 2 to 18%, and at the highest O_2 concentration (18%). The highest ethylene production rate (0.25 μL kg^{-1} h^{-1}) was observed under MA-3 (low O_2 (2%) and low CO_2 (2 %)). However, under MA-2 (2% O_2 + 18% CO_2 + 80% N_2), the production of ethylene was completely eliminated (Fig. 4c). Similarly, the effect of high CO_2 (> 10%) in inhibiting ethylene production in fresh-cut bell peppers was reported by Conesa *et al*. (2007). Abeles *et al*. (2012) also showed that applying low O_2 and elevated CO_2 significantly reduced the ripening and senescence rates of fresh produce primarily by reducing the synthesis and perception of ethylene changes. According to Crisosto *et al*. (2001), ethylene production capacity of pomegranate fruit is limited to 0.1 μL kg^{-1} h^{-1} at 10 °C and 0.2 μL kg^{-1} h^{-1} at 20 °C. Palma *et al*. (2009) reported that 'Primosole' pomegranate arils packed in a polypropylene tray showed a slow increase in ethylene production rate initially and subsequently increased at the end of 8 days in cold (5 °C) storage. No ethylene production was detected under ambient conditions for 'Ganesh' pomegranate fruit (Nanda *et al*., 2001). The mechanism by which atmospheric gas fluctuation in containers affects ethylene production is not clear (Fagundes *et al.,* 2015).

Additionally, the effect of N_2 on RR of arils was significantly greater than the effect of O_2, CO_2, or their interaction, while, the presence of CO_2 showed no effect on the production of ethylene. Generally, there is limited information in the literature about ethylene production capacity of pomegranate arils under various storage and postharvest handling conditions. In this current study, the results showed that ethylene production rate was within the range of 0.00 to 0.25 µL kg^{-1} h^{-1} across all MA conditions. The findings further suggest that pomegranate arils have low ethylene production and physiological activity similar to whole pomegranate fruit (Crisosto *et al.*, 2001).

Effect of modified atmosphere on microbial quality

The total aerobic mesophilic bacteria, yeast and mould growth were evaluated during storage of pomegranate arils as presented in Table 3. These results showed that initial gas modification significantly ($p \leq 0.05$) affected the microbial quality of pomegranate arils. The growth of all the microbes studied (aerobic mesophilic bacteria, yeast and mould) were inhibited under high CO_2 concentration (MA-2 and MA-4). As shown in the ternary contour plots (Fig. 5a, b and c), the green area represents the lowest number of microbial growth. The highest microbial counts (lower inhibitory effect of gases) were found in arils stored under MA-1 and MA-3 conditions. The most likely explanation for the differences observed in the microbiological count results was the impact of CO_2 concentration. Previous studies have shown that high CO_2 concentration reduces microbial growth on pomegranate arils (Belay *et al.*, 2017). Masson *et al.* (2002) also reported that the total microbial load on fruit and vegetables can be modified by the initial CO_2 concentration. This sanitizing effect could be attributed to the formation of carbonic acid due to relative solubility of CO_2 in water, which has been suggested to inhibit microbial growth on produce such as fresh-cut pineapple (Zhang *et al.*, 2013).

The binary and ternary interaction of the gases showed a significant effect on bacterial and yeast count, however the mould count was significantly affected by the interaction of O_2:CO_2. For the other MA conditions with relatively higher CO_2 concentrations (MA-5, MA-6, and MA-7), the growth of microbes were moderate. The advantage of higher CO_2 atmosphere (> 10%) was also clearly observed on total aerobic mesophilic bacteria count. The aerobic mesophilic bacterial count was below the maximum acceptable limit of 7 log CFU mL^{-1} (South African Legislation (FCD Act 54 1979)) for all treatments, with minimal growth observed on arils stored under MA-2 (2% O_2 + 18% CO_2 + 80% N_2) and MA-4 (4.67% O_2 + 12.67% CO_2 + 82.67% N_2). The highest bacterial count (5.83 log CFU mL^{-1}) on arils was observed under MA-1 (18% O_2 + 2% CO_2 + 80% N_2). Similarly, the highest yeast count (5.91 ± 0.08 log CFU mL^{-1}) was observed on pomegranate arils stored at the highest O_2 concentration (MA-1), however the lowest count (3.84 ± 0.03 log CFU mL^{-1}) was found under the highest CO_2 atmosphere (MA-2) followed by MA-4 (4.86 log CFU mL^{-1}) at the end of storage. The yeast count for the remaining modified atmosphere conditions was in the range of 5.04 to 5.62 log CFU mL^{-1}. These results showed that only MA-2 and MA-4 atmospheres maintained the yeast growth below the maximum acceptable limit of yeast count (5 log CFU mL^{-1}) recommended for fresh fruit and vegetable (South African Legislation (FCD Act 54 1979)). Mould count was within the range of 2.15 to 3.63 log CFU mL^{-1}, where the lowest and highest counts were observed at MA-2 and MA-3, respectively. Since O_2 concentration in both modified atmospheres was 2% and the CO_2 concentration was 2% for MA-3 and 18% for MA-2, the significant decrease in mould count at MA-2 was largely attributed to the higher CO_2 concentration.

Mathematical modelling

The first and second-degree polynomials including special cubical models were used to describe the measured response surface for individual parameters assessed (headspace gas concentration, RR, RQ, and microbial quality). The amount of O_2, CO_2 and N_2 were selected as independent variables in a simplex lattice design. Respiration rate of arils, respiration quotient, ethylene concentration, bacteria, yeast and mould were taken as responses. Coefficients of the main effects (β_1, β_2 and β_3) correspond to the average result of changing 1 factor at a time from its lowest to its highest values. The interaction terms (β_{12}, β_{23}, β_{13} *and* β_{123}) show how the responses change when two or more factors are simultaneously changed. The estimates of model parameters are summarized in Table 4.

The cubical model predicted the correlation of single (β_1, β_2, β_3) and interaction (β_{12}, β_{13}, β_{23}) and ternary (β_{123}) effects of gas concentrations on the RQ value with high correlation coefficient value of R^2 = 99%. This means that the model has sufficiently accommodated in accounting for describing the effects of the gas concentration on the RQ value. Based on the magnitude of the parameter estimates (Table 4), CO_2 concentration had the most significant effect on RQ value of pomegranate arils compared to O_2 concentration. The sign of the coefficients for binary (β_{13}) and ternary (β_{123}) terms show that there is a synergetic effect between O_2 and N_2 and between the three gases. The antagonistic effect of the binary interaction (β_{12}, β_{23}) between O_2 and N_2 and between CO_2 and N_2 is shown by the negative parameter estimate values. The binary effect of the interaction of O_2 and CO_2 (β_{12}) and the ternary interaction of the three gas components (β_{123}) showed similar effects on RQ and for both, the model estimates resulted in higher values than the average pure gas mixture. When the binary interactions were studied between O_2 and N_2 (β_{13}) and CO_2 and N_2 (β_{23}), on the other hand, the resulting RQ had a lower average value than the pure gas mixture.

For respiration rate (R_{CO_2}), the correlation of simplex lattice experimental data to predict single, binary and ternary interactions in the cubical model was high (R^2 = 99%). Based on the model parameter estimates shown in Table 4, CO_2 had the most significant effect on the increase in the RR of pomegranate arils for pure gas mixture, followed by the interaction effects of β_{23}. Similarly, the combination of the three gases (β_{123}) was the most synergetic. The sign of the coefficient of all binary interactions (β_{12}, β_{13}, β_{23}) show that there is an antagonistic effect between the O_2 and CO_2, O_2 and N_2, and CO_2 and N_2. Compared to the average estimate of the individual effect, the binary interaction resulted in very low RR values while the combination of the three gas components in a ternary interaction resulted in very high values. Therefore, these results suggested that if lower R_{CO_2} of pomegranate arils is desired, controlling the CO_2 concentration in particular, and the interaction of the three gases are very crucial.

Similar effects of gas modification were observed for ethylene concentration resulting in good fit (R^2 = 95%) between the simplex lattice experimental data and the predicted value of the special cubic model. Values of the coefficients of the special cubic model in Table 4 shows the magnitude of the single parameter estimates, where O_2 and CO_2 had the most synergetic effect on ethylene production rate. However, based on the parameter estimates of the binary gas mixture (β_{12}), the effects of O_2 and CO_2 resulted in higher values than the excepted average, which was the most synergetic interaction to control ethylene production of pomegranate arils. It was evident that ethylene production decreased towards the highest concentrations of CO_2 and O_2. On the other hand, the highest production rate was observed when the concentration of both O_2 and CO_2 decreased to the lowest level (2%) as shown in the contour plot (Fig. 4c). Furthermore, the interaction effect of β_{23} and β_{123} resulted in to lower ethylene production than the estimated average values. However, the effect of the three gas components for ternary interaction (β_{123}) was antagonistic.

For microbial analysis, the correlation of the simplex lattice experimental data to the predicted values in the cubical model fitted well (R^2 > 94%). Based on the magnitude of the parameter estimates (Table 4), the antagonistic effect towards reducing the microbial counts was observed in ternary interaction (β_{123}) for aerobic mesophilic bacteria and yeast, and binary interaction (β_{12}, β_{23} and β_{13}) for mould. However, O_2 concentration in a single component (β_1) resulted in higher microbial counts. From the pure gas mixture, the lower count of microbial growth could be achieved by increasing the CO_2 concentration. The binary interactions (β_{12} and β_{13}) and the ternary interaction (β_{123}) had lower aerobic mesophilic bacterial and yeast counts than the expected average resulting from a single component. For mould, all binary interactions (β_{12}, β_{23} and β_{13}) resulted in lower values and the ternary interaction (β_{123}) resulted in higher values than the average.

In general, according to the second-degree polynomial (Equation 3) which was used to describe the measured responses, adequate fit can be determined by using the sign and relative magnitude of the parameter estimates (Cornel, 2011). For all, physiological and microbial responses (RR, RQ, ethylene, aerobic mesophilic bacteria, yeast and mould), the coefficients of parameter estimates for single components were greater than zero ($\beta_1, \beta_2, \beta_3 > 0$). This means individual component has a direct effect on the increase of all response parameters except β_2 for ethylene, which shows a reduction of ethylene as CO_2 concentration increased. Similarly, binary parameter estimates greater than zero ($\beta_{12}, \beta_{13}, \beta_{23} > 0$) indicate that the interaction of O_2 and CO_2, O_2 and N_2, CO_2 and N_2 has a synergetic effect, whereas estimates less than zero ($\beta_{12}, \beta_{13}, \beta_{23} < 0$) show antagonistic effect. Ternary interaction greater than zero ($\beta_{123} > 0$) for R_{CO_2}, RQ and mould showed that O_2, CO_2 and N_2 have a ternary synergetic effect resulting in the increase of these parameters. The correlation coefficient for special cubical model was $R^2 >$ 99% except for mould which was 94%, which implies that the error variance estimate was less than 10% of the total error variance, indicating high significance of the model. The maximum height of the fitted response (which corresponds to the optimum gas composition) (Van de Velde & Henderickx, 2001) indicated the optimum region on the superimposed ternary contour profile plots in the modified atmospheres.

It was found that when CO_2 concentration increased above from 10%, its effect towards all the response parameters selected for this study improved. However, these parameters responded negatively at very low O_2 (2%) concentration as evident in all the contour plots. Furthermore, the calculated optima indicated that the minimum RR (0.26 mL O_2 kg^{-1} h^{-1} and 0.78 mL CO_2 kg^{-1} h^{-1}), ethylene production rate (0.0 μL kg^{-1} h^{-1}), aerobic mesophilic bacteria (3.9 log CFU mL^{-1}), yeast (3.8 log CFU mL^{-1}) and mould (2.3 log CFU mL^{-1}) can be obtained by using a gas mixture of O_2:CO_2 (2% O_2 and 18% CO_2) as shown in bubble plot in Figure 6. Even though the estimated optimum gas provided an advantage to minimize arils RR, ethylene production rate and microbial load, the experimental results showed that O_2 concentration for this mixture dropped to a level (1.53%) where it could stimulate anaerobic RR during storage. If the aim is to achieve the minimum values of the response variables without negative effect during cold storage, 2% O_2 is therefore recommended for short storage duration (1-3 days). However, for commercialization of pomegranate arils with longer storage life, the concentration of O_2 should be increased within the range of 2 to 4%. This was evident from the maximum RR and RQ values observed under 2% O_2 storage with the elapse of storage time, while the response variables responded positively at 4.67% O_2 atmospheres until the end of storage. This recommendation is supported by results on the ternary contour plots, where the green area in all response variables pointed towards MA-4 (4.67 O_2 and 12.67% CO_2).

Effect of temperature on optimum gas composition

Storage temperature had a significant impact on the change in headspace gas composition and RR ($p \leq 0.05$). Comparing the changes in gas concentration, the highest CO_2 evolution (33%) was observed while arils were stored at 15 °C. Similarly, initial O_2 concentrations were reduced by 5%, 14% and 34% at 5, 10 and 15 °C, respectively after storage for 8 days. The concentration of O_2 was within the tolerance limit (> 2%) at all temperatures at the end of the study. However, fermentation development associated to objectionable odour was detected for arils stored at 15 °C on day 8. RR rate increased significantly with increasing temperature, where the lowest RR was noted at 5 °C and the highest at 15 °C (Fig. 7). R_{O_2} of arils increased from 0.29 mL kg^{-1} h^{-1} to 0.67 mL kg^{-1} h^{-1} at 5 °C and from 2.61 mL kg^{-1} h^{-1} to 3.08 mL kg^{-1} h^{-1} at 15 °C. On the contrary, after 8 days in storage at 10 °C the R_{O_2} reduced form 1.42 mL kg^{-1} h^{-1} h to 0.96 mL kg^{-1} h^{-1}.

Similarly, for R_{CO_2}, a 3 and 2-fold increase was observed when the temperature increased from 5 to 10 and 15 °C, respectively. The observed RR data are in agreement with the findings of Caleb *et al.* (2012) for 'Acco' and 'Herskawitz' pomegranate arils stored under passive atmosphere. The authors reported 3 to 4 fold increase in RR of arils when storage temperature increased from 5 to 15 °C. Similarly, Banda *et al.* (2015b) reported a significant increase in RR with increasing temperature for citric acid treated and untreated 'Wonderful' pomegranate arils, where R_{O_2} increased from 2.6 to 27.2 mL kg^{-1} h^{-1} and R_{CO_2} increased from 2.8 to 28.3 mL kg^{-1} h^{-1}. Gil *et al.* (1996) observed that RR of 'Mollar' pomegranate arils increased from 0.53 mL kg^{-1} h^{-1} to 1.94 mL kg^{-1} h^{-1} when the storage temperature was increased from 1 to 8 °C. Furthermore, Fawole and Opara (2013c) stated that the optimum storage temperature recommended for pomegranate fruit varies from 0-10 °C depending on cultivar. Since pomegranate arils have very low RR at 5 °C and relatively low RR at 10 °C and considering its non-climacteric nature, it can be suggested that storing 'Wonderful' pomegranate arils at 5-10 °C along the cold chain could be effective in reducing the physiological response under modified atmosphere condition MA-4 (4.67% O_2 + 12.67% CO_2 + 82.67% N_2).

Conclusions

This study showed that it is possible to optimize the gas concentration in active modified atmosphere by using the simplex lattice mixture design. The design was effectively used to investigate the effect of individual gas components and their binary and ternary interactions to the selected response variables. All linear and special cubic effects were found to be important for all the parameters assessed, but the correlation of the special cubical model was found to be the most accurate. The model estimates of the special cubic model suggested that CO_2 has a greater significant effect on the degree of physiological response and microbial quality parameters. This study showed the use of high CO_2 MA in packaging or storage of 'Wonderful' pomegranate arils could lower ethylene production rate, RR and microbial count. On the other hand, although lower RR can be achieved by reducing the O_2 concentration, this benefit was limited by the microbial load. Based on the above-mentioned response factors and results obtained, the optimum gas composition without further effect on the change of RR to anaerobic was determined to be MA-4 O_2:CO_2 (4.67% O_2 and 12.67% CO_2). However, in order to maintain this desired optimal atmosphere inside packaged 'Wonderful' pomegranate arils, matching the product physiological data with the packaging material is important.

Results from the second experiment showed the significance of maintaining the optimum cold storage temperature even when the desired atmosphere is established. It showed that pomegranate arils undergo a higher physiological response at 15 °C, and maintained a lower RR at lower temperature (5-10 °C). Furthermore, in the present optimization study, a constant weight of pomegranate arils was used and only one storage temperature was evaluated. Thus, the optimal atmosphere is valid under these conditions, and further experiments are needed to understand the impact of optimal gas concentration on the physicochemical changes and off-odour development at different product weights and temperatures using the simplex lattice design.

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Table 1. Simplex lattice experimental design layout for different gas mixtures.

Gas mixture	Uncoded variables (%)			Pseudo components		
	O_2	CO_2	N_2	O_2	CO_2	N_2
MA-1	18	2	80	1	0	0
MA-2	2	18	80	0	1	0
MA-3	2	2	96	0	0	1
MA-4	4.67	12.67	82.67	⅙	⅔	⅙
MA-5	4.67	4.67	90.67	⅙	⅙	⅔
MA-6	12.67	4.67	82.67	⅔	⅙	⅙
MA-7	7.33	7.33	85.33	⅓	⅓	⅓

Table 2. Effect of different gas mixtures on the respiratory quotient (RQ) of 'Wonderful' pomegranate arils stored at 10 °C for 9 days.

Storage days	Gas mixtures						
	MA-1	**MA-2**	**MA-3**	**MA-4**	**MA-5**	**MA-6**	**MA-7**
1	$0.79 \pm 0.06^{a}_{B}$	$8.47 \pm 7.82^{a}_{AB}$	$5.14 \pm 2.75^{a}_{B}$	2.17 ± 0.15aA	1.82 ± 0.36^{a}	$1.32 \pm 0.46^{a}_{A}$	$1.34 \pm 0.24^{a}_{B}$
3	$6.95 \pm 8.56^{abc}_{AB}$	5.09 ± 0.48b$_{B}$	$4.48 \pm 2.17^{b}_{C}$	3.03 ± 0.11bA	2.56 ± 0.09^{b}	$1.29 \pm 0.35^{c}_{A}$	$1.67 \pm 0.45^{c}_{A}$
6	$0.97 \pm 0.01^{e}_{B}$	$5.78 \pm 0.05^{b}_{B}$	$7.51 \pm 0.35^{a}_{B}$	3.75 ± 1.42cA	2.75 ± 0.29^{c}	$1.13 \pm 0.01^{e}_{A}$	$2.02 \pm 0.06^{d}_{A}$
8	$1.13 \pm 0.17^{e}_{B}$	$6.98 \pm 0.15^{b}_{B}$	$8.64 \pm 0.42^{a}_{A}$	3.98 ± 1.67cA	2.63 ± 0.12^{c}	$1.23 \pm 0.03^{e}_{A}$	$2.05 \pm 0.00^{d}_{A}$

Mean value ± standard deviation in the same rows with different superscript lowercase letters are significantly different ($p \leq 0.05$) along the storage duration, and mean values in the columns with different uppercase subscript letters are significantly different ($p \leq 0.05$).

Table 3. Effect of active modified atmospheres on microbiological quality of 'Wonderful' pomegranate arils stored at 10 °C.

Parameter	**Storage duration**	**Atmospheric conditions**						
		MA-1	**MA-2**	**MA-3**	**MA-4**	**MA-5**	**MA-6**	**MA-7**
Bacteria (log CFU mL^{-1})	Day 1	$2.01 \pm 0.15^{a}_{C}$	$2.01 \pm 0.15^{a}_{B}$	$2.01 \pm 0.15^{a}_{C}$	$2.01 \pm 0.15^{a}_{B}$	$2.01 \pm 0.15^{a}_{C}$	$2.01 \pm 0.15^{a}_{C}$	$2.01 \pm 0.15^{a}_{C}$
	Day 3	$1.60 \pm 0.00^{c}_{D}$	$2.36 \pm 0.05^{a}_{B}$	$2.05 \pm 0.38^{a}_{C}$	$2.24 \pm 0.08^{a}_{B}$	$1.74 \pm 0.37^{bc}_{C}$	$1.93 \pm 0.04^{b}_{C}$	$2.33 \pm 0.04^{a}_{C}$
	Day 6	$4.09 \pm 0.01^{cb}_{B}$	$4.17 \pm 0.02^{b}_{A}$	$4.15 \pm 0.01^{bc}_{B}$	$4.22 \pm 0.05^{b}_{A}$	$4.04 \pm 0.23^{b}_{B}$	$4.71 \pm 0.01^{a}_{B}$	$4.26 \pm 0.03^{b}_{B}$
	Day 8	$5.83 \pm 0.00^{a}_{A}$	$3.95 \pm 0.02^{e}_{A}$	$5.08 \pm 0.01^{c}_{A}$	$4.64 \pm 0.07^{d}_{A}$	$5.55 \pm 0.05^{b}_{A}$	$5.11 \pm 0.05^{c}_{A}$	$5.04 \pm 0.0^{c}_{A}$
Yeast (log CFU mL^{-1})	Day 1	$1.00 \pm 0.00^{a}_{D}$	$1.00 \pm 0.00^{a}_{C}$	$1.00 \pm 0.00^{a}_{D}$	$1.00 \pm 0.00^{a}_{B}$	$1.00 \pm 0.00^{a}_{D}$	$1.00 \pm 0.00^{a}_{C}$	$1.00 \pm 0.00^{a}_{D}$
	Day 3	$1.75 \pm 0.21^{b}_{C}$	$1.87 \pm 0.13^{b}_{B}$	$1.48 \pm 0.00^{c}_{C}$	$2.36 \pm 0.05^{a}_{B}$	$1.65 \pm 0.06^{b}_{C}$	$1.00 \pm 0.00^{d}_{C}$	$1.50 \pm 0.28^{bc}_{C}$
	Day 6	$4.59 \pm 0.09^{b}_{B}$	$4.22 \pm 0.11^{c}_{A}$	$4.07 \pm 0.00^{d}_{B}$	$4.35 \pm 0.01^{c}_{A}$	$4.05 \pm 0.04^{cd}_{B}$	$4.81 \pm 0.05^{a}_{B}$	$4.44 \pm 0.01^{b}_{B}$
	Day 8	$5.91 \pm 0.08^{a}_{A}$	$3.84 \pm 0.03^{f}_{A}$	$5.04 \pm 0.05^{d}_{A}$	$4.86 \pm 0.03^{e}_{A}$	$5.61 \pm 0.01^{b}_{A}$	$5.62 \pm 0.03^{b}_{A}$	$5.39 \pm 0.05^{c}_{A}$
Mould (log CFU mL^{-1})	Day 1	$2.37 \pm 0.07^{a}_{C}$	$2.37 \pm 0.07^{a}_{A}$	$2.37 \pm 0.07^{a}_{B}$	$2.37 \pm 0.07^{a}_{A}$	$2.37 \pm 0.07^{a}_{B}$	$2.37 \pm 0.07^{a}_{B}$	$2.37 \pm 0.07^{a}_{C}$
	Day 3	$1.00 \pm 0.00^{c}_{D}$	$2.31 \pm 0.04^{a}_{A}$	$1.65 \pm 0.07^{b}_{C}$	$1.77 \pm 0.10^{b}_{B}$	$1.65 \pm 0.07^{b}_{C}$	$1.39 \pm 0.13^{c}_{C}$	$1.72 \pm 0.34^{bc}_{D}$
	Day 6	$2.77 \pm 0.10^{b}_{B}$	$2.39 \pm 0.12^{c}_{A}$	$2.39 \pm 0.12^{c}_{B}$	$2.39 \pm 0.12^{c}_{A}$	$2.54 \pm 0.08^{bc}_{B}$	$2.69 \pm 0.30^{bc}_{A}$	$3.73 \pm 0.06^{a}_{A}$
	Day 8	$3.63 \pm 0.07^{a}_{A}$	$2.30 \pm 0.00^{c}_{A}$	$3.26 \pm 0.12^{b}_{A}$	$2.15 \pm 0.21^{c}_{A}$	$2.95 \pm 0.07^{b}_{A}$	$2.94 \pm 0.34^{abc}_{A}$	$2.80 \pm 0.14^{b}_{B}$

Mean value ± standard deviation in the same rows with different superscript lowercase letters are significantly different ($p \leq 0.05$) along the storage duration, and mean values in the columns with different uppercase subscript letters are significantly different ($p \leq 0.05$).

Table 4. Estimated model parameter coefficients with standard error obtained in the linear effect $(\beta_1.\ \beta_2$ *and* $\beta_3)$, binary interaction and the cubic effect $(\beta_{1,2}.\ \beta_{2,3}.\ \beta_{1,3}$ and $\beta_{1,2,3})$ describing the effect of gas combinations on the physiological responses, ethylene production rate and microbial quality.

Constants	R_{CO_2}	**RQ**	**C_2H_4**	**Bacteria**	**Yeast**	**Mould**
β_1	20.54 ± 0.08	1.14 ± 0.21	0.04 ± 0.02	5. 82 ± 0.03	5.90 ± 0.03	3.63 ± 0.12
β_2	26.69 ± 0.08	6.45 ± 0.21	-0.00 ± 0.02	3.95 ± 0.03	3.84 ± 0.03	2.30 ± 0.12
β_3	13.15 ± 0.08	8.92 ± 0.21	0.24 ± 0.02	5.08 ± 0.03	5.04 ± 0.03	3.25 ± 0.12
$\beta_{1,2}$	-27.77 ± 1.26	12.17 ± 3.2	1.39 ± 0.24	-3.36 ± 0.41	0.13 ± 0.46	-5.13 ± 1.78
$\beta_{1,3}$	-0.24 ± 1.26	-29.00 ± 3.2	0.12 ± 0.24	0.82 ± 0.41	1.89 ± 0.46	-1.27 ± 1.78
$\beta_{2,3}$	-4.02 ± 1.26	-18.18 ± 3.2	-0.76 ± 0.24	6.41 ± 0.41	5.15 ± 0.46	-2.71 ± 178
$\beta_{1,2,3}$	55.39 ± 8.89	12.62 ± 22.5	-4.07 ± 1.74	-9.32 ± 2.89	-8.97 ± 3.30	20.2 ± 12.49
R^2	*0.99*	*0.99*	*0.95*	*0.99*	*0.99*	*0.94*

Where, superscript 1, 2 and 3 represents oxygen, carbon dioxide and nitrogen respectively. ± Standard error

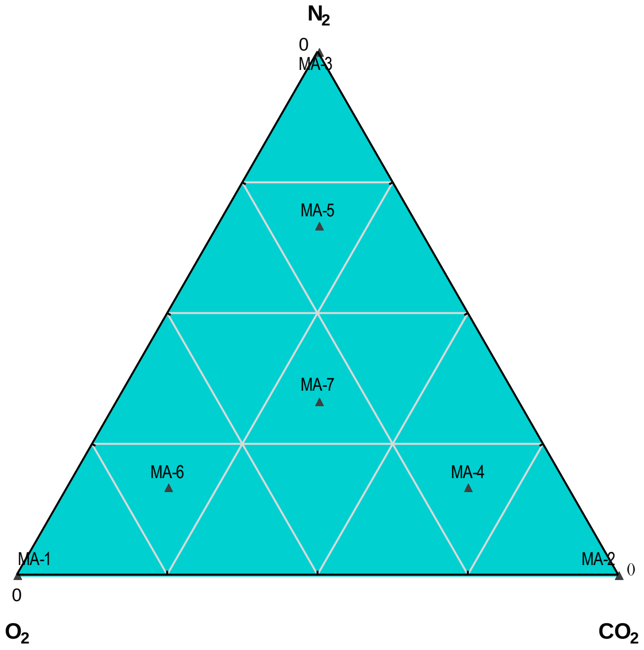

Figure 1. Simplex lattice mixture design for the experimental points (O_2, CO_2, and N_2) for the different gas concentrations (list of gas compositions presented in table 1).

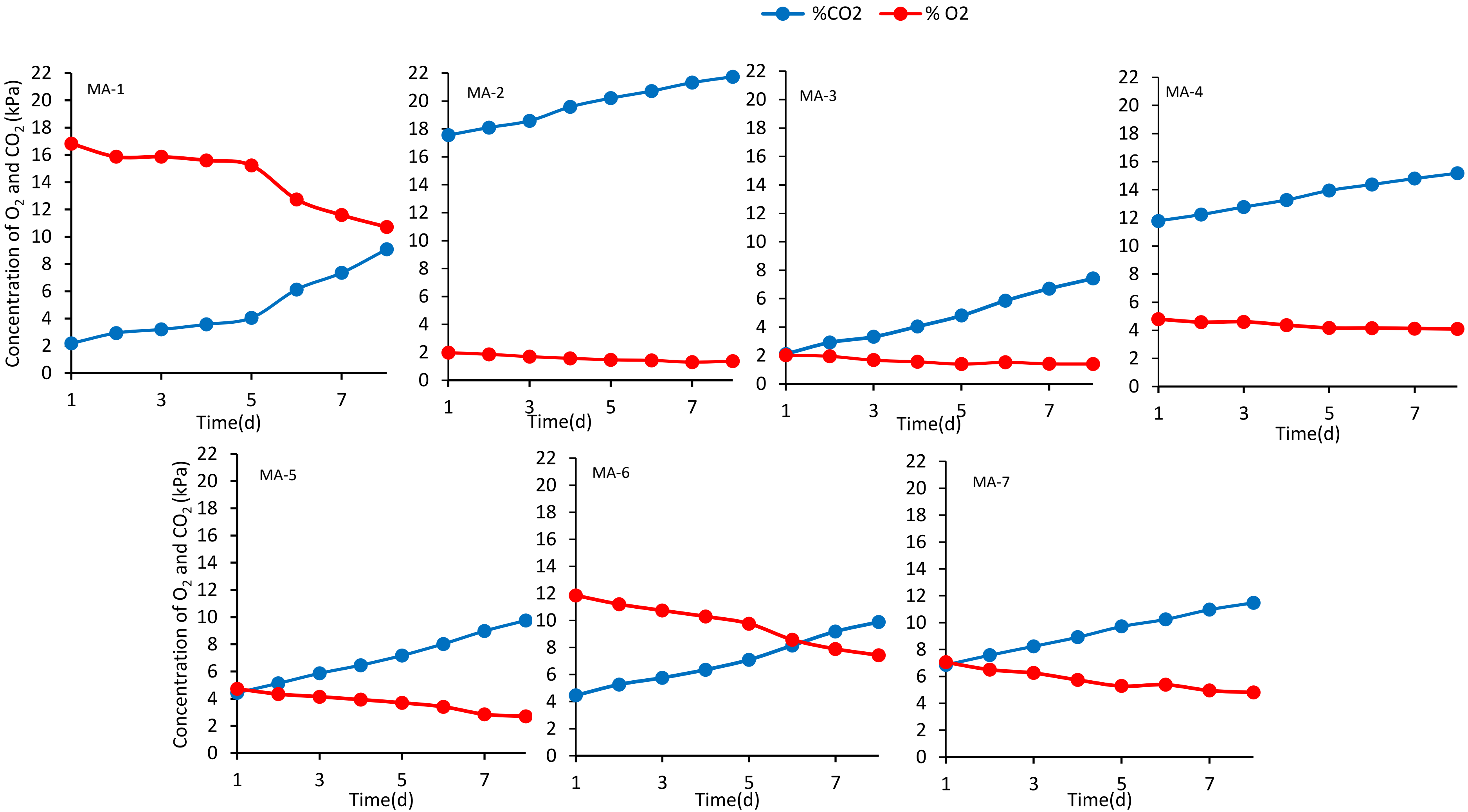

Figure 2. Change in the headspace gas concentration of 'Wonderful' pomegranate arils stored at initially modified atmospheres for 8 days at 10 °C.

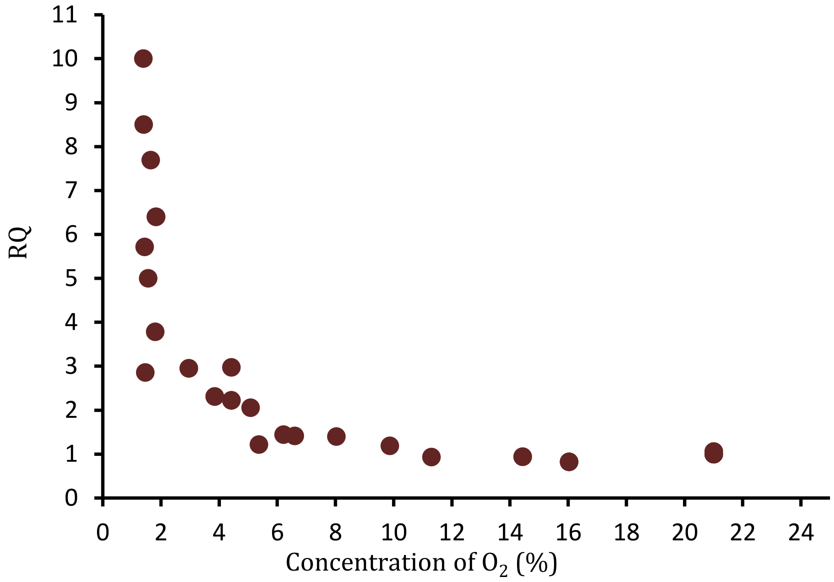

Figure 3. The relationship between the headspace oxygen concentration and respiratory quotient of pomegranate arils at different concentrations (2%–21%).

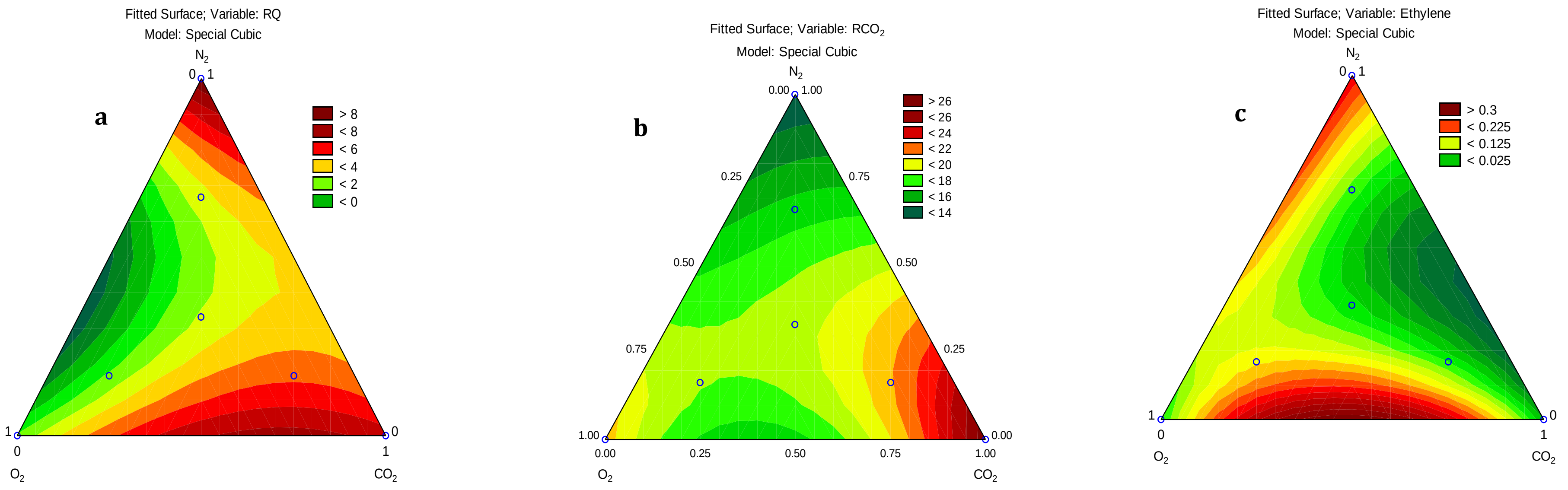

Figure 4. Ternary contour plot for respiration quotient (RQ) (a), respiration rate due to carbon dioxide production (R_{CO_2}) (b), ethylene roduction rate (c).

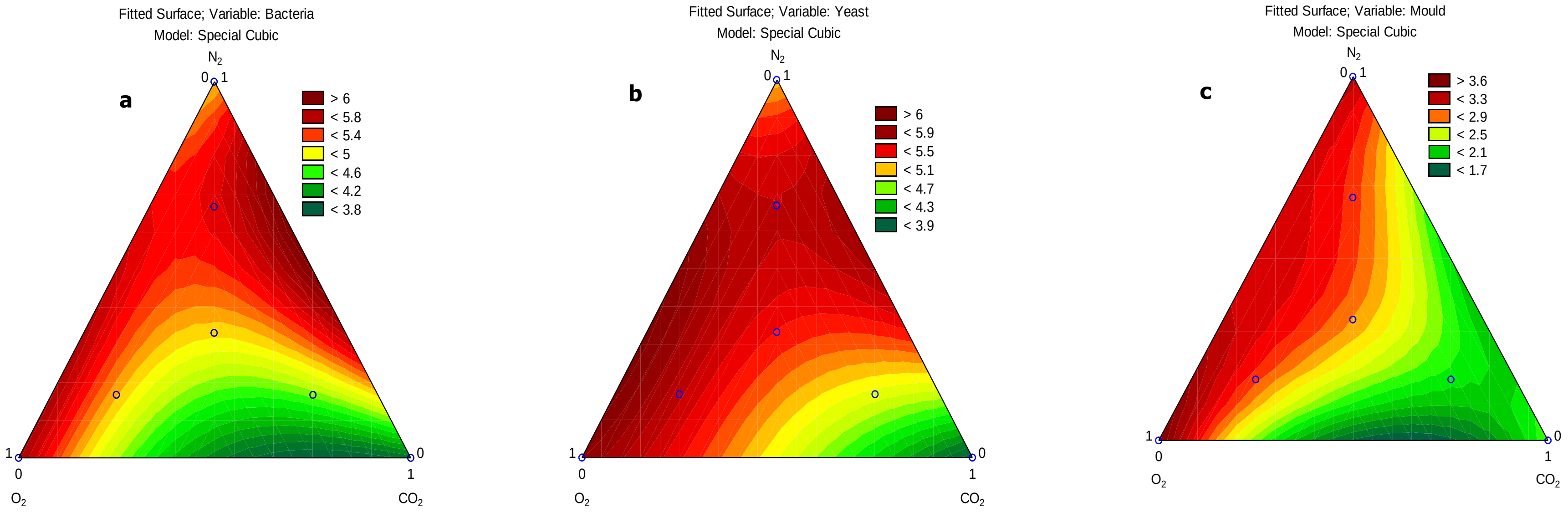

Figure 5. Ternary contour plot for the effect of the initial gas mixture on the microbial count of pomegranate arils: bacteria (a), yeast (b) and mould (c).

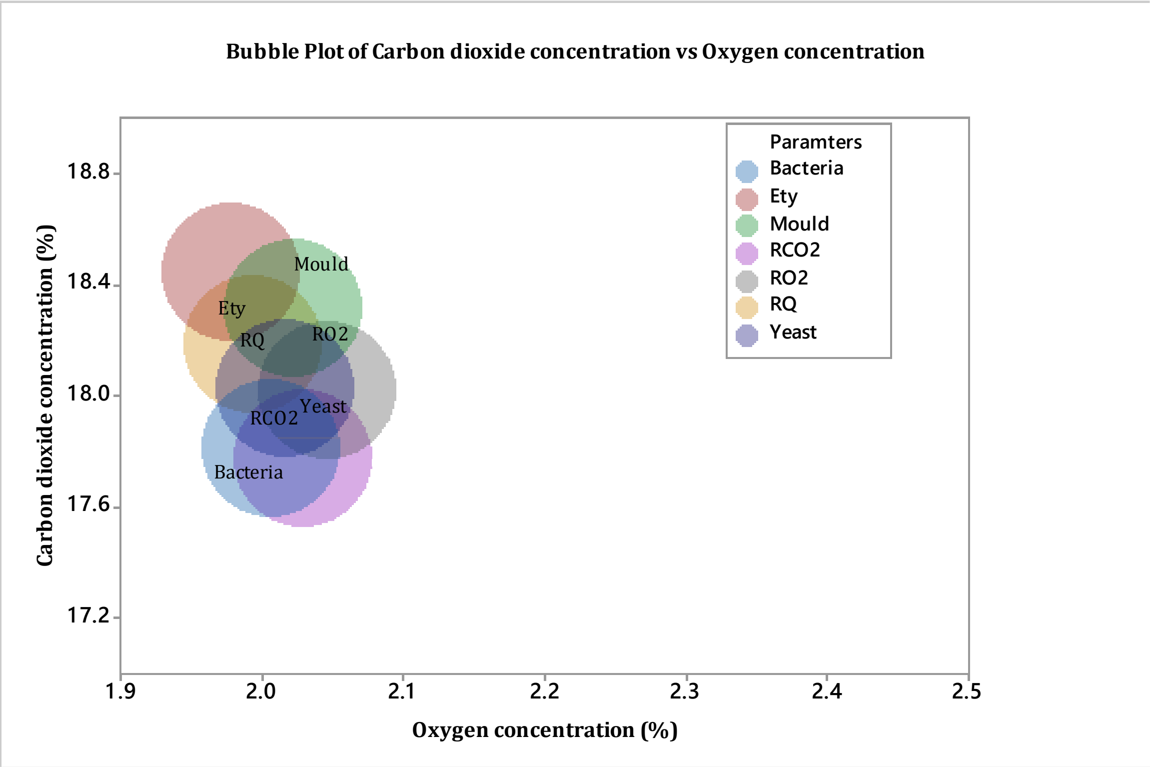

Figure 6. Bubble plots for optimum gas mixture for reducing respiration rate, ethylene production and microbial counts of pomegranate arils.

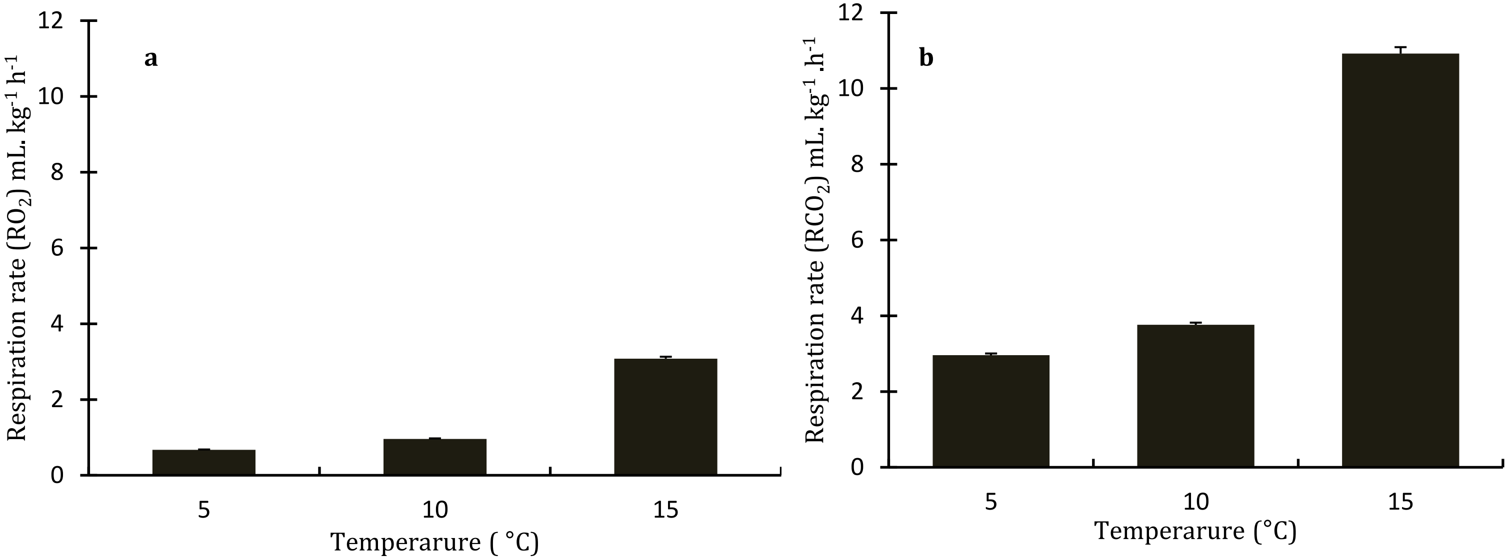

Figure 7. Effect of storage temperatures (5 and 10 °C) on the respiration rate of pomegranate arils stored at optimum gas concentration (4.67% O_2 + 12.67% CO_2 + 82% N_2); Respiration rate due to O_2 consumption (a); Respiration rate due to CO_2 production (b).

Declaration by the candidate:

With regard to Chapter 7 (pp 159-183); the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data analysis and writing of chapter	75

The following co-authors have contributed to Chapter 7 (pp 159-183):

Name	E-mail address	Nature of contribution	Extent of contribution (%)
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Dr O.J. Caleb	CalebO@arc.agric.za	Research input, editorial suggestion and proof reading	10
Dr P.V. Mahajan	PMahajan@atb-potsdam.de	Research input, assistance with optimization, editorial suggestion and proof reading	10

Signature of candidate: Z.A. Belay

Date: 03/11/2017

Declaration by co-authors

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 7 (pp 159-183)

2. no other authors contributed to Chapter 7 (pp 159-183) besides those specified above, and

3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 7 (pp 159-183) of this dissertation.

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Declaration with signature in possession of candidate and supervisor.

CHAPTER 7

A SIMPLEX LATTICE MIXTURE DESIGN TO OPTIMIZE ACTIVE MODIFIED ATMOSPHERE CONDITIONS FOR STORING 'WONDERFUL' POMEGRANATE ARILS: PART II – DETERMINING OPTIMUM GAS COMPOSITION TO MAINTAIN PHYSICOCHEMICAL QUALITY ATTRIBUTES AND VOLATILE ORGANIC COMPOUNDS

ABSTRACT

In this study, a simplex lattice mixture design approach was introduced to select and identify optimum gas composition for individual quality attributes of pomegranate arils under cold storage (10 °C, 91 ± 2% RH). The partial pressure of O_2, CO_2 and N_2 were chosen as factors and visual quality, physicochemical attributes, antioxidant properties and volatile organic compounds (VOCs) were taken as response variables. Special cubical models were generated for the response variables and seven interactive terms were selected. Based on coefficients of model parameter estimates (β_1, β_2, β_3, β_{12}, β_{13}, β_{23} and β_{123}) and ternary contour plots, the component interaction of O_2, CO_2 and N_2 were characterised. Single gas components had a synergetic effect on all response variables, however, binary and ternary interaction effects of gases varied across the responses. CO_2 was the most important factor affecting visual colour, instrumental texture and VOCs (aldehydes, ketones, and monoterpenes). Oxygen played a significant role towards colour attributes (Chroma and hue angle), organic acids, and promoted surface decay and stimulated alcohol VOCs. Variability of optimum gas mixtures for individual quality parameters was observed, where maximizing the concentrations of the individual sugars, organic acids, TSS, and colour were achieved by using a gas mixture of O_2:CO_2(6-7% O_2 and 7-8% CO_2). Maximum release of volatiles responsible for characteristic flavour of the arils was achieved using O_2:CO_2 (2 %O_2 and 18% CO_2). The results showed that the predicted global optima for maximizing the quality parameters of pomegranate arils under modified atmosphere (MA) condition were close to the experimental values.

Key words: Low O_2; Enriched CO_2; Microbial growth; Individual sugars; Antioxidants

Introduction

Pomegranate arils packed under modified atmosphere (MA) is one of the main commercial ready-to-eat products of pomegranate fruit. Pomegranate arils have been extensively studied for its potential health related benefits, such as anti-inflammatory, antimutagenicity, antioxidative and anti-cancer potential (Fawole & Opara, 2013a; Fawole *et al*., 2012a; Ayhan &

Eştürk, 2009) and factors affecting them (Mphahlele *et al.*, 2014a,b). Processor and consumer acceptability of pomegranate depend on a combination of several quality attributes. These include physicochemical, mechanical, microbial and antioxidant properties relating to aril colour, free from physical defects, sugar content, acidity and flavour attributes (Al-Said *et al*, 2009; Fawole & Opara, 2013a,b; Opara *et al.*, 2009). However, due to its relatively short shelf life after processing, the main market for fresh arils is limited (O'Grady *et al.*, 2014). Various studies have reported the benefits of MA conditions based on low O_2 and enriched CO_2 gases in extending the shelf life of minimally-processed ready-to-eat fruit and vegetables (Caleb *et al.*, 2012; Mahajan *et al.*, 2014). In contrast, few efforts have been exerted to develop an optimum active modified atmosphere packaging (MAP) system for pomegranate arils (Ayhan & Eştürk, 2009; Maghoumi *et al.*, 2013, 2014; Banda *et al.*, 2015; Belay *et al.*, 2017).

Ayhan and Eştürk (2009) reported a decrease in antioxidant activity and sensory quality of 'Hicaznar' pomegranate arils under low O_2 atmosphere (5% O_2 + 10% CO_2 + 85% N_2) stored at 5 °C. Higher levels of ascorbic acid, anthocyanins and phenolic contents were observed by Belay *et al.* (2017) for 'Wonderful' pomegranate arils stored at 5 °C under low O_2 concentrations (5% O_2 + 10% CO_2 + 85% N_2 and 10% O_{2+}5% CO_2 + 85% N_2). Similarly, Banda *et al.* (2015) showed that maintaining low O_2 concentration (5% O_2 + 30% CO_2 + 85% N_2) using a low barrier polypropylene plastic film retained the initial anthocyanin concentration of 'Wonderful' pomegranate arils, while a slight increase was observed in a high barrier polylid film. However, these studies did not report on how each gas component and their interactions influenced the measured quality attributes of pomegranate arils investigated. Only the combined effect of gas compositions (O_2, CO_2 and N_2) was evaluated towards maintaining the quality of different pomegranate cultivars. To attain optimal gas composition, it is important that individual gas component and their interaction effects be analysed using a statistical tool (Yilmaz *et al.*, 2015).

Mixture design methodology is very useful to predict the response of any combination of components by investigating the functions of an individual factor and component interaction (Cornell, 2011). This approach is beneficial for solving the problem of searching the optimal proportion of the components (Yilmaz *et al.*, 2015). Furthermore, experimental design can be used as leverage to reduce design costs by speeding up the design process, reducing the complexity in a number of design changes, materials and labour (Hron & Macak, 2015). To the best of our knowledge, no studies have been reported to select and optimize gas compositions of actively MA conditions for storage of pomegranate arils using a mixture design approach.

In chapter 6, we reported the optimization of active modified atmosphere gas mixtures for storing 'Wonderful' pomegranate arils using the simplex lattice mixture design approach concerning physiological response and microbial criteria. In chapter 7, we determined the optimum gas concentration to maintain individual quality parameters based on physicochemical attributes, antioxidant properties and volatile compounds of arils.

Materials and methods

Fresh produce preparation and storage

Pomegranate fruit cv. 'Wonderful were obtained at commercial maturity based on characteristic deep-red skin and arils with mature kernel, total soluble solids (TSS, %) and titratable acidity (TA, g 100 mL^{-1}) (Mphahlele *et al*., 2016). At harvest, the TSS, TA and malic acid contents were 17.5%, 1.1 g 100 mL^{-1} and 2334 mg 100 mL^{-1}, respectively from Sonlia Pack House, Wellington, Western Cape (33°38′23″S, 19°00′40″E), South Africa. Fruit were air-freighted in well-ventilated boxes to the Horticultural Engineering Department Laboratory, Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Germany. On arrival, fruit were stored in a cold storage chamber at 5 °C until processing to extract the arils in the cold room at 5 °C. Damaged fruit were removed and the outer skin of selected healthy fruit was surface disinfected using 70% ethanol prior to processing (Belay *et al*., 2017). Arils were extracted manually by carefully removing the husk. Extracted arils were collected in a tray and mixed to assure uniformity. Table 1 outlines the different gas combinations used to store arils. Arils (350 g) were transferred to 4000 mL airtight glass jars, designed to achieve a completely hermetic seal, and stored at 10 °C for 8 days and sampling was done every two days interval for quality attribute analysis.

Simplex lattice mixture design

The simplex lattice mixture design was used to identify the optimum gas composition in actively modified atmospheres to maintain or improve the individual quality attributes of 'Wonderful' pomegranate arils. Since the standard simplex design is a boundary point design, augmented and centroid points were included to make the prediction with a complete mixture of three gases (O_2, CO_2 and N_2). The selected factors and their levels (three factors, two levels simplex lattices) in terms of coded and uncoded variables are presented in (Table 1). For this study, three different gases, O_2, CO_2 and N_2 were selected as independent components in the mixture design. The analysis was performed using seven gas mixtures (Table 1). Special cubical model using equation 1 as a function of different gas components was fitted for each response:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \qquad (1)$$

where Y represents the measured response, β is the constant coefficient, X_1, X_2 and X_3 are the pseudocomponents of O_2, CO_2 and N_2, respectively. The computational work, including ternary contour plots of the models, was performed using Statistica (version 13, DOE StatSoft Inc. Tulsa, USA). The coefficients of the model parameter estimates were used to compare the influence of storage atmosphere on quality parameters. A positive coefficient value indicates a synergetic effect, while a negative value indicates an antagonistic effect (Cornell, 2011). The individual values obtained from the polynomial equation over the simplex region were used to describe the local optimum gas within the set limits of gases.

Visual quality attributes

A panel of six judges carried out the visual quality evaluation (colour, firmness and surface decay) of pomegranate arils stored at different modified atmospheres according to the method described by Caleb *et al*. (2013). The attributes were evaluated and scored based on a scale of 1 to 5 for visual colour and firmness, where 1 - very poor with severely paled red and no resistance to finger pressure, 2 - poor with half paled red and slight resistance to finger pressure, 3 - fair with slightly paled red and minor sign of softness, 4 - good appearance, completely red with slight loss of turgidity, and 5 - excellent appearance, completely deep red and firm. For decay, 1 was 0% decay; 2 was 1-2% decay; 3 was 26-50% decay; 4 was 51-75% decay and 5 was 76-100% decay. Scores below 3 for visual colour and firmness and above 3 for decay were considered as indicators of unacceptable visual quality.

Physical quality attributes

The surface colour of pomegranate arils was measured and evaluated on the basis of Commission International del' Eclairage (CIE) colour system using a digital Chroma-meter (CM-2600d, Konica Minolta Sensing Inc., Japan) with the method described by Caleb *et al.* (2016a). Calibration of the colour meter was performed against a white and black tile background prior to each measurement. Colour measurements were taken using individual arils and the data was further analysed (Pathare *et al.*, 2013) to describe the measured colour attributes (Chroma (C^*) and hue angle ($h°$)). A mean of 20 measurements for each treatment was used.

Firmness of individual arils was measured using a texture analyser (TA-XT Plus, Stable Micro Systems, Surrey, UK) with a SMSP/2 of 2 mm diameter cylindrical probe. This empirical hardness indicator test was performed according to Szychowski *et al*. (2015). This test included both compression and cutting effort (Chen & Opara, 2013). Penetration rate was 0.3 mm s^{-1} for 5 s after contacting the surface of pomegranate arils and results were expressed in Newton (N). A total of 20 arils were measured per treatment.

Titratable acidity and total soluble solid

Juice of fresh pomegranate arils was used to measure titratable acidity (TA) according to the method described by Caleb *et al*. (2016b). The TA concentration of the juice samples was measured potentiometrically by titration with 0.1 mol L^{-1} NaOH to an end-point of pH 8.2, using an automated T50 M Titrator with Rondo 20 sample changer (Mettler Toledo, Switzerland). The TA value was expressed as g L^{-1} of malic acid, citric acid and acetic acid based on fresh mass. The total soluble solid (TSS) of pomegranate juice samples was measured using a handheld digital refractometer (DR301-95, Krüss Optronic, Hamburg, Germany) and expressed as % Brix.

Individual sugar content

Sugar content (glucose, fructose and sucrose) was determined by the HPLC method (Magwaza & Opara, 2015) using a DIONEX Ultimate 3000 liquid chromatograph fitted with Analytical Auto-sampler WPS-3000TSL (Thermo Fisher Scientific GmbH, Dreieich, Germany) as described by Caleb *et al*. (2016b). The system is equipped with a refractive index detector SHODEX RI-101 (Showa Denko Europe GmbH, Munich, Germany). Sugars were separated on a Eurokat H column (300×8 mm and 10 µm diameter) (KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany), with 0.01 M sulphuric acid as the mobile phase. The injection volume was 10 µL and the flow rate was maintained at 0.8 mL min^{-1} at 35 °C. Sample detection and identification were performed by comparing the retention time of samples with the retention time of the calibration standards and results were quantitatively expressed in g L^{-1} juice.

Antioxidant properties

Total anthocyanin concentration (TAC) was quantified using the pH differential method as described by Fawole *et al*. (2012a) and O'Grady *et al*. (2014). In triplicates, pomegranate juice extracts (1 mL) were mixed with 9 mL of pH 1.0 and pH 4.5 buffers, separately. Absorbance was measured at 510 and 700 nm in pH 1.0 and 4.5 buffers respectively, and results were expressed as cyanidin-3-glucoside equivalents according to equation 2:

$$Total\ anthocyanin\ (mg/L) = \frac{A \times MW \times DF \times 1000}{\varepsilon} \times L \qquad (2)$$

where A is (A_{520}-A_{700}) pH1-(A_{520}-A_{700}) pH4.5; MW is molecular weight of cyanidin-3-glucoside (449.2 g mol^{-1}), DF is dilution factor; ε is molar extinction coefficient (26900) and L is path length in cm. All analysis was carried out in triplicate and results were expressed as mass cyanidin-3-glucoside equivalent per volume of pomegranate juice (mg L^{-1}).

Volatile organic compounds

Volatile compounds were extracted by static headspace sampling (SHS) according to the method described by Caleb *et al.* (2013b; 2016a). Pomegranate arils from each treatment were homogenized into puree and 7 g was placed in a 20 mL glass vial spiked with 100 µL of 3-octanol (diluted in absolute methanol to a concentration of 0.1 g L^{-1}) as internal standard. The vials were tightly capped and allowed to equilibrate at 80 °C for 10 min in the headspace auto-sampler incubator (HS-20 automated-sampler, Shimadzu Europa GmbH, Duisburg, Germany). Analyses were carried out using helium as carrier gas with a constant flow rate of 0.03 mL s^{-1}. The GC temperature was held at 50 °C, then ramped up to 110 °C at 5 °C min^{-1} and further ramped up to 180 at 20 °C min^{-1}, held at this temperature for 2.5 min and finally ramped up to 220 at 10 °C min^{-1}, held at this temperature for 3 min, with a total run time of 24 min. The mass selective detector in this study was operated in full scan mode and mass spectra in the 35-500 m z^{-1} range were recorded. Individual volatile compound was tentatively identified by their retention time and semi-quantification of the identified compounds was estimated using equation 3:

$$RA = \frac{A_{ic}}{A_{itc}} C_{itc} \qquad (3)$$

where RA is the relative abundance of the identified compound (g L^{-1}), A_{ic} is the peak area of the identified compound, A_{itc} is the peak area of the internal standard, C_{itc} is the final concentration of internal standard in the sample (0.1 g L^{-1}).

Statistical analysis and optimisation

The statistical work including designation of experimental points, analysis of variance, fitting of the polynomial model and graphical presentations (ternary contour plots) was performed using a statistical package design expert version STATISTICA (version 13, StatSoft Inc. Tulsa, USA). Post-hoc test (Duncan multi range test) was also used to determine the difference between mean combination values. Optimization of gas composition was determined by using beta values, set constraints, and use of solver to minimize or maximize the respective quality parameters.

Result and discussion

Visual quality assessment

According to the visual quality analysis (colour and decay) and subjective firmness scores, actively modified atmosphere conditions maintained the visual qualities with average scores of > 3 for colour and firmness and < 3 for decay. Based on the coefficient of the model parameter estimates, all single (β_1 β_2 and β_3) and binary ($\beta_{12,}$ β_{23} and β_{13}) components of gases had synergetic significant effects on visual quality attributes, while the ternary interaction effect was antagonistic (Table 2). Comparing these effects, only the effect of single gas components was significant. Among single gas component, higher CO_2 concentration resulted in higher colour value and firmness of pomegranate arils. The occurrence of decay on the surface of the arils was stimulated by higher O_2 among the single components. For instrumental quality analysis (C^*, $h°$ and firmness), only single gas components had significant effects, where the effect of O_2 was the most significant for maintaining both C^* and $h°$ and the effect of CO_2 was the highest for firmness, in which firmness increases when CO_2 concentration increased.

These are evident in the ternary contour plot (Fig. 1a$_1$, a$_2$), where arils colour and firmness slightly increased towards the highest CO_2 atmosphere vertex and decay decreased at the vertexes where the concentration of O_2 and CO_2 were the highest (Fig. 1a$_3$). This study is in line with Maghoumi *et al*. (2013), were 'Mollar de Elche' pomegranate arils treated with UV-C, and high O_2 was acceptable for consumption based on visual quality after 14 days of storage at 5 °C, however, the effect of the treatments was not significant. A significant effect of the low O_2 atmosphere on maintaining sensorial quality (taste and aroma) of 'Wonderful' pomegranate arils has been reported by Banda *et al.* (2015). Similarly, Ayhan and Eştürk (2009) observed that arils cv. 'Hicaznar' packed with air, nitrogen and enriched O_2 were still acceptable on day 18 based on sensory score, whereas, shelf life was limited to 15 days at low O_2 (5%) atmosphere. In addition to storage atmosphere, harvesting dates significantly affected visual quality of 'Mollar Elche' pomegranate arils according to López-Rubira *et al.* (2005). The study reported that visual quality scores were acceptable by consumers until 14 days cold storage for early and 10 days for late harvest fruit. Therefore, according to these studies, it could be stated that the significant effect of modified atmosphere on visual quality of pomegranate arils can differ due to in-package gas concentration, cultivar, and harvesting time.

Instrumental quality assessment

Surface colour

Both colour parameters (Chroma (*C**) and hue angle (*h*°)) increased in increasing storage O_2 concentration at MA-1, MA-5 and MA-6 and decreased towards the highest CO_2 atmospheres (MA-2 and MA-4) as shown in the ternary contour plots (Fig. 1b_1 and b_2). The coefficients of the parameter estimates (Table 2) accounting for the effects of gas compositions on *C** and *h*°, respectively, show that the individual and interaction effects of gas components were not significant ($P \geq 0.05$). Similar to this result, Maghoumi *et al.* (2014) observed no significant effect of active modified atmosphere on *C** value during cold storage of 'Malese-Saveh' pomegranate arils. Artés *et al.* (2000) found slight or no change in *C** and *h*° values of 'Mollar de Elche' pomegranate arils stored under refrigerated air storage. On contrary, Belay *et al.* (2017) observed significant effects of low and super-atmospheric O_2 on colour parameters of pomegranate arils of 'Wonderful' cultivar, where increase in *C** and *h*° was observed. The increase in *h*° indicates a little colour change from reddish-orange to red-magenta during storage, which could probably be due to higher polyphenoloxidase (PPO) activity (Palma *et al.*, 2006).

Aril firmness

Firmness of pomegranate arils during cold storage varied from 1.2 ± 0.42 N to 1.9 ± 0.62 N and increased with increasing CO_2 concentration (MA-2 and MA-4) as shown in the ternary contour plot (Fig. 1b_3). Based on the coefficients of parameter estimates, CO_2 had a greater effect in increasing the firmness of the arils and O_2 had the lowest effect among the gas components. The coefficient values for the single components were equivalent to the experimental data, but the interaction effects of gases resulted in very high and very low values compared to the weighted average. Furthermore, these coefficient values show that the effects of single gas components and the interaction of O_2 and CO_2 were synergetic. The remaining binary and ternary interaction effects of gases were antagonistic, although the effects of all single gas components and the effects of binary and ternary interaction of gases on firmness were not significant (Table 2).

Belay *et al*. (2017) reported similar results, where no significant effect of active modified atmosphere conditions on firmness for 'Wonderful' pomegranate arils stored at 5 °C for 12 days. Ayhan and Eştürk (2009) also found no significant differences between stored active and passive MAP applications until 9 d of storage for Hicaznar pomegranate arils at 5 °C. Contrary to this, Martínez-Romero *et al*. (2013) observed a significant reduction in firmness of arils cv. 'Mollar de Elche' treated with ascorbic and citric acid and stored in refrigerated condition, arils coated with Aloe vera gel delayed softening. Szychowski *et al*. (2015) observed significant effects of pomegranate cultivar and test group on texture parameters of arils. Differences between the results of the current study and literature could be due to the type of modified atmosphere applied, fruit maturity status and cultivars.

Titratable acidity and total soluble solids

In pomegranate arils, the concentration of malic acid (MA) (4.39 to 4.89 g 100 mL^{-1}) was dominant, followed by acetic acid (AA) (1.12 to 1.25 g 100 mL^{-1}) and citric acid (CA) (1.18 to 1.31 g 100 mL^{-1}). Similar results were presented by Ozgen *et al*. (2008), where citric acid (1.78 g 100 mL^{-1}) dominated the organic acid concentration of six pomegranate cultivars studied, followed by a small amount of MA (0.12 g 100 mL^{-1}). Tezcan *et al*. (2009) found MA and CA to be the main organic acids in commercial juice pomegranate. Similarly, Legua *et al*. (2012) showed that CA (0.15-0.22 g 100 mL^{-1}) as the dominant organic acid, followed by MA in the 'Mollar' pomegranate cultivar. Organic acid values displayed in the ternary contour plots (Fig. 2a, b and c) show the tendency of MA, AA and CA to be relatively higher at lower O_2 concentration (5%) under MA-4 and MA-5 atmospheres, while all organic acids decreased at the lowest concentration of O_2 and CO_2 (2%) under (MA-3). According to Ghasemnezhad *et al*. (2013), the reduction in acidity could be attributed to an elevated respiration rate or the fact that organic acid were used as a substrate for respiratory activity.

The coefficients of parameter estimate values (Table 2) illustrated that organic acids (MA, AA and CA), were significantly affected by single and interaction effects of gas concentrations, however, the binary interaction effect of gases was only significant for AA. The coefficients show synergetic effects of single and interaction of binary terms, which implies the direct effect of the gases on the organic acids. On the other hand, the ternary (β_{123}) interaction effects of the gases was antagonistic, indicating an inverse relationship between the concentration of gases and organic acids content. Furthermore, the coefficients of parameter estimate values showed that the effect of individual gas components were similar to the experimental values, however the binary and ternary interaction model estimates resulted in lower values than the weighted average. However, a significant decrease in acidity of storage atmosphere were reported by Martínez-Romero (2013). Since the ratio of sugar and organic acids relate to flavour quality (Magwaza & Opara, 2014; Ozgen *et al*., 2008), using relatively higher CO_2 (> 10%) would maintain the characteristic flavour profile of pomegranate arils.

Individual sugar content

Fructose (7.36 to 7.68 g 100 mL^{-1}) dominated the sugar profile of pomegranate arils, followed by glucose (6.10 to 6.38 g 100 mL^{-1}) and sucrose (1.99 to 2.25 g 100 mL^{-1}). Similar to the current study, Palma *et al*. (2006) reported fructose and glucose as the main sugars of 'Primosole' pomegranate arils stored under passive-MA, which were in the range of 7.59 to 7.75 and 6.42 to 6.76 g 100 mL^{-1}, respectively. The individual sugar content reported in literature for pomegranate arils varies. Melgarejo *et al*. (2000) reported average values of 7.04 and 6.54 g 100 mL^{-1} of fructose and glucose for a sweet Spanish cultivar, and 5.96 and 5.66 of fructose and glucose for a sour Spanish cultivar, respectively. Ozgen *et al*. (2008) found glucose and fructose to be the dominant sugars in different pomegranate cultivars grown in the Mediterranean region of Turkey, with average concentration of 6.80 g 100 mL^{-1} glucose and 6.40 g 100 mL^{-1} fructose, while sucrose concentration was negligible. The highest level of glucose (10.03–11.20 g 100 mL^{-1}) in Spanish pomegranate fruit juices was recorded in 'Mollar' cultivar followed by fructose concentration of 5.53 to 6.42 g 100 mL^{-1} (Legua *et al*., 2012).

The ternary contour plots (Fig. 3 a_1, a_2 and a_3) show lower concentration of all individual sugars towards the highest CO_2 atmosphere (MA-2) while the values were slightly higher at reduced gas concentrations (5% for O_2 and 5 to 12% for CO_2). The decline in sucrose could be due to the consumption of sucrose to support metabolic processes and energy demand for respiration (Caleb *et al*., 2016b). The model parameter estimates (Table 2) showed that the effect of individual gases on sucrose concentration was significant, whereas binary and ternary interaction effect was insignificant except the interaction effect of O_2 and CO_2. This implies that the increase in each gas concentration resulted in a corresponding an increase in individual sugar content. Sucrose concentration was characterized by a synergetic linear effect (β_1, β_2 and β_3). However, none of the interaction effects of gases were able to predict the fructose concentration equal to the average value of fructose concentration predicted by the linear effect.

The TSS content of arils (16.5 to 16.9%); was slightly higher towards the lowest O_2 (2%) atmospheres (MA-2 and MA-3) as shown in the ternary contour plot (Fig. 2d). The increase in TSS value could be associated with a mechanism related to starch breakdown and sugar hydrolysis during the respiration process (Ghasemnezhad *et al*., 2013; Mphahlele *et al*., 2016). The model parameter estimates in Table 2 show that the single gas component effects agreed with experimental values, while the interaction effects were very low compared to the weighted average of the single components. The effect of individual, binary and ternary interaction of gases was not significant. The results from the current study is in agreement with previous studies reported where no marked changes in TSS observed during active modified atmosphere storage of arils from different pomegranate cultivars 'Mollar de Elche' (Maghoumi *et al*., 2013), 'Hicaznar' (Ayhan & Eştürk, 2009), 'Malese-Saveh' (Maghoumi *et al*., 2014), 'Wonderful' (Banda *et al*., 2015), and 'Mollar' (Gil *et al*., 1996).

Ascorbic acid and total monomeric anthocyanins

The ascorbic acid concentration was within the range of 279.5 to 329.5 mg L^{-1}, with the highest value observed in arils stored under MA-3. As shown in the ternary contour plot (Fig. $3b_1$), the concentration of ascorbic acid increased towards a decrease in O_2 and CO_2 concentrations, while lowest concentration of ascorbic acid was observed at the highest O_2 concentration. Overall, results from the current study showed that the effect of CO_2 on ascorbic acid was not clear since ascorbic acid concentration of arils fluctuated under both low and high CO_2 atmospheres. Kader (2009) also reported that low O_2 concentration (1 to 4%) slowed down ascorbic acid degradation through the prevention of oxidation, while the effect of CO_2 concentration could be either positive or negative depending on the commodity, CO_2 concentration, duration of exposure and storage temperature. Based on the model parameter estimates presented in Table 2, individual gas components significantly affected ascorbic acid concentration, whereas the binary ($\beta_{12,}$ β_{23} and β_{13}) and ternary (β_{123}) interaction effects were insignificant. This indicated that individual components were the most important experimental factors leading to increase in ascorbic acid concentration of pomegranate arils. Furthermore, model parameter estimates indicated a similar synergetic effect of individual gas components. These showed that in order to maintain antioxidant properties such as ascorbic acid of arils during storage, emphasis should rather be placed on controlling the individual gas effects rather than the interaction effects.

Anthocyanin levels across all MA treatments ranged from 0.12 to 0.14 mg L^{-1} and were well maintained at highest CO_2 concentrations (MA-2), but reduced slightly when O_2 and CO_2

both reached 5%. This was evident in the ternary contour plot, which showed an insignificant increase in anthocyanin towards the low O_2 and enriched CO_2 atmospheres (Fig. $3b_2$). Similarly, no significant change in anthocyanin concentration was found for UV-C treated, untreated and modified atmosphere packaged 'Mollar de Elche' pomegranate arils (López-Rubira *et al.*, 2005). Belay *et al.* (2017) reported that the effect of low O_2 and high CO_2 concentrations on anthocyanin concentration of 'Wonderful' pomegranate arils stored at 5 °C for 12 days was insignificant, except under super-atmospheric O_2 conditions. Gil *et al.* (1996) reported a general increase in anthocyanin concentration of unpacked 'Mollar' pomegranate arils storage at 1 °C for 7 days; however, arils packed inside POPP bags under modified atmosphere showed a slight but significant increase in anthocyanin concentration. At the end of 12 days in cold storage (5 °C), Banda *et al.* (2015) reported a significant increase in anthocyanin concentration of 'Wonderful' pomegranate arils stored under active and passive modified atmosphere packaging. Ayhan and Eştürk (2009) reported that storing 'Hicaznar' pomegranate arils at 5 °C for 18 days under active modified atmosphere (5 and 70% O_2) significantly affected the reduction of anthocyanin concentration. The difference between the findings could be attributed to several factors including differences in cultivar, fruit maturity status, storage condition and in-package gas composition.

Volatile organic compounds

The identified VOCs comprised of five main group compounds (aldehyde, ketone, alcohol, ester and monoterpene). Considering the individual volatiles compounds identified, ester compounds were dominant (about 33%), followed by ketones (28%) and aldehyde (28%), whereas, alcohol (11%) and monoterpenes (6%) were the least abundant. Individual gas components and both binary and ternary interactions of gases had no significant effect on the evolution of VOCs. According to the model parameter estimates, for single gas components, the effect of CO_2 resulted in higher values of aldehyde, ketone, ester and monoterpenes, whereas the effect of O_2 resulted in highest levels of alcohol VOCs. These findings suggest that the increase in CO_2 or O_2 concentration may have contributed to increase in the VOCs. However, the interaction effect of gases resulted in very high and very low values of VOCs compared to the weighted average for all VOCs identified (Table 2). Furthermore, the ternary interaction effect was antagonistic for all VOCs, except alcohol which was synergetic, which means that the alcohol VOCs increased when the O_2 concentration decreased.

The ternary contour plots (Fig. 4a, d, and e) showed that aldehyde, ketones and monoterpenes increased towards the highest CO_2 concentration and decreased towards the highest and lowest O_2 concentration. Similarly, Belay *et al.* (2017) reported increase in VOCs due to high CO_2 for 'Wonderful' pomegranate arils stored at 5 °C for 12 days. Similarly, Giuggioli *et al.* (2015) reported increase in VOCs in the presence of high CO_2 concentration for raspberry fruit stored at 1 °C for 2 days. The increase in aldehyde VOCs towards the highest CO_2 could be a result of dark fixation of CO_2 to malic acid, which then could be converted to acetaldehydes (Forney *et al.*, 2009). Contrary to this, alcohol decreased towards the highest CO_2 and increased at highest O_2 atmosphere. The decrease in VOCs at low O_2 concentration could be due to the inhibition potential of low O_2 on production of VOCs including alcohols, aldehydes, and esters that contribute to aroma and flavour (Forney *et al.,* 2009). The increase in VOCs at the low O_2 concentration could be associated with exceeding the range of tolerance and induction of anaerobic metabolism that can stimulate production of fermentative compounds (Zhang *et al.*, 2013).

Optimum modified atmosphere

The un-weighted global optimum of gas concentrations varied across the quality attributes of pomegranate arils, which were significantly affected by the active modified atmospheres. According to responses (physicochemical attributes, antioxidant properties and volatile compounds), the subjective weighting yielded four different regions for optimal modified atmospheres O_2:CO_2 of 2:2%, 2:18%, 7:8%, and 18:2% (Fig 5). The optimal region of 7% O_2 and 8% CO_2 resulted in maximum values of selected sugars (sucrose concentration of 2.44 g 100 mL^{-1}), TSS values of 19.19%, ascorbic acid concentration of 348.8 mg L^{-1}, organic acid values (malic acid, 5.02 g 100 mL^{-1}; acetic acid, 1.39 g 100 mL^{-1}; citric acid, 1.33 g 100 mL^{-1}) and Chroma values (32.6). The minimum hue angle value (17.11), higher concentration of ketones (1.25 g L^{-1}), monoterpenes (0.33 g L^{-1}) and the minimum concentration of alcohol (57.65 g L^{-1}) could be obtained from the optimal region of 2% O_2 and 18% CO_2. As shown in Figure 5, the optimum gas mixture (O_2:CO_2) which resulted in minimum concentration of aldehyde (8.86 g L^{-1}) was 2:2%. These results showed that the global optima for maintaining the individual quality parameters of pomegranate arils varied widely. The identified optimum gas mixture concentrations (show the values) for storing pomegranate arils are close to those recommended by previous researchers (Gil *et al.*, 1996; López-Rubira *et al.*, 2005) for CO_2 (5 to 10%) and O_2 (2 to 5%) . However, in the current study, only one storage condition (10 °C, 91± 2% RH) was evaluated and thus the optimized atmosphere is valid under this environment and gas composition.

Conclusions

The study demonstrated that simplex lattice mixture design experiments were suitable for optimization of gas concentrations in active modified atmosphere systems for individual quality attributes of 'Wonderful' pomegranate arils. The special cubical model generated simple parameters that explained interaction effects between gases. The model parameter estimates clearly showed the relationship of the individual gas component and their interaction (synergetic or antagonistic effects) for all response variables. The results identified the concentration of optimum gas mixture as 6-7 % O_2 and 7-8% CO_2, which resulted in the best quality of pomegranate arils with respect to TSS, individual sugars, organic acids, colour attributes and ascorbic acid concentration. Increasing CO_2 concentration in the modified atmosphere up to 18% and reducing O_2 concentration to 2% enhanced the concentration of volatile organic compounds responsible for the characteristic flavour of pomegranate arils. In this study, firmness was the only quality parameter enhanced by storing arils at the highest O_2 concentration (18%). The results of this study provide new information on optimum gas mixture with regard to the target quality parameters for commercialization of modified atmosphere storage of pomegranate arils. However, further studies are required to select the appropriate packaging material to match the optimum gas mixture.

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Table 1. Simplex lattice experimental design layout for different gas mixtures.

Gas mixture	Uncoded variables (%)			Pseudo components		
	O_2	CO_2	N_2	O_2	CO_2	N_2
MA-1	18	2	80	1	0	0
MA-2	2	18	80	0	1	0
MA-3	2	2	96	0	0	1
MA-4	4.67	12.67	82.67	⅙	⅔	⅙
MA-5	4.67	4.67	90.67	⅙	⅙	⅔
MA-6	12.67	4.67	82.67	⅔	⅙	⅙
MA-7	7.33	7.33	85.33	⅓	⅓	⅓

Table 2. Estimated model parameter coefficients with standard error obtained in the linear effect (β_1. β_2 and β_3), binary and ternary effects (β_{12}. β_{13}. β_{23} and β_{123}) describing the effect of gas combinations on selected quality parameters of pomegranate arils stored at 10 °C.

Quality parameters	**Model parameter coefficients**							
	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	β_{123}	R^2
Visual colour	3.7 ± 0.02	4.0 ± 0.02	3.7 ± 0.02	2.6 ± 0.04	4.6 ± 0.02	2.6 ± 0.04	-33 ± 0.02	*0.63*
Chroma	21.0 ± 1.0	16.6 ± 1.0	19.42 ± 1.0	-1.33 ± 15.1	35.0 ± 15.1	29.3 ±15.1	-148 ± 106	*0.66*
Hue	20.5 ± 0.3	17.1 ± 0.3	19.6 ± 0.3	22.6 ± 19.4	-16.7 ± 19.4	52.8 ± 19.4	-118 ± 136	*0.68*
Texture	1.4 ± 0.2	1.7 ± 0.2	1.6 ± 0.2	3.4 ± 2.2	-3.9 ± 2.2	-0.3 ± 2.2	-0.4 ± 15	*0.59*
Malic acid	4.6 ± 0.0	4.5 ± 0.0	4.4 ± 0.0	1.7 ± 0.3	2.3 ± 0.3	4.9 ± 0.3	-22 ± 2.3	*0.98*
Citric acid	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	1.3 ± 0.8	-5.9 ± 0.6	*0.98*
Acetic acid	1.2 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	1.3 ± 0.0	-5.7 ± 0.6	*0.98*
TSS	16.5 ± 0.1	16.8 ± 0.1	16.9 ± 0.1	0.7 ± 1.7	1.6 ± 1.7	-2.6 ±1.7	0.5 ± 11	*0.49*
Sucrose	2.22 ± 0.0	1.98 ± 0.0	2.12 ± 0.0	0.32 ± 0.7	-0.35 ± 0.7	2.59 ± 0.7	-6.96 ± 4.7	*0.79*
Ascorbic acid	2.22 ± 0.0	1.98 ± 0.0	2.12 ± 0.0	0.32 ± 0.7	-0.35 ± 0.7	2.59 ± 0.7	-6.96 ± 4.7	*0.78*
Aldehydes	9.96 ± 1.1	12.73 ± 1.1	8.86 ± 1.1	-5.70 ± 15.7	11.91 ± 15.7	29.70 ± 15.7	-180.94 ± 110	*0.70*
Ketones	0.71 ± 0.2	1.25 ± 0.2	0.63 ± 0.2	1.39 ± 2.6	-1.85 ± 2.6	3.94 ± 2.6	-22.50 ± 2	*0.76*
Alcohols	74.74 ± 3.4	57.65 ± 3.4	73.05 ± 3.4	-21.65 ± 50	-52.25± 50	-51.23 ± 50	646 ± 15	*0.78*
Monoterpenes	0.14 ± 0.1	0.34 ± 0.1	0.16 ± 0.1	1.22 ± 0.8	0.05 ± 0.8	0.02 ± 0.8	-7.69 ± 5.5	*0.73*

Where, superscript 1. 2 and 3 represents oxygen, carbon dioxide and nitrogen respectively. ± Standard error TSS: total soluble solids

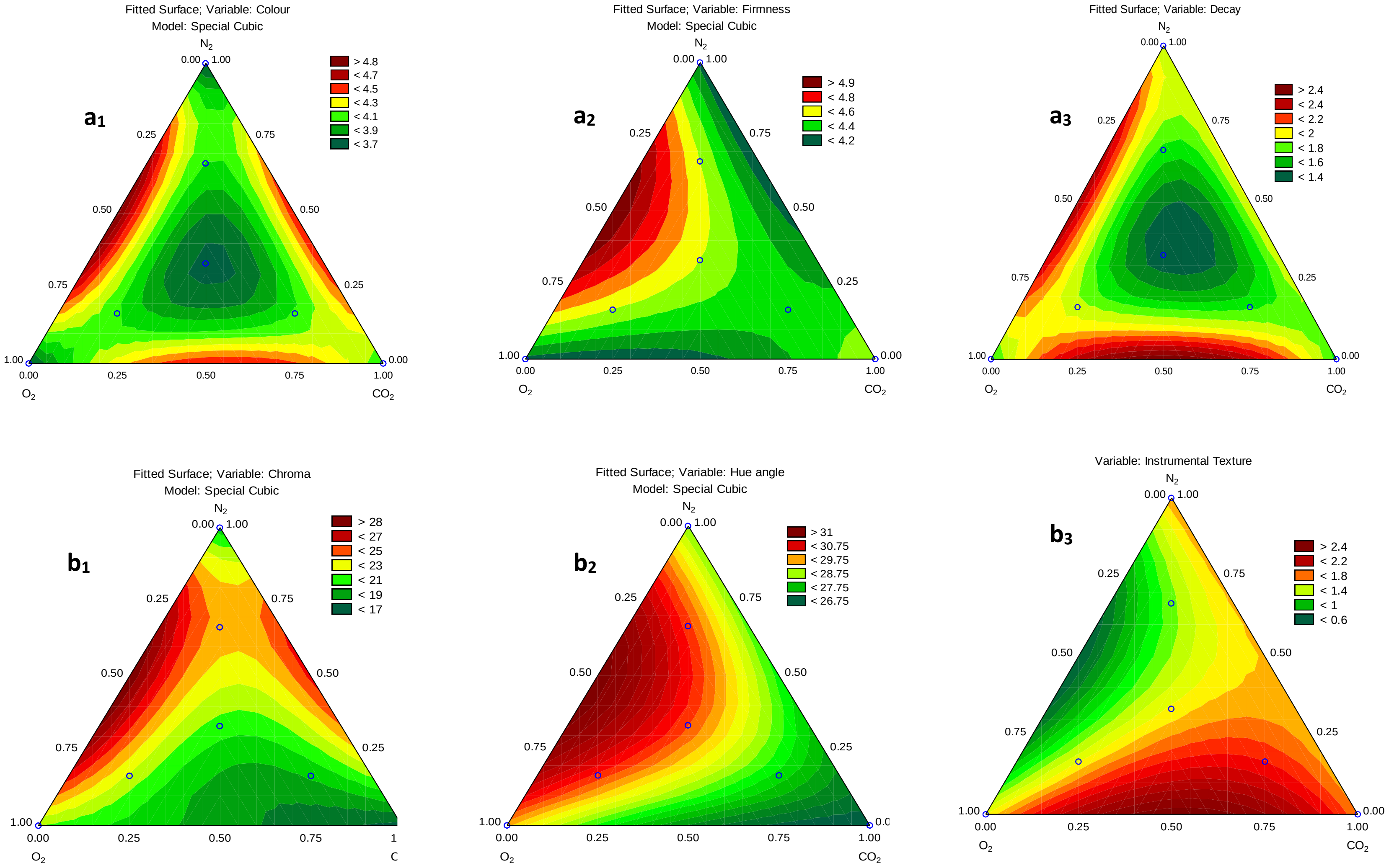

Figure 1. Ternary contour plots for the effect of gas components on the visual and instrumental physical quality of pomegranate arils; colour (a₂), firmness (a₂), decay (a₃) and instrumental physical quality; Chroma (b₁), hue angle (b₂), and texture (b₃)

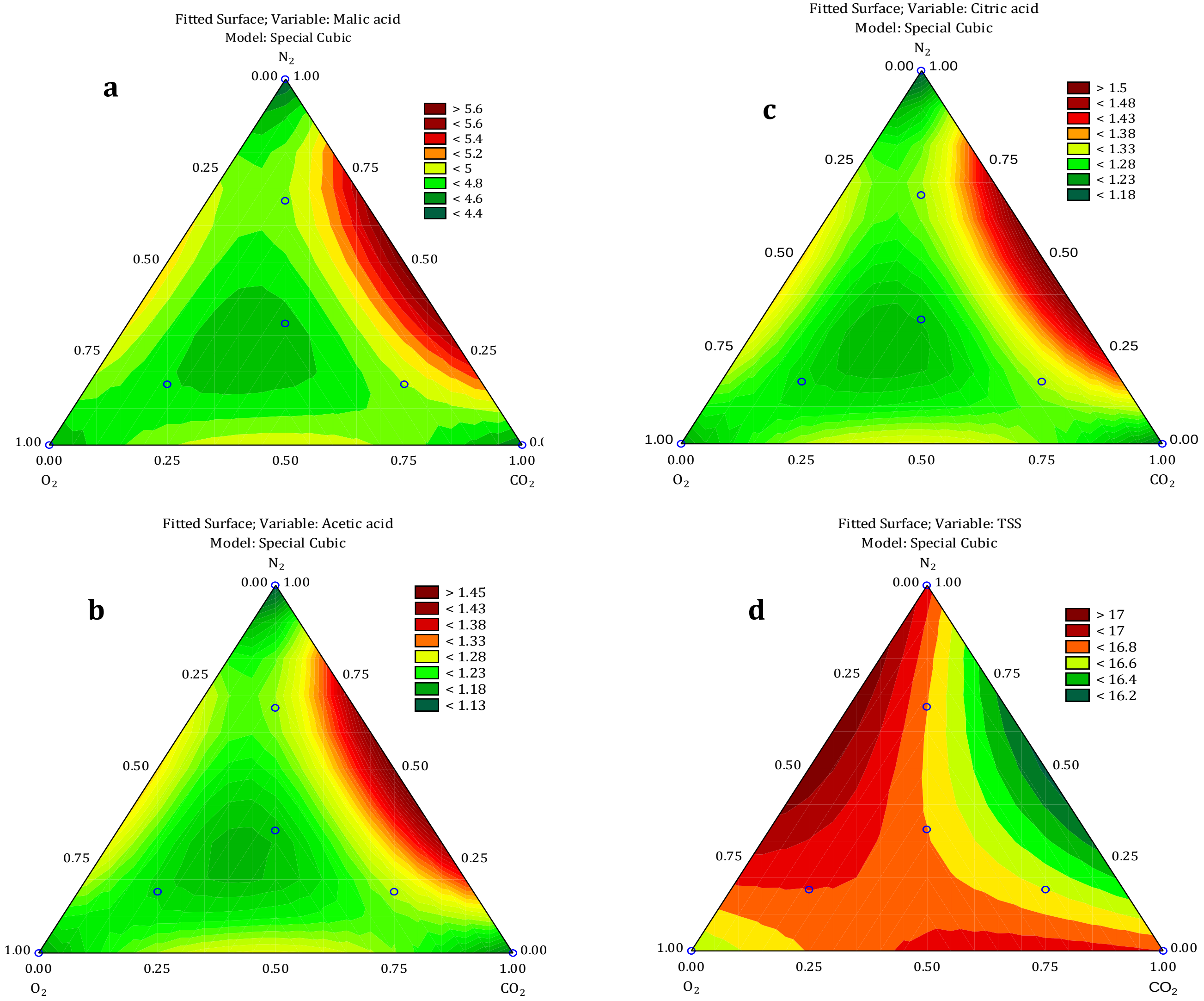

Figure 2. Ternary contour plots for the effect of gas components on titratable acidities and TSS (total soluble solids) of pomegranate arils; malic acid (a), acetic acid (b), citric acid (c) and TSS (d).

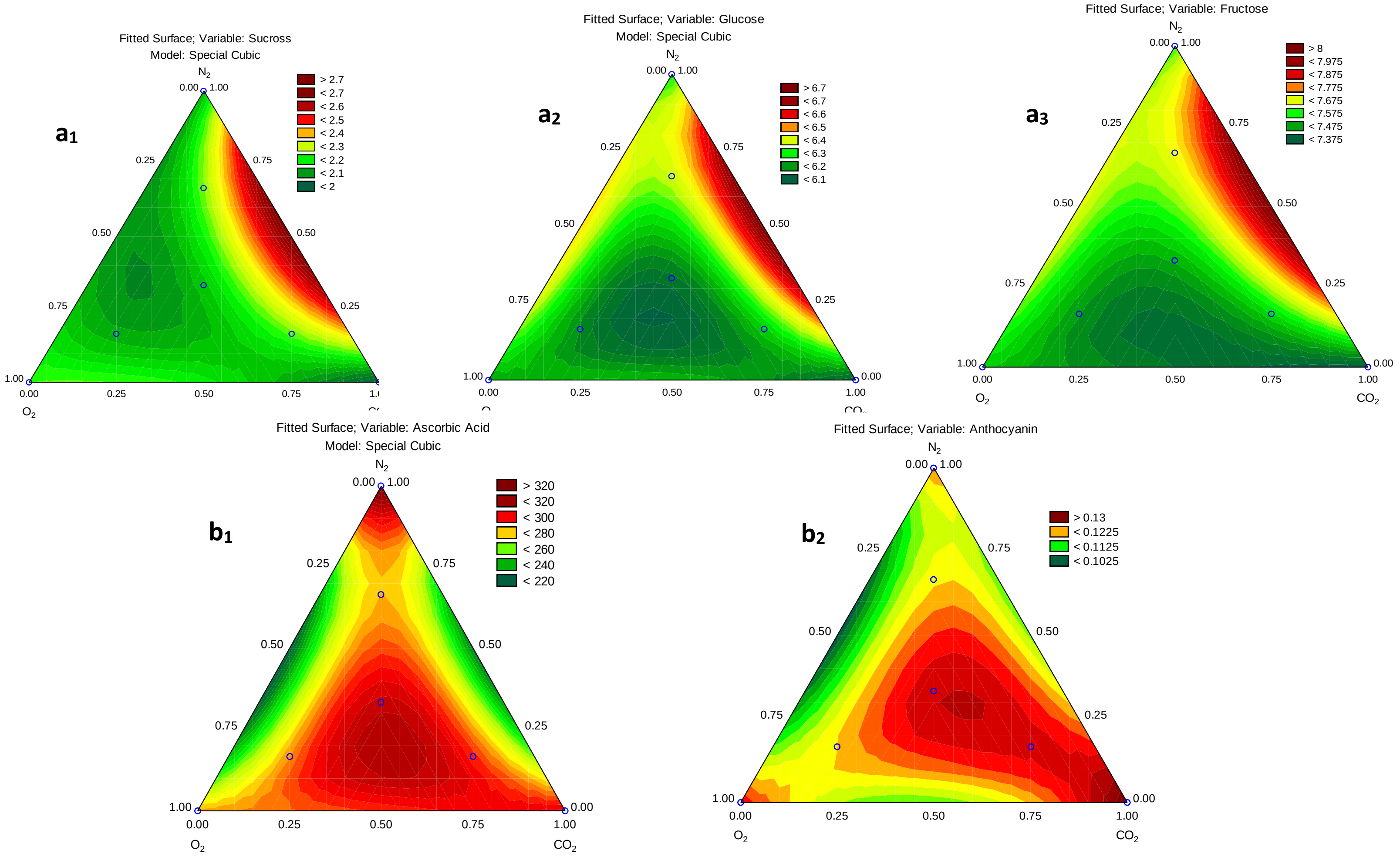

Figure 3. Ternary contour plots for the effect of gas components on individual sugar content of pomegranate arils sucrose; (a_1), glucose (a_2), and fructose (a_3) and antioxidant activity; ascorbic acid (b_1), and anthocyanin (b_2)

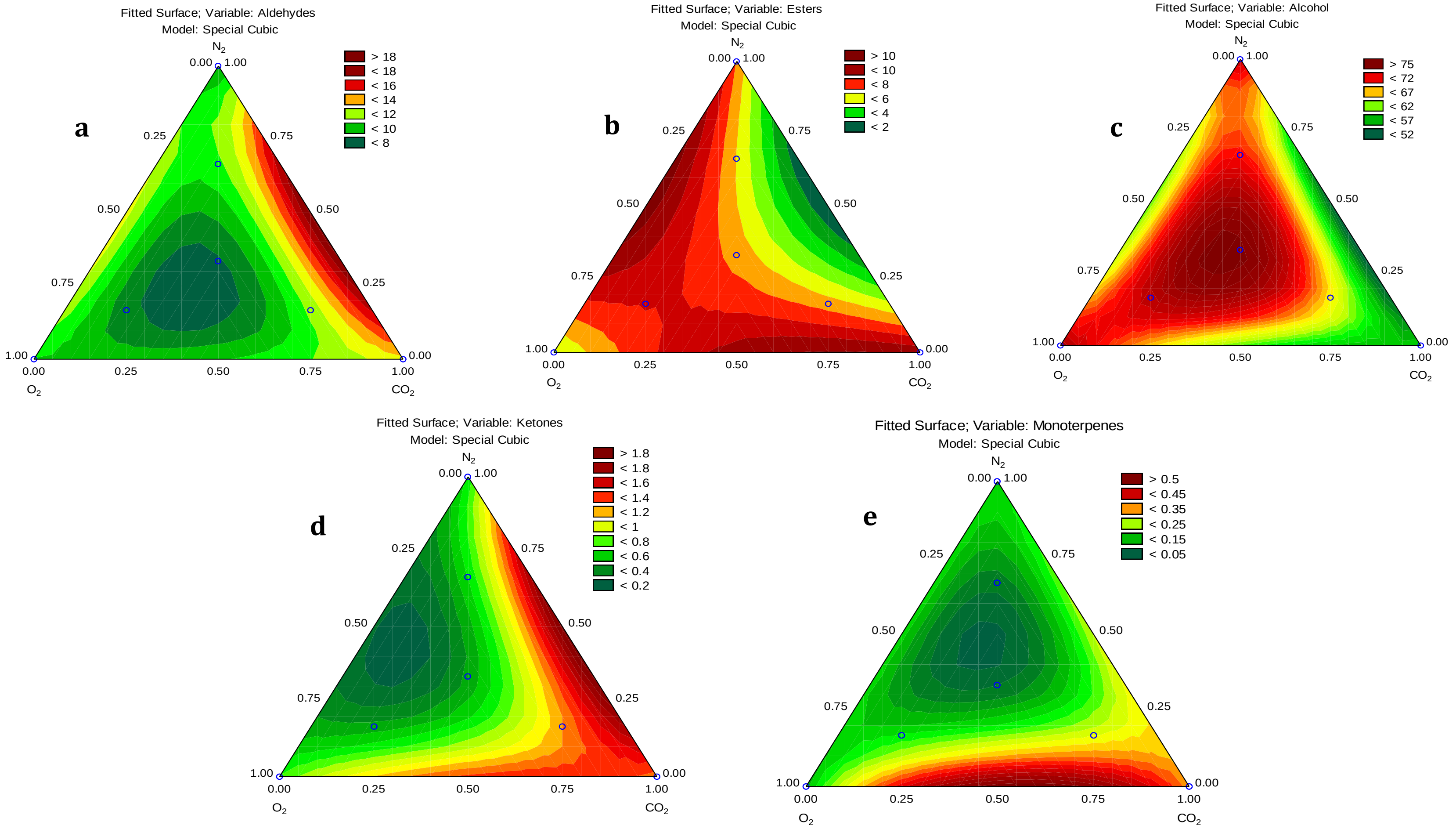

Figure 4. Ternary contour plots for the effect of initial gas components on organic volatile compounds of pomegranate arils; aldehyde (a), ester (b), alcohol (c), ketones (d) and monoterpenes (e).

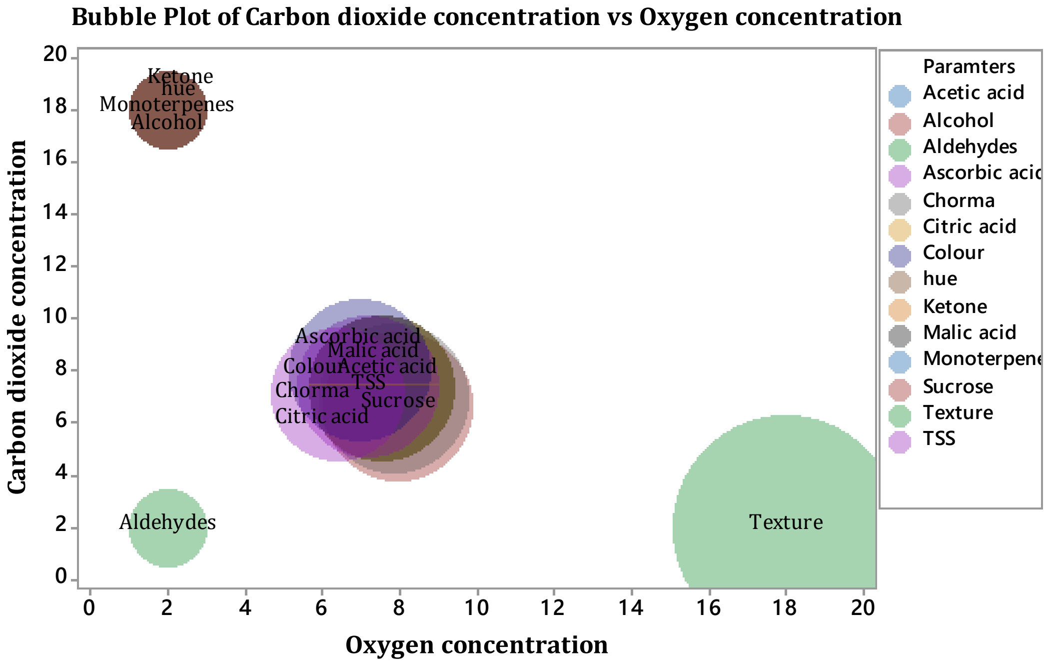

Figure 5. Estimated optimal gas mixture for selected individual quality parameters of pomegranate arils at 10 °C. Oxygen and carbon dioxide concentration (%)

Declaration by the candidate:

With regard to Chapter 8 (pp 185-214); the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Literature search, data analysis and writing of chapter	75

The following co-authors have contributed to Chapter 8 (pp 185-214):

Name	E-mail address	Nature of contribution	Extent of contribution (%)
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Dr P.V. Mahajan	PMahajan@atb-potsdam.de	Research input, editorial suggestion and proof reading	5

Signature of candidate: Z.A. Belay

Date: 03/11/2017

Declaration by co-authors:

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 8 (pp 185-214)
2. no other authors contributed to Chapter 8 (pp 185-214) besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 8 (pp 185-214) of this dissertation.

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Declaration with signature in possession of candidate and supervisor.

CHAPTER 8

DESIGN OF ACTIVE MODIFIED ATMOSPHERE AND HUMIDITY PACKAGING (MAHP) FOR 'WONDERFUL' POMEGRANATE ARILS

ABSTRACT

Modified atmosphere packaging (MAP) systems have been shown as beneficial to prolong postharvest life of pomegranate arils. Current application of such system is limited due to a lack of appropriate packaging films which are able to control in-package moisture condensation. Therefore, the aim of this study was to design a MAP system to balance the optimum gas composition with packaging film permeability as well as optimum in-package relative humidity (RH). The following packaging materials were used: i) 100% cellulose based film (NF), ii) bi-axial oriented polypropylene (BOPP) film (PF), iii) NF-PF (66:33%) film and iv) PF-NF (33:66%) film. The effects of the package design on quality attributes of pomegranate arils stored at 10 °C (91 ± 2% RH) for 9 days were investigated. Package design had significant (($p \leq 0.05$) impact in changing the in-package RH and gas composition. The 100% cellulose based NF package created the lowest RH (60-66%) with highest reduction in O_2 concentration, highest total soluble solids (TSS), hardness, colour change, and high bacterial count. However, the 100% BOPP PF film resulted in highest in-package water vapour condensation and mould growth at the end of storage day 10. The optimized packages using NF-PF and PF-NF films, respectively, maintained quality of pomegranate arils and minimized water vapour condensation better than the control. The results indicated that PF packaging film fitted with NF film window was the best to maintain the quality of pomegranate arils.

Key words: Water vapour condensation; Relative humidity; Sugars; Microbiology; Volatile organic compounds

Introduction

Modified atmosphere packaging (MAP) during postharvest preservation of horticultural commodities has been recognized as one of the important technologies to maintain quality, extend shelf life and reduce losses of fresh fruit and vegetables (Charles *et al*., 2006; Gomes *et al*., 2010; Mangaraj *et al*., 2009). Low O_2 and enriched CO_2 concentrations has been reported as beneficial to reduce respiration rate (RR) and prevent quality changes of pomegranate arils during postharvest handling (Banda *et al*., 2015; Belay *et al*., 2017). Pomegranate arils provide a convenient means to foster the fruit consumption because it is ready to eat, have nutritional benefits, and a reputed health promoting effect related to its antimutagenicity, anti-inflammatory, anti-cancer and antioxidative potential (Caleb *et al*., 2012; Opara *et al*., 2009). However, pomegranate arils are susceptible to quality loss after extracting from the fruit. The perishable nature of the arils then forced to develop appropriate methods for its postharvest consumption in a MAP system. Consequently, a modified atmosphere of 3 to 5% O_2 and 10 to 15% CO_2 was considered optimum for 'Mollar of Elche' pomegranate arils packaging (López-Rubira *et al*. 2005). However, the successful application of MAP not only requires selection of optimum gas but also correct gas permeability and water vapour transmission rate (WVTR) of packaging film to minimize moisture loss and prevent condensation (Somboonkaew & Terry, 2010).

Condensation inside the package of fresh fruit and vegetables represents a treat of product quality and safety (Bovi *et al*., 2016; Hussein *et al*., 2015). Excess of water vapour condensation on the surface will cause an unpleasant package appearance, increase water activity, and consequently create ideal conditions for microbial growth (Song *et al*., 2002). Therefore, for fresh fruit and vegetables, these mass exchanges must be restricted in order to guarantee the quality and safety. Suitable water vapour and gas permeations are needed to maintain fresh produce physiological activities (Del-Valle *et al*., 2004). Controlling in-package humidity via modified atmosphere and humidity (MAHP) has been identified as an important parameter in order to avoid condensation and accelerated degradation of packed fresh produce (Caleb *et al*., 2016a).

Several types of polymeric films have been applied to pomegranate arils packaging, such as bi-axial oriented polypropylene (BOPP) film for cv. 'Mollar de Elche' reported by López-Rubira *et al*. (2005). However, the study provided limited information on the effects of packaging film and packaging film selection. Caleb *et al*. (2013a) reported effects of polylid polymeric packaging film on 'Acco' and 'Herskawitz' pomegranate arils. The authors suggested the need for a polymeric film with high CO_2 permeability but provided no information on in-package RH. In another study, Banda *et al*. (2015) investigated the effect of low and high barrier bi-axial oriented polyester (BOP) polymeric film for Wonderful pomegranate arils. This study reported the necessity of further optimization of packaging material and gas composition. Therefore, the objective of this study was focused on designing a modified atmosphere and humidity packaging for minimally-processed pomegranate arils cv. 'Wonderful', based on integrated designing approach. The study considered the product and packaging film characteristics to design the optimized-package and investigated the effects of such packaging design on the quality attributes.

Materials and methods

Plant material and sample preparation

Pomegranate fruit cv. ‘Wonderful’ were obtained at commercial maturity stage based on characteristic deep-red skin and arils with mature kernel, total soluble solids (TSS, %) and titratable acidity (TA, g 100 mL^{-1}) (Mphahlele *et al*., 2016). At harvest, the TSS and TA contents were 17.5% and 1.1 g 100 mL^{-1}, respectively. The fruit were obtained from Sonlia Pack House, Wellington, Western Cape (33°38′23″S, 19°00′40″E), South Africa. Fruit were air freighted in well-ventilated boxes and transported to Department of Horticultural Engineering, Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Germany. On arrival, the fruit were stored in a cold storage chamber (5 °C) until processing to extract the arils. Damaged fruit were removed and the outer skins of selected healthy fruit were surface disinfected using 70% ethanol (Belay *et al*., 2017). Arils were extracted manually by carefully removing the husk under cool temperature. Extracted arils were collected in a tray and mixed to assure uniformity prior to packaging.

Packaging design and active modified atmosphere storage

In order to identify suitable MA for pomegranate arils, a packaging material designing approach was adopted from Caleb *et al*. (2016a). Pomegranate arils (150 g) was placed into polypropylene plastic tray (102 x 85 x 55 mm^3) and flow wrapped (360 cm^2). Selected polymeric films were BOPP film PropaFilm™ (Innovia films, UK) and cellulose-based NatureFlex™ NVS23 (Innovia films, UK), with distinct permeability to gases and water vapour (Table 1). The films were fitted with a fixed ratio window designed based on desirable in-package gas composition and RH (Table 2). Films were attached to the window using double-sided hermetic sealing tapes to ensure complete airtightness edges of the attached films. Prior to sealing, each package was gas flushed with 4.67% O_2 + 12.67% CO_2 + 82.67% N_2. Packages were heat sealed manually using Kopp HZ sealer (Verpackungsysteme, Reichenbach, Germany) and stored at 10 °C (which reflects average retail market display condition) for 9 days. Three packages were taken per treatment on each sampling days (0, 3, 6 and 9) for analysis.

Prior to opening the packages on each sampling day, O_2 and CO_2 concentrations inside the package were measured using gas analyser (Chechmate 3, PBI Dansensor, Ringstead, Denmark). An additional two packages per treatment were setup with humidity sensors (FHA 646R, Ahlborn, Holzkirchen, Germany) for continuous monitoring of RH inside the packages during the storage period. The performance of each packaging system was measured for the amount of water vapour transmitted through the package and amount of condensation inside the package (Caleb *et al*., 2016a). Water vapour transmission was gravimetrically determined by measuring change in total mass of the packaged product at the end of storage period. The amount of condensation was measured after pomegranate arils were carefully removed from the package.

Physiological response and packaging design

In-packaging atmosphere and respiration rate

The approach for this study was based on the mass balance, accounting for the physiological activity of pomegranate arils, where the transient behaviour of Y_{O_2} and Y_{CO_2} can be obtained by using mass balance equation 1 & 2, respectively:

$$\frac{\partial Y_{O_2}}{\partial t} = \frac{\frac{A_p P_{O_2} P_{atm}}{L}\left[Y_{O_{2i}} - Y_{O_{2f}}\right] - W}{V} \tag{1}$$

$$\frac{\partial Y_{CO_2}}{\partial t} = \frac{\frac{A_p P_{O_2} P_{atm}}{L}\left[Y_{CO_{2f}} - Y_{CO_{2i}}\right] - W}{V} \tag{2}$$

where $\frac{\partial Y_{O_2}}{\partial t}$ and $\frac{\partial Y_{CO_2}}{\partial t}$ are change in concentration of O_2 and CO_2 through time, $P_{O_2}$is permeability to O_2, P_{atm} is atmospheric pressure, A_p is surface area of the package, L is thickness of the packaging film, W is weight of the arils and V is free volume, $Y_{O_{2i}}$and $Y_{CO_{2i}}$are initial O_2 and CO_2 concentration respectively, $Y_{O_{2f}}$ and $Y_{CO_{2f}}$are final O_2 and CO_2 concentration respectively.

Transpiration and permeation rate

The TR model developed for arils by Caleb *et al.* (2013b) was used for estimating the target water vapour transmission rate (WVTR) required to maintain optimal RH inside the package equation 3:

$$\mathrm{TR} = \mathrm{K}_h \times \left(a_{w_i} - a_w\right) \times \left(1 - e^{-aT}\right) \tag{3}$$

where TR is transpiration rate (mL $kg^{-1}h^{-1}$); a_w is the water activity of package headspace (RH/100); a_{wi} is the water activity of the arils (0.984); K_m is the mass transfer coefficient (89.96) and a, is constant coefficient (0.09).

The in-package RH is influenced by TR of produce as well as by the water vapour transmission of the packaging film as shown in equation 4. This is a mass balance equation that describes the rate of change of RH or moisture accumulation in the headspace.

$$\frac{\mathrm{d}H_{\mathrm{i}}}{\mathrm{d}t} = \frac{(\dot{m}_1 - \dot{m}_2)}{W_a} \qquad (4)$$

where $\dot{m}_1$ is the rate of water transpiration from produce to headspace (g kg^{-1} d^{-1}); $\dot{m}_2$ is the rate of water permeation from headspace to surrounding (g kg^{-1} d^{-1}); W_{a} is weight of dry air inside the package (g). At equilibrium when the rate of change in humidity is zero, equation 4 becomes $\dot{m}_1 = \dot{m}_2$. Substituting the product TR and packaging material transmission rate, equation 3 becomes:

$$\mathrm{K}_m \times \left(a_{w_i} - a_w\right) \times (1 - e^{-aT}) \times W = WVTR \times A \qquad (5)$$

where W is weight of pomegranate arils, kg and A is packaging film area (m^2). Re-arranging equation 5 for determining packaging needs for pomegranate arils target WVTR was obtained equation 6. This included an estimation of target WVTR required to achieve 65 to 95% RH inside a package containing 150 g of pomegranate arils (Caleb *et al.*, 2013b):

$$\text{Target WVTR} = \frac{\mathrm{K}_m \times \left(a_{w_i} - a_w\right) \times (1 - e^{-aT}) \times W}{A} \qquad (6)$$

Physicochemical analysis

Mass loss

Mass of each pomegranate arils pack in different packaging conditions taken on sampling day was measured using an electronic balance (CPA10035, Sartorius, Göttingen, Germany) with an accuracy of ± 0.01 g. Mass loss was expressed in percentage equation 7:

$$\mathrm{WL} = \mathrm{W_O} - {}^{\mathrm{W_f}}/_{\mathrm{W_o}} * 100 \qquad (7)$$

where WL is the mass loss (%), W_o is the initial mass (g) and W_f is the final mass (g) on sampling.

Surface colour

The surface colour of pomegranate arils was measured and evaluated on the basis of Commission International del' Eclairage (CIE) colour system using a digital Chroma-meter (CM-2600d, Konica Minolta Sensing Inc., Japan). Calibration of the colour meter was performed against a white and black tile background, respectively, prior to each measurement (Pathare, Opara, & Al-Said, 2013). Colour measurements lightness ($L*$), redness/greenness ($a*$) and yellowness/blueness ($b*$) were taken using individual arils, mean of 20 measurements for each treatment and the data was further analysed to describe Chroma ($C*$) and hue angle ($h°$) according to (Caleb *et al.*, 2016a).

Texture analysis

Softness of individual arils was measured using texture analyser (TA-XT Plus, Stable Micro Systems, Surrey, UK) with a SMSP/2 of 2 mm diameter cylindrical probe. Magness Taylor Test (MTT), which is an empirical hardness indicator test, was performed according to (Szychowski *et al.*, 2015). This test included both compression and cutting effort (Chen & Opara, 2013). Penetration rate was 0.3 mms^{-1} for 5 s after contacting the surface of pomegranate arils and results were expressed in Newton (N). A total of 20 arils were measured per treatment.

Total monomeric anthocyanin

Total *monomeric* anthocyanin concentration (TAC) was quantified using the pH differential method as described by Fawole and Opara (2013). Pomegranate juice extracts (1 mL) were diluted with 9 mL potassium chloride buffer for pH, 1.0 (0.025 M) and sodium acetate buffer for pH, 4.5 (0.4 M), separately. Absorbance was measured at 510 and 700 nm in pH 1.0 and 4.5 buffers after 10 min incubation time and results were expressed as cyanidin-3-glucoside equivalents according to the following equation:

$$Total\ anthocyanin\ (mg\ L^{-1}) = \frac{A \times MW \times DF \times 1000}{\varepsilon} \times L \qquad (8)$$

where A is (A_{520}-A_{700}) pH1-(A_{520}-A_{700}) pH4.5; MW is molecular weight of cyanidin-3-glucoside (449.2 g mol^{-1}, DF is dilution factor; ε is molar extinction coefficient (26900) and L is path length in cm. All analysis was carried out in triplicate and results were expressed as mass cyanidin-3-glucoside equivalent.

Titratable acidity and total soluble solids

Fresh pomegranate arils juice was used to measure titratable acidity (TA) according to the method described by Fawole and Opara. (2013). The TA content of the juice sample was measured potentiometrically by titration with 0.1 mol L^{-1} NaOH, to an end-point of pH 8.2 using an automated T50 M Titrator with Rondo 20 sample changer (Mettler Toledo, Switzerland). The TA value was expressed as g 100 mL^{-1} of malic acid, citric acid and acetic acid based on fresh mass. A total soluble solid (TSS) of pomegranate juice was measured using a hand refractometer (DR301-95, Krüss Optronic, Hamburg, Germany) and expressed as % Brix.

Individual sugar content

Individual sugar contents (glucose, fructose and sucrose) were determined according to the method described by Mphahlele *et al.* (2014). Individual sugar content was determined by HPLC method using a DIONEX Ultimate 3000 liquid chromatograph fitted with Analytical Auto-sampler WPS-3000TSL (Thermo Fisher Scientific GmbH, Dreieich, Germany). The system is equipped with a refractive index detector SHODEX RI-101 (Showa Denko Europe GmbH, Munich, Germany). Sugars were separated on a Eurokat H column (300 × 8 mm and 10 µm diameter) (KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany), with 0.01 M sulphuric acid as the mobile phase. The injection volume was 10 µL and the flow rate was maintained at 0.8 mL min^{-1} at 35 °C. Sample detection and identification were performed by comparing retention time obtained with the retention time of the calibration standards and results were quantitatively expressed in g L^{-1} of juice.

Volatile organic compounds

Volatile compounds were extracted by static headspace sampling (SHS) according to the method described by Caleb *et al*. (2016a). Pomegranate arils from each treatment were homogenized into puree and 7 g was placed in 20 mL glass vial spiked with 100 µL of 3-octanol (diluted in absolute methanol to a concentration of 0.1 g L^{-1}) as internal standard. The vials were tightly capped and allowed to equilibrate at 80 °C for 10 min in the headspace auto-sampler incubator (HS-20 automated-sampler, Shimadzu Europa GmbH, Duisburg, Germany). Analyses were carried out using helium as carrier gas with a constant flow rate of 0.03 mL s^{-1}. The GC temperature was held at 50 °C, then ramped up to 110 °C at 5 °C min^{-1} and further ramped up to 180 at 20 °C min^{-1} held at this temperature for 2.5 min and finally ramped up to 220 at 10 °C min^{-1} held at this temperature for 3 min in total run time of 24 min. The mass selective detector in this study was operated in full scan mode and mass spectra in the 35–500 m/z range were recorded. Individual volatile compound were tentatively identified by their retention time and semi-quantification of the identified compounds was estimated using equation:

$$RA = \frac{A_{ic}}{A_{itc}} C_{itc} \tag{9}$$

where RA is the relative abundances of the identified compound (g L^{-1}), A_{ic} is the peak area of the identified compound, A_{itc} is the peak area of the internal standard, C_{itc} is the final concentration of internal standard in the sample (0.1 g L^{-1}).

Microbial analysis

Microbial quality of pomegranate arils was studied using total plate count method. Total aerobic mesophilic bacterial count was determined using plate count agar (PCA), while yeast and mould counts were determined using rosebengal chloramphenicol agar (RBCA). Pomegranate arils (10 g) was taken for each treatment and transferred into 90 mL peptone buffered water. Samples were homogenized for 2 min at a speed of 4 strokes s^{-1} in a lab blender (BagMixer1400CC1, Interscience, France) and thereafter threefold, serial dilution was made by adding 30 mL of each diluent into 270 mL of PS Rotilabo1-microtest plates (96er U-profile, Carl Roth GmbH & Co KG, Germany) and 100 mL from each dilution was pour-plated on respective growth media. PCA plates for aerobic mesophilic bacterial were incubated at 30 °C for 3 days, and RBCA plate for yeast and mould, respectively, were incubated at 25 °C for 5 days. After incubation colonies between 30 and 300 colonies on each plate were counted (n = 6), and the results were expressed as log colony forming unit per weight (log CFU mL^{-1}).

Statistical analysis

All the data obtained were subjected to analysis of variance (two-way ANOVA), and Duncan Multiple range test was used to determine the difference between mean values at 95 % confident interval. Results were presented as mean ± standard deviation.

Results and discussions

In-package atmosphere and respiration rate

Packaging design and storage duration significantly ($P \leq 0.05$) influenced the evolution of in-package headspace gas composition (Fig. 1). The highest reduction of O_2 concentration (2.8%)

at the end of the storage and highest CO_2 accumulation from the initial concentration was observed for arils packed in NF film. Minimum changes in O_2 and CO_2 concentration were observed for arils packed under PP film. The decrease in O_2 and CO_2 concentration could be attributed to the respiration process in MAP during the storage period. However, due to efficient gas exchange maintained by using films fitted with fixed window (NF-PF and PF-NF); there was an equivalent balance between O_2 consumption and CO_2 production. The NF film has a higher oxygen transmission rate (OTR) and low permeability to CO_2 properties (Somboonkaew & Terry, 2010), which resulted in a higher reduction of O_2 concentration and highest accumulation of CO_2 inside the package throughout the storage period. Packaging materials have been shown in various studies on pomegranate arils to influence in-package headspace gas composition (Ayhan & Esturk, 2009; Banda *et al.*, 2014). Banda *et al.* (2014) reported that higher OTR property of BOP film resulted in higher O_2 concentration (16 to 18%) inside the package above the recommended concentration (2 to 5%). Similarly, a high CO_2 barrier film (polylid) resulted in continuous accumulation of CO_2 (27 to 43%). However, this current study showed that packaging design via incorporation of fixed window films could maintain continuously optimal atmosphere for pomegranate arils.

Transpiration and water vapour transmission rates

Highest TR (0.15 ± 0.01 mg kg^{-1} s^{-1}) of pomegranate arils was observed in samples packed inside NF films, while lowest TR (0.03 ± 0.01 mg kg^{-1} s^{-1}) was found under PF films (Fig. 2). This implies that the RH level in NF film surrounding the arils was lower; where weight loss was greater due to the higher vapour pressure difference between the fruit and the external atmosphere, resulting in higher driving force for water evaporation. However, under PF films the presence of saturated RH (100%) corresponds to the lower TR. On the other hand, TRs of 0.12 ± 0.00 mg kg^{-1} s^{-1} and 0.071 ± 0.01 mg kg^{-1} s^{-1} of arils packed in NF-PF and PF-NF films, respectively, were similar to the predicted TR of 0.0625 mg kg^{-1} s^{-1} at 10 °C under 96% RH.

Based on the application of equation 6, the packaging film with targeted WVTR of 0.009 g hr^{-1} is required to match the TR of pomegranate arils. However, the calculated WVTR obtained was 0.140 g h^{-1}, 0.096 g h^{-1}, 0.053 g h^{-1}, and 0.011 g h^{-1} for NF, NF-PF, PF-NF, and PF films, respectively. The results obtained showed that NF film water vapour permeability was too high, which would lead to product shrivelling, colour change and mass loss. On the hand, the estimated lower WVTR at PF film have resulted in water vapour condensation inside the package. These results were in agreement with the study reported by Caleb *et al.* (2016). Therefore, findings from this study highlight the significance of selecting appropriate packaging films for minimally-processed pomegranate arils.

Package performance

Package designs had significant influence ($p \leq 0.05$) on the in-package RH, moisture condensation on the film and water absorption by the film. In-package RH widely varies across the MAP systems and ranged from 65 to 100% (Fig. 3). Samples packed in PF films RH reached saturation (100%) within 24 h of storage, while the lowest average RH (66%) observed in NF films. Packages fitted with fixed window films (PF-NF and NF-PF) were effective in regulating the in-package RH. These findings were similar to observations reported by Caleb *et al*. (2016), for minimally-processed broccoli under MAHP systems. However, the PF-NF packages better maintained in-package RH at 91 % compared to the NF-PF packages. The capability of PF-NF film to maintain the recommended RH could be associated with the fact that, the low WVTR property of PF film was optimized by incorporating NF window. This helped to increase the WVTR of the package. According to Kader and Rolle (2004), RH range of 90 to 95% was recommended for storage of fresh fruit and vegetables. Thus, this makes the PF-NF package a suitable design for pomegranate arils.

Previous reports have shown that product geometry and structure, storage temperature and RH, as well as attributes of packaging material can affect the intensity of the moisture condensation inside a package (Bovi *et al*., 2016). In this study, package designs had a considerable impact on the water vapour condensation inside the packages as shown in Figure 4. Highest water vapour condensation and lowest water absorption were observed in PF packages. Under this condition, arils were excessively soft due to water vapour condensation both on the arils surface and in the packaging film. Kumar *et al*., 2013) also reported the incidence of moisture accumulation due to the presence of higher RH. On the other hand, highest water absorption was observed for NF film. This resulted in shrivelled arils due to excessive moisture loss at the end of the storage. Meanwhile, PF-NF film was characterised by average water vapour condensation and water absorption characteristics in comparison with NF and PF films. This assures that excess moisture is eliminated in the event that the condensations form with in the package of PP film by fitting NF window film. The moisture uptake depends on water vapour permeability of the packaging material (Dak *et al*., 2014). Water vapour condensation has been identified as the main problem limiting the use of MAP as a postharvest preservation tool (Giuggioli *et al*., 2015). Therefore, this study has shown the ability of PF based NF fitted window film ideally reduce moisture condensation in the package with a minimum weight loss.

Mass loss

The gradual mass loss of pomegranate arils packed under different packaging films stored at 10 °C is shown in Figure 4. Packaging type, storage duration and their interaction significantly affected ($p \leq 0.05$) mass loss of arils. Arils packaged using PF and PF-NF had 0.71% and 2.8% mass loss, respectively after 9 days of storage. On the contrary, mass loss was significantly higher 3.8% and 3.2% for arils packed in NF and NF-PF films, respectively. The most probable explanation of highest moisture loss for NF package is due to high WVTR of the film (Based on Innovia films, UK for NatureFlex) and the presence of significantly lower RH inside the package. Caleb *et al.* (2016) reported similar results of PP film on maintaining the weight loss but resulting water condensation on the film for broccoli branches at 10 °C. Mass loss of pomegranate arils associated with packaging types has been reported by various studies (Caleb *et al.*, 2013a; Hussein *et al.*, 2015). Hussein *et al.* (2015) reported higher mass losses (1.9 to 6.2%) for 'Acco' pomegranate arils under perforated package system. In general, a 3 to 10% loss in mass could have an adverse effect on appearance, saleable weight, texture quality of fresh cut produce (Ben-Yehoshua *et al.*, 1987). Therefore, the mass loss (> 3%) observed by NF and NF-PF fitted window could make these packaging conditions unfavourable for pomegranate arils packaging.

Surface colour

The results of ANOVA for colorimetry analysis indicated that storage duration has a significant effect on all colour parameters (L, C^* and $h°$) (Table 3). There was a significant ($p \leq 0.05$) decrease of L value throughout the storage duration and a slight increase and highest lightness was observed for arils packed under PF and PF-NF films. Pomegranate arils lightness was lower under NF and NF-PF films; however, it was lowest for NF film, meaning that a darker colour was developed through time. Gil *et al.* (1996) reported the increase in the lightness of 'Mollar' pomegranate arils stored at 1, 4 and 8 °C for 7 days under heat-sealed pouches. Packaging type, storage duration as well as their interaction significantly affected the $h°$, C^* *and* $h°$ value significantly decreased throughout the storage with the fewer initial increment. Comparing all packages, highest $h°$ was observed for arils packed in NF films. The highest $h°$ and lower C^* of arils under NF film could show the loss of colour intensity of the arils, and the lowest lightness of arils could be due to losses of high moisture and the presence of very low O_2 (Palma *et al.*, 2006). Furthermore, Li and Kader (1989) reported that 2% O_2 resulted in higher red colour on strawberries. On the other hand, Artés *et al.* (2000) found out the decrease in lightness and C^* value of arils 'Mollar de Elche' at 5 °C and 95% RH.

Palma *et al.* (2006) reported a slight decrease of $h°$ for 'Primosole' pomegranate arils. The study further explained that the increase in $h°$ implies a little colour change from reddish-orange to red-magenta during storage. Ayhan & Eştürk (2009) found out a significant effect of MAP, duration and their interaction on the lightness of 'Hicaznar' pomegranate arils. The decrease of L and $C*$ values and the increase of $h°$ were correlated with browning and loss of red colour due to anthocyanin pigments degradation or polymerization with other phenolic compounds (Maghoumi *et al*., 2014). Furthermore, Caleb *et al*. (2016) found out that RH and modified atmospheres are important factors to maintain colour attributes of broccoli. The difference in fruit colour parameters found in this study may reflect the different physical attributes of the in-package condition (headspace gases changed with time, RH, and moisture condensation) or it could be due to the cultivar.

Aril hardness

Arils hardness followed a declining trend corresponding with advancement in storage duration. Packaging design had significant effects ($p \leq 0.05$) on the textural property of arils at end of the storage duration. Arils hardness was maintained significantly under PF-NF film compared to those packed in PF and NF-PP film (Table 3). For arils packed in PF film, hardness significantly decreased at the end of the storage by 24.8% from the initial value (1.45 N). On the contrary, dryness of the arils was observed under NF film where the hardness increased by 4.4% from the initial value. The difference in hardness between arils in different packaging conditions could be associated with the change in water condensation or water loss during storage (Ayhan & Eştürk, 2009). Change in textural property due to water loss directly related to the decrease in turgor pressure or could be associated with the resistance of outer periderm or removal of the periderm to transpiration movement of water vapour (Ben-Yehoshua *et al*., 1987).

Similarly, Ayhan and Eştürk (2009) reported significant increase (21 to 24%) of 'Hicaznar' pomegranate arils hardness packed in polypropylene tray sealed with biaxial-oriented polypropylene (BOPP) film within 3 days of storage. Similar increase in pomegranate arils hardness from 76.10 N to 77.50 N for 'Acco' and 85.55 N to 102.36 N for 'Herskawitz' packed in polypropylene (PP) tray sealed with polylid films were reported by Caleb *et al.* (2013b). Furthermore, significant reduction of firmness was observed for Aloe vera gel treated 'Mollar de Elche' pomegranate ails (Martínez-Romero *et al*., 2013). However, in the current study, even if the firmness values are different, the effect of storage time and the interaction of storage time and packaging were not significant.

Total monomeric Anthocyanin

The total monomeric anthocyanin concentration of pomegranate arils was affected by packaging design and storage condition (Table 3). The results obtained showed that anthocyanin concentration gradually decreased due to the type of packaging material used, which generated different levels of stress in the arils. Thus arils in PP film had the highest (0.09 mL g^{-1}) anthocyanin concentration, while arils packed in NF film had the lowest (0.07 mL g^{-1}) anthocyanin concentration at the end of the storage duration. This can be linked to the higher O_2 permeability of the film, as it has been reported that reducing the O_2 availability and consequently increasing CO_2 concentration affected the antioxidant capacity by decreasing anthocyanin biosynthesis (Sánchez *et al*., 2014). According to Horbowicz *et al*. (2008), anthocyanin undergoes structural transformations that are pH dependent and were found to be more stable in acidic media (pH < 2 to 3) than in neutral or alkaline media, where the colour goes towards bluish shade at hydroxyl group and reddish in methoxyl groups. In the current study pH measurement has not been done, therefore, reaching a conclusion according to this theory could not be possible.

The presence of higher sugar may also affect the degradation of anthocyanin, in a mechanism associated with inhibition of enzyme activities of phenoloxidase and peroxidase (Delgado-Vargas & Paredes-López, 2002). However, in the current study, the statistical analysis showed that the packaging types and storage duration or their interaction did not have a significant influence on the total monomeric anthocyanin except day 9. This observation was consistent with the study reported by Gil *et al*. (1996), where they observed no significant change of anthocyanin concentration in arils 'Molar' during active MAP storage using OPP film at 1 °C up to 7 days. On the other hand, Ayhan and Eştürk (2009) observed a significant difference of MAP condition, duration and their interaction on the anthocyanin concentration of pomegranate arils 'Hicaznar' stored at different low and super-atmospheric O_2 and nitrogen atmospheres at a PP-BOPP tray for 10 days. However, this variation could be due to fruit cultivar, fruit maturity, production area, and seasonal conditions, but cultivar has the most significant effect (Zaouay *et al*., 2012).

Total soluble solids (TSS)

Total soluble solids (TSS) of arils in NF and PF films increased from its initial concentration by 16.33%. The highest concentration was observed for arils packed in NF film (17.50%), while slight increase (16.66%) of TSS was observed at PF films. Packaging design, storage duration and interaction of both had a significant effect ($p \leq 0.05$). This result is in line with the study reported by Ghasemnezhad *et al*. (2013), where TSS significantly increased for 'Tarom' pomegranate arils stored at 4 °C for 12 days coated with chitosan. In contrast, a significant reduction of TSS was reported for 'Wonderful' pomegranate arils packed in low BOP film at the end of storage day 12 at 5 °C (Banda *et al.*, 2015). On the other hand, Ayhan and Eştürk (2009) reported that different MAP did not affect TSS of arils over 9 days. in the current study, the highest increase in TSS under NF films might be attributed to changes in the solubility of large molecular weight insoluble pectin and non-pectin components such as cellulose, in the cell wall (Ghasemnezhad *et al.*, 2013), due to loss of water and allowing sugar to become more concentrated in the arils (Selcuk & Erkan, 2014).

Titratable acidity

Organic acids are important flavour components; malic acid was the most abundant organic acid for pomegranate arils in all packaging followed by acetic and citric acids. All organic acids concentration decreased initially and increased after day 3. Malic acid (MA) concentration varied across different packaging conditions by the end of the storage (day 9). Highest malic acid concentration (5.46 ± 0.4 g 100 mL^{-1}) was observed for arils packed in PF films and PF-NF fitted window films. Comparing to the initial concentration (5.68 ± 0.4 g. 100 mL^{-1}), highest loss of 7% and 9% were observed in NF-PF and NF films, respectively. However, the effect of packaging films was insignificant. Similar to malic acid, highest concentration of acetic acid (AA) and citric acid (CA) were observed at PF and PF-NF films, where the loss was 4% for both from the initial concentration (AA, 1.52 ± 0.1 g 100 mL^{-1} and CA 45 ± 0.1 g 100 mL^{-1}) (Table 4).

On the other hand, the concentration of AA and CA had shown a similar decline from its initial concentration by 7 and 9% for NF and NF-PF film, respectively. It has been reported that respiration is a major factor that can convert sugars into energy in the presence of O_2, therefore, the immediate increase after day 3 and minimum reduction from the initial values of acids could be due to the presence of low O_2 and low RR. The progressive reduction in acidity with the advancement of storage period might be due to the increased catabolism of organic acids present in the fruit due to respiration process (Mahajan *et al.*, 2015). Furthermore, the lowest loss of the acid under PF films could be associated with the effect of the packaging film for delaying the respiratory process. Organic acids inhibit the growth rate of microorganisms in fruit and their products and affect quality and shelf life (Dafny-Yalin *et al.*, 2010). This could be shown by the lowest growth of bacteria and yeast and highest concentration of organic acids under PF films. However, in this study, statistical correlation of these parameters has not been done.

Individual sugars

All sugar concentrations increased over 9 days, where fructose concentration was the most predominant followed by glucose and sucrose (Table 4). Arils packed under PF films showed the lowest concentrations of all sugars, compared to the other films at the end of storage. In contrast, arils in both PF-NF and NF-PF fitted windows had highest sugar concentrations. The concentration of sucrose increased from the initial 0.27 g 100 mL^{-1} by 33%, 37%, 33% and 18% for NF, NF-PF, PF-NF and PF films, respectively. Similarly, the initial concentration of glucose (5.19 ± 1.4 g. 100 mL^{-1}) and fructose (6.55 ± 1.7 g. 100 mL^{-1}) were increased with the range of 57 to 63%, and 64 to 69%, respectively.

Packaging types did not significantly affect the changes in individual sugars concentration, but storage duration had a significant impact on the observed changes. This result was in line with the study reported by Palma *et al*. (2006) for 'Primosole' pomegranate arils packed in polypropylene film stored at 5 °C for fructose, glucose and sucrose. The authors showed that fructose concentration was the highest (7.59 to 7.75 g.100 mL^{-1}) followed by glucose (6.42 to 6.75 g 100 mL^{-1}). Similarly, Samboonkaew and Terry (2010) reported lowest glucose and sucrose concentration for polypropylene film packaged litchi fruit stored at 13 °C for 9 days. However, there is limited information available on literature about the effect of MAP on the sugar content of pomegranate arils.

Volatile organic compound analysis

A total of 20 volatile compounds (VOCs) were tentatively identified in the fresh and packaged pomegranate arils, which are under seven different functional groups (Table 5). The relative concentration of most of the identified VOCs was significantly influenced ($p \leq 0.05$) by packaging design and storage duration. Prior to packaging and storage, the predominant functional groups on processed arils were alkane derivatives, aldehydes and alcohols. However, aldehyde and alcohol concentrations reduced in the elapse of time during storage. From alkane derivatives, the concentration of pentane was predominant throughout the storage across all the packages. Evolution of ethanol in all packaging was linearly increased except for arils packed in PF and NF films, where the concentration decreased after 6 days of storage. This could be associated with the conversion of alcohol to ester, where it was evident that the ester VOCs increased after day 6. This result was in line with the study done by Rux *et al*. (2017), where the higher accumulation of ethanol was reported for rucola packed under MAP. The presence of high ethanol concentration can be as a result of low O_2 and high CO_2 packaging atmosphere (Belay *et al*., 2017; Caleb *et al*., 20116). On the other hand, several studies demonstrated that many C10 and C15 monoterpenes and sesquiterpenes compose the most abundant group of compounds present in the aroma profile and key compounds determining the characteristic aroma (El-Hadi *et al*., 2013; Edelenbos *et al*., 2009; Giuggioli *et al*., 2015).

Acetaldehyde concentration increased exponentially under NF-PF films and decreased under PF film, while there was no change observed for arils packaged in PF-NF films. On the other hand, β-pinene and D-limonene compound consistently emitted for arils under all packaging conditions throughout the storage time. The retention of these compounds in the packages could be due to the low O_2 effect, which indicates that there was no oxidation. In addition, the condensation of moisture inside the PF film could be a reason to stimulate the leaching out of β-pinene (Edelenbos *et al*., 2009). Giuggioli *et al*. (2015) also reported the possible retention or increase of these VOCs under MAP using low O_2 atmosphere for raspberry fruit.

The higher emission of furan derivatives (3-furaldehyde and 2-furancarboxaldehyde, 5-(hydroxymethyl)-, which can be categorized under lactones was observed throughout the storage. According to Hu *et al*. (2013), the presence of these compounds can affect the flavour, colour and nutritional value of fruit. El-Hadi *et al*. (2013) reported that lactones and esters are responsible for fruity aromas. In the current study, the concentration of ethyl acetate increase slightly from its initial concentration (0.001 g L^{-1}) to the highest (0.013 g L^{-1}) and the lowest (0.006 g L^{-1}) concentrations observed for arils packed in PF and NF films, respectively. This phenomenon indicated the development of off-flavour in-package arils. Esterification of alcohols to esters was observed in this study, wherein alcohol VOCs such as 2-methyl-1-phenyl-1H-benzimidazole, and 3-hydroxy-2,3-dihydro-maltol was not detected after 3 days of storage, while the emission of ester VOCs such as ethyl acetate and acetic acid, hydrazide increased until the end of storage. In line with these results, reduction of alcohol VOCs and increased emission of esters during storage for packaged produce was also reported in the literature (Belay *et al*., 2017; Caleb *et al*., 2013a; Rux *et al*., 2017). In addition, increase in the emission of esters for arils under the NF films, could be associated with the increase in water loss or the presence of relatively lower O_2 which leads to the production of ethyl ester (Giuggioli *et al*., 2015).

Microbial analysis

Initial aerobic mesophilic bacteria, yeast and mould count were 2.53 ± 0.15, 1.74 ± 0.11 and 2.87 ± 0.09 log CFU mL^{-1}, respectively. Microbial load on arils increased through the storage period (Fig. 5). Packaging film, storage duration and their interactions had a significant impact on the microbial growth. The total aerobic mesophilic bacteria and yeast had similar growth trend throughout the storage in all packaging conditions. Higher growth was observed for arils under NF film (bacteria, 6.28 ± 0.013 log CFU mL^{-1} and yeast, 6.19 ± 0.10 log CFU mL^{-1}), while lower growth (5.9 ± 0.22 log CFU mL^{-1}) was observed under PF films. NF and PF fitted window films showed similar growth (6.09 to 6.18 log CFU mL^{-1} and yeast, 5.74 ± 0.29 log CFU mL^{-1}) at the end of the storage. Banda *et al*. (2015) reported similar observation for aerobic mesophilic bacteria for 'Wonderful' pomegranate arils. In the current study, all packaging films were able to maintain aerobic mesophilic bacteria below the maximum acceptable limit (7 log CFU. mL^{-1}) at the end of the storage according to the South African legislation, FCD Act 54 1979. However, yeast count exceeding (5 log CFU mL^{-1}), which was established as a maximum acceptable limit for yeast growth at day 9 in all packaging. The reason for yeast exceeding the maximum acceptable limit could be associated with its capability to grow under lower pH environment comparing with aerobic mesophilic bacteria (Caleb *et al*., 2013a), which can be related to the presence of higher CO_2 in the package. The effect of CO_2 to change the intercellular pH and its bacteriostatic effect which limit the growth of aerobic mesophilic and yeast was reported by Ayhan and Eştürk (2009).

Mould growth followed slightly different growth pattern from yeast and aerobic mesophilic bacteria. It showed an initial decrease until day 6 under all packaging films and the exponential increase was observed at day 9. Lowest mould growth was observed for arils packed under NF films (3.06 ± 0.16 log CFU mL^{-1}) followed by NF based PF fitted window (3.66 ± 0.92 log CFU mL^{-1}). López-Rubira *et al*. (2005) reported similar observation, wherein the yeast count exceeded maximum acceptable limit after 6 days for 'Mollar of Elche' pomegranate arils harvested in December and treated with UV-C at 5 °C. Similarly, Krasnova *et al*. (2012) observed lowest growth of mould under NatureFlex based films and highest growth under PropaFilm for mixed fruit salad. Furthermore, this study reported that PropaFilm based film suppressed bacterial growth. The highest growth of mould in PF film could be a result of higher water vapour condensation inside the package.

Conclusions

The design of modified atmosphere packaging (MAP) had shown significant potential to influence the quality of pomegranate arils stored at 10 °C for 9 days. PF based NF fitted window and NF based PF fitted window films have prevented a build-up of CO_2 and reduction of O_2 concentration. However, the use of NF based PF fitted window film was limited due to low RH inside the package, which affected the product appearance and quality. On the other hand, the use of BOPP based NF window film successfully regulated the recommended in-package RH, thus, assisted to control condensation as well as weight loss. Using absolute cellulose based film has shown highest reduction of O_2 concentration, lowest in-package RH and increased moisture loss, which has resulted in arils hardness and colour change. 100% PF film helped to reduce the weight loss of the arils, but it was not efficient to control the in-package RH. This affected the appearance and aroma of pomegranate arils due to visible surface mould growth and higher concentration of ester compounds at day 9. These results indicated the importance of appropriate design of MAP to maintain the postharvest freshness of pomegranate arils as well as to reduce the economic loss. However, further study is necessary to identify the industrial applicability for compatibility with the packaging machines, saleability and printing qualities of the improved packaging films.

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Table 1. Properties of the packaging materials used in the study

Packaging materials	Characteristics Film thickness (µm)	Permeability ratio to at O_2 at testing conditions	Permeability ratio to at water vapour at testing conditions
BOPP film PropaFilm™ RGP25	25	8.5 x 10^{-12} mol m^{-2} s^{-1} Pa^{-1} at 23 °C and 0% RH	5.7 x 10^{-6} mol m^{-2} s^{-1} Pa^{-1} at 23 °C and 85% RH
Cellulose-based NatureFlex™ NVS23 polymeric film	23.3	8.5-9.4 x 10^{-14} mol m^{-2} s^{-1} Pa^{-1} at 23 °C and 50% RH	9.4 x 10^{-4} mol m^{-2} s^{-1} Pa^{-1} at 25 °C and 75% RH

Table 2. Name and description of packaging designs used in this study

Packaging designs description	Packaging designs Code	Packaging material proportion
NatureFlex film	NF	100% Nature Flex cellulose film
NatureFlex window	PF-NF	66 % PropaFilm window with 33% Nature Flex cellulose film
PropaFilm	PF	100% PropaFilm
PropaFilm window	NF-PF	66 % Nature Flex cellulose film window with 33% PropaFilm

Table 3. Effect of packaging designs and storage duration on physicochemical properties of pomegranate arils stored at 10 °C.

	Storage duration	**Types of packaging material**			
		NF	**NF-PP**	**PP-NF**	**PP**
Chroma	Day-1	17.67 ± 5.0a^{B}	17.67 ± 5.0a^{B}	17.67 ± 5.0a^{B}	17.67 ± 5.0a^{B}
	Day-6	21.46 ± 6.5a^{B}	21.49 ± 5.4a^{AB}	21.05 ± 6.9a^{AB}	23.45 ± 7.7a^{AB}
	Day-3	24.07 ± 6.6a^{AB}	24.98 ± 5.8a^{A}	26.08 ± 6.3a^{A}	25.34 ± 7.9a^{A}
	Day-9	29.26 ± 9.5a^{AB}	26.58 ± 11a^{AB}	24.48 ± 11a^{AB}	29.39 ± 10a^{AB}
Hue	Day-1	23.24 ± 2.0a^{B}	23.24 ± 2.0a^{B}	23.24 ± 2.0a^{B}	23.24 ± 2.0a^{B}
	Day-6	32.66 ± 7.6a^{AB}	29.99 ± 4.5a^{A}	31.17 ± 5.1a^{A}	31.22 ± 6.0a^{A}
	Day-3	34.16 ± 5.0a^{A}	33.66 ± 4.8a^{A}	34.71 ± 5.9a^{A}	33.30 ± 4.2a^{A}
	Day-9	30.78 ± 5.9a^{A}	27.77 ± 8.0a^{AB}	24.16 ± 7.4a^{B}	24.44 ± 7.2a^{B}
Lightness	Day-1	27.88 ± 4.2a^{A}	27.88 ± 4.2a^{A}	27.88 ± 4.2a^{A}	27.88 ± 4.2a^{A}
	Day-6	21.21 ± 3.8a^{B}	20.27 ± 3.9a^{B}	20.27 ± 4.0a^{B}	21.06 ± 3.5a^{B}
	Day-3	20.01 ± 3.5a^{B}	21.55 ± 3.5a^{B}	19.57 ± 4.4a^{B}	20.69 ± 3.7a^{B}
	Day-9	19.99 ± 5.9a^{B}	20.53 ± 8.5a^{B}	22.60 ± 7.9a^{AB}	23.26 ± 4.8a^{AB}
Hardness (N)	Day-1	1.45 ± 0.2a^{A}	1.45 ± 0.2a^{AB}	1.45 ± 0.2a^{A}	1.45 ± 0.2a^{AB}
	Day-6	1.57 ± 0.3a^{A}	1.61 ± 0.5a^{AB}	1.60 ± 0.4a^{A}	1.66 ± 0.2a^{A}
	Day-3	1.51 ± 0.8a^{A}	1.51 ± 0.4ab^{AB}	1.50 ± 0.5a^{A}	1.51 ± 0.4a^{AB}
	Day-9	1.51 ± 0.4abc^{A}	1.36 ± 0.2bc^{B}	1.26 ± 0.4ac^{A}	1.09 ± 0.4c^{B}
Anthocyanin (mg L^{-1})	Day-1	0.11 + 0.0a^{A}	0.11 + 0.0a^{A}	0.11 + 0.0a^{A}	0.11 + 0.0a^{A}
	Day-6	0.11 + 0.0b^{A}	0.10 + 0.0b^{A}	0.12 + 0.0a^{A}	0.10 + 0.0b^{A}
	Day-3	0.10 + 0.0b^{A}	0.12 + 0.0a^{A}	0.10 + 0.0b^{A}	0.09 + 0.0b^{A}
	Day-9	0.08 + 0.0a^{A}	0.08 + 0.0a^{A}	0.07 + 0.0a^{B}	0.09 + 0.0a^{A}
TSS (%)	Day-1	16.33 ± 0.1a^{D}	16.33 ± 0.1a^{B}	16.33 ± 0.1a^{B}	16.33 ± 0.1a^{B}
	Day-6	16.73 ± 0.2a^{C}	16.33 ± 0.4a^{B}	15.70 ± 0.2b^{C}	15.96 ± 0.3b^{C}
	Day-3	17.10 ± 0.0b^{B}	17.20 ± 0.2b^{A}	17.33 ± 0.1ab^{A}	17.13 ± 0.5ab^{AB}
	Day-9	17.50 ± 0.1a^{A}	17.06 ± 0.2b^{A}	17.03 ± 0.2b^{A}	16.66 ± 0.1c^{B}

Description of NF, PF-NF, NF-PF and PF presented in Table 1. Mean value ± standard deviation in the same column and rows with different lower case superscripts are significantly different based on Duncan (Post-hoc test) at $p \leq 0.05$.

Table 4. Effect of packaging designs and storage time in titratable acidity and individual sugar components of pomegranate arils stored at 10 °C for 9 days.

Quality Parameters	Days	NF	NF-PP	PP-NF	PP
Malic acid (g 100 mL^{-1})	1	$5.68 \pm 0.4a^{A}$	$5.68 \pm 0.4a^{AB}$	$5.68 \pm 0.4a^{A}$	$5.68 \pm 0.4a^{AB}$
	3	$5.15 \pm 0.1a^{A}$	$5.16 \pm 0.3a^{B}$	$4.83 \pm 0.3a^{B}$	$4.46 \pm 0.1b^{B}$
	6	$5.32 \pm 0.4a^{A}$	$5.22 \pm 0.3ab^{B}$	$5.06 \pm 0.1b^{B}$	$5.42 \pm 0.1a^{A}$
	9	$5.28 \pm 0.1a^{A}$	$5.17 \pm 0.2a^{B}$	$5.46 \pm 0.4a^{A}$	$5.46 \pm 0.4a^{A}$
Acetic acid (g 100 mL^{-1})	1	$1.52 \pm 0.1a^{A}$	1.52 ± 0.1^{A}	$1.52 \pm 0.1a^{A}$	$1.52 \pm 0.1a^{A}$
	3	$1.38 \pm 0.0a^{B}$	$1.38 \pm 0.1a^{B}$	$1.29 \pm 0.1a^{B}$	$1.19 \pm 0.0b^{B}$
	6	$1.42 \pm 0.1a^{A}$	$1.39 \pm 0.1b^{B}$	$1.36 \pm 0.1b^{B}$	$1.45 \pm 0.0a^{A}$
	9	$1.42 \pm 0.0a^{A}$	$1.38 \pm 0.1a^{B}$	$1.46 \pm 0.1a^{B}$	$1.46 \pm 0.1a^{A}$
Citric acid (g 100 mL^{-1})	1	$1.45 \pm 0.1a^{A}$	$1.45 \pm 0.1a^{A}$	$1.45 \pm 0.1a^{A}$	$1.45 \pm 0.1a^{A}$
	3	$1.32 \pm 0.0a^{B}$	$1.32 \pm 0.1a^{B}$	$1.24 \pm 0.1a^{C}$	$1.14 \pm 0.0b^{B}$
	6	$1.36 \pm 0.1a^{A}$	$1.34 \pm 0.1a^{B}$	$1.29 \pm 0.0b^{B}$	$1.39 \pm 0.0a^{A}$
	9	$1.35 \pm 0.0b^{A}$	$1.32 \pm 0.1b^{B}$	$1.39 \pm 0.1a^{A}$	$1.39 \pm 0.1a^{A}$

Quality Parameters	Days	NF	NF-PP	PP-NF	PP
Sucrose (g 100 mL^{-1})	1	$0.27 \pm 0.0a^{B}$	$0.27 \pm 0.0a^{B}$	$0.27 \pm 0.0a^{B}$	$0.27 \pm 0.0a^{B}$
	3	$0.26 \pm 0.0a^{B}$	$0.25 \pm 0.0a^{c}$	$0.25 \pm 0.0a^{c}$	$0.25 \pm 0.0a^{B}$
	6	$0.25 \pm 0.0b^{B}$	$0.29 \pm 0.0a^{B}$	$0.27 \pm 0.0a^{B}$	$0.25 \pm 0.1b^{AB}$
	9	$0.36 \pm 0.0a^{A}$	$0.37 \pm 0.0a^{A}$	$0.36 \pm 0.1a^{AB}$	$0.32 \pm 0.0b^{A}$
Glucose (g 100 mL^{-1})	1	$5.19 \pm 1.4a^{B}$	$5.19 \pm 1.4a^{B}$	$5.19 \pm 1.4a^{B}$	$5.19 \pm 1.4a^{BC}$
	3	$6.46 \pm 0.1a^{B}$	$6.37 \pm 0.1a^{B}$	$6.15 \pm 0.1b^{B}$	$6.19 \pm 0.1b^{B}$
	6	$6.14 \pm 0.2a^{B}$	$6.04 \pm 1.2abc^{B}$	$6.04 \pm 0.0b^{B}$	$5.85 \pm 0.0c^{C}$
	9	$8.33 \pm 0.5a^{A}$	$8.49 \pm 0.1a^{A}$	$8.46 \pm 0.1a^{A}$	$8.19 \pm 0.1b^{A}$
Fructose (g 100 mL^{-1})	1	$6.55 \pm 1.7a^{BC}$	$6.55 \pm 1.7a^{B}$	$6.55 \pm 1.7a^{B}$	$6.55 \pm 1.7a^{B}$
	3	$8.16 \pm 0.1a^{B}$	$8.05 \pm 0.1a^{B}$	$7.78 \pm 0.2b^{B}$	$7.82 \pm 0.1b^{B}$
	6	$7.78 \pm 0.3a^{C}$	$7.74 \pm 1.6a^{B}$	$7.80 \pm 0.1a^{B}$	$7.61 \pm 0.0a^{B}$
	9	$10.82 \pm 0.7a^{A}$	$11.09 \pm 0.1a^{A}$	$11.16 \pm 0.1a^{A}$	$10.79 \pm 0.1a^{A}$

Description of NF, PF-NF, NF-PF and PF presented at Table 1. Mean value ± standard deviation in the same column and rows with different lower case superscripts are significantly different based on Duncan (Post-hoc test) at $p \leq 0.05$.

Table 5. Groups of volatile organic compounds identified for pomegranate arils packed under different packaging designs at 10 °C.

Functional group	Volatile compounds	RT (min)	Kovats index (NIST library)
Aldehyde	Acetaldehyde[ac]	1.91 - 1.96	480
	Hexanal[a]	10.10 - 10.11	806
	3-Furaldehyde[a]	11.36 - 11.47	831
	2-Furancarboxaldehyde, 5-(hydroxymethyl)-[a]	19.47 - 19.60	1163
Alcohol	Ethanol[a]	2.87 - 2.90	463
	3-Hydroxy-2,3-dihydromaltol[a]	16.75 – 16.84	1269
Ester	Acetic acid methyl ester[b]	3.76	997
	Ethyl acetate[a]	5.57 – 5.60	586
Ketone	Acetone[a]	3.33 - 3.36	806
	3-Octanone[a]	13.803	991
	Pyranone[a]	17.41 – 17.50	1689
Terpenoid	β-Pinene[a]	13.28	945
	D-Limonene[a]	14.03- 14.16	1056
	cis-α-Bergamotene[a]	20.53 - 20.54	1536
Alkane derivatives	Pentane[ac]	2.63	nf
	Decane[ac]	13.20 - 13.30	1015
	3-Ethyl-3-methylheptane[b]	14.27 - 14.32	931
	Eicosane[a]	17.82 – 17.92	nf
	Hexadecane[ac]	18.52 - 18.54	1612
Sulphur compounds	Dimethyl sulphide[a]	3.36 - 3.39	nf

RT, retention time; nf, not found. [a] Primary VOCs. [b] Secondary VOCs.
[c] Primary volatiles that continue accumulate during storage.

Table 6. Volatile organic compounds identified for pomegranate arils packed under different packaging designs and stored at 10 °C for 9 days.

Volatiles	**Day 1**	**Day 3**				**Day 6**				**Day 9**			
		NF	**NF-PF**	**PF-NF**	**PF**	**NF**	**NF-PF**	**PF-NF**	**PF**	**NF**	**NF-PF**	**PF-NF**	**PF**
Acetaldehyde	0.059^{a}	0.106^{a}	0.063^{b}	0.059^{b}	0.088^{a}	0.041^{c}	0.075^{ab}	0.054^{b}	0.074^{a}	0.047^{c}	0.099^{ab}	0.053^{bc}	0.048^{c}
Pentane	0.141^{a}	0.433^{c}	0.815^{a}	0.674^{b}	0.984^{a}	0.679^{b}	0.952^{a}	0.656^{b}	0.973^{a}	0.797^{b}	1.159^{ab}	1.073^{ab}	0.577^{b}
Ethanol	0.002^{a}	0.004^{b}	0.006^{ab}	0.006^{ab}	0.007^{a}	0.004^{c}	0.006^{b}	0.007^{ab}	0.007^{a}	0.002^{b}	0.006^{a}	0.006^{a}	0.002^{b}
Acetone	0.004^{a}	0.007^{c}	0.034^{bc}	0.026^{bc}	0.079^{a}	0.006^{d}	0.015^{c}	0.033^{abc}	0.029^{b}	0.004^{c}	0.027^{a}	0.013^{b}	0.002^{c}
Dimethyl sulphide	0.001^{a}	0.009^{a}	0.006^{b}	0.009^{ab}	0.006^{b}	0.022^{b}	0.006^{cd}	0.042^{abc}	0.002^{d}	0.013^{abc}	0.009^{b}	0.005^{c}	0.004^{c}
Acetic acid methyl ester	nd	Nd	nd	nd	nd	0.001^{a}	nd	nd	nd	0.001^{a}	nd	nd	0.001^{a}
Ethyl Acetate	0.006^{a}	0.027^{ab}	0.009^{b}	0.007^{b}	0.008^{b}	0.006^{d}	0.016^{bc}	0.008^{cd}	0.023^{abc}	0.006^{c}	0.008^{bc}	0.010^{bc}	0.013^{a}
Hexanal	0.001^{a}	Nd	nd	nd	nd	nd	nd	nd	nd	0.001^{a}	nd	nd	0.002^{a}
3-Furaldehyde	0.001^{a}	0.001^{b}	0.001^{b}	0.002^{ab}	0.004^{a}	0.001^{b}	nd	0.002^{b}	0.003^{ab}	0.001^{a}	0.002^{a}	0.002^{a}	0.002^{a}
Decane	0.001^{a}	Nd	0.004^{a}	0.004^{a}	0.004^{a}	nd	0.003^{b}	0.002^{b}	0.011^{a}	0.001^{b}	0.018^{ab}	0.018^{a}	nd
β-Pinene	0.004^{a}	0.005^{a}	0.005^{a}	0.006^{a}	0.006^{a}	0.005^{a}	0.004^{a}	0.005^{a}	0.005^{a}	0.004^{a}	0.005^{a}	0.004^{a}	0.004^{a}
3-Octanone	0.003^{a}	0.003^{b}	0.003^{b}	0.005^{a}	nd	nd	0.001^{a}	0.002^{a}	nd	0.001^{a}	nd	nd	0.001^{a}
D-Limonene	0.002^{a}	0.003^{a}	nd	nd	nd	0.002^{a}	0.001^{a}	nd	0.001^{a}	0.002^{a}	0.001^{a}	nd	0.001^{a}
3-Ethyl-3-methylheptane	nd	0.004^{ac}	0.003^{bc}	0.001^{c}	0.002^{bc}	nd	0.004^{bc}	0.001^{b}	0.010^{a}	0.002^{b}	0.004^{ab}	0.003^{b}	0.004^{a}
3-Hydroxy-2,3-dihydromaltol	0.002^{a}	Nd	0.003^{b}	0.004^{a}	0.005^{a}	nd	nd	nd	nd	nd	nd	0.003^{a}	nd
Pyranone	0.003^{a}	0.004^{ab}	0.001^{b}	nd	0.002^{ab}	0.003^{a}	0.003^{a}	0.001^{b}	0.003^{ab}	0.001^{a}	nd	0.001^{a}	0.001^{a}
Eicosane	0.001^{a}	0.004^{a}	nd	nd	0.001^{b}	nd	nd	0.002^{b}	0.005^{a}	nd	nd	nd	nd
Hexadecane	0.026^{a}	0.063^{b}	0.072^{ab}	nd	0.082^{ab}	0.034^{d}	0.088^{bc}	0.060^{c}	0.164^{a}	0.052^{b}	0.102^{abc}	0.087^{ab}	0.011^{c}
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	0.001^{a}	0.002^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
cis-α-Bergamotene	0.001^{a}	0.003^{a}	nd	0.002^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd

Mean values (n = 3) in the same column along the rows with different lower case superscript are significantly different based on Duncan test at 95% confidence interval. nd, not detected.

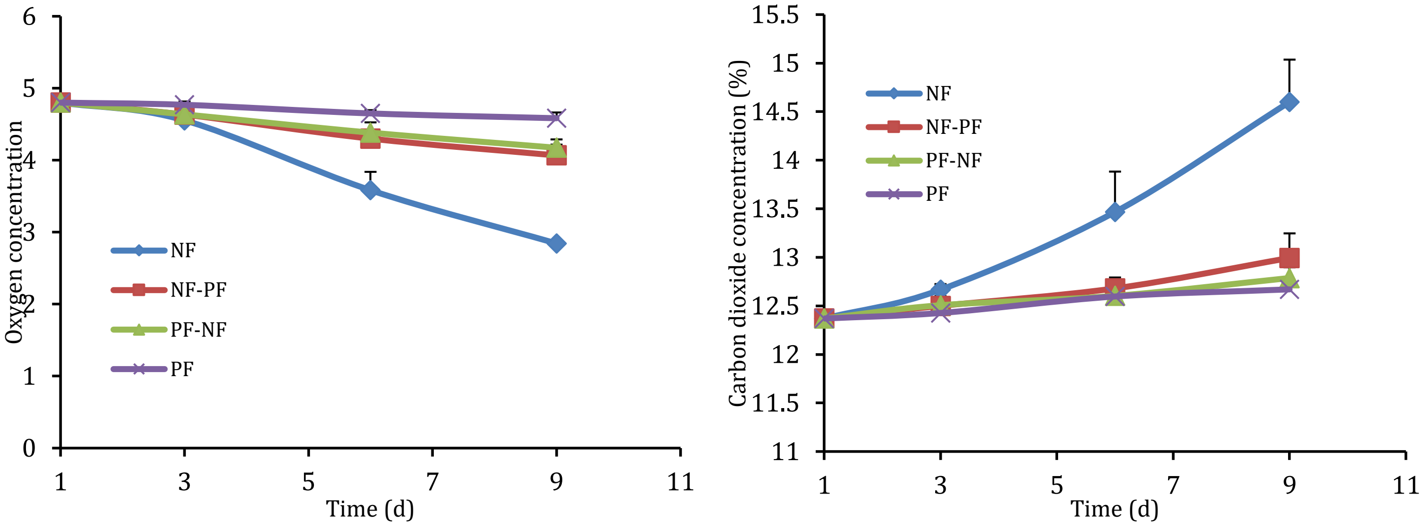

Figure 1. Effect of packaging designs on evolution of headspace gas composition of pomegranate arils over time (a) is O_2 consumption; (b) is CO_2 evolution.

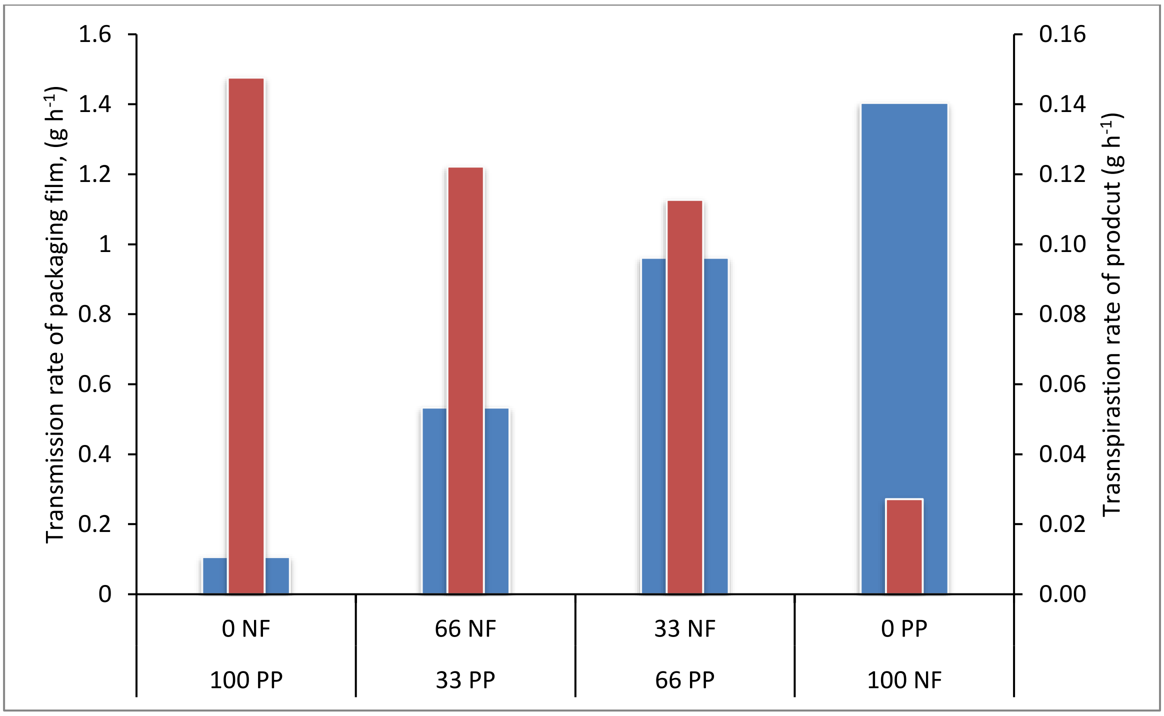

Figure 2. Effect of packaging designs on the water vapour transmission rate of packaging film (red bars) and the transpiration rate (blue bars) of pomegranate arils under different packaging conditions (rows) stored at 10 °C for 9 days.

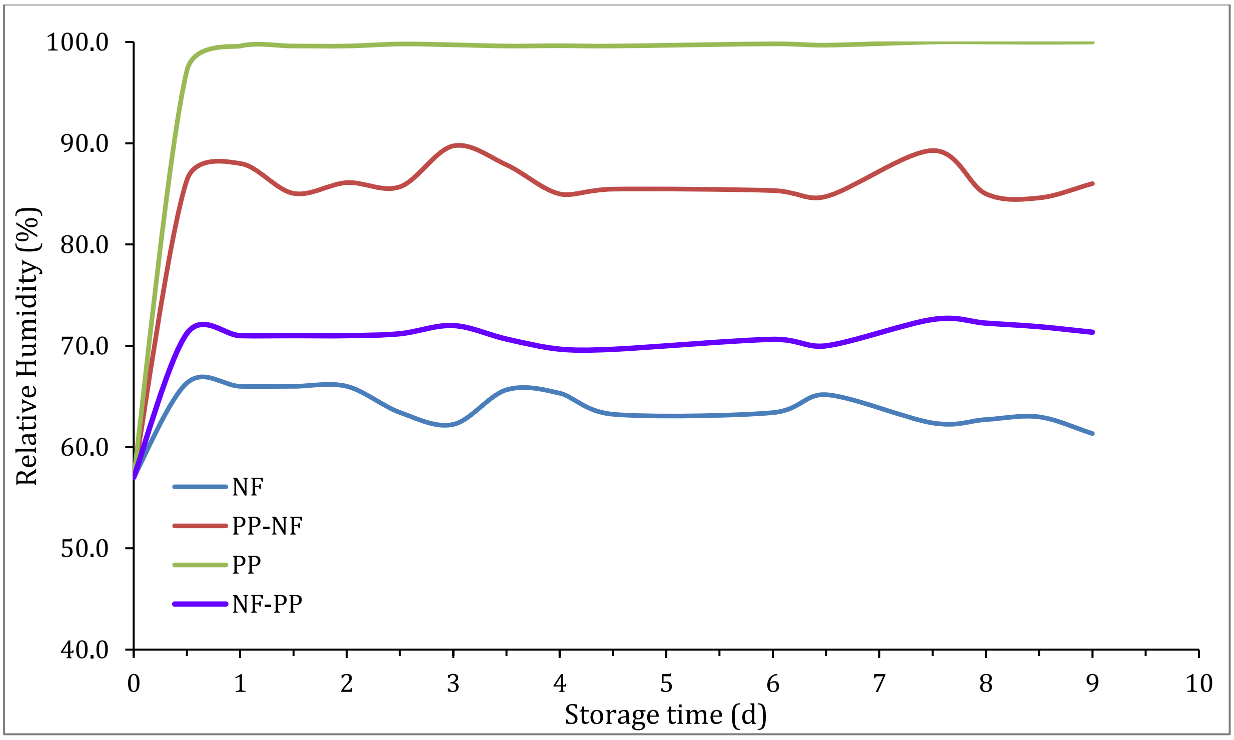

Figure 3. Effect of packaging designs during storage of pomegranate arils at 10 °C for 9 days. Mean RH (n = 2) with less than 0.01 deviations.

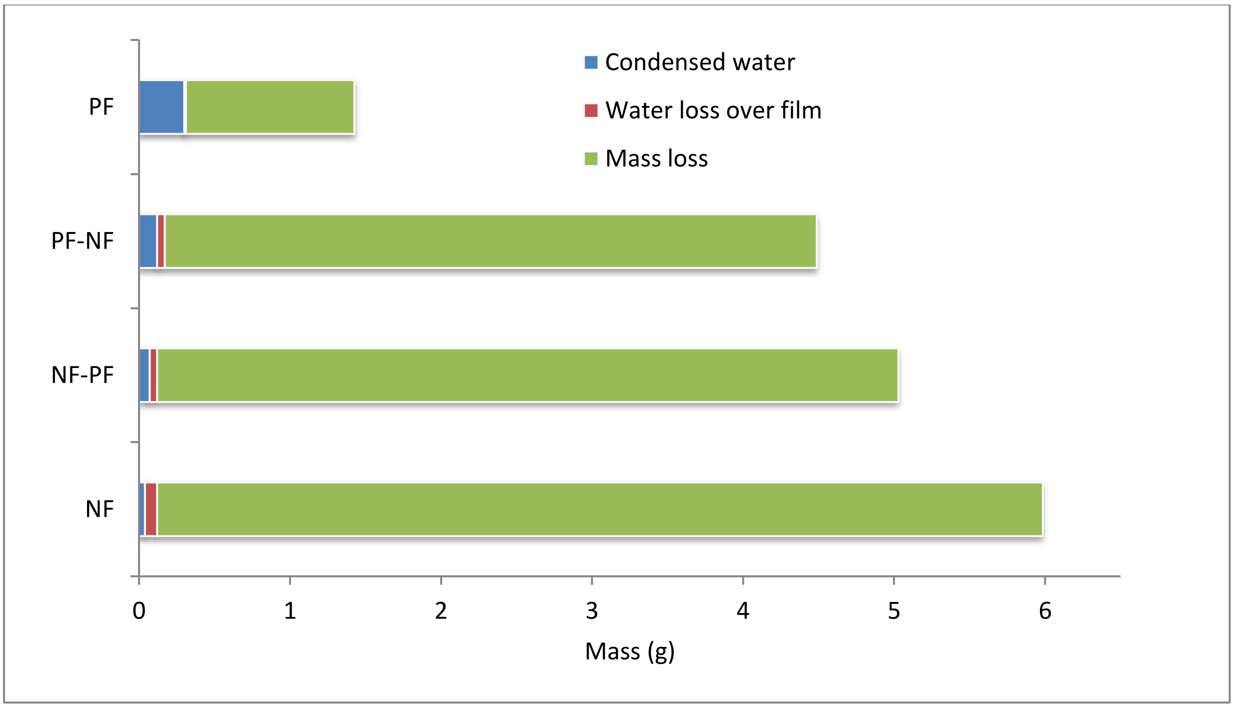

Figure 4. Effect of packaging designs on mass loss of pomegranate arils, water vapour condensed inside the package film, and transmitted through the packaging film after 9 days of storage at 10 °C. Mean values (n=3). Packaging design descriptions are listed in Table 2.

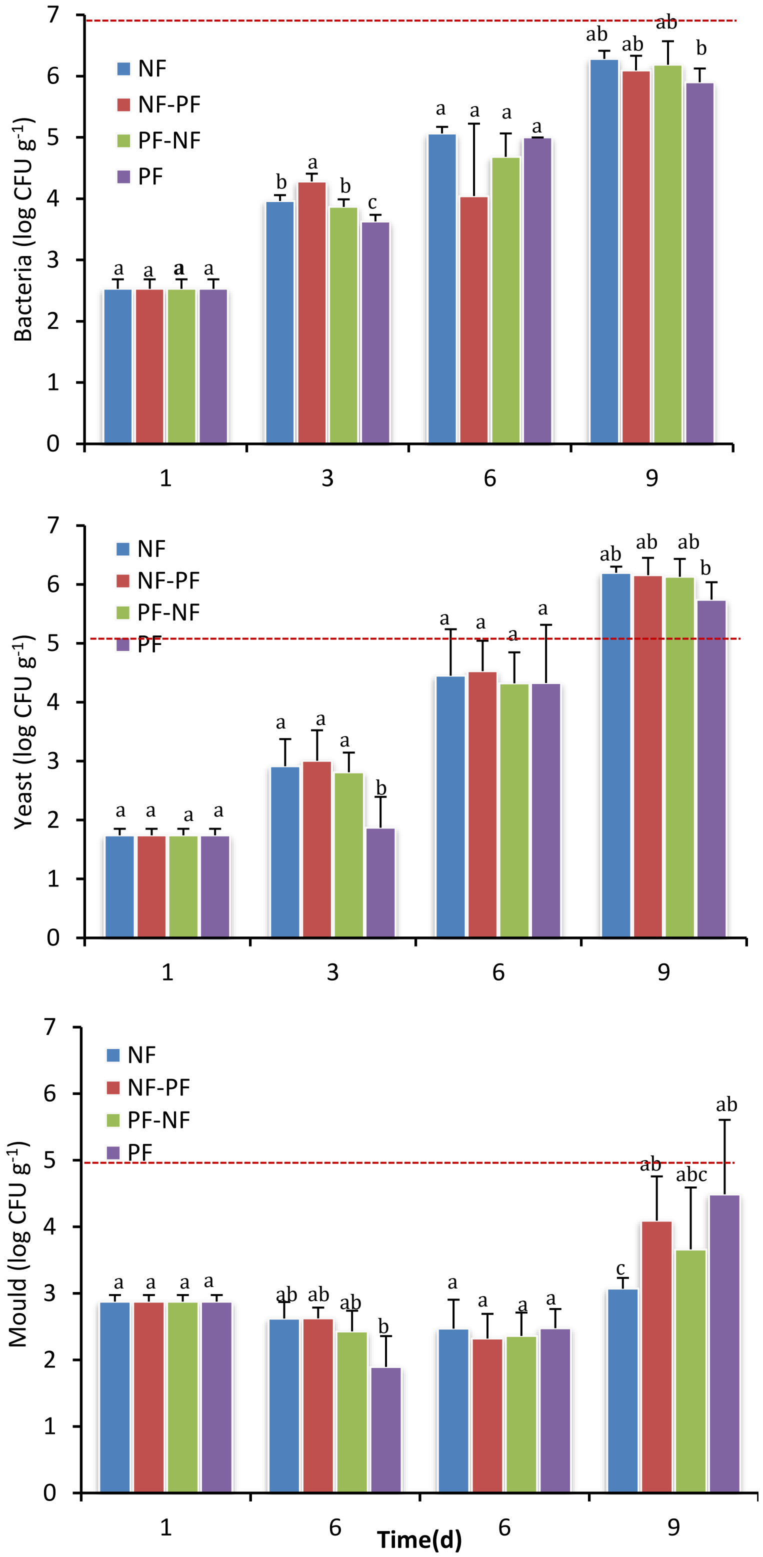

Figure 5. Effect of packaging designs on microbial growth for pomegranate arils (a) aerobic mesophilic bacteria (b) yeast and (c) mould growth on pomegranate arils stored at 10 °C for 9 days. Error bars represent standard deviation (SD) of mean values (n = 6), and bars with similar letters are not significantly different at 95% confident interval ($p \leq 0.05$) according to Duncan's multiple range test.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

The application of modified atmosphere packaging (MAP) in the postharvest preservation of horticultural commodities has been recognized as one the important technologies to reduce postharvest losses, maintain quality and extend shelf life throughout the distribution chain (Charles *et al*., 2006; Mangaraj *et al*., 2009; Azevedo *et al*., 2011). However, the practical benefits of MAP are considered relevant only when the packaging atmosphere, packaging material characteristics and storage temperature are at the optimum level (Gomes *et al*., 2012; Rodriguez-Aguilera & Oliveira 2009; Sandhya, 2010; Sousa-Gallagher *et al*., 2013). Thus, this study aimed to optimize active-MAP for 'Wonderful' pomegranate arils. Optimization requires systematic selection of gas composition using statistical analysis and aided by mathematical modelling (Pintado & Malcata, 2000).

A comprehensive literature review was compiled to asses several mathematical modelling approaches applicable for modified atmosphere packaging (MAP) of fruit and vegetables. The review showed that due to the complexity physiological and metabolic processes of the fresh produce, most of the approaches presented in literature rely on empirical models. However, the application of numerical models in MAP design and performance evaluation offers new opportunities for improved understanding and prediction of the interaction between product characteristics, package performance and environmental conditions. Studies reported the successful application of mathematical models for MAP design to specify and relate input parameters such as respiration rate (RR), transpiration rate (TR), as well as film permeability and storage temperature (Fonseca *et al*., 2002; Guevara *et al*., 2006; González-Buesa *et al*., 2009; Saenmuang *et al*., 2012). Based on the fact that respiratory mechanism is governed by enzymatic reactions, many researchers have used Michaelis-Menten (MM) enzyme kinetics model to describe RR of fresh produce (Fonseca *et al*., 2002), while others reported the application of Arrhenius-type equations to investigate the effect of temperature on RR of fresh produce (Caleb *et al*., 2012). Furthermore, the impacts of active MAP on quality attributes and shelf life of fresh and fresh-cut produce were assessed. The mechanisms by which the storage atmospheres affected the fruit were mostly associated with physiological or enzymatic reactions. Most of the literature reported that low O_2 atmosphere alone or in combination with elevated CO_2 is successful to maintain quality. This review found that the major factor that contributes to the benefit of active MAP includes the storage atmosphere, packaging material, type of fruit, storage temperature and relative humidity (RH). Literature showed that RH plays an important role in the physiological responses of packaged produce and influences product quality (Li *et al*., 2014; Techavises & Hikida, 2008). When high humidity condition prevails in the packages, it causes moisture condensation due to sub-optimal package design (Hussein *et al.*, 2015). This review showed that active-MAP could be considered as a better approach to maintaining the quality of fresh and fresh-cut fruit without any residual gas effects compared to the passive-MAP. However, contradiction exists in the literature regarding the benefit of active-MAPs; which indicates the importance of further research for a particular fruit at different storage conditions.

The fundamental knowledge of respiratory parameters as affected by the low O_2 concentration is essential to establish the physiological limits of the tissue and to design packages aimed at targeted atmosphere condition (Gomes *et al.*, 2012). Therefore, this study aimed to identify the tolerance limit of 'Wonderful' pomegranate arils at different storage temperatures (5 and 10 °C) by using real time gas evolution, RQ, microbial growth and VOCs. The information from the gas evolution was used to identify the final concentrations of the gases and the immediate increase of RQ values at a certain O_2 concentration was used as a shift of respiration to anaerobic respiration. The use of information from the VOCs data and microbial growth was considered to determine either secondary VOCs emitted or microbial resistance observed due to the change from aerobic to anaerobic respiration. The evolution of gases, RQ, microbial growth and emission rate of VOCs were different at the different storage temperatures. Different low O_2 limits were obtained when O_2 concentration reduced at respective storage temperatures, based on the immediate increase of RQ, accumulation of ethanol as well as the yeast growth. Depending on these criteria, pomegranate arils was observed to tolerate up to 2.18% O_2 at 5 °C and 2.28% O_2 at 10 °C. These findings further indicates the potential to use low O_2 atmosphere to maintain the quality of pomegranate arils stored in the range of 5 to 10 °C. However, the results also highlighted the need to select appropriate film permeability to alleviate higher reduction of O_2 at 10 °C.

Different types of gas concentrations have been reported during MAP for different fruit, and response to different storage atmospheres varies with commodity (Beaudry, 2000). Application of low O_2 and enriched CO_2 concentrations has been widely reported (Oms-Oliu *et al.*, 2008; Fonseca *et al.,* 2005; Giuggioli *et al.*, 2015). This atmosphere has shown a lot of benefits to maintain fresh produce quality by reducing the metabolic rate (Ersan *et al.*, 2010; López-Rubira *et al.*, 2005; Weber *et al.*, 2011). However, using low O_2 atmosphere could be risky if the concentration is not controlled and optimized to a level where the product can tolerate, otherwise, the change in respiration to anaerobic stage becomes unavoidable (Ampofo-Asiama *et al.*, 2014; Beaudry *et al.*, 2000). The use of super-atmospheric O_2 has also received attention in efforts to overcome the limitation of low O_2 atmosphere (Ayhan & Esturk, 2009; Maghoumi *et al.*, 2014). The effect of low or super-atmospheric O_2 condition is product specific and there is a need to be studied individual fresh fruit (Conesa *et al.*, 2007).

Furthermore, knowledge of the behaviour of plant products under extreme O_2 and CO_2 levels is crucial for modelling and optimizing MAP design and improving quality and shelf life of minimally-processed commodities (Artés *et al*, 2000).

Therefore, this study was designed to investigate the effects of both low and super-atmospheric O_2 concentrations on quality attributes of 'Wonderful' pomegranate arils during cold storage (5 ℃, 95 ± 2% RH) and ambient storage (20 ℃, 65 ± 2 % RH) conditions. The results showed that MA (5% O_2, 10% CO_2; 10% O_2, 10% CO_2; 70% O_2, 10% CO_2 and air) required for controlling physiological response and maintaining the different quality attributes of pomegranate arils varied during cold storage. These results indicated the postharvest life of pomegranate arils is storage atmosphere dependent. Storage of arils under low O_2 (5-10%) atmosphere reduced the metabolic rate, maintained antioxidant properties, such as phenolic, anthocyanins and ascorbic acid contents. The beneficial effect of low O_2 (5%) in maintaining the quality attributes of 'Wonderful' pomegranate arils was also reported by Banda *et al.* (2015). On the contrary, higher degradation of antioxidants were observed at super-atmospheric O_2 condition. The reduction of antioxidants can be associated with the production of reactive O_2 species during super-atmospheric condition and subsequent oxidation with polyphenol oxidase (PPO) and (POD) activities (Conesa *et al.*, 2007; Ghasemnezhad *et al.*, 2011). Super-atmospheric O_2 was effective for maintaining firmness and colour of the arils and inhibition of microbial growth, as compared to other MA conditions. These advantages of super-atmospheric O_2 could be due to the evolution of the highest CO_2 concentration. Studies have shown that high CO_2 concentration has bacteriostatic effect to inhibit the growth of most aerobic microorganism by the formation of carbonic acid or intercellular pH changes (Banda *et al.*, 2015; Paul & Clarke, 2002).

In this study, enzyme kinetics modelling approach was used to describe the respiration rate (RR) of pomegranate arils. Four types of Michaelis-Menten (MM) equations were applied to identify the effect of CO_2 on the O_2 consumption rate. The effects of both low and super-atmospheric O_2 concentrations on the physiological response of 'Wonderful' pomegranate arils at cold storage (5 °C) and ambient condition (20 °C) were investigated. The study identified that different storage atmospheres and temperatures significantly affected the physiological responses of the arils. Furthermore, the MM enzyme kinetics model effectively predicted the RR of pomegranate arils. This was validated using competitive inhibition type equation to describe the effect of CO_2 on O_2 consumption rate at low O_2 (5%), super-atmospheric O_2 (70%). This result was in line with Ersan *et al*. (2010), where MM enzyme kinetics competitive inhibition model described the RR of 'Hicaz' pomegranate arils at low O_2 and ambient atmosphere. The model provided the primary information for the consecutive studies concerning respiratory metabolism of pomegranate arils. However, from the present study, it was shown that low O_2 (5%) atmosphere reduced the metabolic rate, whereas super-atmospheric O_2 showed little or no benefit in slowing down metabolic process of pomegranate arils. In addition, storing pomegranate arils at ambient condition (20 °C, 65 ± 2 % RH) resulted in higher microbial growth and quality deterioration, thus, highlighting the importance of maintaining the cold chain. Therefore, for the rest of the experiments, the study focused on optimizing cold storage temperature (5 to 15 °C) and atmospheres containing low O_2 and enriched CO_2.

The optimization study was performed using simplex lattice mixture design approach. This experimental mixture design approach assumes that if all the total amount of the factors kept constant, the change made in the value of the response is only due to changes made in the relative proportions of each gas composition making up the mixture (Pintado & Malcata, 2000). The behaviour of the response is thus a function of the joint effects of the gas mixture. The only constant on the design is that for each experiment the sum of all proportions (gas concentration) must add up 1 (Cornell, 2011). For this study, the amount of O_2, CO_2 and N_2 were selected as independent variables in a simplex lattice design. The physiological responses (RR, respiration quotient (RQ), ethylene production rate) and the quality attributes: microbial criteria (bacteria, yeast); physico-chemical properties (visual qualities, colour attributes, firmness, weight loss and mould, soluble solids (TSS), titratable acidity (malic and citric acids)); individual sugars (glucose, fructose and sucrose); antioxidant properties (anthocyanins and ascorbic acid concentration); and volatile organic compounds (VOCs) were taken as responses.

The study showed that the simplex lattice mixture design was suitable to select the experimental points and to optimize the gas concentration for active-MAP. The special cubical model predicted the correlation of single (β_1, β_2, β_3), binary interaction (β_{12}, β_{13}, β_{23}) and ternary interaction (β_{123}) effects of gas concentrations with high correlation coefficient of R^2 = 99%, 99% and 95% for RQ, RR and ethylene production rate, respectively. These correlations indicated that the model sufficiently described the effects of the gas concentration on the physiological response variables. Based on the magnitude of the parameter estimates, CO_2 concentration had the most significant effect on the physiological response (RQ, RR and ethylene production rate) of pomegranate arils compared to O_2 concentration. The results from the coefficients of parameter estimates suggested that, if lower carbon dioxide production rate (RCO_2) is desired for pomegranate arils, controlling the interaction of the three gases are very crucial. On the contrary, the effects of CO_2 was antagonistic for ethylene production rate. This means that ethylene production decreased at highest concentrations of CO_2. Similarly, the model parameter estimates showed that the lowest count of microbial growth can be achieved by increasing the CO_2 concentration (> 10%) with correlation (R^2 > 94%) between the simplex lattice experimental data to the predicted values in the cubical model.

In this study, the maximum height of the fitted response, which corresponds to the optimum gas composition according to Van de Velde and Henderickx (2001), indicated the optimum region on the ternary contour profile plots with a gas mixture of 2% O_2 and 18% CO_2. At this gas concentration (the calculated optimal) for individual response variables indicated that the minimum value of RR, minimum concentration of ethylene production rate as well as lower count of aerobic mesophilic bacteria, yeast and mould can be achieved. The effects of low O_2 and enriched CO_2 to reduce the metabolic rate and microbial growth have been reported for pomegranate arils (Banda *et al.*, 2015; Ersan *et al.*, 2009). However, in the current study O_2 concentration declined to 1.53%, where it could stimulate anaerobic RR during storage. Thus, if the aim of postharvest storage is to reduce RR, ethylene production rate, RQ, aerobic mesophilic bacteria, yeast and mould without negative effect on pomegranate arils during cold storage, 2% O_2 could be recommended for short storage duration (1-3 days). However, for commercialization of pomegranate arils with longer storage life, the concentration of O_2 should be increased within the range of 2 to 4%. This was evident from the maximum RR and RQ values observed under 2% O_2 MA system with the elapse of storage time, while the response variables were positive at 4.67% O_2 atmospheres until the end of storage.

Furthermore, the importance of increasing the O_2 concentration greater than 2% was observed for the other quality attributes, where the results shown the optimum gas mixture of 6-7% O_2 and 7-8% CO_2 to maintain TSS, individual sugars, organic acids, colour attributes and ascorbic acid concentration of pomegranate arils. López-Rubira *et al.* (2005) reported the benefit of 5-10% O_2 for optimum storage of pomegranate arils. On the other hand, if the CO_2 concentration in the MA increased up to 18% and O_2 concentration declined to 2%, VOCs responsible for the flavour of arils could be enhanced. In this study, arils firmness was the only quality parameter enhanced by storing arils at the highest O_2 concentration (18%).

In general, it can be concluded that simplex lattice mixture design can be used as a method to optimize gas concentration for active-MAP. Although various interactions can be evaluated, it was found that the special cubical model generated a term that can explain the interactions between the three gases. The model also described the correlation between the experimental and the predicted values. The results of this study provided new information on optimum gas mixture with regard to the target quality parameters for commercialization of active- MA storage of pomegranate arils. However, further studies were required to select the appropriate storage temperature and packaging material to match the optimum gas mixture, which also requires the knowledge of low O_2 limit of 'Wonderful' pomegranate arils.

Temperature fluctuation during the distribution chain is an avoidable challenge (Tietel *et al.*, 2012). In addition to maintaining the optimum gas composition, storage temperature has been also shown to play an important role in the physiological responses of pomegranate arils

(Caleb *et al*., 2012). Therefore, the effect of storage temperatures (5, 10 and 15 °C) were studied using a non-destructive gas measurement to identify the appropriate storage temperature required to maintain the optimum gas composition. The results showed that storage temperature had a significant impact ($p \leq 0.05$) on headspace gas composition and RR of pomegranate arils. Comparing the changes in gas concentration, the highest CO_2 evolution (33%) was observed for arils stored at 15 °C. Similarly, initial O_2 concentrations were reduced by 5%, 14% and 34% at 5, 10 and 15 °C, respectively, after storage for 8 days. The concentration of O_2 was within the tolerance limit (> 2%) for all temperatures at the end of the study. However, development of objectionable odour was detected for arils stored at 15 °C on day 8. The RR increased significantly with increasing temperature from 5 °C to 15 °C, where the lowest RR was noted at 5 °C and the highest at 15 °C. The observed RR data were in agreement with the findings of Caleb *et al*. (2012) for 'Acco' and 'Herskawitz' pomegranate arils stored under passive atmosphere. Similarly, Gil *et al*. (1996) observed the significant increase in RR of 'Mollar' pomegranate arils from 0.53 mL kg^{-1} h^{-1} to 1.94 mL kg^{-1} h^{-1} when the storage temperature increased from 1 to 8 °C. However, in the current study the RR of arils were not significant different between at 5 °C and 10 °C. These results showed that storage of pomegranate arils at 10 °C could have equivalent advantage as 5 °C, which is a good indication since 10 °C represents a retail market temperature. Therefore, the rest of packaging material designing study have done at 10 °C.

Condensation inside the package of fresh fruit and vegetables represents a threat to product quality and safety (Bovi *et al*., 2016). Improvement on the design of packaging material by matching the optimum gas concentration to avoid condensation was performed. For this study, packaging materials (bi-axial oriented polypropylene (BOPP) film PropaFilm™ (PF) and cellulose-based NatureFlex™ (NF) NVS23) with distinct permeability to gases and water vapour were selected. Films were fitted with a fixed ratio window design based on desirable in-package gas composition and RH. Packages were flushed with 4.67% O_2 + 12.67% CO_2 + 82.67% N_2 gas composition prior to sealing and storage at 10 °C. In this experiment, detailed analysis was performed to quantify product quality and packaging material characteristics. The outcomes of the study showed that the design of MAP had a significant potential to influence the quality of pomegranate arils. Using absolute cellulose based film had the highest reduction of O_2 concentration, lowest in-package RH (> 66%) and increased moisture loss, which resulted in arils hardness and loss of colour. This could be associated with its higher O_2 transmission rate (OTR) and low permeability to CO_2 properties (Somboonkaew & Terry, 2010). On the other hand, arils packed in 100% BOPP film PropaFilm™ had reduced weight loss but, it was not efficient to control the in-package RH (100%). This affected the appearance and aroma of pomegranate arils due to visible surface mould growth and higher concentration of ester compounds. On the other hand, PF based NF window and NF based PF window films prevented the build-up of CO_2 and reduction of O_2 concentration. However, the use of NF based PF window film was limited due to its low in-package RH (< 76%), which affected the product appearance and quality. The use of BOPP based NF window film successfully regulated the recommended in-package RH (> 90%) with minimum changes in O_2 and CO_2 concentrations and also assisted in controlling condensation inside the package. According to Kader and Rolle (2004), RH range of 90 to 95% was recommended for storage of fresh fruit and vegetables. Thus, this makes the PF based NF package design a suitable option for packaging pomegranate arils.

Overall, the results reported in this thesis have demonstrated the importance of system optimization and appropriate MAP design to maintain the postharvest freshness of pomegranate arils as well as to reduce economic loss. However, the commercialization of the optimal MAP solution found still needs to be studied with regard to the environmental impacts of a non-degradable packaging material. Therefore, this study recommends further investigation to replace the BOPP film PropaFilm™ with a biodegradable material.

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Faith ☙ *Hope* ☙ *Love*

Corinthians 13:13