APPLICATION OF POSTHARVEST CHEMICAL TREATMENTS TO ENHANCE STORAGE AND SHELF LIFE OF POMEGRANATE FRUIT (CV. WONDERFUL)

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DECLARATION

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

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ABSTRACT

Pomegranate fruit is susceptible to a number of postharvest quality problems such as external and internal decay, weight loss, internal browning, chilling injury and husk scald. Postharvest treatments offer the possibility of alleviating these challenges and maintain fruit quality. The aim of the study was to investigate the potential of exogenous application of chemical treatments (putrescine (PUT) and fludioxonil (FLU)) in reducing the incidence of postharvest physiological disorders of pomegranate fruit (cv. Wonderful). Fruit were treated at three concentrations (putrescine – 1, 2 and 3 mM; fludioxonil – 150, 300 and 600 mg/L) and stored for 4 months at 5 °C plus an additional 4 days at 20 °C (shelf life). The effects of the chemical treatments and storage duration on fruit physiological response and quality were investigated.

The results showed that treating pomegranate fruit with putrescine at different concentrations (1, 2 and 3 mM) reduced incidence of physiological disorders such as external fruit decay, chilling injury and husk scald during the first 3 months of storage. However, putrescine had no effect on internal disorders such as internal decay and aril browning. Physico-chemical attributes such as peel colour, aril colour, TSS, TA and pH were not significantly ($p > 0.05$) affected by putrescine application. After four months of storage, treated samples had firmer fruit and arils while the control had softer fruit with lower firmness (10.12 ± 0.40 N) and aril hardness (143.20 ± 3.84 N). Fruit treated with 2 mM PUT had the best sensory quality (crispness, sweet taste, juiciness) after 3 months of storage. Although 3 mM PUT effectively reduced physiological disorders, 2 mM PUT had the advantage of both reducing the external disorders and maintaining fruit sensory quality during storage up to 3 months.

Fludioxonil was very effective in reducing decay incidence among treated fruit, with 600 mg/L as the most effective FLU concentration having 15.7 % lower decay than control. However, other physiological disorders such as aril browning, chilling injury and husk scald were more pronounced in treated fruit. Fruit firmness was maintained among treated fruit while aril texture was not significantly ($p > 0.05$) affected. Control fruit had higher aril redness (a*) and intensity (C*) compared to fruit treated with FLU. The chemical attributes TA, TSS and BrimA generally decreased with storage for all FLU concentrations. Fruit treated with 600 mg/L were related to eating attributes for crisp, juicy and sweet fruits. Fruit were successfully stored up to 3 months without adversely affecting quality and 600 mg/L was the most effective FLU concentration.

A further study on the effects of the PUT and FLU treatments on phytochemical and volatile composition of fruit revealed that fruit juice ascorbic acid content decreased slightly while total phenolic content (TPC) significantly decreased during storage for both chemicals. Fruit treated with FLU had higher TPC for the first 3 months while fruit treated with PUT only showed high TPC after month 2 of storage. Total anthocyanin content (TAC) of fruit initially increased to values above harvest regardless of FLU concentration. However, TAC decreased as storage progressed with no significant difference ($p > 0.05$) between FLU concentration at the end of the storage duration. On the other hand, TAC of PUT treated fruit significantly reduced throughout storage (except at month 2), with no significant differences observed among PUT concentrations at the end of storage. In contrast, the antioxidant capacity of both FLU and PUT treated fruit increased throughout the storage duration. Furthermore, a total of 31 and 32 volatile compounds were identified in fruit treated with FLU and PUT, respectively. Six chemical groups (alcohols, aldehydes, acids, ketones, esters and terpenes) were identified among fruit treated with FLU, while five (alcohols, aldehydes, acids, esters and terpenes) were detected in fruit treated with PUT. Volatile compounds evolved with prolonged storage, with new compounds, especially terpenes, detected at later storage durations. Accumulation of terpenes had adverse effects on fruit sensory quality and therefore storage for long duration may result in lower fruit flavour.

Overall, the study provided insightful information on the potential of putrescine and fludioxonil treatments in reducing pomegranate fruit postharvest disorders and their effects on fruit edible and nutritional quality attributes. The application of FLU greatly reduced fruit decay but not chilling injury, husk scald and aril browning while PUT alleviated all these physiological disorders. However, PUT and FLU did not effectively reduce weight loss, and therefore, future studies may focus on combining chemical treatments together with physical treatments such as film wrapping and waxing so as to benefit from the hurdle effect. In addition, combination of FLU and PUT may be explored to harness the full potential of the two chemical treatments.

OPSOMMING

Granate is vatbaar vir 'n aantal na-oes kwaliteit probleme soos uitwendige en inwendige verval, gewigsverlies, koueskade en dopwonde. Na-oes behandelings bied die moontlikheid van die oplossing van hierdie probleme en verbeter ook vrugkwaliteit. Die doel van hierdie studie was om die potensiaal van die uitwendige toediening van chemiese behandelings (putresien (PUT) en fludioksonil (FLU)) in die vermindering van die voorkoms van na-oes fisiologiese afwykings van granate (cv. Wonderful) te ondersoek. Die vrugte is behandel met drie konsentrasies (putresien - 1, 2 en 3 mM is; fludioksonil - 150, 300 en 600 mg/L) en gestoor vir 4 maande by 5 °Cm plus ‘n bykomende 4 dae by 20 °C (raklewe). Die gevolge van chemiese behandelings en stoortydperk op die fisiologiese vrugreaksie en gehalte is ondersoek.

Resultate het getoon dat die behandeling van granate met putresien by verskillende konsentrasies (1, 2 en 3 mM) die voorkoms van fisiologiese afwykings soos uitwendige vrug verval, koueskade en dopwond gedurende die eerste 3 maande van stoor verminder. Putresien het egter geen effek op interne versteurings soos interne verval en saadhuid verbruining gehad nie. Fisiochemiese eienskappe soos die kleur van die skil en saadhuid, TSS, TA en pH was nie beduidend ($p > 0.05$) beïnvloed deur putresien behandeling nie. Na vier maande van stoor het kontrole vrugte verlaagde vrugfermheid (10.12 ± 0.40 N) en saadhuid hardheid (143.20 ± 3.84 N) getoon, terwyl behandelde vrugte se waardes hoër was. Vrugte wat met 2 mM PUT behandel is, het die beste sensoriese kwaliteit na `n 3 maande stoortydperk getoon. Hoewel 3 mM PUT effektief was om fisiologiese versteurings te verminder, was 2 mM PUT voordelig, deurdat dit tydend ’n 3 maansde stoortydperk uitwendige versteurings kon verminder, asook vrug sensoriese kwaliteit handhaaf.

Fludioxonil was baie effektief deur dat dit die verval van behandelde vrugte kon verminder. Die mees doeltreffende FLU konsentrasie was 600 mg/L met `n 15.7 % laer verval as die by die kontrole. Maar ander fisiologiese afwykings soos saadhuid verbruining, koueskade en dopwonde was meer merkbaar in behandelde vrugte. Vrugfermheid was gehandhaaf onder behandelde vrugte, terwyl saadhuid tekstuur nie beduidend ($p > 0.05$) beïnvloed was nie. Hoewel rooiheid (a*) en intensiteit (C*) van die saadhuid in kontrole vrugte hoër was, het FLU-behandelde vrugte ook aanvaarbare rooi kleur vertoon. Die chemiese eienskappe TA, TSS en BrimA was oor die algemeen verlaag tydens stoor onder alle FLU konsentrasies. Vrugte wat met 600 mg/L behandel is, het verbeterde sensoriese eienskappe in terme van varsheid, sappigheid en soetigheid vertoon. Vrugte was suksesvol gestoor tot 3 maande

sonder enige negatiewe invloed op gehalte en 600 mg/L was die mees doeltreffendste FLU konsentrasie.

'n Verdere studie oor die uitwerking van PUT en FLU behandelings op fitochemiese en vlugtige samestelling van vrugte, het gewys dat die askorbiensuur inhoud in vrugte effens afneem, terwyl totale fenoliese inhoud (TPC) aansienlik afneem tydens stoor vir beide chemikalieë. Vrugte onder FLU behandeling het hoër TPC gehad vir die eerste 3 maande, terwyl PUT-behandelde vrugte slegs hoë TPC na maand 2 van stoor gewys het. Algehele antosianien inhoud (TAC) van vrugte het aanvanklik gestyg bo oes, ongeag die invloed van FLU konsentrasie. Maar, TAC het afgeneem namate stoor gevorder het, met geen beduidende verskil ($p > 0.05$) tussen FLU konsentrasie aan die einde van die stoortydperk nie. In teenstelling het die TAC van PUT-behandelde vrugte beduidend verminder tydens stoor (behalwe op maand 2), en geen betekenisvolle verskille is waargeneem onder PUT konsentrasies aan die einde van stoor. In teenstelling hiermee het die antioksidant kapasiteit van beide FLU- en PUT-behandelde vrugte toegeneem gedurende stoor. Verder is 'n totaal van 31 en 32 vlugtige verbindings geïdentifiseer in vrugte onder FLU en PUT behandeling onderskeidelik. Ses chemiese groepe (alkohole, aldehiede, sure, ketone, esters en terpene) is geïdentifiseer in vrugte onder FLU behandeling, terwyl vyf (alkohole, aldehiede, sure, esters en terpene) opgespoor is in vrugte onder PUT behandeling. Vlugtige verbindings he met langdurige stoor ontwikkel, met nuwe verbindings, veral terpene, wat tydens latere stoortydperke opgespoor is. Ophoping van terpene het `n nadelige uitwerking op die sensoriese kwaliteit van vrugte gehad, en dus kan langtermyn stoor lei tot laer vrugsmaak.

In die geheel het hierdie studie insiggewende inligting verskaf oor die potensiaal van putresien en fludioksonil behandelings in die vermindering van granaat versteurings, en die uitwerking daarvan op vrugkwaliteit-eienskappe. Die toediening van die FLU het vrug verval aansienlik verminder, maar nie koueskade, dopwonde en saadhuid verbruining nie, terwyl PUT hierdie fisiologiese versteurings insluitend vrugte verval kon verlig Nietemin kon PUT en FLU nie effektief gewigverlies verminder nie, en dus daar tydens toekomstige studies op die kombinasie van chemiese behandelings met fisiese behandelings soos plastiek verpakking en waslaag toepassing gefokus word ten einde voordeel te trek uit die hekkie effek. Daarbenewens kan’n kombinasie van die FLU en PUT ondersoek word, om voordeel te trek uit die volle potensiaal van die twee chemiese behandelings.

LIST OF PUBLICATIONS

Opara, U.L., **Atukuri, J.** & Fawole, O.A. (2015). Application of physical and chemical postharvest treatments to enhance storage and shelf life of pomegranate fruit- A review. *Scientia Horticulturae*, 197, 41–49.

Atukuri, J., Fawole, O.A. & Opara, U.L. (2017). Effect of exogenous fludioxonil postharvest treatment on physiological response, physico-chemical, textural, phytochemical and sensory characteristics of pomegranate fruit. *Journal of Food Measurement and Characterization*. DOI: 10.1007/s11694-017-9485-6.

Conference presentations

Atukuri, J., Fawole, O.A. & Opara. U.L. (2016). Postharvest chemical treatments to preserve sensory quality and reduce losses of fresh produce. Presented at the 5th African Higher Education Week and RUFORUM Biennial Conference. Cape Town, South Africa, October, 17 – 21.

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TABLE OF CONTENTS

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

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CHAPTER ONE: General Introduction

GENERAL INTRODUCTION

Diet affects human health and unlike genetics, individuals have better control over their own nutrition (McAdams, 2011). The awareness of the role of nutrition on individual health has resulted in interest and development of "functional foods" (American Dietetic Association, 2004; McAdams, 2011). There has been an upsurge of interest in foods that address health issues and in particular, plant-based foods (Espín *et al.*, 2007; McAdams, 2011). Fruit and vegetable consumption can decrease the risk to chronic illnesses, including those that are oxidation-related (Kelawala & Ananthanarayan, 2004). This is because plant phytochemicals have antioxidant activity and antioxidants protect the body against oxidative stress, which is detrimental to cells and biological function (Seeram *et al.*, 2006; Fawole *et al.*, 2011; McAdams, 2011). One of the many plants that have been studied for their potential health benefits is pomegranate. Pomegranate fruit contains high levels of active antioxidants, polyphenols in particular (Kelawala & Ananthanarayan 2004; Adams *et al.*, 2010; Fawole et al., 2011; Mphahlele *et al.*, 2014). The recent global trend of increased demand for pomegranates as fresh fruit or derived products is growing rapidly due to the health benefits of pomegranate (Rymon, 2011; Fawole & Opara, 2013). The health benefits are due to the remarkably high concentration of phenolic compounds (Gil *et al.*, 2000; Fischer *et al.*, 2011). Many clinical studies have shown that consumption of pomegranate contributes to prevention of some diseases such as coronary heart disease and some types of cancer (Aviram *et al.*, 2000; Langley, 2000; Sumner *et al.*, 2005). Consequently, in addition to the traditional markets, new markets are arising based on the manufacture of pomegranate-derived functional food products such as nutraceuticals, dietary and health supplements (Palou *et al.*, 2007).

Pomegranate (*Punica granatum* L.) belongs to the *Punicaceae* family native to areas from Iran to the Himalayans in northern India (Faria & Calhau, 2011). It is a hardy fruit plant extensively produced in the tropical and subtropical regions due to the moderate climatic conditions which are required for fruit maturation (Nanda *et al.*, 2001; Waskar, 2011). The fruit is spherically shaped, crowned with a persistent calyx and leathery pericarp with deep purple-red and glossy appearance (Holland *et al.*, 2009; Wetzstein *et al.*, 2011) (Fig. 1). However, some cultivars such as 'black' pomegranates maintain a black skin colour throughout development of the fruit until fruit ripening (Holland *et al.*, 2009). The edible part of the fruit is the arils which constitutes 55 - 60 % of the fruit weight and contain around 80 % juice and 20 % seed (Al-Maiman & Ahmad, 2002; Al-Said *et al.*, 2009; D'Aquino *et al.*,

2010). The arils are tender, deep crimson with good flavour and the skin is of medium thickness making the fruit well adapted for both fresh consumption and processing for whole arils or juice (Holland *et al.*, 2009). Although pomegranates are mainly grown for consumption of fresh arils, they are also produced as flavouring and colouring agents in the food and beverage industry (Gil *et al.*, 2000; Maestre *et al.*, 2000).

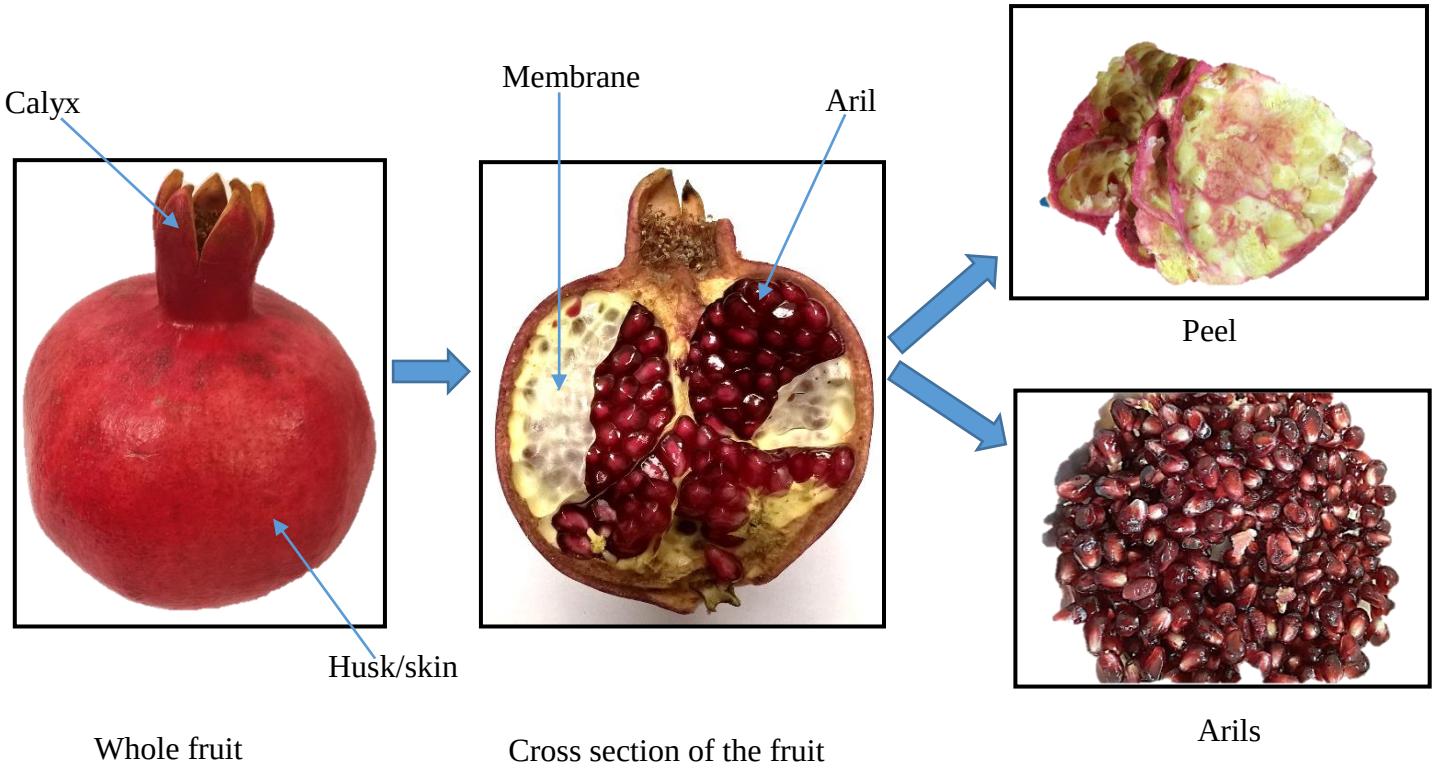

Fig. 1 Pomegranate whole fruit and its parts

Pomegranate has been classified as a non-climacteric fruit which has been attributed to the low respiration and ethylene production rates (Kader *et al.*, 1984). According to Elyatem & Kader (1984), pomegranates kept well when stored at 5 °C as compared to lower temperatures. However, storage at 5 °C caused chilling injury with symptoms increasing with storage time and temperature decrease below 5 °C (Eris & Türk, 1999). Besides chilling injury, other major postharvest conditions contributing to pomegranate fruit loss include fruit decay, bruising, water loss and as well as husk scalding (D'Aquino *et al.*, 2010). A number of physiological and enzymatic disorders lead to quality losses, with the major storage problem being moisture loss leading to browning of both peel and arils (Sayyari *et al.*, 2010). Even though the pomegranate rind appears to be thick, there are numerous minute openings that allow free movement of water vapor which makes the fruit highly susceptible to water loss

and shrivelling (Kader *et al.*, 1984). Another major problem that causes loss and limits pomegranate storability is fruit decay. This is caused by a number of pathogens such as *Alternaria spp*., *Aspergillus spp*., *Penicillium spp*., and especially *Botrytis cinerea* (Roy & Waskar, 1997) which usually develops at the recommended fruit storage condition of 5 - 8 °C and 90 - 95 % RH (Palou *et al.*, 2007). To alleviate these pomegranate postharvest challenges, several postharvest treatments can be applied (Opara *et al.*, 2015). Physical treatments including curing and intermittent warming (Artés *et al.*, 2000), shrink wrapping (Nanda *et al.*, 2001), hot water (Mirdehghan *et al.*, 2007b), gamma irradiation (Shahbaz *et al.*, 2014), among others. Chemical treatments can also be applied. These include applications of fungicides (fludioxonil, carbendazim, thiabendazole, etc), polyamines (putrescine, spermidine, spermine), organic acids and their derivatives (oxalic acid, salicylic acid, methyl jasmonate, methyl salicylate) and calcium chloride (Mirdehghan *et al.*, 2007a; D'Aquino *et al.*, 2010; Sayyari *et al.*, 2010; Ramezanian & Rahemi, 2010; Sayyari *et al.*, 2011; Waskar, 2011) among others.

Pomegranate production in South Africa has increased over the years. Recent local production increased by more than 20 % to 4500 tonnes in 2015 (Kriel, 2015). The number of pomegranate processors has increased in response to rising demand (Kriel, 2015). However, producers are still struggling to achieve the full potential of the fruit through improved postharvest management. According to Kriel (2015), although the pomegranate market is lucrative, production of high-quality fruit remains a challenge. Research efforts have helped to increase the production of pomegranate but the goal of obtaining maximum profit will be served only if the increased production is supplemented with similar efforts to minimize postharvest losses and enhance shelf life (Waskar, 2011).

South Africa is one of the emerging commercial producers in the pomegranate international market (Fawole & Opara, 2013) and to be able to compete globally, there is need for research to reduce postharvest losses and improve fruit quality of the cultivars grown in the country. This is particularly essential because studies have shown that fruit quality of pomegranates differ significantly among growing regions (Schwartz *et al*., 2009; Mditshwa *et al.*, 2013). Furthermore, most of the pomegranates produced in South Africa are sold on the export market, mainly Europe. To reach these markets, fruits spend a long time in transit during which they are susceptible to spoilage leading to losses. Therefore, there is a need to prolong the storability and shelf life such that fruit can reach the destined markets fresh and acceptable so as to fetch premium prices and favourably compete on the international market.

In addition, most research work that have been carried out to date on South African pomegranates focused mainly on treatment of arils to reduce spoilage and extend shelf life, including the application of modified atmosphere packaging (Hussein *et al.*, 2015; Banda *et al.*, 2015a, Caleb *et al.*, 2013), citric acid treatment (Banda *et al.*, 2015b), cold storage (Aindongo *et al.*, 2014) and anti-browning pre-treatments (Caleb *et al.*, 2015). Currently, there is limited information on postharvest treatments to maintain quality and reduce losses of pomegranate whole fruit grown in South Africa.

Research aim and objectives

The aim of this study was to investigate the potential of selected chemicals as postharvest treatments to maintain fruit quality and minimize loses of pomegranate whole fruit (cv. Wonderful).

The research aim was achieved through the following specific objectives;

1. To evaluate the efficacy of putrescine on physiological disorders and impacts on the physiological response, physico-chemical and sensory attributes of pomegranate whole fruit,

2. To determine the effects of fludioxonil on physiological disorders and impacts on the physiological response, physico-chemical and sensory attributes of pomegranate whole fruit, and

3. To investigate the effects of putrescine and fludioxonil on the phytochemicals, antioxidant activity and volatile composition of pomegranate whole fruit.

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CHAPTER TWO: Literature review

APPLICATION OF PHYSICAL AND CHEMICAL POSTHARVEST TREATMENTS TO ENHANCE STORAGE AND SHELF LIFE OF POMEGRANATE FRUIT - A REVIEW

Abstract

There has been recent interest in pomegranate fruit production and research due to its high nutritional and health benefits. The increase in demand of the fruit necessitates the need to improve quality, storability and shelf life to meet consumers' expectations of consistent supply of quality fruit. However, pomegranate fruit is susceptible to various postharvest quality problems including high weight loss, decay and susceptibility to physiological disorders such as chilling injury and husk scald. To improve fruit storability and shelf life, physical and chemical postharvest treatments have been applied. However, these treatments have varied effects on the external and internal quality attributes of fruit. This review therefore discusses the different postharvest treatments applied to enhance storage of pomegranate whole fruit and arils and highlights the effects of the treatments on the fruit quality.

1. Introduction

Pomegranate (*Punica granatum L.*) is an ancient known fruit belonging to the family Lythraceae (Holland *et al.*, 2009). It is an important fruit in the tropical and sub-tropical regions of the world and mainly cultivated in countries with Mediterranean climate. (Özgüven & Yilmaz, 2000; Nanda *et al.*, 2001). As a result of its high adaptability to various soils and climates, pomegranate is now grown in many countries including South Africa, Iran, India, Pakistan, Russia, Turkey, Japan, Greece, Sultanate of Oman, China, Egypt and U.S.A (Elyatem & Kader, 1984; Köksal, 1989; Holland *et al.*, 2009; Fawole & Opara, 2013a,c). The fruit peel colour varies from yellowish-green to deep red depending on cultural practices (like pruning, light penetration), climate, and cultivar (Sepúlveda *et al.*, 2000; Faria & Calhau, 2011). The fruit is made up of a hard leathery outer exocarp (skin), mesocarp (albedo), endocarp (membrane) and many arils. Each aril, which is edible, is surrounded by a translucent sac that contains juice and a seed constituting about 80 % and 20 % (fresh weight) of an aril, respectively (D'Aquino *et al.*, 2010). The fresh juice is mainly made up of water (85 %), sugars (10 % majorly glucose and fructose), ascorbic acid, anthocyanins, polyphenolic flavonoids, pectins, amino acids and minerals (Roy & Waskar, 1997).

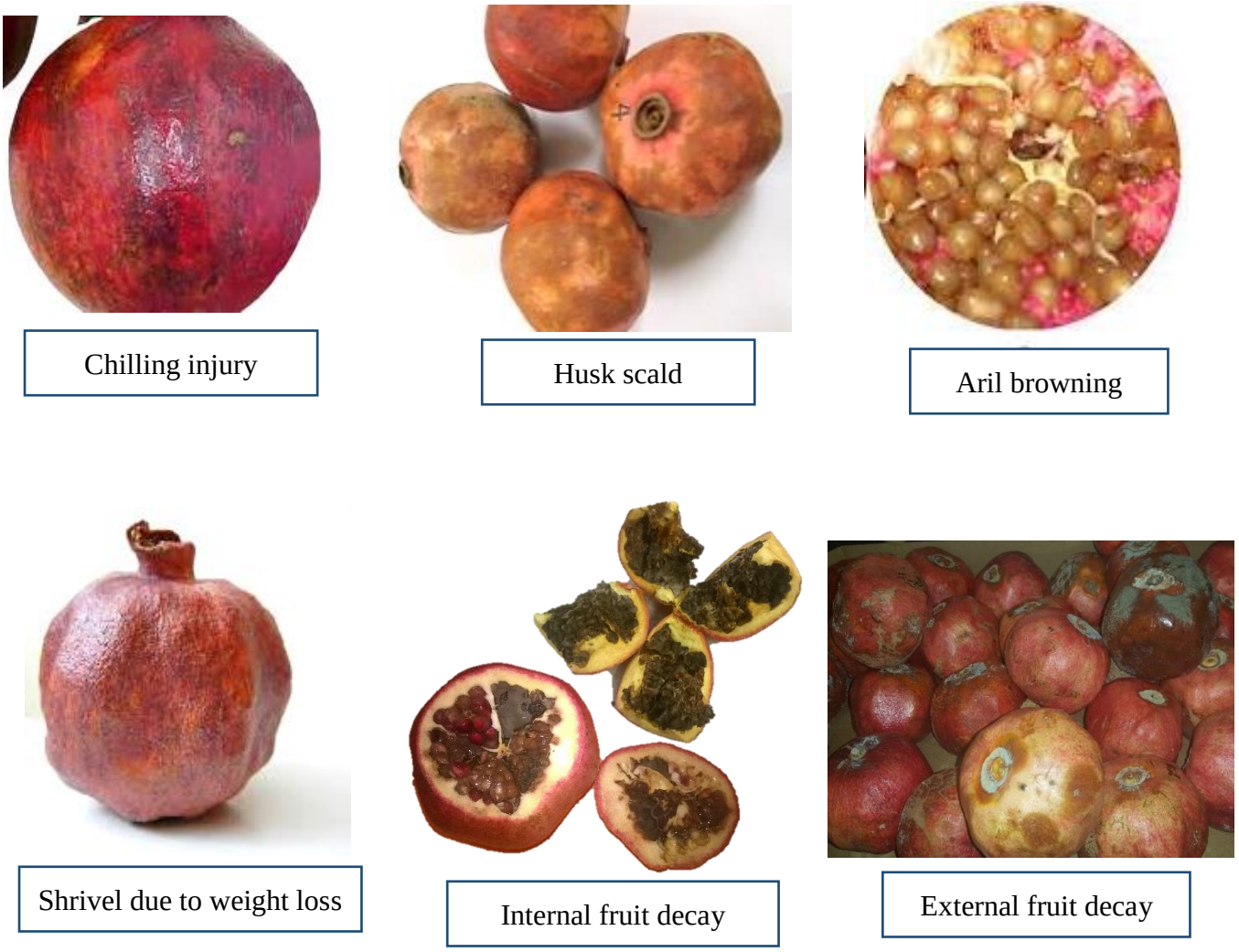

Fig. 1 Pomegranate fruit physiological disorders

According to Kader *et al.* (1984), pomegranate is classified as a non-climacteric fruit due to its low respiration and ethylene production rates after harvest. In spite of its non-climacteric nature, the fruit still undergoes both qualitative and quantitative losses due to postharvest handling processes resulting in chilling injuries, husk scald, weight loss, and decay (Kader *et al.*, 1984) (Fig. 1). Storage of pomegranates at room temperature reduces the shelf life due to increased desiccation and incidence of decay. To prolong storability, there is need to store fruit at low temperatures (Barman *et al.*, 2011; Fawole & Opara, 2013b). However, when fruit are exposed to temperatures below 5 °C, they are susceptible to chilling injury (Sayyari *et al.*, 2010). These symptoms are noticeable as brown discoloration of the peel, surface pitting and susceptibility to decay organisms. In most cases these symptoms reach the arils, which decrease both internal and external quality of the fruit (Elyatem & Kader, 1984; Kader, 2006; Fawole & Opara, 2013b). Husk scald, considered to be a symptom

of chilling injury develops faster and more severely in fruit stored between 6 and 10 °C than in fruit stored at lower temperatures (Ben-Arie & Or, 1986). Decay is another major cause of postharvest loss which limits storability of pomegranate fruit, especially when stored at temperatures above those that cause chilling injury (Palou *et al.*, 2007). Decay usually develops at the recommended storage conditions (5 - 8 °C and 90 - 95 % RH) and is caused by various pathogens such as *Aspergillus spp*., *Alternaria spp*., *Penicillium spp*., and *Botrytis cinerea* (Roy & Waskar, 1997).

Global demand for pomegranate fruit as fresh aril, dried or value-added processed products has increased globally in recent years due partly to reported high content of healthful phytochemicals (Fawole *et al.*, 2012). This increase in demand and popularity among consumers has led to steady increase in production in both Northern and Southern hemisphere countries including the Sultanate of Oman and South Africa (Opara *et al.*, 2009; Mditshwa *et al.*, 2013). A number of treatments have been applied to improve quality and increase the shelf life of pomegranate whole fruit and arils, these include intermittent warming, curing, film wrapping, waxing, polyamines, controlled atmosphere, honey treatments and modified atmosphere packaging, among others (Artés *et al.*, 1998; Nanda *et al.*, 2001; Hess-Pierce & Kader, 2003; Mirdehghan *et al.*, 2007a; Ergun & Ergun, 2009; Waskar, 2011; Caleb *et al.*, 2012a,b; Caleb *et al.*, 2013).

Despite the availability of various postharvest treatments, high incidence of postharvest loss of pomegranates still occurs, often exceeding 30 % for some cultivars in one season (Shete & Workar, 2005). This results in loss of nutritional and quality attributes as well as financial loss which greatly reduces profitability and growth of the industry. Therefore, there is need for more research to increase storability and reduce postharvest loss of pomegranate if the full potential of the fruit is to be realized. Furthermore, there is need for research focusing on the application of postharvest treatments and innovative technologies to maintain or enhance the nutritional and bioactive ingredients of the fruit. The objective of this review was to review current knowledge on the application of postharvest treatments to enhance the storage and shelf life of pomegranate fruit.

2. Physical treatment of pomegranate fruit

2.1. Curing and intermittent warming

2.1.1. Effects on physiological response

Studies have shown that curing of pomegranate fruit results in weight loss, depending on the storage temperature. For instance, pomegranate fruit cured at 33 °C for 3 days resulted in high fruit weight loss, with higher losses observed in fruit stored at 5 °C compared to 2 °C (Artés *et al.*, 2000). However, no symptoms of shrivelling were observed after cold storage and shelf life periods of 6 days at 15 °C (Table 1). This was similar to previous findings that intermittent warming of fruit led to higher weight loss than conventionally stored fruit after cold storage at 0° and 5 °C for 80 days and an additional 7-day shelf life period at 15 °C (Artés *et al.*, 1998). Susceptibility of pomegranate fruit to weight loss after curing or intermittent warming was suggested to be due to easy passage of water vapour through numerous minute openings on pomegranate peel.

Decay incidence in pomegranate can also be reduced by intermittent warming. According to Artés *et al.* (1998), lower decay was observed in fruit subjected to intermittent warming compared to those stored conventionally. However, the choice of cold storage temperature during intermittent warming is important. For instance, after cold storage (0 °C, 2 °C and 5 °C) for 12 weeks and additional shelf life period of 6 days at 15 °C and 75 % RH, fruit stored at 0 °C showed no decay while fruit stored at 2 °C had lower fungal attacks compared to those stored at 5 °C after shelf life (Artés *et al.*, 2000). In the same study comparing curing and intermittent warming (Table 1), it was observed that curing of pomegranate resulted in higher weight loss than a day intermittent warming at 20 °C every 6 days of fruit stored at 2 °C or 5 °C while control fruit had the lowest weight loss (Artés *et al.*, 2000). Curing has also been reported to reduce incidence of decay in pomegranate fruit. After curing pomegranate fruit, Artés *et al.* (2000) observed no symptoms of decay after 12 weeks of cold storage at 2 °C and 5 °C until during a 6-day shelf life at 15 °C and 75 % RH. The authors observed higher decay incidences at higher storage temperature, with decay incidence of 28.7 % at 5 °C as opposed to 8.7 % at 2 °C.

The physical appearance of pomegranate fruit is of great relevance because it affects consumer appeal and purchasability of the produce. Artés *et al.* (2000) observed that curing pomegranate whole fruit at 33 °C for 3 days before storage at 2 °C and 5 °C for 12 weeks did not affect the skin colour, with higher L*, C* and H° colour parameters on the skin than those

obtained on the arils however curing decreased the visual colour appearance of arils after cold storage of fruit (Artés *et al.*, 2000). For intermittent warming however, no changes were observed in fruit lightness (L*) with only slight changes in the colour intensity (C*) and hue angle (*h*°) values of fruit after 80 days of storage at 0° and 5 °C (Artés *et al.*, 1998). According to the authors intermittent warming and storage at 0 °C showed a better result for maintaining the desirable red colour of pomegranate fruit.

2.1.2. Effects on chemical properties

The effect of postharvest treatments on the nutritional and chemical composition of the fruit is of paramount importance in evaluating the relevance of the treatments. Intermittent warming resulted only in a slight decrease in the soluble solids content (SSC) after 80 days of cold storage (0° and 5 °C) and shelf life periods of six additional days at 15 °C and 75 % RH (Artés *et al.*, 1998). Similarly, Artés *et al.* (2000) observed that both curing and intermittent warming of pomegranate whole fruit ('Mollar de Elche') resulted in a slight but non-significant decrease in the soluble solids content after cold storage for 12 weeks at 2 and 5 °C. Generally, curing resulted in slight changes in the SSC, pH and TA (titratable acidity) of pomegranate fruit in comparison to fruit at harvest (Artés *et al.*, 2000). In addition, Artés *et al.* (2000) observed that although anthocyanin concentration decreased with storage, curing maintained the anthocyanin concentration of pomegranate fruit while slight increases were observed in intermittent warmed and control fruit stored at 5 °C for 12 weeks (Artés *et al.*, 2000).

Artés *et al.* (1998) also found that the appearance of arils from intermittent warmed fruit was scored good and remained unchanged during 80 days of storage. This was further supported in a later study that intermittent warming and storage at 5 °C yielded arils with the best visual appearance (Table 1) (Artés *et al.*, 2000). Curing on the other hand resulted in loss of flavour with storage at 5 °C having the lowest flavour (Artés *et al.*, 2000). In comparison with curing, intermittent warming with 2 °C storage was a better treatment for pomegranate whole fruit as it resulted in the best flavour, higher total anthocyanin content, lowest total losses and maintained quality and shelf life for up to 13 weeks (Artés *et al.*, 2000).

2.2. Hot water treatment

2.2.1. Physiological responses

Several studies have reported the use of hot water as a postharvest treatment for pomegranate. Mirdehghan & Rahemi (2005) observed that treatment of 'Malas Yazdi' pomegranate whole fruit with hot water at 50 °C resulted in the least fruit weight loss compared to chemical (Imazalil and Benzyladenine) and control treatments. In addition, fruit weight loss increased with increasing hot water temperature, with significant weight loss and heat injury observed at 65 °C. In another study, it was observed that hot water treatment decreased weight loss by 5.86 % in comparison to the control, with the suggestion that the decrease could be associated with improvement in cells membrane function or in skin cuticular properties (Ramezanian & Rahemi, 2010). Furthermore, a study reported that skin browning was lowest in fruit treated with hot water at 50 °C compared to control and chemical treatments (Imazalil and Benzyladenine) stored at 1.5 °C for 4.5 months (Mirdehghan & Rahemi, 2005). Hot water treatment (HWT) at 45 °C for 2 and 5 min significantly reduced skin browning, however at 65 °C, slight heat injury and increased percentage of browning was observed when fruit were stored for 3 months (Mirdehghan & Rahemi, 2005). Similarly, hot water treatment at 45 °C for 4 min also significantly reduced the rate of browning by 9 % in comparison to control treatment (Ramezanian & Rahemi, 2010). In addition, the authors recommended HWT at 45 °C for 4 min as the best heat treatment to control chilling injury of 'Malas Yazdi' and 'Malas Saveh' whole fruit. This observation is in agreement with a previous study by Mirdehghan *et al.* (2007b) where heat treatment (45 °C for 4 min) significantly reduced chilling injury on 'Malas Yazdi' pomegranate in comparison to control fruit. The authors suggested that heat treatment maintained membrane integrity and unsaturated fatty acids during cold storage thus reducing the incidence of chilling injury.

Electrolyte leakage was significantly reduced with hot water treatment (HWT) at 50 °C for 2 and 5 min when compared to other treatments (Imazalil and Benzyladenine), which had no effect (Mirdehghan & Rahemi, 2005). Mirdehghan *et al.* (2007b) also observed that electrolyte leakage was higher in control than in hot water treated (45 °C) fruit, suggesting that hot water dipping reduced leakage of electrolytes from the fruit. In agreement with other studies, Ramezanian & Rahemi (2010) also found that hot water treatment reduced electrolyte leakage by 20 % in comparison with the control for 'Malas Yazdi' pomegranates stored at 2 °C for 4.5 months plus a further 3 days of shelf life.

2.2.2. Effects on physical and chemical properties

According to Mirdehghan *et al.* (2007b), hot water dipping at 45 °C for 4 min retarded skin browning of pomegranate fruit ('Mollar de Elche') and maintained fruit firmness during storage for 90 days. The author attributed improved fruit firmness to the broad effect of heat on cell wall degrading enzymes (Mirdehghan *et al.*, 2007b). Although hot water treatment was shown to have no significant effects on the TSS, TA, pH and ascorbic acid of fruit after storage for 3 months (Mirdehghan & Rahemi, 2005; Ramezanian & Rahemi, 2010), antioxidant activity increased significantly and this observation was attributed to minimal degradation of phenolic compounds (Ramezanian & Rahemi, 2010). In addition, dipping pomegranate fruit ('Mollar de Elche') in hot water (45 °C for 4 min) preserved the fatty acids concentrations in the juice (Mirdehghan *et al.*, 2007b). It was observed that the concentration of all fatty acids remained significantly higher in fruit treated with hot water than control throughout the storage period. Furthermore, observed decrease in fatty acids in untreated pomegranate fruit was highly correlated with increase in electrolyte leakage (Mirdehghan *et al.*, 2007b).

2.3. Film wrapping

2.3.1. Physiological responses

Respiration rate of 'Ganesh' pomegranate fruit wrapped in shrink film was significantly reduced due to the low permeability of the films used for wrapping (Nanda *et al.*, 2001). Interestingly, after 10 weeks of storage, unwrapped (control) fruit exhibited lower respiration rate than wrapped fruit. This was primarily due to reduced number of living cells in the peel of control fruit resulting from excessive dehydration (D'Aquino *et al.*, 2010). Moreover, weight loss was greatly reduced in shrink wrapped fruit stored at 8, 15 and 25 °C, with BDF and D-955 films having 1.5 and 2.3 % weight loss, respectively compared to 14 % loss in control fruit stored at 25 °C for 25 days (Nanda *et al.*, 2001). This was in agreement with D'Aquino *et al.* (2010), who also reported 0.6 % weight loss in film wrapped 'Primosole' pomegranate as opposed to 5.1 % weight loss in unwrapped fruit after 6 weeks of storage at 8 °C. Film wrapping has also shown to prevent symptoms of husk scald in pomegranate fruit. For instance, wrapping resulted in no signs of scald, discoloration or browning during a 6-week storage period at 8 °C whereas skin of control fruit developed yellow to dark yellow and brown coloration as storage progressed (D'Aquino *et al.*, 2010). Furthermore, Nanda *et*

al. (2001) reported spoilage mainly due to *Penicillium spp.* with 12 % spoilage in unwrapped fruit while the wrapped fruit were fresh with high scores for good appearance (Table 1).

2.3.2. Effects on physical and chemical properties

Fruit firmness of pomegranate ('Ganesh') wrapped with BDF-2001 and D-955 films was maintained throughout a 12-week storage period whereas unwrapped fruit were less firm, tough and desiccated (Nanda *et al.*, 2001). In addition, loss in skin colour was minimized in film wrapped fruit compared to control fruit for 'Primosole' cultivar (D'Aquino *et al.*, 2010). Decrease in acidity was considerably lower in wrapped than those of unwrapped fruit during 12 weeks of storage (Nanda *et al.*, 2001). This was attributed to the higher respiration rate in unwrapped fruit and a concurrent loss in acidity which was attributed to the ongoing metabolism in the fruit. D'Aquino *et al.* (2010) observed a higher increase in pH and decrease in TA of wrapped fruit compared to unwrapped ones during 12 weeks of storage at 8 °C for 'Primosole'. Another advantage of film wrapping on pomegranate is its ability to minimise loss of vitamin C in pomegranate juice. In the study by Nanda *et al.* (2001), film wrapping minimised loss of vitamin C by 3.21- 5.11 % during 12 weeks storage period at 8 °C. On the other hand however, film wrapping has been reported to result to significant reduction in total phenolics and anthocyanin content during storage, resulting to continuous decrease in antioxidant activity of the fruit (D'Aquino *et al.*, 2010).

2.4. Coatings

2.4.1. Physiological responses

The application of coatings on fruits provides a partial barrier to movement of water thus reducing moisture loss from fruit surface and also establishes a modified atmosphere around the fruit thus slowing down respiration and senescence (Mahajan *et al.*, 2014). A number of studies have reported the use of skin coating on pomegranate fruit. Coating of pomegranate whole fruit ('Ganesh') with sucrose polyester (SPE) reduced weight loss during storage at 8 °C and 25 °C (Nanda *et al.*, 2001). Similarly, application of lecithin (Table 1) significantly reduced fruit weight loss as well as incidence and severity of husk scald in 'Primosole' cultivar (D'Aquino *et al.*, 2012). In addition, starch based edible coating (containing cold pressed oil from *Nigella sativa*) had about 6-fold weight loss reduction in pomegranate arils (Table 1). The reduced weight loss was attributed to improved water vapour barrier properties of the coatings by providing hydrophobicity and increased resistance to water transmission

(Oz & Ulukanli, 2012). Furthermore, the use of *Aloe vera* gel (Table 2) has been reported to influence respiration rate of 'Mollar de Elche' arils. This was evidenced by significant increase in CO_2 concentration with a concomitant decrease in O_2 over time (Martínez-Romero *et al.*, 2013). Coating arils with starch and oil has also been reported to reduce browning (Oz & Ulukanli, 2012).

2.4.2. Effects on physical and chemical properties

Coating of arils with either starch or oil significantly reduced the aril softening ratio, with the combination of starch and oil being more effective than starch alone. The softening ratio was 3 % and 5 % when treated with starch and oil, respectively compared to 18 % in control arils (Oz & Ulukanli, 2012). Pomegranate arils treated with either 10 % or 20 % honey solution (Table 2) did not lose firmness as much as those of control samples after 5 days of storage at 4 °C for 'Hicaznar' cultivar (Ergun & Ergun, 2009). Similarly, Martínez-Romero *et al.* (2013) reported that aril firmness was better maintained when arils were treated with *Aloe vera* either alone or in combination with acids. Martínez-Romero *et al.* (2013) also observed decrease in hue angle in arils treated with *Aloe vera* while the control arils showed increased hue angle during storage for cultivar Mollar de Elche. It was suggested that increase in aril colour was due to the increase in anthocyanin pigments during postharvest storage of pomegranate fruit (Sayyari *et al.*, 2011a; Martínez-Romero *et al.*, 2013).

Coating pomegranate fruit ('Primosole') with soy lecithin resulted in slight but significant changes in pH values and decrease in TA over storage while TSS was not affected (D'Aquino *et al.*, 2012). Coating with sucrose polyester (SPE) resulted in a slight decrease in TSS after storage at 8, 15 and 25 °C although the treatment did not minimise loss of vitamin C content during storage (Nanda *et al.*, 2001). Similarly, coating with SPE did not prevent loss of acidity in 'Ganesh' pomegranate between 9 and 12 weeks of storage at 8, 15 and 25 °C (Nanda *et al.*, 2001). Oz & Ulukanli (2011) observed that pomegranate arils ('Silifke aşısı 33 N 16') coated with starch and oil from *Nigella sativa* (Table 2) had 14.2 % loss in TSS content of arils during storage compared with 17 % loss in control fruit. Studies have shown that vitamin C content of pomegranate arils reduces with increasing storage duration (Oz & Ulukanli, 2012; O'Grady *et al.*, 2014). However, the application of oil and starch coating minimized vitamin C loss in 'Silifke aşısı' pomegranates stored for 12 days at 4 °C (Oz & Ulukanli, 2012). According to the authors, vitamin C diminished by 66 % (from 24 to 8 mg/100 g) in control fruit whereas only 12 % (from 58 to 51 mg/100 g) was diminished in

fruit treated with the combination of oil and starch coating. Total antioxidant capacity (TAC) of pomegranate arils treated with the combination of oil and starch was observed to decrease during initial storage (from 4 to 6 days) and stabilized at the later days (6 to 12 days) of storage (Oz & Ulukanli, 2012). Shelf life of pomegranate fruit (Ganesh cultivar) was only marginally extended by coating with a sucrose polyester (Table 1) during storage for 12 weeks (Nanda *et al.*, 2001). Martínez-Romero *et al.* (2013) found that scores for sensory attributes such as colour, aroma, texture, flavor and purchase decision were lowest (below 2) in control arils but arils treated with combination of *Aloe vera* gel and acids had high scores with no detection of off-flavour. Similarly, studies by Ergun & Ergun (2009) showed that aroma scores for control arils declined below the acceptable limit whereas arils treated with honey had excellent aroma scores throughout storage for 10 days (Table 2). Similar findings have been reported by Martínez-Romero *et al.* (2013) who found that arils treated with *Aloe vera* had aroma of fresh fruit while the aroma of control (untreated) arils corresponded with over ripe fruit. The authors concluded that shelf life of arils coated with *Aloe vera* gel could be extended for up to 12 days compared to 8 days for control arils.

2.5. Waxing

2.5.1. Physiological responses

Treatment of 'Mridula' pomegranate fruit with carnauba wax in combination with putrescine (PUT) lowered fruit respiration and ethylene production rates due to reduced gas interchange and low oxygen available for respiration during 60 days of storage at 3 °C (Barman *et al.*, 2011). Combination of carnauba wax and putrescine (Table 1) also reduced fruit weight loss by 10 % in treated compared to 17 % in control fruit due to the overlapping platelets of carnauba wax which act as a barrier for diffusion thus resist penetration of water vapour from the fruit (Barman *et al.*, 2011). This also explains why very low weight loss (0.1 %) was observed for pomegranate fruit ('Bhagawa') coated with wax mixed with carbendazim during 80 days of storage (Waskar, 2011). No decay was observed during storage at 3 °C for 60 days after treatment of 'Mridula' pomegranate fruit with carnauba wax and putrescine (Barman *et al.*, 2011).

2.5.2. Effects on physical and chemical properties

Pomegranate fruit ('Mridula') treated with PUT + carnauba wax and stored for 60 days at 3ºC retained firmness due to less occurrence of dehydration and slower degradation of cell wall

components (Barman *et al.*, 2011). Interestingly, there was lower reduction in juice content for waxed 'Bhagawa' pomegranate as a result of minimal moisture loss and respiration thereby retaining juice percentage (Waskar, 2011). The shelf life of pomegranate fruit ('Bhagawa') treated with waxol (9 %) and carbendazim (9 %) was extended by 30 days at room temperature and by 65 days at 8 °C, with fruit having good acceptance scores and organoleptic rating in terms of colour, flavour and texture (Waskar, 2011).

2.6. Irradiation

2.6.1. Physiological responses

Irradiation has been used as a treatment in a number of fruits but its use is limited due to the concerns surrounding its impact on human health. Irradiation of pomegranate whole fruit is limited as it has been more commonly applied to the juice. However a few studies have reported the effect of irradiating pomegranate fruit (Table 1 and 2). López-Rubira *et al.* (2005) observed that ultraviolet- C (UV-C) irradiation of pomegranate arils ('Mollar') had no effect on respiration rate of on-time and late-harvested fruit although the late harvested fruit generally had a higher respiration rate, which was attributed to increase in metabolic activity as a signal of decay. Likewise, the gas composition of the arils was also not affected by irradiation with increased CO_2 and decreased O_2 levels observed in the packages throughout storage at 5 °C for 16 days (López-Rubira *et al.*, 2005).

2.6.2. Effects on physical and chemical properties

Irradiation has been shown to affect the physical and chemical parameters of pomegranate fruit. Pomegranate juice from irradiated fruit was observed to have a lighter colour compared to the control as redness (a*) and yellowness (b*) increased with increasing irradiation dose due to a decrease in polyphenoloxidase activity by irradiation (Shahba *et al.*, 2014). Chemical compounds in fruit are sensitive to irradiation, in particular bioactive compounds such as anthocyanins have been shown to be affected by irradiation (Maghoumi *et al.*, 2013; Shahba *et al.*, 2014). Lower irradiation doses (0.4 and 1kGy) had no effect on titratable acidity, pH and total soluble solids but losses were reported when higher dosage levels (2 kGy) were applied (Shahba *et al.*, 2014). In addition, total phenolic content and anthocyanin content of juice from irradiated pomegranate fruit (California cultivar) decreased gradually with increase in dosage from 0.4 kGy to 2 kGy due to immediate oxidation of phenolic compounds as these play an antioxidant role by reducing the free radicals and the reactive oxygen species

produced by irradiation (Shahba *et al.*, 2014). Combined use of ultraviolet-C irradiation (UV-C) and high oxygen packing was useful for keeping fresh-cut 'Mollar de Elche' pomegranate arils quality at 5 °C and extending their shelf life to 15 days. However, anthocyanin content declined while total phenolic content remained unchanged with ultraviolet-C irradiation and high oxygen packing during storage of arils ('Mollar de Elche') at 5 °C for 14 days (Maghoumi *et al.*, 2013). Sensory evaluation of juices from low dose irradiated fruit (0.4 and 1 kGy) were preferred among panelists compared to juice from control and high dose treated fruit (2 kGy) as high doses of irradiation can induce an off-odour called "irradiation odour" in fruit juices (Shahba *et al.*, 2014). On the contrary, López-Rubira *et al.* (2005) observed no desirable changes resulting from irradiation of 'California' cultivar.

3. Chemical treatment of pomegranate fruit

3.1. Polyamines

3.1.1. Physiological responses

Polyamines (PAs) are naturally occurring compounds that are involved in many developmental processes of plants. Exogenous application of polyamines such as putrescine, spermidine and spermine on pomegranate has been reported in several studies (Table 1). Barman *et al.* (2011) attributed the reduced ethylene production rate of pomegranate fruit ('Mridula') treated with putrescine to the anti-ethylene function of polyamines because both (PAs and ethylene) use the common precursor SAM (S-adenosyl methionine) for their biosynthesis. While putrescine reduced the respiration rate of 'Mridula' pomegranate fruit (Barman *et al.*, 2011), spermidine did not affect the respiration rate of 'Mollar de Elche' pomegranate during 60 days of storage at 2 °C (Mirdehghan *et al.*, 2007a). Putrescine, either alone or in combination with carnauba wax reduced chilling injury and skin browning of pomegranate ('Mridula') by 65 % due to induced cold acclimation which led to maintenance of membrane fluidity at lower temperatures and consequently reduced electrolyte leakage and skin browning (Barman *et al.*, 2011). Similarly Mirdehghan *et al.* (2007a) also observed that application of putrescine or spermidine either by pressure or immersion reduced skin browning by 25 % and weight loss by 13 % and 15 % for putrescine and spermidine, respectively.

3.1.2. Effects on physical and chemical properties

Treatment of 'Mridula' pomegranate with putrescine (PUT) + carnauba wax maintained the highest fruit firmness after 60 days of storage at 3 °C (Table 1). The effect of polyamines on maintaining fruit firmness was ascribed to their cross-linkage to the carboxyl group of the pectic substances in the cell wall, resulting in rigidification. The binding between PAs and pectin also blocks the access of cell wall degrading enzymes such as pectimethylestearase, pectinesterase and polygalacturonase, thereby reducing the rate of softening during storage (Barmna *et al.*, 2011). This binding effect was evidenced in the report by Mirdehghan *et al.* (2007a) who observed reduction in loss of fruit firmness by application of polyamines during 45 days of storage at 2 °C. Treatment of pomegranate fruit ('Mridula') with PUT + carnauba wax resulted in fruit with higher hue angle and lower chroma values with red shinning colour as opposed to deep tan red dull colour in control fruit after 60 days of storage at 3 °C (Barman *et al.*, 2011). Highest total sugars and TA and lowest TSS were observed in PUT + carnauba wax treated fruit compared to control fruit during a 60-day storage period. This was attributed to lower respiration, maturation process and water loss in treated fruit in comparison to control fruit (Barman *et al.*, 2011). However, on the other hand, Mirdehghan *et al.* (2007a) observed no effect of polyamine treatment on the SSC and acidity during 60 days storage (Table 1). The changes were associated to delayed maturation due to application of PUT or SPD treatments as a result of their anti-senescence properties (Mirdehghan *et al.*, 2007a).

According to Barman *et al.* (2011), total anthocyanin content increased for the first 15 days at 3 °C but later decreased with putrescine treated fruit, having 30 - 40 % higher amounts than control after 60 days of storage (Barman *et al.*, 2011). This was attributed to putrescine protecting the membrane lipids from being converted from liquid-crystalline to a solid-gel state thereby preventing lipid peroxidation. Treatment with PUT + carnauba wax also retained 20 % more ascorbic acid compared to control after storage at 3 °C for 60 days due to the anti-senescence properties of putrescine (Barman *et al.*, 2011).

3.2. Fungicides

3.2.1. Physiological responses

Fungicides have been widely used to control spoilage of pomegranate fruit. A number of studies have shown the effect of these compounds on fruit quality. Fludioxonil (FLU) was effective in reducing decay of pomegranate fruit caused by *Penicilium spp*. At the end of 7

days of shelf life, decay in fruit ('Primosole') treated with FLU alone or in combination with film wrapping was between 8 to 12 %, which was between 2 - 3 fold less than in control fruit stored for 12 weeks at 8 °C (D'Aquino *et al.*, 2010). Furthermore, D'Aquino *et al.* (2012) observed that decay development significantly reduced when 'Primosole' pomegranate was treated with fludioxonil whereas lecithin treatment did not affect fruit decay. Interestingly, fludioxonil when applied alone showed better performance than in combination with lecithin. After one week of storage, no decay was detected in fruit treated with fludioxonil while control fruit showed 35 - 60 % decay, and after 2 weeks of storage there was 100 % decay incidence in control fruit while fruit treated with fludioxonil had only 2.5 - 7.5 % decay even in the third week of storage (D'Aquino *et al.*, 2009). However, fludioxonil had no significant effect on weight loss, husk scald severity and overall appearance (D'Aquino *et al.*, 2012).

3.2.2. Effects on physical and chemical properties

Generally, there is limited information on the effects of fludioxonil on the physical and chemical parameters of pomegranate fruit. Residues of fludioxonil were detected only on the skin but not in the edible flesh part of 'Primosole' pomegranate and residue levels increased with increase in fludioxonil concentration and dipping temperature after 2 weeks at 20 °C (D'Aquino *et al.*, 2009). It was also observed that efficacy of fludioxonil decreased substantially when the infections occurred more than 24 h before treatments due to the fact that fludioxonil is a contact fungicide as opposed to being systemic (D'Aquino *et al.*, 2009).

3.3. Organic acids and their derivatives

3.3.1. Physiological responses

Organic acids are naturally occurring compounds in plants that play different important roles in the survival of the plant. A number of studies have reported the use of these compounds in postharvest treatment of pomegranates (Table 1). Treatment of pomegranate fruit ('Mollar de Elche') with acetyl salicylic acid (ASA) reduced respiration rate by 22 - 38 % compared to control due to retardation of fruit metabolism and reduced chilling injury during 84 days of storage at 2 °C (Sayyar *et al.*, 2011b). In another study, Sayyari *et al.* (2011a) observed that the application of methyl salicylate (MeSa) and methyl jasmonate (MeJa) on 'Mollar de Elche' pomegranates significantly reduced the chilling injury by 2 - 3 folds lower than control fruit during storage for 84 days. The mechanism of MeJa in reducing chilling injury was been attributed to enhancing the activities of superoxide dismutase, catalase and

ascorbate-peroxidase and lowering the activity of lipoxygenase. Similarly, treating fruit ('Mollar de Elche') with oxalic acid reduced respiration, weight loss and electrolyte leakage after 84 days of cold storage at 2 °C (Sayyari *et al.*, 2010). The effects of oxalic acid in reducing the incidence of chilling injury was attributed to the inhibition of polyphenoloxidase and peroxidase activities. Sayyari *et al.* (2009) also observed reduced chilling injury symptoms when fruit were treated with salicylic acid and the effectiveness increased with higher concentration. Similarly, acetyl salicylic acid reduced chilling injury in 'Mollar de Elche' pomegranate, an effect ascribed to its conversion to salicylic acid (Sayyari *et al.*, 2011b).

3.3.2. Effects on physical and chemical properties

Methyl jasmonate (MeJa) and methyl salicylate (MeSa) delayed softening of 'Mollar de Elche' pomegranate fruit, with MeSa being more effective than MeJa (Sayyari *et al.*, 2011a). It was postulated that MeJa reduces pectinmethylesterase (PME) activity, decreasing de-esterification of pectin and thus maintaining fruit texture (Sayyari *et al.*, 2011a). In another study, oxalic acid treatment had no significant effect on fruit firmness after 84 days of storage (Sayyari *et al.*, 2010). Treating fruit with oxalic acid limited the reduction in titratable acidity (TA) but had no effect on the total soluble solids (TSS) content of 'Mollar de Elche' pomegranates after storage at 2 °C for 84 days (Sayyari *et al.*, 2010). Similarly, TSS and TA were not affected by treatment with salicylic acid (Sayyari *et al.*, 2009). Furthermore, decrease in the organic acids during storage was reduced with MeJa and MeSa (Sayyari *et al.*, 2011b). According to the authors, these could be as a result of organic acids being the main respiratory substrates during pomegranate postharvest storage (Sayyari *et al.*, 2011b). Application of oxalic acid reduced loss of phenolics, and significantly increased ascorbic acid during cold storage at 2 °C for 84 days (Sayyari *et al.*, 2010). Moreover, acetyl salicylic acid had no effect on total phenolic content throughout storage for 12 weeks at 2 °C (Sayyari *et al.*, 2011b). On the contrary however, Sayyari *et al.* (2011a) found that total phenolic content increased during storage in fruit treated with MeSa and MeJa. In addition, acetyl salicylic acid increased total anthocyanins during storage by 15 % compared to control during 12 weeks of storage of 'Mollar de Elche' pomegranate (Sayyari *et al.*, 2011b). Increase in anthocyanin concentration due to oxalic acid treatment of pomegranate ('Mollar de Elche') was associated with advancement of the ripening process during storage. According to the authors, exogenous oxalic acid could possibly act as an elicitor of anthocyanin biosynthesis and a natural antioxidant thereby suppressing lipid peroxidation (Sayyari *et al.*, 2010).

4. Application of controlled and modified atmospheres

4.1. Effects on fruit physiological response

In controlled atmosphere storage (CAS) and modified atmosphere packaging (MAP), the gas composition inside the store or package containing produce is altered, and often CO_2 concentration is increased while O_2 concentration is reduced. Research on the effects of applying CA/MA as postharvest on pomegranate fruit is limited. In their study on scald development in 'Wonderful' pomegranates, Defilippi *et al.* (2006) reported that storing fruit under CA effectively controlled the disorder, especially with atmospheres of 15 kPa CO_2 which completely controlled development of scald for up to 6 months at 7 °C storage. Investigating the effects of passive MAP on pomegranate arils ('Acco' and 'Herskawitz'), Caleb *et al.* (2013) observed that headspace O_2 concentration inside the packages decreased while the CO_2 levels increased significantly during storage at different temperatures. To prevent excessive accumulation of CO_2 inside the package, the authors proposed the use of polymeric films with higher permeability to CO_2.

4.2. Effects on physico-chemical quality attributes

Defilippi *et al.* (2006) reported that after 6 months of CA storage, 'Wonderful' pomegranates maintained a lighter red colour relative to control fruit and this effect on CA-stored fruit was attributed to delayed synthesis of anthocyanins and other phenolics responsible for the red colour of the skin. Higher peel colour lightness was also observed in fruit stored under CA. The authors concluded that CA treatments maintained very good fruit visual quality up to 6 months in cold storage.

In their recent study on two pomegranate cultivars ('Acco' and 'Herskawitz') grown in South Africa, Caleb et al. (2013) reported an overall steady weight loss of arils during storage under passive MAP. The initial increase in aril weight observed during the early part of the storage period was attributed to rapid evaporation of moisture from aril surface and condensation inside the package. No significant changes were observed in the firmness and titratable acidity when pomegranate arils stored for 14 days at 5, 10 and 15 °C; however, total anthocyanin content decreased with storage duration. Furthermore, the authors found low total aerobic mesophilic bacterial and fungal counts below detection limits.

5. Conclusions and future prospects

There are several physical and chemical postharvest treatments that can be applied to enhance the quality, storage and shelf life of pomegranate fruit. The use of chemicals like fungicides has been debated over the years because of the potential side effects they impart on both human health and the environment. This review identified natural plant compounds like polyamines such as putrescine, as well as organic acids such as oxalic acid, methyl jasmonate which have been successfully applied to control the incidence of spoilage and physiological disorders in pomegranates. The application of a combination of physical and chemical treatments, often referred to as hurdle technology, results in fruit with better quality. The additive properties of both treatments enhance quality attributes better than when treatments are used individually due to a broad spectrum effect. In addition to postharvest treatments, good crop management strategies should be emphasised if the full potential of the fruit is to be realised as preharvest factors affect the postharvest quality of the fruit.

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Table 1 Effects of postharvest treatment on quality attributes of pomegranate whole fruit

Fruit treatment	Cultivar	Treatment description	Key finding	Reference
Physical				
Curing and intermittent warming	Mollar de Elche	Curing at 33 °C and 95 % RH for 3 days and storage at 2 or 5 °C Cycles of intermittent warming (IW) of 1 day at 20 °C every 6 days at 2 or 5 °C	IW fruits had lowest chilling injury and highest anthocyanin Concentrations, titratable acidity and best visual appearance.	Artés *et al.*, 2000
Shrink film wrapping and skin coating with a sucrose polyester (SPE)	Ganesh	Fruits shrink wrapped with two polyolefin films (BDF-2001 and D-955) and skin coating with a sucrose polyester and stored at 8, 15 and 25 °C	Best obtained with shrink-wrapped with BDF-2001 film and storage at 8 °C.	Nanda *et al.*, 2000
Gamma irradiation	California cultivar	The fruits were irradiated with doses of 0, 0.4, 1 and 2 kGy	Chemical attributes were unaffected up to 1 kGy treatment. Total anthocyanin and phenolic content decreased.	Shahbaz *et al.*, 2013
Heat treatment	Mollar de Elche	Heat treatment (hot water dip at 45 °C for 4 min) and storage at 2 °C for 90 days	Chilling injury (CI) symptoms were reduced. Increase in free putrescine and spermidine observed.	Mirdehghan *et al.*, 2007b

Table 1 continued

Fruit treatment	Cultivar	Treatment description	Key finding	Reference
Chemical				
Application of polyamine by pressure or immersion	Mollar de Elche	Fruits treated with putrescine or spermdine and stored at 2 °C for 60 days	All changes were delayed by polyamine treatments.	Mirdehghan *et al.*, 2007a
Oxalic acid treatment	Mollar de Elche	Oxalic acid applied and fruits stored at 2 °C for 84 days	CI symptoms were reduced. Total phenolics loss reduced and ascorbic acid increased.	Sayyari *et al.*, 2010
Spermidine and calcium chloride treatments	Mala-e-Yazdi	Fruits treated with CaCl and spermidine and stored at 2 °C for 4 months	Treated fruit had higher CAT and SOD activity and lower POX activity.	Ramezanian & Rahemi, 2011
Salicylic acid treatment	Malas saveh	Fruits treated with salicylic acid and stored at 2 °C at 85 % RH for 3 months	2mM was the most effective concentration in reducing CI, EL and for maintenance of AA levels.	Sayyari *et al.*, 2009
Methyl jasmonate and methyl salicylate vapor treatments	Mollar de Elche	Fruits were treated with methyl salicylate (0.1 and 0.01mM) and methyl jasmonate (0.1 and 0.01mM)	The CI symptoms were significantly reduced. Total phenolics and anthocyanins increased.	Sayyari *et al.*, 2010
Lecithin application	Primosole	Fruits dipped in Xedabio (a soy lecithin based formulation) alone or in combination with fludioxonil and stored at 8 °C 90-95 % RH for 6 or 12 weeks	Xedabio maintained the commercial value of fruit. Minor changes occurred in nutritional.	D'Aquino *et al.*, 2012

Table 1 continued

Fruit treatment	Cultivar	Treatment description	Key finding	Reference
Combined				
Fludioxonil application and film wrapping	Primosole	Fruit dipped in fludioxonil, wrapped with a polyolephinic film and stored at 8 °C and 90 % RH for 6 or 12 weeks	Film wrapping maintained freshness for all the storage periods. FLU reduced the incidence of decay.	D'Aquino *et al.*, 2009
Putrescine and carnauba wax	'Mridula'	Fruits treated with putrescine and carnauba wax alone or in combination	All the undesirable changes were delayed by putrescine + carnauba wax application.	Barman *et al.*, 2011
Wax and carbendazim	Bhagwa	Fruits treated with wax (waxol) alone and in combination with carbendazim and stored at 8 °C and 90-95 % RH	Treated fruits stored longer and application of fungicides with wax gave excellent effects.	Waskar, 2011

CAT- catalase; SOD- superoxide dismutase; POX- peroxidase; EL- electrolyte leakage; AA- ascorbic acid ; FLU- fludioxonil

Table 2 Effects of postharvest treatment on quality attributes of pomegranate arils

Aril treatment	Cultivar	Treatment description	Key findings	Reference
Chemical				
Washing with chlorine, ascorbic acid and citric acid and MAP	Mollar de Elche	Arils washed in chlorine, ascorbic acid and/or citric acid, sealed in POPP or OPP and stored at 8, 4 and 1 °C	Best results obtained with chlorine, followed by antioxidant solution and packaged in polypropylene films and storage at 1 °C.	Gil *et al.*, 1996
Honey treatments	Hicaznar	Arils treated with diluted honey solutions for 5min and held at 4 °C for 10 days	Honey extended the fresh-like quality and delayed microbial development.	Ergun & Ergun, 2009
Aloe vera gel coating	Mollar de Elche	Arils treated with aloe vera alone or in combination with ascorbic and citric acid and stored in rigid propylene boxes for 12 days at 3 °C	The combination of A. vera gel at 100 % + ascorbic acid and citric acid at 1 % was the most effective treatment.	Martínez-Romero *et al.*, 2013
Edible starch- based coating (with glycerol plus *Oleum nigella*)	Silifkeasisi	Arils coated with a solution of starch (only) and starch (with oil)	Coating with 300 ppm oil + starch yielded best results.	Oz & Ulukani, 2011
Combined treatment				
Combined heat treatment, UV-C and superatmospheric oxygen packaging	Molar de Elche	Fresh-cut arils subjected to hot water dipping (55 °C), UV-C and passive modified atmosphere packaged or high oxygen packaging	The combination of UV-C and high oxygen maintained the antioxidant compounds.	Maghoumi *et al.*, 2013

Table 2 continued

Aril treatment	Cultivar	Treatment description	Key findings	Reference
UV-C and modified atmosphere packaged	Mollar of Elche	Arils chlorine disinfected, exposed to UV-C radiation, packaged in poly propylene baskets and stored for 13-15 days at 5 °C	No benefits were found with different UV-C radiation doses.	L´opez-Rubira *et al.*, 2005
UV-C treatment	Hicaznar	Arils were illuminated with UV-C and stored at 2 °C for 6 days	UV-C had effect phenolics but not on SSC and citric acid.	Nunes *et al.*, 2010
Hot air treatment, superatmospheric O_2 and elevated CO_2	Malese-Saveh	Arils treated with hot air, packed in PET sealed on top with PE and packaged using different gas compositions and stored at 4 °C, 90 % RH	The combination of HO and HT 45 °C enhanced the benefits of applying each treatment separately obtained the best aril quality.	Maghoumi *et al.*, 2013

MAP- modified atmospherepackaging; POPP- perforated oriented polypropylene; OPP- oriented polypropylene; UVC- ultraviolet-C; HO- high oxygen; HW- hot water

CHAPTER THREE: Postharvest physiological responses of pomegranate fruit (cv. Wonderful) to exogenous putrescine treatment and effects on physico-chemical and sensory quality attributes

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POSTHARVEST PHYSIOLOGICAL RESPONSES OF POMEGRANATE FRUIT (CV. WONDERFUL) TO EXOGENOUS PUTRESCINE TREATMENT AND EFFECTS ON PHYSICO-CHEMICAL AND SENSORY QUALITY ATTRIBUTES

Abstract

Pomegranate fruit (cv. Wonderful) were treated with putrescine (1, 2 and 3 mM) before storage for 4 months at 5 °C and 95 % RH and the effects on postharvest quality were studied. Sampling for fruit quality (physiological, physico-chemical and sensory properties) was carried out on a monthly basis after storing fruit for additional 4 days at 20 °C to simulate market conditions. Results showed that incidence of external decay and physiological disorders such as husk scald, chilling injury and aril browning increased with progressive storage but treating pomegranate fruit with putrescine reduced incidence of most disorders. Treating fruit with 3 mM concentration was the most effective in alleviating the incidence of fruit physiological disorders with regard to decay, weight loss, chilling injury (CI) and husk scald during 4 month storage. Control fruit had higher levels of external decay (1.72 - 33.26 %), CI (10.53 - 38.77 %) and scald (15.04 - 100 %) with less attractive colour during 4 month storage. Variations were observed on other fruit quality parameters although treatment with putrescine at 2 and 3 mM concentration reduced changes in colour, pH, TSS and TA. Peel colour attributes a* (redness) and C* (colour intensity) were higher in untreated fruit after the first 3 months of storage but were lower at the end of storage. Higher a* (20.19 - 21.43) and C* (22.27 - 22.89) were also found for aril colour of control fruit during storage while treated fruit showed lower values of 17.50 - 20.36 and 17.79 - 21.90 for a* and C*, respectively. After 4 months of storage, control fruit had lower fruit firmness (10.12 N) and aril hardness (146.50 N) whereas fruit treated with putrescine showed better results with values of 10.82 - 11.92 N and 155.10 - 159.60 N for firmness and aril hardness, respectively. Sensory parameters were best preserved in fruit treated with 2 mM concentration of putrescine with respect to juiciness and crispness. Treatment of pomegranate fruit with putrescine resulted in improved storability and fruit quality during storage. Therefore, for short term storage, 2 mM concentration of putrescine adequately maintained fruit quality especially in the first three months of storage. However, for longer storage period, 3 mM concentration was the most effective in alleviating disorders in addition to maintaining fruit physico-chemical quality parameters and sensory attributes during storage.

1. Introduction

The explosion of interest in pomegranate fruit and rapid increase in global production and consumption has been credited to its health benefiting properties which in turn are mainly attributed to the content of phytochemicals with high antioxidant activity and functional properties (Opara *et al.*, 2009; Vuida-Martos *et al.*, 2010; Fawole *et al.*, 2013a; Barman *et al.*, 2014). Pomegranate fruit production in South Africa has seen tremendous growth over the years with 40 % and 56 % annual increase in production in 2014 and 2015, respectively, and 31 % rise in total exports in 2015 (Goosen, 2015). The fruit is classified as non-climacteric due to its low respiration, ethylene production rates and the fact that the fruit does not continue ripening off the tree (Kader *et al.*, 1984; Barman *et al.*, 2011; Fawole & Opara, 2013a; Pareek *et al.*, 2015). Despite its non-climacteric nature, pomegranate fruit has short shelf life when stored at ambient temperature (Mirdehghan *et al.*, 2007; Fawole & Opara, 2013a). The factors that contribute to the short shelf life of this fruit include rapid weight loss, incidence of fungal decay and internal browning (Fawole & Opara, 2013a).

Cold storage is commonly used to slow down these processes and extend the storability and shelf life of the fruit, with a recommended optimal storage temperature of 5 °C. However, chilling injury (CI) occurs at 5 °C or lower during prolonged cold storage. Chilling injury is characterized by peel browning, husk scald, aril browning and susceptibility to decay among others (Elyatem & Kader, 1984; Mirdehghan *et al.*, 2007; Barman *et al.*, 2011). This condition limits consumer acceptability, ultimately resulting in economic loss to producers and exporters. In order to mitigate losses caused by these physiological disorders and prolong storage of pomegranate, a number of physical and chemical treatments have been employed (Opara *et al.*, 2015). For example, heat treatments such as intermittent warming, hot water and hot air treatments have been studied for commercial application for extending fruit storage life (Artés *et al.*, 1998; 2000; Mirdehghan & Rahemi, 2005; Mirdehghan *et al.*, 2007). However, due to the presence of numerous micro-openings on pomegranate surfaces (Kader *et al.*, 1984; Nanda *et al.*, 2001; Fawole *et al.*, 2013a), moisture loss becomes problematic in application of heat treatments as the fruit loses moisture rapidly and become unappealing and unmarketable due to excessive shrivel and other skin defects such as browning.

Chilling injury development during low temperature storage of pomegranate fruit involves phase transition of membrane lipids which induces damaging effects on the tissue.

Kramer *et al.* (1989) observed accumulation of putrescine during exposure of apple to chilling stress and proposed that polyamines (PAs) may be involved in reducing chilling injury. PAs are a group of positively charged low molecular weight aliphatic amines that are present in living organisms and have been implicated in a number of biological processes like plant growth, development and response to stress (Smith, 1985). The common polyamines include putrescine (diamine), spermidine (triamine) and spermine (tetramine) (Khosroshahi *et al.*, 2007). Other uncommon polyamines such as homospermidine, 1,3-diaminopropane, cadavarine and canavalmine have also been detected in biological systems of plants, animals, algae and bacteria (Khosroshahi *et al.*, 2007). Concentration of polyamines in cells is regulated by their biosynthesis, breakdown, translocation and conjugation with different compounds (Khosroshahi *et al.*, 2007).

In nature, PAs often occur as free molecular bases and have been reported to bind with negatively charged phospholipids or other anionic sites on membranes. Thus, PAs affect membrane fluidity and indirectly modulate the activities of membrane-associated enzymes (Slocum *et al.*, 1984). Saftner & Baldi (1990) reported that polyamines retarded fruit ripening in tomato and their levels decreased with ripening in most cultivars. Based on the findings, the authors suggested that free polyamines are endogenous anti-senescence agents. Treating fruit with polyamines has been reported to increase fruit firmness in apples (Wang *et al.*, 1993), tomatoes (Law *et al.*, 1991), pomegranate (Barman *et al.*, 2011) and lemons (Valero *et al.*, 1998). These effects of polyamines are associated with their anti-ethylene property because exogenous polyamines have been shown to inhibit ethylene production and activity *in vitro* (Galston & Sawhney, 1990). Application of polyamines can inhibit ethylene biosynthesis by competing with ethylene for the common precursor *S*-adenosyl methionine (Smith, 1985; Pandey *et al.*, 2000). Exogenous application of polyamines impart other beneficial effects such as delayed colour changes, reduced susceptibility to mechanical damage and chilling injury, and increased shelf life of both climacteric and non-climacteric fruit (Serrano *et al.*, 1996; Martínez-Romero *et al.*, 2002; Pe´rez-Vicente *et al.*, 2002). Furthermore, treating pomegranate fruit with putrescine and spermidine has shown to improved fruit quality (Mirdehghan *et al.*, 2007; Barman *et al.*, 2011; Ramezanian & Rahemi, 2011).

The successful broad-spectrum benefits of polyamines such as putrescine in alleviating incidence of physiological disorders and maintaining fruit quality during storage

has been widely studied (Mirdehghan *et al.*, 2007; Barman *et al.*, 2011; Ramezanian & Rahemi, 2011). Response of fruit to postharvest chemical treatments is however dependent on a number of factors such as cultivar, concentration used, mode of application, agro-climactic regions among others. Although the recent years have seen rapid growth of the South African pomegranate industry (Fawole & Opara, 2013b), postharvest losses of the fruit are still high and the application of alternative healthier chemical treatments is limited in the industry. Therefore, the aim of this study was to investigate the physiological responses of pomegranate fruit (cv. Wonderful) to exogenous application of putrescine and to assess the effects on physico-chemical, phytochemical and sensory attributes.

2. Material and methods

2.1. Plant material

Pomegranate fruit (cv. Wonderful) were procured during commercial harvest period from Heinrich F.R. Schaefer (HFR) Properties farm in Western Cape (33º44′26.185″S 18º44′41.193″E), South Africa. Fruit were transported in a ventilated vehicle to the Postharvest Technology Research Laboratory at Stellenbosch University and immediately sorted for presence of physical damage such as cracks, sunburn, decay and bruises. Fruit were equilibrated overnight at ambient room temperature (20 ± 2 °C) prior to treatment.

2.2. Treatments

Fruit were divided into four treatment groups of 108 fruit per group. Fruit were dipped for 2 min in a solution in 15 L of putrescine (Sigma Aldrich, South Africa) containing 2 % Tween-20. A dipping time of 2 min was selected based on preliminary studies in which different dipping times (2, 5 and 8 min) were tested, and 2 min was the most effective. Treatments included; (**1**): Immersion in tap water (control); (**2**) Immersion in 1 mM putrescine for 2 min; (**3**) Immersion in 2 mM putrescine for 2 min; (**4**) Immersion in 3 mM putrescine for 2 min. After immersion, fruit surface was thoroughly dried by holding fruit at ambient room condition (20 ± 2 °C and 65 ± 2 % RH) for 12 h before storage.

2.3. Storage

Fruit were packed in to standard open top ventilated cartons (dimensions: 0.4 m long, 0.3 m wide and 0.133 m high) used for commercial postharvest handling of pomegranates. All the treatment groups were stored at 5° C and 95 % relative humidity for 4 months. Temperature

and relative humidity (% RH) inside the cold room was recorded daily throughout the storage period using Tiny Tag TV-4500 data loggers (Gemini Data Logger, Sussex, UK). At the end of each month, a batch of fruit (n = 20) were removed from 5° C storage and placed at 20 °C and 65 - 70 % RH for additional 4 days in order to simulate a reasonable retail sale period. Fruit were thereafter analysed for incidence of physiological response, physiological disorders, physico-chemical and sensory properties. Measurement of all parameters was carried out on a monthly interval and results were presented as mean ± standard error (S.E).

2.4. Physiological response, decay and physiological disorders

2.4.1. Respiration rate

Fruit respiration was determined using a closed system as described by Caleb *et al.* (2012). In 5 replicates, two fruit were placed in a glass jar containing a rubber septum. The jar was sealed hermetically with vaseline to ensure a vacuum seal. Fruit were incubated for 2 h at 20 °C then gas composition inside each glass jar was measured using a calibrated O_2/CO_2 analyzer (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Carbon dioxide production was determined and results presented as mL CO_2 $kg^{-1}h^{-1}$ of five determinations.

2.4.2. Weight loss

Ten randomly selected fruit per treatment were used for this purpose. Fruit were weighed individually at monthly intervals during storage using an electronic scale (Mettler, Toledo, Switzerland, 0.0001 g accuracy). Cumulative weight loss of each fruit was calculated as:

$$W = \frac{(\mathrm{Wo} - \mathrm{Wi})}{\mathrm{Wo}} \times 100 \qquad (1)$$

Where W is the weight loss (%) of fruit; Wo is the weight (g) of fruit at the beginning of storage; Wi (g) is the weight of fruit at the storage time.

2.4.3. Fruit external and internal decay incidence

Fruit decay incidence was visually assessed as total rots. Fruit with any sign of external rot such as mould and crown rot was considered as external decay. Fruit with external decay appearance were counted and discarded. For internal decay, fruit with rotten arils and heart rot were counted and also discarded. For both external and internal decay, percentage of discarded fruit was calculated using the formula:

$$\text{Decay incidence (\%)} = \frac{\text{(Number of discarded fruit at each sampling date)}}{\text{Total number of fruit}} \times 100 \quad (2)$$

2.4.4. Evaluation of chilling injury, husk scald and aril browning

Incidences of chilling injury, husk scald and aril browning were visually assessed monthly per treatment. The severity of disorders were assessed using a four level scale as described by Fawole and Opara (2013a); where 0 = none (no symptom), 1 = trace (1 – 25 %), 2 = slight (26 – 50 %), 3 = moderate (51 – 75 %) and 4 = severe (76 – 100 %)

A physiological disorder index was calculated by multiplying the scores of severity by the number of affected fruits and dividing by the total number of assessed fruits (Artés *et al.*, 1998; Fawole & Opara, 2013a):

$$\text{Disorder index} = \sum \frac{\text{(Value of scale) x (Number of fruit with the corresponding scale number)}}{\text{Total number of fruit}} \times 100 \quad (3)$$

$$\text{Disorder incidence} = \frac{\text{(Number of affected fruit)}}{\text{Total number of fruit}} \times 100 \quad (4)$$

2.5. Colour and textural attributes

2.5.1. Whole fruit and aril colour

Colour parameters in CIELAB coordinates (L^*, a^*, b^*) were measured using a Chroma meter (CR-400, Minolta Corp, Osaka, Japan). Ten fruit per treatment were used to monitor changes in external colour by measuring peel colour at two opposite spots on individual fruit, while aril colour was determined by placing the arils in a colourless glass Petri dish. Colour intensity or chroma (C^*) and hue angle ($h°$) were calculated using the equations (5) and (6) (Fawole & Opara, 2013a).

$$C* = \sqrt{(a*^2 + b*^2)} \quad (5)$$

$$h° = \arctan(b*/a*) \quad (6)$$

Furthermore, total colour difference (TCD) between the external peel and internal arils components was calculated as;

$$TCD = \sqrt{(L^*_o - L^*)^2 + (a^*_o - a^*)^2 + (b^*_o - b^*)^2} \quad (7)$$

Where L^*_0, a^*_0 and b^*_0 are the colour parameters of the peel (reference value), while L*, a* and b * are the colour values of the aril (Pathare *et al.*, 2013).

2.5.2. Fruit puncture resistance

Fruit puncture resistance was measured using a fruit texture analyzer (GÜSS-FTA, model GS, South Africa). A 5 mm cylindrical probe was programmed to puncture 8.9 mm into the fruit at the speed of 10 mm/s on a steel test platform with the stem calyx axis parallel to the platform. Tests were performed in duplicate on the equilateral region of 10 individual fruit. Puncture resistance was determined as the peak force required to puncture the fruit surface.

2.5.3. Aril firmness

Aril compression test was performed using a texture profile analyzer XT Plus (Stable MicroSystem Ltd., Godalming, UK) equipped with a 35 mm diameter cylindrical compression probe. Compression test was performed on individual arils with the following operating conditions: pre-test speed 1.5 mm/s, probe test speed 1 mm/s, post-test speed 10.0 mm/s, compression force 10 N and compression distance 10 mm (Fawole & Opara, 2013b). Aril hardness (N) (measures the maximum force required to break the aril), elastic modulus (N/mm) (measures the aril's ability to recover from deformation within a given distance), toughness (N mm) (indicates the energy required to completely compress the aril) and bioyield (N) (measures the force required to compress an aril until the juice just exudes without breaking the aril sac) were captured on Exponent v.4 software (Stable MicroSystem Ltd., Godalming, UK). At each storage interval, tests were done using 20 arils extracted from 10 randomly selected fruit for each treatment and results presented as mean ± S.E of 20 determinations.

2.6. Chemical attributes

2.6.1. Titratable acidity, total soluble solids and pH

Titratable acidity (TA) was measured by diluting 2 mL of fresh juice with 70 mL of distilled water and titrating with 0.1 M NaOH to an end point of pH 8.2 using a Metrohm 862 compact titrosampler (Herisua, Switzerland). The results were expressed as percentage of citric acid (% CA). Total soluble solids (TSS, °Brix) was measured using a digital refractometer (Atago, Tokyo, Japan) calibrated with distilled water. The pH values were determined at room temperature using a calibrated pH meter (Crison, Model 00924, Barcelona, Spain). BrimA, a

criterion for consumer acceptance of fruit juice was expressed as BrimA = TSS – *k* * TA, where *k* is the tongue's sensitivity index (k = 2 for pomegranate) (Fawole & Opara, 2013d). All measurements were made on 10 individual fruit juice samples for each treatment.

2.7. Sensory attributes

Sensory evaluation was carried out using a trained panel of 6 members of the Postharvest Technology Research Group at Stellenbosch University who are familiar with the characteristic taste of pomegranate fruit and regular consumers (Caine *et al.*, 2003; Sudha *et al.*, 2007; Chen & Opara, 2013). Panelists received further orientation on pomegranate attributes (Vázquez-Araújo *et al.*, 2011a). Sensory evaluation was carried out on arils (10 g) served at 21 °C on Petri dishes randomly coded (Fawole & Opara, 2013b). The descriptive test required panelists to rate the intensity of the attributes on a scale of 0 – 4 (0 = none, 1 = slight, 2 = moderate, 3 = much, 4 = very much). The descriptive attributes evaluated for the study included sweet taste, sour taste, crispness, astringency, off flavour, juiciness, grittiness and hardness. Sensory evaluation was not carried out beyond 3 months of storage due to decay and limited sample size.

3. Statistical analysis

Statistical analysis was carried out using Statistica software (Statistica version 14.0, StatSoft Inc., Tulsa, USA). Data was subjected to factorial analysis of variance (ANOVA) at 95 % confidence interval. Main effects (putrescine concentration and storage duration) and their interaction effects (concentration*storage duration) were also assessed. Post-hoc test (Duncan's Multiple Range Test) was used to test for statistical significance such that observed differences at $p < 0.05$ were considered significant. Principal component analysis (PCA) was carried out using XLSTAT software version 2012.04.1 (Addinsoft, France).

4. Results and discussion

4.1. Physiological response

4.1.1. Fruit respiration rate

Fruit response showed that respiration rate was majorly dependent on storage duration (Fig. 1). After one month of storage, results showed a decrease in respiration rate from harvest, with no significant difference ($p > 0.05$) between putrescine concentrations. The second

month of storage was, however, characterised by a 1.5 to 2-fold increase in respiration rate both in control and fruit treated with putrescine. This was followed by slight increases in fruit respiration rate after the third month of storage, with fruit treated with 2 mM putrescine having the highest respiration rate (22.49 mL CO_2 kg^{-1} h^{-1}) compared to control with 18.16 mL CO_2 kg^{-1} h^{-1}. At the end of storage, fruit respiration rate declined slightly with no significant differences between the treatment concentrations (Fig. 1). Respiration rate is a good indicator of physiological activity as it affects other quality attributes during storage of fruit. The observed increase in respiration rate of fruit during storage could be an indication of increase in stress including presence of physiological disorders (Fawole & Opara, 2013a). This would also indicate depletion of respiratory substrates such as sugars and organic acids, and concomitant accelerated senescence process (Giménez *et al.*, 2016). Barman *et al.* (2011) also observed increased respiration rate with advancement of storage for 'Mridula' pomegranate stored for 60 days at 3 °C. However, lower respiration rate was reported for fruit treated with a combination of putrescine + carnauba wax compared to sole treatment with putrescine. The authors attributed this to the antisenescence and barrier properties of putrescine and carnauba wax respectively (Barman *et al.*, 2011).

4.1.2. Weight loss

Weight is an important quality parameter as produce price is often determined on weight basis; therefore, it is crucial to ensure low weight loss during storage of fresh produce. Percentage fruit weight loss after the first month of storage was below 10 %, with no significant ($p > 0.05$) differences among treatments (Fig. 2). Fruit continued to lose weight with prolonged storage duration regardless of putrescine concentration. About 1.7 and 2.3-fold increase in weight loss was observed after the second and third months, respectively. However, putrescine concentration slightly but significantly ($p < 0.05$) influenced fruit weight loss after the fourth month of storage, where the highest weight loss of 24.61 % was observed in fruit treated with 1 mM while those treated with 3 mM had the least weight loss (21.49 %) (Fig. 2). This could possibly be because putrescine at 3 mM was high enough to maintain cell membrane integrity. Treating fruit with putrescine was shown to reduce weight loss through consolidation of the cell integrity and permeability of the tissues, changes in biophysical properties of the fruit and ameliorating chilling injury (Barman *et al.*, 2011; Jawandha *et al.*, 2012). As indicated by the results of factorial analysis, storage duration (as opposed to concentration) played a significant ($p < 0.0001$) role on fruit weight loss. High

porosity of the pomegranate peel is responsible for the high fruit susceptibility to weight loss due to increased potential for free water vapour movement from the peel (Elyatem & Kader, 1984; Fawole & Opara, 2013a). The increase in fruit weight loss with storage could be attributed in part to the observed increase in fruit respiration rate with storage duration (Fig. 1). Similar findings were reported by Serrano *et al.* (2003) who observed increase in weight loss with storage of four plum cultivars, although increases were lower in putrescine-treated plums from day 7 until the end of the storage period. However, Khosroshahi *et al.* (2007) reported that putrescine had no significant effect on weight loss of 'Selva' strawberry during storage at 5 °C for 13 days.

4.1.3 External and internal decay incidence

Decay is one of the major challenges faced during storage of pomegranate fruit. Incidence of external fruit decay was low after the one month of storage, affecting less than 10 % of the fruit regardless of putrescine concentration (Fig. 3A). External decay incidence was however apparent after two months of storage, although the incidence remained below 10 % for all treatments except for fruit treated with 3 mM putrescine. In terms of the efficacy of the treatment and concentration thereof, after the third and fourth month of storage, it was clearly observed that treating fruit with putrescine minimized external fruit decay than in control in which fruit decay exceeded 30 % by the end of storage. In addition, 2 mM putrescine treatment showed a better result with the lowest decay incidence at the end of the storage period (Fig. 3A). This could be due to the antipathogenic properties of putrescine. In presence of a pathogen attack, polyamines such as putrescine conjugate with phenolic compounds and hydroxycinamic acid amines and this conjugation has antipathogenic activity (Walters, 2003). In agreement with the results, treating fruit with putrescine reduced decay in fruits such as mango (Jawandha *et al.*, 2012), strawberry (Khosroshahi *et al.*, 2007), among others, and this effect has been attributed to the protective function of putrescine.

Internal decay in pomegranates has been attributed mainly to heart rot (black heart), a pre-harvest disease caused by *Aspergillus niger* and *Alternaria spp.*, characterized by a mass of black arils (Yehia, 2013; Arendse, 2014). The outer peel and the hard rind of infected fruit retain their healthy appearance but when opened, brown (soft) to black (dry) rot of the arils is observed (Tziros *et al.*, 2008; Ezra *et al.*, 2015). After one month of storage, internal decay was only observed in fruit treated with 2 mM putrescine which had 10 % internal decay (Fig. 3B). Interestingly, after the second month of storage, fruit treated with 2 and 3 mM showed

10 % decay while those treated with 1 mM and control had no internal decay. This was interesting because treatment with putrescine reduced external fruit decay (Fig. 3A) and therefore treated fruit were expected to show less internal decay symptoms because of the anti-pathogenic properties of putrescine. After the third and fourth months of storage, internal decay increased with fruit treated with 2 mM having the highest internal decay (20 %) while those treated with 1 mM showed no internal decay incidence (Fig. 3B). It is noteworthy that there is no effect in terms of the efficacy of putrescine in preventing internal fruit decay. This could be becausec aril decay, due to heart rot occurs from infection of fruit in the orchard during flowering (Zhang & McCarthy, 2012; Ezra *et al.*, 2015). Therefore the observed decay incidence could in fact, be due to inherent fruit condition at harvest. Ezra *et al.*, (2015) showed that development of heart rot occurs when a spore (*Alternaria* spp.) penetrates the pistil of an open flower and into the tunnel and then into the loculus, where it remains latent until the ripening fruit can support its growth. In apple (cv. Red Delicious), core rot (mainly associated with *A. alternata*) was found to develop during, rather than prior to fruit development (Shtienberg, 2012). Core rot is characterized by dark brown tissue that appears dry and corky within loculi and contains air pockets when it penetrates the fruit mesoderm (Shtienberg, 2012). Postharvest treatment of pomegranate fruit had no effect on internal decay of pomegranate fruit; therefore, internal decay could best be prevented by ensuring good agricultural practices and application of pre-harvest treatments. Arendse (2014) also observed increased severity of internal decay with prolonged storage and temperature of 'Wonderful' pomegranate fruit. Our results were however lower than those reported by Fawole & Opara (2013a), who observed severe to extremely severe aril decay for 'Bhagwa' and 'Ruby' pomegranates stored for 16 weeks at 5 and 7 °C. This could probably be due to differences in cultivars.

4.1.4. Aril browning

Severity of aril browning increased with prolonged storage regardless of putrescine treatment. The first month of storage was characterised by none to trace levels of aril browning (Fig. 3C). However, after the second month of storage, aril browning became more apparent in fruit treated with putrescine (regardless of concentration) than in control fruit, albeit in trace to slight severity. Aril browning appearance further increased after the third and fourth months of storage to above slight and moderate, respectively, in treated fruit. Visual appearance is essential especially in the pomegranate fresh-cut industry where pomegranate

fruit is minimally processed into ready to eat arils. The colour of arils influences consumer choice (Pathare *et al.*, 2013) and arils with browning above moderate are deemed unmarketable. Overall, external application of putrescine as postharvest treatment of pomegranate fruit did not reduce aril browning in our study. This is could be because putrescine was exogenously applied on fruit surface and therefore had no influence on the internal fractions of the fruit. Arendse (2014) also reported between moderate to severe aril browning after four months of storage of cv. Wonderful pomegranate. However, Fawole & Opara (2013a) observed severe to extremely severe aril browning after four months of storage of 'Bhagwa' and 'Ruby' pomegranates at 5 °C.

4.1.5. Chilling injury incidence and severity

Chilling injury is a physiological disorder that affects chill-sensitive fruits stored at low temperatures. Chilling injury incidence increased with progressive storage of pomegranate fruit irrespective of treatment. The incidence of chilling injury was low after one month of storage with fruit treated with 1 mM putrescine showing the highest (16.35 %) while fruit treated with 3 mM had the lowest (8.08 %) incidence (Fig. 4). A similar trend with treatments was observed after the second month of storage but more fruit became chill-injured, with fruit treated with 1 mM and 3 mM putrescine having the highest and lowest chilling injury incidences, respectively. Although the number of chill-injured fruit increased with prolonged storage, regardless of treatments, the higher concentrations (2 mM and 3 mM) were effective in minimizing incidence of chilling injury when fruit were stored beyond 2 months (Fig. 4). In addition, treatment of fruit with the highest concentration of putrescine (3 mM) evidently maintained the lowest chilling injury incidence throughout the storage period. This could be attributed to the ability of putrescine to enhance cold acclimation. It was important to assess the severity of chilling injury for fruit marketability. Chilling injury severity was well below trace level throughout the storage period (Fig. 4) despite high incidence, suggesting that the fruit could be deemed marketable. With regard to treatments, the severity of chilling injury was again lowest in fruit treated with 3 mM throughout the storage period. During chilling conditions in plant tissues, cell membrane lipids undergo changes in physical state from liquid-crystalline to solid-gel state, which lead to an increase in membrane permeability and ion leakage (Gómez-Galindo *et al.*, 2004). When polyamines (e.g. putrescine, spermidine and spermine) are exogenously applied, they induce cold acclimation, which lead to maintenance of membrane fluidity at low temperatures and thus responsible for reducing electrolyte

leakage and skin browning (Barman *et al.*, 2011) thereby reducing the chilling injury symptoms. Mirdehghan *et al.* (2007) reported that chilling injury developed from the first sampling date but application of putrescine and spermidine significantly reduced skin browning of pomegranate fruit (cv. Mollar de Elche) stored at 2 °C for 60 days. Similarly, application of putrescine, either alone or in combination with carnauba wax significantly reduced chilling injury and skin browning of 'Mridula' pomegranate after storage for 60 days at 3 °C (Barman *et al.*, 2011).

4.1.6. Husk scald incidence and severity

Husk scald is a physiological disorder faced during prolonged storage of pomegranate fruit. It appears as superficial browning that develops from the stem end of the fruit and does not affect the internal quality. However, it affects the visual quality and marketability of fruit and also increases susceptibility of fruit to decay (Defilippi *et al.*, 2006; Fawole, 2013). Husk scald was a major problem observed during long term storage of pomegranate fruit in this study. The incidence and severity of husk scald were low after the first two months of cold storage and subsequent shelf life condition regardless of concentration (Fig. 5). At below trace level, less than 20 % of the fruit were affected by scald incidence in all putrescine concentrations with the exception of fruit treated with 1 mM putrescine after the second month (Fig. 5). However, there were marked increases in scald incidence and severity after the third month of storage, with further exponential increases at the end of the storage period in all treatments. All the remaining fruit developed scald with above moderate severity after the fourth month of storage. There was a very strong positive correlation between husk scalding and weight loss (Table 1). Husk scald in pomegranate has been attributed to enzymatic oxidation of o-dihydroxyphenols which involves breakdown of phenolic compounds in the fruit peel (Ben-Arie & Or, 1986; Defilippi *et al.*, 2006). Although putrescine is reported to have antioxidant properties (Barman *et al.*, 2011), this could probably not have been strong enough to prevent husk scalding as all the remaining fruit developed scald by the end of storage and a further shelf life irrespective of concentration. Furthermore, husk scalding is an oxidative process and can be minimized or controlled in the presence of low oxygen environment (Ben-Arie & Or, 1986). In the current study, fruit were stored under regular atmosphere, with no alteration in atmospheric oxygen more so that the treatment was not a coating. This suggests that there was enough oxygen supply for

enzymatic oxidation hence rendering putrescine ineffective in controlling scalding in pomegranate (in long term stored fruit).

4.2. Physico-textural properties

4.2.1. Peel and aril colour attributes

4.2.1.1. External appearance (peel)

Fruit colour is a vital attribute that influences consumer choice and produce purchasability (Pathare *et al.*, 2013). The current study revealed that changes in fruit peel colour parameters were influenced by the concentration of putrescine applied and storage duration (Fig. 6). In general, fruit peel redness (a*) and colour intensity (C*) decreased gradually throughout the storage duration. This was concomitant with increase in hue angle, suggesting colour loss of pomegranate peel during storage (Fig. 6). Fruit redness and colour intensity did not change significantly among treatments for the first two months of storage except for fruit treated with 1 mM putrescine which had the least peel redness (34.92 ± 1.56). However after the third month, control fruit had the highest peel redness and colour intensity whereas there was no significant difference among treated fruit, and by the end of the storage period (month 4), no differences were observed irrespective of concentration (Fig. 6). The decrease in peel colour during storage could be due to peel browning which was evidenced by development of husk scald. As a result, peel hue angle (*h*°) increased with prolonged storage but there were no significant differences among all concentrations throughout the storage duration (Fig. 6). Hue angle has been shown to increase with a decrease in red colour of pomegranate as it measures colour purity (deviation from saturation). Therefore, the increase in hue could be due to the decrease in peel redness with progressive storage, which could be attributed to the development of physiological disorders such as chilling inujury and husk scald. Arendse (2014) reported initial increase in pomegranate peel colour during the first three months of storage although external appearance deteriorated up till the end of storage (5 months at 5 and 10 °C). Untreated mango fruit (cv. Langra) retained higher values of a* and b* during storage while fruit treated with 2 mmol/L of putrescine recorded minimum values (Jawandha *et al.*, 2012). The authors reported that polyamines may retard chlorophyll degradation in skin tissues by inhibiting peroxidase activity.

4.2.1.2. Internal appearance (aril)

Aril colour is important as it influences consumer choice during purchase of minimally processed pomegranate arils. Changes were observed when fruit were stored for an additional 4 days at 20 °C. Redness of fruit arils (a*) increased slightly with storage and well above the aril colour at harvest. Factorial analysis showed that changes in aril redness and colour intensity were predominantly influenced by storage duration (a* = <0.0001; C* = <0.0001). Again, there was no significant difference among treatments throughout the storage duration (Fig. 6). The increase in aril redness is associated with anthocyanin biosynthesis which has been reported to occur during cold storage of pomegranate fruit (Gil *et al.*, 1995; Arendse, 2014). At the start and end of storage, control fruit had higher aril redness compared to treated fruit. Putrescine has been shown to prevent colour development during storage (Barman *et al.*, 2011; Jawandha *et al.*, 2012; Zafari *et al.*, 2015). It is possible that it could have prevented or retarded the rate of anthocyanin biosynthesis among treated fruit. Decline in hue angle (*h*°) further supported this phenomenon, indicating improved colour development in all treatments throughout the storage period (Fig. 6). The changes were influenced by the storage time and not concentration. Fawole & Opara (2013a) also reported relatively stable aril colour of 'Bhagwa' pomegranate during storage 5 °C for 16 weeks.

Total colour difference (TCD) declined generally during storage of fruit with no difference between the first two months of storage (Fig. 6). After the third month of storage, control fruit had the highest TCD while fruit treated with 1 mM putrescine had the lowest values. A further decrease was observed at month 4 with fruit treated with 3 mM putrescine showing the highest total colour difference (Fig. 6). Treating fruit with 3 mM generally maintained a relatively stable TCD throughout storage. TCD showed a disparity in the colour between the peel and aril. Given the importance of aril and juice redness, lower TCD is desired in the initial stages of storage as it is an indication of smaller differences in peel and aril redness. However, with prolonged storage, higher TCD is desired especially due to the fact that the fruit peel develops physiological disorders such as husk scald and chilling injury. Similar ranges of TCD were observed by Fawole (2013) for 'Arakta', 'Bhagwa', 'Ganesh', 'Mollar de Elche' and 'Wonderful' cultivars of pomegranate. However, Al-said *et al.* (2009) reported higher ranges of TCD (50 - 60) of four cultivars of pomegranate grown in the Sultanate of Oman.

4.2.2. Juice colour

Juice colour is an important quality attribute especially in the juice processing sector as it influences consumer appeal and preference. Juice colour absorbance remained fairly unchanged during storage of pomegranate fruit with no significant ($p < 0.05$) changes among treatments, with significant interaction ($p = 0.0125$) between the main factors (duration and concentration) (Table 1). The changes in juice colour relate to the relatively stable aril redness that was observed during storage of fruit (Fig. 6). This could be because putrescine was applied exogenously and therefore no direct significant effect on the internal components of fruit. According to Shulman *et al.* (1984), pomegranate juice colour absorbance is an indication of anthocyanins which are light-absorbing plant-based pigments. This suggests no significant changes in light-absorbing anthocyanins during storage of pomegranate fruit. No changes were similarly observed in juice colour of 'Wonderful' pomegranate fruit stored for up to 10 weeks at 0 and 5 °C (Elyatem & Kader, 1984). Nanda *et al.* (2001) also reported no statistical differences in juice colour of film wrapped, skin coated and control pomegranate (cv. Ganesh) stored at 8 and 15 °C for 10 weeks.

4.2.3. Fruit puncture resistance (firmness)

Fruit firmness decreased from values at harvest but did not change significantly after the first two months of storage (Table 1). Slight variations were however existent in the last two months of storage with fruit treated with 2 mM having the highest puncture resistance (11.92 ± 0.40 N) after the last month of storage, 15.10 % higher than control fruit and the changes were influenced by the interaction between the putrescine concentrations and storage duration (Table 1). It is possible that putrescine could have maintained the integrity of the fruit peel. Putrescine has been reported to improve fruit firmness of pomegranates (Barman *et al.*, 2011) and plums (Valero *et al.*, 2002) due to its ability to cross link with the pectic substances in the cell wall hence preventing access of cell wall degrading enzymes like polygacturonase, pectinesterase and pectimethylesterase, thereby reducing softening during storage. The beneficial effect of putrescine was also reported by Barman *et al.* (2011) who found 33 % higher fruit firmness in pomegranate fruit (cv. Mridula) treated with putrescine + carnauba wax compared to control.

4.2.4. Aril firmness

Variations were observed in aril hardness during the current study. Aril hardness of treated fruit fluctuated during storage with increase in the last month of storage (Table 1). Aril hardness of untreated fruit on the other hand increased after the second month but thereafter decreased by 3.2 % after the last month of storage. Control fruit significantly had lower aril hardness compared to treatments, with 8.21 % lower hardness at the end of the storage duration. However, no significant changes ($p = > 0.05$) were observed among treated fruit after storage for 4 months and the changes were influenced by interaction between the factors (Table 1). Decrease in aril hardness is due to loss in cell wall integrity of pomegranate arils (Ekrami-Rad *et al.*, 2011; Arendse, 2014). Loss of cell wall integrity could in turn be due to senescence which progresses with storage (Arendse, 2014). Treatment of fruit with putrescine maintained aril hardness during storage (except at month 3), as all treatments had higher aril hardness compared to control fruit. Arendse (2014) found that aril hardness did not differ with storage temperature but decreased with extended storage of 'Wonderful' pomegranate fruit at 5, 7.5 and 10 °C for 5 months. Aril toughness increased in the second month, decreased in the third month and finally increased after the fourth month with significant interaction of concentration and storage duration. The other aril firmness parameters of elastic modulus and bioyield were generally maintained during storage of fruit with no significant differences observed among concentrations (Table 1).

4.3. Chemical attributes

4.3.1. pH

The pH of pomegranate juice determines its sour taste (Zarei *et al.*, 2011). pH of pomegranate juice was characterized as acidic (low below pH 4) and increased as storage progressed for most treatments with some decreases (for concentration 2 and 3 mM) (Table 2). The increase could be explained by the initial decrease in titratable acidity as the two are inversely proportional. Slight differences were observed in pH with fruit treated with 2 mM having the highest and lowest pH in the first two and last two months of storage respectively. The interaction between the two factors played a significant role in the pH of stored fruit ($p < 0.0001$) (Table 2). The increase in pH with storage could be due to utilization of organic acids evidenced by the general reduction in titratable acidity with storage. Putrescine also had no effect on pH of 'Selva' and 'Kamarosa' strawberry fruit (Khosroshahi *et al.*, 2007; Zafari *et*

al., 2015). Similar results were reported for pomegranate fruit (cv. Mollar de Elche) that had undergone curing and intermittent warming prior to storage at 2 °C for 90 days (Artés *et al.*, 2000). Fawole & Opara (2013a) also reported increase in juice pH with storage for 'Ruby' fruit stored at 5 °C, reaching a maximum value of 3.96 after 16 weeks of storage. However, no changes were observed when 'Malas Yazdi' and 'Malas Saveh' pomegranate fruit were treated with hot water (45 °C) and stored at 1.5 °C for 3 months (Mirdehghan & Rahemi, 2005).

4.3.2. Titratable acidity (TA)

Titratable acidity decreased with storage except at month 3 where all treatments showed increases (Table 2). Similarly, no differences were observed during storage with the exception of month 3 where some variations existed with the control fruit showing the highest TA (2.24 ± 0.04). This could most likely be due to concentration of acids from weight loss in control fruit. Storage duration significantly ($p < 0.0001$) influenced the changes in titratable acidity during storage (Table 2). Organic acids (which are the main contributors to titratable acidity) have been reported to be the major substrates for pomegranate respiration during storage (Kader *et al.*, 1984; Fawole & Opara, 2013a). Interestingly, respiration rate of fruit also prominently increased after the third month of storage (Fig. 1) and this could explain the major decrease in titratable acidity by the end of storage. These results are similar to Arendse (2014) who also reported decrease in titratable acidity during storage of pomegranate fruit (cv. Wonderful) at 5 and 7.5 °C for 5 months. Several authors have reported decreases in TA during storage of fruit. Decrease in pH was observed for mango (cv. Langra) treated with putrescine and stored at 13 °C for 4 weeks (Jawandha *et al.*, 2012). After treating four cultivars of plum with putrescine, Serrano *et al.* (2003) found decreased TA during storage at 20 °C for nine days. Barman *et al.* (2011) also reported decrease in TA of pomegranate fruit (cv. Mridula) treated with putrescine and stored for 60 days at 3 °C. On the other hand, increase in TA levels during storage has previously been reported by Gil *et al.* (1996) for the Spanish 'Mollar de Elche' cultivar.

4.3.3. Total soluble solids (TSS)

TSS varied during storage of fruit during this study. TSS decreased from harvest, increased after the second month for some treatments and then finally decreased until end of storage. Fruit treated with 3 mM putrescine had the highest TSS after the first and third months (15.65

± 0.27 and 15.73 ± 0.19 respectively) of storage while no significant differences ($p > 0.05$) were observed after the second and last month of storage (Table 2). The initial increase in TSS could be due to initial concentration of sugars due to loss of moisture whereas the subsequent decrease thereafter could be due to utilization of sugars in fruit metabolic processes (Fawole & Opara, 2013a). Although organic acids have been reported to be the major substrates of pomegranate respiration during storage, the decrease in TSS could be due to utilization of sugars in other metabolic processes with the storage duration showing a significant effect ($p < 0.0001$) (Table 2) on TSS of pomegranate fruit. The changes with time could be due to senescence or increased metabolism with progressive storage. Our findings are in agreement with Fawole & Opara (2013a) who reported decrease in TSS of 'Bhagwa' and 'Ruby' pomegranate with storage duration. On the contrary however, Arendse (2014) reported significant increase in TSS of pomegranate (cv. Wonderful) stored at 5, 7.5 and 10 °C for 5 months.

4.3.4. TSS/TA ratio

The taste of pomegranate is determined mainly by juice TSS level and the ratio between the TSS and TA (Zarei *et al.*, 2011). TSS/TA ratio influences the flavour of products and it measures that balance between the acids and sugars in produce (Fawole & Opara 2013a). As a result of the changes in TSS and TA, fluctuations were observed in the TSS/TA during storage. There was an initial decline in TSS/TA ratio from harvest and thereafter an increase observed in all treatments after 2 months of storage (Table 2). A decrease was observed after the third month followed by ~38.47 % increase after 4 months. However no significant differences ($p > 0.05$) were observed in the TSS/TA during the entire storage duration and the changes were influenced by the storage duration. The increase in TSS/TA ratio could be due to the observed decrease in TA and slight increases in TSS values during storage which then results in higher TSS/TA ratio. The TSS/TA ratio level has been attributed mainly to breakdown of starch into water, soluble sugars, sucrose and glucose (Zafari *et al.*, 2015). The findings in this study are similar to the work by Zafari *et al.* (2015) who observed increase in TSS/TA ratio of strawberry fruit (cv. Kamarosa) treated with putrescine and Aloe vera. Arendse (2014) also reported increase in TSS/TA value during storage of pomegranate fruit (cv. Wonderful) at different temperatures (5, 7.5 and 10 °C). Similarly, Fawole & Opara (2013a) observed significant increases in TSS/TA ratios of 'Bahgwa' and 'Ruby'

pomegranate stored for 16 weeks at 5, 7 and 10 °C from 9.98 at harvest to a maximum of 13.12 after storage.

4.3.5. BrimA

Jordan *et al.* (2001) proposed a new index ("BrimA") based on the TSS/TA ratio to determine acceptability of juices, but added a tongue's sensitivity index ("k") as well. This index, k, takes into account that the tongue has higher sensitivity to acid than to sugar, and has values normally from 2 to 10 depending on the fruit type The authors proposed BrimA as a more sensitive predictor of consumer acceptability in different fruits. During this study, BrimA initially decreased from harvest with no significant differences among treatments except for control fruit that had the lowest values (9.76 ± 0.60) (Table 2). It thereafter increased after the second month, decreased after the third and finally increased by 5.9 % after the fourth month of storage with significant differences. The changes in BrimA were significantly affected by storage duration ($p < 0.0001$) (Table 2). The changes in TSS and TA resulted in significant decreases in BrimA due to storage duration. BrimA decreased in all treatments during storage with the exception of month 2 where increases were observed although no significant differences existed among treatments (Table 2). Overall, treating fruit with 3 mM resulted in the best BrimA compared to other treatments. Similar decrease in BrimA was reported by Fawole & Opara (2013a) for pomegranate (cv. Ruby and Bhagwa) stored at 5, 7 and 10 °C for 16 weeks. Arendse (2014) reported an increase in BrimA from 10.64 at harvest to 14.33, 13.62, 12.96, and 12.30 during storage at 5 °C, 7.5 °C, 10 °C and 21 °C respectively, for 'Wonderful' pomegranate.

4.4. Sensory properties

To evaluate quality of fruits and vegetables, sensory attributes such as appearance, aroma, texture and colour are some of the vital criteria used by a consumer (Nunes *et al.*, 2007; Opara *et al.*, 2007). 'Wonderful' pomegranate is characterized mainly by sour over sweet taste (Vázquez-Araújo *et al.*, 2011b). Sensory attributes of pomegranate fruit changed during storage. After the first month of storage, higher sweet taste was perceived in control fruit (non-treated) compared to treated fruit (Fig. 7A) and this could be related to the higher TSS values that were observed in control fruit. Sour taste was scored higher in fruit treated with 1 and 2 mM putrescine while off flavour was generally low in all treatments with scores of 0.2 - 0.4. Astringency, responsible for the tartness taste in pomegranate especially in the sweet-

sour and sour cultivars, was highest in fruit treated with 1 mM putrescine. Crispness of arils was generally maintained among all treatments while juiciness was highest in control fruit. Grittiness and hardness were scored higher for fruit treated with 3 mM putrescine (Fig. 7A). In general, after one month of storage, control fruit had better sensory quality with regards to sweet taste and juiciness. Jawandha *et al.* (2012) also reported that initially after one week of storage of mango (cv. Langra), highest palatability rating was recorded in untreated (control) fruit compared to fruit that had been treated with putrescine.

After storage of fruit for two months, prominent differences were observed in the sensory attributes, with control and fruit treated with 2 mM showing higher scores while fruit treated with 1 and 3 mM had lower scores (Fig. 7B). As shown by the radar plot (Fig. 7B), control and fruit treated with 2 mM putrescine had better sensory attributes with regards to sweetness, juiciness and crispness compared to the other treatments. Pomegranate fruit (cv. Mridula) treated with putrescine and carnauba wax was reported to have higher sensory scores than control with regard to colour, aroma, taste, juiciness and aril firmness after 60 days storage for storage at 5 °C (Barman *et al.*, 2014).

Moreover, at the end of three months control fruit were scored the highest for aril sweet taste while sour taste was more prominent in fruit treated with 3 mM putrescine. Off flavour increased with storage although fruit with 2 mM putrescine had the lowest scores (Fig. 7C). Astringency was also low in all treatments, even lower than the previous months of storage. This could possibly be attributed to the decrease in T.A and increase in pH among all treatments as storage progressed (Table 2). This is because organic acids (especially tartaric acid) which are responsible for astringency are utilized for metabolism during storage of pomegranate. Fawole & Opara (2013b) also reported low astringency and alcohol taste for 'Ruby' pomegranate fruit. Crispness and grittiness were more pronounced in fruit treated with 2 mM while juiciness was higher in fruit treated with 1 mM putrescine. Additionally, hardness was best maintained in fruit treated with 3 mM putrescine (Fig. 7B). Comparing all treatments at all storage durations, fruit treated with 2 mM putrescine generally had higher sensory attribute ratings compared to other concentration. Jawandha *et al.* (2012) reported highest palatability rating in mango fruit (cv. Langra) treated with putrescine of 2 mM as fruit were still in very good quality after 3 weeks of storage. Similarly, 'Selva' strawberry fruit treated with putrescine had better quality in terms of flesh firmness, appearance, color change and taste especially when fruit were treated with 2 mM putrescine (Khosroshahi *et al.*, 2007).

Treating pomegranate (Mridula) with putrescine and carnauba wax resulted in higher sensory scores compared to control after 60 days of storage at 2 and 5 °C (Barman *et al.*, 2014).

4.5. Multivariate analysis

4.5.1. Pearsons' correlation analysis of physiological responses and disorders

Pearsons' correlation showed a moderate positive correlation between respiration rate and weight loss (r = 0.67) (Table 3). This shows that increase in fruit respiration rate results in increased weight loss of fruit. Fruit weight loss was strongly correlated with fruit decay, chilling injury, scald and aril browning. Therefore, development of physiological disorders result into greater weight loss of fruit. Development of chilling injury was associated with increased respiration rate, weight loss and fruit decay. In addition, husk scald increased fruit susceptibility to decay as there was a strong positive correlation (Table 3). This could be attributed to increased senescence with scalding thus making fruit more prone to fungal attack. Although some studies have reported that husk scald does not affect the internal quality of affected fruit (Defilippi *et al.*, 2006), the strong positive correlation showed that aril browning increased with scald incidence (r = 0.91) and severity (r = 0.93) (Table 3). This indicated the contribution of husk scalding to fruit internal quality through increased enzymatic oxidation activity of the internal components. On the other hand, the presence of internal decay had a weak correlation with the other physiological disorders and this is not suprising because this is a preharvest condition.

4.5.2. Principal component analysis

To obtain a broad view on changes in fruit quality attributes that occurred during storage, the whole data set was subjected to principal component analysis (PCA). An Eigenvalue measures the significance of a factor, with Eigenvalues ≥ 1 considered significant. Therefore the highest eigenvalues are the most significant (Fawole & Opara, 2013c). The total variability was explained by 11 factors (F1-F11), with the first two factors of the PCA showing moderate correlation of 53.22 % (Fig. 8). The first factor (F1) was responsible for 33.36 %, while the second factor (F2) explained 19.86 % of total variation, indicating that the maximum possible variation during fruit storage was explained by the F1 (Fig. 9A). Positive scores along F1 corresponded with long storage duration (2-3 months). Short term storage (1 month) had high negative scores along F1 while fruit stored for 2 months had low positive

scores (Fig. 9A). Negatives scores along F1 corresponded with peel colour, fruit firmness, juice colour, aril hardness, grittiness, TSS, TA and astringency. The peel colour indicated that fruit had better peel appearance during short storage duration but decreased with prolonged storage which can be related to development of physiological disorders especially husk scald. Fruit stored for short duration (1 month) were associated with grittiness, hardness and astringency. The contribution of phenolics to fruit astringency has been previously reported (Kulkarni & Aradhya, 2005). As storage progressed, there was a shift from left to right along F1 (Fig. 9B) with increase in aril redness (a*), sweet taste, crispness and juiciness. This gives a clear indication that fruit stored for short duration (1 month) could clearly be distinguished from fruit stored for long duration (3 months). The increase in aril colour is associated with increased anthocyanin biosynthesis while the increase in sweet taste could be as a result of concentration of sugars due to weight loss with progressive storage of fruit (Fawole & Opara, 2013b). A strong positive relationship between TSS/TA ratio and BrimA was indicated by the short distance between the two attributes on the PCA while a strong negative relationship existed between TA and TSS/TA ratio (Fig 9A). A general view of the PCA showed that short term storage of fruit was associated with grittiness, hardness, fruit firmness, TSS, TA and astringency among others while long term storage resulted in fruit with better aril colour, juiciness, sweet taste and crispiness. The results indicate that storage duration, instead of other factors (such as treatment concentration) contributed to the distinction of sensory and instrumental attributes during the study. This suggests the importance of storage duration in postharvest studies as well as changes in postharvest quality of fresh produce. Arendse (2014) also reported such observation, where storage duration rather than temperatures (5, 7.5 and 10 °C) influenced the storage quality of ‘Wonderful’ pomegranate fruit for up to 4 months of storage.

5. Conclusions

Exogenous application of putrescine on pomegranate fruit, especially at higher concentrations (2 and 3 mM) reduced the incidences of fruit decay and physiological disorders particularly chilling injury severity, with effects more prominent during the last months of storage. In addition, these concentrations resulted in lower husk scald after storage for the first 3 months although 100 % of the fruit developed scald at the end of storage. Husk scald was the major physiological disorder during storage of pomegranate fruit after the chemical treatments

during the study. Since scalding is an enzymatic oxidative process, this highlights the significance of physical treatments as they have been shown to reduce scalding by providing a barrier to oxygen supply. Therefore treating pomegranate fruit with putrescine reduces physiological disorders but only for shorter storage time (2 - 3 months).

Treating fruit with putrescine also reduced changes in physico-chemical properties like colour through reduction of anthocyanin biosynthesis. Despite control fruit having more intense aril red colour, treated fruit had adequate aril colour and the additional advantage of reducing physiological disorders and decay. From the sensory point of view, treating fruit with 2 mM putrescine maintained and in some cases improved the sensory properties of fruit especially after 2 and 3 months of storage. In conclusion, treating 'Wonderful' pomegranate fruit with 2 and 3 mM concentration of putrescine would be recommended to improve postharvest quality of fruit. However, further research is required to combine the benefits of chemical treatment together with physical treatments if the maximum potential of healthier alternative chemicals is to be realized since chemical treatments alone are not substantial enough to cater for all the quality parameters of the fruit.

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Table 1 Changes in juice colour, fruit firmness and aril firmness of pomegranate fruit treated with putrescine during storage for 4 months at 5 °C and additional 4 days at 20 °C

Parameter	Concentration (mM)	Harvest	Storage duration (Month)				Significance level		
			1	2	3	4	Concentration (A)	Duration (B)	A x B
Juice colour absorbance (520nm)		3.28 ± 0.01							
	0 (Control)		3.08 ± 0.14^{a}	3.16 ± 0.07^{a}	3.20 ± 0.01^{a}	3.22 ± 0.01^{a}	0.1758	0.1403	**0.0125**
	1		3.24 ± 0.02^{a}	3.22 ± 0.02^{a}	2.80 ± 0.16^{b}	3.03 ± 0.10^{ab}			
	2		3.20 ± 0.05^{a}	2.97 ± 0.14^{ab}	3.00 ± 0.11^{ab}	3.15 ± 0.07^{a}			
	3		3.26 ± 0.01^{a}	3.23 ± 0.01^{a}	3.20 ± 0.01^{a}	3.02 ± 0.13^{ab}			
Fruit firmness (N)		13.39 ± 0.30							
	0 (Control)		12.04 ± 0.59^{a}	10.96 ± 0.45^{abc}	10.03 ± 0.38^{c}	10.12 ± 0.40^{c}	0.3981	**<0.0001**	**<0.0001**
	1		11.71 ± 0.38^{ab}	10.13 ± 0.46^{c}	11.21 ± 0.50^{abc}	11.17 ± 0.38^{abc}			
	2		11.03 ± 0.48^{abc}	11.06 ± 0.44^{abc}	8.27 ± 0.40^{d}	11.92 ± 0.40^{a}			
	3		12.20 ± 0.23^{a}	10.43 ± 0.34^{bc}	10.20 ± 0.37^{c}	10.83 ± 0.51^{abc}			
Aril firmness									
Hardness (N)		157.20 ± 2.81							
	0 (Control)		91.27 ± 3.05^{f}	142.80 ± 5.42^{de}	151.40 ± 3.57^{abc}	143.20 ± 3.84^{cde}	**<0.0001**	**<0.0001**	**<0.0001**
	1		159.20 ± 4.24^{abc}	158.40 ± 3.36^{ab}	136.50 ± 2.91^{e}	155.10 ± 3.35^{abc}			
	2		92.33 ± 3.03^{f}	158.90 ± 3.71^{ab}	139.00 ± 1.98^{de}	154.20 ± 2.18^{abc}			
	3		157.50 ± 2.80^{ab}	162.50 ± 3.55^{a}	148.40 ± 3.54^{bcd}	160.30 ± 4.77^{a}			
Elastic modulus (N/mm)		8.57 ± 0.49							
	0 (Control)		5.85 ± 0.49^{ab}	6.21 ± 0.46^{ab}	6.41 ± 0.51^{ab}	5.34 ± 0.63^{b}	0.2801	0.2660	0.1662
	1		6.09 ± 0.60^{ab}	5.98 ± 0.49^{ab}	6.28 ± 0.43^{ab}	6.24 ± 0.70^{ab}			
	2		5.96 ± 0.43^{ab}	7.13 ± 0.71^{a}	5.97 ± 0.49^{ab}	5.92 ± 0.46^{ab}			
	3		5.84 ± 0.50^{ab}	5.54 ± 0.38^{ab}	5.78 ± 0.50^{ab}	6.45 ± 0.60^{ab}			
Toughness (N.mm)		169.90 ± 3.83							
	0 (Control)		119.80 ± 4.36^{f}	152.30 ± 8.38^{de}	152.60 ± 5.91^{b-e}	143.30 ± 5.66^{e}	**<0.0001**	0.0616	**0.0039**
	1		170.80 ± 6.90^{abc}	171.00 ± 5.06^{abc}	155.30 ± 5.14^{cde}	162.60 ± 4.63^{a-e}			

	2		117.80 ± 4.09^{f}	171.90 ± 4.15^{ab}	158.70 ± 2.86^{b-e}	164.80 ± 3.06^{a-d}			
	3		165.00 ± 5.23^{a-d}	178.20 ± 5.62^{a}	157.10 ± 5.36^{b-e}	175.70 ± 6.84^{a}			
Bioyield (N)		5.96 ± 0.82							
	0 (Control)		6.17 ± 0.78^{a}	6.25 ± 0.51^{a}	5.96 ± 0.62^{a}	6.53 ± 0.92^{a}	0.9859	0.8250	0.3103
	1		6.32 ± 0.67^{a}	6.40 ± 0.62^{a}	7.50 ± 0.56^{a}	6.62 ± 0.76^{a}			
	2		6.98 ± 0.67^{a}	8.13 ± 0.94^{a}	6.95 ± 0.70^{a}	7.31 ± 0.78^{a}			
	3		6.10 ± 0.64^{a}	6.51 ± 0.68^{a}	6.42 ± 0.61^{a}	7.76 ± 0.92^{a}			

Data presented as mean ± SE. Different letters across concentration and storage duration for each attribute differ significantly ($p < 0.05$) according to Duncan's multiple range test. SE - standard error

Table 2 Chemical attributes of pomegranate fruit treated with putrescine during storage for 4 months at 5 °C and additional 4 days at 20 °C

Parameter	Concentration (mM)	Harvest	Storage duration (Month)				Significance level		
			1	2	3	4	Concentration (A)	Duration (B)	A x B
pH		3.28 ± 0.03							
	0 (Control)		3.00 ± 0.07^{e}	3.25 ± 0.15^{d}	3.49 ± 0.05^{c}	3.61 ± 0.04^{bc}	**0.0002**	**<0.0001**	**<0.0001**
	1		2.58 ± 0.03^{f}	3.23 ± 0.05^{d}	3.68 ± 0.04^{b}	3.87 ± 0.04^{a}			
	2		3.11 ± 0.04^{de}	3.58 ± 0.06^{bc}	3.60 ± 0.05^{bc}	3.49 ± 0.03^{c}			
	3		2.53 ± 0.05^{f}	3.54 ± 0.03^{bc}	3.49 ± 0.05^{c}	3.53 ± 0.06^{bc}			
TA (% citric acid)		1.68 ± 0.08							
	0 (Control)		2.53 ± 0.28^{a}	1.43 ± 0.14^{efg}	2.24 ± 0.15^{ab}	1.26 ± 0.04^{fg}	**0.0261**	**<0.0001**	0.4938
	1		2.40 ± 0.14^{a}	1.45 ± 0.11^{efg}	1.71 ± 0.06^{cde}	1.10 ± 0.07^{g}			
	2		2.21 ± 0.13^{ab}	1.27 ± 0.13^{fg}	1.85 ± 0.09^{cd}	1.09 ± 0.04^{g}			
	3		2.22 ± 0.15^{ab}	1.53 ± 0.08^{def}	1.94 ± 0.11^{bc}	1.14 ± 0.08^{g}			
TSS (°Brix)		16.2 ± 0.16							
	0 (Control)		14.83 ± 0.21^{c-f}	15.21 ± 0.35^{a-d}	14.7 ± 0.41^{c-g}	14.56 ± 0.19^{d-g}	**0.0308**	**<0.0001**	0.1535
	1		15.10 ± 0.23^{a-e}	15.28 ± 0.07^{a-d}	14.86 ± 0.22^{b-f}	13.96 ± 0.31^{g}			
	2		15.5 ± 0.21^{abc}	14.93 ± 0.19^{b-e}	15.08 ± 0.22^{a-e}	14.09 ± 0.21^{fg}			
	3		15.65 ± 0.27^{ab}	15.25 ± 0.26^{a-d}	15.73 ± 0.19^{a}	14.34 ± 0.31^{efg}			
TSS/TA		9.98 ± 0.41							
	0 (Control)		6.40 ± 0.85^{e}	11.33 ± 1.45^{ab}	6.14 ± 0.33^{e}	11.62 ± 0.32^{ab}	0.0935	**<0.0001**	0.4670
	1		6.47 ± 0.41^{e}	11.10 ± 0.80^{ab}	8.79 ± 0.34^{cd}	13.12 ± 0.92^{a}			
	2		7.20 ± 0.42^{de}	12.18 ± 1.25^{ab}	8.30 ± 0.42^{cde}	13.10 ± 0.45^{a}			
	3		7.39 ± 0.59^{de}	10.13 ± 0.70^{bc}	8.25 ± 0.45^{cde}	13.05 ± 0.76^{a}			
BrimA		12.84 ± 0.18							
	0 (Control)		9.76 ± 0.60^{g}	12.75 ± 0.63^{a}	10.23 ± 0.59^{fg}	12.03 ± 0.16^{a-d}	0.1018	**<0.0001**	0.0983
	1		10.29 ± 0.41^{efg}	12.39 ± 0.18^{abc}	11.47 ± 0.25^{bcd}	11.75 ± 0.31^{a-d}			
	2		11.08 ± 0.32^{def}	12.39 ± 0.34^{ab}	11.37 ± 0.32^{b-e}	11.92 ± 0.21^{a-d}			
	3		11.20 ± 0.48^{b-f}	12.14 ± 0.32^{a-d}	11.85 ± 34^{a-d}	12.07 ± 0.27^{a-d}			

Data presented as mean ± SE. Different letters across concentration and storage duration for each attribute differ significantly ($p < 0.05$) according to Duncan's multiple range test. TA - titratable acidity; TSS - total soluble solids; SE - standard error

Table 3 Pearson's correlation coefficient matrix between assessed physiological disorders

Variables	Respiration rate	Weight loss	Fruit decay	CI severity	% CI incidence	Scald severity	% Scald incidence	Aril browning	Internal decay
Respiration rate	**1**								
Weight loss	**0.671**	**1**							
Fruit decay	0.392	**0.868**	**1**						
CI severity	**0.653**	**0.770**	**0.567**	**1**					
% CI incidence	**0.670**	**0.778**	**0.675**	**0.907**	**1**				
Scald severity	0.357	**0.910**	**0.894**	**0.628**	**0.608**	**1**			
% Scald incidence	**0.588**	**0.912**	**0.827**	**0.618**	**0.686**	**0.858**	**1**		
Aril browning	**0.599**	**0.954**	**0.817**	**0.698**	**0.638**	**0.928**	**0.908**	**1**	
Internal decay	0.372	0.425	0.443	-0.027	0.184	0.426	0.495	0.465	**1**

Values in bold are different at significance level of $p < 0.05$. Values in bold have moderate to strong correlation
CI- chilling injury

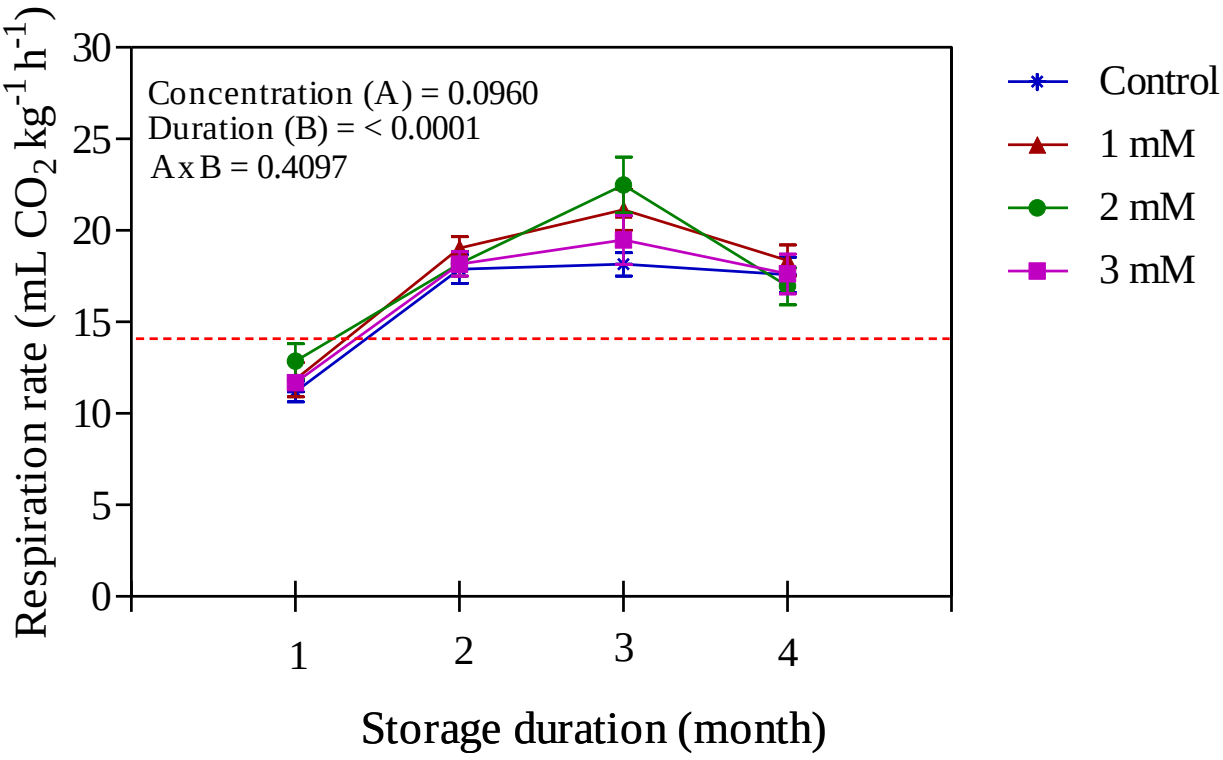

Fig. 1 Respiration rate of control and putrescine treated pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C. Each data point represents mean and error bars designate standard error (SE) of the mean. ------Respiration rate at harvest.

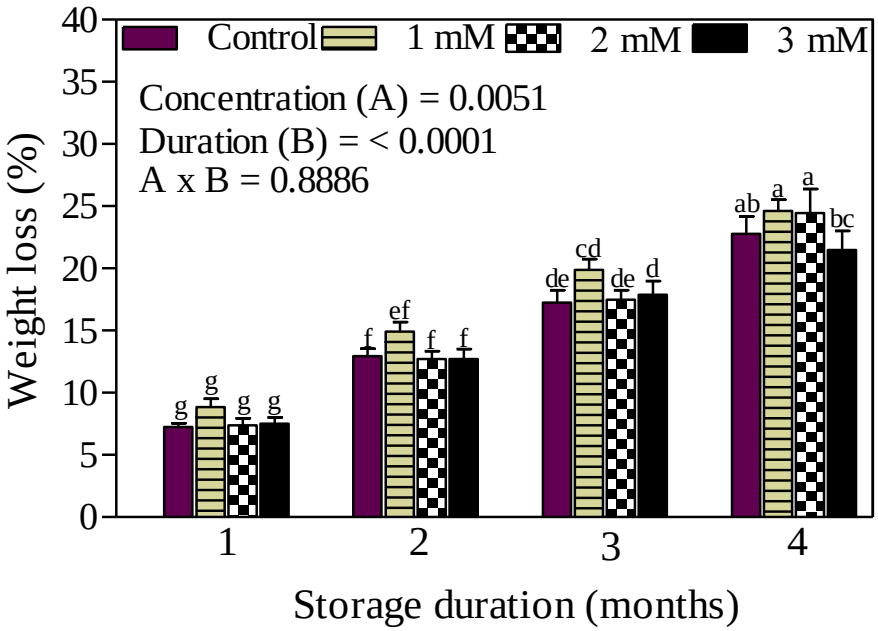

Fig. 2 Cumulative weight loss of pomegranate fruit treated with putrescine during storage for 4 months at 5 °C and additional 4 days at 20 °C. Each bar represents mean and error bars represent standard error (SE) of the mean. Bars followed by different letters are significantly different at $p< 0.05$ according to Duncan's multiple range test.

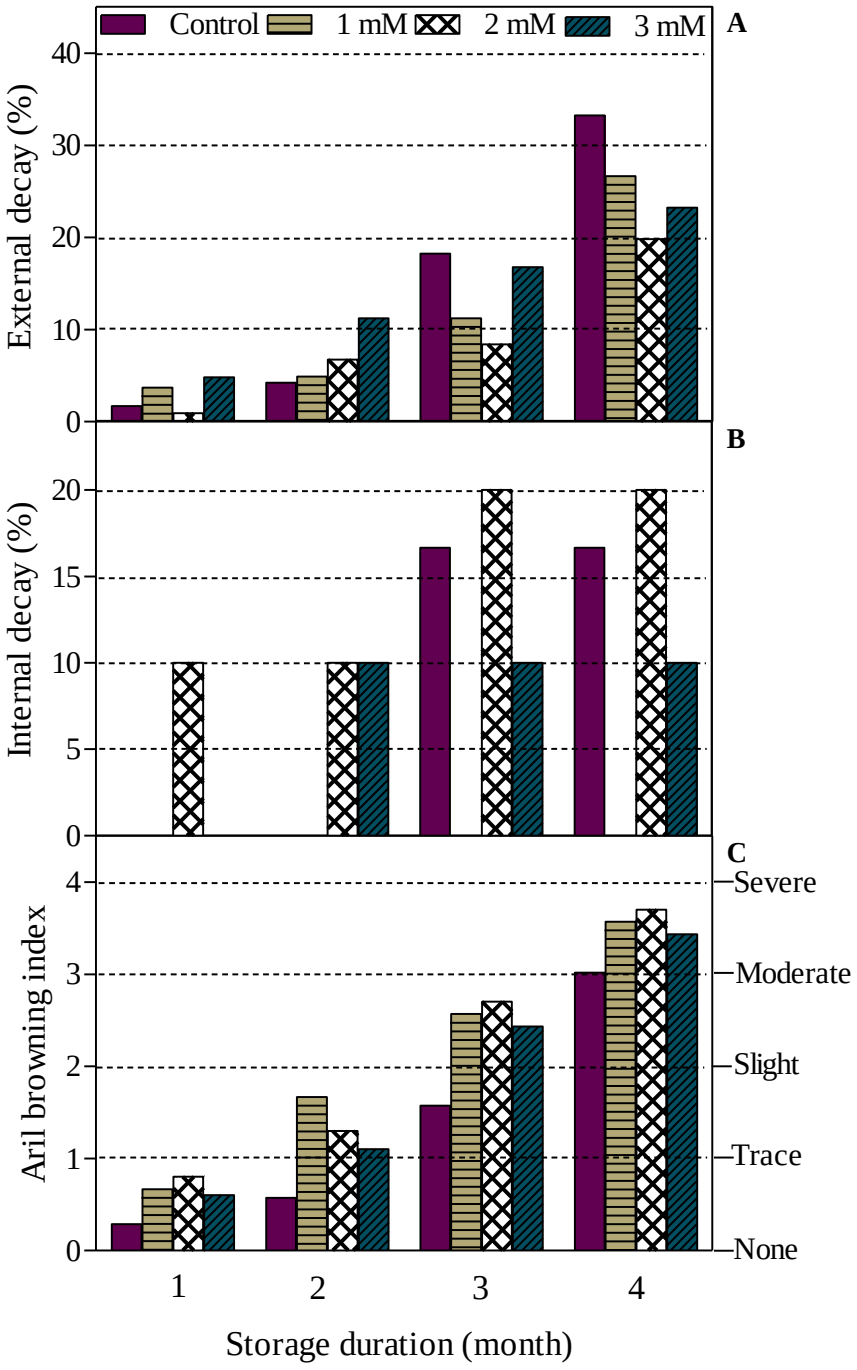

Fig. 3 Effect of putrescine on physiological disorders of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C. External decay (A), internal decay (B), aril browning (C).

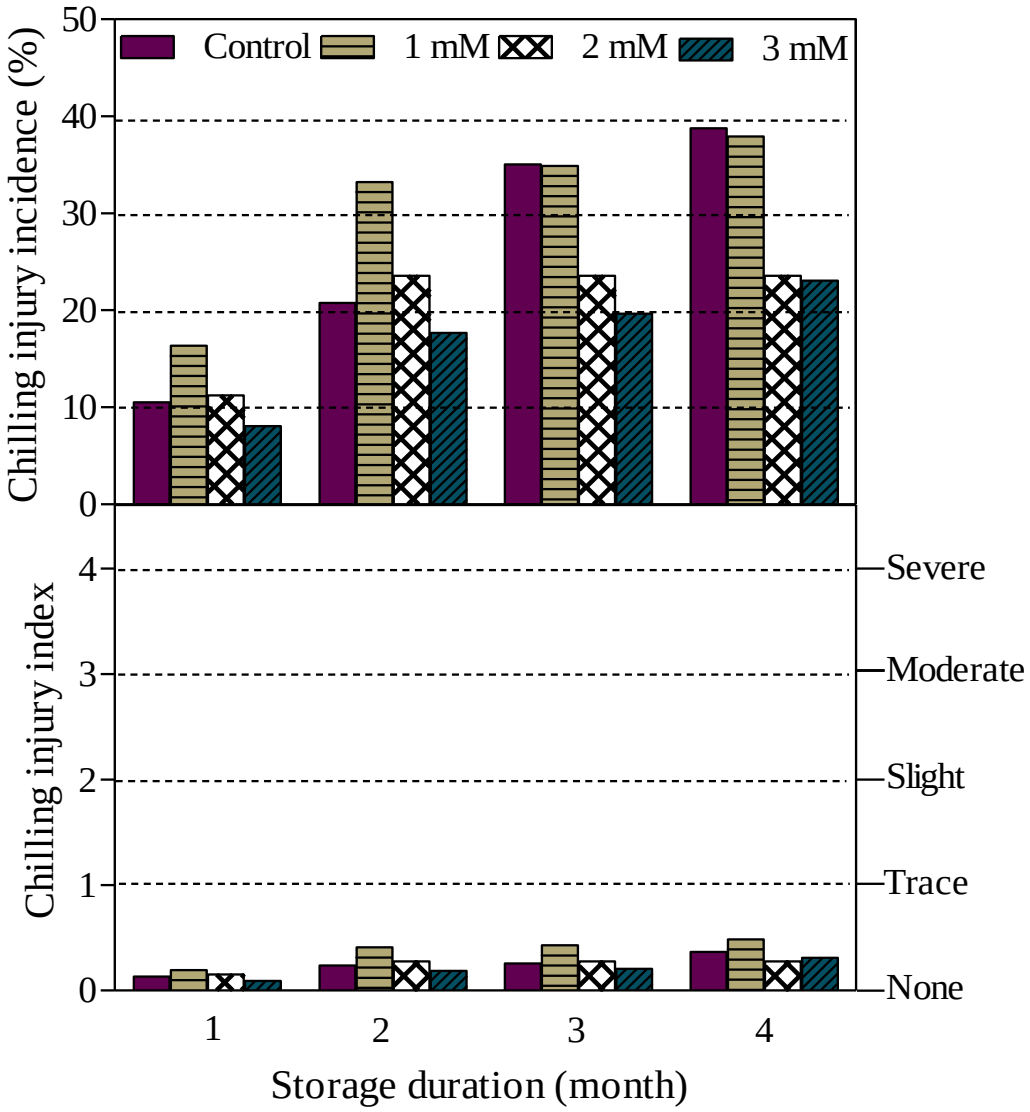

Fig. 4 Effect of putrescine on chilling injury incidence and index of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C.

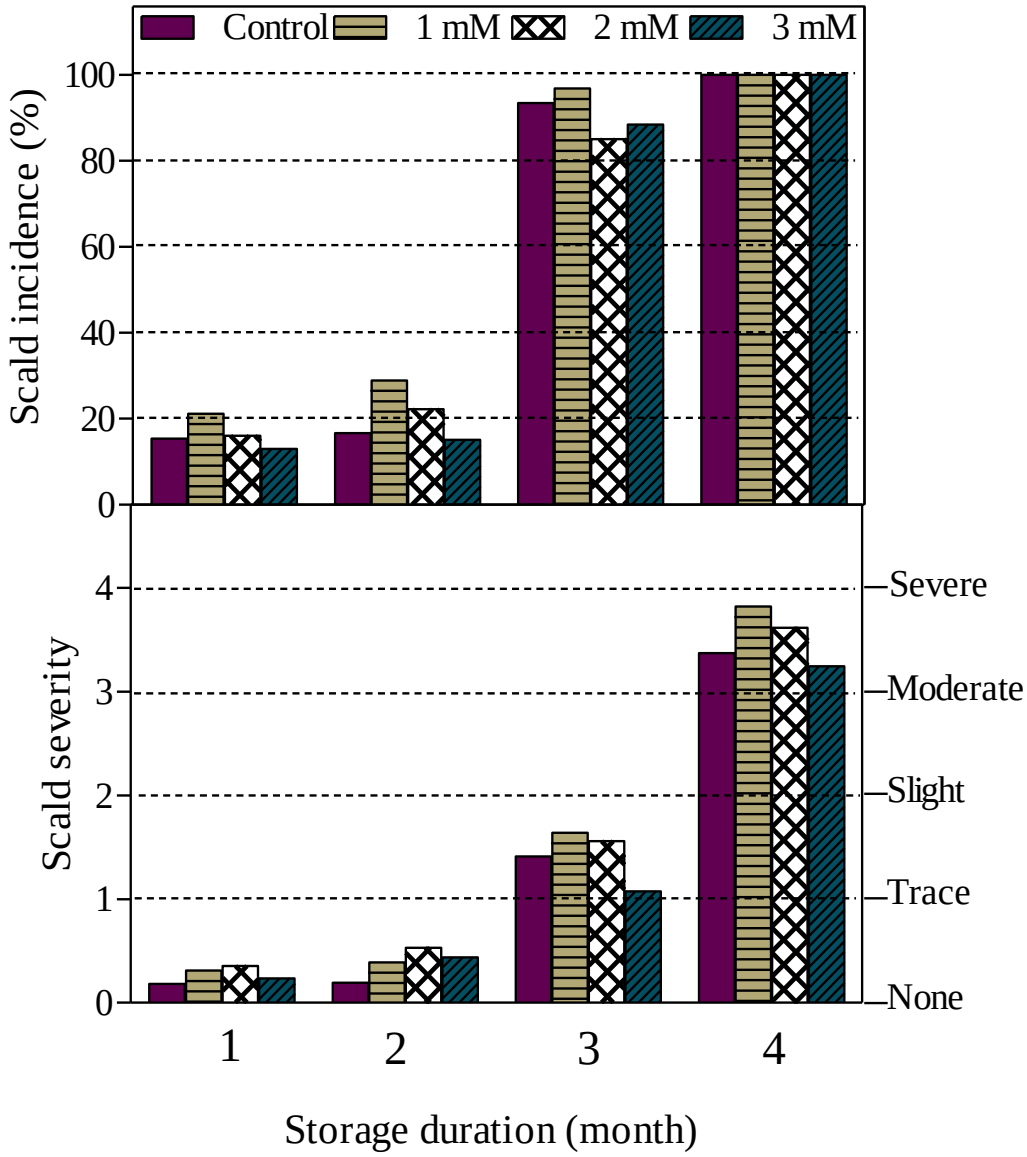

Fig. 5 Effect of putrescine on husk scald incidence and severity of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C.

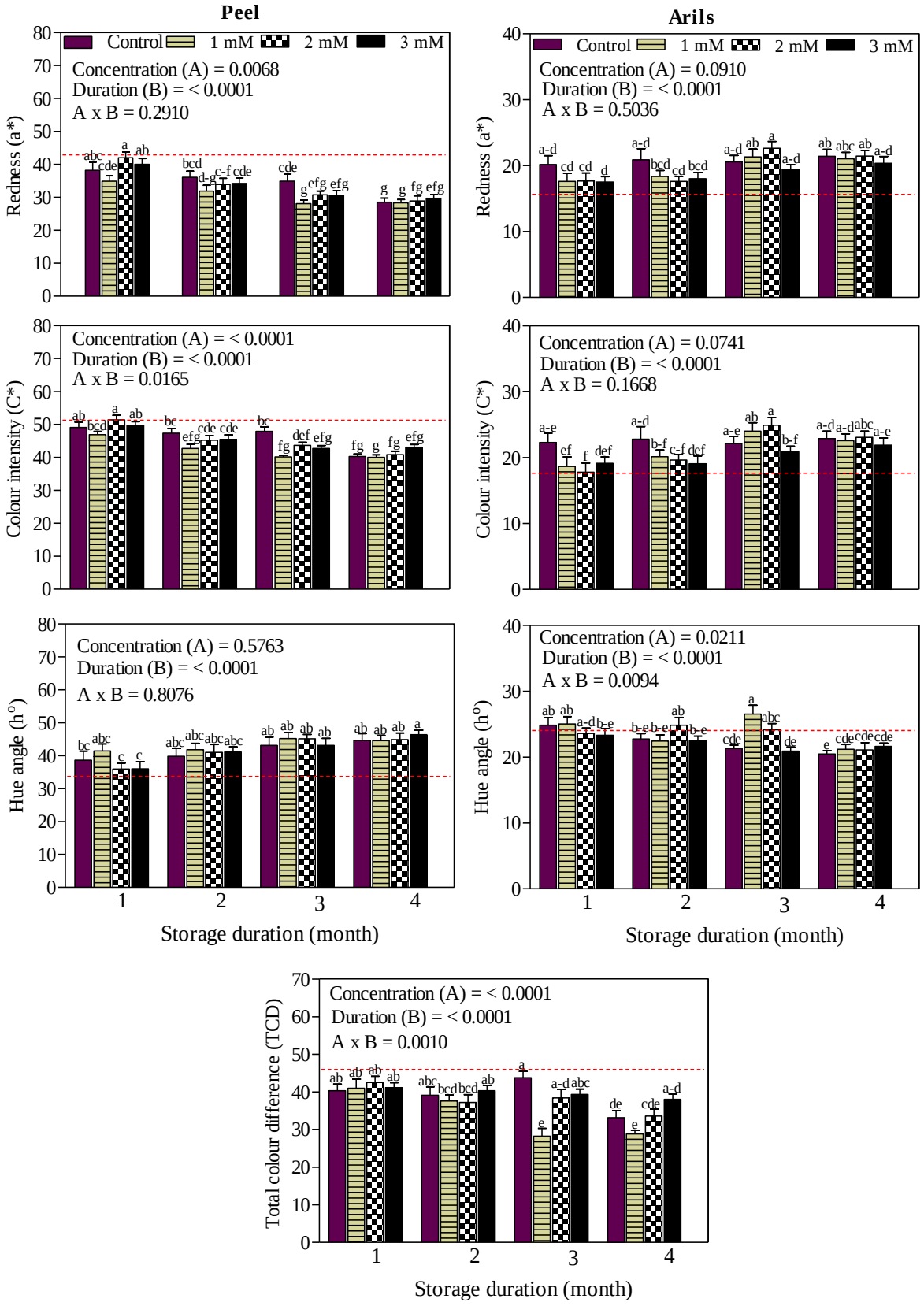

Fig. 6 Changes in colour parameters of pomegranate fruit peel and arils during storage for 4 months at 5 °C and additional 4 days at 20 °C. Each bar represents mean and error bars denote standard error (SE) of the mean. Bars followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test. -----Represents values at harvest.

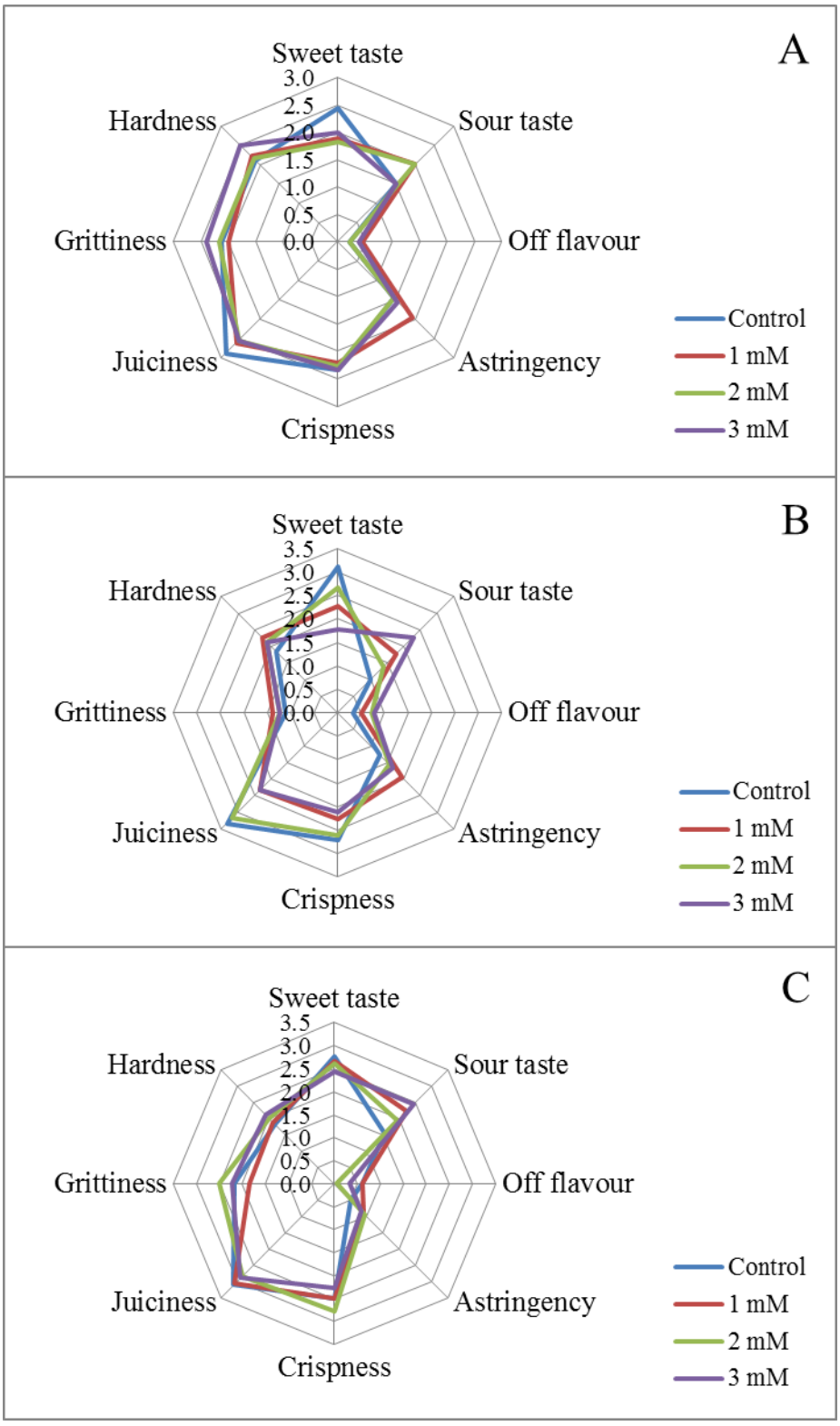

Fig. 7 Average sensory scores of pomegranate fruit treated with putrescine during storage for 3 months at 5 °C and additional 4 days at 20 °C. The plot represents storage at month 1 (A), month 2 (B) and month 3 (C).

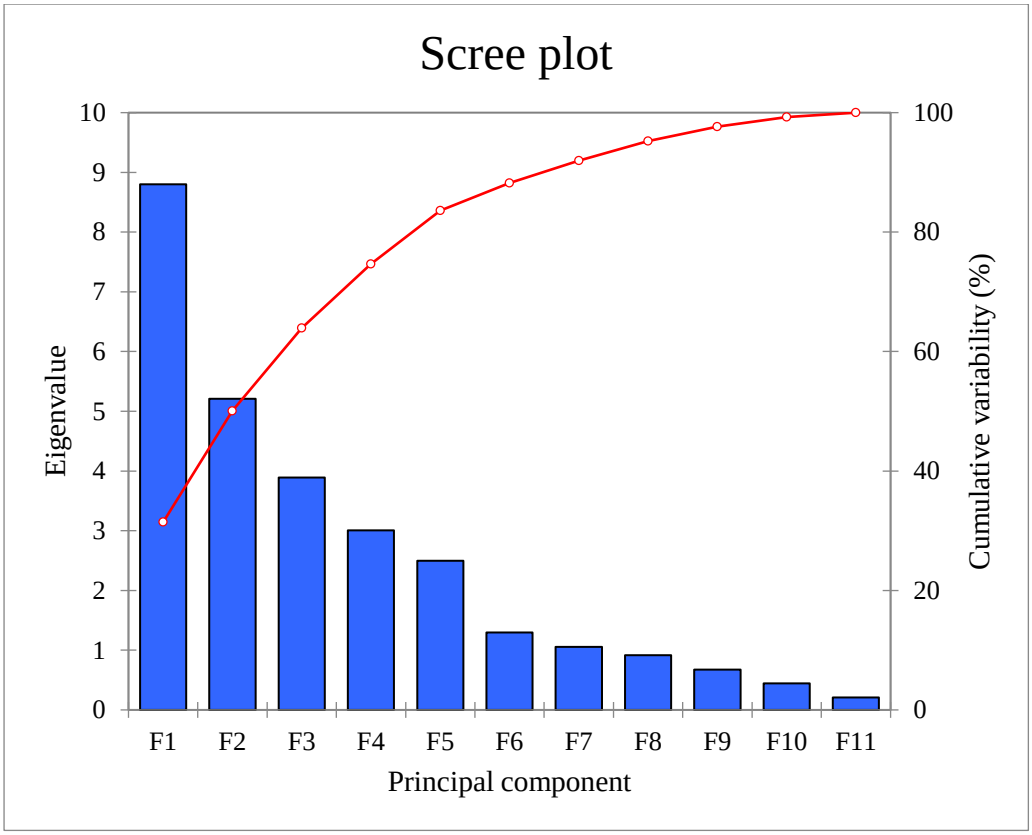

Fig. 8 Scree plot of variance explained by each factor of the principal components. The Eigenvalue indicates the significance of a factor.

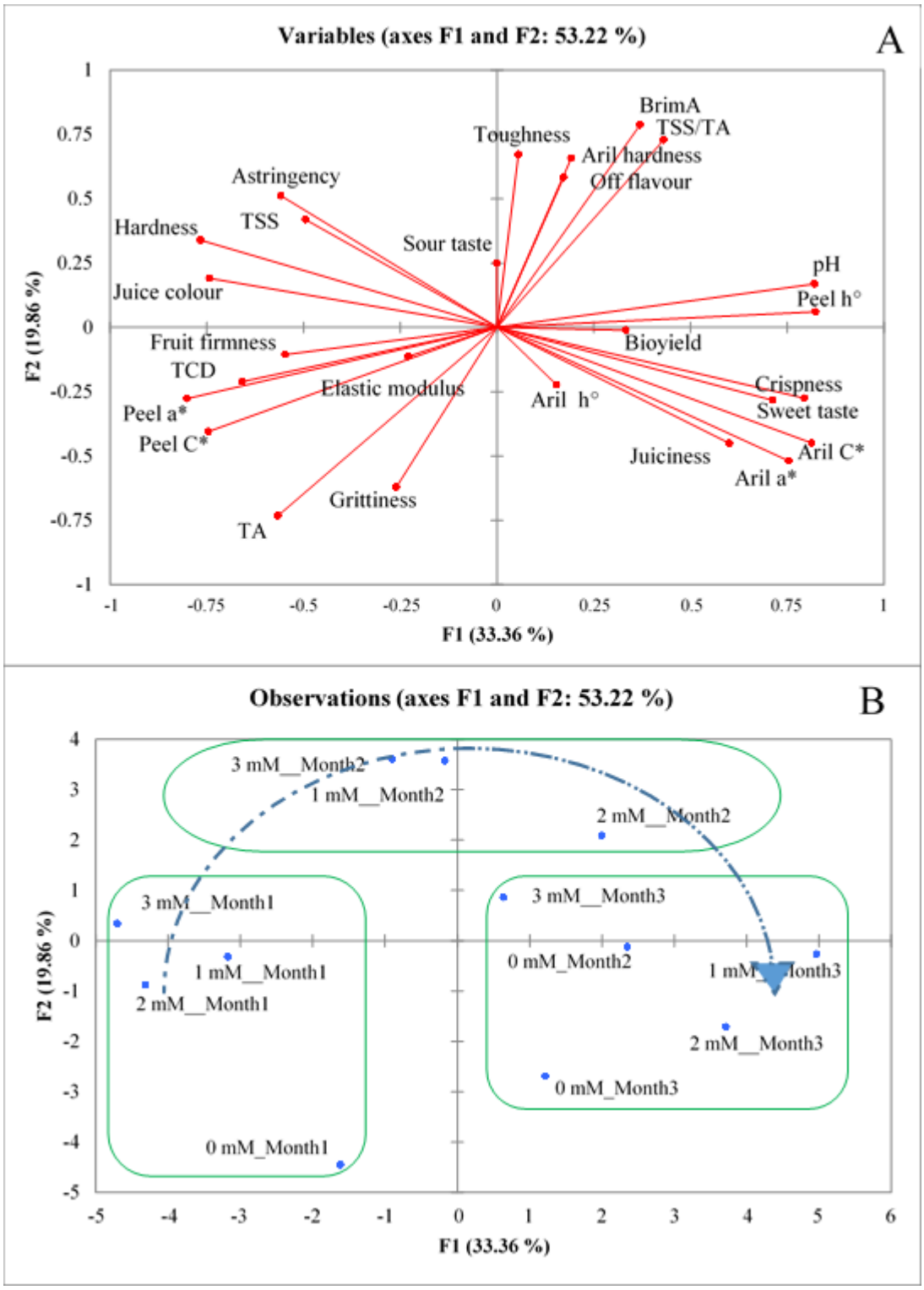

Fig. 9 Principal component analysis showing variables (A) and observations (B) for 'Wonderful' pomegranate fruit using instrumental and sensory attributes of fruit stored for 3 months at 5 °C and additional 4 days at 20 °C. The circulated points indicate the factors and the arrow shows a shift from month 1 to month 3.

CHAPTER FOUR: Effects of exogenous fludioxonil postharvest treatment on physiological response, physico-chemical, textural and sensory characteristics of pomegranate fruit

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EFFECTS OF EXOGENOUS FLUDIOXONIL POSTHARVEST TREATMENT ON PHYSIOLOGICAL RESPONSE, PHYSICO-CHEMICAL, TEXTURAL AND SENSORY CHARACTERISTICS OF POMEGRANATE FRUIT

Abstract

The study investigated the effects of fludioxonil (FLU) postharvest treatment on the postharvest quality of pomegranate fruit (cv. Wonderful). Fruits were dipped in FLU concentrations (control, 150, 300 and 600 mg/L) and stored for 4 months at 5 °C and 90 - 95 % relative humidity (RH) plus an additional 4 days at 20 °C and 65 % RH. Effects of FLU were evaluated on fruit physiological responses, quality and sensory attributes. Results showed that fruit weight loss and decay incidence were reduced by FLU treatment, with fruit treated with 600 mg/L concentration showing the best results. Fruit respiration rate was more influenced by storage duration than FLU concentration. The severity and occurrence of physiological disorders increased with storage duration and were higher in fruit treated with FLU compared to control. FLU treatment enhanced the retention of whole fruit firmness, however, aril texture attributes were not significantly affected. Storage duration influenced fruit colour whereas aril colour was affected by FLU concentration. Untreated fruit showed better aril redness (a*) and chroma (C*) although treated fruit also had acceptable red colour. Chemical quality attributes of fruit juice were not significantly affected by FLU concentrations. Treating fruit with FLU resulted in better sensory attributes with regards to crispness, juiciness and sweetness. The PCA highlighted the contribution of storage duration and FLU concentration on the measured parameters and indicated that fruit quality was majorly affected by storage period as quality deteriorated with time. Overall, this study showed that fruit treated with FLU at 600 mg/L had the best quality with respect to decay incidence, fruit firmness and sensory attributes.

1. Introduction

Pomegranate (*Punica granatum* L.) is a non-climacteric fruit and is an important horticultural crop in many tropical and subtropical regions of the world (Roy & Waskar, 1997; Nanda *et al.*, 2001). The edible portion of the fruit is comprised of arils that can be consumed fresh as seeds, juice or used as flavouring and colouring agents (Gil *et al.*, 2000; Al-Said *et al.*, 2009; Opara *et al.*, 2009). The fruit juice is high in sugars, vitamins, organic acids, polysaccharides, polyphenols and essential minerals (Al-Maiman & Ahmad, 2002; Fawole & Opara, 2013d). Pomegranate fruit has recently captured consumer interest because of its reported beneficial health properties (Heber & Bowerman, 2009; Fawole & Opara, 2013a). Several researchers have reported the chemo-preventative, anti-inflammatory and antibacterial properties of pomegranate fruit (and its derived products) which, have been attributed to its high antioxidant capacity (Kim *et al.*, 2002; Lansky & Newman, 2007; Syed *et al.*, 2007; Opara *et al.*, 2009; Fawole *et al.*, 2012; Mphahlele *et al.*, 2016). Beneficial properties of pomegranates against atherosclerosis, certain cancers and other degenerative diseases have also been reported (Mertens-Talcott *et al.*, 2006; Aviram *et al.*, 2008).

One of the challenges limiting the use of pomegranates is the limited postharvest life. When the fruit is stored under ambient conditions, storage life is limited to only a few weeks and therefore cold storage is recommended, with temperatures between 0 and 10 °C depending on cultivar (Elyatem & Kader, 1984; Fawole & Opara, 2013a). At the same time, relative humidity during storage should be maintained at 90 - 95 % to prevent desiccation of the skin because the fruit is very prone to moisture loss (Roy & Waskar, 1997; Fawole & Opara, 2013a; Arendse *et al.*, 2014). However, high humidity promotes the growth of micro-organisms and enhances fruit decay (D'Aquino *et al.*, 2009). Fruit decay is a major cause of postharvest loss during storage of pomegranate fruit. The major postharvest decay diseases during fruit storage include grey mould – caused by *Botrytis cinerea* (which develops at recommended storage conditions of 5 - 8 °C and 90 - 95 % RH), heart rot – caused by *Aspergillus niger* and *Alternaria* ssp., and Penicillium rot – caused by different species of *Penicillium*, including *P. digitatum* and *P. implicatum* (Roy & Waskar, 1997; Labuda *et al.*, 2004; Pekmezci & Erkan, 2004). To reduce the incidence of fruit decay, fungicides are used both as preharvest and postharvest treatments of fruit. Preharvest fungicides include fenhexamid, azoxystrobin and tebuconazole (Förster *et al.*, 2007; Romanazzi *et al.*, 2014; 2016) and postharvest fungicides include methyl 2-benzimidazole carbamate (BMC),

thiabendazole (TBZ) and fludioxonil (FLU), among others (Errampalli *et al.*, 2006; Romanazzi *et al.*, 2014).

A shift in interest to naturally occurring compounds as antimicrobial agents or as components for chemical synthesis of new active ingredients, together with the understanding of their structures and properties, has motivated the synthesis of new broad spectrum "natural mimetic" fungicides that have different mechanisms of action from other previously registered ones (Gullino *et al.*, 2000; Leroux, 2003; Schirra *et al.*, 2005). To cope with problems associated with resistance development to 'older fungicides' by current and emerging fungal pathogens, a number of novel fungicides have been developed for horticultural crops (Gullino *et al.*, 2000). Among these is fludioxonil, a broad-spectrum fungicide with a different mode of action compared to older registered chemicals (Förster *et al.*, 2007; Schirra *et al.*, 2009). Fludioxonil, together with pyrimethanil and azoxystrobin were categorised as reduced-risk compounds by the United States Environment Protection Agency (US E.P.A) (Adaskaveg *et al.*, 2004). This indicates that these chemicals have more advantageous properties compared to old fungicides and have extensively been assessed for control of green mould in California citrus industry (Adaskaveg *et al.*, 2004; Smilanick *et al.*, 2006). Fludioxonil is a synthetic analogue of pyrrolnitrin belonging to the class of phenylpyrroles (Rosslenbroich & Stuebler, 2000) and is considered a reduced risk pesticide by the US E.P.A (Schirra *et al.*, 2005; D'Aquino *et al.*, 2009). The mode of action is by inhibition of the transport-associated phosphorylation of glucose and the prevention of glycerol synthesis (Vaquero-Fernández *et al.*, 2008; Brycht *et al.*, 2015). This inhibits spore germination, germ tube elongation, and mycelium growth of *B. cinerea* and induces morphological alterations of germ tubes (Leroux, 1996; Rosslenbroich & Stuebler, 2000).

Fludioxonil has been used as postharvest treatment for stone fruit, pome fruit, pomegranates, kiwi fruit, and citrus (Brycht *et al.*, 2015). Developed in the mid-1900s to control *Botryrtis cinerea* in viticulture (Rosslenbroich & Stuebler, 2000), it is also highly effective on a large number of pathogens, including *Botrytis* spp., *Penicillium* spp., *Alternaria* spp, *Sclerotinia* ssp. (D'Aquino *et al.*, 2009). Although the application of fludioxonil has been studied on pomegranate (Adaskaveg & Förster, 2002; D'Aquino *et al.*, 2009; D'Aquino *et al.*, 2010), these studies majorly focused on control of fungal pathogens and decay incidence, as well as crop residue levels. There is limited information on the influence of FLU concentrations on the postharvest quality of pomegranate fruit, a factor that may affect the sensory and antioxidant property of fruit (Feliziani *et al.*, 2014). In addition,

the different cultivars used from different climatic regions in previous studies may also influence the response of fruit to the chemical. Therefore, the aim of this study was to evaluate the effects of treating harvested whole pomegranate fruit with different concentrations of fludioxonil on the physiological response, physico-chemical and sensory quality attributes, and antioxidant properties.

2. Material and methods

2.1. Plant material and chemicals

Pomegranate fruit (cv. Wonderful) were handpicked during commercial harvest from Heinrich F.R. Schaefer (HFR) farm in the Western Cape (33º44′26.185″S 18º44′41.193″E), South Africa and transported in a well-ventilated vehicle to the Postharvest Technology and Research Laboratory at Stellenbosch University. Fruit were selected based on uniform size and colour, and absence of physical damage such as cracks, sunburn and bruises.

The fungicide was a commercial formulation of FLU (Scholar®, Syngenta, South Africa) containing 23 % active ingredient (a.i.).

2.2. Treatments

Upon arrival at the laboratory, fruit were divided into four different treatment groups, each comprised of 108 fruit. Treatments were performed by dipping a batch of fruit in 15 L of scholar solution (a.i. 23 % fludioxonil). The four applied treatments were:

(**1**) Control: immersion in water.

(**2**) Immersion in 150 mg/L fludioxonil.

(**3**) Immersion in 300 mg/L fludioxonil.

(**4**) Immersion in 600 mg/L fludioxonil.

After immersion of each batch for 2 min, fruit surface was left to thoroughly dry at ambient room conditions (20 ± 2 °C and 65 ± 5 % RH) for 12 h before transfer to cold storage (5 ± 0.7 °C and 95 ± 2 % RH).

2.3. Packaging and storage

Fruit were packed into commercial ventilated cartons (0.4 m long, 0.3 m wide and 0.133 m high) used for postharvest handling of pomegranate fruit and put in cold storage for 4 months. Temperature and relative humidity within the cold room was recorded daily using Tiny Tag

TV-4500 data loggers (Gemini Data Logger, Sussex, UK). At the end of cold storage, a batch of fruit (n = 20) from each treatment was placed under ambient storage (20 - 24 °C and 65 - 70 % RH) for a 4-day period to simulate reasonable retail sale period. Fruit were then analysed for incidence of physiological response, physiological disorders, physico-chemical, textural and sensory properties after cold storage and shelf life. Measurement of all parameters was carried out on a monthly interval and results were presented as mean ± standard error (S.E).

2.4. Physiological response, decay and physiological disorders

2.4.1. Respiration rate

Fruit respiration was measured using a closed system as described by Caleb *et al.* (2012). In 5 replicates, two fruit of known weight were placed in a glass jar containing a rubber septum. The jar was sealed hermetically with vaseline to ensure a vacuum seal. Fruit were incubated for 2 h at 20 °C then gas composition inside each glass jar was measured using a calibrated O_2/CO_2 analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Carbon dioxide production was determined and results presented as mL CO_2 $kg^{-1}h^{-1}$ of five determinations.

2.4.2. Weight loss

Ten randomly selected fruit per treatment were used for this purpose. Fruit were weighed individually at monthly intervals during storage using an electronic scale (Mettler, Toledo, Switzerland, 0.0001 g accuracy). Cumulative weight loss of each fruit was calculated as:

$$W = \frac{(\mathrm{Wo} - \mathrm{Wi})}{\mathrm{Wo}} \times 100 \qquad (1)$$

Where W is the weight loss (%) of fruit; Wo is the weight (g) of fruit at the beginning of storage; Wi (g) is the weight of fruit at the storage time.

2.4.3. Fruit external and internal decay incidence

Fruit decay incidence was visually assessed as total rots. Fruit with any sign of external rot such as mould and crown rot was considered as external decay. Fruit with external decay appearance were counted and discarded. For internal decay, fruit with rotten arils and heart rot were counted and also discarded. For both external and internal decay, percentage of discarded fruit was calculated using the formula:

$$\text{Decay incidence (\%)} = \frac{\text{(Number of discarded fruit at each sampling date)}}{\text{Total number of fruit}} \text{ x } 100 \quad (2)$$

2.4.4. Incidence of chilling injury, husk scald and aril browning

Incidences of chilling injury, husk scald and aril browning were assessed monthly. The severity of disorders were assessed using a four-level scale as described by Fawole & Opara (2013a); where 0 = none (no symptom), 1 = trace (1 – 25 %), 2 = slight (26 – 50 %), 3 = moderate (51 – 75 %) and 4 = severe (76 – 100 %)

A physiological disorder index was calculated by multiplying the scores of severity by the number of affected fruits and dividing by the total number of assessed fruits (Artés *et al.*, 1998; Fawole & Opara, 2013a):

$$\text{Disorder index} = \sum \frac{\text{(Value of scale) x (Number of fruit with the corresponding scale number)}}{\text{Total number of fruit}} \text{ x } 100 \quad (3)$$

$$\text{Disorder incidence} = \frac{\text{(Number of affected fruit)}}{\text{Total number of fruit}} \text{ x } 100 \quad (4)$$

2.5. Physico-textural attributes

2.5.1. Whole fruit and aril colour

Colour parameters in CIELAB coordinates (L^*, a^*, b^*) were measured using a Chroma meter (CR-400, Minolta Corp, Osaka, Japan). Ten fruit per treatment were used to monitor changes in external colour by measuring peel colour at two opposite spots on individual fruit, while aril colour was determined by placing the arils in a colourless glass Petri dish. Colour intensity or chroma (C^*) and hue angle ($h°$) were calculated using equations (5) and (6) (Pathare *et al.*, 2013; Fawole & Opara, 2013a).

$$C* = \sqrt{(a*^2 + b*^2)} \quad (5)$$

$$h° = \arctan(b*/a*) \quad (6)$$

Furthermore, total colour difference (TCD) between the peel (external) and arils (internal) was calculated as;

$$TCD = \sqrt{(L*_o - L*)^2 + (a*_o - a*)^2 + (b*_o - b*)^2} \quad (7)$$

Where L^*_0, a^*_0 and b^*_0 are the colour parameters of the peel (reference value), while L*, a* and b* are the colour values of the aril (Al-Said *et al.*, 2009).

2.5.2. Fruit puncture resistance

Fruit puncture resistance was measured using a fruit texture analyser (GÜSS-FTA, model GS, South Africa). A 5 mm cylindrical probe was programmed to puncture 8.9 mm into the fruit on a steel test platform at the speed of 10 mm/s with the stem calyx axis parallel to the platform. Tests were performed in duplicate on the equilateral region of 10 individual fruit. Puncture resistance was determined as the peak force required to puncture the fruit surface.

2.5.3. Aril texture

Aril compression test was performed using a texture profile analyser XT Plus (Stable MicroSystem Ltd., Godalming, UK) equipped with a 35 mm diameter cylindrical compression probe. Compression test was performed on individual arils with the following operating conditions: pre-test speed 1.5 mm/s, probe test speed 1 mm/s, post-test speed 10.0 mm/s, compression force 10 N and compression distance 10 mm (Fawole & Opara, 2013b). Aril hardness/maximum compression force (N), toughness (area under the curve, N mm), elastic modulus (N/mm), and bioyield force (N) were captured using Exponent v.4 software (Stable MicroSystem Ltd., Godalming, UK). At each storage interval, tests were done using 20 arils extracted from 10 randomly selected fruit for each concentration and results presented as mean ± S.E of 20 determinations.

2.6. Chemical quality attributes

2.6.1. Titratable acidity, total soluble solids and pH

Titratable acidity (TA) was measured by diluting 2 ml of fresh juice with 70 ml of distilled water and titrating with 0.1M NaOH to an end point of pH 8.2 using a Metrohm 862 compact titrosampler (Herisua, Switzerland). The results were expressed as percentage of citric acid (% CA). Total soluble solids (TSS, °Brix) was measured using a digital refractometer (Atago, Tokyo, Japan) calibrated with distilled water. The pH values were determined at room temperature using a calibrated pH meter (Crison, Model 00924, Barcelona, Spain). BrimA, a criterion for consumer fruit juice acceptance was expressed as BrimA = TSS – k * TA, where k is the tongue's sensitivity index which normally ranges from 2 to 10. A value of k = 2 was used to avoid a negative BrimA index (Fawole & Opara, 2013c). All measurements were

made on 10 individual fruit juice samples for each treatment.

2.7. Sensory attributes

Sensory evaluation was carried out using a trained panel of 6 members of the Postharvest Technology Research Group at Stellenbosch University who are familiar with the characteristic taste of pomegranate fruit and regular consumers (Caine *et al.*, 2003; Sudha *et al.*, 2007; Chen & Opara, 2013). Panelists received further orientation on pomegranate attributes (Vázquez-Araújo *et al.*, 2011). Sensory evaluation was carried out on arils (10 g) served at 21 °C on Petri dishes randomly coded (Fawole & Opara, 2013b). The descriptive test required panelists to rate the intensity of the attributes on a scale of 0 – 4 (0 = none, 1 = slight, 2 = moderate, 3 = much, 4 = very much). The descriptive attributes evaluated for the study included sweet taste, sour taste, crispness, astringency, off flavor, juiciness, grittiness and hardness. Sensory evaluation was not carried out beyond 3 months of storage due to decay and limited sample size.

3. Statistical analysis

Statistical analysis was carried out using Statistica software (Statistica version 14.0, StatSoft Inc., Tulsa, USA). Data was subjected to factorial analysis of variance (ANOVA) at 95 % confidence interval. Main effects (FLU concentration and storage duration) and their interaction effects (concentration*storage duration) were also assessed. Post-hoc test (Duncan's Multiple Range Test) was used to test for statistical significance such that observed differences at $p < 0.05$ were considered significant. Principal component analysis (PCA) was carried out using XLSTAT software version 2012.04.1 (Addinsoft, France).

4. Results and discussion

4.1. Physiological response

4.1.1. Fruit respiration rate

Results showed that fruit respiration rate was influenced mainly by storage duration ($p < 0.0001$), with upsurge in respiration rate between 1 to 3 months of storage followed by a decline in the last month regardless of FLU concentration (Fig. 1). The first month of storage showed differences in fruit respiration rate, with fruit treated with 150 and 600 mg/L FLU concentration showing the highest and lowest respiration rates, respectively. Thereafter, fruit

respiration rate followed a similar trend with no significant difference among concentrations as storage progressed (Fig. 1). Respiration of pomegranate fruit has been found to increase with advancement of storage (Barman *et al.*, 2011; Fawole & Opara, 2013a). The initial increase in respiration could be due to increased fruit stress as a result of senescence and metabolic reactions as these phenomena have been reported to trigger respiration of pomegranate fruit (Fawole & Opara, 2013a). The decline in respiration at the end of storage could possibly be due to excessive senescence, physiological disorders and cell death of the membrane due to reduced number of living cells (Nanda *et al.*, 2001; D'Aquino *et al.*, 2010). Therefore, treating pomegranate fruit with fludioxonil did not significantly affect the respiration rate of stored fruit since the changes were mainly influenced by the duration of storage (FLU concentration = 0.2760, Duration = < 0.0001).

4.1.2. Weight loss

Weight loss is considered as the main cause of loss of visual quality in horticultural produce as excessive transpiration leads to desiccation, shriveling and hastened senescence (Ben-Yehoshua & Rodov, 2003). In this study, fruit lost weight over the entire storage period but the magnitude was dependent on concentration of fludioxonil treatment as evidenced by the significant effects of concentration ($p < 0.0001$) and storage duration ($p < 0.0001$) (Fig. 2). After cold storage, fruit treated with FLU at 150 mg/L concentration showed the highest weight loss (26.77 %) compared with those treated with 300 mg/L (20.86 %) or 600 mg/L (20.98 %) FLU (Fig. 2). In particular, fruit treated with 150 mg/L FLU concentration had higher weight loss (8.51 %, 15.8 %, 21.81 % and 26.86 %) after storage (1, 2, 3 and 4 months, respectively). Since weight loss is linked to respiration rate (Fawole & Opara, 2013a), the high weight loss could be explained by the high respiration rate that was observed for this treatment after the first and last month storage (Fig. 1). Treatment of pomegranate fruit with higher FLU concentrations (300 and 600 mg/L) reduced weight loss during cold storage (Fig. 2). The reduction in weight loss could possibly be due to the reduction in fruit decay incidence especially for fruit treated with the high concentrations because high decay incidences have been reported to increase weight loss of other types of fruit such as citrus (Montero *et al.*, 2010).

4.1.3. Fruit decay

Fruit decay is a major cause of postharvest loss during storage of pomegranate fruit. Fruit decay increased with storage time, with low decay incidences observed after the first 2

months of storage but later increased with long storage (Fig. 3A). High decay incidence was observed for control fruit especially after 3 and 4 months of storage. It is also worth mentioning that percentage fruit decay seemed to decrease with FLU concentration at the end of the storage period (4 months), with 150, 300 and 600 mg/L having 10.83 %, 12.39 % and 15.76 % lower decay compared to control respectively (Fig. 3A). The decrease in decay among treated fruit is expected since fludioxonil is a fungicide with excellent protective and preventive activity on a large number of pathogens (D'Aquino *et al.*, 2010). Fludioxonil is a reduced-risk fungicide that has a wide spectrum against many pathogens and has been used to control decay in a number of fruits. It has been shown to be effective against decay in other crops like stone fruit (Adaskaveg *et al.*, 2005), pear (Schirra *et al.*, 2009) and citrus (D'Aquino *et al.*, 2010; D'Aquino *et al.*, 2013), and has also been shown to be highly effective at low rates against large spectrum of fungi such as *Botrytis cinerea* and *Penicillium* spp. (D'Aquino *et al.*, 2010). Despite FLU reducing the decay in the fruit, complete control of decay was not achieved. This is possible because FLU is non-systemic and is not able to move deep through the peel of the fruit (D'Aquino *et al.*, 2013). Similar results have been reported with good control of decay of FLU in various fruit including citrus (Zhang, 2007), cactus pear (D'Aquino *et al.*, 2015) and pomegranate (D'Aquino *et al.*, 2010).

4.1.4. Internal decay

The major internal decay disorder observed was heart rot, also known as black heart, caused by *Aspergillus niger* and *Alternaria* spp., which is characterised by a mass of black arils (Yehia, 2013). This is a preharvest disease that affects the postharvest quality of pomegranate fruit and from the results, it increased with storage of fruit during the study (Fig. 3B). Fruit treated with 150 mg/L FLU concentration developed internal decay earlier but maintained the same percentage decay throughout the storage duration whereas 300 mg/L and control fruit developed decay after storage for 2 months. The highest concentration (600 mg/L) developed decay latest and had the lowest percentage internal decay at the end of storage. No direct relationship can be drawn on the effect of fludioxonil on the internal decay of pomegranate fruit as some treatments had higher decay than untreated fruit although it could possibly be that fludioxonil reduced the growth and proliferation of the fungi that caused internal decay after long storage. Internal decay due to heart rot has been reported to occur as a result of infection of fruit during flowering in the orchard (Zhang & McCarthy, 2012; Ezra *et al.*, 2015). The efficacy of FLU on internal decay could be questionable because despite being an excellent protective and preventative fungicide, its curative activity is decreased on old

established latent infections because it is a non-systemic fungicide and also due to the difficulty of the solution in entering the crown of pomegranate fruit (D'Aquino *et al.*, 2010). Therefore, being a preharvest condition (Zhang & McCarthy, 2012; Ezra *et al.*, 2015), this could probably best be prevented by observing good agricultural practices and application of preharvest treatments.

4.1.5. Aril browning

Aril browning increased with progressive storage of fruit with untreated fruit consistently showing lower browning of arils compared to treated fruit throughout the storage duration (Fig. 3C). Aril browning has been associated with enzymatic oxidation and chilling injury in pomegranate fruit (Mirdehghan & Rahemi, 2005). The lower browning of untreated fruit could be related to the higher chilling injury that was observed for treated fruit compared to control (Fig. 4A and B). FLU is a non-systemic fungicide (D'Aquino *et al.*, 2010) and hence does not migrate deep into the fruit. Therefore, its protective effect could be more external with no direct effect on internal (aril) browning. Fawole & Opara (2013a) also observed that internal disorders such as browning and decay increased with progressive storage of 'Bhagwa' and 'Ruby' pomegranate cultivars stored for 16 weeks at 5, 7 and 10 °C.

4.1.6. Chilling injury incidence and severity

Chilling injury (CI) developed from the first sampling date, with an increase in chilling injury percentage incidence as storage advanced indicating an increase in number of affected fruit with advancing storage period. Control fruit had a low incidence of CI while fruit treated with 150 and 300 mg/L FLU concentrations had high incidences throughout the storage period with incidences of 68.58 % and 66.79 % CI, respectively, at the end of storage (Fig. 4A). Despite the occurrence of chilling injury, the severity of the physiological disorder (index) remained below trace level for all concentrations throughout the storage period (Fig. 4B). During chilling conditions, there is a change in state of lipid cell membranes from liquid-crystalline to solid-gel state which causes deleterious effects on the tissues (Mirdehghan *et al.*, 2007; Barman *et al.*, 2011). From the results, it is apparent that fludioxonil does not possess antioxidant properties that could have reduced chilling injury symptoms in treated fruit. Despite its strong antifungal property (D'Aquino *et al.*, 2010), the current study suggests that fludioxonil had no beneficial effect of alleviating chilling injury in pomegranate fruit. A similar observation was reported for citrus (oranges, lemon, grapefruit and clementine mandarins) treated with Fludioxonil and Imazalil at 20 °C (Schirra *et al.*, 2005).

On the contrary, however, fludioxonil has been reported to reduce the typical symptoms of chilling injury and preserve fruit freshness of cactus pear especially when used at 50 °C (D'Aquino *et al.*, 2015). The differences could be due to fruit physiology and morphology, as different fruits may response differently to chemical treatments.

4.1.7. Husk scald incidence and severity

Husk scald together with weight loss and skin pitting are the main physiological disorders responsible for market downgrading in pomegranates (D'Aquino *et al.*, 2012). Husk scald incidence increased with storage, with low incidences observed after the first two months of storage but greatly increased after the last months of storage (Fig. 5A). After the first two months, control fruit had the lowest husk scald incidence while fruit treated with 300 mg/L FLU concentration had the highest incidences (Fig. 5A). After 3 months, 150 mg/L concentration showed the lowest scalding but by the end of storage, regardless of treatment, all fruit had developed scald (Fig. 5A). Husk scald severity (index) was low for the first two months of storage with all treatments having below trace scald (Fig. 5B). After 3 months of storage, scald was between trace and slight with fruit treated with 600 mg/L concentration showing the lowest incidence. The last month of storage was prominently characterised by high scald severity between moderate and severe although control fruit showed the lowest severity (Fig. 5B). This indicates that treating fruit with fludioxonil was not effective in alleviating husk scald in pomegranate fruit. This is in agreement with D'Aquino *et al.* (2012) who reported no significant effect of fludioxonil on husk scald and overall appearance of pomegranate fruit (cv. Primosole) during 12 weeks of storage at 8 °C. However, application of fludioxonil alone or with sodium bicarbonate reduced peel disorders such as scald on cactus pear, with treatments resulting in better fruit appeal compared to untreated fruit (D'Aquino *et al.*, 2015). Husk scald is as a result of enzymatic oxidation of the phenolic compounds in the fruit peel. Tissue browning is reported to be due to oxidation of phenolic compounds into quinone compounds under aerobic conditions by polyphenol oxidase. The quinones then undergo polymerization forming brown pigments thus leading to browning (Kahn, 1983; Zhang & Zhang, 2008). Zhang & Zhang (2008) reported enzyme-mediated denaturation of skin tannins as the basis for pomegranate fruit browning. This was further supported by D'Aquino *et al.* (2010) who observed that husk scald of 'Wonderful' pomegranate at 6 or 10 °C decreased and progressed at a slower rate when the tension of oxygen reduced, however, the incidence of scald increased after transfer of fruit to 20 °C in conventional atmosphere. The development of scald in all fruit at the end of storage (Fig. 5B)

indicates the relevance of combining FLU treatment with other treatments that can control oxygen supply to the fruit during storage, for instance, physical treatments like wrapping, coatings and controlled atmosphere.

4.2. Fruit firmness

As shown in Figure 6, changes in fruit firmness were driven by both FLU concentration ($p = 0.0228$) and storage duration ($p = 0.0023$). Fruit firmness at the end of storage was better maintained among fruit treated with FLU compared to control (Fig. 6). The loss in fruit firmness during storage is due to loss of cell wall integrity resulting from the breakdown of pectic substances, which in turn leads to an increase in soluble pectin and thus a decline in firmness (Sayyari *et al.*, 2011). Generally, the decline in fruit firmness was more evident for control fruit at the later period of storage (3 - 4 months), indicating a faster breakdown of pectic substance in the peel of control fruit (Sayyari *et al.*, 2011).

4.3. Aril texture

In this study, aril elastic modulus decreased from harvest, however, no significant differences were observed during storage as evidenced by the non-significant effect of FLU concentration ($p = 0.3719$), storage duration ($p = 0.3641$) and their interaction ($p = 0.2688$) (Table 1). Aril hardness was however influenced by FLU concentration and storage duration ($p < 0.0001$). Aril hardness increased after the first two months and thereafter decreased after the last two months of storage for control and 150 mg/L FUL concentration while 300 mg/L FLU concentration decreased after two months and then increased after 3 and 4 months of storage with significant interaction of the two factors ($p < 0.0001$). Aril toughness generally decreased compared to values at harvest except for 600 mg/L FLU concentration, which showed highest toughness after 4 months of storage (172.4 ± 6.96 N) (Table 1). Changes in aril toughness were influenced by FLU concentration ($p < 0.0001$) with control fruit having lowest aril toughness values compared to FLU treated fruit all along the storage duration. Decrease in aril toughness could be attributed to softening of arils as a result of membrane deterioration (Bchir *et al.*, 2012; Fawole & Opara, 2013b) indicating that arils of control fruit softened faster than those of FLU treated fruit. Bioyield on the other hand was stable throughout storage with no significant effect of FLU concentration ($p = 0.9988$) or storage duration ($p = 0.8255$) (Table 1).

4.4. Fruit peel and aril colour parameters

4.4.1. Fruit peel colour

Colour of pomegranate is an important quality attribute that is fundamental for consumer preference (Pathare *et al.*, 2013). Generally, peel redness (a*) decreased with storage for all concentrations, with no significant difference in peel redness amongst FLU concentrations except at the end of 3 months in cold storage (Table 2). However, changes in peel redness were influenced by storage duration ($p < 0.0001$). The decrease in redness could be as a result of breakdown of peel colour pigments due to senescence during storage and also peel browning as a result of physiological disorders (Arendse *et al.*, 2014). Peel colour intensity (C*) followed a similar pattern with progressive storage, irrespective of concentration during a short term storage (Table 2). It is, however, worth mentioning that after 3 months of storage, control fruit had the highest colour intensity (C* = 47.88) while fruit treated with 150 mg/L FLU concentration had the lowest values (C* = 38.23) (Table 2). Storage duration affected peel hue angle (h^o) ($p < 0.0001$) which generally increased with fruit storage with no significant differences observed at all storage periods for all concentrations (Table 2). Again, this indicates loss of red colouration in fruit peel with storage duration possibly due to the development of physiological disorders such as husk scalding in fruits and degradation of anthocyanin pigments (Arendse *et al.*, 2014). The findings in this current study corroborate the report by D'Aquino *et al.* (2012) who found that treating 'Primosole' pomegranates fruit with FLU (at 600 mg/L) had no significant effects on the overall appearance after cold storage at 8 °C for 12 weeks.

4.4.2. Aril colour

Results showed a decrease in aril colour redness (a*) for all concentrations after the first 3 months of storage followed by a slight increase with long storage. The effect of FLU concentration was significant ($p < 0.0001$) with untreated fruit having highest aril a* from the second to the last month of storage (Table 2). The initial decrease in aril a* could be attributed to browning of arils due to breakdown of red colour pigments (Arende *et al.*, 2014). A similar trend was observed for aril colour intensity (C*) with untreated fruit having significantly higher aril C* throughout the storage duration and significant effect of concentration ($p < 0.0001$) (Table 2). The high C* in untreated fruit corresponds with the highest aril a* indicating greater anthocyanin biosynthesis in control compared to treated

fruit. The high a* and C* in untreated fruit can be related to the lower aril browning that was observed for untreated fruit (Fig. 3C). However, although arils from treated fruit had lower C* and a* values, the aril colour was sufficient enough for consumption. Aril hue angle (h^o) differed with storage of fruit with no significant differences observed among all concentrations at all storage times (Table 2). The total colour difference (TCD) indicates the colour disparity between the peel and aril and this was influenced by the storage duration. TCD decreased during storage however, no significant differences were observed among concentrations during storage with the exception of month 3 (Table 2). This effect of FLU is similar to a study by Feliziani *et al.* (2015) who reported no significant change in the color luminescence (L*), red tone (a*) and yellow tone (b*) of 'Alba' and 'Romina' strawberry fruit after a fungicide strategy of cyprodinil and fludioxonil was applied as preharvest treatments while benzothiadiazole and chitosan treatments reduced a* and L* values respectively.

4.5. Chemical properties

4.5.1. pH, TA, TSS, TSS/TA and BrimA

There were slight fluctuations observed in pH during storage of fruit (Table 3), with a significant interaction ($p < 0.0001$) between FLU concentration and storage duration. With regard to TA, changes were driven by the combined effect of storage duration ($p < 0.0001$) and FLU concentration ($p = 0.0004$) (Table 3). In general, TA decreased with prolonged storage duration except after month 3 where slight increases were observed probably due to the concentration of acids resulting from weight loss (Fawole & Opara, 2013a). Organic acids (which mainly contribute to titratable acidity) have been reported to be the major substrates for respiration during storage of pomegranate fruit (Kader *et al.*, 1984; Fawole & Opara, 2013a) hence the reduction in TA as storage advanced. The decrease in TA, therefore, could be due to utilisation of organic acids in fruit respiration. Similarly, when 'Montenegrina' tangerine was treated with the fungicide Imazalil or sodium bicarbonate and stored at 5 °C for 20 days, no changes were observed in the TA for Imazalil treatment whereas sodium bicarbonate reduced fruit acidity slightly (Montero *et al.*, 2010). However, Rouchaud *et al.* (1984) reported that preharvest treatment of 'Jonagold' apple trees with fungicides generally increased the fruit citric acid content while total acids were generally not changed after postharvest of fruit and storage at 2 °C for 15 days. In fruit, each acid and sugar have its own taste and acidity enhances fruit flavour (Rouchaud *et al.*, 1984).

There was a general decrease in TSS during storage with no significant ($p = 0.6640$) effect of concentration on TSS values (Table 3). At the end of storage, no significant differences were observed among the treated fruit, which had lower TSS values than control fruit. In addition, a significant interaction ($p = 0.0001$) between concentration and storage duration was observed (Table 3). A similar reduction in TSS during storage of pomegranate fruit has been previously reported (Artés *et al.*, 1998; 2000; Fawole & Opara, 2013a). However with regard to the postharvest treatment of pomegranate fruit, Mirdehghan *et al.* (2007) observed no changes in TSS during storage of pomegranate fruit treated with polyamines. In the current study, the observed reduction in TSS with prolonging storage duration could be attributed to the utilisation of sugars in some metabolic process such as fruit respiration during storage (Fawole & Opara, 2013a).

TSS/TA ratio and BrimA, based on changes in soluble solids and titratable acidity in fruit during storage determine the characteristic taste and flavour of fruit (Zarei *et al.*, 2011; Arendse *et al.*, 2014). Generally, TSS/TA ratio increased with prolong storage duration (month 3 being an exception), suggesting faster depletion of organic acids compared to TSS in the fruit (Table 3). As regard BrimA, there were no significant differences amongst treatments. As indicated by the factorial analysis, the observed changes were influenced by both FLU concentration and storage duration for TSS/TA ratio, while a significant interaction between FLU concentration and storage duration was observed for BrimA (Table 3). It is also worth mentioning that TSS/TA ratio in control fruit was lower compared to treated fruit, indicating a loss in taste and flavour of the fruit.

4.6. Sensory attributes

After storage of fruit for one month, sweet taste of fruit arils was high while sour taste was low for fruit treated at 600 mg/L FLU concentration (Fig. 7A). Off flavour of arils was generally low, less than 0.5 for all concentrations. Astringency was more pronounced for 150 mg/L and 300 mg/L FLU concentration. Crispiness and juiciness were greater for 600 m/L while grittiness and hardness were high for 300 mg/L and control fruit (Fig. 7A). From the results, treating pomegranate fruit with the highest concentration (600 mg/L) of fludioxonil achieved the best sensory characteristics after storage for 1 month.

After two months of storage, fruit treated with 150 mg/L FLU concentration had a high sweet taste (Fig. 7B) which can be related to the higher TSS that was observed for this treatment (Table 2). Sour taste was generally low with similarities among concentrations.

Pomegranate of the 'Wonderful' cultivar is a sweet-sour cultivar and its acidic content contributes the taste of the cultivar. The sweet-sour taste is preferred in some regions of the world depending on consumer preference (Holland *et al.*, 2009). The sour taste is only undesirable when the sourness is extreme as can sometimes happen in some fruits. Off flavour was generally very low less than 0.4 (Fig. 7B). Off flavour indicates spoilage or fermentation in the fruit due to a build-up of alcoholic by-products like ethanol. This is usually more important in minimally processed arils and whole fruit treatments that involve alternating oxygen supply which may cause anaerobic respiration such as MAP, controlled atmosphere storage and film wrapping. Aril crispiness and juiciness was high for 150 mg/L and control fruit. Grittiness and hardness were generally low but highest for 600 mg/L. After storage for 2 months, 150 mg/L FLU concentration maintained the best sensory parameters.

As storage progressed to three months, the untreated fruit had the highest sweet taste which could be due to the concentration of sugars from weight loss (Fig. 7C). Sour taste was high for 150 mg/L and low for untreated fruit while off flavour increased for all concentrations compared to earlier storage periods. Astringency was still quite low among concentrations with 150 mg/L having the highest values while grittiness was high for 300 mg/L (Fig. 7C). Although fruit treated with 600 m/L FLU concentration had the highest aril hardness, the treatment resulted in the best aril crispiness and juiciness. Untreated fruit had good sensory quality although 600 mg/L FLU concentration retained the best characteristics for most sensory attributes.

4.7. Multivariate analysis

4.7.1. Pearsons correlation analysis of physiological responses and disorders

Pearsons' correlation was used to investigate the relationships between the physiological disorders. Fruit weight loss increases with increased respiration and decay and this was indicated by the moderate positive correlation (r = 0.63 and r = 0.77 for respiration rate and weight loss respectively) (Table 4). Weight loss of fruit strongly correlated with fruit decay, chilling injury, scald and aril browning indicating the impact of physiological disorders on fruit weight. Chilling injury correlated strongly with aril browning supporting findings by Elyatem & Kader (1984), who reported that chilling injury affects fruit internal quality. Husk scald strongly associated with fruit decay (Table 4) suggesting that development of scald enhances suspetibilty of fruit to decay possibly due to increased senescence. (Defilippi *et al.*, 2006) reported that scald does not affect internal quality of fruit however, the strong positive correlation showed that development of scald increases aril browning severity (r = 0.93)

(Table 4). Internal decay correlated moderately with respiration rate, weight loss, chilling injury, scakld and aril browning.

4.7.2. Principal component analysis

Examination of eigenvalues and loadings showed that major changes in fruit were dependent on storage duration (Table 5).The factor scores relate with the factor loadings, as the factor loadings indicate the strength of correlation between the variables and factors. The factor scores can be interpreted with factor loadings. Classification of factor loadings is considered 'strong' for values > 0.75, 'moderate' for 0.75 - 0.50 and 'weak' for 0.50 - 0.30 (Liu *et al.*, 2003; Fawole & Opara, 2013d). Along F1, positive factor scores for 150 mg/L FLU concentration (month 1) corresponded moderately to strongly with a crispness, peel a* and C*, aril a* and C* and elastic modulus (Table 5). High negative scores along F1 for 150 mg/L (month 3) correlated with sour taste, off flavour, grittiness, hardness, aril hardness peel h^o and pH (Table 5). Along F2, high positive scores correlated strongly with sweet taste, crispness, juiciness, pH, TSS/TA and BrimA while high negative scores strongly correlated with sour taste, grittiness, aril h^o and TA (Table 5). In general, the study showed that fruit stored for 2 months had the best sensory attributes and described by sweet taste, crispness, juiciness and TSS while fruit stored for a long time (3 months) had sour taste, off flavor, grittiness and hardness (Fig. 8A and B). This indicated quality and sensory deterioration of fruit with long term storage.

5. Conclusions

Treating pomegranate fruit with FLU concentrations improved fruit attributes such as fruit reducing fruit decay, weight loss, fruit firmness and improving sensory quality. The sensory attributes of FLU treated fruit were maintained especially when treated with 600 mg/L FLU concentration. However, the fungicide was not beneficial in reducing the incidence of physiological disorders such as aril browning, chilling injury, husk scald and maintaining aril redness. Therefore, further studies should be carried out on the use of hurdle technology where the fungicide may be used in combination with other technologies such as physical treatments to fully harness the potential of the chemical as postharvest technology for improved handling of pomegranates (cv. Wonderful).

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Table 1 Aril textural parameters of pomegranate fruit treated with fludioxonil during storage for 4 months at 5 °C and additional 4 days at 20 °C

Storage duration (month)	Concentration (mg/L)	Elastic modulus (N/mm)	Hardness (N)	Toughness (N.mm)	Bioyield (N)
Harvest		8.57 ± 0.49	157.20 ± 2.81	169.90 ± 3.83	5.96 ± 0.82
1					
	0 (Control)	5.85 ± 0.49^{ab}	139.31 ± 3.05^{e}	119.80 ± 4.36^{d}	6.17 ± 0.78^{a}
	150	7.21 ± 0.65^{a}	148.80 ± 4.15^{a-d}	154.10 ± 6.60^{bcd}	6.48 ± 0.74^{a}
	300	5.35 ± 0.58^{ab}	155.40 ± 3.55^{ab}	163.40 ± 6.29^{ab}	4.91 ± 0.61^{a}
	600	5.63 ± 0.48^{ab}	154.20 ± 3.32^{abc}	165.20 ± 5.21^{ab}	5.48 ± 0.48^{a}
2					
	0 (Control)	6.21 ± 0.46^{ab}	142.80 ± 5.42^{cd}	152.30 ± 8.38^{bc}	6.25 ± 0.51^{a}
	150	4.99 ± 0.32^{ab}	158.10 ± 4.07^{a}	165.30 ± 5.49^{ab}	5.44 ± 0.39^{a}
	300	6.14 ± 0.41^{ab}	139.60 ± 2.19^{d}	155.30 ± 3.71^{abc}	6.25 ± 0.55^{a}
	600	6.30 ± 0.59^{ab}	154.50 ± 3.95^{abc}	164.90 ± 5.85^{ab}	6.42 ± 0.73^{a}
3					
	0 (Control)	6.42 ± 0.51^{a}	151.40 ± 3.57^{a-d}	152.60 ± 5.91^{bc}	5.96 ± 0.62^{a}
	150	5.02 ± 0.49^{ab}	153.90 ± 3.07^{abc}	160.80 ± 5.41^{abc}	5.79 ± 0.61^{a}
	300	5.47 ± 0.35^{ab}	154.00 ± 3.73^{abc}	164.10 ± 4.74^{ab}	6.05 ± 0.54^{a}
	600	5.54 ± 0.47^{ab}	155.50 ± 3.74^{ab}	162.80 ± 4.34^{ab}	5.90 ± 0.56^{a}
4					
	0 (Control)	5.34 ± 0.63^{ab}	143.20 ± 3.84^{bcd}	143.30 ± 5.66^{c}	6.53 ± 0.92^{a}
	150	4.71 ± 0.43^{b}	145.80 ± 3.23^{a-d}	151.60 ± 3.92^{bc}	5.80 ± 0.59^{a}
	300	5.37 ± 0.33^{ab}	152.50 ± 2.48^{abc}	167.30 ± 3.96^{ab}	6.91 ± 0.56^{a}
	600	5.76 ± 0.51^{ab}	157.50 ± 5.47^{a}	172.40 ± 6.96^{a}	6.91 ± 0.63^{a}
Significance level					
	Concentration (A)	0.3719	**<0.0001**	**<0.0001**	0.9988
	Storage duration (B)	0.3641	**<0.0001**	0.0525	0.8255
	A x B	0.2688	**<0.0001**	**0.0051**	0.3695

Data presented as mean± SE. Different letters across concentration and storage duration for each attribute differ significantly ($p < 0.05$) according to Duncan's multiple range test. SE - standard error

Table 2 Peel and aril colour parameters of pomegranate fruit treated with fludioxonil during storage for 4 months at 5 °C and additional 4 days at 20 °C

Storage duration (month)	Concentration (mg/L)	Peel			Aril			TCD
		a*	C*	*h*°	a*	C*	*h*°	
Harvest		42.54 ± 1.29	51.67 ± 1.03	34.73 ± 1.05	16.33 ± 0.69	18.09 ± 0.78	24.27 ± 1.04	46.15 ± 0.92
1								
	0 (Control)	38.21 ± 2.47^{ab}	49.09 ± 1.59^{a}	38.63 ± 2.75^{bcd}	20.19 ± 1.34^{a-d}	22.27 ± 1.44^{abc}	24.86 ± 1.16^{a}	40.31 ± 1.75^{abc}
	150	39.47 ± 1.44^{a}	48.48 ± 1.18^{a}	35.36 ± 1.43^{d}	18.28 ± 0.98^{b-f}	20.19 ± 1.02^{a-f}	23.33 ± 1.46^{ab}	43.29 ± 1.97^{a}
	300	37.36 ± 2.27^{ab}	46.67 ± 1.45^{ab}	36.52 ± 2.69^{cd}	20.23 ± 0.89^{abc}	21.57 ± 1.10^{a-e}	24.59 ± 1.61^{a}	38.30 ± 1.60^{a-e}
	600	38.27 ± 1.66^{ab}	46.73 ± 1.29^{ab}	35.12 ± 1.53^{d}	17.87 ± 0.98^{b-f}	19.43 ± 1.12^{a-f}	22.17 ± 0.85^{ab}	41.14 ± 1.43^{abc}
2								
	0 (Control)	36.13 ± 1.85^{ab}	47.37 ± 1.38^{ab}	39.86 ± 2.36^{bcd}	20.89 ± 1.68^{ab}	22.78 ± 1.92^{ab}	22.74 ± 0.83^{ab}	39.10 ± 2.31^{a-d}
	150	33.33 ± 1.41^{bcd}	44.92 ± 0.84^{bc}	41.08 ± 1.78^{a-d}	17.85 ± 0.85^{b-f}	19.23 ± 0.97^{b-f}	21.25 ± 0.63^{ab}	40.85 ± 1.41^{abc}
	300	35.29 ± 1.64^{abc}	47.26 ± 1.16^{ab}	41.48 ± 1.94^{a-d}	19.14 ± 0.81^{a-e}	21.00 ± 0.93^{a-e}	23.84 ± 0.94^{ab}	42.27 ± 2.08^{ab}
	600	33.76 ± 1.52^{bcd}	44.40 ± 1.11^{bc}	40.38 ± 1.74^{bcd}	17.09 ± 0.68^{def}	18.86 ± 0.81^{c-f}	23.90 ± 1.38^{ab}	38.22 ± 1.73^{a-e}
3								
	0 (Control)	34.89 ± 2.19^{abc}	47.88 ± 1.42^{ab}	43.14 ± 2.47^{ab}	20.59 ± 0.95^{ab}	22.16 ± 1.09^{a-d}	21.44 ± 0.50^{ab}	43.80 ± 1.67^{a}
	150	29.13 ± 1.01^{def}	38.23 ± 0.77^{ef}	41.96 ± 1.911^{abc}	15.32 ± 0.56^{f}	16.83 ± 0.64^{f}	23.54 ± 1.12^{ab}	35.43 ± 1.55^{cde}
	300	30.45 ± 1.54^{c-f}	41.80 ± 1.11^{cd}	43.18 ± 1.84^{ab}	16.99 ± 0.94^{ef}	18.37 ± 1.11^{ef}	21.28 ± 0.77^{ab}	36.92 ± 0.94^{b-e}
	600	30.94 ± 1.16^{cde}	42.54 ± 1.01^{cd}	43.57 ± 1.54^{ab}	17.09 ± 0.68^{c-f}	18.62 ± 0.83^{def}	21.85 ± 0.50^{ab}	43.55 ± 2.01^{a}
4								
	0 (Control)	28.48 ± 1.29^{ef}	40.30 ± 0.75^{def}	44.61 ± 2.11^{ab}	21.43 ± 1.06^{a}	22.89 ± 1.13^{a}	20.49 ± 0.49^{b}	33.14 ± 1.92^{ef}
	150	26.01 ± 1.06^{f}	35.16 ± 0.72^{f}	41.57 ± 2.12^{a-d}	18.50 ± 0.94^{a-e}	20.43 ± 1.10^{a-e}	24.29 ± 1.09^{a}	28.92 ± 2.31^{f}
	300	26.21 ± 1.01^{ef}	36.06 ± 0.98^{f}	42.65 ± 1.92^{abc}	19.57 ± 1.05^{a-e}	21.55 ± 1.13^{a-e}	24.00 ± 1.38^{ab}	29.76 ± 1.89^{f}
	600	25.96 ± 1.11^{f}	38.45 ± 0.87^{ef}	47.41 ± 1.47^{a}	17.14 ± 0.67^{c-f}	18.33 ± 0.89^{ef}	23.32 ± 1.17^{ab}	33.67 ± 1.60^{def}
Significance level								
	Concentration (A)	0.157	**<0.0001**	0.6152	**<0.0001**	**<0.0001**	0.6146	0.1238
	Storage duration (B)	**<0.0001**	**<0.0001**	**<0.0001**	**0.0483**	0.0598	0.1932	**<0.0001**
	A x B	0.7878	**0.0033**	0.7304	0.6649	0.7566	0.0595	**0.0052**

Data presented as mean ± SE. Different letters across concentration and storage duration for each attribute differ significantly ($p < 0.05$) according to Duncan's multiple range test. SE - standard error

Table 3 Chemical attributes and juice colour of pomegranate fruit treated with fludioxonil during storage for 4 months at 5 °C and additional 4 days at 20 °C

Storage duration (month)	Concentration (mg/L)	pH	TA (% citric acid)	TSS (°Brix)	TSS/TA	BrimA	Juice colour absorbance (520nm)
Harvest		3.28 ± 0.03	1.68 ± 0.08	16.2 ± 0.16	9.98 ± 0.41	12.84 ± 0.18	3.28 ± 0.01
1							
	0 (Control)	2.98 ± 0.07^{h}	2.53 ± 0.28^{a}	14.83 ± 0.21^{d}	6.40 ± 0.85^{d}	9.76 ± 0.60^{g}	3.08 ± 0.14^{abc}
	150	3.05 ± 0.05^{gh}	2.46 ± 0.17^{a}	15.22 ± 0.24^{bcd}	6.43 ± 0.39^{d}	10.31 ± 0.32^{efg}	3.26 ± 0.01^{a}
	300	3.15 ± 0.05^{fg}	2.09 ± 0.11^{bc}	15.81 ± 0.26^{ab}	7.77 ± 0.47^{cd}	11.63 ± 0.33^{bcd}	3.18 ± 0.06^{abc}
	600	3.38 ± 0.04^{cd}	2.04 ± 0.17^{bc}	16.01 ± 0.22^{ab}	8.31 ± 0.69^{cd}	11.92 ± 0.45^{bcd}	3.27 ± 0.03^{a}
2							
	0 (Control)	3.25 ± 0.15^{def}	1.44 ± 0.14^{efg}	15.21 ± 0.35^{bcd}	11.33 ± 1.45^{b}	12.75 ± 0.63^{ab}	3.16 ± 0.07^{abc}
	150	3.76 ± 0.05^{a}	1.46 ± 0.09^{efg}	16.06 ± 0.16^{a}	11.37 ± 0.64^{b}	13.14 ± 0.25^{a}	3.26 ± 0.01^{a}
	300	3.25 ± 0.03^{def}	1.27 ± 0.08^{ghi}	15.64 ± 0.23^{abc}	12.58 ± 0.75^{ab}	13.09 ± 0.37^{a}	3.26 ± 0.01^{a}
	600	3.23 ± 0.03^{def}	1.36 ± 0.08^{fgh}	15.27 ± 0.17^{a-d}	11.59 ± 0.70^{b}	12.55 ± 0.19^{abc}	3.16 ± 0.08^{abc}
3							
	0 (Control)	3.49 ± 0.05^{bc}	2.24 ± 0.15^{ab}	14.70 ± 0.41^{d}	6.14 ± 0.33^{d}	10.23 ± 0.59^{fg}	3.20 ± 0.01^{ab}
	150	3.21 ± 0.07^{ef}	1.67 ± 0.07^{def}	14.74 ± 0.20^{d}	8.95 ± 0.38^{c}	11.39 ± 0.25^{cde}	3.18 ± 0.03^{abc}
	300	3.63 ± 0.03^{ab}	1.75 ± 0.04^{cde}	15.28 ± 0.18^{bcd}	8.80 ± 0.27^{c}	11.78 ± 0.23^{bcd}	3.24 ± 0.01^{a}
	600	3.66 ± 0.05^{a}	1.88 ± 0.08^{cd}	14.91 ± 0.23^{cd}	8.07 ± 0.44^{cd}	11.15 ± 0.37^{def}	3.14 ± 1.11^{abc}
4							
	0 (Control)	3.61 ± 0.04^{ab}	1.26 ± 0.04^{ghi}	14.56 ± 0.19^{d}	11.62 ± 0.32^{b}	12.03 ± 0.16^{a-d}	3.22 ± 0.01^{a}
	150	3.35 ± 0.05^{cde}	1.06 ± 0.06^{hi}	13.74 ± 0.42^{e}	13.19 ± 0.71^{ab}	11.28 ± 0.45^{def}	2.96 ± 0.13^{bc}
	300	3.43 ± 0.04^{a}	0.96 ± 0.05^{i}	13.44 ± 0.29^{e}	13.81 ± 0.89^{a}	11.51 ± 0.36^{cd}	2.96 ± 0.12^{bc}
	600	3.37 ± 0.03^{cde}	0.97 ± 0.08^{i}	13.33 ± 0.19^{e}	14.53 ± 1.11^{a}	11.40 ± 0.28^{cde}	2.95 ± 0.11^{c}
Significance level							
	Concentration (A)	0.1723	**0.0004**	0.664	**0.0018**	**0.0223**	0.8767
	Storage duration (B)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0011**
	A x B	**<0.0001**	0.1826	**0.0001**	0.4049	**0.0020**	0.1135

Data presented as mean ± SE. Different letters across concentration and storage duration for each property differ significantly ($p < 0.05$) according to Duncan's multiple range test. TA - titratable acidity; TSS - total soluble solids; SE - standard error

Table 4 Pearson's correlation coefficient matrix between assessed physiological disorders

Variables	Respiration rate	Weight loss	Fruit decay	CI severity	% CI incidence	Scald severity	% Scald incidence	Aril browning	Internal decay
Respiration rate	**1**	**0.632**	0.247	**0.589**	**0.707**	0.274	**0.562**	**0.509**	**0.595**
Weight loss		**1**	**0.766**	**0.690**	**0.733**	**0.864**	**0.860**	**0.912**	**0.566**
Fruit decay			**1**	0.267	0.348	**0.867**	**0.761**	**0.734**	0.489
CI severity				**1**	**0.970**	**0.573**	**0.573**	**0.741**	**0.577**
% CI incidence					**1**	**0.596**	**0.679**	**0.782**	**0.715**
Scald severity						**1**	**0.848**	**0.926**	**0.524**
% Scald incidence							**1**	**0.917**	**0.691**
Aril browning								**1**	**0.635**
Internal decay									**1**

Values in bold are different at significance level of $p < 0.05$. Values in bold have moderate to strong correlation
CI- chilling injury

Table 5 Factor scores, loadings, eigenvalues and variance (%) for the first two factors (F1 and F2) based on sensory and instrumental attributes of 'Wonderful' pomegranate fruit treated with fludioxonil

Factor loadings		
	F1	F2
Sweet taste	0.196	**0.825**
Sour taste	**-0.596**	**-0.685**
Off flavour	**-0.794**	-0.114
Astringency	0.432	-0.155
Crispness	**0.578**	**0.532**
Juiciness	0.427	**0.633**
Grittiness	**-0.598**	**-0.714**
Hardness	**-0.703**	-0.183
Peel a*	**0.899**	-0.292
Peel C*	**0.934**	-0.103
Peel *h*°	**-0.602**	0.377
Aril a*	**0.745**	-0.104
Aril C*	**0.792**	-0.123
Aril *h*°	0.375	**-0.582**
TCD	0.469	0.112
Juice colour	-0.016	0.407
Fruit firmness	0.042	-0.483
Aril hardness	**-0.551**	0.055
Elastic modulus	**0.617**	-0.125
Toughness	-0.479	0.490
Bioyield	0.220	0.077
pH	**-0.512**	**0.659**
TA	0.235	**-0.783**
TSS	0.242	0.431

TSS/TA	0.005	**0.777**
BrimA	-0.041	**0.809**
Eigenvalue	7.506	6.181
Variability (%)	28.870	23.775
Cumulative (%)	28.870	52.645

Values in bold have moderate to strong correlation.
TCD- total colour difference; TA- titratable acidity; TSS- total soluble solids

Factor scores

Observation	F1	F2
Control_Month1	2.901	-4.102
150 mg/L_Month1	2.954	-2.426
300 mg/L_Month1	0.465	-3.183
600 mg/L_Month1	1.019	0.302
Control_Month2	2.932	2.543
150 mg/L_Month2	0.239	4.847
300 mg/L_Month2	1.596	1.886
600 mg/L_Month2	0.457	1.762
Control_Month3	0.626	-0.203
150 mg/L_Month3	-5.057	-2.088
300 mg/L_Month3	-4.853	0.290
600 mg/L_Month3	-3.278	0.371

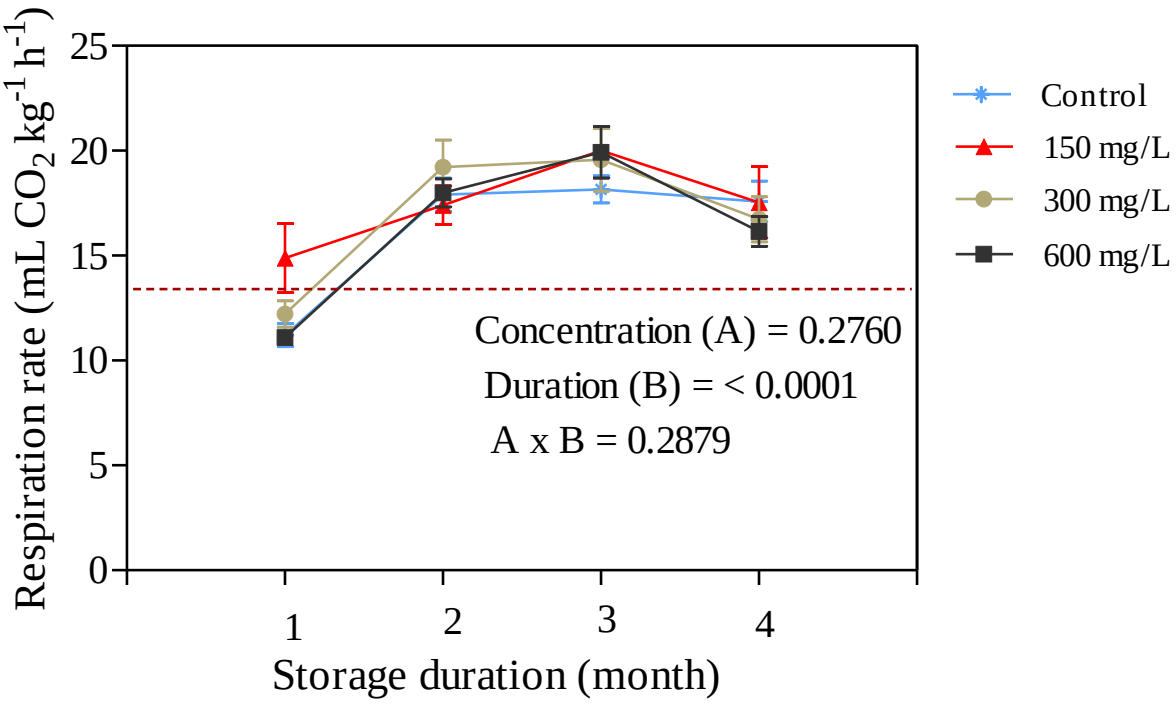

Fig. 1 Effect of fludioxonil concentration on respiration rate of pomegranate fruit during storage for 4 months at 5 °C and an additional 4 days at 20 °C. Each data point represents mean and error bars designate standard error (SE) of the mean. ----- Respiration rate at harvest.

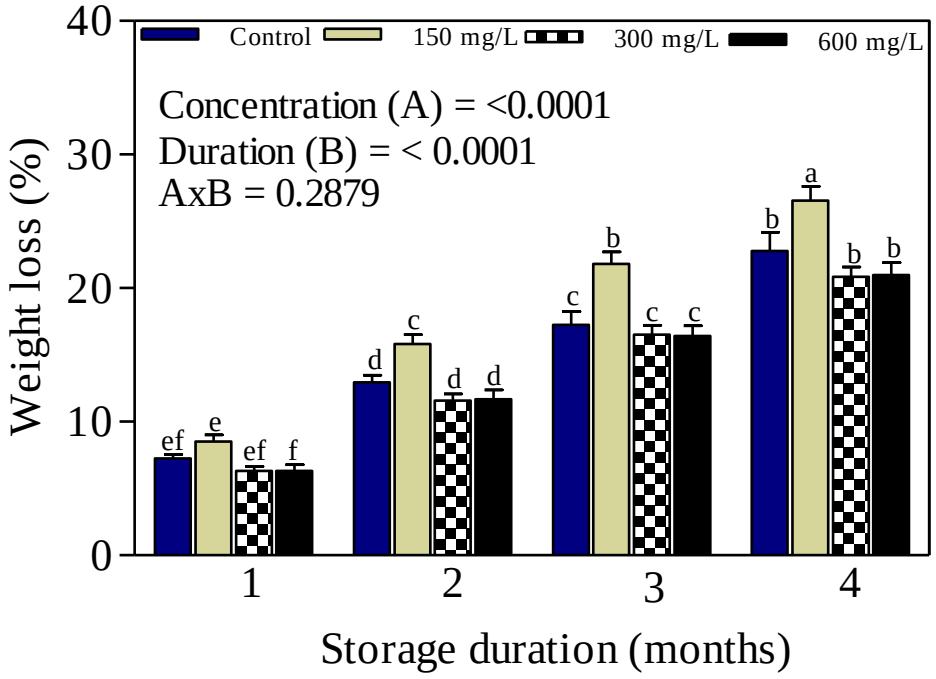

Fig. 2 Weight loss of pomegranate fruit treated with fludioxonil during storage for 4 months at 5 °C and an additional 4 days at 20 °C. Each data point represents mean and error bars designate standard error (SE) of the mean.

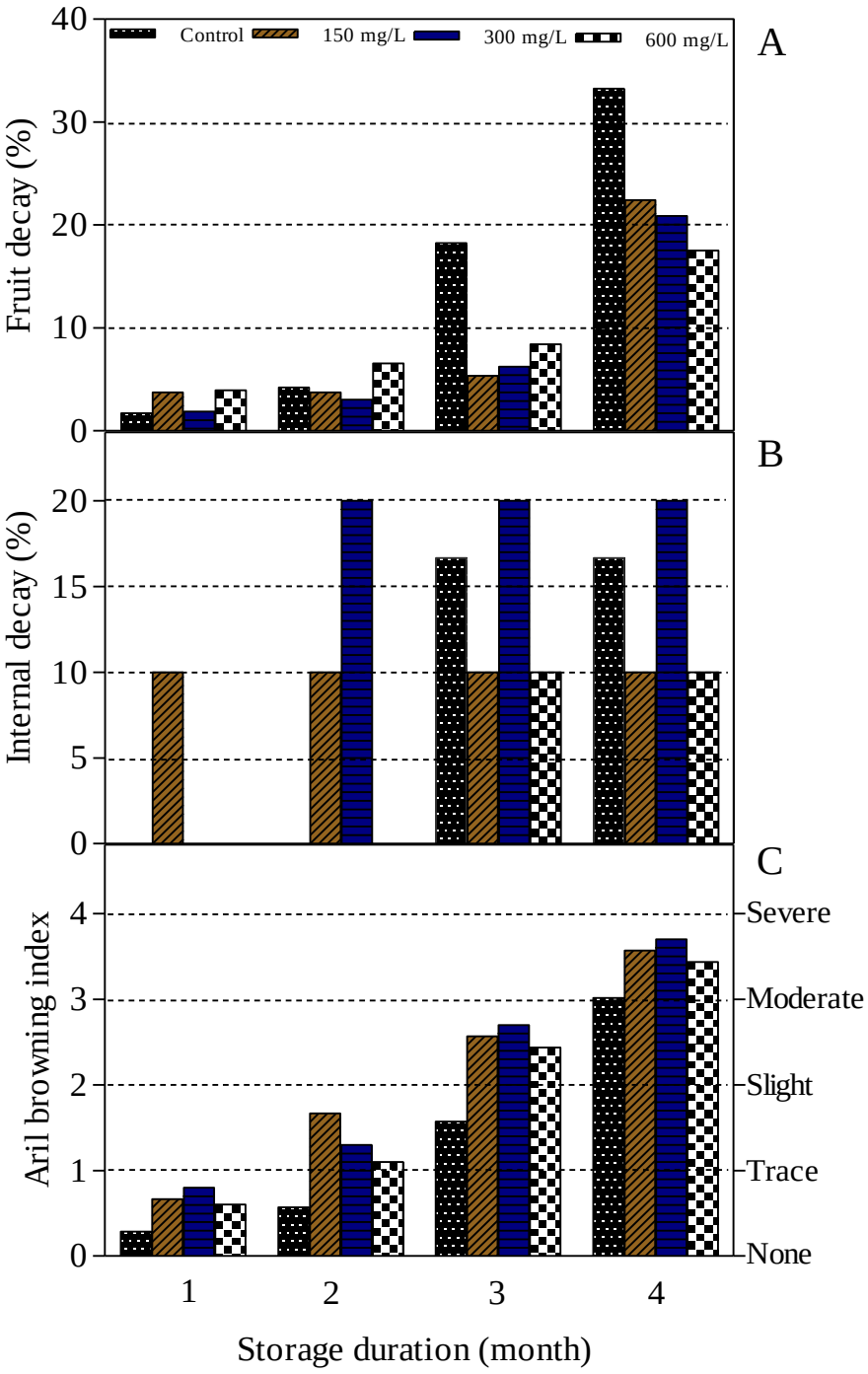

Fig. 3 Influence of fludioxonil concentration on physiological disorders on pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C. External decay (A), internal decay (B), aril browning (C).

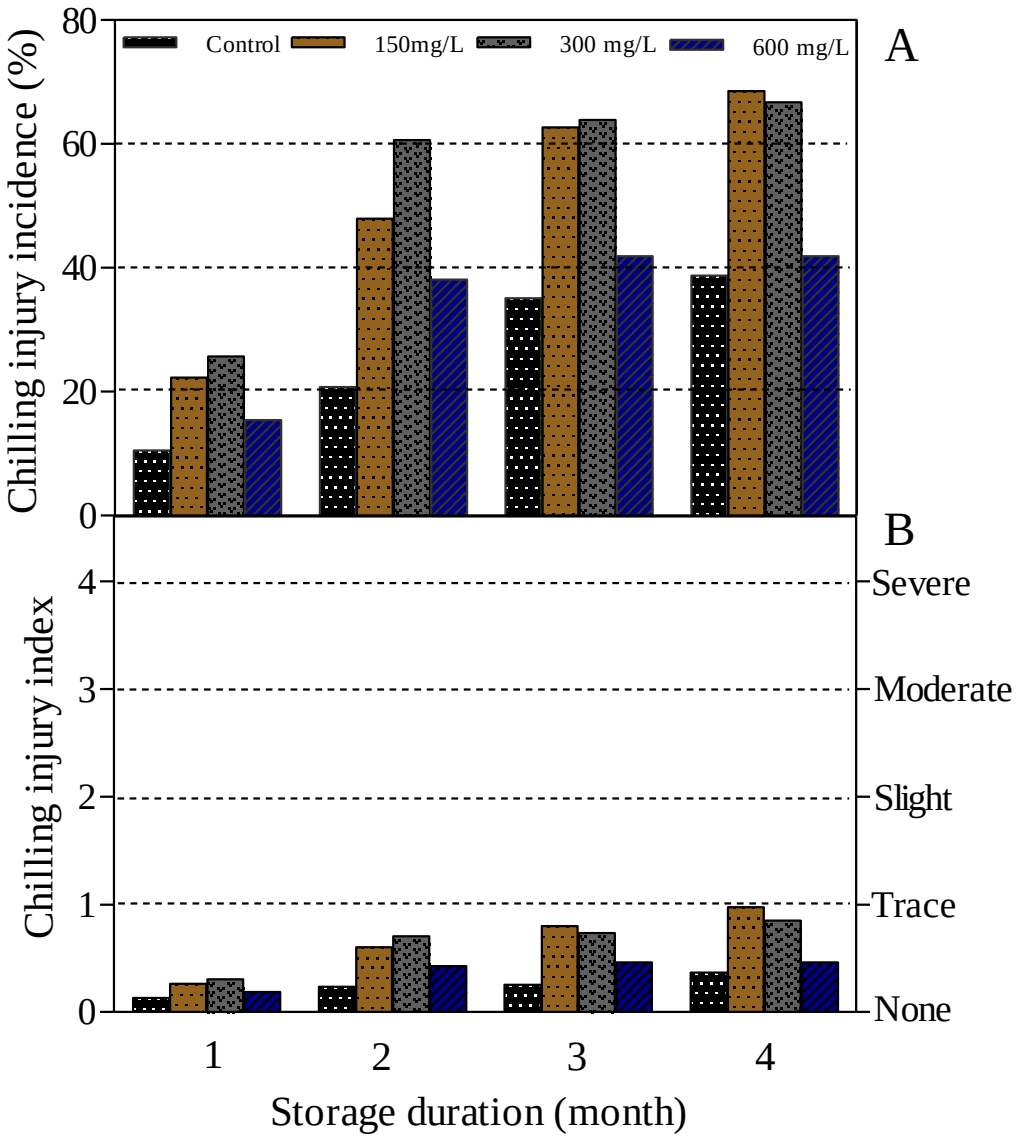

Fig. 4 Effect of fludioxonil on chilling injury incidence and index of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C.

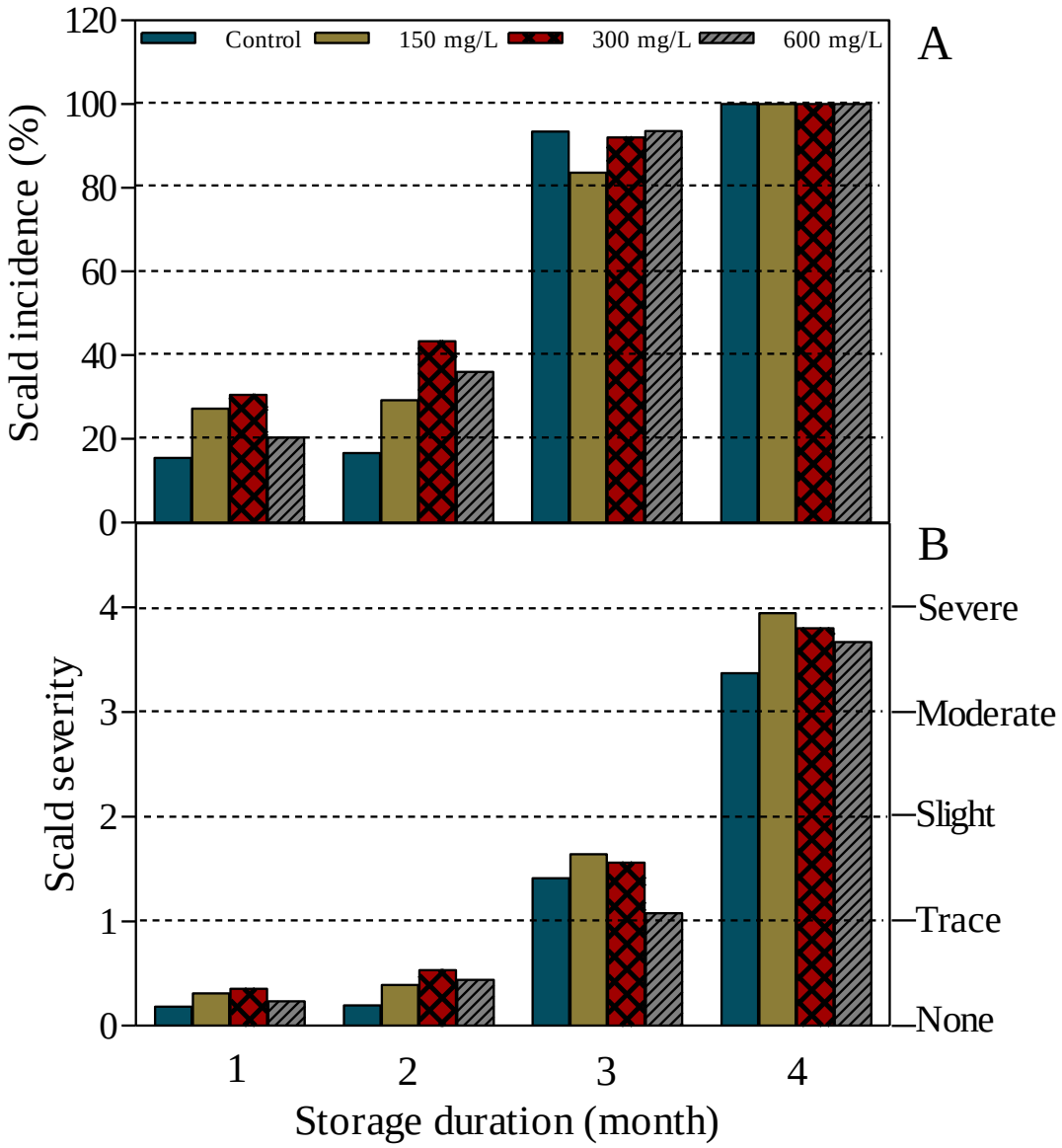

Fig. 5 Effect of fludioxonil on husk scald incidence and severity of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C.

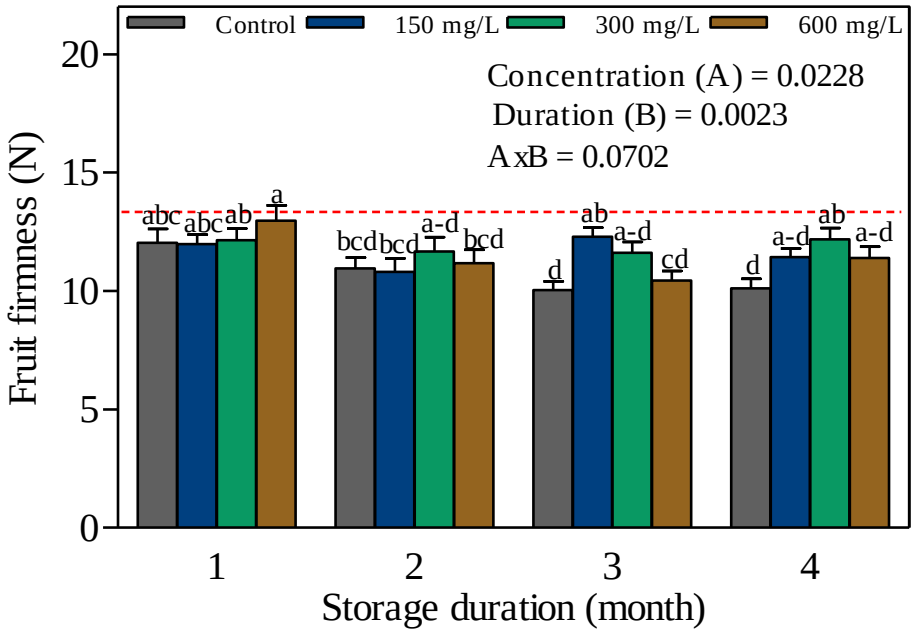

Fig. 6 Changes in fruit firmness of pomegranate fruit treated with fludioxonil and stored for 4 months at 5 °C and additional 4 days at 20 °C. -----Represents values at harvest.

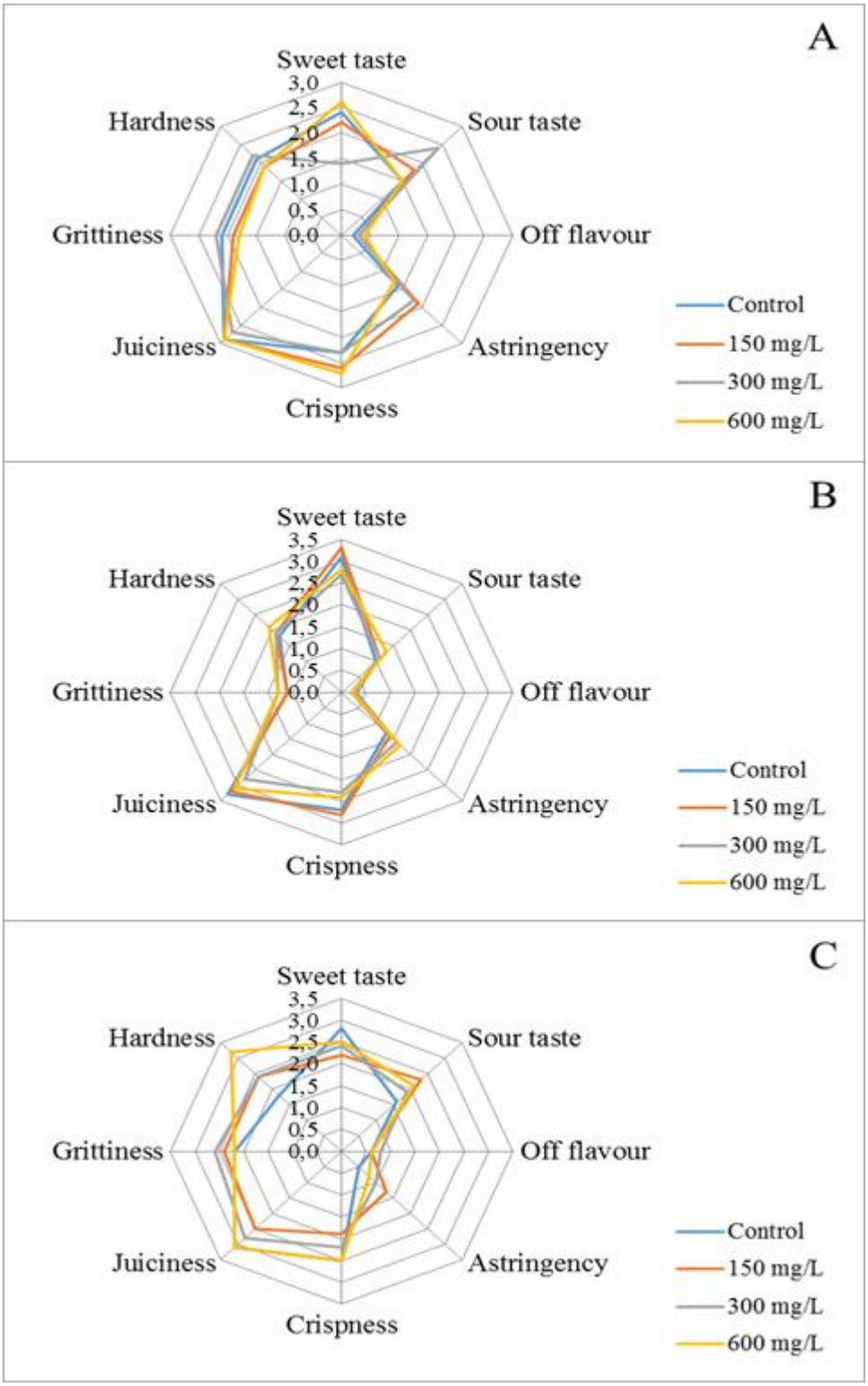

Fig. 7 Radar plot showing averaged sensory scores of pomegranate fruit treated with fludioxonil during storage for 3 months at 5 °C and additional 4 days at 20 °C. The plot represents storage at month 1 (A), month 2 (B) and month 3 (C).

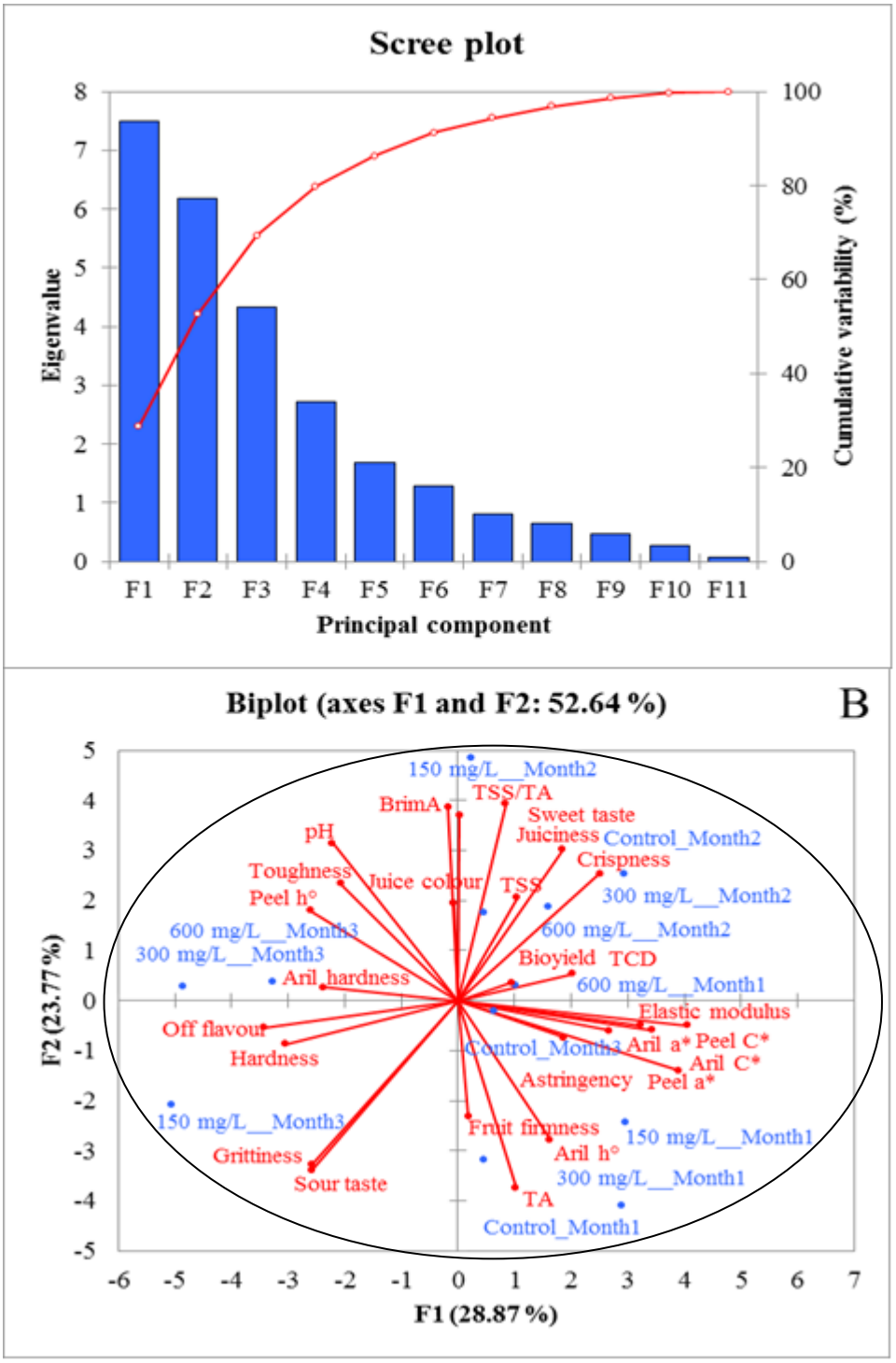

Fig. 8 Instrumental and sensory attributes of fruit stored for 3 months at 5 °C and additional 4 days at 20 °C of 'Wonderful' pomegranate fruit showing Scree plot of variance explained by each factor of the principal components (A) and Principal component analysis showing variables and observations (B).

CHAPTER FIVE: Effects of fludioxonil and putrescine postharvest treatments on phytochemical, antioxidant properties and volatile composition of pomegranate fruit during long-term storage

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EFFECTS OF FLUDIOXONIL AND PUTRESCINE POSTHARVEST TREATMENTS ON PHYTOCHEMICAL, ANTIOXIDANT PROPERTIES AND VOLATILE COMPOSITION OF POMEGRANATE FRUIT DURING LONG-TERM STORAGE

Abstract

The study investigated the effects of postharvest chemical treatments (fludioxonil and putrescine) on the phytochemical, antioxidant properties and volatile composition during storage of pomegranate fruit. Pomegranate whole fruit (cv. Wonderful) were dipped in solutions of different concentrations of fludioxonil (0, 150, 300 & 600 mg/L) and putrescine (0, 1, 2 & 3 mM) for 2 min. Fruit were dried and stored for 4 months at 5 °C and 95 % RH, and analysed monthly for phytochemical, antioxidant properties and volatile composition. Ascorbic acid content of fruit declined slightly for both fludioxonil (FLU) and putrescine (PUT) at different concentrations. For both chemicals used, untreated fruit had the highest ascorbic acid after the storage period (114.70 mg AA/ 100 mL). Total phenolic content significantly decreased as storage progressed. Fruit treated with fludioxonil maintained higher phenolic content (252.40, 165.80, 262.20 mg GAE/ 100 mL) after the first two months of storage compared to control (130.70 mg GAE/ 100 mL) however, no significant differences were observed for the last two months of storage. Putrescine only resulted in higher phenolic content after the second month of storage with no significant differences observed during the other storage periods. Total anthocyanin content (TAC) initially increased for fruit treated with fludioxonil but thereafter decreased as storage progressed. Fruit treated with FLU regardless of concentration had higher anthocyanin content compared to control after the first two months of storage but by the end of the storage duration, no differences existed among concentrations. Generally, putrescine treatment resulted in decrease in fruit TAC during storage, with only slight differences observed among PUT concentrations used. However, increase in antioxidant capacity of fruit was observed with prolonged storage regardless of concentration of FLU and PUT concentration. In total, thirty-two volatile organic compounds belonging to six chemical groups (alcohols, aldehydes, acids, ketones, esters and terpenes) were identified in the investigated fruit, alcohols being the predominant group. However, volatiles belonging to terpene group evolved during later storage (2 - 4 months), indicating changes in fruit flavour with progressive storage.

1. Introduction

The search for natural antioxidants from inexpensive and abundant food sources has attracted global attention (Anahita *et al*., 2015). In the recent years, there has been a spurred interest in consumption of fruits and vegetable due to their health benefiting bioactive constituent. High intake of fruits and vegetable has been linked to diminished morbidity of some diseases such as cardiovascular diseases, neurological damage and certain cancers (Aviram *et al.*, 2001; Lansky & Newman, 2007; Anahita *et al.*, 2015). Epidemiological studies have established the role of antioxidants in disease prevention through mechanisms such as averting chain reactions that generate free radicals and free radical neutralization (Ayala-Zavala *et al.*, 2004; Garcia-Alonso *et al.*, 2004; Stanner *et al.*, 2004). Pomegranate fruit (*Punica granatum* L.) is one of the oldest known edible fruits but has lately become popular because of its high antioxidant activity due to its phytochemicals such as phenolic compounds including anthocyanins (Opara *et al.*, 2009; Fawole *et al.*, 2012a; Mphahlele *et al.*, 2016b). The antioxidant capacity of pomegranate fruit has been linked to polyphenols, ascorbic acid and anthocyanins present in the fruit (Gil *et al.*, 2000; Seeram *et al.*, 2008; Fawole *et al.*, 2012a; Mphahlele *et al.*, 2016b). Other bioactive compounds in pomegranate include catechin, epicatechin, ellagitannins and rutin among others (Mphahlele *et al.*, 2016a). These compounds have diverse biological activities, for example, hindering oxidation through scavenging reactive oxygen species and increasing defense against chronic disease such as cardiovascular disorders and cancers (Fuhrman *et al.*, 2005; Hong *et al.*, 2008).

Some studies have suggested the anti-inflammatory, anti-hypertension and anti-mutagenic properties of polyphenolic compounds of pomegranate and its products (Lansky & Newman, 2007; Viuda-Martos *et al.*, 2010; Fawole *et al.*, 2012b). In local medicine, dried pomegranate fruit fractions have been used for treatment of diarrhoea, wound healing and control of bacterial action (Opara *et al.*, 2009). The importance of pomegranate fruit on health has been appreciated hence the global increase in demand for the fruit. However, pomegranate availability is majorly restricted to the harvesting season due to the high demand (Arendse, 2014). To increase fruit availability beyond its seasonality and improve quality and storability, a number of physical and chemical treatments are applied to the fruit (Opara *et al.*, 2015). These treatments may however have different effects not only on fruit quality but also on phytochemical composition of the fruit. It is therefore important to study the effect of these treatments on health promoting bioactive compounds.

The volatile and phenolic constituents of pomegranate fruit are developed during harvest, postharvest and even during storage, resulting in changes in the overall pomegranate flavour (Fawole & Opara, 2013b,c). Furthermore, fresh pomegranate fruit has low aromatic intensity and flavours of commercial juices are different from that of fresh fruit due to losses from fruit processing (Melgarejo *et al.*, 2011). Previous studies on aroma and flavour of pomegranate have focused on identification of unique volatile compounds produced by the ripe fruit (Calín-Sánchez *et al.*, 2011; Melgarejo *et al.*, 2011; Mayuoni-Kirshinbaum *et al.*, 2012; Fawole & Opara, 2013c; Mphalele *et al.*, 2016b,c) and the effect of modified atmosphere packaging (MAP) on volatile composition (Caleb *et al.*, 2013; 2015). Volatile compounds affect the sensory quality of fresh and processed fruit products (Calín-Sánchez *et al.*, 2011). Together with phenolic compounds, composition of volatile and aroma compounds depends on a number of factors such as climatic conditions, cultivar, harvest, storage conditions, postharvest treatments and processing (Calín-Sánchez *et al.*, 2011).

Considering the changes in quality occurring during long-term storage of pomegranate fruit, and the prospect of extending storage life by applications of postharvest chemical treatments, it is therefore crucial to investigate the potential for modulation of polyphenols and volatile composition of pomegranate by the application of fludioxonil and putrescine. So far, no studies have explored the effect of postharvest chemical treatments on volatile composition of pomegranate fruit. Therefore, the objective of this study was to assess the effects of exogenous application of postharvest chemical treatments (fludioxonil and putrescine) on the phytochemical, antioxidant properties and volatile composition of pomegranate fruit (cv. Wonderful).

2. Materials and methods

2.1. Plant material and chemicals

Pomegranate fruit were obtained during commercial harvest from Heinrich F.R. Schaefer (HFR) farm (33º44′26.185″S 18º44′41.193″E), Western Cape, South Africa. Fruit were transported in well ventilated vehicle to the postharvest technology research laboratory where they were then manually sorted to get rid of the damaged ones (bruise, crack, sunburns) and the remaining fruit used for treatments. Putrescine (PUT) was purchased from Sigma Aldrich, South Africa while the fungicide was a commercial formulation of Fludioxonil (FLU) (Scholar®, Syngenta, South Africa) containing 23 % active ingredient (a.i.).

2.2. Treatments

Fruit were separated into eight treatment groups with 108 fruit per group. Fruit were dipped for 2 min in a solution in 15 L of respective solutions. Fludioxonil and putrescine (containing 20 % Tween-20) solutions were used. Fludioxonil was purchased from Sygenta®, South Africa and putrescine from Sigma Aldrich, South Africa. A dipping time of 2 min was selected based on preliminary studies in which different dipping times (2, 5 and 8 min) were tested, and 2 min was the most effective.

The putrescine treatments included:

(**1**) Immersion in water (control)

(**2**) Immersion in 1 mM putrescine

(**3**) Immersion in 2 mM putrescine

(**4**) Immersion in 3 mM putrescine

The fludioxonil treatments included:

(**1**) Immersion in water (control)

(**2**) Immersion in 150 mg/L fludioxonil

(3) Immersion in 300 mg/L fludioxonil

(**4**) Immersion in 600 mg/L fludioxonil

After dipping in respective solutions, fruit surface was thoroughly dried by keeping fruit at ambient condition (20 ± 2 °C and 65 ± 2 % RH) for 12 h before cold storage.

2.3. Packaging and storage

Fruit were packed inside standard open top ventilated cartons (dimensions: 0.4 m long, 0.3 m wide and 0.133 m high) used for commercial postharvest handling of pomegranates. All the treatment groups were stored at 5 °C and 95 % relative humidity for 4 months. Temperature and relative humidity (% RH) inside the cold room were recorded daily throughout the storage period using Tiny Tag TV-4500 data loggers (Gemini Data Logger, Sussex, UK). At the end of cold storage, a batch of fruit (n = 10) was placed at 20 °C and 65 - 70 % RH for a further 4-day period to simulate a reasonable retail sale period. Fruit were thereafter analyzed for phytochemical, antioxidant properties and volatile composition. Measurements were carried out on a monthly basis. Treatments were independently assessed by comparing concentrations within each chemical treatment used.

2.4. Analysis of phytochemicals

2.4.1. Sample preparation

Pomegranate arils were manually hand extracted from the fruit. Ten (10) fruit from each treatment group were used. Pomegranate juice (PJ) was extracted from the arils of each fruit using a using LiquaFresh juice extractor (Mellerware, South Africa). Crude PJ sample (0.5 mL) was extracted with 14.5 mL of 50 % cold aqueous methanol in centrifuge tubes. The mixture was vortexed and sonicated in ice for 15 min. Thereafter, the mixture was centrifuged at 4000 rpm for 15 min at 4 °C (Merk, Eppendorf AG, Germany) to prevent particle interference when measuring absorbance. The supernatant was carefully collected and subsequently used for analysis of ascorbic acid, total phenolic content, total anthocyanin content and antioxidant capacity. All analyses were carried out in triplicate.

2.4.2. Ascorbic Acid

Ascorbic acid concentration was determined colorimetrically in triplicate using the method described by Barros *et al.* (2007) with some modifications (Fawole *et al.*, 2012a). Pomegranate juice was extracted with 1 % metaphosphoric acid (MPA) (0.5 mL of PJ to 14.5 mL of 1 % MPA). Mixture was vortexed for 2 min and sonicated for 3 min in cold water before centrifugation at 5000 rpm for 10 min at 4 °C. Approximately, 1 mL of the supernatant was mixed with 9 mL of 2, 6-dichlorophenolindophenol (dye), vortexed for 2 min and incubated in the dark for 10 min before the measurement read at absorbance 515 nm against a blank. Ascorbic acid content of each sample was calculated on the basis of the calibration curve of standard L-ascorbic acid. Results were expressed as milligram of ascorbic acid per a hundred millilitres of crude pomegranate juice (PJ) (mg AA/ 100 mL).

2.4.3. Total phenolic content

Folin-Ciocalteu (Folin C) method as described by Makkar *et al.* (2007) was used to determine the total phenolic content in triplicate. About 50 µL of diluted aqueous methanolic juice extracts in the test tube was mixed with 450 µL of 50 % methanol followed by the addition of Folin C reagent (500 µL) and 2.5 mL of sodium carbonate solution after 2 min. The mixture was vortexed and incubated in a dark chamber for 40 min at room temperature (20 °C) before measuring the absorbance at 725 nm using an UV-visible spectrophotometer (Thermo Fisher Scientific, Madson, USA). The results were presented as mean of duplicate analyses and expressed as milligrams of gallic acid equivalent per 100 mL of crude pomegranate juice (mg

GAE/ 100 mL).

2.4.4. Total anthocyanin content

Quantification of total anthocyanin content was determined using the pH differential method as described by Fawole *et al.* (2012a). PJ extract (0.5 mL) was mixed with 4.5 mL of two separate buffers, pH 1.0 and pH 4.5. Absorbance of the two buffers was measured at 510 and 700 nm using a UV-visible spectrophotometer after blanking with 100 % methanol. Total anthocyanin concentration was calculated using equation (1) and expressed as mg cyanidin 3-glucoside equivalent per 100 mL PJ (mg C3gE/ 100 mL PJ).

$$\text{Total monomeric anthocyanin (mg C3gE/ 100 mL)} = \frac{\text{A} \times \text{MW} \times \text{DF} \times 100}{\text{E} \times \text{L}} \quad (1)$$

Where, A = $(A_{510}\text{-}A_{700})_{pH1.0} - (A_{510}\text{-}A_{700})_{pH\ 4.5}$; MW = anthocyanin molecular weight (449.2); DF = dilution factor; E = cyaniding-3-glucoside molar absorbance (26,900); L = cell pathlength (1 cm).

2.4.5. Antioxidant activity

DPPH radical-scavenging activity

The radical scavenging activity of PJ was colorimetrically measured by its ability to scavenge 2,2-diphenyl-1-picryl hydrazyl (DPPH) using a method by Fawole *et al.* (2012a). In triplicate 15 µL PJ methanolic extract were diluted with 735 µL of 100 % methanol followed by addition of 750uL of 0.1mM methanolic DPPH solution. Samples were then vortexed and incubated in a dark chamber for 30 min. thereafter, absorbance was measured at 517 nm using a UV-vis spectrophotometer (Thermo Fisher Scientific, Madson, USA). A standard curve (with varying concentration 0.0-2.0 mM, with linear equation y = -2.433x + 0.5113 and R^2 = 0.9915) was used to compare the absorbance. The free-radical scavenging capacity of PJ was expressed as ascorbic acid (mM) equivalent per 100 mL of crude PJ (mM AAE/100 mL).

2.5. Gas chromatography- Mass Spectroscopy (GC-MS) analysis of volatile composition

Headspace solid-phase micro-extraction (HS-SPME) as described by Melgarejo *et al.* (2011) was used to trap and extract volatile compounds from the sample vial headspace. In triplicate, fresh pomegranate juice (10 mL) was pipetted into a 20 mL SPME vial and 2.5 mL of 20 % Sodium chloride added to enhance the release of volatile compounds into the headspace and inhibit enzymatic degradation. An internal standard of Anisole (50 µL) was added.

Equilibration of the SPME vials was done at 50 °C for 10 min at 250 rpm in a *PAL* COMB-xt autosampler incubator. Thereafter, a fibre coated with 50/30 m divinylbenzene/-carboxen/-polydimethylsiloxane (DVB/CAR/PDMS) was exposed to the sample headspace for 20 min at 50 °C. Volatile desorption from the fibre coating was done in the injection port of CTC at 250 ºC during 6 min in splitless mode. Volatile organic compounds trapped in the fibre were separated, identified and quantified on a gas chromatograph using Agilent 6890 N (Agilent, Palo Alto, CA), coupled with an Agilent mass spectrometer detector Agilent 5975 MS (Agilent, Palo Alto, CA). The GC–MS system was equipped with a polar Agilent Technologies ZB-FFAP capillary column (model Zebron 7HG-G009-11) with dimensions 30 m × 250 mm i.d. and 0.25 μm film thickness. Helium carrier gas with a flow of 1.3 mL/min with average velocity of 42 cm sec^{-1} was used for analyses. The injector temperature was maintained at 250 °C. The oven temperature was started at 40 °C for 5 min and finally increased to 240 °C at 10 °C min^{-1} and held for 6 min. Compounds were identified by comparing the retention times (RI) with mass spectral libraries (NIST, version 2.0). For quantification, the calculated relative percentages were used (Mphahlele *et al.*, 2016d).

3. Statistical analysis

Data was subjected to factorial analysis of variance (ANOVA) at 95 % confidence interval using Statistica software (Statistica version 14.0, StatSoft Inc., Tulsa, USA). Main effects (concentration and storage duration) and their interaction effects (concentration*storage duration) were also assessed. Post-hoc test (Duncan's Multiple Range Test) was used to test for statistical significance such that observed differences at $p < 0.05$ were considered significant. Principal component analysis (PCA) was carried out using XLSTAT software version 2012.04.1 (Addinsoft, France).

4. Results and discussion

4.1. Ascorbic acid content

Ascorbic acid (AA) content of FLU treatment remained steady up to the third month of storage with the exception of control fruit (Fig. 1A), where AA content was significantly higher than in treated fruit. This was followed by significant decreases in AA content in treated fruit below the amount obtained at harvest, however, AA content further increased in controlled fruit (Fig. 1A). In addition, according to factorial analysis, FLU concentration

showed a significant effect on AA content ($p < 0.0001$), however, the magnitude of FLU concentration cannot be established due to significant interaction between FLU concentration. On the other hand, changes in AA content followed a different pattern in fruit treated with PUT, with slight general increases in AA content obtained during storage (Fig.1B). The observed high content of AA content could be attributed to concentration effect due to higher moisture loss in control fruit compared to treated fruit (Fawole *et al.*, 2013a). In comparison with previous studies on postharvest treatments of pomegranate, Sayyari *et al.* (2010) observed that AA content was maintained when 'Mollar de Elche' pomegranate fruit were treated with oxalic acid and stored for 84 days at 2 °C. Furthermore, Barman *et al.* (2014) reported a declining trend in the AA content during storage of 'Mridula' pomegranate fruit although the decline was more pronounced in control as compared to fruit treated with putrescine.

4.2. Total phenolic content

As shown in Fig. 2A, total phenolic content of fruit treated with FLU concentrations significantly reduced with progressive storage for all concentrations and by the end of the storage duration, all concentrations had less than 150 mg GAE/ 100 mL (Fig. 2A). The first three months of storage were characterized by higher total phenolic content among FLU treated fruit than control. Continued storage to 4 months showed no significant differences among FLU concentrations (Fig. 2A). Treating pomegranate fruit with fludioxonil resulted in greater TPC for up to three months of storage. The reduction in phenolic content during storage of pomegranate fruit can be attributed to breakdown of phenolic compounds due to enzymatic activity (Fawole & Opara, 2013a; Arendse *et al.*, 2014). Decrease in phenolic content during storage of arils has also been associated with metabolic processes such as respiration and enzymatic activity for example oxidation of phenolic compounds by phenoloxidase (Shiri *et al.*, 2011). Similarly, decrease in phenolic content of pomegranate fruit has been previously reported (Sayyari *et al.*, 2010, Sayyari *et al.*, 2011b).

Upon treatment with putrescine, total phenolic content (TPC) of fruit similarly decreased with storage. After one month of storage, control fruit showed significantly high phenolic content (233.30 ± 8.58 mg GAE/ 100 mL) while no significant differences ($p > 0.05$) were observed among PUT concentrations (Fig. 2B). As TPC of control fruit declined after 2 months, TPC of treated fruit showed increases with fruit treated with 2 mM having 29.7 % higher TPC than control. The initial increase in phenolic content at month 2 for

treated fruit could be attributed to initial concentration of anthocyanins as a result anthocyanin biosynthesis (Fawole & Opara, 2013a). TPC then gradually decreased during the last two months of storage with no significant differences for all concentrations (Fig. 2B) and significant interaction ($p = 0.0001$) between the factors. The decrease in phenolic content with storage could be attributed to decline in phenolic concentration resulting from enzymatic activities taking place in the fruit as reported in rowanberries and pomegranate fruits (Baltacioğlu *et al.*, 2011; Fawole & Opara, 2013a). Similarly, decrease in phenolic content with storage was reported by Fawole & Opara (2013a) for 'Ruby' pomegranate. Contrary, increases in phenolic content during storage of pomegranate fruit has been reported by Arendse *et al.* (2014) and Mirdehghan *et al.* (2007) and attributed this to accumulation of anthocyanins.

4.3. Total anthocyanin content

Total anthocyanin content (TAC) of fruit initially increased to values above harvest and thereafter, gradually decreased during storage for all FLU concentrations (Fig. 3A). Fruit treated with FLU significantly showed higher TAC after the first three months of storage with 600 mg/L FLU concentration having the highest amounts at month 1 and 3 (138.90 ± 10.21 and 108.90 ± 6.23 mg AAE/ 100 mL respectively). The decrease in TAC during storage could be attributed to enzymatic oxidation of anthocyanin compounds (Jiang & Chen, 1995). Jiang & Chen (1995) reported that anthocyanins are chemically unstable and easily degraded due to enzymatic oxidation resulting from loss of compartmentalization of substrates and enzyme during long-term storage. At the end of the storage duration, untreated fruit exhibited higher TAC however no significant differences were observed compared to untreated fruit. Storage duration was significant ($p < 0.0001$) indicating that the depletion of anthocyanins was influenced by the storage time and not FLU concentration ($P = 0.4658$) (Fig 3A). Artés *et al.* (2000) similarly reported decrease in anthocyanins (delphindin 3-glucoside and delphinidine 3,5-diglucoside) at the end of cold and shelf life storage of 'Mollar de Elche' pomegranate at 2 and 5 °C for 12 weeks. Abd-elghany *et al.* (2012) also observed that anthocyanins content decreased for both untreated and treated (2 % $CaCl_2$ and film wrapped) pomegranate fruit (cv. Wonderful) during 60 days of storage at 5 °C and 85 % RH. Contrary, Sayyari *et al.* (2011a) observed an increase in the total anthocyanin concentration of pomegranate fruit (cv. Mollar de Elche) treated with acetyl salicylic acid (ASA) during storage for 84 days at 2 °C. The effect of fludioxonil (infact fungicides in general) on pomegranate phytochemical composition has not been widely studied however from the

results, the fungicide had no effect ($p = 0.4658$) on the anthocyanin content of pomegranate fruit.

With regards to putrescine treatment, TAC also decreased during storage of fruit with the exception of 1 mM PUT concentration (at month 2) which had significantly high amounts (136.10 ± 2.53) (Fig. 3B). Differences among concentrations were more pronounced at months 2 and 3 where fruit treated with 2 mM and 1 mM PUT concentrations had the lowest TAC (95.02 ± 7.56 and 61.37 ± 9.11 mg AA/ 100 mL respectively). After the storage period, no significant differences were observed among all concentrations and the changes in TAC were influenced by the interaction between the storage duration and concentration (Fig. 3B). As observed for FLU treatment, the decrease in TAC of pomegranate fruit could similarly be attributed to the oxidation of anthocyanin compounds by enzymatic processes (Jiang & Chen, 1995; Champa *et al.*, 2015). Although PUT has been reported to have antioxidant properties, PUT concentration had no significant effect on the anthocyanin content of pomegranate fruit ($p = 0.1396$) during the study. Barman *et al.* (2014) reported that carnauba wax lowered O_2 and therefore contributed to lowering oxidation, which contributed to higher anthocyanins retention in pomegranate when combined with PUT. This indicates the role of O_2/oxidation in lowering anthocyanin content in pomegranate fruit.

Conflicting results have been reported on changes in the total anthocyanin content during storage of pomegranate fruit with some studies showing decreases and others increases. Pérez-Vicente *et al.* (2004) reported decrease in total anthocyanin of pomegranate fruit juices. During storage of cv. 'Mollar' pomegranate, Miguel *et al.* (2007) likewise observed a significant decrease in anthocyanin monoglucosides but not for cv. 'Assaria' pomegranate. Several other studies have similarly reported decrease in pomegranate TAC during storage (Artés *et al.*, 1998; Oz & Ulukanli, 2012; Caleb *et al.*, 2013; Maghoumi *et al.*, 2013). On the other hand, many studies have reported increase in anthocyanin content of fruit during cold storage of pomegranate (Artés *et al.*, 2000; Miguel *et al.*, 2004; Arendse *et al.*, 2014; Fawole & Opara, 2013a; Martinez-Romero *et al.*, 2013), raspberry and strawberry (El Ghaouth *et al.*, 1991; Han *et al.*, 2004). The increase in anthocyanin content has been associated to anthocyanin biosynthetic pathway enzymes (Varasteh *et al.*, 2012). These differences among studies therefore necessitate the need for further research to assess intervarietal differences on changes in bioactive compounds of pomegranate fruit (Artés *et al.*, 1998). Total anthocyanin content during the study was higher than that observed for minimally processed arils (Caleb *et al.*, 2013, 2015; Martinez-Romero *et al.*, 2013; Banda *et*

al., 2015) and this could be due to maintenance of compartmentalization during storage of whole fruit.

4.4. Antioxidant capacity (DPPH radical scavenging activity)

Antioxidant capacity was based on the DPPH assay of juice from pomegranate fruit. Effect of FLU on antioxidant capacity of pomegranate is illustrated in Fig. 4A. The antioxidant capacity of fruit progressively increased during cold storage. Untreated fruit showed higher antioxidant capacity compared to treated fruit after 2 and 3 months of storage while treated fruit showed no statistical differences among FLU concentrations. There was as significant interaction of storage duration and FLU concentration ($p < 0.0001$) with no significant differences observed after the end of the storage period (Fig. 4A). Some studies have attributed the increase in antioxidant capacity of pomegranate to phenolic compounds and anthocyanins (Gil *et al.*, 2000; Fawole & Opara 2013a). However, Barman *et al.* (2014) suggested that plant produce antioxidant capacity is largely due to the presence of pigments vitamins and tannins. In agreement with the results, Ramezanian & Rahemi (2010) observed an increase in the DPPH antioxidant activity of 'Malas Yazdi' pomegranate pretreated with spermidine, calcium chloride and hot water after 4.5 months at 2 °C and 85 % RH. Sayyari *et al.* (2010) similarly reported that the hydrophilic antioxidant activity (H-TAA) of 'Mollar de Elche' pomegranate fruit treated with oxalic acid increased after 84 days of storage at 2 °C. However, when the authors treated the same pomegranate cultivar with acetyl salicylic acid (ASA), a reduction in the antioxidant capacity was observed during fruit storage at 2 °C for 84 days (Sayyari *et al.*, 2011b). Literature on the effect of fungicides on fruit antioxidant capacity is very limited as most studies majorly focus on their role in control of fruit decay. No studies have reported the effect of fludioxonil on the antioxidant activity of pomegranate fruit. Literature on other fruits is also very limited. FLU concentration had a significant effect on fruit antioxidant capacity ($p < 0.0001$) but no conclusion could be made because there was a significant interaction of the two factors ($p < 0.0001$).

The effect of treating pomegranate with putrescine on the antioxidant activity was also determined as shown in Fig. 4B. Similar to results observed for FLU, there was a general increase in the antioxidant capacity of PUT treated fruit with storage. Slight differences were observed after the first two months of storage with 1 mM PUT concentration having the lowest antioxidant capacity at month 2. However, no significant differences were observed between the treated and untreated fruit after 3 and 4 months of storage and the storage

duration ($p < 0.0001$) was the major factor that influenced the changes in antioxidant capacity during storage of PUT treated fruit (Fig. 4B). Increase in total antioxidant activity of heat treated pomegranate (cv. Mollar de Elche) during cold storage at 2 °C for 90 days has also been reported (Mirdehghan *et al.*, 2006) and this was attributed to an increase in the total phenolic content. Similarly, after treating pomegranate with putrescine and spermine, Mirdehghan *et al.* (2007) observed an increase in total antioxidant activity during storage for 60 days. This was attributed to the ability of these polyamines in lowering the losses in phenolic compounds (Mirdehghan *et al.*, 2007). Contrary, the antioxidant activity (expressed as radical scavenging activity) of pomegranate fruit (cv. Wonderful) decreased during storage at various temperatures (21 °C, 10 °C, 7.5 °C, 5 °C) for 5 months (Arendse *et al.*, 2014). Barman *et al.* (2014) first observed an increase and a decrease thereafter in antioxidant activity of 'Mridula' pomegranate during storage (3 and 5 °C for 60 days) after treatment with putrescine and carnauba wax although treated fruit retained more activity than control.

Interestingly, the observed changes in total antioxidant activity did not correlate with the trends observed for TPC, ascorbic acid or total anthocyanins suggesting that other bioactive compounds could have been the major contributors to the antioxidant activity during the study. Some studies have also reported that the trend observed for total phenolic did not correspond to that of content antioxidant activity (Arendse *et al.*, 2014). This may indicate that other bioactive compounds (such as tannins) may also contribute to antioxidant capacity of pomegranate or that antioxidants react differently depending on the assay used (Çam *et al.*, 2009; Arendse *et al.*, 2014). Many studies have however suggested phenolic compounds and anthocyanins as major contributors to the antioxidant capacity of pomegranate fruit (Gil *et al.*, 2000; Mirdehghan *et al.*, 2007; Sayyari *et al.*, 2011b; Fawole & Opara, 2013a). Calín-Sánchez *et al.* (2011) on the other hand reported that the major antioxidant compounds in pomegranate juice are hydrolysable tannins although anthocyanins and ellagic acid are also contributors. Therefore, the increase in antioxidant capacity of fruit during the study could be attributed to migration of tannins from rind to aril (Barman *et al.*, 2014). Pérez-Vicente *et al.* (2004) detected increase in the antioxidant capacity of pomegranate fruit juices during processing and attributed the increase to the hydrolysable tannins in the fruit peel and to increase in ellagic acid. The authors suggested that ellagic structures polymerized into ellagitannins or anthocyanin polymers formed during storage (Pérez-Vicente *et al.*, 2004). Some authors have suggested that the antioxidant activity of some fruits could be attributed to their allo-derivatives contents for example in bananas (García-Alonso *et al.*, 2004). Pérez-

Vicente *et al.* (2004) found no correlation (R^2 < 0.100) between the concentration of anthocyanins, ellagic acid or total phenols and the antioxidant activity of pomegranate juice. The authors insinuated possible synergism phenomena and suggested more research on the bioavailabilty of polymers that could be responsible for the activity.

4.5. Volatile organic composition

In this study, a total of 31 and 32 volatile compounds were identified in pomegranate fruit (cv. Wonderful) treated with FLU and PUT, respectively (Tables 1 & 2). Some of the identified volatiles evolved during storage. Eight compounds were identified at harvest. After 1 month of storage, however, additional 5 and 4 compounds were identified in fruit treated with PUT and FLU, respectively. As storage progressed, more compounds evolved (in particular terpenes) for both treatments (Tables 1 and 2; Figs. 5 and 6). Varying numbers of volatiles have been identified in pomegranate fruit. The number of compounds identified in this study was more than those (between 15 and 23 compounds) reported by Calín-Sánchez *et al.*, 2011; Vázquez-Araújo *et al.*, 2011a; Fawole & Opara 2013c; Caleb *et al.*, 2015. However, more compounds (83 volatiles) than in this study was reported by Beaulie & Stein-Chishol (2016) for 'Wonderful' and commercial pomegranate juices in the U.S. Overall, the abundance of volatile groups was in the order of alcohols>esters>terpenes>aldehydes>acids>ketones between harvest and month 2 for both FLU and PUT. However, this order was altered between month 3 and 4 as terpenes>esters>alcohols>aldehydes>ketones (Tables 1 & 2). There were changes in volatile profile with storage. For instance, the alcohol group was predominant between month 1 and 2 (FLU: 36.43 - 70.62 %; PUT: 22.69 - 55.77 %) (Fig. 5 & 6). This is in agreement with Fawole & Opara (2013c), who reported alcohols as the most dominant volatile group with proportions of 32.5 % and 54.9 % for 'Wonderful' and 'Bhagwa' pomegranate cultivars, respectively, at harvest. Ketones were observed in fruit treated with FLU but not in PUT treated fruit. This could be attributed to the antioxidant properties of putrescine as ketones are formed by oxidation of alcohols (Marko *et al.*, 1996). The alcohol group in this study comprised compounds such as 1-Butanol 2-methyl, 1-hexanol and 3-hexen-1-ol of which 1-hexanol characterized by leafy, fruity and woody aroma was the most abundant for both treatments (FLU: 28.04 - 51.47 % and PUT: 31.07 - 39.79 %).

However, as storage progressed (month 3 and 4), compounds belonging to terpene group were upregulated (FLU: 67.25 - 98.85 %; PUT: 17.53 - 94.98 %). This included monoterpenes (m-cymene, terpineol, limonene, pinene and cineole) and sesquiterpnes

(zingiberene, caryophyllene, farnesene, bisabolene and cadrene) (Fig. 7). Fruit treated with FLU had more volatile compounds than control fruit suggesting its inability to control chilling injury. In particular, m-cymene characterized by citrus, woody, terpenic, spicy and cumin aroma was the most abundant terpene for both treatments, suggesting possible aroma attributes of the pomegranate juice during prolong storage (month 2 - 4) (Tables 1 & 2). In comparison with Mayuoni-Kirshinbaum *et al.* (2013), massive increase in terpenes (majorly sesquiterpenes) and subsequent decrease in flavour preference during long-term storage of 'Wonderful' pomegranate fruit was observed. The observed upregulation in terpenes in this study could be attributed to response of fruit to chilling stress (Mphahlele *et al.*, 2016b).

5. Conclusion

The study revealed that postharvest chemical treatments did not greatly affect the phytochemical and antioxidant properties of pomegranate fruit during cold storage. Treating pomegranate fruit with fludioxonil and putrescine resulted in slight changes in the ascorbic acid content. Ascorbic acid minimally decreased during storage of treated and untreated fruit. Total phenolic content on the other hand significantly declined during storage of fruit and treating fruit with FLU maintained higher values only for short-term storage (2 months). After storage, no significant differences were present irrespective of the concentrations used for both FLU and PUT treatments. Total anthocyanin content slightly increased to values above harvest after the first two months of storage for fruit treated with FLU. However, no differences existed after the end of the storage duration for all FLU concentrations. Putrescine similarly showed decreased anthocyanin content during storage. Both FLU and PUT concentrations had no significant effect on the anthocyanin content of fruit during storage as the changes were majorly influenced by the storage duration. Antioxidant capacity of pomegranate fruit increased as storage progressed for fruit treated with FLU and PUT. This was a surprising result because the trend that was observed did not coincide with those observed for ascorbic acid, total phenolic content and total anthocyanin contents for both FLU and PUT. This indicated that some other bioactive compounds such as tannins might have been the major contributors to the antioxidant capacity of pomegranate fruit during the study. To our knowledge, this is the first study that has reported on the effects of postharvest treatments of fungicides (fludioxonil) on the phytochemical and antioxidant capacity during storage of pomegranate.

A total of 31 and 32 volatile organic compounds were identified during storage of pomegranate fruit treated with fludioxonil and putrescine respectively during the study. The compounds belonged to 6 chemical groups; alcohols, aldehydes, esters, ketones, acids and terpenes for FLU treatment while the PUT treatment had 5 groups (excluding ketones). The alcohols were the most abundant groups during short storage time (2 months) while the terpenes were predominant during prolong e storage duration for both fruit treated with FLU and PUT. Some compounds were only identified in fruit treated with fludioxonil (2-nonane, α-phallendrene and α-cadrene) while others were unique to fruit treated with putrescine (isoamyl alcohol, 1-hexanol, 2-ethyl and phenyl alcohol). Evolution of volatiles with storage was evident as some compounds (alcohols, aldehydes and esters) declined with time while others (terpenes) accumulated with progressive storage. These changes may have influence on the flavour perception of stored fruits, and further studies are warranted in this area.

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Table 1 Effect of fludioxonil on volatile organic composition of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C

Compound	Retention time	Harvest	Month 1				Month 2				Month 3				Month 4			
			Control	150 mg/L	300 mg/L	600 mg/L	Control	150 mg/L	300 mg/L	600 mg/L	Control	150 mg/L	300 mg/L	600 mg/L	Control	150 mg/L	300 mg/L	600 mg/L
Alcohols																		
1-Butanol, 2-methyl-	8.8334	_	_	_	_	_	_	_	_	_	_	2.34±0.19^{e}	2.45±0.57^{e}	2.93±0.60^{e}	_	_	1.51±0.13^{e}	0.86±0.06^{e}
1-Hexanol	11.1371	36.76±9.92^{ab}	_	31.98±2.63^{cd}	51.47±2.81^{ab}	46.72±3.32^{ab}	34.68±5.97^{e}	37.02±3.95^{ab}	28.04±2.16^{bc}	37.30±2.07^{e}	19.39±3.45^{e}	5.14±0.56^{e}	8.73±0.80^{e}	11.21±0.10^{e}	6.34±0.71^{e}	_	1.91±0.12^{e}	3.07±0.29^{e}
3-Hexen-1-ol	11.6104	17.61±2.24^{ab}	18.10±2.69^{a}	10.46±1.18^{cd}	19.15±1.67^{ab}	16.01±0.08^{ab}	10.87±2.45^{e}	6.06±0.38^{ab}	8.39±0.66^{bc}	7.82±0.79^{e}	_	0.97±0.08^{e}	1.56±0.05^{e}	2.19±0.13^{e}	_	_	_	_
Total alcohols		**54.37**	**18.10**	**42.44**	**70.62**	**62.73**	**45.55**	**43.08**	**36.43**	**45.12**	**19.39**	**8.45**	**11.18**	**16.33**	**6.34**	**_**	**3.42**	**3.93**
Aldehydes		_																
Hexanal	5.2919	8.51±1.97^{a}	6.98±1.59^{a}	2.59±0.33^{cd}	5.55±1.94^{ab}	5.55±0.51^{ab}	_	5.12±0.29^{ab}	4.24±0.31^{bc}	_	_	_	_	_	_	0.77±0.08^{de}	_	_
Total aldehydes		**8.51**	**6.98**	**2.59**	**5.55**	**5.55**	**_**	**5.12**	**4.24**	**_**	**_**	**_**	**_**	**_**	**_**	**0.77**	**_**	**_**
Ketones																		
2-Nonanone	11.4261	12.65±1.68^{de}	_	5.87±0.22^{cd}	_	7.64±1.42^{ab}	_	_	_	_	_	_	_	_	_	_	_	_
Total ketones		**12.65**	**_**	**5.87**	**_**	**7.64**	**_**	**_**	**_**	**_**	**_**	**_**	**_**	**_**	**_**	**_**	**_**	**_**
Acids		_																
Acetic acid	12.6492	_	_	_	_	_	_	_	_	_	_	1.70±0.42^{e}	0.79±0.04^{e}	_	_	_	0.44±0.13^{e}	0.70±0.07^{e}
Total acids		**_**	**_**	**_**	**_**	**_**	**_**	**_**	**_**	**_**	**_**	**1.70**	**0.79**	**_**	**_**	**_**	**0.44**	**0.70**
Esters																		
Isoamyl acetate	6.3153	_	2.91±1.25^{a}	1.94±0.13^{bc}	0.88±0.11^{ab}	_	_	_	_	1.30±0.21^{e}	_	9.67±2.28^{e}	3.49±1.57^{e}	7.06±2.24^{e}	_	0.27±0.00^{de}	0.32±0.05^{e}	0.49±0.05^{e}
Acetic acid, hexyl ester	9.3858	2.01±0.61^{e}	28.84±5.07^{a}	21.54±3.72^{cd}	2.99±0.39^{ab}	4.19±1.33^{ab}	24.14±4.83^{e}	2.68±0.84^{ab}	_	5.88±2.56^{e}	15.67±3.15^{e}	5.71±1.4^{e}	7.16±2.32^{e}	7.65±0.94^{e}	_	_	0.51±0.01^{e}	_
3-Hexen-1-ol, acetate	10.2559	_	29.48±9.68^{a}	11.03±1.62^{cd}	1.76±0.18^{ab}	2.05±0.84^{ab}	9.44±2.77^{e}	_	_	2.87±0.40^{e}	4.29±0.63^{e}	1.29±0.37^{e}	1.47±0.35^{e}	1.70±0.23^{e}	_	_	_	_
Total esters		**2.01**	**61.23**	**34.51**	**5.63**	**6.24**	**33.58**	**2.68**	**_**	**10.05**	**19.96**	**16.67**	**12.12**	**16.41**	**_**	**0.27**	**0.83**	**0.49**
Terpenes																		
β-Pinene	5.5194	_	_	_	_	_	_	3.17±0.25^{ab}	2.70±0.50^{bc}	3.57±0.42^{e}	7.17±1.02^{e}	3.01±0.43^{e}	3.21±0.32^{e}	4.19±0.25^{cd}	3.76±0.61^{e}	3.75±0.33^{de}	2.50±0.12^{e}	5.29±0.31^{b}
α-Phellandrene	6.8678	_	_	_	_	_	_	_	0.93±0.21^{e}	_	_	0.41±0.03^{e}	0.46±0.04^{e}	0.81±0.08^{e}	_	_	_	_
Myrcene	7.0139	_	_	_	_	_	_	_	_	1.30±0.1^{e}	1.63±0.12^{e}	1.50±0.12^{e}	1.95±0.07^{e}	3.06±0.35^{e}	1.40±0.07^{e}	1.34±0.02^{de}	0.81±0.07^{e}	1.71±0.17^{e}
α-Terpinene	7.1763	_	_	_	_	_	_	1.15±0.07^{ab}	1.12±0.10^{bc}	_	_	1.24±0.01^{e}	1.66±0.07^{e}	2.99±0.35^{e}	1.12±0.03^{e}	0.70±0.01^{de}	0.46±0.04^{e}	0.94±0.11^{e}
1,4-Cineole	7.3062	_	_	_	1.04±0.33^{ab}	0.85±0.26^{ab}	_	_	_	_	_	_	_	0.48±0.05^{e}	_	_	_	_
Limonene	7.5338	6.50±1.02^{de}	2.41±0.04^{a}	4.63±0.98^{cd}	4.97±0.96^{ab}	4.34±0.62^{ab}	15.04±1.77^{e}	9.20±0.30^{ab}	12.60±0.65^{bc}	0.80±0.12^{e}	15.94±0.77^{e}	6.98±0.29^{e}	8.71±0.37^{e}	12.93±0.63^{e}	8.11±0.17^{e}	6.81±0.36de	4.40±0.32e	8.66±0.40e

β-Phellandrene	7.6961	_	_	_	_	1.89±0.06^{ab}	_	_	3.94±0.63^{bc}	10.67±0.46^{e}	2.22±9.25^{e}	2.01±0.16^{e}	2.37±0.09^{e}	4.08±0.24^{e}	1.85±0.03^{de}	1.68±0.07^{de}	1.09±0.05^{e}	2.16±0.13^{e}
1,8-Cineole	7.9074	13.95±1.63^{ab}	8.57±0.94^{a}	6.37±0.33^{cd}	10.41±1.39^{ab}	10.76±1.52^{ab}	_	17.18±2.75^{ab}	10.92±0.48^{bc}	1.61±0.18^{e}	_	0.58±0.03^{e}	0.73±0.06^{e}	1.22±0.10^{e}	_	0.48±0.05e	0.34±0.02e	0.65±0.04e
γ-Terpinene	8.4273	_	_	_	_	_	_	_	6.51±1.06^{bc}	6.11±0.61^{cd}	10.44±1.57^{a}	2.03±0.21^{e}	3.92±0.71^{e}	4.86±0.38^{cd}	5.65±1.77^{cd}	2.85±0.24^{de}	2.08±0.45^{e}	2.87±0.68^{e}
m-Cymene	9.0932	_	1.08±0.24^{a}	1.37±0.12^{cd}	1.77±0.26^{ab}	_	3.97±1.01^{bc}	4.26±0.25^{ab}	6.49±1.95^{bc}	4.59±1.94^{e}	4.19±0.65^{ab}	23.80±2.89^{e}	_	_	46.25±3.32^{e}	12.94±1.49^{de}	14.14±2.00^{e}	23.43±1.39^{e}
α-terpinolene	9.2883	_	_	_	_	_	_	_	1.31±0.33^{bc}	1.10±0.10^{ab}	_	2.45±0.13^{e}	3.33±0.27^{e}	_	_	1.02±0.03^{de}	1.05±0.07^{ab}	_
α-Zingiberene	13.3066	_	_	_	_	_	_	_	_	_	_	2.05±0.43^{e}	1.41±0.07^{e}	2.12±0.03^{e}	1.52±0.42^{e}	7.10±0.71^{de}	4.62±0.68^{e}	7.48±0.44^{e}
α-Bergamotene	13.9771	_	_	_	_	_	_	9.05±2.37^{ab}	7.61±2.22^{bc}	8.47±1.59^{e}	_	5.19±0.72^{e}	19.93±2.32^{e}	5.90±0.58^{e}	7.39±0.61^{e}	5.89±0.76^{de}	28.31±1.42^{e}	13.11±0.16^{e}
Trans-Caryophyllene	14.9014	_	_	_	_	_	_	_	3.44±0.33^{bc}	_	_	_	5.52±0.49^{e}	2.47±0.04^{e}	2.04±0.37^{e}	5.22±1.09^{e}	_	_
α-Farnesene	14.2709	_	_	_	_	_	_	2.82±0.74^{ab}	_	1.37±0.36^{e}	6.61±1.43^{e}	_	_	_	3.74±0.22^{e}	11.56±0.25^{de}	6.77±0.16^{e}	_
Terpinen-4-ol	14.3681	_	_	_	_	_	1.86±0.21^{e}	_	_	3.49±0.65^{e}	6.66±0.52^{e}	5.75±0.39^{e}	5.98±0.65^{e}	8.99±0.60^{e}	3.06±0.30^{e}	_	2.58±0.31^{e}	4.07±0.29^{e}
Camphene	15.556	2.01±0.09ab	1.62±0.25^{a}	1.64±0.04^{cd}	_	_	_	2.30±0.07^{ab}	1.77±0.38^{bc}	1.76±0.21^{e}	_	_	1.75±0.14^{e}	_	_	_	_	_
β-Bisabolene	15.9439	_	_	_	_	_	_	_	_	_	3.34±0.32^{e}	6.51±1.32^{e}	4.74±0.33^{e}	7.88±0.67^{e}	_	17.85±0.55^{de}	9.63±1.19^{e}	16.12±1.62^{e}
α-Cedrene	16.0771	_	_	_	_	_	_	_	_	_	_	1.48±0.21^{e}	_	_	_	1.40±0.04^{de}	1.02±0.06^{e}	_
β-Farnesene	16.2953	_	_	_	_	_	_	_	_	_	2.45±0.18^{e}	2.01±0.03^{e}	3.09±0.14^{e}	5.27±0.04^{e}	3.01±0.18^{e}	8.58±1.00^{de}	6.49±0.58^{e}	_
β-Sesquiphellandrene	15.4166	_	_	_	_	_	_	_	_	_	_	1.63±0.02^{e}	1.43±0.18^{e}	_	1.59±0.08^{e}	0.93±0.22^{de}	4.37±0.76^{e}	_
α-Curcumene	16.4649	_	_	_	_	_	_	_	_	_	_	4.57±0.11^{e}	4.25±0.50^{e}	_	3.18±0.50^{e}	8.75±0.24^{de}	4.67±0.24^{e}	8.38±0.66^{e}
Total terpenes		**22.46**	**13.68**	**14.01**	**18.19**	**17.84**	**20.87**	**49.13**	**59.34**	**44.84**	**60.65**	**73.20**	**74.44**	**67.25**	**93.67**	**98.85**	**95.33**	**94.87**
Total compounds		100.00	99.99	99.42	99.99	100.00	100.00	100.01	100.01	100.01	100.00	100.02	100.09	99.99	100.01	99.89	100.02	99.99

Data presented as mean ± SE. Different letters across volatile compounds in each row differ significantly ($p < 0.05$) according to Duncan's multiple range test. SE - standard error

Table 2 Effect of putrescine on volatile organic composition of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C

Compound	Retention time	Harvest	Month 1				Month 2				Month 3				Month 4			
			Control	1 mM	2mM	3 mM	Control	1 mM	2 mM	3 mM	Control	1 mM	2 mM	3 mM	Control	1 mM	2 mM	3 mM
Alcohols																		
1-Propanol, 2-methyl-	5.9417	_	_	_	_	_	_	_	_	_	_	3.29±0.57^{d}	3.71±0.17^{d}	2.65±0.11^{d}	_	_	_	_
Isoamyl alcohol	8.8983	_	_	_	_	_	_	_	14.29±4.55^{d}	_	_	18.27±1.23^{d}	24.76±2.40^{d}	_	_	_	_	_
1-Hexanol	11.1371	36.76±9.92^{d}	_	_	_	31.75±1.95^{c}	34.64±5.77^{d}	38.99±1.99^{a}	31.07±3.08^{d}	39.79±3.47^{d}	19.39±3.45^{d}	6.79±1.15^{d}	11.22±0.40^{d}	3.96±0.46^{d}	6.34±0.71^{d}	7.63±1.51^{d}	5.02±0.61^{d}	6.41±0.57^{d}
3-Hexen-1-ol	11.6104	17.61±2.24^{b}	17.29±1.62^{b}	30.53±0.96^{a}	22.69±3.24^{c}	8.24±2.08^{c}	10.89±2.51^{d}	12.15±0.15^{a}	7.61±1.28^{d}	8.73±1.73^{d}	_	1.58±0.31^{d}	2.34±0.08^{d}	_	_	_	_	0.89±0.06^{d}
1-Hexanol, 2-ethyl-	13.0436	_	_	_	_	_	_	2.72±0.19^{a}	2.80±0.47^{d}	2.82±0.06^{d}	_	_	_	_	_	_	_	_
phenyl alcohol	18.0287	_	_	_	_	_	_	_	_	_	_	2.48±0.70^{d}	4.03±1.50^{d}	5.45±0.97^{d}	_	_	_	_
Total alcohols		**54.37**	**17.29**	**30.53**	**22.69**	**39.99**	**45.53**	**53.86**	**55.77**	**51.34**	**19.39**	**32.41**	**46.06**	**12.06**	**6.34**	**7.63**	**5.02**	**7.30**
Aldehydes																		
Hexanal	5.2919	8.51±1.97^{ab}	6.67±0.97^{b}	10.24±1.71^{a}	3.85±0.47^{c}	2.80±0.62^{c}	_	10.48±1.19^{a}	_	_	_	_	_	_	_	_	_	_
2-Hexenal	8.5248	_	4.48±0.65^{b}	9.91±0.51^{a}	15.13±4.73^{c}	_	_	_	_	_	_	_	_	_	_	_	_	_
Total aldehydes		**8.51**	**11.15**	**20.15**	**18.98**	**2.80**	**_**	**10.48**	**_**	**_**	**_**	**_**	**_**	**_**	**_**	**_**	**_**	**_**
Ketones																		
2-Nonanone		12.65±1.68	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Total ketones		**12.65**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Acids																		
Acetic acid	12.6492	_	_	_	_	_	_	_	_	_	_	4.23±0.07^{d}	14.23±4.77^{d}	16.90±4.74^{d}	_	_	_	_
Total acids		_	_	_	_	_	_	_	_	_	_	**4.23**	**14.23**	**16.90**	_	_	_	_
Esters																		
Isoamyl acetate	6.3153	_	2.79±0.77^{d}	_	_	2.17±1.12^{c}	_	_	_	_	_	8.84±0.36^{d}	11.58±0.77^{d}	49.87±1.63^{d}	_	_	_	_
Acetic acid, hexyl ester	9.3858	2.01±0.61^{d}	27.60±3.17^{b}	15.67±5.72^{a}	19.29±8.54^{c}	31.98±1.17^{c}	24.19±4.89d	5.29±0.41a	21.72±1.18d	17.79±6.03d	15.67±3.15^{d}	2.69±0.31d	7.05±0.37d	3.11±0.28d	_	_	_	_
3-Hexen-1-ol, acetate	10.2559	_	28.11±5.82^{b}	12.14±5.64^{a}	18.25±1.56^{c}	6.22±3.76^{c}	9.46±2.78^{d}	1.53±0.11^{a}	8.52±2.11^{d}	6.87±2.73^{d}	4.29±0.63^{d}	0.47±0.05^{d}	1.57±0.05^{d}	0.50±0.08^{d}	_	_	_	_
Total esters		**2.01**	**58.50**	**27.81**	**37.54**	**40.37**	**33.65**	**6.82**	**30.24**	**24.66**	**19.96**	**12.00**	**20.20**	**53.48**	**_**	**_**	**_**	**_**
Terpenes																		
β-Pinene	5.5194	_	_	2.91±0.48^{a}	_	_	_	4.30±0.22^{a}	2.22±0.64^{d}	3.70±0.59^{d}	7.17±1.02^{d}	0.83±0.21^{d}	1.88±0.03^{d}	1.35±0.21^{d}	3.76±0.61^{d}	3.72±0.34^{d}	3.72±0.34^{d}	4.42±0.75^{d}
Myrcene	7.0139	_	_	_	_	_	_	_	_	_	1.63±0.12^{d}	_	_	0.60±0.07^{d}	1.40±0.07^{d}	1.03±0.05^{d}	1.15±0.04^{d}	1.67±0.17^{d}

α-Terpinene	9.2883	_	_	_	_	_	_	_	_	_	_	0.47±0.04^{d}	_	0.61±0.02^{d}	1.12±0.03^{d}	0.78±0.04^{d}	0.93±0.01^{d}	1.26±0.19^{d}
1,4-Cineole	7.3062	_	_	1.18±0.15a	0.83±0.03c	_	_	1.38±0.09a	_	_	_	_	_	_	_	_	_	_
Limonene	7.5338	6.50±1.02^{d}	2.30±0.04^{b}	5.21±0.27^{a}	3.14±0.51^{c}	4.25±1.40^{c}	15.03±1.70^{d}	14.40±1.10^{a}	7.88±0.91^{d}	12.89±0.91^{d}	15.94±0.77^{d}	3.88±0.12^{d}	5.89±0.51^{d}	4.99±0.98^{d}	8.11±0.17^{d}	6.65±0.26^{d}	8.25±0.41^{d}	8.49±0.28^{d}
β-Phellandrene	7.6961	_	_	_	_	_	_	_	_	_	2.22±0.25^{d}	0.77±0.12^{d}	0.92±0.06^{d}	_	1.85±0.03^{d}	1.36±0.11^{d}	1.92±0.02^{d}	1.76±0.23^{d}
1,8-Cineole	7.9074	13.95±1.63^{a}	8.18±0.57^{b}	10.48±0.90^{a}	12.13±0.64^{c}	7.58±1.65^{c}	_	_	_	_	_	0.32±0.02^{d}	_	_	_	_	_	_
γ-Terpinene	8.4273	_	_	_	_	_	_	_	_	_	10.44±1.57^{d}	0.76±0.15^{d}	5.63±0.35^{d}	3.19±0.58^{d}	5.65±1.77^{d}	4.29±0.13^{d}	5.30±1.09^{d}	3.86±0.17^{d}
m-Cymene	9.0932	_	1.03±0.15^{b}	1.73±0.18^{a}	2.42±0.33^{d}	3.90±1.77^{c}	3.94±0.91^{d}	4.68±0.66^{a}	_	2.31±1.16^{d}	4.19±0.65^{d}	43.56±56^{d}	_	_	46.25±3.32^{d}	41.24±2.86^{d}	40.79±0.23^{d}	31.58±0.26^{d}
α-terpinolene	9.1746	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	1.31±0.03^{d}	1.75±0.08^{d}
α-Zingiberene	13.3066	_	_	_	_	_	_	_	_	_	_	_	_	_	1.52±0.42^{d}	1.60±0.14^{d}	_	1.90±0.13^{d}
α-Bergamotene	13.9771	_	_	_	_	_	_	_	_	_	_	_	3.02±0.58^{d}	3.62±0.69^{d}	7.39±0.61^{d}	16.79±2.98^{d}	18.47±1.88^{d}	21.74±1.04^{d}
Trans-Caryophyllene	14.9014	_	_	_	_	_	_	_	_	_	_	_	_	_	2.04±0.46^{d}	_	2.34±0.22^{d}	2.16±0.34^{d}
α-Farnesene	14.2709	_	_	_	_	_	_	_	_	_	6.61±0.43^{d}	_	_	1.12±0.29^{d}	3.74±0.22^{d}	_	3.43±0.25^{d}	2.78±0.91^{d}
Terpinen-4-ol	14.3681	_	_	_	_	_	1.86±0.22^{a}	1.97±0.18^{a}	2.38±0.36^{d}	3.01±0.81^{d}	6.66±0.52^{d}	_	1.17±0.11^{d}	1.27±0.18^{d}	3.06±0.30^{d}	3.14±0.42^{d}	3.72±0.13^{d}	3.64±0.40^{d}
Camphene	15.556	2.01±0.09^{c}	1.55±0.14^{b}	_	2.26±0.35^{c}	1.12±0.32^{c}	_	2.12±0.21^{a}	1.53±0.24^{d}	2.09±0.17^{d}	_	0.77±0.21^{d}	0.98±0.18^{d}	0.78±0.21^{d}	_	_	_	1.03±0.02^{d}
β-Sesquiphellandrene	15.4166	_	_	_	_	_	_	_	_	_	_	_	_	_	1.59±0.08	_	_	_
β-Bisabolene	15.9439	_	_	_	_	_	_	_	_	_	3.34±0.32d	_	_	_	_	4.17±0.42d	1.27±0.27d	1.01±0.03d
β-Farnesene	16.2953	_	_	_	_	_	_	_	_	_	2.45±0.18d	_	_	_	3.01±0.18d	3.45±0.42d	2.38±0.37d	3.63±0.45d
α-Curcumene	16.4649	_	_	_	_	_	_	_	_	_	_	_	_	_	3.18±0.50^{d}	4.13±0.43^{d}	_	_
Total terpenes		**22.46**	**13.06**	**21.51**	**20.78**	**16.85**	**20.83**	**28.85**	**14.01**	**24.00**	**60.65**	**51.36**	**19.49**	**17.53**	**93.67**	**92.35**	**94.98**	**92.68**
Total compounds		100.00	100.00	100.00	99.99	100.01	100.01	98.63	100.02	100.00	100.00	100.00	99.98	99.97	100.01	99.98	100.00	99.98

Data presented as mean ± SE. Different letters across volatile compounds in each row differ significantly ($p < 0.05$) according to Duncan's multiple range test. SE - standard error

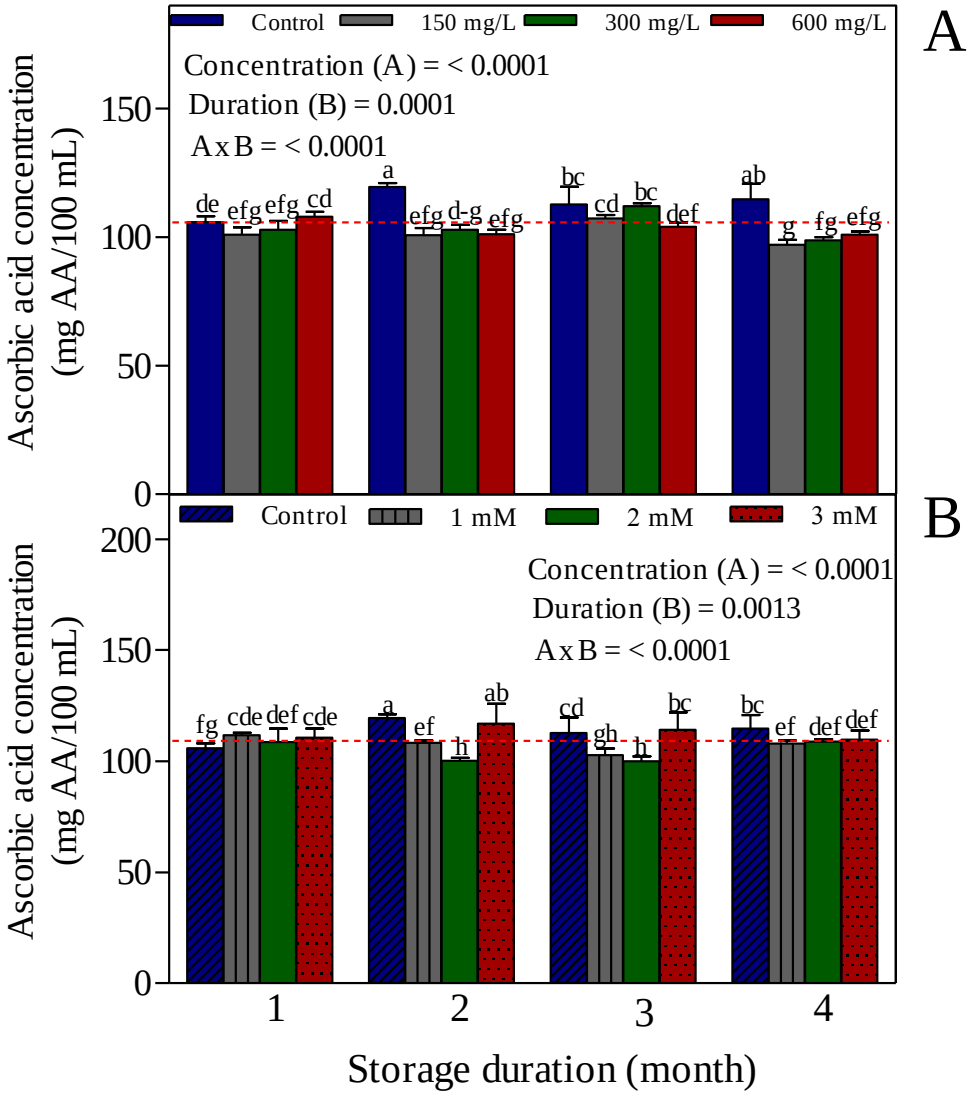

Fig. 1 Ascorbic acid concentration of pomegranate fruit treated with fludioxonil (A) and putrescine (B) during storage for 4 months at 5 °C and additional 4 days at 20 °C. ----- Represents values at harvest. Each bar represents mean and error bars denote standard error (SE) of the mean. Bars followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

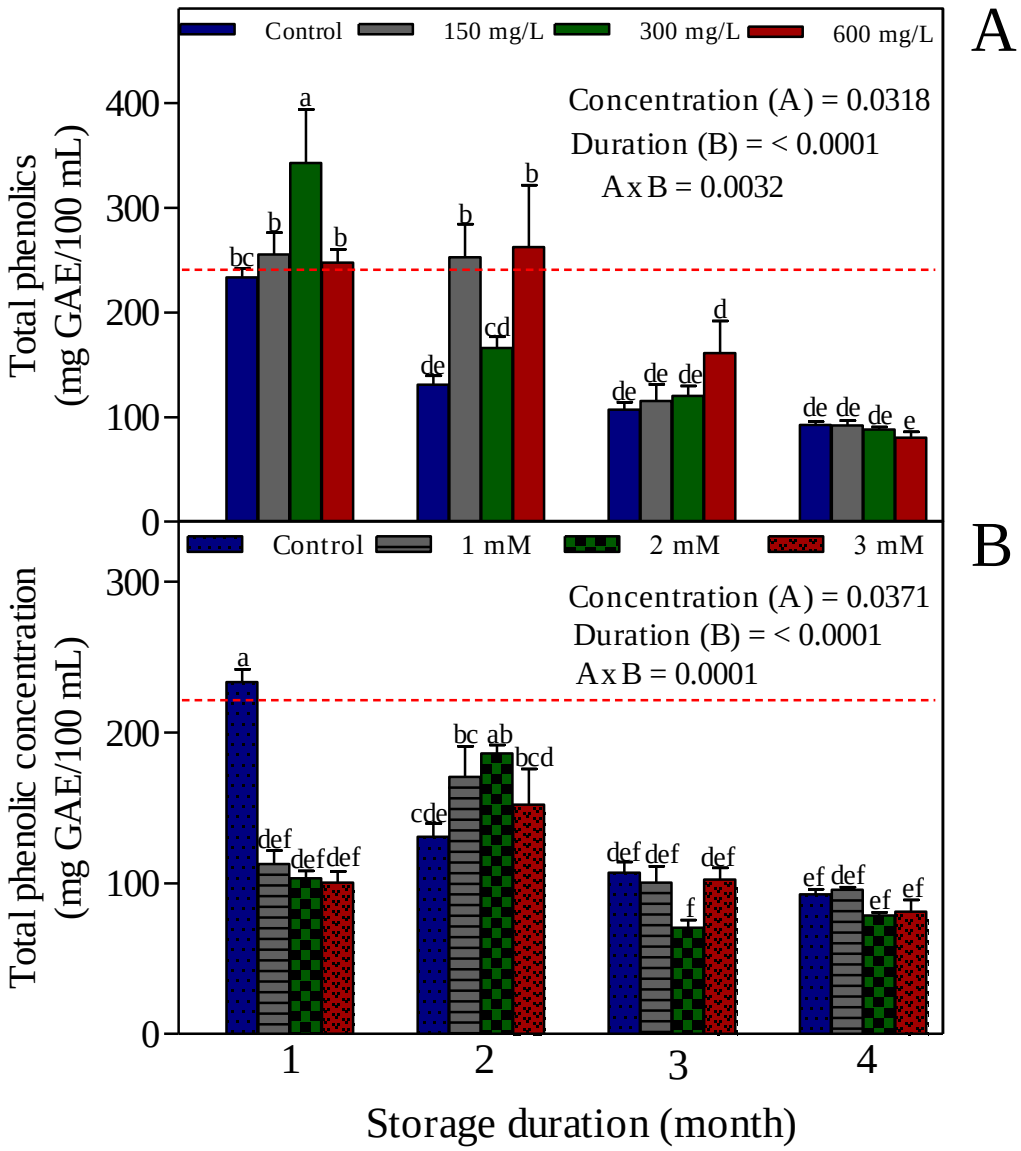

Fig. 2 Changes in total phenolic concentration of pomegranate fruit treated with fludioxonil (A) and putrescine (B) during storage for 4 months at 5 °C and additional 4 days at 20 °C. ---- Represents values at harvest. Each bar represents mean and error bars denote standard error (SE) of the mean. Bars followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

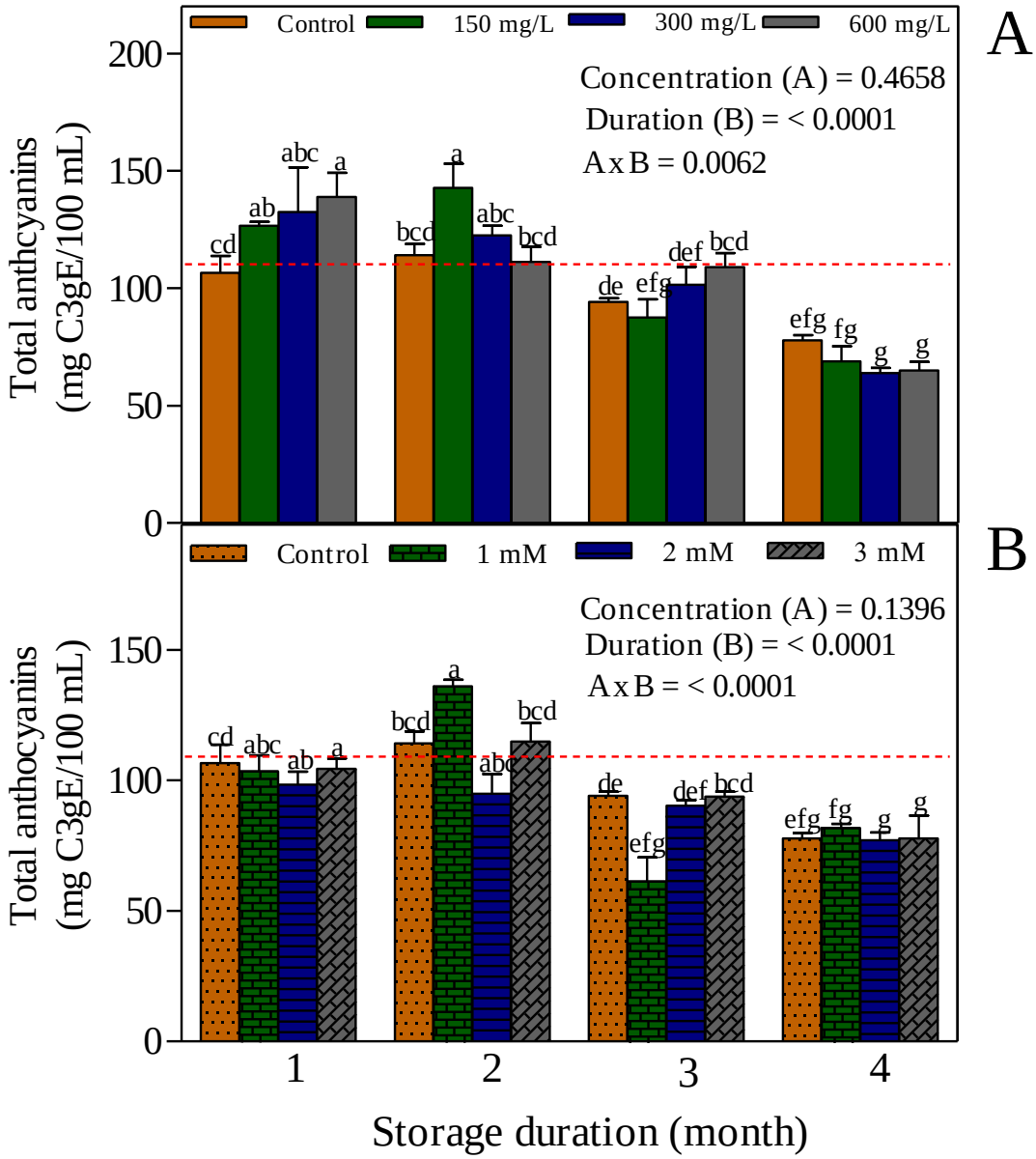

Fig. 3 Effect of fludioxonil (A) and putrescine (B) on total anthocyanin concentration of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C. ----- Represents values at harvest. Each bar represents mean and error bars denote standard error (SE) of the mean. Bars followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

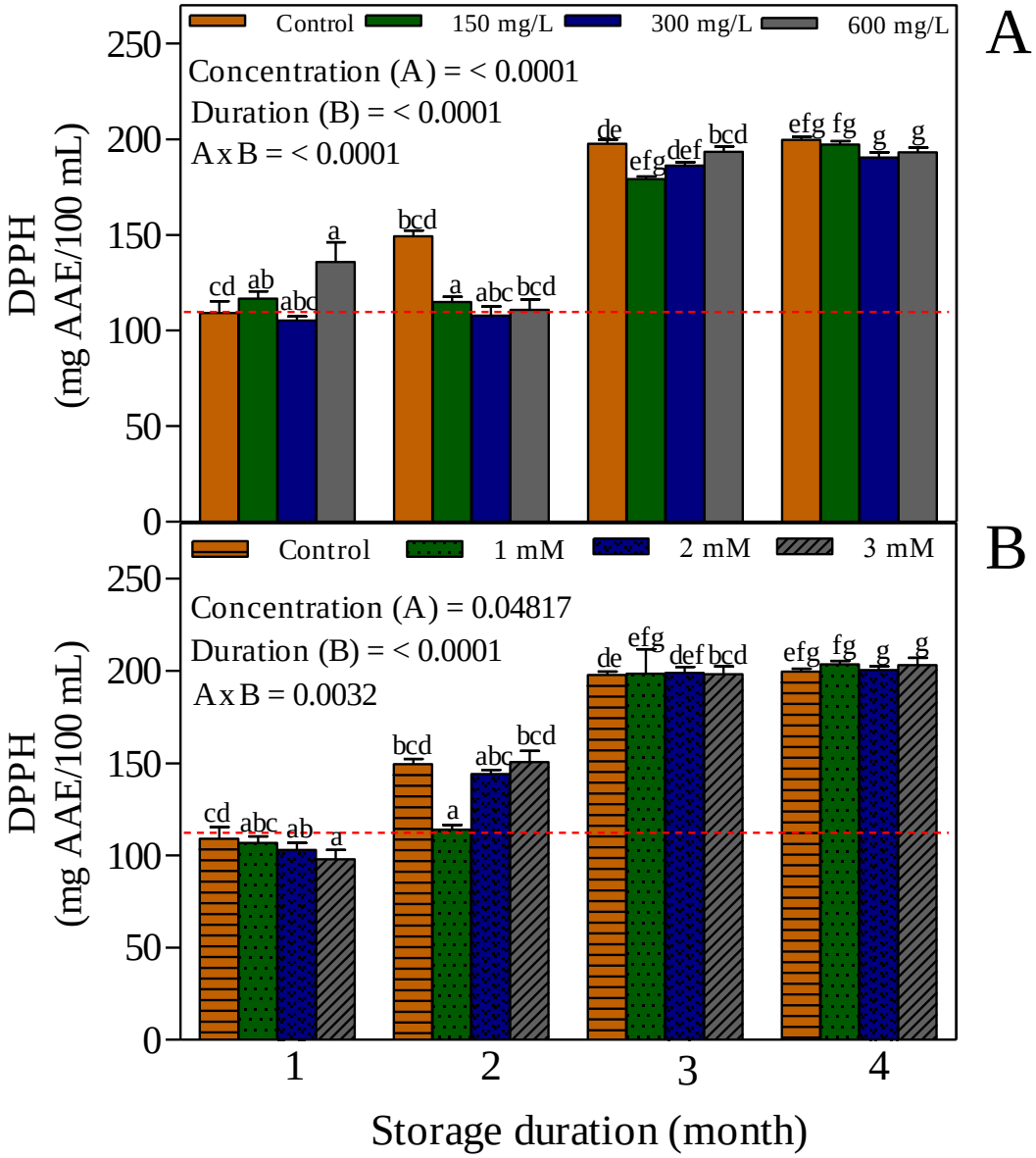

Fig. 4 Effect of fludioxonil (A) and putrescine (B) on DPPH antioxidant capacity of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C. ----- Represents values at harvest. Each bar represents mean and error bars denote standard error (SE) of the mean. Bars followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

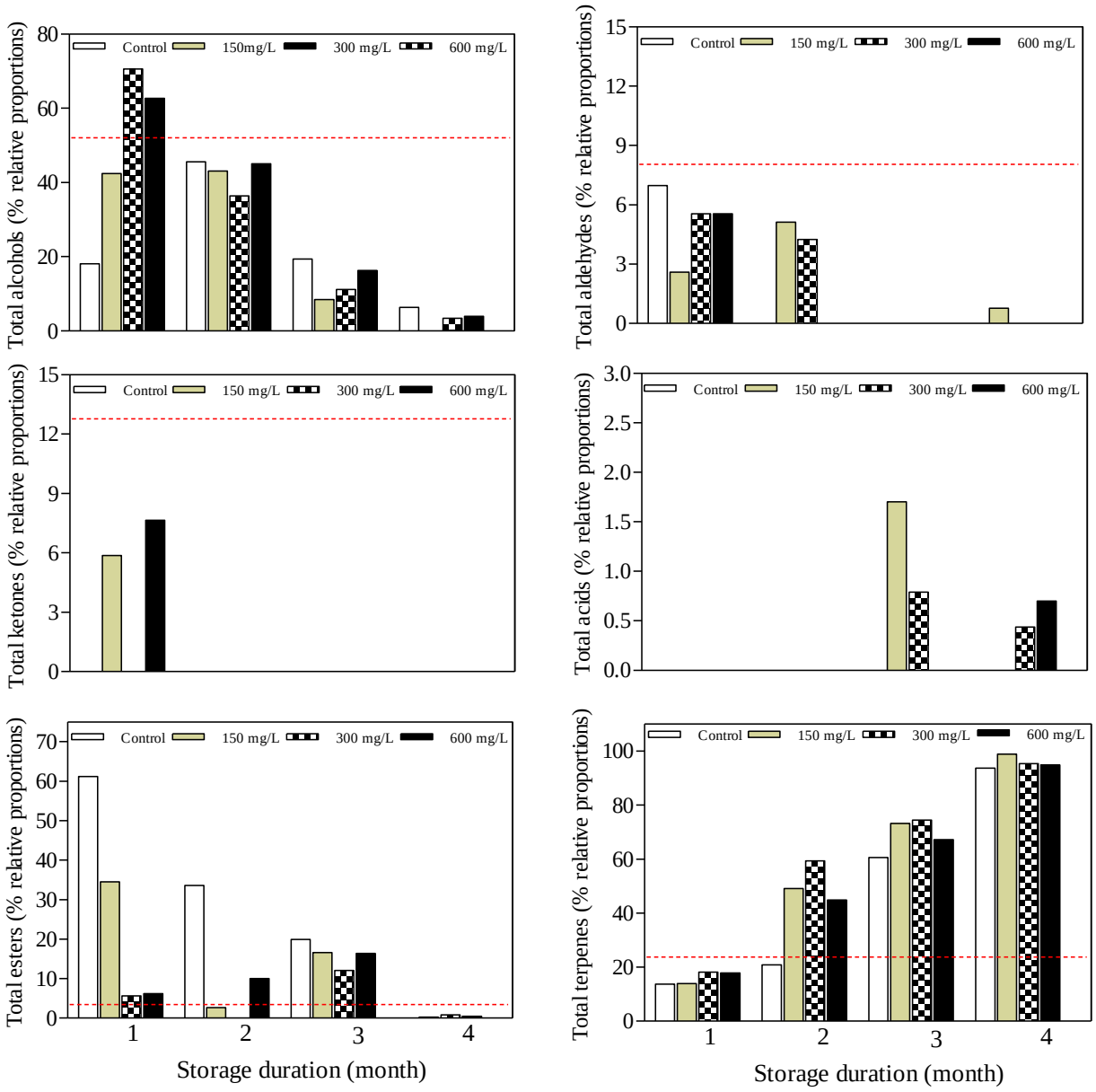

Fig. 5 Changes in chemical groups of volatile compounds in 'Wonderful' pomegranate fruit treated with fludioxonil and stored for 4 months at 5 °C and additional 4 days at 20 °C. ----- Represents values at harvest.

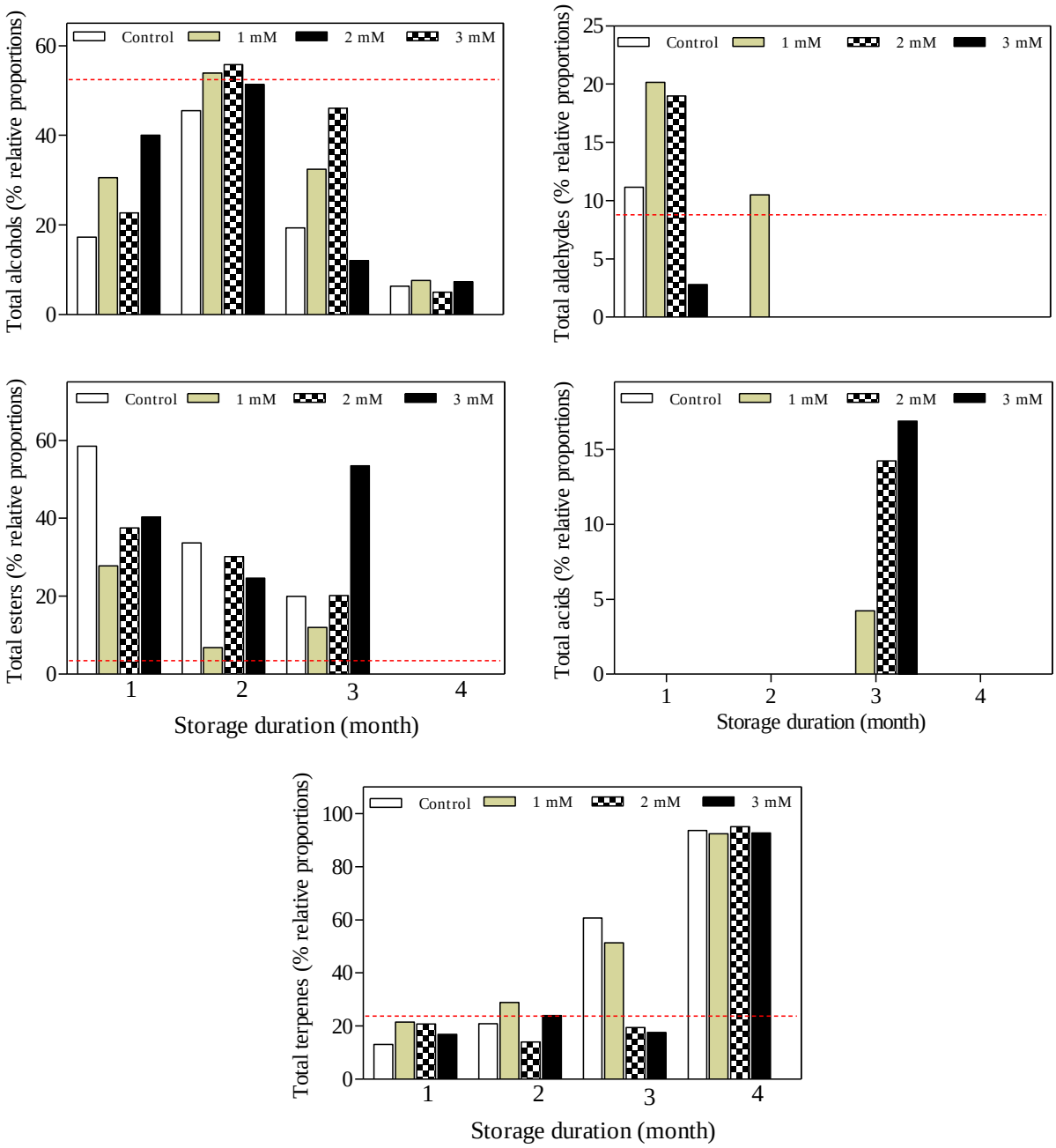

Fig. 6 Changes in chemical groups of volatile compounds in 'Wonderful' pomegranate fruit treated with putrescine and stored for 4 months at 5 °C and additional 4 days at 20 °C. ----- Represents values at harvest.

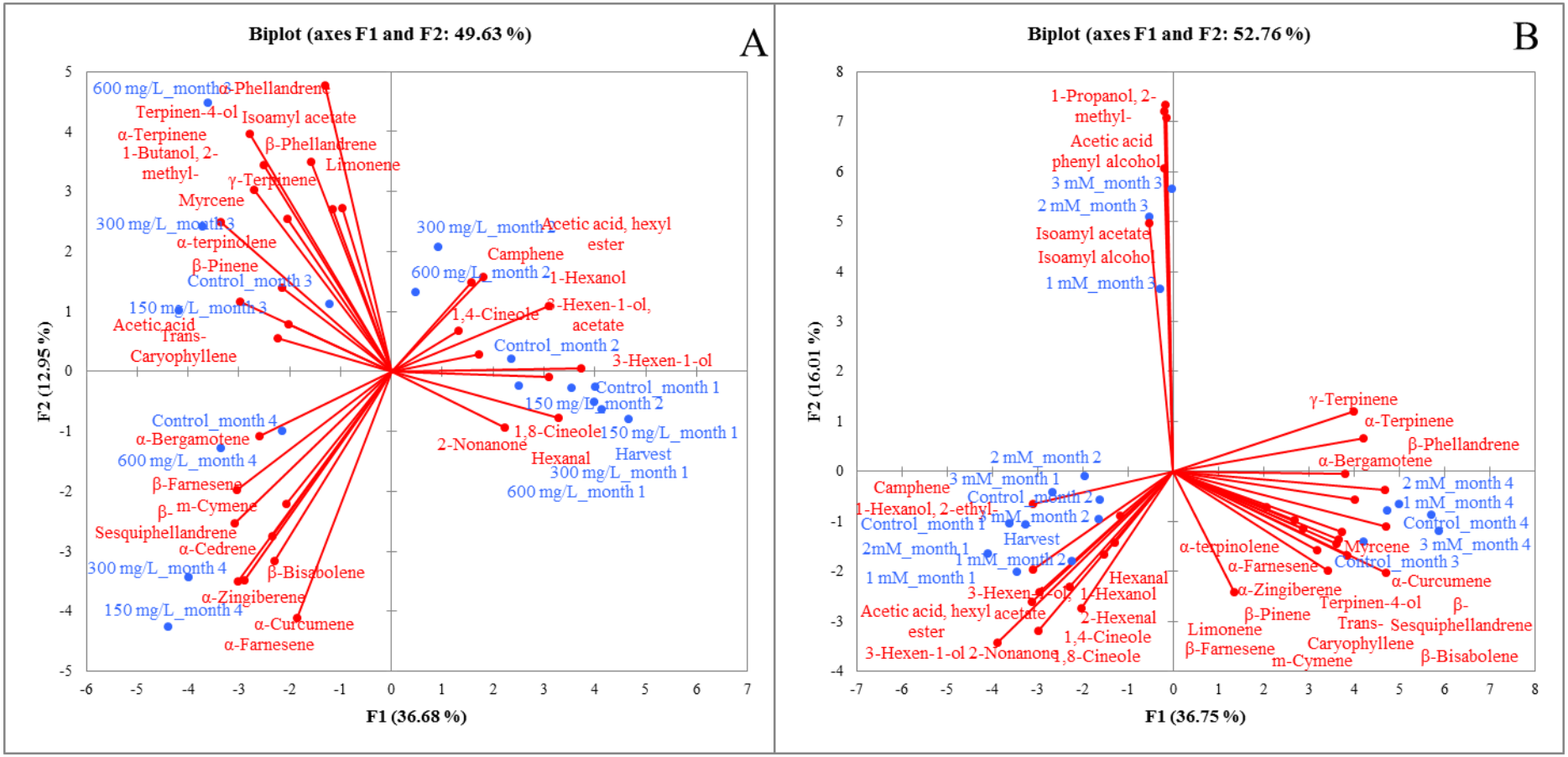

Fig. 7 Principal component analysis biplots showing the effect of fludioxonil (A) and putrescine (B) on volatile organic composition of 'Wonderful' pomegranate fruit stored for 4 months at 5 °C and additional 4 days at 20 °C. Red colour represents the variables while blue colour represents the factors.

CHAPTER SIX: General discussion and conclusions

GENERAL DISCUSSION AND CONCLUSION

Chapter One: General Introduction

Plant phytochemicals have antioxidant activity that protect the body against oxidative stress (Seeram *et al.*, 2006). Therefore, consumption of fruits and vegetables can reduce the risk to chronic illnesses including those that are oxidation-related (Kelawala & Ananthanarayan 2004). Pomegranate in particular has recaptured worldwide consumer interest because of its prominent health benefits (Heber & Bowerman, 2009; Fawole & Opara, 2013a). This has resulted in high global demand for the fruit, and has consequently led to dramatic increase in production and consumption of the fruit (Fawole & Opara, 2013a). Despite the increased demand, pomegranate fruit faces a number of qualitative and quantitative postharvest losses such as chilling injury, weight loss, husk scald and fruit decay (caused by *Aspergillus spp*., *Alternaria spp*., *Penicillium spp*., and especially *Botrytis cinerea*) (Roy & Waskar, 1997). To alleviate these postharvest challenges, a number of postharvest treatments such as fungicide and polyamines may be applied (Opara *et al.*, 2015). It has been shown that quality of pomegranates significantly varies among growing regions (Schwartz *et al.*, 2009; Mditshwa *et al.*, 2013), suggesting that fruit respond differently to different treatments. Most studies on South African grown pomegranates focused on reducing spoilage of arils, which is the edible part of the fruit (Caleb *et al.*, 2013, 2015; Aindongo *et al.*, 2014; Banda *et al.*, 2015a,b; Hussein *et al.*, 2015). Therefore, there is a dearth of information on the postharvest treatments of pomegranate whole fruit grown in South Africa. To supplement the efforts that have helped increase pomegranate production, there is need to minimize postharvest losses and enhance shelf life if the full potential of the emerging pomegranate industry is to be realized. This study investigated the potential of fludioxonil (FLU) and putrescine (PUT) to maintain the quality and reduce postharvest losses of South African pomegranate whole fruit (cv. Wonderful). The study also evaluated the effects of the chemical treatments on the phytochemical properties and volatile organic composition of pomegranate fruit.

Chapter Two: Literature review on application of physical and chemical postharvest treatments to enhance storage and shelf life of pomegranate fruit

To overcome the postharvest challenges faced by the pomegranate fruit industry, a number of postharvest treatments are employed to reduce loss and improve storability of fruit. The

physical and chemical treatments applied to both pomegranate whole fruit and arils were reviewed in this chapter. The chapter explored the effect of these treatments on physiological, physico-chemical, phytochemical and sensory attributes of pomegranate fruit. A detailed discussion of the effects of different postharvest treatments on fruit quality was highlighted. This review emphasised the importance of hurdle technology that involves combining the chemical treatments together with physical treatments to harness the full potential of postharvest technologies. The review showed that despite the availability of various postharvest treatments, high incidence of fruit loss still occurred (Shete & Workar, 2005) which results in loss of nutritional as well as financial loss and growth of the industry. The review therefore highlighted the need for more research focusing on the application of postharvest treatments and innovative technologies to maintain and enhance the nutritional and bioactive components of the fruit.

Chapter Three: Postharvest physiological responses of pomegranate fruit (cv. Wonderful) to exogenous putrescine treatment and effects on physico-chemical and sensory quality attributes.

In this chapter, the potential of putrescine as a postharvest chemical treatment on pomegranate fruit was investigated. Fruit were treated with putrescine (PUT) at 1, 2 and 3 mM concentration and stored for 4 months at 5 °C plus an additional 4 days at 20 °C (shelf life), and the effect on fruit physiological response, quality and sensory attributes evaluated. The results showed that respiration rate increased after the first three months of storage and later decreased after the last month with significant effect of storage duration ($p < 0.0001$). Concentration of putrescine had no significant effect on fruit respiration rate ($p = 0.096$). Fruit weight loss increased over storage with fruit treated with the highest PUT concentration (3 mM) having the lowest weight loss (21.49 %) after the storage duration. This could be due to consolidation of the cell integrity and ameliorating chilling injury (Barman *et al.*, 2011; Jawandha *et al.*, 2012). The results also showed that treating pomegranate fruit with PUT reduced fruit decay especially after long term storage (3 and 4 months), with fruit treated with 2 mM concentration having the least decay incidence. The effect of putrescine on fruit decay could be attributed to the protective function of putrescine through conjugation to phenolic compounds and hydroxycinamic acid amines (Walters, 2003). This showed the potential of PUT in reducing fruit loss during storage. However, putrescine had no effect on internal decay and this was possibly because PUT was exogenously applied and thus did not move

deep into the internal fruit portions to impart its protective benefits. Furthermore, internal decay was due to heart rot, which occurs from infection of fruit during flowering in the orchard (Zhang & McCarthy, 2012; Ezra *et al.*, 2015). PUT was not beneficial in reducing internal decay as treated fruit consistently had higher aril browning compared to control fruit. Therefore, no conclusion on the effect of PUT on aril browning was postulated.

The beneficial effect of PUT on chilling injury (CI) was evident especially after 3 - 4 months of storage such that fruit treated with 2 and 3 mM PUT concentration had lower CI incidence than fruit treated with 1 mM concentration and control. CI index (severity) was generally low among all concentrations. The reduced CI was probably due to the ability of PUT to induce cold acclimation thus maintaining membrane fluidity by preventing changes in lipid cell membrane (Gómez-Galindo *et al.*, 2004; Barman *et al.*, 2011). Lower husk scald was observed among fruit treated with 3 mM PUT while the other concentrations had high incidences. However, after the last storage period (4 months), all fruit had developed husk scald regardless of PUT concentration applied. This was possibly because the antioxidant properties of putrescine were not strong enough to prevent scalding. To control husk scalding in pomegranate, the results highlighted the need to incorporate treatments that ensure a low oxygen environment since scalding in pomegranate is an oxidative process (Ben-Arie & Or, 1986). The lower CI and husk scald indicated that 3 mM PUT concentration resulted in fruit with the best colour and appearance.

External fruit colour decreased with storage duration and the changes in fruit redness (a*) and intensity (a*) were influenced by both PUT concentration and storage duration. The decrease in colour was attributed to peel browning as evidenced by the development of husk scald. Fruit hue angle ($h°$) increased with storage indicating reduction in fruit red colour as storage progressed. Aril colour (a* and C*) on the hand increased with storage for all PUT concentrations and this was probably due to anthocyanin biosynthesis during storage of pomegranate (Gil *et al.*, 1995; Arendse *et al.*, 2014). The decline in aril hue^{o} further buttressed this phenomenon. Fruit treated with PUT may be stored for 4 months without drastic loss of fruit colour. Juice colour of fruit remained stable during storage and was significantly affected by interaction of PUT concentration and duration. Fruit firmness, measured as the fruits ability to resist puncture, decreased with storage with significant interaction between PUT concentration and storage duration. After 4 months of storage, fruit treated with 2 mM had 15.10 % higher firmness than control fruit. This could be attributed to the ability of putrescine to cross link with the pectic substances in the cell wall thus

preventing access of cell wall degrading enzymes and hence reducing softening (Barman *et al.*, 2011). This highlighted role of PUT in reducing senescence and maintaining fruit freshness. There was significant interaction of PUT concentration and duration for aril firmness parameters (hardness, elastic modulus, toughness and bioyield) with treated fruit having higher aril firmness compared to control after storage. Therefore, treating fruit with PUT is recommended as to maintain fruit aril firmness however, the storage period should be put into consideration. Changes in fruit chemical attributes are important because they influence flavour of fruit, which influences consumer acceptability. Increase in pH corresponded with decrease in titratable acidity (TA) during fruit storage and this could be due to utilization of organic acids during metabolic activities such as respiration (Fawole & Opara, 2013a). Initial increase in TSS was probably due to concentration of sugars from moisture loss while the subsequent decrease could be due to utilization of sugars in fruit metabolic processes (Fawole & Opara, 2013a). The changes in TSS and TA influenced the changes in TSS/TA and BrimA parameters, which affect sensory attributes of fruit.

Furthermore, the study evaluated sensory attributes of stored fruit. Results showed that during short-term storage (2 months), control fruit had good sensory quality with regard to sweet taste, crispness and juiciness. However, with longer storage, (2 - 3 months) fruit treated with 1 and 2 mM PUT concentration had better sensory quality (sweet taste, juiciness and crispness). Off flavour was lowest among fruit treated with 2 mM PUT concentration. This therefore, implies that when storing fruit for short period (2 months), chemical treatment may not necessary. However, treating pomegranate fruit with PUT is reccomended when fruit are to be stored for a longer period as it is beneficial in reducing disorders and maintaining sensory quality. The principal component analysis (PCA) explained 53.22 % of the observed variability and separation of parameters was dependent on storage duration. Fruits stored for 1 month were associated with fruit and aril firmness, peel a*, astringency, TA and grittiness. Indicating freshness of fruit with good fruit colour. However, after 3 months of storage, fruit had high sweet taste, crispness, juiciness and aril a*. The high sweet taste was probably due to concentration of sugars and the aril a* was possibly due to biosynthesis of colour pigments, anthocyanins. This therefore elucidates that fruits can be stored for 3 months with good sensory quality.

Overall, this chapter showed the potential of PUT in alleviating physiological disorders during storage of pomegranate fruit with the highest concentration (3 mM) being the most effective. Physico-chemical parameters such as aril colour and fruit firmness were also

maintained after treating fruit with PUT. Sensory quality was best preserved for control and fruit treated with 2 mM PUT concentration. Although 3 mM PUT concentration was the most effective in alleviating physiological disorders, 2 mM PUT concentration had the advantage of both reducing the disorders and also maintaining the sensory quality of the fruit. Therefore, 2 mM is a recommended concentration for treatment of pomegranate fruit with PUT.

Chapter Four: Effects of fludioxonil treatment on physiological response, physico-chemical and sensory properties of pomegranate whole fruit

The postharvest life of fruit is challenged mainly by decay and spoilage during storage. The potential of fludioxonil (FLU) as a postharvest chemical treatment to maintain quality and improve storability of pomegranate whole fruit was explored in this chapter. Fruit were treated with FLU at 150, 300 and 600 mg/L concentration and stored for 4 months at 5 °C plus additional 4 days at 20 °C (shelf life). The effect of FLU on fruit quality was investigated and reported. The results showed increase in fruit respiration rate after the first three months of storage followed by a decrease with changes influenced by storage duration. However, fludioxonil (FLU) concentration had no significantly effect on fruit respiration rate ($p = 0.2760$). The increase in respiration rate was attributed to stress due to ongoing senescence and metabolic activities (Fawole & Opara, 2013a), while the decrease during the last month could be attributed to excessive senescence, physiological disorders and cell disintegration (Nanda *et al.*, 2001; D'Aquino *et al.*, 2010). Weight loss of fruit increased over storage with significant effect of FLU concentration and storage duration. Fruit treated with 150 mg/L FLU concentration consistently had the highest weight loss throughout the storage duration and this was attributed to the high respiration rate that was observed for this concentration especially after months 1 and 4. There were no significant differences observed amongst the other FLU concentrations. This was contrary to the report by D'Aquino *et al.* (2012), who reported no significant effect of FLU treatment on 'Primosole' pomegranate fruit. This conflicting observation further suggested that fruit response to postharvest treatment is dependent on cultivar thus the need for cultivar specific postharvest treatment for pomegranate fruit.

Fruit decay, a major cause of postharvest loss in pomegranate, increased with advanced storage with the highest incidence observed in control fruit. The reduced decay amongst treated fruit could be attributed to the good protective activity of FLU as a fungicide on a

number of pathogens (D'Aquino *et al.*, 2010). FLU has successfully been used to control decay in a number of fruits such as pear, stone fruit and citrus (Adaskaveg *et al.*, 2005; Schirra *et al.*, 2009; D'Aquino *et al.*, 2010; D'Aquino *et al.*, 2013). Internal decay in fruit was majorly due to black heart. Fruit treated with 150 and 600 mg/L FLU concentrations showed low incidences of internal decay while control and 300 mg/L FLU concentration showed high incidences. As FLU application is a contact application, there was no relationship drawn on the effect of FLU on internal decay of pomegranate fruit. It was thus logical to conclude that FLU would not prevent internal decay in pomegranate as such problem results from fruit infection in the orchard during the flowering stage (Zhang & McCarthy, 2012; Ezra *et al.*, 2015). Being a preharvest condition, the study thus suggested use of preharvest treatments and good agricultural practices to control this disorder. Aril browning also increased with storage although untreated fruit had lower browning throughout the storage duration. Since aril browning has been related to CI (Mirdehghan & Rahemi, 2005), it was assumed that the lower incidence in untreated fruit could be attributed to the lower chilling injury incidence that was observed for untreated fruit (Mirdehghan & Rahemi, 2005). Therefore, the study concluded that exogenous application of FLU had no direct effect on the internal components of the fruit.

During the study, chilling injury of fruit developed from the first sampling date and increased as storage progressed. Low CI incidence was observed for control and fruit treated with 600 mg/L FLU while high incidences were observed for 150 and 300 mg/L FLU concentration. CI in pomegranate fruit has been attributed to changes in the state of lipid cell membranes from liquid-crystalline to solid-gel state and this causes injurious effects (Mirdehghan *et al.*, 2007; Barman *et al.*, 2011). The study suggested that treating fruit with FLU did not alleviate chilling injury in pomegranate fruit. Husk scalding was the major physiological disorder observed in the study, with high scalding incidence and severity as storage progressed. After 4 months of storage, all fruit had developed husk scalding regardless of concentration applied, suggesting that treating pomegranate fruit with FLU does not alleviate husk scalding during cold storage. This was possibly because scalding results from enzymatic oxidation of phenolic compounds in the peel of the fruit (Kahn, 1983; Zhang & Zhang, 2008) and since the chemical treatment did not reduce the oxygen tension, fruit developed severe husk scald. Furthermore, control fruit lost aril firmness faster than treated fruit, a phenomenon that results from loss of cell wall integrity due to break down of pectic substances and aril membrane deterioration (Sayyari *et al.*, 2011; Bchir *et al.*, 2012; Fawole & Opara, 2013b).

Aril firmness with regard to hardness declined with storage but there were no significant differences among FLU concentrations. In addition, there were no significant differences found for bioyield, elastic modulus and toughness with significant interaction of the factors (FLU concentration and storage duration).

Colour is an important attribute for consumer acceptability and is used to grade pomegranate fruit. Fruit peel redness (a*) declined as storage progressed, with no significant differences among FLU concentrations. This indicates loss of peel colour pigments due to senescence and peel browning (Arendse *et al.*, 2014). This was also evident by the dynamics in peel hue angle (h^o), which increased with increasing storage duration. Peel colour was mainly influenced by storage duration and fruit may be stored up to 3 months without excessive loss in colour. Aril colour was influenced by FLU concentration with treated fruit having lower aril a* and C* and higher h^o, indicating the negative effect of FLU on colour. Nevertheless, the aril colour from treated fruit was sufficient for consumption. Chemical attributes are of importance as they affect fruit flavour, which influences consumer acceptability and repeat purchase of fruit by consumers. Significant decline in titratable acidity was observed during storage. This was not surprising as organic acids are utilised during fruit respiration (Montero *et al.*, 2010; Fawole & Opara, 2013a). Interestingly however, there was an increase in TA during month 3. This could be due to concentration effect as the fruit continued to loss moisture. Concomitant decline in TSS was also observed as storage progressed possibly due to utilisation of sugars in some metabolic processes during storage (Fawole & Opara, 2013a). Furthermore, there was no significant effect of FLU concentration. The dynamics in TA and TSS reflected different changes on derived parameters; TSS/TA and BrimA. This would have a practical implication on sensory attributes of the investigated fruit.

The study further evaluated the effect of FLU treatment on sensory attributes of fruit. Although control fruit had highest sweet taste after 3 months of storage, fruit treated with 600 mg/L FLU concentration had the better sensory qualities with regard to sweet taste, crispness, juiciness and firmness after 1 and 3 months of storage. The FLU concentration also had lower scores for off flavour, astringency and grittiness. This implies that FLU at 600 mg/L concentration maintained the best sensory quality of fruit for 3 months with fruit having good flavour and firmness and no off flavour. After 3 months, fruit treated with 150 mg/L FLU concentration had the lowest sensory quality (lowest sweet taste, crispness, and juiciness but highest sour taste and astringency). From the principal component analysis (PCA), the effect of storage duration was more prominent as grouping was more distinct with duration than

FLU concentration. Fruit stored for 1 month had high astringency, fruit firmness, TA, peel and aril redness. Implying that fruit were still fresh, firm, more appealing appearance and more acidic, with characteristics of fresh pomegranate fruit. Fruit stored for 2 months were associated with crispness, TSS, sweet taste, juiciness and bioyield. This means that fruit still had their freshness and tasted sweeter probably due to loss of moisture. With further storage to 3 months, fruits had high peel h^o, pH, flavour, grittiness and sour taste indicating loss of fruit colour (due to physiological disorders) and lower acidity but fruit still good flavour. Despite lower colour, fruit were still marketable because after 3 months, the severity of physiological disorders were below moderate. Therefore, fruit can be stored for up to 3 months of storage.

Overall, this chapter showed that FLU was effective in reducing decay of fruit during storage. However, the chemical did not decrease development of physiological disorders; chilling injury, husk scald and aril browning. FLU maintained physico-chemical attributes and had fruit with good sensory quality. Overall, 600 mg/L FLU concentration was the most effective in reducing fruit decay and had fruit with better sensory attributes.

Chapter Five: Effects of fludioxonil and putrescine postharvest treatments on phytochemical, antioxidant properties and volatile composition of pomegranate fruit during long-term storage

The effect of both fludioxonil and putrescine on phytochemicals and volatile composition of pomegranate fruit was assessed in this chapter. With a significant interaction of FLU concentration and duration, there was a slight decline in the ascorbic acid (AA) content of fruit treated with FLU during the study. Control fruit on the other hand had values higher than at harvest for month 2, 3 and 4 (119.50 ± 0.15 and 112.70 ± 0.07 and 114.90 ± 0.60 mg AA/ 100 mL respectively). The higher ascorbic acid content in untreated fruit was attributed to concentration of AA as a result of moisture loss. On treating fruit with PUT, slight changes were observed in the ascorbic acid content of fruit. Interaction of the factors was significant. After storage 4 months of storage, however, control fruit had higher AA content while no significant differences were observed among PUT concentrations and this was also attributed to moisture loss due to concentration. Treating fruit with FLU and PUT did not maintain AA content as treated fruit had lower AA content than control. Total phenolic content (TPC) of fruit significantly declined during storage of fruit treated with FLU with significant

interaction of the factors. Untreated fruit had lower TPC for the first three months of storage compared to treated fruit. The decrease in TPC during fruit storage could be attributed to the breakdown of phenolic compounds due to enzymatic activity (Fawole & Opara, 2013a; Arendse *et al.*, 2014). The study showed that treating pomegranate fruit with FLU resulted into higher TPC for short-term storage up to 3 months thus FLU acted as an elicitor to promote biosynthesis of total phenolics.

With regard to PUT treatment, the trend was reversed as control fruit initially had higher TPC (above harvest). Treated fruit only had higher TPC after month 2. Rapid moisture loss in control fruit resulted in concentration of juice constituents including TPC during the first month. However, TPC declined afterwards probably due to enzymatic breakdown of phenolic compounds (Fawole & Opara, 2013a; Arendse *et al.*, 2014). Therefore, FLU retained more TPC of fruit while PUT had no benefit with regard to mainting TPC of fruit. Treating pomegranate fruit with FLU resulted in an initial increase in the total anthocyanin content (TAC) after the first two months of storage, above harvest. This could be attributed to accumulation due to anthocyanin biosynthesis in pomegranate fruit during storage as previously reported (Miguel *et al.*, 2004; Fawole & Opara, 2013a; Arendse *et al.*, 2014). Anthocyanins have been reported to be unstable and easily degraded due to enzymatic oxidation as a result of loss of compartmentalization of substrates during long storage (Jiang & Chen, 1995). This explains the observed decreased in TAC after prolong storage (3 and 4 months of storage), with no significant differences observed among FLU concentrations. A similar observation was also noted on TAC when fruit were treated with PUT.

The antioxidant capacity of fruit treated with FLU and PUT increased with storage duration. Although previous studies have attributed increase in antioxidant activity to phenolic compounds (Gil *et al.*, 2000; Fawole & Opara 2013a), this was not observed during the study. Some studies have suggested that antioxidant capacity could be due to vitamin C and tannins (Barman *et al.*, 2014). In general, the antioxidant capacity observed did not correlate with the trends observed for TPC, ascorbic acid or total anthocyanin thus suggesting that other bioactive compounds such as tannins could have been the main contributors to the fruit antioxidant capacity found during the study.

On determining the volatile composition during storage of fruit treated with FLU and PUT, 31 and 32 volatile compounds were identified for FLU and PUT respectively. Six chemical groups (alcohols, aldehydes, ketones, acids, esters and terpenes) were detected for fruit treated with FLU while five groups (minus ketones) were detected for PUT treated fruit. The

volatiles evolved during storage with eight compounds identified at harvest but more compounds evolved as storage progressed especially terpenes for both FLU and PUT treatments. The alcohol group was predominant between month 1 and 2 with 1-hexanol (leafy, fruity and woody) as the most abundant compound. As storage progressed to 3 and 4 months, terpenes (monoterpnes and sesquiterpenes) massively accumulated with m-cymene characterised by citrus, woody, terpenic, spicy and cumin aroma being the most abundant compound for both FLU and PUT treatments. Upregulation of terpenes has been attributed to response of fruit to chilling stress (Mphahlele *et al.*, 2016). Accumulation of terpenes may have an impact on flavour of fruit (Mayuoni-Kirshinbaum *et al.*, 2013). Storage of fruit for long duration may result in fruit with undesirable flavour due to accumulation of terpenes. Therefore, irrespective of concentration used fruit treated with FLU and PUT can be stored up to 3 months without significant loss of fruit flavour.

Recommendations and future prospects

The study showed that PUT was effective in alleviating physiological disorders; chilling injury, husk scald and external fruit decay but not internal decay. The chemical maintained physico-chemical and sensory quality of fruit although the benefits on phytochemical properties was not evident. The study highlighted that PUT at 2 mM concentration had the benefit of both reducing physiological disorders and maintaining good sensory fruit quality. With regards to FLU, the chemical significantly reduced fruit decay during storage, but was not effective in alleviating the other physiological disorders (chilling injury, husk scald and aril browning). FLU, however, maintained good sensory quality, physico-chemical attributes and phytochemical properties of fruit. FLU at 600 mg/L concentration was the most effective in reducing fruit decay, had fruit with better sensory attributes and maintained phytochemical properties of stored fruit.

For both FLU and PUT, fruit were stored for up to 3 months without adversely affecting fruit quality. Therefore, FLU and PUT have the potential to be applied as postharvest chemical treatments although further research is required to improve on their efficacy. This information is helpful in reducing postharvest losses, improving storability and maintaining quality by treating fruit at farm and pack-house level. However, husk scald and weight loss remained a challenge during the study especially from 3 months of storage. Therefore, future prospects could focus on the use of physical treatments as complementary postharvest

strategies for reducing fruit spoilage. The use of simple physical treatments such as liners and surface coating (for example waxing) in combination with chemical treatments would be worth investigating. Additionally, further research may also focus on combination of FLU and PUT to benefit from hurdle effect to harness the full potential of the two chemicals since they have different modes of action.

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