Active modified atmosphere packaging, postharvest physiology, quality attributes and shelf life of minimally processed pomegranate arils (cv. Wonderful)

by

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Declaration

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Abstract

Minimally processed fresh products have a short shelf life and marketable period and could therefore benefit from active modified atmosphere packaging (MAP) technology because it allows earlier establishment of suitable equilibrium atmospheres than passive MAP. However, there are limited studies that have explored the application of active MAP in minimally processed pomegranate arils. This study, therefore, investigated the effects of active MAP and storage conditions on postharvest physiology, quality attributes and shelf life of pomegranate arils (cv. Wonderful).

In the first part of the study, the effects of storage temperature (5, 10 and 15 °C), relative humidity (76, 86 and 96%) and citric acid pre-treatment on transpiration rate (TR) of minimally processed pomegranate arils were investigated. In addition, the effects of storage temperature (5, 10, 15 and 20 ± 2 °C, and 90 ± 2 % RH) on respiration rate (RR) and quality attributes of citric acid treated and non-treated arils were determined in order to establish best storage conditions. Citric acid pre-treatment was only effective in reducing aril RR at 15 and 20 °C. Aril RRs were lowest at 5 °C throughout the 5 d storage duration and declined from 4.75 to 2.86 mL CO_2 kg^{-1} h^{-1} and 4.86 to 2.7072 mL CO_2 kg^{-1} h^{-1} for citric acid treated and non-treated arils, respectively. About twofold increase in RR was observed with increase in storage temperature from 5 to 15 °C and threefold when increased to 20 °C. Storing arils under low temperature condition (5 °C and 96 % RH) maintained the lowest transpiration rates (TR), with arils under these conditions suffering negligible moisture loss (~1%) after 9 d compared to 7 and 12% moisture loss for those stored under 86 and 76 % RH, respectively. The study further showed that citric acid pre-treatment had no significant effects on TR of arils at all the temperature and RH combinations.

The effects of active MAP on postharvest physiology, quality attributes and shelf life of minimally processed pomegranate arils at 5 °C and 90 % RH were investigated using two independent experiments. In experiment 1, arils were packed in low barrier bi-axially oriented polyester (BOP) polymeric film under two active MAPs (5% O_2 + 10% CO_2 + 85% N_2; 30% O_2 + 40% CO_2 + 30% N_2), passive MAP and clamshell containers as control. In experiment 2, a high barrier polyethylene polymeric film (polylid) was used with arils packed in three active MAPs (5% O_2 + 10% CO_2 + 85% N_2; 30% O_2 + 10% O_2 + 60% N_2; 100% N_2) and passive MAP as the control. Respiration rate, physico-chemical attributes, antioxidant properties (total anthocyanin, total phenolic and ascorbic acid content, and radical scavenging activity), microbial quality and sensory attributes were monitored every third day over a 12 d storage period.

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Equilibrium O_2 (16-18%) and CO_2 (7%) atmospheres were established after 3 d in the low barrier BOP in experiment 1; however, the recommended levels of gas composition (2-5 % O_2 and 10-20% CO_2) for MAP of minimally processed pomegranate arils were not reached. In contrast, O_2 levels decreased and CO_2 increased continuously, in pomegranate arils packaged in high barrier polylid film in experiment 2.

Respiration rate of arils in both low barrier BOP film and high barrier polylid film were significantly affected by MAP and increased significantly ($p < 0.05$) with storage duration. Arils in clamshell containers maintained lower RR than other MAP treatments, while passive MAP had the highest in experiment 1. Arils in active MAPs with low O_2 (5% O_2 + 10% CO_2 + 85% N_2), high O_2 (30% O_2 + 10% CO_2 + 60% N_2) and passive MAP in the high barrier polylid film generally maintained similar RR levels throughout the 12 d storage duration. In contrast, RR of arils in 100% N_2 was about 40% lower than that in other MAP treatments from day 6 until the end of storage. Furthermore, MAP with 100% N_2 was effective in supressing ascorbic acid oxidation from day 6 until the end of storage. Total anthocyanin content (TAC) of arils fluctuated with storage duration across all the MAP treatments. At the end of 12 d storage duration, anthocyanin content of arils in experiment 1 was highest in clamshell packages (30.7 ± 0.9 mg C3gE/ 100ml) and lowest in passive MAP (26.7 ± 1.8 mg C3gE/ 100 ml). In the high barrier polylid film in experiment 2, arils in 100% N_2 maintained higher TAC levels than other MAP treatments from day 9 until the end of storage. Similarly, radical scavenging activity of arils in the high barrier polylid film in experiment 2 was highest in 100% N_2 while that in passive MAP was lowest from day 6 until the end of storage.

Arils in in 100% N_2 and high O_2 atmospheres in both experiment 1 (30% O_2 + 40% CO_2 + 30% N_2) and 2 (30% O_2 + 10% CO_2 + 60% N_2) maintained lower aerobic mesophilic bacteria counts than other MAP treatments throughout the storage duration. However, shelf life was limited to 9 days for arils in 100% N_2 based on overall acceptability and off-odour sensory scores, while arils in active MAP with high O_2 scored above the acceptable limit by day 9. Arils in passive MAP in both films also remained acceptable until day 9, while those in clamshell packages were not acceptable beyond day 6.

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Opsomming

Vars produkte wat minimaal verwerk is het ’n kort raklewe en kan net vir ’n kort tydperk bemark word. Daar is dus voordeel te trek uit gemodifiseerde atmosfeer verpakking (MAP) tegnologie, want dit maak dit moontlik om vroeër as die geval is met passiewe MAP, ’n toepaslike ewewigatmosfeer te vestig. Tot dusver is daar egter min studies oor die toepassing van MAP op minimaal verwerkte granaat arils gedoen. In hierdie studie was die fokus dus op die effek van aktiewe MAP en stoortoestande op die na-oes fisiologie, gehalte kenmerke en raklewe van granaat arils (Kultivar Wonderful).

In die eerste deel van die studie is die effek van stoortemperatuur (5, 10 and 15 °C), relatiewe humiditeit (76, 86 and 96%) en voorafbehandeling met sitroensuur op die transpirasie-tempo van minimaal verwerkte granaat arils ondersoek. Die effek van stoortemperatuur (5, 10, 15 en 20 ± 2 °C, en 90 ± 2 % RH) op die respirasie-tempo en gehalte kenmerke van sitroensuur behandelde, en nie-behandelde arils is bepaal, om sodoende die beste stoortoestande vas te stel. Behandeling met sitroensuur was net effektief in die verlaging van die arils se respirasie-tempo by 15 en 20 °C. Arils se respirasie-tempo was tydens die 5 dae stoortydperk op sy laagste by 5 °C en het afgeneem van 4.75 tot 2.86 mL CO_2/kg per uur en 4.86 tot 2.7072 mL CO_2/kg per uur vir onderskeidelik behandelde en nie-behandelde arils. Die transpirasie-tempo het ongeveer twee voudig gestyg met ´n vernaging in temperatuur van 5 °C tot 15 °C en drie voudig toegeneem met ´n verderenvelhoging in temperatuur tot 20 °C. Die stoor van arils teen lae temperature (5 °C en 96 % RH) het gelei tot die laagste transpirasie-tempo. Onder hierdie toestande het die arils ook min vog (~1%) na 9 dae verloor, in vergelyking met arils wat 7% en 12% vog verloor het as dit teen 86% and 76 % RH onderskeidelik gestoor is. Daar is verder gevind dat sitroensuur behandeling geen noemenswaardige effek op die transpirasie koers van die arils by al die temperature en lugvoggehalte kombinasies gehad het nie.

Die effek van die aktiewe MAP op die na-oes fisiologie, gehalte kenmerke en raklewe van minimaal verwekte granaat arils teen 5 °C and 90 % RH is deur middel van twee onafhanklike eksperimente ondersoek. In eksperiment 1 is die arils in lae versperring biaksiaal-georiënteerde poliester (BOP) polimeriese film onder twee aktiewe MAP tegnologieë (5% O_2 + 10% CO_2 + 85% N_2; 30% O_2 + 40% CO_2 + 30% N_2), passiewe MAP, en “clamshell” houers as kontrole verpak. In eksperiment 2 is ’n hoë versperring polietileen polimeriese film gebruik (polimeriese deksel) en is met die arils in drie aktiewe MAP tegnologië (5% O_2 + 10% CO_2 + 85% N_2; 30% O_2 + 10% O_2 + 60% N_2; 100% N_2) verpak met ’n passiewe MAP as kontrole. Die respirasie-tempo, fisio-chemiese kenmerke, antioksidant kenmerke (totale antosianien, totale fenoliese en askorbiensuursuur inhoud

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en radikale opruiming aktiwiteit), mikrobiale gehalte en sensoriese kenmerke is elke derde dag oor ’n 12 dae stoortydperk gemonitor.

In eksperiment 1 is ewewig O_2 (16-18%) en CO_2 (7%) atmosfeer na drie dae in die lae versperring BOP atmosfeer bereik; maar die aanbevole gassamestellings vlakke (2-5 % O_2 and 10-20% CO_2) vir die MAP van minimaal verwerkte granaat arils is nie bereik nie. In kontras hiermee het die O_2 en CO_2 vlakke in die granaat arils wat in eksperiment 2 in hoë versperring polietileen film verpak is aanmekaar vermeerder en verminder.

Die respirasie-tempo van die arils in beide die lae versperring BOP film en in die hoë versperring polietileen film is deur MAP ge-affekteer en het heelwat ($p < 0.05$) tydens stoor vermeerder. In eksperiment 1 het arils in “clamshell” houers ’n laer respirasie-tempo behou terwyl passiewe MAP die hoogste telling getoon het. Arils in aktiewe MAP met lae O_2 (5% O_2 +10% CO_2 + 85% N_2), hoë O_2 (30% O_2 + 10% CO_2 + 60% N_2) en passiewe MAP in the hoë versperring polietileen film het gewoonlik dieselfde respirasie-tempo gedurende die 12 dag stoortydperk behou. Die respirasie-tempo van arils in 100% N_2 was vanaf dag 6 tot aan die einde van die stoortydperk omtrent 40% laer as die van die arils wat ander MAP behadelings ondergaan het. Verder was die MAP behandeling met 100% N_2 vanaf dag 6 tot aan die einde van die stoortydperk effektief wat betref die onderdrukking van askorbiensuur oksidasie. Die totale antosianien inhoud (TAC) van arils het ge-fluktueer in die geval van al die MAP behandelings tydens stoor. In eksperiment 1 was die antosianie inhoud van die arils aan die einde van die 12-dag stoortydperk op sy hoogste in die “clamshell” pakette (30.7 ± 0.9 mg C3gE/ 100ml) en op sy laagste in passiewe MAP (26.7± 1.8 mg C3gE/ 100 ml). In die hoë versperring polietileen film in eksperiment 2, het arils in 100% N_2 vanaf dag 9 tot by die einde van die stoortydperk hoër TAC vlakke as by ander MAP behandelings behou. In eksperiment 2 was die vry radikaal opruiming aktiwiteit van die arils in die hoë versperring polietileen film die hoogste in 100% N_2 terwyl dit in die passiewe MAP vanaf dag 6 tot aan die einde van die stoortydperk die laagste was.

Arils in 100% N_2 en hoë O_2 atmosfere in beide eksperiment 1 (30% O_2 +40% CO_2 + 30% N_2) en 2 (30% O_2 + 10% CO_2 + 60% N_2) het laer aerobiese mesofiliese bakterie tellings gedurende die stoortydperk behou. Die raklewe van arils in 100% N_2 was beperk tot 6 dae wat betref algehele aanvaarbaarheid en reuk tellings, terwyl die tellings van arils in aktiewe MAP met hoë O_2 teen dag 9 onaanvaarbaar hoog was. Arils in passiewe MAP het in beide films aanvaarbaar gebly tot by dag 9, terwyl die arils in “clamshell” pakette na dag 6 onaanvaarbaar was.

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The steadfast love of the LORD never ceases, His mercies never come to an end.

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Table of contents

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

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Chapter 1
Introduction

Pomegranate (*Punica granatum* L.) is an ancient fruit belonging to the family *Punicaceae* and the genus *Punica* (Kader, 2006). It has occupied a prominent place in religious symbolism and traditional medicine dating back thousands of years (Viuda-Martos et al., 2010). There is renewed global interest in pomegranate sparked by increasing knowledge of its potential health benefits (Fawole et al., 2013; Opara et al., 2009). Pomegranate therapeutic benefits are attributed to its high antioxidant content and rich pool of polyphenols including flavonoids, condensed tannins and hydrolysable tannins (Seeram et al., 2006). Clinical studies conducted over the past few years suggest the potential therapeutic properties of pomegranate to include treatment and prevention of cancer, cardiovascular diseases, diabetes, dental conditions, erectile dysfunction, diabetes, male sterility, brain ischemia, Alzheimer's disease, arthritis and protection from ultra-violet (UV) radiation (Viuda-Martos et al., 2010; Martínez -Romero et al., 2013).

Minimally processed pomegranate arils represent the edible portion of the fruit and are consumed as fresh fruit or used in preparation of commercial products including juice, wine, jellies, paste and jam (Holland et al., 2009; Al-Said et al., 2009). Pomegranate consumption is limited by difficulties associated with extraction of arils due to the hard fruit husk which is difficult to open. In addition, the phenolic metabolites from the arils and the fruit husk have a staining effect on hands (Caleb et al., 2012). Minimally processed 'ready to eat' pomegranate arils, therefore, provide a more convenient alternative (Ayhan and Eştürk, 2009), however, they are more perishable than the intact pomegranate fruit due to physiological stresses, physical damage and wounding suffered during minimal processing (Rico et al., 2007). Pomegranate arils easily lose quality attributes such as texture, colour and flavour; they also suffer rapid losses in nutritional and microbial quality (Martínez-Romero et al., 2013).

Modified atmosphere packaging (MAP) achieved by sealing fresh respiring produce in polymeric film and low temperature storage has been successfully used to maintain quality and extend the shelf life of minimally processed pomegranate arils (López-Rubira et al., 2005; Caleb et al., 2012). Low O_2 and high CO_2 atmospheres achieved under MAP help to slow down physiological and biochemical processes and retard microbial growth in packaged fresh produce, thereby extending the produce shelf life (Artés et al., 2006). In addition, MAP has been suggested to affect stability and concentration of phytochemical compounds in minimally processed products (Andrés-Lacueva

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et al., 2010), although the specific effects in pomegranate are not well established (Mphahlele et al., 2014).

Modified atmosphere packaging is not a replacement for optimum cold storage conditions (low temperature and high relative humidity) but simply plays a supplementary role (Artés et al., 2006). Temperature management is critical under MAP because it affects both the rate of produce metabolic processes and the permeability characteristics of polymeric packaging film (Charles et al., 2005). Temperature abuse leads to build up of anoxic conditions which may reduce shelf life (Artés et al., 2006). Caleb et al. (2013) reported a decrease in headspace O_2 below the fermentative threshold (2%) in MAP of minimally processed pomegranate arils stored at 10 and 15 °C, which resulted in development of off-odour. MAP is, therefore, most effective if an optimum cold chain is maintained throughout storage.

Other hurdle technologies including gamma and UV-C radiation, thermal treatments, edible coatings and chemical preservative treatments have been used in combination with MAP to enhance its effectiveness in retarding senescence processes and microbial spoilage in minimally processed products (Mahajan et al., 2014). Citric acid is an organic acid that is commercially used as an anti-browning agent in fresh cut fruits and vegetables (Mahajan et al., 2014). It has also been shown to lower the respiration rate (RR) of minimally processed products (Kato-Noguchi and Watada, 1997; Petri et al., 2008). Citric acid has been used as a pre-treatment in minimally processed pomegranate arils alone or in combination with ascorbic acid (López-Rubira et al., 2005; Ayhan and Eştürk, 2009). However, its effects on pomegranate aril physiological responses (respiration and transpiration rates) have not been reported.

Modified atmospheres can be achieved either passively by the interaction of fresh produce respiration and permeability characteristics of packaging film, or actively by replacing the atmosphere within a package with a desired gas mixture (Caleb et al., 2012). Establishment of equilibrium atmospheres in passive or commodity generated MAP takes a long time especially at low temperatures and in produce with low respiration rates (Bai et al., 2003; Rodov et al., 2007). During the period before equilibrium is reached, produce is exposed to non-optimal atmospheres and continues deteriorating (Rodov et al., 2007). In active MAP, however, the desired atmospheres are created immediately inside the package by flushing pre-mixed gases or by using gas scavenging and emitting systems (Kader and Watkins, 2000; Charles et al., 2006). Studies by Sivakumar et al. (2008) showed that equilibrium conditions in litchi packaged under active MAP were established almost from the first day of storage, while it took 6 days under passive MAP. Similarly, equilibrium atmospheres in minimally processed pomegranate arils (cv. Hicaznar) stored at 5 °C for 18 days

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were established earlier in packages initially flushed with low and super atmospheric oxygen than in those under passive MAP (Ayhan and Eştürk, 2009).

Minimally processed fresh products generally have a shorter marketable period than intact produce and could therefore benefit from earlier establishment of equilibrium atmospheres under active MAP (Bai et al., 2003). Cantaloupe packaged in initially modified atmospheres achieved by gas flushing maintained colour, visual quality and microbial quality compared to those stored under passive MAP and air (Bai et al., 2003). Active MAP in low O_2 (5 and 8%) atmospheres suppressed RRs, browning and microbial growth in fresh-cut cabbage packaged in perforated film packages and oriented polypropylene stored at 5 °C for 4 days (Hu et al., 2007). Microbial growth in minimally processed 'Wonderful' pomegranate arils stored at 5 °C for 16 days was suppressed by packaging under active MAP in enriched CO_2 (15 and 20 %) atmospheres (Hess–Pierce and Kader, 1997). In addition, pomegranate arils in these modified atmosphere conditions were still above the limit of marketability by day 16.

Despite the potential benefits of active MAP, few studies have investigated its effects on minimally processed pomegranate arils (Ayhan and Eştürk, 2009). Most studies with minimally processed pomegranate arils have focused on the use of passive MAP. López-Rubira et al. (2005) investigated the effects of passive MAP and UV-C treatment on quality, anthocyanin content and antioxidant activity of minimally processed 'Mollar of Elche' pomegranate arils harvested at two different dates at 5 °C for up to 15 days. The authors reported inconclusive results on the effects of UV-C radiation on microbial quality of minimally processed pomegranate arils. In addition, harvest dates were reported to have significant effects on quality and shelf life of arils. Caleb et al. (2013) investigated the effects of passive MAP on quality attributes, compositional changes and microbial quality of minimally processed pomegranate arils 'Acco' and 'Herskawitz' at 5, 10 and 15 °C for 14 days. Quality of modified atmosphere packaged pomegranate arils were best maintained at 5 °C with arils retaining physico-chemical attributes and microbial quality up to 10 days. Pomegranate aril flavour life was, however, limited to 7 days.

Ayhan and Eştürk (2009) investigated the effects of active MAP on minimally processed pomegranate arils (cv. Hicaznar) with low and super atmospheric O_2 at 5 °C for 18 days and reported slight or no significant changes in chemical and physical attributes of the arils despite equilibrium atmospheres being established earlier in active MAP (day 6) than passive MAP (day 9). However, the authors did not investigate the effects of active MAP on RR of arils despite results from studies on other minimally processed fresh products suggesting that active MAP suppresses RR (Ersan et al., 2000; Rattanapanone et al., 2001). Furthermore, research has shown that the

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response of pomegranate to MAP is cultivar dependent (Caleb et al., 2013). 'Wonderful' is one of the most important pomegranate cultivars grown and marketed globally. Nevertheless, studies on the effects of active MAP on 'Wonderful' pomegranate arils are still limited. Hess-Pierce and Kader (1997) investigated the effects of carbon dioxide enriched atmospheres (10, 15 and 20% CO_2) on postharvest life of 'Wonderful' pomegranate arils at 5 and 10 °C. The authors recommended packaging of arils in air flushed with 20% CO_2. However, the effects of O_2 on aril postharvest life were not investigated.

This study, therefore, investigated the effects of active MAP and storage conditions on the overall quality and shelf life of pomegranate arils (cv. Wonderful). The specific objectives of the study were to: (i) determine the physiological responses (respiration and transpiration rates) of pomegranate arils to different storage conditions (temperature and RH) and citric acid pre-treatment, (ii) evaluate the effects of active MAP on physiological responses, quality and shelf life of minimally processed pomegranate arils, and (iii) investigate the effects of active MAP on phytochemical properties of pomegranate arils.

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Chapter 2
Postharvest preservation of minimally processed pomegranate arils

Introduction

There has been growing commercial interest in pomegranate fruit, sparked by increasing knowledge of its health-related benefits (Viuda-Martos et al., 2010). Pomegranate arils, which are the edible portion of the fruit, are a rich source of bioactive phytochemical compounds such as phenolics, flavonoids and tannins (Teixeira da Silva et al., 2013). These bioactive phytochemical compounds are responsible for pomegranates therapeutic properties which include anti-inflammatory, antioxidant and anti-cancer activity (Lansky and Newman, 2007; Viuda-Martos et al., 2010; Martínez -Romero et al., 2013). Consumption of pomegranate is however limited by difficulties associated with peeling the fruit to obtain the arils. Minimally processed pomegranate arils, therefore, provide a more convenient and appealing alternative (Ergun and Ergun, 2009).

Minimally processed pomegranate arils, like all other fresh-cut or minimally processed fresh fruit, suffer accelerated deterioration in quality due to enhanced enzymatic and metabolic activity as well as microbial spoilage (Martínez-Romero et al., 2013). Modified atmosphere packaging (MAP), combined with low temperature storage, offers the possibility to maintain quality and extend shelf life of minimally processed fruit and vegetables (Artés et al., 2006). Studies have reported the successful application of MAP technology in maintaining desired quality attributes and shelf life for minimally processed pomegranate arils (Gil et al., 1996; Sepulveda et al., 2000; López-Rubira et al., 2005; Palma et al., 2009). The low oxygen (O_2) and high carbon dioxide (CO_2) atmospheres attained in MAP have been shown to slow down physiological processes, retard compositional changes and microbial proliferation (Jacxsens et al., 2002; Rico et al., 2007; Sandhya, 2010). The success of MAP in maintaining product quality, however, depends on the rapid establishment of suitable equilibrium atmospheres within a package, failure to which may result in hastened product deterioration and a shortened shelf life (Artés et al., 2006; Mangaraj et al., 2009).

The objective of this review is to discuss the effects of minimal processing on physiological properties and quality attributes of pomegranate arils. The review also highlights the various hurdle technologies employed to maintain quality and extend shelf life of fresh-cut produce including the application of MAP technology in minimally processed pomegranate arils.

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Origin and production of pomegranate

Pomegranate (*Punica granatum* L.) is a popular fruit of tropical and subtropical regions, belonging to the family Punicaceae. It is native to the area stretching from Iran to the Himalayas in northern India and has been naturalised in the Mediterranean region since ancient times (Viuda-Martos et al., 2010). Pomegranate has the ability to adapt to varying climatic and soil conditions, and has a wide genetic diversity consisting of more than 500 cultivars. This has resulted in its being cultivated globally across different climatic regions (Teixeira da Silva et al., 2013).

The largest commercial producer of pomegranate is India, accounting for more than 50 % of global production, and second only to Iran in exports (Teixeira da Silva et al., 2013). Other important commercial producers include Pakistan, Israel, Afghanistan, Egypt, China, Japan, USA, Russia, Saudi Arabia, South Africa, Australia, Chile, Peru and Argentina (Fawole and Opara, 2013b). South Africa has recently emerged as one of the recognised producers of pomegranate in the southern hemisphere, competing with countries such as Chile, Australia, Peru and Argentina (Fawole and Opara, 2013b). Pomegranate exports in South Africa increased by 40%, from 2524.1 metric tonnes in 2013 to 3434.74 metric tonnes in 2014 (Pomegranate Association of South Africa, 2014).

Morphological characteristics of pomegranate

The pomegranate tree is an evergreen shrub or small tree that can grow to a height of 6 to 10 m at maturity (Stover and Mercure, 2007; Fawole and Opara, 2013a). It begins to set fruit 2 to 3 years after propagation, but generally reaches good commercial production by the 5^{th} to 6^{th} year (Stover and Mercure, 2007). The fruit is described as ‘berry-like’ (Fig.1), with a thick leathery, woody husk that varies in colour from yellow overlaid with light or dark pink to bright red depending on the variety and stage of maturity (Kader, 2006; Holland et al., 2009; Fawole and Opara, 2013a). It is crowned with a tubular calyx which is maintained to maturity and is a distinct feature of pomegranate (Teixeira da Silva et al., 2013).

The seeds, which consist the edible portion of the fruit, are enclosed within the fruit husk (Stover and Mercure, 2007; Teixeira da Silva et al., 2013).They account for 55-60% of the total fruit weight and are surrounded by a juicy pulp (aril) which varies in colour from deep red to virtually colourless depending on the cultivar and stage of development (Teixeira da Silva et al., 2013; Al-Said et al., 2009; Kader, 2006). The seeds are organised in locules separated by membranous walls and a spongy mesocarp. They are also distinguished as hard or soft depending on their sclerenchyma tissue content (Stover and Mercure, 2007). This trait is cultivar dependent and is suggested to

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influence consumer preference as 'soft' seeds are more appealing than hard seeds (Fawole and Opara, 2014).

Economic importance of pomegranate

Pomegranate has been popular since ancient times serving as a source of nutrients in the human diet as well as satisfying the medicinal and spiritual needs of many cultures (Fawole and Opara, 2013a; Viuda-Martos et al., 2010). Pomegranate seeds and extracts from the bark, leaves, flowers and the fruit husk have been used traditionally to treat diarrhoea, diabetes, leprosy, haemorrhages, snake bites, dysentery, ulcers, acidosis, microbial infections, and as contraceptives (Stover and Mercure, 2007; Viuda-Martos et al., 2010; Lansky and Newman, 2007; Larrosa et al., 2010; Lee et al., 2010).

Recent scientific findings have shown that apart from being a rich dietary source of sugars, organic acids, fatty acids and lipids, protein, crude fibres, vitamins and minerals (Viuda-Martos et al., 2010; Fawole and Opara, 2013a), pomegranate constituents are also a rich source of phenolics, flavonoids and tannins, bioactive phytochemicals that confer medicinal properties (Teixeira da Silva et al., 2013). Pomegranate juice has high polyphenol content and is reported to have up to three times higher antioxidant activity compared to other polyphenol rich beverages such as red wine, grape juice and green tea (Rosenblat and Aviram, 2006). The fruit rind is also an important source of bioactive compounds including phenolics, ellagitannins and proanthocyanidin (Rosenblat and Aviram, 2006; Viuda-Martos et al., 2010) and is therefore utilised in the food and pharmaceutical industry (Stover and Mercure, 2007; Teixeira da Silva et al., 2013). These findings have provided a credible basis for some of the traditional ethno medicinal uses of pomegranate (Gözlekç et al., 2011).

The potential therapeutic properties of pomegranate are wide-ranging and include treatment and prevention of cancer, cardiovascular diseases, diabetes, dental conditions, erectile dysfunction, diabetes, male sterility, brain ischemia, Alzheimer's disease, arthritis and protection from ultra-violet (UV) radiation (Jurenka, 2008; Viuda-Martos et al., 2010; Martínez-Romero et al., 2013). A study by Aviram et al. (2004) showed that consumption of pomegranate juice by 10 carotid artery stenosis patients for 1 year resulted in 21% reduction in systolic blood pressure. Continued intake of the juice for 3 years by 5 of the patients, however, did not result in further blood pressure reduction. Recent clinical evaluation studies by Asgary et al. (2014) also showed that pomegranate juice consumption reduced both systolic and diastolic blood pressure in 21 hypertensive patients. The study further recommended the use of pomegranate juice as an adjunct to anti-hypertensive medication and as a constituent of daily regime for patients who are at high risk for hypertension

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and cardiovascular disease. Zhang et al. (2005) demonstrated through cell culture studies that the pomegranate constituents cyanidin, delphinidin and petunidin, were able to inhibit the growth of breast cancer cells. Further studies are, however, required in order to determine the bioavailability, metabolism and safety of the bioactive compounds derived from pomegranate (Viuda-Martos et al., 2010)

The increasing knowledge of the potential health benefits of pomegranate have sparked commercial growth of pomegranate derived products on the market including; pomegranate juice, canned beverages, jellies, wine, jam, paste and food seasonings (Viuda-Martos et al., 2010). Pomegranate is also known for its non-food value, with almost every part of the plant being utilised. Tannin extracts from the bark are used for curing leather; extracts from the flowers and fruit husks are used as dyes in textiles and the dwarf pomegranate trees serve as ornamental plants (Stover and Mercure, 2007; Teixeira da Silva et al. 2013).The ability of various extracts from the fruit to facilitate skin repair has also resulted in its use in cosmetics (Teixeira da Silva et al., 2013).

Pomegranate postharvest challenges

Pomegranate ripens 5 to 6 months after fruit set and is characterised by a sequence of quality changes including fruit size, colour, acidity and total soluble solids that are cultivar dependent (Kader, 2006; Fawole and Opara, 2013a). It is non-climacteric and therefore does not ripen off the tree even with ethylene treatment and should be picked when fully ripe to ensure its best flavour. It can be stored for 2 months at 5 ℃ and up to 5 months under controlled atmospheres (Kader, 2006). Pomegranate postharvest life is limited by physiological disorders, moisture loss and decay (Caleb et al., 2012a). In addition, the fruit husk is susceptible to damage as a result of sunburn, bruising and cracking (Ghasemnezhad et al., 2013; Caleb et al., 2012a). This review will focus on pomegranate physiological disorders and decay.

Physiological disorders

Pomegranate fruit are susceptible to chilling injury when stored longer than one month at temperatures between their freezing point (-3 °C) and 5 °C, or longer than two months at 5 °C. (Kader, 2006; Mirdehghan et al., 2006; Selcuk and Erkan, 2014). Chilling injury symptoms which are visible when fruit is moved to higher temperatures manifest as brown discolouration and pitting of rind, paleness of the arils and increased susceptibility to decay. This condition is aggravated by prolonged cold storage (Kader, 2006; Mirdehghan et al., 2006). Hurdle technologies such as conditioning before storage, intermittent warming and modified atmosphere packaging have been

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shown to reduce the incidence and severity of chilling injury symptoms (Artés et al., 2000; Artés and Tomás-Barberan, 2000; Kader, 2006). Artés et al. (2000) studied the efficacy of intermittent warming and curing in reducing chilling injury and its subsequent effect on changes in pigmentation and keeping quality of sweet pomegranate (cv. Mollar de Elche) stored at 2 and 5 °C for 13 weeks. Intermittent warming (20 °C every 6 days) significantly reduced chilling injury symptoms and resulted in fruit with better visual quality.

Husk scald is another physiological disorder that limits storage of pomegranate fruit (Kader, 2006). It manifests as superficial browning of the husk, initiating from the stem end of the fruit and spreading towards the blossom end as severity increases. It is limited to the external part of the fruit and is thought to be caused by oxidation of phenolic compounds on the husk at temperatures higher than 5 °C (Kader, 2006). Controlled atmosphere (CA) storage and MAP have been shown to be effective in reducing husk scald symptoms (Artés et al., 2000; Defillipe et al., 2006; Selcuk and Erkan, 2014). Defillipe et al. (2006) evaluated the efficacy of pre-storage treatment with diphenylamine (DPA) and/or 1-methylcyclopropene (1-MCP) and low oxygen atmospheres (1 kPa O_2, 1 kPa O_2 + 15 kPa O_2 and 5 kPa O_2 + 15 kPa CO_2) in controlling scald incidence and severity in 'Wonderful' pomegranates stored at 7 °C for 6 months. The treatments DPA and 1-MCP were not effective in reducing scald incidence and severity. In contrast, controlled atmospheres significantly reduced scald incidence and severity on pomegranates for up to 6 months. However, the CA treatments with lower O_2 levels (1 kPa O_2, 1kPa O_2 + 15 kPa O_2) resulted in accumulation of fermentative metabolites.

Postharvest decay

Gray mould caused by *Botrytis cinerea* is the most economically important postharvest fungal disease of pomegranate (Selcuk and Erkan, 2014; Kader, 2006). Most infections occur through the flowers or calyx while the fruit is still in the field but remain latent until after harvest (Kader, 2006). Disease development is favoured by storage temperatures between 5 to 10 °C and RH above 90%. The disease spreads from the blossom end of the fruit, causing discoloration and toughing of the husk, followed by the appearance of gray mycelial as it progresses (Kader, 2006; Palou et al., 2007). Controlled atmosphere and modified atmosphere treatments combined with anti-fungal treatments such as fludioxonil have been shown to be effective against *B. cinerea* (Tedford et al., 2005; Palou et al., 2007). Palou et al. (2007) studied the effects of combined treatments; food additives, fungicide fludioxonil and CA on the control of gray mould in pomegranate (cv.

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Wonderful) stored at 7.2 °C. The study revealed that CA conditions and the anti-fungal treatments synergistically controlled growth and sporulation of *B. cinerea* on artificially inoculated fruit. .

Heart rot also referred to as black heart caused by *Aspergillus* spp. and *Alternaria* spp. is another postharvest fungal disease of pomegranate (Zhang and McCarthy, 2012). Infection takes place pre-harvest when the fruit is still in the orchard during early fruit set and continues to grow and spread as the fruit develops (Kader, 2006; Zhang and McCarthy, 2012). The fungi causes decay of arils without obvious external symptoms on the fruit except for a slightly abnormal skin colour (Zhang and McCarthy, 2012; Yehia, 2013). In some instances, the mass of blackened arils reaches the rind, causing softening of the affected area (Kader, 2006). The lack of obvious external symptoms of heart rot makes identification a challenge during sorting and packaging (Zhang and McCarthy, 2012; Yehia, 2013).

Non-destructive techniques such as nucleic magnetic resonance (NMR) relaxometry and magnetic resonance imaging (MRI) were used by Zhang and MacCarthy (2012) to characterise and detect heart rot in 'Wonderful' pomegranate. NMR relaxometry showed cell water redistribution among cell compartments in fruit, indicating tissue damage as a result of infection. Heart rot infection was also visualised by magnetic resonance imaging. The study concluded that these non-destructive techniques had the potential to be used in identification of heart rot.

Minimal processing of pomegranate fruit

Minimal processing has been extensively utilised in fresh fruit and vegetables, in order to meet the growing consumer demand for fresh and safe 'ready-to-eat' produce. Minimal processing involves cleaning, peeling, cutting, slicing, shredding, trimming and/or coring, washing, drying and packaging (Gil et al., 1996a; Watada, 1996). Minimal processing ensures convenience and also provides consumers with a high value product, while avoiding costs associated with transporting whole fruit or vegetables which are bulky. The market has recently seen an increase in minimally processed pomegranate arils. This has been necessitated by the need to provide a convenient 'ready to eat or use' fresh form since pomegranate consumption is limited by difficulties associated with aril extraction (Artés and Tomás-Barberan, 2000; López-Rubira et al., 2005; Caleb et al., 2012a). The fruit husk is hard and difficult to open and the phenolic metabolites in the husk have a staining effect on the hands (Caleb et al., 2012a; Gil et al., 1996). Pomegranate minimal processing also allows utilization of bruised, cracked, sunburnt, small sized and physiologically damaged fruit that would otherwise not be marketable on the fresh market despite the superior internal quality (Artés and Tomás-Barberan, 2000; Ghasemnezhad et al., 2013; Caleb et al., 2012a).

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Tissue wounding from processing procedures results in enhanced respiration rates, enzymatic and microbial activity and moisture loss in minimally processed pomegranate arils (Rico et al., 2007; Toivonen and Brummell, 2008), which accounts for their shorter shelf life compared to the intact fruit. Pomegranate arils easily lose quality attributes such as texture, colour and flavour, as well as suffer microbial spoilage (Martínez-Romero et al., 2013). The shelf life of arils based on the visual quality attributes such as colour, browning and dehydration was limited to about 10 days for the late harvested pomegranate cv. Molar of Elche stored at 1 °C (López-Rubira et al., 2005). A similar shelf life was reported for pomegranate arils (cv. Primosole) stored in polypropylene film at 5 °C (Palma et al., 2009). Caleb et al. (2013b) suggested a shorter shelf life of 7 days for modified atmosphere packaged pomegranate arils cv. 'Acco' and 'Herskawitz', when taking into account changes in volatile compounds and flavour life.

Physiological responses of pomegranate fruit to minimal processing

Respiration rate

Respiration is the oxidative breakdown of stored organic materials such as starch, sugars and organic acids into simple end products including carbon dioxide (CO_2) and water coupled with the release of energy (Fonseca et al., 2002). In the absence of or under excessively low oxygen (O_2), energy is obtained by fermentative metabolism or anaerobic respiration in which pyruvate is broken down to ethanol and CO_2. Despite respiration being essential for the survival of living plants, it is also a degradative process for harvested fruit and vegetables that results in loss of quantitative and qualitative food value (Rico et al., 2007). Respiration rate (RR) is determined by the rate at which O_2 is consumed and/or the rate at which CO_2 evolved. It is associated with the rate at which compositional changes take place within plant tissue and is, therefore, an indicator of product potential shelf life (Kader et al., 1989; Martínez-Ferrer et al., 2002; Hu et al., 2007; Rojas-Graü et al., 2009). Apart from being a measure of the rate at which finite energy supplies are depleted within a product, RR could also serve as an indicator of the presence of spoilage micro-organisms (Garcia et al., 2000).

Fresh produce RR is affected by both intrinsic and extrinsic factors (Table 1). Minimal processing and cutting operations often result in enhanced RR in fresh horticultural commodities, due to increased surface area and enhanced permeability of respiratory gases (Manolopoulou et al., 2012). Sliced peaches, pears, banana, kiwi fruit and tomato had about 65% higher RRs than their corresponding intact fruit (Kader, 2002). Cutting of mango and pineapple was also reported to

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drastically increase RR (Martínez-Ferrer et al., 2002). Manolopoulou et al. (2012) studied the physiological behaviour of fresh-cut green peppers packaged in impermeable high density film at 0 and 5 °C. The study showed that cutting increased RR of unpackaged fresh-cut bell peppers by 24% compared to that of the whole peppers at 5 °C. Cutting operations however did not significantly alter RR of peppers at 0 °C. This highlights the influence of temperature on RR.

Respiratory response to minimal processing however varies depending on the type of fresh horticultural commodity and the extent of minimal processing. Some commodities exhibit very minimal increase or even a decrease in RR. Removal of hulls from strawberry and stems from seedless grapes resulted in a minimal change in RR and this was attributed to the minimal damage sustained during these operations (Artés et al., 2007). Minimally processed pomegranate arils exhibit relatively lower RR values partly due to their non-climacteric nature and also as a result of the minimal mechanical damage and wounding they suffer during minimal processing compared to other fruits (Garcia et al., 2000). Garcia et al. (2000) compared the respiratory intensity of minimally processed pomegranate arils and orange slices packaged in semi-permeable film at 4 °C. Respiratory intensity of the orange slices was found to be two times higher (57.1 mL CO_2/kg h) than that of the minimally processed pomegranate arils (30.8 mL CO_2/kg). These differences were attributed to the greater mechanical damage suffered by the orange slices compared to the pomegranate arils which were almost intact. Ersan et al. (2010) reported a minimum RR of 0.5 mL CO_2/kg h for pomegranate arils (cv. Hicaz) stored under modified atmosphere condition 2% O_2 + 10% CO_2 at 4 °C. While, RR of pomegranate arils (cv. Mollar Elche) stored at 5 °C in air was about 1.2 mL CO_2/kg h (López-Rubira et al., 2005). In addition, studies by Caleb et al. (2012) showed that RRs of minimally processed pomegranate arils cvs. 'Acco' and 'Herskawitz' stored at temperatures 5, 10 and 15 °C, were 2 to 3 times lower than those reported for the whole fruit stored at similar temperatures. This was attributed to the minimal injury suffered in arils and the presence of numerous micro pores on the fruit husk which allow easy diffusion of gases. The varying reports on pomegranate aril RR values in literature may also be attributed to differences in the degree of damage suffered during minimal processing, cultivars, maturity stages and storage conditions.

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Enzymatic activity

Cutting and peeling operations during minimal processing lead to rapture of cells and the release of exudates rich in enzymes, which hasten deterioration through tissue softening, cut-surface browning and enhanced biochemical processes (Artés et al., 2007). The exudates are also rich in nutritional components which accelerate the growth of spoilage microorganisms (Artés et al., 2006). Minimally processed fruits and vegetables, therefore, suffer loss of quality attributes colour, texture and microbial quality faster than whole products (Artés et al., 2007).

Tissue browning is a physiological disorder that is caused by the oxidation of phenolic compounds on the cut surface of fruits and vegetables (Toivonen and Brummell, 2008; Ergun and Ergun, 2009). Wounding and cell rupture from minimal processing procedures lead to interaction of polyphenols and oxygen with polyphenol oxidase (PPO), an enzyme that catalyzes the browning reactions in tissues of minimally processed fruits and vegetables (Artés et al., 2006; Toivonen and Brummell, 2008). Other enzymes, such as phenol peroxidases, have also been implicated in these oxidation reactions, although PPO remains the most dominant (Toivonen and Brummell, 2008). Browning disorders are most obvious in white fleshed fruits such as apple and pear and also in products rich in polyphenols (Artés et al., 2007). Browning has also been reported in minimally processed pomegranate arils (Sepúlveda et al., 2000; Ergun and Ergun, 2009; Maghoumi, 2013) and is attributed to the high phenolic content. Browning results in loss of sensory quality of pomegranate arils since they are known for their attractive red colour (Ergun and Ergun, 2009).

Moisture loss

Moisture loss poses a major challenge in both whole and minimally processed pomegranate fruit. The fruit appears hardy but is highly susceptible to water loss through the numerous minute pores on the fruit husk (Kader, 2006). Moisture loss is also accelerated by high temperatures and low relative humidity (RH) resulting in loss of saleable weight, shrivelling of the fruit and in extreme cases browning, hardening and drying of the husk and arils (Kader, 2006; Caleb et al., 2013a). Storage at 5 °C and 90 to 95 % RH has been recommended as optimal for minimising moisture loss and prolonging shelf life (Artés et al., 2007).

A study by Nanda et al. (2001) investigated the use of shrink film wrapping and coating with a sucrose polyester on moisture loss and quality retention in pomegranates at 8, 15 and 25 °C. The study showed that unpackaged fruit had up to 13% higher weight loss after 15 days of storage at 25 °C than the shrink wrapped fruit. In addition shrink film wrapping and low temperatures were

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effective in maintaining fruit firmness and quality. Removal of the outer protective husk as a result of minimal processing further predisposes pomegranate arils to moisture loss which results in weight loss, shrivelling and loss of textural quality. Sepúlveda et al. (2000) reported significant dehydration in arils packaged in perforated polyethylene bags compared to those packaged in semi-permeable film. Similarly, unpackaged storage of pomegranate arils at 8, 4 and 1 °C for 7 days led to significant moisture loss and shrivelling, whereas moisture loss in arils under MAP conditions was negligible (Gil et al., 1996).

Microbial deterioration

Shelf life of minimally processed pomegranate arils is limited by microbial spoilage caused by proliferation of yeasts and moulds as well as bacteria (López-Rubira et al., 2005). Minimally processed foods are at risk of contamination at various points including processing, packaging, storage and distribution (Gorny, 2003). In addition, exposed cut-surfaces and increased moisture content in minimally processed fresh products provide conditions ideal for microbial proliferation (Artes et al., 2007).

Studies conducted with pomegranate (cv. Wonderful) revealed that arils which suffered mechanical damage during extraction appeared soft and aqueous and were much more susceptible to microbial spoilage (Hess-Pierce and Kader, 1997). Minimizing mechanical damage during extraction, washing, drying, packaging and storage at low temperatures ensures microbial safety of pomegranate arils (Kader, 2006)

Preservation of minimally processed pomegranate arils

Storage condition

Optimum storage temperature and RH are critical in maintaining quality of fresh fruits and vegetables (Kader, 2002). Previous studies have demonstrated that temperature is the most important factor in controlling the respiratory activity, transpiration and development of microbial pathogens (Artés and Tomás-Barberán, 2000; Barbosa et al., 2011). Every 10 °C increase in temperature accelerates deterioration and rate of loss in nutritional quality by two to threefold (Kader, 2002). In addition, high temperatures and temperature fluctuations in fresh products packaged under MAP conditions results in changes in RR and package permeability characteristics. This affects the effectiveness of the modified atmosphere systems and in some instances, may even result in a shortened product shelf life (Artés et al., 2006). Caleb et al. (2013b) reported a decrease

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in headspace O_2 below the fermentative threshold (2%) in MAP for minimally processed pomegranate arils stored at 10 and 15 °C, which resulted in development of off-odour. Maintenance of low temperatures in MAP is therefore critical for maintaining product quality.

Pomegranate is susceptible to chilling injury when stored at temperatures below 5 °C (Kader, 2006). In contrast, minimally processed pomegranate arils have been shown to be tolerant to chilling temperatures and therefore should be stored between 0 to 5 °C (Kader 2006). Studies by Gil et al. (1996) revealed that pomegranate arils (cv. Mollar) stored at 1°C maintained lower RRs and better quality than those stored at 4 and 8 °C.

Chemical and physical preservation treatments

Minimal processing renders fresh produce susceptible to desiccation, discoloration or browning, tissue softening and microbial spoilage (Peter et al., 2002). In addition, handling during processing operations increases the risk of contamination and cross-contamination which poses a health hazard especially in the case of fruits and some vegetables which are not heated prior to consumption (Ahvenainen, 1996). Chemical and physical preservation treatments, some of which are summarized in Table 2, are usually applied on fresh fruit and vegetables in order to retard the microbial spoilage and biochemical quality changes associated with minimal processing.

Washing fruit and vegetables with sterile water or chlorine based solutions such as sodium hypochlorite (NaCIO) removes dirt and pesticide residues and also reduces the microbial load resulting from processing operations (Gil et al., 1996; Artés et al., 2009). Washing also allows removal of juice leaking from wounded tissue, which if left unchecked provides ideal conditions for microbial proliferation (Ahvenainen, 1996). Sodium hypochlorite (NaCIO) is the most commonly used disinfectant for both minimally processed fresh products and processing equipment (Artés et al., 2009; Mahajan et al., 2014) as it provides a cheap, yet potent disinfectant (Artés et al., 2009). Its efficacy, however, increases with increasing chlorine concentration (Artés et al., 2009; Mahajan et al., 2014) and it has been reported to react with organic food constituents to produce unhealthy carcinogenic compounds which are harmful to the liver (Artés et al., 2009). The use of NaCIO in minimally processed products has, therefore, been restricted in certain European countries, and alternatives such as peroxyacetic acid, chlorine dioxide, ozone, trisodium phosphate and hydrogen peroxide are being explored (Artés et al., 2009).

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Several other chemical preservatives have been used in combination with or as an alternative to chlorine based solutions to retard the biochemical and quality changes that result from minimal processing operations. Organic acids, in particular citric and ascorbic acids, as well as calcium based solutions have been used to control physiological and quality changes in fresh cut tissues of minimally processed fresh fruits and vegetables (Mahajan et al., 2014). Citric acid dips of 1mM or higher concentration reduced RRs of shredded carrots by 50% (Kato-Noguchi and Watada, 1997). Similarly ascorbic acid dips reduced the RR of 'Fuji' apple slices stored in a 0% O_2 atmosphere (Gil et al., 1998). Ascorbic acid alone and/or in combination with citric acid has also been used to retard cut surface browning and microbial proliferation in minimally processed products (Sepúlveda et al., 2000). A combination of citric and ascorbic acids added to chlorinated water was suggested as a suitable wash solution for pomegranate arils in order to prevent microbial development and browning (Artés et al., 2009, Gil et al., 1996). Similarly, Sepulveda et al. (2000) observed a significant reduction in browning and population of spoilage micro-organisms in minimally processed pomegranate arils that had been treated with a combination of chlorine and antioxidants (citric and ascorbic acids) compared to those that had been washed with chlorinated water only. Calcium is associated with maintaining cell wall structure and firmness of plant commodities by combining with pectin to form calcium pectate. Calcium chloride (CaCl) and calcium lactate dips have successfully been used in retarding tissue softening and have also been found effective in inhibiting enzymatic browning in minimally processed fresh products (Artés et al., 2009)

Edible coatings have been explored extensively as preservation treatment for minimally processed fresh fruits and vegetables because of their ability to minimize moisture loss, inhibit enzymatic browning, reduce RR and ethylene production, as well as confer antimicrobial properties (Olivas and Barbosa-Cánovas, 2005). They comprise one or more major components; polysaccharides, proteins, resins, waxes or oils, forming a thin layer of protective material on the surface of fresh cut fruits and vegetables (Valencia-Chamono et al., 2011). Chitosan, aloe vera gel and honey have been successfully used as edible coatings in minimally processed pomegranate arils (Ergun and Ergun, 2009; Ghasemnezhad et al., 2013; Martínez-Romero et al., 2013). Chitosan coating significantly reduced bacterial and fungal counts in minimally processed pomegranate arils after 12 days of storage at 4 °C (Ghasemnezhad et al., 2013). Martínez-Romero et al. (2013) investigated the effect of pre-treatments with aloe vera gel alone and in combination with ascorbic and citric acids on quality of minimally processed pomegranate arils stored under MAP at 3 °C. The study showed that the pre-treatments were effective in inhibiting growth of aerobic mesophillic bacteria, yeast and moulds. In addition, aloe vera gel and the acid treatments inhibited RRs and delayed softening of

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minimally processed pomegranate arils. Ergun and Ergun (2009) investigated the efficacy of honey dip treatments in maintaining the fresh-like quality and extending the shelf life of minimally processed pomegranate arils (cv. Hicaznar) at 4 °C. Honey treated arils exhibited better aroma and flavour during 9 day storage period than the untreated arils. Honey treatments were also effective in delaying aril softening and inhibiting microbial growth and enzymatic browning.

Other physical treatments including low temperature storage, modified atmosphere packaging, heat treatments, gamma radiation and UV-C light treatments have also been explored for use in retarding tissue softening, cut surface browning, moisture loss and microbial growth. Maghoumi et al. (2013) reported a reduction in mesophilic bacteria, mould and yeast growth in minimally processed pomegranate arils treated with hot water (HW) alone or in combination with UV-C and high oxygen (HO) atmospheres. Although RR was highest under HO, all the treatments did not significantly alter product RR. López-Rubira et al. (2005) also investigated the effect of UV-C light on quality and shelf life of minimally processed pomegranate arils. The study however found inconclusive results regarding the effect of UV-C on microbial growth. Modified atmosphere packaging (MAP) has seen increasing application in the past few years as a result of increase in minimally processed fresh produce. The next section focuses on MAP in line with the scope of the thesis

Modified atmosphere packaging (MAP)

Fundamentals of MAP

Modified atmosphere packaging (MAP) is a technique in which the normal composition of air (O_2-21%; CO_2-0.01%; N_2-78%) around a product is altered within a package (Al-Ati and Hotchkiss, 2002; Waghmare and Annapure, 2013). This is achieved by hermetically sealing actively respiring fresh produce within a polymeric film under normal air conditions and allowing the atmosphere to be modified naturally by the interplay of produce respiration and film permeability or by actively replacing the atmosphere within a package with a desired gas mixture (Kader and Watkins, 2000; Al-Ati and Hotchkiss, 2002; Rico et al., 2007; Mangaraj et al., 2009; Brandenburg and Zagory, 2009).

Low levels of O_2 (1-5%) and high levels of CO_2 (3-10%) are desirable under MAP to reduce RR, delay senescence and extend the shelf life of fresh produce (Jacxsens et al., 2002; Rico et al., 2007; Sandhya, 2010). MAP also improves moisture retention, which can have a greater influence on preserving quality than levels of O_2 and CO_2 (Mangaraj et al., 2009). The lack of continuous and strict control of gases in MAP compared to controlled atmosphere (CA) conditions limits its use to

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temporary storage and/or transportation of fresh and minimally processed produce (Brandenburg and Zagory, 2009). It is extensively applied to retail level packages but is also used in bulk packaging containers and as individual produce coatings.

Active modified atmosphere packaging, achieved by flushing an initial desired amount of gas into a package, can provide an earlier state of equilibrium and help to keep an adequate atmosphere for longer. (Gorny, 2003; Kader and Watkins, 2000). Passive or commodity generated MAP on the other hand takes a long time to establish equilibrium because it depends on gradual modification of atmospheres within a package by the produce. Cameron et al. (1995) suggested that it can take up to 2 to 3 weeks at low temperatures depending on produce RR and the available gaseous space within the package. During the period before equilibrium is attained, the product is exposed to non-optimal atmospheres and continues deteriorating (Rodov et al., 2007). Equilibrium atmospheres in litchi cultivars 'Mauritius' and 'McLeans Red' packaged under active MAP were established almost from the first day of storage, whereas those in passive MAP were established 6 to 10 days after packaging (Sivakumar et al., 2008). Active modified atmospheres are especially useful for non-climacteric products such as pomegranate which have a low respiratory intensity and therefore take long to reach atmospheric equilibrium. In addition, the beneficial effects of active MAP can be utilised in fresh cut/minimally processed products which have a relatively short marketing period (Bai et al., 2003).

Initial atmosphere composition does not, however, affect the final steady state or equilibrium atmosphere attained within a package, but only determines the time necessary to reach equilibrium. Costa et al. (2011) studied the effects of passive and active MAP (5 % O_2 +3% CO_2, 5 % O_2 +3% CO_2, 5 % O_2 +3% CO_2) on quality retention of table grapes and reported similar O_2 and CO_2 levels at equilibrium in films with similar barrier properties irrespective of initial gas composition. The further the initial O_2 and CO_2 levels are from the steady state values achievable within a package, the longer it will take to reach equilibrium. Therefore, if a package is flushed with an initial atmosphere corresponding to the steady state gas levels attainable within a given packaging film, there will be little or no change in package gas composition. Fresh-cut honeydew packaged under active MAP (5 % CO_2 + 5 % CO_2) and stored at 5 °C retained a steady atmosphere immediately after packaging with proportions of gas similar to those initially flushed into the package during the entire storage period (Bai et al., 2003). In contrast, O_2 gradually decreased and CO_2 increased in passively modified atmospheres and did not reach equilibrium by the end of the storage period.

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Although active MAP is commonly proposed to be more beneficial than passive MAP, variable results on its effect on product quality have been reported in literature. Cantaloupes stored in rapidly flushed modified atmospheres maintained better quality in terms of colour retention, visual quality and microbial quality compared to those stored under passive MAP and air (Bai et al., 2003). In addition, MAP was shown to be effective in inhibiting an increase in RR, with samples stored under active MAP maintaining the lowest RR throughout the storage period. RR, browning and microbial growth was suppressed in fresh-cut cabbages packaged in perforated film packages and oriented polypropylene under initially modified oxygen atmospheres (5 and 8% O_2) at 5 °C (Hu et al., 2007). Microbial growth in minimally processed 'Wonderful' pomegranate arils was suppressed by packaging under active MAP in enriched CO_2 (15 and 20 %) atmospheres (Hess–Pierce and Kader, 1997). Shelf life of minimally processed pomegranate arils was suggested as 16 days when stored under CO_2 enriched atmospheres (20%) at 5 °C. In contrast, studies by Sivakumar et al. (2008) on the effects of passive and active atmospheres on oxidation enzymes and quality attributes of litchi cultivars 'Mauritius' and 'McLeans Red', packaged in 3 different lidding films at 2 °C, showed that initial atmospheres did not have significant effects on the quality of the packaged product. Instead, quality was influenced by the type of lidding film. Despite equilibrium atmospheres being attained earlier in the initially flushed packages, polyphenol oxidase activity, anthocyanin levels, membrane integrity, browning index, flavour and overall appearance were all not affected by initial gas composition. Costa et al. (2010) also reported that although RR was generally lower in table grapes packaged under active MAP across all packaging films, active MAP did not seem to promote any further enhancement in grape shelf life. In fact, samples under active MAP maintained an overall quality score lower than that of similar samples stored under passive MAP. This was attributed to evaporation of moisture from product during gas flushing and sealing. The study, however, showed that packaging films significantly prevented product decay and promoted substantial shelf life prolongation compared to unpackaged product. Ayhan and Estürk (2009) studied the effect of initially modified atmospheres, including low and super atmospheric oxygen on the quality and shelf life of pomegranate arils (cv. Hicaznar), and also reported slight or no significant changes in chemical and physical attributes of the arils despite equilibrium atmospheres being established earlier in active MAP. These findings also seem to suggest that product response to active MAP is dependent on product type and cultivar, gas proportions used and storage conditions (Artés et al., 2006)

The success of MAP in maintaining quality of packaged produce depends on the creation of suitable equilibrium or steady state conditions within a package (Mangaraj et al., 2009). Steady state or

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equilibrium conditions are achieved when the rate at which O_2 allowed through a package is offset by the rate of consumption by the product. Similarly, CO_2 must be vented out of the package to offset the production of CO_2 by the product (Kader, 2002). Levels of O_2 and CO_2 at equilibrium are determined by the interaction of the fresh product respiratory behaviour, packaging film permeability characteristics and the storage temperature (Kader, 2002; Mangaraj et al., 2009; Caleb et al., 2012a; Charles et al., 2003).

The wide variability in respiratory behaviour of minimally processed fresh products, and the limited availability of packaging film with suitable permeability characteristics, poses a great challenge in establishing suitable equilibrium atmospheres (Artés et al., 2006). In addition, the limits of tolerance to low O_2 and/or elevated CO_2 varies in different products, often resulting in anaerobic respiration and production of ethanol (Soliva-Fortuny and Martín-Belloso, 2003; Artés et al., 2006). Recommendations of CA and modified atmosphere (MA) conditions for a number of fruits and vegetables have been made and some have been adapted for minimally processed products (Table 3). However, the optimum atmosphere varies with product cultivar, growing region and storage time before processing (Gorny, 2003).

Gases used in MAP

Oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) are the most common gases used in MAP. Other gases including carbon monoxide, sulphur dioxide and noble gases are also used but to a limited extent. A single gas or combination of gases can be used in MAP depending on the product to be packaged (Velu et al., 2013). Oxygen and carbon dioxide are both biologically active molecules that play dynamic roles in the primary and secondary metabolic processes in plant organs (Artés et al., 2006). Nitrogen on the other hand is used as a filler gas to avoid package collapse (Sandhya, 2010). The role of these gases is discussed further in the following sub-sections.

Oxygen

Oxygen makes up about 21% of normal air. It is a reactive gas that is responsible for most of the degradative processes that occur in fresh produce. It also encourages the growth of aerobic micro-organisms involved in food spoilage (Sandhya, 2010). It is therefore the objective of most MAP systems to maintain oxygen levels as low as possible. Slight decreases in O_2 are usually not effective in imparting the beneficial effects of MAP and must be lower than 10-12 % (Artés et al., 2006). On the other hand, very low O_2 levels (< 2%) alone or in combination with very high CO_2 levels (> 20%) result in injurious effects to fresh products, such as off-flavour development, due to

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anaerobic respiration, initiation or aggravation of physiological disorders and in some cases, decay (Gorny, 2003; Artés et al., 2006).

Super atmospheric oxygen atmospheres (O_2 > 21%) have been explored as alternatives to low oxygen atmospheres in MAP of fresh products because of their ability to prevent anaerobic fermentation, inhibit enzymatic discolouration and microbial growth (Jacxsens et al., 2001). Studies by Jacxsens et al. (2001) on the effect of high oxygen atmospheres on microbial and sensory properties of fresh cut vegetables, grated celeriac, shredded chicory and mushroom showed that high oxygen atmospheres of 80 and 92% inhibited the growth of moulds *A. flavus* and *B. cinerea.* Studies by Ayhan and Esturk (2009) reported an increase in antioxidant activity in arils stored under super atmospheric oxygen (70%) atmospheres compared to those stored under low oxygen (5%) and in normal air. The arils under these atmospheres also had the lowest aerobic mesophilic bacteria counts compared to those in other MAPs.

Carbon dioxide

Carbon dioxide (CO_2) makes up only 0.03% of the normal air. It is a colourless gas, with a slightly offensive smell at high concentrations (Sandhya, 2010). It is desirable in high concentrations (10-20%) in MAP because it plays antagonistic roles in respiratory degradative processes by slowing down respiration rate, although its effects are not as drastic as reduced oxygen (Brandenburg and Zagory, 2009). Studies by Hess-Pierce and Kader (1997) showed that CO_2 enriched atmospheres (20% CO_2 + Air) extended the postharvest life of minimally processed pomegranate arils to 16 days at 5 °C. Irtwange (2006) also recommended 15-20% CO_2 as conditions ideal for best quality of minimally processed pomegranate arils. Carbon dioxide at high concentrations also reduces the sensitivity of plant tissue to the ripening hormone ethylene; this is especially useful in climacteric fruit which is sensitive to ethylene (Artés et al., 2006).

Carbon dioxide is highly soluble at high concentrations and low temperatures forming carbonic acid when it dissolves in water (Sandhya, 2010).The accumulation of carbonic acid accumulation n tissues of minimally processed products is in part responsible for the bacteriostatic properties of CO_2 (Church and Parsons, 1994; Sandhya, 2010). However, it also causes changes in organoleptic properties of some minimally processed products. Solubility of CO_2 also results in package collapse in some instances due to a change in the volume of gas within the package (Church and Parsons, 1994).

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Nitrogen

Nitrogen is a colourless, tasteless, odourless, inert gas that makes up the largest proportion of the atmosphere at 79% (Sandhya, 2010). It has a relatively lower permeability across films compared to oxygen and carbon dioxide (Church and Parsons, 1994). It is, therefore, useful as a filler gas to limit package collapse and to exclude more active gases (Church and Parsons, 1994; Brandenburg and Zagory, 2009). Nitrogen displaces O_2 and therefore helps to retard oxidative processes as well as growth of aerobic spoilage microorganisms but does not prevent the growth of anaerobic bacteria (Sandhya, 2010).

Several studies have examined the use of 100% N_2 packaging as a means of modified atmosphere packaging for extending the shelf life of fresh cut products (Koseki and Itoh, 2002; Ayhan and Estürk, 2009; Ahmed et al., 2011). 100% N_2 was used as packaging atmosphere for fresh cut vegetables (lettuce and cabbage) stored at 1, 5 and 10 °C for 5 days (Koseki and Itoh, 2002). Although there was initially no O_2 and CO_2 in these packages, the levels of these gases increased to 1.2-5% and 0.5-3.5% respectively, by the end of the storage period due to permeability of the packaging film. The N_2 atmospheres also maintained quality and appearance of the fresh cut vegetables stored at 1 and 5 °C by the end of the storage period. Firmness, colour and chemical properties were maintained and shelf life extended in persimmon fruit packaged in 100% N_2, stored at 0 °C and 85-95 % RH for 90 days (Ahmed et al., 2011). Studies by Ayhan and Estürk (2009) in which 100% N_2 was used as the packaging atmosphere for minimally processed pomegranate arils (cv. Hicaznar) also showed that although there was initially no O_2 in the packages, it increased to 2.7 % by day 18 of storage. Although N_2 was not found to be effective in inhibiting the growth of aerobic microbes, arils still maintained their colour, taste and texture.

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Modified atmosphere packaging of minimally processed pomegranate arils

The beneficial effects of MAP combined with low temperature storage have been extensively utilised in fresh-cut fruits and vegetables due to their susceptibility to suffer hastened deterioration (Gorny, 1997; Bai et al., 2001; Artés et al., 2006). MAP has been successfully used to extend the shelf life of minimally processed fresh pomegranate arils (Gil et al., 1996; Sepulveda et al., 2000; López-Rubira et al., 2005; Palma et al., 2009; Caleb et al., 2012a). MAP technology extends shelf life and maintains quality of fresh-cut produce by slowing down physiological processes, reducing moisture loss, retarding development of physiological disorders and proliferation of spoilage microbes (Artés et al., 2006).

A significant reduction in RRs was observed in minimally processed pomegranate arils (cv. Hicaznar) stored in modified atmospheres (2 and 10% O_2, and 10 and 20% CO_2) at 4 °C compared to those stored under normal air conditions (Ersan et al., 2000). The lowest aril RR values (1.5 mL O_2/kg h and 0.52 ml CO_2/kg h) were observed at atmosphere combinations 2% O_2 + 10% CO_2. The ability for modified atmospheres to slow down the rate of physiological processes, including RR, has also been noted in other minimally processed fruit and vegetables. Respiration rates of peach slices were reduced in low O_2 and/or elevated CO_2 atmospheres (Gorny et al., 1999). Studies by Rattanapanone et al. (2001) on the effects of modified atmospheres (4 % O_2 and 10% CO_2) on RR and quality changes in 'Kent' and 'Tommy Atkins' mango slices at 5 and 10 °C, also showed suppression of RR at 10 °C. RR values in these samples increased only slightly (17%) by day 5 of storage compared to a 66% rise in air-stored samples. In addition, the marketable period of mango slices under MAP conditions at both temperatures was extended with samples retaining their aroma and colour.

Studies by Gil et al. (1996) which investigated the effects of storage temperature, pre-treatments and MAP on keeping quality of minimally processed pomegranate arils at 1, 4 and 8 °C also revealed that arils under MAP in oriented polypropylene film maintained their chemical quality attributes (total soluble solids and titratable acidity) and colour by the end of a 7 day storage period. In contrast, chemical attributes in control arils packaged in perforated orientated film (POPP) which allowed free diffusion of gases increased with storage. Unpackaged arils also suffered dehydration and shrivelling. The study recommended washing in chlorine followed by antioxidant solution and MAP at 1 °C as conditions ideal for maintaining quality of arils. Physico-chemical attributes, titratable acid, total soluble solids, colour and sensory quality attributes, aroma, firmness, appearance and taste were preserved in 'primosole' pomegranate arils packaged under passive MAP

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in polypropylene film at 5 °C (Palma et al., 2009). Although the study showed that in-package ethylene levels also increased with storage, they did not exceed 0.12 ppm and did not induce senescence.

While the beneficial effects of atmosphere modification are many, cases have been reported in which CA and MA storage has resulted in detrimental effects. These detrimental effects are mainly caused by too low O_2 (< 2%) or too high CO_2 (> 10) in the storage atmosphere (Soliva-Fortuny and Martín-Belloso, 2003), and are further influenced by type of commodity and physiological age, storage temperature and duration, among other factors (Kader, 2002). Initiation or aggravation of physiological disorders, irregular ripening and development of off-flavours are some of the most common detrimental effects of atmosphere modification (Kader, 2002). Moisture loss in MAP packaged shiitake mushroom was significantly suppressed. However, mushrooms packaged under active MAP (15 or 25% O_2) developed off-flavour after 12 d of storage with O_2 concentrations falling below 5% (Antmann et al., 2008). Agar et al. (1999) studied the influence of low O_2 atmospheres on the respiratory metabolism of fresh cut kiwi slices and also reported a rise in acetaldehyde and ethanol contents after 12 d storage, especially in slices kept under 0.5 kPa O_2. Qi et al. (1998) reported rapid depletion of O_2 and onset of anaerobic respiration in fresh-cut honeydew melons stored in modified air (2% O_2 and 10% CO_2) at 10°C. Hess Pierce and Kader (2003) also reported accumulation of fermentative metabolites in 'Wonderful' pomegranate stored in CO_2 enriched atmospheres at 5, 7.5 and 10 °C. The high CO_2 concentrations caused anaerobic respiration, even though off-flavours were not detected in the produce. The study recommended 5 kPa O_2 + 15 kPa CO_2 as the optimal CA conditions for 'Wonderful' pomegranate at 7.5 °C for 5 months. Produce response to MAP depends on a number of factors including variety, growing conditions, harvesting system, physiological age, postharvest handling, gas composition, storage temperature and time (Artés et al., 2006). These factors explain the wide variability of results and recommendations in literature on the response of a given product to MAP conditions.

Conclusions

Minimally processed pomegranate arils provide a more convenient and 'ready to eat/use' product than the intact fruit. The arils, however, suffer rapid deterioration due to enhanced biochemical and enzymatic processes, moisture loss and microbial decay. Chemical and physical preservation treatments including washing with chlorine based solutions and antioxidant solutions, low temperature storage, thermal treatments, irradiation and UV-C treatments and modified atmosphere packaging have been successfully used to maintain quality and extend shelf life of minimally

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processed fresh pomegranate arils. Storage temperature is the most important factor in controlling respiratory activity, transpiration rate and microbial quality in fresh produce and therefore needs to be maintained at optimum. Temperatures between 0 and 5 °C have been suggested as optimal for storage of pomegranate arils, since arils do not suffer chilling injury under these conditions. Modified atmosphere packaging, combined with low temperature, has been reported to slow down physiological processes associated with senescence, reduce moisture loss and delay microbial growth. The success of MAP in maintaining quality of fresh produce, however, depends on rapid creation of suitable equilibrium atmospheres which is achieved by proper matching of film permeability characteristics, produce RRs and storage temperature. For a wide range of produce, active MAP has been shown to be more beneficial for maintaining quality and extending shelf life of minimally processed fresh produce than passive MAP because it allows immediate establishment of equilibrium atmospheres. Nonetheless, research on the effects of active MAP on physiological and biochemical processes, and quality attributes of pomegranate is still limited.

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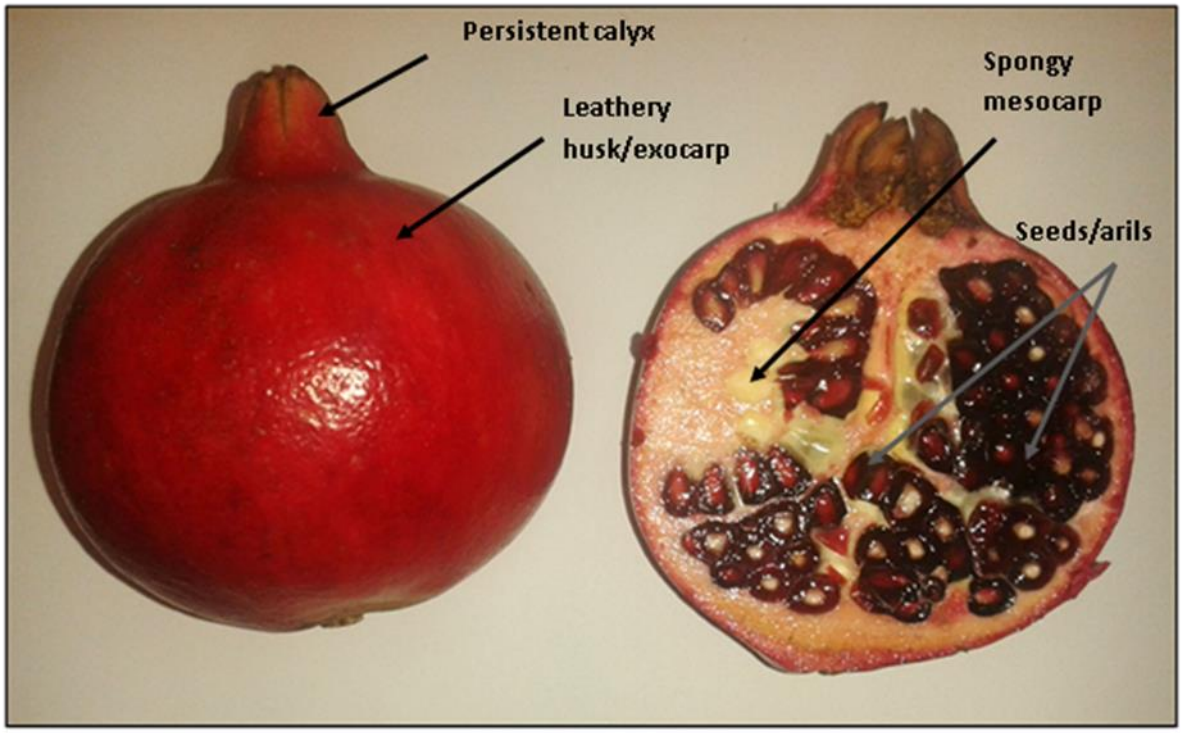

Figure 1. A typical anatomic structure of pomegranate fruit

Table 1. Factors affecting respiration rate of fresh horticultural products (Caleb et al., 2013)

Intrinsic factors	**Extrinsic factors**
Type of produce	Temperature
Cultivar	Level of O_2
Growing season	Level of CO_2
Farming system	Storage duration
Growing region	Extent of minimal processing (type and size of cuts)
Produce maturity level	Pre-treatments

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Table 2. Postharvest treatments applied to minimally processed produce

Type of treatment	Examples of treaments	Type of produce	References
Anti-browning agents	citric acid, ascorbic acid, tartataric acid, lactic acid, malic acid, phosphoric acid, hydrochloric acid, L-cysteine, EDTA, sporix, cyclodextrins, 4-hexylresorcinal, sodium chloride	Apple slices, Pomegranate arils, Avocado slices, fresh-cut lettuce, potato slices, fresh-cut melon slices	Dorantes-Alvarez et al., 1998; Sepúlveda et al.,2000; Rico et al., 2007; Mosneaguta et al., 2012; Mahajan et al., 2014; Pace et al., 2014
Antimicrobial agents	Benzoic acid, Sodium benzoate, Potassium sorbate, Propionic acid, organic acids (lactic acid, citric acid acetic acid tartaric acid), essential oils	Pomegranate arils; fresh-cut pineapple; fresh-cut melons	Gil et al.,1996; Sepúlveda et al., 2000; Azarakhsh et al., 2013; Mahajan et al., 2014
Texture enhancers	Calcium chloride, calcium lactate	Fresh-cut mangoes, apple slices	Artés et al., 2009; Wu et al., 2012; Ngamchuachit et al., 2014
Edible films and coatings	Honey, aloe-vera, chitosan, whey protein	Pomegranate arils; fresh-cut melons, plums, apple slices	Olivas and Barbosa-Cánovas, 2005; Qi et al., 2011; Martínez-Romero et al., 2013, Ghasemnezhad et al., 2013; Ahmed and Butt, 2014
Physical treatments	MAP, Thermal treatments, Irradiation, UV-C treatments	Pomegranate arils, table grapes, apple slices	Rico et al., 2007, López-Rubira et al., 2005 Costa et al., 2011; Maghoumi et al., 2013; Siroli et al., 2014.

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Table 3. CA and MA recommendations for fresh-cut and minimally processed fruit (Gorny, 2003)

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

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Chapter 3
Effects of pre-treatment with citric acid and storage conditions on respiration and transpiration rate of pomegranate arils (cv.Wonderful)

Abstract

Postharvest preservation of fresh produce requires maintaining optimum storage conditions that keep physiological processes such as respiration, moisture loss and microbial growth at a minimum. This study investigated the effects of citric acid pre-treatment (10g/L) and storage conditions (temperature and relative humidity) on transpiration rate (TR), respiration rate (RR), and physicochemical attributes of minimally processed pomegranate arils (cv. Wonderful). TR of citric treated and non-treated arils was determined over a 9-day storage period at 5, 10 and 15 °C, and 76, 86, 96% relative humidity (RH), while RR was measured over a 5-day storage period at 5, 10, 15 and 20 ± 2 °C, and 90 ± 2 % RH. Citric acid treatment did not have a significant effect ($p > 0.05$) on aril TR while both storage temperature and duration had significant effects. Regardless of pre-treatment, lowest TR (1.26 g/kg/day) occurred at storage conditions characterised by low temperature and high RH (5 °C, 96 % RH), while the highest TR (24.77 g/kg/day) occurred at high temperature and moderate RH storage condition (15 °C, 76% RH). The interaction of storage temperature, duration and pre-treatment had a significant ($p < 0.05$) effect on RR. Lowest RR was maintained at 5 °C decreasing from 4.75 to 2.82 mL CO_2 kg^{-1} h^{-1} and 4.86 to 2.71 mL CO_2 kg^{-1} h^{-1} for citric acid treated and non-treated arils, respectively, over the 5 day storage period. Citric acid treatment had no significant effect on RR of arils stored at 5 and 10 °C; however, the treatment was effective in reducing RR of arils stored at higher temperatures after 3 days of storage. No significant change was observed in redness colour and firmness of arils across all the treatments over the 5 day storage period. In contrast, TSS:TA ratio increased significantly ($p < 0.05$) with storage across all the treatments.

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Introduction

Minimally processed fresh fruit and vegetables have a physiology that is different from that of intact produce, characterised by enhanced respiration rate, enzymatic and microbial activity and moisture loss (Rico et al., 2007; Barbosa et al., 2011). These responses result from tissue wounding, and in some cases, from the removal of protective epidermal tissue during minimal processing (Waghmare and Annapure, 2013). Respiration is a degradative process that involves the oxidative breakdown of complex organic compounds such as carbohydrates, lipids and organic compounds into simpler molecules, including CO_2 and water with the release of energy (Fonseca et al., 2002). This results in weight loss, deterioration in overall quality and senescence of the produce (Rico et al., 2007). Rate of respiration is associated with the rate at which compositional changes take place within a plant product. It is therefore a useful measure of fresh product potential shelf life (Kader et al., 1989; Martínez-Ferrer et al., 2002).

Most minimally processed fruit and vegetables are characterised by an increase in RR (Tovar et al., 2001; Martínez-Ferrer et al., 2002). In contrast, RR of minimally processed pomegranate arils has been reported to be lower than that of intact fruit. This has been attributed to the minimal injury suffered in arils and the presence of numerous micro pores on the fruit husk which allows easy diffusion of gases (Garcia et al., 2000; Caleb et al., 2012). Caleb et al. (2012) reported the RR of pomegranate arils (cvs. Acco and Herskawitz) stored at 5, 10 and 15 °C as ranging from 2.51 to 7.59 mL CO_2 kg^{-1} h^{-1} and 2.72 to 9.01 mL CO_2 kg^{-1} h^{-1}, respectively. These RR values were two to three times lower than those observed for whole fruit at similar temperatures. Ersan et al. (2010) reported a minimum RR of 0.5 mL CO_2 kg^{-1} h^{-1} for pomegranate arils (cv. Hicaz) stored under modified atmosphere condition 2% O_2 + 10% CO_2 at 4 °C. While RR of pomegranate arils (cv. Mollar Elche) stored at 5 °C in air was about 1.2 mL CO_2 kg^{-1} h^{-1} (López-Rubira et al., 2005). These differences in aril RRs among different pomegranate cultivars and under different storage conditions warrant further studies to characterise the RR of specific cultivars.

Minimally processed pomegranate arils also suffer moisture loss and shrivelling due to removal of the outer protective husk of the whole fruit and hence exposure of arils (Caleb et al., 2013a). Sepúlveda et al. (2000) reported significant dehydration in unpackaged pomegranate arils and in those packaged in perforated polyethylene bags compared to those packaged in non-perforated semi-permeable film. Similarly, significant moisture loss and shrivelling was observed in unpackaged pomegranate arils stored at 1, 4 and 8 °C (Gil et al., 1996). The rate of moisture loss (transpiration rate) is driven by the water vapour pressure deficit between a product and its

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environment (Artés and Tomás-Barberan, 2000). An unpackaged product will, therefore, suffer more moisture loss compared to a packaged one. Temperature and RH play complementary roles in regulating the vapour pressure difference between a product and its environment (Watada et al., 1996; Artés and Tomás-Barberan, 2000) and, subsequently, product transpiration rate (Caleb et al., 2013a). Thus, the objective in minimising moisture loss in fresh-cut produce is to maintain low temperatures and a high RH, without increasing microbial development growth and decay (Artés and Tomás-Barberan, 2000).

Low temperature storage is used in combination with modified atmosphere packaging (MAP) to slow down RR (Saltveit, 2003; Torrieri et al., 2010), moisture loss (Habibunnisa et al., 2000) and microbial growth (Kader, 1995; Sivakumar et al., 2008), leading to shelf life extension of minimally processed fresh produce. Sanitizing agents are also used to reduce produce initial microbial load and to enhance the beneficial effects of MAP (Sepulveda et al., 2000). Citric acid is a naturally occurring organic acid that is commonly used as an antimicrobial agent and preservative (Pao and Petracek, 1997; Rico et al., 2007). It has been used as a pre-treatment in minimally processed pomegranate arils. However, the effect of citric acid on physiological response of arils has not been reported (López-Rubira et al., 2005; Ayhan and Eştürk, 2009). Therefore, the objective of this study was to investigate the effects of storage temperature, relative humidity and citric acid pre-treatment on the physiological responses (RR and TR) and quality of minimally processed pomegranate arils (cv. Wonderful).

Materials and Methods

Sample preparation

Pomegranate fruit (cv. Wonderful) was obtained at commercially ripened stage from Houdconstant packhouse in Porterville, Western Cape (33°01′00″S, 18°59′00″E), South Africa. Fruit were then transported to the Postharvest research laboratory at Stellenbosch University, where they were stored at 7.5 °C until the next day. Damaged and cracked fruit were discarded and those free from visible defects were opened using sharpened knives and the arils were carefully extracted manually. Extracted arils were collected on a tray and mixed to ensure uniformity and then divided into two equal portions. One portion of the arils was dipped in citric acid (52 mM) for 1 minute (Gil et al., 1996) followed by air drying on sterile paper towels, while the other portion was left untreated. Processing and pre-treatment of arils was done in a sterilized cold room at 7 °C.

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Transpiration rate of citric acid treated and non-treated pomegranate arils was subsequently determined at combinations of temperature (5, 10 and 15 °C) and relative humidity (76, 86 and 96 % RH) over a 9-day storage period. In addition, respiration rate and physico-chemical attributes of arils were determined at 5, 10, 15 and 20 ± 2 °C over a 5-day storage period.

Transpiration rate

Transpiration rate was determined by the weight loss approach (Caleb et al., 2013a). The experimental setup consisted of sterile airtight plastic containers in which four Petri dishes containing approximately 15 g of arils each, were placed and stored at 5, 10 and 15 °C with different combinations of relative humidity (76, 86 and 96 % RH) inside the containers, which was independently controlled and maintained using saturated salt solutions of sodium chloride, potassium chloride and potassium nitrate, respectively. Petri dishes containing the arils where kept above the salt solutions within the containers by mounting them on a wire mesh support (Figure 1). Temperature and RH data loggers (Tinytag, TV-4500, Hastings Data Loggers, Australia) were placed inside the test containers to monitor RH. Both temperature and RH was kept fairly constant during the storage duration. Due to the small quantity of arils used in this experimental set up, physicochemical attributes were not measured, but visual observation for decay was conducted. Weight loss measurements were discontinued for arils that had visual signs of decay or mould growth.

Arils were weighed on a daily basis for 9 days using an electronic balance with ± 0.01g accuracy (Mettler, Telodo, Switzerland). Transpiration rate (TR) was calculated from the changes in weight of the arils over time and expressed as weight change (g/kg/day) using equation (1):

$$TR = \frac{Mi - M}{t\left(\frac{Mi}{1000}\right)} \quad (1)$$

where; TR is the transpiration rate (g/kg/day); Mi is the initial mass of samples (g); M is the mass of sample (g) after time t (days).

Respiration rate

Respiration rate was determined using the closed system method, by measuring carbon dioxide accumulation and oxygen depletion in hermetically sealed jars containing arils (Fonseca et al., 2002). RR was determined in triplicate at four temperatures 5, 10, 15 °C and room temperature (20 ± 2 °C). Approximately 150 g per replicate of both the citric treated and non-treated arils was separately weighed in to 1100 mL airtight glass jars using an electronic balance (Mettler, Telodo,

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Switzerland). The glass jars were hermetically sealed by incorporating Vaseline petroleum jelly in the gap between the lid and the jar. Samples were equilibrated at the respective temperatures for an hour prior to the experiment. Gas samples were drawn at hourly intervals for five hours per day through a rubber septum and the gas composition monitored by an O_2/CO_2 gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark) as shown in figure 2. Measurements were repeated over a five day storage period in order to determine the effect of storage duration on pomegranate aril respiration rate. Glass jars were left slightly open overnight to avoid build-up of sub-optimal gases over the 5 day storage period (Caleb et al., 2012).

Respiration rate was calculated by fitting experimentally obtained data using equations 2 and 3:

$$y_{O_2} = y_{O_2}^{i} - \frac{R_{O_2} W}{V_f}(t - t_i) \times 1000 \quad (2)$$

$$y_{CO_2} = y_{CO_2}^{i} + \frac{R_{CO_2} W}{V_f}(t - t_i) \times 1000 \quad (3)$$

Where R_{O_2} and R_{CO_2} is the oxygen and carbon dioxide respiration rate (RR) in (mL kg^{-1} h^{-1}) respectively; $y^i o_2$ *and* yo_2 is oxygen concentration (%) at the initial time t_1 (hours, h) (time zero) and at time t (h), respectively; and $y^i co_2$ and yco_2 is the carbon dioxide concentration (%) at the initial time t_1 (hours, h) (time zero) and at time t (h), respectively. W is the total weight of product (kg) and V_f is the free volume inside jar (mL); determined by subtracting volume of product from the total volume of the glass jar.

Aril colour and texture

Physicochemical properties of the arils were measured at the beginning and end of the RR experiment. Aril colour measurements were performed using a colorimeter (Minolta Chroma Meter, CR-300, Minolta, Japan). Approximately 30 g of arils per replicate were weighed on to a Petri dish and five readings of each colour index in the CIE L^* (Lightness/darkness), a^* (redness/greenness) and b^*(yellowness/blueness) were taken. Colour parameter Chroma (C^*) and the hue angle ($h°$) were calculated according to the following equations (Pathare and Opara, 2013):

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (4)$$

$$h° = arctan\,(b^*/a^*) \quad (5)$$

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Aril hardness was determined using a texture analyser (Tensilon mode UTM-4L, Toyo Measuring Instruments Co., Tokyo). Arils were crushed using a 35 mm diameter cylindrical probe. Maximum compression force (N) was used as a measure of aril hardness. Test speed of 1.0 mm/s and penetration distance of 9.5 mm was used. Each aril was tested individually and an average of 20 arils was tested for each treatment.

Total soluble solids (TSS), titratable acidity (TA) and pH

Arils were juiced separately for citric acid treated and non-treated air at the start and end of the RR experiment using a LiquaFresh juice extractor (Mellerware, South Africa). Juice was used to determine pH using a pH meter (Crison, Barcelona) and TSS was measured using a digital refractometer (Atago, Tokyo) and expressed as °Brix. Titratable acidity (TA) was measured by titration to an endpoint of pH 8.2 using a Metrohmn 862 compact titrosampler (Herisau, Switzerland) and expressed as g of citric acid per 100g of juice. All values are presented as mean ± standard deviation (SD).

Statistical analysis

A factorial analysis of variance (ANOVA) was performed to determine the effects of temperature, citric acid pre-treatment and storage duration, and means separated using Fisher's least significant differences (LSD) test at 95% confidence interval using Statistica software (Statistica 10.0, Statsoft Inc., USA). All values are presented as mean ± standard deviation (SD).

Results and Discussion

Transpiration Rate

Storage condition (combination of temperature and RH) had a significant effect on TR of pomegranate arils (Fig. 3). TR was lowest in arils stored at 5 °C and 96 % RH (1.26 g/kg/day) and highest in samples at 15 °C and 76% RH (24.77 g/kg/day). Citric acid pre-treatment, however, had no significant effect on aril TR (data not shown, refer to Appendix A, Figure 1). Results from this study are comparable to those reported by Caleb et al. (2013a) and Aindongo et al. (2014). Caleb et al. (2013a) reported that TR of pomegranate arils (cv. Acco) stored at 5, 10 and 15 °C and 76, 86 and 96 % RH ranged from 1.14 to 16.75 g/kg/day, while Aindongo et al. (2014) observed that TR of arils and aril-sacs ranged from 1.42 to 15.23 g/kg/day and 0.63 to 9.95 g/kg/day, respectively, under similar storage conditions.

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Other studies have shown that RH is the variable with the greatest influence on TR in pomegranate arils (Caleb et al., 2013a; Aindongo et al., 2014). Caleb et al. (2013a) reported a decrease in TR by up to 83.5% at 5 °C when RH was increased from 76 to 96% RH, compared to a 68.9% decrease in TR when temperature was reduced from 15 to 5 °C.

Aril weight loss was lowest at 96 % RH across all the temperature regimes with almost 100% aril weight retained (Fig. 4). Arils at 10 and 15 °C, however, developed mould before the end of the 9 d storage period. Weight loss in arils stored at 5 °C and 76 % RH was up to 12% by day 9 of storage and the arils appeared shrivelled. Previous studies have shown that pomegranate arils are susceptible to moisture loss and shrivelling (Sepulveda et al., 2009; Gil et al., 1996). Unpackaged pomegranate arils stored at 8, 4 and 1 °C suffered shrivelling and weight loss, with almost half of the water present in the arils lost by day 7 of storage (Gil et al., 1996). From our study 5 °C and 96% RH was found to be the best storage condition with arils suffering very minimal weight loss and remaining free from mould.

Respiration rate

RR increased significantly with increase in storage temperature ($p < 0.05$), and was lowest at 5 °C across all the treatments (Fig 5). It decreased from 4.75 to 2.86 mL CO_2 kg^{-1} h^{-1} and 4.86 to 2.7072 mL CO_2 kg^{-1} h^{-1} for citric acid treated and non-treated arils, respectively, at the end of the 5 day storage period. This observation is consistent with literature, which showed that an increase in temperature promotes increase an in metabolic activity (Barbosa et al., 2011). Results from this study are in agreement with values reported by Caleb et al. (2012) who found an average RR of 2.5 and 2.72 mL CO_2 kg^{-1} h^{-1} for pomegranate arils 'Acco' and 'Herskawitz' stored at 5 °C. Similar results were also reported by Hess-Pierce and Kader (1997) for 'Wonderful' pomegranate arils under CO_2 enriched atmospheres, with RR of the arils ranging from 1.5 - 3 mL CO_2 kg^{-1} h^{-1} and 3 - 6 mL CO_2 kg^{-1} h^{-1} at 5 and 10 °C, respectively. In contrast, lower RRs (1.15 and 2.11 mL CO_2/kg h) were reported by López-Rubira et al. (2005) for the early and late harvested pomegranate arils (cv. Mollar Elche) at 5 °C. These differences may be attributed to differences in cultivars, maturity stage, growing and storage conditions (Lopez-Rubira et al., 2005; Ersan et al., 2010; Caleb et al., 2012).

Temperature is an important factor influencing the rate of physiological activities in minimally processed products (Artés and Tomás-Barberan, 2000; Barbosa et al., 2008). Every 10 °C increase in temperature has been reported to result in a two to three fold increase in RR of most minimally processed products (Iqbal et al., 2008). Studies by Torrieri et al. (2010) on the effects of

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temperature, O_2 and CO_2 on the RR of minimally processed broccoli also showed that temperature had a greater effect on RR than gas composition. The authors reported that RR of air-stored minimally processed broccoli increased by 84% with increase in temperature from 3 to 20 °C compared to a 35% increase in RR of arils stored at constant temperature and 1% O_2. Similarly, Fonseca et al. (2002) reported that temperature reduction from 20 to 1 °C resulted in 90% reduction in RR of 'Galega' Kale compared to 80% reduction when the atmosphere was modified to 1% O_2 and 20% CO_2 at 20 °C. In our study, RR of both citric treated and non-treated arils stored at 15°C was twofold higher during the first 3 days of storage compared to those at 5 °C (Fig. 5). Maintenance of low temperature cold chain is therefore critical in maintaining product quality.

Citric acid is commercially used as an anti-browning agent in fresh cut fruits and vegetables. It has also been shown to lower the RR of minimally processed products (Kato-Noguchi and Watada, 1997; Petri et al., 2008). In our studies citric acid (52 mM) treatment had no significant effect ($p < 0.05$) on RR of the arils stored at 5 and 10 °C, but it was effective in reducing RR of arils at 15 and 20 °C. Kato-Noguchi and Watada (1997) also reported a decrease in RR of citric acid treated shredded carrots stored at 15 °C. RR of the carrots treated with 1 mM citric acid reduced by 18% while those treated with 100 mM citric acid had 69% reduction in RR. It was suggested that citric acid lowers RR by inhibiting the action of phosphofructokinase, an enzyme that catalyses the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate in the glycolytic pathway of respiratory metabolism. This phenomenon could explain the observation in our study. Furthermore, Petri et al. (2008) also showed that both citric and acetic acid enhanced the effects of sodium metabisulfite in reducing RR of minimally processed potato (cv. Monalisa).

Aril colour and texture

No significant change ($p > 0.05$) was observed in a^*(redness) and b*(yellowness/blueness) values at the end of the 5 d storage period (Table 1). These results are in agreement with those reported in literature in which pomegranate aril colour attributes are not significantly altered with storage (Caleb et al., 2013b; Ayhan and Eştürk, 2009; Sepúlveda et al., 2000). Sepúlveda et al. (2000) studied the effect of semi-permeable films and the use of an antioxidant mixture solution on the shelf life of minimally processed pomegranate arils (cv. Wonderful) stored at 4 °C and reported that dark red colour of arils was maintained after 14 days of storage.

Hue angle ($H°$) is a qualitative attribute of colour by which colours are described as reddish or greenish whereas chroma (C^*) is a quantitative attribute which indicates colour intensity (Pathare et al., 2013). Hue angle and C^* ranged from 15.41 ± 1.30 to 16.87 ± 0.26 and 24.31 ± 1.93 to 24.14 ±

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2.59, respectively, and was not significant ($P > 0.05$) affected by citric acid treatment, storage temperature and duration. Aril hardness expressed as maximum compression force required to crush the arils was also maintained (Table 1). Aril firmness obtained in the present study is similar to that reported by Ayhan and Eştürk (2009) for pomegranate arils (cv. Hicaznar) stored under MAP conditions at 5 °C.

Titratable acidity, total soluble solids and pH

Total soluble solids (TSS), titratable acidity (TA), TSS:TA and pH values are shown in Figure 6. Initial values for chemical attribute pH (3.29 ± 0.05), TSS (16.73 ± 0.05° Brix) and TA (0.86 ± 0.01 g CA/100 mL) are similar to those reported by Sepúlveda et al. (2000) for 'Wonderful' pomegranate arils pre-treated with an antioxidant mixture of ascorbic and citric acid.

TSS was maintained in the citric acid treated arils at both 5 and 10 °C, while it increased significantly in the non-treated arils. In contrast, TA decreased significantly ($p < 0.05$) with storage in all the treatments, while pH, was maintained in all the treatments except in the non-treated arils at 10 °C in which it decreased significantly ($p < 0.05$). TSS:TA ratio increased with storage in all the treatments, a result of the decrease in TA in the treatments (Artés et al., 2000). A decrease in acidity after storage was also reported for minimally processed pomegranate arils 'Hicaznar' (Ayhan and Eştürk, 2009). The decrease in TA could be attributed to utilization of organic acid in metabolic processes of arils during storage.

Conclusions

Temperature and RH had significant effects on TR of both citric acid treated and non-treated arils (cv. Wonderful), with lowest TR occurring at 5 °C and 96 % RH (1.26g/kg/day) and the highest at 15 °C and 76 % RH (24.77g/kg/day). Citric acid pre-treatment, however, had no significant effect on TR of arils. Storage temperature was shown to have a significant effect on RR of arils. Increasing storage temperature from 5 to 15 °C resulted in a two fold increase in RR of both citric acid treated and non-treated arils. Aril RR was lowest at 5 °C, decreasing from 4.75 to 2.86 mL CO_2 kg^{-1} h^{-1} and 4.86 to 2.7072 mL CO_2 kg^{-1} h^{-1} for citric acid treated and non-treated arils, respectively, after 5 d storage period. Citric acid pre-treatment did not alter RR of pomegranate arils at 5 and 10 °C but was effective in reducing RR of arils at 15 and 20 °C. Therefore it may be necessary to pre-treat arils with citric acid in order to retard increase in aril RR in instances where temperature abuse occurs. Physico-chemical attributes (colour, firmness and pH) were not significantly affected by citric acid treatment, temperature and storage duration, while TSS:TA increased significantly due to

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a decrease in TA as storage duration increased. The study showed that maintaining optimum cold storage condition is critical in keeping physiological processes at a minimum to maintain aril quality.

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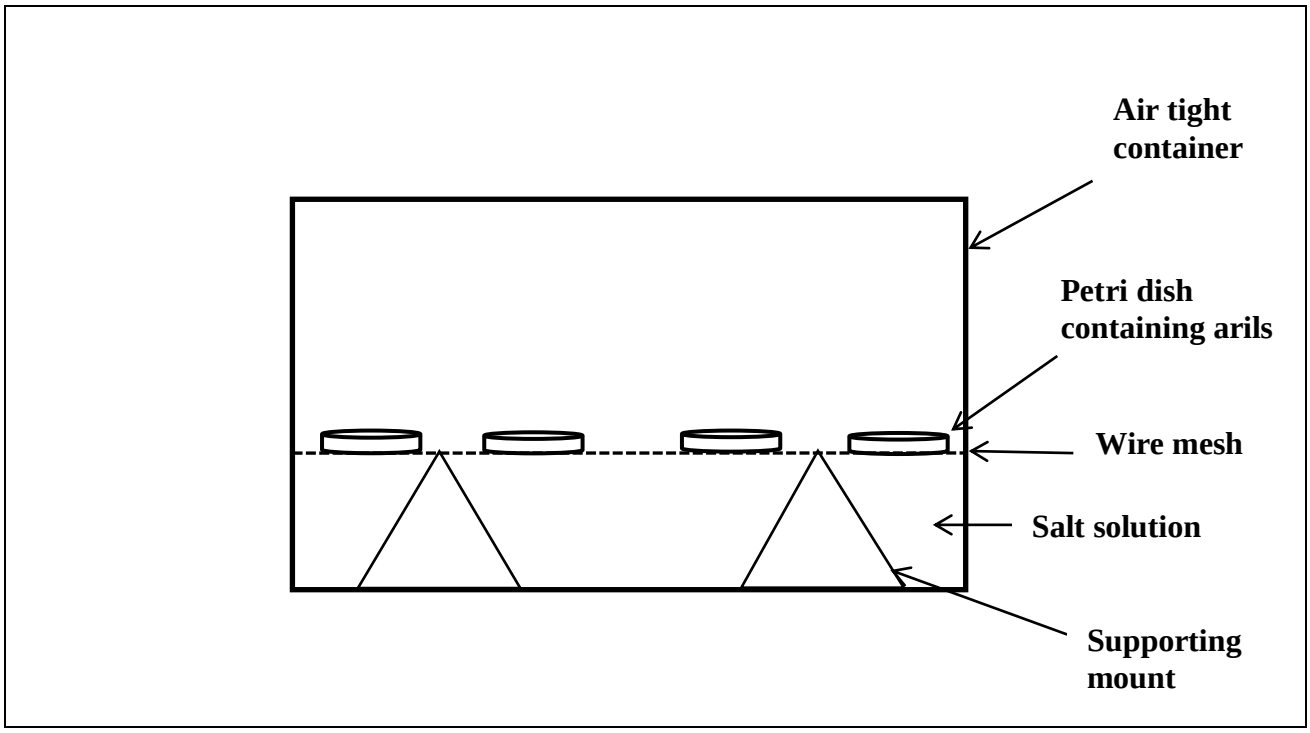

Figure 1. Experimental set-up for transpiration study.

Figure 2. Gas sampling using Checkmate 3, PBI Dansensor gas analyser.

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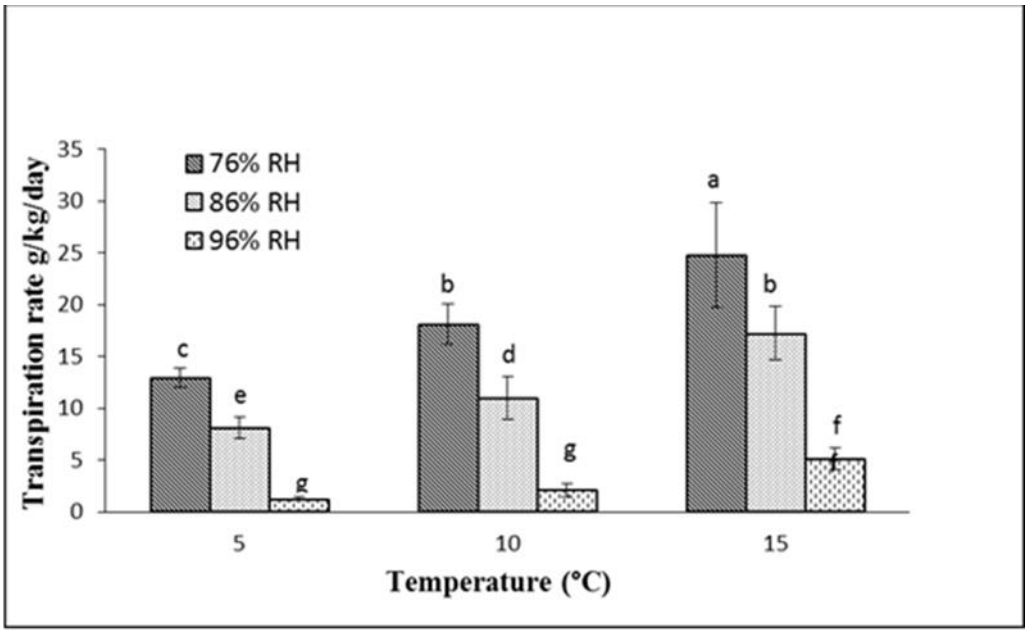

Figure 3. Effects of temperature and relative humidity (RH) on transpiration rate of pomegranate arils. Different letters indicate a significant difference in mean values ± SD; temperature*RH ($p = 0.0003$).

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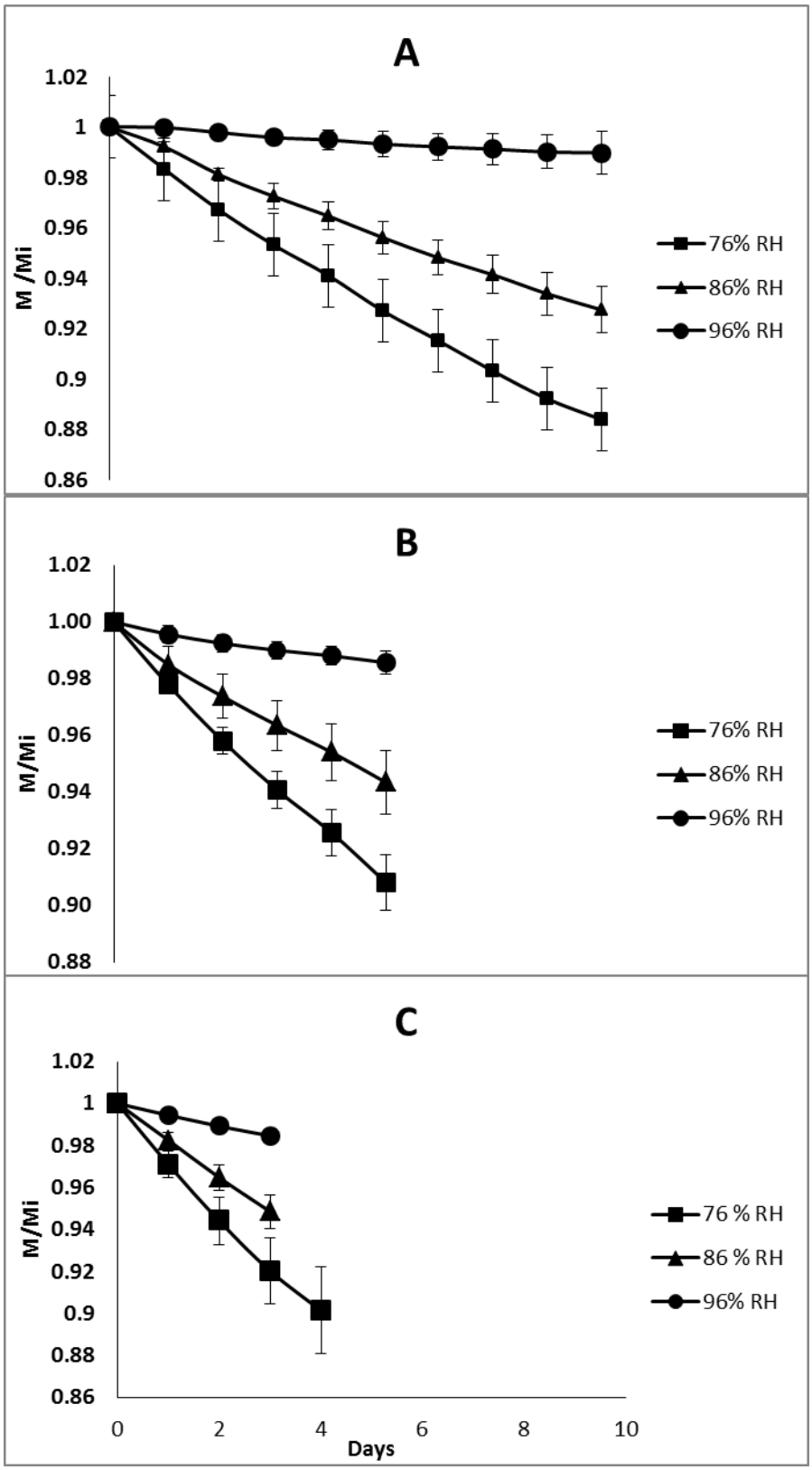

Figure 4. Effect of relative humidity (76, 86 and 96%) and temperature (**A**) 5 °C (**B**) and 10 °C and (**C**) 15 °C on weight loss of pomegranate arils. The values are normalised with respect to initial weight of pomegranate arils.

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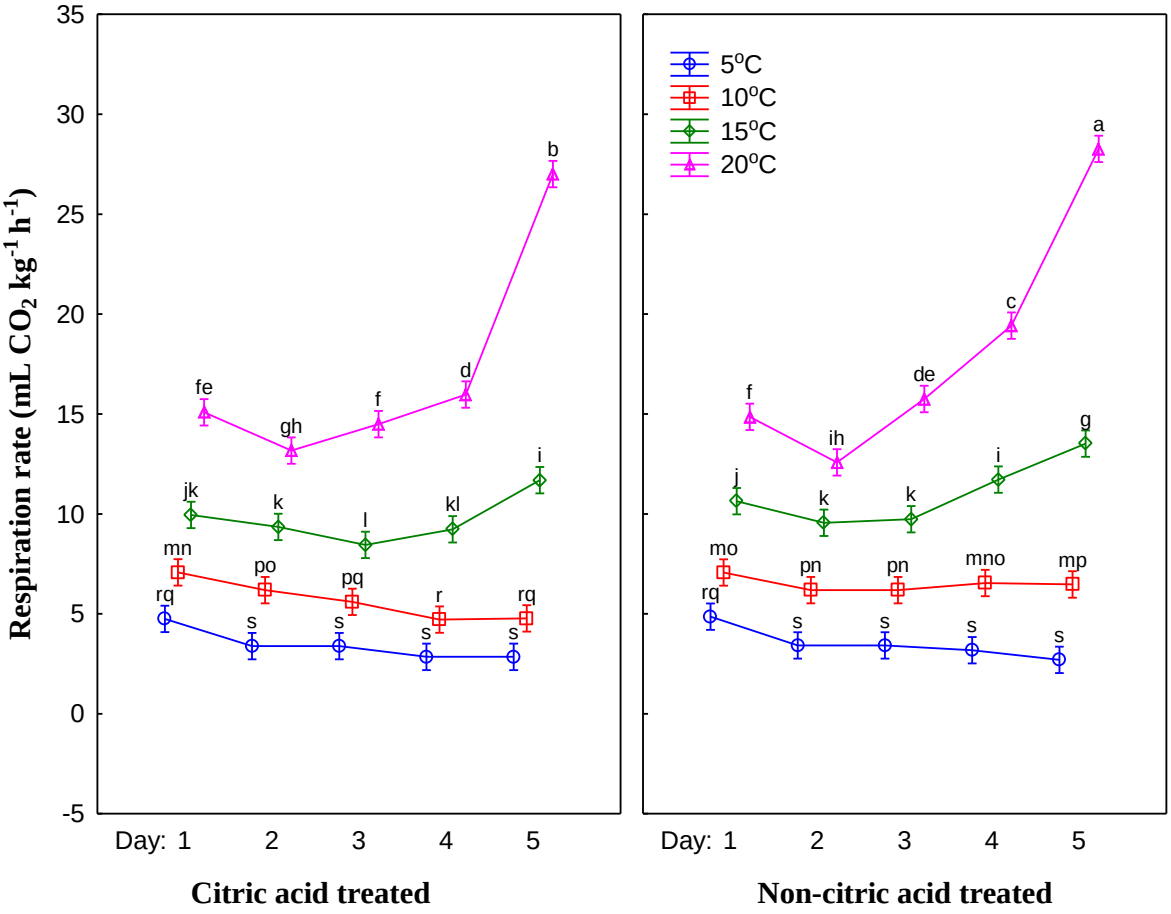

Figure 5. Effects of temperature, citric acid treatment and storage duration on respiration rate of pomegranate arils. Vertical bars denote SD of mean values; temperature*day*citric acid (p = 0.001)

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Table 1. Changes in colour and textural attributes of pomegranate arils (cv. Wonderful) as affected by storage temperature and citric acid treatment at day 0 and the end of 5 day storage. Data represents mean values ± SD

Treatments	CIELAB colour index					
	L*	***a****	***b****	***C****	***H°***	**Aril hardness (N)**
Control (Day 0)	12.35 ± 3.73^{ab}	14.02 ± 1.19^{a}	5.71 ± 0.54^{a}	15.14 ± 1.28^{a}	22.17 ± 0.99^{a}	201.62 ± 14^{a}
Non-treated_5°C	13.66 ± 1.25^{ab}	14.03 ± 1.16^{a}	6.35 ± 0.78^{a}	15.41 ± 1.30^{a}	24.31 ± 1.93^{a}	203.17 ± 22^{a}
Non-treated_10°C	15.74 ± 0.61^{a}	14.79 ± 0.26^{a}	6.53 ± 0.44^{a}	16.17 ± 0.39^{a}	23.80 ± 1.17^{a}	195.09 ± 48^{a}
Treated_5°C	10.52 ± 2.38^{b}	12.97 ± 1.46^{a}	5.83 ± 0.84^{a}	14.23 ± 1.67^{a}	24.14 ± 0.88^{a}	201.76 ± 12^{a}
Treated_10°C	15.42 ± 1.09^{ab}	15.28 ± 0.47^{a}	7.11 ± 0.65^{a}	16.87 ± 0.26^{a}	24.14 ± 2.59^{a}	200.83 ± 13^{a}

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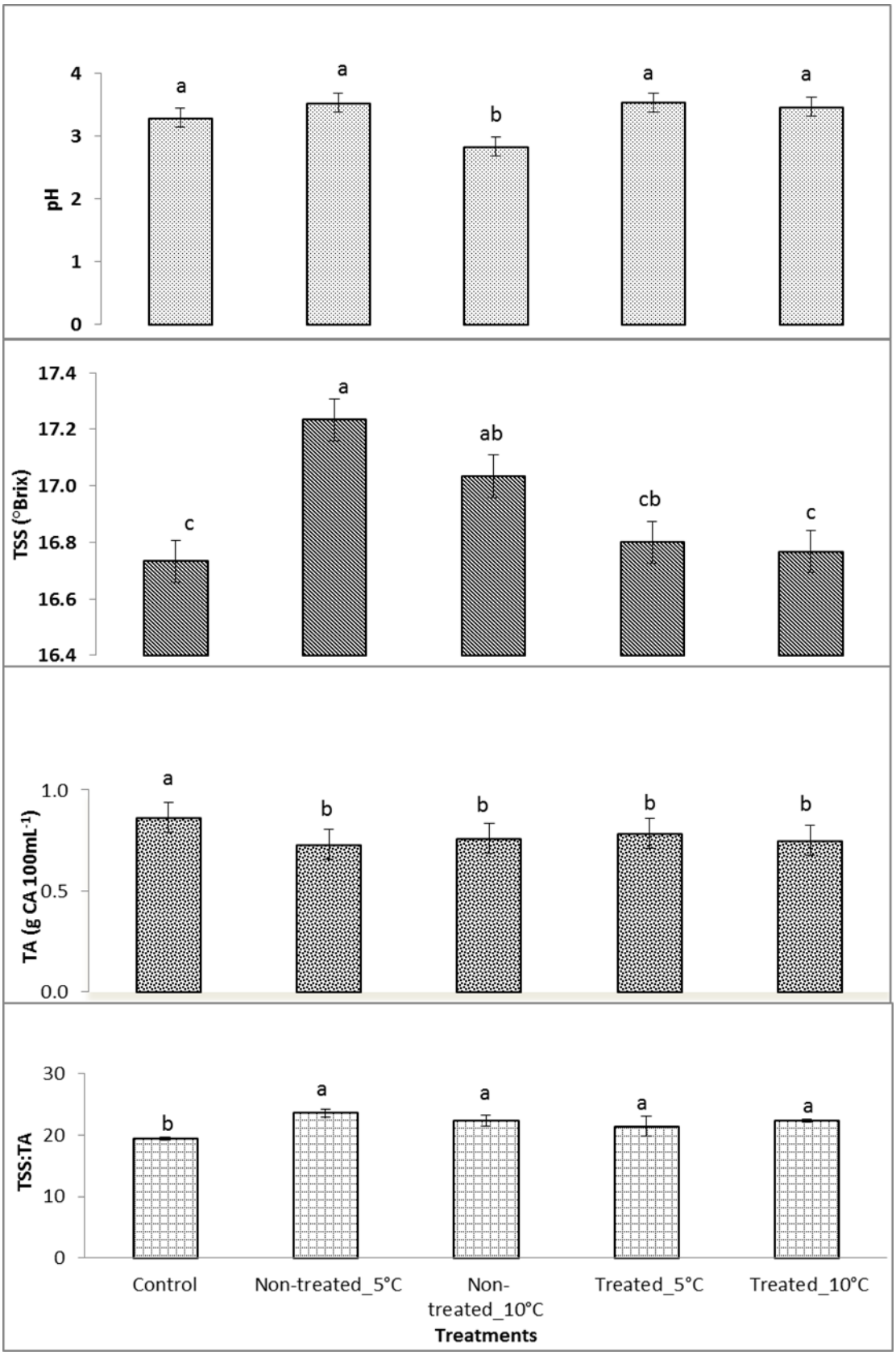

Figure 6. Total soluble solids (TSS), pH and titratable acidity (TA) and TSS: TA ratio of arils after 5 days of storage compared to initial values. Bars with the same letter are not significantly different ($p < 0.05$).

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Chapter 4
Active modified atmosphere packaging of pomegranate arils (cv. Wonderful)

Abstract

Two experiments were conducted to investigate the effects of active and passive modified atmosphere packaging on respiration rate and quality attributes of minimally processed pomegranate arils (cv. Wonderful) stored at 5 °C for 12 days. In experiment 1, pomegranate arils were packaged in low barrier bi-axially oriented polyester (BOP) film under active modified atmospheres (5% O_2 + 10 % CO_2, 30 % O_2 + 40 % CO_2), passive modified atmosphere and clamshell container as control. A high barrier polylid film was used in experiment 2 with arils packaged under three active modified atmospheres (5% O_2 + 10% CO_2 + 85% N_2, 30% O_2 +10% O_2 + 60% N_2, 100% N_2) and passive modified atmospheres. Arils packed in clamshell trays maintained the lowest RR compared to the other MAP treatments in experiment 1 throughout the storage duration, ranging from 3.4 mL CO_2 kg^{-1} h^{-1} on day 3 to 19.6 mL CO_2 kg^{-1} h^{-1} on day 12. Respiration rate of arils packaged in the high barrier polylid film in experiment 2 was significantly affected by MAP treatments and arils packaged in 100% N_2 atmosphere maintained significantly lower respiration rates throughout the storage duration. Physico-chemical attributes (colour, texture, TSS, TA and pH) of arils were not significantly affected by the MAP treatments in both experiments. Packaging arils in low barrier BOP film with high O_2 atmosphere (30% O_2 +40% CO_2) was effective in extending the lag phase of aerobic mesophilic bacteria by 6 days. Similarly, arils packaged under high oxygen atmosphere (30% O_2 + 10% CO_2) and 100% N_2 atmosphere in high barrier polylid film maintained significantly lower aerobic mesophilic bacteria counts throughout the storage duration. Based on overall acceptability sensory scores and absence of microbial spoilage, shelf life was limited to 6 and 9 days for arils packaged in clamshell containers and passive MAP, respectively. On the other hand, those packaged under high O_2 atmospheres scored above the acceptable limit by day 9 in both BOP and polylid films.

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Introduction

Modified atmosphere packaging (MAP) combined with low temperature storage has been successfully used to prolong the shelf life of fresh fruit and vegetables (Artés et al., 2006). Modified atmospheres are achieved by hermetically sealing fresh respiring produce in polymeric film and allowing the atmosphere within the package to be modified passively by the interplay of produce respiration rate (RR) and the film permeability properties, or actively by flushing the desired gas mixtures inside a package before sealing (Kader and Watkins, 2000; Al-Ati and Hotchkiss, 2002; Rico et al., 2007; Mangaraj et al., 2009; Brandenburg and Zagory, 2009). Modified atmosphere packaging slows down physiological and biochemical processes and retards senescence (Jacxsens et al., 2002; Rico et al., 2007). In addition, sealing fresh products in polymeric film provides a barrier against moisture loss and microbial contamination (Mangaraj et al., 2009). The success of MAP in maintaining quality and prolonging shelf life of fresh produce depends on the creation of suitable equilibrium atmospheres around the produce. Failure to create this suitable atmosphere may result in a shortened shelf life (Mangaraj et al., 2009). Suitable equilibrium atmospheres are achieved by proper matching of fresh produce RR and film permeability characteristics (Kader, 2002; Mangaraj et al., 2009; Caleb et al., 2012; Charles et al., 2003). The selection of packaging films with suitable barrier properties is, therefore, of crucial importance in developing a suitable gas composition to maintain quality and assure a long shelf life for packaged fresh produce (Martinez-Romero et al., 2013).

Low O_2 (2-5%) and/ or moderate CO_2 (~10%) atmospheres are desired in MAP (Rico et al., 2007; Sandhya, 2010). Super-atmospheric oxygen atmospheres (> 21%) have also been used in MAP of minimally processed products because of their ability to prevent anaerobic fermentation, inhibit enzymatic discolouration and microbial growth (Jacxsens et al., 2001). Ayhan and Eştürk (2009) reported an increase in antioxidant activity and lower mesophilic bacteria counts in minimally processed pomegranate arils (cv. Hicaznar) stored under super atmospheric O_2 (70%) atmospheres compared to those stored under low O_2 (5%) and in normal air at 5 °C. Oxygen concentrations > 25% are nonetheless considered highly explosive and, as they pose a hazard should be used with caution (Jacxsens et al., 2001). Nitrogen (N_2) is a non-reactive gas that is used to exclude more reactive gases from packages and acts as a filler gas to prevent package collapse (Brandenburg and Zagory, 2009). Several studies with minimally processed products have explored the use of 100 % N_2 atmospheres in MAP (Koseki and Itoh, 2002; Ayhan and Estürk, 2009; Ahmed et al., 2011) because of their ability to maintain fresh produce quality. Firmness, colour and chemical properties were maintained and shelf life extended in persimmon fruit packaged in 100% N_2, stored at 0 °C

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and 85-95% RH for 90 days (Ahmed et al., 2011). Similarly, fresh-cut cabbage and lettuce in packages initially flushed with 100% N_2 atmospheres at 1 and 5 °C maintained their quality and appearance by the end of the 5 day storage period (Koseki and Itoh, 2002).

The success of MAP does not only depend on creation of a suitable equilibrium atmosphere around a product; the time taken to establish these atmospheres is also critical especially in minimally processed products which have a short marketable life (Bai et al., 2003). Active modified atmosphere packaging achieved by flushing desired gas mixtures into packages allows earlier establishment of equilibrium atmospheres than passive MAP and has, therefore, been recommended for minimally processed products (Bai et al., 2003; Rodov et al., 2007). Equilibrium atmospheres in active MAP of litchi (cvs. Mauritius and McLeans Red) were established almost from the first day of storage, whereas those in passive MAP were established 6 to 10 days after packaging (Sivakumar et al., 2008). In the period before equilibrium is reached, the product is exposed to non-optimal atmospheres and continues deteriorating (Rodov et al., 2007).

Despite the successful application of active MAP in a wide range of fresh-cut and minimally processed products, few studies have investigated the effects of active MAP on minimally processed pomegranate arils. The objective of this study was, therefore, to determine the effects of different initial packaging atmospheres achieved by gas flushing on respiration rate (RR), quality attributes and shelf life of minimally processed pomegranate arils (cv. Wonderful) packaged in low barrier BOP and polylid film and stored at 5 °C and 90 ± 2 % RH.

Materials and Methods

Sample preparation and packaging

Pomegranate fruit (cv. Wonderful) was obtained at commercially ripened stage from Houdconstant packhouse in Porterville, Western Cape (33°01′00″S, 18°59′00″E), South Africa. Fruit were sorted, cleaned and minimally processed at the farm pack house. Fruit free from visible physical defects were washed in sterilised water and arils extracted using a commercial extraction machine (Arilsystem, Juran Metal Works, Israel). Extracted arils were bulk packaged in sterilized polyethylene bags and transported in ice boxes to the postharvest research laboratory at Stellenbosch University. Arils (300g) were packaged in polyethylene terephthalate (PET) trays with dimensions 28 x 19 cm (ZIBO containers, PTY, LTD. Kuilsrivier, South Africa) and flushed with food grade gas mixtures (Air Products Pty; Kempton Park, South Africa) using a tray sealer (Model T200 Multivac, Wolfertschwenden, Germany).

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Two experiments were conducted consecutively. In the first experiment a low barrier bi-axially oriented polyester (BOP) polymeric film supplied by Knilam Packaging (Pty) Ltd. (Cape Town, South Africa) was used to heat-seal the PET trays. The properties of the films were: 26µm thickness, 75 ml/m^2/day O_2 and 15-20 ml/m^2/day CO_2 transmission rates and 20 g/m^2/day water vapour transmission rate at 25°C and 50% RH. The following gas mixtures were applied: MAP-A (5% O_2 + 10% CO_2 + 85% N_2), MAP-B (30% O_2 + 40% CO_2 + 30% N_2), MAP-C (passive MAP). Control arils were packaged in polyethylene terephthalate (PET) clamshell containers (420 µm thickness) and dimensions of 11.5 × 11.5 × 3.5 cm. In the second experiment, a high barrier polymeric film Polylid® 107 HB55 (55µm thickness, 21-23 ml/m^2/day O_2 and 15-20 ml/m^2/day CO_2 transmission rates and 5-7g/m^2/day water vapour transmission transmission rates at 25°C and 50% RH) supplied by Barkai Polyon industries Ltd. (Tel Aviv, Isreal) was used. The following gas mixtures were applied: MAP-D (5% O_2 + 10% CO_2 + 85% N_2), MAP-E (30% O_2 + 10% CO_2 + 60% N_2), MAP-F (100% N_2) and MAP-G (passive MAP).

Samples were stored at 5 °C and 90 ± 2 % RH for 12 days and analyses were conducted in triplicate on days 0, 3, 6, 9, and 12. Physico-chemical attributes (colour, firmness, total soluble solids, titratable acidity, pH), microbial quality and sensory attributes of arils were evaluated.

Headspace gas composition

Headspace O_2 and CO_2 composition of packaged pomegranate arils was determined using an O_2/CO_2 gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Gas analysis was done by inserting a needle attached to the gas analyser through a rubber septum on the packaging film. Gas sampling was done before opening the package to remove the arils. Three additional replications per treatment were used to monitor in-package head space gas composition during the entire storage period.

Respiration rate

Post-storage respiration rate (RR) of pomegranate arils was determined using the closed system method. On each sampling day, 150 g pomegranate arils from each of the MAP treatments were separately weighed into 1100 mL glass jars using a balance (Bosch SAE200, GmbH). The glass jars were hermetically sealed by incorporating Vaseline petroleum jelly in the gap between the lid and the jar. The hermetically sealed jars were stored at 5 °C and left for 1 h before taking the first measurement. Gas samples were drawn at hourly intervals over a period of 4 h through a rubber septum fitted on the jar and the gas composition was monitored by gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Measurements were repeated on each of the sampling days using

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fresh samples each time in order to determine the effect of modified MAP and storage duration on pomegranate arils RR (Fonseca et al., 2002; Bhatia et al., 2013).

Respiration rate was calculated by fitting experimentally obtained data in the following equations 1 and 2:

$$y_{O_2} = y_{O_2}^{i} - \frac{R_{O_2} W}{V_f}(t - t_i) \times 1000 \quad (1)$$

$$y_{CO_2} = y_{CO_2}^{i} + \frac{R_{CO_2} W}{V_f}(t - t_i) \times 1000 \quad (2)$$

Where R_{O_2} and R_{CO_2} is the oxygen and carbon dioxide respiration rate (RR) in (mL kg^{-1} h^{-1}); $y^{i}o_2$ *and* yo_2 is oxygen concentration (%) at the initial time t_1 (hours, h) (time zero) and at time t (h) respectively and $y^{i}co_2$ and yco_2 is the carbon dioxide concentration (%) at the initial time t_1 (hours, h) (time zero) and at time t (h) respectively. W is the total weight of product (kg) and V_f is the free volume inside jar (mL); determined by subtracting volume of product from the total volume of the glass jar (Caleb et al., 2012)

Aril colour and firmness

Aril colour measurements were performed using a colorimeter (Minolta Chroma Meter, CR-300, Minolta, Japan). Approximately 30 g of arils were weighed onto a Petri dish and five readings of each colour index in the CIELAB *L** (Lightness/darkness), *a** (redness/greenness) *and* *b**(yellowness/blueness) were taken. This was done in triplicate for each of the treatments. Colour parameter Chroma (*C**) and the hue angle (*h*°) were calculated according to the following equations (Pathare and Opara, 2013):

$$C^{*} = (a^{*2} + b^{*2})^{1/2} \quad (3)$$

$$h° = arctan\,(b^{*}/a^{*}) \quad (4)$$

In addition, total colour difference (ΔE) was calculated using the equations;

$$\Delta E = \sqrt{(a - a_0^{*})^2 + (b - b_0^{*})^2 + (L - L_0^{*})^2} \quad (5)$$

where L_0*,a_0* b_0* are control values for the unpackaged initial pomegranate arils at day 0, and the *L**,*a** *b** are the values for the arils from the different treatments at each sampling time during the storage period.

Differences in colour can be analytically classified as very distinct $\Delta E > 3$, distinct $1.5 < \Delta E < 3$ and small differences $\Delta E < 1.5$ (Pathare and Opara, 2013).

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Aril firmness was determined using a texture analyser (Tensilon mode UTM-4L, Toyo Measuring Instruments Co., Tokyo). Each aril was compressed using a 35 mm diameter cylindrical probe. Maximum compression force (N) was used as a measure of aril hardness. Test speeds of 1.0 mm/s and penetration distance of 9.5 mm were used. Each aril was tested individually and an average of 20 arils was tested for each treatment.

Total soluble solids, titratable acidity and pH

Arils were juiced separately for each of the replicates on each sampling day using a LiquaFresh juice extractor (Mellerware, South Africa). Pomegranate juice (PJ) was used to determine pH using a pH meter (Crison, Barcelona), TSS expressed as °Brix using a digital refractometer (Atago, Tokyo) and TA measured by titration to an endpoint of pH 8.2 using a Metrohmn 862 compact titrosampler (Herisau, Switzerland) and expressed as (g of citric acid per 100 mL of juice). All values are presented as mean ± SD.

Microbial analysis

Microbial quality of arils was screened on days 0, 6 and 12 of storage. Approximately 10 g of arils were weighed and put into 100 mL of physiological salt solution and shaken for 5 min. This was done in triplicate for each MAP treatment. Total bacterial count was determined using plate count agar (PCA) incubated at 30 °C for 48 hours, while yeast and mount counts were determined using potato dextrose agar (PDA) modified by adding streptomycin (100 ppm) and chloramphenicol (50 ppm) and incubated at 26 °C for 5 days.

Sensory evaluation

Sensory evaluation of arils was done by a panel of 6 untrained judges who are regular consumers of pomegranate and were familiar with its quality attributes. Aril quality attributes, red colour, browning, firmness, taste, off-odour, flavour, aroma and overall acceptability were scored on scale of 0 to 5. The score 0 corresponded to poor/none and 5 to excellent/prominent. Scores below 3 were considered the cut-off point for quality attributes red colour, firmness, taste, flavour and overall acceptability, while scores above 3 were used as indicators of the end of acceptable quality for browning and off-odour.

All parameters were measured in triplicate except for texture determinations which were repeated 20 times for each treatment.

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Statistical analysis

A factorial analysis of variance (ANOVA) was performed to determine the effects of MAP and storage duration and means separated using Fisher's least significant differences (LSD) test at 95% confidence interval using Statistica software (Statistica 10.0, Statsoft Inc., USA). All values are presented as mean ± standard deviation (SD).

Results and Discussion

Headspace gas composition

In the first experiment with the lower barrier film, equilibrium O_2 and CO_2 levels were attained by day 3 in packaged arils across all MAP treatments (Fig. 1 A and B). However, the steady state gas composition of O_2 observed in this study (16-18%) was above the recommended level of 2-5% O_2 (Lopez-Rubira et al., 2005). This could be attributed to the high oxygen transmission rate of the BOP film used in the study. Carbon dioxide levels attained were also slightly lower (~7%) than those recommended (10-20%) for pomegranate arils (Hess-Pierce and Kader, 1997; Irtwange, 2006)

In the second experiment, O_2 decreased and CO_2 increased continuously, across all the treatments regardless of MAP treatment. Oxygen levels in packages flushed with low O_2 (2% O_2 +10% CO_2 + 85% N_2) went below the critical limit of 2% by day 12 (Fig. 1C), a condition that is known to be ideal for occurrence of anaerobic respiration (Gorny, 2003; Artés et al., 2006). The presence of anaerobic respiration results in development of off-flavours and renders products undesirable. Carbon dioxide levels also accumulated (27-43%) beyond levels recommended for MAP of pomegranate arils across all MAP treatments (Fig. 1D). Carbon dioxide is soluble at high concentrations and low temperatures forming carbonic acid which has bacteriostatic effects on tissues of fresh-cut produce. On the other hand, accumulation of carbon acid causes changes in organoleptic properties of some minimally processed products. (Sandhya, 2010). The barrier films used in both experiments did not create suitable equilibrium O_2 and CO_2 levels for pomegranate arils.

Respiration rate

Modified atmospheres, storage duration and their interaction had significant effects ($P < 0.05$) on RR of pomegranate arils packaged in the low barrier BOP film and clamshell trays (Fig. 2A). RR of arils reduced initially with increase in storage duration from 7.39 ± 0.73 mL CO_2 kg^{-1} h^{-1} on day 0 to a range of 3.38 -7.1 mL CO_2 kg^{-1} h^{-1} on day 3 across the treatments, and then increased

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significantly ($P < 0.05$) from day 3 until the end of the storage period. Arils packed in clamshell trays maintained the lowest RR throughout the storage duration, ranging from 3.4 mL CO_2/kg h on day 3 to 19.6 mL CO_2 kg^{-1} h^{-1} on day 12.

Respiration rate of arils packed in the high barrier film (polylid) were generally higher than those of low barrier film experiment (Fig. 2B). This may be attributed to differences in headspace gas composition created inside the barrier films. Aril RR were similar to those reported by Bhatia et al. (2013), for minimally processed 'Mridula' pomegranate arils under MAP at 5 °C for 15 days. The authors reported a progressive increase in RR of arils during the storage duration, ranging from 25.6 mL CO_2 / kg h to 80.5 mL CO_2 kg^{-1} h^{-1}. Similarly, RR of arils in experiment 2 of our study increased throughout the storage duration across all the treatments. By day 3, arils in MAP-F (100% N_2) and MAP-G (passive) had significantly lower RRs than the other treatments. Arils packed in 100% N_2 maintained significantly lower RRs than the other MAP treatments from day 6 to the end of the storage period. The low RRs could be attributed to the low levels of O_2 maintained in the 100% N_2 packages compared to the other MAP treatments (Fig. 1C).

Other studies have shown that MAPs with low O_2 (2-5%) and high CO_2 (10-20%) levels reduce RR of minimally processed fresh produce (Gorny et al., 2003; Ersan et al., 2010; Rattanapanone et al., 2001). Ersan et al. (2010) investigated the effects of varying combinations of O_2 (2, 10, 21%) and CO_2 (0, 10, 20%) concentrations on RR of minimally processed pomegranate arils (cv. Hicaznar) stored at 4 °C. The studies revealed that combinations of low O_2 (2-5%) and high CO_2 (10 and 20%) significantly reduced RRs of arils. The use of high O_2 atmospheres (>21%) has been suggested as an alternative to low O_2 in order to prevent anaerobic respiration (Jacxsens et al., 2001). However, respiratory response to high O_2 atmospheres varies in different products (Kader and Ben-Yehoshua, 2000). High O_2 atmospheres (100% O_2, 95% O_2 + 5% CO_2, 80% O_2 + 20% CO_2, 75% O_2+25% CO_2) significantly reduced respiration rates of fresh-cut onions stored at room temperature for 9 days (Chunyang et al., 2010). In contrast, Maghoumi et al. (2013) reported an increase in RR of minimally processed pomegranate arils (cv. Molar de Elche) packaged under high oxygen atmospheres (90 kPa O_2) compared to those under passive MAP at 5°C. The study suggested that the high O_2 levels enhanced the production of reactive O_2 species and caused respiratory stress leading to increased aril RR.

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Aril colour and firmness

Chroma (*C**) is the quantitative attribute for colourfulness and corresponds to the colour intensity of samples perceived by humans. While total colour difference (ΔE) indicates the magnitude of colour between the stored samples and the initial or control samples (Pathare and Opara, 2013).

In the first experiment with the low barrier film, no significant differences ($p > 0.05$) were observed in aril colour attributes under the different MAPs investigated (data not shown, Refer to appendix B, Table. 1A). However, the colour attributes fluctuated signicantly with storage duarion ($P < 0.05$). Chroma (*C**) values increased significantly ($P < 0.05$) with storage duration across all the treatments from day 0 until day 9, after which they decreased slightly on day 12 (data not shown, Refer to appendix B, Table. 1A). Similarly, ΔE values increased significantly with storage duration across all the treatments (data not shown, Refer to appendix B, Table. 1A). Arils in MAP treatments MAP-B (30% O_2 + 40% CO_2 + 30% N_2), MAP-C (passive) and clamshell containers had ΔE values ranging from 2.64 to 2.84 indicating distinct visual colour differences with the initial samples. Pomegranate arils packaged in MAP with low O_2 (5 % O_2 + 10% CO_2 + 85% N_2) had the highest ΔE (3.76) by the end of the storage duration indicating very distinct visual colour differences with initial aril samples.

Similarly, colour attributes of arils packaged in high barrier polylid film were not significantly ($P > 0.05$) affected by MAP treatments (data not shown, Refer to appendix B, Table.1B). Chroma values and total colour difference (ΔE) fluctuated across all the treatments during the entire storage duration. By the end of the storage duration, arils packaged in MAP treatments, MAP-D (5% O_2 + 10% CO_2 + 85% CO_2), MAP-E (30% O_2 + 10% CO_2 + 60% N_2) and MAP-F (100% N_2) maintained minimal visual colour differences ($\Delta E < 1.5$). In contrast, arils packaged under passive MAP (MAP-G) had the highest ΔE (2.28) by the end of the storage period.

Colour of fresh produce is affected by chemical, biochemical, physical and microbial changes occurring during postharvest handling, processing and storage (Pathare and Opara, 2013). Colour is, therefore, used as a visual indicator of freshness (Kader, 2002; Pathare and Opara, 2013). Pomegranate arils are known for the attractive red colour due to the presence of anthocyanins (Martínez-Romero et al., 2013). Previous studies have shown that MAP and storage duration have had no significant effects on colour attributes of pomegranate arils. Minimally processed pomegranate arils (cv. Wonderful) packaged in semi-permeable films and stored at 4 °C, maintained their deep red colour throughout the 14 day storage period (Sepúlveda et al., 2000). Similarly, Caleb et al. (2013) reported that passive MAP and storage time had no significant effects

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on colour parameters *a** and *b** of minimally processed pomegranate arils cv. 'Acco' and 'Herskawitz' stored at 5 °C for 14 days. Colour attributes of minimally processed pomegranate arils (cv. Hicaznar) packaged under active and passive MAP and stored at 5 °C for 18 days, were not significantly affected by the MAP and storage duration (Ayhan and Estürk, 2009). Similarly, in our studies, instrumental measurements of aril colour attributes *L**, *a** and *b** fluctuated with storage but they were not significantly affected by MAP (data not shown, Refer to appendix B, Table 1 A and B). However, ΔE results suggest visual colour differences in arils in MAP-A (5% O_2 +10% CO_2 + 85% CO_2), MAP-B (30% O_2 + 40% CO_2 + 30% N_2) and MAP-C (passive).

Interaction of storage duration and MAP had a significant impact on firmness of arils ($P < 0.05$) packaged in low barrier BOP film (Table 1). Arils packaged in high O_2 atmospheres (MAP-B) maintained the highest firmness values from day 9 until the end of storage, while MAP-C (passive) had the lowest.

Similarly, firmness of arils packaged in the high barrier polylid film was not significant affected by MAP ($P < 0.05$). However, increased significantly with storage ($p = 0.002$) from an initial value of 126.4 ± 7.77 N, across all the treatments and was highest on day 9 across all the treatments with values ranging from 218.3 ± 10.37 N to 229.4 ± 10.84 N and then decreased slightly on day 12 (Table 1). Our results were similar to those reported by Ayhan and Estürk (2009) for pomegranate arils (cv. Hicaznar) stored under passive and active MAP at 5 °C for 18 days. The authors reported an increase in aril firmness with storage from an initial 157.6 ± 23.9 N to values ranging from 183.1 ± 10.6 N to 216 ± 14.9 N across the treatments by the end of the storage period. The study further reported a significant increase in firmness in arils packaged in enriched oxygen atmospheres (70% O_2) after day 15 of storage.

The observed fluctuations in aril firmness and the large variability between individual aril mechanical attributes in our study have also been reported in other studies and are attributed to the non-uniform flesh characteristics of arils (Ayhan and Estürk, 2009; Caleb et al., 2013). Changes in aril firmness have been suggested to be as a result moisture loss (Ayhan and Estürk, 2009). Bhatia et al. (2013) reported a progressive reduction in firmness of 'Mridula' pomegranate arils packaged in polypropylene (PP), low density polyethylene (LDPE) and cryovac KPA bags at 5 °C. The authors observed that arils packaged in KPA bags had the highest moisture loss and consequently suffered the highest loss in firmness. This highlights the role of packaging as a barrier against moisture loss, which helps to maintain textural integrity of fresh produce. However, accumulation of moisture within a package can be detrimental and result in tissue softening and proliferation of spoilage micro-organisms. Caleb et al. (2013) reported deterioration in firmness of pomegranate

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arils cv. 'Acco' and 'Herskawitz' packaged in clamshell containers at 5 °C and attributed it to accumulation of moisture within the containers which resulted in softening of membranes of arils. Ergun and Ergun (2009) reported a delay in softening of honey treated pomegranate arils (cv. Hicaznar) after 5 days of storage at 4 °C and attributed this to preservative and osmotic effects of honey treatment.

Total soluble solids (TSS), titratable acidity (TA) and pH

Chemical attributes, TSS and TA are responsible for flavour and therefore it is desirable that they are not altered with storage. MAP had no significant effects ($P > 0.05$) on chemical attributes of minimally processed arils in both experiment 1 and 2, except for TSS in arils packaged in low barrier BOP film (Table 2). Initial TSS (17.13 ± 0.32° Brix) and TA (1.61 ± 0.20 g CA/100 mL) of minimally arils packaged in low barrier BOP film (experiment 1) was indicative of good maturity indices as recommended for 'Wonderful' pomegranate arils (Kader, 2002). TSS of arils in low barrier BOP film (experiment 1) reduced significantly ($P < 0.05$) with storage and ranged from 15.33 ± 0.55 to 16 ± 0.46° Brix across all the treatments by the end of the storage period. Arils packaged in passively modified atmospheres (MAP-C) and clamshell containers maintained significantly higher ($p < 0.05$) TSS than MAP-A (5% O2 + 10% CO_2 +85% N_2) and MAP-B (30% O_2 + 40 CO_2 + 30% N_2), throughout the storage duration. Titratable acidity (TA) fluctuated with storage but did not differ significantly ($P < 0.05$) with the initial values across all the treatments. Arils packaged under passive MAP (MAP-C) had the highest TA (1.78 ± 0.27 g CA/100 mL) by the end of storage duration, while those in high O_2 atmospheres (MAP-B) had the lowest (1.36 ± 0.04). Interaction of MAP and storage duration had a significant effect ($P<0.05$) on pH of arils in experiment 1. The pH levels were highest on day 9 across all the treatments (3.2 ± 0.01 - 3.3 ± 0.02) and then they reduced significantly to an average of 2.9 by day 12 across all the treatments. This decrease in pH could have been caused by accumulation of a carbonic acid in the aril tissues resulting from accumulation of CO_2 in the MA packages.

Pomegranate arils packaged in the high barrier polylid film (experiment 2) had a slightly higher initial TSS:TA than those packaged in the low barrier BOP film, but it was well within the range recommended for 'Wonderful' pomegranate (Table 3). Chemical attributes TA and TSS were not significantly ($P > 0.05$) affected by MAP treatments. Total soluble solids (TSS) fluctuated with storage duration across all the treatments, but values by the end of the storage period (16.07 ± 0.49 - 17.77 ± 2.67° Brix) did not differ significantly ($P > 0.05$) from the initial. Titratable acidity reduced initially across all the treatments until day 6 after which it increased significantly until day 12. The increase in TA could have been an indication of the onset of anaerobic RR due to depletion of O_2

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and build-up of CO_2 in packages with the low barrier polylid film (Fig 1 C and D). Anaerobic respiration constituents include acids which could have caused an increase in TA. Sivakumar et al. (2008) also reported a lower TSS:TA ratio in litchi packaged in non-perforated polypropylene punnets compared to the perforated ones and attributed it to increased acidity caused by fermentation as a result of accumulation of CO_2.

The range of values of TSS, TA and pH found in our studies are similar to those reported by Sepúlveda et al. (2000) for 'Wonderful' pomegranate arils. The authors studied the effects of semi-permeable films (PE, BB4 and BE) and antioxidant mixture solutions on shelf life of minimally processed pomegranate arils and reported initial values of pH, TSS and TA as 3.1, 15.8° Brix and 1.1 g CA/100 mL respectively. TSS values increased to 17° Brix in PE bag, while it remained unchanged in the other packages. In contrast pH values decreased only slightly to a range of 2.92-2.98 by the end of storage across all the treatments. Similarly in our studies, pomegranate aril chemical attributes were generally not affected by MAP and storage duration. Other studies have also reported minimal changes in chemical attributes TA, TSS and pH in modified atmosphere packaged pomegranate arils (Gil et al., 1996; Ayhan and Estürk, 2009; Maghoumi et al., 2013). These observations have been attributed to the beneficial effects of MAP and the relatively low RR of arils due to their non-climacteric nature. Studies by Ayhan and Estürk (2009) reported minimal differences in chemical attributes in minimally processed pomegranate arils (cv. Hicaznar) packaged in passive MAP, low O_2 (5% O_2 + 10% CO_2), enriched O_2 (70% O_2 +10% CO_2) and 100% N_2 atmospheres at 5 °C. Similarly, minimally processed pomegranate arils (cv. Mollar) packaged in OPP bags under different initial atmospheres (140 mL/L O_2 + 80 mL/L CO_2 and 20 mL/L O_2 + 0 mL/L CO_2) at 1 °C showed minimal differences in colour, TSS and TA by the end of the storage duration (Gil et al., 1996). Maghoumi et al. (2013) also reported minimal changes in chemical attributes of minimally processed 'Mollar of Elche' pomegranate arils packaged in high O_2 atmospheres (100 kPa O_2) at 5 °C, despite their RR being higher than that of arils in passive MAP.

Microbial analysis

Modified atmospheres packaging (MAP), storage duration and interaction of MAP and storage duration had significant effects ($P < 0.05$) on total aerobic mesophilic bacteria counts in arils in low barrier BOP film (Table 4). High O_2 atmospheres (MAP-B) extended the lag phase of total aerobic mesophilic bacteria until day 6. Total aerobic mesophilic counts in this treatment (MAP-B) increased threefold from the initial count (1.25 log CFU g^{-1}) on day 0 compared to a fivefold increase in passive MAP conditions (MAP-C). However, by the end of the storage, aerobic

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mesophilic counts in high O_2 atmospheres (MAP-B) had increased to the same levels as the other MAP treatments. In contrast, yeast and mould counts did not differ significantly ($P > 0.05$) across the MAP treatments in the low barrier BOP film. The counts were below detection limit by day 0 and increased significantly with storage to a range of 4.16 ± 0.28 and 4.53 ± 0.25 log CFU g^{-1} by day 12 across all the treatments.

Aerobic mesophilic bacteria counts in arils packaged in the high barrier polylid film increased significantly with storage from an initial 1.62 log CFU g^{-1} on day 0 to a range of 5.53 ± 0.08 to 5.61 ± 0.07 log CFU g^{-1} across all the treatments by the end of the storage duration. Pomegranate arils packaged in 100% N_2 (MAP-F) and high oxygen O_2 atmospheres (MAP-E) maintained significantly lower aerobic mesophilic bacteria counts throughout the storage duration compared to those packaged in MAP-D (5 % O_2 + 10% CO2 + 85% N_2) and passive MAP (Table 5). Yeast and mould counts were below the detection limit on day 0 and increased significantly with storage across all the treatments with values ranging from 4.49 ± 0.20 to 4.73 ± 0.15 log CFU g^{-1} by the end of the storage duration. Total aerobic bacteria and yeast and mould counts were below the maximum limits of 7 log CFU g^{-1} and 5 log CFU g^{-1} respectively, for fresh cuts in the South African legislation (FCD, Act 57 1979) in both experiment 1 and 2 by the end of the storage period.

The range of values of aerobic mesophillic bacteria, and yeast and mould counts in the present study are similar to those reported by López-Rubira et al. (2005) for minimally processed pomegranate arils (cv. Mollar of Elche) stored at 5 °C. The ability of high O_2 atmospheres to suppress aerobic mespohilic growth in arils packaged in low barrier polylid film corroborates findings by Ayhan and Estürk (2009). The authors reported the lowest aerobic mesophilic counts in high O_2 atmospheres (70% O_2 +10% CO_2). High O_2 atmospheres have been suggested to lead to intracellular generation of reactive oxygen species (O_2^-, H_2O_2, OH), which damage vital cell components and reduce cell viability when oxidative stresses overwhelm cellular protection systems (Kader and Ben-Yehoshua, 2000). The inhibitory effects of high O_2 atmospheres on growth of micro-organisms are more pronounced when combined with high CO_2 (10-20%) levels.

In contrast to the studies by Ayhan and Estürk (2009), however, 100% N_2 atmospheres (MAP-F) in our studies was also found effective in suppressing aerobic mespohilic growth in arils packaged in low barrier polylid film. Nitrogen displaces O_2 and therefore helps to retard growth of aerobic spoilage microorganisms (Sandhya, 2010).

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Sensory evaluation

In experiment 1, all the quality attributes assessed were not significantly ($p > 0.05$) altered by MAP. However, scores for overall acceptability of arils in clamshell containers fell below the acceptance limits of 3 out of 5 by day 6 (data not shown, refer to Appendix B, Table 2A). In addition, Sensory evaluation scores for taste and aroma of arils in this treatment (clamshell) also fell below acceptable limits by the end of the storage duration (data not shown, refer to Appendix B, Table 2A). Arils packaged in low O_2 atmospheres (MAP-A) had the highest scores for off-odour by the end of the storage duration and were not acceptable by day 9 (data not shown, refer to Appendix B, Table 2 A). In contrast, arils in passive MAP and MAP-B (30% O_2 + 40% CO_2 + 30% N_2) scored above the acceptance limit by day 9.

Arils in low O_2 atmospheres (5% O_2 +10% CO_2 + 85% N_2) and 100% N_2 (MAP-F) in the high barrier polylid film fell below the acceptance limit by day 9 with overall acceptability scores of 2.83 ± 0.37 and 2.5 ± 0.5 respectively (data not shown, refer to Appendix B, Table 2B). However, arils in passive MAP (MAP-G) remained acceptable until day 9 and those in O_2 atmospheres (MAP-E) scored above the acceptable limit for overall consumer acceptability by day 9. The extremely low O_2 levels in the low oxygen (MAP-D) and 100% N_2 (MAP-F) packages (Fig. 1C), could have resulted in fermentative metabolism in the arils, making them undesirable for consumption as observed by the off-odour and flavour scores (data not shown, Refer to appendix A, Table. 2B). Studies by Ayhan and Estürk (2009) also reported a lower shelf life for minimally processed pomegranate arils packaged in low oxygen atmospheres (5% O_2 + 10% CO_2) compared to those packaged in air, nitrogen and enriched oxygen.

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Conclusions

Equilibrium O_2 (16-18%) and CO_2 (7%) levels were established in the low barrier BOP film but they were not within the recommended levels (2-5 % O_2 and 10-20% CO_2) for minimally processed pomegranate arils. In contrast, O_2 decreased and CO_2 increased continuously in the high barrier polylid film during storage. Carbon dioxide accumulated to high levels (27-43%) that might have been detrimental to the packaged arils across all the treatments, while O_2 levels in packages initially flushed with low O_2 reduced below the recommended critical level (2%). Aril RRs increased significantly with storage duration across all the treatments in both types of polymeric films. In experiment 1, pomegranate arils packaged under passive modified atmospheres in low barrier BOP film maintained the highest RR from day 3 to the end of the storage duration, while those packaged in clamshell containers maintained the lowest RRs throughout the storage duration. Arils packed in modified atmospheres that were initially flushed with 100% N_2 in the high barrier polylid film (experiment 2) maintained significantly lower RRs than the other MAP treatments from day 6 until the end of the storage period. The low O_2 atmosphere attained in MAP initially flushed with 100% N_2 were effective in inhibiting increase in RR. High O_2 atmospheres (30% O_2 + 40% CO_2) were effective in prolonging the lag phase of total aerobic bacteria in arils packaged in low barrier BOP film until day 6. Similarly, pomegranate arils packed in high oxygen (30% O_2 + 40% CO_2) and 100% N_2 MAP in the high barrier polylid film maintained significantly lower aerobic mesophilic bacteria counts than the other MAP treatments throughout the storage duration. Shelf life based on overall acceptability sensory scores was limited to 6 and 9 days for arils in clamshell containers and passive MAP respectively, while those in high O_2 atmospheres in both the low barrier BOP and high barrier polylid film were still acceptable beyond day 9. Although they are commonly used in industry for passive MAP of fresh horticultural produce, including pomegranate arils, this study has shown that both the low barrier BOP and high barrier polylid polymeric films did not create suitable equilibrium atmosphere conditions for the minimally processed pomegranate arils. Therefore, further studies using more suitable barrier films are recommended.

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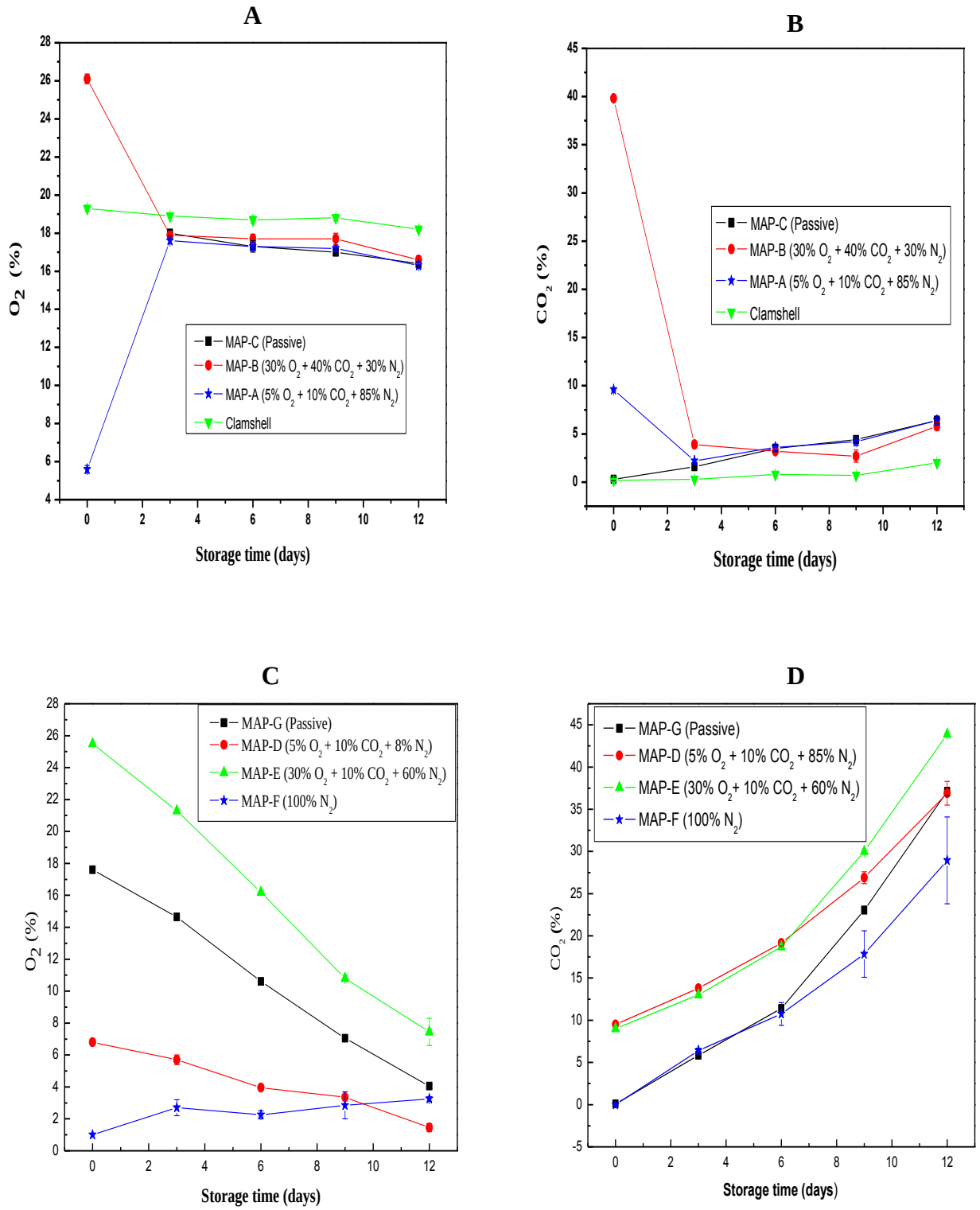

Figure 1. Changes in headspace gas composition in minimally processed pomegranate arils packaged in PET trays and sealed with different polymeric films at 5 °C. (**A**) Changes in O_2, and (**B**) CO_2 levels for low barrier BOP film and clamshell packages; (**C**) Changes in O_2 and (**D**) CO_2 levels in high barrier Polylid film. Error bars represent standard deviation (P = 0.05).

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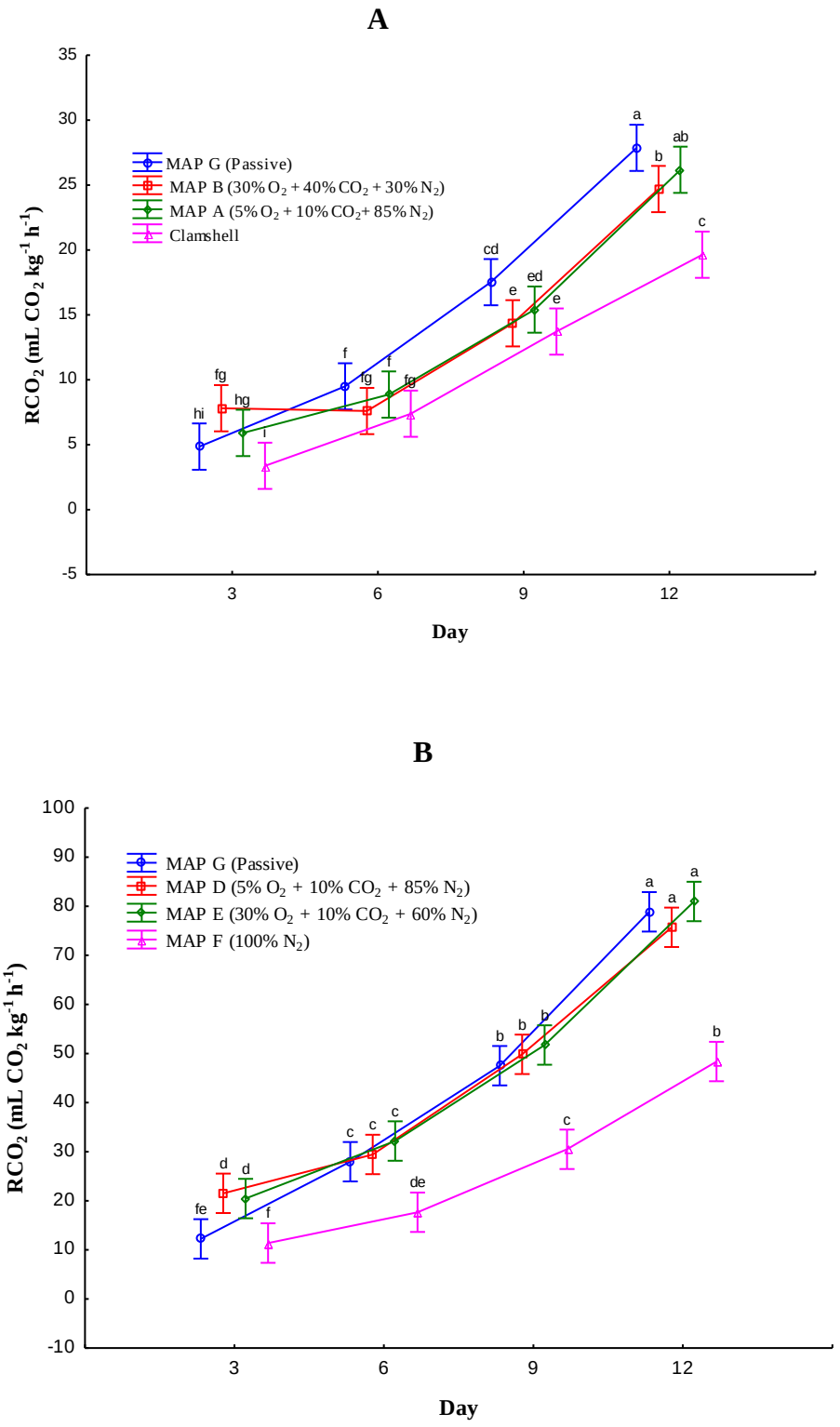

Figure 2. Respiration rate (RCO_2) of minimally processed pomegranate arils packaged under passive and active modified atmospheres in (**A**) low barrier BOP film and clamshell packages (**B**) high barrier POLYLID film. Vertical bars denote SD of mean values. MAP*Storage duration ($P < 0.05$)

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Table 1. Effects of active and passive MAP and storage duration on firmness of minimally processed pomegranate arils packaged in low barrier BOP film, clamshell trays and high barrier polylid film at 5 °C. Means with the same letters across each column and row are not statistically different according to Fisher LSD test (P = 0.05)

	Treatments	Storage duration (days)			
		3	**6**	**9**	**12**
BOP fim	**MAP-A** (5% O_2+10%CO_2)	116.07 ± 4.78^{efc}	114.09 ± 2.80^{efd}	116.00 ± 0.36^{efc}	121.39 ± 7.77^{abcd}
	MAP-B (30% O_2+40%CO_2)	119.65 ± 4.36^{ae}	118.65 ± 1.40^{eb}	118.13 ± 4.7^{ec}	127.67 ± 6.43^{a}
	MAP-C (Passive)	127.33 ± 1.02^{ab}	124.11 ± 0.02^{abc}	107.64 ± 5.34^{f}	115.96 ± 2.75^{efc}
	Clamshell	120.12 ± 5.09^{ae}	118.59 ± 1.27^{eb}	111.72 ± 5.75^{ef}	120.28 ± 0.65^{ae}
Polylid film	**MAP-D** (5% O_2+10%CO_2)	115.35 ± 4.60^{ec}	118.28 ± 2.44^{a}	222.29 ± 11.66^{bcd}	125.54 ± 11.32^{ec}
	MAP-E (30% O_2+10%CO_2)	109.20 ± 4.68^{be}	121.75 ± 3.02^{a}	229.35 ± 10.84^{be}	121.35 ± 7.59^{e}
	MAP-F(100% N_2)	111.16 ± 4.32^{ec}	117.60 ± 2.38^{a}	218.32 ± 10.37^{b}	133.77 ± 10.69^{ed}
	MAP-G(Passive)	111.89 ± 4.23^{bcd}	124.03 ± 0.76^{a}	218.94 ± 10.38^{bc}	126.17 ± 7.29^{ec}

Treatment	**Effect**	***p***
BOP and clamshell	MAP (A)	0.285
	Storage duration (B)	0.004
	A*B	0.031
Polylid	MAP (A)	0.999
	Storage duration (B)	0.002
	A*B	0.536

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Table 2. Effect of active and passive MAP and storage duration on chemical attributes (pH, TA, TSS, TA:TSS)of minimally processed pomegranate arils packaged in BOP low barrier film at 5 °C. Means with the same letters across each column and row are not significantly different (P = 0.05)

Parameter	Application	Day 0	Day 3	Day 6	Day 9	Day 12
pH	**MAP-A** (5% O_2 + 10% CO_2)	3.10 ± 0.03^{d}	2.91 ± 0.04^{f}	3.08 ± 0.02^{d}	3.29 ± 0.02^{a}	2.89 ± 0.02^{f}
	MAP-B (30% O_2 + 40% CO_2)	3.10 ± 0.03^{d}	2.91 ± 0.02^{f}	3.07 ± 0.02^{d}	3.24 ± 0.03^{cb}	2.90 ± 0.06^{f}
	MAP-C (Passive)	3.10 ± 0.03^{d}	2.96 ± 0.03^{e}	3.07 ± 0.02^{d}	3.22 ± 0.01^{c}	2.92 ± 0.02^{ef}
	Clamshell	3.10 ± 0.03^{d}	2.92 ± 0.02^{ef}	3.1 ± 0.02^{d}	3.27 ± 0.01^{ab}	2.85 ± 0.04^{g}
Titratable acidity (%)	**MAP-A** (5% O_2 + 10% CO_2)	1.61 ± 0.20^{ac}	1.46 ± 0.05^{cb}	1.41 ± 0.05^{c}	1.88 ± 0.44^{a}	1.63 ± 0.23^{ac}
	MAP-B (30% O_2 + 40 CO_2)	1.61 ± 0.20^{ac}	1.46 ± 0.23^{cb}	1.40 ± 0.02^{c}	1.48 ± 0.25^{cb}	1.36 ± 0.04^{c}
	MAP-C (Passive)	1.61 ± 0.20^{ac}	1.66 ± 0.38^{ac}	1.38 ± 0.05^{c}	1.45 ± 0.05^{cb}	1.78 ± 0.27^{ab}
	Clamshell	1.61 ± 0.20^{ac}	1.43 ± 0.05^{cb}	1.43 ± 0.05^{cb}	1.43 ± 0.06^{cb}	1.63 ± 0.39^{ac}
TSS (°Brix)	**MAP-A** (5% O_2 + 10% CO_2)	17.13 ± 0.32^{a}	16.1 ± 0.66^{fb}	16.37 ± 0.25^{abcd}	15.57 ± 1.11^{fd}	15.47 ± 0.58^{fe}
	MAP-B (30% O_2 + 40%CO_2)	17.13 ± 0.32^{a}	15.97 ± 0.38^{fb}	16.13 ± 0.40^{fb}	15.73 ± 0.81^{fc}	15.6 ± 0.40^{fd}
	MAP-C (Passive)	17.13 ± 0.32^{a}	16.67 ± 0.12^{ab}	16.3 ± 0.44^{abcde}	16.67 ± 0.35^{ab}	16 ± 0.46^{fb}
	Clamshell	17.13 ± 0.32^{a}	17.03 ± 0.46^{a}	16.6 ± 0.53^{abc}	16.07 ± 0.31^{fb}	15.33 ± 0.55^{f}
TSS:TA	**MAP-A** (5% O_2 + 10% CO_2)	10.75 ± 0.88^{abcd}	11.01 ± 0.4^{abcd}	11.62 ± 0.4^{abc}	8.60 ± 1.88^{e}	9.62 ± 1.20^{ec}
	MAP-B (30% O_2 + 40% CO_2)	10.75 ± 0.88^{abcd}	11.07 ± 1.1^{abcd}	11.50 ± 0.15^{abc}	10.76 ± 1.1^{abcd}	11.47 ± 0.11^{abc}
	MAP-C (Passive)	10.75 ± 0.88^{abcd}	10.39 ± 1.76^{ae}	11.84 ± 0.12^{ab}	11.50 ± 0.21^{abc}	9.16 ± 1.22^{ed}
	Clamshell	10.75 ± 0.88^{abcd}	11.89 ± 0.14^{a}	11.64 ± 0.10^{abc}	11.24 ± 0.24^{abc}	9.81 ± 1.94^{eb}

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Table 2 continued

Effect	*p-value*			
	pH	**TA**	**TSS**	**TA:TSS**
MAP(A)	0.337	0.221	0.04	0.202
Storage duration(B)	0.000	0.158	0.007	0.018
A*B	0.000	0.253	0.211	0.188

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Table 3. Effect of active and passive MAP and storage duration on chemical attributes (pH, TA, TSS, TA:TSS) of minimally processed pomegranate arils packaged in high barrier Polylid film at 5 °C. Means with the same letters across each column and row are not significantly different (P = 0.05)

Parameter	Application	Day 0	Day 3	Day 6	Day 9	Day 12
pH	**MAP-D** (5% O_2 + 10% CO_2)	3.10 ± 0.2^{ef}	2.06 ± 0.04^{g}	3.70 ± 0.01^{a}	3.33 ± 0.10^{bcd}	3.24 ± 0.06^{ec}
	MAP-E (30 % O_2 + 10% CO_2)	3.10 ± 0.2^{ef}	2.14 ± 0.04^{g}	3.71 ± 0.02^{a}	3.41 ± 0.19^{b}	3.11 ± 0.01^{ef}
	MAP-F (100% N_2)	3.10 ± 0.2^{ef}	2.09 ± 0.04^{g}	3.71 ± 0.01^{a}	3.11 ± 0.04^{ef}	3.35 ± 0.13^{bc}
	MAP-G (Passive)	3.10 ± 0.2^{ef}	2.11 ± 0.04^{g}	3.65 ± 0.01^{a}	3.20 ± 0.01^{ed}	3.03 ± 0.02^{f}
Titratable acidity (%)	**MAP-D** (5% O_2 + 10% CO_2)	1.34 ± 0.26^{ac}	1.19 ± 0.04^{cb}	1.17 ± 0.03^{c}	1.66 ± 0.32^{a}	1.33 ± 0.11^{ac}
	MAP-E (30 % O_2 + 10% CO_2)	1.34 ± 0.26^{ac}	1.20 ± 0.06^{cb}	1.13 ± 0.02^{c}	1.37 ± 0.50^{cb}	1.67 ± 0.24^{c}
	MAP-F (100% N_2)	1.34 ± 0.26^{ac}	1.27 ± 0.09^{cb}	1.13 ± 0.02^{cb}	1.54 ± 0.44^{cb}	1.60 ± 0.33^{ac}
	MAP-G (Passive)	1.34 ± 0.26^{ac}	1.34 ± 0.13^{ac}	1.19 ± 0.14^{c}	1.43 ± 0.34^{cb}	1.41 ± 0.08^{ab}
TSS (°Brix)	**MAP-D** (5% O_2 + 10% CO_2)	16.60 ± 0.1^{abc}	15.77 ± 0.23^{cb}	16.17 ± 0.72^{ac}	17.13 ± 0.55^{ab}	17.77 ± 2.67^{a}
	MAP-E (30 % O_2 + 10% CO_2)	16.60 ± 0.1^{abc}	15.80 ± 0.36^{cb}	15.43 ± 0.29^{cb}	14.47 ± 2.15^{c}	16.20 ± 0.62^{ac}
	MAP-F (100% N_2)	16.60 ± 0.1^{abc}	15.77 ± 0.61^{cb}	15.57 ± 0.15^{cb}	16.97 ± 0.32^{ab}	15.70 ± 1.73^{c}
	MAP-G (Passive)	16.60 ± 0.1^{abc}	16.10 ± 0.53^{ac}	16.00 ± 0.40^{cb}	15.63 ± 0.40^{cb}	16.07 ± 0.49^{ac}
TSS:TA	**MAP-D** (5% O_2 + 10% CO_2)	12.48 ± 1.05^{ac}	13.22 ± 0.35^{ac}	13.85 ± 0.28^{a}	10.58 ± 2.17^{ac}	13.50 ± 3.20^{ab}
	MAP-E (5% O_2 + 10% CO_2)	$12..48 \pm 1.05^{ac}$	13.15 ± 0.57^{ac}	13.66 ± 0.35^{a}	11.75 ± 5.09^{ac}	9.84 ± 1.88^{ab}
	MAP-F (100% N_2)	12.48 ± 1.05^{ac}	12.48 ± 0.88^{ac}	13.82 ± 0.23^{a}	11.56 ± 2.91^{ac}	10.11 ± 2.70^{cb}
	MAP-G (Passive)	12.48 ± 1.05^{ac}	12.06 ± 1.20^{ac}	13.54 ± 1.75^{ab}	11.24 ± 2.11^{ac}	11.43 ± 0.36^{ac}

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Table 3 continued

Effect	*p-value*			
	pH	**TA**	**TSS**	**TA:TSS**
MAP (A)	0.053	0.963	0.054	0.776
Storage duration (B)	0.000	0.001	0.448	0.016
A*B	0.001	0.586	0.239	0.753

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Table 4. Effect of active and passive MAP and storage duration on total aerobic mesophilic bacteria, and mould and yeast counts of minimally processed pomegranate arils packaged in low barrier BOP and clamshell packages at 5 °C. Means with the same letters across each column and row are not significantly different (P = 0.05)

Parameters	Treatments	Storage duration		
		0	6	12
Total aerobic mesophilic bacteria counts (mean log CFU g^{-1})	**MAP-A** (5%O_2+10%CO_2)	1.25± 0.03^{g}	5.42 ± 0.05^{e}	6.11 ± 0.02^{b}
	MAP-B (30%O_2+40%CO_2)	1.25 ± 0.03^{g}	4.13 ± 0.04^{f}	6.20 ± 0.05^{a}
	MAP-C (Passive)	1.25 ± 0.03^{g}	6.02 ± 0.05^{c}	6.17 ± 0.02^{ab}
	Clamshell	1.25 ± 0.03^{g}	5.50 ± 0.02^{d}	6.05 ± 0.03^{c}
Yeast and mould counts (mean log CFU g^{-1})	**MAP-A** (5%O_2+10%CO_2)	below detection	3.58 ± 0.05^{c}	4.16 ± 0.28^{b}
	MAP-B (30%O_2+40%CO_2)	below detection	3.56 ± 0.24^{c}	4.53 ± 0.25^{a}
	MAP-C (Passive)	below detection	3.63 ± 0.13^{c}	4.20 ± 0.35^{ab}
	Clamshell	below detection	3.53 ± 0.12^{c}	4.36 ± 0.10^{ab}

Effects	*p*-value	
	Total aerobic mesophilic count	Yeast and mould count
MAP(A)	0.000	0.538
Storage duration(B)	0.000	0.000
A*B	0.000	0.315

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Table 5. Effect of active and passive MAP and storage duration on total aerobic mesophilic bacteria, and mould and yeast counts of minimally processed pomegranate arils packaged in high barrier polylid film at 5 °C. Means with the same letters across each column and row are not significantly different (P = 0.05)

Parameters	Treatments		Storage duration	
			6	12
Total aerobic mesophilic bacteria counts (mean log cfu g^{-1})	**MAP-D** (5%O_2+10%CO_2)	1.62 ± 0.10^{e}	5.77 ± 0.14^{ab}	5.84 ± 0.12^{a}
	MAP-E (30%O_2+10%CO_2)	1.62 ± 0.10^{e}	5.51 ± 0.15^{cd}	5.53 ± 0.08^{cb}
	MAP-F(100%N_2)	1.62 ± 0.10^{e}	5.43 ± 0.06^{d}	5.61 ± 0.07^{cb}
	MAP-G (passive)	1.62 ± 0.10^{e}	5.72 ± 0.06^{ab}	5.82 ± 0.15^{a}
Yeast and mould counts (mean log cfu g^{-1})	**MAP-D** (5%O_2+10%CO_2)	below detection	3.13 ± 0.12^{c}	4.73 ± 0.15^{a}
	MAP-E (30%O_2+40%CO_2)	below detection	3.79 ± 0.17^{b}	4.49 ± 0.20^{a}
	MAP-F(100%N_2)	below detection	3.10 ± 0.17^{c}	4.55 ± 0.13^{a}
	MAP-G (Passive)	below detection	3.39 ± 0.09^{c}	4.69 ± 0.27^{a}

Effects	*p*- value	
	Total aerobic mesophilic count	Yeast and mould count
MAP (A)	0.000	0.031
Storage duration (B)	0.000	0.000
A*B	0.013	0.002

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Chapter 5

Phytochemical properties and radical scavenging activity of pomegranate arils (cv. Wonderful) as affected by active modified atmosphere packaging

Abstract

This study investigated the effects of modified atmosphere packaging (MAP) and storage duration on total anthocyanin content, total phenolic content, ascorbic acid content and radical scavenging activity of minimally processed pomegranate arils stored at 5 °C for 12 days. Two separate experiments were conducted. In experiment (1) pomegranate arils were packaged in low barrier bi-axially oriented polyester (BOP) film in active modified atmospheres (5% O_2 + 10 % CO_2 + 85 %N_2; 30 % O_2 + 40 % CO_2 + 30 % N_2), passive MAP and clamshell containers as control. In experiment (2) a high barrier polymeric (polylid) film was used and arils were packaged in three active modified atmospheres (5% O_2 + 10% CO_2 + 85% N_2; 30% O_2 + 10% CO_2 + 60% N_2; 100% N_2) and passive MAP. Total anthocyanin content of arils in experiment 1 was significantly affected by MAP and fluctuated with storage duration across all the MAP treatments. At the end of 12-day storage duration, anthocyanin content was highest in clamshell packages (30.7 ± 0.9 mg C3gE/ 100ml) and lowest in passive MAP (26.7 ± 1.8 mg C3gE/ 100 ml). Similarly, total anthocyanin content (TAC) of arils in high barrier polylid film fluctuated with storage across all the MAP treatments, and arils packaged in active MAP with 100% N_2 maintained higher TAC levels from day 9 until the end of storage. Total phenolic content (TPC) was not significantly altered by MAP in both low barrier BOP and high barrier polylid film. However, TPC of arils in low barrier BOP increased significantly with storage across all MAP treatments. Ascorbic acid content of pomegranate arils in both experiments decreased significantly with storage duration across all the MAP treatments. Active MAP with 100% N_2 was effective in supressing ascorbic acid oxidation from day 6 until the end of storage. Similarly, although radical scavenging activity (RSA) of minimally processed pomegranate arils was not significantly affected by MAP and storage duration in both experiments, RSA of arils in 100% N_2 in experiment 2 was relatively higher than other MAP treatments from day 6 until the end of storage.

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Introduction

Pomegranate arils and extracts from the bark, leaves, flowers and the fruit husk have been used traditionally since ancient times to treat various ailments (Lansky and Newman, 2007; Larrosa et al., 2010; Lee et al., 2010). Recent scientific findings have shown that pomegranate is a rich reservoir of phytochemical compounds, flavonoids, phenolic acids and tannins that confer medicinal properties (Fawole and Opara, 2013; Mphahlele et al., 2014). The functional properties of pomegranate include anti-microbial, antioxidant, anti-mutagenic, anti-inflammatory, anti-hypertension and antitumor (Opara et al., 2009; Viuda-Martos et al., 2010).

Minimally processed pomegranate arils provide a convenient, fresh and healthy food (Ayhan and Eştürk, 2009). However, they suffer accelerated deterioration in quality attributes and nutrients compared to the intact fruit due to physiological stresses, physical damage and wounding suffered during minimal processing (Rico et al., 2007; Martínez-Romero et al., 2013). Modified atmosphere packaging (MAP), a technique in which the normal composition of air (O_2-21%; CO_2-0.01%; N_2-78%) around packaged fresh produce is altered (Al-Ati and Hotchkiss, 2002; Waghmare and Annapure, 2013; Caleb et al., 2013) offers the possibility to extend shelf life of minimally processed pomegranate arils (Gil et al., 1996; López-Rubira et al., 2005; Palma et al., 2009; Caleb et al., 2013). Modified atmosphere packaging slows down the rate of physiological and biochemical processes and retards senescence (Artés et al., 2006). The stability and concentration of bioactive compounds in pomegranate is affected by MAP but the specific effects are not well established (Mphahlele et al., 2014).

Anthocyanin content of pomegranate arils (cv. Mollar of Elche) harvested in October and stored under passive MAP at 5 °C for 13 days remained unaltered (López-Rubira et al., 2005). In contrast, Caleb et al. (2013) reported a decrease in anthocyanin content of minimally processed pomegranate arils 'Acco' and 'Herskawitz' packaged in passive modified atmospheres at 5, 10 and 15 °C for 14 days. Similarly, Ayhan and Eştürk (2009) reported a decrease in anthocyanin content of pomegranate arils (cv. Hicaznar) packaged in active MAP and stored at 5 °C for 18 days. The authors reported a higher retention of TPC in pomegranate arils in high O_2 atmospheres (70%) than those in other MAP treatments. In contrast, TPC of minimally processed pomegranate arils packaged in high oxygen (90 kPa O_2) at 5 °C decreased with storage (Maghoumi et al., 2013).

The variation in bioactive compounds reported in these studies could imply that the response of bioactive compounds to MAP is dependent on cultivar and the in-package atmospheric compositions. Furthermore, the limited scientific information on the effects of MAP on

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phytochemicals and radical scavenging activity of minimally processed pomegranate arils warrants further studies. This study, therefore, investigated the effects of MAP on phytochemical and radical scavenging activity of minimally processed pomegranate arils (cv. Wonderful) stored at 5 °C for 12 days.

Materials and Methods

Sample preparation and packaging

Pomegranate fruit (cv. Wonderful) was obtained at commercially ripened stage from Houdconstant packhouse in Porterville, Western Cape (33°01′00″S, 18°59′00″E), South Africa. Fruits were sorted, cleaned and minimally processed at the farm pack house. Fruits free from defects were washed in sterilised water and arils extracted using a commercial extraction machine (Arilsystem, Juran Metal Works, Israel). Extracted arils were bulk packaged in sterilized polyethylene bags and transported in ice boxes to the postharvest research laboratory at Stellenbosch University. Arils (300g) were packaged in polyethylene terephthalate (PET) trays with dimensions 28 x 19 cm (ZIBO containers, PTY, LTD. Kuilsrivier, South Africa) and flushed with food grade gas mixtures (Air Products Pty; Kempton Park, South Africa) using a tray sealer (Model T200 Multivac, Wolfertschwenden, Germany).

Two experiments were conducted consecutively. In the first experiment a low barrier polymeric film, bi-axially oriented polyester (BOP) (26µm thickness, 75 cc/m^2/day O_2 and 15-20 ml/m^2/day CO_2 transmission rates, respectively, and 20 g/m^2/day water vapour transmission rate transmission rates) supplied by Knilam Packaging (Pty) Ltd. (Cape town, South Africa) was used to heat-seal PET trays. The following gas mixtures were applied: MAP-A (5% O_2 + 10% CO_2 + 85% N_2), MAP-B (30% O_2 + 40% CO_2 + 30% N_2), MAP-C (passive MAP). Control arils were packaged in polyethylene terephthalate (PET) clamshell containers (420 µm thickness) and dimensions of 11.5 × 11.5 × 3.5 cm 3.5 cm^3. In the second experiment, a high barrier polymeric film Polylid® 107 HB55 (55µm thickness, 21-23 ml/m^2/day O_2 and 5-7g/m^2/day water vapour transmission rate transmission rates) supplied by Barkai Polyon industries Ltd. (Tel Aviv, Isreal). The following gas mixtures were applied: MAP-D (5% O_2 + 10% CO_2 + 85% N_2), MAP-E (30% O_2 + 10% CO_2 + 60% N_2), MAP-F (100% N_2) and MAP-G (passive MAP).

Samples were stored at 5 °C for 12 days and analyses conducted in triplicate on sampling days 0, 3, 6, 9, and 12. Pomegranate arils were juiced separately for each of the treatments on the sampling days using a LiquaFresh juice extractor (Mellerware, South Africa) and used to determine total

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phenolic content (TPC), total anthocyanin content (TAC), ascorbic acid content and radical scavenging activity (RSA).

Total anthocyanin concentration

Total anthocyanin concentration (TAC) was quantified using the pH differential method (Fawole et al., 2011). In triplicates, pomegranate juice (PJ) sample (1 mL) was mixed with 9 mL of pH 1.0 and pH 4.5 buffers, separately. The absorbance of the mixtures was measured at 520 and 700 nm using a UV-vis spectrophotometer. TAC was expressed as cyaniding 3-glucoside using the following equations

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.0} \quad (1)$$

$$\text{Total monomeric anthocyanin (mg/mL)} = (A \times MW \times DF)/(\varepsilon \times L) \quad (2)$$

where A = Absorbance, ε = Cyd-3-glucoside molar absorbance (26,900), MW = anthocyanin molecular weight (449.2), DF = dilution factor, L = cell path length (1 cm). Results were expressed as mean ± S.D cyaniding 3-glucoside equivalents per 100 mL PJ (mg C_3gE/100 mL PJ).

Total phenolic content

Total phenolic (TP) concentrations in juice samples were determined using the Folin Ciocalteu (Folin-C) colorimetric method (Fawole et al., 2011). Total phenolic concentrations were determined spectrophotometrically at 750 nm (Thermo Scientific Technologies, Madison, Wisconsin). by adding Folin Ciocalteu reagent to the pomegranate juice (PJ) sample, and expressed as mean ± S.D (mg) gallic acid equivalents per 100 mL crude juice.

Ascorbic acid content

Ascorbic acid was determined by the colorimetric method described recently by Arendse et al. (2014). Approximately 1 mL of PJ was diluted with 1% metaphosphoric acid at room temperature and voltexed and sonicated for 5 min in cold water followed by centrifugation at 10,000 rpm for 5 min at 4 °C. The supernatant (1 mL) was diluted with 9 mL of 2,6-dichlorophenolindophenol dye (0.0025%) and incubated in a dark environment for 10 min. Ascorbic acid concentration was measured spectrophotometrically at 510 nm (Thermo Scientific Technologies, Madison, Wisconsin). The concentration of ascorbic acid in PJ was quantified using a standard curve (R^2 >

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0.95) of known concentration of l-ascorbic acid (Sigma) and final results expressed as mean ± S.D. (milligrams) ascorbic acid per 100 ml of crude juice.

Radical scavenging activity

Radical scavenging activity of the PJ was determined by the DPPH method as described by Fawole et al. (2013). Methanolic extracts of pomegranate juice sample (15 µL) was diluted with methanol (735 µL) in test tubes followed by the addition of methanolic DPPH solution (750 µL, 0.1 mM). The mixtures were incubated at room temperature for 30 min in the dark, and the absorbance was measured at 517 nm using UV-vis spectrophotometer (Thermo Scientific Technologies, Madison, Wisconsin). Absorbance was compared with the standard curve ($R^2 > 0.9$) (ascorbic acid, 0 – 2.0 mM). The free-radical capacity of PJ was expressed as ascorbic acid (mM) equivalents per mL PJ (mM AAE/mL).

Statistical analysis

A factorial analysis of variance (ANOVA) was performed to determine the effects of MAP and storage duration and means separated using Fisher's least significant differences (LSD) test at 95% confidence interval using Statistica software (Statistica 10.0, Statsoft Inc., USA). All values are presented as mean ± standard deviation (SD).

Results and Discussion

Total anthocyanin

Anthocyanins are water soluble polyphenolic compounds responsible for the red colouration in pomegranate fruit peel and arils (Alighourchi et al., 2008; Arendse et al., 2014). MAP and storage duration had significant effects ($P < 0.05$) on total anthocyanin content (TAC) of minimally processed pomegranate arils in experiment 1 (Table 1). Total anthocyanin content fluctuated with storage in MAP-A (5% O_2 + 10% CO_2 +85% N_2), MAP-B (30% O_2 + 40% CO_2 + 85% N_2) and clamshell packages, throughout the storage. On the other hand, arils in passive MAP generally maintained steady levels of total anthocyanins, increasing slightly during storage. By the end of storage, TAC of arils across all MAP treatments was significantly higher ($P < 0.05$) than those at day 0. The lowest TAC values were observed in arils in passive MAP (26.7 ± 1.8 mg C3gE/ 100 ml) and the highest in clamshell packages (30.7 ± 0.9 mg C3gE/ 100 ml). Interaction of MAP and storage duration had significant effects ($p = 0.005$) on TAC of minimally processed pomegranate in experiment 2 (table 2). TAC fluctuated with storage across all the MAP treatments and by the end

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of storage, 100% N_2 atmospheres had the highest TAC (21.2 ± 1.9 mg C3gE/ 100 ml) and MAP-E (30% O_2 + 10% CO_2 + 60% N_2) had the lowest (17± 1.2 mg C3gE/ 100ml).

The range of TAC values found in our study are similar to those reported by Caleb et al. (2013) for minimally processed pomegranate arils 'Acco' and 'Herskawitz' packaged in passive MAP at 5 °C, 10 and 15 °C for 14 days. The authors reported a decrease in TAC with storage duration across all the treatments from 21.1 to 13.3 mg C3gE/ 100 ml for 'Acco' and 20.4 to 12.3 mg C3gE/ 100 ml for 'Herskawitz'. In addition, higher TAC was observed in arils packaged under passive MAP compared to clamshell containers across all the temperatures, which could suggest that MAP is effective in retarding anthocyanin degradation. Similarly, TAC of 'Molar of Elche' pomegranate arils harvested in October and stored in passive MAP at 5 °C for 13 days was maintained (López-Rubira et al., 2005). Total anthocyanin content of 'Primosole' pomegranate arils packaged under passive MAP at 5 °C was also maintained after 10 days of storage (Palma et al., 2009). Gil et al. (1996) investigated the effects of washing treatments and active and passive MAP on quality attributes of 'Molar' pomegranate arils at 1 °C for 7 days and reported minimal changes in TAC of arils across all MAP treatments. Studies by Ayhan and Eştürk et al. (2009) also showed that MAP did not significantly alter TAC of 'Hicaznar' arils packaged in active and passive MAP at 5 °C for 18 days. Similarly, TAC of arils in experiment 2 of our study was not significantly altered by MAP and storage duration. However, TAC of arils in experiment 1 increased significantly with storage across all the MAP treatments. This might have been as a result of the low barrier characteristics of the packaging film used in experiment 1, which provided a poor barrier to gas and water vapour diffusion. In addition, the highest increase in TAC was observed in arils in clamshell containers which might have suffered more moisture loss compared to those packaged in polymeric film. Gil et al. (1996) also reported an increase in TAC with storage in unpackaged 'Molar' pomegranate arils at 1, 4 and 8 °C and attributed it to enhanced moisture loss.

Total phenolic content

Interaction of MAP and storage duration had no significant effects ($p > 0.05$) on the total phenolic content (TPC) of pomegranate juice in both experiments. Total phenolic content of pomegranate in experiment 1 fluctuated with storage across all the MAP treatments from 633.9 ± 27.7 mg/100 mL on day 0 to values ranging from 822.2 ± 7.8 to 966.32 ± 29.19 mg/100 ml by the end of storage (Table 1). Similarly, TPC of minimally processed arils in experiment 2 fluctuated with storage across all the MAP treatments. By the end of the storage duration, TPC of pomegranate across all the MAP treatments was not significantly different from that at day 0 (544.9 ± 20.8 mg/100 mL),

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except for a significant increase in arils in Passive MAP (Table 2). The range of TPC values in our studies are similar to those reported by Fawole et al. (2013) for 'Bhagwa' and 'Ruby' pomegranate. The authors reported a significant reduction in TPC of pomegranate stored beyond 8 weeks at 5 °C.

In contrast to the findings in the present study, Palma et al. (2009) reported significant reduction in TPC of 'Primosole' pomegranate arils packaged in polypropylene trays and stored at 5 °C, from 1492 mg/L on day 0 to 1392 mg/L by day 10. Similarly, TPC of pomegranate arils packaged in rigid polypropylene boxes and stored at 3 °C for 12 days decreased with storage from 107.1 ± 2.3 mg $100g^{-1}$ to 94.8 ± 4.1 mg $100g^{-1}$ after 8 days of storage (Martínez-Romero et al., 2013). Fawole et al. (2013) also reported significant reductions in TPC of 'Bhagwa' and 'Ruby' pomegranate with prolonged storage at 5 °C. Reduction of total phenolics with storage in minimally processed produce is attributed to mechanical damage suffered during minimal processing operations which triggers phenolic oxidation by the enzymes polyphenol oxidase (PPO) and peroxidase (Andrés-Lacueva et al., 2010).

Modified atmospheres with low O_2 and high CO_2 have been suggested to result in higher retention of TPC due to a reduction in biological activity in tissues of minimally processed products and reduced activity of PPO (Saxena et al., 2009). Fresh-cut jackfruit bulbs packaged in low O_2 (3 kPa) and high CO_2 (5 kPa) at 6 °C for 35 days had a higher retention of TPC than those packaged in passive MAP (Saxena et al., 2009). The study proposed that the low O_2 atmospheres and high CO_2 reduced the rate of phenolic oxidation by PPO. On the other hand, high O_2 atmospheres have been shown to result in enhanced oxidation of phenolic compounds in minimally processed fresh produce (Oms-Oliu et al., 2008). Total phenolic content of minimally processed pomegranate arils packaged in high O_2 (90 kPa) at 5 °C decreased during the first week of storage (736.8 mg ChAE kg^{-1}FW), then increased in the last 7 days of shelf life (881.4 mg ChAE kg^{-1}FW) when the O_2 concentration decreased (Maghoumi et al., 2013). Similarly, fresh-cut 'Flor de Invierno' pears under high O_2 (70kPa) atmospheres underwent substantial loss of phenolic compounds (chlorogenic acid) throughout the storage duration compared to the low O_2 atmospheres (2.5 kPa O_2 +7 kPa CO_2) and passive MAP (Oms-Oliu et al., 2008). However, TPC of minimally processed pomegranate arils in our study was not significantly affected by MAP in both experiment 1 and 2. Instead, TPC fluctuated inconsistently with storage across all the MAP treatments. Ayhan and Eştürk (2009) also observed fluctuations in TPC of minimally processed pomegranate arils (cv. Hicaznar) packaged in low O_2 (5%), high O_2 (70%), 100% N_2 and passive MAP at 5 °C. The authors further reported that alterations in TPC resulting from storage duration were more pronounced than those resulting from MAP application.

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Ascorbic acid

Modified atmospheres had no significant effects on ascorbic acid content of arils in experiment 1 ($p > 0.05$). However, ascorbic acid content decreased significantly ($P = 0.000$) with storage across all the treatments until day 9, after which it increased slightly. Arils in clamshell containers had the lowest ascorbic acid content from days 9 to 12. Modified atmospheres and storage duration had significant effects ($p < 0.05$) on ascorbic acid content of arils in experiment 2. Ascorbic acid in MAP-E (30% O_2 + 10% CO_2 + 60% N_2), MAP-F (100% N_2) and MAP-G (Passive) increased during the first three days of storage and then reduced significantly until day 9 before increasing slightly on day 12 (Table 2). Arils in passive MAP (MAP-G) had the lowest ascorbic acid content from day 6 to the end of the storage duration. On the other hand, minimally processed pomegranate arils in 100% N_2 maintained the highest ascorbic acid content from day 6 until the end of storage. Ascorbic acid content values found in our study are similar to those reported by Opara et al. (2009), who found significant variation in ascorbic acid content among 5 pomegranate cultivars ranging from 52.8 mg/100g (fresh weight) FW to 72.0 mg/100g FW.

Ascorbic acid plays an important role as a phytochemical due to its functionality as an antioxidant besides its vitamin C activity (Saxena et al., 2009). Extended storage, especially at high temperatures, has been reported to result in significant loss in ascorbic acid in pomegranate (Arendse et al., 2014; O'Grady et al., 2014). O'Grady et al. (2014) reported an initial increase in ascorbic acid content in pomegranate 'Arakta', 'Bhagwa' and 'Ruby' at 1, 4 and 8 °C until day 7 after which it decreased across all treatments. Physiological stress imposed upon fresh-cut or minimally processed products further hastens ascorbic acid loss. Modified atmospheres with low O_2 concentrations have been reported to result in higher retention of ascorbic acid content in fresh-cut commodities (Odriozola-Serrano et al., 2008). Ascorbic acid content in honey pomelo (*Citrus grandis* L.) packaged in low O_2 (3 kPa O_2 + 5 kPa CO_2) at 4 °C reduced by 13.4% compared to 25.2% reduction under passive MAP (Li et al., 2012). Similarly, ascorbic acid oxidation in fresh-cut 'Flor de invierno' pears was favoured more by high O_2 atmospheres (70 kPa O_2) than low O_2 (2.5 kPa O_2 + 7 kPa CO_2) atmospheres (Oms-Oliu et al., 2008). Higher ascorbic acid content was observed in arils packaged in 100% N_2 atmospheres (MAP-F) from day 6 until the end of storage in experiment 2 of our study. The lower O_2 levels achieved in MAP initially flushed with 100% N_2 may have inhibited ascorbic acid oxidation which could explain the higher ascorbic acid content compared to other MAP treatments.

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Radical scavenging activity

Pomegranate exhibits good antioxidant capacity primarily due to its high levels of phenolic acids, flavonoids and other polyphenolic acids (Ayhan and Eştürk, 2009). The antioxidant capacity of pomegranate juice by radical scavenging activity was determined by the DPPH assay. Interaction of MAP and storage duration had significant effects on radical scavenging activity (RSA) of arils in both experiments ($p = 0.000$). Radical scavenging activity of arils in experiment 1 of our study fluctuated with storage across all the MAP treatments, decreasing between day 3 and 6 before increasing again on day 9. By the end of the storage duration, RSA had decreased slightly from values on day 0 (159.7 ± 9.4 mg/100ml) to values ranging from 150.9 ± 13.2 to 154.3 ± 5.24 mg/100ml. Similarly, RSA of arils in experiment 2 fluctuated in an inconsistence pattern across all MAP treatments except MAP-E (30% O_2 + 10% CO_2 + 60% N_2) in which it decreased throughout the storage duration. By the end of the storage duration, RSA was highest (84.47 ± 14.66 mg/100 mL) in arils in MAP-F (100% N_2) and lowest (61.1 ± 24 mg/100mL) in MAP-E (30% O_2 + 10% CO_2 + 60% N_2). The RSA values observed in our studies are similar to those reported by Arendse et al. (2014) for 'Wonderful' pomegranates at harvest (146 mg/100ml).

Studies have shown that the response of antioxidant content and bioactivity to modified atmospheres varies depending on the type of product and atmosphere composition (Ayala-Zavala et al., 2005). Fresh-cut jackfruit packaged in GFPE bags with low O_2 atmospheres (3Kpa O_2 + 5kPa CO_2) showed significantly higher retention of RSA compared to passive MAP (Saxena et al., 2009). Antioxidant capacity of fresh-cut 'Flor de Invierno' pears in low O_2 (2.5 kPa O_2 +7 kPa CO_2) atmospheres was also significantly higher than those observed in pears under high O_2 (70kPa) atmospheres and passive MAP. Similarly, in our studies, although MAP had no significant effect on RSA in both barrier films, arils in MAP with 100% N_2 in experiment 2 maintained relatively higher RSA than other MAP treatments from day 6 until the end of storage. This may have been as a result of the lower O_2 levels maintained in this MAP treatment compared to the other treatments. On the other hand, the highest loss in RSA was observed in arils in MAP-E (30% O_2 + 10% CO_2 + 60% N_2) from day 6 until the end of the storage duration. High O_2 levels increase production of free radicals and cause oxidative stress in fruit tissue, triggering responses of the antioxidant system and affecting phytochemical content and activity (Ayala-Zavala et al., 2007). Maghoumi et al. (2013) reported an initial decrease in antioxidant activity of pomegranate arils (cv. Mollar de Elche) in high O_2 atmospheres (100 kPa O_2) at 5 °C but as O_2 concentrations decreased in the packages towards the end storage, pomegranate aril antioxidant activity increased. The authors suggested that high O_2 atmospheres were not favourable for maintaining antioxidant activity in minimally processed

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pomegranate arils. In contrast, minimally processed pomegranate arils (cv. Hicaznar) in high O_2 atmospheres (70%) at 5 °C maintained higher antioxidant activity than those in low O_2 (5%), passive MAP and 100% N_2 during the first 15 days of storage, after which it decreased significantly (Ayhan and Eştürk, 2009).

Conclusions

Ascorbic acid content of pomegranate arils reduced significantly with storage duration across all MAP treatments. Ascorbic acid of arils in the high barrier polylid film in experiment 2 was highest in 100% N_2 while that in passive MAP was lowest from day 6 until the end of storage. These observations suggest that low O_2 atmospheres were effective in retarding ascorbic acid oxidation. Total anthocyanin content (TAC) of minimally processed pomegranate arils in experiment 1 increased with storage across all the MAP treatments. It was highest in clamshell packages (30.7 ± 0.9 mg C3gE/ 100 ml) and lowest in passive MAP (26.7± 1.8 mg C3gE/ 100 ml) by the end of the storage period. On the other hand, TAC of minimally processed pomegranate arils in experiment 2 was maintained with storage across all the MAP treatments. Total phenolic content (TPC) was not significantly altered by MAP and storage duration in both experiments, but it increased slightly with storage in arils in experiment 1 and decreased in experiment 2. MAP had no significant effect on RSA in both barrier films, however, RSA of arils in MAP with 100% N_2 in experiment 2 maintained relatively higher RSA than other MAP treatments from day 6 until the end of storage. The polymeric barrier films used in this study did not create suitable equilibrium O_2 and CO_2 levels for minimally processed pomegranate arils and, therefore, posed a limitation to the study. Further studies with more suitable barrier films for pomegranate arils are therefore recommended.

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Table 1. Effects of active and passive MAP on phytochemical properties (total anthocyanin content, total phenolic content, vitamin C and radical scavenging activity) of minimally processed pomegranate arils packaged in BOP low barrier film and clamshell containers at 5 °C.

Parameter	Application	Day 0	Day 3	Day 6	Day 9	Day 12
Total anthocyanin content	**MAP-A** ($5\%O_2+10\%CO_2$)	21.52 ± 0.56^{e}	26.70 ± 5.22^{ad}	30.34 ± 4.03^{ab}	22.76 ± 3.19^{de}	27.15 ± 1.31^{ad}
(mg C3gE/ 100ml)	**MAP-B** ($30\%O_2+40\%CO_2$)	21.52 ± 0.56^{e}	24.28 ± 2.15^{dec}	25.62 ± 1.34^{db}	20.54 ± 2.28^{e}	28.59 ± 0.96^{abc}
	MAP-C(Passive)	21.52 ± 0.56^{e}	25.07 ± 1.743^{dec}	24.83 ± 4.88^{dec}	25.37 ± 3.04^{deb}	26.70 ± 1.84^{ad}
	Clamshell	21.52 ± 0.56^{e}	27.15 ± 1.31^{ad}	30.33 ± 0.20^{ab}	25.95 ± 5.77^{ad}	30.71 ± 0.87^{a}
Total phenolic content	**MAP-A** ($5\%O_2+10\%CO_2$)	633.9 ± 27.7^{d}	818.10 ± 11.85^{b}	882.03 ± 19.07^{ba}	708.53 ± 14.10^{cd}	829.10 ± 13.0^{b}
(mg/100mL)	**MAP-B**($30\%O_2+40\%CO_2$)	633.9 ± 27.7^{d}	816.86 ± 16.89^{b}	920.41 ± 20.73^{a}	717.27 ± 15.13^{cd}	844.27 ± 18.7^{b}
	MAP-C(Passive)	633.9 ± 27.7^{d}	827.20 ± 32.72^{b}	931.21 ± 30.90^{a}	773.01 ± 12.20^{bc}	866.32 ± 29.19^{ba}
	Clamshell	633.9 ± 27.7^{d}	801.85 ± 2.94^{b}	929.10 ± 39.54^{a}	769.61 ± 11.63^{bc}	822.21 ± 7.78^{b}
Ascorbic acid	**MAP-A**($5\%O_2+10\%CO_2$)	47.10 ± 2.69^{a}	43.24 ± 1.67^{abc}	34.91 ± 4.25^{def}	27.72 ± 2.45^{gh}	32.98 ± 1.24^{ge}
(mg/100mL)	**MAP-B**($30\%O_2+40\%CO_2$)	47.10 ± 2.69^{a}	45.70 ± 1.67^{a}	37.28 ± 3.83^{dce}	30.00 ± 3.10^{ghf}	30.88 ± 1.32^{ghf}
	MAP-C(passive)	47.10 ± 2.69^{a}	43.33 ± 5.66^{abc}	39.21 ± 4.95^{db}	31.86 ± 4.22^{ge}	32.37 ± 0.26^{ge}
	Clamshell	47.10 ± 2.69^{a}	44.12 ± 1.19^{ab}	38.25 ± 5.85^{dbe}	25.70 ± 2.81^{h}	27.72 ± 2.45^{gh}
Radical scavenging	**MAP-A** ($5\%O_2+10\%CO_2$)	159.76 ± 9.36^{ab}	158.34 ± 7.87^{ab}	147.42 ± 6.64^{ab}	152.46 ± 7.54^{ab}	154.28 ± 5.24^{ab}
activity	**MAP-B** ($30\%O_2+40\%CO_2$)	159.76 ± 9.36^{ab}	168.14 ± 8.47^{ab}	153.16 ± 5.49^{ab}	165.62 ± 6.83^{ab}	152.60 ± 12.95^{ab}
(mg/ 100ml)	**MAP-C**(Passive)	159.76 ± 9.36^{ab}	167.58 ± 9.88^{a}	151 ± 7.36^{ab}	167.16 ± 9.78^{ab}	150.92 ± 13.23^{ab}
	Clamshell	159.76 ± 9.36^{ab}	153.58 ± 9.74^{ab}	139.44 ± 4.95^{b}	151.62 ± 9.24^{ab}	153.30 ± 10.00^{ab}

Means with the same letters across each column and row are not significantly different (P = 0.05).

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Table 1 Continued

Effect	*p-value*			
	TAC	**TPC**	**Ascorbic acid**	**RSA**
MAP(A)	0.024	0.000	0.413	0.000
Storage duration(B)	0.003	0.000	0.000	0.000
A*B	0.424	0.000	0.149	0.009

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Table 2. Effects of active and passive MAP on phytochemical properties (total anthocyanin content, total phenolic content, vitamin C and radical scavenging activity) of minimally processed pomegranate arils packaged in polylid high barrier film at 5 °C.

Parameter	Application	Day 0	Day 3	Day 6	Day 9	Day 12
Total anthocyanin content	**MAP-D** (5%O_2+10% CO_2)	18.55 ± 1.9^{ab}	22.14 ± 2.30^{a}	22.23 ± 4.01^{a}	19.42 ± 2.01^{ab}	17.92 ± 0.06^{ab}
(mg C3gE/ 100ml)	**MAP-E** (30%O_2+10% CO_2)	18.55 ± 1.9^{ab}	18.27 ± 1.32^{ab}	19.85 ± 0.98^{ab}	15.83 ± 4.22^{ab}	16.98 ± 1.18^{ab}
	MAP-F (100% N2)	18.55 ± 1.9^{ab}	20.52 ± 2.84^{a}	19.59 ± 0.10^{ab}	21.22 ± 3.56^{a}	21.21 ± 1.92^{a}
	MAP-G(Passive)	18.55 ± 1.9^{ab}	21.14 ± 1.10^{a}	19.60 ± 2.12^{ab}	11.94 ± 2.39^{b}	20.06 ± 3.71^{ab}
Total phenolic content	**MAP-D**(5%O_2+10% CO_2)	544.9 ± 20.8^{d}	645.76 ± 20.86^{b}	647.45 ± 16.4^{b}	688.17 ± 15.63^{b}	535.47 ± 18.20^{d}
(mg/100mL)	**MAP-E**(30%O_2+10% CO_2)	544.4 ± 20.8^{d}	745.86 ± 15.14^{a}	672.90 ± 18.60^{ab}	657.63 ± 15.63^{b}	564.31 ± 18.20^{d}
	MAP-F(100% N_2)	544.4 ± 20.8^{d}	699.94 ± 29.82^{ab}	625.40 ± 12.70^{bc}	674.60 ± 19.07^{ab}	584.67 ± 19.27^{d}
	MAP-G(Passive)	544.4 ± 20.8^{d}	727.20 ± 18.68^{a}	554.13 ± 18.35^{d}	649.15 ± 10.62^{b}	625.40 ± 16.41^{bc}
Ascorbic acid	**MAP-D**(5%O_2+10% CO_2)	84.9 ± 1.4^{b}	82.81 ± 7.12^{cb}	75.09 ± 5.62^{cd}	71.58 ± 1.32^{de}	75.26 ± 2.85^{cd}
(mg/100mL)	**MAP-E**(30%O_2+10% CO_2)	84.9 ± 1.4^{b}	91.75 ± 3.58^{ab}	76.23 ± 3.83^{cd}	71.40 ± 3.07^{de}	80.35 ± 2.81^{cd}
	MAP-F(100 % N_2)	84.9 ± 1.4^{b}	91.93 ± 7.28^{ab}	81.40 ± 1.90^{c}	78.60 ±0.92^{cd}	85.65 ± 1.85^{b}
	MAP-G(Passive)	84.9 ± 1.4^{b}	95.00 ± 4.92^{a}	72.72 ± 0.16.3^{de}	70.00 ± 1.47^{e}	74.21 ± 4.47^{de}
Radical scavenging	**MAP-D**(5%O_2+10% CO_2)	114 ± 2.7^{ab}	87.09 ± 0.98^{ac}	86.14 ± 2.88^{ac}	71.69 ± 2.37^{cb}	80.13 ± 8.19^{ac}
activity	**MAP-E**(30%O_2+10% CO_2)	114 ± 2.7^{ab}	91.96 ± 19.63^{ab}	82.69 ± 11.56^{ac}	71.84 ± 28.54^{cb}	61.16 ± 24.05^{c}
(mg/100mL)	**MAP-F**(100% N_2)	114 ± 2.7^{ab}	87.87 ± 13.49^{ab}	93.53 ± 12.68^{ab}	100.92 ± 11.18^{a}	83.47 ± 14.66^{ac}
	MAP-G(Passive)	114 ± 2.7^{ab}	80.64 ± 11.86^{ac}	89.60 ± 10.34^{ab}	81.74 ± 17.60^{ac}	79.54 ± 10.51^{ac}

Means with the same letters across each column and row are not significantly different (P = 0.05).

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Table 2 continued

Effect	*p-value*			
	TAC	**TPC**	**Ascorbic acid**	**RSA**
MAP (A)	0.063	0.069	0.002	0.000
Storage duration (B)	0.191	0.000	0.000	0.000
A*B	0.005	0.000	0.074	0.000

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Chapter 6
General discussion and conclusions

Minimally processed pomegranate arils have gained commercial importance in the recent past due to consumer demand for fresh, healthy and convenient food (Ayhan and Eştürk, 2009). Pomegranate is a rich source of phytochemicals which have been shown to have health promoting benefits (Fawole and Opara, 2013; Teixeira da Silva et al., 2013). The therapeutic properties of pomegranate include antioxidant, anti-inflammatory and anti-cancer activity (Lansky and Newman, 2007; Viuda-Martos et al., 2010; Martínez-Romero et al., 2013). However, minimally processed pomegranate arils are more perishable than the intact fruit. Shelf life of intact pomegranate has been shown to be 2- 5 months in comparison to 7-18 days reported for pomegranate arils (López-Rubira et al., 2005; Ayhan and Estürk, 2009; Caleb et al., 2013). The arils suffer softening, shrivelling, browning and microbial decay as a result of injury suffered from minimal processing procedures (Martínez-Romero et al., 2013).

The use of modified atmosphere packaging (MAP) in combination with low temperature storage has the potential to maintain quality and prolong shelf life of minimally processed pomegranate arils (Sepulveda et al., 2000; López-Rubira et al., 2005; Palma et al., 2009). Low O_2 (2-5%) and high CO_2 (10-20%) atmospheres are recommended for MAP of minimally processed pomegranate arils and have been shown to slow down respiration rates, retard aerobic microbial growth and maintain quality attributes (López-Rubira et al, 2005). The success of MAP in maintaining product quality, however, depends on the rapid establishment of suitable equilibrium atmospheres within a package, failure of which may result in accelerated product deterioration and reduced shelf life (Artés et al., 2006; Mangaraj et al., 2009). Active MAP achieved by flushing desired gas mixtures in to a polymeric film before sealing fresh produce ensures rapid establishment of equilibrium atmospheres (Rodov et al., 2007). Active MAP is recommended for minimally processed products because they generally have a short marketable life and cannot benefit from MAP unless suitable equilibrium atmospheres are established rapidly (Bai et al., 2003).

The effects of passive MAP on minimally processed pomegranate arils have been studied extensively but there have been very few studies on the application of active MAP (Caleb et al., 2012; Ayhan and Eştürk, 2009). Palma et al. (2009) investigated the effects of passive MAP on 'Primosole' pomegranate arils at 5 °C. The authors reported that physico-chemical and sensory quality attributes were preserved in arils under MAP. Caleb et al. (2013) also investigated the effects of passive MAP on quality attributes, compositional changes and microbial quality of

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minimally processed pomegranate arils 'Acco' and 'Herskawitz' at 5, 10 and 15 °C for 14 days. Quality of modified atmosphere packaged pomegranate arils were best maintained at 5 °C with arils maintaining physico-chemical attributes and microbial quality up to 10 days.

Furthermore, studies applying active MAP have focused on its effects on physical, chemical and microbial quality of minimally processed pomegranate arils and not on the physiological effects including respiration rate (RR). Ayhan and Eştürk (2009) investigated the effects of active MAP on minimally processed pomegranate arils (cv. Hicaznar) with low and super atmospheric O_2 at 5 °C for 18 days. However, the study was limited to the effects of active MAP on aril physico-chemical, microbial and sensory quality. Postharvest produce RR is important because it gives an indication of the rate at which finite energy supplies are depleted within a product and is therefore related to shelf life (Rojas-Graü et al., 2009). Another aspect that has not been covered adequately is how the phytochemical properties and antioxidant capacity of pomegranate arils are affected by MA and storage. The aim of this study, therefore, was to investigate the effects of active modified atmospheres on postharvest physiology, quality and shelf life of minimally processed pomegranate arils.

In chapter 3 the effects of storage conditions (temperature and RH) and citric acid pre-treatment on postharvest physiology (respiration and transpiration rate) of minimally processed pomegranate arils were investigated in order to determine the optimum storage conditions. Storage temperature and relative humidity (RH) can be manipulated in order to slow down physiological and biochemical processes, moisture loss and microbial decay. Pomegranate aril RR was lowest at 5 °C throughout the storage duration and about two-fold lower than that at 15 °C. Temperature is the most important factor that controls the rate of metabolic activities in fresh produce and has been reported to cause a 2-3 fold increase in RR with every 10 °C rise; therefore it is critical that it is maintained as low as possible (Iqbal et al., 2008). Citric acid is commonly used in minimally processed fresh products alone or in combination with ascorbic acid to slow down physiological and quality changes (Mahajan et al., 2014). It has been suggested that citric acid lowers RR of fresh-cut produce by inhibiting the action of phosphofructokinase, an enzyme that catalyses the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate in the glycolytic pathway of respiratory metabolism (Kato-Noguchi and Watada, 1997). However, in our study citric acid (52 mM) pre-treatment did not significantly reduce pomegranate aril RR at 5 and 10°C, but was effective at 15 and 20°C. Therefore it may be necessary to pre-treat arils with citric acid in order to retard increase in aril RR in instances were temperature abuse occurs. Pomegranate arils TR was lowest (1.26 g/kg/day) at 5 °C and 96% RH and arils in these conditions suffered negligible weight

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loss (~1%) after 9 days compared to 7 and 12% weight loss for those stored under 86% and 76% RH, respectively. Storage temperature of 5 °C and 96% RH were therefore selected as optimum for maintaining pomegranate aril RR and TR at a minimum and were used in subsequent studies.

The effects of active MAP using different gas combinations on pomegranate aril physiological processes, phytochemical properties, physico-chemical attributes, sensory quality attributes and microbial quality were studied in chapters 4 and 5. Minimally processed pomegranate arils packed in 100% N_2 MAP maintained significantly lower RR than those in other MAP treatments. This might have been as a consequence of the lower O_2 (< 4%) levels maintained in this treatment compared to the other MAP treatments. Ersan et al. (2010) also reported significant reduction in RR of minimally processed pomegranate arils at low O_2 (2-5%) and high CO_2 (10-20%). In addition, 100% N_2 maintained lower aerobic mesophilic bacterial counts compared to other MAP treatments throughout the storage duration and was effective in suppressing ascorbic acid loss from day 6 until the end of storage. In addition, arils in 100% N_2 maintained the highest radical scavenging activity in the high barrier polylid film in experiment 2 from day 6 until the end of storage, while that in passive MAP was lowest. However, pomegranate arils in this MAP treatment (100% N_2) had unacceptable sensory scores for off-odour and overall acceptability by day 9 which could have been an indication that anaerobic respiration might have occurred under these conditions.

Modified atmospheres initially flushed with high O_2 (30%) were also effective in suppressing aerobic mesophillic bacteria proliferation in minimally processed pomegranate arils in our study. Ayhan and Estürk (2009) also reported lower aerobic mesophilic counts in minimally processed pomegranate arils in high O_2 atmospheres (70% O_2 +10% CO_2) compared to other MAP treatments. High O_2 atmospheres have been suggested to cause intracellular generation of reactive oxygen species which may damage vital cell components and reduce cell viability of microbial organisms (Kader and Ben-Yehoshua, 2000). High O_2 atmospheres were also effective in preventing development of off-odour in minimally processed pomegranate arils during the storage duration. The arils in this MAP treatment had the longest shelf life (> 9 days) based on overall acceptability scores.

Pomegranate aril physical attributes (colour and texture), chemical attributes and total phenolic content were not significantly altered by MAP and storage duration. This is consistent with reports by other authors (Ayhan and Esturk, 2009; Maghoumi et al., 2013) who reported minimal alterations in physical and chemical attributes in arils under MAP. This behaviour has been

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attributed to pomegranates non-climacteric nature which distinguishes it from climacteric fruits which exhibit a rapid deterioration in physical and chemical attributes during postharvest storage.

The polymeric barrier films used in this study provided a limitation because they did not create suitable equilibrium atmospheres recommended for pomegranate arils. Equilibrium atmospheres were achieved in low barrier BOP film but O_2 levels in this film accumulated to higher levels (16-18%) than those recommended for pomegranate arils across all the MAP treatments. This could account for the minimal differences observed in quality attributes in pomegranate arils packaged in this barrier film. Similarly, CO_2 levels accumulated to very high levels (27-43%) in the high barrier BOP film which could have had negative effects on pomegranate arils packaged in this film. On the other hand, O_2 decreased to critical levels (< 2%) that might have caused anaerobic respiration in MAP treatments flushed with low O_2. The use of a barrier film with more suitable O_2 and CO_2 permeability characteristics is, therefore, recommended for further studies. Permeability of high barrier films can be improved by use of micro perforations in order to avoid accumulation of CO_2 or depletion of O_2 to detrimental levels. Future studies are also recommended to characterise the volatile compounds in MAP stored pomegranate arils; the outcome of such a study would assist in determining the occurrence and onset of anaerobic respiration. In addition, the identification of specific microbial organisms affecting arils packed inside in MAP would be useful to determine the effects of different atmospheric conditions on specific microbes in pomegranate arils.

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Appendix A. Selected data of Chapter 3

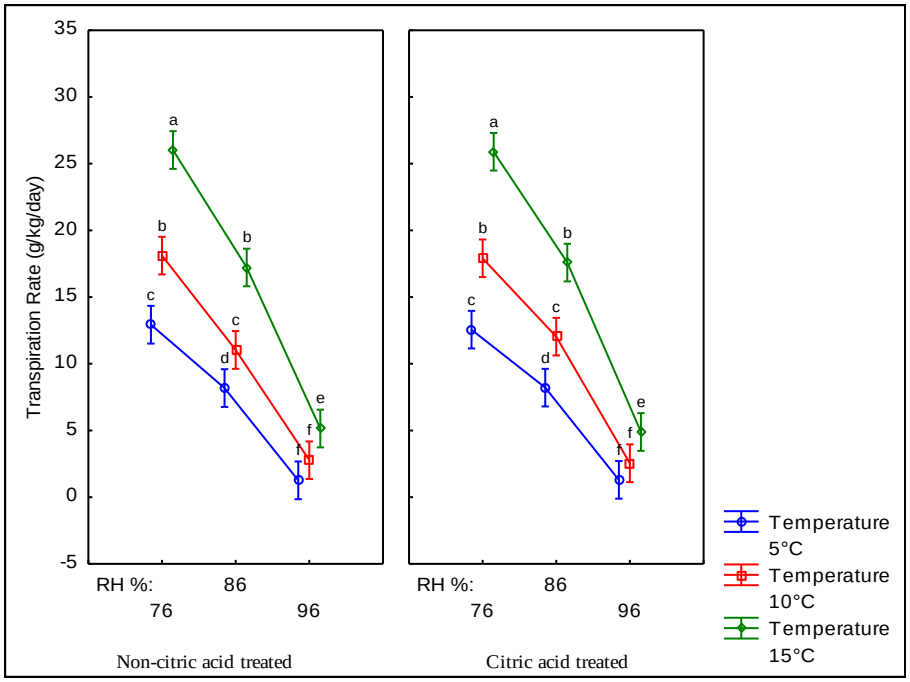

Figure 1. Effects of citric acid pre-treatment, storage temperature (5, 10 and 15 °C) and RH (76%, 86% and 96%) on transpiration rate of minimally processed pomegranate arils (cv. Wonderful). Different letters indicate a significant difference in mean values ± SD; tem ($p = 0.964$)

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Appendix B. Selected data of Chapter 4

Table 1A. Effects of active and passive MAP on colour of minimally processed pomegranate aril packaged in low barrier BOP film and clamshell containers at 5 °C. Means with the same letters across each column and row are not significantly different (P .= 0.05)

Colour attributes	Treatments	Day 0	Day 3	Day 6	Day 9	Day 12
L*	MAP-C (Passive)	11.96 ± 0.10^{eb}	14.31 ± 0.34^{a}	9.41 ± 1.17^{gf}	12.19 ± 0.66^{db}	11.5 ± 1.86^{dce}
	MAP-A (5% O_2+10%CO_2+85%N_2)	11.96 ± 0.10^{eb}	13.05 ± 1.17^{ad}	8.87 ± 0.42^{g}	13.64 ± 1.81^{ab}	12.72 ± 2.26^{ad}
	MAP-B (30%O_2+40%CO_2+30%N_2)	11.96 ± 0.10^{eb}	12.74 ± 0.30^{ad}	9.50 ± 0.75^{gf}	13.73 ± 0.50^{ab}	11.77 ± 0.59^{ad}
	Clamshell	11.96 ± 0.10^{eb}	12.48 ± 0.52^{ad}	9.75 ± 0.74^{ge}	13.45 ± 0.69^{abc}	11.11 ± 2.13^{def}
a*	MAP-C (Passive)	10.99 ± 0.54	13.68 ± 1.42^{abcd}	11.30 ± 0.68^{e}	13.83 ± 0.55^{abc}	13.28 ± 1.22^{eb}
	MAP-A (5% O_2+10%CO_2+85%N_2)	10.99 ± 0.54	12.16 ± 0.55^{ec}	11.44 ± 0.79^{e}	15.53 ± 2.15^{a}	14.37 ± 2.25^{ab}
	MAP-B (30%O_2+40%CO_2+30%N_2)	10.99 ± 0.54	11.74 ± 0.59^{ed}	11.78 ± 0.43^{ec}	14.83 ± 0.58^{ab}	13.77 ± 0.40^{abcd}
	Clamshell	10.99 ± 0.54	11.86 ± 1.46^{ec}	11.55 ± 0.67^{e}	14.43 ± 1.18^{ab}	12.08 ± 2.08^{ec}
b*	MAP-C (Passive)	2.47 ± 0.24^{dc}	3.06 ± 0.45^{ab}	2.35 ± 0.19^{d}	2.61 ± 0.09^{db}	2.70 ± 0.31^{db}
	MAP-A (5% O_2+10%CO_2+85%N_2)	2.47 ± 0.24^{dc}	2.66 ± 0.14^{db}	2.51 ± 0.19^{dc}	3.34 ± 0.57^{a}	12.93 ± 0.61^{abc}
	MAP-B (30%O_2+40%CO_2+30%N_2)	2.47 ± 0.24^{dc}	2.49 ± 0.14^{dc}	2.59 ± 0.10^{db}	2.82 ± 0.29^{ad}	2.78 ± 0.05^{db}
	Clamshell	2.47 ± 0.24^{dc}	2.66 ± 0.38^{db}	2.37 ± 0.17^{d}	2.86 ± 0.43^{ad}	2.53 ± 0.14^{dc}
C*	MAP-C (Passive)	11.26 ± 0.58^{f}	14.01 ± 1.35^{abc}	11.54 ± 0.62^{d}	14.07 ± 0.56^{abc}	13.55 ± 1.24^{db}
	MAP-A(5% O_2+10%CO_2+85%N_2)	11.26 ± 0.58^{f}	12.45 ± 0.42^{dc}	11.71 ± 0.80^{d}	15.89 ± 2.21^{a}	14.67 ± 2.32^{ab}
	MAP-B (30%O_2+40%CO_2+30%N_2)	11.26 ± 0.58^{f}	12.00 ± 0.59^{dc}	12.06 ± 0.40^{dc}	16.00 ± 0.64^{ab}	14.04 ± 0.39^{abc}

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	Clamshell	11.26 ± 0.58^{f}	12.15 ± 1.46^{dc}	11.79 ± 0.67^{d}	14.71 ± 1.25^{ab}	12.35 ± 1.89^{dc}

Table 1A Continued

H°	MAP-C (Passive)	12.67 ± 0.96^{a}	12.58 ± 1.47^{ae}	11.76 ± 3.99^{ab}	10.70 ± 0.95^{e}	11.49 ± 1.75^{eb}
	MAP-A (5% O_2+10%CO_2+85%N_2)	12.67 ± 0.96^{a}	12.37 ± 3.79^{abcd}	12.34 ± 2.20^{a}	12.11 ± 1.93^{e}	11.47 ± 1.74^{ed}
	MAP-B (30%O_2+40%CO_2+30%N_2)	12.67 ± 0.96^{a}	11.96 ± 1.84^{ab}	12.42 ± 1.71^{ab}	10.76 ± 0.69^{e}	11.41 ± 0.86^{ec}
	Clamshell	12.67 ± 0.96^{a}	12.61 ± 3.92^{a}	11.58 ± 2.37^{abc}	11.16 ± 0.72^{e}	11.99 ± 9.75^{ab}
TCD (ΔE*)	MAP-C (Passive)	0	3.752 ± 1.03^{abc}	2.619 ± 1.14^{db}	2.894 ± 0.64^{db}	2.824 ± 1.13^{db}
	MAP-A (5% O_2+10%CO_2+85%N_2)	0	1.761 ± 1.04^{dc}	3.181 ± 0.30^{ad}	5.047 ± 2.56^{a}	3.761 ± 2.78^{abc}
	MAP-B (30%O_2+40%CO_2+30%N_2)	0	1.235 ± 0.83^{d}	2.604 ± 0.66^{db}	4.285 ± 0.514^{ab}	2.843 ± 0.36^{db}
	Clamshell	0	1.346 ± 1.73^{d}	2.357 ± 0.53^{db}	3.846 ± 1.15^{ab}	2.643 ± 1.09^{db}

Effect	***P*-value**					
	L*	***a****	***b****	***C****	***H°***	***ΔE***
MAP(A)	0.893	0.369	0.249	0.359	0.382	0.337
Storage duration(B)	0.000	0.000	0.013	0.000	0.000	0.004
A*B	0.326	0.356	0.204	0.344	0.511	0.283

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Table 1B. Effects of active and passive MAP on colour of minimally processed pomegranate aril packaged in high barrier polylid film at 5 °C. Means with the same letters across each column and row are not significantly different (P = 0.05)

Colour attributes	Treatments	Day 0	Day 3	Day 6	Day 9	Day 12
L*	MAP-G (Passive)	9.61 ± 0.90^{c}	10.21 ± 0.52^{ac}	9.24 ± 1.20^{c}	11.84 ± 2.17^{ab}	10.72 ± 1.91^{ac}
	MAP-D ($5\%O_2+10\%CO_2+85\%N_2$)	9.61 ± 0.90^{c}	12.12 ± 1.89^{a}	10.00 ± 0.55^{cb}	10.38 ± 0.58^{ac}	9.98 ± 0.48^{cb}
	MAP-E($30\% O_2+10\%CO_2+60\%N_2$)	9.61 ± 0.90^{c}	10.62 ± 0.48^{ac}	9.62 ± 0.48^{c}	9.23 ± 0.48^{c}	8.73 ± 0.69^{c}
	MAP-F ($100\%N_2$)	9.61 ± 0.90^{c}	9.58 ± 0.54^{c}	10.12 ± 1.58^{ac}	9.64 ± 1.88^{c}	9.19 ± 1.41^{c}
a*	MAP-G (Passive)	11.95 ± 0.14^{abc}	9.10 ± 2.11^{c}	11.25 ± 1.87^{ab}	12.77 ± 1.94^{a}	11.99 ± 1.79^{ab}
	MAP-D ($5\%O_2+10\%CO_2+85\%N_2$)	11.95 ± 0.14^{abc}	12.7 ± 0.93^{a}	12.70 ± 1.35^{a}	11.92 ± 0.47^{ab}	10.79 ± 0.62^{ac}
	MAP-E($30\% O_2+10\%CO_2+60\%N_2$)	11.95 ± 0.14^{abc}	11.71 ± 0.75^{ab}	11.46 ± 1.74^{ab}	10.81 ± 0.65^{ac}	11.94 ± 0.30^{ab}
	MAP-F ($100\% N_2$)	11.95 ± 0.14^{abc}	12.91 ± 0.42^{a}	11.62 ± 1.64^{ab}	10.54 ± 0.52^{cb}	10.90 ± 1.05^{ac}
b*	MAP-G (Passive)	3.70 ± 0.16^{abcd}	2.96 ± 0.83^{d}	$3.90 \pm 0.0.36^{ab}$	3.91 ± 0.81^{ab}	3.55 ± 0.38^{ad}
	MAP-D ($5\% O_2+10\%CO_2+85\%N_2$)	3.70 ± 0.16^{abcd}	3.98 ± 0.16^{ab}	3.87 ± 0.27^{abc}	3.45 ± 0.28^{db}	$3.19 \pm 0.0.27^{dc}$
	MAP-E ($30\%O_2+10\%CO_2+60\%N_2$)	3.70 ± 0.16^{abcd}	3.62 ± 0.52^{ad}	3.68 ± 0.30^{abc}	3.45 ± 0.30^{db}	3.84 ± 0.25^{abc}
	MAP-F ($100\% N_2$)	3.70 ± 0.16^{abcd}	4.22 ± 0.35^{a}	3.52 ± 0.39^{db}	3.21 ± 0.12^{dc}	3.37 ± 0.18^{db}
C*	MAP-G (Passive)	12.51 ± 0.19^{abc}	9.57 ± 2.27^{d}	11.92 ± 1.85^{abc}	13.35 ± 2.09^{ab}	12.51 ± 1.80^{abc}
	MAP-D ($5\%O_2+10CO_2+85\%N_2$)	12.51 ± 0.19^{abc}	13.31 ± 0.93^{ab}	13.28 ± 1.37^{ab}	12.41 ± 0.53^{abc}	11.25 ± 0.67^{db}
	MAP-E ($30\%O_2+10\%CO_2+85\%N_2$)	12.51 ± 0.19^{abc}	12.25 ± 0.87^{abc}	12.04 ± 1.73^{abc}	11.34 ± 0.70^{db}	12.54 ± 0.36^{abc}
	MAP-F ($100\%N_2$)	12.51 ± 0.19^{abc}	13.58 ± 0.46^{a}	12.14 ± 1.67^{abc}	11.01 ± 0.52^{dc}	11.41 ± 1.05^{ad}

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Table 1B Continued

H°	MAP-G (Passive)	17.20 ± 0.53^{cb}	17.94 ± 0.92^{ac}	19.30 ± 2.29^{a}	16.93 ± 1.00^{cb}	16.61 ± 1.55^{cb}
	MAP-D (5%O_2+10CO_2+85%N_2)	17.20 ± 0.53^{cb}	17.40 ± 0.66^{cb}	16.96 ± 0.58^{cb}	16.15 ± 0.74^{c}	16.48 ± 0.40^{cb}
	MAP-E (30%O_2+10%CO_2+85%N_2)	17.20 ± 0.53^{cb}	17.12 ± 1.33^{cb}	17.94 ± 1.52^{ac}	17.69 ± 0.57^{ac}	17.83 ± 0.68^{ac}
	MAP-F (100% N_2)	17.20 ± 0.53^{cb}	18.10 ± 1.18^{ab}	16.92 ± 0.93^{cb}	16.98 ± 0.40^{cb}	17.22 ± 0.97^{cb}
TCD (ΔE*)	MAP-G (Passive)	0	3.143 ± 2.05^{a}	1.720 ± 1.28^{ac}	3.04 ± 1.94^{ab}	2.279 ± 1.06^{ac}
	MAP-D (5%O_2+10CO_2+85%N_2)	0	2.666 ± 2.05^{ac}	1.10± 1.23^{cb}	1.024 ± 0.247^{c}	1.350 ± 0.75^{ac}
	MAP-E (30%O_2+10%CO_2+85%N_2)	0	1.190 ± 1.17^{ac}	1.434 ± 0.77^{ac}	1.266 ± 0.77^{ac}	1.038 ± 0.46^{c}
	MAP-F (100% N_2)	0	1.202 ± 0.46^{ac}	1.931 ± 0.60^{ac}	2.174 ± 0.35^{ac}	1. 449 ± 1.43^{ac}

Effect	***P*-value**					
	L*	***a****	***b****	***C****	***H°***	***ΔE***
MAP(A)	0.081	0.533	0.971	0.587	0.150	0.062
Storage duration(B)	0.201	0.919	0.328	0.878	0.167	0.647
A*B	0.236	0.017	0.013	0.015	0.308	0.696

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Table 2A. Scores for sensory quality attributes of minimally processed pomegranate arils stored under active and passive MAP in low Barrier BOP film at 5 °C for 9 days. Means with the same letters across each column and row are not significantly different (P = 0.05)

Quality parameters	Treatments	Storage duration (days)			
		0	3	6	9
Aril redness	MAP-C (Passive)	4.83 ± 0.37^{a}	4.5 ± 0.55^{ab}	4.0 ± 1.10^{cb}	3.50 ± 0.5^{c}
	MAP-A (5%O_2+10%CO_2+85%N_2)	4.83 ± 0.37^{a}	4.4 ± 0.55^{ab}	4.5 ± 0.55^{cb}	3.5 ± 0.50^{c}
	MAP-B (30%O_2+40%CO_2+30%N_2)	4.83 ± 0.37^{a}	4.83 ± 0.41^{a}	4.0 ± 0.63^{cb}	3.5± 0.76^{c}
	Clamshell	4.83 ± 0.37^{a}	4.5 ± 0.55^{ab}	3.5 ± 0.84^{c}	3.5± 0.58^{c}
Browning	MAP-C (Passive)	0	0.33 ± 0.82^{a}	0.66 ± 0.82^{a}	0.83 ± 0.69^{a}
	MAP-A (5%O_2+10%CO_2+85%N_2)	0	0.17 ± 0.41^{a}	0.67 ± 0.82^{a}	0.83 ± 0.69^{a}
	MAP-B (30% O_2 + 40%CO_2+30%N_2)	0	0.17 ± 0.41^{a}	0.50 ± 0.83^{a}	0.67 ± 0.74^{a}
	Clamshell	0	0.17 ± 0.41^{a}	0.50 ± 0.56^{a}	0.50 ± 0.50^{a}
Firmness	MAP-C (Passive)	5.00 ± 0.00^{a}	4.33 ± 0.51^{b}	4.17 ± 0.84^{ab}	3.33 ± 0.75^{b}
	MAP-A (5%O_2+10%CO_2+85%N_2)	4.67 ± 0.47^{ab}	4.50 ± 0.84^{ab}	3.83 ± 1.17^{b}	3.67 ± 0.94^{b}
	MAP-B (30%O_2+40%CO_2+60%N_2)	4.67 ± 0.47^{ab}	3.67 ± 1.03^{b}	4.0 ± 0.63^{b}	3.83 ± 0.37^{b}
	Clamshell	4.67 ± 0.47^{ab}	3.83 ± 0.98^{b}	3.17 ± 1.17^{b}	3.00 ± 0.81^{b}
Taste	MAP-C (Passive)	5.00 ± 0.00^{a}	4.33 ± 0.51^{b}	3.5 ± 0.84^{c}	3.33 ± 0.75b^{c}
	MAP-A (5%O_2+10%CO_2+85%N_2)	5.00 ± 0.00^{a}	3.5 ± 1.38^{c}	3.83 ± 1.69^{b}	3.67 ± 1.11b^{c}
	MAP-B (30%O_2+40%CO_2+60%N_2)	5.00 ± 0.00^{a}	4.00 ± 0.63^{b}	3.33 ± 0.52b^{c}	3.17 ± 0.37b^{c}
	Clamshell	5.00 ± 0.00^{a}	3.83 ± 1.17^{b}	2.50 ± 0.55^{c}	2.33 ± 0.47^{c}

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Table 2A.continued

Off-odour	MAP-C (Passive)	0	0	0.5 ± 0.84^{a}	0.67 ± 0.75^{a}
	MAP-A ($5\%O_2+10\%CO_2+85\%N_2$)	0	0.17 ± 0.41^{a}	0.83 ± 0.98^{a}	1.00 ± 0.82^{a}
	MAP-B (30%O2+40%+85%N2)	0	0.83 ± 2.04^{a}	0.50 ± 0.55^{a}	0.67 ± 0.47^{a}
	Clamshell	0	0.33 ± 0.82^{a}	1.17 ± 1.47^{a}	1.33 ± 1.25^{a}
Flavour	MAP-C (Passive)	4.83 ± 0.37^{a}	4.17 ± 0.75^{a}	3.5 ± 0.84^{ab}	3.33 ± 0.75^{ab}
	MAP-A ($5\%O_2+10\%CO_2+85\%N_2$)	4.83 ± 0.37^{a}	4.0 ± 1.26^{a}	3.75 ± 0.88^{ab}	3.58 ± 0.84^{ab}
	MAP-B ($30\%O_2+40\%CO_2+85\%N_2$)	4.83 ± 0.37^{a}	3.83 ± 0.98^{ab}	3.50 ± 0.55^{ab}	3.33 ± 0.47^{ab}
	Clamshell	4.83 ± 0.37^{a}	4.00 ± 1.26^{a}	2.83 ± 0.41^{c}	2.67 ± 0.47^{c}
Aroma	MAP-C (Passive)	5.00 ± 0.00^{a}	3.67 ± 0.82^{b}	3.33 ± 0.52^{b}	3.17 ± 0.37^{b}
	MAP-A ($5\%O_2+10\%CO_2+85\%N_2$)	5.00 ± 0.00^{a}	3.5 ± 1.22^{b}	3.83 ± 1.17^{ab}	3.67 ± 0.94^{b}
	MAP-B ($30\%O_2+40\%CO_2+85\%N_2$)	5.00 ± 0.00^{a}	3.67 ± 1.21^{b}	3.67 ± 0.52^{b}	3.5 ± 0.5^{b}
	Clamshell	5.00 ± 0.00^{a}	3.33 ± 1.63^{b}	2.83 ± 0.41^{b}	2.67 ± 0.47^{b}
Overal aceptability	MAP-C (Passive)	5.00 ± 0.00^{a}	4.33 ± 0.52^{b}	3.75 ± 0.42^{bc}	3.08 ± 0.19^{c}
	MAP-A ($5\%O_2+10\%CO_2+85\%N_2$)	5.00 ± 0.00^{a}	4.17 ± 0.98^{b}	3.92 ± 1.02^{bc}	2.92 ± 0.19^{c}
	MAP-B ($30\%O_2+40\%CO_2+85\%N_2$)	5.00 ± 0.00^{a}	4.17 ± 0.75^{b}	3.67 ± 0.52^{bc}	3.50 ± 0.5^{bc}
	Clamshell	5.00 ± 0.00^{a}	3.83 ± 1.17^{bc}	3.00 ± 0.63^{c}	2.33 ± 0.47^{c}

Effect	***p-value***							
	Aril redness	**Browning**	**Firmness**	**Taste**	**Off-odour**	**Flavour**	**Aroma**	**Overall acceptability**
MAP(A)	0.528	0.913	0.416	0.244	0.678	0.605	0.465	0.186
Storage duration(B)	0.001	0.057	0.187	0.022	0.184	0.027	0.674	0.023
A*B	0.755	0.986	0.538	0.166	0.554	0.605	0.759	0.843

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Table 2B. Scores for sensory quality attributes of minimally processed pomegranate arils stored under active and passive MAP in high barrier polylid film at 5 °C for 9 days. Means with the same letters across each column and row are not significantly different (P = 0.05)

Quality parameters	Treatments	Storage duration (days)			
		0	3	6	9
Redness	MAP-G (Passive)	4.83 ± 0.37^{a}	4.33 ± 0.52^{a}	4.33 ± 0.82^{a}	4.33 ± 0.75^{a}
	MAP-D ($5\%O_2+10\%CO_2+85\%N_2$)	4.83 ± 0.37^{a}	4.33 ± 0.52^{a}	4.16 ± 0.98^{a}	4.00 ± 0.82^{a}
	MAP-E ($30\%O_2+10\%CO_2+60\%N_2$)	4.83 ± 0.37^{a}	4.67 ± 0.52^{a}	4.50 ± 0.84^{a}	4.17 ± 0.69^{a}
	MAP-F (100% N_2)	4.83 ± 0.37^{a}	4.67 ± 0.52^{a}	4.33 ± 0.52^{a}	4.5 ± 0.5^{a}
Browning	MAP-G (Passive)	0	0	1.00 ± 1.1^{a}	1.17 ± 0.90^{a}
	MAP-D ($5\%O_2+10\%CO_2+85\%N_2$)	0	0.5 ± 0.84^{ab}	0.83 ± 0.75^{ab}	0.83 ± 0.69^{ab}
	MAP-E ($30\%O_2+10\%CO_2+60\%N_2$)	0	0.17 ± 0.41^{ab}	1.00 ± 0.89^{a}	1.00 ± 0.58^{a}
	MAP-F ($100\%N_2$)	0	0.17 ± 0.41^{ab}	0.67 ± 0.82^{ab}	1.00 ± 0.82^{a}
Firmness	MAP-G (Passive)	4.83 ± 0.37^{a}	3.67 ± 1.03^{a}	3.67 ± 0.52^{a}	3.5± 0.5^{a}
	MAP-D ($5\%O_2+10\%CO_2+85\%N_2$)	4.83 ± 0.37^{a}	4.7 ± 1.17^{a}	3.67 ± 0.52^{a}	3.83± 0.37^{a}
	MAP-E ($30\%O_2+10\%CO_2+60\%N_2$)	4.83 ± 0.37^{a}	4.33 ± 0.52^{a}	4.00 ± 0.63^{a}	3.83± 0.37^{a}
	MAP-F ($100\%N_2$)	4.83 ± 0.37^{a}	3.83 ± 0.75^{a}	4.00 ± 0.63^{a}	3.5± 0.5^{a}
Taste	MAP-G (Passive)	5.00 ± 0.00^{a}	3.67 ± 0.18^{b}	3.17 ± 0.75^{b}	3.00 ± 0.58^{b}
	MAP-D ($5\%O_2+10\%CO_2+85\%N_2$)	5.00 ± 0.00^{a}	3.5 ± 1.22^{b}	3.33 ± 1.03^{b}	3.17 ± 0.90^{b}
	MAP-E ($30\%O_2+10\%CO_2+60\%N_2$)	5.00 ± 0.00^{a}	3.67 ± 1.03^{b}	3.67 ± 0.82^{b}	3.67 ± 0.75^{b}
	MAP-F ($100\%N_2$)	5.00 ± 0.00^{a}	3.67 ± 1.03^{b}	3.33 ± 1.21^{b}	3.00 ± 0.82^{b}

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Table 2B Continued

Off-odour	MAP-G (Passive)	0	0.5 ± 0.55^{b}	2.50 ± 1.05^{a}	2.67 ± 0.47^{a}
	MAP-D (5%O_2+10%CO_2+85%N_2)	0	0	2.67 ± 1.51^{a}	2.67 ± 0.94^{a}
	MAP-E (30%O_2+10%CO_2+60%N_2)	0	0	2.33 ± 1.37^{a}	2.17 ± 0.68^{a}
	MAP-F (100%N_2)	0	0.17 ± 1.0.41b	2.50 ± 1.05^{a}	2.33 ± 0.74^{a}
Flavour	MAP-G (Passive)	5.00 ± 0.00^{a}	3.67 ± 0.52^{ab}	3.67 ± 0.52^{ab}	3.67 ± 0.47^{ab}
	MAP-D (5%O_2 + 10%CO_2+85%N_2)	5.00 ± 0.00^{a}	4.00 ± 0.63^{ab}	3.83 ± 0.41ab	3.67 ± 0.47^{ab}
	MAP-E (30% O_2+10%CO_2+60%N_2)	5.00 ± 0.00^{a}	3.83 ± 0.75^{ab}	3.50 ± 0.55^{ab}	3.5 ± 0.5^{ab}
	MAP-F (100%N_2)	5.00 ± 0.00^{a}	3.83 ± 0.75^{ab}	3.50 ± 0.84^{ab}	3.17 ± 0.69^{b}
Aroma	MAP-G (Passive)	5.00 ± 0.00^{a}	4.50 ± 0.55^{a}	3.67 ± 0.52^{b}	3.67 ± 0.47^{b}
	MAP-D (5%O_2+10%CO_2+85%N_2)	5.00 ± 0.00a	4.17 ± 0.41^{ab}	3.83 ± 0.75^{ab}	3.67 ± 0.47^{b}
	MAP-E (30%O_2+10%CO_2+60%N_2)	5.00 ± 0.00^{a}	4.5 ± 0.55^{a}	4.17 ± 0.75^{ab}	3.83 ± 0.69^{ab}
	MAP-F (100%N_2)	5.00 ± 0.00^{a}	4.5 ± 0.55^{a}	3.83 ± 0.98^{ab}	3.67 ± 0.74^{b}
Overall acceptability	MAP-G (Passive)	5.00 ± 0.00^{a}	3.67 ± 0.82^{b}	3.50 ± 0.55^{b}	3.00 ± 0.00^{b}
	MAP-D (5%O_2+10%CO2+85%N_2)	5.00 ± 0.00^{a}	4.00 ± 0.63^{b}	3.50 ± 0.55^{b}	2.83 ± 0.37^{bc}
	MAP-E (30%O_2+10%CO_2+60%N_2)	5.00 ± 0.00^{a}	4.00 ± 0.63^{b}	3.83 ± 0.75^{b}	3.33 ± 0.47^{b}
	MAP-F (100%N_2)	5.00 ± 0.00^{a}	4.0 ± 0.89^{b}	3.33 ± 0.82^{b}	2.5 ± 0.5^{c}

Effect	***p-value***							
	Aril redness	**Browning**	**Firmness**	**Taste**	**Off-odour**	**Flavour**	**Aroma**	**Overall acceptability**
MAP(A)	0.389	0.853	0.465	0.919	0.873	0.709	0.640	0.702
Storage duration(B)	0.672	0.003	0.450	0.393	0.000	0.263	0.007	0.077
A*B	0.980	0.670	0.696	0.936	0.873	0.903	0.727	0.048