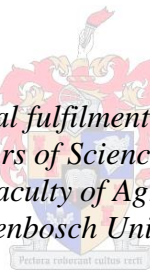


# **Determining optimum storage conditions for pomegranate fruit (cv. Wonderful)**

by

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degree of Masters of Science in Food Science  
in the Faculty of AgriSciences  
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## **DECLARATION**

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## SUMMARY

The development of science-based management tools and appropriate postharvest handling protocols are required for the determination of optimal storage performance of pomegranate fruit. The South African pomegranate industry experiences considerable fruit quality losses due to the lack of knowledge on optimal storage and handling practices. The cultivar ‘Wonderful’ is the widely grown in South Africa; however, to date there is currently limited scientific knowledge on the storage requirements. To develop quality standards for the export market, knowledge of optimum storage conditions are required to provide an understanding of postharvest quality attributes and consumer organoleptic perceptions. The overall aim of this research was to provide science-based management tools for the storage performance of pomegranate fruit (cv. Wonderful).

The research reported in Chapter 3 focused on the physiological responses of pomegranate fruit at different storage temperatures. Commercially harvested fruit were stored at  $5\pm 0.7^{\circ}\text{C}$ ,  $7.5\pm 0.3^{\circ}\text{C}$  and  $10\pm 0.5^{\circ}\text{C}$  with  $92\pm 2\%$  RH and at room temperature ( $21\pm 3^{\circ}\text{C}$ ,  $65\pm 6\%$  RH) for 5 months. Fruit respiration and physiological disorders during long term storage were investigated. During storage, low temperatures evidently resulted in lower respiration rates; however, respiration rate increased gradually after 2 months resulting in higher respiration rates at  $5^{\circ}\text{C}$  than  $7.5^{\circ}\text{C}$  after 3 month storage period. Overall, fruit became more susceptible to internal and external disorders as storage period progressed. Storage of fruit longer than 2 months at  $5^{\circ}\text{C}$  resulted in chilling injury and this was observed over the 5 month storage period. Fruit stored at  $21^{\circ}\text{C}$  and  $10^{\circ}\text{C}$  were discarded after 1 and 4 months, respectively, due to complete fruit loss to decay and peel shrinkage. Furthermore, the severity of browning increased with storage temperatures, although this became more severe at  $5^{\circ}\text{C}$  after 3 months. Therefore, to maintain a relatively low respiration rate and minimize physiological disorders, the cv. Wonderful should be stored at  $5^{\circ}\text{C}$  and  $>92\%$  RH for storage period up to 3 months.

In Chapter 4, the effects of temperature and storage duration on pomegranate fruit quality and mechanical properties were conducted. This study revealed that weight loss increased with rise in temperature and storage duration with the primary source of moisture loss being the fruit skin (peel), which resulted in significant reduction in peel thickness with prolonged storage period. The CIE ( $L^*$ ,  $a^*$ ,  $b^*$  and  $C^*$ ) colour parameters of fruit and arils decreased during storage. However, the hue ( $h^{\circ}$ ) for whole fruit increased as a result of browning incidence, and decreased in arils suggesting an increase in redness. Significant increases in total soluble solids (TSS), pH, TSS:TA and BrimA were observed with significant decreases in titratable acidity (TA) occurring throughout the storage period. Storage temperature and duration significantly affected majority

of the investigated mechanical properties. Puncture resistance, fruit and aril compression strength decreased with storage temperature and duration. These findings showed that fruit may be stored between 2 to 3 months at 5°C to ensure the best internal and external quality attributes.

The studies in Chapter 5 investigated the effects of storage temperature and duration on phytochemical and antioxidant properties. Fresh pomegranate juice was assessed for concentrations of total phenolic compounds, total anthocyanin and ascorbic acid. The antioxidant property of the fruit juice was tested against 2, 2-diphenyl-1-picryl hydrazyl (DPPH). The results showed that total phenolic and total anthocyanin concentration increased up to 3 months of storage at 5°C, 7.5°C, 10°C and 21°C and decreased gradually over time. For antioxidant activity, storage of fruit at 5°C, 7.5°C and 10°C significantly ( $p < 0.05$ ) reduced the radical scavenging activity of juice by more than 56% when stored beyond 2 months. Furthermore, ascorbic acid concentration gradually declined with increasing storage duration, resulting in reduced juice antioxidant capacity. These findings are beneficial to pomegranate export industries, especially where fruit are stored for long for use in health-promoting purposes.

The research conducted in Chapter 6 focused on determining suitable storage conditions based on the combination of instrumental measurements and sensory attributes. During storage, individual fruit were evaluated by trained sensory panel based on the overall appearance, taste and aril texture. Discriminant analysis at different storage temperatures was used to distinguish fruit from each other at 2 months of storage with sensory attributes such as overall pomegranate flavour ( $R^2 = 0.56$ ), total anthocyanin ( $R^2 = 0.46$ ) and Chroma ( $C^*$ ) colour index ( $R^2 = 0.37$ ). Discriminant analysis further showed that storage time rather than storage temperature led to the reduction in overall quality when storing fruit beyond 2 months. Based on sensory attributes, suitable storage temperature and duration were found to be 5°C and 2 months when overall flavor were highly rated; thereafter, significant reductions in overall appearance, aril and kernel texture were observed. Furthermore, the proposed storage conditions were supported with instrumental measurements, which revealed a decline in important fruit attributes such as total phenolics, total anthocyanin, aril colour and aril texture after 2 months of storage.

Overall, this study provides science-based tools required for developing cold chain handling protocols needed to manage the long supply chain of 'Wonderful' pomegranate fruit grown in South Africa.

## OPSOMMING

Die ontwikkeling van wetenskap-baseerde beheerinstrumente en toepaslike na-oes hanteringsmetodes is nodig vir die vasstelling van die optimale stoorprestasie van granate. Die Suid-Afrikaanse granaatindustrie ondervind groot vrug kwaliteit verliese as gevolg van die gebrek aan kennis oor optimale stoor en hantering praktyke. Die kultivar Wonderful is die wyd gegroei in Suid-Afrika, maar tot hede daar is tans beperk wetenskaplike kennis oor die stoor vereistes. Om gehaltestandaarde vir die uitvoermark te ontwikkel word kennis van die optimale stoortoestande benodig sodat 'n begrip van die na-oes gehalte-kenmerke en verbruiker se organoleptiese persepsies gevorm kan word. Die oorhoofse doelwit van die navorsing is om wetenskap-baseerde beheerinstrumente vir die stoor van granate (bv. Wonderful) te verskaf.

Die navorsing wat in Hoofstuk 3 beskryf word is gerig op die fisiologiese respons van granate op verskillende bergingtemperatuur. Kommersieel-gekweekte vrugte is by  $5\pm 0.7^{\circ}\text{C}$ ,  $7.5\pm 0.3^{\circ}\text{C}$  en  $10\pm 0.5^{\circ}\text{C}$  met  $92\pm 2\%$  RH en by kamertemperatuur by ( $21\pm 3^{\circ}\text{C}$ ,  $65\pm 6\%$  RH) vir 5 maande gestoor. Die respirasie van die vrugte en die fisiologiese ongesteldhede gedurende langtermyn stoor word ondersoek. Gedurende stoor het die laer temperature gelei tot laer respirasie koerse; maar respirasie koers het geleidelik na 2 maande verhoog wat lei tot hoër respirasie koerse by  $5^{\circ}\text{C}$  as teen  $7.5^{\circ}\text{C}$  na 'n 3-maande stoorperiode. Algehele, vrugte het egter meer vatbaar geword vir interne en eksterne ongesteldhede hoe langer die stoortydperk geduur het. Die stoor van vrugte langer as 2 maande teen  $5^{\circ}\text{C}$  lei tot skade as gevolg van verkoeling en dit is oor die 5 maande stoor tydperk waargeneem. Vrugte wat teen  $21^{\circ}\text{C}$  en  $10^{\circ}\text{C}$  gestoor is moes na onderskeidelik 1 tot 4 maande as gevolg van verlies wat die gevolg was van swam skade en skil krimpings, weggegooi word. Die erns van die verbruining het verhoog toe die stoortemperature verhoog, alhoewel dit meer geraak het teen  $5^{\circ}\text{C}$  na 3 maande. Om dus 'n betreklik lae respirasie koers en min fisiologiese probleme te verseker, moet die kultivaar Wonderful teen  $5^{\circ}\text{C}$  en  $>92\%$  RH vir 3 maande gestoor word.

In Hoofstuk 4 word die effek van temperatuur en die duur van stoor op die gehalte van die granate en die meganiese eienskappe gemeet. Daar is bevind dat gewigsverlies met verhoogte toename in temperatuur en langer stoorperiodes toeneem en dat die hoofbron van verlies aan vog die skil van die vrug is. Die gevolg hiervan is 'n betekenisvolle reduksie in die dikte van die skil na 'n lang stoorperiode. Die CIE ( $L^*$ ,  $a^*$ ,  $b^*$  and  $C^*$ ) kleur parameters van vrugte en granaatpitte het tydens stoor verminder. Die tint, ( $h^{\circ}$ ) van die hele vrug het as gevolg van verbruining, verhoog en het verminder in granaatpitte wat daarop dui vermeerdering in rooiheid. Daar was betekenisvolle verhogings in die totale oplosbare vaste stowwe (TSS), pH, TSS:TA en BrimA is opgemerk met betekenisvolle vermindering in asiditeit waarvan die waarde bepaal kan word

(TA) en wat tydens die stoortydperk plaasvind. Stoortemperatuur en die duur van die stoor het 'n groot invloed gehad op die meganiese kenmerke wat ondersoek is. Weerstand teen priken die kompressie krag van die vrugte en die granaatpitte het met verhoogde temperatuur en duur van stoor afgeneem. Hierdie bevindinge het getoon dat vrugte kan gestoor word tussen 2 tot 3 maande by 5°C die beste interne en eksterne kwaliteit eienskappe om te verseker.

In hoofstuk 5 is die effek van stoortemperatuur en duur op die fitochemiese en antioksidant kenmerke ondersoek. Vars granaatsap is ondersoek en ramings is gemaak t.o.v. totale konsentrasies van fenoliese samestellings, totale antosianiene en askorbinesuur. Die antioksidant kenmerke van die vrugtesap is getoets vir met 2, 2-diphenyl-1-picryl hydrazyl (DPPH). Daar is bevind dat die totale fenoliese en totale antosianiene konsentrasies tot by 3 maandemaande van stoor teen 5°C, 7.5°C, 10°C and 21°C toegeneem het en toe mettertyd afgeneem het. Wat betref antioksidant aktiwiteit, is daar gevind dat die stoor van vrugte teen 5°C, 7.5°C en 10°C die radikale reinigingsaktiwiteite van die sap betekenisvol ( $p < 0.05$ ) met meer as 56% verminder as dit vir meer as 2 maande gestoor word. Verder, askorbiensuur konsentrasie geleidelik afgeneem met toenemende stoor duur, wat lei tot verlaagde sap antioksidant kapasiteit. Hierdie bevinding is van belang vir die granaatuitvoerindustrie, veral waar vrugte vir 'n lang tydperk gestoor vir gebruik in gesondheids-bevordering doeleindes.

Die navorsing wat in hoofstuk 6 beskryf is, het gefokus op die vasstelling van geskikte stoortoestande baseer op 'n kombinasie van instrumentale meting en sensoriese kenmerke. Gedurende stoor word individuele vrugte deur 'n opgeleide panel evalueer t.o.v. voorkoms, smaak en tekstuur van die granaatpitte. Diskriminantontleding teen verskillende stoor temperature is gebruik om vrugte na 2 maande stoor vrugte t.o.v sensoriese kenmerke soos algehele granaat smaak. ( $R^2 = 0.56$ ), totale antosianiene ( $R^2 = 0.46$ ) en Chroma ( $C^*$ ) kleur indeks ( $R^2 = 0.37$ ) te onderskei. Diskriminantontleding het verder getoon dat die duur van die stoor en nie die stoortemperatuur nie, gelei het tot die reduksie in algehele gehalte as die vrugte vir langer as 2 maande gestoor word. Gegronde op sensoriese eienskappe is geskik stoor temperatuur en duur gevind word by 5°C en 2 maande wanneer algehele geur was as hoog beoordeel; en daarna, is aansienlike vermindering in die algehele voorkoms, en die tekstuur van die granaatpitte afgeneem. Hierdie voorgestelde stoortoestande word ook ondersteun deur instrumentele meting, wat 'n afname in belangrike kenmerke soos totale fenologie, totale antosianiene en die kleur en tekstuur van die granaatpitte na 'n 2 maande stoorperiode toon.

In die geheel verskaf die bevindinge van hierdie studie wetenskap-baseerde instrumente vir die ontwikkel van koue-ketting hantering protokol vir die bestuur van die lang verskaffingsketting van Wonderful granate wat in Suid-Afrika gekweek word.

**LIST OF PAPERS TO BE SUBMITTED FOR PUBLICATION**

1. **Arendse, E.**, Fawole, O.A. Opara U.L. Postharvest biology and storage behaviour of pomegranate fruit (*Punica granatum* L.): a review. Prepared to be submitted to *Journal of the Science of Food and Agriculture*
2. **Arendse, E.**, Sigge G.O. Fawole, O.A. Opara U.L. Influence of storage temperature and duration on postharvest physico-chemical and mechanical properties of pomegranate fruit and arils. Prepared to be submitted to *CyTA Journal of Food*
3. **Arendse, E.**, Fawole, O.A. Opara U.L. Effects of postharvest storage conditions on phytochemical and antioxidant properties of pomegranate (cv. Wonderful). Prepared to be submitted to *Scientia Horticulturae*
4. **Arendse, E.**, Fawole, O.A. Opara U.L. Postharvest physiological response of pomegranate (*Punica granatum*) fruit at different temperature regimes. Prepared to be submitted to *International Journal of Fruits Science*
5. **Arendse, E.**, Fawole, O.A. Opara U.L. Discrimination of pomegranate fruit quality by instrumental and sensory measurements during storage at three temperature regimes. Prepared to be submitted to *Journal of Stored Products*

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# Chapter 1

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## Introduction

## GENERAL INTRODUCTION

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### 1. Background

Pomegranate (*Punica granatum* L.) belongs to the *Punicaceae* family. It is native to Persia (Iran) and widely cultivated in the Mediterranean region (Holland *et al.*, 2009). The edible part (aril) of the fruit is consumed as fresh arils or as processed products such as jams, jellies, wine, and beverages (Aarabi *et al.*, 2008; Mousavinejad *et al.*, 2009; Opara *et al.*, 2009). Scientific evidence has linked increasing consumption of pomegranate fruit to improved human health as a result of active phenolic compounds which have potent pharmacological activities, including, antioxidant, anti-mutagenic, anti-hypertension, anti-inflammatory activities (Gil *et al.*, 2000; Kaur *et al.*, 2006; Duman *et al.*, 2009; Viuda-Martos *et al.*, 2010; Fawole *et al.*, 2012).

At present ninety percent of the world's pomegranate production occurs in the Northern Hemisphere. The main producers are India, Iran, USA, Turkey, Spain and Israel (Citrogold, 2011; Pomegranate Association of South Africa, 2012). A growing exporting opportunity exists for countries in the Southern Hemisphere to provide fruit to these markets during the counter season. South Africa is one of the major producers of pomegranates in the Southern Hemisphere, competing with countries such as Chile, Argentina and Australia (Brodie, 2009). Currently, South Africa's commercial production of pomegranate fruit stands at 198,000 tons/440,000 cartons, which is a dramatic increase from 2009/2010 exporting season of 315 tons/70,000 cartons (Brodie, 2009; Perishable Products Export Control Board, 2012). South African pomegranates are mainly cultivated in the Northern Cape, Western Cape, Gauteng, Mpumalanga and Limpopo provinces (Wohlfarter *et al.*, 2010). The harvesting period for pomegranates in Western Cape is from February to late May. The main cultivars that are produced are 'Bhagwa', 'Mollar de Elche', 'Ruby', 'Arakta', 'Ganesh' and 'Wonderful' (Brodie, 2009).

Consumption and the availability of pomegranate fruit in the market are largely restricted to the harvesting season due to a high demand and lack of appropriate postharvest technology to extend the storage life and maintain fruit quality. Postharvest handling practices such as packaging and postharvest conditions such as temperature and relative humidity could be used to maintain fruit quality to prolong storage periods (Nanda *et al.*, 2001; Bayram *et al.*, 2009). Storage temperature and relative humidity are important environmental factors

affecting postharvest life of fresh fruit because they regulate the rate of all associated physiological processes, biochemical reactions and microbial growth (Li & Kader, 1989; Al-Mughrabi *et al.*, 1995). Previous reports have shown that physiological, physicochemical, phytochemical, mechanical, microbial and sensory qualities of pomegranate fruit are influenced by storage temperature, packaging and atmospheric conditions (Elyatem & Kader, 1984; Küpper *et al.*, 1994; Gil *et al.*, 1996; Artés *et al.*, 2000; Bayram *et al.*, 2009; Ekrami-Rad *et al.*, 2011; Fawole & Opara, 2013).

Pomegranates are classified as non-climacteric fruits and therefore cannot continue the ripening process once detached from the plant (Kader, 2006). The fruit may be stored for several months at temperatures below 10°C to extend the marketing value (Artés *et al.*, 2000; Kader, 2006; Ghafir *et al.*, 2010). However, several postharvest disorders could occur during short or long term storage. Apart from the external postharvest quality defects, such as moisture loss, leading to appearance of husk scald (browning of the skin surface), and the development of decay (Elyatem & Kader, 1984; Ben-Arie & Or, 1986), changes in the internal quality of the fruit could also occur (Fawole & Opara, 2013). Many authors have reported decline in the total soluble solids and titratable acidity (Elyatem and Kader, 1984; Artés *et al.*, 1998; Aarabi *et al.*, 2008; Fawole & Opara, 2013). In addition, loss in pomegranate fruit colour, as a result of degradation of anthocyanins has been reported (Gil *et al.*, 1995). Furthermore, optimum storage conditions have been reported to range between 0 to 10°C, depending on fruit cultivar (Fawole & Opara, 2013). According to Kader (2006), the Californian grown ‘Wonderful’ fruit was susceptible to quality loss and chilling injury when stored longer than 1 month at temperatures between -3°C and 5°C or upon transfer from cold storage to 20°C. These findings highlight the need to study specific cultivars in order to determine their optimal postharvest storage performance.

The South African pomegranate industry is currently plagued with fruit quality loss as a result of inappropriate storage and handling. To date, there is currently limited scientific knowledge on the storage requirements for the ‘Wonderful’ cultivar. In order to take full advantage of the existing export market, there is a need to develop postharvest handling practices to maintain fruit quality and reduce postharvest losses. Therefore in order to develop quality standards for the export market optimum storage conditions are required to provide an understanding of postharvest quality attributes and consumers organoleptic perception.

## **2. Aim and objectives**

### *2.1. Aim*

The overall aim of this research study was to provide science-based management tools for improving the storage performance of pomegranate fruit (cv. Wonderful).

### *2.2. Objectives*

The specific objectives of this study were to

- a. investigate fruit physiological responses under different storage temperatures
- b. determine the effects of storage handling practices (temperature, relative humidity and duration) on the quality and mechanical attributes of pomegranate fruit
- c. evaluate the effects of storage temperature and duration on fruit phytochemical and antioxidant capacities during postharvest storage
- d. evaluate the effect of postharvest storage conditions and duration on sensory attributes of pomegranate fruit

## **3. Thesis structure**

This dissertation is structured into 7 chapters (1-7) each addressing a specific research subject

- Chapter 1: contains a brief background, overall research aim and objectives (Introduction)
- Chapter 2: gives a descriptive review on the existing knowledge on the effects of post-harvest handling practices on storage behavior of pomegranate fruit
- Chapter 3: reports on the physiological responses of pomegranate fruit under different storage temperatures
- Chapter 4: reports the effect of storage temperature on postharvest quality attributes and mechanical properties of pomegranate fruit and arils
- Chapter 5: focuses on phytochemicals and antioxidant capacities of pomegranate arils
- Chapter 6: discusses postharvest quality of pomegranate fruit under different storage temperatures based on the combination of sensory and instrumental attributes

- Chapter 7: gives a general discussion on the results from all chapters. It highlights the practical contribution of the studies which would help to provide science-based management tools for the storage performance of the investigated cultivar

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# **Chapter 2**

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## **Literature Review**

## REVIEW OF LITERATURE: POSTHARVEST BIOLOGY AND STORAGE BEHAVIOUR OF POMEGRANATE FRUIT

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### 1. Introduction

Pomegranate (*Punica granatum* L.) belongs to the *Punicaceae* family; it is a tropical and subtropical deciduous or evergreen shrub capable of growing in different soil types and climatic conditions (Sepúlveda *et al.*, 2000). Due to its multifunctional and nutritional benefit in the human diet (Lansky & Newman, 2007; Opara *et al.*, 2009; Fawole & Opara, 2013a), there has been a considerable increase in commercial farming of pomegranate fruit globally, satisfying the nutritional and medicinal needs of consumers in various countries (Holland *et al.*, 2009). Several studies have reported potent anti-mutagenic, anti-hypertension, and anti-inflammatory properties in pomegranate fruit. These properties are due to several groups of therapeutic compounds in the fruit, majorly polyphenols which are reported to have strong antioxidant and other biological activities (Gil *et al.*, 2000; Lansky & Newman, 2007; Elfalleh *et al.*, 2009; Viuda-Martos *et al.*, 2010; Fawole *et al.*, 2012a).

Despite the increasing consumer awareness of the health benefits of pomegranate, consumption of the fruit is still limited due to the difficulty in extracting arils from the fruit. Occurrence of physiological disorders such as husk scalds, splitting, and chilling injury is other challenge which reduces marketability and consumers acceptance (Ben-Arie & Or, 1986; Saxena *et al.*, 1987). During transportation and storage of pomegranate fruit, a number of physiological, biochemical and textural processes occur, which result in changes in colour, taste, texture, and ultimately decline in nutritional quality and sensory attributes. Furthermore, shrivelling which leads to hardening and browning of fruit rind and arils and increased fruit susceptibility to decay also occurs during storage (Caleb *et al.*, 2012a).

Pomegranate fruit quality assessment is based on several important external and internal attributes. External attributes include fruit size, shape and skin appearance (colour, free of cracks, sun scalds, bruises), while internal attributes include total soluble solids, titratable acidity and flavour (sugar/acid ratio) and tannin content (Citrogold, 2011). These attributes vary depending on cultivar differences, degree of maturity and growing region (Fawole *et al.*, 2012b). Hence, the choice of postharvest handling and storage practices should consider delivery of harvested fruit to consumers in the most excellent condition for desirable organoleptic, nutritional, and antioxidant attributes (Kader, 2008; Fawole & Opara, 2013b).

In the recent years, several studies have focused on quality attributes, physiological response and antioxidant capacities of pomegranate fruit (Labbé *et al.*, 2010; Hasnaoui *et al.*, 2011). However, optimum storage conditions differ depending on cultivars (Opara *et al.*, 2008; Fawole & Opara, 2013c). There is a need for the application of the knowledge acquired over the years towards the development of optimum postharvest handling and storage conditions for specific cultivars. This review discussed current knowledge on the effects of storage temperature and duration on quality and physiological attributes of pomegranates.

## **2. Physiological and quality attributes of pomegranates**

### *2.1. Sensory quality*

Sensory quality attributes and nutritional value of fruit play an important role in consumer satisfaction and repeated purchase (Fawole & Opara, 2013d). However like other fruits, pomegranate also experiences postharvest quality losses during handling and storage. Quality assessment of pomegranate fruit at harvest is based on a wide range of physico-chemical characteristics including fruit colour, TSS, TA, TSS/TA and texture (Fawole & Opara, 2013e). The flavour sensation and aroma produced from non-volatile compounds generates a characteristic sweetness, saltiness, bitterness, sourness and pungent or astringent feeling in the mouth (Coulter, 2007). Pomegranate flavour has been attributed to a combination of sweetness and sourness. This combination is often derived from the ratio between TSS and TA (TSS: TA). The overall sensory sweetness of pomegranate juice depends on sugars types namely fructose, glucose, sucrose, whereas its acidic tastes is as a result of its organic acids, majorly; malic, tartaric, citric acids (Melgarejo *et al.*, 2000). Sweet cultivars are reported having high sugar content and low organic acid levels whereas sour cultivars have high organic acid and low sugar content levels (Melgarejo *et al.*, 2000).

### *2.2. Nutritional quality*

The pomegranate is a highly nutritional fruit consisting of several compounds beneficial to human health. The edible part of the fruit consists of 40% arils and 10% seeds (Viuda-Martos *et al.*, 2010). The arils contain average of 85% water, 10% sugars, mainly fructose and glucose, and 1.5% pectin, organic acid such as ascorbic acid, citric acid, and malic acid, vitamins, polysaccharides, and important minerals compounds (Miguel *et al.*, 2010; Viuda-Martos *et al.*, 2010). Fruit juice nutritional content varies depending on cultivar types, as well as agroclimatic region and degree of fruit maturity. For instance, at commercial harvest,

Tehraniifar *et al.* (2010) reported vitamin C content ranged between 9 and 20 mg/100ml for 20 cultivars grown in Iran. These values were higher than the range for 3 Saudi Arabian cultivars ('Taeifi', 'Manfaloti' and 'Ganati') with vitamin C content ranging between 2 and 8 mg/100ml (Al-Mughrabi *et al.*, 1995). Furthermore, Fawole & Opara (2012) reported that aril mineral content for 7 South African cultivars ranges between 0.14 and 6.9 mg/kg fresh matter with the general mineral composition of pomegranate includes calcium, iron, magnesium, phosphorous, potassium, sodium, zinc, copper, nickel, selenium and manganese. Thus, this study shows that consuming pomegranate arils is a good source of mineral elements in human diet.

Pomegranate seeds are a great source of lipids; seed oil consists between 12 to 20% of total seed weight. Pomegranate seeds are a rich source of essential polyunsaturated (n-3) fatty acids such as linolenic, linoleic, and punicic acid (Ozgul-Yucel, 2005; Fadavi *et al.*, 2006). In addition, seeds also contain considerable amount of protein, crude fibers, vitamins, minerals, pectin, sugars, polyphenols, isoflavones, the phytoestrogen coumestrol, and the sex steroid, estrone (El-Nemr *et al.*, 2006; Viuda-Martos *et al.*, 2010).

### 2.3. Functional properties

The pomegranate fruit have several different classes of phytochemical compounds which have been identified in pomegranate fruit parts (arils, rind, pith, pericarp, and seeds). Classes of phytochemicals include active phenolic compounds which can range from simple molecules such as phenolic acids, catechin, procyanidins, anthocyanins, anthocyanidins, flavonols to highly polymerized compounds like ellagitannins and gallotannins (Seeram *et al.*, 2006). Furthermore, these phytochemicals differ in concentration depending on cultivar types (Gil *et al.*, 2000, Shwartz *et al.*, 2009, Fawole *et al.*, 2012b).

Several studies have linked increase consumption of pomegranate fruit to improved human health as a result of phenolic compounds in the fruit, which have shown to possess potent pharmacological activities including, antioxidant, anti-mutagenic, anti-hypertension, anti-diabetic and anti-inflammatory activities (Gil *et al.*, 2000; Kaur *et al.*, 2006; Duman *et al.*, 2009; Xu *et al.*, 2009; Viuda-Martos *et al.*, 2010; Kim *et al.*, 2002). Seeram *et al.* (2004) conducted a study where human subjects consumed pomegranate juice containing ellagic acid (25 mg) and hydrolyzable ellagitannins (318 mg as punicalagins); plasma antioxidant status was observed to be higher than that of control subjects. This observation suggests that pomegranate polyphenolic compounds are able to elevate the antioxidant capacity of the

body. In another study, Lansky *et al.* (2005) observed that a combination of three pomegranate components (juice, seeds and peel) had synergistic interactions in the inhibition of prostate cancer cell proliferation. Reddy *et al.* (2007) demonstrated that pomegranate water and ethanol extracts showed antimicrobial activity when assayed against *E. coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Cryptococcus neoformans*, methicillin-resistant and *Streptococcus aureus*. Guo *et al.* (2008) reported that several phenolic compounds present in pomegranate juice enhanced antioxidant function and reduced oxidative damage in elderly patients when compared to apple juice. The phytochemistry and pharmacological actions of pomegranate derivatives suggest that bioactive phenolic compounds in pomegranate juice have an array of clinical applications for the prevention and treatment of several diseases.

#### 2.4. Microbial quality

Postharvest handling and transport can favor the development of postharvest diseases; especially the level of latent microbial infection at the time of harvest is high. Several types of moulds and bacteria are associated with pomegranate fruit affecting its overall quality; these include *Botrytis cinerea*, *Aspergillus niger*, *Penicillium spp.*, *Alternaria spp.*, *Nematospora spp.*, *Coniella granati*, or *Pestalotiopsis versicolor* (Palou *et al.*, 2007; Yehia, 2013). These pathogens are one of the major factors limiting storage potential of pomegranate fruit. Infection usually occurs through skin breaks caused by cracks, insect punctures, mechanical injuries located on the fruits surface or with abusive temperatures resulting in increased microbial infestation during postharvest storage. Furthermore, storage of fruit at 5°C or lower temperatures could result in several postharvest disorders such as husk scald and chilling injury increasing fruit susceptibility to decay. Therefore, the suitability of cultivars to postharvest handling and storage may be the primary factor affecting the quality of pomegranate.

#### 2.5. Volatile and flavour composition

Volatile compounds could be used to characterize the aroma intensity and odour in fruit (Visai & Vanoli, 1997; Melgarejo *et al.*, 2011). The fruit has trace amounts of volatile compounds, leading to low intensities of both odour and aroma of the fruit parts (Carbonell-Barrachina *et al.*, 2012; Fawole & Opara, 2013b). Current research on volatile and aroma composition are limited, however, few scientific studies have reported on several volatile compounds in fresh pomegranate juice and the evolution of these compounds during

packaging and storage. More recently, however, Fawole & Opara (2014) reported a total of 15 aromatic volatile compounds in 8 commercially grown South African pomegranates, whereas, Caleb *et al.* (2013) identified 18 different aromatic compounds in 2 commercially grown cultivars, several of which evolved over a 10 day storage period at temperatures ranging between 5°C and 15°C. Furthermore, the most predominant volatile compounds found in fruit juice according to these authors were trans-3-hexen-1-ol and 1-hexanol, while several other volatiles identified were present in very low concentrations (Caleb *et al.*, 2013). Melgarejo *et al.* (2011) identified 21 aroma volatile compounds in fruit juice of nine different Spanish pomegranate cultivars using gas chromatography-mass spectrometry (GC-MS). Two aldehydes and one ethanol were predominant compounds in Spanish samples. These studies suggest that alcohols and aldehydes are the most important volatile groups present in fruit juice that could be used for the classification of pomegranate cultivars.

### **3. Effects of storage on physical properties of pomegranate fruit**

#### *3.1. Colour dynamics*

Colour is an important quality attribute in the food and bioprocess industries, and it influences consumer's choice and preferences (Pathare *et al.*, 2012). Artés *et al.* (1998) reported that the CIE L\*, a\*, and b\* colour parameters were higher in pomegranate fruit husk than in arils and juice at harvest period. However, the authors observed no significant colour difference in fruit husk and arils after 80 days of cold storage at 0°C and 5°C, respectively. For the Spanish 'Mollar de Elche' fruit stored at 25°C for 150 days of storage, Marti *et al.* (2001) reported a decrease in juice lightness (L\*), and increases in C\* and h° values, indicating loss of desirable red colouration. Similarly, Fawole & Opara (2013c) reported significant decreases in the CIE a\* significantly decreased during storage of 'Bhagwa' cultivar when stored between 5°C and 10°C for up to 16 weeks of cold storage. The authors also reported significant decreases in fruit colour intensity (C\*) with increasing storage temperature and duration. However, for 'Ganesh', changes in colour of fruit stored at 8°C, 15°C and 25°C over a 12-week period was not significant (Nanda *et al.*, 2001).

#### *3.2. Textural properties*

Several studies have shown that textural properties of pomegranate fruit changed depending on storage conditions. According to Nanda *et al.* (2001), storage of 'Ganesh' fruit at 25°C, 15°C and 8°C resulted in decreases in fruit firmness after 1, 5 and 7 weeks,

respectively. 'Mollar de Elche' fruit stored at 2°C and 90% RH exhibited a significant decrease in firmness after 90 days (Mirdehghan *et al.*, 2006a). Mansouri *et al.* (2011) studied fruit firmness of two Iran cultivars ('Hondos-e-Yalabad' and 'Malas-e-Saveh'). According to the authors, fruit became less firm after 30 days of storage at 5°C. However, Ekrami-Rad *et al.* (2011) reported an initial increase in firmness after a month of storage for 'Wonderful', but a decline in firmness was observed thereafter. Research has shown that increase in firmness during storage could be due to moisture loss from the fruit resulting to hardening and increase in mechanical strength of fruit peel (Ekrami-Rad *et al.*, 2011).

#### **4. Biochemical response of pomegranate fruit during storage**

##### *4.1. Total soluble solids (TSS)*

Reports on changes in TSS contents in pomegranate during storage varied, depending on storage conditions, cultivar types, agro-climatic regions and fruit maturity at harvest (Kader *et al.*, 1984; Gil *et al.*, 1996; Fawole & Opara, 2012). Fawole & Opara (2013c) reported significant decrease in TSS contents with prolonged storage period for two South African grown 'Bhagwa' and 'Ruby' pomegranates stored at 5°C, 7°C, 10°C and 92% RH for 12 weeks. The authors findings are in agreement with those described by Artés *et al.* (1998) for Spanish 'Mollar de Elche' stored at 0°C and 5°C and 95% RH for 80 days. Similarly, Kader *et al.* (1984), reported significant decrease with increasing temperature and prolonged duration for Californian 'Wonderful' stored at 5°C for 16 weeks. Decrease in TSS content during these studies could be attributed to degradation of sugars with prolonged storage period. On the contrary however, TSS content in Californian 'Wonderful' fruit remained relatively constant for 10 weeks when stored at 0°C, 10°C, 20°C, 30°C (Elyatem & Kader, 1984). Similar findings were reported by Gil *et al.* (1996) for 'Mollar de Elche', where no significant changes were observed in TSS for 'Mollar de Elche' stored at 5°C and 95%RH for 7 weeks.

Interestingly, some studies have reported increases in TSS content of pomegranate during postharvest storage. According to Ghafir *et al.* (2010), there was a significant increase in TSS for 'Shlefy' when stored at 5°C and 7°C for 4 months. In addition, Al-Mughrabi *et al.* (1995) observed an increase in TSS content for 'Taeifi', 'Manfaloti' and 'Ganati' after 8 weeks of cold storage at 5°C, 10°C and 22°C. Increase in TSS has been attributed to moisture loss, leading to concentration of sugars inside the fruit (Köksal, 1989).

#### 4.2. Titratable acidity (TA) and pH

Generally, TA in pomegranate juice differed depending on the cultivar, growing region, maturity at harvest and postharvest handling practices (Fawole & Opara, 2012). Kader *et al.* (1984) reported that pomegranate 'Wonderful' cultivar has a high acidity content, ranging between 1.11 to 1.58%. TA decreased at temperatures ranging between 0°C and 10°C for 16 weeks. For Spanish grown 'Mollar de Elche', Artés *et al.* (1998) reported no significant changes in TA during storage at 5°C for 80 days of cold storage, however, after 7 days of shelf-life period TA decreased significantly. According to Artés *et al.* (2000a) significant decrease in TA was reported for 'Mollar de Elche' stored at 5°C for 90 days and shelf-life period of 6 day at 15°C and 75% RH. These studies are in agreement with Fawole & Opara (2013c), who reported decreases in TA for two South African grown pomegranates ('Bhagwa' and 'Ruby') at 5°C, 7°C and 10°C for 4 months. In contrast, Mirdehghan *et al.* (2006b) reported a significant increase in organic acids for 'Mollar de Elche' stored at 2°C for 90 days. These findings are comparable to those reported by Bayram *et al.* (2009), who reported an increase in TA levels for untreated 'Hicaznar' fruit when stored at 6°C and 90% RH for 6 months.

There is an inverse relationship between pH and acidity of pomegranate juice (Zarei *et al.*, 2011), thus pH values could describe its acidic taste. Kader *et al.* (1984) observed increase in pH values for 'Wonderful' stored at 0°C and 10°C for 4 months. In addition, for 'Ruby' fruit stored at 5°C, Fawole & Opara (2013c) observed that juice pH increased with storage duration, reaching a maximum pH value of 3.96 after 16 weeks of storage. However, the study by Artés *et al.* (1998) showed no significant difference in pH values for 'Mollar de Elche' fruit stored at 5°C and 95% RH for 80 days. Similar findings were reported by Gil *et al.* (1996) for 'Mollar' stored under similar storage conditions.

#### 4.3. Brix-acid ratio

Brix:acid ratio (TSS:TA) determines the taste and flavour of pomegranate fruit at harvest and during postharvest handling. Changes in Brix:acid ratio is dependent on changes in both TSS and TA contents in fruit juice. According to Artés *et al.* (1998), there was no significant difference in juice TSS:TA ratio in 'Mollar' fruit stored at 5°C for 80 days, whereas the ratio increased significantly after 7 days of shelf-life period. Fawole & Opara (2013c) observed a decrease in TA and TSS during postharvest storage, resulting in a significant increase in TSS/TA ratio at most storage temperatures for 'Bhagwa' and 'Ruby'.



#### 4.4. Phenolic concentration

Phenolic compounds are responsible for most functional properties of many fruits including pomegranate (Gomez-Caravaca *et al.*, 2013). According to Mirdehghan *et al.* (2006b), juice of heat treated fruit ('Mollar de Elche') showed a higher phenolic content (108.39 mg equivalent gallic acid 100 g<sup>-1</sup>) compared to control (92.05 mg equivalent gallic acid 100 g<sup>-1</sup>) stored at 2°C for 90 days. In addition, total phenolic concentration declined in Chilean 'Codpa' fruit stored at 5°C for 12 weeks (Labbe *et al.*, 2010). Similarly, the study by Sayyari *et al.* (2011) on untreated fruit 'Mollar de Elche' showed that total phenolic concentration decreased from 261.19 mg/100g before storage to 234.10 mg/100g after 84 days under 2°C and 90% RH conditions. This agreed with the study by Fawole & Opara (2013c), who reported significant reduction in total phenolic concentration for 'Bhagwa' and 'Ruby' stored at 5°C beyond 8 weeks. On the contrary however, an opposite trend was observed during the storage of 'Chaca' at 5°C for 12 weeks (Labbe *et al.*, 2010).

#### 4.5. Anthocyanin

Anthocyanin compounds are responsible for the characteristic red colouration in pomegranate fruit peel and juice (Gil *et al.*, 1996; Artés *et al.*, 1998). The total anthocyanin concentration in untreated fruit 'Mollar de Elche' increased between harvest and shelf-life when stored for 12 weeks at 0°C and 5°C in 95% RH (Artés *et al.*, 1998). Similarly, Fawole & Opara (2013c) reported an increase in juice total anthocyanin concentration between harvest and after 4 months of storage at 5°C, 7°C and 10°C for 'Bhagwa' and 'Ruby'. These studies agreed with Miguel *et al.* (2004), who reported an increase in anthocyanin concentration after the first month of storage at 5°C for 'Assaria' fruit grown in Portugal. However, report by Artés *et al.* (2000b) was on the contrary, where no change in anthocyanin concentration was observed for 'Mollar de Elche' between harvest and shelf-life after 12 weeks. These studies give an indication that cultivar difference may play a role in the postharvest biosynthesis of anthocyanins in pomegranate fruit.

#### 4.6. Vitamin C

For 'Taeifi', 'Manfaloti' and 'Ganati' ascorbic acid concentration in pomegranate juice was not significantly affected by storage temperature, but gradually declined with storage period (Al-Mughrabi *et al.*, 1995). This was comparable to the report by Küpper *et al.* (1995) for untreated 'Hicaz' fruit stored at 6°C, 8°C and 10°C for > 6 months. These studies agreed with Opara *et al.* (2008), who reported that refrigeration significantly enhanced vitamin C

retention during the first 2 weeks of storage. The authors reported rapid decline in ascorbic acid concentration after 6 weeks at 7°C and 21°C was observed. In contrast, ascorbic acid concentration increased during storage at 5°C and 90-95% RH for 3 months of ‘Assaria’ and ‘Mollar’ pomegranates grown in Portugal (Miguel *et al.*, 2006). A decrease in vitamin C may be related to the irreversible oxidation of dehydro-L-ascorbic acid (DHAA) to 2,3-diketo-L-gulonic acid (Coultate, 2007). Furthermore, ascorbic acid is affected and its activity is reduced by the presence of oxygen, alkalinity and high temperatures (Coultate, 2007).

## 5. Postharvest Physiology

### 5.1. Weight loss

One of the major problems associated with pomegranate fruit is excessive weight loss which may result in hardening of the husk and browning of the rind and arils (Artés *et al.*, 2000b; Caleb *et al.*, 2012a). Even in the absence of shrivelling, water loss can cause undesirable textural and flavour changes, ultimately resulting to loss of visual appeal. The storage potential of pomegranate fruit at 21°C and 82% RH may not be more than 15 days (Waskar, 2011). However, under refrigerated conditions and high RH, most cultivars can be stored for prolonged periods (Elyatem & Kader, 1984). Storage trials conducted on ‘Hicaz’ cultivar stored at 6°C showed that weight loss (9%) increased with increasing temperature and prolonged storage duration (Küpper *et al.*, 1995). Al-Mughrabi *et al.* (1995) observed that weight loss increased with storage temperature and time for ‘Taeifi’, ‘Manfaloti’, ‘Ganati’ pomegranates. The authors reported significantly higher weight loss at 22°C than at 5°C and 10°C, with average weight losses of 18.32%, 21.93% and 32.83% at 5°C, 10°C and 22°C, respectively, after 8 weeks of storage.

This is in agreement with Opara *et al.* (2008), who reported weight losses of 3.85% in ‘Halow’ pomegranate stored at 7°C and 95% RH for 6 weeks, whereas at 21°C and 65% RH the weight loss was significantly higher (16.42%). The dramatic increase in weight loss at ambient temperatures could probably due to a lower relative humidity during storage resulting in a higher percentage weight loss compared to cold storage temperatures. Similarly, Fawole & Opara (2013c) observed that storage of both ‘Bhagwa’ and ‘Ruby’ at 5°C, 7°C, 10°C and 22°C showed increase in weight loss of with storage temperatures and duration of up to 16 weeks. However, on the contrary, Köksal (1989) studied weight loss on Turkish ‘Gok Bahce’, the author reported that weight loss in untreated fruit at 5°C (16.5%) were higher than fruit stored at 1°C (8%), 10°C (6.1%) and 21°C (14%) after 4 months storage

duration. This clearly showed the importance of low storage conditions in reducing weight loss in pomegranate fruit.

### 5.2. Respiration rate and ethylene production

Fruits and vegetables are living hence they continue the respiratory process (Maguire *et al.*, 2001). This process is essential to maintain biochemical, cellular organization and membrane integrity. Pomegranates are classified as non-climacteric fruits and therefore cannot continue the ripening process once detached from the plant (Kader, 2006). Furthermore, pomegranates are sensitive to inconsistent or abusive temperature which triggers and increases respiration enhancing microbial proliferation and deterioration during postharvest handling.

Elyatem & Kader (1984) reported a relatively low respiration rate (8 ml CO<sub>2</sub>/kg/h) for 'Wonderful' stored at 0°C and 10°C for 3 months, while trace amount (less than 0.2 µL/kg/h) of ethylene was detected when stored at 20°C for 2 weeks. Contrary to these findings, Koksall (1989) reported for 'Gok Bache' grown in Turkey, where respiration rate was reduced from first month of storage (7.8, 4.3, 2.4 ml CO<sub>2</sub>/kg/h) to (0.9, 1.3, 0.9 ml CO<sub>2</sub>/kg/h) after 4 months at 1°C, 5°C and 10°C. For South African grown 'Herskowitz' and 'Acco', Caleb *et al.* (2012b) reported a decline in respiration rate of about 67% and 68%, respectively, for whole fruit when temperature was reduced to 5°C with an average production of 14.67 ml CO<sub>2</sub>/kg/h. These studies agreed with Fawole & Opara (2013c) who reported lower respiration rates at harvest than during storage at 5°C and 10°C for 'Bhagwa' and 'Ruby'. In contrast, Opara *et al.* (2008) showed that the respiration rate (3.4 CO<sub>2</sub>/kg/h) and ethylene production (< 0.1 µL/kg/h) of 'Helow' increased when stored at 21°C and 65% RH for 6 weeks. However, the authors reported that cold storage conditions (7°C and 95% RH) significantly suppressed the rate of ethylene production by over 63%. These studies highlight the importance of understanding the physiological responses of pomegranate cultivars under different storage conditions to assist in developing optimal postharvest handling processes.

### 5.3. Response to ethylene treatment

According to Elyatem & Kader (1984), the 'Wonderful' pomegranate fruit were not sensitive to ethylene exposure, although it was observed that ethylene at  $\geq 1$  µl/kg/h stimulated respiration. The stimulated increase in fruit respiration as a result of ethylene treatment was temporary for 'Wonderful' (Ben-Arie *et al.*, 1984). Exposure of 'Wonderful' fruit to ethylene treatment at 20°C resulted to an increase in respiration rate, however no

significant effects on fruit and juice colour, soluble solids, pH or acidity were observed (Kader *et al.*, 1984). Treatment of 'Wonderful' fruit with 10, 100 or 1000 ppm ethylene for 2, 4 or 7 days at 20°C had no significant effect on fruit external and internal attributes (Elyatem & Kader, 1984). These studies indicate that pomegranate fruit are non-climacteric and do not ripen after harvest. They should be picked when fully ripe to ensure the best eating quality for desirable organoleptic and nutritional value for consumers.

## 6. Physiological Disorders

### 6.1. Chilling Injury

The 'Wonderful' pomegranate has been reported having high susceptibility to chilling injury if stored at temperatures below 5°C, or more than 2 months at 5°C (Elyatem & Kader, 1984; Kader *et al.*, 1984). However, chilling injury may become more noticeable when transferred to 20°C after 2 months of cold storage (Kader, 2006). Mirdehghan *et al.* (2006a) reported that storage at 2°C plus 3 days shelf-life for 2 weeks results in chilling injury for 'Mollar de Elche'. External symptoms of chilling injury include brown discolouration of fruit peel, cracking, necrotic pitting and increased susceptibility to decay (Elyatem & Kader, 1984). Internal symptoms include reduction in aril colour, aril browning and discolouration of white membrane segments (Elyatem & Kader, 1984; Kader *et al.*, 1984, Köksal, 1989). Depending on cultivar types, pomegranate fruit can be successfully stored for 2 to 7 months between temperatures ranging from 0°C to 10°C (Köksal, 1989; Onur *et al.*, 1992).

Intermittent warming of pomegranate fruits has been reported to reduce chilling injury symptoms and fruit decay (Artés *et al.*, 2000b). Similarly, Mirdehghan & Rahemi (2005) showed that dipping in water at 50°C temperature for 5 min significantly reduced chilling injury for 'Malas Yazdi' and 'Malas Saveh' stored for 4.5 months at 1.5°C and 85±3% RH. These studies are comparable with Mirdehghan *et al.* (2006b) who reported that heat treatment such as water dipping at 45°C for 4 min reduced chilling injury symptoms. You-lin & Run-guang (2008) reported that intermittent warming at 15°C for 24 h reduced browning of the husk and could prevent chilling injury when fruits were stored for 120 days for the 'Ganesh' pomegranate.

### 6.2. Husk scald

Husk scald is a common physiological disorder appearing as a superficial (peel) browning of the husk, which generally develops from the stem end of the fruit and spreads

towards the blossom end as severity increases (Ben-Arie & Or, 1986; Defilippi *et al.*, 2006). This disorder is suggested to be due to the oxidation of phenolic compounds on the husk of the fruit when stored at temperatures exceeding 5°C (You-lin & Run-guang, 2008). The severity of scald incidence increases when pomegranates are harvested late in the season, indicating that this disorder may be associated with senescence (Kader, 2006). At advanced stages, scalded areas may become susceptible to decay (Kader, 2006). Pekmezci *et al.* (1998) reported that scald symptoms become evident after 8 weeks storage at 2°C. For the 'Wonderful', Ben-Arie & Or (1986) reported that husk scald can be effectively controlled when fruit were stored at 2% oxygen at 2°C. However, it was observed that this treatment leads to build-up of ethanol which produced off-flavours in the fruit.

### 6.3. Decay

The major cause limiting the storage potential of pomegranates is the development of decay which are caused by various pathogens such as *Aspergillus* spp, *Cladosporium* spp, *Colletotrichum* spp, *Epicoccum* spp, *Penicillium* spp, *Pestalotia* and *Botrytis cinerea* (Maclean *et al.*, 2011; Caleb *et al.*, 2012a). Several postharvest diseases are mainly associated with pomegranate fruit include gray mold (*Botrytis cinerea*) rot, green mold (*Penicillium digitatum*) rot, blue mold (*P. expansum*) rot and heart (*Aspergillus niger*) rot (Roy & Waskar, 1997; Palou *et al.*, 2007). *B. cinerea* is able to infect stored pomegranates by mycelial spread from infected fruit to adjacent healthy fruit, causing 'nests' of decay. *B. cinerea* mainly infects fruit through the crown (calyx) of young fruit on the tree, remains latent and after harvest forms a characteristic grey mycelium on the affected area under humid conditions (Caleb *et al.*, 2012a). Grey mold rot usually starts from the calyx, spreading onto the skin causing an apparent brown discoloration, making the peel tough and leathery (Ryall & Pentzer, 1974). Furthermore, *B. cinerea* are able to infect stored pomegranates by spreading from infected fruit to adjacent healthy fruit, causing 'nests' of decay (Palou *et al.*, 2007). In heart rot, with *A. niger* fruit show no external symptoms except for slight abnormal peel colour or soft spot with a blackened mass of arils (Yehia, 2013).

Padule & Keskar (1988) reported that treating pomegranate fruit with aqueous Topsin-M (0.1%) and Bavistin (0.05 - 0.1%) significantly suppressed the growth of *A. niger*. When pomegranate 'Wonderful' were inoculated in the crown with *B. cinerea*, stored for 15 weeks at 7.2°C and 95% RH and treated with an antifungal fludioxonil, decay were shown to be significantly reduced when compared to untreated fruits (Palou *et al.*, 2007). Hence, it is

necessary to develop control methods to control postharvest decay and extend the marketing life of pomegranate fruits.

## **7. Storage recommendations**

Storage recommendations for pomegranate fruit are summarized in Table 1. Optimum storage conditions have been reported to range between 0°C to 10°C, depending on cultivar difference, production area and postharvest treatment (Onur *et al.*, 1995; Fawole & Opara, 2013c). Overall, control of the relative humidity is critical to fruit storage performance, as low relative humidity causes fruit peel to desiccate, resulting in hardening of the husk, and subsequent shrivelling which are unattractive and reduces marketability (Pekmezci *et al.*, 1998). Therefore maintaining postharvest quality of pomegranate fruit requires a high relative humidity and low temperature to control respiration rate, reduce decay and maintain fruit quality. Furthermore, several cultivars have been susceptible to chilling injury. Fruit could be stored and maintained at temperatures ranging from 2°C to 10°C for up to several weeks depending on the cultivar type.

## **8. Conclusions and future prospects**

Comprehensive review of literature showed that various pomegranate cultivars are available globally and are distinguished by distinctive characteristics such as fruit size, weight, sweetness, acidity, flavour as well as aril and peel colour. Clearly, different pomegranate cultivars respond differently to optimum storage conditions. Furthermore, inconsistent and abusive temperature contributes to increased respiration and transpiration rates, which results in increased perishability and loss of organoleptic, nutritional and antioxidant attributes. For successful postharvest handling and storage of pomegranate fruit, further studies should be carried out separately for each commercially grown cultivar with a more informative output on the physiological response, for example, respiration rates, disorders as well as fruit phytochemicals (phenolics, anthocyanins, tannins) under different storage conditions. Microbial infestation and development of physiological disorders such as chilling injury and husk scald leads to postharvest losses in pomegranate during cold storage. More studies are also needed in the areas addressing reduction of postharvest loss and improvement of marketability of pomegranate fruit. This holistic approach would help in the development of appropriate science based management tools for optimal storage performance of pomegranate fruit.

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Table 1

Recommended storage requirements for various pomegranate cultivars

Cultivar	Temperature (°C)	Relative humidity (%)	Storage period (month)	References
Bhagwa	5	>92	2-3	Fawole & Opara, 2013c
Banati	5	80-90	2	Al-Mughrabi <i>et al.</i> , 1995
Hicaz	6	85-90	5	Küpper <i>et al.</i> , 1995
Hicaz	8-10	85-90	<2	Küpper <i>et al.</i> , 1995
Helow	7	90-95	1	Opara <i>et al.</i> , 2008
Molas Torsh	0-1.7	85-95	3	Pantastico, 1975
Manfaloti	5	80-90	2	Al-Mughrabi <i>et al.</i> , 1995
Ruby	5	>92	2-3	Fawole & Opara, 2013c
Shirin Paezeh	1	90-95	4.50	Askary & Shahedi, 1994
Taeifi	5	80-90	2	Al-Mughrabi <i>et al.</i> , 1995
Wonderful	5	95	2	Kader <i>et al.</i> , 1984
Wonderful	7.2	90-95	>2	Kader, 2006

# Chapter 3

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## **Postharvest physiological responses of pomegranate fruit at different temperature regimes**

## POSTHARVEST PHYSIOLOGICAL RESPONSES OF POMEGRANATE FRUIT AT DIFFERENT TEMPERATURE REGIMES

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### Summary

Pomegranate fruit (cv. Wonderful) were procured at commercial maturity and stored at  $5\pm 0.7^{\circ}\text{C}$ ,  $7.5\pm 0.3^{\circ}\text{C}$ ,  $10\pm 0.5^{\circ}\text{C}$  with  $92\pm 2\%$  relative humidity (RH) and  $21\pm 3^{\circ}\text{C}$  with  $65\pm 6\%$  RH for 5 months. Fruit respiration and incidence of physiological disorders were measured at monthly intervals. The results showed fruit respiration rate declined at all storage regimes, with the highest respiration rate occurring in freshly harvested fruit ( $7.93 \text{ CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ). The severity and the occurrence of decay was lowest in fruit stored at  $5^{\circ}\text{C}$ . However, fruit became more susceptible to internal and external disorders as storage period progressed. Storing fruit longer than 2 months at  $5^{\circ}\text{C}$  resulted in chilling injury. Furthermore, the severity of peel and aril browning increased with storage temperatures, although the occurrence of browning became more severe at  $5^{\circ}\text{C}$  after 3 months. Therefore, to maintain a relatively low respiration rate and minimize physiological disorders, the 'Wonderful' pomegranates should be stored at  $5^{\circ}\text{C}$  and  $>92\%$  RH for up to 3 months.

### 1. Introduction

The pomegranate (*Punica granatum* L.) fruit has captured consumer interest worldwide due to its health promoting benefits (Heber & Bowerman, 2009). The highly nutritious fruit contains considerable amount of sugars, vitamins, polysaccharides, and important minerals (Miguel *et al.*, 2010). Due to its multifunctional and nutritional benefit in the human diet (Lansky & Newman, 2007; Opara *et al.*, 2009; Fawole & Opara, 2013a), there has been a considerably increase in commercial farming of pomegranate fruit. Despite the health benefits of pomegranate, consumption of the fruit is limited due to lack of appropriate postharvest operations required to extend the storage life of fruit. The occurrence of physiological disorders such as husk scalds, splitting, and chilling injury are challengers which reduce marketability and consumers acceptance (Ben-Arie & Or, 1986; Saxena *et al.*, 1987).

Pomegranates are classified as non-climacteric fruits and therefore cannot continue the ripening process once detached from the plant (Kader, 2006). The fruit may be stored for several

months at temperatures below 10°C to extend the marketing value (Artés *et al.*, 2000; Kader, 2006; Ghafir *et al.*, 2010). However, pomegranates are highly perishable and several postharvest disorders could occur during short or long term storage. These include development of chilling injury when fruit is stored below its suboptimum of 5°C or below, appearance of husk scald (browning of the skin surface) with prolong storage period, and the development of decay. Optimum storage conditions have been reported to range between 0°C to 10°C with storage life ranging between 2 weeks to 6 months (Onur *et al.*, 1995; Fawole & Opara, 2013b). Storage period may also be influenced by cultivar difference, geographical region, postharvest treatment and degree of fruit maturity (Koksal, 1989; Gil *et al.*, 1996; Waskar, 2011; Fawole & Opara, 2013b). For the ‘Hicaz’, Küpper *et al.* (1995) recommended storage temperature of 6°C and 85-90% RH for 5 month, while Fawole & Opara (2013b) recommended 2-3 months storage duration for ‘Bhagwa’ and ‘Ruby’ cultivars stored at 5°C and 90-93% RH. Kader (2006) reported that ‘Wonderful’ pomegranates can be successfully stored for 2 months at 5°C and 90-95% RH, thereafter, for long-term cold storage temperature of 7.2°C and 90-95% for 5 months has been recommended as the Wonderful cultivar.

The South African pomegranate industry is currently challenged with fruit quality loss as a result of occurrence and incidence of decay and postharvest disorders during storage. To date, there is currently lack of scientific knowledge on the storage requirements for the ‘Wonderful’ cultivar grown in South Africa. The objective of this was to study the storage performance of ‘Wonderful’ pomegranate based on physiological responses during prolong storage, and also to determine the optimal storage condition and duration in order to maintain fruit quality and reduce losses.

## **2. Materials and Methods**

### *2.1. Plant material and storage conditions*

Commercially ripe pomegranate fruit (cv. Wonderful) were obtained from Sonlia Pack-house (33°34'851"S, 19°00'360"E) in Western Cape, South Africa and transported in an air-conditioned vehicle to the Postharvest Technology and Research Laboratory, Stellenbosch University. Fruit without any physical defects were selected and equilibrated at ambient temperature (21±3°C). A total of 640 fruit samples were packed inside open carton boxes with the following dimensions:

width 0.3 m, length 0.4 m, height 0.133 m and a total of 22 perforations. Fruit samples were randomly divided into 4 lots. Three lots were stored at each of the following conditions  $5\pm 0.7^{\circ}\text{C}$ ,  $7.5\pm 0.3^{\circ}\text{C}$  and  $10\pm 0.5^{\circ}\text{C}$  with  $92\pm 2\%$  RH. Another lot comprising of 40 fruit was stored at ambient conditions of  $21\pm 3^{\circ}\text{C}$  and  $65\pm 6\%$  relative humidity (RH). Temperature ( $^{\circ}\text{C}$ ) and RH (%) were monitored on an hourly base using Tiny Tag TV-4500 data loggers (Gemini Data Logger, Sussex, UK).

## 2.2. Fruit respiration

A closed system method was used to determine the rate of carbon dioxide ( $\text{CO}_2$ ) production in whole pomegranate fruit as described by Caleb *et al.* (2012). In three replicates, each fruit was placed in air tight glass jars each containing a rubber septum in the middle. Vaseline was incorporated into the gaps between the lid and jars to ensure a vacuum seal. Gas composition was taken from the head space through the rubber septum hourly for 5 hours over a period of 5 days before storage trials and every month during storage. Gas composition inside each glass jar was measured using a calibrated  $\text{O}_2/\text{CO}_2$  analyzer (Checkmate 3, PBI Dansensor, Ringstead, Denmark) with accuracy of 0.5%. Carbon dioxide production was determined by using equation 1.

$$Y_{\text{CO}_2} = Y^i_{\text{CO}_2} + \frac{R_{\text{CO}_2} * W}{V_f} \cdot (t - t_i) \times 100 \quad (1)$$

Where  $y^i_{\text{CO}_2}$  and  $y_{\text{CO}_2}$  are  $\text{CO}_2$  concentration (%) at the initial time  $t_i$  (h) and time at sampling  $t$  (h), respectively.  $R_{\text{CO}_2}$  are RR in  $\text{mL kg}^{-1} \text{h}^{-1}$  and  $W$  is the weight of the fruit (g),  $t_i$  (h) is the initial time and  $t$  (h) is time of sampling,  $V_f$  is the free volume in the jar which is the total volume minus the mass (g) occupied by the fruit.  $\text{CO}_2$  production was presented as mean  $\pm$  S.E. ( $\text{ml CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ).

## 2.3. Physiological disorders

Incidence of decay, aril browning, chilling injury, dehydration and husk scald were assessed for each storage regime. The severity of physiological disorders was evaluated subjectively on a monthly basis using a hedonic scale, where 0 = none; 1 = trace; 2 = slight; 3 = moderate; 4 = severe; 5 = extremely severe. Only severe injuries could be considered as commercially unacceptable (Artés *et al.*, 1998). Physiological disorders were subjectively calculated by

multiplying the scores of severity by the number of fruit affected and dividing by the total number of fruit (Fawole & Opara, 2013b).

#### 2.4. Browning index

The browning index (BI) is used to characterise the overall changes in browning colour (Quitão-Teixeira *et al.*, 2008). BI was calculated from the Hunter's  $L^*$ ,  $a^*$ ,  $b^*$  values as described by Pathare *et al.* (2012). The CIE  $L^*$ ,  $a^*$ ,  $b^*$  coordinates were measured with a calibrated Minolta Chroma Meter (Model CR-400/410; Minolta Corp, Osaka, Japan). Peel colour measurements were taken along the equatorial axis of each fruit at three marked spots. BI was calculated using the following expression:

$$BI = 100 \times \frac{(x-0.31)}{0.17} \quad (2)$$

where  $x$  = chromaticity coordinate calculated from the  $L^*$ ,  $a^*$ ,  $b^*$  values

$$x = \frac{(a^* + 1.75L^*)a^*}{(5.645L^* + a^* - 3.012b^*)} \quad (3)$$

$L^*$ ,  $a^*$ ,  $b^*$  values represent the lightness, redness, and yellowness of the sample. Results were expressed as mean  $\pm$  S.E. (n=10)

### 3. Statistical Analysis

Statistical analysis was carried out using Statistica software (Statistica version 10, StatSoft Inc., Tulsa, USA). Data obtained was subjected to one-way analysis of variance (ANOVA) at 95% confidence interval according to Duncan's multiple range test. GraphPad Prism software version 4.03 (GraphPad Software, Inc., San Diego, USA) was used for the graphical presentations.

### 4. Results and Discussion

Experimental trials for fruit stored at 21°C and 10°C were terminated after 1 month and 4 months, respectively, due to complete fruit loss to decay.

#### 4.1. Fruit respiration

Respiration rates of fruit at all storage regimes were significantly ( $p < 0.05$ ) lower at most storage temperatures than those measured at harvest period (Fig. 1). However, amongst the storage temperatures, respiration rates were significantly higher in fruit stored at 21°C for 1 month storage. This may be attributed to the onset of anaerobic respiration at 21°C resulting from microbial growth of the fruit which led to the termination of the storage trials at 21°C after 1 month. A comparable trend was reported by Opara *et al.* (2008) for ‘Hellow’ pomegranate stored at 21°C and 65% RH for 1 month. In addition, when compared to other cold storage regimes, fruit stored at 10°C for 2 months exhibited a higher respiration rate  $>4.3 \text{ ml CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  during cold storage and remained high afterwards. Furthermore, after 3 months, a rise in fruit respiration rate was observed at 5°C, 7.5°C and 10°C, with further increases in fruit respiration beyond 3 months (Fig. 1.).

Fruit stored at 5°C respired more after and beyond 3 months in storage compared to those stored at 7.5°C (Fig. 1.). This respiratory behavior might be attributed to the occurrence of physiological disorders such as chilling injury and lower relative humidity which was confirmed when fruit were examined for external and internal disorders. In addition the respiration rate of fruit stored at 5°C and 7.5°C was non-significant ( $p < 0.05$ ) after 5 months of storage. These results are in agreement with previous findings by Elyatem & Kader (1984), who reported that storage at 5°C resulted in higher respiration rate compared to lower storage temperatures of 0-2.2°C for 84 days.

#### 4.2. Physiological disorders

The severity and occurrence of physiological disorders (shriveling, aril browning, and chilling injury) were generally low in fruit stored at lower temperatures, however the severity of disorders increased with prolonged storage (Fig. 2A-G). Storage of fruit at 21°C for 1 month resulted in the highest incidence and occurrence of physiological disorders. About 40% of fruit were affected by internal disorders such as aril browning and decay, whereas 98% of all fruit stored at 21°C displayed external disorders such as shriveling and fruit decay, resulting in discoloration and complete fruit loss after 1 month (Fig. 3A and Fig. 3B). No external disorders were observed at 5°C in the first month of storage, with less than 5% of the fruits affected by internal disorders (Fig. 2A and Fig. 2B). These results agree with those reported by Fawole &

Opara (2013b), who found no visible decay in ‘Bhagwa’ and ‘Ruby’ pomegranate fruits stored at 5°C for 4 weeks. With increase in storage temperature and duration, the severity of external disorders became more obvious with increase in storage temperature and duration.

The severity of shrinkage was higher in fruit stored at 5°C than those stored at 7.5°C after 3 months of storage, and the percentage of fruit affected by shrinkage at 5°C were relatively lower than those stored at 10°C (Fig. 2A). In addition, it was observed that fruit decay increased with temperature and storage period (Fig. 2B). A similar observation was observed for aril decay, where the severity of internal decay was enhanced with increasing storage temperature and prolonged duration (Fig. 2C). Similarly, internal disorders such as aril browning on fruit stored at 5°C and 7.5°C after 4 months followed a similar trend for fruit decay (Fig. 2D).

Furthermore, chilling injury became noticeable after 2 months of storage at 5°C (below 5% incidence) and was observed afterwards till the end of the trials (Fig. 2E). This is in agreement with Kader *et al.* (1984) who reported chilling injury on fruits of pomegranate ‘Wonderful’ cultivar when stored at 5°C and 95% RH for longer than 1 month. The percentage of external and internal disorders were higher in fruit stored at 10°C compared to 5°C and 7.5°C, and the proportion of fruit affected by internal and external disorders was 63% and 49% after 4 months (Fig. 3A and Fig. 3B).

These observations in our study suggest that the investigated cultivar are more susceptible to disorders when stored at temperature of 5°C for longer than one month. Storage of fruit at low temperatures of 5°C and 7.5°C for up to 3 months or beyond clearly became more susceptible to external and internal disorders.

#### 4.3. Browning index (BI) of husk

The BI of fruit peel during different storage temperatures are presented in Fig. 4. There were significant ( $p < 0.05$ ) changes in BI with storage temperatures and storage duration. BI values increased significantly with increasing storage temperatures although for fruit storage at 5°C and 7.5°C were non-significant ( $p > 0.05$ ) for 3 months of storage. Storage of fruit at 21°C exhibited the highest BI (126) when compared to fruit stored at lower temperatures during the first month of storage. Similarly, storage of fruit at 5°C resulted in lower BI than those stored at higher temperatures for 2 months. The reduced browning at 5°C may be related to the lower storage



temperature; as such temperatures inactivate the enzymes responsible for browning in fruit (Mohapatra *et al.*, 2010). However, after 3 months, browning was significantly higher (122) in fruit stored at 5°C than those stored at 7.5°C (113) (Fig. 4). The dramatic increase in browning at 5°C could be attributed to chilling injury. This is in agreement with previous findings by Zhang & Zhang (2008), who observed that storage of pomegranate ‘Mollar de Elche’ at 2°C resulted in significant increase in peel browning after fruits received chilling injury. Overall, browning index at 10°C was higher than those at lower temperatures throughout storage.

## 5. Conclusions

The results of this study showed that postharvest physiological response for the investigated cultivar was affected by storage temperature and duration. The investigated cultivar showed a decline in respiration rate during storage with the highest respiration rate measured at harvest time (7.9 ml CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>), however the respiration rate gradually increased after 2 months storage and did not vary significantly ( $p < 0.05$ ) between the last month of storage for 5°C and 7.5°C. As observed in this study, the incidence and occurrence of physiological disorders was reduced at lower temperatures but progressively increased with storage period. Furthermore, the investigated cultivar was susceptible to chilling injury when stored at 5°C for 2 months and was observed afterwards till the end of the storage period. Given that fruit stored at 5°C and 92% RH showed significant reduced respiration rate and lower incidence of decay compared to other investigated temperatures up to 3 months, it could be recommended that the investigated cultivar be stored at 5°C and >92% RH for up to 3 months. Overall, there is a need for further studies focused postharvest treatments of fruit before storage to minimise physiological disorders and fruit decay, especially for prolonged storage of the investigated cultivar.

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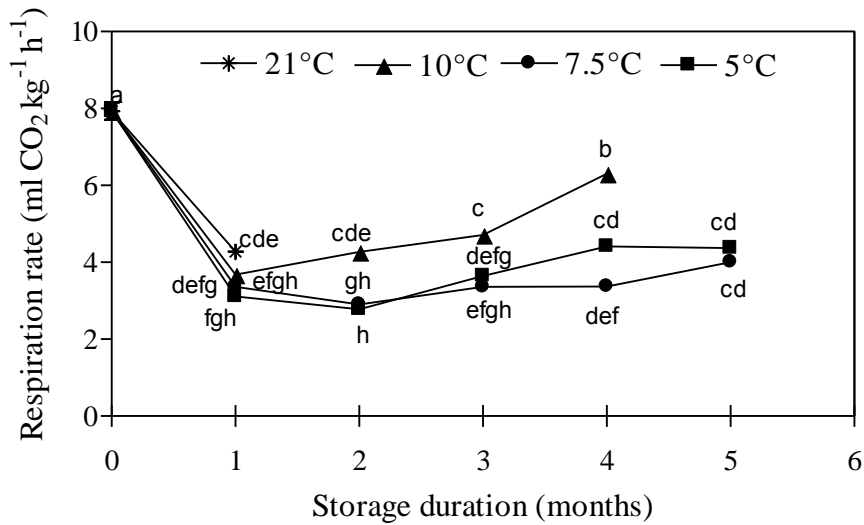


Fig.1. Respiration rate for pomegranate fruit (cv. Wonderful) stored at different temperatures. Data points each represent the mean of 15 replicates, vertical bars denotes the standard error (S.E.). Different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test (a - h). Experiments with fruit stored at 21°C and 10°C were discontinued after 1 and 4 months, respectively, due to complete fruit loss.

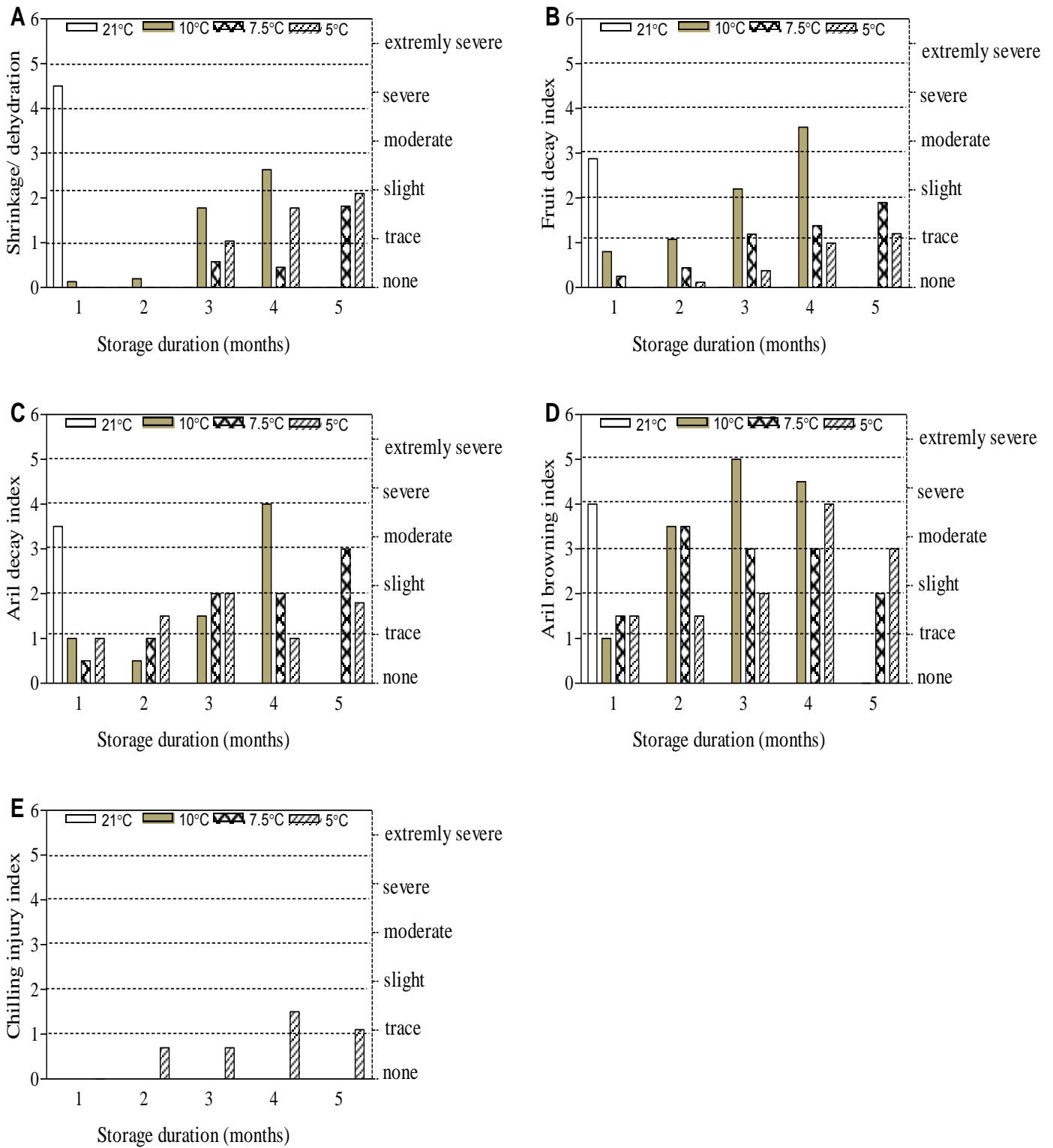


Fig. 2. Occurrence of physiological disorders in pomegranate fruit stored at different temperatures: shrinkage/dehydration (A), fruit decay (B), aril decay (C), aril browning (D), chilling injury (E). Experiments with fruit stored at 21°C and 10°C were discontinued after 1 and 4 months, respectively, due to complete fruit loss.

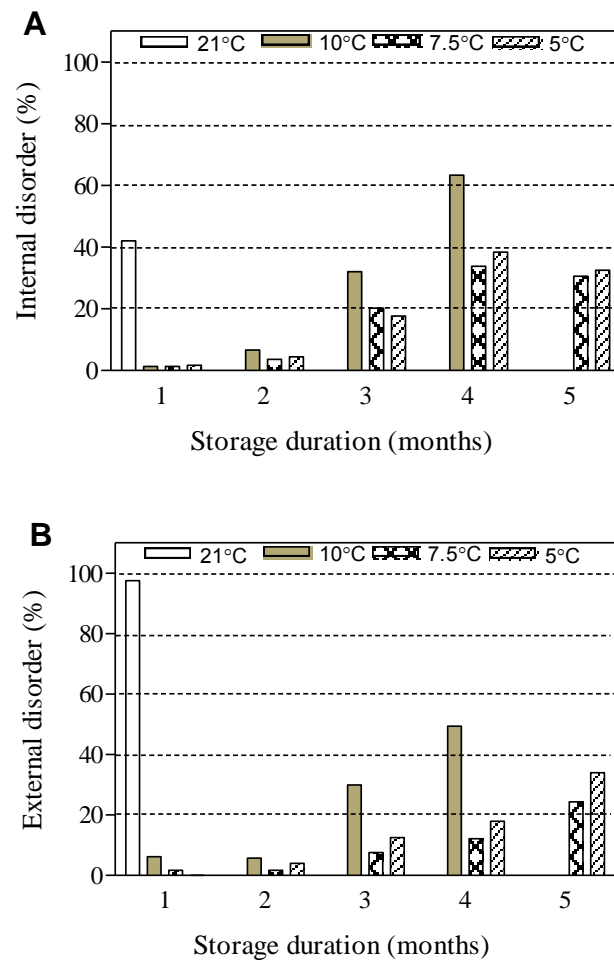


Fig.3. Percentage of fruit affected by internal disorders (A) and external disorders (B) Experiments with fruit stored at 21°C and 10°C were discontinued after 1 and 4 months, respectively, due to complete fruit loss.

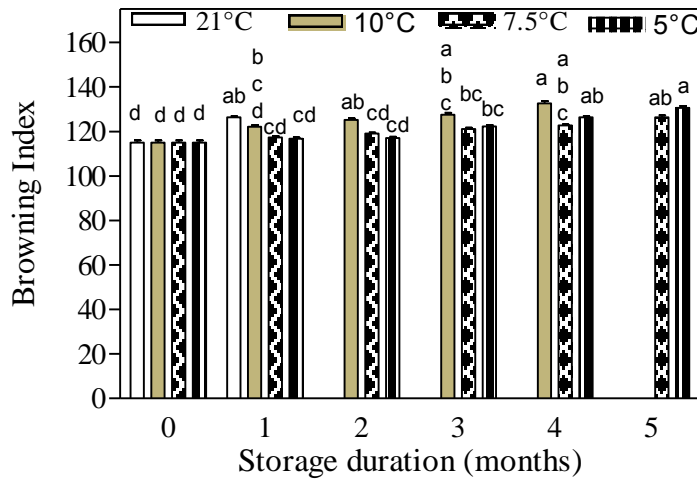


Fig.4. Browning index based on CIE L\*, a\*, b\* coordinates for pomegranate fruit 'Wonderful' cultivar during storage at different temperatures. Data points each represent the mean of 15 replicates, vertical bars denotes the standard error (S.E.). Different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test (a - d). Experiments with fruit stored at 21°C and 10°C were discontinued after 1 and 4 months, respectively, due to complete fruit loss.



# **Chapter 4**

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**Influence of storage temperature and duration on  
postharvest physico-chemical and mechanical  
properties of pomegranate fruit**

## INFLUENCE OF STORAGE TEMPERATURE AND DURATION ON POSTHARVEST PHYSICO-CHEMICAL AND MECHANICAL PROPERTIES OF POMEGRANATE FRUIT

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### Summary

Knowledge of postharvest physico-chemical and mechanical properties of pomegranate fruit at harvest and during storage period are important in the design and management of optimum postharvest handling including packaging, transportation and storage systems. Physico-chemical and mechanical properties for pomegranate (cv. 'Wonderful') were determined over a period of 5 months of storage at 5°C (92% RH), 7.5°C (92% RH), 10°C (92% RH) and 21°C (65% RH). Fresh pomegranate juice of each temperature regime was assessed for total soluble solids (TSS), titratable acidity (TA), pH and BrimA index. Fruit mechanical properties such as puncture resistance, cutting force, and fruit and aril compression were determined during storage. Aril and peel moisture content, peel thickness and colour dynamics were also investigated. The results showed that the primary source of moisture loss was the fruit skin (peel), and this resulted in significant ( $p < 0.05$ ) reduction in peel thickness with prolonged storage period. The CIE ( $L^*$ ,  $a^*$ ,  $b^*$  and  $C^*$ ) colour parameters of fruit and arils decreased during storage. However, the hue ( $h^\circ$ ) values for whole fruit increased as a result of browning incidence, whereas hue values for arils decreased suggesting increase in aril redness. Furthermore, TSS, pH, TSS:TA and BrimA increased significantly ( $p < 0.05$ ) throughout the storage period. On the contrary, there were significant ( $p < 0.05$ ) decreases in titratable acidity (TA) throughout the storage period at all storage temperatures, with the exception of 10°C. Storage temperature and duration significantly ( $p < 0.05$ ) affected majority of the investigated mechanical properties. Puncture resistance significantly ( $p < 0.05$ ) decreased with storage temperature and duration. Similarly, temperature and duration resulted in a significant ( $p < 0.05$ ) decrease in fruit compression parameters such as firmness, toughness and bioyield. Furthermore, the force and energy required to cut pomegranate fruit did not significantly change at majority of the storage temperatures. These findings showed that fruit should be stored between 2 to 3 months and maintained at 5°C to ensure the best internal and external quality attributes.

## 1. Introduction

Pomegranates (*Punica granatum* L.) are commercially cultivated in many subtropical countries such as Tunisia, Turkey, Egypt, Spain, Morocco Iran, Afghanistan, India, Pakistan and USA (Stover & Mercure, 2007; Holland *et al.*, 2009). Nearly all parts of the fruit can be utilized. The edible part (aril) contains sugars, vitamins, polysaccharides, polyphenols and important minerals (Miguel *et al.*, 2010). The peel is a rich source of natural antioxidants and has been used in the Middle East as colorants for textiles due to the high tannin and phenolic content (Al-Said *et al.*, 2009; Li *et al.*, 2006). The high antioxidant activities of pomegranate fruit are attributed to high levels of polyphenolic compounds, which act as good free radical scavengers (Fawole *et al.*, 2012). In the last few years there has also been increase in the demand for industrial processing of pomegranate arils for fresh consumption and processed products such as food colorants, tannins for leather, jellies, jams and wines (Maclean *et al.*, 2011; Zaouay *et al.*, 2012; Caleb *et al.*, 2012).

Despite the nutritional and health benefits of consuming pomegranate, there are difficulties in extracting the arils, and this requires adequate knowledge of the fruit mechanical properties such as toughness, firmness, cutting force and shear strength (Ekrami-Rad *et al.*, 2011). Knowledge of the physical, chemical and mechanical properties of pomegranate fruit and arils are useful for the design and operation of machines and new processes for harvesting, handling and postharvest operations (Ekrami-Rad *et al.*, 2011).

Postharvest mechanical tests such as fruit compression simulate static load which is the most common method of deriving stress-strain properties of fruit during handling and storage (Bentini *et al.*, 2009). In the horticultural industry, fruit firmness is used in combination with other maturity indices to identify optimal harvest dates to ensure good storage potential and acceptable sensory quality. Therefore, mechanical properties such as compression and puncture resistance could be important quality indicators of pomegranate, However, there is limited understanding of the effects of temperature on firmness of pomegranate fruit (Johnston *et al.*, 2001).

During storage and transportation, significant changes in organic acids, sugars, fruit colour and texture have been reported by various authors (Koksal, 1989; Gil *et al.*, 1996; Waskar, 2011). Studies have shown that optimal storage conditions are influenced by cultivar, growing region and degree of fruit maturity (Koksal, 1989; Gil *et al.*, 1996; Waskar, 2011). Quality after harvest can greatly be altered during supply chain. Storage temperature,

humidity and duration have a considerable effect on changes in fruits quality and mechanical properties (Ekrami-Rad *et al.*, 2011). For instance, Mansouri *et al.* (2011) reported a decrease in firmness during storage period for two Iranian pomegranate cultivars ‘Hondos-e-Yalabad’ and ‘Malas-e-Saveh’. In addition, Johnston *et al.* (2001) reported an increase in the firmness and tensile strength for ‘Gala’ and ‘Granny Smith’ apples when stored for 100 days at 3°C. On the contrary, Masoudi *et al.* (2007) reported that storage period resulted in decrease in firmness for ‘Golden Delicious’ and ‘Granny Smith’ apples stored at 5°C and 70% RH. Yurtlu & Erdoğan (2005) established that storage time resulted in a decrease in firmness and increase in bruising susceptibility for pears when stored up to 4 months at 0°C and 90% RH. Singh & Reddy (2006) found that temperature and storage time significantly decreased effect on firmness in ‘Nagpur Mandarin’ oranges at 7°C and 78% RH for 10 days of storage.

Pomegranate products are new and rapidly increasing in South Africa. Scientific literature on quality attributes, physiological response and antioxidant capacities of various pomegranate cultivars are voluminous. However, scientific knowledge on mechanical properties of pomegranate fruits is lacking, especially during postharvest storage. Most research on mechanical properties of pomegranates focused mainly on textural properties of arils. There is a need for better understanding of relevant mechanical properties of whole fruit and how they relate to storage and processing. This information would be helpful in design of optimum postharvest handling including packaging, transportation and storage systems to minimize damage of fruit and maintain quality of arils. The objective of this study was therefore to investigate the effects of storage temperature and duration on postharvest physico-chemical and mechanical properties of pomegranate fruit and arils.

## **2. Materials and Methods**

### *2.1. Plant material and storage conditions*

Fruit sampling and storage conditions were performed as described in chapter 3, section 2.1.

### *2.2. Sample preparation and fruit processing*

Postharvest physico-chemical and mechanical properties were determined with respect to storage temperature and duration in both ambient and cold storage conditions. Over a period of 5 months, fruit were sampled at 1 month interval. For physico-chemical properties, each fruit was hand-peeled and 100 g of arils were juiced (without crushing the kernels) using a

Liquafresh juice extractor (Mellerware, South Africa) and ten milliliters of juice from each fruit was used for chemical analysis. All analysis was performed in triplicate at room temperature.

### 2.3. *Physical properties*

#### 2.3.1. *Weight loss*

Cumulative weight loss for pomegranate fruit was determined for each storage condition, ten fruit samples of similar size were randomly selected, numbered and weighed, at one month intervals. Fruit weight loss was measured with respect to storage period using an electronic scale (Mettler Toledo, model ML3002E, Switzerland, 0.0001 g accuracy). The loss in weight was calculated as:

$$W = [(W_i - W_f) \div W_i] \times 100 \quad (1)$$

where  $W$  = cumulative weight loss (%) of fruit;  $W_i$  = initial weight (g) of the fruit at the beginning of storage;  $W_f$  = final weight (g) of the fruit at the time of sampling during storage. Weight loss was calculated for each storage temperature on 10 individual fruit and values were presented as mean  $\pm$  S.E.

#### 2.3.2. *Aril and peel moisture content*

In triplicate, ten grams of arils and 50 g of peel samples were taken into glass petri dishes. Samples were dried in an oven (Prolab, model OTE 160, South Africa) at 80°C for 24 hours. Dried samples were weighed with an electronic balance scale (Mettler Toledo, model ML3002E, Switzerland, 0.0001 g accuracy). Moisture content was calculated similar to equation 1 but on a dry weight bases (db) as:

$$db = [(W_i - W_f) \div W_i] \times 100 \quad (2)$$

#### 2.3.3. *Peel thickness*

Peel thickness were measured using a Vanier caliper (Mitutoyo, model CD-6 CX, Japan) with 0.01 mm accuracy on opposite sides of 20 pieces of fruit peel. All analysis was performed at room temperature. Peel thickness was measured using 10 randomly selected fruit stored at each temperature and the average values were reported as mean  $\pm$  S.E.

#### 2.3.4. *Colour attributes*

The colour change in pomegranate fruit and arils was measured using the CIE  $L^*$ ,  $a^*$ ,  $b^*$  coordinates with a calibrated Minolta Chroma Meter (Model CR-400/410 (Minolta Corp,

Osaka, Japan). Peel colour measurements were taken along the equatorial axis of each fruit at three marked spots. Similarly, three measurements of aril colour were taken in a petri dish. The hue angle ( $h^\circ$ ) was calculated using (Pathare *et al.*, 2012):

$$h^\circ = \tan^{-1} (b^*/a^*) \quad (3)$$

Chroma was calculated according to using:

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

Results were presented as mean  $\pm$  S.E. (n=10)

## 2.4. Chemical properties

### 2.4.1. Titratable acidity, total soluble solids and pH

Titrate acidity (TA) was measured by diluting 2 ml of fresh juice with 80 ml of distilled water and titrated 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 g 100 ml/citric acid. Total soluble solid (TSS, °Brix) was measured using a digital refractometer (Atago, Tokyo, Japan). The pH values were determined at room temperature using a calibrated pH meter (Crison, Model 00924, Barcelona, Spain). All measurements were made on 10 individual fruit juice samples for each storage temperature and results were presented as mean  $\pm$  S.E.

TSS/TA values were also calculated. Also, BrimA index, a variant of the TSS/TA and a criterion for the acceptance of fruit juice, was calculated using  $TSS - k \cdot TA$ . Where k is the tongue's sensitivity index normally ranging from 2 to 10 (Fawole & Opara, 2013a; Jordan *et al.*, 2001). To avoid a negative BrimA index k value of 2 was used (Fawole & Opara, 2013a).

## 2.5. Mechanical properties

### 2.5.1. Fruit puncture resistance test

Fruit texture analyzer (GÜSS-FTA, model GS, South Africa) was used to measure fruit puncture resistance. 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. Duplicate test were performed on opposite sides on the equilateral region of 10 individual fruit for each storage regime. Peak force required to puncture the fruit surface was taken as puncture resistance and the values were presented as mean  $\pm$  S.E.

### 2.5.2. *Fruit compression test*

Fruit compression was performed using a texture profile analyzer XT Plus (Stable MicroSystem, Godalming, UK) with a 70x70 mm, P70 compression platen probe. The texture profile analyzer was calibrated with a 10 kilogram load cell. The operating conditions for the profile analyzer were as follows: pre-test speed 1.5 mm/s, probe test speed 1 mm/s, post-test speed 10.0 mm/s, compression force 1000 N, deformation distance 20 mm. A single fruit was placed on a steel test platform with the stem calyx axis parallel to the platform and a force deformation curve was obtained for each test. Two variables, force (N) and distance (mm) was obtained using the force deformation curve and the data was interpreted using texture profile analyzer software Exponent v.4 (Stable MicroSystem Ltd., Godalming, UK). The elastic modulus ( $\text{N}/\text{mm}^2$ ), force (N), toughness (N mm), and bioyield force (N) were calculated by running macro software. The elastic modulus or young modulus could be defined as the initial slope which gives an indication of the fruits tendency to deform elastically when a force is applied. The firmness was expressed as the maximum force (N) required to compress the fruit to a distance of 20 mm. The toughness (energy) required to compress the fruit was determined by calculating the area under the force displacement curve. The bioyield point was considered as the force under prescribed conditions to cause permanent deformation. Fruit compression test was carried out on opposite sides of 10 individual fruit, similar in size for each temperature regime and values for 20 determinations were presented as mean  $\pm$  S.E.

### 2.5.3. *Fruit cutting test*

Texture profile analyser XT Plus (Stable MicroSystem, Godalming, UK) were used with a blade set knife. For each test, a single pomegranate fruit was positioned with its stem calyx axis parallel to the platform. The operating conditions for the profile analyzer were as follows: pre-test speed 1 mm/s, test speed 1 mm/s, post-test speed 10 mm/s, cutting force 1000 N and cutting distance 20 mm. The data obtained from the textural profile analyzer was interpreted using software Exponent v.4. The software was used to run macro which was used to evaluate the cutting force and energy. Fruit cutting test was carried out on opposite side of 10 randomly selected fruit, similar size for each temperature regime and values for 20 determinations were expressed as mean  $\pm$  S.E.

#### 2.5.4. Aril compression test

Aril compression test was performed using a texture profile analyzer XT Plus (Stable MicroSystem Ltd., Godalming, UK), 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). The data obtained from the textural analyzer was interpreted using software Exponent v.4 (Stable MicroSystem Ltd., Godalming, UK). The software was used to run macro which gave the elastic modulus ( $\text{N/mm}^2$ ), rupture force (N), toughness (N mm), and bioyield force (N). Aril compression test were done on 10 randomly selected fruit for each storage regime and the results were presented as the mean ( $\pm$  S.E) of 40 determinations are reported.

#### 2.6. Statistical analysis

Statistical analysis was carried out using Statistica software (Statistical version 10, StatSoft Inc., Tulsa, USA). One-way analysis of variance (ANOVA) was used to evaluate the effects of storage temperature and duration on physico-chemical and mechanical properties. The difference between mean values of parameters was investigated by using Duncan's Multiple Range Test.

### 3. Results and discussion

#### 3.1. Physical Properties

##### 3.1.1. Weight loss

The percentage cumulative weight loss of pomegranate fruit during storage under ambient ( $21\pm 3^\circ\text{C}$  with  $65\pm 6\%$  RH) and cold storage conditions ( $5^\circ\text{C}$ ,  $7.5^\circ\text{C}$ ,  $10^\circ\text{C}$  with  $92\pm 5\%$  RH) over 5 months are presented in Fig. 1. Pomegranate is highly susceptibility to weight loss is due to high porosity of the fruit peel which permits free water vapour movement (Elyatem & Kader, 1984), and the susceptible could depend on storage conditions. Our present study showed significant ( $p < 0.0001$ ) differences in fruit weight loss among the storage temperatures and durations. Weight loss increased with increasing storage temperature and prolonged storage. After one month of storage, fruit stored at ambient temperature ( $21^\circ\text{C}$ ) almost 20% of fruit weight obtained before storage, resulting to more than 5 folds compared to other storage conditions at the same period. High temperature



coupled with low relative humidity at ambient conditions could be responsible for the observed weight loss as such conditions induce high respiration and transpiration in pomegranate fruit (Opara *et al.*, 2008). Weight loss remained below 10% at 7.5°C and 5°C and no sign of shriveling was observed on fruit even after 2 months of storage. Weight losses obtained after one month of storage are comparable with those reported by Elyatem & Kader (1984). The authors reported weight loss of 1.0%, 1.4%, 1.6% and 2.7% at 0°C, 5°C, 10°C and 20°C, respectively after 5 weeks of storage. Interestingly, weight losses in fruit stored at 5°C and 7.5°C did not differ significantly until after 3 months. Only fruit stored at 5°C and 7.5°C lasted for 5 months, with weight loss of 27.67% and 45.67%, respectively. Weight loss obtained at 5°C after 5 months of storage clearly suggests the importance of low temperature and high relative humidity for pomegranate fruit storage. This viewpoint is buttressed by Fawole & Opara (2013c), who reported that both storage temperature and relative humidity had a significant interaction effect on weight loss in ‘Bhagwa’ and ‘Ruby’.

### *3.1.2. Aril and peel moisture content*

Aril and peel moisture content for pomegranate fruit during storage under ambient (21±3°C, 65±6% RH) and cold storage conditions (5°C, 7.5°C, 10°C with 92±5% RH) for up to 5 months of storage are presented in Table 1. Aril moisture content did not change significantly over time under the investigated storage temperatures. However, peel moisture content decreased significantly ( $p<0.05$ ) over time. Initial moisture content fruit peel was 78.58% and decreased to 73.08% after 1 month at 21°C. Similarly, moisture content decreased to 66.16% after 4 months at 10°C, and 66.18% and 59.49% after 5 months of storage at 5°C and 7.5°C, respectively. The observed moisture content is an indication that the pomegranate fruit moisture loss was primarily from fruit peel, with negligible moisture loss from aril. This information is important in fruit handling where appropriate storage temperature is required for long time storage.

### *3.1.3. Peel thickness*

There were decreases in peel thickness at all the investigated storage conditions over time (Table 1). After 1 month of storage, peel thickness decreased significantly ( $p<0.05$ ) at 21°C, whereas the decrease was not significant at 5°C, 7.5°C and 10°C. Drastic decrease in peel thickness at ambient temperature may be due to low relative humidity coupled with high temperature. Peel thickness gradually decreased from the initial 5.3 mm to 3.61 mm after 4 months at 10°C and to 3.69 mm and 3.66 mm after 5 months of storage at 5°C and 7.5°C,

respectively. The decrease in peel thickness may be attributed to moisture loss from fruit peel as storage period progressed. However, moisture loss was minimized at 5°C compared to other storage regimes.

#### 3.1.4. Peel and aril colour

Colour of pomegranate fruit is an important quality attributes affecting marketability, consumer's acceptance and commercial value (Gil *et al.*, 1996). The colour attributes of whole fruit (Table 2) and arils (Table 3) changed significantly ( $p < 0.05$ ) at all the investigated storage conditions. Increase in peel colour of fruit stored at 5°C was obvious during the first three months in storage. The intense colouration was evident by increases in the CIE  $a^*$  and  $C^*$  values as well as decrease in the hue angle ( $h^\circ$ ). The increase in  $C^*$  values could be as a result of biosynthesis and accumulation of anthocyanin pigments in the peel, resulting in intense red colouration (Gil *et al.*, 1995). However fruit external appearance deteriorated after 3 months up till the end of the storage trials (5 months), possibly as a result of breakdown or browning of the husk. In contrast, aril colour declined at varying degrees under the investigated storage temperatures throughout the storage period (Table 2). Overall results indicate that colour of fruit peel and aril was better maintained at 5°C for between 2 and 3 months, when red colouration ( $a^*$ ) and intensity ( $C^*$ ) for peel and arils were considerably higher than the perceived fruit colour at harvest.

#### 3.2. Chemical properties

Chemical parameters like TSS, TA and TSS/TA have been used to describe taste (flavour) with regards to the sweetness and acidity; it has been used as a quality criterion for the formulation of pomegranate products and its juice (Al-Said *et al.*, 2009). As shown in Table 4, there were significant differences ( $p < 0.05$ ) in the juice chemical properties at different storage conditions. The lowest total soluble solids (TSS, °Brix) content was recorded at commercial harvest (week 0). TSS increased significantly ( $p < 0.05$ ) during storage at the investigated temperature regimes. For instance, after one month storage TSS increased from 13 °Brix to 16.22 °Brix, 15.36 °Brix, 14.84 °Brix and 14.35 °Brix at 5°C, 7.5°C, 10°C and 21°C, respectively. TSS level remained relatively steady up till the end of the experiment, TSS content being 16.22 °Brix in fruit stored at 5°C for 5 months (Table 3). In contrast to our present study Kader *et al.* (1984) and Artés *et al.* (1998) previous study done on other pomegranate cultivars showed a decline in TSS as storage period progressed (Kader *et al.* 1984; Artés *et al.* 1998). Overall, storage temperature of 5°C would have the best keeping

potential for the investigated cultivar in terms of TSS.

Titrateable acidity (TA) significantly ( $p < 0.05$ ) decreased at all storage conditions, with the exception of 10°C which significantly ( $p < 0.05$ ) increased at 1 month of storage and gradually decreased with storage duration. This increase in TA may be attributed to water loss which increases with storage temperature. Furthermore, the significant ( $p < 0.05$ ) decrease at a higher temperature of 21°C may be attributed to low RH and the rapid breakdown of organic acids. Increases in TA levels during storage have previously been reported by Gil *et al.* (1996) for the Spanish ‘Mollar de Elche’ cultivar. On the contrary, several authors have reported a decline in TA levels for pomegranate fruits (Artés *et al.*, 2000; Fawole & Opara, 2013c); the storage behavior of TA would differ depending on the cultivar, growing region and storage conditions (Gil *et al.*, 1996; Martinez *et al.*, 2012). TA for the investigated cultivar ranged during storage between 0.87-1.59 (% citric acid). The pH values increased as storage period progressed at all storage regimes. The increase in pH was accompanied by a decline in acidity levels. Similarly Fawole & Opara (2013c) observed an increase in pH values for two South African grown cultivars ‘Bhagwa’ and ‘Ruby’.

As a result of the changes in TSS and TA contents, TSS/TA ratio increased from 10.59 to 19.03 during storage with significant changes observed at different storage temperatures. The calculated TSS/TA values in the present study are similar to those reported by Ben-Arie *et al.* (1984), with TSS/TA values ranging from 11-16 for ‘Wonderful’ cultivar.

In a quest to exploring chemical changes related to flavour we used BrimA index adopted from Jordan *et al.* (2001). This index allows for small amounts of acid than sugar to make the same numerical changes to BrimA index as observed in the present study Table 4. BrimA increased from 10.64 at harvest to 14.33, 13.62, 12.96, and 12.30 for 5°C, 7.5°C, 10°C and 21°C, respectively, during storage. Our result is contrary to Fawole & Opara (2013c) who reported a decrease in BrimA for ‘Ruby’ cultivar stored at 5°C, 7°C, 10°C and 21°C for 4 months of storage. Overall, storage temperature of 5°C for 2 months seems to be the best suitable for the calculation of BrimA compared to other storage regimes.

### 3.3. Mechanical Properties

Ambient conditions (21±3°C and 65 ±6% RH) were not studied as storage of fruit up to 1 month resulted in decay and were limited.

### 3.3.1. Puncture resistance

Fruit puncture resistance increased in fruit stored at all storage regimes in the first month of storage indicating hardening of the peel (Table 4). The increase in puncture resistance was more pronounced under 10°C (138.64 N) than that under 5°C (130.32 N) and 7.5°C (133.52 N), respectively. Increase in puncture resistance could be due to moisture loss from the fruit which resulted to the hardening of the pomegranate peel. However, after 1 month of storage a decrease in puncture resistance was observed. The overall decreases in puncture resistance with prolong storage duration, suggest softening of fruit and its arils occurs during storage. Similar results were reported by Mansouri *et al.* (2011) who showed the loss of puncture resistance in fruit during storage at 5°C for 30 days. This fruit property could be used with several other quality indices to predict fruit firmness for optimum storage potential.

### 3.3.2. Fruit compression

Fruit compression results of pomegranate fruit in cold storage conditions is presented in Table 5. There was a significant ( $p < 0.05$ ) difference in the force required to compress fruit at different temperatures. The force required to compress fruit decreased significantly ( $p < 0.05$ ) from harvest (295.24 N) and continued to decline with extend storage duration resulting in the lowest observed force at 5°C (162.64 N) for 5 months. This observation indicates that extended storage duration results in a decline in fruit firmness. The decrease in firmness may be due to the loss in cell-wall integrity of the pomegranate arils (Ekrami-Rad *et al.*, 2011). Another reason for the significant decrease for the 'Wonderful' fruit stored at 5°C for 5 months of storage could be as a result of chilling injury, which ultimately leads to loss of cell-wall integrity in pomegranate (Elyatem & Kader, 1984). Our results with regards to fruit firmness, is in agreement with Ekrami-Rad *et al.* (2011) who reported a reduction in firmness for 'Wonderful' stored at 5°C for 5 months. Similarly, the influence of temperature and storage duration on fruit toughness (energy) was significantly ( $p < 0.05$ ) evident, were the amount of energy to compress fruit was reduced with increasing storage duration. Furthermore, Holt (1970) reported that several factors affects fruit compression test results this may depend on the mechanical strength of the skin, firmness of the flesh, juice viscosity, and size of the fruit.

The bioyield point declined at all the investigated storage temperatures when compared with fruit at harvest (32.81 N). However, the decline was not significant ( $p > 0.05$ ) amongst majority of storage temperatures but rather affected by storage duration. A similar trend was

reported by Singh *et al.* (2006) for oranges. The reduction in bioyield demonstrates increase deformability of fruit to compression test as storage period progressed. The Young modulus did not significantly ( $p > 0.05$ ) change during temperature or storage duration storage. As observed with the investigated cultivar, the reduction of Young's modulus at 5°C for 5 months showed that fruit moisture in fruit peel is still retained. Overall, the best suitable temperature for fruit firmness would be at 5°C for the investigated cultivar.

### 3.3.3. Fruit cutting test

The cutting force and energy of pomegranate fruit in cold storage conditions are presented in Table 6. Cutting force did not differ significantly ( $p < 0.05$ ) as a result of storage temperature particularly between 1 and 2 months, but rather declined after harvest (229.56 N); although the influence of temperature and storage duration was significantly ( $p < 0.05$ ) apparent on the cutting energy. Moreover, the energy required to cut pomegranate fruit declined and gave a similar trend observed by cutting force. The decrease in cutting force may be attributed to peel thinning and gradual changes to the inner portions of the fruit occurring during storage leading to softening Ekrami-Rad *et al.* (2011). Similar results were reported by Ekrami-Rad *et al.* (2011) who showed a decrease in cutting force and energy during storage 'Wonderful' at 5°C for 5 months.

### 3.3.4. Aril compression test

The textural property is an important quality attribute in the pomegranate industry (Fawole & Opara, 2013b). The results obtained showed that aril hardness, elastic modulus, energy and bioyield changed significantly during storage (Table 7). After 2 months, aril hardness did not differ significantly amongst storage temperatures. Although, aril hardness showed a significant ( $p < 0.0001$ ) decreasing trend with extended storage duration, resulting in the lowest force being observed at 10°C (112.29 N) and 7.5°C (112.22 N) for 4 months. The decrease in aril hardness has been attributed to loss in cell-wall integrity of pomegranate arils (Ekrami-Rad *et al.*, 2011). The reduction in the Young modulus demonstrates the increasing deformability of fruit arils with increase storage temperature and duration. Similarly it was observed that the energy required to compress aril declined with a reduction in arils hardness. This behavior for pomegranate aril demonstrates a tendency for elasticity to decrease with storage temperature and period. Bioyield showed slight variation, however no significant difference was observed for 7.5°C and 10°C for 4 and 3 months of storage.

Similar results were reported by Fawole & Opara (2013c) who showed a loss of firmness in arils during fruit storage at 5°C for 6 weeks with shelf life period of 5 days at 20°C.

#### 4. Conclusions

Changes in physico-chemical and mechanical properties of pomegranate fruit 'Wonderful' cultivar at different storage temperatures were investigated which would provide useful information regarding quality changes during transportation and storage. It can be concluded that the investigated cultivar should be stored at low temperatures and high in other to minimize weight loss, slow down chemical depreciation, and maintain overall fruit quality. Our study showed that weight loss, colour attributes, chemical attributes and textural quality of the fruit can be optimally maintained at 5°C for 2 months of storage. These findings may be of value for the development of optimal storage conditions for handling and processing of pomegranate fruit for food and industrial use.

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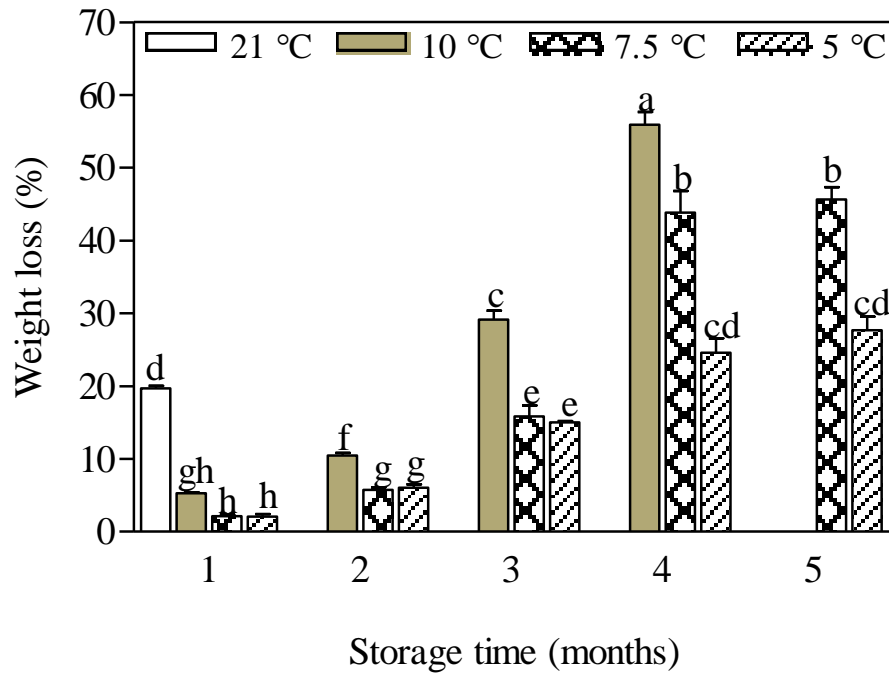


Fig.1. Cumulative weight loss for pomegranate fruit ('Wonderful' cultivar) at 21°C (65% RH), 10°C (92% RH), 7.5°C (92% RH) and 5°C (92% RH) for 5 months

Table 1

Peel thickness and moisture content of 'Wonderful' fruit stored at 21°C (65% RH), 10°C (92% RH), 7.5°C (92% RH) and 5°C (92% RH) for 5 months

Duration (month)	Temperature (°C)	Peel thickness (mm)	Moisture content	
			Aril (%)	Peel (%)
Initial		5.30±0.02ab	81.24±0.34a	78.58±0.48a
1	21	4.20±0.05d	83.49±0.10a	73.08±0.50bc
	10	5.32±0.03a	80.64±0.32a	75.18±0.89ab
	7.5	5.30±0.02ab	81.34±0.20a	76.02±0.35ab
	5	5.30±0.02ab	81.07±0.16a	76.56±0.32ab
2	10	4.61±0.02d	82.45±0.24a	68.29±0.73cde
	7.5	4.57±0.02d	79.74±0.04a	70.60±0.24cd
	5	4.67±0.02cd	83.06±0.14a	73.64±0.31bc
3	10	4.58±0.02cd	80.10±0.41a	68.46±0.20def
	7.5	4.84±0.02cd	80.66±0.12a	70.19±0.30cd
	5	4.57±0.02bc	80.92±0.09a	68.53±0.49def
4	10	3.61±0.02e	81.13±0.07a	66.16±0.44dfg
	7.5	3.87±0.02e	85.11±0.64a	64.55±1.40fg
	5	4.50±0.02d	81.05±0.04a	66.18±0.32defg
5	7.5	3.66±0.02e	82.72±0.26a	59.49±0.59g
	5	3.69±0.02e	81.69±0.16a	65.13±0.17efg
<i>p</i> -value		< 0.0001	0.99945	< 0.0001

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test. Storage trials for fruit stored at 21°C and 10°C were discontinued after 1 month and 4 months, respectively, due to complete fruit loss to decay

Table 2

Peel colour dynamics of 'Wonderful' fruit stored at 21°C (65% RH), 10°C (92% RH), 7.5°C (92% RH) and 5°C (92% RH) for 5 months

Duration (month)	Temperature (°C)	$L^*$	$a^*$	$C^*$	$h^\circ$
Initial		48.27±0.24ef	39.81±0.21bc	47.09±0.12cd	32.02±0.19cdef
1	21	42.46±0.14g	38.75±0.11cd	44.10±0.11ef	28.27±0.12f
	10	48.38±0.28ef	39.56±0.25bc	46.47±0.15de	31.42±0.26cdef
	7.5	46.75±0.25fg	40.87±0.18bc	47.23±0.13cd	29.72±0.23ef
	5	45.86±0.23fg	41.91±0.13bc	47.80±0.13bcd	28.29±0.18f
2	10	64.15±0.37a	39.27±0.32c	47.30±0.25cd	33.53±0.33bcde
	7.5	61.38±0.34ab	42.32±0.22bc	49.59±0.15bc	30.86±0.29cef
	5	58.41±0.29bc	46.13±0.17a	53.57±0.15a	30.03±0.22ef
3	10	56.45±0.36cd	33.27±0.24ef	41.25±0.20fgh	35.60±0.32abc
	7.5	55.59±0.31cd	41.02±0.22bc	49.08±0.13bcd	32.59±0.31bcdef
	5	51.91±0.27de	43.27±0.17ab	50.54±0.15b	30.40±0.24ddef
4	10	46.99±0.26efg	29.66±0.21g	38.63±0.17h	39.24±0.30a
	7.5	46.23±0.27fg	33.42±0.16ef	41.57±0.14fg	35.57±0.29abc
	5	44.26±0.22fg	35.83±0.15de	43.25±0.13f	32.34±0.15bcdef
5	7.5	44.67±0.23fg	30.84±0.17fg	39.82±0.12gh	37.30±0.32ab
	5	42.88±0.22g	34.97±0.17e	42.90±0.14f	35.45±0.31abcd
<i>p</i> -value		< 0.0001	< 0.0001	< 0.0001	< 0.0001

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test. Storage trials for fruit stored at 21°C and 10°C were discontinued after 1 month and 4 months, respectively, due to complete fruit loss to decay.

Table 2

Aril colour dynamics of 'Wonderful' fruit stored at 21°C (65% RH), 10°C (92% RH), 7.5°C (92% RH) and 5°C (92% RH) for 5 months

Duration (month)	Temperature (°C)	$L^*$	$a^*$	$C^*$	$h^\circ$
Initial		25.83±0.28abc	23.94±0.21abc	26.68±0.23a	25.90±0.10ab
1	21	18.63±0.27efg	20.40±0.21def	22.72±0.24bcd	25.76±0.16ab
	10	24.82±0.34abcd	22.12±0.19bcd	24.98±0.21ab	26.94±0.19a
	7.5	23.17±0.35bce	18.75±0.19ef	21.27±0.19cde	27.02±0.30a
	5	19.24±0.29efg	21.08±0.17cdef	23.40±0.19bc	25.03±0.23ab
2	10	26.05±0.31abc	24.91±0.18ab	27.23±0.21a	23.03±0.15bcd
	7.5	28.50±0.36a	24.59±0.19abc	25.40±0.20ab	21.08±0.20cde
	5	26.84±0.39ab	25.27±0.10a	27.34±0.11a	22.31±0.08bcd
3	10	24.24±0.30abcd	21.36±0.19dce	23.48±0.21bc	23.83±0.20abc
	7.5	20.89±0.29def	18.63±0.15ef	19.86±0.18def	18.38±0.22e
	5	21.81±0.19cdef	21.93±0.14bcd	23.11±0.15bcd	17.90±0.10e
4	10	14.86±0.21g	17.97±0.16fg	19.56±0.18ef	22.30±0.17bd
	7.5	15.74±0.21g	19.04±0.15def	20.47±0.17cef	20.98±0.12cde
	5	15.68±0.15g	19.56±0.19def	20.95±0.21cef	19.85±0.15de
5	7.5	17.71±0.21fg	15.69±0.21g	17.64±0.22f	25.06±0.50ab
	5	19.19±0.26eg	19.10±0.16def	20.62±0.15cef	20.31±0.18cde
<i>p</i> -value		< 0.0001	< 0.0001	< 0.0001	< 0.0001

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test. Storage trials for fruit stored at 21°C and 10°C were discontinued after 1 month and 4 months, respectively, due to complete fruit loss to decay.

Table 3

Chemical attributes of 'Wonderful' fruit stored at 21°C (65% RH), 10°C (92% RH), 7.5°C (92% RH) and 5°C (92% RH) for 5 months

Duration (month)	Temperature (°C)	TSS	TA	pH	TSS:TA	BrimA
Initial		13.38±0.11g	1.37±0.04abc	3.09±0.02abc	10.59±0.33f	10.64±0.16e
1	21	14.35±0.13cdef	1.03±0.03ef	3.11±0.01a	14.75±0.32f	12.30±0.13de
	10	14.84±0.12ef	1.59±0.04a	3.04±0.01ef	10.16±0.37bde	10.64±0.15cd
	7.5	15.36±0.11abcde	0.96±0.02ef	3.22±0.01ef	16.80±0.42abc	13.45±0.12abc
	5	16.22±0.13a	0.95±0.03ef	3.37±0.02ef	18.04±0.51ab	14.33±0.11a
2	10	15.80±0.07abc	1.42±0.03ab	3.02±0.01ab	11.55±0.23ef	12.96±0.08bc
	7.5	15.95±0.12ab	1.16±0.03bcde	3.35±0.01bcde	14.32±0.33cde	13.62±0.17ab
	5	15.51±0.08abcd	1.01±0.02ef	3.17±0.01ef	14.69±0.25bcde	13.64±0.08ab
3	10	15.42±0.12abcde	1.43±0.04ab	3.19±0.02ab	11.50±0.33ef	12.56±0.13bcd
	7.5	15.84±0.08abc	1.19±0.03bcde	3.29±0.01bcde	14.14±0.39cde	13.46±0.12abc
	5	15.84±0.08abc	1.09±0.02def	3.42±0.01def	14.93±0.26bcde	13.24±0.11ab
4	10	16.02±0.13ab	1.31±0.03bcd	3.18±0.01bcd	12.88±0.36def	11.66±0.17abc
	7.5	14.71±0.11def	1.01±0.02ef	3.53±0.01ef	15.19±0.31bcd	12.71±0.12bcd
	5	16.20±0.10a	0.87±0.01f	3.56±0.01f	19.03±0.34a	12.74±0.40bcd
5	7.5	14.12±0.11fg	0.94±0.02ef	3.50±0.01ef	15.63±0.36bcd	12.23±0.12bc
	5	15.07±0.09bcdef	1.11±0.02cdf	3.49±0.01cdef	14.07±0.33cde	12.85±0.12bcd
<i>p</i> -value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test. TSS- total soluble solids; TA- titratable acidity. Storage trials for fruit stored at 21°C and 10°C were discontinued after 1 month and 4 months, respectively, due to complete fruit loss to decay.

Table 4

Puncture resistance for 'Wonderful' fruit stored at 21°C (65% RH), 10°C (92% RH), 7.5°C (92% RH) and 5°C (92% RH) for 5 months

Duration (month)	Temperature (°C)	Force (N)
Initial		127.94±1.19ab
1	10	138.64±1.29a
	7.5	133.52±1.45a
	5	130.29±1.36ab
2	10	126.83±0.98ab
	7.5	122.66±1.01abc
	5	114.00±1.29bcd
3	10	122.00±1.19abc
	7.5	112.03±1.54bcd
	5	109.36±0.77cde
4	10	97.50±1.13def
	7.5	104.38±1.28def
	5	95.69±0.97ef
5	7.5	97.50±1.13def
	5	86.25±1.08f
<i>p</i> -value		< 0.0001

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test. Storage trials for fruit stored at 21°C and 10°C were discontinued after 1 month and 4 months, respectively, due to complete fruit loss to decay.

Table 5

Fruit compression property for 'Wonderful' fruit stored at 21°C (65% RH), 10°C (92% RH), 7.5°C (92% RH) and 5°C (92% RH) for 5 months

Duration (month)	Temperature (°C)	Elastic Modulus (N/mm)	Firmness (N)	Toughness (N mm)	Bioyield (N)
Initial		355.77±12.18a	295.24±2.28a	1236.12±15.28a	32.81±0.52a
1	10	315.91±10.67a	250.39±2.57bc	1111.68±12.39ab	26.34±0.26bc
	7.5	254.22±12.71a	233.65±12.52bcd	1042.40±55.58bc	23.55±1.32bcdef
	5	221.95±9.57a	231.15±2.27cd	977.76±11.33bcd	23.26±0.23bcdef
2	10	277.42±9.49a	255.99±2.05ab	1065.65±10.69ab	27.66±0.30b
	7.5	231.85±12.30a	211.18±3.93de	825.27±20.41d	21.68±0.31cdef
	5	252.41±12.21a	204.47±2.21de	834.76±13.10d	21.53±0.31cf
3	10	321.02±15.30a	232.67±2.92bcd	960.79±13.91bcd	24.83±0.45bcde
	7.5	310.25±10.17a	207.46±2.18de	893.18±9.09cd	20.75±0.22def
	5	302.96±10.92a	189.80±2.41ef	795.51±11.63d	19.00±0.24f
4	10	360.39±19.65a	220.89±3.43cde	882.54±13.60cd	24.20±0.53bcdef
	7.5	329.88±12.62a	213.05±2.34de	868.25±9.89cd	23.41±0.49bcdef
	5	308.76±14.42a	200.16±2.66de	837.91±12.03d	20.35±0.26dcdef
5	7.5	379.79±18.70a	228.20±2.66cd	870.93±13.07cd	25.30±0.40bcd
	5	192.68±9.52a	162.64±1.78f	574.07±10.14e	19.42±0.39ef
<i>p</i> -value		0.05463	< 0.0001	< 0.0001	< 0.0001

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test.



Table 6

Fruit cutting test for 'Wonderful' fruit stored at 21°C (65% RH), 10°C (92% RH), 7.5°C (92% RH) and 5°C (92% RH) for 5 months

Duration (month)	Temperature (°C)	Force (N)	Toughness (N mm)
Initial	-	229.56±3.08a	1425.43±29.55abcd
1	10	203.29±2.08abc	1269.95±17.94bcde
	7.5	193.69±2.29abc	1108.50±16.56de
	5	203.82±3.20abc	1535.42±38.89abc
2	10	191.15±2.41bc	1272.48±27.20bcde
	7.5	153.81±2.33abc	909.21±23.27de
	5	194.71±2.63abc	1433.98±26.83abcd
3	10	191.75±1.81bc	1433.98±25.92abcd
	7.5	185.41±2.06bcd	1152.08±15.98cde
	5	190.74±3.05cd	1343.88±36.07bcde
4	10	200.34±2.26d	1113.32±21.10e
	7.5	184.66±1.66bcd	1297.75±21.25bcd
	5	222.30±2.62ab	1709.68±30.14a
5	7.5	198.53±2.93abc	1549.95±30.18ab
	5	201.13±1.56abc	1459.37±35.03abcd
<i>p</i> -value		0.0512	0.00025

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test. Storage trials for fruit stored at 21°C and 10°C were discontinued after 1 month and 4 months, respectively, due to complete fruit loss to decay.

Table 7

Aril compression property of 'Wonderful' fruit stored at 21°C (65% RH), 10°C (92% RH), 7.5°C (92% RH) and 5°C (92% RH) for 5 months

Duration (month)	Temperature (°C)	Elastic Modulus (N/mm)	Hardness (N)	Toughness (N mm)	Bioyield (N)
Initial		4.15±0.10a	127.30±0.97a	157.46±1.51a	18.76±0.14ab
1	10	3.18±0.08bcd	125.80±0.84a	154.82±1.19ab	18.67±0.11ab
	7.5	2.55±0.08def	121.03±0.68abcd	146.45±1.13abcdef	17.66±0.15abc
	5	4.52±0.08a	123.24±0.93abc	147.28±1.10abcdef	18.69±0.13ab
2	10	4.38±0.10a	124.35±0.98ab	156.21±1.15a	18.15±0.13abc
	7.5	2.80±0.08cdf	124.41±0.71ab	148.38±0.84abcde	18.60±0.12ab
	5	3.77±0.10ab	123.62±0.54ab	149.20±0.71abcd	18.10±0.09abc
3	10	2.16±0.08ef	117.26±0.67be	136.23±0.79ef	18.24±0.10ac
	7.5	2.76±0.09df	119.52±0.72ae	143.02±1.12bf	18.22±0.12ac
	5	2.94±0.08bdf	117.26±0.90be	142.22±1.60cf	18.65±0.16ab
4	10	3.07±0.05bde	112.29±0.38e	139.41±0.62df	17.50±0.06ac
	7.5	3.20±0.07bd	112.22±0.79e	135.56±1.10f	16.91±0.14c
	5	4.01±0.10abc	119.93±0.73ab	148.18±1.35abc	18.43±0.13a
5	7.5	2.12±0.03f	113.20±0.29de	140.84±0.52df	17.01±0.06c
	5	2.70±0.59df	115.37±0.20ce	132.01±0.78bf	17.66±0.13bc
<i>p</i> -value		< 0.0001	< 0.0001	< 0.0001	< 0.0001

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test. Storage trials for fruit stored at 21°C and 10°C were discontinued after 1 month and 4 months, respectively, due to complete fruit loss to decay.

# Chapter 5

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**Effects of postharvest storage conditions on  
phytochemical and antioxidant properties of  
pomegranate (cv. Wonderful)**

# EFFECTS OF POSTHARVEST STORAGE CONDITIONS ON PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF POMEGRANATE

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## Summary

This study was conducted to investigate the effects of storage temperature and duration on phytochemical and antioxidant properties of pomegranate fruit (cv. Wonderful). Commercially ripe fruit were stored at  $5\pm 0.7^{\circ}\text{C}$ ,  $7.5\pm 0.3^{\circ}\text{C}$ ,  $10\pm 0.5^{\circ}\text{C}$  with  $92\%\pm 2\%$  relative humidity (RH) and  $21\pm 3^{\circ}\text{C}$  with  $65\pm 6\%$  RH for 5 months. Fresh pomegranate juice was assessed for concentrations of total phenolic compounds (TP), total anthocyanin (Acy) ascorbic acid. The antioxidant property of the fruit juice was tested against 2, 2-diphenyl-1-picryl hydrazyl (DPPH). The investigated parameters differed significantly ( $p < 0.05$ ) at different temperatures and duration. Results showed that TP increased with storage temperatures and duration with the maximum levels measured at  $10^{\circ}\text{C}$  (364.47 mg/100ml) for 3 months storage period. However, the concentration declined thereafter at all storage regimes, the lowest concentration was 196 mg/100ml in fruit stored at  $7.5^{\circ}\text{C}$  for 5 months. Similarly, Acy concentration increased with storage temperatures in the first month, and gradually declined thereafter. Furthermore, ascorbic acid concentration gradually declined with storage period, with the lowest concentration (12.69 mg/100ml) measured at  $7.5^{\circ}\text{C}$  after 5 month storage. For antioxidant activity, storage of fruit at  $5^{\circ}\text{C}$ ,  $7.5^{\circ}\text{C}$ , and  $10^{\circ}\text{C}$  significantly ( $p < 0.05$ ) reduced the radical scavenging activity of juice by more than 56% when stored beyond 2 months. This study highlights the need to consider the effects of different temperatures and duration on health promoting compounds in pomegranate fruit, especially where fruit are stored for long term and primarily used for health-promoting purposes.

## 1. Introduction

Fresh fruit and vegetables play an essential role in human nutrition and health because they contain high concentration of beneficial phytonutrients, dietary fiber and other micro-nutrients (Kader 2002; Opara & Al-Ani, 2010). Pomegranate fruit (*Punica granatum* L.) is known as a highly nutritional fruit, consisting of considerable amount of sugars, vitamins, polysaccharides

and important minerals (Miguel *et al.*, 2010). In addition, the fruit contain several important medicinal ingredients that are beneficial to human health. Such ingredients include several groups of phytochemical compounds, in particular, phenolic compounds which have high correlation with juice antioxidant capacity (Fawole *et al.*, 2012a). Several studies suggest that polyphenolic compounds in pomegranate fruit and derived products may exhibit anti-mutagenic, anti-hypertension, and anti-inflammatory properties (Gil *et al.*, 2000; Lansky & Newman, 2007; Elfalleh *et al.*, 2009; Viuda-Martos *et al.*, 2010; Fawole *et al.*, 2012b).

As a result of the multi-functionality and great nutritional benefit of pomegranate in human diet, global commercial production and consumption of pomegranate fruit have increased remarkably (Fawole & Opara, 2013a). At present, ninety percent of the world's pomegranate productions are in the Northern Hemisphere, India, Iran, USA, Turkey, Spain and Israel being the main producers (CitroGold, 2011, Pomegranate Association of South Africa, 2012). This has consequently spurred a growing export opportunity for countries in the Southern Hemisphere to provide fruit to international markets during the counter season (Fawole & Opara, 2013b). South Africa is one of the major producers of pomegranates in the Southern Hemisphere, competing with countries such as Chile, Argentina and Australia (Brodie, 2009). However, consumption and the availability of pomegranate fruit in the market are largely restricted to the harvesting season due to a high demand and lack of appropriate postharvest handling practices to extend the storage life and maintain fruit quality.

Postharvest handling conditions and practices like storage temperature, relative humidity and packaging could be used to maintain fruit quality for prolong storage (Nanda *et al.*, 2001; Bayram *et al.*, 2009). Scientific assessment effects of storage condition on health beneficial phytochemicals and antioxidant attributes are lacking for South African commercially grown 'Wonderful' cultivar. The objective of this study was to investigate the effects of storage conditions phytochemical and antioxidant properties of pomegranate (cv. Wonderful) grown in South Africa. Such information would be useful in determining the postharvest handling of pomegranate with regards to health benefiting phytochemicals and antioxidant capacity of the fruit.

## 2. Material and Methods

### 2.1. Plant material and storage conditions

Fruit sampling and storage conditions were performed as described in chapter 3, section 2.1.

### 2.2. Preparation of sample

Crude pomegranate juice (1 ml) was mixed with 29 ml of 50% methanol in a centrifuge tube. The mixture was vortexed using a mixer (Model. G560E, Scientific Industries, USA) and sonicated using an ultrasonic bath (Ultrasonic Cleaner DC400H, MRC Ltd. Israel) in cold water for 10 min and then centrifuged at 10000 rpm for 10 min at 4°C in a centrifuge (Eppendorf Model 5810 R, Merck, Hamburg, Germany). The supernatant was collected into clean vials and stored at 4°C for further use.

### 2.3. Phytochemical analysis

#### 2.3.1. Determination of total phenolic concentration

The total phenolic (TP) concentration was determined in triplicate using Folin-Ciocalteu (Folin C) method as described by Makkar *et al.* (2007). TP concentration was measured spectrophotometrically at 750 nm by adding 500 µl of 1N Folin C and 2.5 ml of 2% sodium carbonate to 50 µl fruit juice sample. TP concentration was expressed as mean ± SE (milligrams) gallic acid equivalent (GAE) per 100 ml of crude juice.

#### 2.3.2. Determination of total anthocyanin concentration

Total anthocyanin concentration (Acy) was determined according to Wrolstad (1993) using the pH differential method. In triplicate, juice extract (1 ml) was diluted with 9 ml of pH 1 (potassium chloride, 0.025M). Similarly, juice extract (1 ml) was diluted with 9 ml of pH 4.5 (sodium acetate, 0.4M) buffer in separate vials. Acy was measured spectrophotometrically against 50% blank aqueous methanol at 510 nm and 700 nm. Total Acy was expressed as cyanidin-3-glucoside and was calculated using the following equation:

$$\text{Total anthocyanins (mg } 100L^{-1}) = \frac{A \times MW \times DF \times 100}{\epsilon \times 1} \quad (1)$$

where A = difference in absorbance at pH 1 ( $A_{520} - A_{700}$ ) – pH 4.5 ( $A_{520} - A_{700}$ ); MW (molecular weight) for cyanidin-3-glucoside = 449.2 g mol<sup>-1</sup>; DF = dilution factor; 1 = path-length in cm;  $\epsilon$  = 26900 molar extinction coefficient.

Final results were expressed as mean ± S.E (mg/100 ml) crude juice.

#### 2.4. Quantification of ascorbic acid concentration

Ascorbic acid concentration was determined in triplicate using colorimetric method as described by Barros *et al.* (2007) with some modifications. PJ (1 ml) was diluted with 1% metaphosphoric acid (MPA), vortexed, sonicated for 5 min in cold water and centrifuged at 10000 rpm for 5 min at 4°C. The samples were diluted with 0.0025% 2, 6-dichlorophenolindophenol dye and incubated in a dark environment for 10 min. Ascorbic acid concentration was measured spectrophotometrically at 510 nm. The concentration of ascorbic acid in PJ was quantified using a standard curve of known concentration of L-ascorbic acid (Sigma) and final results expressed as mean  $\pm$  SE (milligrams) ascorbic acid per 100 ml of crude juice.

#### 2.5. Radical-scavenging activity

Radical scavenging activity (RSA) of PJ was determined according to Fawole *et al.* (2012a). PJ was tested against the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). In triplicate, extracted sample (15 $\mu$ l) was diluted with 735  $\mu$ l of 100% methanol followed by the addition of 750  $\mu$ l methanolic DPPH solution under dim light. Samples were incubated in the dark at room temperature for 30 min before the absorbance was measured at 517 nm with a spectrophotometer (Thermo Fisher Scientific, USA). The RSA of PJ was expressed as ascorbic acid mg per 100ml of crude juice.

### 3. Statistical Analysis

Results of all studied variables are presented as mean ( $\pm$ S.E.) in replicates. One-way analysis of variance (ANOVA) was performed using SPSS software (version 10, SPSS Inc. Chicago, USA).

### 4. Results and Discussion

Fruit stored at 21°C and 10°C were discarded after 1 and 4 months of storage, respectively due to complete fruit loss to decay.

#### 4.1. Total phenolic content

Total phenolic (TP) concentration increased in fruit stored at all storage regimes in the first month of storage, however there were no significant differences among the cold storage

temperature Table 1. After 2 months, there were further significant ( $p < 0.05$ ) increases in TP in fruit stored at 5°C, 7.5°C, and 10°C. The increase may be related to the continued accumulation of anthocyanins at lower temperatures (Fawole & Opara, 2013a). Furthermore, in the second month of storage, TP concentrations showed a significant ( $p < 0.05$ ) increasing trend with increase in temperatures, the highest phenolic concentration being 364 mg/100 ml at 10°C in the third month. Storage of fruit for 4 months resulted in decline in TP concentration at all storage regimes with fruit stored at 7.5°C having the lowest concentration (196 mg/100 ml) after 5 months. In addition, storage of fruit at 5°C for 4 months or longer resulted to higher TP concentration as compared to 7.5°C (Table. 1). Fawole & Opara (2013a) reported that a decline in total phenolic concentration in pomegranate fruit may be related to the breakdown of phenolic compounds as result in enzymatic activity occurring during storage.

Our findings are in agreement with the report by Labbe *et al.* (2010) where increase in TP compounds was observed in ‘Chilean Chaca’ cultivar at 5°C for 12 weeks. Contrary to our findings, Sayyari *et al.* (2011) reported a decrease in TP concentration after harvest period for untreated ‘Mollar de Elche’ stored at 2°C for 84 days.

#### 4.2. Total anthocyanin concentration

Anthocyanin compounds are responsible for the characteristic red colouration in pomegranate fruit peel and juice (Gil *et al.*, 1996; Artés *et al.*, 1998). Total anthocyanin concentration increased in fruit at all storage regimes in comparison to the concentration at harvest (73.64 mg/100 ml) (Table. 1). The increase in anthocyanin concentration at all the investigated storage temperatures may be attributed to increase in biosynthesis and accumulation of anthocyanin, which is known to be induced in pomegranates at lower temperatures (Miguel *et al.*, 2004). Fruit kept at 21°C had the highest anthocyanin concentration (139.52 mg/100 ml) than those stored at 5°C, 7.5°C, and 10°C after 1 month (Table 1). Further increases in anthocyanin concentrations were measured in fruit stored at 10°C, 7.5°C and 5°C until 3 months before the concentration started to decline. Our findings are in agreement with those reported by Fawole & Opara (2013a), who increase in total anthocyanin concentration in ‘Bhagwa’ and ‘Ruby’ stored at 5°C, 7.5°C and 10°C for 16 weeks. However, the report by Artés *et al.* (2000) was on the contrary, the author observed no change in anthocyanin concentration for ‘Mollar de Elche’ pomegranate between harvest and shelf life period after 12 weeks of storage.



#### 4.3. Radical scavenging activity

Several studies have linked regular consumption of pomegranate fruit to high levels of antioxidant activity, primarily as a result of bioactive phenolic compounds in the juice (Gil *et al.*, 2000; Kim *et al.*, 2002; Viuda-Martos *et al.*, 2010). In this study PJ extract was tested against radical scavenging activity (RSA) at different storage temperatures (Table 2). There were significant ( $p < 0.0001$ ) differences in RSA values among storage temperatures and duration. In comparison to fruit at harvest (146 mg/100ml), fruit RSA values declined at all storage regimes with storage time. In addition, between 2 and 3 months of storage, RSA declined by over 56% in fruit stored at 5°C, 7.5°C and 10°C. Furthermore, RSA levels in fruit stored at 7.5°C declined significantly ( $p < 0.05$ ) to the lowest levels (78%) after 5 months of storage. Our results are in agreement with those reported by Fawole & Opara (2013b) for Ruby cultivar stored at 5°C, 7°C and 10°C for 16 weeks. However, the observed trend for total phenolic content did not corresponded with the trends observed for antioxidant activity displayed by the fruit during postharvest storage, suggesting that antioxidants may react in different ways depending on the type of antioxidant assays (Çam *et al.*, 2009).

#### 4.4. Ascorbic acid

Ascorbic acid concentration declined at all the investigated storage temperatures when compared with the concentration at harvest (16.82 mg/100 ml), however, the decline was not significant ( $p > 0.05$ ) amongst the storage temperatures in the first month (Table 2). In addition, ascorbic acid concentration decreased with prolonged storage period, with significant ( $p < 0.05$ ) decline at 10°C (16.61 mg/100 ml) and 5°C (16.68 mg/100 ml) after 3 months. Ascorbic acid concentration was at the lowest at 5°C (13.97 mg/ 100 ml) and 7.5°C (12.96 mg/100 ml) after 5 months of storage. The decrease in ascorbic acid during postharvest storage may be related to the irreversible oxidation of dehydro-L-ascorbic acid (DHAA) to 2,3-diketo-L-gulonic acid (Coulate, 2007). Similarly, Al-Mughrabi *et al.* (1995) reported gradual decrease in ascorbic acid concentration for ‘Taeifi’, ‘Banati’, ‘Manfaloti’ pomegranates stored at 5°C, 10°C and 22°C for 8 weeks with no significant changes in ascorbic acid amongst storage temperatures. However, on the contrary, Miguel *et al.* (2006) observed a significant increase in ascorbic acid concentration in ‘Mollar de Elche’ and ‘Assaria’ fruit stored in the dark at 5°C for 4 months. Decline in

ascorbic acid may be an indication of less antioxidant properties in the investigated pomegranate juice.

## 5. Conclusions

Significant differences in total phenolic, total anthocyanin, free radical scavenging activity and ascorbic acid concentrations were found in 'Wonderful' pomegranate at different storage temperatures. Fruit total phenolic concentration increased with storage temperature and duration, resulting in the highest phenolic concentration of 364 mg/100 ml at 10°C for 3 months. Reduction in phenolic concentration was observed after the third month with the lowest concentration measured at 7.5°C of 196mg/100 ml for 5 months. In addition, total anthocyanin concentration increased after 1 month storage period and thereafter a gradual decrease beyond 3 month storage period was observed. Furthermore, fruit radical scavenging activity was severely affected by storage duration, resulting in over 50% reduction in its scavenging activity after 3 month of storage period. Ascorbic acid concentration was affected at by storage duration with the highest loss being at 7.5°C after 5 months, indicating a significant loss in nutritional quality. This study highlights effects of different temperatures and duration on health promoting compounds in pomegranate fruit. These findings are beneficial towards optimal fruit quality management in the pomegranate export industry, especially where fruit are stored for long term and primarily used for health-promoting purposes.

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Table 1

Total phenolic concentration (gallic acid equivalent) and Total anthocyanin concentration (cyanidin-3-glucoside equivalent) for pomegranate fruit juice during stored at 21°C, 10°C, 7.5°C and 5°C.

Duration (month)	Temperature (°C)	Total phenolic concentration	Total anthocyanin concentration
		Gallic acid (mg/100ml)	cyaniding-3-glucoside (mg/100ml)
Initial	-	226.27±2.82fg	73.64±1.95g
1	RT	246.07±2.40fg	139.52±0.96b
	10	238.97±2.10fg	107.46±2.65cdef
	7.5	228.87±2.02fg	99.49±2.54cdef
	5	230.52±1.45fg	101.83±3.08cdef
2	10	287.96±1.91de	162.80±9.75a
	7.5	299.13±3.39bcd	114.45±2.19bcde
	5	228.90±1.60fg	134.38±1.52b
3	10	364.47±5.52a	127.52±1.37bc
	7.5	292.69±5.23bcd	120.85±3.29bcd
	5	264.04±4.06ef	87.67±3.63fg
4	10	332.50±3.46b	104.60±3.14cdef
	7.5	269.73±2.87de	82.74±1.19fg
	5	312.53±8.11bc	108.51±0.92cdef
5	7.5	196.00±3.12g	94.32±1.38defg
	5	266.65±3.15de	96.84±1.31efg
<i>p</i> -value		< 0.0001	< 0.0001

Data points each represent the mean of 6 replicates, vertical bars denotes the standard error (S.E.). Different letter(s) indicate significant difference ( $p < 0.0001$ ) according to Duncan's multiple range test (a - g). Experimental trials for fruit stored at 21°C and 10°C were discontinued after 1 and 4 months respectively, due to complete fruit loss.

Table 2

Radical scavenging activity (RSA) (ascorbic acid mg/100ml) and ascorbic acid concentrations (ascorbic acid mg/ 100ml) for pomegranate fruit juice during stored at 21°C, 10°C, 7.5°C and 5°C.

Duration (month)	Temperature (°C)	Radical scavenging activity	Ascorbic acid concentration
		Ascorbic acid (mg/100ml)	Ascorbic acid (mg/100ml)
Initial	-	146.33±2.61a	16.82±0.03a
1	RT	113.36±5.20d	16.14±0.04ab
	10	121.15±3.66cd	16.56±0.03ab
	7.5	120.62±2.14cd	16.67±0.04a
	5	124.06±2.38bcd	16.77±0.04ab
2	10	142.71±2.50ab	16.59±0.07ab
	7.5	142.09±1.12abc	16.23±0.03bc
	5	133.01±3.03abcd	16.42±0.02ab
3	10	63.11±3.29e	16.62±0.03bc
	7.5	61.70±1.12e	16.58±0.05c
	5	50.75±0.65ef	16.68±0.01ab
4	10	46.81±1.60ef	15.79±0.03d
	7.5	35.94±2.87f	16.25±0.05c
	5	47.59±1.66ef	16.24±0.01c
5	7.5	31.45±3.51f	12.69±0.03f
	5	49.58±3.38ef	13.97±0.06e
<i>p</i> -value		< 0.0001	< 0.005

Data points each represent the mean of 6 replicates, vertical bars denotes the standard error (S.E.). Different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test (a - f). Experimental trials for fruit stored at 21°C and 10°C were discontinued after 1 and 4 months respectively, due to complete fruit loss.



# Chapter 6

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**Discrimination of pomegranate (cv. Wonderful) fruit  
quality by instrumental and sensory measurements  
during storage at three temperature regimes**

## DISCRIMINATION OF POMEGRANATE FRUIT QUALITY BY INSTRUMENTAL AND SENSORY MEASUREMENTS DURING STORAGE AT THREE TEMPERATURE REGIMES

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### Summary

Discrimination of ‘Wonderful’ pomegranate fruit quality was carried out by simulating storage duration at different temperature regimes. Commercial harvested fruit were stored at 5°C, 7.5°C, and 10°C with 92% RH for 4 months in order to determine the suitable storage conditions based on the combination of instrumental measurements and sensory attributes. Instrumental measurements such as aril colour, total soluble solids (TSS), titratable acidity (TA), TSS: TA, juice content as well as phytochemical components including total phenolics and anthocyanins were measured. In addition, aril textural properties such as Young’s modulus of elasticity, aril hardness, toughness and bioyield point were investigated. During storage, individual fruit were evaluated by trained sensory panel based on the overall appearance, taste and aril texture. Discriminant analysis was used to determine the relationship between the instrumental and sensory descriptive attributes for storage temperatures and duration. Overall pomegranate flavor, total anthocyanin, juice content and C\* were the most influential attributes distinguishing storage temperatures at 2 months. Discriminant analysis showed that storage time rather than storage temperature led to the reduction in overall quality when storing fruit beyond 2 months. Based on sensory attributes suitable storage temperature and duration was at 5°C for 2 months when overall flavor was highly rated. Furthermore, the proposed storage conditions was supported when instrumental measurements, which showed a reduction in overall quality parameters (total phenolics, total anthocyanin, aril colour and aril texture) after 2 months of storage. The proposed temperature and storage duration could be used as a guideline to establish reliable postharvest handling protocols for the investigated cultivar.

### 1. Introduction

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family; it’s a tropical and subtropical deciduous or evergreen shrub capable of growing in different soil types and climatic conditions (Sepúlveda *et al.*, 2000). The fruit is highly valued due to its exceptional and unique

sensory and nutritional properties (Lopez-Rubira *et al.*, 2005). The edible portion (aril) can be consumed fresh or used for the preparation of fresh juice, canned beverages, jelly, jam, and paste and also for flavoring and coloring beverage products (Lansky & Newman, 2007; Holland *et al.*, 2009; Opara *et al.*, 2009). Due to the global awareness of the potential health benefits derived from consumption of pomegranate fruit there has been a considerably increase in commercial farming of pomegranate fruit worldwide (Holland *et al.*, 2009). Nearly 90 percent of global commercial production of pomegranate fruit is produced in the Northern Hemisphere. The main producers are India, Iran, USA, Turkey, Spain and Israel (Citrogold, 2011; Pomegranate Association of South Africa, 2012). During the counter season, countries in the Southern Hemisphere provide fruit to the international markets. South Africa, being one of the main producers in the Southern Hemisphere competes with several other countries including Chile, Peru, and Argentina (Fawole & Opara, 2013a). Currently, South Africa's commercial production of pomegranate fruit stands at 198,000 tons/440,000 cartons, which is a dramatic increase from 2009/2010 exporting season of 315 tons/70,000 cartons (Brodie, 2009; Perishable Products Export Control Board, 2012).

To provide good quality fruit to consumers, particularly for the long supply chain, postharvest handling and management practices like storage temperature and duration should be optimal. During transportation and storage of pomegranate fruit, a number of physiological, biochemical and textural processes occur, which result in changes in colour, taste, texture, and ultimately decline in nutritional quality and sensory attributes. Hence, the choice of storage temperature and duration should consider the delivery of harvested fruit to consumers in the most excellent condition for desirable organoleptic, nutritional, and antioxidant attributes (Kader, 2008; Fawole & Opara, 2013a). The objective of this study was to determine suitable storage temperature and duration for optimal postharvest storage performance of pomegranate fruit (*cv.* Wonderful) based on combination of instrumental measurements and sensory attributes.

## **2. Materials and Methods**

### *2.1. Fruit sampling and storage conditions*

Fruit were packed according to industry practice were fruit samples was placed in open carton boxes with the following dimensions: width 0.3 m, length 0.4 m, height 0.133 m and a

total of 22 perforations. A total of 144 fruits were packed in boxes, each consisting of 10-12 fruits. The boxes were stacked on pallet and stored at  $5\pm 0.7^{\circ}\text{C}$ ,  $7.5\pm 0.3^{\circ}\text{C}$  and  $10\pm 0.5^{\circ}\text{C}$  with  $92\pm 2\%$  RH. Fruit were sampled at 2 months coinciding with the commercial exporting trade, thereafter fruit were sampled at 1 month intervals. Instrumental and sensory measurements were carried out on 8 fruit at each storage temperature for the purpose of correlation and discriminant analysis. Fruit samples were manually peeled and arils were placed into labelled glass beakers. Extracted arils were weighed individually and split into ratio of 3:1, where larger portion (75%) were used for sensory evaluation and smaller portion (25%) used for instrumental analysis. This procedure was followed according to Fawole & Opara (2013a).

## *2.2. Preparation of sample*

Sample extraction for phenolic and anthocyanin analysis was performed as described in chapter 5, section 2.2.

## *2.3. Instrumental measurements*

### *2.3.1. Measurement of total soluble solids, titratable acidity and juice content*

Fruit juice extraction was performed with a Liquafresh juice extractor (Mellerware, South Africa). Juice content (ml) was determined by extracting juice from 100g arils. Total soluble solids (TSS) and titratable acidity (TA) were performed as described in chapter 4, section 2.4.1.

### *2.3.2. Colour attributes*

Aril colour change was measured using the CIE  $L^*$ ,  $a^*$ ,  $b^*$  coordinates with a calibrated Minolta Chroma Meter (Model CR-400/410 (Minolta Corp, Osaka, Japan) as described in chapter 4 section 2.3.4.

### *2.3.3. Determination of total phenolic concentration*

Total phenolic concentration was determined as described in chapter 5, section 2.1.1. Results were expressed as the mean  $\pm$  SE (milligrams) of gallic acid equivalent (GAE) per 100 ml of crude juice.

#### 2.3.4. Total anthocyanin concentration

Total anthocyanin concentration was quantified as described in chapter 5, section 2.1.2. Total anthocyanin concentration was expressed as mean  $\pm$  S.E (milligrams) cyanidin-3-glucoside equivalent per 100 ml of crude juice.

#### 2.3.5. Aril texture dynamics

Aril compression test was performed using texture profile analyzer XT Plus (Stable MicroSystem Ltd., Godalming, UK), with a 35 mm diameter cylindrical compression probe as described in chapter 4, section 2.5.4.

### 2.4. Sensory measurements

#### 2.4.1. Training panel

Panel members were trained using consensus method as described by Koch *et al.* (2012). The eight panel members (1 male and 7 female) were sensory analysts. Each panel member received a range of arils from various storage conditions. Descriptive and scores were adopted from Fawole & Opara (2013a) with few modifications, where each panelist was required to assess sensory attributes (appearance, flavour, and texture). A score sheet was developed which included a scale for each attribute, ranging from 0 mm (lowest level) corresponding to the word “none” to 100 mm (highest level) corresponding to the word “prominent”. Two training sessions were performed before each sensory evaluation of pomegranate arils.

#### 2.4.2. Sensory evaluation

Sensory evaluation was carried out at ambient temperature (21°C) in Sensory Laboratory, Department of Food Science, Stellenbosch University, at the same time for all three storage temperatures. Evaluation of pomegranate arils were performed at individual booths, each booth was equipped, server windows and adequate light. Sensory assessment was recorded using Compusense<sup>®</sup> five sensory data program (Guelph, Ontario, Canada) where pomegranate arils ( $\pm 20$  g) for the three temperatures were given 3 random digit codes for each storage temperature and placed in a clean labelled Petri-dishes (Fawole & Opara, 2013a).

### 3. Statistical Analysis

All instrumental and sensory measurements were performed on 8 individual fruit for each storage temperature. Statistical analysis was carried out using Statistica software (Statistica version 10, StatSoft Inc., Tulsa, USA). Sensory data was assessed using Panel Check Software (Version 1.3.2, [www.panelcheck.com](http://www.panelcheck.com)). To distinguish if the three temperatures were distinctly dissimilar from each other, Discriminant analysis (DA) was done using (XLStat, version 7.5.2, Addinsoft, New York, USA) using both instrumental measurements and sensory attributes.

### 4. Results and Discussion

#### 4.1. Changes in instrumental measurements during storage

##### 4.1.1. Total soluble solids (TSS), titratable acidity (TA), TSS:TA ratio and juice content

Total soluble solids (TSS), titratable acidity (TA) and TSS:TA were significantly influenced by temperature and storage duration. After 2 months no significant increases in TSS were observed at 5°C and 7.5°C, however after 2 months, there were further significant ( $p < 0.0001$ ) increases in TSS in fruit stored at 5°C, 7.5°C, and 10°C with the highest TSS values (16.1 °Brix) observed at 5°C after 4 months (Table 1). The observed changes in TSS values during storage are in agreement with Ghafir *et al.* (2010), who reported significant increase in TSS for ‘Shlefy’ when stored at 5°C and 7°C for 4 months. A decreasing trend for titratable acidity (TA) levels was observed at 5°C and 7.5°C with prolong storage, whereas TA levels first increased significantly ( $p < 0.00018$ ) between harvest (1.38) and 2 months (1.48) at 10°C and declined afterwards until 4 month (1.10) storage period (Table 1). This study shows that fruit acidity levels would decrease during shipping and handling period when fruit are stored at temperatures below 10°C. The decline in acidity levels during storage may be attributed to the breakdown in organic acids required to maintain ongoing metabolism in the fruit during storage (Fawole & Opara, 2013a). Our findings are in agreement with Fawole & Opara (2013b), where significant decrease in TA was reported for two South African grown pomegranates (‘Bhagwa’ and ‘Ruby’) at 5°C, 7°C and 10°C for 4 months.

As a result of the changes in TSS and TA contents during storage, TSS:TA ratios significantly ( $p < 0.0001$ ) increased with prolong storage period (Table 1). The highest TSS:TA ratios were observed at 5°C (20.28) and 7.5°C (20.09) for 4 months of storage, with no

significant differences observed between the two temperatures (Table 1). These results agree with Fawole & Opara (2013b), who reported significant increase in TSS:TA ratio at 5°C, 7°C and 10°C for 4 months in ‘Bhagwa’ and ‘Ruby’. Although, not statistically significant ( $p > 0.05$ ) juice content decreased at all storage temperatures in comparison to fruit at harvest (66.80ml/100g).

#### 4.1.2. Total phenolic and anthocyanin concentration

In general, significant changes were observed in total phenolic (TPC) and anthocyanin concentration (Acy) between harvest and storage period (Table 2). The TPC were significantly higher at harvest (223.27 mg/ 100ml) and declined throughout storage at all storage temperatures with the exception of 5°C (236.14) for 3 months. In addition, Acy increased significantly ( $p < 0.0001$ ) throughout storage, with the highest concentration at 10°C for 3 months, before declining afterwards. However, no significant changes were observed at 5°C (87.24 mg/ 100ml) and 7.5°C (85.46 mg/ 100ml) for 4 month storage. The observed increase may be attributed to the biosynthesis and accumulation of anthocyanin, which is known to be induced in pomegranates at lower temperatures (Miguel *et al.*, 2004). These results agree with those reported by Fawole & Opara (2013b) where increase in juice total anthocyanin concentration was observed between harvest and after 4 months of storage at 5°C, 7°C and 10°C for ‘Bhagwa’ and ‘Ruby’.

#### 4.1.3. Aril colour dynamics

Significant ( $p < 0.05$ ) variation in aril colour property was observed when measured CIE  $L^*$ ,  $a^*$ ,  $C^*$  and  $h^\circ$  values at (Table 3). Significant ( $p = 0.0102$ ) changes in aril redness ( $a^*$ ) were only observed at 7.5°C (17.82) and 10°C (21.24) for 2 months, this suggest that aril redness were no longer influenced by temperature. Likewise, colour intensity ( $C^*$ ) also differed significantly ( $p = 0.0055$ ) after 2 months, with no significant differences found between storage temperatures. However, slight significant variation was observed at 5°C for 4 months. In addition,  $h^\circ$  values showed variation during storage, with a significant ( $p = 0.0017$ ) declined perceived after 2 months. These observations are in agreement with Artés *et al.* (1998), who reported no significant differences in aril color for ‘Mollar de Elche’ pomegranates stored at 5°C for 80 days of storage. Our findings indicate that pomegranate arils showed higher red colouration when stored at 5°C for 2 months.

#### 4.1.4. Aril texture properties

Aril hardness showed no significant difference among temperatures and storage period, but rather a decline in aril hardness was observed throughout storage (Table 4). In addition, aril toughness declined during storage showing significant variation among temperatures. Similarly, arils displayed slight variation in bioyield, however no significant differences ( $p = 0.062$ ) was observed for majority of storage temperatures. Significant ( $p < 0.05$ ) changes were observed for Young's modulus of elasticity during storage, although storage of fruit for 3 months showed no significant changes in Young's modulus at 5°C, 7.5°C and 10°C. Moreover, a gradual decrease in young's modulus was observed at harvest (4.15 N/mm) and continued to decline throughout storage. These results are similar to those reported by Labbe *et al.* (2010), where loss in firmness was reported for fruit arils stored at 5°C for 8 weeks. Overall, decrease in textural property for the investigated cultivar shows that softening of arils occur with prolong storage. This is an indication that fruit arils may increase in deformability with increasing temperature and storage duration which could be attributed to cell membrane degradation due to higher temperatures and prolong storage (Bchir *et al.*, 2012).

#### 4.2. Sensory attributes during cold storage

There were significant ( $p < 0.05$ ) differences observed among sensory attributes (Fig 2). Fruit sensory attributes such as overall appearance, sweet taste, sour taste, overall flavor, crispness and crunchiness were noticeably distinguished at different temperature regimes and storage periods. Overall appearance was higher at 2 months of storage (Fig 2), with significant ( $p < 0.05$ ) decrease in visual appearance observed beyond 3 months of storage (Fig. 3 and Fig.4). In addition, there were significant decreases in aril and kernel texture. Aril texture indicated reduction in juice content, crispness and crunchiness as storage period progressed. Furthermore, kernel texture intensity was reduced at all temperatures and with increasing storage period. The decrease in aril texture may be attributed to loss in cell wall integrity of pomegranate arils (Ekrami-Rad *et al.*, 2011). The most significant differences among the temperatures and storage duration were found in sweet taste and sour taste (Fig. 1 and Fig. 3). Overall, fruit stored at 10°C for 2 months showed at low intensity (32.83) in sweet taste and high intensity (58.24) of sour taste (on a scale from 0 - 100) compared to 7.5°C (sweet taste = 47.82 and sour taste = 42.19) and 5°C (sweet taste = 49.36 and sour taste = 38.67).



Furthermore, the values decreased significantly ( $p < 0.05$ ) with storage period, which is an indication of breakdown of sugars and organic acids required for the ongoing metabolism in the fruit during storage (Fawole & Opara 2013b). Several sensory attributes such as astringency, alcoholic taste, bitterness and off-flavour were rated extremely low among the temperature regimes and storage durations.

#### 4.3. Discriminant analysis (DA)

After two months of storage, ten quality attributes showed good correlation (values  $> 0.5$ ) as shown in Table 5. The stepwise model revealed four attributes (overall pomegranate flavor, total anthocyanin, juice content and C\*) showing significant contribution to the separation of the temperature regimes. Pomegranate flavor showed the highest importance with partial regression ( $R^2$ ) of 0.564 at  $p < 0.0001$  (Table 6). Total anthocyanin and C\* described the differences between fruit stored at 5°C and those stored at 7.5°C and 10°C along F1, while F2 separated fruit stored at 7.5°C from stored at 10°C fruit with overall pomegranate flavour (Fig. 4). The DA model separated fruit into three classes with 95.83% prediction. The observed confusion was between fruit stored at 10°C and 7.5°C (Fig. 4). This gives a clear indication that fruit stored up to 2 months at the investigated storage temperature could clearly be differentiated from each other using unique attributes to distinguish them.

After three month storage duration, seven quality attributes showed correlation values  $> 0.5$  (Table 5). Three quality attributes (total anthocyanin, titratable acidity and crunchiness) contributed significantly to the separation of the storage temperatures (Table 6). Total anthocyanin contributed the most with partial regression ( $R^2$ ) of 0.472 ( $p < 0.01$ ). Total anthocyanin and titratable acidity described the difference between fruit stored at 10°C and those from stored at 7.5°C and 5°C along F1 and F2 region (Fig. 5). Furthermore, F1 separated fruit stored at 5°C from those stored at 7.5°C fruit using the crunchiness attribute. The DA model only separated fruit into three classes with 70.83% prediction (Fig. 5). The observed confusion was between fruit stored at 7.5°C, 5°C and 10°C. These results suggest that fruit stored up to three months could not clearly be distinguished among the investigated storages. In addition, storage duration resulted in reduction in overall fruit quality.

After four month storage duration, seven fruit quality attributes showed correlation values  $> 0.5$  (Table 5), where sweet taste and  $h^{\circ}$  values gave significant contribution to separation of

storage temperatures (Table 6). Sweet taste gave a partial regression ( $R^2$ ) of 0.560 ( $p < 0.0001$ ) compared to  $h^\circ$  0.430 ( $p < 0.04$ ), suggesting that the major contributor to separation of fruit after 4 months storage is sweet taste. Sweet taste and  $h^\circ$  described the differences between fruit stored at 5°C from those stored at 7.5°C and 10°C on the F1 and F2 (Fig. 6). Furthermore, the DA model separated the fruit into three classes, with 79.17% prediction and with the observed confusion was between fruit stored at 10°C and 7.5°C. Moreover, the DA model correctly predicted 100% of fruit stored at 5°C, where fruit stored at 7.5°C and 10°C could not be distinguished. This indicates that storage of fruit for up till 4 months at 7.5°C and 10°C were not affected by temperature but rather by storage duration. Overall, fruit quality is reduced mainly by extended storage periods rather than storage temperature if fruit are stored beyond 2 months.

## 5. Conclusions

This study showed that overall fruit quality was primarily influenced by temperature and storage duration as demonstrated by the instrumental quality parameters. However, postharvest storage temperature played a significant role in several quality parameters (TSS, TA, total anthocyanin) during 2 month storage life. Overall quality of fruit was reduced with prolonged storage as evident in both sensory and instrumental attributes. Discriminant analysis showed that fruit stored for 2 months could be distinguished from each other based on quality attributes such as overall pomegranate flavor, total anthocyanin and  $C^*$ . For desirable sensory attributes, optimum storage temperature and duration were found to be 5°C and 2 months, when overall flavor and overall appearance values were highly rated. Likewise, according to the instrumental measurements, fruit could be successfully stored for 2 months without significant reduction in health-benefitting and colour parameters (total phenolics, total anthocyanin and aril colour). Furthermore, prolonged storage led to a reduction in overall quality and may cause development of undesirable off flavours if fruits are stored longer than 2 months. The storage conditions proposed in the current study for the investigated cultivar could be used as a guideline to establish suitable postharvest handling and storage conditions to ensure best fruit quality attributes for long supply chains.

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Table 1

Chemical attributes of 'Wonderful' fruit stored at 10°C, 7.5°C and 5°C with 92±2% RH for 4 months

Duration (month)	Temperature (°C)	TSS	TA	TSS:TA	Juice content (ml)100g arils
Initial	-	13.38±0.11d	1.37±0.04ab	10.59±0.33d	66.80±0.51a
2	10	15.18±0.11ac	1.48±0.07a	11.46±0.47cd	61.77±0.45b
	7.5	14.7625±0.10c	1.22±0.03abc	12.56±0.32bcd	62.670.44b
	5	15.10±0.15bc	1.10±0.03bcd	14.13±0.31bcd	60.05±0.59b
3	10	15.19±0.10abc	1.39±0.04ab	11.62±0.42cd	59.47±0.34b
	7.5	14.9±0.10bc	0.96±0.02cd	16.06±0.39b	61.05±.35b
	5	15.88±0.10ab	1.05±0.03bcd	15.93±0.56b	60.78±0.54b
4	10	14.84±0.12c	1.10±0.05bcd	14.94±0.58bc	59.67±0.26b
	7.5	15.61±0.08abc	0.83±0.03d	20.09±0.69a	58.87±0.65b
	5	16.1±0.08a	0.82±0.02d	20.28±0.53a	62.74±0.36b
<i>p</i> -value		<0.0001	0.00018	<0.0001	0.064

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test. TSS- total soluble solids; TA- titratable acidity.

Table 2

Total phenolic concentration (gallic acid equivalent) and total anthocyanin concentration (cyanidin-3-glucoside equivalent) of 'Wonderful' fruit stored at 10°C, 7.5°C and 5°C with 92±2% RH for 4 months

Duration (month)	Temperature (°C)	Phytochemical components	
		Total phenolics (mg/ 100 ml)	Total anthocyanins (mg/ 100 ml)
Initial	-	226.27±2.82ab	73.64±1.95d
2	10	213.03±4.09abc	108.62±3.06abc
	7.5	213.28±5.22abc	118.24±2.10ab
	5	187.36±5.25bcd	98.20±3.04bc
3	10	164.36±2.58d	123.87±1.37a
	7.5	171.59±3.21cd	118.06±3.29ab
	5	236.14±10.82a	89.11±3.63cd
4	10	187.59±3.10bcd	116.17±4.60ab
	7.5	167.03±2.78d	85.46±6.71cd
	5	175.73±4.39cd	87.24±5.54cd
<i>p</i> -value		0.0014	<0.0001

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test.

Table 3

Arl colour dynamics of 'Wonderful' fruit stored at 10°C, 7.5°C and 5°C with 92±2% RH for 4 months

Duration (month)	Temperature (°C)	L*	C*	h°	a*
Initial					
	-	25.83±0.28ab	26.68±0.23a	25.90±0.10ab	23.94±0.21a
2					
	10	25.30±0.31ab	22.95±0.32abc	21.83±0.25bc	21.24±0.30ab
	7.5	24.44±0.52ab	20.13±0.22c	25.77±0.50ab	17.82±0.22b
	5	30.59±0.45a	26.40±0.27ab	23.55±0.27abc	24.04±0.25a
3					
	10	20.10±0.40bc	20.98±0.25bc	22.01±0.29abc	19.26±0.22ab
	7.5	22.80±0.42b	21.2±0.22abc	21.04±0.27c	19.61±0.21ab
	5	24.27±0.45ab	22.43±0.23abc	21.07±0.29c	20.80±0.21ab
4					
	10	18.79±0.38bc	23.94±0.22abc	25.89±0.20ab	21.87±0.18ab
	7.5	19.89±0.37bc	21.94±0.23abc	26.21±0.26a	19.57±0.21ab
	5	14.95±0.27c	20.82±0.21c	22.06±0.15abc	19.25±0.19ab
<i>p</i> -value		0.00037	0.0055	0.0017	0.01022

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test.



Table 4

Aril compression property of 'Wonderful' fruit stored at 10°C, 7.5°C and 5°C with 92±2% RH for 4 months

Duration (month)	Temperature (°C)	Elastic Modulus (N/mm)	Hardness (N)	Toughness (N mm)	Bioyield (N)
Initial	-	4.15±0.10a	127.30±0.97a	157.46±1.51ab	18.76±0.14a
2	10	4.10±0.12ab	120.13±1.94ab	121.23±1.58d	18.11±0.17b
	7.5	3.10±0.16bcd	118.64±1.80ab	146.70±3.23abc	17.78±0.19b
	5	4.13±0.12ab	124.02±1.70ab	148.34±2.40abc	18.44±0.23ab
3	10	4.03±0.12abc	121.23±1.05ab	155.94±1.95ab	17.04±0.14b
	7.5	3.56±0.07abc	117.87±0.96ab	148.08±2.04abc	17.36±0.10b
	5	4.04±0.15abc	124.91±1.74ab	163.95±3.19a	18.3±0.06ab
4	10	3.80±0.07abcd	113.40±1.14b	134.46±1.61bd	17.99±0.29ab
	7.5	3.35±0.22cd	118.89±2.20ab	126.02±4.42ab	19.62±0.17ab
	5	2.77±0.10d	112.14±0.88b	131.42±1.35cd	17.15±0.15b
<i>p</i> -value		0.0061	0.274	0.001	0.06169

Mean ± S.E. presented. Mean values in the same column followed by different letter(s) indicate significant difference ( $p < 0.05$ ) according to Duncan's multiple range test.

Table 5

Variables and factors (F1 and F2) correlations of discriminant analysis for the sensory and instrumental data

Attributes	2 months		3 months		4 months	
	F1	F2	F1	F2	F1	F2
Aril colour	0.335	0.150	-0.147	0.041	<b>0.514</b>	-0.044
Overall appearance	0.386	0.158	0.438	0.154	0.247	-0.285
Overall pomegranate flavour	0.189	<b>0.943</b>	0.043	-0.069	0.421	0.045
Sweet taste	0.128	<b>0.865</b>	-0.153	-0.304	<b>0.860</b>	<b>0.510</b>
Sour taste	-0.094	<b>-0.863</b>	0.304	0.235	<b>-0.707</b>	-0.453
Alcohol	-0.187	-0.016	<b>-0.511</b>	-0.106	0.140	0.080
Astringency	-0.218	<b>-0.787</b>	0.185	0.110	<b>-0.662</b>	<b>-0.510</b>
Bitterness	-0.219	<b>-0.664</b>	0.208	-0.113	-0.263	-0.381
Off-Flavours	-0.344	0.153	<b>-0.660</b>	-0.196	0.239	0.192
Crispness	-0.085	0.157	<b>0.766</b>	0.161	-0.405	-0.158
Crunchiness	-0.073	0.233	<b>0.832</b>	0.018	<b>-0.554</b>	-0.078
Juiciness	0.089	<b>0.645</b>	-0.287	-0.090	0.448	0.353
Grittiness	-0.149	<b>-0.696</b>	0.344	0.267	-0.377	-0.334
Hardness of kernel	-0.127	-0.488	0.402	0.249	-0.301	-0.042
TSS	-0.334	-0.003	0.498	-0.220	0.471	-0.065
TA	0.074	-0.301	<b>-0.679</b>	<b>-0.535</b>	-0.369	-0.301
TSS:TA	-0.250	0.299	<b>0.684</b>	0.404	0.351	0.157
Juice content	0.297	-0.132	0.181	-0.249	0.244	-0.068
Total phenolics	0.152	-0.425	0.427	-0.349	0.015	-0.423
Total anthocyanin	<b>0.552</b>	-0.140	<b>-0.727</b>	<b>0.635</b>	-0.340	-0.277
L*	-0.281	0.256	0.436	-0.023	<b>-0.548</b>	0.086
C*	<b>-0.637</b>	0.189	0.044	0.010	-0.444	-0.277
h°	0.165	-0.056	0.080	0.105	<b>-0.763</b>	<b>0.646</b>
a*	<b>-0.661</b>	0.172	0.045	0.004	-0.374	-0.453
Bioyield	-0.220	-0.338	0.299	-0.389	-0.248	-0.343
Aril hardness	-0.189	-0.050	-0.096	-0.128	-0.081	0.441

Correlations was at  $p < 0.05$ . Values highlighted in bold indicate strong to moderate correlations between variables and their corresponding factors.

Table 6

Summary of variable selection table showing attributes that contribute most to the different temperatures using a stepwise (forward) analysis

2 months

No. of variables	Variable IN/OUT	Status	Partial R <sup>2</sup>	F	Pr > F
1	Overall pomegranate flavour	IN	0.564	13.604	0.000
2	C*	IN	0.374	5.977	0.009
3	Total anthocyanin	IN	0.463	8.200	0.003
4	Juice content	IN	0.331	4.443	0.027

3 months

No. of variables	Variable IN/OUT	Status	Partial R <sup>2</sup>	F	Pr > F
1	Total anthocyanin	IN	0.472	9.399	0.001
2	TA	IN	0.352	5.444	0.013
3	Crunchiness	IN	0.293	3.928	0.037

4 months

No. of variables	Variable IN/OUT	Status	Partial R <sup>2</sup>	F	Pr > F
1	Sweet taste	IN	0.560	13.343	0.000
2	h°	IN	0.430	7.532	0.004

Partial R<sup>2</sup> = determination coefficient; F statistic = F ratio test; Pr > F = P-value at significance level of 0.05.

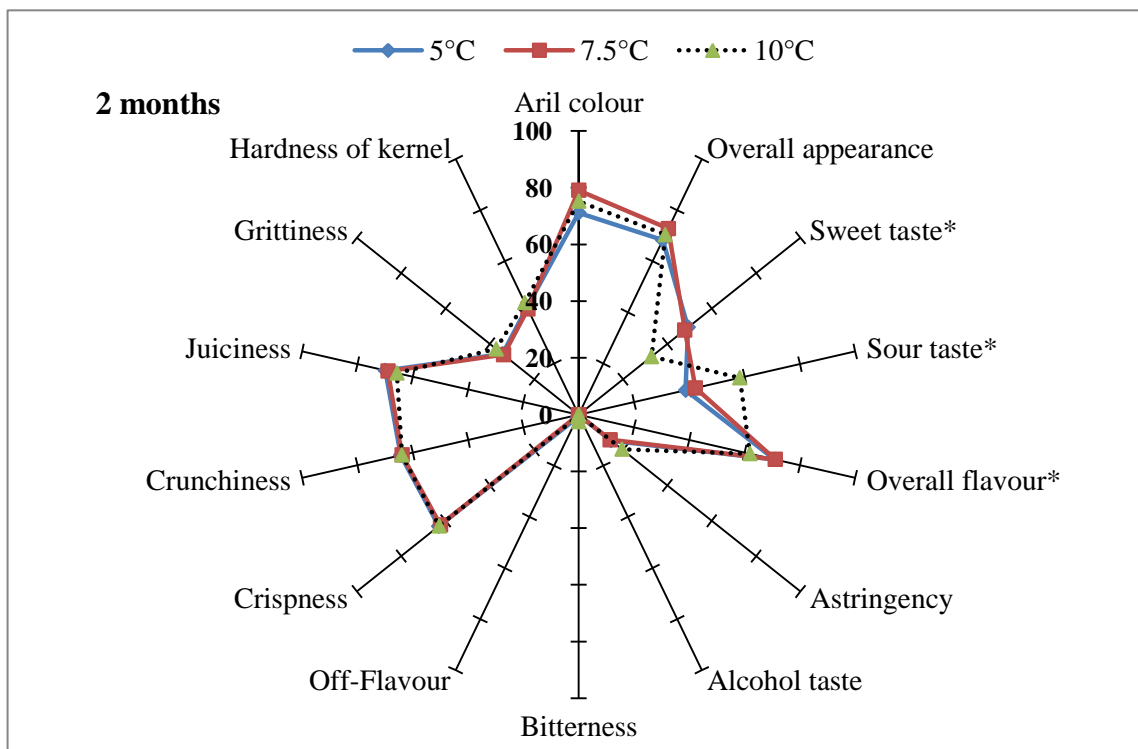


Fig. 1. Radar plot showing averaged sensory scores (scale = 0 - 100; n = 72) of pomegranate fruit stored at 10°C, 7.5°C and 5°C with 92±2% RH analyzed for ‘Wonderful’ cultivar for 2 months. \* Indicates significant difference ( $p < 0.05$ ) between temperatures.

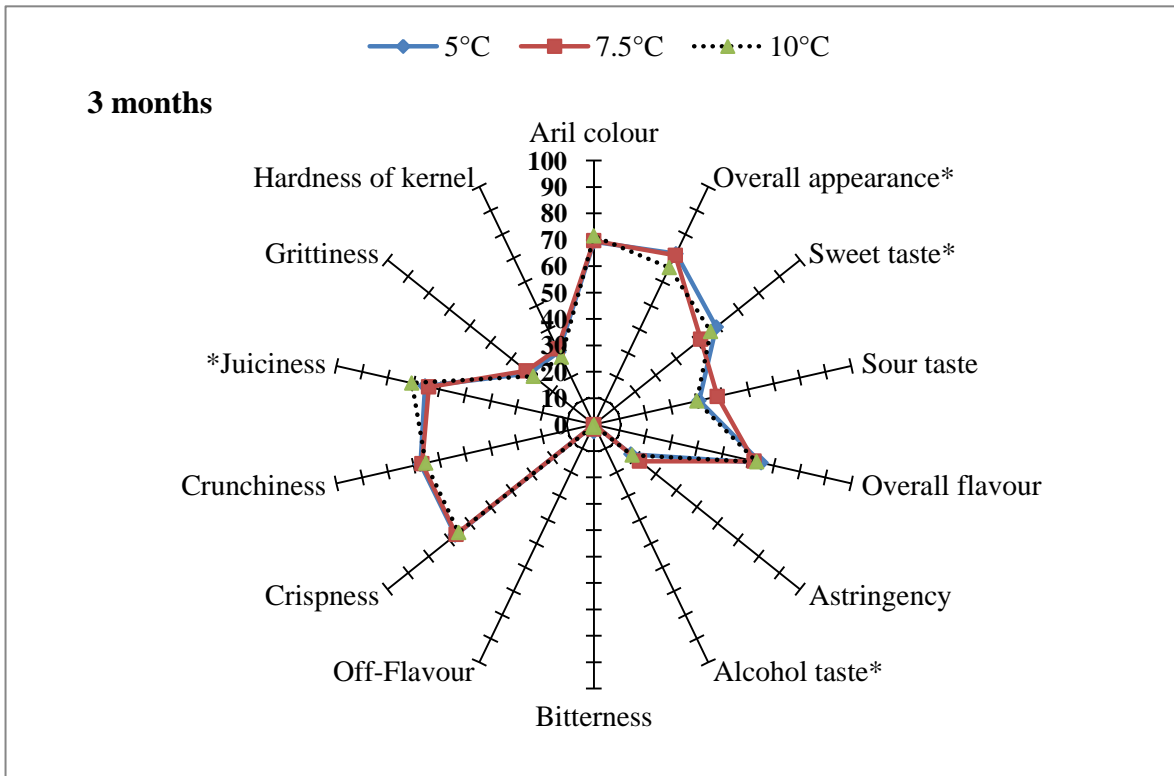


Fig. 2. Radar plot showing averaged sensory scores (scale = 0 - 100; n = 72) of pomegranate fruit stored at 10°C, 7.5°C and 5°C with 92±2% RH analyzed for ‘Wonderful’ cultivar for 3 months. \* Indicates significant difference ( $p < 0.05$ ) between temperatures.

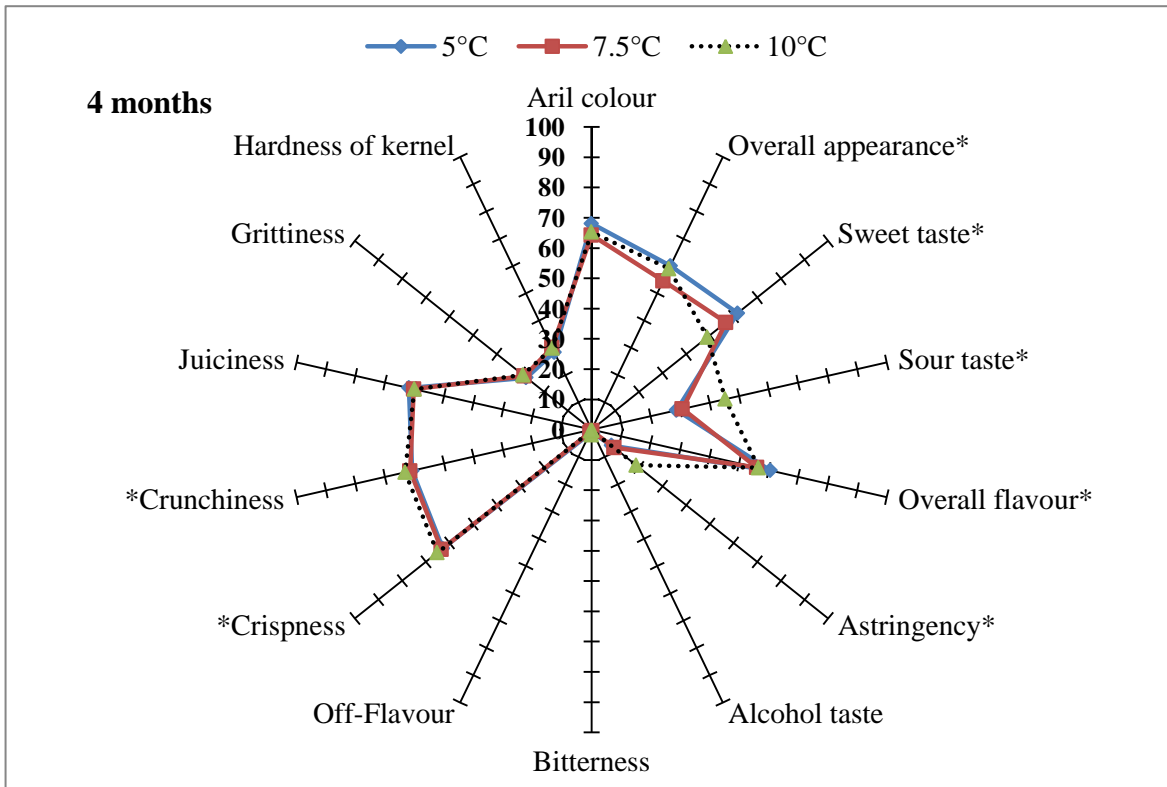


Fig. 3. Radar plot showing averaged sensory scores (scale = 0 - 100; n = 72) of pomegranate fruit stored at 10°C, 7.5°C and 5°C with 92±2% RH analyzed for ‘Wonderful’ cultivar for 4 months. \* Indicates significant difference ( $p < 0.05$ ) between temperatures.

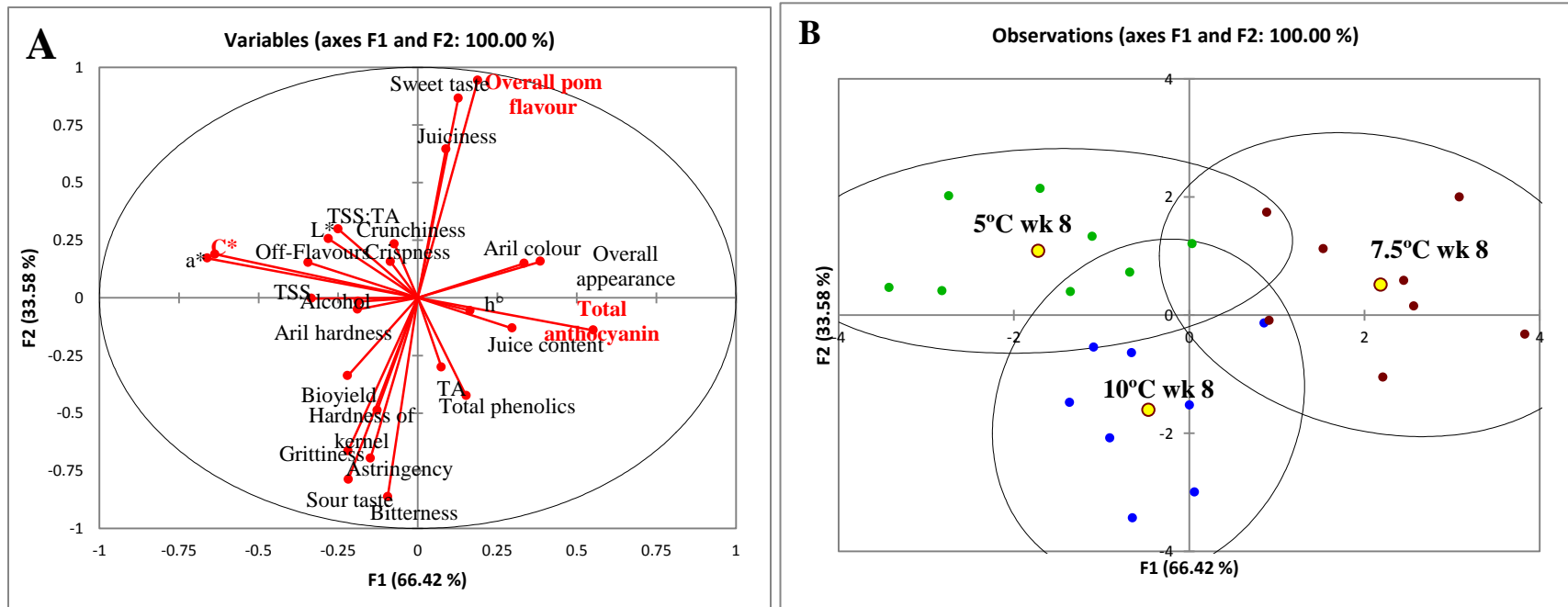


Fig. 4. Discriminant analysis (DA) showing variables chart (A) and observations chart (B) for ‘Wonderful’ cultivar using instrumental and sensory attributes stored 10°C, 7.5°C and 5°C with 92±2% RH for 2 months

Confusion matrix for 2 months:

from \ to	10°C	5°C	7.5°C	Total	% correct
10 month 2	7	0	1	8	87.50%
5 month 2	0	8	0	8	100.00%
7.5 month 2	0	0	8	8	100.00%
Total	7	8	9	24	95.83%

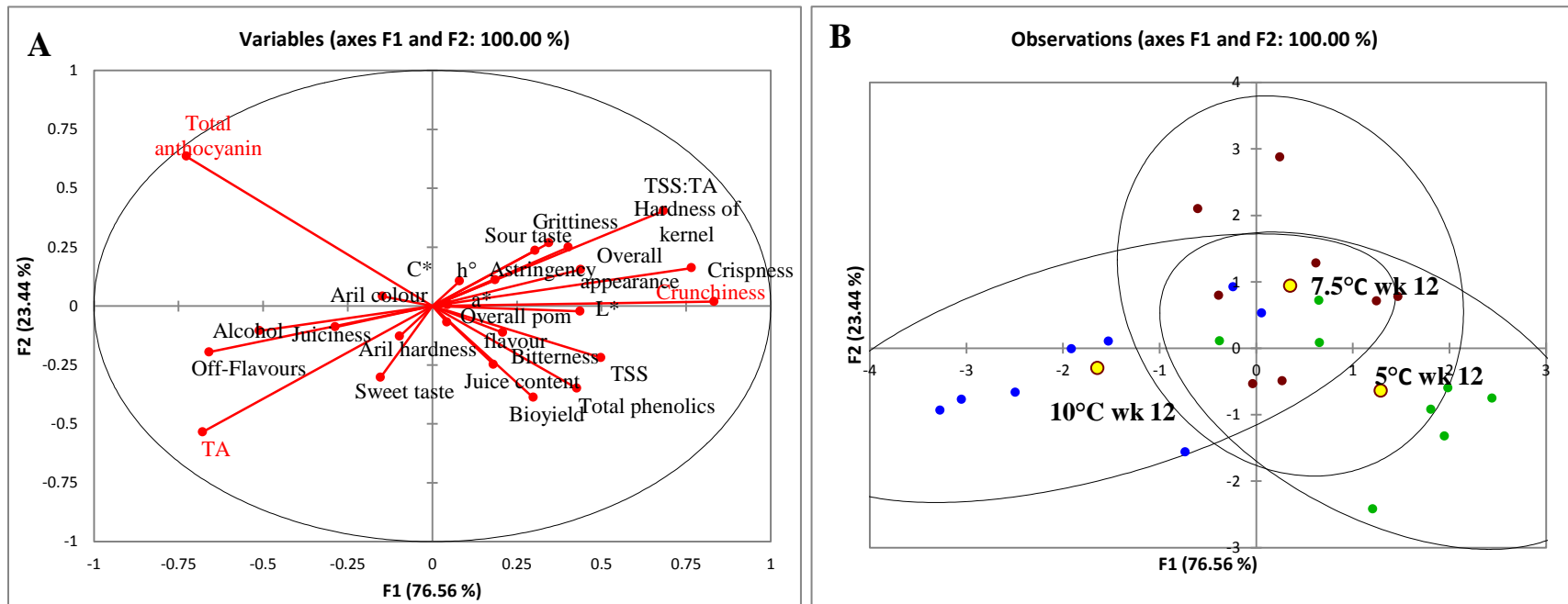


Fig. 5. Discriminant analysis (DA) showing variables chart (A) and observations chart (B) for ‘Wonderful’ cultivar using instrumental and sensory attributes stored 10°C, 7.5°C and 5°C with 92±2% RH for 3 months

Confusion matrix for 3 months

from \ to	10°C	5°C	7.5°C	Total	% correct
10 month 3	6	0	2	8	75.00%
5 month 3	0	5	3	8	62.50%
7.5 month 3	0	2	6	8	75.00%
Total	6	7	11	24	70.83%



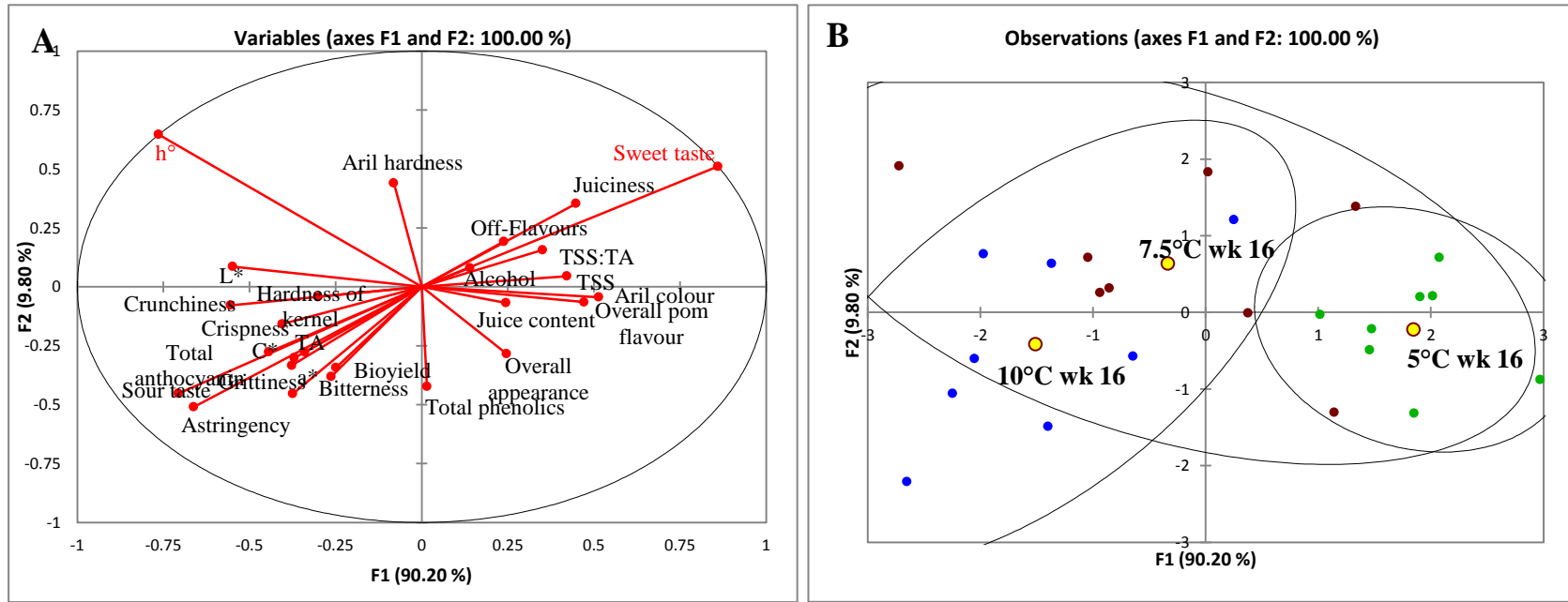


Fig. 6. Discriminant analysis (DA) showing variables chart (A) and observations chart (B) for ‘Wonderful’ cultivar using instrumental and sensory attributes stored 10°C, 7.5°C and 5°C with 92±2% RH for 4 months

Confusion matrix for 4 months

from \ to	10°C	5°C	7.5°C	Total	% correct
10 month 4	6	0	2	8	75.00%
5 month 4	0	8	0	8	100.00%
7.5 month 4	1	2	5	8	62.50%
Total	7	10	7	24	79.17%

# Chapter 7

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## GENERAL DISCUSSION AND CONCLUSIONS

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### 1. Introduction

The South African pomegranate export industry is expected to grow rapidly in the next few years as consumers become more aware of the potential health benefits of consuming fresh and processed products. Consumer demand for fruit is principally governed by important external and internal attributes. External quality attributes include fruit size, shape and skin appearance (colour, free of cracks, sun scalds, bruises), while internal attributes include total soluble solids, titratable acidity and flavour (sugar/acid ratio). Hence, the choice of postharvest handling and storage practices should consider delivery of fruit to consumers in the most excellent condition for desirable organoleptic, nutritional and antioxidant attributes (Kader, 2008; Opara, 2009; Fawole & Opara, 2013b). The overall aim of this research was to provide science-based management tools for the storage performance of pomegranate fruit (cv. Wonderful).

### 2. General Discussion

#### 2.1. Literature review on postharvest biology and storage performance of pomegranate fruit

This chapter reviewed previous research on storage performance of pomegranate fruit. The objective of the review was to discuss current knowledge on the effects of storage temperature and duration on quality and physiological attributes of pomegranates. Scientific literature on postharvest quality of pomegranates is voluminous; however, limited information is available on the storage performance of commercially grown cultivars in South Africa.

Postharvest handling conditions and practices like storage temperature, relative humidity and packaging could be used to maintain fruit quality during prolonged storage (Nanda *et al.*, 2001; Bayram *et al.*, 2009). However, like other fresh produce, pomegranate fruit undergoes quality losses resulting in changes in physiological, biochemical and textural properties.

Pomegranate cultivars respond differently at different optimum storage conditions, these storage conditions have been reported to range between 0°C to 10°C (Onur *et al.*, 1995; Fawole & Opara, 2013a). In addition, the storage performance of pomegranate fruit is dependent on the cultivar type, growing region and degree of maturity. Literature review revealed that control of the relative humidity is critical to fruit storage performance, as low relative humidity usually

causes fruit desiccation. This causes hardening and shrivelling of the husk, subsequently making the fruit unattractive and of poor market value (Pekmezci *et al.*, 1998). In order to maintain quality and reduce losses, fruit could be stored and maintained at temperatures ranging from 2°C to 10°C for up to several weeks, depending on the cultivar type. Moreover, the storage performance of commercially grown cultivars should be studied to develop postharvest handling protocols which will ensure supply of, nutritious and desirable fruit in the long supply chain.

## *2.2. Postharvest physiological response of pomegranate fruit at different temperature regimes*

The effects of postharvest physiological response of ‘Wonderful’ pomegranate were discussed in chapter 4. Fruit respiration and physiological disorders during long term storage were investigated. During storage cold temperatures evidently resulted in lower respiration rates, however the respiration rates gradually increased after 2 months. In addition, fruit stored at 5°C respired more after and beyond 3 months in storage compared to those stored at 7.5°C. This respiratory behavior might be attributed to the occurrence of physiological disorders such as chilling injury and lower relative humidity which was confirmed when fruit were examined for external and internal disorders. Generally, the severity and occurrence of physiological disorders were low in fruit stored at lower temperatures. After 1 month storage period fruit at 5°C showed no signs of external disorders, whereas storage of fruit at 21°C evidently resulted to the highest incidence and occurrence of physiological disorders. Fruit stored at 21°C and 10°C were discarded after 1 and 4 months, respectively, due to complete fruit loss to decay and shrinkage. Furthermore, it was observed that fruit decay increased with temperature and storage period. A similar observation was made for aril decay, where the severity of internal decay was enhanced with increasing storage temperature and prolonged duration. As observed in this current study, chilling injury only occurred in fruit stored at 5°C for 2 months and was observed afterwards till the end of the storage period, although the severity of chilling injury was below five percent.

Fruit stored at 21°C exhibited the highest browning index compared to fruit stored at lower temperatures; however, significant increases in browning were observed after 3 months particularly at 5°C in comparison to those stored at 7.5°C. This increase in browning could be related to the occurrence of physiological disorders such as chilling injury. These results suggest that the investigated cultivar may be sensitive to temperature abuse, where increases in temperature and storage period could elevate respiration rates, ultimately leading to biochemical

changes, increasing physiological disorders and quality loss. Furthermore, to prevent temperature abuse low temperatures and high relative humidity should be maintained throughout storage period. This would reduce the very high occurrence of moisture loss that can disfigure fruit and make them become unacceptable and undesirable to consumers. Therefore, it is recommended that fruit be stored at 5°C with >92% RH for up to 3 months.

### *2.3. Effect of storage temperature on postharvest quality attributes and mechanical properties of pomegranate fruit*

Practical information on reliable postharvest handling practices of South African grown pomegranates, specifically the ‘Wonderful’ cultivar, is lacking. During storage and transportation to market destinations, significant changes in organic acids, sugars, fruit colour and texture have been reported by various authors (Koksal, 1989; Gil *et al.*, 1996; Waskar, 2011). The overall objective of this study was to investigate the effects of storage temperature and duration on physical, chemical and textural properties of fruit and aril for ‘Wonderful’ cultivar. Commercially harvested fruit were stored at 5±0.7°C, 7.5±0.3°C and 10±0.5°C with 92±2% RH and at room temperature (21±3°C, 65±6% RH) for 5 months. This current study showed that major changes occurred in fruit internal and external quality during storage. A gradual increase in weight loss was observed with increasing storage temperatures and prolonged storage. The observed increase in weight loss could be due to high porosity of the fruit peel which permits free water vapour movement, as previously reported (Elyatem & Kader, 1984). Furthermore, susceptibility of fruit was dependent on storage temperature and relative humidity. A similar observation was observed for pomegranates stored at different temperatures (Elyatem & Kader, 1984; Küpper *et al.*, 1994; Fawole & Opara, 2013a). Interestingly, the investigated cultivar showed a significant loss in moisture from fruit peel, with negligible moisture loss found from aril. Furthermore, the investigated cultivar showed a significant reduction in peel thickness with increasing storage period.

This study showed that a significant change in chemical composition occurred during storage. Total soluble solids increased whereas titratable acidity declined with storage duration at 21°C, 10°C, 7.5°C and 5°C. The increase in total soluble solids may be associated with active hydrolysis of starch to sugars in pomegranate fruit. The decrease in titratable acidity may be related to the breakdown of organic acids during the respiration process. Furthermore, increase in

TSS/TA with storage temperature and duration was observed. This is in agreement with previous findings on 'Wonderful' cultivar (Ben-Arie *et al.*, 1984). Similarly, BrimA index a variant of the TSS/TA showed increases up to 3 months of storage. Furthermore, it was observed that fruit kept at 5°C had a reduced weight loss, and best in maintaining the chemical composition (TSS and TA, BrimA). Overall, storage temperature of 5°C for 2 months seems to be the best suitable for the chemical composition compared to other storage regimes. Additionally, it is recommended that fruit should not be stored at 21°C or 10°C due to excessive weight loss resulting in complete fruit loss to decay. This study provides valuable information (such as fruit and aril colour, total soluble solids, and acidity) suitable for the development of optimal postharvest handling.

The study reported in chapter 3 on mechanical properties showed that storage conditions influence the textural properties of fruit. Puncture resistance increased after 1 month storage, primarily due to moisture loss, which resulted in hardening and toughening of the fruit husk. As storage period progressed, puncture resistance decreased, an indication of loss in firmness. This study revealed that when the investigated fruit cultivar was subjected to compression force, a decrease in fruit firmness was evident with prolonged storage duration. Similarly, the amount of energy required to compress fruit decreased with extent of loss in firmness. These results are in agreement with those reported by Ekrami-Rad *et al.* (2011), who reported a reduction in firmness when for 'Wonderful' pomegranate was subjected to compression force at 5°C after 5 months storage. Practicable mechanical properties assessed included aril hardness, energy, bioyield point and Young's modulus of elasticity. Results showed that storage temperature and duration caused significant changes in aril texture. Aril hardness, toughness, bioyield and Young modulus all declined after harvest. The decrease in aril texture may be related to loss in cell wall integrity of pomegranate arils (Ekrami-Rad *et al.*, 2011). Fruit should not be stored at temperatures of 21°C or 10°C due to complete fruit loss to decay.

The present study showed that physico-chemical properties related to fruit quality can be optimally maintained at 5°C for 2 months of storage. Textural quality of fruit can be maintained at 5°C for up to 1 month; thereafter, decreases in fruit firmness, puncture resistance and aril hardness were evident especially with extended storage duration. For transportation of commercially harvested fruit to destination markets that takes just few weeks, maintaining the cold chain 5°C storage may be advisable. However, if fruit are harvested to be stored in order to capture a window period when the demand will be high, it should be warned that prolong storage

> 2 months at 5°C may result in chilling injury. These findings showed that fruit should be stored between 2 to 3 months and maintained at 5°C to ensure the best internal and external quality attributes. These studies may be of value for the development of optimal storage conditions for handling and processing products of pomegranate fruit for food and industrial use.

#### *2.4. Effects of postharvest storage conditions on phytochemical and antioxidant properties of pomegranate*

This study was conducted to investigate the effects of storage temperature and duration on phytochemical and antioxidant properties of pomegranate fruit (cv. Wonderful). Overall, results of this chapter provide relevant information on effects of storage temperatures and duration on beneficial health promoting compounds which could be used by the exporting industry especially where fruit are stored for long term and primarily used for health-promoting purposes. There were increases in total phenolic concentrations at all storage regimes before declining at 4 months of storage, whereas the highest concentration (364 mg/100 ml) was found at 10°C for 3 months. Similarly, total anthocyanin concentration increased after 1 month storage at all storage temperatures, with further increase observed at 10°C, 7.5°C and 5°C until 3 months before the concentration began to decline. The related increases in these two phytochemical concentrations may be due to the accumulation and biosynthesis of anthocyanin content, which is known to be induced in pomegranates at lower temperatures (Miguel *et al.*, 2004). The decrease in phenolic concentration including anthocyanins in pomegranates could be attributed to the change of enzyme activities resulting to phenolic degradation (Fawole & Opara, 2013a).

The antioxidant activity of fruit juice extract was tested against radical scavenging activity (RSA). Results showed that RSA was significantly reduced with prolonged storage periods particularly beyond 2 months of storage, where over 56% reduction in its activity was observed at all storage regimes. The trend in RSA is in agreement with previous studies on 'Ruby' pomegranate stored at 5°C, 7°C and 10°C for 16 weeks. Furthermore, ascorbic acid concentration declined significantly after 2 months at all temperatures with the lowest concentrations being at 5°C and 7.5°C after 5 months. Additionally, it is recommended that fruit should not be stored at 21°C or 10°C due to excessive weight loss resulting in complete fruit loss to decay. This study clearly shows that extended storage of fruit causes substantial losses in fruit phytochemical components and antioxidant activity.

### 2.5. Discrimination of pomegranate fruit quality by instrumental and sensory measurements during storage at three temperature regimes

Research discussed in this chapter addressed the need for improved methods for determining suitable storage conditions for ‘Wonderful’ pomegranate for long supply chain. This approach for establishing suitable storage conditions was based on a combination of instrumental and sensory methods. Instrumental measurements demonstrated that fruit quality was primarily influenced by temperature and storage duration with increases in TSS, TSS:TA and decrease in TA, total phenolic and anthocyanin concentration. Furthermore, no substantial change in aril colour was observed during storage. Aril texture such as hardness, toughness, Young’s modulus of elasticity and bioyield decreased with prolonged storage period. These results provide evidence that fruit arils may increase in deformability with increasing temperature and storage duration which could be attributed to cell membrane degradation due to higher temperatures and prolong storage (Bchir *et al.*, 2012).

Sensory attributes such as appearance, flavour and texture were assessed by a trained panel. Sensory attributes that had significant differences among the temperatures and storage duration were overall appearance sweet taste, sour taste and overall favour. Furthermore, aril texture showed a decrease in juice content, crispness and crunchiness as storage period progressed. In addition, kernel texture (kernel hardness and grittiness) declined with increasing storage period at all temperatures. The decrease in aril texture may be attributed to loss in cell wall integrity of pomegranate arils (Ekrami-Rad *et al.*, 2011). Discriminant analysis at different temperatures was used to distinguished fruit from each other at 2 months of storage with sensory attributes such as overall pomegranate flavor ( $R^2 = 0.564$ ), total anthocyanin ( $R^2 = 0.463$ ) and  $C^*$  ( $R^2 = 0.374$ ). Furthermore, discriminant analysis showed that storage of fruit for 3 months and beyond could clearly not be separated at different temperatures. Therefore, it could be deduced that the overall fruit quality of the investigated cultivar declined primarily due to storage duration beyond 2 months of storage, irrespective of storage temperature regime. For desirable sensory attributes, optimum storage temperature and duration were found to be 5°C and 2 months, when overall flavor and overall appearance values were highly rated. Similarly, according to the instrumental measurements, fruit could be successfully stored for 2 months without significant reduction in health-benefitting and colour parameters (total phenolics, total anthocyanin and aril colour).



Furthermore, extending storage duration beyond 2 months may increase physiological disorders which could ultimately lead to the development of undesirable off flavours. Therefore, the preference of storage temperature and duration should consider the market life of fruit and their use as either fresh or processed products. The storage conditions proposed in the current study for the investigated cultivar could provide growers and exporters with science-based management tools which could be used as a guideline to establish suitable postharvest handling and storage conditions to ensure best fruit quality attributes for long supply chains.

### 3. General Conclusions

This study provides science-based cold chain management tools to maintain postharvest quality of pomegranate fruit grown in the South African. These guidelines could add value to the pomegranate export industry, especially for long term storage to meet the increasing for fresh consumption as well as industrial manufacture of pharmaceutical, cosmeceutical and health-promoting products. In addition, this study contributes valuable information to the current knowledge on pomegranate postharvest research and encourages future studies towards the development of optimum postharvest handling protocols for other commercially grown cultivars in South Africa.

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