Maturity Indexing, Pharmacological Properties and Postharvest Performance of Pomegranate Fruit Grown in South Africa

By

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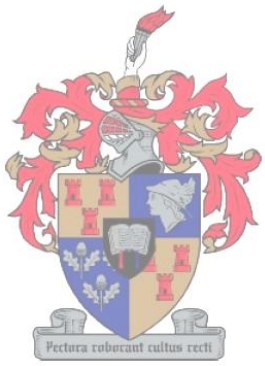

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own original work, and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

The development and application of science-based tools for determining optimum fruit maturity and postharvest handling protocols to maintain quality and reduce losses during postharvest handling and marketing is essential to maintain the competitiveness of the emerging pomegranate industry in South Africa. Currently, there are no quality standards for the South African pomegranate industry, neither is there a general consensus on the optimal harvest maturity indices for fruit cultivars. These information are important to ensure the delivery of good quality fruit to consumers, particularly for long supply chains. The overall aims of this study were (a) to develop science-based management tools for determining optimum maturity indices and storage performance of pomegranate fruit cultivars grown in South Africa, and (b) to characterise the physico-chemical and pharmacological properties of selected cultivars relevant to postharvest handling and industrial applications.

In Section II, seasonal studies on pomegranate (‘Baghwa’ and ‘Ruby’) fruit growth and the evolution of maturity indices during development were conducted. Significant increases in total soluble solids (TSS), sugars (glucose and fructose) and anthocyanin composition, coupled with significant decline in titratable acidity (TA), organic acids and total phenolics (TP) occurred with advancing fruit maturity. Fruit at advanced maturity stages were characterized by intense pigmentation of peel and aril, which coincided with maximum accumulation of anthocyanins. Among all the major maturity indices investigated, TSS, BrimA and anthocyanins did not show significant (p<0.05) seasonal variability, and strong correlations were found among the indices. In combination, these indices accounted for fruit juice sugar content, acidity and colour and could serve as reliable markers to determine optimal maturity for both pomegranate cultivars.

The studies in Section III focused on characterization of postharvest quality including nutritional, medicinal and antioxidant properties of fruit parts. Quality attributes of eight commercial cultivars were analysed by cluster analysis, which enabled the cultivars to be separated into two clusters (cluster 1 = ‘Ruby’, ‘Arakta’ and ‘Ganesh’; cluster 2 = ‘Bhagwa’, ‘Acco’ and ‘Herskawitz’) and two ungrouped cultivars (‘Molla de Elche’ and ‘Wonderful’) based on important quality attributes (size, texture, colour, soluble solids, acidity, juiciness and phenolics). Furthermore, pomegranate fruit peel extracts were studied to highlight their potential for value-adding in pharmaceutical and other industrial applications. The results showed that

fruit peels of the investigated cultivars possess strong antibacterial, antioxidant and anti-tyrosinase activities, and hence could be exploited as potential sources of natural antimicrobial and antioxidant agents, as well as a potential tyrosinase inhibitor.

The research reported in Section IV investigated the effects of harvest maturity and storage conditions on postharvest quality and nutritional value of 'Bhagwa' and 'Ruby' cultivars. Fruit harvested at commercial maturity were stored at 5±0.3°C, 7±0.5°C and 10±0.4°C with 92±3% RH and at room temperature (20±2.2°C, 65±5.5% RH) for 16 weeks. Fruit physiological responses and quality were affected by storage condition, with the maximum levels of respiration occurring at higher temperature and extended storage duration. Fruit colour and antioxidant capacity varied slightly among storage temperatures, with total soluble solids and titratable acidity decreasing gradually over time at different temperatures. Considering that fruit stored at 5°C and 92% RH had significantly reduced weight loss, low incidence of physiological disorders and best results in maintaining flavour attributes (TSS and TA, TSS:TA ratio), the investigated cultivars may be stored at 5°C and >92% RH for 8 - 12 weeks.

In paper 9 (Section IV), the research investigated the relationships between instrumental and sensory measurements of pomegranate fruit at different harvest maturities during storage and shelf life. Mature 'Bhagwa' fruit harvested at different times could not be discriminated by sensory attributes assessed by a trained panel. However, TSS (R^2 = 0.677) and juice content (R^2 = 0.512) were the two most decisive quality attributes at shelf life related to harvest maturity status. For 'Ruby', however, a combination of instrumental and sensory attributes appeared to be influential in discriminating mature fruit harvested at different times, with TSS:TA ratio being the most decisive (R^2 = 0.654) in distinguishing different fruit harvests, followed by sweet taste (R^2 = 0.474) and hue angle (R^2 = 0.431). The results showed that to ensure the best post-storage quality of 'Bhagwa', the optimum harvest maturity was between 167 - 175 DAFB (H2 and H3) when fruit reached maximum TSS level (>16°Brix; H3) and juice content (>65 mL/100 g aril; H2). However, for 'Ruby', this study indicated that the optimum harvest date was at 143 DAFB (H2) when TSS:TA ratio was >55, which coincided with significantly higher sensory rating for sweet taste after shelf life of fruit at H2 than H1 and H3, respectively.

The results from this thesis provide new understanding and better insights on fruit characteristics of major pomegranate cultivars grown in South Africa. Overall, the study provides new knowledge on science-based tools for assessing fruit readiness for harvest as well

as storage conditions to maintain fruit postharvest quality and reduce losses. It also provides scientific information on phytochemical contents and antioxidant compounds in fruit to promote value-adding of pomegranate as a good raw material with potential applications in health food products and other industrial applications such as pharmaceuticals and cosmetics.

OPSOMMING

Die ontwikkeling en toepassing van wetenskapgegronde instrumente vir die bepaling van optimale vrugrypheid en naoes-hanteringsprotokolle om gedurende die naoes-hantering en -bemarking van vrugte gehalte te behou en verliese te verminder, is noodsaaklik om die mededingendheid van die ontluikende granaatbedryf in Suid-Afrika te verseker. Tans is daar nie enige gehaltestandaarde vir die Suid-Afrikaanse granaatbedryf óf algemene eenstemmigheid oor die optimale oesrypheidsaanwysers vir vrugtekultivars nie. Hierdie inligting is belangrik om die naoes-lewering van uithalervrugte aan verbruikers te verseker, veral vir lang verskaffingskettings. Die oorkoepelende doelwitte van hierdie studie was (a) om wetenskapgegronde bestuursinstrumente te ontwikkel vir die vasstelling van optimale rypheidsaanwysers en bergingsprestasie van granaatkultivars wat in Suid-Afrika verbou word, en (b) om die fisiko-chemiese eienskappe en farmakologiese kenmerke van gekose kultivars te tipeer.

In deel II is seisoenale studies oor granaatgroei en die ontwikkeling van rypheidsaanwysers gedurende groei onderneem. Namate vrugte ryp geword het, is beduidende toenames in totale oplosbare vaste stowwe (TSS), suikers (glukose en fruktose) en antosianien-samestelling opgemerk, sowel as ’n beduidende afname in titreerbare suur (TA), organiese suur en totale fenol (TP). Vrugte in gevorderde stadia van rypheid is gekenmerk deur intense pigmentasie van die skil en aril, wat met maksimum opbou van antosianien verband gehou het. Van ál die belangrike rypheidsaanwysers wat ondersoek is, het TSS, BrimA en antosianien onbeduidende (p<0.05) seisoenale veranderlikheid getoon, en is sterk verbande tussen die aanwysers opgemerk. Gesamentlik sou die aanwysers kon rekenskap gee van sapsuikerinhoud, -suurgehalte én -kleur, en sou dit dus as betroubare rypheidsmerkers kon dien om optimale rypheid vir albei granaatkultivars te bepaal.

Die studies in deel III het gekonsentreer op die tipering van die naoes-kenmerke, onder meer die voedings-, medisinale en antioksidant-kenmerke van vrugtedele. Kenmerke van agt kommersiële kultivars is deur middel van groepsontleding bestudeer, waarvolgens die kultivars op grond van belangrike kenmerke (grootte, tekstuur, kleur, oplosbare vaste stowwe, suurgehalte, sappigheid en fenol) in twee groepe (groep 1 = ‘Ruby’, ‘Arakta’ en ‘Ganesh’; groep 2 = ‘Bhagwa’, ‘Acco’ en ‘Herskawitz’) en twee niegegroepeerde kultivars (‘Molla de Elche’ en

'Wonderful') ingedeel is. Ten einde die toegevoegde waarde van granaatskille vir farmaseutiese en kosmetiese doeleindes te bevorder, is skilekstrakte ook bestudeer. Die resultate toon dat die vrugteskille van die bestudeerde kultivars oor sterk antibakteriese, antioksidant- en anti-tirosinase-eienskappe beskik. Daarom kan die skil van die granaatkultivars as moontlike bron van natuurlike antimikrobiese en antioksidant-agense sowel as 'n moontlike tirosinase-inhibitor ontgin word.

Die navorsing in deel IV het ondersoek ingestel na die uitwerking van oesrypheid en bergingsomstandighede op die naoes-gehalte en -voedingswaarde van die kultivars 'Bhagwa' en 'Ruby'. Vrugte wat op kommersiële rypheid geoes is, is vir 16 weke by 5±0.3 °C, 7±0.5 °C en 10±0.4 °C met 92±3% RH, sowel as by kamertemperatuur (20±2.2 °C, 65±5.5% RH) geberg. Die bergingsomstandighede het die fisiologiese reaksies en gehalte van die vrugte beïnvloed: Maksimum vlakke van respirasie het teen hoër temperature en met verlengde berging voorgekom. Die kleur en antioksidantvermoë van die vrugte het effens tussen bergingstemperature verskil, en totale oplosbare vaste stowwe en titreerbare suur het mettertyd geleidelik by verskillende temperature afgeneem. Gedagtig daaraan dat die vrugte wat teen 5 °C en 92% RH geberg is beduidend minder gewigsverlies, 'n lae voorkoms van fisiologiese afwykings en die beste resultate in blywende geurkenmerke (TSS en TA, TSS:TA-verhouding) getoon het, kan die bestudeerde kultivars vir 8 tot 12 weke teen 5 °C en >92% RH geberg word (navorsingstuk 8).

In navorsingstuk 9 (deel IV) is daar ondersoek ingestel na die verhouding tussen instrument- en sintuiglike metings van granate in verskillende stadia van oesrypheid gedurende berging en raklewe. Geen verskil in sintuiglike kenmerke kon bespeur word by ryp 'Bhagwa'-vrugte wat op verskillende tye geoes is nie. Tog was TSS (R^2 = 0.677) en sapinhoud (R^2 = 0.512) die twee bepalendste gehaltekenmerke wat betref oesrypheidstatus gedurende raklewe. By 'Ruby' kon 'n kombinasie van instrument- en sintuiglike kenmerke egter wél tussen stadia van oesrypheid onderskei, met die TSS:TA-verhouding die bepalendste (R^2 = 0.654) in die onderskeid tussen verskillende vrugteoeste, gevolg deur 'n soet smaak (R^2 = 0.474) en skakeringshoek (R^2 = 0.431). Die resultate toon dat die beste nabergingsgehalte vir 'Bhagwa' verkry word by 'n optimale oesrypheid van 167–175 DAFB (H2 en H3), wanneer vrugte die maksimum TSS-vlak (>16°Brix; H3) en sapinhoud (>65 mL/100 g aril; H2) bereik het. Vir 'Ruby' dui hierdie studie op 'n optimale oesdatum van 143 DAFB (H2) met 'n TSS:TA-

verhouding van >55, wat verband gehou het met ’n beduidend hoër telling vir soet smaak by H2 eerder as by H1 en H3 ná raklewe.

Die resultate van hierdie tesis bied ’n beter begrip van, en insig in, die vrugtekenmerke van granaatkultivars wat in Suid-Afrika verbou word. Oor die algemeen bied die studie wetenskaplike inligting om moontlik die toegevoegde waarde van granate as ’n goeie bron van minerale elemente sowel as farmaseutiese, kosmetiese en antioksidant-verbindings te bevorder. Dit bied ook kennis oor die ontwikkeling van wetenskapgegronde instrumente vir die vasstelling van optimale vrugrypheid en naoes-hanteringsprotokolle om gedurende die naoes-hantering en -bemarking van granate vruggehalte te behou en verliese te verminder.

PUBLICATIONS AND CONFERENCE PRESENTATIONS FROM THIS THESIS

Published/submitted articles

1. **Fawole, O.A.,** Opara, U.L. **2013**. Harvest discrimination of pomegranate fruit: postharvest quality changes and relationships between instrumental and sensory attributes during shelf life. *Journal of Food Science*, 78, S1264–S1272. IF: 1.66

2. **Fawole, O.A.,** Opara, U.L., **2013**. Developmental changes in maturity indices of pomegranate fruit: A descriptive review. *Scientia Horticulturae*, 159, 152–161. IF: 1.53(ranked **12th of ScienceDirect TOP25 Hottest Articles**; July - September 2013)

3. **Fawole, O.A.,** Opara, U.L., **2013**. Fruit growth dynamics, respiration rate and physico-textural properties during pomegranate development and ripening. *Scientia Horticulturae*, 157, 90 – 98. IF: 1.53

4. **Fawole, O.A.,** Opara, U.L., **2013**. Effects of maturity status on biochemical concentration, polyphenol composition and antioxidant capacity of pomegranate fruit arils (cv. 'Bhagwa'). *South African Journal of Botany*, 85, 23 – 31. IF: 1.66

5. **Fawole, O.A.,** Opara, U.L., **2013**. Changes in physical properties, chemical and elemental composition and antioxidant capacity of pomegranate (cv. 'Ruby') fruit at five maturity stages. *Scientia Horticulturae*, 150, 37 – 46. IF: 1.53 (ranked **13th of ScienceDirect TOP25 Hottest Articles**; April - June 2013)

6. **Fawole, O.A.,** Opara, U.L., **2013**. Effects of storage temperature and duration on physiological responses of pomegranate fruit. *Industrial Crops and Products*, 47, 300 – 309. IF: 2.47

7. **Fawole, O.A.,** Opara, U.L., **2013**. Seasonal variation in chemical composition, aroma volatiles and antioxidant capacity of pomegranate during fruit development. *African Journal of Biotechnology*, 12, 4006 – 4019. IF: 0.50

8. **Fawole**, **O.A.,** Makunga, N.P., Opara, U.L., **2012**. Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract. *BMC Complementary and Alternative Medicine*, 12, 200: 1-11. IF: 2.24 (qualified as "**highly assessed**")

9. **Fawole**, **O.A.,** Opara, U.L., **2012**. Composition of trace and major minerals in different parts of pomegranate (*Punica granatum*) fruit cultivars. *British Food Journal*, 114, 1518 – 1532. IF: 0.70

10. **Fawole**, **O.A.,** Opara, U.L., Theron, K.I., **2012**. Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa. *Food Bioprocess and Technology*, 5, 2934 – 2940. IF: 4.12

11. **Fawole, O.A.,** Opara, U.L. Physico-mechanical, phyto-chemical, and free radical scavenging properties and volatile compounds in eight pomegranate cultivars and classification by principal component and cluster analyses. *British Food Journal*, 116 (In press). IF: 0.70

12. **Fawole, O.A.,** Opara, U.L. Instrumental and sensory measurements to determine harvest index of pomegranate fruit in consideration of long supply chains. *Postharvest Biology and Technology* (under review). IF: 2.41

List of conference presentations

1. **Fawole, O.A.,** Opara, U.L., 2012. Harvest maturity discrimination of pomegranate fruit (cv. 'Bhagwa') by instrumental and sensory measurements during storage and shelf life conditions (oral). Presented at 7th International CIGR Technical Symposium. "***Innovating the Food Value Chain***" Stellenbosch, South Africa, November 25-29.

2. **Fawole, O.A.,** Opara, U.L., 2012. Seasonal variation in physicochemical properties and antioxidant capacity of pomegranate fruits (cv. 'Ruby') during maturation (oral). Presented at 7th International CIGR Technical Symposium. "***Innovating the Food Value Chain***" Stellenbosch, South Africa, November 25-29.

3. **Fawole, O.A.,** Makunga, N.P., Opara, U.L., 2012. Investigation of antibacterial, antioxidant and tyrosinase-inhibition activities of South African grown pomegranate fruit peel extract (poster). Presented at 7th International CIGR Technical Symposium. "***Innovating the Food Value Chain***" Stellenbosch, South Africa, November 25-29.

4. Arendse, E.E., **Fawole, O.A.,** Opara, U.L., 2012. Influence of storage temperature on postharvest mechanical properties of pomegranate fruit and arils (poster). Presented at 7th International CIGR Technical Symposium. "***Innovating the Food Value Chain***" Stellenbosch, South Africa, November 25-29.

5. **Fawole**, **O.A.,** Opara, U.L., Theron, K.I., 2012. Influence of fruit developmental and maturity stages on chemical, phytochemical and antioxidant properties of pomegranate juice (oral). Presented at 2nd All African Horticulture Congress 2012. "***Horticulture for Humanity***", Kruger National Park, South Africa, January 15-20.

   Proceeding published as: **Fawole, O.A.,** Opara, U.L., Theron, K.I. **2013**. Influence of fruit developmental and maturity stages on chemical, phytochemical and antioxidant properties of pomegranate juice. *Acta Horticulturae*, 1007, 461-469.

6. **Fawole**, **O.A.,** Opara, U.L., Theron, K.I., 2011. Comparative study of phytochemical contents and antioxidant properties of juice of three pomegranate fruit cultivars grown in South Africa (poster). Presented at 6th International CIGR Technical Symposium. "***Towards a Sustainable Food Chain Food Process, Bioprocessing and Food Quality Management***", Nantes, France, April. **Awarded the Prize for Third Best Poster.**

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SECTION I

- General Introduction
- Paper 1: Developmental changes in maturity indices and cold-storage responses of pomegranate fruit: A descriptive review[1]

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[1] Sci. Hort. 159: 152–161.

GENERAL INTRODUCTION

1. Background

Pomegranate (*Punica granatum* L.) is an emerging crop in South Africa. The fruit has long been valued for its flavourful and juicy edible part (aril), and more lately for commercial juice production (Wetzstein et al., 2011). Increasing consumer interest and awareness on pomegranate as a medicinal food has spurred global increase in production and marketing of the fruit and its products. Commercial orchards of different pomegranate cultivars are grown in different countries (Holland et al., 2009), with about 90% of the world pomegranate production occurring in the Northern Hemisphere (Holland et al., 2009; Citrogold, 2011; Pomegranate Association of South Africa, 2012). The health benefits of consuming pomegranate fruit have been attributed to the exceptionally high antioxidant capacity which is strongly linked to the high concentration and unique composition of fruit phenolic compounds (Gil et al., 2000; Fischer et al., 2011).

South Africa is one of the producers of pomegranate in the Southern Hemisphere, competing with countries such as Chile, Australia, Peru and Argentina. Consequently, the growing export opportunity has encouraged large scale production to increase exports, allowing producers to fill the counter-season window during spring and early summer months in the Northern Hemisphere (Brodie, 2009; Pomegranate Association of South Africa, 2012). Pomegranate production in South Africa stands at about 1,000 ha, and fruit export has increased drastically from about 70,000 cartons (315 tonnes) in 2009/2010 to over 440,000 cartons 198,000 tonnes) in 2011/2012 season, mainly to European countries, Far Eastern countries and Canada (Brodie, 2009; Citrogold, 2012; Perishable Products Export Control Board, 2012; Pomegranate Association of South Africa, 2012). Pomegranate production in South Africa is projected to grow by 189% by 2017 (Pomegranate Association of South Africa, 2012). To fully harness the opportunity of existing and future competitive export markets, there is a need to characterize commercial pomegranate cultivars grown in South Africa for the purpose of selection and marketing of cultivars with better quality as fresh fruit or processed products.

Commercial pomegranate production in South Africa is challenged by the lack of science-based tools for determining optimum fruit maturity and postharvest handling protocols to maintain quality and reduce losses during postharvest handling and marketing.

Currently, there are no established fruit quality standards in the South African pomegranate industry and there is a lack of knowledge on the optimal storage requirements for cultivars that are commercially grown in the country. Based on current practice among growers, harvest periods are determined by calendar dates or when fruit calyx has closed and the fruit can produce a metallic sound when tapped (Brodie, 2009; Citrogold, 2011; Oliver, F., pers. comm., 2011). There is no scientific evidence on the relevance of these maturity indices to storage performance and sensory perception of quality of harvested pomegranate fruit.

Pomegranate fruit are non-climacteric and thus cannot continue the ripening process after detachment from the parent plant (Kader, 2006). Harvesting pomegranate at an early stage of maturity may result in fruit that has good appearance and can withstand postharvest handling, but with poor aril colour intensity and unacceptable flavour. On the other hand, fruit harvested at late maturity are more susceptible to spoilage and have short storage potential. Apart from the common external postharvest quality defects such as decay and water loss, leading to browning symptoms in the peel and arils (Kader et al., 1984), internal quality losses also occur. Other researchers have reported colour loss as a result of degradation of anthocyanin (Turfan et al., 2011), as well as decreases in total soluble solids and titratable acidity, which are accompanied by a reduction in consumer acceptability in terms of freshness, taste and loss of potential medicinal properties (Gil et al., 1996; Artes et al., 2000; Nanda et al., 2001; Labbé et al., 2010; Turfan et al., 2011).

A number of parameters can be used to assist in determining fruit harvest maturity. Pomegranate quality parameters such as size and colour are physical attributes used for grading purposes, but the most important maturity indices for quality assessment to meet market requirement include internal attributes such as aril colour, total soluble solids content and titratable acidity (Chace et al., 1981; Ben-Arie et al., 1984; Cristosto et al., 2000; Kader, 2006; Martinez et al., 2006). For instance, maximum titratable acidity may be 1% in sweet cultivars and 1.5 to 2% in sweet-sour cultivars, with minimum total soluble solids between between 15 and 17% (Kader, 2006). For the Wonderful cultivar grown in California (USA), the combination of red juice colour equal to or darker than Munsel colour chart 5R-5/12, titratable acidity below 1.85%, and total phenolics contents below 0.25% was considered desirable for optimal levels of sweetness and stringency (Kader, 2006). Furthermore, the optimum storage temperature recommended for pomegranates varied from 0 to 10°C, with storage life ranging from 2 weeks to 5 months (Pantastico et al., 1975; Kupper et al., 1994; Kader, 2006). These findings highlight the need to study specific cultivars grown in particular

regions in order to determine the relevant harvest maturity indices for optimal postharvest performance and sensory quality.

To maintain fruit quality and reduce losses during postharvest handling to distant markets, good understanding of the effects of fruit maturity status and postharvest storage conditions on quality is warranted. Similarly, better understanding of the physical, biochemical and physiological changes that occur during fruit growth and development is necessary to assist in identifying potential maturity indices that relate to postharvest performance. To develop quality standards for fruit marketing, it is also necessary to identify reliable quality cues based on combined understanding of postharvest quality attributes and consumer perception of the organoleptic attributes.

2. Aims and objectives

2.1. Aims

The overall aims of this research were to develop science-based management tools for determining optimum maturity indices and storage performance of pomegranate fruit cultivars grown in South Africa, and to characterise the physico-chemical and pharmacological properties of major selected cultivars.

2.2. Objectives

The specific objectives of this study were to:

a. study fruit growth and related changes in physical, biochemical and physiological properties during development of ‘Bhagwa’ and ‘Ruby’ cultivars,
b. characterise postharvest quality attributes and pharmacological properties of selected cultivars,
c. determine the physiological responses and changes in quality of fruit under different storage temperatures, and
d. determine potential harvest maturity indicators for selected cultivars based on a combination of sensory and instrumental quality attributes.

3. Thesis structure

This dissertation is structured into five Sections (**I - V**), with each section addressing a particular research theme.

- **Section I**: provides a brief background and discusses the research aims and objectives (*General introduction*). It also provides a descriptive review of current knowledge on the changes which occur in pomegranate fruit maturity indices during development and postharvest storage (*Paper 1*)
- **Section II**: focuses on fruit growth and evolution of maturity indices of Bhagwa and Ruby pomegranate cultivars (*Papers 2 & 3*)
- **Section III**: reports the studies to characterise postharvest quality attributes and antioxidant capacities of fruit parts of selected commercial pomegranate cultivars (*Papers 4 - 6*). Special emphasis was given to the promotion of value-adding and health-benefiting properties by investigating the antibacterial, antioxidant, and tyrosinase-inhibition activities of fruit peel extracts (*Paper 7*)
- **Section IV**: discusses postharvest quality attributes of Bhagwa and Ruby pomegranate cultivars in relation to harvest maturity and storage conditions (*Papers 8 & 9*)
- **Section V**: presents a general discussion which integrates the results from all the eight papers. It also highlights practical contribution of the studies to the South African pomegranate industry

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PAPER 1

Developmental changes in maturity indices and cold storage responses of pomegranate fruit: A descriptive review

1. Introduction

Pomegranate (*Punica granatum* L.) has gained popularity in recent years due to its multi-functionality and nutritional value in human diet. The fruit is grown globally in different geographical regions, satisfying the nutritional and medicinal needs of populations of various countries (Holland et al., 2009). During pomegranate fruit development, advancing maturity stages correspond to a number of coordinated physiological, biochemical, and structural processes that result in changes in size, colour and flavour, ultimately making the fruit desirable for consumption (Ben-Arie et al., 1984; Al-Maiman and Ahmad, 2002). Quality assessment of pomegranate fruit is based on important external attributes such as size, shape and colour (Kader, 2006; Holland et al., 2009). However, because fruit skin colour does not indicate the extent of ripening or its readiness for consumption, internal attributes such as colour, total soluble solids and acidity are also considered in assessing readiness for harvest to meet market requirements (Ben-Arie et al., 1984; Kader, 2006; Holland et al., 2009).

The timing of harvest is of utmost importance if fruit, either for immediate fresh market or for storage, are to reach the customer in prime condition. Studies have shown the effects of cultivar differences, growing region and maturity status on pomegranate fruit maturity indices (Al-Maiman and Ahmad, 2002; Shwartz et al., 2009). In other studies, authors have explored individual fruit physico-chemical parameters and the relationships among these parameters to assist in identifying consistent fruit indices for reliable prediction of fruit maturity indicators (Ben-Arie et al., 1984; Shwartz et al., 2009; Al-Maiman and Ahmad, 2002). Furthermore, many studies have shown that the optimum storage condition for pomegranate fruit is dependent on cultivar as well as fruit responses during cold storage (Kader et al., 1984; Gil et al., 1996b; Artes et al., 2000; Alighourchi et al., 2008). However, like other types of fruit, pomegranate also undergoes postharvest quality losses during handling and storage. Apart from common external postharvest quality losses such as colour degradation (Kader, 2006; Mirdehghan et al., 2006), internal quality losses also occur. These include loss of colour as a result of degradation of anthocyanin (Turfan et al., 2011), as well

as decrease in vitamin C content, total soluble solids (TSS) and titratable acidity (TA), which are accompanied by a reduction of acceptability in terms of freshness, juiciness and taste (Gil et al., 1996b; Artes et al., 2000; Nanda et al., 2001; Labbé et al., 2010).

The choice of a reliable maturity index should reflect the quality requirements of harvested fruit and it should also enable the delivery of harvested fruit to consumers in the best condition in terms of desirable organoleptic, nutritional and antioxidant attributes (Kader, 2008). Literature on quality indices of pomegranate fruit at commercial harvest is voluminous; however, research applying changes that occur during fruit development and storage to determine objective criteria for assessing fruit readiness to harvest are lacking.

The objective of this review was to discuss current knowledge on the changes which occur in fruit maturity indices during development and storage of pomegranates.

2. Characteristics of pomegranates

2.1. Plant description and distribution

The pomegranate (*Punica granatum* L.) is a tropical and subtropical attractive deciduous or evergreen shrub belonging to the Punicaceae family. A fully grown tree is between 6 - 10 m tall, much-branched, more or less spiny, and extremely long-lived (Morton, 1987). The plant is considered either a small tree or a large shrub and its fruit is often considered to be a large berry (Faria and Calhau, 2010). It has glossy and leathery leaves that are narrow and lance-shaped. The attractive scarlet, white or variegated flowers have 5 to 8 petals. The red, fleshy, tubular calyx produces the delicious fruit crowned at the base by the prominent calyx. It is one of the hardest fruit crops, round or spherical in shape, with a tough, leathery skin or exocarp often deep pink or rich red in colour (Holland et al., 2009). A picture of a typical pomegranate fruit is shown in Figure 1.

Pomegranate fruit is non-climacteric, with a low respiration rate and is generally harvested when fully ripe (Ben-Arie et al., 1984; Shulman et al., 1984). The interior is separated by membranous walls and white spongy tissue into compartments packed with transparent sacs filled with fleshy, juicy, red, pink or whitish pulp called the arils. In each aril sac, there is one white or red, angular, soft or hard seed. The arils account for about 52% of the weight of the whole fruit (Biale, 1981; Morton, 1987; Al-Said et al., 2009; Holland et al., 2009), and have long been valued for their flavour (Wetzstein et al., 2011) and good consumer preference for the sweet acidic and refreshing juice. They are also consumed as

fresh arils or as processed food materials and other industrial products (Al-Maiman and Ahmad, 2002; Jalikop, 2007).

Over 1000 cultivars of *P. granatum* have been identified globally (Levin, 1994). The pomegranate is native to the area stretching from Iran to the Himalayas in northern India and has been cultivated and naturalized over the whole Mediterranean region since ancient times. It has been reported to be grown commercially in several regions including in India, Pakistan, Israel, Afghanistan, Iran, Egypt, China, Japan, USA, Russia, Australia, South Africa and Saudi Arabia and in the subtropical areas of South America (Holland et al., 2009). Although pomegranate is tolerant of a wide range of different soil conditions, it thrives well under sunlight and mild winters with minimal temperatures not lower than -12ºC, and hot dry summers (Levin, 2006). India is the global principal producer, with more than 100,000 ha of pomegranate (Stover and Mercure, 2007).

2.2. Economic importance, enthobotany and functional properties

The pomegranate is a highly nutritious fruit with considerable amounts of acids, sugars, vitamins, polysaccharides, polyphenols and important minerals (Al-Maiman and Ahmad, 2002). It is currently ranked 18th in terms of annual global fruit consumption (Brodie, 2009), with increasing demand especially in developed countries due to its reported health benefits (Lansky and Newman, 2007; Opara et al., 2009). Pomegranate fruit is used for the preparation of numerous commercial fresh products including juice which is the most popular, jellies, wine, jam, paste and colouring beverages, as well as salad dressing and seed oils (Holland et al., 2009).

Pomegranate has accompanied mankind over hundreds of years. Its existence is woven in many religious beliefs and rituals, including its symbol of life, health, spirituality, morality, and longevity or even immortality in Judaism, Christianity, Islam and Buddhism (Mahdihassan, 1984). Pomegranate arils are also used as natural symbol of fertility in many cultures (Mahdihassan, 1984). In traditional medicine, the fruit is used as a blood tonic. Due to the strong astringency of pomegranate hulls, it is used for the treatment of dysentery and diarrhoea as well as an antiparasitic agent (Boukef et al., 1982; Caceres et al., 1987; Nagaraju and Rao, 1990; Naqvi et al., 1991). Other reported traditional uses include the treatment of snakebite, diabetes, burns and leprosy (Siang, 1983; Jain and Puri, 1984; Singh, 1986).

There is a renewed global interest in pomegranate consumption following recent studies showing that the fruit has high amounts of antioxidants and medicinal characteristics

which are beneficial to human health. The phytochemistry and pharmacological actions of pomegranate components suggest a wide range of clinical applications for the treatment and prevention of several diseases. Several comprehensive reviews have been published recently on the current knowledge, basic principles and concepts, as well as clinical nutrition evidence on the effects of pomegranate consumption and application for the prevention and treatment of diseases (Lansky and Newman, 2007; Basu and Penugonda, 2009; Viuda-Martos et al., 2010; Faria and Calhau, 2010).

3. Fruit development

3.1. Flowering and fruit set

The flowering period of pomegranates occurs about one month after bud-break, and varies from variety to variety and is governed by agro-climatic conditions (Holland et al., 2009). Flowering time corresponds with mean temperature for the 'Mule's Head' and 'Wonderful' cultivars grown in Israel (Shulman et al., 1984). The flowers may be solitary or grouped and can be classified as male, hermaphroditic, and intermediate. Differences between male and hermaphrodite flowers are apparent in the shape and colour of the calyx, and the number of the hermaphrodite flowers determines fruit set capacity and yield in pomegranates. The ovary of a male flower is rudimentary whereas those of intermediate flowers are of the degenerating type. If fruit set occurs in such flowers, they may drop before reaching maturity, and even if some fruits reach maturity they are often deformed. The time of dehiscence of the anther varies in different cultivars and no general sequence is found at the time of anthesis (Nath and Randhawa, 1959; Josan et al., 1979; Melgarejo et al., 1997; Mir et al., 2012).

The percentage of flowers that are male in pomegranate can be significantly high, exceeding 60 to 70% depending on variety and season (Holland et al., 2009; Wetzstein et al., 2011). For example, studies have shown that the number of male flowers ranged between 43 and 66% for Israeli 'Wonderful' (Assaf et al., 1991) and 78 to 86% for 'Hicaznar' grown in Turkey (Gozlekçi and Kaynak, 2000). The pomegranate flower is self- and cross-pollinated mainly by bees (Morton, 1987), but cross pollination is reported to have higher rates of fruit set (Derin and Etis, 2001; Mir et al., 2012). Fruit development starts after flowering of the ovary, with flowering and fruit set lasting about one month (Holland et al., 2009).

3.2. Fruit growth patterns

Pomegranate fruit growth pattern has been characterized as a simple sigmoidal curve from fruit set to maturity (Ben-Arie et al., 1984; Gozlekçi and Kaynak, 2000; Varasteh et al., 2008). According to Kumar and Purohit (1989), there are periods of fast fruit growth rate which alternate with periods of slow growth rate. The initial rapid increment in fruit growth occurs during cell division, which is characterized by growing kernel tissue and the increment in testa hardness (Shulman et al., 1984), after which a slowdown in growth occurs (Gozlekçi and Kaynak, 2000). However, while the kernel stops growing, the aril continues to grow steadily as the fruit increase to its final size through cell enlargement during maturation (Ben-Arie et al., 1984; Shulman et al., 1984; Melgarejo et al., 1997).

Pomegranate fruit growth pattern appears to be cultivar dependent. A slow growth phase was noticed in the 'Wonderful' when fruit diameter reached 52.5 mm (Ben-Arie et al., 1984). In the 'Malas-e-Torsh-e-Saveh' grown in Iran, the average fruit weight and volume increased rapidly until 45 days after fruit set (DAFS) and then continued more slowly until harvest (Varasteh et al., 2008). According to Shulman et al. (1984), the 'Mule's Head' cultivar followed a simple sigmoid curve whereas the growth pattern of 'Wonderful' was linear. However, for the Omani cultivars grown in the Al-Jabal Al-Akhadar area (Al-Yahyai et al., 2009) and 'Wonderful' grown in Australia (Weerakkody et al., 2010), the researchers reported linear fruit growth pattern. In both studies, the measurement of fruit growth started several weeks after fruit set.

It has been reported that differences in ripening time among pomegranate cultivars is not derived from the differences in flowering dates but rather dependent on the time from anthesis (Holland et al., 2009). The time taken for fruit to reach harvest maturity varies with cultivar, growing location and season (Gil et al., 1995; Shulman et al., 1984). The study by Shulman et al. (1984) showed that in the hot valley region in Israel, fruit matured more rapidly than in the coastal plain. Fruit ripens 5 to 8 months from fruit set, involving a sequence of changes in fruit characteristics from flowering to maturity and senescence. These changes include physical, structural, biochemical, physiological and elemental changes, reflecting differences in fruit appearance during maturation and ripening among cultivars (Ben-Arie et al., 1984; Shulman et al., 1984; Al-Maiman and Ahmad, 2002; Holland et al., 2009; Shwartz et al., 2009).

4. Changes in maturity indices during fruit development and ripening

A wide range of methods used to evaluate quality and physiological attributes of pomegranate fruit are summarized in Table 1.

4.1. Physical changes

4.1.1. Fruit parts and juice content

Physical properties of fruit such as weight, volume, and juice content are important from a marketing viewpoint because the attributes influence consumer preference (Hess-Pierce and Kader, 2003; Holland et al., 2009). During the early, middle and late stages of fruit development of 'P-23' grown in India, increase in fruit size, weight and volume were observed throughout fruit development (Khodade et al., 1990). For the Wonderful cultivar grown in Condobolin, Australia, Weerakkody et al. (2010) reported linear increase in fruit mass, which reached a maximum value of 675 g per fruit after 14 weeks of fruit set. Fruit length and diameter increased while length/diameter ratio (shape index) decreased during development and maturation, indicating that fruit diameter increases faster than the length. Data on typical changes in physical attributes of fruit during growth and development are shown in Table 2. According to Shwartz et al. (2009), these changes in physical characteristics of pomegranate fruit indicate that the fruit continues to grow even after the optimum harvesting stage, presumably due to cell expansion from uptake of water and other nutrients. The study by Borochov-Neori et al. (2011) on four Israeli grown pomegranate accessions ('PG 128-29', 'PG 130-31', 'EG1' and 'EG 2') showed that fruit that ripened in early summer and during the winter period were significantly smaller than those which ripened in late summer and autumn. For Malas Yazdi cultivar grown in Iran, the weight of peel contributed more to fruit mass early in the season while the weight of arils became dominant from the middle of season until fruit harvest, with mean dry weight of 35.03 and 22.33 g in arils and peel, respectively, during early and late parts of the season (Mirdehghan and Rahemi, 2007). Shulman et al. (1984) reported that fruit arils constituted about 50% of the fruit weight during most stages of fruit development for Mule's Head and Wonderful cultivars, while 57 - 66% of fruit weight was accounted for arils in Spanish 'Mollar' (Gil et al., 1996a).

The average yield of fruit juice at commercial harvest was 37% of the total fruit weight for 'Wonderful' grown in Condobolin, Australia (Weerakkody et al., 2010). Fruit

juice content was less than 25% during late immature fruit maturity stages but continued to increase until harvest time, constituting 35 - 40% in 'Mule's Head' and 40 - 45% in 'Wonderful' (Shulman et al., 1984). Lower juice content at a wider range (18 - 40%) was reported for for 'Wonderful' grown in Israel, with differences in climatic conditions accounting for the large variation (Shulman et al., 1984). In Israeli grown pomegranate cultivars, early summer fruit had the highest juice content compared to late summer, autumn and midwinter fruit at mature stage (Borochov-Neori et al., 2011). Similarly, Al-Said et al. (2009) reported juice content ranging between 57 - 67% for Omani pomegranate cultivars, with 'Jabal 2' having the highest amount of juice compared to 'Jabal 1' and 'Jabal 3'.

4.1.2. Colour dynamics

Colour is an important factor affecting marketability and consumer preference of fruit, including pomegranates (Opara et al., 2009; Pathare et al., 2012). However there is no correlation between the outer skin (peel) colour and the colour of arils inside fruit (Al-Said et al., 2009; Holland et al., 2009). Depending on cultivar and maturity stage, fruit external colour varies considerably from yellow, green, or pink overlain with pink to deep red or indigo to fully red (Holland et al., 2009). During fruit development and ripening of Spanish cultivar 'Mollar de Elche', the fleshy arils changed from white to pinkish-red whereas the peel of the fruit changed from green to greenish yellow, and finally to brownish yellow with reddish patches (Melgarejo et al., 1997). Studies on different accessions of 'Wonderful' cultivar showed that red pigmentation increased significantly in fruit parts during ripening (Ben-Arie et al., 1984; Shulman et al., 1984; Shwartz et al., 2009).

The colour of pomegranate juice is influenced by a wide range of pre- and postharvest factors, including growing conditions. According to Shulman et al. (1984), fruit grown under coastal plain regions developed more intense colour than those grown in warmer valley regions in Israel. The study conducted by Borochov-Neori et al. (2009) showed that the intensity of red colour of arils was inversely related to heat units accumulated during fruit development and ripening. In a mid-season cultivar, the CIE a^* (redness) value for arils increased while the luminosity (L^*) value declined concurrently, corresponding to a gradual change in aril colour from white to pink during the ripening period (Borochov-Neori et al., 2009). Similarly, colour parameters for 'Codpa' grown in Chile showed that fruit at green maturity stage had the highest L^* and $h°$ values (Labbé et al., 2010). According to Ben-Arie

et al. (1984), juice colour continued to increase in intensity even when the maximum soluble solids content was reached in the Wonderful cultivar.

4.2. Biochemical changes

4.2.1. Total soluble solids (TSS)

Total soluble solids (TSS), which is mostly made of sugar, increased significantly during three major fruit developmental stages in 'Rabbab-e-Fars' (Zarei et al., 2011). The TSS content increased from 10.30°Brix in immature fruit at 20 days after fruit set (DAFS) to 19.56°Brix in fully ripe fruit at 140 DAFS (Zarei et al., 2011). Similarly, for 'Ganesh' grown in India, TSS content of 13% in 40 day-old fruit increased but this was not significant until the 100th day of fruit development when TSS exceeded 15%. The highest TSS content of 16.3% was recorded in 140 day-old fruit (Kulkarni and Aradhya, 2005). A different pattern of TSS accumulation in pomegranate fruit was reported for 'Taifi', where TSS value was 16.4 °Brix in green unripe fruit, with very small increments in TSS during the remaining stages of fruit development resulting in 16.9°Brix at full-ripe stage (Al-Maiman and Ahmad, 2002). This finding corroborates the results reported by Kader et al. (1984) on Wonderful cultivar grown in California, USA.

TSS content remained constant throughout the ripening process in mature fruit for Spanish clones 'ME5', 'ME17' and 'MO6' (Legua et al., 2000) and the sweet 'Mollar' (Gil et al., 1995). The results agree with those reported by Shulman et al. (1984) for 'Mule's Head' and 'Wonderful', where significant differences in TSS content occured in the later stages of fruit ripening. Ben-Arie et al. (1984) studied 'Wonderful' grown in two different regions and over two seasons in Israel, and observed that TSS content increased during the first 4.5 months after flowering period, and remained fairly constant afterwards. At TSS level of 15% the fruit were considered fully ripe in terms of eating quality.

Weerakkody et al. (2010) observed inconsistent TSS levels in 'Wonderful' over two growing seasons. In the first season, the authors reported increase in TSS content from 8% at 30 DAFS to 15.5% at harvest, whereas in the second season the fruit had a lower TSS content of 12.2% at harvest. On the contrary, ripening season did not influence TSS level in four Israeli grown pomegranate accessions 'PG 128-29', 'PG 130-31', 'EG1' and 'EG 2' (Borochov-Neori et al., 2011). Shwartz et al. (2009) reported high correlation between TSS

content and sugar components in the 121-2 and 101-2 accessions of the Wonderful cultivar during fruit development.

4.2.2. Sugars

Juice from fully mature pomegranate fruit has 12 - 16% sugar content, consisting mainly of glucose and fructose (Nerd, 1965; Gil et al., 1996a; Al-Maiman and Ahmad, 2002; Shwartz et al., 2009). According to Legua et al. (2000), glucose was the more predominant sugar than fructose. This is in agreement with Al-Maiman and Ahmad (2002), who reported that the concentration of glucose was higher than fructose in unripe, half-ripe and full-ripe fruit of 'Taifi' cultivar. Apart from glucose and fructose, Legua et al. (2000) found negligible levels of saccharose and maltose in Spanish pomegranates clones 'ME5', 'ME17' and 'MO6' during fruit development. Fructose and glucose concentration increased significantly during fruit maturation in '121-2' and '101-2' accessions of the 'Wonderful' (Shwartz et al., 2009). According to the authors, the sweet accession '121-2' had similar levels of glucose and fructose with accession 101-2, which has a sweet-sour taste. In 'Ganesh', the content reducing sugars remained unchanged during the first 80 DAFS but significantly increased at 140 DAFS when fruit became full ripe (Kulkarni and Aradhya, 2005).

4.2.3. pH

The pH value of pomegranate juice characterizes its acidic taste, the former having an inverse correlation with the latter (Zarei et al., 2011). In 'Taifi', the pH of the juice increased with maturity, reaching a maximum value of 3.57 at the full-ripe stage (Al-Maiman and Ahmad, 2002). During fruit development of Israeli grown pomegranates, Borochov-Neori et al. (2009) found that pH values in early- and late-cultivars ranged from 3.8 - 4.2 and 3.2 - 3.4, respectively. However, Gil et al. (1996a) did not find any significant changes in pH of 'Mollar' fruit harvested at different maturity stages (This is contrary to the findings of Zarei et al. (2011), who reported significant increases in pH value during fruit ripening of Rabbab-e-Fars cultivar.

4.2.4. Titratable acidity (TA)

Titratable acidity (TA) is an important quality attribute of pomegranate juice (Shwartz et al., 2009). High acidity content in juice of Wonderful cultivar during fruit development was used to classify the fruit as a late cultivar (Shulman et al., 1984). Generally, TA in

pomegranate juice decreases with advancing fruit maturation but the rate of decline differs among cultivars and growing region. For Israeli grown pomegranate cultivars, Shwartz et al. (2009) reported a significant decline in TA content in juice of accession '101-2' while a decrease in TA for accession '121-22' was not significant. TA levels in pomegranate grown in warmer valley region were lower than those grown in the coastal plain region of Israel (Shulman et al., 1984). According to Varasteh et al. (2008), TA increased at the beginning of fruit set and decreased until the end of growing season for 'Malas-e-Torsh-e-Saveh' grown in Iran. Reduction in TA during fruit growth and development was also found in juice of 'Wonderful', 'Taifi', 'Codpa' and 'Ganesh' (Ben-Arie et al., 1984; Gil et al., 1995; Al-Maiman and Ahmad, 2002; Labbé et al., 2010; Kulkarni and Aradhya, 2005). Chace et al. (1981) studied the 'Wonderful' over three seasons and considered a TA value of 1.8% as the most satisfactory maturity standard.

4.2.5. Brix-acid ratio (TSS:TA)

Decrease in TA levels during fruit development with concomitant increase in TSS content is an inherent process during growth and development of pomegranate which imparts the characteristic juice flavour (Kulkarni and Aradhya, 2005). The Brix:acid ratio (TSS:TA), also referred to as maturity index (MI) by Hernandez et al. (1999), is commonly used to define the 'taste' of pomegranate fruit during development (Shwartz et al., 2009), although Ben-Arie et al. (1984) observed that the ratio did not correlate satisfactorily with taste for Israeli grown 'Wonderful'. TSS:TA ratio increased by 48.5% from 0.66 at unripe stage to 0.87 at full-ripe stage of 'Taifa' (Al-Maiman and Ahmad, 2002). Zarei et al. (2011) highlighted the importance of TSS:TA value as a good indicator of fruit maturity of 'Rabbab-e-Fars' because it increased significantly during fruit ripening. The authors reported TSS:TA values of 3.73, 7.93 and 14.48 in fruit at 20, 80 and 140 DAFS, respectively. The TSS:TA value recorded for 'Wonderful' grown in Israel varied between locations and growing seasons (Ben-Arie et al., 1984).

4.2.6. Organic acids

The composition and concentration of organic acids are important factors that determine consumer perceptions of both sweetness and sourness in pomegranate fruit (Jalikop, 2007; Holland et al., 2009). Several organic acids have been reported in pomegranate fruit juice but the major acid accounting for titratable acidity is citric acid

(Melgarejo et al., 2000; Shwartz et al., 2009). The concentrations of different acids and total organic acid significantly differed among clones (Legua et al., 2000). In pomegranate clones 'ME5', 'ME17' and 'MO6', malic acid was the most predominant, followed by citric acid. However, in two accessions of Wonderful cultivar grown under the same agro-climatic conditions, citric acid was predominant in accession '101-2' and lowest in accession '121-2' compared to other organic acids (Shwartz et al., 2009). Furthermore, oxalic and succinic acids were only found in accession '121-2' and the concentrations decreased significantly with advancing maturity. During fruit development of both accessions, citric acid concentration decreased and malic acid increased (Shwartz et al., 2009). Several studies have shown strong correlations between organic acids and titratable acidity in harvested fruit (Shwartz et al., 2009; Hasnaoui et al., 2011; Mena et al., 2011).

4.2.7. Ascorbic acid

For Ganesh cultivar, ascorbic acid concentration declined rapidly in pomegranate juice during initial stages of fruit maturity, and further declined gradually until fruit reached advanced maturity (Kulkarni and Aradhya, 2005). A similar trend was previously reported by Al-Maiman and Ahmad (2002) for Taifi cultivar. These studies are in agreement with Zarei et al. (2011), who reported a significant decrease from 25.84 g/100 g in 20 day-old fruit to 9.78 g/100 g in 140 day-old fruit of Rabbab-e-Fars cultivar. On the contrary, however, an opposite trend was observed during the development of two accessions of Wonderful cultivar grown in Israel (Shwartz et al., 2009).

4.2.8. Phenolics concentrations

The loss of astringency is one of the desirable changes that occur during maturation and ripening of many pomegranate cultivars and this is primarily due to the decline in phenolic compounds (Al-Maiman and Ahmad, 2002; Kulkarni and Aradhya, 2005). Kulkarni and Aradhya (2005) reported 54.5% reduction in total phenolics during the initial stage of fruit development between 20 and 40 DAFS, and the decrease continued until the 140th day, when fruit were considered full-ripe. Similarly, the lowest total phenolic concentration in 'Rabbab-e-Fars' fruit (786.20 mg/100 g) was measured at commercial harvest (Zarei et al., 2011). Weerakkody et al. (2010) reported a decline of about 50% in total phenolic concentration in the first growing season studied for Wonderful cultivar; however during the second season, phenolic concentration initially increased for 10 weeks after fruit set and then

declined rapidly afterwards. Similarly, Mirdehghan and Rahemi (2007) observed an increase in phenolics for 'Malas Yazdi' at early maturity stage in both fruit peel and arils, which later decreased with advancing maturation. Another study on Israeli grown pomegranate cultivars showed that midwinter ripened fruit had the highest concentration of total phenolics compared to early summer, late summer and autumn ripened fruit (Borochov-Neori et al., 2011). The influence of maturity stage and growing region on the phenolic concentration of Chilean grown Codpa pomegranate cultivar was investigated by Labbé et al. (2010) and the results showed that the highest total phenolic concentration was found in fruit juice at green maturity stage, which significantly decreased with advancing maturity.

In terms of human health, the higher the level of phenolic compounds the higher the total antioxidant activity of pomegranate fruit juice and its relative human health benefit (Aviram et al., 2000; Gil et al., 2000; Tzulker et al., 2007). Although juice containing very high concentrations of phenolic compounds could be less desirable due to high astringency (Kader, 2006), several reports have demonstrated that a significant reduction in phenolic compounds in pomegranate coincides with a sharp decline in juice antioxidant capacity during fruit development (Borochov-Neori et al., 2009; Shwartz et al., 2009; Labbé et al., 2010; Weerakkody et al., 2010). Common phenolic compounds in pomegranate juice include ellagic acid derivatives and hydrolysable tannins (Gil et al., 2000; Shwartz et al., 2009).

4.2.9. Anthocyanin composition

The red colour of pomegranate juice is due to the presence of anthocyanins (Shulman et al., 1984). Generally, studies have shown that the anthocyanin profile is identical in many pomegranate cultivars irrespective of the growing region, but the relative amounts of individual anthocyanins differ among cultivars (Gil et al., 1995). Increase in total anthocyanin was not observed in 'Ganesh' until the 80 DAFS, but the increase was significant with advancing maturation (Kulkarni and Aradhya, 2005). Similarly, Gil et al. (1996a) observed a slow increase in anthocyanin during the first stages (26 -28 weeks) of fruit ripening, followed by very fast increment at the middle stages (29-30 weeks); however no increment in juice pigmentation was detected during the late stages between 31and 32 weeks after fruit set. This agrees with the patterns of anthocyanin accumulation in pomegranate fruit reported by Zarei et al. (2011) and Shwartz et al. (2009). Borochov-Neori et al. (2011) studied the anthocyanin concentration in pomegranate cultivars during early summer, late summer, autumn and winter

seasons, and reported over 200-fold concentration of anthocyanin in winter ripened fruit compared to fruit harvested in summer season.

The anthocyanin profile of pomegranate juice is known to constitute six anthocyanins, namely the 3-glucoside and 3, 5-diglucosides of pelargonidin, cyanidin and delphinidin (Gil et al., 1995, 1996a; Hernandez et al., 1999; Borochov-Neori et al., 2011). The study conducted by Gil et al. (1995) on Tunisian grown pomegranate ('Chelfi', 'Mekki' and 'Zehri') cultivars showed the same anthocyanin profile in all the cultivars, but the total amount of anthocyanins was largely affected by differences in cultivar, maturation stage and the geographical source of the fruit. In Spanish pomegranate clones 'ME16', 'VA1', 'PTO8', 'BA1' and 'MA2', Hernandez et al. (1999) reported between 5- and 10-fold increases in anthocyanins in fruit from immature to mature maturity stages. Delphinidin 3,5-diglucoside was identified as the dominant pigment in early ripening stages while the monoglucoside derivatives of cyanidin 3-glucoside and delphinidin 3-glucoside increased considerably in the later stages.

4.2.10. Proximate compositions

Moisture content varies significantly from unripe to full-ripe fruits with an average value of 84.57% during pomegranate fruit development. However, moisture content did not differ significantly in arils at different fruit maturity stages for 'Taifi' (Al-Maiman and Ahmad, 2002). Gozlekçi et al. (2011) measured moisture content of 21.14% at immature stage, 74.85% at unripe stage and 75.99% in full-ripe fruit juice for 'Hicaznar'.

For 'Ganesh', highest concentration (209 mg/100 g) of total protein was found in fruit aril at 20 DAFS. The total protein decreased rapidly by 66.9% at 80 days and then increased between 80 and 120 DAFS by 58.7%, followed by a decrease of 6.3% at 140 DAFS (Kulkarni and Aradhya, 2005). On the contrary, Al-Maiman and Ahmad (2002) found no significant changes in juice and aril protein contents during ripening and no significant changes were observed in both saturated and unsaturated fats contents at different developmental stages.

4.2.11. Volatiles

There are very few reported studies on volatile composition of fresh pomegranates or processed juices, and no information was found on the evolution of volatile compounds in pomegranate during fruit development. Pomegranate fruit has low concentrations of volatile compounds, leading to low intensities of both odour and aroma of the fruit parts (Carbonell-Barrachina et al., 2012). Only nine compounds were found in juices of 'Berit Kazeroon'

grown in Iran (Raisi et al., 2008). Vazquez-Araujo et al. (2010) studied pomegranate and berry juices and only identified 10 volatiles. The only common volatile compound found in the two studies was 1-hexanol. More recently, however, Carbonell-Barrachina et al. (2012) reported 18 aromatic compounds in Spanish pomegranates whereas Calín-Sánchez et al. (2011) and Melgarejo et al. (2011) identified 21 different aromatic compounds. Two aldehydes and one alcohol were predominant compounds in Spanish samples, and several of the volatiles identified by the authors were present in very low concentrations. In addition, 23 volatile compounds, including aldehydes, alcohols and terpenes were identified in pomegranate juice of 'Wonderful', with terpene derivatives constituting the major volatile compounds in the juice samples (Vázquez-Araújo et al., 2011).

4.3. Changes in mineral nutrient concentration

There is limited information on the elemental changes in pomegranate fruit parts during maturation. Al-Maiman and Ahmad (2002) reported that K was the most abundant element, followed by Na and Ca in the fruit of 'Taifi'. The highest concentration of K in arils and juice was found in unripe and full-ripe fruit, respectively. In addition, the concentrations of P, Na and Ca in arils increased significantly while Mg, Na and Ca in juice decreased with advancing maturity. The concentration of micronutrients studied was in the decreasing order of Zn > Fe > Cu. Mirdehghan and Rahemi (2007) studied the relationship between time and mineral nutrient accumulation in fruit arils and peel of 'Mala Yazdi'. The authors observed increases in the accumulation of macronutrients at successive harvests throughout fruit development, whereas the concentration of all micronutrients (with the exception of B) in both arils and peel decreased from a maximum value to a minimum value between 10 and 140 DAFB.

In their study of 'Hicaznar', Gozlekçi et al. (2011) reported that K was the major element present in all the fruit parts. In fruit peel, P was the highest at unripe maturity stage, while Mg, Mn Zn and Cu were the highest at immature stage. However, both mature and unripe stages had equal amount of K, and at full mature stage Ca and Fe were the highest. According to the authors, the concentrations of minerals in aril showed that mature fruit stage contained the highest concentration of P, K, Mg, Mn, Zn, Cu, Ca and Na while Fe was the highest at full mature stages of fruits. Moreover, in fruit kernel and juice, K, Mg, Mn, Zn and Cu were the highest at unripe stage, while P and Ca were the highest at full mature stage in fruit kernel. In the juice, concentrations of P, Ca, Mg and Na were similar at both unripe and

full-ripe stages and Mn, Zn and Cu were the highest at unripe stage. The concentration of minerals in fruit parts investigated, at each maturity stage followed the order of K > P > Ca > Mg > Na > Mn > Zn > Fe > Cu with exceptions of Ca higher than P in fruit peel.

The composition and concentration of mineral nutrients at fruit developmental stages have been implicated in cracking incidence in pomegranate fruit. The disorder was reported to be associated with B and Ca deficiency (Mir et al., 2012). Mirdehghan and Rahemi (2007) highlighted the association of significant accumulation of Ca during early growth to the fruit structural properties (thick peel and hard kernel) of 'Mala Yazdi'.

4.4. Physiological changes

4.4.1. Respiration and ethylene production

The non-climacteric nature of pomegranate fruit during development and ripening was first reported by Lee et al. (1974), who observed a decline in respiration rate during fruit development. Further studies by Ben-Arie et al. (1984) and Shulman et al. (1984) on Israeli grown pomegranates provided more information on the respiratory behavior of the fruit at different maturity stages.

For Wonderful cultivar, Ben-Arie et al. (1984) observed a gradual decline in fruit respiration during and after 1 month of fruit set, both on the day of harvest and thereafter. In young fruit initial rise in CO_2 output occurred for a day, and then gradually declined. Furthermore, with advancing maturity the production of CO_2 became progressively less pronounced, indicating the non-climacteric nature of the fruit. Only a trace of ethylene production was detected at all maturity stages, even when fruit were sealed in respiration jars for 4 h. However, the treatment of fruit with exogenous ethylene induced higher respiration rate by 30% and 100% in 1 and 5 month-old fruit, respectively. Respiration rate was 2-fold higher in peel than arils, while intact fruit harvested at the same time had the same respiration rate as the arils.

The respiratory pattern in Wonderful and Mule's Head pomegranate cultivars also confirmed the non-climacteric nature of the fruit. Shulman et al. (1984) observed a decrease in fruit respiration rate with advancing maturity. Low, but uniform CO_2 evolution was measured in fruit from July to October (mature and ripening period) of the growing season; between 15 and 25 mL kg^{-1} h^{-1} in 'Wonderful' and 25 and 35 mL kg^{-1} h^{-1} in 'Mule's Head'. When fruit at different maturity stages were kept at 22°C for 11 days, CO_2 evolution was relatively uniform and decreased only slightly with time in 'Wonderful', while in 'Mule's

Head' a constant level of CO_2 was observed for six days. No measurable ethylene production was found in both cultivars.

5. Fruit responses during cold storage

5.1. Weight loss, physical and textural changes

The storage life of pomegranate fruit is not more than 10 - 15 days at room temperature (Waskar, 2011). However, fruit can be stored for extended periods if held under refrigeration in air and high relative humidity (Elyatem and Kadar, 1984). Storage trials conducted on 'Wonderful' stored at 0, 2.2, 5 and 10°C showed that weight loss increased with storage temperature and time (Elyatem and Kadar, 1984). The authors found no significant temperature effects on changes in peel or juice colour during storage for up to 8 weeks, but fruit stored at 5°C and 10°C maintained red peel colour better than those kept at lower temperatures. This is in agreement with Gil et al. (1996b), who found no significant differences in aril colour of pomegranate ('Mollar') stored at 5°C for 1.5 months. For 'Taeifi', 'Banati' and 'Manfaloti', Al-Mughrabi et al. (1995) observed significantly higher weight loss in fruit stored at room temperature than those stored at 5°C and 10°C. Differences between weight loss of fruits stored at 5°C and 10°C were significant only after 2 weeks of storage and after 6 weeks of storage weight loss was 18.32%, 21.93% and 32.83% in fruit stored at 5°C, 10°C and room temperature, respectively. According to Opara et al. (2008), refrigerated fruit lost about 4% in weight after 2 weeks of storage and weight loss remained below 4% throughout the 6-week storage period, whereas weight loss of fruit stored in the normal room condition increased linearly from 9% to 16.5% for 'Helow' pomegranate. Aside from weight loss, the authors observed a significant decrease in diameter of fruit stored at room temperature than those stored at 5°C, presumably due to peel dehydration. After eight weeks, fruit weight and length and juice content were significantly lower in fruit stored at 10°C than those at 5°C.

Nanda et al. (2001) studied peel weight loss of non-wrapped fruit at stored at 8°C, 15°C and 25°C for 'Ganesh'. Fruit became tough, desiccated and less firm after 1, 5 and 7 weeks of storage at 25, 15 and 8°C, respectively. Juice colour changed slightly during storage at different temperatures. Bchir et al. (2012) reported that arils obtained from frozen fruit lost their textural property when compared to those from fresh fruit. The study on two Chilean

pomegranate cultivars showed loss of firmness in aril during fruit storage at 5°C for 8 weeks and decrease in aril colour stored at 5°C over 12 weeks (Labbé et al., 2010).

5.2. Changes in biochemical quality attributes

In pomegranate fruit (cv. 'Wonderful') stored at 5°C for 12 weeks before transfer to 20°C for four days, decrease in TSS and TA values coincided with increased pH value in fruit (Kader et al., 1984). However, Elyatem and Kader (1984) reported no significant differences in TSS, pH and TA among fruit ('Wonderful') stored for 8 weeks at 5°C, although a decrease in TA was noted after 8 weeks of storage. This was similar to the findings by Gil et al. (1996b) on 'Mollar', who reported no significant decrease in TSS and TA values when pomegranate fruit harvested at two maturity stages were stored for 6 weeks at 5°C. According to Artes et al. (2000), decrease in TSS content was not significant whereas TA decreased significantly in fruit ('Molla de Elche') stored at 5°C for 90 days and 6 additional days at 15°C and 75% RH. Contrarily, Mirdehghan et al. (2006) reported a significant increase in organic acid and sugar concentrations in the same 'Mollar de Elche' during cold storage for 90 days at 2°C and 3 additional days at 20°C. For pomegranate fruit grown in Libya, Ghafir et al. (2010) reported a significant increase in TSS and significant decrease in TA and pH values in fruits stored at 5 and 7°C over 120 days.

These findings are similar to those of Al-Mughrabi et al. (1995), who reported an increase in TSS levels in pomegranate fruit ('Taeifi', 'Banati' and 'Manfaloti') during cold storage. After four weeks of storage, the authors observed significantly lower TSS levels in fruit stored at 5°C and 10°C treatments, respectively, compared to fruit stored at room temperature, whereas fruit acidity (%) did not follow a consistent trend during storage period in all treatments. In another study on two Chilean pomegranate cultivars stored at 5°C over 12 weeks, Labbé et al. (2010) reported significant decrease in TSS value until the eighth week, followed by significant increase in TSS on the 12th week, while TA decreased throughout the storage period.

In 'Mollar de Elche' fruit stored at 2°C and 90% RH, the concentration of total phenolic compounds at harvest was 261.19 mg/100g which declined throughout storage in untreated fruit with a final concentration of 234.10 mg/100g after 84 days (Sayyari et al., 2011). For 'Assaria' cultivar grown in Portugal, Miguel et al. (2004) reported an increase in juice anthocyanin concentration after first month of fruit storage at 5°C. However, Artes et al.

(2000) found no differences in anthocyanin concentration between harvest and shelf life of pomegranate fruit stored at 5°C for 12 weeks.

5.3. Respiration rate and ethylene production during cold storage

The non-climacteric nature of pomegranate fruit was reported by Elyatem and Kader (1984). Fruit of 'Wonderful' cultivar had a relatively low respiration rate, which declined with time after harvest (Elyatem and Kader, 1984). The ranges of respiration rates for California-grown 'Wonderful' pomegranates were 2 - 4, 4 - 8 and 8 - 18 mL CO_2/kg/h at 5°C, 10°C and 20°C, respectively, while trace quantities of ethylene production (less than 0.2 µL/kg/h) were measured in fruit stored at 20°C for 2 weeks (Elyatem and Kader, 1984; Kader, 2006). In South African grown 'Acco' and 'Herskawitz', a reduction in temperature from 15 to 5°C decreased respiration rate by about 68 and 67% for whole fruit and, 67 and 70% for fresh arils, respectively, with an average of 14.67 mL CO_2/kg/h respiration rate in whole fruit (Caleb et al., 2012). López-Rubira et al. (2005) reported a respiration rate of 14.45 nmol CO_2/kg/s (1.15 mL CO_2/kg/h) for pomegranate arils ('Mollar') stored at 5°C in air, similar to the study by Gil et al. (1996a), who reported respiration rate of 14.77 nmol CO_2/kg/s (1.30 mL/kg h) for fresh arils ('Mollar') stored at 4°C.

5.4. Common physiological disorders during cold storage

Pomegranate fruit is highly susceptible to chilling injury if stored longer than 1 month at temperatures below 5°C, or longer than 2 months at 5°C (Elyatem and Kader, 1984; Kader et al., 1984; Kader, 2006). Chilling injury became more noticeable in fruit transferred to 20°C for 3 to 4 days after 8 weeks of cold storage. However, no sign of chilling injury was observed in fruit stored at 10°C even after 12 weeks of storage. According to Kader et al. (1984), external symptoms of chilling injury in pomegranate California grown 'Wonderful' included browning of the peel and increased susceptibility to decay, while internal symptoms included loss in red colour of the arils and brown discolouration of the white membrane.

Husk scald is also a common problem in pomegranate fruit. It is a superficial browning which generally develops from the stem end of the fruit and then spreads towards the blossom end as it increases in severity (Ben-Arie and Or, 1986; Defilippi et al., 2006). Scalding has no observable internal changes on the arils or on the white astringent membrane as observed with chilling injury, and fruit stored at temperatures above 5°C are more

susceptible to the disorder (Ben-Arie and Or, 1986). Fruit kept in regular atmosphere exhibited some scald after 4 to 6 months at 7°C (Defilippi et al., 2006).

Internal breakdown is another physiological disorder in pomegranates. It results in the arils having a "light streaky" appearance and a "flat" taste (Pantastico, 1975). The cause of this disorder is not known.

6. Existing recommendations on fruit maturity index and storage

6.1. Maturity index

With experience, correct harvest maturity for pomegranate fruit can be determined by tapping the fruit and listening for a metallic sound (Mir et al., 2012). However, a science-based maturity index for pomegranate cultivars other than Wonderful cultivar is not currently available or well established.

In general, for 'Wonderful,' the acids should be lower than 1.85%, soluble sugar content greater than 15 - 17% and the sugar:acid ratio greater than 18.5. Red juice colour equal to or darker than Munsell colour 5 R 5/12 is also a requirement in the USA market (Kader, 2002; 2006). In Iran, it was reported that the optimum harvesting time was when the soluble solids content reached 17.5% (Sherafatian, 1994).

6.2. Storage recommendations

Recommended storage conditions for pomegranate fruit are summarized in Table 3. Overall, pomegranate can best be stored at low temperature and high relative humidity.

7. Conclusion and future prospects

In recent years, the search for an objective, easy, reliable and simple determination of maturity has occupied the attention of many pomegranate growers in the different production regions. In practice, it is the duty of the fruit industry to establish a minimum maturity index based on available scientific information. Currently, literature relating to the various quality attributes of pomegranates at commercial harvest is voluminous, yet limited information is available on the pattern of maturity indices and the timing of fruit harvest of several commercial pomegranate cultivars.

A science-based maturity index for pomegranate cultivars other than 'Wonderful' is not currently available. Current scientific knowledge that has been accumulated on the

physico-chemical and physiological attributes of fruit maturity need to be harnessed towards the development of reliable maturity indices by correlating changes in fruit physico-chemical attributes during maturation with optimum eating quality and postharvest storage performance of fruit. Extensive review of scientific literature showed that lack of publications in this area. This approach should be thoroughly studied to develop suitable tools for harvest maturity management of fruit to provide consumers with consistent supply of good quality, tasty, flavourful and healthful produce.

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Table 1

Summary of methods used for evaluating maturity, quality and physiological attributes of pomegranate fruit

Index	Method of determination	Subjective	Objective	Destructive	Non-destructive	Selected reference
Elapsed fruit maturation description	Computational (Days/months after full bloom)		x		x	Ben-Arie et al.,1984; Mirdehghan and Rahemi, 2007
	Computational (Days/weeks after fruit set)		x		x	Shwartz et al., 2009; Zarei et al., 2011
	Maturity estimation (Immature, half-ripe & full-ripe stages)	x			x	Al-Maiman and Ahmad, 2002; Gozlekçi et al., 2011
Fruit heat units	Computational from weather data (Temperature)		x		x	Melgarejo et al., 1997; Borochov-Neori et al., 2009
Physico-textural property						
Fruit mass	Weight measuring devices		x		x	Al-Said et al., 2009; Gozlekçi et al., 2011
Fruit length & diameter	Linear dimension measurement (Vernier caliper)		x		x	Al-Said et al., 2009; Al-Yahai et al., 2009
Fruit volume	Mathematical measurement		x		x	Al-Yahai et al., 2009
	Water displacement method		x		x	Al-Maiman and Ahmad, 2002; Zarei et al., 2011

Table 1 contd.

Index	Method of determination	Subjective	Objective	Destructive	Non-destructive	Selected reference
Peel colour	Light reflectance (Chromameter)		x		x	Opara et al., 2009; Shwartz et al., 2009
	Visual (Colour charts)	x			x	Ben-Arie et al., 1984; Shulman et al., 1984
Aril colour	Light reflectance (Chromameter)		x	x		Opara et al., 2009; Shwartz et al., 2009
Textural property	Compression test (Texture profile analyzer)		x	x		Al-Said et al., 2009; Sayyari et al., 2011
Chemical composition						
Juice content/free water	Extraction		x	x		Al-Said et al., 2009;Weerakkody et al., 2010
	Magnetic Resonance Imaging (MRI)		x		x	Khoshroo et al., 2009
Soluble solids content	Light reflectance (Refractometer)		x	x		Shwartz et al., 2009; Fawole et al., 2012
Juice colour	Light reflectance (Spectrophotometer/chromameter)		x	x		Fawole et al., 2012; Zaouay et al., 2012
Sugar composition	High performance liquid chromatography		x	x		Shwartz et al., 2009; Dafny-Yalin et al., 2010
Titratable acidity	Titration (Manual or automatic techniques)		x	x		Ben-Arie et al., 1984; Fawole et al., 2012
Organic acids	High performance liquid chromatography		x	x		Shwartz et al., 2009; Dafny-Yalin et al., 2010
Juice pH	pH meter		x	x		Al-Said et al., 2009; Fawole et al., 2012
Total phenolics	Chemical test (Folin C assay)		x	x		Shwartz et al., 2009; Fawole et al., 2012

Table 1 contd.

Index	Method of determination	Subjective	Objective	Destructive	Non-destructive	Selected reference
	High performance liquid chromatography		x	x		Gil et al., 2000; Fischer et al., 2011
Anthocyanins	Chemical test (pH differential assay)		x	x		Shwartz et al., 2009; Fawole et al., 2012
	Light absorbance (Spectrophotometer)		x	x		Shulman et al., 1984
	High performance liquid chromatography					Gil et al., 1995; Borochov-Neori et al.,2011
Ascorbic acid	High performance liquid chromatography		x	x		Kulkarni and Aradhya, 2005;Shwartz et al., 2009
	Titration technique		x	x		Opara et al., 2009; Zarei et al., 2011
Kernel oil content	Solvent extraction		x	x		Fadavi, et al., 2005; Hassan et al., 2012
Flavour						
Juice taste	Sensory measurement (Panel test)	x	x	x		Gadže et al., 2011; Calín-Sánchez, et al., 2011
Aroma and volatiles	Gas chromatography		x	x		Vazquez-Araujo et al., 2011; Calín-Sánchez, et al., 2011
	Sensory measurement (Panel test)	x	x	x		Nanda et al.,2001; Calín-Sánchez, et al., 2011
Mineral nutrients	Spectrophotometry (Atomic and flame tests)		x	x		El Kar et al., 2011; Fawole and Opara, 2012

Table 1 contd.

Index	Method of determination	Subjective	Objective	Destructive	Non-destructive	Selected reference
Physiological attributes						
Respiration rate	Gas chromatography		x		x	Ben-Arie et al., 1984; Elyatem and Kader, 1984
	Headspace gas analysis (PBI-Dansensor)		x		x	Caleb et al., 2012
Ethylene production	Gas chromatography		x		x	Ben-Arie et al., 1984; Elyatem and Kader, 1984
Internal quality/structure	Nuclear Magnetic Resonance (NMR)		x		x	Zhang and McCarthy, 2012
	Magnetic Resonance Imaging (MRI)		x		x	Khoshroo et al., 2009
	Visual examination	x		x		Elyatem and Kader, 1984; Nanda et al., 2001

Table 2

Changes in physical attributes of fruit during growth and maturity of two pomegranate fruit cultivars

Cultivar	Characteristics	Developmental stages			Area	Reference
		Immature	Unripe/half-ripe	Mature		
'Taifi'	Length (mm)	66.1	67.6	65.5	Riyadh, Saudi Arabia	Al-Maiman and Ahmad, 2002
	Diameter (mm)	35.4	36.1	36.7		
	Length/diameter (L/D)	1.87	1.87	1.79		
	Volume (cm3)	126.74	161.02	156.74		
	Densities of fruit	1.29	1.2	1.38		
	Fruit weight (g)	163.51	193.82	216.5		
	Skin weight (g)	48.34	54.43	69.02		
	Aril weight (g)	90.01	111.95	129.27		
	Fruit peel (%)	29.56	28.08	31.87		
	Aril yield (%)	55.05	57.77	59.71		
	Juice weight (g)	59.99	67.60	81.03		
	Juice yield per fruit (%)	30.57	30.32	32.88		
'Rabbab-e-Fars'	Length (mm)	53.11	65.27	71.15	Yazd Province, Iran	Zarei et al., 2011
	Diameter (mm)	47.06	66.64	83.16		
	Length/diameter (L/D)	1.13	0.97	0.85		
	Volume (cm3)	78.71	159.2	250.15		

Table 2 contd.

Cultivar	Characteristics	Developmental stages			Area	Reference
		Immature	Unripe/half-ripe	Mature		
'Rabbab-e-Fars'	Densities of fruit	0.97	0.94	0.94	Yazd Province, Iran	Zarei et al., 2011
	Fruit weight (g)	76.71	150.32	235.09		
	Skin weight (g)	56.19	83.93	98.96		
	Seed weight (g)	20.52	66.38	136.13		
	Fruit peel (%)	73.33	56.13	42.13		
	Aril yield (%)	26.66	43.86	57.86		
	Juice weight (g)	5.80	50.94	112.00		
	Juice yield per fruit (%)	7.56	33.78	48.01		

Table 3

Recommended storage requirements for some pomegranate cultivars

Cultivar	Temperature. (°C)	RH (%)	Storage period (month)	References
'Hicaz'	6	85 - 90	5	Kupper et al., 1994
'Hicaz'	8 - 10	85 - 90	< 2	Kupper et al., 1994
'Khandhar'	0 - 1.7	85 - 95	< 3	Pantastico et al., 1975
'Molas Torsh', 'Saveh Hendes', 'Shirin Paezeh', 'Sefi Torsh'	1	90 - 95	4.5	Askary and Shahedi, 1994
'Wonderful'	5	90 - 95	2	Kader, 2006
'Wonderful'	7	90 - 95	>2	Kader, 2006

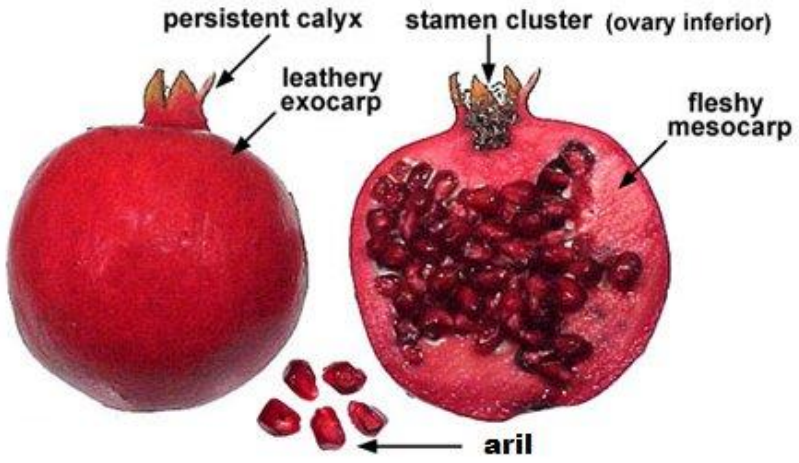

Figure 1. A typical anatomic structure of pomegranate (*Punica granatum*) fruit

Source: http://waynesword.palomar.edu/termfr4.htm (modified, 13/02/2013)

SECTION II

FRUIT GROWTH AND EVOLUTION OF MATURITY INDICES OF 'BHAGWA' AND 'RUBY' POMEGRANATE CULTIVARS *

- Paper 2: Fruit growth dynamics, respiration rate and physico-textural properties during pomegranate development and ripening[1]
- Paper 3: Seasonal variation in aroma volatiles, chemical composition, antioxidant capacity and mineral elements of pomegranate during fruit development [2,3,4&5]

* Studies were done during the 2010/11 and 2011/12 growing seasons

1. Sci. Hort. 157, 90 – 98.
2. Sci. Hort. 150, 37 – 46.
3. S. Afr. J. Bot. 85, 23 – 31.
4. Afr. J. Biotechnol. 12, 4006 – 4019.
5. Acta Hort. 1007, 461– 469.

This section presents a compilation of manuscripts where each paper is an individual entity and some repetition between papers, therefore, has been unavoidable.

PAPER 2

Fruit growth dynamics, respiration rate and physico-textural properties during pomegranate development and ripening

Abstract

The time course and pattern of fruit growth, and changes in physical and physiological properties and texture dynamics of pomegranate fruit (cvs. 'Bhagwa' and 'Ruby') were studied over two seasons under South African agro-climatic conditions. Significant variations in fruit growth, respiration rate and physico-textural properties of fruit were found at five maturity stages (S1-S5). Fruit lineal dimensions (length and diameter) and weight exhibited a linear growth pattern. All fruit cultivars showed a decline in respiration rate during fruit development, with the highest respiration rate measured in immature fruit ('Bhagwa', 66.83 mL CO_2 $kg^{-1}h^{-1}$; 'Ruby', 51.17 mL CO_2 $kg^{-1}h^{-1}$) and minimum rates in fully ripe fruit ('Bhagwa', 23.84 mL CO_2 $kg^{-1}h^{-1}$; 'Ruby', 19.16 mL CO_2 $kg^{-1}h^{-1}$). No ethylene gas was detected throughout fruit development. Fruit pigmentation increased with advancing maturity and the lowest total colour difference (TCD) between fruit peel and arils was noted in immature fruit. Aril yield remained less than 50% until the middle of the season (S3), and increased until commercial harvest for 'Ruby', but remained below 50% throughout the season for 'Bhagwa'. Fruit peel thickness increased with advancing maturity as the cells underwent periclinal growth, while fruit calyx opening reduced significantly (p<0.05) with advancing maturity for both cultivars. The kernel index (KI=kernel weight/aril weight) ranged from 20.72 (S1) - 13.46% (S5) for 'Bhagwa' and from 19.21 (S1) - 11.83% (S5) for 'Ruby'. This study showed that textural dynamics of pomegranate fruit peel and arils change with fruit maturity. Textural dynamics of aril revealed increasing trend in bioyield force and elasticity with advancing maturity. The results indicated that fruit reached mature stage between the 132 - 139 DAFB for 'Ruby' and 140- 165 DAFB for 'Bhagwa'. This period could be regarded as the physiological mature stage of the fruit for the investigated cultivars. These findings could be used as a tool to assist growers in assessing fruit readiness for harvest.

Keywords: pomegranate, growth, respiration, texture, South Africa

1. Introduction

The pomegranate (*Punica granatum* L.) is a tropical and subtropical attractive deciduous or evergreen shrub belonging to Punicaceae family. It is native to the area from Iran to the Himalayas in northern India and has been cultivated and naturalized over the whole Mediterranean region since ancient times (Holland et al., 2009). The pomegranate plant is tolerant of many different soil conditions, but thrives well under sunlight and mild winters with minimal temperatures not lower than -12 ºC and hot dry summers (Levin, 2006). Pomegranate is one of the hardest fruit crops, round or spherical in shape, with a fleshy, tubular calyx and leathery skin often deep pink or rich red in colour (Morton, 1987). The interior of the fruit is separated by membranous walls and white spongy tissue into compartments packed with transparent sacs filled with fleshy, juicy, red, pink or whitish pulp called the arils. In each aril sac, there is one white or red, angular, soft or hard seed (Biale, 1981; Morton, 1987; Holland et al., 2009; Levin, 2006).

In recent years, pomegranate has gained popularity due to its multi-functionality and great nutritional benefit in the human diet. The fruit is now grown globally under different climatic regions, satisfying the nutritional and medicinal needs of populations of various countries (Holland et al., 2009; Wetzstein et al., 2011). Major pomegranate producers include India, Israel, Afghanistan, Iran, Egypt, China, Japan, USA, Russia, Australia, South Africa and Saudi Arabia and in the subtropical areas of South America (Elyatem and Kader, 1984; Artes et al., 2000; Holland et al., 2009; Faria and Calhau, 2010).

Like most fruit, the pomegranate has many generally well-defined stages of growth and development, and advancing maturity corresponds to a number of coordinated physiological, biochemical, and structural processes that result in changes in size, colour and flavour (Nunes et al., 2009; Opara, 2000). It has been demonstrated that fruit of pomegranate cultivars vary considerably in their physico-chemical and textural properties (Gozlekci and Kaynak, 2000; Martinez et al., 2006; Al-Said et al., 2009; Opara et al., 2009; Hasnaoui et al., 2011). In addition, fruit quality attributes such as size, colour, juiciness, taste, and seed hardness, among others, are influenced primarily by genetics, but are also influenced by the environment in which the crop is grown (Jalikop, 2007; Holland et al., 2009). For pomegranate fruit maturity assessment, physical attributes are as important as biochemical indices. Several studies have assessed pomegranate

fruit maturity status based on a combination of physical, physiological and biochemical indices (Ben-Arie et al., 1984; Cristosto et al., 2000; Martinez et al., 2006).

Commercial production of pomegranates is fairly new and increasing rapidly in South Africa and the cultivars ‘Bhagwa’ and ‘Ruby’ are amongst the most widely grown in the country and globally (Holland et al., 2009). However, producing higher yield with good fruit quality is an important challenge for the South African pomegranate industry. The aim of this work was to study the time course and pattern of fruit growth as well as changes in physical and physiological properties and texture dynamics of pomegranates (cvs. ‘Bhagwa’ and ‘Ruby’) grown in South Africa. The results obtained could contribute towards defining the optimal time of fruit harvestfor best postharvest peformance.

2. Materials and methods

2.1. Geographic data and climatic parameters

Two pomegranate cultivars ‘Ruby’ and ‘Bhagwa’ grown on a commercial orchard in Porterville, 33°01′00″S, 18°58′59″E (Western Cape, South Africa) were used in this study. The orchards were located on sandy loam soil, and the trees received the same fertilizer program and irrigation delivering about 32 $L.ha^{-1}.day^{-1}$. Values of maximal, minimal and mean daily air temperatures (°C) and rainfall (mm) were collected from a nearby meteorological station between October to May for the 2010/11 and 2011/12 fruit growing seasons. Mean daily air temperature was 19.2°C for the two seasons, with the highest mean daily air temperature of 26.2°C and 25.2°C in February 2011 and January 2012, respectively. The heat units were measured as the sum of the differences between mean daily temperatures and a base temperature of 10°C (Melgarejo et al., 1997). Average monthly rainfall was 24.7mm in 2010/11and 17.6 mm in 2011/12, the highest rainfall being in May (47.0 mm) and April (32.0 mm) in 2011 and 2012, respectively (Figure 1).

2.2. Fruit growth attributes

2.2.1. Plant material, samplings and measurements

The trees (6 years old) used were at planting distance of 5 m x 3 m, with same row orientation and tree training. Ten trees per cultivar were randomly selected for studying fruit growth measurement and analysis. Ten sun-exposed fruits (~20 mm diameter) per tree, were tagged after fruit set and monitored by measuring lineal fruit growth parameters (length and diameter) at 2-week intervals starting from 28 days after full bloom (DAFB) in December to full-ripe stage (139 DAFB for 'Ruby' and 160 DAFB for 'Bhagwa') in the two seasons. Fruit length (L) and diameter (D) were measured at two opposite longitudinal and equatorial fruit perimeters (excluding the fruit calyx) using a digital Vernier caliper (Mitutoyo, Kawasaki, Japan, ± 0.01 mm). Using a mathematical relationship assuming spherical shape, fruit volume (V, cm^3) was determined using equation (1) and shape index (S) was calculated using equation (2) according to Al-Yahyai et al. (2009):

$$\text{Fruit volume (V)} = [(4/3) \times \pi \times r^3] \quad (1)$$

where r is the average of the fruit radius of the fruit diameter and length.

$$\text{Shape index (SI)} = (L/D) \quad (2)$$

2.3. Fruit maturity evaluation

Fruit maturity was evaluated at five distinct developmental stages (Table 1). For each cultivar, twenty healthy and identical fruit from ten randomly selected trees (not from tagged trees for fruit growth studies) were collected monthly and transported in an air-conditioned vehicle to the Postharvest Technology Research Laboratory, Stellenbosch University. Fruit were evaluated for respiration rate and physico- textural attributes.

2.3.1. Fruit respiration and ethylene production

Fruit respiration rate was measured at intervals using the closed system method as described by Caleb et al. (2012). Respiration rate was measured by the amount of CO_2 evolved

and based on fresh weight unit. In 5 replicates, one fruit was placed in a 3 L glass jar hermetically sealed for 3 h with a lid containing a rubber septum in the middle. CO_2 production within the glass jar was measured from the head space through the rubber septum using an infrared O_2/CO_2 gas analyzer (Checkmate 3, PBI Dansensor, Denmark). Measurements were done over 10 days after harvest and results are presented as mean ± S.E (mL CO_2 $kg^{-1}h^{-1}$) of five determinations. Similarly, using a static system, the production of ethylene was checked with a gas chromatograph (Model N6980, Agilent Inc., Wilmington, U.S.A.) fitted with a Porapak® Q column, and flame ionization and thermal conductivity detectors.

2.3.2. Colour attributes

Fruit peel colour along the equatorial axis at two opposite spots were recorded in CIE coordinates (L^*, a^*, b^*) using a Minolta Chroma Meter CR-400 (Minolta Corp, Osaka, Japan) after calibration with a white tile background. Similarly, duplicate colour measurements (L^*, a^*, b^*) were made on arils placed in a colourless glass Petri dish. The colour parameter Chroma (C^*_{ab}) which describes the length of the colour vector in the plane formed by a^* and b^*, and the hue angle (h^o) that determines the position of such vector were calculated according to the following equations:

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

$$h^o = \arctan (b^*/ a^*) \quad (4)$$

Total colour difference (TCD) between the fruit skin (external) and arils (internal) components was calculated as:

$$TCD = \sqrt{((L^*_0 - L^*)^2 + (a^*_0 - a^*)^2 + (b^*_0 - b^*)^2)} \quad (5)$$

where L^*_0, a^*_0 and b^*_0 are the colour parameters of the skin (reference value), while L^*, a^* and b^* are the colour values of the aril (Al-Said et al., 2009; Pathare et al., 2012). Twenty fruit were used and results are presented as mean ± S.E. (n = 20).

2.3.3. Fruit morphological characteristics

The following parameters were investigated for fruit morphological characteristics: fruit and total aril weight (g); calyx diameter (Cd, mm); peel thickness (Pt, mm); single aril weight (Aw, g); aril length (Al, mm); aril width (Awd, mm); kernel weight (Kw, mm); kernel length (Kl, mm); kernel width (Kwd, mm) and kernel index (K-index). Fruit and aril weight measurements were evaluated using an electronic balance (Mettler, Toledo, Switzerland) with an accuracy of 0.01 g, while an analytical balance (Mettler, Toledo, Switzerland, 0.0001 g accuracy) was used for single aril and kernel weight measurements. For each cultivar, 10 g of arils from 20 fruit were mixed in a bowl, and the weight and lineal dimensions were measured using 20 randomly selected arils. Kernel measurements were made after manually removing the pulp of the randomly selected arils. All lineal dimensions were done using a digital Vernier caliper (Mitutoyo, Kawasaki, Japan, ± 0.01 mm). Aril yield (Ay) was calculated using equation 6 and kernel index (K-index) was calculated using equation 7 (Martinez et al., 2006):

$$\text{Aril yield (Ay)} = [(\text{total aril weight}/\ \text{fruit weight}) \times 100\%] \quad (6)$$

$$\text{K-index} = [(\text{Kw} / \text{Aw}) \times 100\%] \quad (7)$$

2.3.4. Textural properties

Fruit puncture resistance of whole fruit was determined using a firmness analyzer with a 5 mm cylindrical probe (GÜSS-FTA, South Africa) programmed to penetrate 8.9 mm into the sample at a speed of 10 $mm.s^{-1}$. Duplicate puncture tests were performed on opposite sides of the equatorial region of each fruit. Peak force required to puncture fruit skin was taken as puncture resistance. A sample of 10 fruit was used and results were expressed as the means ± S.E (n = 10).

Aril compression test was performed using a texture profile analyzer (TA.XT *plus*, Stable MicroSystems Ltd., Godalimng, UK) with 10 kg loading cell and a 35 mm diameter cylindrical compression probe. Test was run per aril on the compression platform in 10 replicates. The textural profile was interpreted using force (N) and distance (mm) as the two fundamental

variables. The operating conditions of the instrument were: pre-test speed 1.5 mm/s, 0.5 mm/s test speed, 10.0 mm/s post-test speed, and 0.20 N trigger force (Al-Said et al., 2009). The data acquisition carried out on the textural profiles was operated by the software Exponent v.4 (Stable MicroSystems Ltd., Godalimng, UK) provided with the TA.XT *plus* texture profile analyzer. The software was used to run a macro for the bioyield force (N) defined as the point on the force-deformation curve at which the juice content in compressed aril just oozed without tearing the aril sac. The Young's modulus or elasticity (N/mm^2) was calculated from the slope of the force-distance curve and rupture force (N) was calculated as the maximum force required to completely break an aril (Al-Said et al., 2009; Bchir et al., 2010).

2.4. Statistical analysis

Mean (± S.E) values of all the studied variables are presented. Graphical presentations were made using GraphPad Prism software version 4.03 (GraphPad Software, Inc. San Diego, USA). Analysis of variance (ANOVA) was carried out using Statistica software (Statistica 11.0, StatSoft Inc., Tulsa, OK, USA) according to Duncan's multiple range test. Where appropriate, 2-way ANOVA was carried out.

3. Results and discussion

3.1. Fruit growth pattern

Changes in size of pomegranate fruit during growth and development based on increases in lineal dimension (length and diameter), volume and fruit shape are shown in Figure 2 ('Bhagwa') and Figure 3 ('Ruby'). Fruit length, diameter and volume of both cultivars increased until the last maturity stage during the two growing seasons. The linear regression model provided the most satisfactory fit to describe the growth traits investigated (Table 2), which is a strong indication that both fruit cultivars exhibited linear growth pattern. A steady increase in fruit diameter, length volume and occurred during growth and development development. The growth pattern for the investigated cultivars differed from a single sigmoidal growth pattern previously reported for several pomegranate cultivars (Ben-Arie et al., 1984; Shulman et al., 1984; Gozlekçi and Kaynak, 2000; Varasteh et al., 2008). According to Shulman et al. (1984), the growth of 'Wonderful' was linear whereas the 'Mule's Head' followed a single sigmoid

curve. Other studies have reported linear growth pattern of pomegranate fruit, including Omani pomegranate (Al-Yahyai et al., 2009) and 'Wonderful' grown in Australia (Weerakkody et al., 2010). In the present study, both 'Baghwa' and 'Ruby' showed marked changes in fruit shape index [length/diameter], resulting from growth dynamics of fruit during development, especially in the days after full bloom (Figure 2). At the early stages of fruit set, fruit conformed more closely to a sphere shape (length/diameter = ~1); however, fruit sphericity declined as maturity advanced, indicating that fruit diameter grew faster than fruit length during the later stages of development and ripening for both cultivars (Figure 2 and Figure 3).

3.2. Fruit respiration and ethylene production

The highest respiration rate occurred on the day of harvest (day 0) for both pomegranate cultivars. In most cases, the respiration rates declined for about 3 - 4 days, followed by a short spike in respiration rates (which lasted for 1 - 2 days) before the rates declined further (Figure 4).

The highest mean respiration rates were obtained with small immature fruit ('Bhagwa', 66.83 mL CO_2 $kg^{-1}h^{-1}$; 'Ruby', 51.17 mL CO_2 $kg^{-1}h^{-1}$), which decreased as fruit advanced in maturity (Figure 5). Although fruit respiration rate decreased at S2 maturity stage, the decrease was only significant ($p<0.05$) for 'Bhagwa'. As fruit matured through to S3, respiration rate for 'Ruby' declined further but remained fairly steady until S5 with a respiration rate of 19.16 mL CO_2 $kg^{-1}h^{-1}$. However, the respiration rate 'Bhagwa' fruit declined significantly between S3 and S4 stages, and remained stable through to S5 with respiration rate of 23.84 mL CO_2 $kg^{-1}h^{-1}$. Although the respiration rate of climacteric fruit such as arazá (*Eugenia stipitata* Mc Vaugh) also decreases during fruit development (Hernandez et al., 2007), the fact that pomegranate fruit did not show an upsurge of respiration rate at advanced fruit maturity stages S4 and S5 confirms that they are non-climacteric. The overall decline in respiration rates for 'Bhagwa' and 'Ruby' were 64.33% and 62.55%, respectively, between S1 and S5 maturity stages. These results agree with previous findings by Ben-Arie et al. (1984) for 'Wonderful'. The non-climacteric nature of the fruit was characterized by a gradual decline in respiration rate of during and after one month of fruit set, which declined markedly with advancing maturity. Similarly, Shulman et al. (1984) observed a decline in respiration rate during fruit development of 'Wonderful' and 'Mule's Head' pomegranate cultivars. The production of CO_2 in matured fruit was low, ranging between 15 and 25 mL kg^{-1} h^{-1} for 'Wonderful' and 25 and 35 mL kg^{-1} h^{-1} for 'Mule's Head'. In the

present study, ethylene gas was not detected in both cultivars during for the period of growth and development investigated. This corroborates the findings of Shulman et al. (1984) with 'Wonderful' and 'Mule's Head' pomegranates, but contrary to Ben-Arie et al. (1984) who reported a trace of ethylene production at all maturity stages.

3.3. Colour attributes

Significant differences (p<0.05) were found among the developmental stages in the colour of fruit peel and arils (Table 3). Fruit peel lightness (L*) was significantly (p< 0.0001) influenced by the interaction between maturity stage and season (maturity*season) for both cultivars, with a similar extent of interaction effect showed for aril lightness (Table 3). In the two growing seasons, the L* values increased from S1 through S3 and then gradually decreased up to S5 as fruit advanced in maturity, whereas the value decreased in arils with advancing maturity for both cultivars. In contrast, the CIE a* (redness) increased from S1 to S5, with the greatest change occurring during advanced fruit maturity stages S3 - S5 for both cultivars in both seasons. For 'Ruby', a significant interaction was observed between maturity and season for peel and aril redness. Regarding peel and aril redness (CIE a*) for 'Bhagwa', there was significant interaction between the main factors for peel redness (Table 3).

Furthermore, the h^o colour index showed declined with advancing maturation as fruit changed from green (peel) or white (aril) to red colour, probably resulting from the accumulation of anthocyanins (Shulman et al., 1984) in fruit parts. The observed colour dynamics in this study contradict those reported for Spanish 'Molla de Elche', where change in fruit arils from white to pinkish-red and fruit peel from green to greenish-yellow and finally to brownish yellow with reddish patches during fruit development was reported (Melgarejo et al., 1997). In the present study, the h^o colour index was significantly influenced by the interaction between the maturity and season (Table 3). In particular, colour saturation or intensities (C*) of fruit peel and aril were significantly influenced by the interaction between the maturity and season for 'Bhagwa'. However, this was not true for fruit peel and aril of 'Ruby' (p> 0.05), where any significant differences in fruit peel and aril colour intensity can be explained by variations in fruit maturity stage (p< 0.0001) and seasons (p= 0.0174) possibly due to climatic factors (Table 3).

Interestingly, fruit peel had higher C* and lower h^o values than observed in arils during the ripening stages (S3 - S5), showing a disparity in colour dynamics in different fruit parts in

both seasons. As a result, the desirable red colouration occurred earlier in fruit peel than in aril and the intensity of the colour differed in peel and arils for both cultivars. These results suggest that pomegranate peel colour does not correspond with that of the arils for the investigated cultivars. Similar observations have been reported for other cultivars in different countries (Al-Said et al., 2009; Holland et al., 2009). This phenomenon clearly confirmed that pomegranate peel colour intensity alone cannot be used to predict optimum aril colour intensity for harvest maturity management. For both cultivars, fruit at S1 exhibited the lowest TCD value, which increased to the highest at S3 and S4 for 'Ruby' and 'Bhagwa', respectively. Given the importance of aril and juice redness as a desirable quality attribute, lower TCD between fruit peel and aril could provide a valuable marker of fruit maturation and ripening status, especially for the studied cultivars. Unfortunately, however, the interaction between maturity stage and season was evident in both cultivars (see appendix: paper 1), and this deserves further attention in future studies.

When both seasons were compared, it was clearly evident that fruit peel and arils had better red colouration in 2012 than 2011 season for both cultivars. These seasonal effects might be related to the prevailing temperature during the growing season. According to Shulman et al. (1984) and Borochov-Neori et al. (2009), temperature is known to exert a strong influence on development of fruit colour in pomegranates. Analysis of meteorological data in the pomegranate growing area used in the present study showed that the number of heat units available between blooming and harvesting was lower by 12.73% in the 2012 growing season than 2011 (Figure 1). Low heat condition is known to be an optimum factor for the biosynthesis of red anthocyanins compounds in pomegranate fruit. According to Shulman et al. (1984), 'Wonderful' pomegranate fruit grown under coastal plain regions developed more intense colour than those grown in warmer valley region. Moreover, the study conducted by Borochov-Neori et al. (2009) showed that the intensity of the red colour of fruit aril was inversely related to heat units accumulated during fruit development and ripening.

3.4. Fruit morphometric characteristics

Fruit weight increased with advancing maturity in both cultivars for each season and nearly followed a linear pattern ('Bhagwa', $R^2 > 0.96$; 'Ruby', $R^2 > 0.97$) similar to the growth of fruit lineal dimensions. For 'Bhagwa', fruit weight increased from 82.7 to 319.1 g and 93.91

to 344.5 g in 2011 and 2012, respectively, while 'Ruby' fruit increased from 99.84 to 302.9 g and 106.4 to 321.5 g in 2011 and 2012, respectively (Figure 6). In both seasons fruit growth was characterized by a weight pattern which increased significantly between S1 and S2 and proceeded with a rapid weight increase at S3 until S4 before attaining its maximum weight at S5 (Figure 6). Similar growth pattern was reported by Weerakkody et al. (2010) for 'Wonderful' grown in Australia, where increase in fruit weight followed a linear pattern throughout development and maturation, reaching a maximum value of 675 g per fruit after 14 weeks of fruit set. Differences in fruit weight patterns could be due to many preharvest factors including differences in climatic condition (Ben-Arie et al., 1984; Shulman et al., 1984). The study by Borochov-Neori et al. (2011) on four Israeli grown pomegranate accessions ('PG 128-29', 'PG 130-31', 'EG1' and 'EG 2') showed that fruit that ripened in early summer and during the winter period were significantly smaller than late summer and autumn ripened fruit. Results from the present study showed that there was a significant combined effect of maturity status and season on the development of fruit weight ($p < 0.0001$ for 'Bhagwa' and $p = 0.0025$ for 'Ruby') (Figure 6). 'Baghwa' fruit weight was higher in 2011 than 2012 while 'Ruby' fruit weight was higher during the 2012 season. The seasonal shift in fruit weight is of economic importance, especially given that fruit is sold on weight basis. The reasons for this shift are not clear from the present study and warrants further investigation.

Increase in fruit size could be attributed to an increase in aril size and juice content as well as a result of the development of the peel structure (Mirdehghan and Rahemi, 2007). Therefore, optimizing the quality attributes of these fruit parts is the goal of growers, breeders and processors (Martinez et al., 2006; Hasnaoui et al., 2011). Result obtained during the 2011 season showed that aril yield (fresh weight basis) remained less than 50% of total fruit weight until middle of the season (S3), and increased until commercial harvest for 'Ruby', whereas for 'Bhagwa' it remained below 50% of total fruit weight throughout the season (Table 4). Shulman et al. (1984) observed that arils constituted about 50% of the fruit weight during most stages of fruit development for 'Mule's Head' and 'Wonderful', while 57 - 66% of fruit weight was accounted for arils in Spanish 'Mollar' (Gil et al., 1996). In comparison to fruit at commercial harvest, the aril yield of 55.6% in Moroccan grown 'Ruby' reported by Martínez et al. (2012) was comparable to that found in this study.

It is well known that peel cells undergo periclinal growth, which is accompanied by an increase in peel thickness during fruit development but interestingly, a decrease in peel thickness was observed at S2, probably due to fruit dehydration which occurred during this period (February) when temperature was highest during 2010/2011 growing season (Figure 1). Fruit peel ranged from 4.49 to 4.94 mm for 'Bhagwa' and 4.88 to 4.94 mm for 'Ruby'. This is contrary to Zarei et al. (2011) who reported a significant decrease in peel thickness during fruit development of Rabbab-e-Fars pomegranate cultivar. Pomegranate fruit crown (calyx) is a very important characteristic of the fruit. In this study, fruit calyx opening (diameter) reduced significantly (p<0.0001) between immature and full-ripe stages for both cultivars. Calyx diameter reduced from 23.55 mm (S1) to 6.96 mm (S5) for 'Bhagwa' and from 23.12 mm (S1) to 11.04 mm (S5) for 'Ruby' (Table 4). According to Zarei et al. (2011), calyx diameter showed significant increase during fruit development of 'Rabbab-e-Fars'.

Increases in individual aril and kernel sizes followed the overall fruit development pattern for both cultivars (Table 3). Increase in aril size could be attributed to increase in juiciness as the fruit advanced in maturation (Mirdehghan and Rahemi, 2007). The weight of individual aril increased significantly (p<0.0001) throughout the maturity stages whereas individual aril length and width only showed significant increase at the mid-ripe stage (S3). According to Shulman et al. (1984), immature fruit stage is characterized by growing kernel tissue and increment in testa hardness. The KI obtained in the present study ranged from 20.72 (S1) - 13.46% (S5) for 'Bhagwa' and from 19.21 (S1) - 11.83% (S5) for 'Ruby' (Table 3). In comparison with fruit at commercial harvest, the KI values for the investigated cultivars were higher than those reported in the literature. The kernel index of Moroccan cultivars varied between 6.2% for 'Rouge Marrakech' to 10.7% for 'Bouaâdime'. The KI for Spanish cultivars varied from 6.1% in PTO2 to 9.7% in CRO1, while it was 5.4% in the Italian 'Dente di Cavallo' (Barone et al., 2001) and 71.8% for 'Alandi' (Purohit, 1985). Kernel index (KI) is a good parameter that quantifies the woody portion in edible part of pomegranate fruit (Martinez et al., 2006). K-index is relevant in pomegranate maturity determination because it is directly linked to the edible part of the fruit. Since K-index decreased with advancing maturity in this study, K-index could be used in combination with other parameters to determine the developmental status of the investigated pomegranate cultivars.

3.5. Textural dynamics

There was a significant difference (p<0.05) in the force required to puncture fruit at the different maturity stages (Table 5). The trend in puncture force (N) was similar for both cultivars, although 'Bhagwa' required higher puncture force than 'Ruby' in the 2012 season. The force required to puncture fruit decreased significantly (p<0.05) as the fruit advanced in maturity from S1 to S2, and this might related to the concurrent decrease observed in fruit peel thickness at S2 stage, resulting in low puncture resistance. However, the puncture force increased dramatically from S2 to maximum value at S4, probably as a result of the strengthening of the structural integrity of fruit peel with advancing maturity.

There were significant differences (p<0.05) in the textural properties (rupture force, bioyield point and modulus of elasticity) of arils among the maturity stages investigated for both cultivars (Table 5). Aril rupture force increased from S1 to a maximum value at S3 for 'Ruby' and S4 for 'Bhagwa', and significantly decreased thereafter until commercial harvest. Both cultivars had the same trend in bioyield force although higher values were recorded in 'Ruby'. Bioyield force (N) increased with fruit maturity, starting with an initial steady increment between S1 and S2, which became significantly higher through to S5. Dynamics of elastic modulus of fruit arils at different maturity stages showed that aril elasticity reduced significantly at S2, followed by a significant increase at S3 which then remained steady until physiological maturity in 'Ruby'. However, it further increased significantly to a maximum value at S5 for 'Bhagwa' (Table 5). The significance of the use of textural properties in characterizing maturity of pomegranate fruit has not been well studied. As turgidity, rather than hardness is the most important texture component in pomegranate aril, textural properties such as bioyield force and elasticity are most related to internal turgor pressure within the arils and could be used to discriminate pomegranate maturity stages. Previous studies have shown that factors such as growing conditions and genetics could influence textural properties of pomegranate (Al-Said et al., 2009). In order to have a broad and more exhaustive view of pomegranate fruit textural changes during development further studies are necessary.

4. Conclusion

Significant variations in fruit morphology, respiration and physico-textural properties of fruit were found in 'Bhagwa' and 'Ruby' at major maturity stages. Fruit lineal dimensions exhibited a linear growth pattern. Fruit sphericity decreased with maturity, with young fruit having the most rounded shape. The results of this study support the non-climacteric nature of pomegranate fruit which was characterized by undetectable levels of ethylene gas throughout fruit development. In addition, there was no upsurge in fruit respiration at advanced maturity stages (S4 and S5). The fruit cultivars showed a decline in respiration rate during fruit development with the highest respiration rate measured in immature fruit ('Bhagwa', 66.83 mL CO_2 $kg^{-1}h^{-1}$; 'Ruby', 51.17 mL CO_2 $kg^{-1}h^{-1}$) and did not vary significantly (p>0.05) between the last two advances maturity stages (S4 and S5).

Fruit pigmentation increased with advancing maturity and the lowest total colour difference (TCD) between fruit peel and aril was lowest in immature fruit. Fruit weight also increased with advancing maturity and followed a linear growth pattern similar to that observed in fruit lineal dimensions. 'Bhagwa' fruit weight increased from 82.7 to 319.1 g and 93.91 to 344.5 g in 2011 and 2012, respectively, and increased from 99.84 to 302.9 g and 106.4 to 321.5 g for 'Ruby' in 2011 and 2012, respectively. Aril yield remained less than 50% until the middle of the season (S3), and continued increasing (59%) until commercial harvest for 'Ruby', whereas aril yield remained below 50% throughout the season for 'Bhagwa'. Peel thickness increased from 4.49 to 4.94 mm for 'Bhagwa' and 4.88 to 4.94 mm for 'Ruby', while fruit calyx opening reduced significantly between immature and full-ripe stages for both cultivars.

Weight of individual aril increased significantly up to commercial fruit harvest (S5) and kernel index (KI) ranged from 20.72 (S1) - 13.46% (S5) for 'Bhagwa' and from 19.21 (S1) - 11.83% (S5) for 'Ruby'. This study showed that textural dynamics of pomegranate fruit peel and arils change with fruit maturity. Textural properties such as aril bioyield force and elasticity, which are most related to internal turgor pressure within the arils, could be used to discriminate pomegranate maturity stages. The results indicate that 'Ruby' pomegranate reached maturity between the 132 - 139 DAFB and 'Bhagwa' 140 - 165 DAFB. This period could be regarded as the physiological mature stage of fruit. Good knowledge of fruit maturity indices is important to assist growers in managing harvest maturity to meet market demand and export quality

standards. Information on maturity attributes such as fruit respiration rate, fruit pigmentation, aril yield and aril textural properties contribute to better understanding of the physiological maturity status of fruit, and hence provide basic information to assist in determing reliable markers to assess fruit readiness for harvest.

However, it is noteworthy to acknowledge the limitation of this current study. The maturity results obtained are only representative for orchards around Porterville (Western Cape) and only over two seasons; hence the results can at this stage be used only as a guideline and as a basis for future studies. In addition, other indicators such as the changes in biochemical fruit attributes at maturity stages should be taken into account when assessing readiness to harvest to ensure optimum income returns to growers. This information would allow pomegranate growers to have a tool to assess fruit maturity before harvesting and packaging for exportation in other to meet the maturity characteristics set by purchasers or export markets.

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Table 1

Description of fruit maturity at different sampling stages along days after full bloom (DAFB)

Stage	DAFB (Month)		Description of maturity stages
	'Bhagwa'	'Ruby'	
S1	54 (Jan)	54 (Jan)	Immature: Green skin, immature white arils with immature kernels
S2	82 (Feb)	82 (Feb)	Mature/unripe: Light-red skin, mature white arils with mature kernels
S3	110 (Mar)	110 (Mar)	Mature/semi-ripe: Red skin, mature pink arils with mature kernels
S4	140 (Apr)	132 (Apr)	Mature/ripe: Red skin, mature red arils with mature kernels
S5	165 (May)	139 (Apr)	Mature/full-ripe: Deep-red skin, deep-red arils with mature kernels

Table 2

Linear models fitted for different growth traits against days from full bloom (DAFB) of 'Bhagwa' and 'Ruby' during 2010/2011 and 2011/2012 growing seasons

Cultivar	Dependent variable (Y)	Linear model	Season	Regression (R^2)
'Bhagwa'	Diameter	Y = 0.3043x + 36.412	2011	0.994
		Y = 0.3384x + 41.144	2012	0.991
	Length	Y = 0.2642x + 36.539	2011	0.995
		Y = 0.2963x + 35.603	2012	0.995
	Volume	Y = 1.8638x - 29.137	2011	0.965
		Y = 2.3856x - 33.652	2012	0.993
	Shape index (L/D)	Y = -0.0004x + 0.987	2011	0.659
		Y = -0.0001x + 0.888	2012	0.181
'Ruby'	Diameter	Y = 0.3389x + 38.223	2011	0.990
		Y = 0.3359x + 36.905	2012	0.991
	Length	Y = 0.2227x + 43.038	2011	0.992
		Y = 0.2842x + 31.603	2012	0.990
	Volume	Y = 1.8109x + 0.628	2011	0.962
		Y = 1.9421x - 30.873	2012	0.963
	Shape index (L/D)	Y = -0.0015x + 1.063	2011	0.836
		Y = -0.0002x + 0.8738	2012	0.009

Table 3

Influence of maturity stages and seasons and their interaction on peel and aril colour properties (*L*, C*, h°, a*, TCD*) of 'Bhagwa' and 'Ruby' during 2010/2011 and 2011/2012 growing seasons.

	Peel				Aril				
Maturity stage	*L**	*C**	*h°*	*a**	*L**	*C**	*h°*	*a**	TCD
'Bhagwa'									
2011_S1	50.4±1.50bc	31.8±0.94e	64.4±2.49b	13.5±1.33d	58.7±1.21a	19.4±0.38d	88.7±0.73a	0.5±0.25e	19.9±1.75de
2011_S2	52.5±1.00b	33.2±0.38e	64.0±2.00b	14.3±0.95d	48.3±1.76bc	19.9±0.61d	60.3±3.81c	9.6±0.63d	21.1±0.64cde
2011_S3	56.3±1.37a	36.6±0.89cd	62.0±1.96b	16.9±1.05dc	43.5±2.01cd	21.0±1.18d	44.6±4.27d	15.2±1.80c	26.1±1.30c
2011_S4	46.2±0.74de	58.4±0.80a	35.2±0.62c	47.7±0.67a	27.6±1.83ef	29.0±1.01b	28.9±0.72f	25.4±0.96a	36.0±1.14ab
2011_S5	45.3±0.56de	52.8±0.50b	27.2±0.32d	46.9±0.49a	21.7±1.34g	29.9±1.00b	26.4±0.45f	26.8±0.89a	32.3±0.28b
2012_S1	43.7±1.01e	34.7±0.61ed	76.6±2.81a	7.3±1.56f	50.9±1.55b	16.2±0.34e	76.4±2.35b	3.6±0.63e	23.1±1.55cd
2012_S2	48.6±1.27cd	38.3±1.02c	60.8±2.10b	18.6±1.50d	39.2±3.35d	20.8±0.98d	37.2±1.74de	15.7±1.09c	31.2±1.94b
2012_S3	51.8±1.08bc	53.0±1.40b	35.1±0.95c	43.4±1.45b	29.8±1.48e	19.0±1.57d	38.2±1.91d	16.1±1.07c	38.7±1.72a
2012_S4	39.5±0.95f	51.2±0.84b	27.2±0.74d	45.9±0.67ab	29.7±1.57e	24.0±1.45c	29.9±1.15ef	20.8±1.33b	33.5±1.27b
2012_S5	26.1±2.92g	28.0±3.41f	25.8±0.79cd	46.8±2.91c	23.3±1.82fg	32.7±2.21a	29.8±2.42ef	28.5±2.39a	27.7±1.33e
Prob. > F									
Maturity (A)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Season (B)	< 0.0001	0.0615	< 0.0001	0.8322	< 0.0001	0.0501	< 0.0001	0.0682	0.1762
A * B	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0024**	**< 0.0001**	**< 0.0001**	**< 0.0001**

Table 3 contd.

Maturity stage	Peel *L**	Peel *C**	Peel *h°*	Peel *a**	Aril *L**	Aril *C**	Aril *h°*	Aril *a**	TCD
'Ruby'									
2011_S1	48.0±1.37b	28.9±1.09f	76.7±3.73a	5.7±1.80e	51.2±1.81a		86.2±2.30a	1.0±0.61g	16.9±1.80f
2011_S2	49.9±1.18ab	31.9±1.12ef	67.9±3.22b	11.5±1.80d	45.4±1.31b		64.5±3.75b	8.0±1.49f	20.4±1.77ef
2011_S3	51.8±1.23a	46.7±1.92bc	52.2±3.41c	29.0±3.12b	40.5±2.26b		50.5±3.76c	11.4±1.4de	37.0±2.97a
2011_S4	46.5±1.13bc	50.4±0.89ab	33.1±1.48e	40.3±1.28a	30.9±2.01cd		37.2±1.80d	16.1±1.06c	35.9±2.84a
2011_S5	44.2±1.04cd	48.4±1.06abc	30.6±1.30ef	43.1±0.89a	30.5±2.44cd		32.1±1.78de	23.1±1.85ab	30.3±1.11bc
2012_S1	41.3±0.81ef	34.8±1.11e	55.3±2.78c	19.7±1.71c	40.5±1.46b		59.2±3.14b	8.7±1.07ef	21.8±1.34def
2012_S2	38.9±1.03fg	39.6±1.34d	41.2±2.28d	29.7±1.74b	32.7±1.63c		38.4±1.91d	13.9±0.9cd	26.1±1.45cd
2012_S3	43.0±0.91de	46.1±1.82c	29.6±0.75ef	40.0±1.60a	30.07±2.29cd		40.7±2.28d	15.7±0.93c	33.6±2.52ab
2012_S4	37.4±0.64fg	51.1±0.85a	29.8±0.92ef	44.3±0.99a	29.4±1.89cd		34.19±2.73de	19.9±1.67b	30.9±2.33bc
2012_S5	36.7±0.91g	49.6±1.43abc	25.5±0.83f	44.6±1.11a	25.0±1.57d		28.2±0.70e	24.5±1.10a	24.8±1.32de
S1*						17.29±0.67c			
S2						18.82±0.62c			
S3						17.95±0.67c			
S4						22.05±0.77b			
S5						27.03±0.75a			
Seasonal average						2011 = 19.22b 2012 =21.40a			
Prob. > F									
Maturity (A)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	**< 0.0001**	< 0.0001	< 0.0001	< 0.0001
Season (B)	< 0.0001	0.0117	< 0.0001	< 0.0001	< 0.0001	**0.0174**	< 0.0001	< 0.0001	**0.5728**
A * B	**0.0038**	**0.0052**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.1292	**< 0.0001**	**0.0188**	**0.0003**

Factorial ANOVA was performed for Factor A (maturity stage) and Factor B (season). Different letter(s) on column indicate statistical significant (p<0.05) differences according to Duncan's multiple range test. *2011 and 2012 data were pooled for parameter(s) where interaction effects were not significant (p<0.05).

Table 4

Changes in aril yield (Ay), peel thickness (Pt), calyx diameter (Cd), aril weight (Aw), aril length (Al), aril width (Awd), kernel width (Kw), kernel length (Kl), kernel width (Kwd) and kernel index (K-index) of 'Bhagwa' and 'Ruby' pomegranate fruits harvested at five maturity stages (Mean ± S.E for 2011 season presented).

DAFB	Ay (%)	Pt (mm)	Cd (mm)	Aw (g)	Al (mm)	Awd (mm)	Kw (g)	Kl (mm)	Kwd (mm)	K- index
'Bhagwa'										
S1	39.60±2.39c	4.49±0.14ab	23.55±1.39a	0.07±0.001e	7.41±0.16d	4.72±0.15d	nd	nd	nd	nd
S2	45.46±1.32b	4.03±0.17b	21.98±1.06ab	0.14±0.001d	8.02±0.12c	6.09±0.18c	0.04±0.001b	6.70±0.09a	2.81±0.06b	20.72±0.97a
S3	49.29±0.73a	4.46±0.20ab	17.99±2.02b	0.18±0.01c	8.69±0.20b	6.43±0.16b	0.04±0.001b	6.70±0.11a	3.15±0.05b	19.88±0.71a
S4	46.76±1.19b	4.82±0.26a	6.96±1.45c	0.31±0.01b	9.79±0.14a	7.37±0.18a	0.05±0.002a	6.92±0.08a	3.88±0.12a	15.74±0.98b
S5	45.95±2.71b	4.94±0.25a	6.74±1.48c	0.34±0.02a	10.14±0.22a	7.22±0.26a	0.05±0.001a	6.41±0.12a	3.49±0.12a	13.46±0.84b
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0045	0.0147	0.0034	0.0022
'Ruby'										
S1	47.02±2.68c	4.88±0.24a	23.12±1.66a	0.10±0.11d	4.49±0.14c	4.81±0.24c	nd	nd	nd	nd
S2	39.01±2.57d	3.31±0.16b	21.86±1.19ab	0.18±0.004c	8.82±0.13b	6.13±0.11b	0.03±0.002b	6.07±0.10a	3.21±0.08b	19.21±1.62a
S3	51.42±3.78b	4.13±0.25a	20.57±0.97ab	0.22±0.01b	9.37±0.15a	7.17±0.23a	0.03±0.001b	6.55±0.12a	3.10±0.08b	14.99±1.07b
S4	58.45±2.59a	4.32±0.25a	18.50±2.13b	0.40±0.02a	9.73±0.14a	7.13±0.23a	0.04±0.004a	6.85±0.12a	3.35±0.08a	11.27±1.45b
S5	58.82±3.24a	4.94±0.62a	11.04±0.88c	0.42±0.003a	9.77±0.23a	7.38±0.23a	0.05±0.002a	6.64±0.02a	3.68±0.23a	11.83±1.49b
p-value	< 0.0001	0.0047	< 0.0001	< 0.0001	0.0044	0.0041	0.0024	0.0831	0.0039	0.0047

Different letter(s) on column per cultivar indicate statistically significant differences ($p<0.05$) according to Duncan's multiple range test. nd = not determinable.

Table 5

Changes in mechanical properties of 'Bhagwa' and 'Ruby' harvested at five maturity stages (Mean ± S.E for 2012 season presented)

Cultivar	Property	S1	S2	S3	S4	S5	*p*-value
'Bhagwa'							
	Whole fruit						
	Puncture force (N)	142.9±6.85b	75.8±3.85d	107.6±5.62c	182.3±6.30a	144.7±7.03b	< 0.0001
	Aril						
	Rupture force (N)	42.4±3.05d	75.8±3.85c	90.9±2.63b	99.2±2.96a	81.6±2.04c	< 0.0001
	Bioyield force (N)	4.4±0.57b	4.3±0.33b	5.2±0.57ab	5.6±0.50ab	6.46±0.43a	0.0021
	Modulus of elasticity (N/mm)	4.4±0.66b	2.8±0.28c	4.4±0.48b	4.2±0.29b	5.2±0.41a	0.0011
'Ruby'							
	Whole fruit						
	Puncture force (N)	123.6±5.62b	72.26±2.45d	104.6±3.49c	143.6±5.07a	124.6±6.12b	< 0.0001
	Aril						
	Rupture force (N)	71.41±3.36b	72.26±2.45b	87.48±1.60a	71.71±2.29b	67.44±3.14b	0.0021
	Bioyield force (N)	5.01±0.43c	5.05±0.46c	6.07±0.67b	6.24±0.41b	7.40±0.41a	0.0037
	Modulus of elasticity (N/mm)	4.10±0.38a	2.67±0.36b	4.72±0.37a	4.46±0.40a	5.24±0.41a	0.0428

Different letter(s) on rows indicate statistically significant differences ($p<0.05$) according to Duncan's multiple range test.

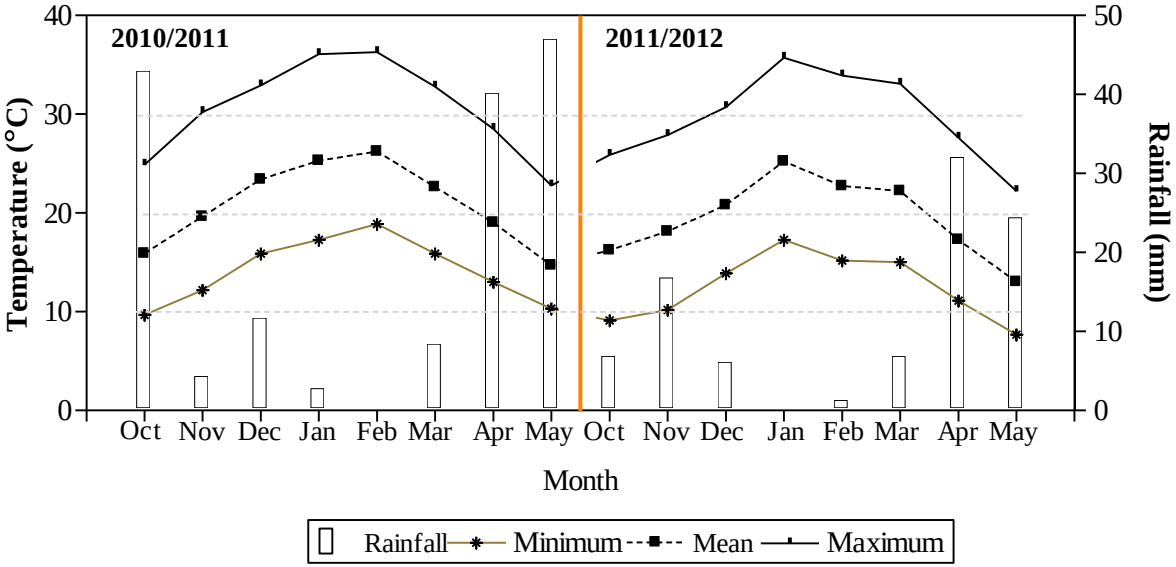

Figure 1. Mean values of maximum, minimum and average temperatures and total rainfall as recorded monthly at Porterville region between October and May in 2010/11 and 2011/12 growing seasons. Heat units (HU) during 2010/2011 season = 2620.5 H.U; 2011/2012 season = 2287 H.U. The heat units were measured as the sum of the differences between mean daily temperatures and a base temperature of 10°C.

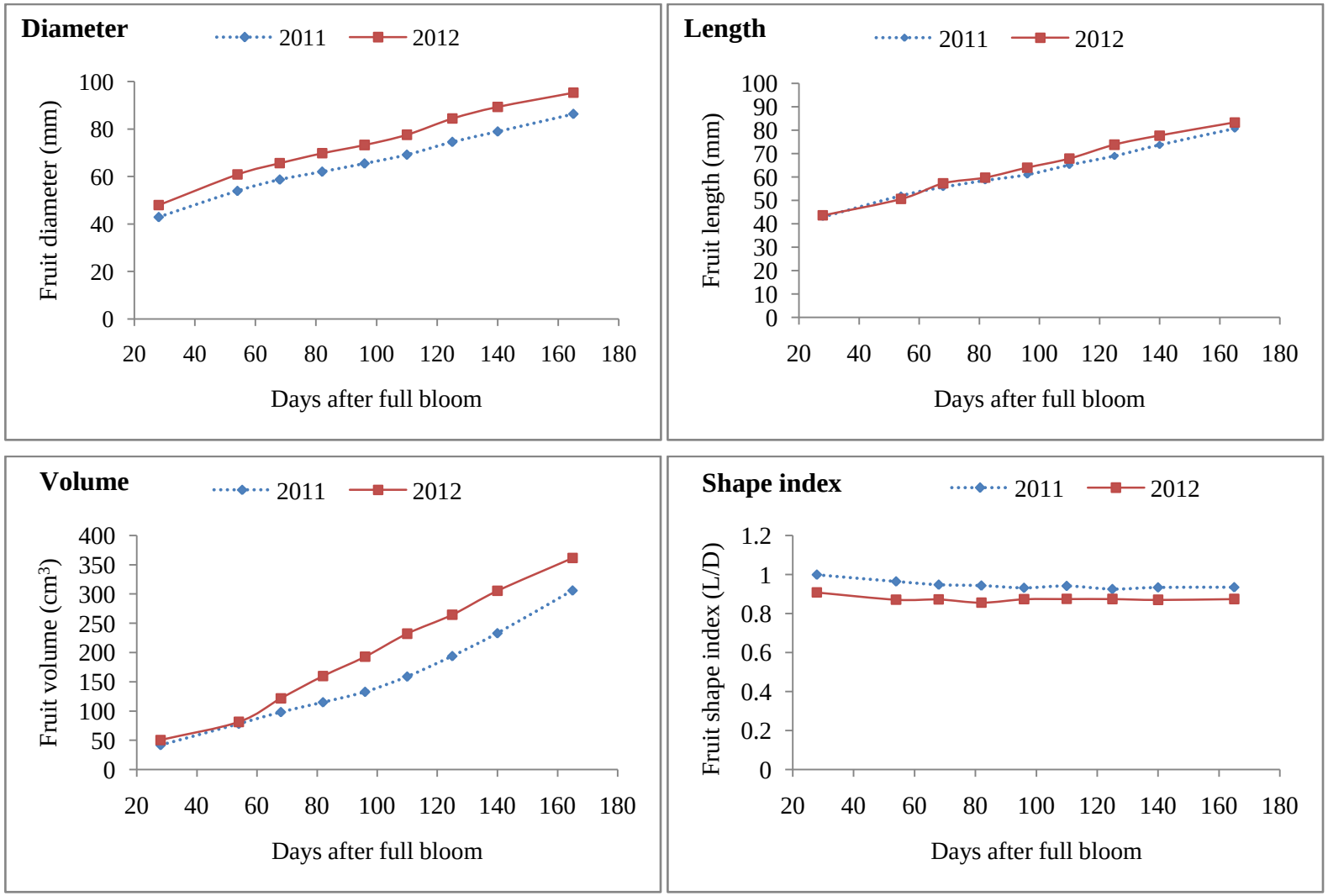

Figure 2. Cumulative growth attributes of **'Bhagwa'** pomegranate fruit from 28 days after full bloom (DAFB) until harvest (165 DAFB) during 2010/2011 and 2011/2012 growing seasons. Shape index (Length/Diameter). Growth curves were best fitted with linear model. Model equations are provided in Table 2.

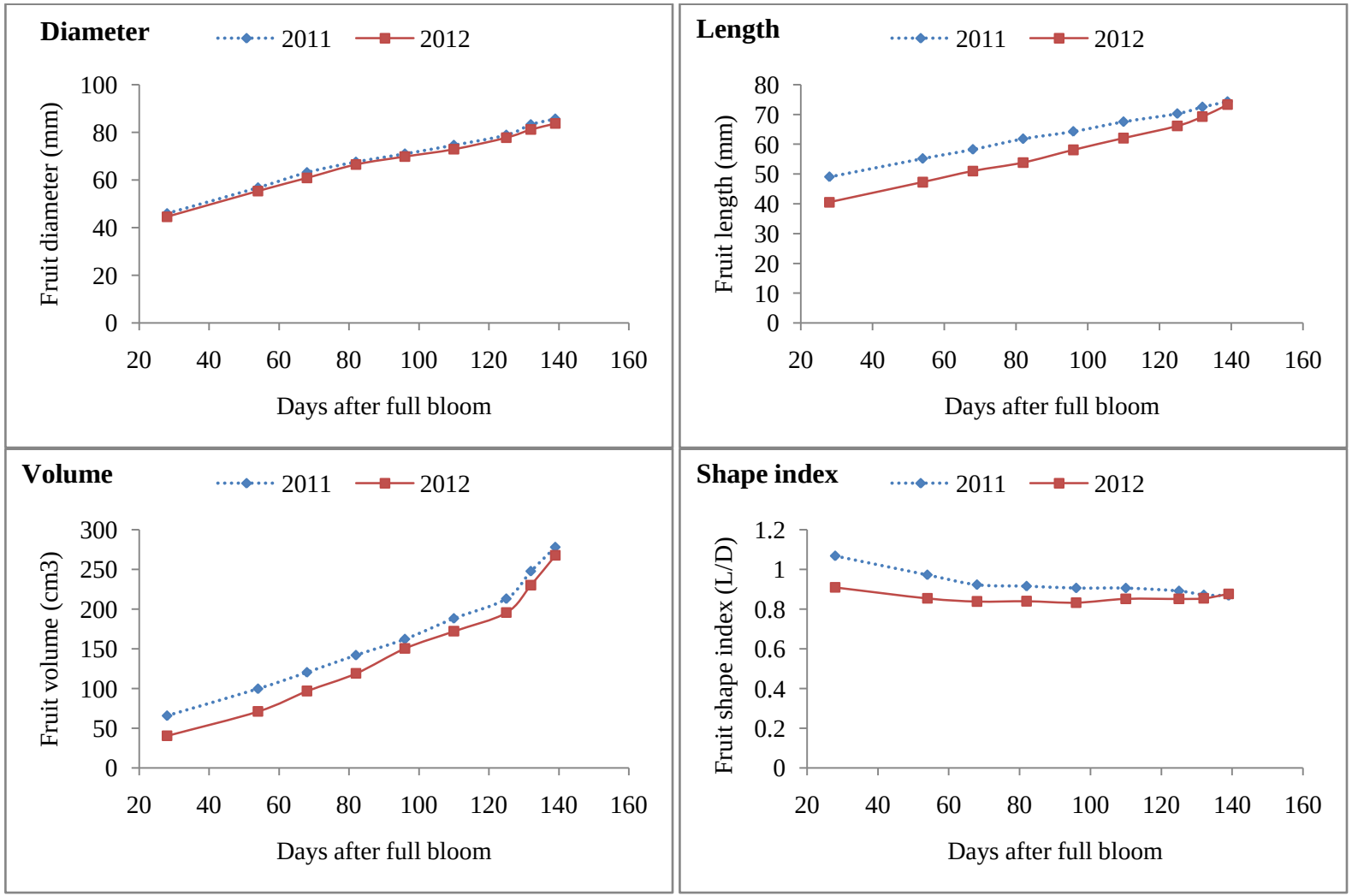

Figure 3. Cumulative growth attributes of **'Ruby'** pomegranate fruit from 28 days after full bloom (DAFB) until harvest (139 DAFB) during 2010/2011 and 2011/2012 growing seasons. Shape index (Length/Diameter). Growth curves were best fitted with linear model. Model equations are provided in Table 2.

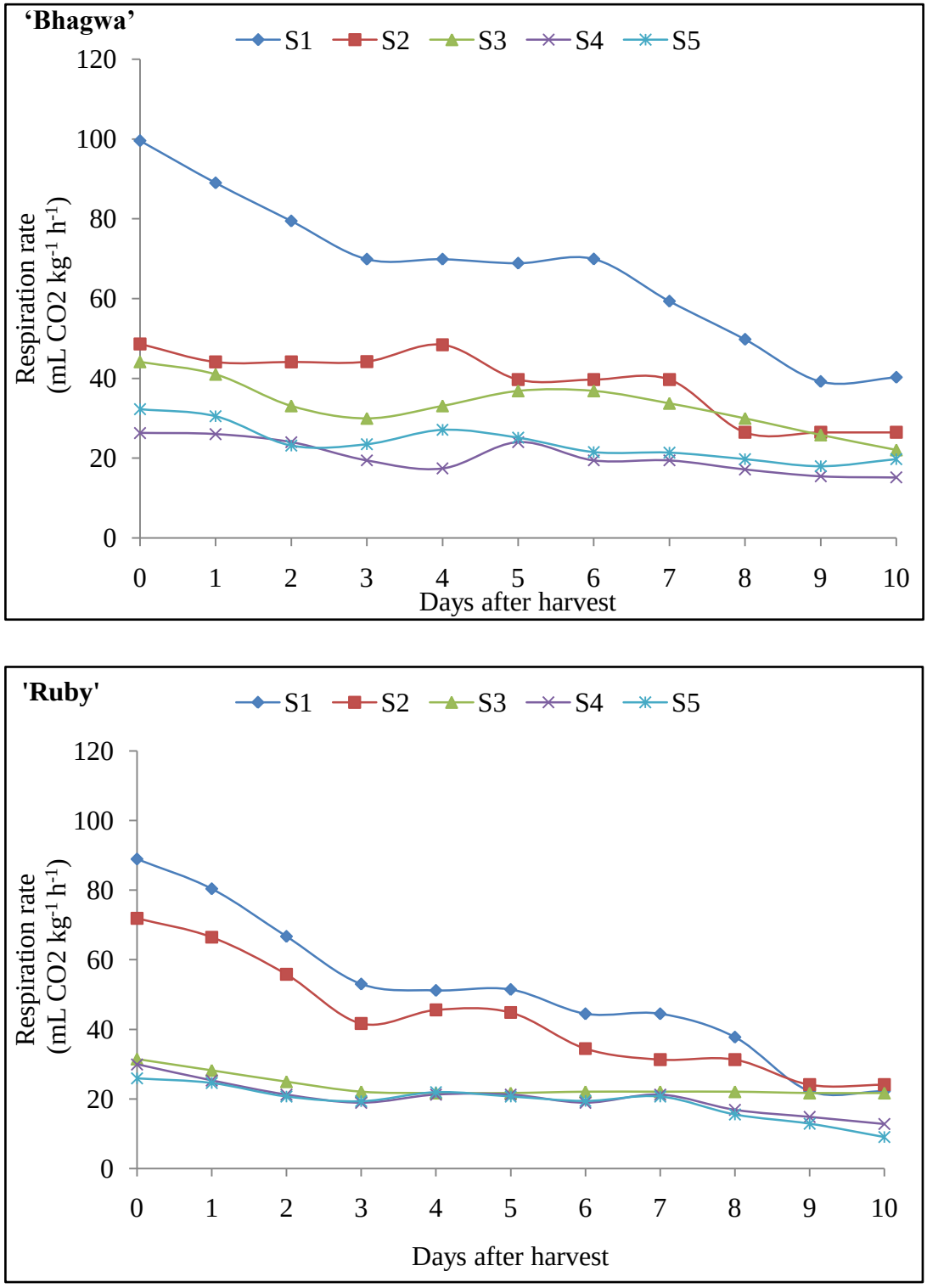

Figure 4. Changes in respiration rates of pomegranate cultivars over 10 days after harvest at 20°C. Five different maturity stages (S1 - S5) were studied in 2012 growing season.

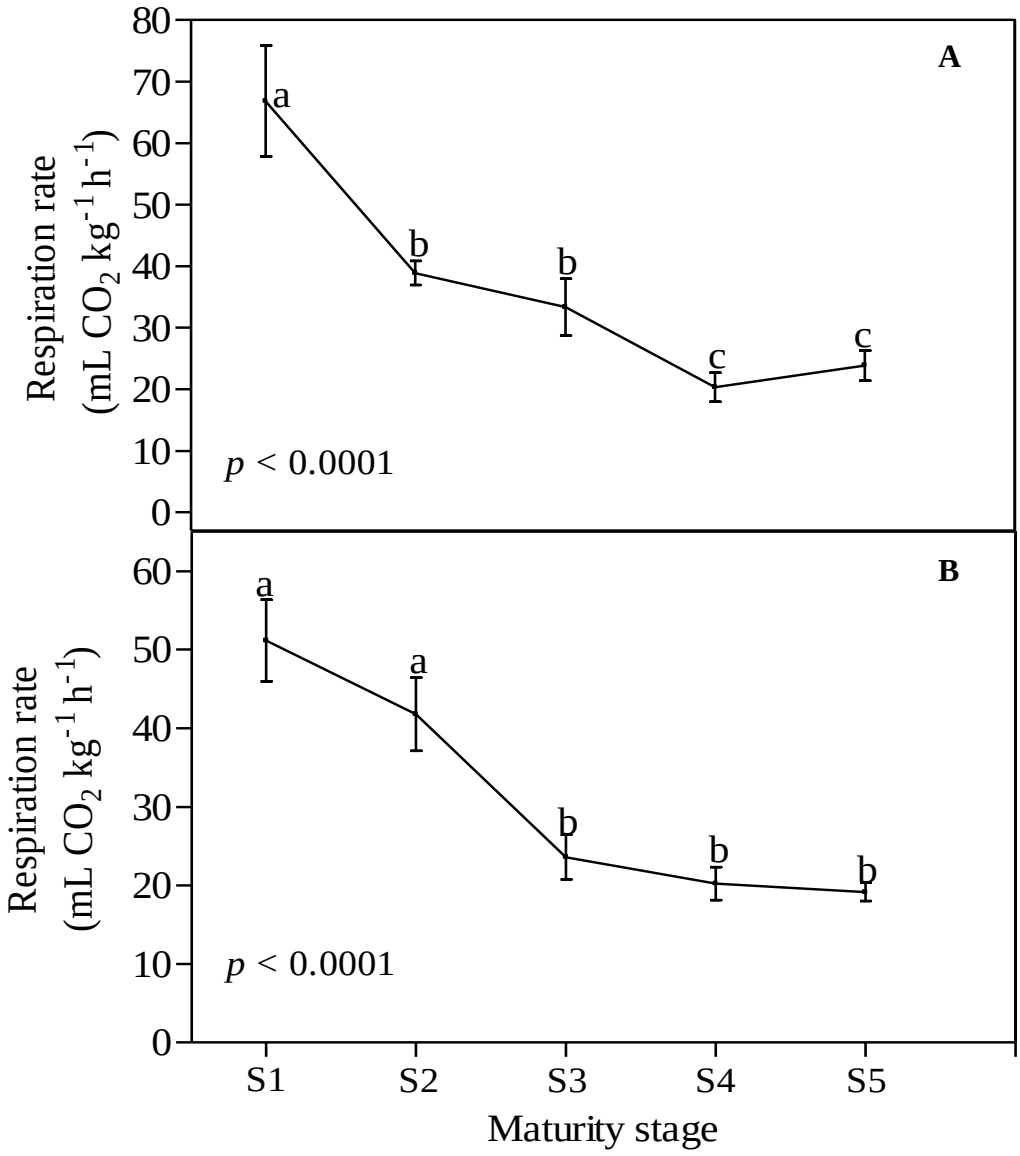

Figure 5. Respiration rate of pomegranate cultivars harvested at five maturity stages in 2012 growing season. Mean ± S.E presented. Different letter(s) on data point indicate statistically significant differences. 'Bhagwa' (**A**) and 'Ruby' (**B**).

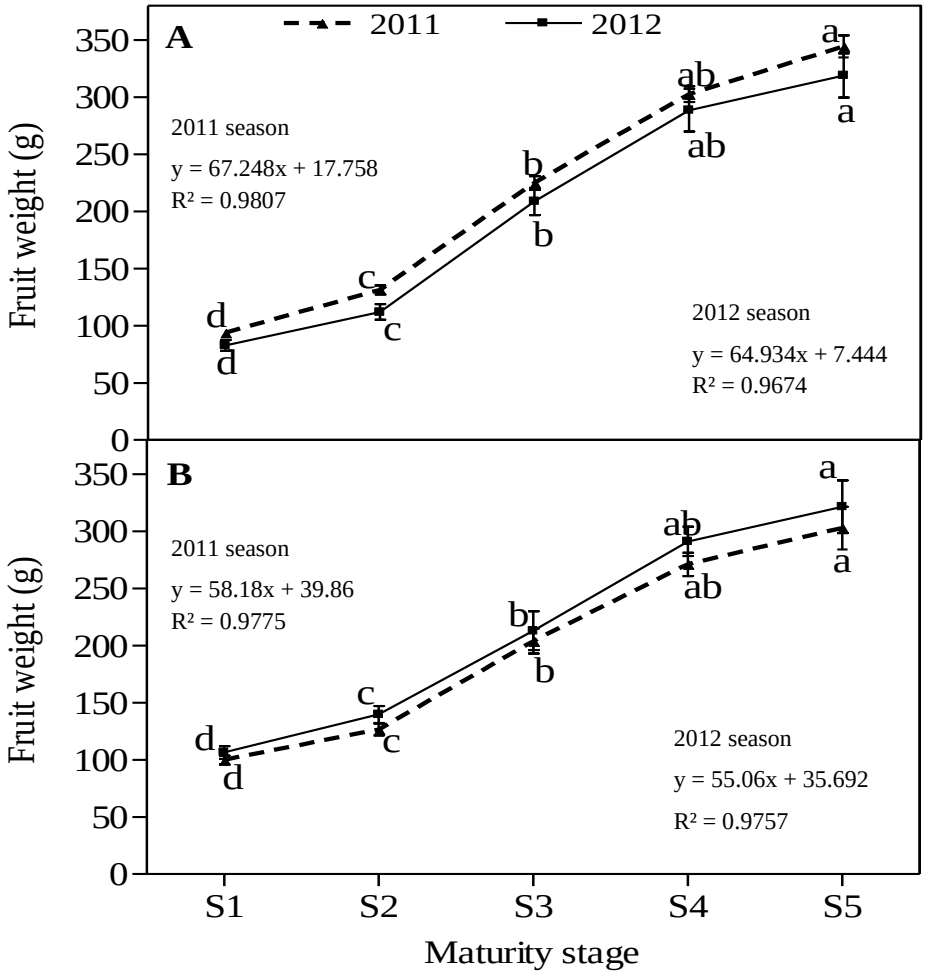

Figure 6. Changes in weights of fruit of 'Bhagwa' (**A**) and 'Ruby' (**B**) harvested at five maturity stages (Mean ± S.E for 2011 and 2012 seasons presented). Different letter(s) indicate statistically significant differences according to Duncan's multiple range test.

Cultivar	Source	*Prob. > F*
'Bhagwa'	Maturity stage (*A*)	0.0021
	Season (*B*)	0.0941
	*A*B*	< 0.0001
'Ruby'	Maturity stage (*A*)	0.1529
	Season (*B*)	0.0410
	*A*B*	0.0025

PAPER 3

Seasonal variation in chemical composition, aroma volatiles, antioxidant capacity and mineral elements of pomegranate during fruit development

Abstract

This study was conducted to investigate compositional changes and antioxidant capacities of pomegranate fruit ('Bhagwa' and 'Ruby') at five distinct stages of maturity and ripening over two growing seasons. Total soluble solids (TSS), pH, titratable acidity (TA), phenolic concentrations, antioxidant capacities, individual organic acid and sugar concentrations, aroma volatile constituents and mineral element composition were investigated. Principal component analysis (PCA) and Pearson correlation were used to visualize the changes in major chemical indices and the relationship among them. Results showed that major compositional changes in fruit are developmentally regulated. Significant increases in total soluble solids (TSS), sugars (glucose and fructose) and anthocyanin composition, coupled with significant decline in titratable acidity (TA), organic acids and total phenolics (TP) occurred with advancing maturity. Fruit at advanced maturity stages were characterized by intense pigmentation of peel and aril, which coincided with maximum accumulation of anthocyanins. TSS and TA showed strong relationships with most of the chemical indices, each showing significantly (p<0.05) strong correlations with the compositions of sugar, organic acid and phenolic indices as well as with antioxidant capacity (FRAP and DPPH) measured.

There were no significant (p <0.05) seasonal effects on juice absorbance (colouration) and TSS for 'Bhagwa' as well as juice absorbance, TSS, TSS/TA and BrimA for 'Ruby'. In combination, these maturity indices account for juice colour evolution as well as juice flavour and taste evolution for both cultivars. The maturity indices identified for each cultivar could serve as reliable maturity markers to determine fruit readiness for harvest and optimal harvest maturity for the pomegranate cultivars.

Keywords: pomegranate, maturity, antioxidant capacity, anthocyanins.

1. Introduction

A large number of physiological, biochemical, and structural changes occur during fruit maturation and ripening (Nunes et al., 2009). Biochemical components such as sugars, organic acids, mineral elements, volatile constituents and polyphenol concentrations are mostly considered parameters for quality assessment of many types of fruit and vegetables, due to the roles they play in determining nutritive value and making the produce desirable for consumption (Ashoor and Knox, 1982; Glew et al., 2003). In pomegranate fruit handling and marketing, important quality attributes include size, skin and aril colour, juiciness, taste and flavour (Jalikop, 2007; Holland et al, 2009; Opara et al., 2009). The edible part of the fruit (aril), which is usually consumed as fresh aril or as processed products, contains considerable amounts of acids, sugars, polyphenol and important minerals (Al-Maiman and Ahmad, 2002).

Pomegranate fruit peel colour is not a reliable indicator of the degree of ripening or readiness for consumption (Holland et al., 2009); therefore, harvesting fruit at optimum maturity is crucial for maintaining high sugar content, good colour and overall flavour for fresh market and juice industries. Fruit maturity status of some pomegranate cultivars has previously been assessed based on a combination of indices including external (peel) colour, aril pigmentation, total soluble solids contents and titratable acidity (Ben-Arie et al., 1984; Cristosto et al., 2000; Martinez et al., 2006). Aside from the influence of phenolics on the sensory quality of the fruit colour (anthocyanins) and taste (tannins), phenolic compounds in pomegranate have beneficial health effects. In recent years, due to increasing consumer interest and awareness on the health benefits of the fruit juice, the importance of evaluating fruit health-benefiting phenolic compounds as part of target traits for accurate fruit quality evaluation is well recognised (Holland et al., 2009). The health benefits of consuming pomegranates has been attributed to the exceptionally high antioxidant capacity that strongly correlates with high concentration and unique composition of phenolic compounds (Gil et al., 2000; Borochov-Neori et al., 2011; Fischer et al., 2011; He et al., 2011). However, the phytochemicals differ in concentration and are dependent on cultivar (Gil et al., 2000; Shwartz et al., 2009; Tezcan et al., 2009; Elfalleh et al., 2011). Furthermore, studies have also shown that differences in fruit maturity and agro-climatic regions may substantially influence the antioxidant capacity and major chemical indices, such as juice total soluble solids (TSS), sugar and organic acid composition, titratable acidity

(TA), total phenolics and anthocyanin concentration, as well as volatile constituents and mineral elements composition (Al-Maiman and Ahmad, 2002; Mirdehghan and Rahemi, 2007; Shwartz et al., 2009; Melgarejo et al., 2011; Vázquez-Araújo et al., 2011).

Commercial production of pomegranates is fairly new in South Africa, and 'Bhagwa' and 'Ruby' are among the most widely grown in the country and globally. Practically, it is the responsibility of the fruit industry to establish minimum maturity indices based on available scientific information and its needs (Cristosto et al., 2000). Unfortunately, at present there are no established quality/maturity standards in the South African pomegranate fruit industry. Furthermoreinformation is lacking on the compounds responsible for fruit organoleptic and health-promoting properties during fruit development and ripening of South African grown pomegranate cultivars. In the absence of objective maturity indices, calendar dates are commonly used to determine harvest periods by farmers (Brodie, 2009; Olivier, F., pers. comm., 2011). Unfortunately, maturity indices established in other pomegranate growing countries may not be directly applicable under South Africa conditions due to differences in cultivar types and agro-climatic regions. For example, Chace et al. (1981) established a maturity standard for 'Wonderful' grown in California based on 1.8% titratable acidity (TA) level and total soluble solids (TSS) content above 17%. From the point of view of flavour, these values are too high for most of the pomegranate cultivars grown in South Africa. More so, that the choice of a reliable harvest index should reflect consumer sensory quality requirements of harvested fruit that permits the postharvest delivery of the fruit to consumers to meet their demand for organoleptic, nutritional, and antioxidant attributes (Kader, 2008).

To determine the optimal accumulation period of desirable compounds, it was imperative to study the changes that occur in the various fruit metabolites such as sugar, organic acid, mineral element compositions and volatile constituents. This could be useful for two major reasons; first, to provide information on the behavior of the fruit during development and ripening, and second, it will enable the identification of most consistent indices relating to desirable organoleptic attributes, which are those with the best potential for the development of a reliable maturity index.

Based on these considerations, the aim of this work was to study the evolution of fruit biochemical properties, mineral element composition volatile constituents and antioxidant capacity during development and ripening over two growing seasons.

2. Materials and methods

2.1. Fruit sample

The study was carried out over two seasons during 2010/2011 and 2011/2012. Pomegranate fruit ('Ruby' and 'Bhagwa') grown in commercial orchards located in the Porterville regions (South Africa, 33°01′00″S, 18°58′59″E) were studied. The orchards were located on sandy loam soil, and the trees received the same fertilizer program and irrigation delivering about 32 $L.ha^{-1}.day^{-1}$. The trees were about 6 years old at planting distance of 5 m x 3 m, with same row orientation and tree training. The rainfall and temperature data were recorded at a nearby local meteorological station during the growing seasons. A sample of twenty fruits of the same size and without physical defect was randomly collected from different positions of 10 randomly selected trees per orchard, and transferred to the laboratory in an air-conditioned car. Sampling was done monthly, with the first samples being in January (54 days after full bloom; DAFB) when it was possible to squeeze juice from the arils till April for 'Ruby'; 139 DAFB or May for 'Bhagwa'; 165 DAFB. Each harvest date corresponded to a different maturity stage (S) ranging from S1 to S5 during fruit development. A detailed description of fruit at these stages is presented in Table 1. Arils were removed manually from fruits, and then followed by extraction of juice from the arils (without crushing the kernels) using a blender (Mellerware, South Africa).

2.2. Chemical composition

2.2.1. Juice absorbance, pH, titratable acidity and total soluble solids

Pomegranate juice (PJ) colour absorbance was measured at 520 nm using a Helios Omega UV-vis spectrophotometer (Thermo Scientific technologies, Madison, USA). The pH of PJ was determined at room temperature using a pH meter (Crison, Barcelona, Spain). All measurements were made on individual fruit samples. Titratable acidity (TA) was determined using a Metrohm 862 compact titrosampler (Herisau, Switzerland), and the results were expressed as percentage tartaric acid. Total soluble solids (TSS) were measured using a digital refractometer (Atago, Tokyo, Japan). BrimA index, a variant of TSS/TA and a criterion for acceptance of fruit juice, which is expressed as BrimA = TSS – k * TA, where k is the tongue's

sensitivity index normally ranging from 2 - 10 (Jordan et al., 2001; Jaya and Das, 2003). In this study *k* value of 2 was used to avoid negative BrimA index.

2.2.2. HPLC analysis of individual sugars and organic acids

Sugar and organic acid concentrations in PJ at each maturity stage were analyzed using high performance liquid chromatography (HPLC) (Agilent 1100 Series, Waldron, Germany) equipped with a diode array detector (DAD). A sample of ten microlitres of extracted juice sample was injected into the HPLC and optimal separation was performed in an isocratic mobile phase of 5mM H_2SO_4 (560 µl of H_2SO_4 in 2L) using an HPX 87H column (Aminex, 300 mm x 7.78 mm). A refraction index detector was utilized at 55°C at a flow rate of 0.5 mL/min with UV detection set at 210 nm. Sample preparation and chromatographic procedure were based on the method of Castellari et al. (2000). Identification and quantification of sugar and organic acid composition were made by comparison of peak retention times, peak areas and spectra with those of external standards. Total sugar and organic acid composition was calculated by summation of individual sugar and acids, respectively (Melgarejo et al., 2000). Measurements were conducted in triplicate on pooled fruit samples.

2.3. Phytochemical composition

2.3.1. Sample preparation

Crude PJ sample (1 mL) was extracted with 29 mL of cold 50% aqueous methanol. The resulting mixture was vortexed, and then sonicated in ice for 20 min in a cold water bath followed by centrifuging at 10000 rpm for 5 min at 4ºC (Merk, Eppendorf AG, Germany). The supernatant was subsequently collected and assayed for phenolic components and antioxidant capacity.

2.3.2. Determination of total phenolic compounds

Total phenolic concentration (TPC) was determined in triplicates by the Folin-Ciocalteu (Folin-C.) colourimetric method (Makkar et al., 2000) and results were expressed as gallic acid equivalents (GAE) per 100 mL PJ.

2.3.3. Total flavonoids concentration

The total flavonoids concentration (TFC) was determined using the method described by (Yang et al., 2009) and results were expressed as catechin equivalents (CAE) per 100 mL PJ.

2.3.4. Rhodanine assay for total gallotannins

Determination of total gallotannins concentration (TGC) in PJ was carried out as described by Makkar (2000). In triplicate, diluted extracts (50 µL) were mixed with 150 µL of 0.4 N sulphuric acid followed by 600 µL rhodanine. After 10 min, 200 µL of 0.5 N KOH were added and subsequently distilled water (4 mL) after 2.5 min. The absorbance was read at 520 nm against a blank that contained aqueous methanol instead of sample after 15 min incubation at room temperature. Gallic acid was used for the standard curve. TGC was calculated from the standard curve and expressed as gallic acid equivalent (GAE) per 100 mL PJ.

2.3.5. Total anthocyanin concentration

Total anthocyanin concentration (TAC) was quantified using the pH differential method (Wrolstad, 1993). In triplicate, PJ extracts (1 mL) were mixed with 9 mL of pH 1.0 and pH 4.5 buffers, separately. Absorbance was measured at 520 and 700 nm in pH 1.0 and 4.5 buffers and the result expressed as cyanidin 3-glucoside using the following equations:

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH4.0} \quad (1)$$

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

where A = Absorbance, ε = Cyd-3-glucoside molar absorbance (26,900), MW = anthocyanin molecular weight (449.2), DF = dilution factor, L = cell pathlength (1 cm). Final results were expressed as cyanidin 3-glucoside equivalents per 100 mL PJ (mg C_3gE/100 mL PJ).

2.3.6. Identification of individual anthocyanins using LC-MS^n

Liquid chromatography-mass spectrometry (LC-MS) analysis of anthocyanin components in PJ at different maturity stages was performed according to Fischer et al. (2011)

with slight modification, using a Synapt G_2 mass spectrometer UPLC^{TM} system (Waters Corp., Milford, USA) connected to a photo diode array detector and a BEH C18 column (1.7µm particle size, 2.1x100 mm, Waters Corp.).The mobile phases were 5% formic acid in water (v/v) as eluent A and 95% acetonitrile, 5% formic acid (v/v) as eluent B. The flow rate was fixed at 0.2 mL/min and the column temperature was set 40°C. The electrospray ionization (ESI) probe was operated in the positive mode with capillary voltage, 3 kV; and cone voltage, 15 V. The injection volume was 10 µL and the diode array detector set at between 200-600 nm. Individual anthocyanins were identified by comparison of the mass spectra with those reported in literature (Fischer et al., 2011) and were quantified as equivalents of standard cyanidin 3, 5-diglucoside (Sigma-Aldrich, St. Louis, USA).

2.4. Multi-element determination by ICP-OES technique

Mineral analysis was carried out at Bemlab Analytical Laboratory, Strand, South Africa. This determination was investigated only during the 2011 season. Ash content of fruit peel and arils was first recovered according to Kalra (1998), followed by the determination of concentration of 6 major elements (N, P, K, Ca, Mg and Na) and 5 trace minerals (Mn, Fe, Cu, Zn and B) using an inductively coupled plasma optical emission spectrometer (ICP-OES) calibrated with different concentrations of standard solutions of the minerals. Working conditions of the ICP-OES were:

Instrument:	ICP-OES (Varian-Vista; Australia)
RF power:	0.7 - 1.5 kW (1.2 - 1.3 kW for axial)
Plasma gas flow rate (Ar):	10.5 - 15 L per min (radial); 15 L per min (axial)
Auxiliary gas flow rate (Ar):	1.5 L per min
Viewing height:	5 - 12 mm
Copy and reading time:	1 - 5 s (max. 60 s)
Copy time:	3 s (max. 100 s)

Data was obtained from three analytical replicates for each mineral element.

2.5. GC-MS determination of volatile constituents

Volatile compounds in PJ samples were profiled by subjecting juice samples to headspace solid phase micro-extraction (HS-SPME). Five millilitres of fresh juice sample were dispensed into 22 mL crimp cap headspace vials, followed by the addition of 1 g of table salt (NaCl, Sigma, St. Louis, USA) to enhance the release of the volatile compounds (Lachenmeier et al., 2006). A 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) fiber was used for all the analysis. Pre-incubation and extraction times to 50°C were 10 and 20 min, respectively. Volatile organic compounds trapped in the fibre were analysed by an Agilent GC (6890 N, Agilent Technologies, Santa Clara, CA, USA) coupled with a mass spectrometer (5975 N, Agilent Technologies, Santa Clara, CA, USA), and equipped with an Rxi®-5Sil MS column (30 m length, 0.25 mm i.d., 0.25 µm film thickness). Oven temperature was maintained at 40°C for 2 min and then programmed to maximum temperature of 250°C at 5°C /min, where it was isothermally held for 10 min. Helium was the carrier gas at a constant flow rate of 1.2 mL/min. Tests were run in duplicate per sample, each sample comprising three individual fruit. Volatile compounds were identified by comparing their mass spectra with the mass spectra of libraries (NIST, 95) with comparison quality >90%. Semi-quantification of compounds identified was achieved by calculating the relative proportions (%) of each volatile compound as percentage ratio of peak area of each compound to the total peak area of all identified compounds. Measurements were conducted in triplicate on pooled fruit samples.

2.6. Antioxidant capacity

2.6.1. DPPH radical-scavenging activity

The DPPH assay was carried out in triplicate, according to the method used by Karioti et al. (2004) with some modifications. Methanolic extract of PJ sample (15µL) was diluted with methanol (735 µL) in test tubes followed by the addition of methanolic DPPH solution (750 µL, 0.1 mM). The mixtures were incubated at room temperature for 30 min in the dark, and the absorbance was measured at 517 nm using a UV-vis spectrophotometer. Absorbance was compared with the standard curve (ascorbic acid, 0 - 2.0 mM). The free-radical capacity of PJ was expressed as ascorbic acid (mM) equivalents per mL PJ (mM AAE /mL).

2.6.2. Ferric ion reducing antioxidant power (FRAP)

The antioxidant power of PJ was measured colorimetrically according to the method of Benzie and Strain (1996) with a few modifications (Fawole et al., 2012). The FRAP working solution containing mixtures of 300 mM acetate buffer (50 mL), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) (5 mL) and 20 mM ferric chloride (5 mL) was freshly prepared and incubated in a water bath at 37ºC before being used. In triplicates, diluted aqueous methanolic PJ extracts (150 µL) were added to 2850 µL of the FRAP working solution before incubation in the dark for 30 min. Trolox (100 - 1000 µM) was used for the calibration curve, and the results were expressed as trolox (mM) equivalents per mL PJ (mM TE/mL PJ).

2.7. Statistical analysis

The results of all studied variables are presented a mean (± S.E) values. Analysis of variance (ANOVA) was carried out using Statistica software (Statistica 11.0, StatSoft Inc., Tulsa, OK, USA) according to Duncan's multiple range test. Where appropriate, 2-way ANOVA was also carried out. Correlation coefficients (r) were determined by the Pearson correlation matrix method using SPSS for Windows. Principal component analysis (PCA) was carried out using the statistical software XLSTAT Version 2012.4.01 (Addinsoft, France).

3. Results and discussion

3.1. Juice absorbance, pH, titratable acidity (TA) and total soluble solids (TSS)

Changes in juice absorbance, pH, TA and TSS during pomegranate fruit development of the two pomegranate cultivars are reported in Table 2. For both cultivars, a gradual increase in juice absorbance value with advancing maturation occurred in each season. The absorbance values were notably higher at advanced stages (S4 and S5) when the fruit aril colour had changed from pink to the desirable red colouration. For both cultivars, no interactions were found between fruit maturity and growing season for juice absorbance ('Bhagwa': $p = 0.1552$; 'Ruby': $p = 0.3736$). However, juice absorbance was significantly influenced by fruit maturity for 'Bhagwa' ($p< 0.0001$) while the main differences for 'Ruby' were evidently due to fruit maturity and growing season (maturity; $p< 0.0001$, season; $p = 0.0254$) (Table 2). These results confirm

the findings of Gil et al. (1995) on Spanish cultivars. The observed changes in juice absorbance could be explained by increase in biosynthesis and accumulation of red anthocyanins in fruit. Indeed, according to Shulman et al. (1984), pomegranate juice absorbance is a reflection of anthocyanins which are light-absorbing plant-based pigments.

The pH of pomegranate juice characterizes its acidic taste (Zarei et al., 2011). Changes in juice pH during fruit development was significantly (p <0.05) influenced by the interaction between maturity stage and growing season. In the 2011 season, significant (p <0.05) increase in pH value occurred with advancing maturity in 'Bhagwa', whereas the changes in pH value for 'Ruby' did not follow a significant (p<0.05) pattern. In 2012 season, however, for both cultivars, immature fruit (S1 - S2) were characterized by low pH values (more acidic) while at later maturity stages (S3 - S5) had higher pH values (less acidic) (Table 2). The fluctuations observed in juice pH could be a reflection of within-season changes such as orchard management practices which could affect juice acidity during fruit development.

Titratable acidity (TA, expressed as % tartaric acid) in both cultivars decreased during fruit development and ripening, especially at S3 stage where a significant decline occurred, and then followed by gradual decrease until the fully-ripe maturity stage (S5) (Table 2). During maturity stages investigated, TA levels in the 'Bhagwa' decreased primarily due to fruit maturity and season (p<0.0001). The interaction between fruit maturity and season was evident on TA levels for 'Ruby' (Table 2). The TA level decreased from 0.39% to 0.31% in 2011 season and from 0.49% to 0.31% in the subsequent season (Table 2). The observed trends in TA levels in our study are in agreement with those reported by several researchers during fruit development of 'Ganesh', 'Taifi' and 'Wonderful' pomegranates (Ben-Arie et al., 1984; Gil et al., 1995; Kulkarni and Aradhya, 2005; Shwartz et al., 2009).

Considerable variation was observed in total soluble solid (TSS) content at all the maturity stages for both cultivars (Table 2). TSS content increased more rapidly during early stages of fruit development (S1 - S3) for both cultivars in each season, a phenomenon associated with active hydrolysis of starch to sugars in maturing fruit (Kulkarni and Aradhya, 2005). High TSS content is highly desirable in pomegranate fruit juice as it enhances sweetness and flavour especially if accompanied by a decrease in juice acidity and tannin concentration (Shwartz et al., 2009; Zarei et al., 2011). TSS content in both cultivars at full-ripe stage (S5) reached at least 15°Brix in each season. TSS contents increased in 'Bhagwa' from 10.33 to16.18°Brix in 2011

season and from 9.28 to 15.56°Brix in 2012 season. The differences in TSS accumulation may be attributed to the significant (p <0.0001) interaction between maturity stage and growing season, although seasonality effect alone was slightly noticeable but not significant (p = 0.0516; see appendix: paper 2). In contrast, the significant differences observed in TSS contents in 'Ruby' were particularly due to the influence of fruit maturity (p <0.0001, see appendix: paper 2). These results showed the significant influence of fruit maturity stage in TSS content, which suggests that it might be possible to define TSS content in fruit at a certain maturity stage regardless of growing season. These findings were in agreement with previous studies for other pomegranate cultivars grown under different agro-climatic regions and in different growing seasons (Ben-Arie et al., 1984; Shulman et al., 1984).

As a result of changes in TSS and TA contents, the ratio of TSS/TA increased considerably in both cultivars during the two seasons. TSS/TA ratio increased significantly at the first three maturity stages (S1 - S3) for both cultivars. Further the increase between the last two maturity stages (S4 - S5) was not significant except for 'Bhagwa' in 2012. TSS/TA has been reported as one of the most reliable indicators of fruit maturity in some pomegranates although it is largely dependent on cultivar (as fruit juice ranges from sweet to sweet-sour or sour) and agro-climatic conditions (Ben-Arie et al., 1984; Al-Maiman and Ahmad, 2002; Kulkarni and Aradhya, 2005; Shwartz et al., 2009). Fruit of the Wonderful cultivar (sour type) which is regarded as 'tasty' had a TSS/TA ratio range of 11 - 16 (Ben-Arie et al., 1984). According to Chace et al. (1981), a TSS value of 17% and a TA value of 1.8% (TSS/TA = 9.44) was recommended as having minimum maturity for commercial harvesting of pomegranates grown in California. Various TSS/TA values have been successfully used to classify of pomegranate cultivars of Spanish and Italian origins (Hernandez et al., 1999; Martinez et al., 2006). Furthermore, TSS/TA value is an important criterion also for evaluating pomegranate juice quality in the processing industry for formulation of food and beverage products (Al-Said et al., 2009). Results obtained in the present study have confirmed cultivar differences in TSS/TA ration as evidenced by the significant interaction effects between fruit maturity and growing season for 'Bhagwa' (p = 0.0082), whereas changes in TSS/TA values for 'Ruby' were mainly due to fruit maturity (p <0.0001) (Table 2).

To further explore the relationship between TSS and TA as a potential maturity indicator, BrimA index (Jordan et al., 2001) was calculated. This index allows smaller levels of acidity in

juice than sugar content to make the same numerical change to BrimA (Jordan et al., 2001; Jaya and Das, 2002). This observation was also valid in the present study (Table 2). Specifically, the main differences in BrimA were due to the interactions (p <0.0001) between fruit maturity and season for 'Bhagwa', whereas only fruit maturity had significant (p <0.0001) influence on BrimA for 'Ruby' (Table 2).

3.2. Sugars and organic acids concentrations

Figure 1 and Table 3 show the effects of fruit maturity stages on sugar and organic acid composition of juice during the 2011 season. Glucose and fructose were the major soluble sugars found in both cultivars. This result is in agreement with previous studies on other pomegranate cultivars (Melgarejo et al., 2000; Al-Maiman and Ahmad, 2002; Shwartz et al., 2009; Tezcan et al., 2009; Mena et al., 2011). Although appreciable amounts of sucrose were reported in some Tunisian pomegranate cultivars (Hasnaoui et al., 2011), the amount of sucrose in the investigated cultivars were less than the level of quantification (LOQ) throughout the developmental stages (data not shown). The concentration of both sugars increased significantly and more rapidly until S3 stage in both cultivars, and further sugar accumulation was not significant in 'Ruby' unlike in 'Bhagwa' where further accumulation was significant at advanced maturity stages. Total sugars increased from 626.05 (S1) to 1514.11 (S5) mg/100 mL juice in 'Bhagwa', and 799.98 (S1) to 1288.07 (S5) mg/100 mL juice in the 'Ruby'. The accumulation of simple sugars is one of the processes occurring during the final developmental stages of fruit, resulting in increases in sweetness as fruit approach ripeness (Shwartz et al., 2009; Zarei et al., 2011). In both cultivars, fructose concentration was more than glucose, and the glucose-fructose ratio (G/F) ranged from 0.72 to 0.86 among the maturity stages. This is noteworthy as fructose is twice as sweet as glucose (Nookaraju et al., 2010), and could be used as a measure of degree of juice sweetness during fruit ripening (Al-Maiman and Ahmad, 2002).

Organic acid concentration is another important maturity parameter of pomegranate fruit because it plays a major role in the development of juice flavour. The findings in the present study showed that the major organic acids in juice of both cultivars were tartaric, citric and malic acids. Of the total acids quantified at late maturity (S5), tartaric acid concentration contributed to more than 70% of total acids in each cultivar. The organic acids decreased significantly during fruit early maturity stages in both cultivars and decline steadily afterwards in 'Bhagwa', while in

'Ruby' the concentrations of citric and malic acids declined below the quantification level between S3 and S5 maturity stages (Table 3). The decline in total organic acids accounted for aboutbetween immature (S1) and fully ripe (S5) fruit was 64% and 73% in 'Bhagwa' and 'Ruby', respectively. This result corroborates the general phenomenon that organic acids accumulate during early fruit maturity stage and are used rapidly as respiratory substrates in mature fruit (Diakou et al., 2000). Furthermore, the dilution effect associated with increase in fruit size during growth and development also contributes to reduction of acidity levels (Moing et al., 2001).

3.3. Phytochemical compounds in fruit during development and ripening

Seasonal changes in total phenolics (TPC), total flavonoid concentration (TFC), gallotannin concentration (GTC) and total anthocyanin concentration (TAC) in the investigated cultivars are presented in Table 4. Generally, total phenolic concentration (TPC) has been reported to occur at high concentrations at early stages of fruit development and decline with advancing maturation (Al-Maiman and Ahmad, 2002; Kulkarni and Aradhya, 2005; Shwartz et al., 2009). During 2011 for 'Bhagwa' the highest TPC was detected at S1 (2027.46 mg GAE /100 mL) and declined by 71% at late maturity stage (S5). Similarly, for 'Ruby', TPC declined by 54% between S1 (1051.60 mg GAE/100 mL) and S5 (483.31 mg GAE /100 mL). In the following season (2012), the TPC in both cultivars were lower than those observed the previous season, with a reduction of about 82% ('Bhagwa') and 49% ('Ruby') between S1 and S5. The significant (p<0.05) interaction between maturity stage and growing season revealed that total phenolic concentration in both cultivars was influenced by fruit maturity (Table 4). Decrease in phenolic concentration during fruit development might result from the oxidation of polyphenols (Amiot et al., 1995; Kulkarni and Aradhya, 2005; Shwartz et al., 2009). The reduction of phenolic concentraton during fruit development has also been attributed to the decline and eventual end of polyphenols biosynthesis during fruit maturation (Kulkarni and Aradhya, 2005; Shwartz et al., 2009).

The decline in TPC corresponded with significant (p<0.05) reduction in total flavonoids and gallotannin concentrations in fruit of both cultivars. Since flavonoids and gallotannins are phenolic compounds, it was hypothesized that TFC and GTC would contribute to the total phenolic concentration of fruit juice (Gil et al., 2000; Fischer et al., 2011). A considerable

decline in total flavonoids, including condensed and hydrolysable tannins, is desirable in pomegranate juice. Moderate concentration of these compounds contribute to the typical pomegranate juice flavour, whereas an excessive flavonoids concentrations, like those measured in immature and unripe fruit stage, confers an unpleasant and astringent taste (Al-Said et al., 2009; Zarei et al., 2011). Comparable values of TFC were observed between the two harvest seasons for both cultivars. For 'Bhagwa' at S5 stage, the TFC value was 201.57 mg CAE/100 mL in 2011 and 165.99 mg CAE/100 mL in 2012. TFC concentrations in 'Ruby' were significantly higher at 395.27 and 309.93 mg CAE/100 mL in 2011 and 2012 seasons, respectively. The significant (p<0.05) interactions between fruit maturity and growing season was limited to significant (p<0.05) influence of maturity for both cultivars. In contrast, for GTC, both main factors contributed to the significant (p<0.05) interactions observed between the factors (Table 4).

Anthocyanins are phenolic compounds that give the characteristic red colouration to pomegranates. Total anthocyanin concentration (TAC) increased with advancing fruit maturity during each season (Table 4). In both cultivars TAC was detected at very low concentration at S1 stage and steadily increased until S3 stage, but the rate of accumulation increased rapidly thereafter. However, there was no significant difference (p<0.05) between the last two maturity stages of 'Ruby' in 2012, a possible indication of fruit readiness for harvest between S4 and S5 maturity stages. Fruit maturity stage interacted significantly (p<0.05) with growing season for 'Bhagwa' but overall, significant variability was noted for fruit maturity status (p <0.0001). Furthermore, this study showed that anthocyanin concentration in 'Ruby' was primarily influenced by fruit maturity (p <0.0001) (Table 4). For both cultivars, the increase in anthocyanin concentration was more marked between S4 and S5 maturity stages. These results confirmed previous literature evidence. Hernández et al. (1999) reported that anthocyanin concentration in ripe fruit of Spanish cultivars was eightfolds higher than the early stages of fruit development. The accumulation of anthocyanins in plant tissue is directly linked to the contribution of phenolic compounds to the biosynthesis of the flavylium ring of anthocyanins (Kulkarni and Aradhya, 2005). Deep colour formation is one of the most important parameters used in assessing pomegranate fruit aril quality. Indeed, high anthocyanin concentration results in high fruit red colouration. This attribute is, in particular, desirable in pomegranate arils and juice. The results from the present study demonstrate that anthocyanin concentration in fruit juice

offers a potential tool to assess fruit maturation in pomegranate grown in the Porterville area since its accumulation continued throughout fruit development and did not decrease when physiological maturity was reached during two the seasons studied.

3.4. Individual anthocyanins

The biosynthesis of individual anthocyanin in pomegranate juice was measured at the maturity stages studied. Total anthocyanin composition represents the summation of the individual anthocyanins compounds identified in each stage. Six major anthocyanins were identified: cyanidin 3,5-diglucoside, cyanidin 3-glucoside, delphinidin 3,5-diglucoside, delphinidin 3-glucoside, pelargonidin 3,5-diglucoside and pelargonidin 3-glucoside (Figure 2). Anthocyanin levels at the first sampling stage (S1) in both cultivars were below the detection limit in both seasons. As fruit development progressed in S2, three different anthocyanins were identified in both cultivars. In 'Bhagwa', dephinidin-3,5-diglucoside, cyanidin-3,5-diglucoside and pelargonidin-3,5-diglucoside were identified, while the mono- and di-glucosylated derivatives of delphinidin, as well as cyanidin 3,5-diglucoside were identified in 'Ruby'. In 'Bhagwa', anthocyanin composition increased due to the addition cyanidin-3-glucoside at S3 stage, delphinidin-3-glucoside at S4 stage, and pelargonidin-3-glucoside at the last maturity stage. Anthocyanin concentration in 'Ruby' increased further due to the presence of pelargonidin-3,5-diglucoside and cyanidin-3-glucoside at S3 stage, and at S4, all the six anthocyanin compounds were identified (Figure 2B). The amount of individual anthocyanins in fruit juice varied in relation to different maturity stages. The most abundant anthocyanin compound throughout fruit developmental stages was cyanidin 3,5-diglucoside. Although, the same anthocyanins can give rise to different colours depending on factors such as pH, concentration and copigmentation (Brouillard,1988), it has been reported that the derivatives of pelargonidin are responsible for the orange and red colours, while the derivatives of cyanidin are responsible for red, and crimson and delphinidin derivatives account for violet and blue colour (Harborne, 1982). Hernández et al. (1999) reported that delphinidin 3,5-diglucoside was the main pigment in the juice of the Spanish pomegranate cultivars studied, while cyanidin 3-glucoside was the predominant pigment in the Israeli 'Wonderful' (Borochov-Neori et al.,2011).

3.5. Changes in mineral element concentration

Changes in mineral element concentration in fruit aril and peel of the investigated cultivars at different developmental stages are presented in Table 5. Fruit maturity stage was a significant (p<0.05) factor for all the mineral elements in both aril and peel for both cultivars. The results showed that mineral element concentrations (with the exception of N, P and Zn) in aril for both cultivars were the highest at the first maturity stage (S1), and then declined in subsequent stages before slightly increasing in the last maturity stage (S5). Throughout fruit development, amongst the macro-elements, N was predominant in aril while K was predominant in peel for both cultivars. On the other hand, amongst the micro-elements, Fe and B were predominant in aril and peel, respectively. Previous studies have reported similar findings, where K and Fe were predominant in pomegranate aril (Al-Maiman and Ahmad, 2002; Mirdehghan and Rahemi, 2007; Gozlekci et al., 2011). Most of the mineralelements measured decreased between the first and last maturity stages in the fruit parts of both cultivars (Table 5), although in the case of Mn, Fe and Zn in 'Ruby' peel there were clearly no significant differences in the pattern throughout the season. The early stages of fruit growth and development were characterized by rapid and extensive changes in mineral elements. In a previous study, Mirdehghan and Rahemi (2007) reported sharp decreases in mineral concentrations in arils during the first 40 DAFB, which was followed by gradual decrease in the following days after full bloom. The authors suggested that decrease in mineral element concentration may occur when fruit growth rate increases faster that rate of mineral accumulation.

Late harvest fruit (S5), the relative concentration of the macro-elements in fruit parts were; 'Bhagwa' aril = N > K > P > Ca > Mg > Na; peel = K > N > Ca > P > Mg > Na, for 'Ruby' aril = N > K > P > Mg > Ca > Na, peel = K > N > P > Ca > Mg > Na. For Iranian 'Malas Yazdi', Mirdehghan and Rahemi (2007) reported similar order of macronutrient concentration to the present study for arils, and peel as K > N > Ca > P > Mg > Na. Similarly, Gozlekci et al. (2011) reported a decreasing order of K > P > Ca > Mg > Na in arils of 'Hicaznar' grown in Turkey. The general orders of concentration of the micro-elements in mature (S5) fruit parts were Fe > Zn > B > Mn > Cu (aril) and B > Mn > Fe > Cu = Zn (peel) for 'Bhagwa', and Fe > B > Zn > Cu > Mn (aril) and B > Fe > Zn > Mn > Cu (peel) for 'Ruby'. The concentrations of mineral elements in found in the present study were comparable to those reported by Al-Maiman

and Ahmad (2002). However, Mirdehghan and Rahemi (2007) reported lower values, while Gozlekci et al. (2011) reported higher values of mineral element concentrations during fruit development. This variation could be attributed to differences in cultivar and preharvest factors such as plant nutrition, climate and soil conditions (Hamurcu et al., 2010).

3.6. Volatile constituents

The relative contents (%) of volatile compounds in juice of 'Bhagwa' and 'Ruby' are presented in Table 6. A total of only 10 aroma compounds were detected in the headspace of juices from both cultivars. This was not surprising because pomegranate fruit are known to have very low odour and aroma intensity (Carbonell-Barrachina et al., 2012) which makes it difficult to study the aroma composition. Factors such as sample preparation and method of determination could influence the values obtained. Raisi et al. (2008) used pervaporation to recover more aroma compounds from juice of Iranian pomegranate cultivars but were able to identify only nine compounds. However, in a recent study by Calín-Sánchez et al. (2011), a total of 18 compounds were identified in Spanish pomegranate juices using the hydrodistillation technique.

In this study, the identified compounds belong to 4 chemical groups including alcohols, esters, ketones and terpene (Table 6). In general, the composition and relative proportions of the aroma volatiles were different among fruit maturity stages for both cultivars. Only two aroma volatile compounds (hexanol and limonene) were detected at the first maturity stage (S1) in both cultivars. Hexanol belongs to the alcohol group, constituting 52.4% in 'Bhagwa' and 95.2% in 'Ruby', while limonene belongs to the terpenes group and constituted 47.6% and 4.8% of total aroma in 'Bhagwa' and 'Ruby', respectively. In each of the subsequent maturity stages (S2 - S5), 'Bhagwa' had six compounds while 'Ruby' had seven compounds. At maturity stage S2, the alcohol group had the highest proportion and increased in 'Bhagwa' but decreased in 'Ruby' when compared to the previous stage. As fruit advanced to maturity stage S3, the ketone group became prominent in 'Bhagwa' accounting for 34.7% of the total volatile compounds. The identified ketones comprised of 2-heptanone (7.8%) and 2-octanone (27.7%). In 'Ruby', however, the esters group contributed to 23.7% of the total volatiles at S3, comprised of hexylacetate, 2-ethyl acetate and buty acetate, and the group dominated the rest of the maturity stages (S4 = 49%; S5 = 33.4%). In 'Bhagwa', maturity stage S4 was characterized by the

dominance of esters with total proportion of 36.4%, while in S5 stage the ethanol group (44.5%) was dominant.

The odour threshold of each volatile compound present in fruit could be used to characterize aroma intensity (Visai and Vanoli, 1997; Melgarejo et al., 2011). Quantitatively, the alcohol group and limonene were identified in all the maturity stages in both cultivars (Table 6). The alcohol group was mainly represented by hexanol and 3-hexen-1-ol in 'Bhagwa' and hexanol, 3-methyl butanol and phenyl ethanol in 'Ruby'. Limonene has previously been identified in pomegranate juice (Melgarejo et al., 2011), and describes the mild, citrus, sweet, orange and lemon sensory attributes in fruits (Melgarejo et al., 2011). In the present study, limonene content decreased between S1 and S5 stages. Further studies on this compound could possibly be linked to fruit maturity. The esters group was another important group of aroma volatile compounds identified in large proportion in fruit juice at advanced maturity stages (S4 and S5). The hexyl acetate is a major representative in this group and is known to be responsible for fruity and pineapple odours in fruit (Visai and Vanoli, 1997). Megarejo et al. (2011) reported the aldehydes group as predominant in Spanish cultivars. This group was not identified in the present study on 'Baghwa' and 'Ruby' grown in South Africa.

3.7. Antioxidant capacity

The antioxidant capacity of pomegranate juice at different stages of fruit maturation is shown in Figure 3. Antioxidants may act in various ways in different antioxidant assays (Çam et al., 2009). Antioxidant capacity has been determined by several methods based on both the free radical scavenging and the oxidation-reduction mechanisms, although the mechanism of action set in motion by the antioxidant activity of these compounds is still not clearly understood (Viuda-Martos et al., 2010). In this study, antioxidant capacity was measured using DPPH radical scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP). There was a significant ($p<0.05$) decrease in the antioxidant capacity (DPPH and FRAP assays) of both cultivars with advancing maturity stages in both seasons. For 'Bhagwa', ferric reducing power (FRAP) decreased from 1.67 to 0.74 mM TE/mL (2011 season) and from 1.48 to 0.76 mM TE/mL (2012 season), while the radical scavenging activity (DPPH) decreased from 1.57 to 0.39 mM AAE/mL and from 1.16 to 0.33 mM AAE/mL in 2011 and 2012 seasons, respectively. For 'Ruby', FRAP decreased in 2011 and 2012 seasons from 1.43 to 0.71 mM TE/mL and from 0.97

to 0.52 mM TE/mL, respectively, while DPPH decreased from 0.89 to 0.34 AAE/mL in 2011 season and from 0.52 to 0.27 AAE/mL in 2012 season. Fruit maturity stage and growing season had significant (p<0.0001) interaction effects on antioxidant capacity in both FRAP and DPPH assays for both cultivars. It appeared that the variation in antioxidant capacity was brought about by the significant (p<0.05) effects of fruit maturity and growing seasons. The reduction in antioxidant capacity during fruit development may be associated with the decrease in quantity of polyphenols in fruit as shown in Table 4. This contribution of phenolic compound to total antioxidant capacity in pomegranate fruit has been reported in previous studies (Gil et al., 2000; Fischer et al., 2011). In particular, fruit harvested during early maturity stages (S1 and S2) showed markedly higher antioxidant values. This further supports the higher polyphenol concentrations such as flavonoid concentrations found in fruit juice (Table 4). Although anthocyanins are known to be antioxidant compounds, their increase during fruit development constituted only a small proportion of total flavonoid concentration of fruit juice; hence the change in flavonoid has a much greater influence than anthocyanins on juice antioxidant capacity. Fully ripe 'Ruby' fruit (S5) had higher (but not significant in some cases) antioxidant capacity than fruit at the preceding maturity stage (S4) for both seasons, although this was not the case in 'Bhagwa' (Figure 3). Higher values antioxidant capacity at the last maturity stage could, in part, be due to a relatively higher accumulation of anthocyanin compounds, highlighting the important contribution of anthocyanins in total antioxidant capacity of fruit at harvest maturity of the investigated cultivars.

3.8. Correlation between maturity indices

Pearson correlation was used to investigate the interrelationships between selected chemical indices of fruit maturity including phenolic components and the antioxidant capacity over the two seasons for both cultivars (Tables 7 and 8). Significantly (p<0.05) strong relationships were revealed among some of the parameters assessed. TSS and TA showed strong negative correlation in both cultivars (r = -0.98). This relationship clearly showed that decrease in fruit titratable acidity may also bring about an increase in TSS during fruit development regardless of fruit maturity stage or season. Furthermore, the significant positive correlations between TSS and the main sugars (glucose and fructose) indicated that increase in TSS could be as a result of increase in the composition of sugar. It is therefore reasonable to hypothesize that

TSS could be a suitable predictor of sugars in the investigated cultivars. The hypothesis could also be applied to the significant positive correlations between TA and organic acids concentration in both cultivars (Table 7 and 8). Another interesting relationship was the strong positive correlation between juice absorbance and anthocyanin concentration in both cultivars. This suggests that high anthocyanin concentration would contribute to better red colouration of arils, which is a desirable attribute in pomegranate marketing. Considering the reported health benefits of consuming fruit high in phenolic compounds (Gil et al., 2000; Tzulker et al., 2007), its therefore not surprising that antioxidant capacity (both DPPH and FRAP) showed a positive correlation with total phenolic concentration but not with the concentration of anthocyanins. Therefore, it is plausible that the changes in the antioxidant capacity of fruit during maturity are largely dependent on the total phenolic concentration. This is suppoted by evidence from results of the study on 'Wonderful' grown in Israel (Shwartz et al., 2009).

3.9. Principal component analysis

To elucidate the metabolic changes that occur during pomegranate fruit maturation, key maturity indices measured in the two seasons were subjected to principal component analysis (PCA). An eigenvalue gives a measure of the significance of the factor; thus the factors with the highest eigenvalues are the most significant and eigenvalues ≥1 are considered significant (Shrestha and Kazama, 2007; Garizi et al., 2011). PCA of the data sets yielded two principal factors (F1 and F2) with eigenvalues >1, explaining more than 80% of the total variance. Acceptable explanations can be drawn from the first factor (F1) which accounted for over 75% of the total variance in both cultivars (Figure 4 A and B). The relationships between the indices were evidenced by short distances between juice absorbance and anthocyanin concentration and between TSS and BrimA. Also, short distances between phenolic groups and antioxidant capacity suggest significant contribution of phenolics to the antioxidant capacity measured by both DPPH and FRAP assays. According to Shwartz et al. (2009), during the early fruit maturity stage, when high acidity and total phenolics prevailed, fruit antioxidant activity was high. However, both concentration of phenolics and antioxidant activity decreased with advancing fruit maturation. In the present study, the decrease in total phenolics and acidity from immature to ripe fruit was also characterized by a shift from right to left on the PCA plot, reflecting the beginning of the ripening process in the cultivars between S2 and S3 (Figure 4 A and B).

Close examination of eigenvalues, loadings and significant maturity parameters in each cultivar showed that major compositional changes in fruit are developmentally regulated (Table 9). The factor loadings obtained corresponded with the strength of correlation between the original variables and the factors. According to Liu et al. (2003), classification of factor loading is considered 'strong', 'moderate' and 'weak' corresponding to absolute loading values of >0.75, 0.75 - 0.50 and 0.50 - 0.30, respectively. In both seasons, positive scores of F1 corresponded to immature and unripe fruit (S1 - S2). Ripe and full-ripe fruit at maturity stages S4 and S5 had high negative scores along F1, while fruit at S3 that had low negative scores were semi-ripe for both cultivars (Figure 4 and Table 9). The scores can be interpreted by the factor loadings (Table 9), with F1 showing strong positive correlations for TA, TPC, TFC, GTC, FRAP and DPPH, and negative loadings for juice absorbance, pH, TSS, TSS:TA, BrimA and anthocyanin concentration. In general, this study showed that young (immature) fruit had higher acidity (resulting from higher organic acid concentrations) and phenolic concentrations, while mature but semi- mature to full-ripe fruit had higher anthocyanin concentration and TSS (which correlated with fructose and glucose).

4. Conclusion

Seasonal changes in chemical composition, aroma volatiles and mineral elements as well as antioxidant properties of pomegranate fruit at different maturity stages were investigated during the time course of fruit development and ripening. Results obtained showed that major compositional changes in the fruit are developmentally regulated. Fruit organic acids (which influence TA content) and phenolics declined with advancing maturity, suggesting a decrease in juice sourness, while sugar concentration and red colour intensity (anthocyanin) increased in both cultivars. Principal component analysis (PCA) was used to characterize the relationships between the major maturity indicators. Fruit at advanced maturity stages (S4 and S5) were characterized by higher TSS as well as intense fruit and aril pigmentation, which coincided with highest accumulation of anthocyanins.

The interactions between fruit maturity stage and growing season did not influence juice colouration (absorbance) and TA for 'Bhagwa', and juice colouration, TSS, TSS:TA and BrimA for 'Ruby'. However, the effects fruit maturity and or season were clearly evident. Moreover, significant correlations were found between some of the maturity indices. For instance, results

showed that TSS could be used as a measure of glucose and fructose levels in the fruit cultivars during maturity, while TA could reflect the contents of organic acids in maturing fruit. For 'Bhagwa', juice colouration and TSS were not influenced by growing season but rather by fruit maturity stage. Similarly, TSS, TSS:TA and BrimA level in 'Ruby' did not show significant seasonality during fruit maturation.

In combination, the identified maturity indices (absorbance, TSS, TSS:TA and BrimA level) would account for the evolution of juice colour, flavour and taste. The identified maturity indices for each cultivar could aid the search for reliable maturity markers to determine fruit readiness for harvest. However, it is noted that the findings reported in this thesis are only representative for 'Bhagwa' and 'Ruby' grown in Porterville area in South Africa and only over two seasons; hence further studies will have to be conducted in more growing areas and seasons in other to make general recommendations of fruit maturity and readiness for harvest. In addition, studies on sensory and postharvest storage quality of fruit at different harvest dates should be conducted to assist in establishing optimum maturity standards.

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Table 1

Description of fruit maturity at different sampling stages along days after full bloom (DAFB)

Stage	DAFB (Month)		Description of maturity stages
	'Bhagwa'	'Ruby'	
S1	54 (Jan)	54 (Jan)	Immature: Green skin, immature white arils with immature kernels
S2	82 (Feb)	82 (Feb)	Mature/unripe: Light-red skin, mature white arils with mature kernels
S3	110 (Mar)	110 (Mar)	Mature/semi-ripe: Red skin, mature pink arils with mature kernels
S4	140 (Apr)	132 (Apr)	Mature/ripe/ harvest 1: Red skin, mature red arils with mature kernels
S5	165 (May)	139 (Apr)	Mature/full-ripe/harvest 2: Deep-red skin, deep-red arils with mature kernels

Table 2

Changes in chemical composition in pomegranate juice at major maturity stages over two seasons

Maturity stage	Juice abs.	pH	TSS (°Brix)	TA (% tartaric)	TSS:TA	BrimA (TSS- k*TA)
'Bhagwa'						
2011_S1		3.18±0.03^{e}	10.33±0.35^{f}		16.68±0.35^{e}	9.10±0.20^{f}
2011_S2		3.22±0.05^{de}	11.97±0.19^{e}		21.23±0.58^{e}	10.83±0.14^{e}
2011_S3		3.24±0.23^{de}	13.83±0.29^{d}		30.89±0.59^{d}	12.94±0.17^{d}
2011_S4		3.35±0.04^{cde}	15.12±0.25^{bc}		39.19±1.31^{c}	14.42±0.06^{bc}
2011_S5		3.57±0.13^{abc}	16.18±0.21^{a}		41.83±2.21^{c}	15.38±0.06^{a}
2012_S1		3.44±0.02^{abcd}	9.28±0.13^{g}		18.96±0.26^{e}	8.30±0.13^{f}
2012_S2		3.37±0.01^{bcd}	14.52±0.10^{cd}		30.11±0.31^{d}	13.55±0.1^{cd}
2012_S3		3.58±0.02^{ab}	14.90±0.07^{bc}		47.69±0.41^{b}	14.27±0.06^{bc}
2012_S4		3.61±0.03^{a}	15.31±0.06^{abc}		49.68±0.78^{b}	14.69±0.06^{ab}
2012_S5		3.54±0.02^{abc}	15.56±0.03^{ab}		56.70±0.66^{a}	15.00±0.03^{ab}
S1*	0.06±0.24^{c}			0.55±0.02^{a}		
S2	0.29±0.25^{c}			0.53±0.04^{a}		
S3	0.71±0.27^{c}			0.38±0.01^{b}		
S4	1.71±0.20^{b}			0.35±0.06^{b}		
S5	2.91±0.19^{a}			0.34±0.01^{b}		
2011	1.22$^{n.s}$			0.50^{a}		
2012	1.02$^{n.s}$			0.37^{b}		
Prob. > F						
Maturity (A)	**< 0.0001**	0.0563	< 0.0001	**< 0.0001**	< 0.0001	< 0.0001
Season (B)	0.2747	0.0061	0.0516	**< 0.0001**	< 0.0001	0.0017
A * B	0.1552	**0.0067**	**< 0.0001**	0.4707	**0.0082**	**< 0.0001**

Table 2 contd.	Juice abs.	pH	TSS (°Brix)	TA (% tartaric)	TSS:TA	BrimA (TSS- k*TA)
'Ruby'						
2011_S1		3.30±0.03^{b}		0.39±0.03^{b}		
2011_S2		3.11±0.04^{b}		0.37±0.01^{bc}		
2011_S3		3.25±0.09^{b}		0.33±0.04^{cd}		
2011_S4		3.18±0.05^{b}		0.33±0.01^{cd}		
2011_S5		3.28±0.05^{b}		0.31±0.01^{d}		
2012_S1		3.25±0.02^{b}		0.49±0.01^{a}		
2012_S2		3.27±0.02^{b}		0.50±0.01^{a}		
2012_S3		3.58±0.02^{a}		0.32±0.004^{d}		
2012_S4		3.61±0.02^{a}		0.32±0.02^{d}		
2012_S5		3.62±0.01^{a}		0.31±0.03^{d}		
S1*	0.14±0.02^{c}		10.95±0.29^{c}		25.25±1.89^{b}	10.06±0.29^{c}
S2	0.34±0.03^{c}		13.23±0.30^{b}		31.17±1.92^{b}	12.31±0.38^{b}
S3	0.59±0.12^{bc}		14.23±0.27^{b}		44.51±1.90^{a}	13.58±0.24^{b}
S4	1.53±0.18^{b}		15.03±0.20^{a}		48.68±1.30^{a}	14.40±0.20^{a}
S5	2.40±0.19^{a}		14.97±0.21^{a}		46.79±1.29^{a}	14.32±0.19^{a}
2011	0.86^{b}		13.41$^{n.s}$		39.34$^{n.s}$	12.71$^{n.s}$
2012	1.22^{a}		13.37$^{n.s}$		37.39$^{n.s}$	12.62$^{n.s}$
Prob. > F						
Maturity (A)	**<0.0001**	0.0015	**<0.0001**	<0.0001	**<0.0001**	**<0.0001**
Season (B)	0.0254	**<0.0001**	0.8760	0.0003	0.0996	0.8634
A * B	0.3736	0.0046	0.1523	**<0.0001**	0.3152	0.2489

Factorial ANOVA was performed for Factor A (maturity stage) and Factor B (season). Different letter(s) on column indicate statistically significant differences (p<0.05) according to Duncan's multiple range test. *2011 and 2012 data were pooled for parameter(s) where interaction effects were not significant (p<0.05). n.s – non-significant.

Table 3

Changes in organic acid composition (mg/100 mL fresh juice) of pomegranate fruit at major maturity stages (2011 season)

Stage	Tartaric acid	Citric acid	Malic acid	Total acids
Bhagwa				
S1	378.04±12.63[a]	180.77±6.14[a]	397.64±19.38[a]	956.45±38.15[a]
S2	345.35±3.95[a]	141.67±6.82[a]	184.94±9.32[b]	671.96±20.09[b]
S3	284.25±11.75[b]	123.39±21.97[abc]	38.59±2.49[c]	446.23±36.21[c]
S4	271.33±19.46[b]	102.75±42.15[bc]	27.91±3.67[c]	401.99±65.28[c]
S5	262.41±28.02[b]	54.50±8.00[c]	26.77±1.85[c]	341.68±37.87[c]
Ruby				
S1	290.85±7.54[a]	176.26±33.60[a]	233.44±32.75[a]	700.55±68.84[a]
S2	271.71±6.77[ab]	52.25±5.90[b]	142.46±21.48[b]	466.41±28.41[b]
S3	237.44±9.84[b]	< LOQ [c]	28.40±1.08[c]	265.84±4.69[c]
S4	240.70±26.36[b]	< LOQ [c]	< LOQ [d]	240.69±18.58[c]
S5	188.00±8.74[c]	< LOQ [c]	< LOQ [d]	188.00±8.74[c]

[y]< LOQ - Less than level of quantification (< 25 mg/100 mL). Mean±S.E presented. Different letter(s) on column per cultivar indicate statistically significant differences (p< 0.05) according to Duncan's multiple range test.

Table 4

Changes in phenolic concentration (mg/100 mL) in pomegranate juice at major maturity stages over two seasons

	'Bhagwa'				'Ruby'			
Stage	Total phenolics	Total flavonoids	Gallotannins	Anthocyanins	Total phenolics	Total flavonoids	Gallotannins	Anthocyanins
2011_S1	2027.46±17.60^{a}	1459.94±106.91^{a}	125.33±5.78^{a}	0.01±0.00^{d}	1051.60±36.35^{a}	752.18±38.49^{a}	64.80±4.81^{a}	
2011_S2	1335.57±10.14^{c}	749.89±109.60^{c}	61.07±6.37^{cde}	6.47±0.80^{cd}	772.59±14.94^{b}	621.11±47.12^{ab}	35.20±1.22^{bcd}	
2011_S3	675.83±76.46^{ef}	568.16±119.86^{cd}	53.60±6.84^{e}	13.11±0.63^{bc}	409.75±11.25^{de}	253.19±18.69^{ef}	38.13±0.41^{bc}	
2011_S4	550.25±7.47^{g}	462.25±23.68^{d}	58.30±3.61^{cde}	40.84±0.31^{a}	429.63±29.99^{d}	356.35±20.51^{de}	21.07±0.82^{e}	
2011_S5	583.72±43.2^{gf}	201.57±29.32^{e}	56.53±2.97^{de}	48.61±0.01^{a}	483.31±4.31^{cd}	397.27±26.48^{cde}	29.07±0.62^{cd}	
2012_S1	1499.35±2.56^{b}	1045.62±1.96^{b}	91.46±0.10^{b}	0.89±0.48^{d}	771.00±22.55^{b}	529.19±9.39^{bc}	41.23±0.64^{b}	
2012_S2	1076.36±39.60^{d}	1002.34±4.91^{b}	89.34±0.24^{b}	4.59±0.27^{d}	707.07±13.73^{b}	531.911±15.43^{bc}	41.41±1.05^{b}	
2012_S3	753.36±3.56^{e}	697.60±23.55^{c}	80.96±0.75^{b}	13.99±0.84^{bc}	624.07±14.89^{bc}	472.41±10.19^{bcd}	37.38±0.69^{bc}	
2012_S4	257.83±5.53^{g}	465.21±31.56^{d}	76.11±1.29^{bc}	19.42±1.44^{b}	268.79±39.85^{e}	229.34±27.27^{f}	20.89±1.85^{e}	
2012_S5	265.54±13.46^{h}	150.99±24.35^{e}	52.59±3.98^{e}	45.79±4.46^{a}	386.59±50.24^{de}	309.93±34.38^{ef}	26.36±2.33^{ed}	
S1*								0.50±0.15^{d}
S2								4.44±0.59^{cd}
S3								9.67±0.70^{c}
S4								16.46±1.32^{b}
S5								25.86±1.82^{a}
Prob. > F								
Maturity (A)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	**< 0.0001**
Season (B)	< 0.0001	0.7121	0.0085	0.3028	0.0075	0.0542	0.0179	0.0589
A * B	**< 0.0001**	**0.0011**	**0.0018**	**0.0026**	**0.0003**	**0.0022**	**0.0028**	0.1535

Factorial ANOVA was performed for Factor A (maturity stage) and Factor B (season). Different letter(s) on column indicate statistically significant differences (p<0.05) according to Duncan's multiple range test. Total phenolics - mg GAE/100 mL juice; Total flavonoids - mg CAE/100 mL juice, Gallotannins - mg GAE/100 mL juice and Anthocyanins - mg Cy3dE/100 mL juice. GAE - gallic acid equivalent; CAE - catechin equivalent; Cy3dE - cyanidin-3-glucoside equivalent. *2011 and 2012 data were pooled for parameter(s) where interaction effects were not significant (p<0.05).

Table 5

Mineral element concentrations in pomegranate peel and aril at major maturity stages in 2010/11 season

Element	Bhagwa					Ruby				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Aril										
(mg/100 g fresh mass)										
N	423.0±3.53^{a}	380.7±3.17^{b}	372.0±2.52^{b}	334.8±2.26^{c}	260.3±2.17^{a}	280.0±15.13^{a}	261.3±14.12^{ab}	261.7±10.48^{ab}	244.2±9.78^{ab}	239.3±8.17^{b}
P	76.5±1.60^{a}	68.9±1.44^{ab}	65.8±1.59^{ab}	59.3±1.43^{b}	59.6±3.40^{b}	51.9±2.76^{a}	48.4±2.58^{ab}	45.6±2.23^{abc}	42.6±2.08^{bc}	40.4±1.47^{c}
K	206.0±0.33^{a}	185.4±0.30^{ab}	197.3±1.95^{ab}	177.6±1.76^{b}	178.0±9.39^{b}	189.0±17.24^{a}	176.4±16.09^{a}	190.0±4.04^{a}	177.3±3.77^{a}	176.3±3.48^{a}
Mg	21.8±0.35^{a}	19.6±0.31^{ab}	18.9±0.44^{ab}	17.0±0.39^{b}	16.7±0.87^{b}	13.8±0.43^{a}	12.9±0.40^{ab}	13.3±0.20^{ab}	12.4±0.19^{b}	12.0±0.64^{b}
Ca	23.9±0.54^{a}	21.5±0.49^{ab}	16.4±0.29^{b}	14.8±0.26^{b}	17.6±1.43^{ab}	18.4±0.80^{a}	17.1±0.75^{a}	16.4±0.33^{a}	10.7±1.18^{b}	9.6±0.83^{b}
(mg/kg fresh mass)										
Na	10.3±0.61^{a}	7.5±0.55^{b}	7.5±0.18^{b}	6.8±0.17^{b}	8.4±0.32^{ab}	12.3±0.69^{a}	11.5±0.65^{ab}	9.8±0.41^{bc}	9.2±0.38^{c}	11.1±0.52^{ab}
Mn	2.5±0.07^{a}	2.3±0.06^{a}	1.9±0.06^{b}	1.7±0.05^{b}	1.83±0.07^{b}	1.8±0.03^{a}	1.7±0.03^{a}	1.7±0.13^{a}	1.6±0.13^{a}	1.6±0.18^{a}
Fe	6.2±0.20^{a}	5.6±0.18^{ab}	6.2±0.18^{a}	5.6±0.16^{ab}	4.7±0.38^{b}	5.4±0.03^{a}	5.1±0.03^{a}	5.0±0.06^{a}	5.1±0.06^{a}	5.3±0.34^{a}
Cu	2.7±0.09^{a}	2.4±0.08^{a}	1.7±0.05^{b}	1.5±0.05^{b}	1.5±0.07^{b}	2.3±0.09^{a}	2.1±0.08^{ab}	1.8±0.22^{ab}	1.7±0.2^{b}	1.8±0.12^{ab}
B	3.5±0.07^{ab}	3.2±0.06^{bc}	3.3±0.03^{cd}	3.0±0.03^{d}	3.8±0.05^{a}	5.0±0.09^{a}	4.6±0.08^{b}	3.6±0.07^{d}	3.4±0.06^{e}	4.4±0.07^{c}
Zn	6.2±0.19^{a}	5.6±0.17^{ab}	5.0±0.21^{bc}	4.5±0.19^{bc}	4.3±0.24^{c}	4.7±0.26^{a}	4.4±0.24^{a}	4.2±0.22^{a}	4.0±0.20^{a}	3.9±0.41^{a}
%Water	76.7±0.27^{b}	77.5±0.23^{ab}	77.8±0.38^{ab}	78.6±0.35^{ab}	79.4±0.38^{a}	80.3±0.26^{a}	74.9±0.24^{c}	78.8±0.16^{b}	73.5±0.15^{d}	79.8±0.52^{a}
Peel										
(mg/100 g fresh mass)										
N	228.7±1.39^{a}	178.3±1.35^{b}	160.5±1.21^{c}	115.3±1.58^{d}	97.0±0.58^{e}	240.7±2.69^{a}	216.6±2.43^{b}	213.7±1.84^{b}	192.3±1.65^{c}	156.0±0.67^{d}
P	50.2±0.20^{a}	32.4±0.74^{b}	29.1±0.66^{bc}	27.05±0.77^{c}	15.9±0.49^{d}	37.8±0.16^{a}	34.0±0.14^{b}	28.0±0.55^{c}	25.2±0.49^{d}	18.6±0.33^{e}
K	317.3±2.01^{a}	264.0±5.81^{b}	237.6±5.23^{bc}	227.7±2.41^{c}	184.0±9.24^{d}	321.3±5.80^{a}	289.2±5.22^{b}	257.3±4.29^{c}	231.6±3.86^{cd}	214.7±7.18^{d}

Table 5 contd.

Element	Bhagwa S1	Bhagwa S2	Bhagwa S3	Bhagwa S4	Bhagwa S5	Ruby S1	Ruby S2	Ruby S3	Ruby S4	Ruby S5
Peel										
Mg	30.1±0.15^{a}	17.3±0.21^{b}	15.6±0.19^{c}	16.4±0.06^{d}	11.4±0.08^{e}	25.3±015^{a}	22.7±0.14^{b}	20.4±0.07^{c}	18.4±0.06^{d}	12.7±0.10^{e}
Ca	65.5±0.15^{a}	53.7±0.96^{b}	48.4±0.87^{c}	51.0±0.69^{bc}	33.7±0.59^{d}	65.4±0.81^{a}	58.8±0.73^{b}	50.0±1.01^{c}	45.0±0.91^{d}	39.0±0.11^{e}
(mg/kg fresh mass)										
Na	57.9±0.72^{a}	24.1±0.30^{d}	21.7±0.27^{d}	39.4±0.61^{b}	28.5±0.10^{b}	29.6±0.94^{a}	22.6±0.80^{c}	29.53±0.60^{a}	25.1±0.51^{bc}	26.1±0.27^{ab}
Mn	3.5±0.08^{a}	2.2±0.03^{b}	2.0±0.03^{bc}	1.8±0.07^{cd}	1.63±0.05^{d}	3.37±0.05^{a}	3.0±0.05^{b}	2.8±0.01^{c}	2.5±0.01^{d}	1.5±0.06^{e}
Fe	2.7±0.08^{a}	2.5±0.04^{b}	2.2±0.03^{b}	2.2±0.04^{b}	1.5±0.02^{c}	2.6±0.03^{cd}	2.7±0.04^{bc}	3.0±0.08^{ab}	3.3±0.09^{a}	2.3±0.03^{d}
Cu	2.2±0.20^{a}	1.5±0.07^{b}	1.4±0.06^{b}	1.3±0.05^{b}	1.0±0.03^{b}	2.0±0.08^{b}	2.2±0.08^{ab}	2.5±0.10^{ab}	2.8±0.11^{a}	1.3±0.12^{c}
B	6.5±0.07^{a}	4.5±0.02^{b}	4.1±0.02^{c}	3.2±0.01^{d}	2.8±0.02^{e}	9.1±0.08^{a}	8.2±0.07^{b}	5.2±0.02^{c}	4.7±0.02^{d}	3.3±0.03^{e}
Zn	2.2±0.19^{a}	1.9±0.07^{ab}	1.7±0.06^{b}	1.3±0.02^{c}	1.1±0.03^{c}	2.6±0.07^{b}	2.9±0.08^{ab}	2.9±0.02^{b}	3.2±0.02^{a}	1.6±0.00^{c}
%Water	62.3±0.03^{e}	71.4±0.08^{d}	73.5±0.08^{c}	74.4±0.04^{b}	78.2±0.07^{a}	62.9±0.04^{e}	66.1±0.04^{d}	68.5±0.10^{c}	71.9±0.11^{b}	76.9±0.03^{a}

Mean ± S.E with different letter(s) in the same row per cultivar indicate statistically significant differences (p<0.05) according to Duncan's multiple range test.

Table 6

Aroma volatile compounds (peak area %) composition analysed in 'Bhagwa' and 'Ruby' fruit at five maturity stages in 2011/12 season

		Bhagwa					Ruby				
Compound	Code	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Alcohols											
3-Hexen-1-ol	3hOH	n.d	17.4^{c}	29.3^{b}	16.2^{c}	44.5^{a}	n.d	n.d	n.d	n.d	n.d
Hexanol	hexOH	52.4^{a}	53.7^{b}	n.d	n.d	n.d	95.2^{a}	68.3^{b}	8.5^{d}	18.4^{c}	5.7^{d}
3-methyl-1-butanol	3mebOH	n.d	n.d	n.d	n.d	n.d	n.d	n.d	11.2^{b}	19.4^{a}	6.7^{c}
Phenyl ethanol	phenOH	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1.3
Total		52.4	71.1	29.3	16.2	44.5	95.2	68.3	19.7	37.8	12.4
Ketones											
2-Heptanone	2heONE	n.d	12.0^{ab}	7.0^{b}	7.8^{b}	16.4^{a}	n.d	16.9^{a}	6.4^{b}	n.d	n.d
2-Octanone	2octONE	n.d	4.1^{b}	27.7^{a}	2.9^{c}	5.5^{b}	n.d	8.2^{a}	5.6^{b}	11.2^{a}	2.5^{c}
Total		n.d	16.1	34.7	10.7	21.9	n.d	25.1	12.0	11.2	2.5
Esters											
Hexyl acetate	hexATE	n.d	n.d	0.9^{c}	34.4^{a}	27.8^{b}	n.d	n.d	22.7^{c}	46.0^{a}	32.8^{b}
2-Ethyl acetate	2eATE	n.d	n.d	n.d	n.d	n.d	n.d	5.1^{a}	0.7^{b}	0.1^{b}	n.d
Butyl acetate	butATE	n.d	2.2^{b}	4.4^{a}	2.0^{b}	1.3^{c}	n.d	n.d	0.3^{b}	2.9^{a}	0.6^{b}
Total		n.d	2.2	5.3	36.4	29.1	n.d	5.1	23.7	49.0	33.4
Terpene											
Limonene	lim	47.6^{a}	10.6^{b}	10.3^{b}	3.8^{b}	1.7^{c}	4.8^{a}	1.5^{b}	1.1^{b}	1.9^{b}	0.4^{c}
Total		47.6	10.6	10.3	3.8	1.7	4.8	1.5	1.1	1.9	0.4

Mean values with different letter(s) in the same row indicate statistically significant differences (p<0.05) according to Duncan's multiple range test; n.d- not detected.

Table 7

Pearson correlation coefficient matrix between chemical indices measured in 'Bhagwa' during 2011 and 2012 seasons

	Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	juice abs	**1**																	
2	pH	**0.95**	**1**																
3	TSS	**0.93**	0.87	**1**															
4	TA	**-0.89**	-0.79	**-0.98**	**1**														
5	TSS:TA	**0.94**	0.86	**0.99**	**-0.99**	**1**													
6	BrimA	**0.92**	0.86	**1.00**	**-0.99**	**0.99**	**1**												
7	Glucose	**0.92**	0.83	**0.99**	**-0.99**	**0.99**	**1.00**	**1**											
8	Fructose	**0.93**	0.85	**0.99**	**-0.98**	**0.99**	**0.99**	**1.00**	**1**										
9	G/F	0.77	0.68	**0.95**	**-0.95**	**0.92**	**0.95**	**0.96**	**0.95**	**1**									
10	Malic	-0.72	-0.63	**-0.92**	**0.93**	**-0.89**	**-0.93**	**-0.93**	**-0.92**	**-1.00**	**1**								
11	Tartaric	-0.84	-0.76	**-0.98**	**0.99**	**-0.97**	**-0.98**	**-0.98**	**-0.97**	**-0.98**	**0.96**	**1**							
12	Citric	**-0.94**	**-0.94**	**-0.97**	**0.91**	**-0.94**	**-0.96**	**-0.95**	**-0.96**	-0.88	0.85	**0.91**	**1**						
13	TPC	-0.76	-0.66	**-0.94**	**0.96**	**-0.93**	**-0.95**	**-0.96**	**-0.94**	**-1.00**	**0.99**	**0.98**	0.86	**1**					
14	TFC	-0.81	-0.78	**-0.95**	**0.91**	**-0.90**	**-0.94**	**-0.95**	**-0.95**	**-0.96**	**0.96**	**0.93**	**0.94**	**0.95**	**1**				
15	Anthocyanin	**1.00**	**0.92**	**0.94**	**-0.92**	**0.96**	**0.94**	**0.94**	**0.95**	0.81	-0.76	-0.87	**-0.93**	-0.80	-0.83	**1**			
16	GTC	-0.54	-0.48	-0.77	0.75	-0.70	-0.77	-0.79	-0.78	**-0.91**	**0.93**	0.80	0.73	**0.89**	**0.92**	-0.57	**1**		
17	FRAP	-0.85	-0.74	**-0.96**	**0.98**	**-0.98**	**-0.97**	**-0.97**	**-0.96**	**-0.95**	**0.93**	**0.99**	0.87	**0.96**	**0.88**	**-0.88**	0.74	**1**	
18	DPPH	-0.78	-0.68	**-0.95**	**0.95**	**-0.92**	**-0.95**	**-0.96**	**-0.95**	**-1.00**	**0.99**	**0.97**	**0.88**	**0.99**	**0.97**	-0.82	**0.92**	**0.94**	**1**

Correlation values in **bold** are significant at $p < 0.05$

Table 8

Pearson correlation coefficient matrix between chemical indices measured in 'Ruby' during 2011 and 2012 seasons

	Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	Juice abs	**1**																	
2	pH	0.19	**1**																
3	TSS	0.72	-0.05	**1**															
4	TA	-0.78	-0.12	**-0.98**	**1**														
5	TSS:TA	0.76	0.08	**0.99**	**-0.99**	**1**													
6	BrimA	0.72	-0.04	**1.00**	**-0.98**	**0.99**	**1**												
7	Glucose	0.72	-0.08	**0.99**	**-0.97**	**0.98**	**0.99**	**1**											
8	Fructose	0.71	-0.17	**0.99**	**-0.95**	**0.96**	**0.99**	**0.99**	**1**										
9	G/F	0.30	-0.35	0.86	-0.78	0.81	0.87	0.86	**0.88**	**1**									
10	Malic acid	-0.69	0.07	**-0.99**	**0.97**	**-0.98**	**-0.99**	**-0.98**	**-0.99**	**-0.88**	**1**								
11	Tartaric acid	**-0.92**	-0.21	**-0.88**	**0.94**	**-0.92**	**-0.89**	**-0.90**	-0.87	-0.57	0.86	**1**							
12	Citric acid	-0.58	0.31	**-0.95**	**0.89**	**-0.91**	**-0.952**	**-0.96**	**-0.98**	**-0.94**	**0.95**	0.78	**1**						
13	TPC	-0.55	0.12	**-0.97**	**0.93**	**-0.95**	**-0.98**	**-0.96**	**-0.97**	**-0.95**	**0.98**	0.78	**0.97**	**1**					
14	TFC	-0.46	0.09	**-0.94**	**0.90**	**-0.92**	**-0.94**	**-0.94**	**-0.93**	**-0.96**	**0.94**	0.74	**0.94**	**0.99**	**1**				
15	Anthocyanin	**0.97**	0.25	0.84	**-0.90**	**0.88**	0.84	0.85	0.82	0.47	-0.81	**-0.99**	-0.71	-0.71	-0.65	**1**			
16	GTC	-0.60	0.51	-0.87	0.77	-0.79	-0.86	-0.87	**-0.92**	-0.84	0.87	0.67	**0.92**	0.85	0.78	-0.64	**1**		
17	DPPH	-0.60	0.05	**-0.97**	**0.94**	**-0.96**	**-0.97**	**-0.957**	**-0.96**	**-0.91**	**0.99**	0.79	**0.93**	**0.98**	**0.96**	-0.74	0.84	**1**	
18	FRAP	-0.48	0.11	**-0.94**	**0.89**	**-0.92**	**-0.95**	**-0.93**	**-0.94**	**-0.95**	**0.96**	0.71	**0.93**	**0.99**	**0.98**	-0.64	0.83	**0.99**	**1**

Correlation values in **bold** are significant at $p<0.05$

Table 9

Factor scores, loadings, eigenvalues and variance (%) for the first two factors (F1 and F2) based on selected indices and antioxidant capacity in 2011 and 2012 seasons for both cultivars

	Factor scores			
	'Bhagwa'		'Ruby'	
Observation	F1	F2	F1	F2
S1_2011	5.663	-0.218	5.468	1.717
S2_2011	2.385	-1.436	2.497	-0.340
S3_2011	-0.229	-1.465	-1.316	-1.102
S4_2011	-2.382	-1.325	-1.936	-1.399
S5_2011	-3.543	-0.559	-2.498	0.511
S1_2012	3.299	0.994	3.499	-1.125
S2_2012	1.638	0.446	2.026	-0.893
S3_2012	-0.690	1.814	-0.306	1.161
S4_2012	-1.988	1.704	-3.564	0.244
S5_2012	-4.152	0.044	-3.869	1.227
	Loadings			
	F1	F2	F1	F2
juice abs	-0.885	-0.194	-0.844	0.344
pH	-0.682	0.684	-0.518	0.604
TSS	-0.892	0.028	-0.928	-0.057
TA	0.730	-0.537	0.731	-0.392
TSS/TA	-0.902	0.333	-0.921	0.189
BrimA	-0.908	0.060	-0.936	-0.021
TPC	0.956	-0.097	0.955	0.241
TFC	0.949	0.015	0.915	0.307
GTC	0.794	0.417	0.904	0.301
Anthocyanins	-0.900	-0.222	0.868	0.307
FRAP	0.906	0.315	-0.904	0.237
DPPH	0.915	0.359	0.938	0.278
Eigenvalue	9.126	1.371	9.196	1.147
Total variance (%)	76.052	11.421	76.636	9.558
Cumulative (%)	76.052	87.473	76.636	86.195

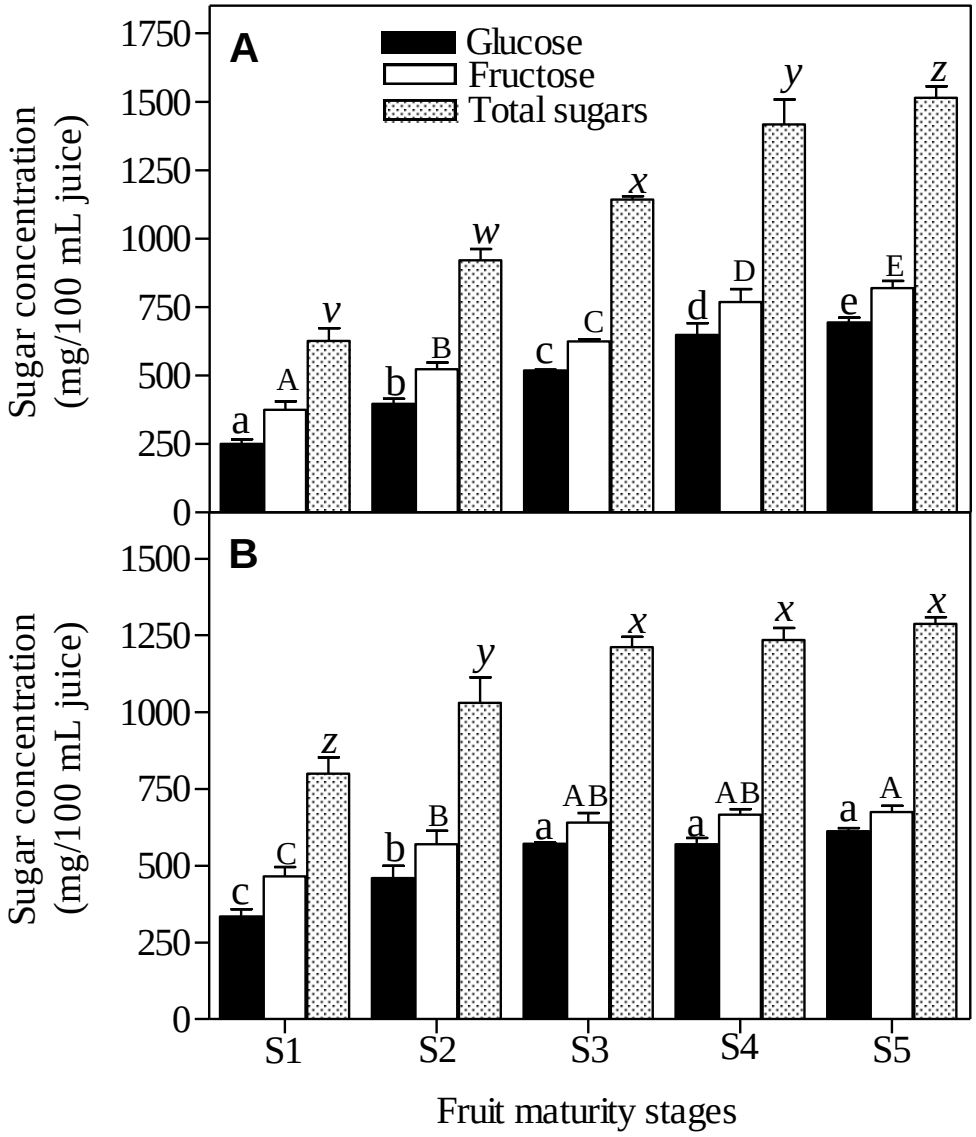

Figure 1. Sugar composition (mg/100 mL fresh juice) of pomegranate fruit at major stages of development and maturation for 'Bhagwa' (**A**) and 'Ruby' (**B**) during 2011 season. Different letters on bars mean statistically significant differences ($p<0.05$) according to Duncan's multiple range test. Each sugar parameter statistically analysed separately as 1-way anova per cultivar.

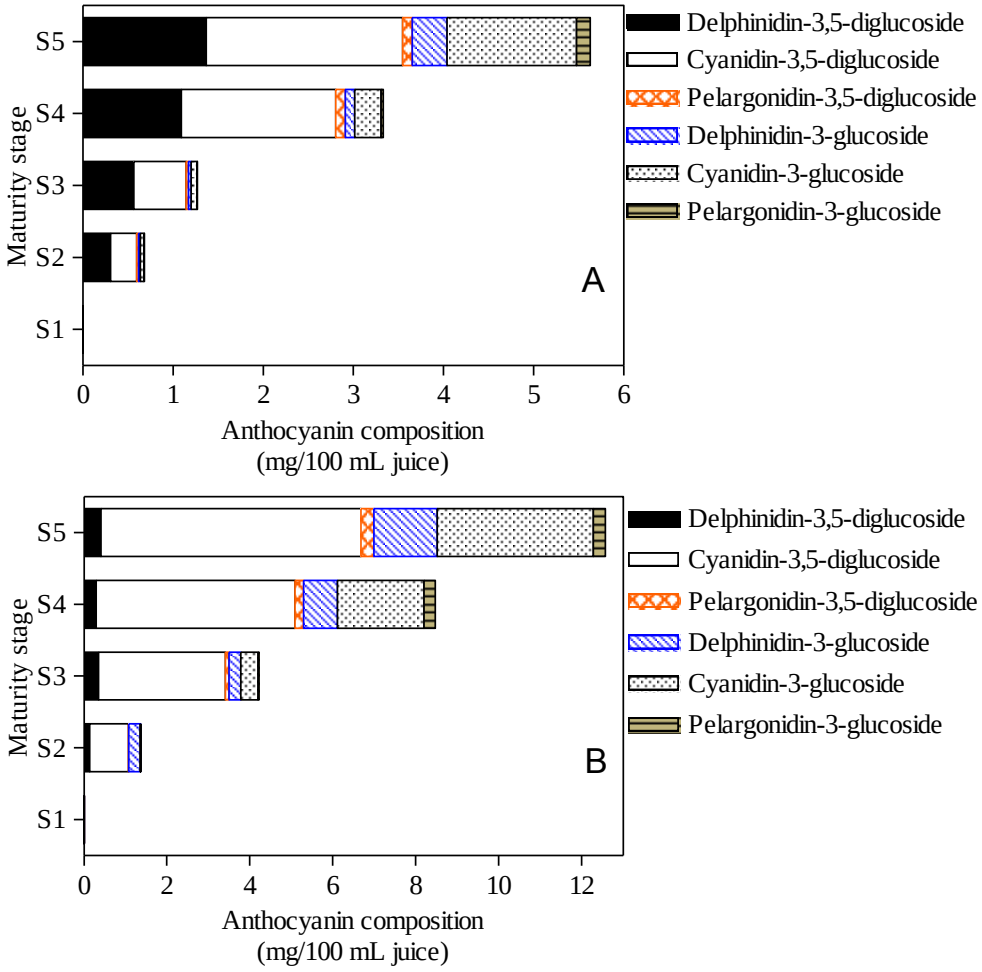

Figure 2. Anthocyanin composition in pomegranate juice at major maturity stages in the 2010/11season. 'Bhagwa' (**A**) and 'Ruby' (**B**).

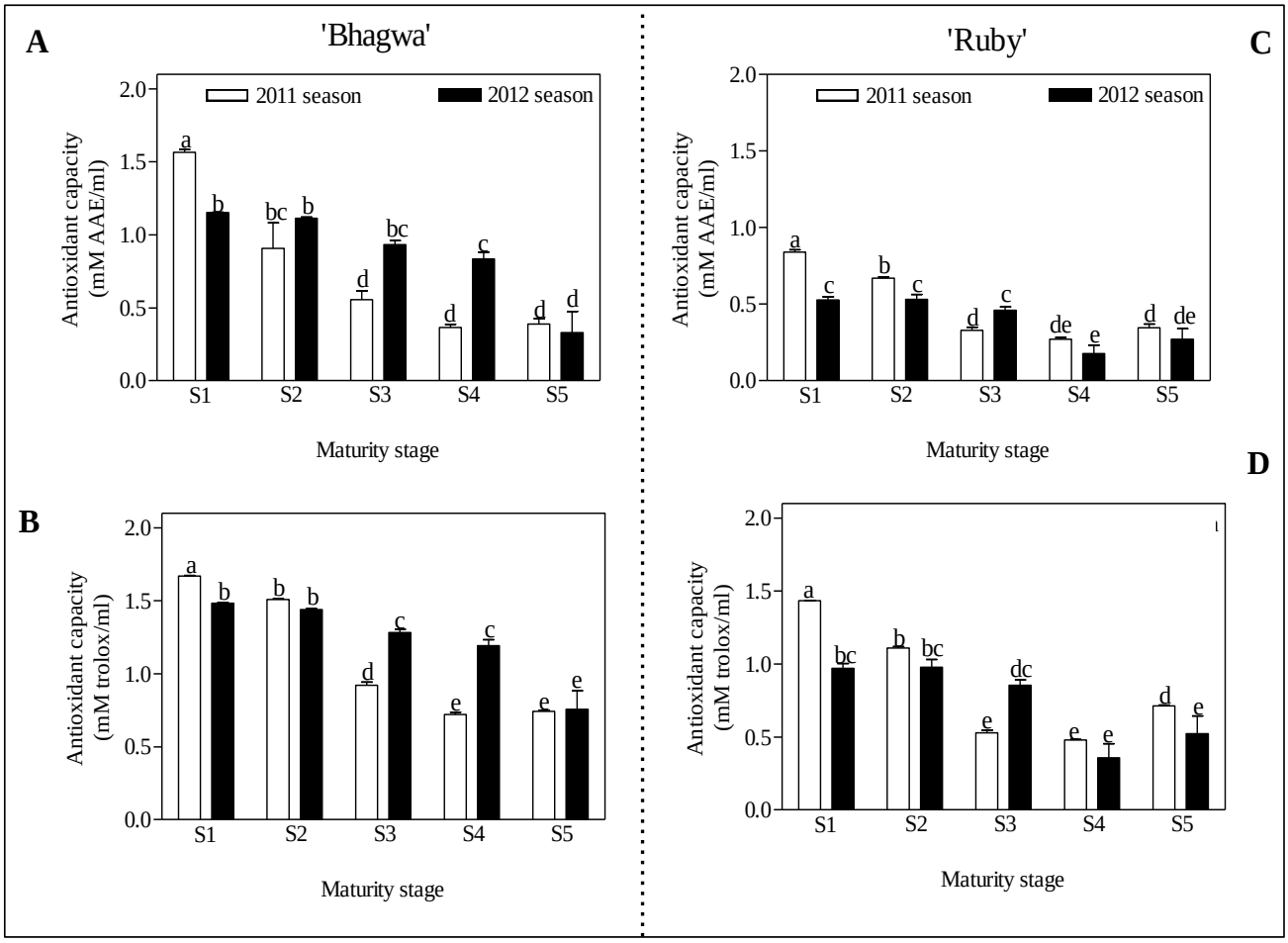

Figure 3. Antioxidant capacity (DPPH and FRAP) of pomegranate juice at major maturity stages over two seasons (2011 and 2012). 'Bhagwa': DPPH assay (**A**) and FRAP assay (**B**); 'Ruby': DPPH assay (**C**) and FRAP assay (**D**). Different letters on bars mean statistically significant differences ($p<0.05$) according to Duncan's multiple range test. AAE- Ascorbic acid equivalent.

Significance level

Cultivar	Source	*Prob. > F*	
		DPPH	FRAP
'Bhagwa'	Maturity stage (*A*)	< 0.0001	< 0.0001
	Season (*B*)	0.0286	0.0004
	*A*B*	**0.0001**	**< 0.0001**
'Ruby'	Maturity stage (*A*)	< 0.0001	< 0.0001
	Season (*B*)	0.0002	0.0031
	*A*B*	**< 0.0001**	**< 0.0001**

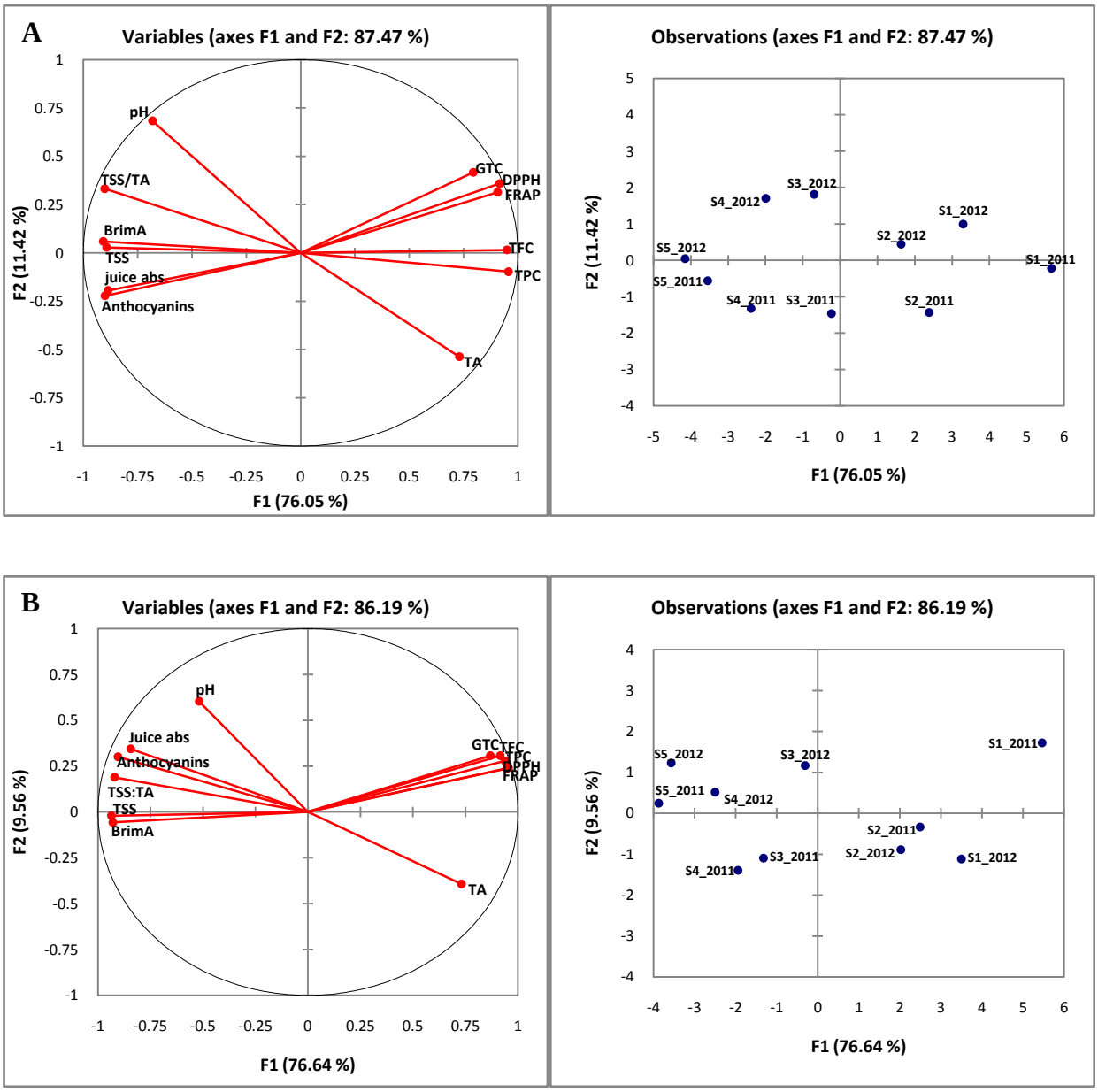

Figure 4. Variables and observations chart using key maturity indices and antioxidant capacity in 2011 and 2012 seasons. 'Bhagwa' (**A**); 'Ruby' (**B**)

SECTION III

CHARACTERIZATION OF QUALITY ATTRIBUTES AND PHARMACOLOGICAL PROPERTIES OF SELECTED CULTIVARS *

- Paper 4: Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa [1]
- Paper 5: Composition of trace and major minerals in different parts of pomegranate (*Punica granatum* L.) fruit cultivars [2]
- Paper 6: Physico-mechanical, phytochemical, and free radical scavenging properties and volatile compounds in eight pomegranate cultivars and classification by principal component and cluster analyses[4]
- Paper 7: Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract [3]

* Studies were done during the 2009/10 harvest season

1. Food Bioprocess Tech. 5, 2934 – 2940.
2. British Food J. 114, 1518 – 1532.
3. BMC Complement. Altern. Med. 12, 200.
4. British Food J. 116, (In press).

This section presents a compilation of manuscripts where each paper is an individual entity and some repetition between papers, therefore, has been unavoidable.

PAPER 4

Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa

Abstract

A comparative study of chemical concentrations and antioxidant activities of three pomegranate cultivars ('Arakta', 'Bhagwa' and 'Ruby') grown in South Africa was conducted. Fresh pomegranate juice (PJ) of each cultivar was assessed for soluble solids content (SSC), pH and titratable acidity (TA), while extracted juice samples were evaluated for total phenolics (TP), including total tannins (TT), proanthocyanidins (Pcy), total flavonoids (TF), anthocyanins (Acy) and gallic acid (GA) using spectrophotometric methods. The antioxidant properties of the juice samples were evaluated against stable 2, 2-diphenyl-1-picryl hydrazyl (DPPH), as well as in ferric reducing ability of plasma (FRAP) and QuantiChrom^{TM} (TAC) antioxidant assays. There were significant differences in the chemical properties of the cultivars. SSC, TA and pH varied between the range of 14.07 - 15.10°Brix, 0.22 - 0.28 g/100 mL and 3.32 - 3.64, respectively. 'Bhagwa' had the highest TP (449.9 mg/100 mL), 1.3-fold and 1.6-fold higher than 'Arakta' and 'Ruby', respectively. The strongest total antioxidant activity was exhibited by 'Bhagwa' with an antioxidant index of 95.7%, followed by 'Arakta' (93.2%) and 'Ruby' (79.9%). PJ phytochemical properties (TP, TT, Pcy, GA) and antioxidant activity (FRAP and TAC) were significantly correlated (r = 0.509 - 0.885) with each other.

Keywords: Pomegranate, Total soluble solids, Antioxidant, Anthocyanins, South Africa

1. Introduction

Several epidemiological and intervention studies have reported positive correlations between fruit and vegetable intake and prevention of non-communicable diseases such as cardiovascular disease, many forms of cancer, type 2 diabetes mellitus and slowing of the aging process (Lampe, 1999; WCRF/AICR, 2007). Scientific evidence linking increasing consumption of fresh fruit and vegetables to improved human health have been attributed mainly to their

concentrations of beneficial phytochemicals and other micro-nutrients (Opara et al., 2010) which act as antioxidants by scavenging the oxidation caused by free radicals that lead to cell and DNA damage and many degenerative disorders (Lampe, 1999; Boyer and Liu, 2004). The phytochemistry and pharmacological actions of pomegranate (*Punica granatum* L.) components suggest a wide range of clinical applications for the treatment and prevention of cancer, as well as other diseases where chronic inflammation is believed to play an essential etiologic role (Lansky and Newman, 2007). These bioactivities are attributed to the high level of antioxidant polyphenols concentration in pomegranate.

Increasing large scale commercialisation and awareness of pomegranate fruit as a medicinal food and dietary supplement has tremendously facilitated its availability and interest among consumers. Commercial orchards of pomegranate are grown in countries such as Iran, India, Egypt, China, Israel, Tunisia, Syria, Lebanon, Turkey, Greece, Cyprus, Italy, France, Spain, Chile, Portugal, USA, Oman and most recently in South Africa (Al-Said et al., 2009; Holland et al., 2009; Opara et al., 2009; Bchir et al., 2010a). The commercialisation of pomegranate has consequently resulted in the introduction and spread of different cultivars and selections throughout different regions and continents. Due to inherent genetic variability among pomegranate cultivars and the influence of climatic conditions on the chemical and antioxidant values of the fruits (Al-Said et al., 2009; Holland et al., 2009; Opara et al., 2009), scientific assessment of commercially important cultivars grown in different countries is essential for the proper selection and marketing of fruit with desirable quality attributes for fresh consumption as fresh or processed products.

The goal of the present study was to investigate the chemical and antioxidant properties of three commercially grown pomegranate cultivars in South Africa. The specific objectives were to evaluate the chemical (soluble solids contents, pH and acidity) and phenolic concentrations, and the antioxidant capacity of fruit juice.

2. Materials and methods

2.1. Plant materials and fruit processing

Commercially ripe pomegranate fruit (‘Arakta’, ‘Bhagwa’ and ‘Ruby’) were collected during commercial harvest period (2010 season) from Houdconstant Pack-house Porterville,

Western Cape (33°01′00″S, 18°58′59″E) and transported in an air-conditioned vehicle to the Postharvest Technology Laboratory, Stellenbosch University. Three replicate samples, each containing three fruit, were used for all analyses. Each fruit was hand-peeled and the arils juiced using a LiquaFresh juice extractor (Mellerware, South Africa).

2.2. Titratable acidity, soluble solids content and pH

TA was measured by titration to an endpoint of pH 8.2 using a Metrohm 862 compact titrosampler (Herisau, Switzerland). SSC (°Brix) was measured using a digital refractometer (Atago, Tokyo, Japan). The pH values were measured at room temperature using a pH meter (Crison, Barcelona, Spain). All values were presented as mean ± S.E.

2.3. Preparation of fruit sample

Juice samples for phenolic compositions and antioxidant activity were prepared by mixing 2 mL of PJ with 10 mL of cold 50% aqueous methanol in a centrifuge tube. The mixture was vortexed and sonicated in cold water for 5 min before being centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was gently collected into a clean tube and stored at 4°C.

2.4. Phytochemical analysis

2.4.1. Determination of total phenolic compounds

Total phenolic (TP) concentrations in juice samples were determined using the Folin-Ciocalteu (Folin-C) colourimetric method as described by Makkar et al. (2007). TP concentrations were determined spectrophotometrically at 750 nm by adding Folin-Ciocalteu reagent to the juice sample, and expressed as the mean ± S.E (mg) of gallic acid equivalents (GAE) per 100 mL crude juice for three replicates.

2.4.2. Quantification of total tannins

Total tannins (TT) concentration was quantified according to Makkar et al. (2007). TT concentration was expressed as the mean ± S.E (mg) gallic acid equivalents (GAE) per 100 mL crude juice for three replicates.

2.4.3. Butanol-HCl assay for proanthocyanidins

Proanthocyanidin (Pcy) concentration was determined as described by Vermerris and Nicholson (2006). Briefly, PJ sample was mixed with Butanol-HCl-Iron reagent and heated in a water bath at 95ºC for 60 min. The absorbance was read at 530 nm using a UV-vis spectrophotometer (Thermo Scientific technologies, Madison, USA). Pcy concentrations were expressed as mean ± S.E cyanidin equivalents (mg CyE/ 100 mL crude juice) from the standard curve.

2.4.4. Determination of total flavonoids

PJ samples (250 µL) were mixed with 5% sodium nitrite solution (75 µL), and subsequently with aluminium chloride (10%, 150 µL), sodium hydroxide (1 M, 500 µL) and distilled water (775 µL). The absorbance of the mixture was measured spectrophotometrically at 510 nm (Yang et al., 2009). Total flavonoids (TF) concentration was expressed as the mean ± S.E (mg) of gallic acid equivalents (GAE) per 100 mL crude juice in triplicates.

2. 4.5. Rhodanine assay for gallic acid concentration

The amount of gallic acid (GA) concentration in PJ samples was carried out as described by (Makkar, 2000). Absorbance was read at 520 nm using a UV-vis spectrophotometer. GA concentration was calculated from the standard curve of analytical gallic acid and expressed as gallic acid equivalents (GAE) per 100 mL crude juice.

2.4.6. Quantification of total anthocyanins

Total anthocyanin concentration (Acy) was quantified according to Wrolstad (1993) using the pH differential method. In triplicates, diluted PJ sample (1 mL) was mixed with 9 mL of pH 1.0 and pH 4.5 buffers, separately. The absorbance of the mixtures was measured at 520 and 700 nm using a UV-vis spectrophotometer. Acy was expressed as cyanidin 3-glucoside (molar extinction coefficient of 26,900 and a molecular weight of 449.2) equivalent per 100 mL crude juice (mg C_3gE/100 mL PJ).

2.5. Antioxidant capacity

2.5.1. Determination of free radical scavenging activity

PJ samples were tested against stable 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in the DPPH assay according to Karioti et al. (2004). Briefly, under dim light, PJ sample (15µL) was diluted with methanol (735 µL) followed by the addition of methanolic DPPH solution (0.1 mM, 750 µL). The mixtures were incubated in the dark at room temperature for 30 min, before the absorbance was measured at 517nm. The free-radical scavenging activity of PJ was expressed as ascorbic acid (mM) equivalent per mL crude PJ (mM AAE /mL; n = 3).

2.5.2. Ferric ion reducing antioxidant power (FRAP)

The method of Benzie and Strain (1996) was employed for measuring the FRAP of PJ sample with few modifications. In triplicates, diluted PJ samples (150 µL) were added to 2850 µL of the FRAP solution (300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM ferric chloride) before being incubated in a dark condition for 30 min. The reduction of the Fe^{3+}-TPTZ complex to a coloured Fe^{2+}-TPTZ complex at low pH by the PJ sample was monitored by measuring the absorbance at 593 nm using a UV-vis spectrophotometer. Results were expressed as trolox (mM) equivalents per mL crude PJ (mM TE/mL PJ).

2.5.3. QuantiChrom™ antioxidant assay for total antioxidant capacity

For the measurement of the total antioxidant capacity (TAC) of PJ samples, the QuantiChrom™ antioxidant assay kit (DTAC-100) from BioAssay Systems (BioAssay Systems, Hayward, CA) was used. The assay kit is designed for the quantitative colourimetric determination of total antioxidant capacity in the food and drug industry. In the assay, the total antioxidant capacity is a measure of the amount of copper (II) (Cu^{2+}) reduced by antioxidants to copper (I) (Cu^{+}) such that the resulting Cu^{+} specifically forms a deep blue complex with a dye reagent. The colour intensity at 570 nm is proportional to the TAC of the sample being assayed. The absorbance was measured using a Multiskan FC plate reader (Thermo scientific

technologies, China). Results were expressed as Trolox (mM) equivalents per mL crude PJ (mM TE/mL PJ) for three replications.

2.6. Statistical analysis

One way analysis of variance (ANOVA) and correlation coefficients (r) were carried out using SPSS software (version 10, SPSS Inc. Chicago, USA). Bar chats were plotted using GraphPad Prism software version 4.03 (GraphPad Software, Inc. San Diego, USA).

3. Results and discussion

3.1. Titratable acidity, soluble solids content and pH

In general, juice characteristics of the pomegranate cultivars investigated were significantly (p<0.05) different (Table 1). The soluble solids content ranged from 14.07 - 15.1°Brix, the highest being in 'Arakta'. This result is within the range (12.36 - 16.32°Brix) reported by Martinez et al. (2006) for five Spanish cultivars and also similar to those reported by Tehranifar et al. (2010) and Borochov-Neori et al. (2009) for cultivars grown in Iran and Israel, respectively. The highest acidity was found in 'Bhagwa' (0.32 g CA/100 mL), while the lowest was found in 'Ruby' (0.22 g citric acid/100 mL). The low acidity levels observed were accompanied by relatively high pH values ranging from 3.43 to 3.64 (Table 1). These pH values were within the ranges (2.75 - 4.14) reported by Akbarpour et al. (2009). The maturity index (MI) (SSC/TA) were in the order of 'Bhagwa' < 'Arakta' < 'Ruby'. Based on the MI classification of Spanish pomegranate cultivars by Martinez et al. (2006), all the pomegranate cultivars evaluated in the present study could be categorized as sweet cultivars (14.07 - 15.1°Brix).

3.2. Phytochemical analysis

3.2.1. Total phenolic compounds

The total phenolic composition expressed as milligrams of gallic acid equivalent (GAE) per 100 mL juice ranged from 289 - 450 mg, 'Bhagwa' having 1.6 and 1.3 folds of TP concentration than 'Ruby' and 'Arakta', respectively (Figure 1 X). Pomegranate fruit is

traditionally a rich source of polyphenols. Considerable varying amounts of total phenolic concentrations have been reported by various authors (Gil et al., 2000; Poyrazoglu et al., 2002; Mousavinejad et al., 2009; Tezcan et al., 2009; Tehranifar et al., 2010).

3.2.2. Total tannin concentration

The amount of tannins contained in fruit determines the level of astringency (Ozawa et al., 1987). The amount of TT found varied between 50.23 - 128.16 mg GAE/100 mL (Figure 1 Y). There was no significant difference between the amount of TT contained in 'Arakta' and 'Bhagwa'. This compared to previous studies conducted by Mousavinejad et al. (2009) and Zarei et al. (2010) on Iranian cultivars. The authors reported that the total tannins concentrations varied between 15 - 32 mg and 18.77 - 38.21 mg/100 g juice, respectively.

3.2.3. Proanthocyanidin concentration

Proanthocyanidin (condensed tannins) concentration found ranged from 39.69 to 52.07 mg cyanidin/100 mL (Figure 1 Z). No significant difference was observed between the amounts found in 'Arakta' and 'Ruby'. The amounts found in this study were higher than those reported by Guo et al. (2008) and Zarei et al. (2010), and lower than that (530 mg catechin/100 g) reported by Li et al. (2006).

3.2.4. Total flavonoids, total anthocyanin and gallic acid concentrations

The total flavonoid concentration (catechin equivalent) observed in the pomegranate cultivars was significantly different ($p<0.05$), ordered as 'Bhagwa' > 'Ruby' > 'Arakta' with approximately 1.6-fold difference between the highest and the lowest concentrations (Table 1). The results revealed considerable amounts of total anthocyanins expressed as cyanidin 3-glucoside equivalents (mg C_3gE/100 mL) in the investigated pomegranate cultivars. Total Acy ranged between 16.53 - 26.93 mg C3gE/100 mL, 'Bhagwa' having approximately 1.6-fold more than in 'Arakta'. The findings in the present study are comparable with the Acy reported in previous studies (Gil et al., 2000; Tehranifar et al., 2010). Gallic acid constitutes a predominate part of total phenolic acids in pomegranate juice (Poyrazoglu et al., 2002). The colourimetric quantification of amounts of total GA after a partial hydrolysis in the rhodanine assay ranged

between 47.03 - 86.97 mg GAE/100 mL with a 1.8-fold difference between the lowest ('Arakta') and the highest ('Bhagwa') concentration (Table 1).

3.3. Antioxidant capacity

The antioxidant potentials of pomegranate juice showed significant differences among the cultivars ($p<0.05$) as presented in Table 2. The radical scavenging power of polyphenolic juice ranged between 0.15 to 0.17 mM/mL ascorbic acid equivalent. 'Bhagwa' showed the highest antioxidant potentials in both DPPH and FRAP assays, whereas the highest value by QuantiChrom^{TM} assay (1.23 mM TE/mL) was found in 'Arakta'. Varying degrees of antioxidant capacities by pomegranate juice in all the antioxidant assays could, in part, be due to differences in reaction kinetics and steady state antioxidant potentials of various reductive substrates in the juice (Ozgen et al., 2008).Calculation of an overall antioxidant index (AI, %) as described by Madrigal-Carballo et al. (2009), showed that AI was in the order of Bhagwa > Arakta > Ruby (Table 2). The results are in agreement with previous reports which showed high antioxidant capacity in some pomegranate cultivars (Gil et al., 2000; Kulkarni and Aradhya, 2005; Madrigal-Carballo et al., 2009; Shwartz et al., 2009; Zarei et al., 2010; Bchir et al., 2010b).

3.4. Correlations

Correlation tests indicated significantly ($p<0.01$) high relationship between TP and FRAP ($r = 0.885$) and a lower correlation between TP and TAC ($r = 0.660$) (Table 3). The correlation test showed a positive but weak relationship between FRAP and TAC ($r = 0.577$, $p<0.05$). High correlation was found between TT and FRAP ($r = 0.817$), correlation tests revealed moderate relationships between TT and TAC ($r = 0.624$), Pcy and FRAP ($r = 0.644$), GA and TAC ($r = 0.610$) at $p<0.01$, while correlation between GA and FRAP ($r = 0.526$) at $p<0.05$ was relatively lower (Table 3).

4. Conclusion

The results obtained showed that there were significant cultivar variations on the chemical and phytochemical properties of three pomegranate cultivars grown in South Africa. This study also showed that the evaluated pomegranate cultivars could exhibit different

antioxidant capacity. Total phenolic composition of fruit juice correlated considerably with the antioxidant capacity. The MI and pH values found in the evaluated cultivars could be useful in characterizing the taste and flavour of the investigated pomegranate fruit cultivars. Collectively, the evaluated characteristic components reported in this study provide the first scientific evidence that could be used towards phytochemical and antioxidant profiling of pomegranate cultivars grown in South Africa which could assist in the development of objective indices for cultivar selection for such nutrients.

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Table 1

Soluble solids, Titratable acidity, pH and phytochemical concentrations of three pomegranate cultivars grown in South Africa

Cultivar	SSC (°Brix)	TA (g CA/100 mL PJ)	pH	Maturity index (SSC/TA)	Flavonoids (mg CE/100 mL PJ)	Anthocyanins (mg C3gE/100 mL PJ)	Free gallic acid (mg GAE/100 mL PJ)
Arakta	15.10±0.12^{a}	0.28±0.01^{b}	3.43±0.02^{b}	53.41±1.91^{b}	46.38±1.36^{c}	16.53±0.57^{b}	47.03±3.80^{b}
Bhagwa	14.43±0.27^{ab}	0.32±0.01^{a}	3.32±0.03^{c}	45.69±1.72^{c}	72.28±1.48^{a}	26.93±0.46^{a}	86.97±3.25^{a}
Ruby	14.07±0.28^{b}	0.22±0.01^{c}	3.64±0.04^{a}	63.13±2.73^{a}	57.25±3.11^{b}	18.14±1.31^{b}	54.59±2.40^{b}

Values (mean ± S.E) in the same column followed by different letter(s) are significantly different (p<0.05) according to Duncan's multiple range test.

Table 2

Total antioxidant capacity of phytochemical juice of three pomegranate cultivars grown in South Africa

Cultivar	DPPH [a] (mM AAE /mL PJ)	FRAP [b] (mM TE/mL PJ)	TAC [c] (mM TE/mL)	Antioxidant index (%)
Arakta	0.15±0.010^{ab}	0.32±0.007^{b}	1.23±0.11^{a}	93.2±3.50^{ab}
Bhagwa	0.17±0.004^{a}	0.35±0.002^{a}	1.07±0.07^{ab}	95.7±4.34^{a}
Ruby	0.15±0.004^{b}	0.28±0.006^{c}	0.88±0.03^{b}	79.9±4.80^{b}

[a] DPPH = antioxidant capacity based on DPPH assay; [b] FRAP = antioxidant capacity based on FRAP assay; [c] TAC = antioxidant capacity based on QuantiChrom™ assay. AAE - Ascorbic acid equivalent; TE - Trolox equivalents. Values (mean ± S.E) in the same column followed by different letter(s) are significantly different (p<0.05) according to Duncan's multiple range test.

Table 3

Pearson's correlation coefficients (*r*) among the investigated parameters of three pomegranate juice cultivars grown in South Africa [a]

Variable	TP	TT	Pcy	TF	Acy	GA	DPPH	FRAP
TT	0.779**							
Pcy	0.768**	0.539*						
TF	0.346 [ns]	0.061 [ns]	0.179 [ns]					
ACY	-0.386[ns]	-0.525*	-0.421 [ns]	-0.107 [ns]				
GA	0.774**	0.355 [ns]	0.691**	0.509*	-0.233 [ns]			
DPPH	0.303 [ns]	0.222 [ns]	0.311 [ns]	0.393 [ns]	-0.025 [ns]	0.306 [ns]		
FRAP	0.885**	0.817**	0.644**	0.192 [ns]	-0.323 [ns]	0.526*	0.251 [ns]	
TAC	0.660**	0.624**	0.512*	-0.004 [ns]	-0.244 [ns]	0.610**	0.363 [ns]	0.577*

[a] 95% confidence interval. TP = total phenolics, TT = total tannins, Pcy = proanthocyanidins, TF = total flavonoids, Acy = total anthocyanins, GA = gallic acid, DPPH = antioxidant capacity based on DPPH assay, FRAP = antioxidant capacity based on FRAP assay, TAC = antioxidant capacity based on QuantiChrom^{TM} antioxidant assay. [ns] = non significant, * = $p<0.05$ and ** = $p<0.01$ (2-tailed).

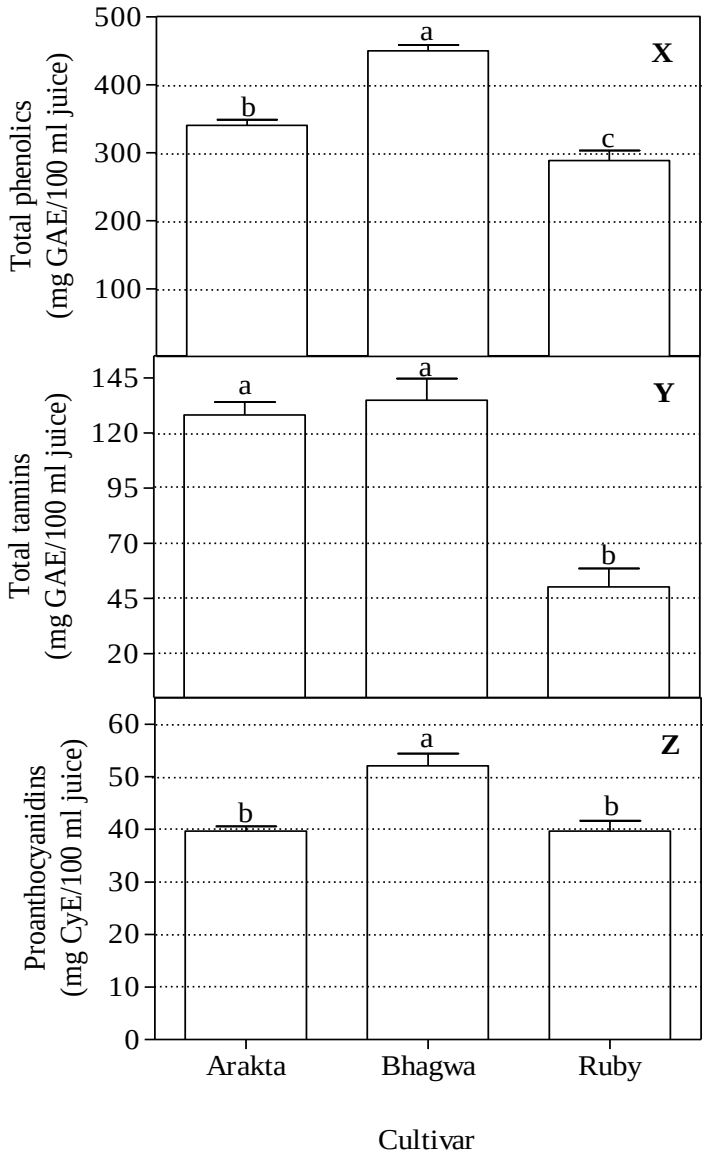

Figure 1. Total phenolic compounds (**X**), total tannins (**Y**) and proanthocyanidins (**Z**) concentration of methanolic extracts of three pomegranate cultivars grown in South Africa. GAE - Gallic acid equivalent; CyE - Cyanidins equivalent. Bars (mean ± S.E) with no letter in common within an analysis are significantly different (p<0.05) according to Duncan's multiple range test.

PAPER 5

Composition of trace and major minerals in different parts of pomegranate (*Punica granatum* L.) fruit cultivars

Abstract

A comparative study on elemental composition of seven fruit pomegranate cultivars was determined. Concentration of major elements (N, P, K, Ca, Mg, S, Cl and Na) and trace elements (Mn, Fe, Cu, Zn, B, Ni, Co, Cr, Pb, Cd, Se, Al, As, Li, Sr, Ti and V) were determined in fruit rind, mesocarp and arils of all the investigated pomegranate cultivars. Among the cultivars, the highest amounts of N, P, S and Cl were found in 'Bhagwa', while 'Arakta', 'Ruby' and 'Wonderful' had the highest amount of Mg, Ca, and Na, respectively. P, Fe, Zn, Ti, V were more in quantity in the edible portion, while other mineral elements were in larger proportions or in some cases, were only found (Co, Al, As) in the non-edible fractions of the investigated pomegranate fruit cultivars. Among the major mineral elements investigated, P had the highest covering of the RDA, ranging from 6.78 to 8.53% contribution to the RDA, followed by K (4.53 to 4.95% RDA), S (1.25 to 1.54% RDA), Ca (1.04 to 1.54% RDA), Mg (4.33 to 5.26% RDA), and Na (0.15 to 0.17% RDA). The consumption of fresh arils of pomegranate irrespective of the cultivar may supply considerably amounts of mineral elements to human diet. This work attempts to contribute to knowledge of the nutritional properties of pomegranate cultivars grown in South Africa.

Keywords: mineral elements, pomegranate, recommended daily allowance

1. Introduction

Mineral components of fruit and vegetables contribute to their nutritive value. A number of mineral elements are required in varying amounts by humans for proper growth, function and overall well-being (Obiajunwa et al., 2005; Hussain et al., 2010). In recent years, food analysts have been interested in the amounts of various nutritive components and their changes in fruits, and the importance of dietary minerals in the prevention of several diseases such as bone demineralization, arterial hypertension, and overall cardiovascular risk (Glew et al., 2003; Ozcan, 2004; Segura et al., 2006). Mineral nutrients are divided into two broad groups; major and trace elements. Major minerals represent 1% of bodyweight and are required in amounts greater than 100 mg per day, while trace minerals make up less than

0.01% of bodyweight and are essential in much smaller amounts (Ozcan, 2004). It is estimated that 70 biological elements are needed by all living things for the normal function of their metabolism, reproductive and immune system (Ozcan, 2004; Obiajunwa et al., 2005).

Pomegranate (*Punica granatum* L.) is an important tree of the tropical and subtropical regions which is valued for its delicious edible fruit. The fruit has the leathery exocarp and the interior is separated by membranous walls and white spongy tissue into compartments packed with transparent sacs filled with fleshy, juicy, red, pink or whitish pulp called the arils. The arils may be consumed directly, as fresh juice, or used for the preparation of numerous industrial food products (Al-Maiman and Ahmad, 2002; Al-Said et al., 2009; Holland et al., 2009; Mousavinejad et al., 2009). In recent times, the fruit has attracted renewed global interest for its nutritional and antioxidant characteristics (Holland et al., 2009). Due to the influence of environmental and cultivar differences on the nutritional values and elemental characteristics of the fruits, several studies on medicinal properties of pomegranate fruit have been reported (Gil et al., 2000; Mousavinejad et al., 2009; Opara et al., 2009; Fawole et al., 2012). However, information on the concentration of major, minor and trace elements in pomegranate cultivars is scarce in the literature. A few studies on some major and trace element concentration of pomegranate fruit cultivars have shown that different pomegranate fruit parts are rich in vital mineral elements (Al-Maiman and Ahmad, 2002; Waheed et al., 2004; Dumlu and Gürkan, 2007; Fadavi et.al., 2005; Mirdehghan and Rahemi, 2007; Samadloiy et al., 2008; Orak, 2009; El Kar et al., 2011; Gozlekci et al., 2011). For instance, Al-Maiman and Ahmad (2002) reported high levels of Cu, Fe, Zn, Mg, P, Na, Ca and K in the seed and juice of 'Taifi' grown in Saudi Arabia, while Mirdehghan and Rahemi (2007) emphasized the high amount of some mineral nutrients in both peel and arils, especially microelements in the arils of Iranian 'Malas Yazdi'. According to the authors, the average concentrations of Zn, Cu and B determined in arils were greater than those found in 'Navel' oranges.

Adequate knowledge of the elemental composition of pomegranate fruit parts may be useful as dietary information in food composition databases and may better assess their contribution to macronutrient and micronutrient requirements in human diet. The objective of this study was to evaluate the composition and distribution of mineral elements (8 macro-elements and 17 microelements) in seven pomegranate fruit cultivars cultivated in South Africa. The distribution of mineral elements in fruit fractions (rind, mesocarp and arils) was determined and estimates of the contribution of major mineral elements in arils to recommended dietary allowance (RDA) were made.

2. Materials and methods

2.1. Fruit sampling and preparation

Seven pomegranate cultivars cultivated in the Western Cape Province, South Africa, were studied. Commonly available ('Arakta', 'Bhagwa' and 'Ruby') were procured from the same orchard in Porterville (33°01′00″S, 18°58′59″E), while other cultivars ('Ganesh', 'Herskawitz', 'Molla de Elche' and 'Wonderful') were procured from Piketberg (32° 54′ 00″ S, 18° 46′ 00″ E). The sampling locations are 27 km apart and are representative sites for the studied pomegranate cultivars within the Province. Fruit samples were randomly collected at maturity from 5-year old trees between March and June 2010, and transported in an air-conditioned vehicle to the Postharvest Technology Laboratory at Stellenbosch University.

For each cultivar, a sample of ten fruits without any physical damage was selected, washed with distilled water and air-dried. In order to determine the elemental composition of fruit fractions, fruits were manually separated into three parts; (*i*) rind (the outer leathery fruit peel), (*ii*) mesocarp (fleshy albedo and thin membrane), (*iii*) the aril (containing juice and seed). Each fruit fraction was bulked separately and stored immediately at -85 ºC in zip-lock plastic bags until required for analysis.

2.2. Mineral analysis

Mineral analyses were carried out at Bemlab Analytical Laboratory, Strand, South Africa. The laboratory is accredited by the South African National Accreditation System (SANAS) in accordance with recognized International Standards. Moisture content was determined using the direct drying method (Isaac and Johnson, 1998). A portion of the dried samples were digested using hydrochloric acid (HCl, 3 mL) in a crucible before being incinerated at 480 ± 20 °C for 8 h in a muffle furnace. Ash was dissolved with 3 mL HCl in a flask and diluted with water (1:1 by volume) (Kalra, 1998). Concentration of major elements; Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), chlorine (Cl) and sodium (Na), as well as trace elements; manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), boron (B), nickel (Ni), cobalt (Co), chromium (Cr), lead (Pb), cadmium (Cd), selenium (Se), aluminium (Al), arsenic (As), lithium (Li), strontium (Sr), titanium (Ti), Vanadium (V) were determined using an inductively coupled plasma optical emission spectrometry (ICP-OES) calibrated with different concentrations of standard solutions of the minerals. Data was obtained from three analytical replicates for each mineral element.

Working conditions of the ICP-OES were:

Instrument:	ICP-OES (Varian-Vista; Australia)
RF power:	0.7 - 1.5 kW (1.2 - 1.3 kW for axial)
Plasma gas flow rate (Ar):	10.5 - 15 L per min (radial)
	15 L per min (axial)
Auxiliary gas flow rate (Ar):	1.5 L per min
Viewing height:	5 - 12 mm
Copy and reading time:	1 - 5 s (max. 60 s)
Copy time:	3 s (max. 100 s)

2.3. Data analysis

All data are presented as mean values ± standard error (S.E). Analysis of variance (ANOVA) was also performed using SPSS 10.0 for Windows (SPSS Inc. Chicago, USA) to assess differences among cultivars and fruit fractions. Where there was statistical significance ($p<0.05$), the means were further separated using Duncan's multiple range test. Bar charts were developed using GraphPad Prism software version 4.03 (GraphPad Software, Inc. San Diego, USA).

3. Results and discussion

3.1. Major mineral elements composition

The major mineral element concentration of rind, mesocarp and arils are shown in Table 1. Water content varied within fruit fractions and among fruit cultivars, and was significantly ($p<0.05$) the least in fruit rind while the mesocarp contained the highest in most of the fruit cultivars. On average, among all the fruit cultivars, the moisture content in fruit fractions ranged between 64.19% ('Wonderful') - 73.91% ('Ganesh') for rind, 77.92% ('Herskawitz') - 83.02% ('Ganesh') for mesocarp, and 74.92% ('Wonderful') - 82.04% ('Molla de Elche') for arils. Ash content of the fruit fractions varied markedly for most of the investigated fruit cultivars. Ash content in fruit rind, mesocarp, and arils of the investigated cultivars ranged between 3.05% ('Ganesh') - 4.51% ('Arakta'), 5.50% ('Bhagwa') - 11.90% ('Wonderful'), and 2.20% ('Wonderful') - 3.10% ('Molla de Elche'), respectively. Variations in moisture and ash content have been attributed to the influence of climatic, genetic and

geographical factors as well as differences in harvest year and growth conditions of plants (Haciseferogullari et al., 2007).

The amount of major mineral nutrients in fruit fractions of the seven pomegranate cultivars is shown in Table 1. These results indicated significant differences in fruit fractions, and among pomegranate cultivars. Potassium, Ca (except in 'Arakta'), Cl, and Na were the highest in rind while N (except in 'Molla de Elche'), P, Mg (except in 'Arakta') and S (except in 'Molla de Elche') were the highest in the arils. The concentration of K in all investigated cultivars followed a significant (p<0.05) descending order: rind > mesocarp > arils (Table 1).

Overall, major mineral nutrient concentrations in the rind of investigated cultivars ranged from 146 ('Arakta') to 351.50 mg/kg ('Bhagwa') for N, 12.31 ('Ganesh') to 33.03 mg/kg ('Bhagwa') for P, 323.5 ('Ganesh') to 554.0 mg/kg ('Bhagwa') for K, 16.15 ('Arakta') to 60.65 mg/kg ('Ruby') for Ca, 10.50 ('Ganesh') to 26.4 mg/kg ('Arakta') for Mg, 9.10 ('Ganesh') to 19.43 mg/kg ('Bhagwa') for S, 39.78 ('Ruby') to 122.96 mg/kg ('Bhagwa') for Cl, and 40.40 ('Ganesh') to 117.40 mg/kg ('Wonderful') for Na. Mineral concentrations were determined in lower quantities in the mesocarp ranging between 108.5 ('Arakta') - 205.5 mg/kg ('Herskawitz') for N, 13.83 ('Ganesh') to 23.33 mg/kg ('Herskawitz') for P, 254.0 ('Molla de Elche') to 394.5 mg/kg ('Herskawitz') for K, 7.5 ('Molla de Elche') to 23.65 mg/kg ('Arakta') for Ca, 5.10 ('Molla de Elche') to 10.35 mg/kg ('Arakta') for Mg, 6.91 ('Ganesh') to 10.79 mg/kg ('Bhagwa') for S, 22.98 ('Molla de Elche') to 57.68 mg/kg ('Bhagwa') for Cl, while Na concentration ranged between 24.75 ('Ganesh') and 40.15 mg/kg ('Wonderful').

Mineral concentrations in the arils varied markedly with average values for N, P, K, Ca, Mg, S, Cl and Na ranging between 222.0 ('Molla de Elche') to 406.50 mg/kg ('Ganesh'), 36.15 ('Molla de Elche') to 65.17 mg/kg ('Bhagwa'), 192.5 ('Molla de Elche') to 242.0 mg/kg ('Bhagwa'), 7.40 ('Molla de Elche') to 22.80 mg/kg ('Arakta'), 15.15 ('Molla de Elche') to 24.0 mg/kg ('Bhagwa'), 14.44 ('Molla de Elche') to 25.74 mg/kg ('Ganesh'), 21.18 ('Ganesh') to 51.08 mg/kg ('Arakta'), and 21.05 ('Ganesh') to 27.65 mg/kg ('Herskawitz'), respectively. Mineral nutrient distribution in the evaluated fruit fractions could probably be due to plant nutrition, climate and soil conditions (Hamurcu et al., 2010). Irrespective of type of fruit part, K was the most abundant mineral element present in all the investigated fruit cultivars, with the total concentration in fruits of the seven cultivars ranging between 799.50 ('Ganesh') to 1180 mg/100 g FW ('Bhagwa'). This level is comparable to K level in pomegranate fruit cultivars studied by previous authors (Al-Maiman and Ahmad 2002; Mirdehghan and Rahemi 2007; Gozlekci et al., 2011), but generally higher than those

reported by Fadavi et.al. (2005). In comparison with other fruits, the K level determined was the higher than those found in Mexican apple, avocado, grapes, lime, lemon, mango, plum, orange, papaya and pineapple (Sanchez-Castillo et al., 1998).

Among the cultivars assessed, the highest amounts of N, P, S and Cl were found in 'Bhagwa', while 'Arakta', 'Ruby' and 'Wonderful' had the highest amount of Mg, Ca, and Na, respectively. The pattern of concentration of the investigated major elements in all the seven pomegranate cultivars was in a decreasing order of K > N > Cl > P > Ca > Mg > S > Na for 'Arakta', 'Ruby' and 'Wonderful', and K > N > Cl > P > Ca > S >Mg > Na for 'Bhagwa', 'Ganesh', 'Herskawitz' and 'Molla de Elche'. These trends are similar to those reported for pomegranate fruit cultivars grown in different countries. Mirdehghan and Rahemi (2007) reported similar order of macronutrient concentration in pomegranate arils and rind as K > N > Ca > P > Mg > Na for Iranian 'Malas Yazdi', while Gozlekci et al. (2011) reported a decreasing order of K > P > Ca > Mg > Na for 'Hicaznar' grown in Turkey.

Average mineral composition for each element in the investigated pomegranate is shown in Table 3. Regardless of cultivar differences, among the fruit parts, N (350.57±16.33 mg/100 g), P (53.60±2.84 mg/100 g), Mg (20.14±0.91 mg/100 g) and S (20.92±1.00 mg/100 g) are significantly the highest in fruit arils, while fruit rind had the highest values for K, Ca, Cl and Na (Table 3).

3.2. Trace mineral elements composition

The composition of trace elements in the investigated fruit cultivars is presented in Table 2. Trace elements concentrations differed considerably between the fruit parts of the same cultivar and between the fruit cultivars. Generally, relatively high amounts of Mn, Fe, Cu, Zn, B, Ni, Se, Al and Sr were determined in most of the fruit cultivars, while the amounts of Ni, Co, Pb, Cd, As, Li, Ti and V in the fruit parts were low or often below detection level. The highest and lowest values in trace elements in the edible fraction (arils) of fruit cultivars are 2.10 mg/kg ('Bhagwa') and 1.05 mg/kg ('Molla de Elche') for Mn; 7.35 mg/kg ('Wonderful') and 4.95 mg/kg ('Molla de Elche') for Fe; 2.10 mg/kg ('Bhagwa') and 1.15 mg/kg ('Molla de Elche') for Cu; 5.10 mg/kg ('Ganesh') and 2.80 mg/kg ('Molla de Elche') for Zn; 5.25 mg/kg ('Arakta') and 1.65 mg/kg ('Molla de Elche') for B; 0.92 mg/kg ('Ruby') and 0.14 mg/kg ('Herskawitz') for Ni; 1.15 mg/kg ('Ruby') and 0.08 mg/kg ('Ganesh') for Cr; 2.34 mg/kg ('Ganesh') and 0.07 mg/kg ('Herskawitz') for Pb; 0.11 mg/kg ('Wonderful') and 0.02 mg/kg ('Bhagwa') for Cd; 14.32 mg/kg ('Wonderful') and 0.76 mg/kg ('Ruby') for Se; 0.39 mg/kg ('Bhagwa') and 0.06 mg/kg ('Arakta') for Li; 3.94 mg/kg ('Arakta') and 0.06

mg/kg (‘Ganesh’) for Sr; 0.03 mg/kg (‘Arakta’) and 0.01 mg/kg (‘Ganesh’) for Ti as well as between 0.29 mg/kg (‘Bhagwa’) and 0.03 mg/kg (‘Ruby’) for V. No detections of Co, Al and Co were observed in the arils of all the cultivars.

Regardless of fruit cultivar, average values of Fe (5.85±0.38 mg/kg), Cu (1.71±0.12 mg/kg) and Zn (4.17±0.29 mg/kg) were significantly (p<0.05) the highest in the edible part of pomegranate fruit (arils) while Sr (12.51±2.94 mg/kg) was the highest in the rind (Table 3). Values for Fe, Cu and Zn in this study were below those reported by Gozlekci et al. (2011) but higher that those reported by Fadavi et.al. (2005). Differences in pomegranate cultivar, soil type, agro-climatic and environmental conditions as well as soil contamination and bioavailability of trace elements, have been implicated in the variability of trace elements and heavy metals in pomegranate fruit cultivars (Fadavi et.al. 2005).

3.3. Distribution of mineral elements in edible and non-edible fruit parts

After combining the values for each of the investigated mineral elements in non-edible fractions (rind and mesocarp) of the seven pomegranate cultivars, the content of mineral elements in edible and non-edible fruit portions were calculated (Figure 1). Among the twenty five mineral elements studied, the quantity of five elements (P, Fe, Zn, Ti, V) were higher in the edible portion, while other mineral elements (Co, Al, As) were in larger proportions and in some cases, were only found in the non-edible fractions of the investigated cultivars.

3.4. Implications of mineral concentration on recommended dietary allowance (RDA)

Table 4 shows the range of mineral element compositions determined in arils (edible portion) of the pomegranate cultivars, as well as the contribution (%) of the elements to the recommended dietary allowance (RDA). Among the major mineral elements investigated, P had the highest covering of the RDA, ranging from 6.78 to 8.53% contribution to the RDA, followed by K (4.53 to 4.95% RDA), S (1.25 to 1.54% RDA), Ca (1.04 to 1.54% RDA), Mg (4.33 to 5.26% RDA), and Na (0.15 to 0.17% RDA) (Table 4). The coverings of RDA among the investigated major elements were relatively lower for Ca and Na. Major mineral elements such as phosphorus, potassium, calcium, magnesium, sulphur, chloride and moderate sodium are required in amounts greater than 100 mg per day (Ozcan, 2004). Intake of Ca, P, Mg and K, together with a moderate Na intake, is associated with protection against bone demineralization, arterial hypertension, insulin resistance, and overall cardiovascular risk (Segura et al., 2006). The results obtained in the present study indicate the levels of major

mineral element concentrations that make pomegranate arils a beneficial source to the consumer.

Pomegranate is highly rich in Se, a physiologically essential trace element. Therefore the importance of the appreciable quantity of Se found in arils cannot be overemphasized because it offers protective actions on many toxicological effects of Cd and Hg. It also acts by metabolic interference with As, and plays an active role as a modulator in inflammatory and immune responses (Neve, 1991). In summary, pomegranate arils can be considered as a good source of minerals which are essential for human health, consuming a fruit per day may cover considerable amounts of the daily requirement of many mineral elements for an average adult.

4. Conclusions

This study has provided information on the distribution of major and traces mineral elements among pomegranate cultivars and fruit fractions. The findings showed that most of the mineral elements were considerably accumulated in the non-edible fruit fraction (rind and mesocarp). Phosphorus, Fe, Zn, Ti and V were accumulated in higher concentrations in edible portion for all the investigated cultivars. Overall, the results presented in this work show that consuming pomegranate arils is a good source of mineral elements in human diet.

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Table 1

Concentration (fresh matter basis) of different major mineral nutrients in fruit fractions of the seven pomegranate cultivars

Cultivar	Part	Water (%)	Ash (%)	(mg/100 g fresh matter)							
				N	P	K	Ca	Mg	S	Cl	Na (mg/kg)
'Arakta'	Rind	72.09^{c}	4.51^{b}	146.00^{b}	16.38^{b}	435.50^{a}	16.15^{b}	26.40^{a}	11.60^{b}	97.70^{a}	49.50^{a}
	Mesocarp	81.38^{a}	6.53^{a}	108.50^{c}	16.15^{b}	271.00^{b}	23.65^{a}	10.35^{c}	7.01^{c}	39.12^{b}	29.45^{b}
	Arils	77.73^{b}	2.85^{c}	350.50^{a}	48.81a	226.00^{c}	22.80^{ab}	18.00^{b}	19.81^{a}	51.08b	25.05^{b}
	Total			605.00	81.34	932.50	62.60	54.75	38.42	187.90	104.00
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.006	<0.0001	<0.0001	<0.0001	<0.0001
'Bhagwa'	Rind	68.77^{c}	3.80^{b}	351.50^{b}	33.03^{b}	554.00^{a}	48.35^{a}	12.10^{b}	19.43^{b}	122.96^{a}	46.30^{a}
	Mesocarp	80.58^{a}	5.50^{a}	196.00^{c}	22.51^{c}	384.50^{b}	17.75^{b}	7.05^{c}	10.79^{c}	57.68^{b}	39.50^{a}
	Arils	76.00^{b}	2.40^{c}	386.50^{a}	65.17^{a}	242.00^{c}	16.20^{b}	24.00^{a}	24.50^{a}	37.24^{c}	21.35^{b}
	Total			934.00	120.71	1180.50	82.30	43.15	54.72	217.88	107.15
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
'Ganesh'	Rind	73.91^{b}	3.05^{b}	173.00^{b}	12.31^{b}	323.50^{a}	31.90^{a}	10.50^{b}	9.10^{b}	87.50^{a}	40.40^{a}
	Mesocarp	83.02^{a}	9.27^{a}	131.00^{b}	13.83^{b}	243.00^{b}	11.95^{c}	5.85^{b}	6.91^{c}	30.57^{b}	24.75^{b}
	Arils	76.34^{b}	2.23^{c}	406.50^{a}	61.06^{a}	233.00^{c}	17.05^{b}	22.40^{a}	25.74^{a}	21.18^{b}	21.05^{b}
	Total			710.50	87.2	799.50	60.90	38.75	41.74	139.25	86.20
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 1 contd.

Cultivar	Part	Water (%)	Ash (%)	(mg/100 g fresh matter)							
				N	P	K	Ca	Mg	S	Cl	Na (mg/kg)
'Herskawitz'	Rind	66.12^{b}	3.70^{b}	247.50^{b}	26.52^{b}	477.00^{a}	51.55^{a}	17.00^{b}	14.12^{b}	52.50^{a}	65.10^{a}
	Mesocarp	77.92^{a}	11.25^{b}	205.50^{c}	23.33^{b}	394.50^{b}	16.35^{b}	7.30^{c}	9.92^{b}	46.23^{a}	31.15^{b}
	Arils	77.38^{a}	2.70^{c}	379.00^{a}	58.68^{a}	233.50^{c}	11.15^{b}	21.00^{a}	21.43^{a}	30.44^{b}	27.65^{b}
	Total			832.00	108.53	1105.00	79.05	45.30	45.47	129.16	123.90
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001	<0.0001
'Molla de Elche'	Rind	64.34^{b}	3.60^{b}	273.00^{a}	27.38^{b}	490.00^{a}	28.65^{a}	12.50^{b}	16.29^{a}	103.43^{a}	78.35^{a}
	Mesocarp	81.59^{a}	7.10^{a}	158.50^{c}	20.33^{c}	254.00^{b}	7.50^{b}	5.10^{c}	8.08^{b}	22.98^{b}	25.95^{b}
	Arils	82.04^{a}	3.10^{b}	222.00^{b}	36.15^{a}	192.50^{c}	7.40^{b}	15.15^{a}	14.44^{a}	27.84^{b}	24.60^{b}
	Total			653.50	83.86	936.50	43.55	32.75	38.81	154.24	128.90
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
'Ruby'	Rind	70.51^{b}	3.55^{b}	244.00^{b}	23.36^{b}	406.50^{a}	60.65^{a}	17.65^{a}	14.38^{b}	39.78^{a}	45.85^{a}
	Mesocarp	79.69^{a}	8.13^{c}	176.50^{c}	20.37^{b}	285.50^{b}	18.60^{b}	9.50^{b}	9.67^{c}	31.49^{ab}	28.10^{b}
	Arils	78.69^{a}	2.75^{c}	333.00^{a}	45.27^{a}	228.50^{c}	20.60^{b}	17.70^{a}	18.99^{a}	23.51^{b}	24.50^{b}
	Total			753.50	88.99	920.50	99.85	44.80	43.04	94.78	98.45
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.006	<0.0001	<0.0001	0.02	<0.0001
'Wonderful'	Rind	64.19^{c}	3.65^{b}	258.50^{b}	32.92^{b}	442.50^{a}	39.50^{a}	18.80^{b}	12.67^{b}	58.02^{a}	117.40^{a}
	Mesocarp	78.62^{a}	11.90^{a}	194.00^{c}	26.41^{c}	307.00^{b}	15.25^{b}	9.25^{c}	10.41^{b}	37.41^{b}	40.15^{b}
	Arils	74.92^{b}	2.20^{b}	376.50^{a}	60.05^{a}	204.50^{c}	12.85^{c}	22.80^{a}	21.53^{a}	31.46^{b}	21.40^{c}
	Total			829.00	119.37	954.00	67.60	50.85	44.61	126.88	178.95
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.01	<0.0001

Means in each column followed by different letter (s) for each cultivar are significantly different (p<0.05) according to Duncan's multiple range test. Major elements were expressed (mg/100 g fresh matter), Na (mg/kg fresh matter). Fruit were analyzed at commercial harvest during 2010 season.

Table 2

Concentration of different trace mineral nutrients (mg/kg fresh matter) in fruit fractions of the seven pomegranate cultivars. Fruit were evaluated at commercial harvest during the 2010 season

Cultivar	Part	Mn	Fe	Cu	Zn	B	Ni	Co	Cr	Pb	Cd	Se	Al	As	Li	Sr	Ti	V
'Arakta'	Rind	2.25	1.70	0.80	1.80	5.70	nd	0.10	0.56	nd	nd	2.27	10.23	1.12	0.27	23.42	nd	0.03
	Mesocarp	0.85	1.85	0.95	1.45	8.10	1.13	0.03	0.55	0.13	0.73	nd	1.31	1.77	0.07	8.77	nd	nd
	Arils	1.80	5.15	1.75	4.10	5.25	0.22	nd	0.28	nd	nd	2.76	nd	nd	0.06	3.94	0.03	nd
	Total	4.90	8.70	3.50	7.35	19.05	1.34	0.13	1.39	0.13	0.73	5.03	11.54	2.88	0.40	36.12	0.03	0.03
'Bhagwa'	Rind	2.50	1.45	0.75	1.25	4.55	0.22	nd	2.05	0.53	nd	3.30	8.15	nd	0.33	22.01	nd	nd
	Mesocarp	1.00	1.55	1.00	1.30	6.25	1.37	0.09	1.02	0.41	0.02	0.91	7.26	0.60	0.11	10.85	nd	nd
	Arils	2.10	6.40	2.10	4.65	4.05	0.23	nd	0.27	nd	0.02	1.5	nd	nd	0.39	2.31	nd	0.29
	Total	5.60	9.40	3.85	7.20	14.85	1.82	0.09	3.34	0.94	0.04	5.71	15.41	0.60	0.83	35.16	nd	0.29
'Ganesh'	Rind	0.90	1.70	0.70	1.80	4.00	0.23	0.02	0.16	0.35	0.20	2.78	6.37	nd	0.24	4.04	nd	0.02
	Mesocarp	0.50	1.55	0.95	1.75	4.75	nd	nd	1.47	0.37	nd	0.68	6.87	nd	0.22	1.48	0.06	nd
	Arils	2.05	5.25	1.90	5.10	5.30	nd	nd	0.08	2.34	0.06	0.92	nd	nd	0.19	0.66	0.01	0.23
	Total	3.45	8.50	3.55	8.65	14.05	0.23	nd	1.70	3.05	0.26	4.37	13.24	nd	0.65	6.18	0.07	0.25
'Herskawitz'	Rind	1.40	3.00	0.75	2.45	4.50	0.04	nd	0.36	0.41	nd	2.49	6.44	0.52	0.32	10.40	nd	nd
	Mesocarp	0.65	1.60	1.25	1.65	5.15	1.26	nd	0.06	0.71	nd	2.63	6.73	0.16	0.26	5.08	nd	nd
	Arils	1.80	6.90	1.45	4.15	2.45	0.14	nd	0.10	0.07	nd	1.71	nd	nd	0.31	0.97	nd	nd
	Total	3.85	11.50	3.45	8.25	12.10	1.43	nd	0.52	1.19	nd	6.82	13.17	0.67	0.88	16.44	nd	nd

Table 2 contd.

Cultivar	Part	Mn	Fe	Cu	Zn	B	Ni	Co	Cr	Pb	Cd	Se	Al	As	Li	Sr	Ti	V
'Molla de Elche'	Rind	1.05	3.30	0.55	3.65	3.60	nd	nd	1.09	0.70	nd	2.78	12.05	nd	0.29	6.56	nd	0.03
	Mesocarp	0.40	1.65	0.75	1.20	3.20	1.13	0.11	0.26	nd	0.08	5.62	6.43	0.64	0.29	2.08	nd	0.03
	Arils	1.05	4.95	1.15	2.80	1.65	0.69	nd	0.24	nd	nd	1.12	nd	nd	0.10	1.04	nd	0.14
	Total	2.50	9.90	2.45	7.65	8.45	1.82	0.11	1.58	0.70	0.08	9.51	18.48	0.64	0.68	9.67	nd	0.20
'Ruby'	Rind	2.15	2.85	1.15	3.45	3.80	2.02	nd	0.26	0.43	nd	4.87	7.28	nd	0.25	14.67	nd	0.03
	Mesocarp	0.85	1.90	1.10	1.70	3.70	0.13	0.05	0.76	0.19	0.25	3.89	14.98	0.21	0.24	6.33	nd	nd
	Arils	1.60	4.95	1.75	3.70	3.35	0.92	nd	1.15	0.86	nd	0.76	nd	nd	0.17	3.28	nd	0.03
	Total	4.60	9.7	4.00	8.85	10.85	3.06	0.05	2.16	1.47	0.25	9.52	22.26	0.21	0.65	24.27	nd	0.06
'Wonderful'	Rind	1.25	2.25	0.70	1.80	3.20	3.18	0.07	0.83	nd	nd	1.43	9.16	nd	0.31	6.46	nd	nd
	Mesocarp	0.50	1.80	1.65	2.50	3.90	2.32	0.08	2.68	0.44	1.75	2.56	7.79	0.26	0.30	3.53	nd	nd
	Arils	1.90	7.35	1.90	4.70	2.40	nd	nd	0.91	nd	0.11	14.32	nd	nd	0.23	1.08	nd	0.03
	Total	3.65	11.40	4.25	9.00	9.50	5.50	0.15	4.41	0.44	1.85	18.31	16.95	0.26	0.83	11.07	nd	0.03

nd, not detected.

Table 3

Weighted average of each mineral elements in fruit fractions of all the investigated pomegranate cultivars*

Part	N	P	K	Ca	Mg	S	Cl	Na	Mn
Rind	207.93±14.75[b]	22.28±1.84[b]	401.14±21.67[a]	30.81±4.56[a]	14.56±1.86[b]	12.10±0.82[b]	65.92±28.26[a]	58.44±8.02[a]	1.64±0.24[a]
Mesocarp	167.14±9.39[c]	20.42±1.12[b]	305.64±15.92[b]	15.86±1.32[b]	7.77±0.51[c]	8.97±0.44[c]	37.92±11.60[b]	31.29±1.66[b]	0.68±0.09[b]
Aril	350.57±16.33[a]	53.60±2.84[a]	222.86±4.62[c]	15.44±1.39[b]	20.14±0.91[a]	20.92±1.00[a]	31.82±10.19[b]	23.66±0.75[b]	1.76±0.13[a]
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

	Fe	Cu	Zn	B	Ni	Co	Cr	Pb	Cd
Rind	2.32±0.28 [b]	0.77±0.07 [b]	2.31±0.35 [b]	4.19±0.31 [a]	0.81±0.48 [a]	0.03±0.01 [b]	0.76±0.25 [a]	0.34±0.10 [a]	0.03±0.02 [a]
Mesocarp	1.70±0.05 [b]	1.09±0.11 [b]	1.65±0.16 [b]	5.01±0.64 [a]	1.05±0.30 [a]	0.05±0.02 [a]	0.97±0.34 [a]	0.32±0.09 [a]	0.40±0.25 [a]
Arils	5.85±0.38 [a]	1.71±0.12 [a]	4.17±0.29 [a]	3.49±0.54 [a]	0.31±0.13 [a]	0.00±0.00 [c]	0.43±0.16 [a]	0.47±0.33 [a]	0.03±0.02 [a]
p-value	<0.0001	<0.0001	<0.0001	0.146	0.308	0.039	0.352	0.868	0.130

	Se	Al	As	Li	Sr	Ti	V
Rind	2.84±0.40 [a]	8.52±0.80 [a]	0.23±0.16 [a]	0.29±0.01 [a]	12.51±2.94 [a]	0.00±0.00 [a]	0.01±0.004 [b]
Mesocarp	2.20±0.80 [a]	7.34±1.51 [b]	0.52±0.23 [a]	0.21±0.03 [a]	5.44±1.31 [b]	0.01±0.001 [a]	0.004±0.003 [a]
Arils	3.08±1.90 [a]	0.00±0.00 [c]	0.00±0.00 [a]	0.21±0.04 [a]	1.90±0.49 [b]	0.01±0.003 [a]	0.10±0.04 [a]
p-value	0.867	<0.0001	0.171	0.187	0.003	0.542	0.033

Mean ± S.E in each column followed by different letter (s) are significantly different ($p<0.05$) according to Duncan's multiple range test. *Major elements (mg/100 g fresh matter), Na and trace elements (mg/kg fresh matter). Mineral elements from pooled data for mineral element contents for 'Arakta', 'Bhagwa', 'Ganesh', 'Herskawitz', 'Molla de Elche', 'Ruby' and 'Wonderful' at commercial harvest during 2010 season.

Table 4

Major mineral element concentration in pomegranate arils and the covering (%) of RDA for adults

Mineral	Concentration (mg/100 g)	RDA* (mg/day)	Covering of RDA (%)
P	47.47 - 59.72	700	6.78 - 8.53
K	212.89 - 232.8	4700	4.53 - 4.95
S	18.75 - 23.09	1500	1.25 - 1.54
Ca	12.43 - 18.44	1200	1.04 - 1.54
Mg	18.18 - 22.11	420	4.33 - 5.26
Na	2.20 - 2.53	1500	0.15 - 0.17

*RDA - Recommended Dietary Allowances (Park et al., 2008; Hussain et al., 2010).

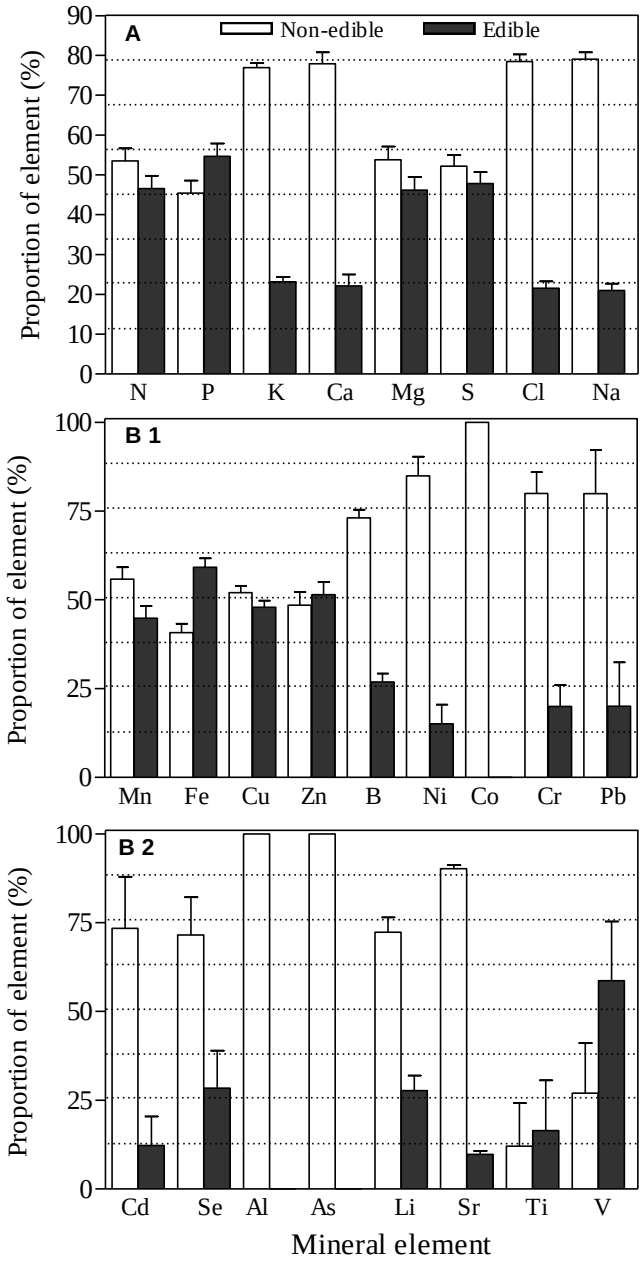

Figure 1. Proportion (%) of elements determined in non-edible and edible portions of all the investigated pomegranate fruit cultivars (A) major elements, (B 1 & B 2) trace elements. Means and standard error presented.

PAPER 6

Physico-mechanical, phytochemical, and free radical scavenging properties and volatile compounds in eight pomegranate cultivars and classification by principal component and cluster analyses

Abstract

In this study, fruit properties of South African grown pomegranate cultivars were characterized for food and industrial purposes. The physico-textural and chemical properties as well as the volatile profile and free radical scavenging capacity of eight cultivars ('Acco', 'Arakta', 'Bhagwa', 'Ganesh', 'Herskawitz,' 'Molla de Elche', 'Ruby', and 'Wonderful') were investigated to demonstrate the diversity among commercially grown cultivars, and to provide information that could assist in selecting cultivars for commercial purposes. Statistically significant differences were found between cultivars for most of the evaluated characters, suggesting genetic diversity among the cultivars. Classification of cultivars based on important quality traits (size, texture, colour, soluble solids, acidity, juiciness and phenolics) showed the potential of the cultivars for fresh market and processing. Relationship among these quality traits was analysed by principal component analysis (PCA) resulting in the separation of the investigated cultivars into two groups (cluster 1: 'Ruby', 'Arakta' and 'Ganesh'; cluster 2: 'Bhagwa', 'Acco' and 'Herskawitz') and two ungrouped cultivars ('Molla de Elche' and 'Wonderful') based on combinations of their physico-chemical and nutritional properties.

Keywords: Pomegranate; physico-chemical properties; textural properties; postharvest handling; processing; South Africa

1. Introduction

Pomegranate (*Punica granatum* L.) belongs to the family Punicaceae, and has gained popularity in recent years due to its multi-functionality and great nutritional benefit in the human diet. The fruit is grown globally in many different geographical regions, satisfying the nutritional

and medicinal needs of populations of various countries (Holland et al., 2009). The fruit is rich in several groups of medicinal substances, in particular, phenolic compounds which have high antioxidant capacity, and several biological actions (Lansky and Newman, 2007; Seeram et al., 2005; Martınez et al., 2006; Jaiswal et al., 2010). The pomegranate fruit is spherical, crowned with persistent calyx, and covered with a leathery pericarp (Holland et al., 2009; Wetzstein et al., 2011). The aril of fruit is about 55 - 60% of the total fruit weight and consists of about 75 - 85% juice and 15 - 25% seeds (Al-Maiman and Ahmad, 2002). The fruit is usually consumed as fresh arils, or as processed products such as fruit juice, jams, wine, beverages (Elyatem and Kader, 1984; Martinez et al., 2006; Lansky and Newman, 2007; Opara et al., 2009).

Pomegranate cultivars differ in their taste, ranging from sweet to sour, as well as in seed hardness ranging from soft to hard (Holland et al., 2009). However, the desired pomegranate traits vary in different countries and regions (Ferrara et al., 2011). Commercial orchards of diverse pomegranate cultivars are grown in countries such as Iran, India, Egypt, China, Israel, Tunisia, Syria, Lebanon, Turkey, Greece, Cyprus, Italy, France, Spain, Chile, Portugal, USA, Oman and most recently in South Africa (Al-Said et al., 2009; Holland et al., 2009; Fawole et al., 2012). It has been demonstrated that a wide diversity in several physico-chemical and textural properties exists among pomegranate cultivars grown in many countries (Gozlekci and Kaynak, 2000; Martinez et al., 2006; Al-Said et al., 2009; Opara et al., 2009; Tehranifar et al., 2010; Hasnaoui et al., 2011). In addition, it is been established that fruit quality attributes such as size, colour, juiciness, taste, and seed hardness among others are influenced primarily by genetics, but could also be influenced by the environment in which the crop is grown (Jalikop, 2007; Holland et al., 2009).

Due to vast number of factors which may affect consumer acceptability and cultivar promotion, as well as industrial use of pomegranate fruit for processing, it is important to consider not only fruit phytochemical and antioxidant properties, but also mechanical properties that are relevant in cultivar characterization, marketing and postharvest handling. Despite several studies published in major productive regions across the globe to assess the variation of several pomegranate fruit traits and characterization of pomegranate cultivars, there is a dearth of information on the assessment and characterization of fruit cultivars grown in South Africa. The objective of this study was to investigate the physico-textural and chemical properties, volatile profile and antioxidant capacity of eight pomegranate cultivars. Such data will assist in cultivar

selection for commercial production to meet market demand, and provide information on fruit quality properties relevant to postharvest handling and processing.

2. Materials and methods

2.1. Fruit samples

Eight commercially grown pomegranate cultivars including 'Arakta', 'Bhagwa', 'Ruby', 'Acco', 'Ganesh', 'Herskawitz', 'Molla de Elche' and 'Wonderful' were studied. Fruits (10 kg) were harvested at commercial harvest between March and April (2010) from Western Cape regions (Porterville; 33°01′00″S, 18°58′59″E, Piketberg; 32° 54′ 00″ S, 18° 46′ 00″ E) in Western Cape Province, South Africa. The sampling locations are 27 km apart and are representative sites for the studied pomegranate cultivars in the region. Fruit were transported in an air-conditioned vehicle to the postharvest laboratory and then sorted by selecting those without blemish, cracks, cuts and sunburn before being stored at 5°C and 91±2.5% relative humidity for 72 h before analysis.

2.2. Fruit physical properties

2.2.1. Whole fruit

Physical measurements were carried out on 15 randomly selected fruit of each cultivar. Individual fruit weight (W_F) was measured using an electronic balance (Mettler, Toledo, Switzerland) with an accuracy of 0.01 g. Linear dimensions, the length (L) (fruit polar axis) and diameter (D) (perpendicular distance to the polar axis) of the fruits were measured using a digital Vernier caliper (Mitutoyo, Kawasaki, Japan, ± 0.01 mm,). Fruit geometric mean diameter (D_g, mm), sphericity , and surface area (S_F, cm^2) were determined according to Mohsenin (1986) using equations 1, 2 and 3, respectively:

$$D_g = (L \times D^2)^{1/3} \quad (1)$$

$$Ø = Dg/L \quad (2)$$

$$S_F = \Pi \times D_g^2 \quad (3)$$

Fruit volume (V, cm^3) was determined using a mathematical relationship assuming spherical shape (Al-Yahyai et al., 2009):

$$V = [(4/3) \times \Pi \times r^3 \quad (4)$$

Each fruit was manually peeled and the peel thickness on opposite sides of each fruit was measured using a digital Vernier caliper. Total number of arils per fruit (*Aril no.*) was estimated by counting the number per 20 g aril sample and then multiplied the number by total aril mass per fruit (Al-Said et al., 2009). The moisture contents of each individual fruit peel and arils (50 g) were then determined in triplicates using a standard method (Kalra, 1998).

2.2.2. Aril and kernel

Weight (g) of arils per fruit (total aril wt.) was recorded using an analytical balance (Mettler, Toledo, Switzerland, 0.0001 g accuracy). Ten grams of arils from 15 fruit samples per cultivar were mixed in a bowl and the following linear dimensions were measured using 20 randomly selected arils: weight per aril, length and maximum width. After manually removing the pulp of the arils, 20 kernels were randomly selected and the weight, length and maximum width per kernel were measured. All linear measurements were carried out using a digital Vernier caliper (0.01 mm, Mitutoyo, Kawasaki, Japan). Kernel index (KI) was calculated according to Martinez et al. (2006) using equation 5:

$$KI = (\text{kernel width} \div \text{aril width}) \times 100\% \quad (5)$$

2.2.3. Fruit colour

Fruit skin colour along the equatorial axis of each fruit at two opposite spots were recorded in (CIELAB) coordinates (L^*, a^*, b^*) using a Minolta Chroma Meter CR-400 (Minolta Corp, Osaka, Japan) after calibration with a white tile background. Similarly, duplicate colour measurements (L^*, a^*, b^*) were made on the arils placed in a colourless glass Petri dish. The colour parameter Chroma (C^*_{ab}) which describes the length of the colour vector in the plane

formed by a^* and b^*, and the hue angle (h^o) that determines the position of such vector were calculated according to the following equations:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (6)$$

$$h^o = \arctan (b^*/ a^*) \quad (7)$$

The total colour difference (TCD) between the fruit skin (external) and arils (internal) components was calculated as:

$$TCD = \sqrt{((L^*_0 - L^*)^2 + (a^*_0 - a^*)^2 + (b^*_0 - b^*)^2)} \quad (8)$$

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

2.3. Fruit textural properties

2.3.1. Fruit puncture resistance

Fruit skin strength based on resistance to puncture was measured using a firmness analyzer with a 5 mm diameter cylindrical probe (GÜSS-FTA, South Africa) programmed to penetrate 8.9 mm into the test fruitat speed of 10 mm/s . Duplicate puncture tests were performed on opposite sides of equatorial region of each fruit. Peak force required to puncture skin was taken as fruit puncture resistance. Ten fruit per cultivar were measured and results were expressed as means ± S.E. of determinations obtained.

2.3.2. Aril and kernel texture

Tests were carried out on twenty randomly arils and kernels, respectively, which were selected from a pool extracted from 15 fruit. Compression test was performed on individual arils and kernels using a texture profile analyzer (TA.XT *plus*, Stable Micro System, UK) with a 35 mm cylindrical probe. The operating conditions of the instrument were as follows: pre-test speed 1.5 mm/s, 0.5 mm/s test speed, 10.0 mm/s post-test speed, and 0.20 N trigger force and each test sample was aligned horizontally on the platform (Al-Said et al., 2009). Aril and kernel

hardness (N) was calculated as the maximum force required for complete breakage of the test sample while the toughness (N) of the arils was determined from the area under the curve (Al-Said et al., 2009; Bchir et al., 2010a). The means (± S.E) of 20 determinations are reported.

2.4. Fruit chemical property

2.4.1. Titratable acidity, total soluble solids and pH

Juice was extracted (without crushing the kernels) using a blender (Mellerware, South Africa). Titratable acidity (TA) was determined by titration using a Metrohm 862 compact titrosampler (Herisau, Switzerland). Fresh juice (2 mL) was diluted with Milli-Q water (70 mL) and titrated with 0.1 N NaOH to an endpoint of pH 8.2 and results expressed as g citric acid/ 100 mL of juice. Total soluble solids TSS (°Brix) were measured at room temperature using a digital refractometer (Atago, Tokyo, Japan) calibrated with Milli-Q water. The pH values were measured at room temperature using a pH meter (Crison, Barcelona, Spain). Analyses were carried out on each of the 15 randomly selected fruit per cultivar.

2.5. Gas chromatography-mass spectrometry (GC-MS) for volatile profiling

Volatile fruit compounds were profiled by subjecting juice samples to headspace solid phase micro-extraction (HS-SPME). Juice samples were bulked into three replicates, each containing five fruit per cultivar. Extraction was carried out by taking 5 mL of each sample into 22 mL crimp cap headspace vials in duplicates. A 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) fiber was used for all the analysis. Pre-incubation and extraction times to 50˚C were 10 and 20 min, respectively. Volatile organic compounds trapped in the fiber were analysed by an Agilent GC (6890 N, Agilent Technologies, Santa Clara, CA, USA) coupled with a mass spectrometer (5975 N, Agilent Technologies, Santa Clara, CA, USA), and equipped with an Rxi®-5Sil MS column (30 m length, 0.25 mm i.d., 0.25 µm film thickness). Oven temperature was maintained at 40°C for 2 min and then programmed to rise to 250°C at 5°C/min, where it was isothermally held for 10 min. Helium was the carrier gas at a constant flow rate of 1.2 mL/min. Compounds were identified by comparing the MS for each putative compound with those in the National Institute of Standards and Technology (NIST) 2005 Mass Spectral library and then confirmed by GC retention time (RT) and Mass Spectra custom library generated using

commercially available compounds at the central analytical facility of Stellenbosch University. The relative proportions (%) of volatile compounds were expressed as their percentage peak area ratio to total peak area of all identified compounds.

2.6. Free radical scavenging capacity

The scavenging capacity of 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) was tested on both hydrophilic and lipophilic fractions of juice samples of each cultivar using the DPPH assay. Analysis was done on bulked samples, each containing five fruit per cultivar. Extraction procedure of both fractions was carried out according to Legua et al. (2011) using 50 mM phosphate buffer (pH 7.8) and ethyl acetate as extraction solvent for the separation of hydrophilic and lipophilic fractions. The DPPH assay was carried out in triplicate according to the method used by Karioti et al. (2004) with some modifications as previously reported (Fawole et al., 2010). Juice fraction (15µL) was diluted with methanol (735 µL) followed by the addition of methanolic DPPH solution (0.1 mM, 750 µL) under a dim light. The mixtures were incubated in the dark at room temperature for 30 min, before the absorbance was measured at 517 nm. Ascorbic acid was used as a standard curve and free radical scavenging capacity was expressed as ascorbic acid (mg) equivalents per 100 mL juice fraction (mg AAE /100 mL).

2.7. Total phenolic concentration (TPC)

Total phenolic (TP) concentration was determined in triplicates by the Folin-Ciocalteu (Folin-C.) colourimetric method (Makkar, 2000) and results represented as gallic acid equivalents (GAE) per 100 mL juice. Analysis was done in triplicates on bulked samples, each containing five fruit per cultivar.

2.8. Statistical analysis

Mean (±S.E) values of all the studied variables were presented. Analysis of variance (ANOVA) was also performed using SPSS for windows (SPSS Inc., Chicago, IL, USA). Differences between cultivar means were tested with Duncan's multiple range test. In order to find the main variation trends between physico-textural, chemical and antioxidant characters of the investigated fruit cultivars and to evaluate their correlation, data were processed according to

principal component analysis (PCA) using using XLSTAT software Version 2012.4.01 (Addinsoft, France). Furthermore, XLSTAT was used to carry out cluster analysis on the standardized data to obtain hierarchical associations employing Euclidean distance and Ward's method as dissimilarity measure and amalgamation rule, respectively.

3. Results and discussions

3.1. Physical properties

3.1.1. Whole fruit

Physical properties of the eight pomegranate cultivars are shown in Table 1. Significant differences were observed for fruit length, diameter, weight, sphericity, geometric mean diameter, volume and surface area. These properties were the highest for 'Wonderful' cultivar, while the lowest dimensions were found in 'Acco'. Fruit weight is one of the important characteristics in pomegranate fruit production and marketing because it influences consumer preference and income to growers (Hess-Pierce and Kader, 2003; Holland et al., 2009). In general, fruit weight of the pomegranate cultivars studied varied between 274.04 g ('Acco') to 509.82 g ('Wonderful'). These values are within those (196.89 and 524.02 g) reported in previous studies for different pomegranate cultivars in different producing countries (Amoros et al., 2000; Gozlekci and Kaynak, 2000; Opara et al., 2009; Zarei et al., 2010; Tehranifar et al., 2010). Similarly, the fruit volumes showed statistical differences among some of the cultivars, ranging between 222.52 to 509.82 cm^3; however, there were no significant differences between 'Arakta', 'Bhagwa' and 'Herskawitz', and between 'Molla de Elche' and 'Ruby'. Fruit sphericity was highest (p<0.05) for 'Bhagwa' and lowest for 'Molla de Elche'. In terms of fruit size, 'Ganesh' and 'Wonderful' seem the most promising for their bigger fruit. It is worth noting that the small but random sample size (15 fruit) used in this study is limitation in making conclusive statements on the relative sizes of fruit among the investigated cultivars. Furthermore, it is well documented that fruit grown using normal commercial production methods could exhibit a wide range of sizes which are usually influenced by factors such as tree age, fruit maturity and micro-climate (Wetzstein et al., 2011).

3.1.2. Arils and kernel

The edible portion of pomegranate fruit consists of the aril which contains the juice and the kernel (woody portion). Optimizing the quality attributes of these fruit parts is therefore the goal of growers, breeders and processors (Martinez et al., 2006; Hasnaoui et al., 2011). As shown in Table 2, many physical properties of the arils and kernels of the investigated pomegranate cultivars were found to be statistically (p<0.05) different.These differences could be attributed to the inherent (genetic) properties of the pomegranate fruit cultivars (such as fruit size) and environmental conditions (Shulman et al., 1984; Tehranifar et al., 2010). Mean values for aril content (g) per fruit varied significantly, ranging from 123.88 ('Molla de Elche') to 305.33 g ('Ganesh') (Table 2). This range is close to those reported by several researchers on pomegranates cultivars grown in other producing countries such as Iran and Oman (Zarei et al., 2010; Al-Said et al., 2009; Tehranifar et al., 2010). Aril yield is a desirable property from a consumer point of view as well for industrial juice processing. 'Ruby' had the highest (68.05%) aril yield, while the lowest value (47.49%) was in 'Wonderful'. These values are comparable with those (57.86 - 75.48%) reported by Zarei et al. (2010) for Iranian cultivars. There were less variation in the mean values for length and width of aril and kernel among the studied cultivars (Table 2). Similar observation was reported by Hasnaoui et al. (2011) on pomegranate fruit grown in Tunisia. According to Martinez et al. (2006), the kernel index (KI) is a good parameter that quantifies the woody portion in edible part of pomegranate fruit. The KI obtained in our study ranged between 6.08 - 14.81%. Some values obtained in our study were higher than those reported in the literature. Hasnaoui et al. (2011) reported values ranging from 3.35 to 6.50%, while Martinez et al. (2006) reported values ranging from 7.80 to 9.68%.

3.3. Skin and aril colour

The colour attributes of the investigated pomegranate cultivars are shown in Table 3. Fruit skin and aril colour varied significantly (p<0.05) in the colour parameters L^*, a^*, b^*, C^* and h^o among the pomegranate cultivars. The observed variation was not surprising as the colours and intensity of fruit peel and aril visibly differed. Pomegranate fruit colour is another important factor affecting marketability and consumer preference (Opara et al., 2009). The CIE a^* (+) value, which indicates the redness of the fruit skin ranged between 16.30 and 47.67. These

values corresponded with the visual variation observed among the studied cultivars, ranging from pink ('Molla de Elche'), light red to dark red ('Acco'). 'Acco' showed the highest *a** value while 'Molla de Elche' showed the least value. There was no significant difference ($p>0.05$) between the redness of 'Arakta' and 'Herskawitz', as well as between 'Bhagwa' and 'Wonderful' fruit skin. Skin lightness (*L**) was the highest for 'Molla de Elche' while the lowest was measured for 'Ruby'. Fruit skin colour intensity (*C**) varied markedly among the cultivars; the highest and the least red colour intensity were found in 'Acco' and 'Ruby', respectively. On the other hand, in comparison to the fruit peel, lower values were obtained for the aril colour components. This is in agreement with previous studies that there is no correlation between the outer skin colour and the colour of the arils (Al-Said et al., 2009; Celik and Ercisli, 2009; Holland et al., 2009). These studies showed that the *L** value of arils of 'Ganesh' was the highest among the cultivars reported, which corresponded with its good visual appeal.

The variation in redness of fruit peel was higher than the aril among the cultivars. 'Herskawitz' arils had the highest *a** value while the least was exhibited by 'Ganesh'. The calculated total colour difference values (TCD) showed the disparity in the colour between the peel and aril. There were significant differences ($p<0.05$) between the TCD of the evaluated pomegranate cultivars, ranging from 20.53 in 'Ruby' to 52.59 in 'Acco'. Similar ranges in TCD between fruit peel and arils were reported by Al-Said et al. (2009) for four pomegranate cultivars grown in Oman. Given the importance of aril and juice redness as a desirable quality attribute, lower TCD between fruit peel and aril (such as in 'Ruby') could provide a valuable index of maturation and ripening status, especially for pomegranate cultivars with marked changes in peel redness during fruit growth and development.

3.4. Textural properties

The mechanical properties of the pomegranate cultivars are presented in Table 4. There were significant ($p<0.05$) differences in whole fruit puncture force among the pomegranate cultivars, ranging between 68.89 ('Herskawitz') to 130.98 N ('Bhagwa'). These differences in fruit resistance to external puncture may be affected by cultivar differences in peel properties such as thickness and moisture content. Peel resistance to compression force is a direct measure of the interfacial toughness of fruit peel (Thouless and Yang, 2008). The peel of some pomegranate cultivars is relatively thinner and softer than others, thus making them more

susceptible to physical damage in the form of punctures and cracks (Holland et al., 2009). Knowledge of mechanical properties of pomegranate fruit peel (in terms of puncture resistance) could be used in the improvement of harvest practices, transport and postharvest handling of pomegranate fruit cultivars.

There were significant differences in the textural properties (hardness and toughness) of both arils and kernels. The aril and kernel of 'Wonderful' were the hardest and toughest, respectively, while 'Ruby' had the least hardness and toughness. The woody part (kernel) is the major trait used in the classification of pomegranate fruit cultivars as 'soft' or 'hard' seed. Kernel hardness is known to influence consumer preference for pomegranate cultivars, with 'soft' seed being more appealing than 'hard' seed (Al-Said et al., 2009).

3.5. Chemical properties

The juice content and the chemical composition of the pomegranate cultivars are presented in Table 5. High juice content is a desirable attribute from industrial point of view. There were significant differences in the juice content of the studied pomegranate cultivars, ranging from 42 to 63.19 (mL per 100 g arils), with 'Ruby' having the highest amount of juice while 'Acco' had the lowest juice content. The juice content results obtained in this study are within the limits of those reported for other cultivars (Martinez et al., 2006; Al-Said et al., 2009; Tehranifar et al., 2010; Zarei et al., 2010). Fruit juice sugar content varied significantly from 14.04 to 16.32 (TSS, ºBrix), with the highest content in 'Wonderful'. The sugar content observed in different cultivars ('Aghaye', 'Faroogh', 'Rabbab-e-Fars', 'Shahvar', 'Shirin-e-Bihaste' and 'Shirin-e-Mohali') grown in Iran were much higher, which ranged from 15.77 to 19.56ºBrix (Zarei et al., 2010). Our data are however, within the limits of those reported in a recent study conducted with 'Mollar de Elche', 'Valenciana' and 'Wonderful' grown in Spain, with TSS values which ranged from 13.23 to 17.6 ºBrix (Mena et al., 2011).

The pH values ranged between 2.96 ('Wonderful') and 4.26 ('Molla de Elche'). Similarly, titratable acidity (citric acid) was highest (1.16%) in 'Wonderful', which was more than 6-fold more acidic than 'Molla de Elche' (0.19%). The acidity level has been linked to the genetic make-up of fruit cultivars and determines consumer perceptions of both sweetness and sourness in pomegranate fruit cultivars (Jalikop, 2007; Holland et al., 2009). The considerable variation observed in acid concentration in the studied cultivars suggests a diverse genetic make-

up or biological variation. The sugar:acid ratio (TSS:TA) is another important parameter that plays a major role perceptions of pomegranate juice taste (Martinez et al., 2006; Hasnaoui et al., 2011). Different pomegranate cultivars have been classified as sweet, sweet-sour and sour based on this parameter (Martinez et al., 2006; Tehranifar et al., 2010; Hasnaoui et al., 2011). Moreover, the TSS:TA ratio has been reported to play a major role in determination of fruit quality and ripeness thereby making it a commonly considered parameter for pomegranate fruit quality (Hasnaoui et al., 2011). This ratio is an important quality considered for cultivar selection in the juice processing industry as fruit cultivars with very low TSS:TA ratios are more desirable for formulation of a wide range of food and beverage products (Al-Said et al., 2009). In our study, there were significant differences (p<0.05) between the values of the TSS:TA ratio among the studied cultivars. The values obtained ranged from 14.28 to 75.77, the lowest value being in 'Wonderful'. Interestingly, the TSS:TA values in this study are within the limits of those recently reported for 'Molla de Elche' and 'Wonderful' grown in Spain (Mena et al., 2011). Based on this attribute, the studied pomegranate cultivars can be classified into three major groups, with 'Wonderful' as a 'sour' cultivar (TSS:TA ratio = 14.28), 'Acco', 'Arakta', 'Bhagwa', 'Ganesh', 'Herskawitz', and 'Ruby' as 'sweet-sour' (TSS:TA ratio; 37.48 - TSS:TA ratio = 55.48), while 'Molla de Elche' classified as a 'sweet' cultivar (75.77).

3.6. Gas chromatography-mass spectrometry (GC-MS) for volatile profiling

Table 6 summarizes the relative proportions (%) as well as the sensory descriptors of the volatile compounds identified in the investigated cultivars. There were significant differences in the proportion of the identified volatiles among the fruit cultivars. In total, fifteen volatile constituents belonging to 6 groups including aldehydes, alcohol, terpenes, ketones, carboxylic acid and esters were identified. More volatile compounds than in our study have previously been reported in the juice of other pomegranate cultivars (Calin-Sanchez et al., 2011; Vázquez-Araújo et al., 2011). The odour threshold of each volatile compound present in fruit could be used to characterize its aroma intensity (Visai and Vanoli, 1997; Calin-Sanchez et al., 2011; Melgarejo et al., 2011). Melgarejo et al. (2011) reported aldehydes group as the predominant in Spanish cultivars; on the contrary however, the alcohol group was the predominant group in our study. Total alcohol proportion ranged from 32.5 ('Wonderful') to 54.9% ('Bhagwa'). These findings suggest that alcohols could be one of the important volatile groups that could be used for the

classification of pomegranate fruit cultivars. The group contains mainly propanol, isobutanol, 4-penten-1-ol, 1-hexanol and 3-hexen-1-ol, with ethanol (sweet) being the major compound in the group. Volatile compounds such as limonene (citrus, mint), acetic acid (sour) and ethyl acetate (pineapple) were identified in all the investigated cultivars, while compounds such as α-pinene, β-caryophyllene, octanone, propanol, 4-penten-1-ol and decanal were either present in low proportion or absent in the most of the investigated cultivars.

3.7. Free radical scavenging capacity

The free radical scavenging capacities of both hydrophilic and lipophilic fractions are presented in Figure 1A. The results showed the power of the antioxidant compounds functioning as proton radical scavengers or hydrogen donors in both juice fractions of the fruit cultivars. There were differing degrees of scavenging capacities among the investigated fruit cultivars for both fractions. 'Herskawitz' and 'Wonderful' exhibited the highest scavenging capacities for lipophilic (629.25 mg/100 mL) and hydrophilic (693.17 mg/100 mL) fractions, respectively. The lowest lipophilic scavenging capacity was exhibited by both 'Bhagwa' and 'Molla de Elche'. The antioxidant capacities obtained in this study for both lipophilic and hydrophilic fractions were higher than those recently reported for Moroccan varieties (Legua et al., 2012) although it was difficult to directly compare as a different antioxidant assay was employed. Overall, it has been established that antioxidant capacity of pomegranate could be cultivar dependent (Gil et al., 2000; Kulkarni and Aradhya, 2005; Li et al., 2006; Cam et al., 2009; Shwartz et al., 2009; Zarei et al., 2010; Legua et al., 2012). The radical scavenging capacities exhibited by the investigated cultivars demonstrate inter-cultivar significant differences that could be used for cultivar classification.

3.8. Total phenolics

The amount of extractable total phenolic concentration significantly varied among the fruit cultivars with total phenolic concentrations ranging from 140.08 to 530.55 mg/100 mL, 'Herskawitz' having about 4-fold of phenolic concentration than 'Ganesh' (Figure 1 B). The fruit cultivars with highest concentration, in decreasing order (p<0.05), were 'Herskawitz' > 'Bhagwa' > 'Acco' = 'Arakta' = 'Ruby' > 'Wonderful' = 'Molla de Elche' > 'Ganesh'. Variations of phenolic concentrations in fruit cultivars may arise due to many factors including

genotypes, climatic conditions and fruit maturity (Castrejon et al., 2008). Varying amounts of total phenolics have previously been reported to characterize pomegranate cultivars grown in different regions. Values ranging between 14.4 - 1008.6 mg/100 g were used to characterize Turkish cultivars (Tezcan et al., 2009); while a wide range of phenolic concentration (238 - 985.32 mg/100 g) was reported for Iranian cultivars (Mousavinejad et al., 2009; Tehranifar et al., 2010). The results obtained for the investigated cultivars fell within those reported for Turkish and Iranian cultivars, however differed considerably from those recently reported (41.01 - 83.43 mg/100 g) for Moroccan pomegranate cultivars (Legua et al., 2012). Our results confirm a variation in the phenolic concentration among the South African grown pomegranate cultivars.

3.9. Principal component analysis

Principal component analysis resulted in seven principal components, out of which only PCs 1-4 showed good correlations and accounted for 84.65% of the total variability (Table 7). The cumulative variance (%) showed that almost all the variations in fruit physico-textural, chemical and antioxidant characters can be explained either strongly or moderately by these four principal components. Acceptable explanations can be drawn from the first three PC plots (PCs 1, 2 and 3) which accounted for 71.74% of the total variance (Figure 2). Considering factor loadings, principal component 1 (PC1) showed a negative correlations for TSS:TA and was characterized by positive loadings from peel thickness, kernel weight, fruit chroma (*C**), titratable acidity (TA), hardness and toughness for arils and kernels as well as hydrophilic radical scavenging capacity H-DPPH as the main contributors of total variance. Principal component 2 (PC2) had positive component loadings from fruit length, aril weight and total phenolic concentrations as well as a negative loading from kernel index (K.I). PC3 however was negatively correlated to total soluble solids (TSS) whereas it had positive loadings from aril width, CIE *a** and *b** (fruit) and total colour difference (TCD). PC4 exhibited less variable correlations but was dominant for aril colour parameter; chroma (*C**) and hue angle (h˚) with the positive correlations.

3.10. Cluster analysis

Cluster analysis discriminated the investigated cultivars into two clusters and two ungrouped individuals (Figure 3). Two major clusters of cultivars were differentiated based on

physico-textural, chemical and antioxidant characters investigated. Cluster 1 consisted of 'Ruby', 'Arakta' and 'Ganesh' while 'Bhagwa', 'Acco' and 'Herskawitz' were placed in cluster 2. 'Molla de Elche' and 'Wonderful' were plotted ungrouped on the quadrant hence can be rather considered as singular. Further characterization using the individual scores of the first four PCAs (Table 8) for each of the investigated pomegranate cultivars revealed specific quality characteristics that could be used to improve the industrial exploitation for some of the investigated fruit cultivars. In summary, 'Wonderful' with the high PC1 score might be defined as the cultivar having thick peel, hard arils and kernels, as well as very high acidity (TA) content, suggesting the cultivar is a sour cultivar and might be suitable for blending variety of juices, more so in that the score corresponded to high hydrophilic antioxidant capacity (H-DPPH). 'Ganesh' has a high PC2 score and could be defined as cultivar having big fruits and arils weight, while 'Molla de Elche' with high scores for PC3 as well as a low score for PC1, might be defined as a cultivar with high lightness (L^*) for its yellow appearance, a low PC1 score might classify the fruit as a sweet cultivar due to and very weak acidity (TSS/TA > 75). Additionally, a very low PC2 score for 'Herskawitz' could be used to classify the cultivar as having high phenolic concentration. The presence of bioactive phenolic compounds in the fruit juice may be desirable for potential health benefits. Furthermore, because of low PC1 scores for 'Arakta', 'Ganesh' and 'Ruby' that corresponded with high PC1 loading for sugar:acid ratio, the cultivars could be classified as sweet or sweet-sour cultivars.

3.11. Correlation analysis

Pearson correlation was conducted per cultivar on important fruit attributes for industrial and processing purposes (Table 9). Interesting significant correlation coefficients ($p<0.05$) were obtained. All of the investigated cultivars (except 'Ruby') showed no significant correlations between fruit peel and aril for redness (a^*) and colour intensity (C^*) and between fruit h° vs. h° aril. Practically, this shows that high fruit external colouration might not neccessarily translate to high colour intensity of arils. 'Herskawitz' and 'Wonderful' did not show significant correlations between acidity and soluble solids (TA vs. TSS), probably because the cultivars are regarded as 'sour cultivar' due to their high acid content. However, there were moderate but negative correlation coefficients among the traits in other cultivars. 'Bhagwa' and 'Herskawitz' showed positive significant correlation between fruit mass and aril yield (Fruit mass vs. aril yield), this is

practically logical as pomegranate aril weight could contribute substantially to total fruit weight. There were no significant correlations between fruit peel and puncture resistance regardless of cultivar types. Although peel moisture contents was not determined in this study, it could be hypothesized that fruit puncture resistance could more dependent on peel moisture content than peel thickness. Future study is required to validate this hypothesis. In all the cultivars, phenolic concentration correlated positively with TSS content (except in 'Arakta'), whereas phenolic correlated negatively with juice acidity (except in 'Bhagwa'). The obtained correlation coefficients might be useful in selecting fruit cultivars for both food and industrial purposes.

4. Conclusions

The present findings on the characterization of South African pomegranate cultivars based on physico-textural, chemical, antioxidant and volatile attributes could be the basis of research and development programme, preferentially aimed at exploiting pomegranate fruit suitable for industrial use as fresh arils, or as processed products such as fruit juice. However, the results obtained can at this stage be used only as a guideline for fruit cultivars grown in various agro-climatic regions in South Africa. In addition, future studies should focus on the development and evolution of the investigated attributes during fruit growth and maturation.

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Table 1

Physical characteristics of eight pomegranate fruit cultivars at commercial harvest maturity grown in South Africa

Property [q]	'Acco'	'Arakta'	'Bhagwa'	'Ganesh'	'Herskawitz'	'Molla de Elche'	'Ruby'	'Wonderful'
Length (mm)	68.7 ± 1.75^{e}	75.0 ± 1.22^{cd}	77.6 ± 1.58^{c}	76.7 ± 0.78^{b}	68.7 ± 1.50^{c}	73.5 ± 1.55^{de}	77.8 ± 1.50^{c}	88.7 ± 1.13^{a}
Diameter (mm)	75.0 ± 1.34^{d}	79.9 ± 1.48^{c}	80.6 ± 1.56^{c}	93.4 ± 2.43^{b}	81.2 ± 1.64^{c}	81.7 ± 1.52^{c}	84.2 ± 1.74^{c}	99.7 ± 1.58^{a}
Weight (g)	274.0 ± 19.09^{c}	302.4 ± 14.77^{bc}	290.1 ± 16.48^{bc}	455.6 ± 46.24^{a}	295.3 ± 9.23^{bc}	274.3 ± 13.16^{c}	352.7 ± 16.73^{b}	509.8 ± 21.41^{a}
Volume (cm^3)	222.5 ± 12.12^{d}	271.5 ± 14.70^{cd}	277.6 ± 16.37^{cd}	433.0 ± 34.64^{b}	283.3 ± 7.84^{cd}	291.5 ± 16.05^{c}	316.2 ± 18.33^{c}	506.1 ± 30.19^{a}
Density (g/cm^3)	1.23 ± 0.11^{a}	1.12 ± 0.00^{ab}	1.05 ± 0.00^{bc}	1.04 ± 0.00^{bc}	1.02 ± 0.01^{bc}	0.94 ± 0.00^{c}	1.13 ± 0.00^{ab}	1.06 ± 0.02^{bc}
Geo. mean diameter (mm)	72.5 ± 1.39^{d}	77.9 ± 1.31^{c}	79.2 ± 1.50^{c}	89.6 ± 2.44^{b}	79.3 ± 1.12^{c}	78.1 ± 1.42^{c}	81.8 ± 1.72^{c}	94.6 ± 1.18^{a}
Sphericity	1.06 ± 0.01^{bc}	1.04 ± 0.01^{cd}	1.02 ± 0.01^{d}	1.07 ± 0.01^{ab}	1.04 ± 0.02^{cd}	1.10 ± 0.01^{a}	1.05 ± 0.01^{cd}	1.08 ± 0.01^{ab}
Surface area (cm^2)	165.6 ± 6.36^{d}	191.5 ± 6.40^{c}	197.9 ± 7.53^{c}	253.3 ± 14.09^{b}	197.8 ± 5.59^{c}	192.8 ± 6.96^{c}	211.3 ± 8.60^{c}	281.3 ± 6.92^{a}
Peel thickness (mm)	4.6 ± 0.66^{bc}	2.7 ± 0.23^{c}	3.9 ± 0.34^{bc}	4.1 ± 0.14^{bc}	5.8 ± 1.24^{b}	3.2 ± 0.36^{c}	2.9 ± 0.21^{c}	8.0 ± 0.49^{a}

[q] Means (±SE) in each row followed by different letter (s) are significantly different (p<0.05) according to Duncan's multiple range test.

Table 2

Physical characteristics of aril and kernel of eight pomegranate fruit cultivars at commercial harvest maturity grown in South Africa

Property [r]	'Acco'	'Arakta'	'Bhagwa'	'Ganesh'	'Herskawitz'	'Molla de Elche'	'Ruby'	'Wonderful'
Aril								
Total aril wt.(g)	180.9 ± 21.31^{cde}	187.0 ± 26.68^{cd}	166.2 ± 21.03^{de}	305.3 ± 9.13^{a}	180.3 ± 19.31^{cde}	123.9 ± 8.10^{e}	233.6 ± 12.50^{bc}	260.3 ± 17.86^{ab}
Aril yield (%)	60.1 ± 7.08^{abc}	64.2 ± 2.82^{ab}	54.1 ± 2.86^{bcd}	58.9 ± 1.40^{abc}	61.3 ± 6.57^{abc}	51.7 ± 1.11^{cd}	68.1 ± 0.68^{a}	47.5 ± 2.09^{d}
Aril no.	626.3 ± 73.77^{abc}	655.8 ± 111.55^{ab}	433.8 ± 54.92^{cd}	815.5 ± 55.24^{a}	635.3 ± 70.32^{ab}	271.6 ± 17.24^{d}	534.2 ± 35.52^{bc}	592.2 ± 37.62^{bc}
Aril wt. (g)	0.29 ± 0.02^{e}	0.32 ± 0.01^{de}	0.34 ± 0.01^{cd}	0.38 ± 0.001^{c}	0.32 ± 0.001^{de}	0.47 ± 0.01^{a}	0.43 ± 0.001^{ab}	0.43 ± 0.001^{b}
Aril length (mm)	10.5 ± 0.24^{bc}	9.8 ± 0.14^{c}	9.9 ± 0.18^{c}	10.2 ± 0.71^{c}	10.5 ± 0.24^{bc}	11.9 ± 0.18^{a}	11.4 ± 0.24^{ab}	10.7 ± 0.16^{bc}
Aril width (mm)	7.9 ± 0.16^{a}	6.4 ± 0.17^{b}	8.0 ± 0.20^{a}	6.5 ± 0.49^{b}	7.6 ± 0.31^{a}	8.0 ± 0.29^{a}	7.0 ± 0.29^{ab}	7.9 ± 0.19^{a}
Kernel								
Kernel wt. (g)	0.04 ± 0.001^{b}	0.03 ± 0.001^{b}	0.04 ± 0.001^{ab}	0.04 ± 0.001^{b}	0.04 ± 0.001^{ab}	0.03 ± 0.001^{b}	0.03 ± 0.001^{b}	0.04 ± 0.001^{a}
Kernel length (mm)	7.1 ± 0.16^{b}	7.0 ± 0.10^{a}	7.0 ± 0.09^{a}	7.0 ± 0.14^{a}	7.0 ± 0.19^{a}	7.1 ± 0.18^{a}	7.5 ± 0.11^{a}	7.2 ± 0.16^{a}
Kernel width (mm)	3.0 ± 0.08^{b}	3.2 ± 0.07^{a}	3.1 ± 0.11^{a}	3.3 ± 0.09^{a}	3.1 ± 0.13^{a}	3.3 ± 0.09^{a}	3.3 ± 0.14^{a}	3.2 ± 0.10^{a}
KI (%)	14.8 ± 1.08^{a}	8.9 ± 0.62^{abc}	10.6 ± 0.36^{a}	11.4 ± 0.45^{a}	11.6 ± 0.59^{a}	6.1 ± 0.28^{c}	6.8 ± 0.47^{bc}	9.5 ± 0.55^{ab}

[r] KI- kernel index. Means (± SE) in each row followed by different letter (s) are significantly different (p<0.05) according to Duncan's multiple range test.

Table 3

Fruit skin and aril colour of eight pomegranate cultivars at commercial harvest maturity grown South Africa

Property [x]	'Acco'	'Arakta'	'Bhagwa'	'Ganesh'	'Herskawitz'	'Molla de Elche'	'Ruby'	'Wonderful'
Skin								
*L**	46.7 ± 1.56^{b}	35.8 ± 0.43^{d}	41.1 ± 1.26^{c}	41.1 ± 2.2^{c}	34.2 ± 0.37^{d}	54.2 ± 1.82^{a}	27.5 ± 0.84^{e}	44.9 ± 1.43^{bc}
*a**	47.7 ± 2.07^{a}	40.8 ± 0.62^{b}	44.8 ± 1.02^{ab}	34.0 ± 1.99^{c}	43.0 ± 1.16^{b}	16.3 ± 1.69^{e}	24.6 ± 1.82^{d}	43.9 ± 1.31^{ab}
*b**	25.8 ± 0.86^{a}	13.8 ± 0.30^{d}	18.5 ± 0.61^{c}	16.8 ± 1.23^{c}	17.2 ± 0.67^{c}	24.0 ± 0.78^{ab}	6.7 ± 0.73^{e}	22.6 ± 1.08^{b}
Chroma (*C**)	54.6 ± 1.43^{a}	43.0 ± 0.65^{c}	48.5 ± 1.05^{b}	38.7 ± 1.11^{d}	46.3 ± 1.28^{bc}	29.3 ± 1.28^{e}	25.5 ± 1.94^{f}	49.5 ± 1.51^{b}
Hue angle (*h*°)	29.1 ± 2.03^{c}	18.7 ± 0.33^{ef}	22.4 ± 0.65^{de}	27.5 ± 3.33^{cd}	21.8 ± 0.47^{de}	56.7 ± 3.27^{a}	14.7 ± 0.66^{f}	49.5 ± 1.51^{b}
Aril								
*L**	13.0 ± 1.39^{c}	15.6 ± 1.45^{bc}	13.5 ± 1.08^{c}	22.0 ± 1.45^{a}	16.6 ± 1.76^{bc}	20.3 ± 1.99^{ab}	13.2 ± 1.42^{c}	11.0 ± 1.01^{c}
*a**	16.5 ± 1.61^{abc}	15.1 ± 1.85^{bc}	13.5 ± 0.85^{bc}	12.5 ± 1.05^{c}	20.5 ± 2.29^{a}	13.2 ± 1.28^{bc}	12.7 ± 0.95^{bc}	17.6 ± 0.93^{ab}
*b**	6.3 ± 0.77^{bc}	5.2 ± 0.71^{bc}	4.6 ± 0.32^{c}	6.1 ± 0.23^{bc}	11.0 ± 0.72^{a}	5.8 ± 0.53^{bc}	4.4 ± 0.26^{c}	7.1 ± 0.63^{b}
Chroma (*C**)	17.7 ± 1.77^{bc}	16.0 ± 1.98^{bc}	14.3 ± 0.90^{c}	13.9 ± 0.98^{c}	23.4 ± 2.09^{a}	14.7 ± 1.19^{bc}	13.5 ± 0.68^{c}	19.0 ± 1.08^{b}
Hue angle (*h*°)	20.8 ± 0.91^{b}	18.9 ± 0.63^{b}	18.5 ± 0.41^{b}	26.5 ± 1.97^{ab}	29.3 ± 3.45^{a}	25.6 ± 3.27^{ab}	19.0 ± 0.72^{b}	21.7 ± 1.04^{ab}
TCD	52.6 ± 1.63^{a}	33.4 ± 2.52^{cd}	42.5 ± 2.29^{bc}	30.9 ± 1.49^{d}	29.9 ± 3.33^{d}	40.7 ± 3.18^{bc}	20.5 ± 2.38^{e}	43.8 ± 3.40^{ab}

[x] Means (± SE) in each row followed by different letter (s) are significantly different (p<0.05) according to Duncan's multiple range test.

TCD = total colour difference

Table 4

Mechanical properties of fruits arils and kernels of eight pomegranate cultivars at commercial harvest maturity grown in South Africa

Property [z]	'Acco'	'Arakta'	'Bhagwa'	'Ganesh'	'Herskawitz'	'Molla de Elche'	'Ruby'	'Wonderful'
Fruit								
Puncture resistance (N)	111.0 ± 5.84^{abc}	94.3 ± 8.29^{c}	131.0 ± 6.05^{a}	94.5 ± 12.64^{c}	68.9 ± 4.63^{d}	117.9 ± 10.06^{ab}	98.7 ± 5.43^{bc}	120.5 ± 5.14^{ab}
Aril								
Hardness (N)	74.5 ± 2.21^{cd}	83.3 ± 2.72^{bc}	85.9 ± 2.51^{b}	85.9 ± 2.45^{b}	79.8 ± 2.48^{bcd}	80.9 ± 2.70^{bcd}	72.4 ± 2.77^{d}	118.4 ± 2.35^{a}
Toughness (N mm)	67.8 ± 1.94^{de}	78.1 ± 1.34^{c}	77.8 ± 1.74^{c}	78.5 ± 1.36^{c}	71.6 ± 2.39^{cd}	90.3 ± 3.18^{b}	60.6 ± 2.98^{e}	118.0 ± 3.57^{a}
Kernel								
Hardness (N)	70.7 ± 2.74^{c}	74.8 ± 1.60^{bc}	79.7 ± 2.52^{b}	67.3 ± 3.64^{c}	67.2 ± 1.66^{c}	69.0 ± 2.66^{c}	66.6 ± 2.22^{c}	103.6 ± 2.52^{a}
Toughness (N mm)	39.2 ± 1.77^{c}	41.0 ± 2.71^{c}	49.4 ± 1.60^{b}	38.7 ± 2.83^{c}	35.2 ± 1.42^{c}	39.5 ± 2.22^{c}	36.0 ± 2.30^{c}	65.6 ± 2.35^{a}

[z] Means (± SE) in each row followed by different letter (s) are significantly different (p<0.05) according to Duncan's multiple range test.

Table 5

Juice content and chemical properties of fruit of eight pomegranate cultivars at commercial harvest maturity grown in South Africa

Cultivar	Juice (mL)/100 g arils	TSS (°Brix)	pH	TA (Citric acid %)	TSS:TA
'Acco'	42.0 ± 3.06^{d}	14.9 ± 0.41^{bc}	3.4 ± 0.00^{b}	0.46 ± 0.03^{b}	32.4 ± 2.32^{e}
'Arakta'	62.4 ± 8.84^{ab}	15.6 ± 0.20^{ab}	3.2 ± 0.00^{bc}	0.34 ± 0.00^{bc}	46.4 ± 0.92^{c}
'Bhagwa'	51.7 ± 2.02^{abcd}	14.0 ± 0.26^{c}	3.1 ± 0.00^{cd}	0.36 ± 0.02^{bc}	39.4 ± 1.57^{cde}
'Ganesh'	48.5 ± 1.48^{cd}	14.8 ± 0.21^{bc}	3.5 ± 0.00^{b}	0.34 ± 0.00^{bc}	43.1 ± 0.42^{cd}
'Herskawitz'	49.7 ± 1.67^{bcd}	15.5 ± 0.35^{bc}	3.5 ± 0.01^{b}	0.42 ± 0.04^{bc}	37.5 ± 2.54^{de}
'Molla de Elche'	51.7 ± 1.11^{abcd}	14.1 ± 0.36^{c}	4.3 ± 0.12^{a}	0.19 ± 0.00^{d}	75.8 ± 3.24^{a}
'Ruby'	63.2 ± 4.38^{a}	16.3 ± 0.01^{a}	3.5 ± 0.00^{b}	0.29 ± 0.00^{cd}	55.5 ± 0.91^{b}
'Wonderful'	57.0 ± 0.70^{abc}	16.3 ± 0.12^{a}	3.0 ± 0.00^{d}	1.16 ± 0.17^{a}	14.3 ± 0.90^{f}

TSS- Total soluble solids, TA- Titratable acidity. Means (± SE) in each column followed by different letter (s) are significantly different (p<0.05) according to Duncan's multiple range test.

Table 6

Relative proportions (%) of aromatic compounds found in the headspace of investigated pomegranate juice samples

Compound	LRI [a]	Odour descriptor	Cultivar							
			'Acco'	'Arakta'	'Bhagwa'	'Ganesh'	'Herskawitz'	'Molla de Elche'	'Ruby'	'Wonderful'
Aldehydes										
Pentanal	732	almond, malt, pungent	3.7 [b]	tr	tr	19.6 [a]	4.7 [b]	tr	tr	4.1 [b]
Hexanal	801	grass, tallow, fat	19.7 [b]	7.5 [c]	1.1 [e]	nd	26.8 [a]	18.2 [b]	18.2 [b]	4.2 [d]
Decanal	1209	soap, orange peel, tallow	tr	2.9 [a]	tr	nd	nd	2.6 [a]	nd	3.2 [a]
Total Aldehydes			23.4	10.4	1.1	19.6	31.5	20.8	18.2	11.5
Alcohols										
Propanol	536	alcohol, pungent	nd	nd	7.9 [b]	tr	14.9 [a]	tr	nd	nd
Isobutanol	647	wine, solvent, bitter	tr	6.6 [b]	nd	9.7 [a]	1.9 [c]	nd	2.5 [c]	nd
Ethanol	668	sweet	20.0 [b]	13.8 [cd]	13.0 [d]	36.5 [a]	7.4 [e]	13.6 [cd]	16.0 [cd]	17.3 [bc]
4-Penten-1-ol	686	butter, pungent	nd	nd	3.6 [a]	nd	3.9 [a]	nd	2.0 [ab]	nd
1-Hexanol	851	resin, flower, green	10.9 [bc]	17.7 [a]	12.7 [b]	tr	11.1 [bc]	12.1 [bc]	14.3 [ab]	8.6 [c]
3-Hexen-1-ol	858	grass	13.8 [bc]	14.1 [b]	17.7 [a]	nd	9.3 [cd]	11.9 [bc]	6.9 [d]	6.6 [d]
Total Alcohols			44.7	52.2	54.9	46.2	48.5	37.6	41.7	32.5
Terpenes										
α-Pinene	939	pine, turpentine	5.5 b	tr	3.8 [c]	tr	tr	5.6 [b]	tr	20.2 [a]
Limonene	1030	citrus, mint	3.8 [e]	8.4 [cd]	6.8 [de]	7.5 [de]	4.5 [e]	16.3 [b]	20.4 [a]	11.3 [c]
β-Caryophyllene	1467	wood, spice	nd	nd	nd	2.9 [b]	nd	3.8 [a]	nd	nd

Table 6 contd.

Compound	LRI [a]	Odour descriptor	Cultivar							
			'Acco'	'Arakta'	'Bhagwa'	'Ganesh'	'Herskawitz'	'Molla de Elche'	'Ruby'	'Wonderful'
Total Terpenes			9.3	8.4	10.6	10.4	4.5	23.7	20.4	31.5
Ketone										
Octanone	965	herb, butter, resin	0.8 [c]	7.1 [a]	4.4 [b]	5.0 [b]	nd	nd	tr	tr
Total			0.8	7.1	4.4	5.0	nd	nd	tr	tr
Carboxylic acid										
Acetic acid	600	sour	6.9 [cd]	19.8 [a]	16.1 [b]	10.6 [c]	5.4 [d]	5.5 [d]	8.8 [cd]	17.9 [ab]
Total Carboxylic			6.9	19.8	16.1	10.6	5.4	5.5	8.8	17.9
Ester										
Ethyl Acetate	628	pineapple	13.2 [b]	0.8 [e]	11.2 [b]	6.9 [cd]	10.0 [bc]	15.6 [a]	9.6 [bc]	5.6 [de]
Total Ester			13.2	0.8	11.2	6.9	10.0	15.6	9.6	5.6
Total overall			98.3	98.1	98.3	98.7	99.9	100.2	98.7	99.0

Percentages obtained by FID peak area normalisation (Rtx®-5MS column); [a] Linear retention indices; tr < 0.5%; trace

Table 7

Factor loadings, eigenvalues and cumulative variance (%) for the first four principal components based on physico-textural, chemical and antioxidant characters

	Loadings			
Variables	PC1	PC2	PC3	PC4
Length	0.539	0.736	-0.327	-0.174
Diameter	0.569	0.632	-0.353	0.254
Weight	0.588	0.530	-0.470	0.135
Volume	0.588	0.622	-0.337	0.237
Density	0.057	-0.448	-0.350	-0.598
Peel thickness	0.937	-0.013	-0.001	0.297
Fruit weight	0.332	0.252	-0.687	0.049
Aril yield	-0.640	-0.451	-0.592	-0.038
Aril no.	0.263	-0.366	-0.633	0.047
Aril wt.	-0.107	0.915	0.070	0.307
Aril length	-0.319	0.508	0.292	0.424
Aril width	0.381	-0.015	0.711	-0.001
Kernel wt.	0.705	-0.400	0.104	0.000
Kernel length	-0.192	0.418	-0.426	-0.033
Kernel width	-0.390	0.799	-0.274	0.308
KI	0.419	-0.744	0.088	-0.203
peel *L**	0.245	0.285	0.871	-0.044
peel *a**	0.663	-0.636	-0.137	-0.343
peel *b**	0.495	-0.054	0.818	-0.022
peel *C**	0.726	-0.575	0.173	-0.304
peel *h*°	0.352	0.578	0.647	0.256
aril *L**	-0.477	0.129	0.243	0.462
aril *a**	0.586	-0.575	0.003	0.441
aril *b**	0.396	-0.500	0.060	0.766
aril *C**	0.538	-0.568	0.032	0.545

Table 7 contd.

	Loadings			
Variables	PC1	PC2	PC3	PC4
aril h°	0.049	-0.148	0.237	0.888
TCD	0.507	-0.123	0.707	-0.416
Juice content	-0.193	0.463	-0.624	-0.056
TSS	0.282	0.076	0.785	0.198
pH	-0.613	0.234	0.534	0.483
TA	0.938	0.208	-0.174	0.004
TSS:TA	-0.889	0.276	0.301	0.179
Puncture resistance	0.225	0.509	0.469	-0.649
Aril hardness	0.856	0.466	-0.065	0.036
Aril toughness	0.725	0.579	0.210	0.122
Kernel hardness	0.853	0.383	-0.040	-0.211
Kernel toughness	0.829	0.444	0.025	-0.253
Phenolic concentration	-0.020	-0.783	-0.086	0.250
L-DPPH	0.207	-0.623	-0.148	0.688
H-DPPH	0.818	-0.134	-0.072	0.461
Eigenvalue	12.17	9.51	7.01	5.17
Cumulative %	30.42	54.20	71.74	84.65

KI- kernel index, TCD- Total colour difference, TSS- Total soluble solids, TA- Titratable acidity

Table 8

Scores within each principal component (PC1- PC5) of pomegranate juices

Cultivar	PC1	PC2	PC3	PC4
'Acco'	0.92	-4.09	2.21	-2.31
'Arakta'	-1.75	-0.95	-2.09	-1.59
'Bhagwa'	0.44	-0.63	1.62	-3.22
'Ganesh'	-0.54	1.25	-1.48	0.83
'Herskawitz'	0.87	-4.99	-0.36	0.35
'Molla de Elche'	-4.41	3.81	5.02	1.82
'Ruby'	-3.36	0.92	-3.99	-0.23
'Wonderful'	7.84	3.67	-0.92	0.33

Table 9

Correlation coefficients between major investigated fruit properties for each pomegranate cultivar

	'Acco'	'Arakta'	'Bhagwa'	'Ganesh'	'Herskawitz'	'Molla de Elche'	'Ruby'	'Wonderful'
Fruit *a** vs. *a** aril	n.s	n.s	n.s	n.s	n.s	n.s	-0.58	n.s
Fruit *C** vs. *C** fruit	n.s	n.s	n.s	n.s	n.s	n.s	-0.58	n.s
Fruit h° vs. h° aril	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Fruit mass vs. aril yield	-0.66	n.s	0.71	n.s	0.80	n.s	n.s	n.s
Juice content vs. aril yield	0.86	0.53	n.s	0.52	-0.97	n.s	0.94	n.s
Peel thickness vs. puncture force	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
pH vs. TSS	0.76	n.s	n.s	0.87	n.s	0.72	-0.84	n.s
pH vs. TA	-0.87	0.65	-0.60	n.s	-0.81	-0.72	0.97	-0.84
TA vs. TSS	-0.54	-0.50	-0.84	-0.72	n.s	-0.58	-0.94	n.s
Aril hardness vs. aril length	n.s	n.s	-0.55	n.s	n.s	n.s	n.s	n.s
Aril hardness vs. aril width	n.s	n.s	n.s	n.s	0.63	n.s	0.52	n.s
Kernel hardness vs. kernel length	-0.53	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Kernel hardness vs. kernel width	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Aril width vs. kernel width	n.s	n.s	n.s	n.s	n.s	n.s	0.65	n.s
Aril length vs. kernel length	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Phenolic vs. TSS	0.88	n.s	0.88	0.97	0.52	n.s	0.77	0.71
Phenolic vs. TA	-0.74	-0.99	n.s	-0.54	-0.95	-0.56	-0.52	-0.56
Phenolics vs. L-DPPH	0.59	-0.93	-0.80	0.89	0.52	-0.92	n.s	-0.87
Phenolics vs. H-DPPH	-0.59	0.93	-0.80	-0.89	0.52	-0.92	n.s	-0.87

n.s; not significant at $p<0.05$, v.s.; versus

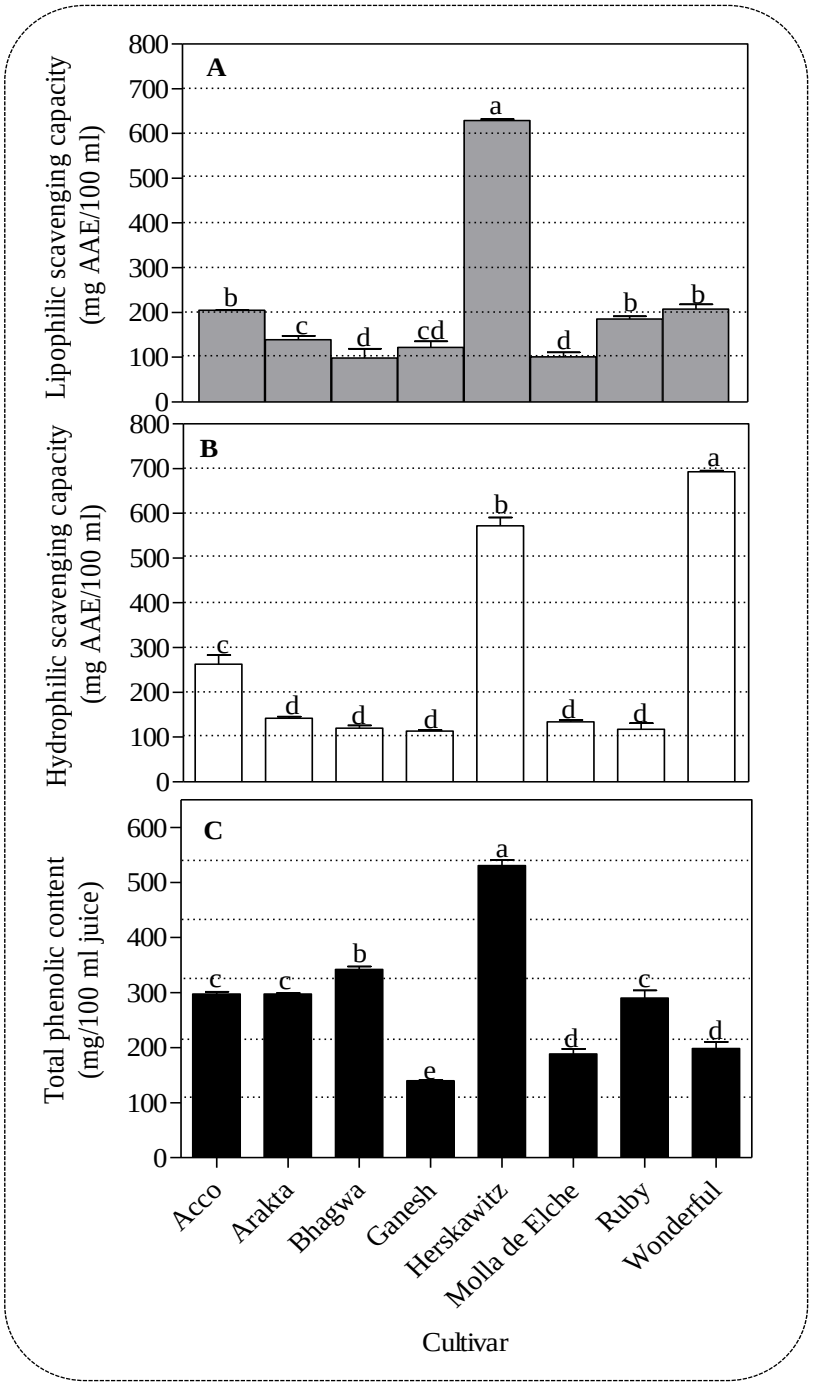
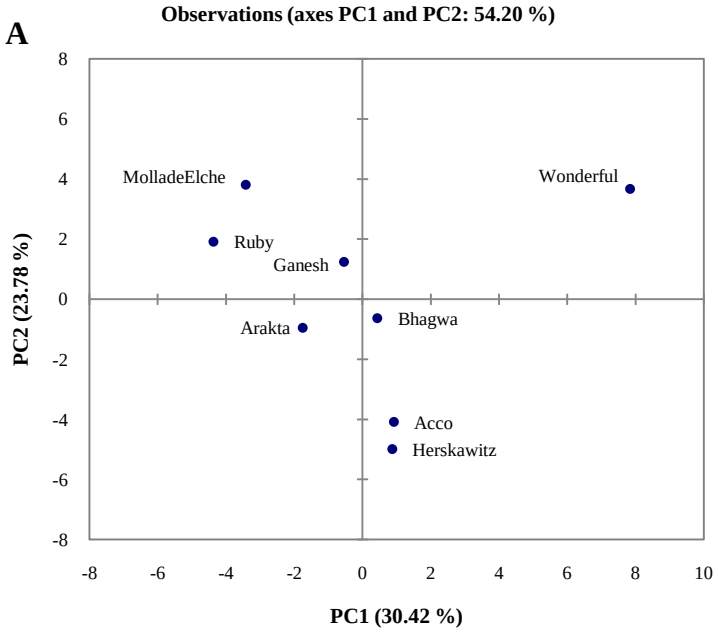


Figure 1. Pomegranate radical scavenging capacity of lipophilic fractions (**A**), hydrophilic (**B**) and total phenolic concentrations of pomegranate juices (**C**). Bars (Mean±SE) with different letters within measured parameters are significantly different (p<0.05) according to Duncan's multiple range test.



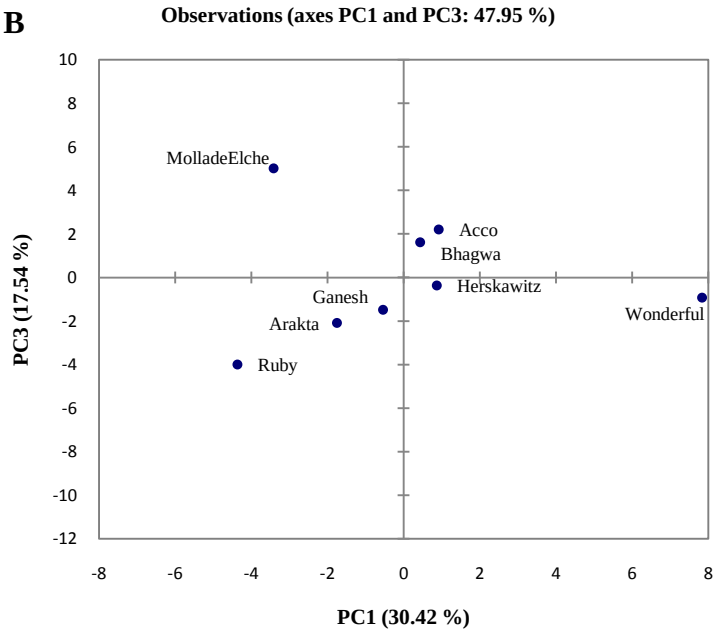


Figure 2. Plot of PC1vs. PC2 (**A**) and PC1 vs. PC3 (**B**) resulting from a PCA of the pomegranate cultivars using physico-textural, chemical and antioxidant characters.

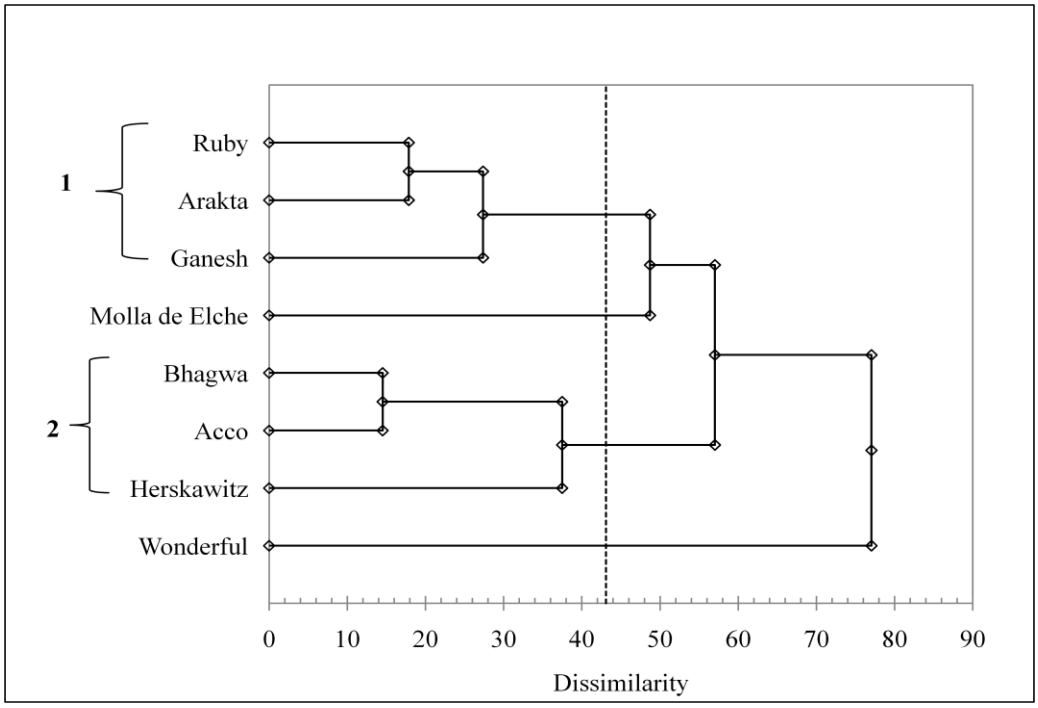


Figure 3. Dendogram of cluster analysis of eight investigated pomegranate cultivars based on physico-textural, chemical and antioxidant characters.

PAPER 7

Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract

Abstract

This study evaluated, using *in vitro* assays, the antibacterial, antioxidant, and tyrosinase-inhibition activities of methanolic extracts from peels of seven commercially grown pomegranate cultivars. Antibacterial activity was tested on Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) using a microdilution method. Several potential antioxidant activities, including radical-scavenging ability (RSA), ferrous ion chelating (FIC) and ferric ion reducing antioxidant power (FRAP), were evaluated. Tyrosinase enzyme inhibition was investigated against monophenolase (tyrosine) and diphenolase (DOPA), with arbutin and kojic acid as positive controls. Furthermore, phenolic concentrations including total flavonoid concentration (TFC), gallotannin concentration (GTC) and total anthocyanin concentration (TAC) were determined using colourimetric methods. HPLC-ESI/MS^n analysis of phenolic composition of methanolic extracts was also performed. Methanolic peel extracts showed strong broad-spectrum activity against Gram-positive and Gram-negative bacteria, with the minimum inhibitory concentrations (MIC) ranging from 0.2 to 0.78 mg/mL. At the highest concentration tested (1000 µg/mL), radical scavenging activities were significantly higher in 'Arakta' (83.54%), 'Ganesh' (83.56%), and 'Ruby' (83.34%) ($p< 0.05$). Dose dependent FIC and FRAP activities were exhibited by all the peel extracts. All extracts also exhibited high inhibition (>50%) against monophenolase and diphenolase activities at the highest screening concentration. The most active peel extract was the 'Bhagwa' against monophenolase and the 'Arakta' against diphenolase with IC_{50} values of 3.66 µg/mL and 15.88 µg/mL, respectively. High amounts of phenolic compounds were found in peel extracts with the highest and lowest total phenolic concentrations of 295.5 ('Ganesh') and 179.3 mg/g dry extract ('Molla de Elche'), respectively. Catechin, epicatechin, ellagic acid and gallic acid were found in all cultivars, of which ellagic acid was the most abundant comprising of more than 50% of total phenolic compounds detected in each cultivar. The present study showed that the tested pomegranate peels exhibited strong antibacterial, antioxidant and tyrosinase-inhibition activities. These results suggest that pomegranate fruit peel could be exploited as a potential source of natural antimicrobial and antioxidant agents as well as tyrosinase inhibitors.

Keywords: Antibacterial activity, tyrosinase-inhibition, phenolics, pomegranate, South Africa

1. Introduction

Numerous epidemiological studies suggest that diets rich in phytochemicals and antioxidants have protective roles in health and disease (Lampe, 1999). These natural antioxidants might play an important role in combating oxidative stress associated with degenerative diseases such as cancer, cardiovascular diseases, diabetes, Alzheimer's disease and aging (Naczk and Shahidi, 2006; Wong et al., 2006). The antioxidative phytochemicals, especially phenolic compounds, found in vegetables and fruits have received increasing attention for their potential role in the prevention of human diseases (Cai et al., 2004; Hazra et al., 2008, 2010; Abdel-Hameed, 2009; Opara and Al-Ani, 2010).

Pomegranate (*Punica granatum* L.; Punicaceae) has gained popularity in recent years due to its multi-functionality and nutritional benefit in the human diet. The fruit is rich in tannins and other biochemicals, particularly phenolics, which have been reported to reduce disease risk (Martínez et al., 2006; Jaiswal et al., 2010). Pomegranate fruit peel constitutes about 50% of the total fruit weight (Al-Said et al., 2009) and it is often discarded as waste. However, the fruit peel contains higher amounts of polyphenol compounds than the juice, and it possesses stronger biological activities (Li et al., 2006; Hajimahmoodi et al., 2008; Gözlekçi et al., 2011). Studies have shown that pomegranate peel extract had markedly higher antioxidant capacity than juice extract in scavenging against superoxide anion, hydroxyl and peroxyl radicals and it inhibited $CuSO_4$-induced LDL oxidation (Li et al., 2006). Besides high antioxidant capacity, pomegranate peel extracts have been reported to possess a wide range of biological actions including anti-cancer activity (Ackland et al., 2005; Brusselmans et al., 2005; Kowalski et al., 2005), antimicrobial activity (McCarrell et al., 2008; Endo et al., 2009), anti-diarrheal activity (Olapour et al., 2009), apoptotic and anti-genotoxic properties (Seeram et al., 2005; Lin et al., 1999), anti-tyrosinase activity (Yoshimura et al., 2005), anti-inflammatory and anti-diabetic activities (Lansky and Newman, 2007; Althunibat et al., 2010). Polyphenol compounds such as ellagic tannins, flavonols, anthocyanins, catechin, procyanidins, ellagic acid and gallic acid have been implicated in various pharmacological activities in the fruit peel (Lansky and Newman, 2007; Viuda-Martos et al., 2010). However, the levels of these compounds in the pomegranate peel may vary among pomegranate cultivars which may result in differing levels of bioactivity (Holland et al., 2009).

In South Africa, more than ten pomegranate cultivars are commercially cultivated (Fawole et al., 2012). To date, there is no available information on bioactivities of fruit peels

of pomegranate cultivars grown under South African agro-climatic conditions. If fruit peels of pomegranate cultivars show potential to improve human health, their utilisation should be encouraged in food processing and other industrial applications. In the quest to promote the development of functional foods with health-benefiting properties, we investigated the antibacterial, antioxidant, and tyrosinase-inhibition activities of extracts from peels, using *in vitro* assays, of seven commercially pomegranate cultivars grown in the Western Cape, South Africa. Furthermore, total phenolic concentration including flavonoid, gallotannin and anthocyanin concentration, and individual phenolics were quantified.

2. Materials and Methods

2.1. Plant material

The studies were performed on peels of seven pomegranate fruit cultivars (‘Arakta’, ‘Bhagwa’, ‘Ganesh’, ‘Herskawitz’, ‘Molla de Elche’, ‘Ruby’, and ‘Wonderful’) which are commercially grown in South Africa. Fruit were procured from a commercial pomegranate pack house in Porterville (Western Cape Province). Fruit were harvested between February and May 2010, packed in paperboard cartons and transported in an air-conditioned car to the Postharvest Research Laboratory. Immediately on arrival in the laboratory, ten fruit per cultivar were washed and manually peeled. The peels were freeze-dried, ground into powder, and stored in airtight containers at 7°C in the dark.

2.2. Preparation of peel extract

For each cultivar, each sample (2 g) of finely-powdered peel was extracted separately with 10 mL of 80% (v/v) methanol (MeOH) and distilled water (aqueous) by sonication for 1 h (Al-Zoreky, 2009). The extract was filtered under vacuum through Whatman No.1 filter paper, and the residue was re-extracted further following the same procedure. Extracts were dried under a stream of air and first tested in the antibacterial assay to determine which extracts would be worth subjecting to further pharmacological investigations. Only the methanol extract was tested further as it recorded highest antibacterial activity.

2.3. Antibacterial property

2.3.1. Microdilution antibacterial assay

The antibacterial activity of peel extract was tested using the microdilution antibacterial assay for the minimum inhibitory concentration (MIC) values (Eloff, 1998) as detailed by Fawole et al. (2009), except that in the present study, the initial concentration (50 mg/mL) of the sample was prepared by dissolving dried extracts in 80% (v/v) methanol. Two Gram-negative bacteria (*Escherichia coli* ATCC 11775 and *Klebsiella pneumonia* ATCC 13883) and two Gram-positive bacteria (*Bacillus subtilis* ATCC 6051 and *Staphylococcus aureus* ATCC 12600) were used. The extract was serially diluted two-fold with sterile distilled water in a 96-well micro-plate in triplicate for each of the four bacteria used. Streptomycin (0.1 mg/mL) was used as positive control, while water and bacteria-free broth were included as negative controls under the same conditions. Methanol (80%) was also included to check for false antibacterial activity. The final concentration of pomegranate extract ranged from 0.097 - 12.5 mg/mL, reducing the methanol content in the test extract to between 0.19 and 20%, whereas streptomycin was between 0.78 and 100 µg/mL.

2.4. Antioxidant property

2.4.1. Radical-scavenging ability

The scavenging ability of stable free radicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a known mechanism for antioxidation. The DPPH assay was carried out in triplicate, according to the method reported by Karioti et al. (2004). Extracts of different concentrations (10, 100 and 1000 µg/mL) were tested in triplicate for free-radical scavenging activity. The scavenging activity of the extract was compared with ascorbic acid (1000 µg/mL). A blank containing methanol instead of the test sample or ascorbic acid was also included under the same condition. The free radical scavenging activity (RSA) as determined by the decolouration of the DPPH solution was measured at 517 nm and calculated according to the formula:

$$\text{RSA (\%)} = [1 - (A_{test}/A_{blank}) \times 100] \quad (1)$$

where A_{test} is the absorbance of the reaction mixture containing the standard antioxidant or extract, and A_{blank} is the absorbance of the blank test.

2.4.2. Ferrous ion chelating (FIC) assay

The FIC activity assay of Singh and Rajini (2004) was adopted. Briefly, 0.1 mM $FeSO_4$ (0.5 mL) was mixed with the extract (0.5 mL) of different concentrations (10, 100 and 1000 µg/mL) in triplicate, followed by adding 0.25 mM ferrozine (1 mL). The reaction mixtures were incubated for 10 min and the absorbance (*A*) was measured at 562 nm. Ascorbic acid (1000 µg/mL) was included as the positive control. A blank test containing methanol instead of the test sample or ascorbic acid was also included under the same conditions. The ability of extracts to chelate ferrous ions was calculated using the following equation:

$$\text{Chelating ability (\%)} = [(A_{blank} - A_{test})/A_{blank}] \times 100 \quad (2)$$

where A_{test} is the absorbance of the reaction mixture containing extract or ascorbic acid and A_{blank} is the absorbance of the blank test.

2.4.3. Ferric ion reducing antioxidant power (FRAP) assay

The reducing power of extracts was measured according to the colourimetric method reported by Benzie and Strain (1996) with a few modifications. In triplicates, methanolic extract (150 µL) of different concentrations at 10, 100 or 1000 µg/mL was added to 2850 µL of FRAP solution that consisted of 300 mM acetate buffer, 50 mL; 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), 5 mL; and 20 mM ferric chloride, 5 mL. Following the same procedure, a blank test containing 80% methanol instead of extract was included, while trolox at 10 µg/mL served as the positive control under the same condition. The reaction mixtures were incubated in the dark for 30 min. The reduction of the Fe^{3+}-TPTZ complex to a coloured Fe^{2+}-TPTZ complex by the extract was monitored by measuring the absorbance at 593 nm using a Helios Omega UV-vis spectrophotometer (Thermo Scientific technologies, Madison, USA). The changes in absorbance values of test reaction mixtures from the initial blank reading were considered as FRAP activity.

2.5. Tyrosinase inhibition property

Tyrosinase inhibition activity was determined as described by Momtaz et al. (2008), with L-3,4-dihydroxyphenylalanine (L-DOPA, Sigma) and tyrosine as substrates. Samples were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 20 mg/mL, and further

diluted in potassium phosphate buffer (50 mM, pH 6.5) to 600 µg/mL. Assays were carried out in a 96-well micro-titre plate and a Multiskan FC plate reader (Thermo scientific technologies, China) was used. All the steps in the assay were conducted at room temperature. In triplicate, each prepared sample (70 µL) was mixed with 30 µL of tyrosinase (333 Units/mL in phosphate buffer, pH 6.5). After 5 min incubation, 110 µL of substrate (2 mM $_{L}$ -tyrosine or 12 mM L-DOPA) was added to the reaction mixtures and incubated further for 30 min. The final concentration of the extract was between 2.6 - 333.3 µg/mL. Arbutin (1.04 - 133.33 µg/mL) was used as a positive control while a blank test was used as each sample that had all the components except $_{L}$ -tyrosine or $_{L}$-DOPA. Results were compared with a control consisting of DMSO instead of the test sample. Absorbance values of the wells were then determined at 492 nm. The percentage tyrosinase inhibition was calculated as follows:

$$\% \text{ inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (3)$$

where $A_{control}$ is the absorbance of DMSO and A_{sample} is the absorbance of the test reaction mixture containing extract or arbutin. The IC_{50} values of extracts and arbutin were calculated.

2.6. Phenolic concentration determination

2.6.1. Total phenolic concentration (TPC)

The total phenolic (TP) concentration was determined in triplicate by the Folin-Ciocalteu (Folin-C.) colourimetric method (Singleton and Rossi, 1965) as modified by Makkar (2000) and calculated as gallic acid equivalents (GAE) per gram dry matter.

2.6.2. Total flavonoid concentration (TFC)

Total flavonoid concentration (TFC) was determined using the method described by Yang et al. (2008) and the results expressed as catechin equivalents (CAE) per gram dry weight.

2.6.3. Rhodanine assay for gallotannin concentration (GTC)

Determination of the gallotannin concentration in peel methanolic extracts was carried out as described by Makkar (2000). Samples (50 µL) were mixed with 150 µL of 0.4 N sulphuric acid followed by 600 µL rhodanine. After 10 min, 200 µL of 0.5 N KOH was added and subsequently distilled water (4 mL) after 2.5 min. The absorbance was read at 520 nm

(room temperature) against a blank test that contained aqueous methanol instead of the sample after 15 min incubation. The GTC was calculated from the standard curve (gallic acid) and expressed as gallic acid equivalents (GAE) per gram DM.

2.6.4. Total anthocyanin concentration (TAC)

Total anthocyanin concentration (TAC) was quantified using the pH differential method described by Wrolstad (1993). In triplicate, each extract (1 mL) was mixed with 9 mL of pH 1.0 and pH 4.5 buffers, in separate test tubes. Absorbance of the reaction mixture was measured at 510 and 700 nm in pH 1.0 and 4.5 buffers, respectively. The total absorbance was calculated from equation 4, while total anthocyanin concentration was calculated from equation 5. The result was expressed as cyanidin 3-glucoside:

$$A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH4}} \quad (4)$$

$$\text{Total anthocyanin (µg/mL)} = [(A \times MW \times DF)/\varepsilon \times L] \quad (5)$$

A = Absorbance, ε = Cyd-3-glucoside molar absorbance (26,900), MW = anthocyanin molecular weight (449.2), DF = dilution factor, L = cell path-length (1 cm). Final results are expressed as µg Cyd-3-glucoside equivalents (C_3gE) per gram dry matter (µg C_3gE/g DM).

2.7. HPLC-ESI/MS^n analysis of phenolic composition

The LC-MS analysis of phenolics and anthocyanin components in the pomegranate peel extract was performed according to Fischer et al. (2011) with slight modification, using a Synapt G_2 mass spectrometer UPLC^{TM} system (Waters Corp., Milford, USA) connected to a photo diode array detector and a BEH C18 column (1.7 µm particle size, 2.1x100 mm, Waters Corp.). The mobile phases were 5% formic acid in water (v/v) as eluent A and 95% acetonitrile, 5% formic acid (v/v) as eluent B. The flow rate was fixed at 0.2 mL/min and the column temperature was set at 40°C. The electrospray ionization (ESI) probe was operated in the positive mode with the capillary voltage of 3 kV; and cone voltage of 15 V. The injection volume was 10 µL and the detection was the diode array detector set at between 200 - 600 nm. Individual phenolic compounds were quantified by comparison with a multipoint calibration curve obtained from the corresponding standard (catechin, epicatechin, protocatechuic acid, gallic acid, ellagic acid) from Sigma Aldrich (Germany), while

anthocyanins were quantified by an external standard cyanidin 3,5-diglucoside (Sigma Aldrich, Germany).

2.8. Statistical analysis

All data are presented as mean values (± S.E). Analysis of variance (ANOVA) was performed using SPSS 10.0 for Windows (SPSS Inc. Chicago, USA). Where there was statistical significance ($p<0.05$), the means were further separated using Duncan's Multiple Range Test. Graphical analysis carried out using GraphPad Prism software version 4.03 (GraphPad Software, Inc. San Diego, USA). The IC_{50} values for the tyrosinase assay were calculated from the logarithmic non-linear regression curve derived from the plotted data using GraphPad Prism software version 4.03 (GraphPad Software, Inc., San Diego, USA).

3. Results and discussion

3.1. Antibacterial activity

The antibacterial activities of methanol and aqueous peel extracts of all the investigated pomegranate cultivars are presented in Table 1. None of the aqueous extracts exhibited good antibacterial activity at the highest screening concentration (>12.5 mg/mL). The methanol extract, however, showed varying broad-spectrum antibacterial activity at statistically different MIC values ($p<0.05$) against the test bacteria. Although it is ideal to test plant extracts against a wide range of target microorganisms, taxonomically representative bacterial species were used in this test to avoid handling numerous pathogenic microorganisms. The minimum inhibitory concentrations (MIC) were obtained for extract concentrations ranging from 0.78 to 0.20 mg/mL. In this study, MIC values less than 1.0 mg/mL were considered active for crude extracts (van Vuuren, 2008). Similar to the findings reported by Opara et al. (2009) on peels of pomegranates grown in Oman, all peel extracts of the investigated pomegranate cultivars showed activity against the Gram negative and positive bacteria used. These findings are contrary to the work of Kanatt et al. (2010), which reported that pomegranate extracts showed little or no effect with regards to Gram negative bacteria. The concentration of methanol used in the assay was inactive against tested bacteria in the assay (data not shown). It is worth noting that although 80% methanol was used to dissolve the extracts, methanol concentration was <1.25% in all the extracts where the MIC values were record. The total antibacterial index (TAI) was calculated to determine the overall effects of the peel extracts of each cultivar of pomegranate studied against test bacteria. The

most active cultivar was 'Herskawitz' with the highest TAI value (6.25), while the lowest TAI value was exhibited by 'Bhagwa'; clearly indicating that activity was cultivar dependent.

Pomegranate peel polyphenols, especially tannins, are the major components in the pomegranate peel extract that have been implicated in antimicrobial potential (for example, antiviral, antifungal and antibacterial activities; Miguel et al., 2010). Vasconcelos (2009) studied the antibacterial activity of methanolic peel extracts of pomegranate cultivars against both Gram negative and positive bacteria strains and reported MIC values ranging from 0.25 to 4.0 mg/mL against the test bacteria. The author reported a two-fold MIC value against a Gram positive bacterium (*S. aureus*) than against a Gram negative bacterium (*E. coli*). It has been suggested that the antimicrobial activity of tannins may be due to the ability of tannin compounds to precipitate proteins, therefore causing leakage of cell membrane of the microorganism (Endo et al., 2010), and aiding cell lysis which ultimately leads to cell death.

As the peel extracts are complex mixtures of metabolites (Figure 1), it is difficult to pinpoint all the metabolites that are responsible for pharmacological activity. Other researchers have postulated that punicalagin, along with a combination of various phytochemicals, plays a positive role in perceived bacterial inhibition (Opara et al., 2008; Al-Zoreky, 2009). Considering the broad spectrum antibacterial property exhibited by the methanolic extracts, the fruit peel of the investigated pomegranate cultivars can be considered an effective antibacterial agent. Several reasons may explain varying antibacterial potency of the extracts tested here. Differences in antibacterial activities in the peel extracts may be linked to inter-genetic cultivar variability which is also associated with the inherent chemical composition of the fruit peel. Also, gene-to-biosynthetic modifications are well established in plants as they respond plastically to geo-environmental spatial variation (Viljoen et al., 2005). Secondary metabolites play a key role as defence chemicals for many plants, and so, their accumulation is often dependent on environmental factors. Chemical heterogeneity ultimately leads to differences in the bioactivity of extracts derived from plants growing in different microclimatic areas as these plants face different abiotic and biotic challenges altering gene expression and secondary metabolite synthesis (Field and Lake, 2011).

3.2. Antioxidant activity

Negi and Jayaprakasha (2006) extracted antioxidants from pomegranate peel with the use of methanol, acetone or water and found that methanol gave maximum antioxidant yield. In this study, some degree of radical scavenging activity (RSA) was observed in all the evaluated extracts, with considerable increase in RSA with increasing in concentration level.

Considering RSA of 50% as good activity, poor RSAs were exhibited by all the evaluated samples at concentrations of 100 µg/mL and 10 µg/mL (Table 2). However at the highest concentration tested (1000 µg/mL), the RSA was superior in all the fruit cultivars. The RSA values were high in 'Arakta' (83.54%), 'Ganesh' (83.56%), and 'Ruby' (83.34%) (p<0.05), while the lowest activity was exhibited by 'Molla de Elche' (71.65%). The RSA of ascorbic acid (67.02%), used in this study as a positive control, was lower than the plant extracts at 1000 µg/mL.

The chelating ability of methanolic extracts of pomegranate peel on ferrous ion is presented in Table 2. Although low FIC activity was exhibited at the lowest concentration (10 µg/mL) assayed, extracts of 'Molla de Elche', 'Ruby' and 'Wonderful' showed relatively good FIC activity, ranging from 47.24 to 49.65%. At 100 µg/mL the FIC activity of all extracts (except 'Arakta') increased above 50%, with 'Herskawitz', 'Molla de Elche' and 'Wonderful', showing highest values of 69.97%, 70.57% and 71.02%, respectively. Moreover, FIC activities exhibited by methanolic extracts of most of the cultivars investigated at 100 µg/mL were higher than that of the positive control (ascorbic acid at 1000 µg/mL). Dose dependent FIC activity exhibited by all the investigated extracts indicated that the pomegranate fruit peel contains constituents that inhibit oxidation through a different mechanism other than free radical scavenging.

The FRAP assay measures the ability of an antioxidant to reduce ferric (III) to ferrous (II) in a redox-linked colourimetric reaction that involves single electron transfer (Li et al., 2006). The reducing power of a compound serves as a significant indicator of its potential antioxidant activity. All the investigated extracts showed dose-dependent reducing power (Table 2). Interestingly, there was no significant difference (p>0.05) in the reducing capacities among all the cultivars at the highest concentration (1000 µg/mL).

Antioxidant capacity based on both the free radical scavenging and the oxidation-reduction mechanisms may be determined by several methods, although the mechanism of action set in motion by antioxidant compounds is still not clearly understood (Viuda-Martos et al., 2010). Previous studies have shown strong antioxidant activity in pomegranate fruit peel extracts (Li et al., 2006; Hajimahmoodi et al., 2008). In comparison with other fruit peels, Okonogi et al. (2009) studied the radical scavenging activity on DPPH and ABTS of pomegranate peel extract with other fruit types including rambutan, mangosteen, banana, coconut, dragon fruit, passion fruit as well as long-gong fruit. In the study, the highest scavenging activity was reported in pomegranate peel extract. The observed antioxidant property in the peel extract in this study could be attributed to polyphenol compounds such as

ellagic tannins, ellagic acid and gallic acid (Gil et al., 2000; Lansky and Newman, 2007). The results show that pomegranate peel may have great relevance in the prevention and therapies of diseases in which oxidants or free radicals are implicated, hence could serve as an economic source of natural antioxidants.

3.3. Tyrosinase inhibition activity

Tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions such as food browning and melanisation of human skin (Yoshimura et al., 2005). Results of tyrosinase inhibition activity of pomegranate methanol peel extract at different concentrations (2.6 - 333.3 µg/mL) are presented in Figure 2 A and B against monophenolase (tyrosine) and diphenolase (DOPA), respectively. Activities were assessed in terms of dopachrome formation. Although the extracts were initially dissolved in 100% DMSO, the final content of the DMSO in the reaction mixture was between 0.14% and 18.3%. Also, DMSO was used as a control in the assay; therefore, any effect of DMSO would have been taken care of in the calculation. Generally, tyrosinase inhibition was displayed in a dose-dependent way and there was higher monophenolase inhibition than diphenolase inhibition. In this study, inhibition activity percentage above 50% was described as good tyrosinase inhibition. All extracts exhibited good inhibition against monophenolase and diphenolase activities at the highest screening concentration (Figure 2).

Furthermore, there were significant (p<0.05) differences in the concentrations of 50% tyrosinase inhibition (IC_{50}) by the fruit peel. The most active peel extract was the 'Bhagwa' against monophenolase and the 'Arakta' against diphenolase with IC_{50} values of 3.66 µg/mL and 15.88 µg/mL, respectively (Table 3). The inhibitory activities of most of the peel extracts were higher than the positive control (arbutin) which is a known tyrosinase inhibitor. The IC_{50} values obtained in this study were lower than those reported by Yoshimura et al. (2005) where IC_{50} value of 182.2 µg/mL was reported for 50% aqueous ethyl alcohol extract of pomegranate rind. Polyphenols are also the largest groups in tyrosinase inhibitors until now (Chang, 2009). The pomegranate fruit peel is rich in polar substances such as flavonoid constituents and tannins. In solution, the defining characteristic of tannins is the ability to precipitate mainly proteins, and the structure of flavonoid is compatible with the roles of both substrates and inhibitors of tyrosinase (Chang, 2009). It could be suggested that tannin concentration in pomegranate peel could precipitate tyrosinase enzyme, thereby inhibiting enzymatic activity in the reaction medium. These constituents are readily soluble in methanol

and show high tyrosinase inhibitory activity in different plants (Kubo et al., 2000; Meda et al., 2005).

According to Yoshimura et al. (2005), ellagic acid in pomegranate rind showed an inhibitory effect on tyrosinase *in vitro* and a whitening effect *in vivo* on UV-induced pigmentation of brownish guinea pig skin. On the contrary, however, Chang (2009) argued that some phenolic compounds could be mistakenly classified as tyrosinase inhibitors due to their role as alternative enzyme substrates whose quinoid reaction products absorb in a spectral range different from that of dopachrome. As a result, when the phenolics show a good affinity for the enzyme, dopachrome formation is prevented.

3.4. Phenolic compounds analysis

3.4.1. Total phenolics, flavonoid, gallotannin and anthocyanin

Pomegranate fruit components are rich in phenolic compounds which have synergistic and/or additive effects on its pharmacological properties (Seeram et al., 2005). Phenolic constituents in pomegranate peel have been implicated in bioactivities such as antimicrobial, antioxidant, and anti-tyrosinase activities (Yoshimura et al., 2005; Lansky and Newman, 2007; Miguel et al., 2010). Results obtained in this study revealed significant cultivar differences ($p<0.05$) in the levels of phenolic compounds (Table 4). The 'Ganesh' had the highest amount of total phenolics (295.5 mg/g DM) whereas 'Molla de Elche' had the lowest phenolic concentrations (179.3 mg/g DM). These results corroborate the levels of total phenolic concentration (249.4 mg/g) reported by Li et al. (2006), who found that total phenolic concentration of pomegranate peel extract was 10-fold as high as that of the juice extract. Similarly, the highest (126 mg/g DM) and lowest (97.8 mg/g DM) concentrations of total flavonoid were measured in 'Ruby' and 'Wonderful', respectively. This result was expected as flavonoid is a major phenolic group in pomegranate and should contribute to the total phenolic concentration in the peel extracts. Flavonoid concentrations found in the investigated cultivars were higher than the value reported by Li et al. (2006). Gallotannin concentration in 'Arakta' (783.6 µg/g DM) was higher in other cultivars, but not significantly higher than that of 'Ganesh' (777.2 µg/g DM). Anthocyanin is one of the most important groups of flavonoid which is responsible for red colouration of pomegranate fruit (Afaq et al., 2005). Total anthocyanin was the highest in 'Wonderful' (322.2 µg/g DM) whereas the lowest was contained in the 'Molla de Elche' (58.5 µg/g DM).

3.5. HPLC-MS^n analysis of phenolic composition

Individual phenolic compounds in pomegranate fruit peel such as punicalagin, ellagic acid, gallic acid, caffeic acid, protocatechuic acid and *p*-coumaric acid have received considerable attention due to their potent antibacterial, antioxidant and anti-tyrosinase activities (Gil et al., 2000; Kubo et al., 2000; Meda et al., 2005; Yoshimura et al., 2005; Lansky and Newman, 2007; Miguel et al., 2010). High-performance liquid chromatography-mass spectrometry (HPLC-MS) was used to determine the individual concentrations of the most prominent phenolic compounds in the methanolic peel extract of pomegranate cultivars studied. As shown in Figure 3, seven individual phenolics were identified and quantified, namely; anthocyanins: delphinidin 3,5-diglucoside, cyanidin 3,5-diglucoside, flavonoids: catechin, epicatechin and rutin; hydrolysable tannin: ellagic acid; and hydroxybenzoic acid: gallic acid. Phenolic profile and concentration varied amongst the fruit cultivars. Catechin, epicatechin, ellagic acid and gallic acid were found in all cultivars, of which ellagic acid was the most abundant comprising of more than 50% of total phenolic compounds detected in each cultivar. The concentration of ellagic acid ranged from 46.87 µg/mL (‘Ruby’) to 209.44 µg/mL (‘Ganesh’). The anthocyanin types; delphinidin 3,5- diglucoside and cyanidin 3,5-diglucoside, were detected in ‘Arakta’, ‘Bhagwa’ and ‘Herskawitz’, while ‘Ganesh’, ‘Ruby’ and ‘Wonderful’ contained only cyanidin 3,5- diglucoside (Figure 3).

Noda et al. (2002) reported that cyanidin, pelargonidin, and delphinidin were the principal anthocyanins in pomegranate peel. In the present study, rutin was found in all cultivars except in ‘Wonderful’, and catechin was the highest in ‘Molla de Elche’ with a concentration of 28.85 µg/mL. Overall, the total concentration of the identified phenolic compounds was in the order of ‘Ganesh’ > ‘Herskawitz’ > ‘Molla de Elche’ > ‘Bhagwa’ > ‘Arakta’ > ‘Wonderful’ > ‘Ruby’. The presence of these polyphenols in the pomegranate peel may be responsible for the bioactivities observed in the methanol extracts. Phenolic contained in plants influence antimicrobial activity of the plants (Rauha et al., 2000). For instance, flavone, quercetin and naringenin were reported showing strong inhibitory activity on the growth of *Aspergillus niger*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, while gallic acid inhibited only *P. Aeruginosa*. No inhibitory activity was exhibited by rutin and catechin on the tested microorganisms (Rauha et al., 2000). Major chemicals identified through LCMS may not be the only compounds responsible for bioactivity in the pomegranate peel extracts. Other compounds not identified may play a more significant role in the biological activities exhibited by the peel extracts.

4. Conclusion

This study has shown that the peel of the investigated pomegranate fruit cultivars possess strong antibacterial, antioxidant and anti-tyrosinase activities. Therefore, this waste product from pomegranate processing could be exploited as a potential source of natural antimicrobial and antioxidant agents, as well as a potential tyrosinase inhibitor. The findings provide scientific basis to promote value-adding of pomegranate fruit peels for pharmaceutical and cosmetic purposes. Further studies on the isolation of active ingredients, determination of cytotoxicity and genotoxicity effects as well as the mode of action of tyrosinase-inhibitory, antibacterial and antioxidant properties in pomegranate peel extracts are warranted.

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Table 1

Antibacterial activity of fruit peel methanol extract of pomegranate cultivars (at commercial maturity) cultivated in South Africa

	Minimum inhibitory concentration (MIC; mg/mL)				
Cultivar	B.s	E.c	K.p	S.a	TAI
'Arakta'	0.39^{b}	0.78^{b}	0.20^{a}	0.39^{b}	6.00^{b}
'Bhagwa'	0.39^{b}	0.78^{b}	0.20^{a}	0.78^{c}	5.70^{a}
'Ganesh'	0.39^{b}	0.39^{a}	0.20^{a}	0.78^{c}	6.00^{b}
'Herskawitz'	0.20^{a}	0.39^{a}	0.20^{a}	0.78^{c}	6.25^{c}
'Molla de Elche'	0.39^{b}	0.78^{b}	0.39^{b}	0.26^{a}	5.92^{ab}
'Ruby'	0.39^{b}	0.39^{a}	0.39^{b}	0.39^{b}	6.00^{b}
'Wonderful'	0.39^{b}	0.59^{ab}	0.33^{b}	0.39^{b}	5.99^{b}
Significance level	<0.0001	0.001	<0.0001	<0.0001	0.004
Streptomycin (µg/mL)	3.13	3.13	2.60	5.21	
Methanol (control)	>12.50	>12.50	>12.50	>12.50	

Mean values in the same column followed by different letter(s) represent statistical different (p<0.05) using the Duncan's multiple range test. MIC - Minimum inhibitory concentration, TAI - Total antibacterial index, the higher the TAI the higher antibacterial activity. B.s - *Bacillus subtilis*, E.c - *Escherichia coli*, K.p - *Klebsiella pneumonia*, S.a - *Staphylococcus aureus*. For all of the investigated cultivars, MIC and TAI values were >12.50 mg/mL and <1.00, respectively against the test bacteria.

Table 2

Antioxidant activity of fruit peel methanol extracts of seven pomegranate cultivars (at commercial harvest maturity) cultivated in South Africa

	DPPH (%)			FIC (%)			FRAP (abs. at 593 nm)		
Cultivar	1000 µg/mL	100 µg/mL	10 µg/mL	1000 µg/mL	100 µg/mL	10 µg/mL	1000 µg/mL	100 µg/mL	10 µg/mL
'Arakta'	83.54±0.31^{d}	13.35±0.98^{ab}	5.55±0.06^{c}	79.44±0.21^{a}	49.94±0.89^{a}	37.32±1.82^{b}	1.19±0.03^{ns}	0.52±0.02^{b}	0.11±0.003^{c}
'Bhagwa'	73.02±0.26^{ab}	12.34±0.73^{ab}	1.37±0.34^{a}	84.96±1.43^{bc}	65.54±1.09^{c}	18.83±0.22^{a}	1.03±0.28	0.38±0.00^{ab}	0.04±0.01^{a}
'Ganesh'	83.56±0.05^{d}	16.70±0.83^{bc}	2.42±0.99^{ab}	82.98±0.18^{b}	65.82±0.51^{c}	15.80±0.52^{a}	1.47±0.04	0.73±0.12^{c}	0.08±0.01^{bc}
'Herskawitz'	78.06±0.71^{c}	15.18±0.97^{abc}	2.71±0.77^{ab}	87.82±0.57^{de}	69.97±0.25^{d}	34.32±2.45^{b}	1.29±0.04	0.34±0.01^{ab}	0.08±0.02^{bc}
'Molla de Elche'	71.65±0.08^{a}	10.59±0.18^{a}	1.61±0.08^{a}	86.59±0.90^{cd}	70.57±0.43^{d}	47.24±1.34^{c}	1.47±0.11	0.33±0.05^{a}	0.03±0.004^{a}
'Ruby'	83.34±0.51^{d}	19.67±2.24^{c}	4.10±2.24^{bc}	83.58±0.62^{b}	53.39±1.29^{b}	47.25±0.66^{c}	1.18±0.02	0.38±0.004^{ab}	0.08±0.01^{bc}
'Wonderful'	74.19±1.05^{b}	12.22±3.13^{ab}	3.01±0.47^{ab}	89.67±0.72^{e}	71.02±0.38^{d}	49.65±1.26^{c}	1.32±0.16	0.33±0.01^{a}	0.06±0.002^{ab}
Ascorbic acid	67.02±0.06			62.15±0.98					
Trolox									0.82±0.03

Means in the same column followed by different letters represent statistical significance (p<0.0001) according to the Duncan's multiple range test. Positive controls: Ascorbic acid and trolox. Antioxidant activities of peel methanol extract for all the investigated cultivars were statistically within the same test concentration.

Table 3

Effective inhibition concentration (EC_{50}) of fruit peel methanol extracts against tyrosinase

Cultivar	IC_{50} Monophenolase (µg/mL)	IC_{50} Diphenolase (µg/mL)
'Arakta'	11.03±0.08[c]	15.88±0.10[a]
'Bhagwa'	3.66±0.11[a]	21.16±0.09[a]
'Ganesh'	25.38±0.06[f]	40.93±0.12[b]
'Herskawitz'	7.56±0.08[b]	59.03±0.07[c]
'Molla de Elche'	25.56±0.06[f]	27.11±0.09[ab]
'Ruby'	20.33±0.07[d]	114.9±0.08[e]
'Wonderful'	23.67±0.06[e]	27.26±0.07[ab]
Arbutin	34.66±0.05[g]	98.66±0.12[d]
Significance level	<0.0001	<0.0001

Mean values in the same column followed by different letters represent statistical different (p<0.0001) using the Duncan's multiple range test.

Table 4

Phenolic concentration in fruit peel methanol extracts of seven pomegranate cultivars (at commercial harvest maturity) cultivated in South Africa

Cultivar	Total phenolics	Total flavonoid	Total gallotannin	Total anthocyanin
	mg GAE/ g DM	mg CAE /g DM	µg GAE/ g DM	µg C_3gE /g DM
'Arakta'	187.4±6.44^{ab}	103.0±1.86^{a}	783.6±65.11^{d}	289.7±1.63^{d}
'Bhagwa'	224.1±6.86^{c}	112.6±1.51^{b}	697.7±42.92^{cd}	312.6±1.25^{e}
'Ganesh'	295.5±23.91^{d}	121.1±3.12^{c}	777.2±34.28^{d}	65.1±1.00^{a}
'Herskawitz'	198.1±9.22^{abc}	101.0±1.02^{a}	530.1±33.86^{b}	195.9±2.25^{c}
'Molla de Elche'	179.3±4.60^{a}	99.5±2.94^{a}	560.3±62.08^{bc}	58.5±1.27^{a}
'Ruby'	218.2±4.53^{bc}	126.0±0.57^{c}	326.0±35.28^{a}	111.7±3.51^{b}
'Wonderful'	189.1±3.79^{ab}	97.8±2.10^{a}	466.3±69.4^{ab}	322.2±11.90^{f}

GAE, gallic acid equivalent; CAE, catechin equivalent; C_3gE, cyanidin-3glucoside equivalent; DM, dry matter. Mean values in the same column followed by different letters represent statistical different (p<0.0001) using the Duncan's multiple range test.

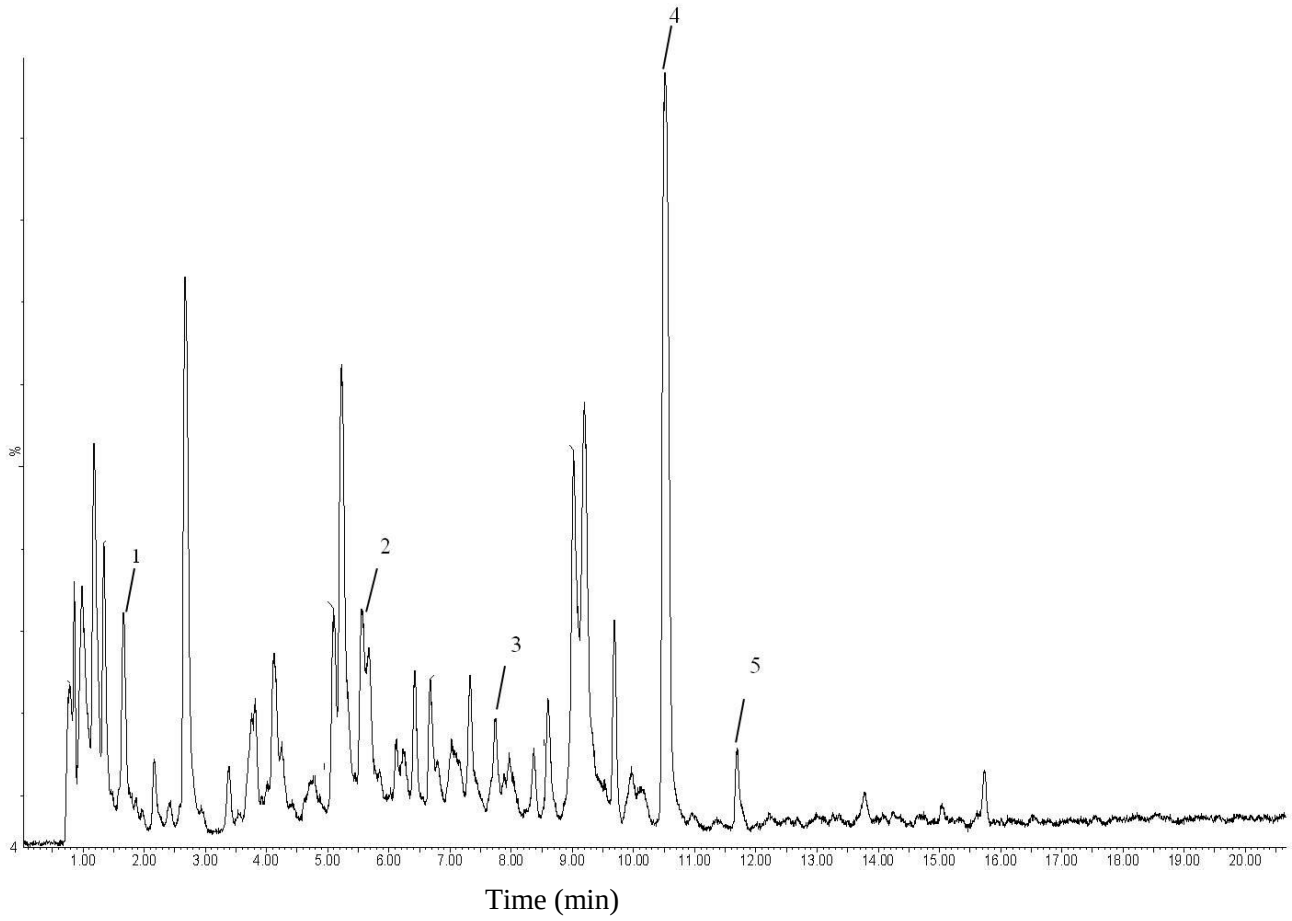

Figure 1. Typical HPLC-MS chromatogram of methanolic peel extract of pomegranate fruit. (1) Gallic acid; (2) Catechin; (3) Epicatechin; (4) Ellagic acid; (5) Rutin.

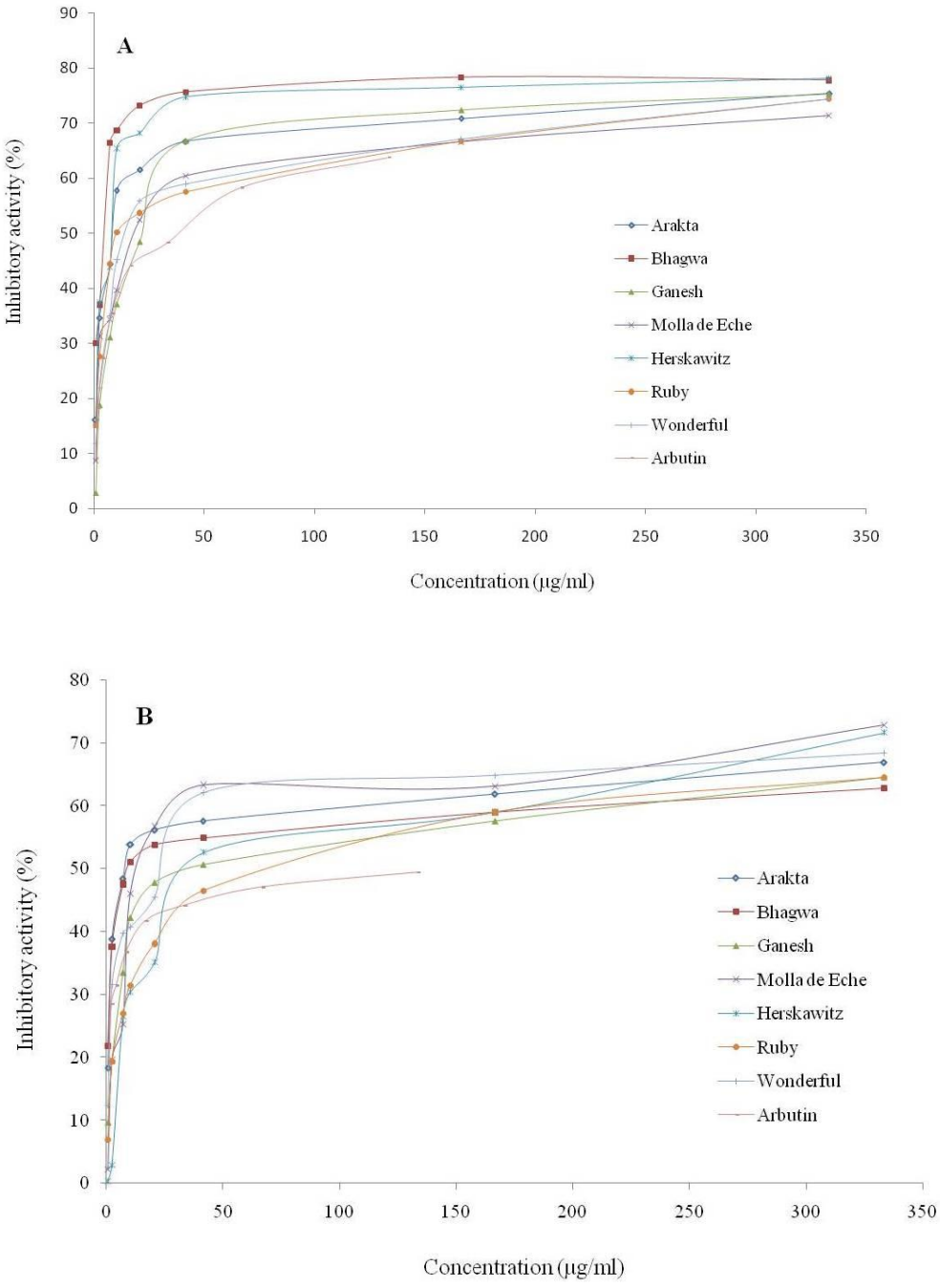

Figure 2. Tyrosinase enzyme inhibitory activity of fruit peel methanol extracts of seven pomegranate cultivars (at commercial harvest) cultivated in South Africa. Monophenolase inhibition (**A**) and Diphenolase inhibition (**B**). Arbutin was used as a positive control.

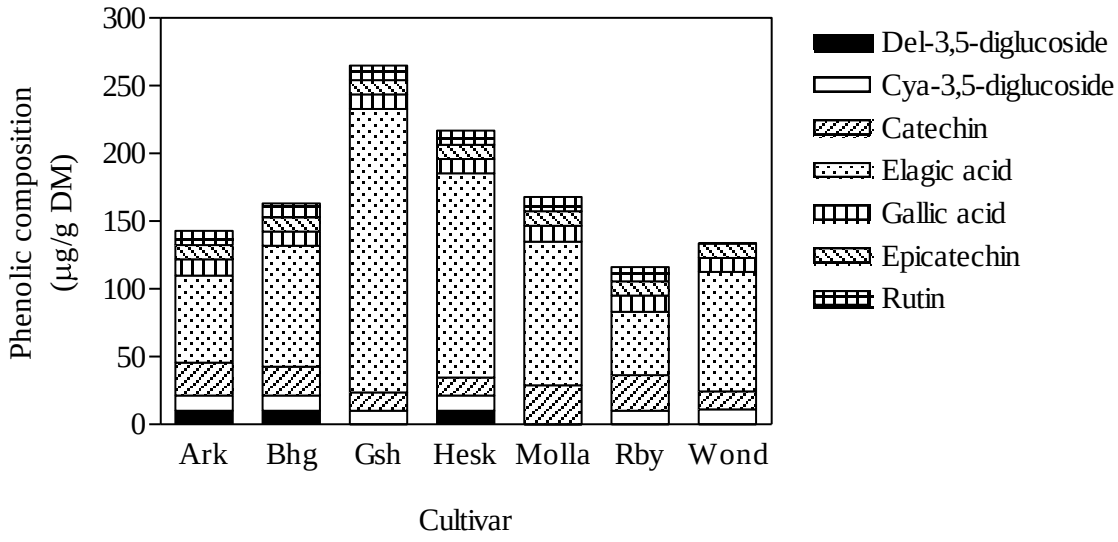

Figure 3. Phenolics composition in peel methanol extracts of seven pomegranate cultivars. Ark- 'Arakta', Bhg- 'Bhagwa', Gsh- 'Ganesh', Hesk- 'Herskawitz', Molla- 'Molla de Elche', Rby- 'Ruby' & Wond- 'Wonderful'.

SECTION IV

POSTHARVEST QUALITY ATTRIBUTES OF 'BHAGWA' AND 'RUBY' POMEGRANATE CULTIVARS IN RELATION TO HARVEST MATURITY AND STORAGE CONDITIONS *

- Paper 8: Effects of storage temperature and duration on physiological response, quality and antioxidant capacities of pomegranate fruit [1]
- Paper 9: Harvest maturity discrimination of pomegranate fruit by instrumental and sensory measurements during storage and shelf life conditions[2]

* Studies were done during the 2010/11 and 2011/12 harvest seasons

1. Ind. Crop Prod. 47, 300 – 309.

2. J. Food Sci. 78, S1264–S1272.

This section presents a compilation of manuscripts where each paper is an individual entity and some repetition between papers, therefore, has been unavoidable.

PAPER 8

Effects of storage temperature and duration on physico-chemical attributes and physiological responses of pomegranate fruit

Abstract

Pomegranate fruit ('Bhagwa' and 'Ruby') harvested at commercial maturity were stored at 5±0.3°C, 7±0.5°C and 10±0.4°C with 92±3% relative humidity (RH), and at room temperature (20±2.2°C, 65±5.5% RH) for 16 weeks during which fruit respiration, physico-chemical attributes, antioxidant capacities and incidence of physiological disorders were measured at 4-week intervals. Results showed that the physiological responses and quality of fruit were affected by storage condition, with the maximum levels of respiration occurring at higher storage temperature and extended duration. Fruit colour and antioxidant capacity varied slightly among storage temperatures, and total soluble solids and titratable acidity decreased gradually over time at different temperatures. Although storage at 5°C significantly reduced the total phenolic content of fruit when stored beyond 8 weeks, levels of antioxidant activity in the fruit were not affected. Fruit weight loss during storage was particularly high at room temperature for 4 weeks, ranging between 20 - 25% for both cultivars. Furthermore, the severity and occurrence of physiological disorders were lower in fruit stored at low temperature but increased with extended duration. Considering that fruit stored at 5°C and 92% RH had significantly reduced weight loss, low incidence of physiological disorders and best results in maintaining flavour attributes (TSS and TA, TSS:TA ratio), it is recommended that the investigated cultivars be stored at 5°C and >92% RH for 8 - 12 weeks.

Keywords: Antioxidant capacity, pomegranate, respiration, South Africa, Storage temperature

1. Introduction

Global production and consumption of pomegranate (*Punica granatum* L.) has gained popularity in recent years due to its multi-functionality and great nutritional benefit in the human diet. The fruit is grown globally in many different geographical regions, satisfying the nutritional

and medicinal needs of populations of various countries (Holland et al., 2009). South Africa has recently emerged as one of the recognized producers of pomegranate fruit in the Southern Hemisphere, competing with countries such as Chile, Australia, Peru and Argentina. Consequently, the production and export opportunity has increased to meet the counter-season market window during the spring and early summer months in the Northern Hemisphere (Brodie, 2009). Pomegranate production in South Africa stands at about 1,000 hectares, with an estimate of about 2,250 tons of fruit exports in 2012 (Citrogold, 2012; Perishable Products Export Control Board, 2012).

Pomegranate fruit is highly susceptible to weight loss and decay during postharvest handling and storage. For instance, storage time for 'Wonderful' pomegranate kept at 20°C or 30°C was about 1 to 4 weeks, with high incidence of quality losses (Elyatem and Kader, 1984). Apart from the common external postharvest quality defects such as decay and water loss, leading to browning symptoms in the peel and arils (Kader et al., 1984), internal quality losses also occur. Other researchers have reported colour loss as a result of degradation of anthocyanin (Turfan et al., 2011), as well as a decrease in total soluble solids and titratable acidity, which are accompanied by a reduction in consumer acceptability in terms of freshness, taste and loss of potential medicinal properties (Gil et al., 1996; Artes et al., 2000a; Nanda et al., 2001; Labbé et al., 2010; Turfan et al., 2011). Incidence and severity of sensory quality losses and physiological disorders in pomegranate have been linked to pre-harvest temperatures, fruit maturity and cultural practices (Elyatem and Kader, 1984).

The optimum storage temperature recommended for pomegranates vary from 0 to 10°C with storage life ranging from 2 weeks to 5 months, depending on cultivar. For 'Molas Torsh', Pantastico et al. (1975) recommended storage temperatures between 0 - 1.7°C and 85 - 95% RH for 3-month storage, while Kader (2006) recommended 2-month storage duration for 'Wonderful' pomegranate stored at 5°C and 90 - 95% RH. Kupper et al. (1994) reported that 'Hicaz' pomegranates can be stored at 6°C and 85 - 90% RH for 5 months. Unfortunatley, the production of pomegranate in South Africa is currently plagued by high incidence of postharvest losses especially during handling is a major problem affecting pomegranate production in South Africa, and there is currently limited knowledge on storage requirements of several commercial cultivars such as 'Bhagwa' and 'Ruby'. The objectives of this work were (a) to study storage performance based on fruit physiological responses and changes in physico-chemical properties

and antioxidant capacity during long term cold storage of 'Bhagwa' and 'Ruby', and (b) to determine suitable storage conditions and duration to maintain fruit quality and reduce losses.

2. Materials and methods

2.1. Fruit sampling and storage conditions

Fully ripe fruit were handpicked during commercial harvest period (March for 'Ruby' and April for 'Bhagwa') from an orchard in Porterville (33°01′00″S, 18°58′59″E) South Africa during the 2010/2011 growing season, and transported in an air-conditioned vehicle to the Postharvest Technology Laboratory at Stellenbosch University. Fruit free from blemishes and visible external damage were sorted for uniformity of colour and size and stored overnight at 20°C, 70% RH. A total of 800 fruit per cultivar was selected, and randomly divided into four lots, each comprised of 200 fruit, and packed inside standard open top cartons with dimensions of 0.4 m long, 0.3 m wide and 0.133 m high and a total of 22 perforations. Each fruit lot (10 cartons, twenty fruit per box) was stored at one of the following conditions: 5±0.3°C, 7±0.5°C and 10±0.4°C with 92±3% RH, and at room temperature (20±2.2°C, 65±5.5% RH). Temperature (°C) and relative humidity (% RH) inside the cold rooms were recorded on hourly basis throughout the storage period using Tiny Tag TV-4500 data loggers (Gemini Data Logger, Sussex, UK) with a functional range of -40°C to +85°C and 0% to 100% RH.

2.2. Fruit respiration

Fruit respiration rate was measured as the amount of CO_2 evolved by fruit under different storage temperatures using the closed system method as described by Caleb et al. (2012). In 5 replicates, each fruit was placed in a 3 L hermetically sealed glass jar for 4 h with a lid containing a rubber septum in the middle. After 4 h incubation, CO_2 production inside each glass jar was measured from the head space through the rubber septum using an O_2/CO_2 gas analyzer (Checkmate 3, PBI Dansensor, Denmark). Results for CO_2 production were presented as mean ± S.E (ml CO_2 $kg^{-1}h^{-1}$). Measurements were taken at harvest and every month during storage trials.

2.3. Weight loss and physiological disorders

For each storage condition, 15 fruit were randomly selected, weighed individually at intervals during storage using an electronic scale (Mettler, Toledo, Switzerland, 0.0001 g accuracy). Weight loss of each fruit was calculated as:

$$w = [(Wi - Wt) \div Wi] \times 100 \quad (1)$$

where w is the weight loss (%) of the fruit; Wi is the weight (g) of the fruit at the beginning of storage; Wt (g) is the weight of the fruit at the storage time (monthly). For each cultivar, weight loss was calculated as the mean of 15 fruit per treatment.

Incidence of decay, aril browning, dehydration and husk-scald were assessed. Monthly evaluation of severity of physiological disorders was carried out subjectively using a hedonic scale, where 0 = none, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, and 5 = extremely severe. Only severe injuries were considered as commercially unacceptable. An index of severity of physiological disorders was calculated by multiplying the scores of severity by the number of fruit affected and dividing by the total number of fruit (Artés et al., 1998).

2.4. Physico-chemical properties

2.4.1. Colour attributes

Aril colour was assessed in CIELAB coordinates (L^*, a^*, b^*) using a Minolta Chroma Meter CR-400 (Minolta Corp, Osaka, Japan). For each cultivar, 20 fruit were used per storage temperature. Changes in external colour was monitored by measuring peel colour at two marked surface areas, while aril colour from sampled fruit was measured in duplicate in a Petri dish. The colour parameters chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($h^o$ = arctan (b^*/ a^*)) were calculated (Al-Said et al., 2009; Pankaj et al., 2012). Measurements were carried out monthly and results were expressed as means ± S.E. of determinations obtained (n = 40).

2.4.2. pH, titratable acidity and total soluble solids

Twenty fruit were sampled per storage temperature for each cultivar on a monthly basis. Juice from arils of each fruit was extracted using a blender (Mellerware, South Africa) without crushing the kernels. Juice pH value was determined at room temperature using a pH meter

(Crison, Barcelona, Spain). Titratable acidity (TA) was determined using a Metrohm 862 compact titrosampler (Herisau, Switzerland), and the results were expressed as percentage citric acid. Total soluble solids (TSS) were measured using a digital refractometer (Atago, Tokyo, Japan). TSS/TA ratio and BrimA index, a criterion for acceptance of fruit juice expressed as BrimA = TSS – *k* * TA, were calculated. Where *k* is the tongue's sensitivity index normally ranging between 2 - 10 (Jordan et al., 2001; Jaya and Das, 2002). In this study *k* value of 2 was used according to Fawole and Opara (2012). All measurements were made on individual fruit sample.

2.5. Phytochemical contents and antioxidant capacities

2.5.1. Sample preparation

Four replicates of crude pomegranate juice (PJ) samples were each prepared from five fruit. PJ (1 ml) was extracted with 29 ml of cold 50% aqueous methanol. The resulting mixture was vortexed, and then sonicated in ice for 20 min in a cold water bath followed by centrifuging at 11963 G-force for 5 min at 4 ºC. The supernatant was subsequently collected and assayed for phenolic components and antioxidant capacity.

2.5.2. Total phenolic compounds

Total phenolic content (TPC) was determined in triplicates by the Folin-Ciocalteu (Folin-C.) colorimetric method (Makkar et al., 2000) and results were expressed as gallic acid equivalents (GAE) per litre PJ.

2.5.3. Total anthocyanins content

Total anthocyanins content (TAC) was quantified in triplicates using the pH differential method (Wrolstad, 1993). Final results were expressed as equivalent per litre PJ (mg C_3gE/L PJ).

2.5.4. Radical-scavenging activity (RSA)

The 2, 2-diphenyl-1-picryl hydrazyl (DPPH) assay was carried out in triplicate, according to the method used by Karioti et al. (2004) with some modifications according to Fawole et al.

(2012). The free-radical capacity of PJ was expressed as ascorbic acid (mM) equivalent per ml PJ (mM AAE /ml).

2.5.5. Ferric ion reducing antioxidant power (FRAP)

The antioxidant power of PJ was measured calorimetrically according to the method of Benzie and Strain (1996) with a few modifications (Fawole et al., 2012). Results were expressed as trolox (mM) equivalents per ml PJ (mM TE/ml PJ).

2.6. Statistical analysis

The results of all the studied variables are presented as mean (± S.E). Analysis of variance (ANOVA) was carried out using Statistica software (Statistica 11.0, StatSoft Inc., Tulsa, OK, USA) according to Duncan's multiple range test. Mean values for different temperatures over time were separated, and where appropriate, 2-way ANOVA was carried out. Graphical presentations were made using GraphPad Prism software version 4.03 (GraphPad Software, Inc., San Diego, USA).

3. Results and discussion

Storage trials for fruit stored at room temperature and 10°C were discontinued after 4 weeks and 12 weeks, respectively, due to complete fruit loss.

3.1. Fruit respiration rate

Temperature is a crucial variable influencing the respiration rate of s during storage of fruit including pomegranates (Elyatem and Kader, 1984). Results of the present study showed that fruit respiration rate depended on storage temperature in both cultivars (Figure 1). Respiration rates of fruit after storage at all the temperatures were lower than those measured at harvest period. Fruit respired significantly (p<0.05) slower at lower storage temperature (5°C) in both pomegranate cultivars, with respiration rate of ≤5 ml CO_2 $kg^{-1}h^{-1}$ during storage (except after 8 week storage for 'Ruby'). There was an elevation in respiration rate of fruit stored at 7°C and 10°C for 8 weeks for both cultivars, probably triggered by the severity of physiological stress; however fruit respiration rate declined after 12 weeks at the storage temperatures. This

respiratory behavior is unclear but previous studies have shown that increase in stress and physiological disorders could trigger respiration rate in pomegranate fruit (Elyatem and Kader, 1984). The higher respiration rate of fruit stored at room temperature for 4 weeks in our study might be attributed to the onset of anaerobic respiration resulting from microbial infestation of fruit, which led to the discontinuation of experiment at room temperature after 4 weeks. A similar observation was reported by Opara et al. (2008) for 'Helow' pomegranate stored at the room temperature of 22°C. Storage temperature and storage duration significantly ($p< 0.0001$) affected fruit respiration rate for both cultivars (Figure 1); however, the interaction effects between the two factors were not significant ($p = 0.0740$ for 'Bhagwa' and $p = 0.4279$ for 'Ruby'). This suggests that the effects of storage temperature on respiration are more critical than duration for the studied pomegranate cultivars. This assumption is partly corroborated by the findings of Caleb et al. (2012) on South African grown 'Acco' and 'Herskawitz', where a reduction in storage temperature from 15 to 5°C decreased fruit respiration rate by about 67% .

3.2. Physico-chemical properties

3.2.1. Color dynamics

The values for CIE a^* (redness) and C^* (colour intensity) for fruit peel and aril during storage at different temperatures are presented in Figure 2 and 3 for 'Bhagwa' and 'Ruby', respectively.

External appearance (peel): There were significant changes ($p = 0.0002$ for 'Bhagwa' and $p = 0.0006$ for 'Ruby') in fruit peel redness (a^*) with storage temperatures and duration. The peel a^* values decreased significantly ($p<0.05$) with increasing storage temperature in both cultivars (Figure 2 and Figure 3). Colour intensity (C^*) of fruit also differed significantly ($p < 0.0001$ for 'Bhagwa' and $p = 0.0005$ for 'Ruby') with storage temperature as well as duration, and followed a similar trend observed for a^* values (Figure 2 and Figure 3). In previous studies, Nanda et al. (2001) reported non-significant changes in colour of fruit stored at 8°C, 15°C and 25°C over a 12-week for 'Ganesh'. In our study however, storage temperature of 5°C had the better fruit colour maintenance throughout the storage duration as evidenced by higher a* and C* compared to other temperature regimes (Figure 2 and Figure 3).

Internal appearance (aril): For 'Bhagwa', although slight fluctuation was observed, aril redness (a^*) showed no significant differences for both cultivars ($p = 0.2287$ for 'Bhagwa' and $p = 0.9154$ for 'Ruby') among the storage temperatures and storage durations (Figs. 2 and 3). In addition, there were no significant ($p = 0.822$) differences in aril colour intensity (C^*) for 'Bhagwa' (Figure 2). This suggests that pomegranate aril colour might be relatively stable irrespective of the storage conditions. Although changes in aril colour intensity (C^*) with storage temperature and storage durationwere significant ($p = 0.0175$) for 'Ruby' (Figure 3), the changes may not have a significant effect on aril colour quality since aril redness (a^*) did not change significantly ($p > 0.05$) during the same period. In fact, the characteristic red colour of extracted arils was reported to be stable when stored at 5°C in different packaging materials over 18 days (Ayhan and Eştürk, 2009). However, on the contrary, Labbé et al. (2010) reported a decrease in aril colour in two Chilean pomegranate varieties stored at 5°C over 12 weeks. The discrepancies between the study by Labbé et al. (2010) and our results may be due to cultivar types.

3.2.2. Changes in chemical quality attributes

Tables 1 and 2 present the chemical properties of pomegranate fruit stored at different temperatures for 16 weeks. There were significant ($p<0.0001$) differences in pH among storage temperature and duration. Generally, fruit pH values increased with increasing storage time, whereas in most cases the values decreased with increasing storage temperature in both cultivars (except at 4 weeks in 'Ruby'). In 'Bhagwa', this behaviour was significantly ($p<0.0001$) influenced by both storage temperatures and storage durations but there was no significant ($p = 0.1943$) interaction between the two factors (Table 1). However, it appeared that the interaction between storage temperatures and durations played a significant role on fruit pH for 'Ruby'. The taste of pomegranate is determined mainly by juice TSS level and the ratio between the TSS and TA (Zarei et al., 2011). In this study, TA decreased significantly ($p < 0.05$) at all temperatures over time (Table 1 and Table 2). In 'Bhagwa', the highest TA level (0.33%) was record at room temperature after 4 weeks of storage (Table 1). This could be as a result of loss of moisture resulting to concentration of juice acidity in the fruit stored at room temperature. However, there were no significant differences between the TA levels in fruit stored at 5°C and 7°C between 8 and 12 weeks of storage. Similarly, in 'Ruby', TA level of 0.32% measured in fruit stored at room temperature and 10°C was significantly ($p <0.05$) higher than those stored at 5°C and 7°C

after 4 weeks of storage, whereas fruit acidity decreased with storage temperature and storage duration between 8 and 12 weeks of storage periods. This is in agreement with previous studies on different pomegranate cultivars stored under different conditions (Kader et al., 1984; Artes et al., 2000b; Nanda et al., 2001). TA levels in fruit stored at 5°C declined significantly (p <0.05) to the lowest levels (0.18% in 'Bhagwa' and 0.20% in 'Ruby') after 16 week storage. The observed changes in TA levels are a strong indication of the ongoing metabolism in the fruit during storage and the utilisation of organic acids during the respiratory process (Kader et al., 1984). Moreover, changes in acidity levels in 'Ruby' were significantly (p = 0.0036) influenced by the interaction between storage temperatures and storage periods (Table 2). On the other hand, for 'Bhagwa' the interaction between storage temperature and storage duration on juice acidity was not significant (p > 0.005). These results suggest that storage temperature could play a significant role on juice acidity given the significance effect (p = 0.0514) found in this study (Table 1). Further studies are required to confirm this relationship.

When compared with values at harvest, other than at 5°C between 4 and 8 weeks for 'Bhagwa', TSS content significantly (p < 0.005) decreased with storage duration, irrespective of storage temperatures for both cultivars (Table 1 and Table 2). During the first 4 weeks of storage, fruit stored at RT exhibited a marked decrease in TSS content and the least decrease was observed in fruit stored at 5°C in 'Ruby'. These findings are in agreement with Elyatem and Kader (1984), who reported a decrease in TSS content in 'Wonderful' with increasing storage temperature and storage duration. On the contrary, Koksal (1988) and Ghafir et al. (2010) reported significant increases in TSS content in pomegranate fruit during storage. Koksal (1988) attributed this effect to the loss of moisture resulting in concentration of sugars in inside fruit. Possible reason for the observed decrease in TSS content could be as a result of degradation of sugars over time. Furthermore, statistical analysis showed highly significant (p = 0.0013 for 'Bhagwa' and p < 0.0001 for 'Ruby') influence of storage duration on TSS content (Tables 1 and 2). Decreases in TA and TSS during postharvest storage resulted in significant (p < 0.0001) increases in TSS/TA ratio in most cases. The changes were primarily influenced by the interaction between storage temperatures and storage durations (p = 0.0041 for 'Bhagwa' and p = 0.0360 for 'Ruby'). In addition, changes in TSS and TA resulted in significant (p<0.05) decreases in BrimA index due mainly to storage duration in both cultivars (Tables 1 and 2).

3.2.3. Total phenolic and anthocyanin concentrations

Total phenolic (TP) concentration in juice did not vary significantly (p<0.05) among storage temperatures after 4 weeks of storage in 'Bhagwa', whereas in 'Ruby' TP concentrations declined in fruit stored at RT and 10°C during the same period (Figure 4). After 8 weeks of storage, TP concentration in 'Bhagwa' increased significantly (p<0.05) in fruit stored at 10°C and 7°C compared to the first 4 weeks of storage, probably due to the continued accumulation of anthocyanins, whereas TP concentration decreased in fruit stored at 5°C with prolonged storage time in the same period. In 'Ruby' however, TP concentrations first decreased significantly (p<0.05) at RT and 10°C after 4 weeks of storage and afterwards followed a significant (p<0.05) decreasing trend with decreasing temperatures (Figure 4). These findings are in agreement with the report by Sayyari et al. (2011), where decrease in total phenolic compounds was observed in untreated pomegranate fruit ('Mollar de Elche') stored at 2°C for 84 days. Reduction is total phenolic concentrations during postharvest storage could be attributed to phenolic degradation as a result of enzymatic activities occurring in the fruit as previously reported in other fruit such as rowanberries (Baltacioğlu et al., 2011).

Generally, total anthocyanin concentration in juice increased in comparison to total anthocyanin concentrations at harvest for both cultivars (Figure 4). As indicated previously, this suggests that all the investigated storage temperatures favoured the continued biosynthesis of anthocyanins, a process which is known to be induced in pomegranates at lower temperatures compared to the prevailing field temperature at harvest (Miguel et al., 2004). Fruit kept at 10°C had higher anthocyanin concentrations than those stored at 7°C and 5°C after 8 weeks of storage (Figure 4). In 'Bhagwa', significant (p<0.05) decreases in anthocyanin concentrations were observed after 12 weeks until the end of the experiment, whereas in 'Ruby', further increases in anthocyanin concentrations were observed after 12 weeks of storage at 10°C and 7°C before declining after 16 weeks of storage (Figure 4). This result is contrary to earlier report by Artes et al. (1998), where anthocyanin concentrations remained unaltered in 'Mollar de Elche' pomegranate between harvest and shelf life when stored at 5°C for 12 weeks. This indicates that cultivar differences may be a key factor influencing postharvest biosynthesis of anthocyanins in pomegranates as previously indicated by Turfan et al. (2011).

3.2.4. Antioxidant capacity

Pomegranate exhibits good antioxidant capacity primarily due to its high levels of total phenolic concentrations including flavonoids, anthocyanins and other polyphenol compounds (Kulkarni and Aradhya, 2005; Ayhan and Eştürk, 2009). In comparison to freshly harvested fruit, the radical scavenging activity (in DPPH assay) and ferric ion reducing antioxidant power (FRAP assay) increased in both cultivars at all the storage temperatures after 4 weeks (Figure 5). Subsequently, however, the antioxidant capacities declined gradually at most of the storage temperatures (Figure 5). The radical scavenging activity declined with increasing storage temperature whereas ferric reducing antioxidant power declined with increasing storage temperature (Figure 5). In terms of human health, the higher the level of phenolic compounds the higher the total antioxidant activity of pomegranate fruit juice and its relative human health benefit (Aviram et al., 2000; Gil et al., 2000; Tzulker et al., 2007). Individual phenolic components such as ellagic acid derivatives and hydrolysable tannins as well as anthocyanins have been implicated in antioxidant capacity of pomegranate fruit (Gil et al., 2000; Shwartz et al., 2009). Interestingly, the observed changes in total anthocyanins coincided with the trends observed in the antioxidant capacity exhibited by fruit during postharvest storage, suggesting that anthocyanins could make important contribution to the exhibited antioxidant capacity.

3.2.5. Weight loss and incidence of storage disorders and decay

Weight loss: There were significant ($p < 0.0001$) differences in fruit weight loss among the storage temperatures (Figure 6). In the first 4 weeks of storage in both cultivars, fruit weight loss was higher at room temperature compared to other temperatures. The combination of higher temperature and lower relative humidity in room storage contributed to the high fruit weight loss between 20 - 25%. The experiment at room temperature had to be discontinued due to complete loss of fruit after 4 weeks (Figure 6). Weight loss in fruit stored at 10°C was significantly higher than those stored at 7°C and 5°C after 4, 8 and 12 weeks of storage, and this might be due to higher fruit respiration at 10°C. After 8 weeks of storage, a notable increase in weight loss was found at all the storage temperatures. At the same period, weight loss ranging between 12 - 15% was observed in fruit stored at 10°C in both cultivars, resulting in noticeable shriveling. However, weight loss remained below 10% at 7°C and 5°C and no sign of shriveling was

observed at the same period. These observations contrast with the study by Kader et al. (1984), who reported that weight loss of 5% or more resulted in visible shriveling on 'Wonderful' pomegranate fruit during cold storage. Pomegranate is highly susceptibility to weight loss due to high porosity of the fruit peel which permits free water vapour movement (Elyatem and Kader, 1984), and the susceptibility is affected by storage condition and type of cultivar. When compared with previous studies, 'Bhagwa' and 'Ruby' lost more weight after 4 weeks of storage than 'Wonderful' under similar storage conditions and duration (Elyatem and Kader, 1984). The authors reported fruit 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. However, the findings from the present study were comparable to those communicated by Al-Mughrabi et al. (1995), who reported weight loss of 18.32%, 21.93% and 32.83% in fruit stored at 5°C, 10°C and room temperature, respectively, after 6 weeks of storage. Weight loss in fruit stored at 5°C and 7°C did not differ significantly ($p < 0.05$) until 12 weeks and 16 weeks of storage for 'Ruby' and 'Bhagwa', respectively (Figure 6). However, significant differences were observed afterwards, with higher weight loss in fruit stored at 7°C than at 5°C. Overall, weight loss increased with increasing storage temperature and prolonged storage duration, both with significant effects ($p < 0.0001$) (Figure 6). The higher relative humidity (>92%) in the 5°C storage would have also contributed to the lower weight loss of fruit due to the combined effects of lower respiration and transpiration.

Storage disorders and decay: The severity and occurrence of physiological disorder (aril browning and decay, husk scalding and external decay) were generally low in fruit stored at lower temperature but the disorders clearly became severe with prolonged storage (Figure 7). Fruit stored at room temperature for 4 weeks exhibited the highest incidence and occurrence of physiological disorders, with rapid deterioration of visual appeal. About 60% of 'Bhagwa' and 40% of 'Ruby' fruit were affected by internal disorders such as browning and decay, whereas all fruit exhibited external disorders mainly due to shriveling and discolouration as well as rots for both cultivars resulting in complete loss of fruit after 4 weeks of storage (Figure 7 D & E). No external defects were observed on fruit stored at 5°C and the proportion of fruit affected by internal disorders was below 5%. Furthermore, the incidence of disorders was below the moderate level for both cultivars (Figure 7A, B, C & D). This is in agreement with Kader et al. (1984), who found no visible decay in 'Wonderful' pomegranate fruit stored at 5°C for 4 weeks. Although the severity of aril browning and decay was higher in 'Bhagwa' fruit stored at 5°C than

those stored at 7°C after 8 weeks of storage, the proportion of fruit affected at 5°C was relatively lower than on fruit stored at 7°C (Figure 7A, B, C & D). A similar observation was also observed for external decay and scalding on fruit stored at 5°C and 7°C for 'Ruby' after 8 weeks of storage (Figure 7C). After 12 weeks of storage however, scalding and shivering increased in all the fruit and deterioration became more noticeable in both cultivars.

Fruit stored at 10°C were discarded after 12 weeks of storage due to high incidence of decay and fungal attacks. In both cultivars, about 40% and over 60% of fruit were affected by internal and external defects, respectively, (Figure 7A, B, C & D). Fruit stored at 5°C and 7°C for up to 12 weeks clearly became more susceptible to both internal and external decay and fungal attacks. In contrast to our findings, Artes et al. (1998) reported visible decay in 'Mollar de Elche' pomegranate fruit after 12 weeks of storage at 5°C. Differences in decay incidence and severity in pomegranate could be due to the level of infection by pathogens at harvest, since the fruit were not treated with postharvest fungicides before storage. This may also be related to pre-harvest temperatures, fruit maturity and cultural practices (Elyatem and Kader, 1984).

4. Conclusion

The results of this study showed that postharvest physiological responses, quality and antioxidant activities of pomegranate fruit are affected by storage temperature and duration. Increase in fruit respiration with increasing storage temperature suggested that the investigated cultivars might be susceptible to temperature abuse and even small increases in temperature could increase respiration rates, which ultimately could lead to fruit compositional changes and quality loss. Pomegranate fruit colour, composition and antioxidant capacity were affected by the storage temperatures investigated. A gradual decrease in total soluble solid content and titratable acidity was observed over time at different temperatures. In addition, it was clearly shown that weight loss and decay are major postharvest problems affecting the investigated pomegranate fruit cultivars during a prolonged storage period. Fruit weight loss was significantly affected at all temperatures throughout the storage durations investigated, with the highest weight loss occurring at elevated temperatures.

Although total phenolic concentration declined when fruit were storage at 5°C when stored beyond 8 weeks, the antioxidant capacities in fruit did not decline dramatically. The severity and occurrence of physiological disorder such as husk scald were generally low in fruit

stored at lower temperatures but fruit deterioration and decay increased with increasing storage duration. Storing fruit at 5°C and 92% RH clearly resulted in reduced weight loss, lower incidence of physiological disorders, no chilling injury, and maintenance of quality parameters that relate to fruit flavor. Hence, it is recommended that the investigated cultivars grown in the sampling area may be stored at 5°C and >92% RH for 8 to 12 weeks without severe deterioration in quality attributes and market value.

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Table 1

Changes in chemical quality attributes of pomegranate fruit ('**Bhagwa**') during storage at different temperatures during 2011 season

Parameter	Temp. (°C)	Harvest	Storage duration (week)					Significance level[1]		
			4	8	12	16	0+4+8+12	Temp. (A)	Storage week (B)	A*B
pH		3.35^{e}								
	5		3.80^{b}	3.85^{b}	3.88^{b}	4.08^{a}	3.43^{b}	**< 0.0001**	**< 0.0001**	0.1943
	7		3.66^{c}	3.81^{b}	3.91^{b}	3.86^{b}	3.79^{a}			
	10		3.30^{ef}	3.51^{d}	3.48^{d}	-	3.84^{a}			
	RT		3.19^{f}	-	-	-				
TA (% citric acid)		0.34 ^{a}								
	5		0.30^{abc}	0.25^{cde}	0.24^{de}	0.18^{f}	0.27^{a}	0.0514	0.3732	0.0563
	7		0.28^{cd}	0.25^{cde}	0.24^{de}	0.22^{ef}	0.26^{a}			
	10		0.26^{cde}	0.29^{bc}	0.27^{cde}	-	0.26^{a}			
	RT		0.33^{ab}	-	-	-				
TSS (°Brix)		16.18 ^{a}								
	5		16.25^{a}	16.14 ^{a}	15.66 ^{ac}	14.40 ^{d}	15.79^{a}	0.1473	**0.0013**	0.9255
	7		16.02^{ab}	15.84 ^{abc}	15.10 ^{cd}	15.24 ^{bc}	15.65^{b}			
	10		16.03 ^{ab}	15.88 ^{abc}	15.46 ^{bc}	-	16.02^{a}			
	RT		15.08 ^{cd}	-	-	-				
TSS/TA		48.53 ^{d}								
	5		55.63 ^{cd}	64.37 ^{bc}	64.30 ^{bc}	78.45 ^{a}		0.3406	0.2975	**0.0360**
	7		58.62 ^{c}	63.14 ^{bc}	62.71 ^{bc}	70.13 ^{b}				
	10		62.15 ^{bc}	55.32 ^{cd}	58.84 ^{c}	-				
	RT		45.57 ^{d}	-	-	-				

BrimA		15.50 [ab]							
	5		15.69 [a]	15.64 [a]	15.17 [abc]	14.03 [d]	15.25[a]	0.1495	**0.0022**	0.9036
	7		15.46 [ab]	15.34 [abc]	14.62 [cd]	14.80 [cd]	15.14[a]			
	10		15.51 [ab]	15.30 [abc]	14.92 [cd]	-	15.49[b]			
	RT		14.22 [cd]	-	-	-				

Different letters across storage temperatures and storage durations for each fruit attribute differ significantly (p<0.05) according to Duncan's multiple range test. TA- titratable acidity; TSS- total soluble solids. RT = Room temperature. Storage trials for fruit stored at room temperature and 10°C were discontinued after 4 weeks and 12 weeks, respectively, due to complete fruit loss. [1]In other to determine the interaction effects between storage temperatures (A) and storage durations (B), data for fruit stored at 10°C, 7°C and 5°C for week 4, week 8 and week 12 storage periods were used.

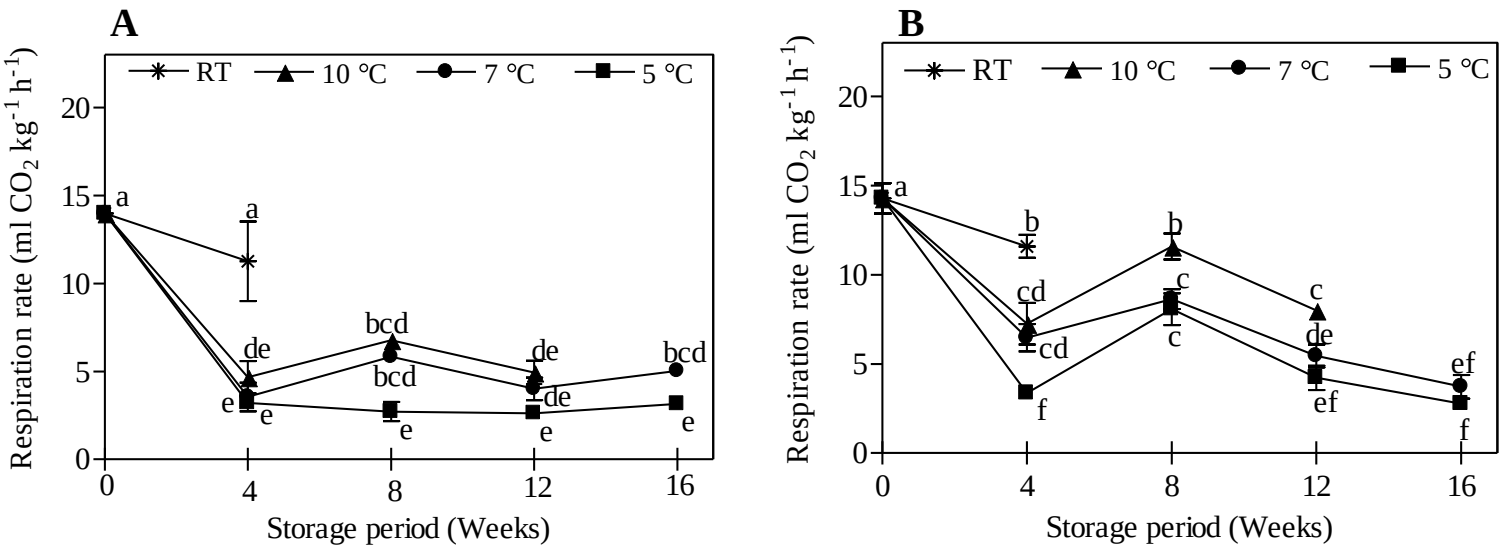
Table 2

Changes in chemical quality attributes of pomegranate fruit (**'Ruby'**) during storage at different temperatures

Parameter	Temp. (°C)	Harvest	Storage duration (week)					Significance level[1]		
			4	8	12	16	0+4+8+12	Temp. (A)	Storage week (B)	A*B
pH		3.18[e]								
	5		3.49 [dc]	3.65 [b]	3.40 [d]	3.96 [a]		0.7178	0.3187	**<0.0001**
	7		3.48 [dc]	3.44 [d]	3.66 [b]	3.70 [b]				
	10		3.68 [b]	3.39 [d]	3.40 [d]	-				
	RT		3.61 [bc]	-	-	-				
TA (% citric acid)		0.33[a]								
	5		0.30 [abc]	0.28 [abc]	0.22 [ef]	0.20 [f]		0.0139	<0.0001	**0.0036**
	7		0.29 [abc]	0.26 [cd]	0.24 [de]	0.21 [ef]				
	10		0.32 [ab]	0.23 [def]	0.22 [ef]	-				
	RT		0.32 [ab]	-	-	-				
TSS (°Brix)		17.07 [a]								
	5		17.00 [ab]	16.15 [abc]	15.32 [de]	14.90 [e]	16.16[a]	0.5702	**<0.0001**	0.7582
	7		16.98 [ab]	15.72 [cd]	15.42 [cde]	14.78 [e]	16.04[a]			
	10		16.85 [ab]	15.85 [bc]	15.20 [de]	-	15.97[a]			
	RT		15.90 [bc]	-	-	-				
TSS/TA		52.12 [e]								
	5		57.18 [de]	54.72 [e]	70.89 [bc]	77.21 [a]		0.0067	0.0011	**0.0041**
	7		59.07 [de]	61.14 [cde]	64.40 [cd]	71.91[ab]				
	10		51.93 [e]	69.13 [bc]	70.01 [bc]	-				
	RT		49.69 [e]	-	-	-				

BrimA		16.40 [a]							
	5		16.42 [a]	15.56 [bc]	14.88 [cde]	14.51 [de]	16.59 [a]	0.7511	**<0.0001**	0.7564
	7		16.37 [a]	15.20 [cd]	14.94 [cde]	14.37 [e]	15.51 [b]			
	10		16.20 [ab]	15.39 [bc]	14.76 [cde]	-	15.45 [a]			
	RT		15.28 [bc]	-	-	-				

Different letters across storage temperatures and storage durations for each fruit attribute differ significantly (p<0.05) according to Duncan's multiple range test. TA- titratable acidity; TSS- total soluble solids. RT = Room temperature. Storage trials for fruit stored at room temperature and 10°C were discontinued after 4 weeks and 12 weeks, respectively, due to complete fruit loss. [1]In other to determine the interaction effects between storage temperatures (A) and storage durations (B), data for fruit stored at 10°C, 7°C and 5°C for week 4, week 8 and week 12 storage periods were used.



See appendix: paper 8 for pooled mean values

Significance level[1]

Cultivar	Source	*Prob. > F*
'Bhagwa'	Storage temperature (*A*)	**0.0221**
	Storage duration (*B*)	**< 0.0001**
	*A*B*	0.0740
'Ruby'	Storage temperature (*A*)	**< 0.0001**
	Storage duration (*B*)	**< 0.0001**
	*A*B*	0.4279

Figure 1. Respiration rate of pomegranate fruits during storage at different temperatures; 'Bhagwa' (**A**) and 'Ruby' (**B**). Different letters on lines represent statistical differences (p<0.05) using Duncan's multiple range test. RT = Room temperature. Storage trials for fruit stored at room temperature and 10°C were discontinued after 4 weeks and 12 weeks, respectively, due to complete fruit loss. [1]In other to determine the interaction effects between storage temperature and storage duration, data for fruit stored at 10°C, 7°C and 5°C for week 4, week 8 and week 12 storage periods were used.

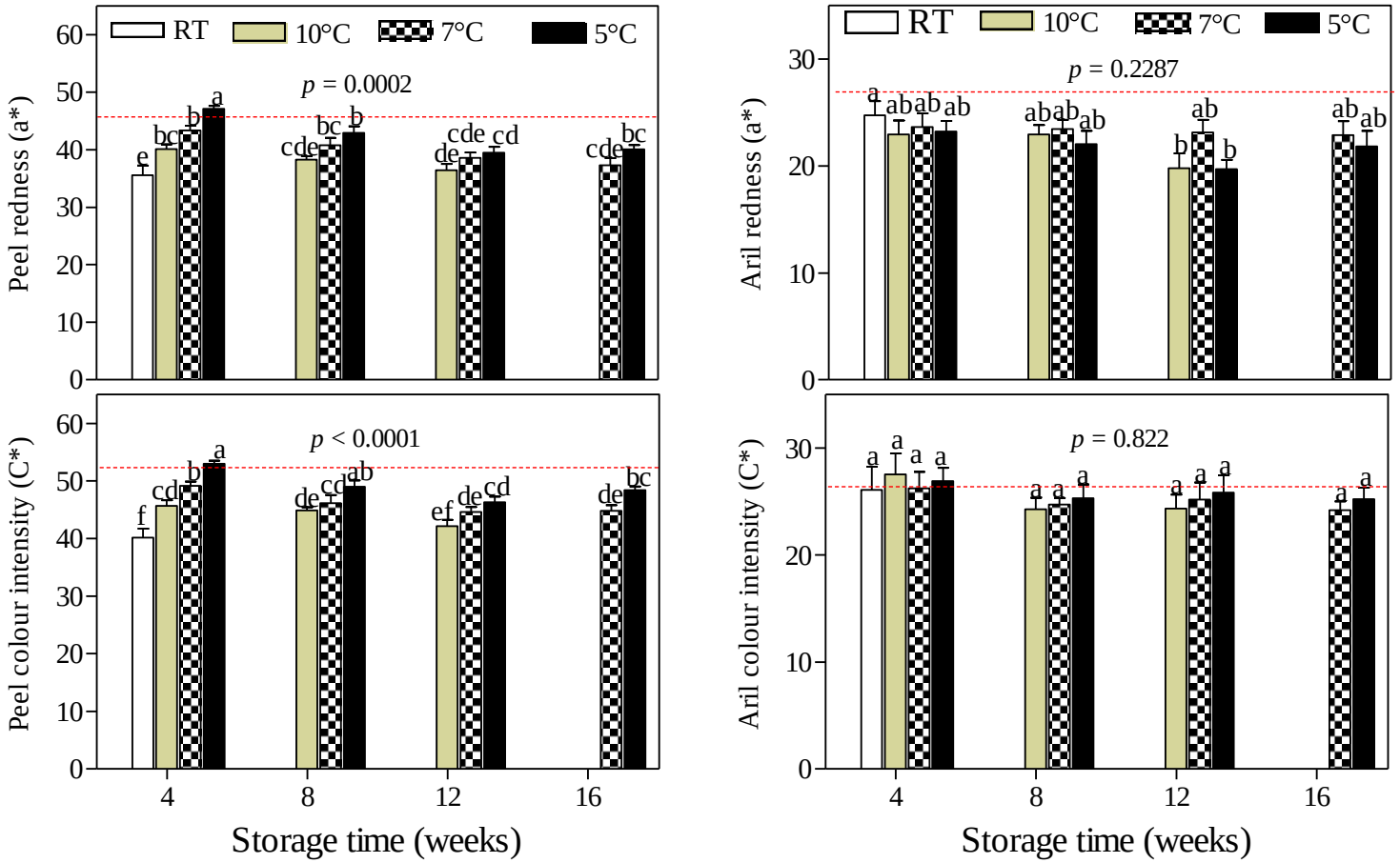


Figure 2. Changes in the CIE *a** and *C** colour parameters on pomegranate fruit peel and aril during storage at different temperatures (**'Bhagwa'**). Different letters on bars represent statistical differences ($p<0.05$) using the Duncan's multiple range test. RT = room temperature. Storage trials for fruit stored at room temperature and 10°C were discontinued after 4 weeks and 12 weeks, respectively, due to complete fruit loss. --- represents measurements at harvest.

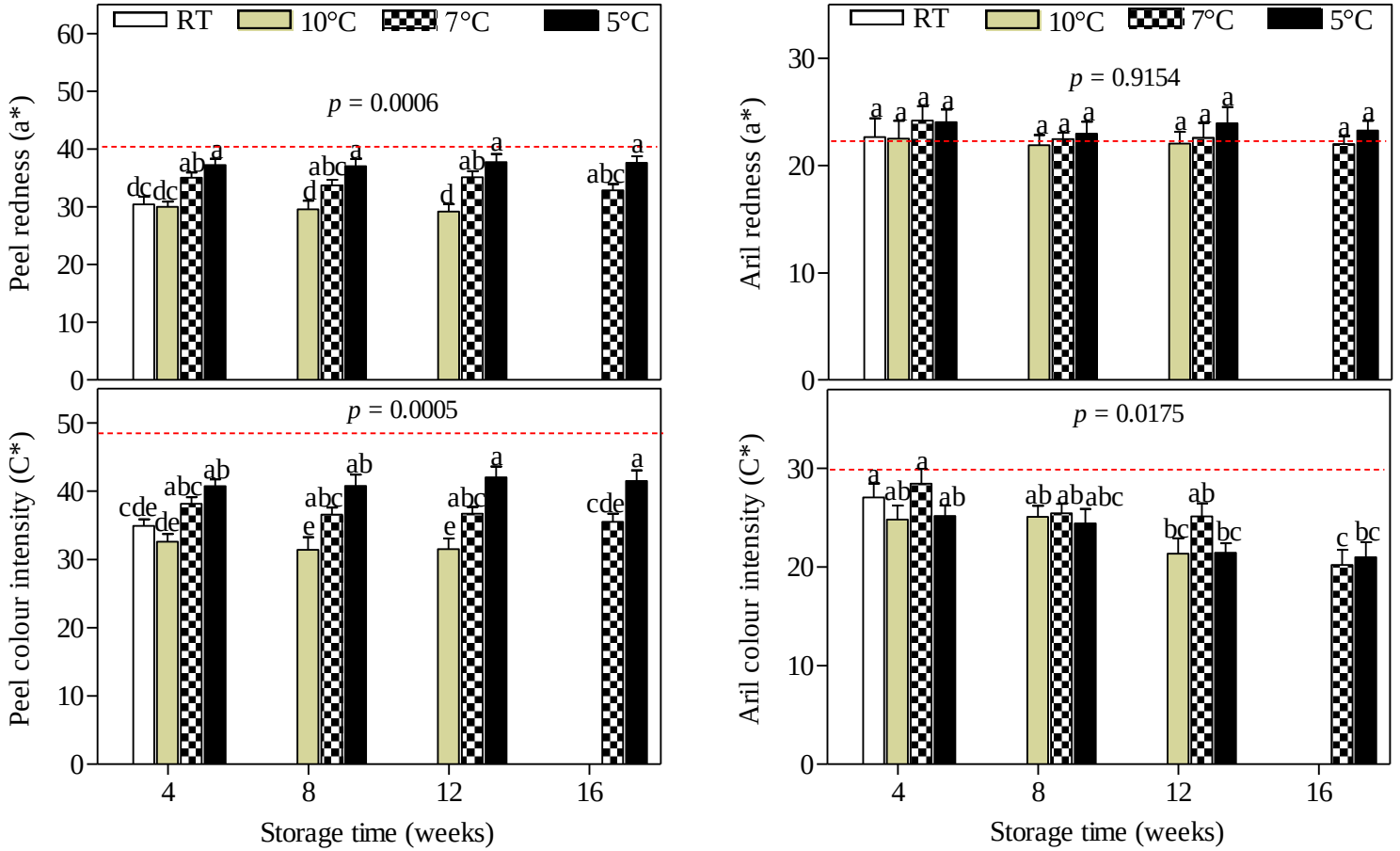


Figure 3. Changes in the CIE *a** and *C** colour parameters on pomegranate fruit peel and aril during storage at different temperatures (**'Ruby'**). Different letters on bars represent statistical differences ($p<0.05$) using the Duncan's multiple range test. RT = room temperature. Storage trials for fruit stored at room temperature and 10°C were discontinued after 4 weeks and 12 weeks, respectively, due to complete fruit loss. --- represents measurements at harvest.

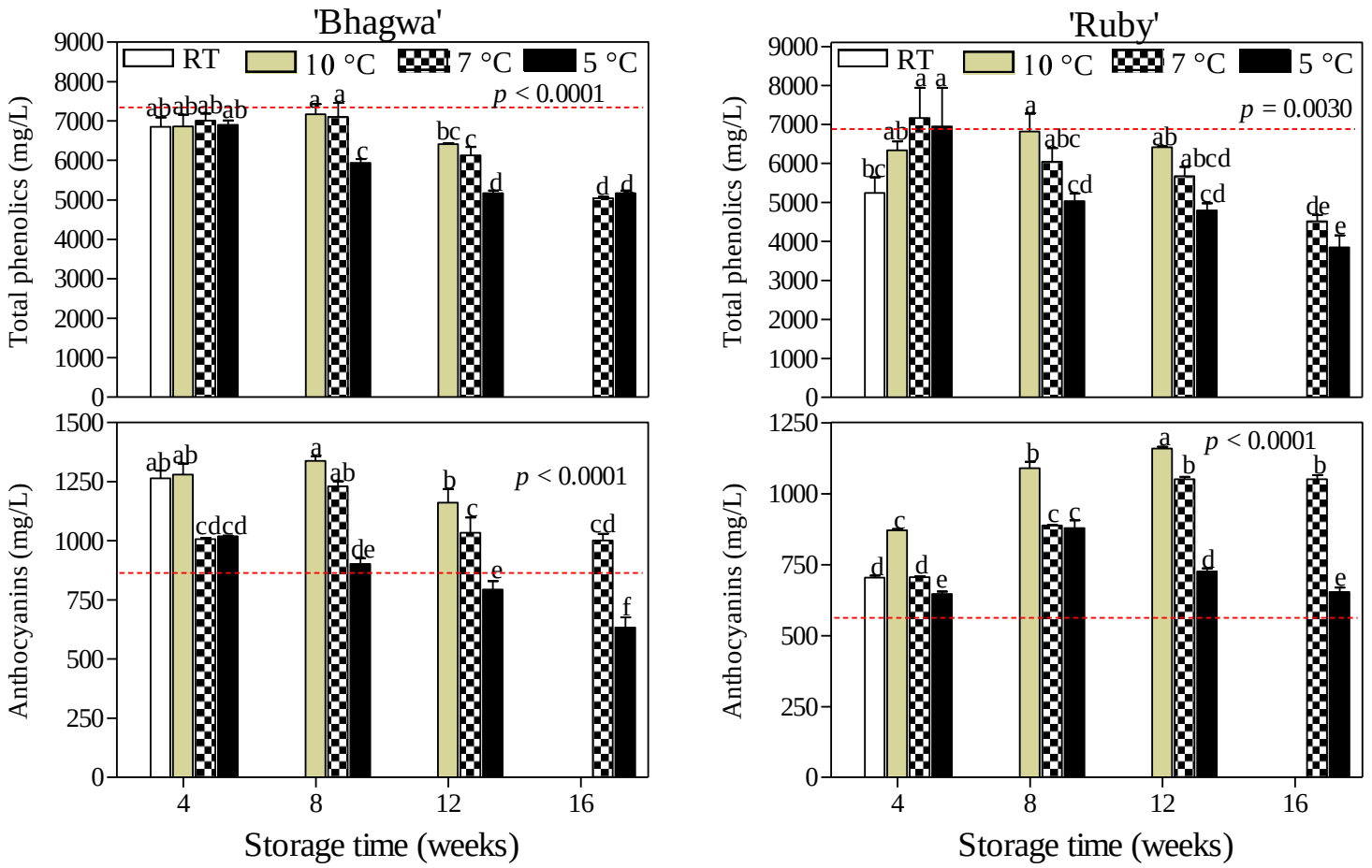


Figure 4. Change in total phenolic (gallic acid equivalent) and anthocyanin (cyanidin 3-glucoside equivalent) concentrations of pomegranate fruit juice ('Bhagwa' and 'Ruby') during storage at room temperature (RT), 10°C, 7°C and 5°C. Different letters on bars represent statistical differences (p<0.05) using Duncan's multiple range test. Storage trials for fruit stored at room temperature and 10°C were discontinued after 4 weeks and 12 weeks, respectively, due to complete fruit loss. ---- represents measurements at harvest.

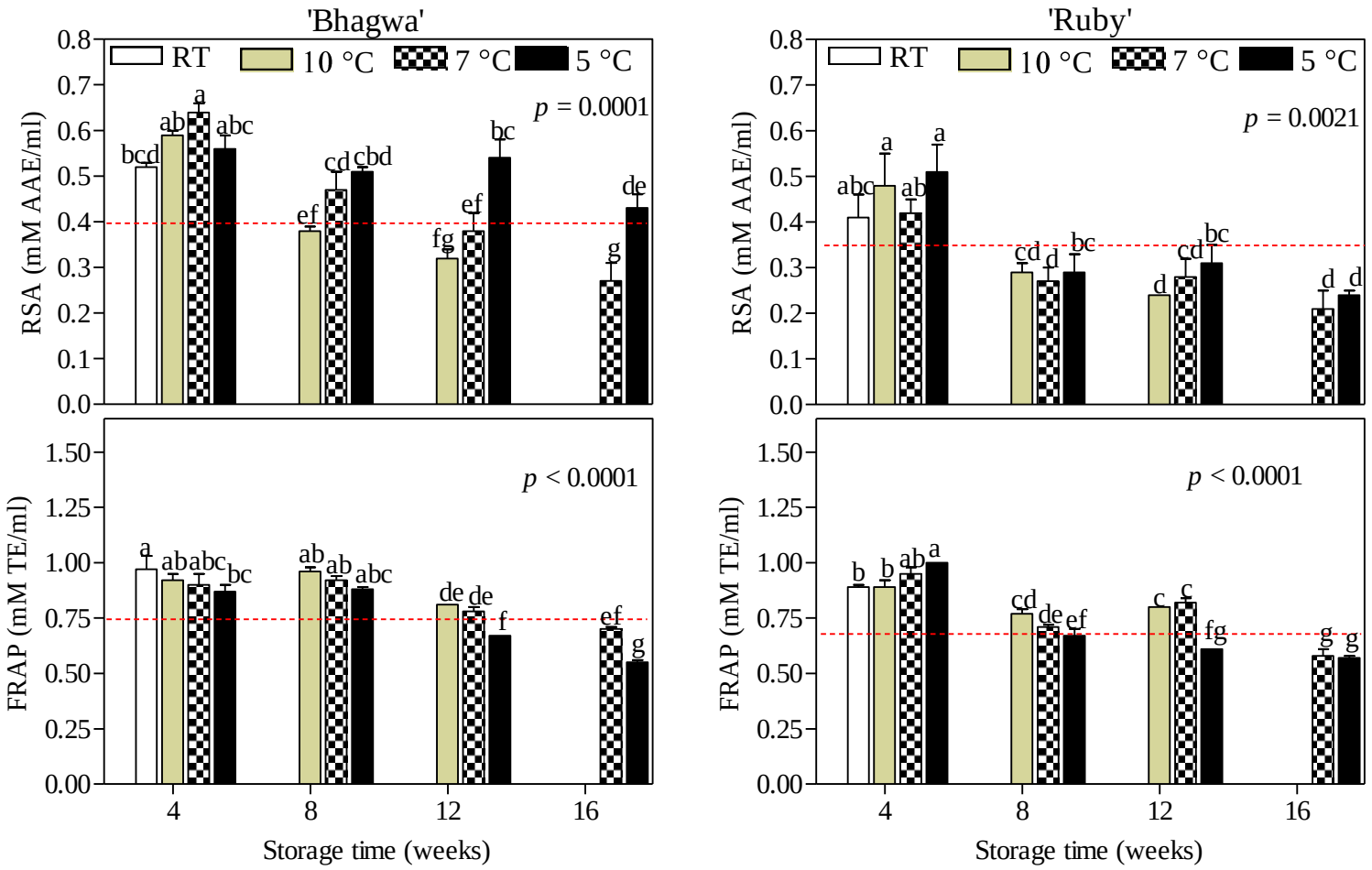
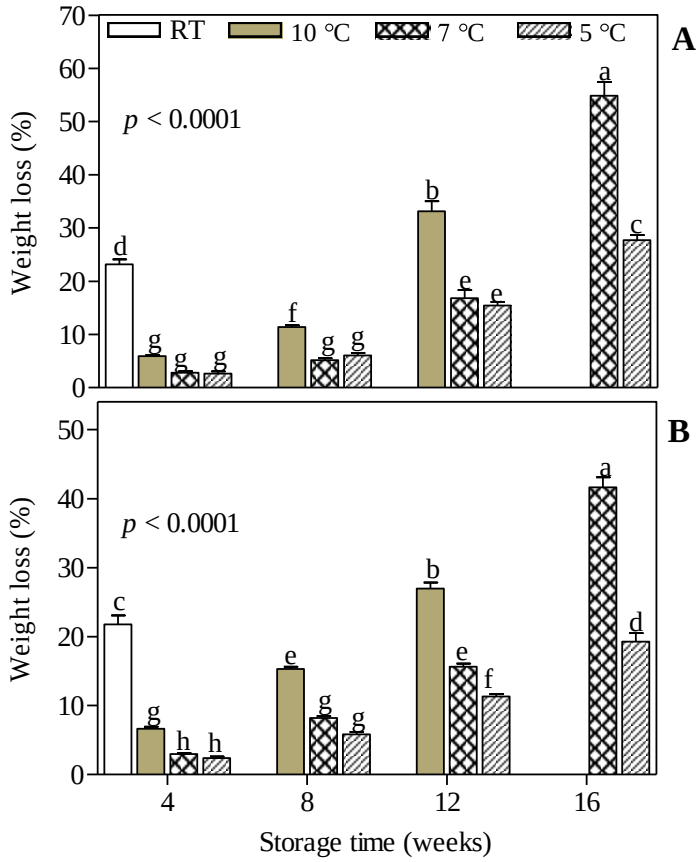


Figure 5. Change in antioxidant capacity of pomegranate fruit juice ('Bhagwa' and 'Ruby') during storage at room temperature (RT), 10°C, 7°C and 5°C. RSA - Radical scavenging activity; FRAP - Ferric reducing antioxidant power. Different letters on bars represent statistical differences (p<0.05) using Duncan's multiple range test. Storage trials for fruit stored at room temperature and 10°C were discontinued after 4 weeks and 12 weeks, respectively, due to complete fruit loss. ---- represents measurements at harvest.



Significance level

Cultivar	Source	*Prob. > F*
'Bhagwa'	Storage temperature (*A*)	< 0.0001
	Storage duration (*B*)	< 0.0001
	*A*B*	**< 0.0001**
'Ruby'	Storage temperature (*A*)	< 0.0001
	Storage duration (*B*)	< 0.0001
	*A*B*	**< 0.0001**

Figure 6. Weight loss of pomegranate fruits during storage at different temperatures for 'Bhagwa' (**A**) and 'Ruby' (**B**). Different letters on bars represent statistical differences ($p<0.05$) using Duncan's multiple range test. Storage trials for fruit stored at room temperature and 10°C were discontinued after 4 weeks and 12 weeks, respectively, due to complete fruit loss. [1]In other to determine the interaction effects between storage temperature and storage duration, data for fruit stored at 10°C, 7°C and 5°C for week 4, week 8 and week 12 storage periods were used.

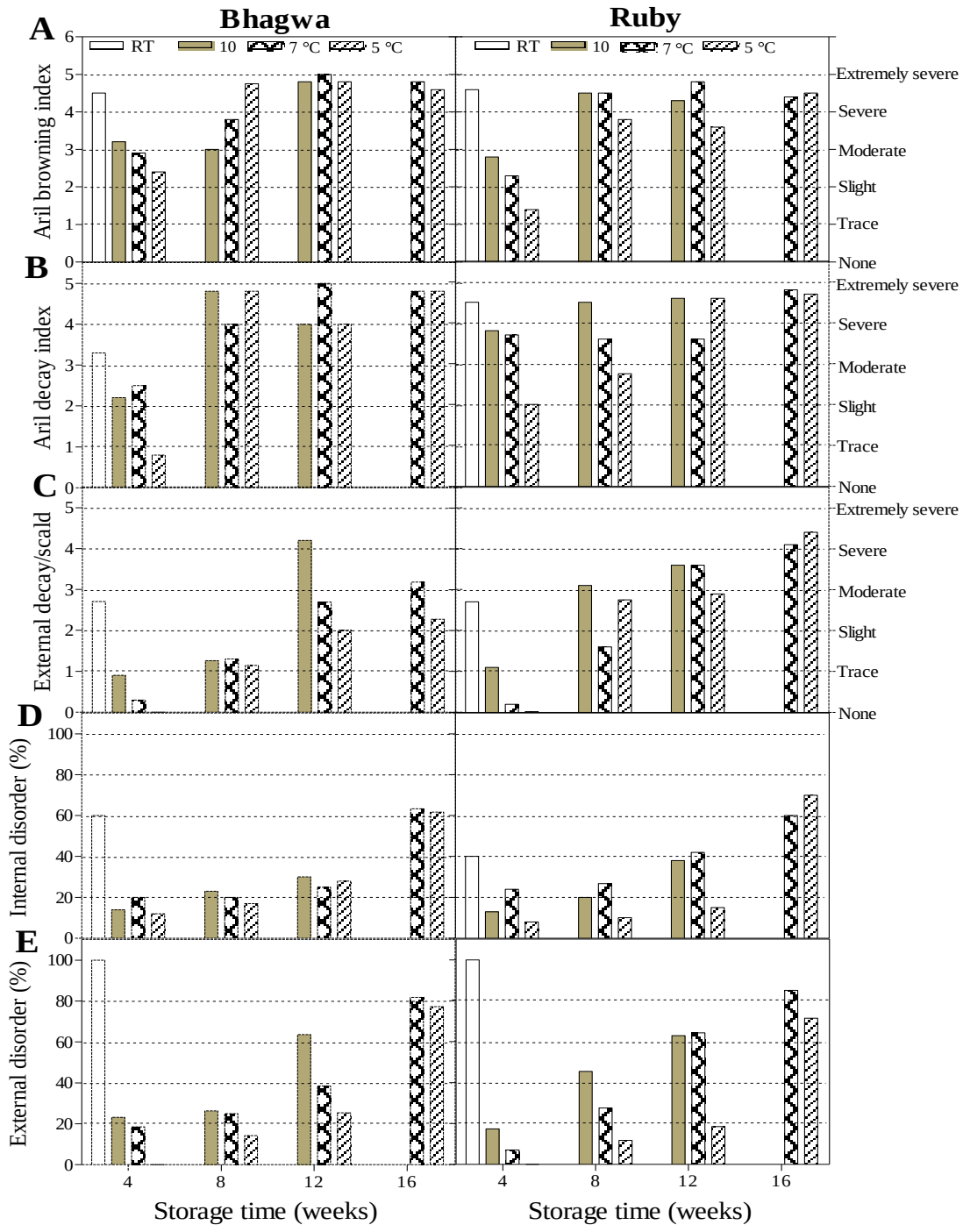


Figure 7. Occurrence of physiological disorders in pomegranate ('Bhagwa' and 'Ruby') during storage at different temperatures. Disorder indices: aril browning (**A**), aril decay (**B**), external decay/scalding (**C**). Percentage of fruit affected by: internal disorders (**D**), external disorder (**E**).

PAPER 9

Harvest maturity discrimination of pomegranate fruit by instrumental and sensory measurements during storage and shelf life conditions

Abstract

Harvest maturity discrimination was carried out for two pomegranate cultivars ('Bhagwa' and 'Ruby') in simulated handling conditions for long distant supply chains. Fruit were harvested at three different maturities (H1: 157 days after full bloom (DAFB), H2: 167 DAFB, H3; 175 DAFB for 'Bhagwa' and H1: 133 DAFB, H2; 143 DAFB, H3: 157 DAFB for 'Ruby'). The effects of harvest maturity on fruit quality attributes during a 6-week period of cold storage (5°C, 95% RH) plus subsequent 5 days of shelf life (20°C, 75% RH) were investigated. Instrumental evaluation of aril colour, juice content, juice absorbance (520 nm), total soluble solids (TSS), pH, titratable acids (TA) and phytochemical components including total phenolics, flavonoids and anthocyanins were carried out. Textural properties of arils which included hardness, toughness, bioyield point and Young's modulus were also investigated. After the shelf life period, arils from individual fruit were rated by a trained sensory panel based on appearance, taste and texture. The combination of instrumental and descriptive sensory data was used to discriminate fruit harvest maturity using discriminant analysis. Among the attributes evaluated, TSS, juice content, aril hardness and anthocyanin for 'Bhagwa' and TSS:TA, sweet taste and the CIE hue angle ($h°$) for 'Ruby' were the most decisive attributes distinguishing the harvest maturities. The results showed that to ensure the best post-storage quality of 'Bhagwa', the optimum harvest maturity was between 167 - 175 DAFB (H2 and H3) when fruit had reached maximum TSS level (> 16°Brix; H3) and juice content (> 65 mL/100 g aril; H2). However, for 'Ruby', this study indicated that the optimum date for harvesting was at 143 DAFB (H2) when fruit TSS:TA ratio was > 55, which coincided with significantly higher rating for sweet taste in fruit at H2 than at H1 and H3 after shelf life. In addition, discriminant analysis (DA) showed the possibility of combining these decisive harvest parameters with aril colour intensity to form a potential co-index suitable for distinguishing fruit harvest maturity. The harvest index proposed in this study could be used as a guide to establish a reliable harvest maturity index to assist in assuring fruit quality in consideration of long supply chains for the investigated cultivars.

Keywords: Pomegranate, Sensory attributes, Storage, Harvest index, Discriminant analysis

1. Introduction

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family. The fruit has long been valued for its flavourful and juicy edible part (aril), and more lately for commercial juice production (Wetzstein et al., 2011). Rising consumer interest and awareness on pomegranate as a medicinal food has spurred global increase in production and marketing of pomegranate fruit and its products. Commercial orchards of different pomegranate cultivars are grown in different countries (Al-Said et al., 2009; Holland et al., 2009; Opara et al., 2009; Bchir et al., 2010), with 90% of the world pomegranate production occurring in the Northern Hemisphere (Holland et al., 2009; Citrogold, 2011). South Africa is one of the recognized producers in the Southern Hemisphere, competing with countries such as Chile, Australia, Peru and Argentina. Consequently, the export opportunity has, in recent years, encouraged large scale production to increase exports, allowing producers to fill the counter-season window during spring and early summer months in Northern Hemisphere (Brodie, 2009). Over 400,000 cartons of pomegranate fruit were exported from South Africa during the 2012 harvest season, mainly to European countries, Far Eastern countries and Canada (Brodie, 2009; Citrogold, 2012; Perishable Products Export Control Board, 2012).

Pomegranate fruit is non-climacteric and thus cannot continue the ripening process after detachment from the parent plant (Kader, 2006). Fruit maturity indices are usually based on a wide range of physico-chemical characteristics that are judged either objectively or subjectively. Although quality parameters such as fruit size, shape and peel colour are important external attributes for grading and marketing, fruit peel colour does not indicate the extent of ripening or readiness of the arils for consumption (Kader, 2006; Holland et al., 2009); hence, additional maturity indices such as peel colour, total soluble solids and acidity are commonly used in fruit quality assessment to meet market requirements (Ben-Arie et al., 1984; Cristosto et al., 2000; Kader, 2006; Martinez et al., 2006). According to Kader (2006), the maximum titratable acidity of fruit may be 1% in sweet cultivars and 1.5 to 2% in sweet-sour cultivars, while minimum soluble solids vary from 15 to 17%. Chace et al. (1981) established a maturity standard for

'Wonderful' grown in California based on their finding that 1.8% titratable acidity level and total soluble solids content above 17% was the most satisfactory maturity standard. Other authors proposed that the minimum harvest maturity indices for Califonia-grown 'Wonderful' pomegranates were red juice colour equal to or darker than Munsel colour chart 5R-5/12 and titratable acidity below 1.85% (Elyatem and Kader, 1984; Kader et al., 1984). These characteristic quality attributes are acquired during fruit maturation and ripening and are cultivar dependent (Ben-Arie et al., 1984; Al-Maiman and Ahmed, 2002; Gil et al., 1996). As a result, the choice of a reliable harvest index should reflect the quality requirements of harvested fruit and also enable postharvest delivery of fruit to consumers based on desired nutritional, sensory and antioxidant attributes (Kader, 2008).

Sensory quality attributes and nutritive value of fruit play an important role in consumer satisfaction and repeat purchase. Juice taste, aroma, aril texture and appearance are generally considered important sensory attributes (Gadze et al., 2011). However like other fruits, pomegranate also undergoes postharvest quality losses during handling and storage. Apart from common external postharvest quality defects such as bruises, decay and water loss, leading to browning symptoms in both peel and arils (Kader, 2006; Mirdehghan et al., 2006), internal quality losses also occur. These include loss of colour as a result of degradation of anthocyanin (Turfan et al., 2011), and decrease in vitamin C concentration, total soluble solids (TSS) and titratable acidity (TA), which are accompanied by a reduction of acceptability in terms of freshness, juiciness and taste (Gil et al., 1996; Artes et al., 2000; Nanda et al., 2001; Labbé et al., 2010; Turfan et al., 2011). In addition, pomegranate fruit is highly susceptible to chilling injury if stored at low temperatures (Elyatem and Kader, 1984; Kader et al., 1984; Kader, 2006). Chilling injury often results to external symptoms including browning of the peel and increased susceptibility to decay, while internal symptoms included loss in red colour of the arils and brown discolouration of the white membrane depending on storage temperature and duration (Kader et al., 1984). Husk scald is also a common problem in pomegranate fruit. It is a superficial browning which generally develops from the stem end of the fruit and then spreads towards the blossom end as it increases in severity (Ben-Arie and Or, 1986; Defilippi et al., 2006). Internal breakdown is another physiological disorder in pomegranates, resulting to light streaky appearance and a 'flat' taste of the arils (Pantastico, 1975).

Currently, there are no quality standards for the South African pomegranate industry. Neither is there a general consensus on the optimal harvest maturity indices for fruit cultivars. This information is important to ensure postharvest delivery of good quality fruit to consumers, particularly for long supply chains. In the absence of objective maturity indices, calendar dates are commonly used to determine harvest periods (Brodie, 2009; Olivier, F., pers. comm., 2011), or when fruit calyx closes (Citrogold, 2011). To ensure an appropriate balance between harvest maturity and postharvest quality, it is necessary to define picking maturity standards (Brown and Walker, 1990). In order to determine this, it is important to investigate the effects of narrow but different maturity stages at harvest on changes in physico-chemical and textural properties of fruit in consideration of long supply chains.

The objective of this study was to determine reliable harvest maturity indicators for optimum postharvest performance of pomegranate fruit based on a combination of sensory and instrumental quality attributes.

2. Materials and methods

2.1. Fruit

Two pomegranate 'Ruby' and 'Bhagwa' grown on a commercial pomegranate orchard in Porterville (Western Cape, South Africa) were investigated. In other to ensure comparable crop load and fruit size, flower and fruit thinning was carried out on tagged trees at the beginning of the season. Fruit were harvested at three harvest maturities (H1 - H3) between March and April in 2012 from 4 year-old trees during the usual harvest season. For 'Ruby', first harvest (H1) occurred at 133 days after full bloom (DAFB) on 16th March, the second harvest (H2) on 26th March (143 DAFB) and the third harvest (H3) on 9th April (157 DAFB). Harvest times of 'Bhagwa' occurred as follows; H1- 9th April (157 DAFB); H2- 19th April (167 DAFB); H3- 27th April (175 DAFB). During each harvest, a sample of 150 fruit was harvested randomly, taking care to avoid fruit with sunburn, blemish and cracks. Harvested fruit were transported in an air-conditioned vehicle to the Postharvest Technology Laboratory at Stellenbosch University where they were further sorted for colour and size uniformity before cooling overnight at room temperature. The same experimental and handling procedure was conducted in the same manner at the three separate harvests for both cultivars.

2.2. Fruit storage

The study took into account a storage period that simulated the time that elapses from harvest to consumption in the commercial export chain. Fruit were packed in single layers in 4.5 kg open top boxes with no lids according to industry practice (Citrogold, 2011). Fruit did not receive any postharvest chemical treatments. Packed boxes (15 fruits per box) were stacked on a pallet and stored at 5°C with 95% relative humidity (RH) based on current industry practice. Cold storage was terminated after 6 weeks followed by shelf life period of five days at 20°C and 75% RH to simulate a reasonable retail period (Artes et al., 2000). Temperature (°C) and relative humidity RH (%) inside the cold room were recorded on hourly basis using three Tiny Tag TV-4500 data loggers (Gemini Data Logger, Sussex, UK) with functional ranges of -40°C to +85°C and 0% to 100% RH, respectively.

2.3. Fruit quality evaluation

For each cultivar, fifteen fruit per harvest were evaluated at 2-week intervals during cold storage and at the end of shelf storage for key quality indices (aril colour, juice absorbance, total soluble solids, pH and titratable acidity). Other quality parameters such as textural and phytochemical attributes were evaluated before cold storage and during the shelf life period. Descriptive sensory evaluation was conducted only during shelf-life, when fruit was assumed to be available to consumers. Instrumental and sensory measurements were carried out using eight individual fruit at shelf life for the purpose of correlation and discriminant analyses.

2.4. Instrumental analyses

2.4.1. Colour analysis

Aril colour was assessed in CIELAB coordinates (L^*, a^*, b^*) using a Minolta Chroma Meter CR-400 (Minolta Corp, Osaka, Japan). Duplicate colour measurements were made on arils (per fruit) placed in a colourless glass Petri dish. The colour parameters chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($h^o$ = arctan (b^*/ a^*)) were calculated (Pathare et al., 2012; Al-Said et al., 2009). Pomegranate juice (PJ) colour absorbance was measured at 520 nm using a Helios Omega UV-vis spectrophotometer (Thermo Scientific technologies, Madison, USA). The data on colour

attributes are presented only for fruit before storage and after shelf life, as the changes during 6 week cold storage were negligible.

2.4.2. Measurement of pH, titratable acidity and total soluble solids

Juice extraction (without crushing the kernels) was done on the remaining arils using a blender (Mellerware, South Africa). Juice pH value was determined at room temperature using a pH meter (Crison, Barcelona, Spain). Titratable acidity (TA) was determined by titration (to a pH 8.2) using a Metrohm 862 compact titrosampler (Herisau, Switzerland), and the results were expressed as percentage tartaric acid equivalents. Total soluble solids (TSS) were measured using a digital refractometer (Atago, Tokyo, Japan). TSS/TA ratio and BrimA index, a criterion for acceptance of fruit juice expressed as BrimA = TSS - k * TA, were calculated, where k is the tongue's sensitivity index normally ranging between 2 - 10 (Jordan et al., 2001; Jaya and Das, 2003). In this study k = 2 was used (Fawole and Opara 2013a). All measurements were made on individual fruit samples.

2.4.3. Determination of total phenolic concentration

Total phenolic concentration (TPC) was determined in triplicates by the Folin-Ciocalteu colourimetric method (Makkar et al., 2000) and results were expressed as gallic acid equivalents (GAE) per 100 mL PJ.

2.4.4. Total flavonoids concentration

The total flavonoids concentration (TFC) was determined using the method described by (Yang et al., 2009) and results were expressed as catechin equivalents (CAE) per 100 mL PJ.

2.4.5. Total anthocyanin concentration

Total anthocyanin concentration (TAC) was quantified using the pH differential method (Wrolstad, 1993). In triplicates, PJ extracts (1 mL) were mixed with 9 mL of pH 1.0 and pH 4.5 buffers, separately. Absorbance was measured at 510 and 700 nm in pH 1.0 and 4.5 buffers and result was expressed as cyanidin-3-glucoside equivalents using the following equations:

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH4.0} \quad (1)$$

Total anthocyanin concentration (mg/mL) = (A x MW x DF) ÷ (ε x L) (2)

where A = Absorbance, ε = Cyd-3-glucoside molar absorbance (26,900), MW = anthocyanin molecular weight (449.2), DF = dilution factor, L = cell pathlength (1 cm). Final results are expressed as cyanidin-3-glucoside equivalents per 100 mL PJ (mg C_3gE/100 mL PJ).

2.4.6. Texture dynamics of pomegranate aril

Compression tests were performed using a texture profile analyzer (TA.XT Plus, Stable Microsystems, England) with a 35 mm compression probe. A total of 10 arils extracted from each fruit were used, and a test was run per aril aligned horizontally on the compression platform. The operating conditions of the instrument were: pre-test speed 1.5 mm/s, 0.5 mm/s test speed, 10.0 mm/s post-test speed, and 0.20 N trigger force (Al-Said et al., 2009). The force-deformation curves generated were analysed for bioyield point (N) that is defined as the point on the force-deformation curve at which the juice content in compressed aril just oozed without the tearing of the aril sac. When loading is continued beyond the bioyield point, a rupture occurs (Mohsenin, 1986). Young's modulus or elasticity (N/mm^2) was calculated as the slope from the start of the compression to that of the bioyield point. Rupture force or hardness (N) was calculated as the maximum force required for complete breakage of the test sample while the rupture energy or toughness (N.mm) was determined from the area under the curve (Al-Said et al., 2009; Bchir et al., 2010). To evaluate the influence of harvest dates on texture parameter of pomegranate aril between harvest and shelf life, a storage index (SI) was computed below according to Costa et al. (2012).

$$SI = \log_2 (T_iSL/T_iH) \quad (3)$$

where T_iH is the value of the texture property (rupture force, toughness, young modulus and bioyield) measured at harvest, and T_iSL is the value of the same property measured after shelf life. Positive SI values indicate a texture property enhancement, while negative values point to a loss of textural property at shelf life. SI equal to zero means stable maintenance of the textural trait under investigation (Costa et al., 2012).

2.5. Instrumental and sensory measurements after shelf life period

2.5.1. Sample preparation

Each fruit was manually peeled in a sterile condition and arils were placed into clean labeled beakers. After peeling, the extracted aril from each fruit was weighed and divided unequally into the 2:1 ratio, the larger portion (75%) for sensory evaluation and the other (25%) for instrumental analysis.

2.5.2. Instrumental analysis

The same experimental protocols were followed as previously described for all instrumental measurements.

2.5.3. Panel training

The training of the panel was conducted according to the consensus method (Lawless and Heymann, 1998; Koch et al., 2012). The panel was comprised of 9 members (7 women and 2 men) with previous formal sensory evaluation and descriptive analysis experience. The panelists were provided with a range of pomegranate arils at various maturity levels, followed by the development of descriptors and scores. Some of the descriptor and attribute definitions were developed through brainstorming and round-table consensus. The panelists were required to test the attributes in the order of appearance, flavour and texture. A score sheet was then developed which was used by the panel to scale the intensity of each of the descriptors on a 100 mm unstructured line scale ranging from low intensity (0 mm) corresponding to the word anchor "none" to high intensity (100 mm) corresponding to the word anchor "prominent". Descriptors that were not suitable (for instance, aroma) for the product were eliminated. Retention of a descriptor in the initial list was on the basis that the attribute should be relevant to the product and be easily perceived by each panel member. Sensory attributes were adopted from Koppel and Chambers (2010). During the final training session, the panel practised intensity rating of the individual attributes on the line scales and panel performance assessment was carried out.

2.5.4. Sensory evaluation

All sensory assessments were conducted on the same date for the harvest maturities at ambient room temperature (20°C) in the Sensory Laboratory, Food Science Department, Stellenbosch University, South Africa. Individual testing booths equipped with serving windows and controlled lighting were used. Pomegranate arils (~ 10 g per cultivar) of the three harvest times were randomized and coded with three-digit random numbers in clean Petri dishes. Sensory ratings were recorded using the Compusense® five sensory data acquisition program (Guelph, Ontario, Canada).

2.6. Statistical analysis

Mean values of all the studied variables are presented. Analysis of variance (ANOVA) was carried out using Statistica software (Statistica 11.0, StatSoft Inc., Tulsa, OK, USA) according to Duncan's multiple range test. Mean values for different harvests with storage duration were separated, and where appropriate, 2-way ANOVA was carried out. Graphical data presentations were performed using GraphPad Prism software version 4.03 (GraphPad Software, Inc. San Diego, USA). Sensory data were pre-processed for application in multivariate analyses. Panel performance was monitored using Panel Check Software (Version 1.3.2, www. panelcheck.com). In the event of significant non-normality (p<0.05) outliers were identified and removed (Koch et al., 2012). Discriminant analysis (DA) was performed (using XLStat, version 7.5.2, Addinsoft, New York, USA) on the three harvest times for each cultivar to establish if the harvest times were indeed distinctly dissimilar with both instrumental and sensory attributes investigated.

3. Result and discussion

3.1. Initial measurements of major maturity indices at fruit harvest dates

Pomegranate fruit harvested at different maturity dates showed degrees of heterogeneity in some of the key quality attributes. For both cultivars ('Bhagwa' and 'Ruby'), among the CIE aril colour parameters, only the colour intensity (C^*) statistically distinguished the harvest maturities investigated. The C^* increased significantly (p<0.05) with increasing harvest dates (Table 1 and Table 2). This suggests that the desirable red colouration in pomegranate aril

increased with delayed fruit harvest, probably due to the continued accumulation of anthocyanins with advancing maturity as previously reported for both cultivars (Fawole and Opara, 2013a; 2013b). In addition, juice contents and absorbance increased also increased with prolonged harvest dates in both cultivars, with fruit juice at H1 having significantly (p<0.05) lower values than later harvested fruit (Table 1 and Table 2).

Titratable acidity (TA) decreased with prolonged harvest dates and was significantly higher in H1 fruit (Figure 1A and Figure 1B). Drastic decline in juice acidity levels in H1 compared to H2 and H3 is an indication that fruit was properly still actively undergoing the maturity process. The observed decline in fruit acidity may be attributed to the cumulative effects of increasing fruit juice content and the use of organic acids as respiratory substrates that occur during fruit maturation (Diakou et al., 2000; Moing et al., 2001). The TA level decreased further between H2 and H3 for 'Bhagwa' (Figure 1A) whereas in 'Ruby', an increase in TA was observed between H2 and H3 (Figure 1B).

Changes in total soluble solids (TSS) showed that fruit maturity indices follow different patterns. Contrary to juice acidity pattern, total soluble solids (TSS) increased significantly (p<0.05) between H1 and H3 in both cultivars (Figure 1A and Figure 1B). TSS values increased from 15.33 °Brix (H1) to 16.88°Brix (H3) and from 15.29°Brix (H1) to 17.24°Brix (H3) in 'Bhagwa' and 'Ruby', respectively (Figure 1A and Figure 1B). TSS content in H1 'Bhagwa' fruit did not differ significantly (p>0.05) from that of H2 (Fig 1A), whereas all the harvest dates in 'Ruby' differed significantly (p<0.05) (Figure 1B). Ripening of pomegranates is closely associated with increase in TSS (Fawole and Opara, 2013a; 2013b). The observed increase in TSS with prolonged harvest dates could possibly be due to the continued hydrolysis of starch to sugars with increased ripening time (Kulkarni and Aradhya, 2005). As a result of changes in TSS and TA, fruit TSS:TA ratios and BrimA also increased significantly (p<0.05) with prolonged harvest in both cultivars (Figure 1A and Figure 1B). Previous studies have shown the effects of different harvest dates on pomegranate fruit chemical attributes (Gil et al., 1995; Shwartz et al., 2009; Borochov-Neori et al., 2011). For example, in two accessions of 'Wonderful' grown in Israel, Shwartz et al. (2009) reported significant difference in titratable acidity, pH and total soluble solids contents of mature fruit harvested at weekly intervals.

3.2. Change in aril colour properties after storage

Differences in aril colour property measured as CIE *L**, *a**, *C**, and *h*° during cold storage at 5°C for 6 weeks did not change significantly ($p<0.05$) among the harvest dates for both cultivars (data not shown). A similar observation was reported by Gil et al. (1996), who found no significant differences in aril colour of pomegranate fruit harvested at different maturities after storage at 5°C for 1.5 months. However, overall aril colour depreciated between the initial harvest and shelf life periods (Tables 1 and 2). It appeared that aril colour depreciation was brought about by the significant ($p<0.05$) effect of harvest times on aril colour intensity (*C**) in 'Bhagwa' (Table 1). On the otherhand, depreciation in colour was significantly ($p<0.05$) influenced by fruit harvest time and storage period on colour parameters *L** and *h*° in 'Ruby' (Table 2).

The decline in juice absorbance values corroborated the decline in aril colour properties in 'Ruby' (Table 2). Absorbance values measured at 520 nm declined between initial measurements at harvest and shelf period, whereas juice absorbance did not change over time in 'Bhagwa' (Table 1). However, during shelf period, absorbance value was significantly higher in H3 compared to H1 and H2 for both cultivars (Table 1 and Table 2), suggesting H3 fruit juice remained the most red in colour. Anthocyanins are light-absorbing plant-based pigments (Shulman et al., 1984) and are responsible for the red colour of pomegranate juice (Miguel et al., 2004). Our findings indicated that fruit harvested at later dates had higher red colouration which was better maintained during postharvest handling than in early harvested fruit.

3.3. Change in pH, titratable acidity (TA) and total soluble solids (TSS) during storage and shelf life

There was a significant ($p<0.0001$) interaction effect between harvest date and storage duration on juice pH value for both cultivars (Figure 1A and Figure 1B). In 'Bhagwa', pH values declined significantly ($p<0.05$) between the initial fruit harvest date until 2 weeks and 4 weeks of cold storage in H1 and H2, respectively, having later storage times higher values of pH. However, in H3, pH values increased significantly ($p<0.05$) between the initial harvest until 6 weeks of storage. In general, there were no significant differences between all the harvest dates in pH values after 6 weeks storage (for H2 and H3) and during shelf life period (Figure 1A). In

'Ruby', however, pH values increased significantly (p<0.05) in H1 and H2 between the initial harvest (week 0) until 2 weeks of cold storage, and then increased significantly (p<0.05) during shelf life period (Figure 1B). An opposite trend was observed in H3, where pH values declined significantly (p<0.05) between fruit harvest through to shelf period. This resulted to lower pH value in H3 than in H2 and H3 after 6 weeks and during shelf life (Figure 1B). Fruit pH value is dependent on the dissociation and release of ionic hydrogen from the carboxyl group of organic acids in the fruit (Ding and Ong, 2010). The observed changes in fruit pH values during storage could be an indication of the content of and dynamics in fruit organic acids. Our study contradicts the study by Elyatem and Kader (1984), who reported no significant difference in fruit pH values in fruit ('Wonderful') stored for 8 weeks at 5°C.

In 'Bhagwa', titratable acidity (TA) decreased significantly (p<0.05) with prolonged harvest dates and during storage period. These differences remained among harvest dates in fruit during storage and shelf life period (Figure 1A). In 'Ruby' however, H1 exhibited a decreasing trend in TA level with prolonged storage, whereas in H2 and H3, TA levels first increased significantly (p<0.05) between the initial harvest period and 4 weeks and declined afterwards until shelf life period (Figure 1B). This shows that irrespective of harvest dates, fruit acidity would decline during shipping period as demonstrated in this study. The observed decline in TA could probably be due to the ongoing metabolism in the fruit during storage (Kader et al., 1984). Our findings agree with Artes et al. (2000), who reported significant decreases in pomegranate fruit (cv. 'Molla de Elche') stored at 5°C for 90 days and six additional days at 20°C. The interaction effect between harvest dates and storage duration played a significant ('Bhagwa': p = 0.0012; 'Ruby': p<0.001) role on fruit TA levels during the simulated storage 6-week storage at 5°C plus subsequent shelf life of 5days at 20°C (Figure 1A and Figure 1B).

In general, H3 had higher total soluble solids (TSS) contents than in earlier harvests and the difference were maintained during storage and shelf life period in both cultivars (Figure 1A and 1B). In 'Bhagwa', total soluble solids (TSS) in H1 and H3 was unaffected until 4 weeks of storage and then decreased significantly (p<0.05) afterwards, whereas in H2, the TSS did not change until shelf life period (Figure 1A). Similarly, in 'Ruby', TSS contents decreased significantly (p<0.05) in all the harvests from 2 weeks of storage through to shelf life period (Figure 1B). There were significant differences among fruit harvests after shelf life, H3 offering

significantly (p<0.05) higher TSS contents at the end of market life for both cultivars (Figure 1B).

In comparison to a similar study on 'Mollar', after 6 weeks of storage at 5°C, decreases in TSS contents in harvested at two maturity stages were not significant (Gil et al., 1996). The interaction between harvest date and storage period had a significant (p= 0.0004) effect on TSS content in 'Bhagwa' (Figure 1A), whereas both harvest time and storage duration significantly influenced TSS content for 'Ruby' (Figure 1B). Such cultivar differences need to be considered when assessing fruit maturity and potential for storage.

The changes in TA and TSS values were reflected in the TSS/TA ratio, which increased with storage period among harvests of both cultivars. It is noteworthy that in H2 and H3 the TSS/TA ratio remained steady during storage (Figure 1A and Figure 1B), probably suggesting that the TSS and TA contents declined in equal proportions during postharvest storage. According to Ben-Arie et al. (1984), TSS/TA ratio is a more reliable indicator than TSS to differentiate quality and characterize flavour of different harvest dates of pomegranates. The observed sharp decline in TA level, with a rather gradual decrease in TSS resulted to significant (p<0.05) fluctuations in TSS/TA ratio in fruit. In 'Ruby' later harvests (H2 and H3), the TSS/TA ratio first declined significantly (p<0.05) between the initial harvest dates and then increased afterwards (Figure 1B). During shelf life period, the values of TSS/TA in early harvested fruit (H1) were significantly lower than in later harvests for both cultivars (Figure 1A and Figure 1B), suggesting lower flavour quality. The interaction effect between harvest time and storage duration played a significant ('Bhagwa': p = 0.011; 'Ruby': p<0.001) on the changes in TSS/TA ratio in both cultivars. Postharvest storage duration and harvest dates had significant (p<0.05) influences on BrimA values in both cultivars (Figure 1A and Figure 1B), resulting to significant decreases in BrimA between harvest dates and shelf life period. The changes in BrimA value were a reflection of the levels of TSS and TA in fruit during storage, with early harvested fruit (H1) having the lowest BrimA index.

3.4. Changes in phenolic concentration after storage

In general, there were no significant (p>0.05) interaction effects between harvest dates and storage duration on total phenolic concentration (TPC), total flavonoids concentration (TFC) and total anthocyanin concentration (TAC) in both cultivars (with the exception of TFC for

'Ruby'; p = 0.002) (Table 1 and Table 2). The TPC and TFC in fruit harvested early in the season were significantly (p<0.05) higher than in H2 and H3 and the differences were maintained during shelf life in both cultivars (Table 1 and Table 2). Anthocyanin concentrations in 'Bhagwa' increased with harvest dates and the concentrations did not differ significantly (p<0.05) between harvest and shelf life (Table 1). However in 'Ruby', the highest anthocyanin concentration was found in H2 and the concentration decreased significant during shelf life period (Table 2). There are very few studies on changes in pomegranate phenolic concentration during fruit storage. Labbé et al. (2010) studied the behavior of two Chilean pomegranate varieties stored at 5°C over 12 weeks. The authors reported the decrease in aril colour (degradation of anthocyanins) and fruit quality as well as a decrease in the antioxidant capacity of the investigated cultivars. In comparison with other fruit such as rowanberry fruits, Baltacioğlu et al. (2011) reported about 50% and 30% reduction of phenolic concentration in fruit stored over 20 days at 22°C and 4°C, respectively. The authors associated phenolic degradation in rowanberries with enzymatic activities occurring in the fruit. This reason was supported by Makris and Rossiter (2002), who pointed out the adverse effect of postharvest handling and storage temperature of plant tissues and products on the overall fruit quality. The sharp decrease in polyphenols including anthocyanins in pomegranate during shelf life period could be attributed to the change of enzyme activities resulting to phenolic degradation. According to Oren-Shamir (2009), the degradation process begins with intracellular decompartmentation and cell layer damage in fruit during storage, hence exposing the pigments to micro-environmental conditions that differ from those in on-tree fruit, including enzymes that are not located in the vacuoles when the fruit cells are intact.

3.5. Change in aril texture after storage

Textural property of arils is an important quality attribute in the pomegranate industry. The mechanical properties related to texture that were evaluated included hardness, rupture energy or toughness, bioyield and Young's modulus of elasticity. These parameters are illustrated in a typical force-distance graph in Figure 2. In 'Bhagwa', aril hardness and toughness decreased significantly (p<0.05) with prolonged harvest dates and during shelf life period, and maintaining the differences among harvest dates during shelf life period (Table 1). Aril hardness changed significantly with harvest time and storage duration whereas change in aril toughness

was due to storage duration (Table 1). However in 'Ruby', aril toughness showed no significant (p<0.05) changes among harvest and during storage period, with the exception of H1 during shelf life (Table 2). The influence of harvest time and storage duration on aril toughness in 'Ruby' was significantly evident (Table 2). In addition, aril hardness was significantly (p<0.05) influenced by harvest time (Table 2). Furthermore, decline in bioyield was significantly influenced by harvest time (Table 1), whereas the Young modulus remained unaltered among harvests during storage and the subsequent shelf period for both cultivars (Table 1 and Table 2).

The overall decreases in aril textural property after shelf life for both cultivars suggest the softening of arils during the simulated shelf period. This could be attributed to cell membrane deterioration due to higher temperature under shelf life condition (Bchir et al., 2011). Similar results were reported by Labbé et al. (2010) who showed the loss of firmness in arils during fruit storage at 5°C for 8 weeks. The calculated storage index (SI) provide information on the texture dynamics of aril measured between harvests and after storage at 5°C for 6 weeks plus a subsequent 5 days of shelf life period at 20°C (Figure 3). The texture dynamics clearly showed the extent at which all the textural parameters were lost in arils at different harvests for both cultivars. It is noteworthy that although textural parameters decreased in arils at all the harvests, texture dynamics showed that arils from H2 for 'Bhagwa' and H3 for 'Ruby' lost the least textural property compared to the other harvest dates (Figure 3). This could be as a result of significantly higher juice contents in arils of H2 and H3 (Table 1 and Table 2), resulting in higher turgor pressure within the arils.

3.6. Sensory characteristics during shelf-life

This study showed that long term storage and shelf life condition of pomegranate arils inevitably led to reduction in visual appearance and flavour. Differences in sensory attributes of arils at different harvests after 5 days of shelf life are shown in Figure 4A and Figure 4B for 'Bhagwa' and 'Ruby', respectively. Significant differences were observed in some of the sensory attributes evaluated. Fruit harvests for 'Bhagwa' differed significantly (p<0.05) in aril colour, overall flavour and overall appearance, with arils from late harvested fruits (H3) rated higher than earlier harvests (Figure 4A). For 'Ruby', sensory attributes such as overall flavour, sweet taste, sour taste and grittiness clearly distinguished the different harvests. Grittiness intensity was significantly (p<0.05) higher in early harvested (H1) fruit (Figure 4B). In addition, overall

flavour and sweet taste were significantly (p<0.05) higher in H2 than H1 and H3 (Figure 4B). For both cultivars, the most significant difference found among fruit harvests was the overall flavour attribute. The intensity of the attribute increased (on a scale of 0 - 100) from 66.10 in H1 fruit to 68.1 and 70.5 in fruit of H2 and H3, respectively for 'Bhagwa', whereas for 'Ruby' it increased from 63.5 in H1 to 67 in H2 and then decreased to 64.9 in H3. The observed differences in shelf life flavour intensities of fruit from different harvests might be attributed to the variability in fruit chemical attributes such as total soluble solids, acidity and phenolics (Ben-Arie et al., 1984; Kader, 2006).

In general, sensory attributes such as sour taste, astringency, bitterness and alcohol taste were rated extremely low in the harvests, followed by kernel hardness and grittiness for both cultivars (Figure 4A.and Figure 4B). Furthermore, the panel scored kernel hardness and grittiness with the same rating of 'Bhagwa' fruit harvests (Figure 4A), and the same rating for crunchiness and crispness of 'Ruby' fruit harvests (Figure 4B). Although not statistically different, ratings for 'Bhagwa' showed that sweetness increased from H1 through to H3 while sour taste decreased with increasing harvest dates (Figure 4A). It is also worth noting that for 'Ruby', the intensity of aril colour was scored lower (although not statistically different) in fruit from H1 compared to those from later harvests.

3.7. Pearson's correlation between instrumental and sensory measurements

3.7.1. Correlation between attributes for 'Bhagwa'

Table 3 showed strong positive correlations between aril colour and overall appearance (r = 0.950), aril colour and overall flavour (r = 0.802), and crunchiness and crispness (r = 0.906). Sweet taste showed moderate but positive relationships with aril colour, overall appearance, overall flavour, and negative correlations with sour taste and juiciness. Correlation between sour taste and astringency was positive and moderate (r = 0.726). This is contrary to Koppel and Chambers (2010), who reported a negative correlation between sour taste and astringency for thirty-three pomegranate juices. Strong relationships among instrumental attributes were positive between TSS and BrimA, chroma (C^*) and redness (a^*) and between aril hardness and toughness. TA and TSS:TA as well as total phenolic and anthocyanin were strong but negatively correlated. Strong correlations were found between TSS and BrimA, and this relationship

suggests that BrimA is mainly influenced by TSS. Also, strong relationship found between TA and TSS:TA suggests a predominant influence of TA in TSS:TA ratio. This is in accordance with the findings of Mena et al. (2011), who reported strong relationship between TA and TSS:TA. The relationships found between sensory and instrumental data showed that most of the sensory attributes could not be explained by instrumental attributes (Table 3). Relationships found between sensory and instrumental attributes showed that while sensory sweetness had poor relationship with TSS or BrimA, moderate but positive correlations related overall flavour with TSS (r = 0.508) and BrimA (r = 0.501). This suggests that overall flavour could possibly be predicted by TSS and BrimA. Given that BrimA is an index derived from TSS and TA, it could also describe fruit flavor (Gunness et al., 2009). According to Williams (1979), flavour is the result of the perception of a combination of many components resulting in sensory aroma and taste sensation. Flavour in pomegranate fruit is mainly dependent on sugar and acid, which varies among cultivars (Kader, 2006).

3.7.2. Correlation between attributes for 'Ruby'

Significant relationships that exist among attributes measured are presented in Table 4. There were strong positive correlations between overall appearance and aril colour (r = 0.803), crispness and crunchiness (r = 0.785), while juiciness strongly related with crunchiness (r = 0.778) and crispness (r = 0.807). The relationships juiciness shared with crunchiness and crispness is noteworthy and suggests that juiciness could be an integrated representation of the textural properties of the pomegranate aril. A similar observation was highlighted by Harker et al. (2002), who referred to juiciness as a textural attribute that defines many fruits. Furthermore, there was a significant but negative correlation between sweet taste and sour taste. This is well in agreement with Koppel and Chambers (2010) who reported significant negative correlation between sweet and sour taste of thirty-three pomegranate juices. Strong relationships found amongst instrumental measurements included positive correlations amongst TSS, BrimA and TSS:TA as well as between C^* and a^* (r = 0.974) and strong negative correlations between TA and TSS:TA (r = -0.945), TSS:TA and phenolic (r = -0.754) and anthocyanin and phenolic (r = -0.765). Moderate correlations were found between some sensory and instrumental attributes but none of the relationship seems to be applicable in practice. For instance, a moderate positive correlation (r = 0.548) was found between overall flavour and chroma (C^*). In practice, no

relevant prediction of pomegranate juice flavour could possibly be made using aril colour intensity since colour measurement technique apply only to colour of aril tissues. Other relationships found were moderate correlation between sour taste and lightness (L^*), grittiness and TA, and those of sensory hardness with $h°$ and C^* (Table 4). The overall quality of the investigated pomegranate fruit clearly depends on complex interactions of different parameters, of which only the interactions amongst the instrumental parameters seem promising and practicable. This raises the question on the reliability of prediction of pomegranate sensory attribute by instrumental measurements. It has been showed in other pomegranate cultivars grown in western Herzegovina that analytical measurements cannot be substituted for sensory evaluation (Gadze et al., 2011).

3.8. Discriminant analysis (DA)

Discriminant analysis of the sensory and instrumental data (Figure 5) with the first and second factors (F1 and F2) revealed dominant discriminants that separated the harvest times for both cultivars. Table 5 revealed the variables and factors (F1 and F2) correlations of the discriminant analysis, while in Table 6 the summary of variable selection showing attributes that contribute most to the harvest groups are revealed. Confusion matrixes showing the number of correct and incorrect predictions made by the model compared with the actual classifications in the instrumental and sensory data for both cultivars were presented in Table 7.

3.8.1. DA of attribute for 'Bhagwa' harvests

Discriminant analysis (DA) of both sensory and instrumental attributes for 'Bhagwa' is presented in Figure 5. The DA revealed that eight attributes showed correlation values >0.5 (Table 5). None of the sensory attributes evaluated was loaded on the factors. However, seven instrumental attributes (TSS, BrimA and anthocyanins, total phenolics, h°, hardness and toughness) were loaded along FI, while juice content was the only attribute loaded along F2 (Table 5). Fruit from H1 were located on the left along F1 and separated from H2 and H3 fruit (Figure 5), most likely due to higher negative correlations for total phenolics, h°, hardness and toughness (Table 6). Furthermore, F2 seemed to separate H2 and H3 harvests with juice content. The stepwise model indicated that, of the seven attributes having correlation values > 0.5, only four (TSS, juice content, hardness and anthocyanin) contributed significantly to the separation of

the three harvests (Table 6), with TSS having the highest importance (partial R^2 =0.677; p<0.0001). The confusion matrix indicated that 100% of all the fruits could be separated correctly in the three classes (Table 7), confirming the clarity of the separations and the significant differences in the distinguishing attributes.

3.8.2. DA of attribute for 'Ruby' harvests

Thirteen quality attributes showed correlation values > 0.5 (Table 5), with the stepwise model indicating that three of the attributes (TSS:TA, sweet taste and h°) contributed significantly to the separation of the harvests (Table 6). TSS:TA showed the highest importance with partial regression (R^2) of 0.654 (p<0.0001). Moreover, TSS:TA and h° described the differences between H1 fruit and those from H1 and H2 along F1, while F2 separated H2 and H3 fruit with sweet taste attribute (Figure 5). The DA model predicted 95.83% of the fruit into three classes with the observed confusion being between H2 and H3 fruit (Table 7). The results suggest that each of the harvests had at least, a unique attribute that could be used to differentiate the harvests during shelf life.

4. Conclusion

Harvest maturity status of pomegranate fruit (cvs. 'Bhagwa' and 'Ruby') were characterized by differences in instrumental and sensory quality attributes of fruit during storage and shelf-life. Analysis of variance showed significant differences in TSS, TA, BrimA, TSS/TA, juice absorbance, juice content, hardness and toughness as well as in phenolic compounds for both cultivars. Pearson correlation clearly showed that substitution of instrumental measurements for sensory evaluation might not be reliably applicable in practice for both cultivars. Discriminant analysis showed that harvest maturity was crucial for the postharvest quality classification of individual fruit during shelf life period. The results indicated that mature 'Bhagwa' fruit harvested at different times could not be discriminated by sensory attributes; however, instrumental parameters such as TSS (R^2 = 0.677) and juice content (R^2 = 0.512) were the two most decisive quality attributes describing harvest maturity after shelf life. Aril hardness and anthocyanin concentration also had significant influence in distinguishing fruit harvest maturities. However, for 'Ruby', a combination of instrumental and sensory attributes seemed influential in discriminating fruit harvests, with TSS:TA ratio being the most decisive (R^2

=0.654) in distinguishing the fruit harvests, followed by sweet taste (R^2 = 0.474) and hue angle (R^2 = 0.431).

For both cultivars, there was a significant decline in fruit quality attributes such as TA and an increase in TSS during storage. However, although aril colour was significantly higher at late (H2 and H3) than early (H1) harvest, colour did not change significantly during cold storage for both cultivars. Instrumental measurements showed that significant reduction in quality attribute became more evident during shelf life, H1 fruit being the most susceptible to losses in terms of flavour parameters; TA, TSS, TSS/TA, and BrimA, as well as the colour attributes for both cultivars. However, sensory assessments of fruit arils during shelf life period indicated significantly higher ratings for aril colour, overall appearance and overall flavour for 'Bhagwa' fruit at H3. For 'Ruby', fruit at H2 were significantly rated higher for overall flavour and sweet taste, fruit at H1 as more gritty, whereas fruit at H3 were classified as higher in sourness, contradicting the instrumental measurement which showed that the fruit had lower acidity during shelf life period (Fig 1B). No significant correlation was between sourness and TA. This raises the question on the reliability of prediction of pomegranate sensory attribute by instrumental measurements.

Based on the results of this study, it can be deduced that the overall quality of the investigated pomegranate cultivars is influenced by the time of harvest as demonstrated by the instrumental quality parameters at harvest. However, postharvest handling time also played a significant role in distinguishing fruit harvests in both sensory and instrumental characteristics, both during storage and subsequent shelf life. Results of the discriminant analysis showed that to ensure the best postharvest quality of 'Bhagwa' fruit, the optimum harvest maturity was between 167 - 175 days after full bloom (H2 and H3) when fruit had reached maximum TSS level >16°Brix (H3) and juice content >65 mL/100 g aril (H2). However for 'Ruby', the optimum date for harvesting was at 143 DAFB (H2) when fruit TSS:TA value was >55 units. The markers also coincided with significantly higher ratings for sweet taste in fruit at H2 than at H1 and H3 after shelf life. In addition, discriminant analysis showed the possibility of combining the decisive harvest parameters with aril colour intensity to form a potential co-index suitable for distinguishing fruit at different harvest maturities to meet consumer sensory preference. However, it must be noted that, according to the instrumental measurement, late harvest fruit may have shorter postharvest or market life. Furthermore, late harvest fruit may develop

undesirable off flavours even without any visible postharvest physiological disorders if stored longer than the 6 weeks storage period. Therefore, selection of harvest times should also consider fruit market life and utilization as fresh fruit or processed products. The harvest index proposed in this study provides a technological tool to assist in assessing fruit readiness at harvest to meet consumer expectation of good postharvest quality fruit in consideration of long supply chains. However, it should be noted that these conclusions are representative for orchards around Porterville (Western Cape) and only applicable to 'Bhagwa' and 'Ruby'. Hence the results can at this stage be used only as a guideline and a basis for future studies.

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Table 1

Effect of harvest maturity on changes in colour properties, phenolic concentrations and mechanical properties of pomegranate arils (**'Bhagwa'**) at harvest dates and during simulated shelf-life of 5 days at 20°C

	Harvest			Shelf life			Significance level		
Attribute	H1	H2	H3	H1	H2	H3	A	B	A*B
*L**	22.29a	20.42a	22.37a	20.85a	18.71a	18.39a	**0.082**	0.464	0.712
*C**	24.51bc	26.72b	30.83a	20.11dc	20.27d	20.23dc	**<0.0001**	0.118	0.118
h°	22.02ab	21.47b	20.38b	29.89a	27.07ab	24.99b	**0.078**	**0.096**	0.264
*a**	20.60a	22.56a	26.37a	17.69a	18.02a	20.01a	0.654	**0.079**	0.610
Juiciness (mL/100 g aril)	63.55d	66.73ab	65.80b	64.26cd	66.05a	64.63c	0.815	**<0.0001**	**0.010**
Juice abs.	1.85b	2.63a	2.62a	1.78b	2.55a	2.43a	0.341	**<0.0001**	0.878
TAC (mg/100 mL)	31.94b	45.02a	45.10a	30.71b	43.71a	41.76a	0.297	**<0.0001**	0.867
TPC (mg/100 mL)	465.19a	263.65b	303.08b	411.67a	257.47b	280.63b	0.226	**<0.0001**	0.694
TFC (mg/100 mL)	297.20a	147.95b	180.13b	263.01a	144.48b	162.28b	0.457	**<0.0001**	0.551
Hardness (N)	102.66a	81.73c	73.68d	90.85b	79.35c	68.18d	**0.002**	**<0.0001**	0.205
Toughness (N.mm2)	111.73a	95.51b	82.08dc	108.39a	90.79bc	73.95d	**0.095**	**<0.0001**	0.710
Bioyield point (N)	6.01ab	6.56ab	6.71a	5.78ab	5.43b	5.74ab	**0.014**	0.658	0.478
Young modulus (N/mm)	3.71a	3.82a	4.14a	3.53a	3.51a	3.83a	0.184	0.315	0.959

Mean values with different letter(s) across harvest and shelf life period indicate statistically significant differences (p<0.05) according to Duncan's multiple range test. H 1, H2 and H3 = Harvest dates 1, 2 and 3. TAC – total anthocyanin concentration, TPC – total phenolic concentration, TFC – total flavonoid concentration.

Table 2

Effect of harvest maturity on changes in colour properties, phenolic concentrations and mechanical properties of pomegranate arils (**'Ruby'**) at harvest dates and during simulated shelf-life of 5 days at 20°C

	Harvest			Shelf life			Significance level		
Attribute	H1	H2	H3	H1	H2	H3	A	B	A*B
*L**	22.07b	20.21b	20.10b	26.95a	20.62b	20.31b	0.006	<0.0001	**0.004**
*C**	23.94ab	24.72ab	27.86a	21.34b	22.91ab	23.07ab	**0.021**	0.212	0.624
h°	30.19b	24.82d	24.28d	34.98a	29.27c	29.20cd	**<0.0001**	**<0.0001**	0.249
*a**	21.93c	23.73b	26.52a	17.79e	20.35d	20.59d	**<0.0001**	**<0.0001**	0.020
Juiciness (mL/100 g aril)	61.18b	66.56a	67.90a	61.12b	65.08ab	67.20a	<0.0001	<0.0001	**0.005**
Juice abs.	1.99c	3.10a	3.00a	1.79c	2.74b	2.91a	<0.0001	<0.0001	**0.031**
TAC (mg/100 mL)	21.08c	31.58a	30.47ab	18.99c	27.95b	29.58ab	**0.015**	**<0.0001**	0.425
TPC (mg/100 mL)	489.32a	293.27b	233.38b	440.83a	259.53b	226.58b	0.105	**<0.0001**	0.632
TFC (mg/100 mL)	385.19a	251.96b	206.44b	347.02a	222.97b	200.43b	0.104	<0.0001	**0.002**
Hardness (N)	90.80a	92.11a	90.83a	81.80b	85.05b	85.27b	**<0.0001**	0.403	0.062
Toughness (N.mm2)	88.82a	93.71a	90.18a	80.02b	88.24a	87.55a	**0.003**	**0.016**	0.386
Bioyield point (N)	7.45ab	7.54ab	7.86a	5.81c	6.67bc	6.81b	**<0.0001**	0.088	0.481
Young modulus (N/mm)	4.90a	5.06a	4.94a	4.41a	4.48a	4.50a	**0.022**	0.912	0.967

Mean values with different letter(s) across harvest and shelf life period indicate statistically significant differences ($p<0.05$) according to Duncan's multiple range test. H 1, H2 and H3 = Harvest times 1, 2 and 3. TAC – total anthocyanin concentration, TPC – total phenolic concentration, TFC – total flavonoid concentration.

Table 3

Pearson correlation coefficients among instrumental and sensory attributes of 'Bhagwa' pomegranate fruits at shelf life [a]

Sensory attributes		*r*	***Instrumental attributes***		*r*
Aril colour	Overall appearance	**0.950**	TSS	BrimA	**0.998**
	Overall flavour	**0.802**		h°	-0.515
Sweet taste	Aril colour	0.710	TA	TSS:TA	**-0.868**
	Overall appearance	0.742	T. phenolic	Anthocyanin	**-0.789**
	Overall flavour	0.717	*C**	*a**	**0.894**
	Sour taste	-0.598	Hardness	Toughness	**0.898**
	Juiciness	0.583			
	Grittiness	-0.524	***Sensory vs. instrumental***		
Sour taste	Astringency	0.726	Overall flavour	TSS	0.508
	Juiciness	-0.636	Crispness	TA	-0.540
Astringency	Crispness	0.521	Crunchiness	TA	-0.505
	Crunchiness	0.532	Crispness	TSS:TA	0.532
	Bitterness	0.508	Overall flavour	BrimA	0.501
Crunchiness	Crispness	**0.906**			
Grittiness	Overall flavour	-0.545			
Hardness	Sweet taste	-0.646			
	Sour taste	0.535			
	Astringency	0.592			
	Grittiness	**0.746**			
Bitterness	Overall flavour	-0.514			
	Alcohol	0.506			

[a] Relationships with correlation coefficients >0.5 are presented. Values highlighted in **bold** represent strong (>0.75) correlations.

Table 4

Pearson correlation coefficients among instrumental and sensory attributes of 'Ruby' pomegranate fruits at shelf life [a]

Sensory attributes		*r*	***Instrumental attributes***		*r*
Overall appearance	Aril colour	**0.803**	TSS	TA	-0.531
	Overall flavour	0.547		TSS:TA	**0.757**
Sweet taste	Sour taste	-0.643		BrimA	**0.998**
Juiciness	Crunchiness	**0.778**		Juice content	0.571
	Crispness	**0.807**		Anthocyanin	0.601
Crispness	Crunchiness	**0.785**		T. phenolic	-0.629
			TA	TSS:TA	**-0.945**
Sensory vs. instrumental				BrimA	-0.584
Overall flavour	*C**	0.548		Anthocyanin	-0.605
Sour taste	*L**	-0.517		T. phenolic	0.689
Grittiness	TA	0.510	TSS:TA	BrimA	**0.796**
Grittiness	h°	0.579		Anthocyanin	0.702
Hardness	h°	0.569		T. phenolic	**-0.754**
	*C**	-0.502	BrimA	Juice content	0.566
				T. phenolic	-0.648
				Anthocyanin	0.601
			Anthocyanin	T. phenolic	**-0.765**
				h°	-0.537
			T. phenolic	h°	0.537
			*C**	h°	-0.552
				*a**	**0.974**
			h°	Hardness	-0.728

[a] Relationships with correlation coefficients >0.5 are presented. Values highlighted in **bold** represent strong (>0.75) correlations.

Table 5

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

	'Bhagwa'		'Ruby'	
Variables	F1	F2	F1	F2
Sensory rating				
Aril colour	0.271	-0.352	0.447	0.060
Overall appearance	0.342	-0.304	0.465	0.131
Overall flavour	0.397	-0.218	**0.500**	0.204
Sweet taste	0.319	0.034	0.376	**0.909**
Sour taste	-0.260	-0.171	-0.225	**-0.694**
Astringency	-0.142	-0.142	-0.100	-0.365
Crispness	0.158	0.087	-0.138	-0.113
Crunchiness	0.124	0.080	-0.104	-0.144
Juiciness	-0.046	0.293	-0.002	-0.084
Grittiness	-0.215	-0.069	**-0.678**	0.083
Sensory hardness	-0.379	-0.082	**-0.503**	0.174
Instrumental measurement				
pH	-0.044	-0.297	-0.034	-0.267
TSS	**0.879**	-0.132	**0.707**	-0.263
TA	0.051	-0.314	**-0.816**	0.086
TSS:TA	0.376	0.245	**0.870**	-0.219
BrimA	**0.881**	-0.115	**0.739**	-0.258
Juice content	0.268	**0.914**	0.429	-0.378
Anthocyanin	**0.544**	0.317	**0.696**	-0.295
Phenolics	**-0.524**	-0.317	**-0.696**	0.295
*L**	-0.416	-0.096	**-0.537**	0.362
*C**	-0.039	-0.015	0.283	-0.256
h°	**-0.645**	0.140	**-0.677**	0.382
*a**	0.206	-0.179	0.422	-0.312
Hardness	**-0.801**	0.232	0.242	-0.024
Toughness	**-0.795**	0.061	0.424	0.143

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 harvest groups using a stepwise (forward) analysis [a]

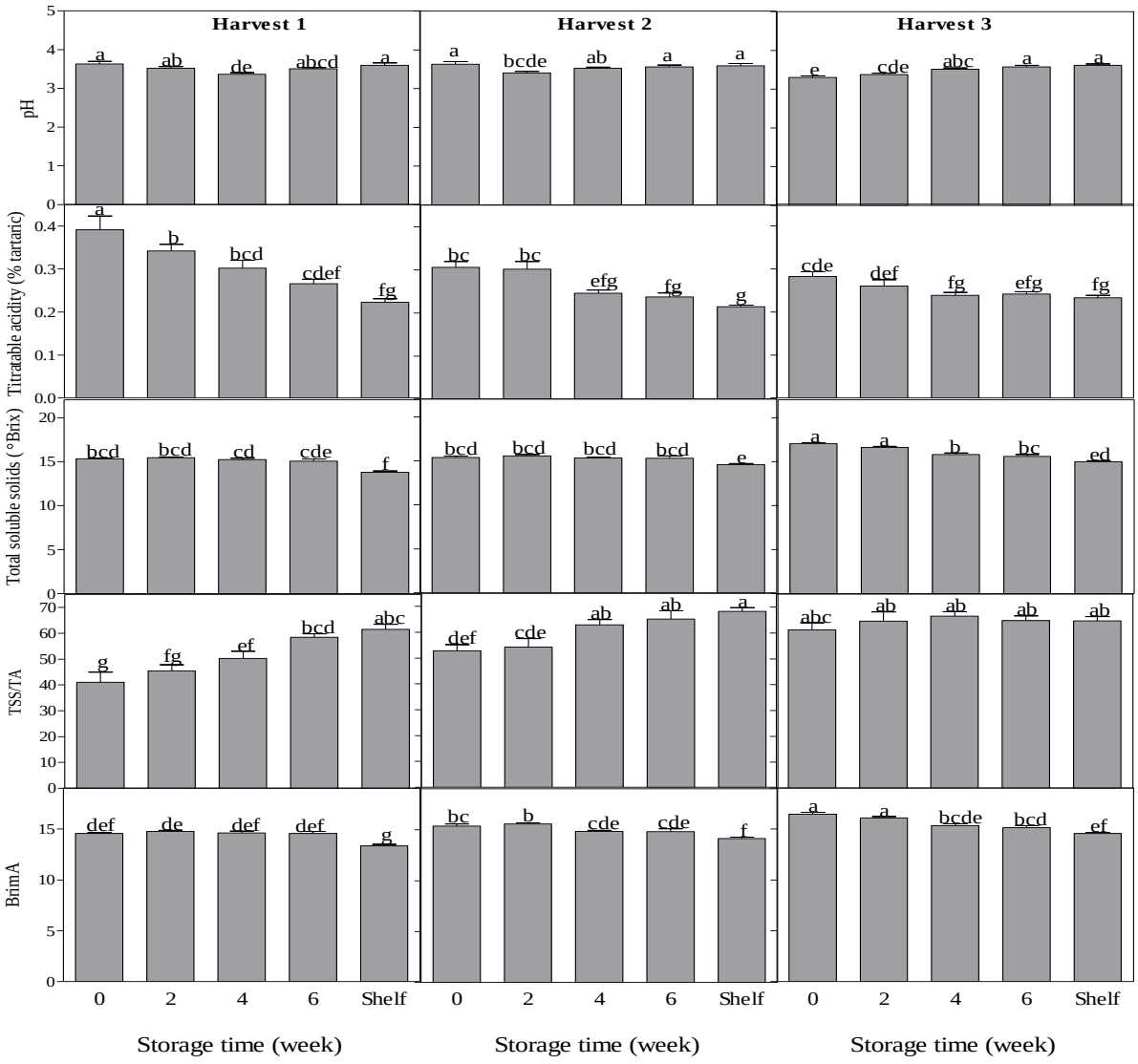
Cultivar	Variable IN/OUT	Status	Partial R^2	F statistic	Pr > F
'Bhagwa'	TSS	IN	0.677	22.034	< 0.0001
	Juice content	IN	0.512	10.499	0.001
	Hardness	IN	0.429	7.134	0.005
	Anthocyanin	IN	0.301	3.879	0.040
'Ruby'	TSS:TA	IN	0.654	19.838	< 0.0001
	Sweet taste	IN	0.474	9.024	0.002
	h°	IN	0.431	7.188	0.005

[a] Partial R^2 - determination coefficient; F statistic - F ratio test; Pr > F - *p* value at significance level of 0.05.

Table 7

Confusion matrixes showing the number of correct and incorrect predictions model made by the model compared with the actual classifications in the instrumental and sensory data

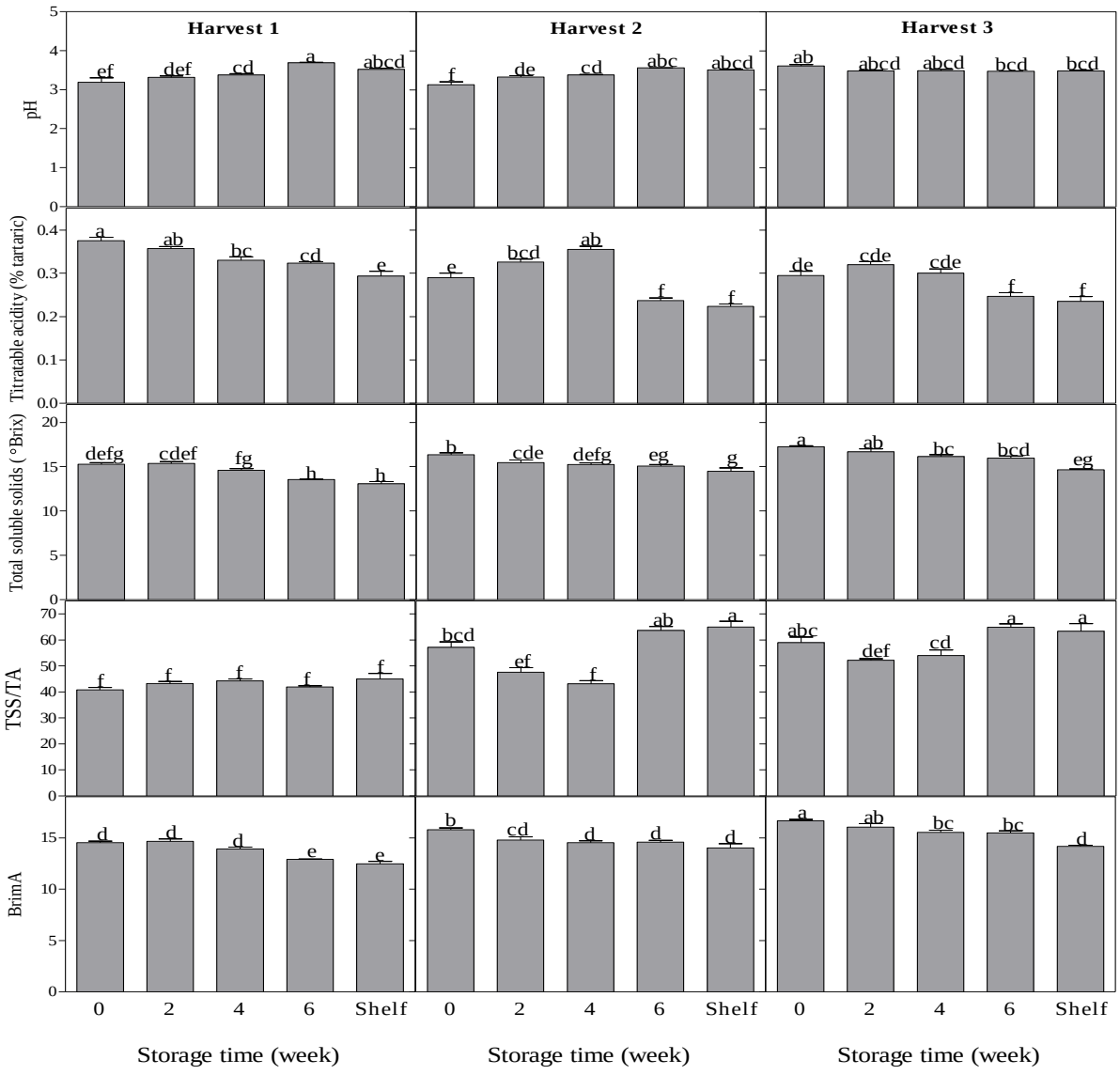
Cultivar	From \ To	Harvest 1	Harvest 2	Harvest 3	Total	% Correct
'Bhagwa'	Harvest 1	8	0	0	8	100.00
	Harvest 2	0	8	0	8	100.00
	Harvest 3	0	0	8	8	100.00
	Total	8	8	8	24	100.00
'Ruby'						
	Harvest 1	8	0	0	8	100.00
	Harvest 2	0	8	0	8	100.00
	Harvest 3	0	1	7	8	87.50
	Total	8	9	7	24	95.83



Significance level

Parameter	Harvest (A)	Storage duration (B)	A * B
pH	0.0035	0.0688	**<0.0001**
Titratable acidity (TA)	<0.0001	<0.0001	**0.0012**
Total soluble solids (TSS)	<0.0001	<0.0001	**0.0004**
TSS/TA	<0.0001	<0.0001	**0.011**
BrimA	<0.0001	<0.0001	**0.0085**

Figure 1A. Chemical changes in '**Bhagwa'** pomegranate as influenced by harvest maturity during simulated 6-week storage at 5°C plus subsequent shelf life of 5 days at 20°C. Different letters on bars across harvests mean statistically significant differences (p<0.05) according to Duncan's multiple range test.



Significance level

Parameter	Harvest (A)	Storage duration (B)	A * B
pH	<0.0001	0.0057	**<0.0001**
Titratable acidity (TA)	<0.0001	<0.0001	**<0.0001**
Total soluble solids (TSS)	<0.0001	<0.0001	**0.079**
TSS/TA	<0.0001	<0.0001	**<0.0001**
BrimA	<0.0001	<0.0001	**0.0383**

Figure1B. Chemical changes in '**Ruby'** pomegranate as influenced by harvest maturity during simulated 6-week storage at 5°C plus subsequent shelf life of 5 days at 20°C. Different letters on bars across harvests mean statistically significant differences (p<0.05) according to Duncan's multiple range test.

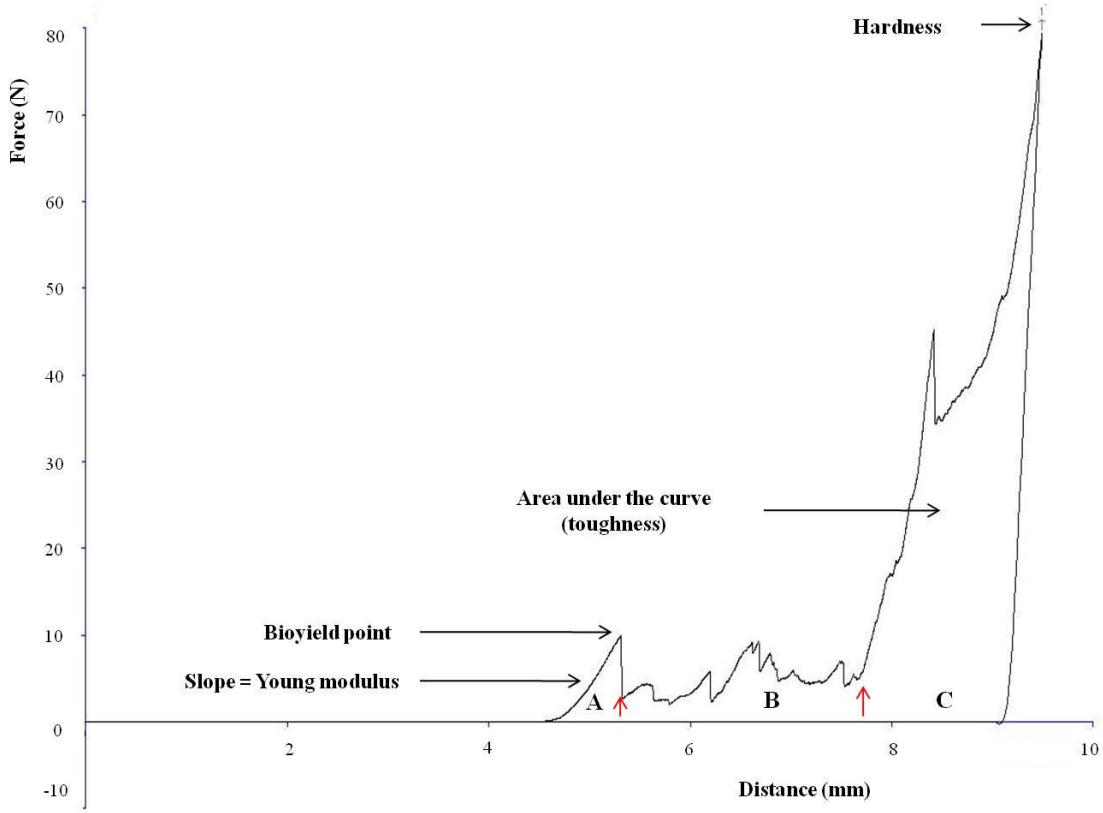


Figure 2. A typical force-distance curve for texture dynamics of pomegranate aril. Area (**A**) = Aril resistance; Area (**B**) = aril rupture zone; Area (**C**) = crushing of kernel

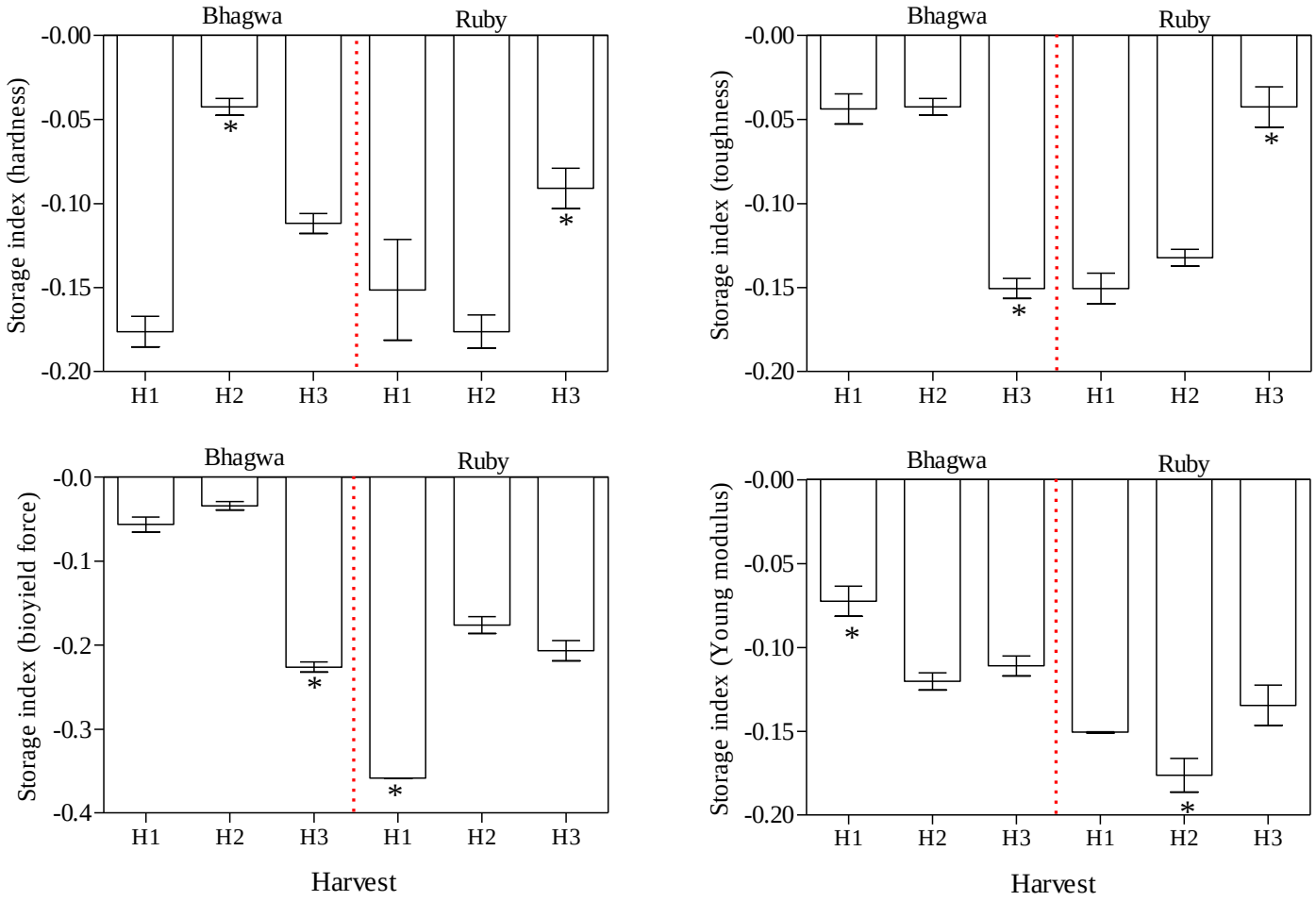


Figure 3. Storage Index (SI) for the texture profile of arils from fruits harvested at three harvest dates and stored at 5°C for 6 weeks plus a 5 day shelf life period at 20°C. H1, H2 and H3 = Harvest times 1, 2 and 3. * Indicates significant difference (p<0.05) among harvest dates

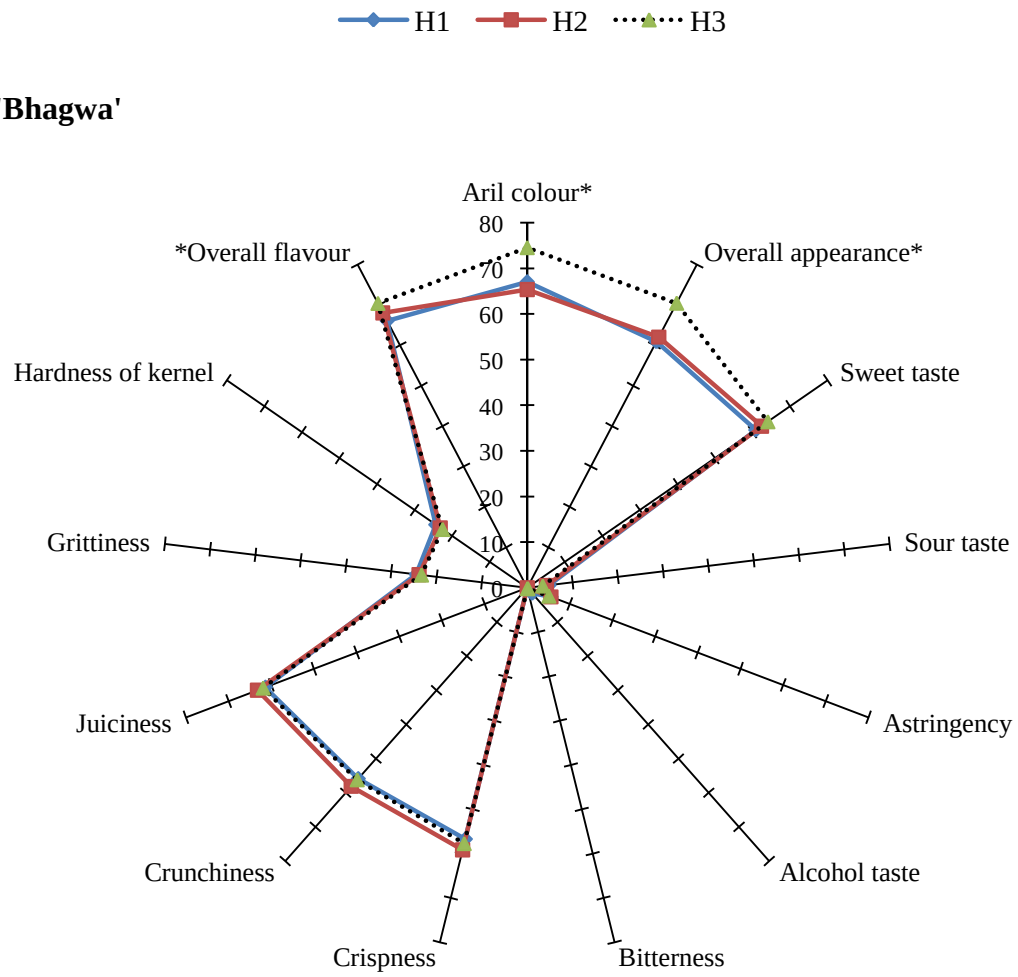


Figure 4A. Radar plot showing averaged sensory scores (scale=0 - 100; n = 72) of pomegranate fruit at three different harvest dates analyzed after storage, 'Bhagwa'. *Indicates significant difference (p<0.05) between harvest dates. Fruits were stored at 5°C for 6 weeks plus a 5 day shelf life period at 20°C before sensory evaluation. H1, H2 and H3 = Harvest times 1, 2 and 3.

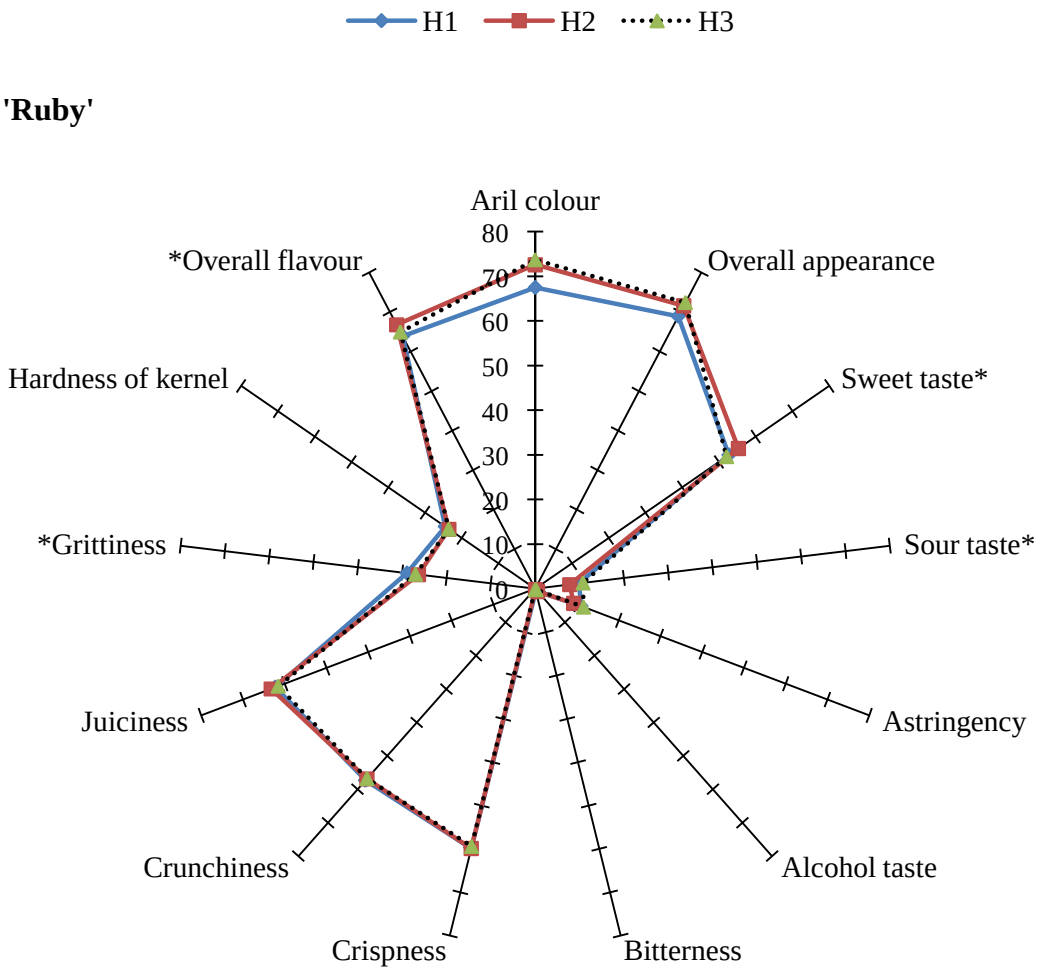


Figure 4B. Radar plot showing averaged sensory scores (scale=0 - 100; n = 72) of pomegranate fruit at three different harvest dates analyzed after storage for 'Ruby'. *Indicates significant difference (p<0.05) between harvest dates. Fruits were stored at 5°C for 6 weeks plus a 5 day shelf life period at 20°C before sensory evaluation. H1, H2 and H3 = Harvest times 1, 2 and 3.

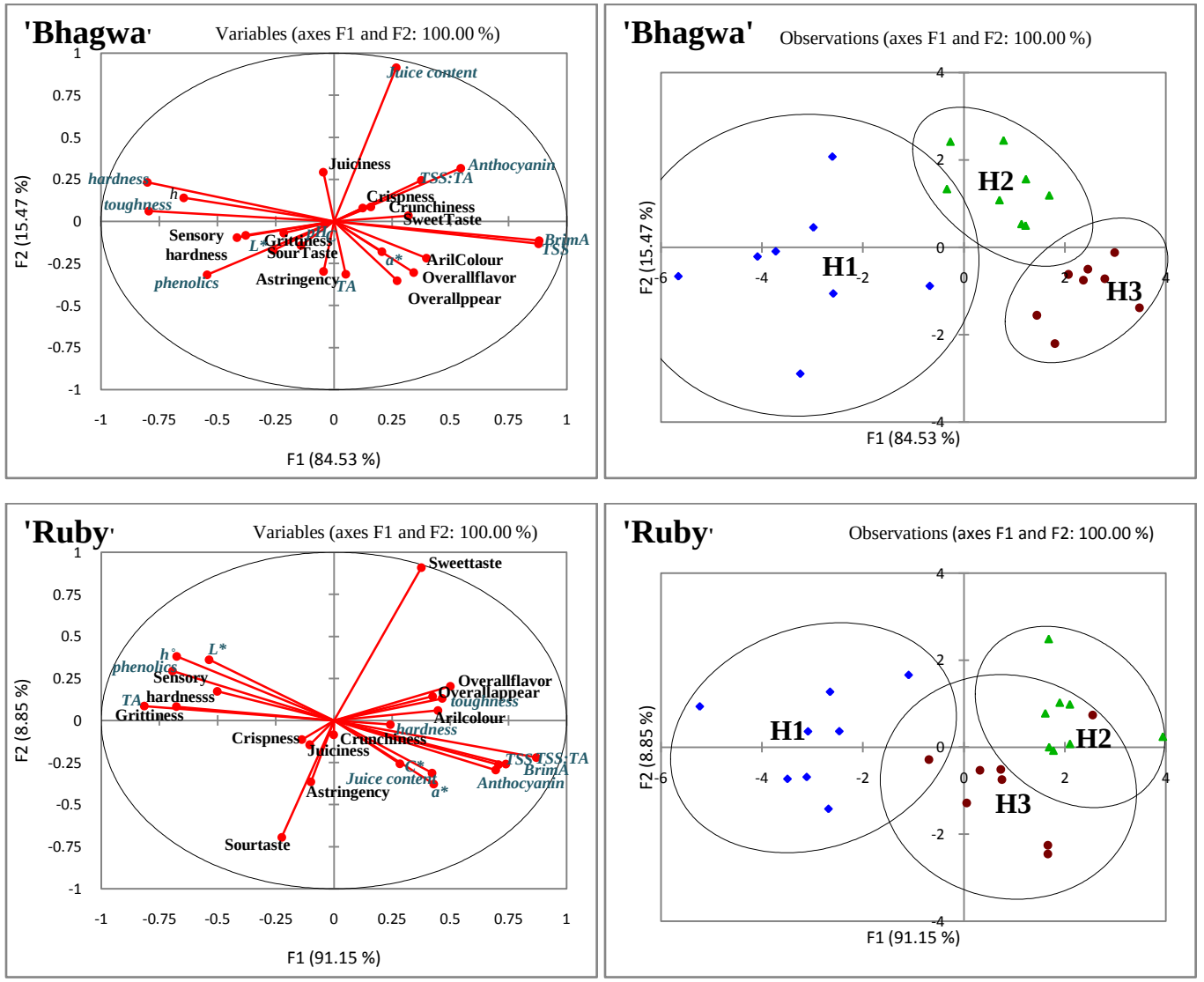


Figure 5. Variables and observations charts of discriminant analysis (DA) for 'Bhagwa' and 'Ruby' using instrumental (*italicized*) and sensory (**bold**) attributes. H1, H2 & H3 = Harvest dates 1, 2 and 3.

SECTION V

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS

1. Introduction

Pomegranate production and export from South Africa is projected to continue its current rapid growth as consumers become increasingly aware of the health and dietary benefits of consuming fresh arils or processed pomegranate products. The health benefits of pomegranate consumption have been attributed to its exceptionally high antioxidant capacity, as a result of high concentration and unique composition of the fruit phenolic compounds (Gil et al., 2000; Fischer et al., 2011). However, consumer demand is still principally governed by fruit appearance as well as desirable taste and flavour; hence, fruit attributes such as size, fruit peel and aril colour, total soluble solids and acidity are commonly used in fruit quality assessment and classification (Ben-Arie et al., 1984; Martinez et al., 2006).

The prospects for a competitive South African pomegranate industry may be achieved not only through exportation of improved cultivars, but also through the development and application of science-based tools for determining optimum maturity and handling protocols to maintain quality and reduce losses during postharvest handling and marketing. The overall aims of this research were (a) to develop science-based management tools for determining optimum maturity indices and storage performance of pomegranate fruit cultivars grown in South Africa, (b) to characterise the physico-chemical properties and pharmacological properties of selected cultivars.

Accordingly, this dissertation was structured into sections, with each section comprised of research papers that address a particular research theme. The themes include;

- Section I: Review of the literature on maturity indexing and storage of pomegranates
- Section II: Pomegranate fruit growth and evolution of maturity indices during development of ‘Bhagwa’ and ‘Ruby’
- Section III: Characterization of fruit postharvest and pharmacological properties of selected cultivars grown in South Africa
- Section IV: Postharvest quality attributes of ‘Bhagwa’ and ‘Ruby’ in relation to harvest maturity, storage conditions and storage durations

2. General discussion

*2.1. Literature review on maturity indexing and storage of pomegranates (**Section I**)*

This section introduced the thesis and reviewed the literature on maturity indexing and storage of pomegranates. The objective of **Paper 1** was to discuss current knowledge on the changes which occur in fruit maturity indices during development and postharvest storage of pomegranate. Pomegranates ripen 5 to 8 months from fruit set, and this involves a sequence of changes in fruit characteristics from flowering to maturity and senescence (Ben-Arie et al., 1984; Al-Maiman and Ahmad, 2002; Holland et al., 2009). Fruit maturity indices are usually based on a wide range of physico-chemical characteristics that are judged either objectively or subjectively. Quality parameters such as fruit size, shape and peel colour are important external attributes for grading and marketing, meanwhile fruit skin colour does not indicate the extent of ripening or readiness for consumption (Kader, 2006; Holland et al., 2009); hence, maturity indices such as aril colour, total soluble solids and acidity are commonly used in fruit quality assessment to meet market requirements (Ben-Arie et al., 1984; Cristosto et al., 2000; Kader, 2006; Martinez et al., 2006). For instance, the maximum titratable acidity may be 1% in sweet cultivars and 1.5 to 2% in sweet-sour cultivars, while minimum total soluble solids vary between 15 and 17% (Kader, 2006).

However, like other types of fruit, pomegranate also undergoes postharvest quality losses during handling and storage. Postharvest response and quality of pomegranate are highly be influenced by maturity at harvest and postharvest storage condition (Kader, 2006). The storage life of pomegranate fruit is generally about 10-15 days at room temperature (Waskar, 2011); however, fruit can be stored for extended periods if held under refrigerated air and high relative humidity (Elyatem and Kadar, 1984). The optimum storage temperature recommended for pomegranates vary from 0 to 10°C with storage life ranging from 2 weeks to 5 months, depending on cultivar (**Paper 1**).

Currently, literature relating to the various quality attributes of pomegranates at commercial harvest is voluminous, yet limited information is available on the pattern of maturity indices and the timing of fruit harvest of several commercial cultivars. This is surprising given the influence of maturity level on the quality attributes of pomegranate fruit. A workable

science-based maturity index for pomegranate cultivars other than 'Wonderful' is currently unavailable. The knowledge that has been accumulated on physico-chemical and physiological attributes of fruit maturity needs to be channeled towards the development of a reliable maturity index by correlating changes in fruit physico-chemical attributes during maturation with the best eating quality and postharvest storage performance of fruit. This approach should be thoroughly studied to develop applications for the supply chain management of fruit and to provide consumers with reliable supply of good quality, tasty, flavourful and healthful pomegranate fruit (**Paper 1**).

*2.2. Fruit growth and evolution of maturity indices of pomegranate cultivars (**Section II**)*

Pomegranate harvest quality and marketability are somewhat challenged by to lack of scientific information on reliable maturity indicators that can assist in determining fruit harvest maturity of many important commercial cultivars. **Papers 2** and **3** provide useful information on fruit behaviour during growth and development. Studies on the changes that occurred in fruit along the days after full bloom included common quality parameters such as fruit size, colour, as well as juice acidity and total soluble solids. Changes in fruit phytochemicals, evolution of aroma volatiles and antioxidant properties were also investigated.

Both 'Bhagwa' and 'Ruby' followed a linear growth pattern from 25 days after full bloom (DAFB) (**Paper 2**). At the beginning of fruit set, fruit shape conformed more closely to a sphere; however, the sphericity declined as growth progressed due to faster growth in diameter than in length between 28 DAFB and 68 DAFB. This knowledge is useful for the development of effective fertigation scheduling and improvement of orchard management depending on the prevailing environmental conditions. Furthermore, the study supported the non-climacteric nature of pomegranate fruit, with undetectable level of ethylene production throughout fruit development, as well as decline in respiration rate with advancing fruit maturity.

Interestingly, in the two season investigated, desirable red colouration occurred earlier in fruit peel than in the aril and the intensity of the colour differed in peel and arils for the cultivars. The disparity between peel and aril colouration was noticeable during ripening stages (110 - 139 DAFB for 'Ruby' and 110 - 165 DAFB for 'Bhagwa'). These periods corresponded to days when decrease in temperature was observed (cool days) in the fruit growing area. Fruit peel and

arils developed more intense red colouration in 2012 season than in 2011 season, suggesting seasonal variability. This could be attributed to lower number of heat units available between blooming and harvesting in the 2012 growing season than in 2011. Temperature is known to exert a strong influence on the colour of pomegranate fruit (Shulman et al., 1984; Borochov-Neori et al., 2009), with low temperature being an important factor for optimal biosynthesis of anthocyanins in pomegranate fruit. This suggests that cool temperatures might be necessary for the development of intense red colouration of pomegranate peel and arils at advance maturity stages, as it appeared to be the case in this study (**Paper 2**). However, there were significant (p<0.05) interaction between fruit maturity and growing seasons on the CIE colour parameters (L^*, a^*, C^* $h°$) in most cases hence at this stage it would be misleading to suggest that fruit maturity or season influence fruit colour dynamics.

Furthermore, increase in fruit weight with advancing maturity in both cultivars during each season followed a linear growth pattern similar to that observed for fruit lineal dimensions. Similar growth patterns have been reported for Australian grown 'Wonderful' (Weerakkody et al., 2010). Optimizing quality attributes of fruit parts such as arils and juice content is one of the primary goals of growers, breeders and processors (Martinez et al., 2006; Hasnaoui et al., 2011). Results obtained during the 2011 season showed that total aril yield (fresh weight basis) was less than 50% of total fruit weight until the middle of the season, and then increased until fruit harvest for 'Ruby', whereas for 'Bhagwa' it remained below 50% of total fruit weight throughout the season. The weight of individual arils increased significantly up to fruit harvest, which has been attributed to increasing juiciness with advancing maturation (Mirdehghan and Rahemi, 2007).

Kernel index (KI) is as important parameter that quantifies the woody portion in the edible part (aril) of pomegranate fruit (Martinez et al., 2006). The KI values obtained in this study were higher than those reported for Moroccan cultivars ('Rouge Marrakech' and 'Bouaâdime'), Spanish cultivars ('PTO2' and 'CRO1') and Italian 'Dente di Cavallo' and 'Alandi' (Purohit, 1985; Barone et al., 2001).

Aside being the first study on South African grown pomegranate cultivars, the novelty of the study in **Paper 2** involved the textural dynamics of pomegranate at different developmental stages. Textural properties such as aril bioyield force and elasticity, which are related to internal

turgor pressure within the arils, and also important in sensory perception of quality, were found to be potential textural makers to discriminate pomegranate maturity levels.

Most of previous research on pomegranate fruit maturity indices considered mainly changes in fruit biochemical and phenolic composition during one season. In the present study, seasonal changes in biochemical and phenolic composition including aroma volatiles, antioxidant capacity and mineral elements were studied. Results obtained showed that major compositional changes in the fruit are developmentally regulated. A gradual increase in juice absorbance value with advancing maturation corroborated the observed aril colour dynamics for both cultivars. A similar observation was reported by Gil et al. (1995) for Tunisian 'Chelfi', 'Kalai', 'Mekki', 'Zehri' and 'Gabsi'. Considerable variation was observed in total soluble solids (TSS) content at all the maturity stages for both cultivars, with more rapid increases in TSS during early stages of fruit development. This phenomenon is associated with active hydrolysis of starch to sugars in maturing pomegranate fruit (Kulkarni and Aradhya, 2005). The identification of glucose and fructose as the major soluble sugars in both cultivars corroborates the findings of previous studies in different pomegranate cultivars (Melgarejo et al., 2000; Mena et al., 2011). The accumulation of simple sugars is one of the processes occurring during the final developmental stages of fruit, resulting in increase in sweetness as fruit approach ripeness (Shwartz et al., 2009; Zarei et al., 2011). Both glucose and fructose sugars increased significantly and more rapidly until 110 DAFB in both cultivars. Additionally, fructose concentration was more than that of glucose. This finding is noteworthy given that fructose is twice as sweet as glucose (Nookaraju et al., 2010), and therefore could be used as a measure of juice sweetness during fruit ripening of the investigated cultivars.

The findings reported in the study (**Paper 3**) further showed that major organic acids in juice of both cultivars during the investigated developmental stages were tartaric, citric, and malic acids. Fruit organic acids (and titratable acidity) and total phenolics declined with advancing maturity, suggesting that sourness decreased in fruit juice. Six major anthocyanins (cyanidin 3,5-diglucoside, cyanidin 3-glucoside, delphinidin 3,5-diglucoside, delphinidin 3-glucoside, pelargonidin 3,5-diglucoside and pelargonidin 3-glucoside) were identified in juice of both cultivars at advanced maturity stages. This appeared to be the case for 'PG 128-29','PG 130-31', 'EG 1' and 'EG 2' accessions as well (Borochov-Neori et al., 2011). The most abundant anthocyanin compound throughout the fruit developmental stages was cyanidin 3,5-diglucoside,

as opposed to Hernández et al. (1999) who reported delphinidin 3,5-diglucoside as the main pigment in Spanish pomegranate cultivars.

A total of 10 aromatic compounds were detected in the headspace of the juices from both cultivars. This low number of aromatic compounds compared to other types of fruit was not surprising given that pomegranates are known to have very low odour and aroma intensity (Carbonell-Barrachina et al., 2012), hence making it difficult to study the fruit volatiles. In addition, factors such as sample preparation and method of determination could have influenced the results of the study. Nevertheless, the dynamics in limonene composition during fruit maturity seems promising as it decreased with advancing fruit maturity. Review of the literature showed that this was the first report on the evolution of limonene in pomegranate during fruit development. There, the results could be used as a guideline and basis for future studies on aroma evolution in pomegranate during fruit development. In addition, future studies should investigate alternative methods, such as hydrodistillation technique (Calín-Sánchez et al., 2011) or pervaporation techniques (Raisi et al., 2008) for the investigation of aroma composition in pomegranate fruit.

Finally, based on the results reported in this section (**Papers 2** and **3**) 'Ruby' appeared to reach harvest maturity between 132 and 139, while 'Bhagwa' between 140 and 165 DAFB. For 'Bhagwa', maturity indices such as juice absorbance and TSS were not influenced by growing seasons but rather fruit maturity stage. Similarly, TSS, TSS:TA and BrimA did not show significant seasonality during fruit maturation. The identified maturity indices would account for the evolution of juice colour, flavour and taste attributes. If the combination of these indices is considered, it should be practicable to develop a reliable maturity index for determining optimum harvest maturity of 'Bhagwa' and 'Ruby'.

*2.3. Characterization of fruit postharvest quality attributes and pharmacological properties of selected pomegranate cultivars grown in South Africa (**Section III**)*

Increasing consumer awareness of the health-promoting properties of pomegranate has spurred interest among growers and researchers to identify cultivars that possess desirable health promoting attributes. Due to the vast number of factors which may affect consumer acceptability and industrial use of pomegranates, it was expedient to consider not only fruit phytochemical and

antioxidant properties, but also physico-textural properties relevant in cultivar characterization, marketing and postharvest handling. In addition, little was known about the distribution of mineral element in different parts of pomegranate fruit. More importantly, from the health point of view, there was a need to explore the value-adding properties of pomegranate peel for the development of functional foods with health-benefitting properties. Overall, results in this section (**Papers 4, 5, 6** and **7**) provided valuable information to enhance cultivar selection and value addition of both the edible and non-edible fractions of pomegranate fruit.

The study reported in **Paper 4** on three commercial pomegranate cultivars ('Arakta', 'Bhagwa' and 'Ruby') were aimed towards method adoption and modification for evaluating a wide range of attributes in terms of chemical, phytochemical properties and total antioxidant capacity of various cultivars and to develop an understanding of the relationships that exist among the investigated parameters. In agreement with the findings of previous studies on pomegranates (Gil et al., 2000; Shwartz et al., 2009), the investigated cultivars exhibited high but varying antioxidant capacity among the cultivars. 'Bhagwa' showed the highest antioxidant capacity in both DPPH and FRAP assays, whereas the highest value was found in 'Arakta' using the QuantiChrom^{TM} assay. In addition, as reported in previous studies (Gil et al., 2000; Shwartz et al., 2009) high correlations between juice phenolics and antioxidant capacity evidently suggests that phenolic compounds are the main antioxidants in pomegranate juice.

In two other studies (**Papers 5** and **6**), more pomegranate cultivars were used. In the first trial, fruit from seven pomegranate cultivars were characterized based on the composition of trace and major minerals in fruit parts including the rind, mesocarp and aril (**Paper 5**). The second trial (**Paper 6**) however, studied the classification of eight commercial pomegranate cultivars based on physico-mechanical, phytochemical and volatiles composition. The hydrophilic and lipophilic free radical scavenging activities was also conducted. The findings in **Paper 5** showed that the concentrations of most of the mineral elements were higher in the non-edible fruit fractions (rind and mesocarp). The novelty of this study involved the estimation of the covering of recommended dietary allowances when pomegranate aril is consumed. Over all, these findings showed that pomegranate edible portion is a good source of mineral elements in human diet. The fruit part is especially rich in P, Fe, Zn, Ti and V (**Paper 5**).

Further, the findings discussed in **Paper 6** could be used as a guideline for further studies preferentially aimed at characterizing commercial pomegranate fruit cultivars for industrial use,

either as fresh arils or as processed products such as fruit juice. With the use of cluster analysis, the investigated cultivars were clustered into two groups (cluster 1 = 'Ruby', 'Arakta' and 'Ganesh'; cluster 2 = 'Bhagwa', 'Acco' and 'Herskawitz') and two ungrouped cultivars ('Molla de Elche' and 'Wonderful'). The grouping was based on important quality traits such as fruit size, texture, colour, soluble solids, acidity, juiciness and total phenolics. The study also provides valuable cultivar-specific information (such as aril colour, kernel hardness, juice content and acidity) useful for the development of optimal postharvest handling and processing parameters for each of the investigated cultivars.

Results obtained suggest that all the cultivars investigated have considerable potential for industrial use as fresh arils, or as processed products. 'Wonderful' could be classified as a cultivar with thick peel, hard arils and kernels, as well as very high acidity (TA) content (TSS/TA < 15), suggesting that it is a sour cultivar and might be suitable for blending variety of juices. 'Ganesh' could be defined as cultivar having big fruit size with high aril yield. Furthermore, 'Molla de Elche' should be regarded as a sweet cultivar due to its exceptionally high TSS/TA (> 75), suggesting its suitability for blending variety of juice. The cultivar receiving the highest assessment for phenolic concentration and total antioxidant capacity was 'Herskawitz', followed by 'Bhagwa'. Owing to the multi-functionality and nutritional benefit of pomegranate phenolics in the human diet, from a consumer quality point of view, high bioactive phenolic compounds in fruit juice of the cultivars suggests that they could be the most health-beneficial among the cultivars investigated. Furthermore, 'Arakta', 'Ganesh' and 'Ruby' could be classified as sweet or sweet-sour cultivars considering their moderate (TSS/TA; 40 – 55) sugar:acid ratio.

The research study conducted in **Paper 7** was aimed at promoting the development of functional foods with health-benefiting properties. This study was conducted from a practical point of view that, if fruit peels of the selected cultivars show potential to improve human health, their utilization should be encouraged during fruit processing. Indeed, results obtained showed that the peel of the investigated cultivars possess strong antibacterial, antioxidant and anti-tyrosinase activities. Also, differing bioactivities were observed among the investigated cultivars, suggesting cultivar differences. The findings could provide a novel insight into the utilization of pomegranate fruit peel for value-added and medicinal purposes. The next step in this study would be to isolate and identify the active ingredients in the fruit peel extracts and also to

determine the mode of action of tyrosinase-inhibitory, antibacterial and antioxidant properties of the extracts. However, it is noteworthy that spraying of pomegranate with various chemicals (for disease prevention/control) is a common practice among South African pomegranate farmers. Owing to this reason, it is necessary that further studies be conducted on potential cytotoxicity and genotoxicity effects of pomegranate fruit peel before they can be considered acceptable for human consumption.

*2.4. Postharvest quality attributes of 'Bhagwa' and 'Ruby' pomegranate cultivars in relation to harvest maturity and storage conditions (**Section IV**)*

The effects of harvest maturity, storage temperature and storage duration were discussed in **Section IV**. During the 2011 season, fruit physiological responses and changes that occurred in physico-chemical properties and antioxidant capacity during long term storage at room temperature, 10°C, 7°C and 5°C plus 92% RH were investigated (**Paper 8**). Subsequent research discussed in **Paper 9** was conducted in an attempt to address the urgent need for improved methods to determe pomegranate harvest maturity for long supply chain.

Increasing storage temperature evidently resulted in higher respiration rates and weight loss. This suggests that the fruit cultivars might be susceptible to temperature abuse, and even small increases in temperature could increase respiration rates, which could ultimately lead to fruit compositional changes and quality loss. Fruit stored at room temperature were discarded after 4 weeks due to excessive weight loss (20 - 25%), decay and high browning index. According to Kader et al. (1984), weight loss of 5% or more resulted in visible shrivelling on 'Wonderful' pomegranate fruit during cold storage. Furthermore, fruit weight loss was between 6 - 8% at 7°C and 5°C after 8 weeks for both cultivars, and, interestingly, no sign of shrivelling was observed. Fruit stored at 5°C and 7°C for up to 12 weeks became more susceptible to both internal and external decay as well as fungal attacks. In contrast to our findings, Artes et al. (1999) observed decay in 'Mollar de Elche' stored at 5°C only after 12 weeks. Fruit stored at 5°C and 92% RH had significantly reduced weight loss, low incidence of physiological disorders and best results in maintaining flavour attributes (TSS and TA, TSS:TA ratio).

It was found storage of fruit at 5°C for up to 8 weeks would be adequate to prevent drastic quality changes (loss of TSS, TA and antioxidants) and most of the physiological disorders (weight loss and scalding) which occur in 'Bhagwa' and 'Ruby'.

The novel approach for determining maturity indices of fruit based on a combination of objective (instrumental) and sensory methods discussed in **Paper 9**, is at the core of this dissertation. The approach carried out was aimed at evaluating and exploring the relationships between instrumental and sensory measurements of individual pomegranate fruit at different harvest maturities after shelf life. The relationships found between sensory and instrumental attributes showed that while sensory sweetness had poor relationship with TSS or BrimA, moderate but positive correlations linked overall flavour with TSS (r = 0.51) and BrimA (r = 0.50). This suggests that overall flavour for 'Bhagwa' could possibly be predicted by TSS and BrimA. Given that BrimA is an index derived from TSS and TA, it could also describe fruit flavour. A similar observation was reported on strawberry fruit (Gunness et al., 2009). For 'Ruby', however, an increase in overall aril appearance correlated with an increase in aril colour (r = 0.803). Crispness and crunchiness (r = 0.785) as well as crunchiness (r = 0.778) and crispness (r = 0.807) also showed the same relationships.

The overall quality of the investigated pomegranate fruit clearly depends on complex interactions between different parameters, of which only the interactions amongst the instrumental parameters seemed promising and practicable. This raised the question on the reliability of the prediction of pomegranate sensory attributes using instrumental measurements. However, juiciness correlated well with crunchiness and crispness. This is noteworthy, suggesting that juiciness could be an integrated representation of the textural properties of pomegranate aril. This is supported by the work of Harker et al. (2002), who referred to juiciness as a textural attribute that defines many fruits.

Furthermore, mature 'Bhagwa' fruit harvested at different times could not be discriminated by any of the sensory attributes; however, TSS (R^2 = 0.677) and juice content (R^2 = 0.512) were the two most decisive quality attributes describing harvest maturity after shelf life. However, for 'Ruby', a combination of instrumental and sensory attributes seemed influential in discriminating fruit harvests. Interestingly, TSS:TA ratio was the most decisive (R^2 = 0.654) in distinguishing the fruit harvests, followed by sweet taste (R^2 = 0.474) and hue angle (R^2 = 0.431). This also coincided with significantly higher rating for sweet taste in fruit at Harvest 2

(H2) than those at Harvest 1 (H1) and Harvest 3 (H3) after shelf life. In addition, discriminant analysis showed the possibility of combining the decisive harvest parameters with aril colour intensity to form a potential co-index suitable for distinguishing fruit at different harvest maturities to meet consumer preference.

It can be deduced that the overall quality of the investigated pomegranate cultivars is dependent on harvest times, as evident in the instrumental parameters. However, postharvest handling and storage duration played a significant role on fruit sensory and instrumental characteristics. The interaction between harvest time and storage duration was significant especially for pH, total soluble solids, titratable acidity, TSS:TA and BrimA. According to the discriminant analysis, to ensure the best post-storage quality of 'Bhagwa' fruit, the optimum harvest maturity was between 167 - 175 days after full bloom (H2 and H3) when fruit had reached maximum TSS level (>16°Brix; H3) and juice content (>65 mL/100 g aril; H2). However for 'Ruby', the optimum date for harvesting was at 143 DAFB (H2) when fruit TSS:TA value was >55 unit. In addition, discriminant analysis showed the possibility of combining the decisive harvest parameters with aril colour intensity to form a potential co-index suitable for distinguishing fruit at different harvest maturities to meet consumer sensory preference. However, it must be noted that, according to the instrumental measurement, if fruit are stored longer than the 6 weeks storage period reported in this thesis, late harvest fruit (for instance, H3) in the season may have a short postharvest or market life. Also, the fruit may develop undesirable off flavours even without any visible postharvest physiological disorders. Therefore, the preference among fruit from different harvests should also consider fruit market life and their use, either as fresh fruit or processed products.

It is noted that the results reported in this thesis are only representative of the orchards around Porterville (Western Cape) and applicable to 'Bhagwa' and 'Ruby', and hence, the results can at this stage be used only as a guide in harvest maturity management. This approach could provide growers and exporters with a tool that would ensure high quality in consideration of long supply chains for South African grown pomegranate cultivars.

3. General conclusions

In conclusion, this study represents the findings of a pilot research aimed at providing science-based tools to support the management of harvest maturity and postharvest quality of fruit in the South African pomegranate industry. This dissertation covered topics relevant to the characterization of commercial fruit cultivars for the purpose of selection and marketing of quality fresh fruit. Furthermore, it provides scientific guidelines to promote value-adding of South African grown pomegranate fruit as a good source of pharmaceutical, cosmetical and antioxidant compounds as well as mineral elements. In addition, it provides a basis for future studies towards the development of science-based tools for determining optimum fruit maturity and postharvest handling protocols for pomegranate cultivars grown in South Africa and globally.

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APPENDIX: PAPER 1

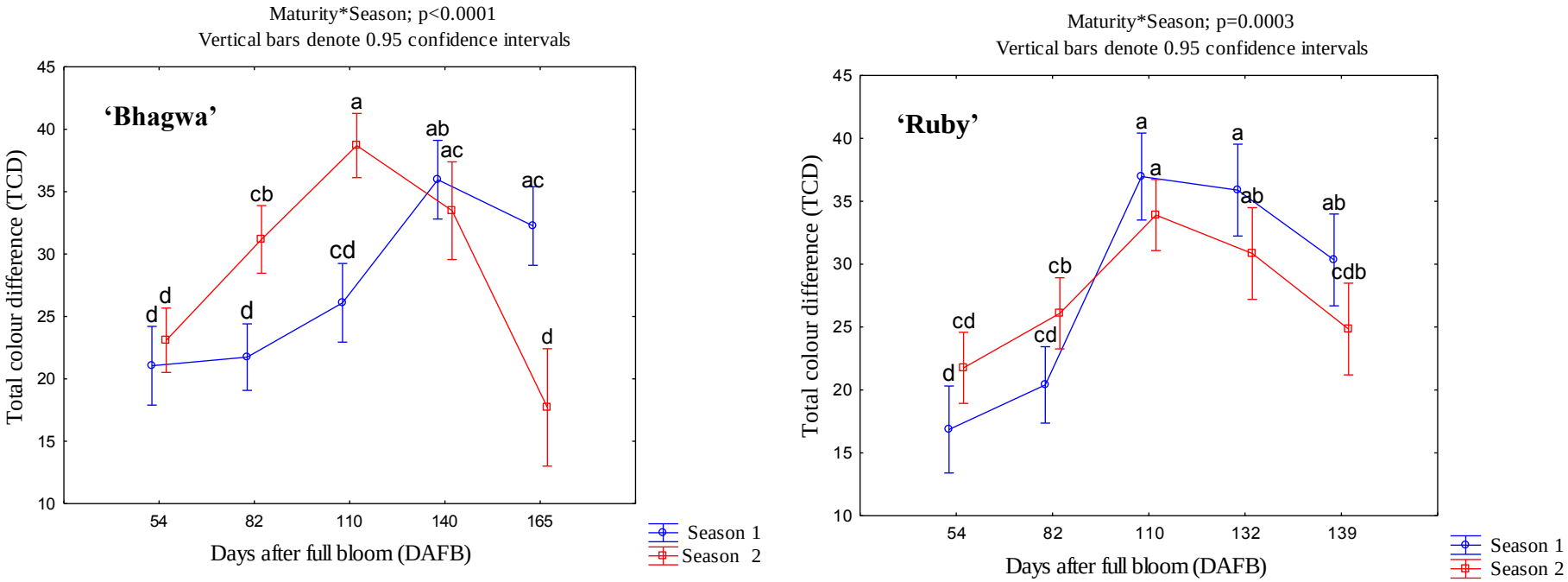

Figure 1: Significant interaction effects (maturity*season) on total colour difference for 'Bhagwa' and 'Ruby' (2011 and 2012)

APPENDIX: PAPER 2

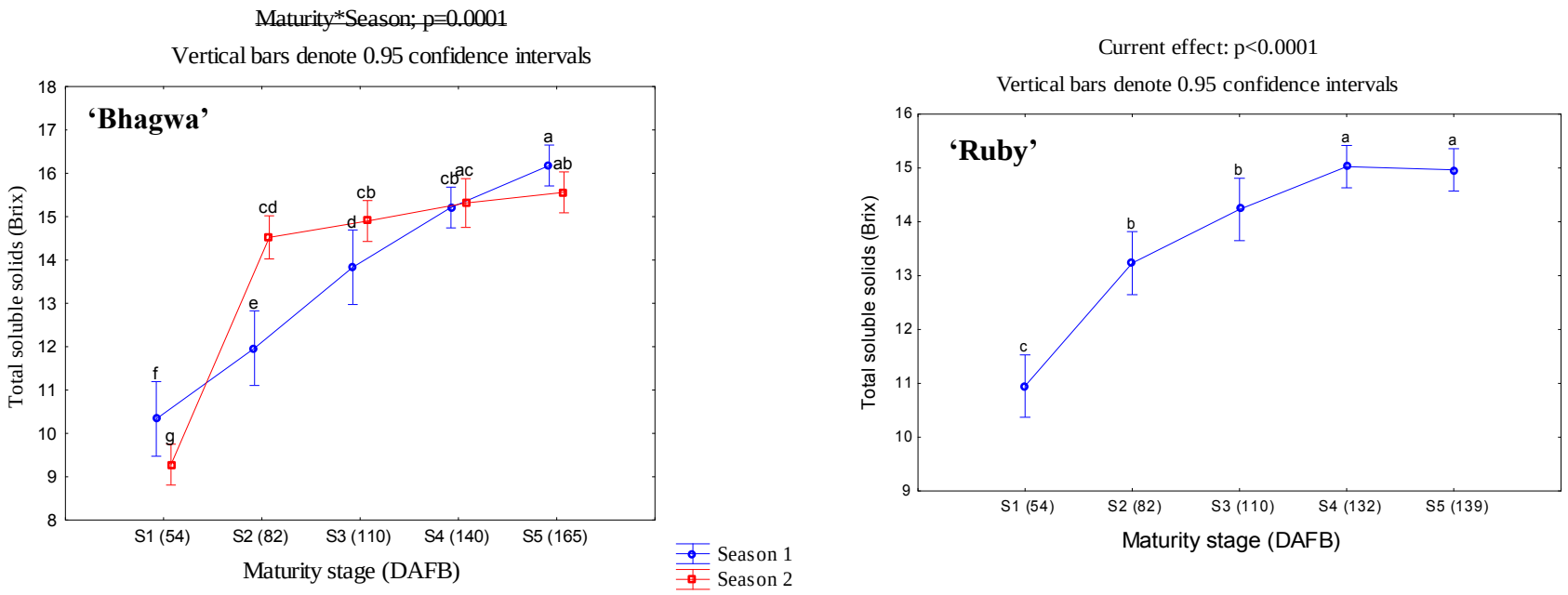

Figure 1: Significant interaction effects (for 'Bhagwa') and maturity effect (for 'Ruby) on total soluble solids content during 2011 and 2012 seasons.

APPENDIX: PAPER 8

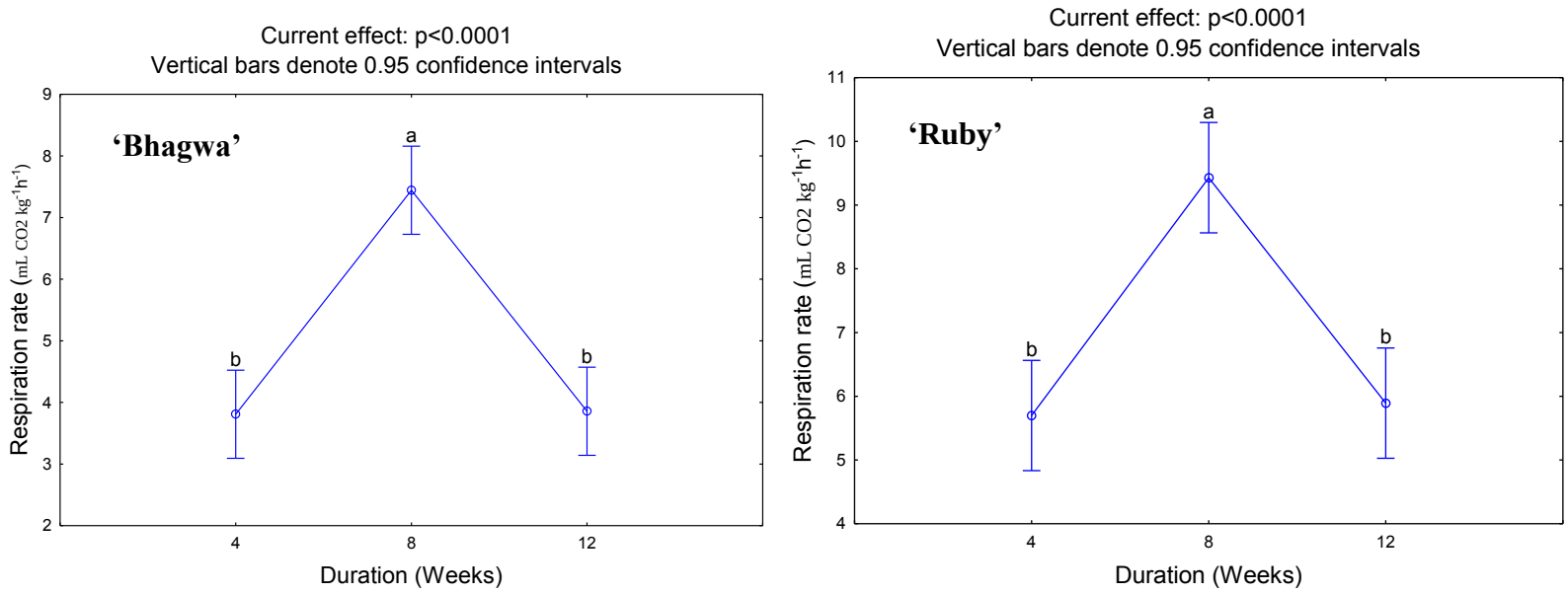

Figure 1: Respiration rate of pomegranate fruits during storage at different temperatures; 'Bhagwa' and 'Ruby'. Storage temperature (10ºC, 7 ºC and 5 ºC) data were pooled due to non-significant (p<0.05) interaction.