Effects of Postharvest Handling on Nutritional Quality of Pomegranate (*Punica granatum*) Arils

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DECLARATION

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

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ABSTRACT

The aim of this study was to investigate the effects of storage temperature and duration on the proximate composition, physico-chemical properties and selected bioactive components (vitamin C, anthocyanins and β-carotene) of arils from three pomegranate cultivars ('Arakta', 'Bahgwa' and 'Ruby'). Pomegranates were hand-peeled and stored at three different temperatures (1°, 4° and 8°C) at 95% relative humidity (RH) for 14 days, with an additional day at ambient conditions (~21°C). Physico-chemical attributes, anthocyanins, ascorbic acid and proximate composition was measured on day 0, 7, 14 and 15. O_2 consumption and CO_2 production increased at elevated temperatures. No visual mould growth was detected in 'Arakta' and 'Bahgwa' arils after 14 days at 1°C 95% RH and after 7 days at 4°C 95% RH. Higher storage temperature negatively affected the proximate composition, physico-chemical attributes and bioactive components. The physico-chemical properties and selected bioactive components (anthocyanins, ascorbic acid, β-carotene) of pomegranate arils ('Arakta', 'Bahgwa' and 'Ruby') packed in three punnets made of polyethylene terephthalate (PET1 - clampshell; PET2 - tub and lid) or polypropylene (PP - tub and lid) material were studied for a period of 14 days at 5°C 95% RH. Packaging did not have a major effect on the physico-chemical and bioactive components of pomegranate arils, although PET2 had relatively stable headspace gas composition within the punnets. Storage duration caused a rise in pH and a decline in titratable acidity irrespective of packaging type. No visual mould growth was detected in 'Arakta' arils after 7 days irrespective of the type of packaging, while mould growth was detected in all 'Ruby' in all types of packaging. The earlier onset of visual microbial growth lead to a baseline microbiological study evaluating the effect of pre-storage water dipping of whole fruit on the microbiological quality of pomegranate arils stored for 8 days at 5°C 95% RH. Freshly harvested pomegranate fruit ('Bahgwa') were dipped in distilled water and air-dried (dipped fruit) or stored without postharvest dipping (dry fruit) at 7°C 95% RH for 15 weeks. Arils were extracted, packaged and stored at 5°C 95±8.34% RH for 8 days. Total viable aerobic mesophillic bacteria, yeasts and moulds, *Escherichia coli* and *Staphylococcus aureus* were enumerated. After 8 days at 5°C 95% RH no microbial growth was detected on arils from 'dry fruit', while 'dipped fruit' showed increased yeast and mould counts (4.74 log cfu.g^{-1}) and total viable aerobic mesophillic bacteria count (3.73 log cfu.g^{-1}). In conclusion storage temperature affects the nutritional quality of pomegranate arils and is best maintained at 1°C for 14 days or 4°C for 7 days at 95% RH. Current South African packaging used to market pomegranate arils don't have a major effect on the nutritional quality of pomegranate arils, although the headspace gas composition was most stable in PET2 packaging. Pre-storage water dipping of whole pomegranates should be avoided as this could reduce the shelf life of extracted pomegranate arils.

SAMEVATTING

Die doel van hierdie studie was om die gevolge van bergingstemperatuur en -tydperk op die proksimale samestelling, fisies-chemiese eienskappe en geselekteerde bio-aktiewe komponente (vitamien C, antosianiene en β-karoteen) van granaatpitte van drie kultivars ('Arakta', 'Bahgwa' en 'Ruby') te ondersoek. Granaatpitte is geberg by drie temperature (1°, 4° en 8°C) 95% RH vir 14 dae plus 'n addisionele dag by kamertemperatuur (~21°C). Fisies-chemiese eienskappe, antosianiene, askorbiensuur en proksimale samestelling is gemeet op dag 0, 7, 14 en 15. Die granaatpitte se O_2-verbruik en CO_2-produksie het toegeneem by hoër bergingstemperature. Geen swamgroei was sigbaar na 7 dae by 1°C 95% RH sowel as 14 dae 4°C 95% RH. Hoër bergingstemperatuur het die proksimale samestelling, fisies-chemiese eienskappe en bio-aktiewe komponente negatief beïnvloed. Die fisies-chemiese eienskappe en geselekteerde bio-aktiewe komponente (antosianiene, askorbiensuur, β-karoteen) van granaatpitte ('Arakta', 'Bahgwa' en 'Ruby') is verpak in drie bakkies vervaardig van polyethyleentereftalaat (PET1 – klamp-bakkie, PET2 – bakkie en deksel) of polipropileen (PP – bakkie en deksel) en bestudeer vir 14 dae by 5°C 95% RH. Heel granate was onderworpe aan 10-14 weke bergingstydperk by 7°C 95% RH voor die granate geskil en verpak is. Die bakkie tipe het nie 'n duidelike uitwerking op die fisies-chemiese en bio-aktiewe komponente van die granaatpitte gehad nie, alhoewel die gas samestelling in die kopspasie van PET2 bakkies relatief onveranderd gebly het. Gedurende die bergingstydperk het die pH gestyg en die titreerbare suur gedaal ongeag die bakkie tipe. Geen visuele swamgroei was sigbaar in 'Arakta' granaatpitte na 7 dae by 5°C 95% RH terwyl 'Ruby' granaatpitte wel swamgroei getoon het ongeag die bakkie tipe. Die vroeë aanvang van sigbare swamgroei het tot 'n verdere mikrobiologiese basislynstudie gelei. Die uitwerking van voorbergingswaterdompeling van heel granate is geëvalueer op die mikrobiologiese gehalte van die granaatpitte wat gestoor was vir 8 dae by 5°C 95% RH. Vars ge-oesde 'Bahgwa' granate is in water gedompel en gelugdroog (gedompelde granate) of glad nie in water gedompel nie (droë granate) en geberg by 7°C 95% RH vir 15 weke. Granate is ontpit, verpak en geberg by 5°C 95±8.34% RH vir 8 dae. Totale lewensvatbare aërobiese mesofilliese bakterieë, giste en swamme, *Escherichia coli* en *Staphylococcus aureus* telling is vervat. Granaatpitte van 'droë granate' was vry van enige mikrobiese groei na 8 dae. Die 'gedompelde granate' het 'n toename in giste en swamme (4,74 log cfu.g^{-1}) en totale lewensvatbare aërobiese mesofilliese bakterieë (3,73 log cfu.g^{-1}) getoon. Hierdie studie maan dus teen waterdompeling van heel granate voor 'n bergingsperiode van 10-15 weke. Ten slotte word die voedingswaarde van granaatpitte wel beïnvloed deur 'n hoër bergingstemperatuur en sal die granaatpitte se gehalte behou word by 1°C vir 14 dae of 4°C vir 7 dae by 95% RH. Die voedingswaarde van granaatpitte word nie beïnvloed deur kommersiële verpakking wat tans in Suid-Afrika gebruik word om granaatpitte te adverteer

nie, alhoewel PET2 bakkies se gas samestelling in die kopspasie onveranderd gebly het. Waterdompeling van heel granate voor ‘n verlengde bergingstydperk moet eerder vermy word aangesien dit die raklewe van granaatpitte verminder.

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Pomegranate

Oh Jewel of winter

So gracious and fair

The colour becomes you

Whatever you wear

Mysteriously crafted

At the beginning of time

Crowned with endurance

And goodness divine

Beneath a leathery surface

In beds snuggled up tight

Lies intricate rubies

Of extraordinary delight

dedicated to my beloved father and mother,

brothers, sisters, nephew and niece

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CHAPTER 1

INTRODUCTION

Times have changed from a retailer's perspective to a consumer's perspective. The retail market is driven by consumer demands and trends that involve greener and healthier lifestyles. These days' mealtimes are no longer just a necessary part of your life, but a culinary experience altogether. The combination of unique colours, textures and flavours create a space for the consumer to experience food through all senses. Consumers eat food to satisfy not only their appetite, but their senses and health consciousness too. There is an increasing awareness of the preventative effect that a healthy diet and lifestyle could have against various diseases such as cancers, obesity, type II diabetes and osteoporosis (Faria & Calhau, 2010). Therefore it is most satisfactory to consume food that is safe, nutritious, attractive and beneficial to one's health. Fresh fruit and vegetables are a source of various vitamins, minerals, dietary fibre and phytonutrients that play an important role in human nutrition and health (Kader *et al.,* 2002). Recently, one fruit in particular has recaptured consumer interest worldwide due to its health promoting benefits: the pomegranate (Heber & Bowerman, 2009).

The pomegranate fruit has been venerable throughout centuries and symbolises life, health, longevity, femininity, fertility, knowledge, morality, immortality and spirituality (Mahdihassan, 1984). Pomegranate (*Punica granatum* L.) is part of the Punicaceae family and originated between the areas of Iran and the Himalayas in northern India before being cultivated over the whole Mediterranean region (Faria & Calhau, 2010). Pomegranates are commercially available as fresh (whole fruit, conveniently packed arils), processed (salads, juice, yoghurts), preserved (jellies, glazes) or in numerous pomegranate derived products (pharmaceutical supplements, cosmetics).

The main global pomegranate producers are India and Iran followed by USA, Turkey, Spain and Israel (Brodie, 2009). Pomegranate research and cultivation are also established in other countries like Saudi-Arabia, Egypt, Sultanate of Oman, Afghanistan, Taifi, China, Japan, Russia, Bangladesh, Greece and South Africa. South African pomegranates are mostly cultivated in Western Cape, Northern Cape, North West, Mpumalanga and Limpopo provinces (Wohlfarter *et al.*, 2010) (Fig. 1).

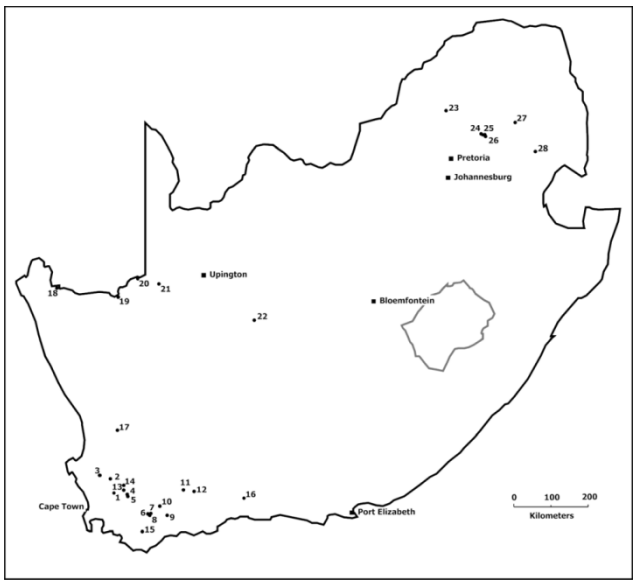

Figure 1 Distribution of pomegranate production in South Africa (Wohlfarter *et al.*, 2010)

The South African harvesting season for pomegranates is from March to late May. The most popular cultivars in South Africa are 'Bahgwa', 'Arakta', 'Ruby', 'Ganesh', 'Mollar de Elche' and 'Wonderful' that are exported to the UK, Europe, Canada, Far East and Middle East (Brodie, 2009; Citrogold, 2011). About 5,700 metric tons from 1,000 ha pomegranates are produced in South Africa of which 40% is absorbed by the local market and no more than 5% is organically certified (Turner, P. 2012, director, Citrogold, Stellenbosch, South Africa, personal communication, 16 November).

Many studies suggest that pomegranate fruit and its derived products may have chemo preventative and anti-inflammatory properties due to its high antioxidant activity (Kim *et al.*, 2002; Lansky *et al*., 2007; Syed *et al*., 2007). Gil *et al.* (2000) reported the antioxidant activity of pomegranate juice to be three times that of green tea or wine. Neurath *et al.* (2004) reported the possible use of pomegranate in producing a topical anti-HIV-1 microbicide to help control the HIV pandemic. Anthocyanins, ascorbic acid and β-carotene are some known antioxidant compounds reported in pomegranate arils in variable proportions (Curl, 1963; Drogoudi & Constantinos, 2005; Dumlu & Gürkan, 2007; Tzulker *et al*. 2007). Pomegranate leaves has been recommended to treat diarrhoea by rural traditional healers in the Limpopo region of South Africa, and was justified by Mathabe and others (2006) who confirmed the antibacterial effects of pomegranate leaf extracts.

Postharvest handling practices like storage temperature and packaging material could be used to preserve qualities that render the fruit so desirable. Literature showed physiological, physico-chemical, phytochemical and microbial quality of pomegranate fruit are influenced by storage temperature, atmosphere conditions and packaging (Elyatem & Kader, 1984; Gil *et*

al. 1996; Artés *et al.*, 2000; Nanda *et al.*, 2001; Lopez-Rubira *et al*., 2005; Ayhan & Eştürk, 2009; Ergun & Ergun, 2009; Ghafir *et al*., 2010).

Pomegranate fruit have a very low respiration rate comparable to other non-climacteric fruits like strawberry and grape (Elyatem & Kader, 1984). The whole fruit can be stored for 3 to 4 months at temperatures below 10°C (Elyatem & Kader, 1984; Artés *et al.*, 2000; Nanda *et al.*, 2001; Ghafir *et al*., 2010), but when peeled the arils will only last a week or up to two weeks under modified atmosphere packaging (MAP) conditions at temperatures of 5°C and below (Gil *et al.*, 1996; Artés *et al.*, 2000; Sepulveda *et al.*, 2000; López-Rubira *et al.*, 2005; Ayhan & Eştürk, 2009). The shelf life of South African grown pomegranate arils stored at 0-2°C 95% RH is between 12-14 days, however no common consensus has been reach regarding the recommended storage temperature of pomegranate arils yet (Olivier, F. 2009, Chief Executive Officer, Pomegranate Fruit SA, Porterville, South Africa, personal communication, 14 April).

Pomegranate fruit (Californian 'Wonderful') are subjected to chilling injury when stored at very low temperatures (-1°C) and recommended storage temperatures are above 5°C (Elyatem & Kader, 1984). When pomegranate fruits were removed from storage temperatures of 0°C or 2.2°C the quality of the fruits degraded very quickly and therefore had to be consumed directly (Elyatem & Kader, 1984). However Artés *et al.* (2000) reported Spanish 'Mollar de Elche' whole pomegranate fruit was stored at 2°C under modified atmospheric conditions with minimum loss in quality. The two aforementioned studies illustrated the impact of cultivar, geographical location and season on the physiological response to storage conditions in agreement to the review study done by Kays (1999). Other studies also reported variations in physico-chemical and phytochemical properties of pomegranates due to seasonal, agro-climatic and cultivar differences (Borochov-Neori *et al*., 2009; Opara *et al.*, 2009; Schwartz *et al*., 2009; Fawole *et al.*, 2011).

Ghafir *et al*. (2010) reported that packaging material and storage temperature had little effect on the chemical properties of whole pomegranate fruit, but by using polyethylene packaging the weight loss was reduced and ascorbic acid and anthocyanin levels better retained than using a wax coating. Wrapping individual pomegranates with heat-shrinkable films for 12 weeks at 8°C preserved the chemical composition of pomegranate juice while also reducing both the respiration rate and weight loss of individual pomegranate fruits compared to non-wrapped fruit (Nanda *et al.*, 2001).Therefore storage temperature and packaging play an important role to preserve the quality of pomegranate fruit depending on different cultivar, season or geographical regions (Elyatem & Kader, 1984; Gil *et al*. 1996; Artés *et al.*, 2000; Nanda *et al.*, 2001; Lopez-Rubira *et al*., 2005; Ayhan & Eştürk, 2009; Ergun & Ergun, 2009; Ghafir *et al*., 2010;). Pomegranate arils in South Africa are currently packaged using a wide

range of polyethylene terephthalate punnets under normal air atmospheric conditions and without using any films (Olivier, F. 2009, Chief Executive Officer, Pomegranate Fruit SA, Porterville, South Africa, personal communication, 14 April). No research has been reported on the effects of postharvest handling practices on the nutritional quality attributes of pomegranate fruit grown in South Africa. Therefore the research questions are:

1) What are the effects of postharvest handling practices (storage temperature and duration, packaging) on the nutritional quality of commercially grown cultivars for minimally processed pomegranates in South Africa? and

2) What is effect of pre-storage water dipping (~21°C) of whole pomegranate fruit on the microbial quality of minimally processed pomegranate arils after eight days of cold storage at 5°C?

To address these questions the specific research objectives were to:

1) Investigate the effects of storage temperature and duration on physico-chemical properties, proximate composition and selected bioactive components (vitamin C and anthocyanins) of the arils of three pomegranate cultivars (‘Arakta’, ‘Bahgwa’ and ‘Ruby’).

2) Determine the effects of packaging material on physico-chemical properties and selected bioactive components (vitamin C and anthocyanins) of the arils of three pomegranate cultivars (‘Arakta’, ‘Bahgwa’ and ‘Ruby’) during cold storage.

3) Evaluate the effect of pre-storage water dipping treatment of whole fruit on the microbial quality of pomegranate arils stored for 8 days at 5°C.

References

Adams, L.S., Seeram, N.P., Aggarwal, B.B., Takada, Y., Sand, D. & Heber, D. (2006). Pomegranate Juice, Total Pomegranate Ellagitannins, and Punicalagin Suppress Inflammatory Cell signaling in Colon Cancer Cells. *Journal of Agricutural and Food Chemistry,* **54**, 980-985.

Artés, F., Villaescusa, R. & Tudela, J.A (2000). Modified Atmosphere Packaging of Pomegranate. *Journal of Food Science,* **65**, 1112-1116.

Ayhan, Z & Eştürk (2009). Overall Quality and Shelf Life of Minimally Processed and Modified atmosphere Packaged "Ready-to-Eat" Pomegranate Arils. *Journal of Food Science,* **74,** C399-C405.

Bielsalski, H., Dragsted, L. O., Elmadfa, I., Grossklaus, R., Müller, M., Schrenk, D., Walter, P. & Weber, P. (2009). Bioactive compounds: Definition and assessment of activity. *Nutrition*. 1202-1205.

Borochov-Neori, H., Judeinstein, S., Tripler, E., Harari, M., Greenberg, A., Shomer, I & Holland, D. (2009). Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum L.*) fruit. *Journal of Food Composition and Analysis*, **22**, 189-195.

Brodie, L. (2009). Pomegranate Production in South Africa. *South African Fruit Journal,* **8**, 30-35.

United States Department of Agriculture (USDA). (2010). National Nutrient Database for Standard Reference, Release 23, Food Group: Fruit and Fruit Juices. Pomegranates, raw [WWW document]. URL http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/SR23/reports/sr23fg09.pdf. Accessed 19/11/2010.

Citrogold. (2011). Producing Pomegranates in South Africa. URL. http://www.citrogold.co.za/Producing%20Pomegranates%20in%20South%20Africa%20Citrogold%202011.pdf. Accessed 17/11/2012.

D'Aquino, S., Palma, A., Schirra, M., Continella, A., Tribulato, E. & La Malfa, S. (2010). Influence of film wrapping and fludioxonil application on quality of pomegranate fruit. *Postharvest Biology and Technology*, **55**, 121-128.

Dumlu, M.U. & Gürkan, E. (2007). Elemental and nutritional analysis of *Punica granatum* from Turkey. *Journal of Medicinal Foods,* **10**, 392-395.

Elyatem, S. M. & Kader, A. A. (1984). Post-harvest physiology and storage behaviour of pomegranate fruits. *Scientia Horticulturae,* **24**, 287-298.

Faria, A. & Calhau, C. (2010). Pomegranate in Human Health: An Overview. In: *Bioactive Foods in Promoting Health: Fruits and Vegetables* (edited by R.R. Watson & V.R. Preedy). Pp. 551-563. Amsterdam: Academic Press.

Ghafir, S.A.M., Ibrahim, I.Z. & Zaied, S.A. (2010). Response of Local Variety 'Shlefy' Pomegranate Fruits to Packaging and Cold Storage. Acta Horticulturae, **877** 427-432.

Gil, M. I., Martínez, J. A. & Artés, F. (1996). Minimally Processed Pomegranate Seeds. *Lebensmittel-Wissenschaft und-Technologie*, **29**, 708-713.

Gil, M. I., Tomas-Barberan, F.A., Hess-Pierce, B., Holcroft, D.M. & Kader, A.A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural Food Chemistry,* **48**, 4581-4589.

Heber, D. & Bowerman, S. (2009). Californian Pomegranates: The ancient fruit is new again. *Nutrition Today,* **44***,* 180-184.

Kader, A.A. (2002). Quality parameters of fresh-cut fruit and vegetable products. In: *Fresh-cut fruits and vegetables: science, technology, and market* (edited by O. Lamikanra). Pp. 13. Florida: CRC Press.

Lei, F., Zhang, X.N., Wang, W., Xing, D.M., Xie, W.D., Su, H. & Du, L.J. (2007). Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice. *International Journal of Obesity*, **31**, 1023-1029.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 74-185.

Madrigal-Carballo, S., Rodriguez, G., Krueger, C.G., Dreher, M. & Reed, J.D. (2009). Pomegranate (*Punica granatum*) supplements: Authenticity, antioxidant and polyphenol composition. *Journal of Functional Foods*, **1,** 324-329.

Mahdihassan, S. (1984). Outline of the beginnings of alchemy and its antecedents. *American Journal Chinese Medicals*, **12**, 32–42.

Mathabe, M.C., Nikolova, R.V., Lall, N. & Nyazema, N.Z. 2006. Antibacterial activities of medicinal plants used for the trseatment of diarrhoea in Limpopo Province, South Africa. *Journal of Ethnopharmacology*, **105**, 286-293.

Nanda, S., Sudhakar Rao, D.V. & Krishnamurthy, S. (2001). Effects of shrink film wrapping and storage temperature on the shelf life and quality of pomegranate fruits cv. Ganesh. *Postharvest Biology and Technology.* **22**, 61-69.

Neurath, A. R., Strick, N.,Li, Y. & Debnath, A.K. (2004). Punica granatum (Pomegranate) juice provides an HIV-I entry inhibitor and candidate topical microbicide. *BMC Infectious Diseases,* **4**,

Opara, L.U., Al-Ani, M.R. & Al-Shuaibi, Y.S. (2009). Physico-chemical Properties, Vitamin C Content and Antimicrobial Properties of Pomegranate Fruit (*Punica granatum* L.). *Food and Bioprocess Technology*, **2,** 315-321.

Sepúlveda, E., Galletti, l., Sáenz, C. & Tapia, M. (2000). Minimal Processing of pomegranate var. Wonderful. In: *Symposium on production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology*. CIHEAM – IAMZ p 237-242. Zaragoza, Spain.

Schwartz, E., Tzulker, R., Glazer, I., Bar-Ya' Akov, I., Wiesman, Z., Tripler, E., Bar-Ilan, I., Fromm, H., Borochov-Neori, H., Holland, D. & Amir, R. (2009). *Journal of Agricultural and Food Chemistry*. **57,** 9197-9209.

Syed, D.N., Afaq, F. & Mukhtar, H. (2007). Invited article: Pomegranate derived products for cancer chemoprevention. *Seminars in Cancer Biology*, **17**, 377-385.

Wohlfarter, M., Giliomee, J.H. & Venter, E. 2010. A Survey of the Arthropod Pests Associated with Commercial Pomegranates, *Punica granatum* (Lythraceae), in South Africa. *African Entomology* **18**, 192-199.

CHAPTER 2

LITERATURE REVIEW

The Pomegranate Fruit

The pomegranate (*Punica granatum*) is also known as the seeded apple or 'jewel of winter' and originates from the area between Iran and the Himalayas in northern India (Faria & Calhau, 2010). The history of pomegranates is filled with symbolism, religion and mythology. Roman soldiers brought the fruit from the Middle East to Europe and Spanish settlers introduced the fruits to America (Heber & Bowerman, 2009). Over 1,000 cultivars exist and are widely cultivated and studied in India, Afghanistan, China, Japan, Russia, USA (Faria & Calhau, 2010) and recently in South Africa (Fawole *et al.*, 2011; Caleb *et al.*, 2012). Scientists are constantly seeking innovative solutions to address pre-harvest and postharvest problems facing the pomegranate industry.

Pomegranate fruit consist of a hard leathery outer skin, an albedo, septa, membranes, aril and seed (Fig. 2.1). The arils are the edible part of the fruit and consist of fleshy ruby-like jewels surrounding a white seed. The colour of arils varies in red intensity from white to pink to wine red depending on the cultivar (Stover & Mecure, 2007). Arils are embedded in the septa in a tightly packed manner and are enfolded by membranes into different compartments. The albedo of the pomegranate is the white rubbery flesh underneath the crown-shaped calyx of the fruit (Fig. 2.1). The colour of the outer skin is cultivar-dependent and although usually red it could vary from yellow to purple colour (Stover & Mecure, 2007).

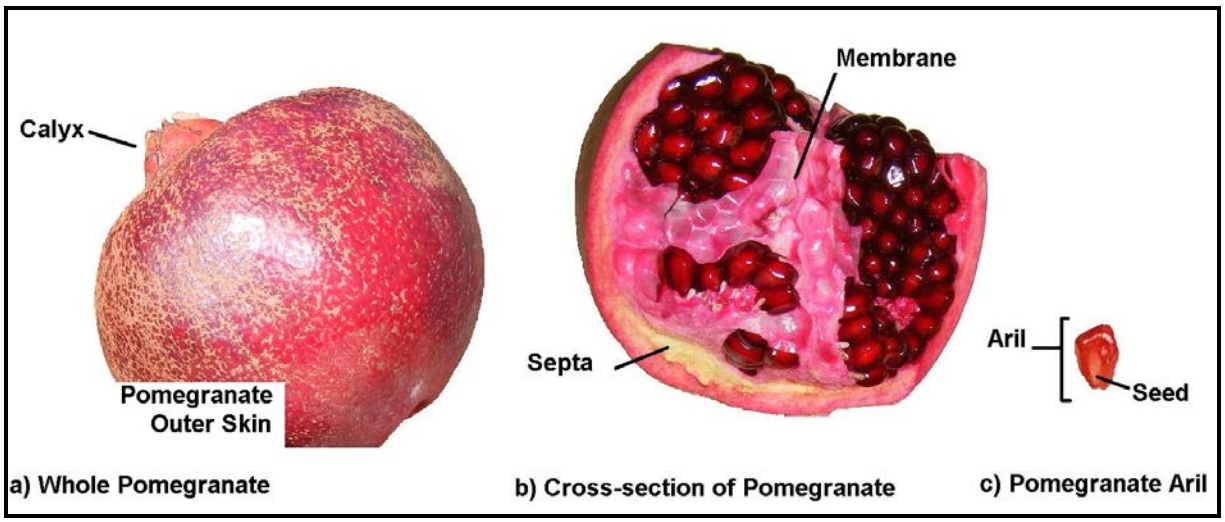

Figure 2.1 **Basic structure of a pomegranate showing the whole fruit (a), a cross section of the fruit (b) and an individual aril (c).**

Nutritional Composition of Pomegranate Arils

Table 2.1 shows the nutritional information of pomegranate arils (raw peeled pomegranate) according to South African national food composition tables of the Medical Research Council (MRC, 2010). Pomegranates have high water content (more than 75%), are low in protein and virtually fat free (less than 0.5 mg per 100g). Dietary fibre and total sugars form the carbohydrate component of the fruit, the latter being the main source of energy. Nutritional composition tables varied between countries of origin: American nutritional composition table (USDA, 2010) showed Californian 'Wonderful' pomegranate arils was a source of dietary fibre (4 g.100 g^{-1}) while South African MRC table showed very low levels of dietary fibre in pomegranate fruit (0.6 g.100 g^{-1}) (MRC, 2010). The vitamin C was also 70% lower and no mention was made of vitamin K content in MRC compared to the USDA compositional table. General mineral composition of pomegranate includes calcium, iron, magnesium, phosphorous, potassium, sodium, zinc, copper and manganese (MRC, 2010; USDA, 2010).

Proximate Composition

MOISTURE

Pomegranate arils contain 78% juice and 22% seed according to El-Nemr *et al*. (1990). Table 2.2 shows moisture content differ according to the different parts of the pomegranate fruit: aril (76-81%), juice (84-85%), seeds (5-9%) and peels (66-76%).

ASH

Pomegranate arils contain about 0.47-0.60 g.100 g^{-1} ash (total mineral) content (Table 2.2). Most prominent minerals reported in pomegranate (100 g^{-1}) from highest to lowest levels: 259.0 mg potassium, 8.0 mg phosphorous 3.0 mg calcium, 3.0 mg magnesium, 0.3 mg iron, 3.0 mg sodium, and 0.2 mg manganese (Table 2.1).

PROTEIN

Protein content has been found to fluctuate during fruit development, ripening and senescence according to the requirements of the fruit (Kulkarni *et al*., 2005). During fruit development the protein level dropped sharply, followed by a gradual increase during ripening and declined once again during senescence when the enzymes were broken down (Kulkarni *et al*., 2005). However Al-Maiman & Ahmad (2002) did not find any change in protein levels during ripening. Protein content vary across different parts of the fruit, the seed being highest in protein (13.2-13.5 mg.100 g^{-1}), followed by the aril (1.0-4.5 mg.100 g^{-1}) and juice (1.0 mg.100 g^{-1}) (Table 2.2).

Table 2.1 Nutritional information of Pomegranate arils (MRC, 2010)

Typical Nutritional Information (Information refers to raw peeled pomegranate)	Per 100g	Per 87 g single serving
Proximate Composition		
Moisture (g)	81.0	70.5
Energy (kJ)	321.0	279.3
Total Protein (g)	1.0	0.9
Total Fat (g)	0.3	0.3
Available Carbohydrate (g)	16.6	14.4
Of which total sugars	16.6	14.4
Glucose (g)	9.0	7.8
Fructose (g)	7.3	6.4
Sucrose (g)	0.3	0.3
Total dietary fibre (g)	0.6	0.5
Insoluble dietary fibre (g)	0.5	0.4
Soluble dietary fibre (g)	0.1	0.1
Ash (g)	0.6	0.5
Organic acids		
Malic acid (mg)	399.0	347.1
Citric acid (mg)	1357.0	1180.6
Vitamins and Bioactive Components		
Vitamin A (µg retinol equivalents)	6.0	5.2
Niacin (mg)	0.3	0.3
Folate (µg)	6.0	5.2
Panthothenic acid (mg)	0.6	0.5
Biotin (µg)	3.8	3.3
Vitamin C (mg)	6.0	5.2
Vitamin E (mg)	0.6	0.5
Total carotenoids (µg)	33.0	28.7
Of which β-carotene (µg)	26.0	22.6
Minerals		
Calcium (mg)	3.0	2.6
Iron (mg)	0.3	0.3
Magnesium (mg)	3.0	2.6
Phosphorous (mg)	8.0	7.0
Potassium (mg)	259.0	225.3
Sodium (mg)	3.0	2.6
Manganese (µg)	197.0	171.4

Table 2.2 Proximate composition table of pomegranate fruit of different cultivars and countries

Cultivar	Part of the fruit	Moisture%	Protein%	Ash%	Carbohydrate%	Total lipid%	Dietary fibre%	Reference
Saudi-Arabia	Juice	83.7	1.03	0.32				Al-Maiman & Ahmad, 2002
Egypt	Juice	85.4		0.05				El-Nemr *et al.,* 1990
Saudi-Arabia	Arils	77.7	4.45	0.47		0.25		Al-Maiman & Ahmad, 2002
Sultanate of Oman	Arils	76.0 - 79.9						Al-Said *et al.,* 2009
Bangladesh	Arils	77.1	1.40		9.90	0.10	0.50	Paul & Shaba, 2004
South Africa	Arils	81.0	1.00	0.60	16.6	0.30	0.60	MRC, 2010
USA	Arils	77.9	1.67	0.53	18.7	1.17	4.00	USDA, 2010
	Arils	81.3					2.80	Ramulu *et al.*, 2003
Turkey	Dried seeds	5.38**		1.83**				Uçar *et al.,* 2009
Turkey	Seeds		13.2*			2.41 - 3.73*		Dumlu & Gürkan, 2007
Iran	Seeds					9.74 - 14.8*		Fadavi *et al.,* 2006
Egypt	Seeds	8.60*	13.5*	2.00*		27.2*	35.3***	El-Nemr *et al.,* 1990
Sultanate of Oman	Peels	66.0 - 75.6						Opara *et al.,* 2009

*Values were determined on a dry weight basis. **Values were determined on a fresh weight basis. ***Value refers to crude fibres also determined by dry weight basis.

LIPID

The seeds of pomegranate fruit amount to almost a quarter of the fruit on fresh weight basis and are discarded in the industry as waste material (Wiesman *et al.*, 2008). Fadavi and others (2006) noted that pomegranate yields too little oil for industrial use and would be more feasible for medicinal or personal purposes. Wiesman and others (2008) studied the fatty acid profile of pomegranate seeds and uncovered chemical properties that will benefit its use in the cosmetic and pharmaceutical industry. The seeds were rich in essential C18:3 linoleic acid fatty acid (80%), antioxidant α-tocopherol (2700 $mg.kg^{-1}$) and β-sitosterol (4000 $mg.kg^{-1}$) compared to almond oil (Wiesman *et al.*, 2008). The individual fatty acid profile and ratio of saturation of different pomegranate seed oil have been studied by various authors and are depicted in Table 2.3 in comparison to the aforementioned almond oil.

Table 2.3 Percentage (%) fatty acid composition of *Punica granatum* dried seeds and seed oils from different countries

Fatty Acids (%)		Pomegranate seed (dwt)	Pomegranate seed (oil)	Almond oil	Reference
Saturated					
C6:0	Caproic	-	2.2	-	1
C8:0	Caprylic	-	36.3	-	1
C10:0	Capric	-	1	-	1
C12:0	Lauric	<0.1 - 3.08	6.6	-	1, 2
C14:0	Myristic	<0.1 - 4.70	7.6	0.1	1, 2, 3, 5,
C16:0	Palmitic	2.8 - 22.6	3.0 - 7.5	8.4	1, 3 - 6,
C18:0	Stearic	0.3 - 9.9	1.6 - 22.5	2.2	3, 4, 5, 6
C20:0	Arachidic	0.2 - 2.8	0.6 - 0.7	0.2	2, 5, 6
C22:0	Behenic	0.2 - 3.9	-	0.1	2, 3, 4, 5
C24:0	Lignoceric	0.0 1.9	-	<0.1	2, 4, 5
Monounsaturated					
C14:1	Myristoleic	-	0.4	-	1
C16:1	Palmitoleic	0.1 - 2.7	0.1	0.6	1 - 6
C18:1	Oleic	0.4 - 31.3	5.1 - 9.4	-	1
C20:1	Arachidoic	0.4 - 0.9	0.4 - 0.7	0.1	2, 4, 5, 6
Polyunsaturated					
C18:2	Linoleic	4.7 - 38.6	5.0 - 12.3	19.5	1 - 6
C18:3	Linolenic	0.6 - 86.6	59.3 - 61.0	-	2, 3, 6
C18:3	Punicic	59.3 - 83.1	66.8 - 79.3	3.3	4, 5, 6
				-	
Saturated fatty acids (SFA)		83.6	4.6 - 33.9	-	1, 2, 3, 6
Unsaturated fatty acids (UFA)		16.3	66.1 - 95.1	-	1, 2, 3, 6
SFA: UFA		5.1:1	0.1 - 0.5	-	1, 2, 3, 6

*(1) El-Nemr *et al.*, 1990; (2) Melgarejo *et al.*, 1995; (3) Fadavi *et al.*, 2006; (4) Abbasi *et al.*, 2008; (5) Wiesman *et al.*, 2008; (6) Liu *et al.*, 2009.

Melgarejo and others suggested an association between lipid content and sweetness of 6 Spanish pomegranate cultivars: sweet-sour (51-66 $g.kg^{-1}$) < sweet (98-108 $g.kg^{-1}$) < sour

cultivars (135-152 g.kg^{-1}). Fadavi and others (2006) reported a similar association in 25 Iranian pomegranate cultivars, however the lipid content of sweet-sour cultivars differed from the previous authors: sweet (66-134 g.kg^{-1}) < sour (112-176 g.kg^{-1}) < sweet-sour cultivars (124-193 g.kg^{-1}). This confirms a possible association between lipid content and sweetness, as well as the observation that oriental and Mediterranean pomegranate cultivars vary in lipid content (Melgarejo *et al.*, 1995).

Linoleic and linolenic acids are essential fatty acids, necessary for physiological functioning of the body but cannot be synthesised by humans and should therefore be incorporated in the diet through vegetable oils, nuts and seeds (Whitney & Rolfes, 2005). The fatty acid profile of pomegranate cultivars studied in Israel, China, Iran showed punicic acid (confugated linolenic acid) contributed to most (60-80%) of the fatty acid profile followed by linoleic (5-12%) and oleic (6-10%) fatty acid (Fadavi *et al.*, 2006; Abbasi *et al.*, 2008; Wiesman *et al.*, 2008; Liu *et al.*, 2009). This was contrary to Melgarejo and others who reported highest levels of linoleic (31-39%), followed by oleic (25-31%) and palmitic (18-23%) fatty acid. El-Nemr and others (1990) reported caprylic acid (36%) to be the most abundant fatty acid, followed by stearic (22%) and linoleic acid (10%) in dried seeds of a sweet Egyptian pomegranate cultivar. Apart from the study by El-Nemr and others (1990), most other pomegranate cultivars seem be low in saturated (palmitic, stearic) and high in unsaturated (linolenic, linoleic, oleic) fatty acid content (Table 2.3).

CARBOHYDRATE

According to Kader & Barrett (2005) the quality of fresh fruit depends on its carbohydrate content. Carbohydrates have two very simplistic functions in plants; to provide an energy reserve as well as serve as structural support. In the same basic way available carbohydrates provide our diet with energy, while complex carbohydrates keep our bowel movement regular amongst many other potential health promoting activities (Coultate, 2007). Carbohydrates can be classified as available (sugars and starch) and unavailable (complex) carbohydrates also known as non-starch polysaccharides (Coultate, 2007).

Fructose and glucose are the primary simple sugars present in pomegranate fruit (Melgarejo *et al.*, 2000; Al-Maiman & Ahmad, 2002; Ozgen *et al.*, 2008; Tezcan *et al.*, 2009). Pomegranate juice (100 mL^{-1}) contains slightly less sugar (10-16 g total sugar: 5-9 g fructose and 4-8 g glucose) than arils (11-23 g total sugar: 6-7 g fructose and 6-8 g glucose) (Table 2.4).

Complex carbohydrates are non-starch polysaccharides (NSP) and commonly referred to as fibre. The American Association of Cereal Chemists (AACC, 2001) structured a new definition for dietary fibre that also focuses on physiological activity of dietary fibre:

'Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysacccharies, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation and/or blood cholesterol attenuation, and/or blood glucose attenuation.'

Table 2.4 Sugar content of pomegranate juice (g.100 mL^{-1}) and arils (g.100 g^{-1}) of various cultivars from different countries

Country	Sample	Unit	Total sugars	Fructose	Glucose	Sucrose	Reference
Turkey	Juice	g.100 mL^{-1}	11.6-14.3	5.8-7.1	5.8-7.6		Ozgen *et al.,* 2008
Turkey	Juice	g.100 mL^{-1}	8.6-16.3	4.6-9.4	4.0-6.9		Tezcan *et al.,* 2009
Egypt	Juice	g.100 mL^{-1}	10.6				El-Nemr *et al.,* 1990
Spain	Arils	g.100 g^{-1}	11.4-13.5	6.0-7.0	5.7-6.5	0.0-0.1	Melgarejo *et al.,* 2000
Taifi	Arils	g.100 g^{-1}	14.6	6.7	7.7		Al-Maiman & Ahmad, 2002
Iran	Arils	g.100 g^{-1}	13.2-21.7				Teharanifar *et al.,* 2010
Iran	Arils	g.100 g^{-1}	16.9-22.8				Zarei *et al.,* 2010

The different fibre classes include lignin, insoluble cellulose, hemi-cellulose, soluble non-cellulosic polysaccharides (pectins, gums and seaweed polysaccharides) and resistant starch (Coultate, 2007).

Dietary fibre has been reported to protect against cardiovascular diseases and type II diabetes due to its reducing effect on blood cholesterol and glucose levels (Whitney & Rolfes, 2005). Californian 'Wonderful' pomegranate arils were found to be a source of dietary fibre (USDA, 2010). Pectin and cellulose in pomegranate arils form part of soluble and insoluble dietary fibre, respectively. Dietary fibre has many health attributes such as relieving constipation, reducing type II diabetes, maintaining a healthy heart and weight (Whitney & Rolfes, 2005). Furthermore dietary fibre cannot be digested by the human digestive system and is therefore passed right through the system to produce short chain fatty acids through fermentation of bacteria in the colon (Whitney & Rolfes, 2005). These fatty acids reduce the risk of cancer by decreasing the pH of the colon (Whitney & Rolfes, 2005). Dietary fibre reduces the risk of diabetes type II by keeping the food in your system for longer that in effect reduces the glycaemic response of foods (Whitney & Rolfes, 2005). It also makes one feel more satiated because it expands when water from digestive juices is absorbed. Bile acids bind to soluble fibres instead of cholesterol, lowering blood cholesterol

and therefore the risk of heart disease too (Whitney & Rolfes, 2005). There might be a relationship between hardness and dietary fibre content of different cultivars due to the different aril hardness of arils (Table 2.11).

Pomegranate arils contain about 2.8 g.100 g^{-1} total dietary fibre, of which 17.8% is soluble and 82.2% insoluble dietary fibre (Ramulu *et al.*, 2003). When the lignin composition of pomegranate and tomato seeds was compared, pomegranate seeds contained more polysaccharides formed part of a lignocarbohydrate complex with sugars such as glucose, xylose, arabinose and galactose (Dalimov *et al.*, 2003).

Vitamins

VITAMIN C (ASCORBIC ACID)

Dehydro-L-ascorbic acid (DHAA) is the oxidised form of Vitamin C (also known as ascorbic acid or L-ascorbate) and they have relatively the same vitamin activity, which is irreversibly lost when DHAA is rapidly converted to 2,3-diketo-L-gulonic acid (Coultate, 2007). Ascorbic acid is an unstable vitamin and is destroyed in the presence of oxygen (especially when fruits are damaged), light, alkalinity, enzyme phenolase and elevated temperatures (Coultate, 2007). Whitney & Rolfes (2005) explained the antioxidant role of ascorbic acid to preserve the body against oxidative stress: Oxidative stress occurs in the body due to an imbalance of unpaired electrons (free radicals); free radicals are highly unstable and reactive, taking electrons from other compounds and transforming them into free radicals; Vitamin C offers two hydrogens with their electrons to restore unpaired electrons of free radicals (Whitney & Rolfes, 2005). Ascorbic acid was discovered to cure scurvy in 1753, and has been under scrutiny ever since to uncover its effect on common colds, atherosclerosis, hypertension, iron absorption and cancer (Patil *et al*., 2009).

In the Sultanate of Oman dried pomegranate arils and especially the peels are known for its healing properties and used as traditional medicine to treat common ailments such as diarrhoea and bacterial infection (Opara *et al*., 2009). These authors found that ascorbic acid content of pomegranate arils were cultivar dependent, however the pomegranate peels contained higher ascorbic acid levels than the arils. Superior antioxidant activity of pomegranate peels compared to pomegranate arils were also linked with four major hydrolyzable tannins (ellagic acid, gallagic acid, punicalin, punicalagin isomers). Pomegranate peels are dried using two traditional drying methods in the Sultanate of Oman: sun-drying (duration of 4 days at an average day temperature of 40°C and night temperature of 28°C) and conventional oven drying (overnight drying at 100°C) Opara *et al.*, 2009). Drying pomegranate peels at a lower temperature for a longer duration (sun-drying) preserved the

vitamin C content of the pomegranate peels more than conventional oven drying which used a higher temperature for a shorter duration (Opara *et al.,* 2009).

The presence of vitamin C in pomegranate juice was confirmed when the vitamin C plasma levels of rats increased after a 7 week administration period of pomegranate juice (Türk *et al.*, 2008). Vitamin C content of pomegranate arils varies between cultivars (0.18-312 mg. 100 g^{-1}) and depends on cultivar and the country of cultivation amongst other factors (Table 2.5).

ALPHA-TOCOPHEROL

Vitamin E (α-tocopherol) is a fat soluble vitamin with antioxidant properties to protect polyunsaturated fatty acids and other lipid compounds from oxidation (Whitney & Rolfes, 2005). Vitamin E has been associated with a reduced risk of chronic heart diseases and haemolytic anaemia (Whitney & Rolfes, 2005). Pomegranate seed oil from fruit cultivated in harsh desert conditions showed a significantly greater alpha-tocopherol concentration (270 mg.100 g^{-1}) than soy bean oil (8 mg.100 g^{-1}) according to Wiesman *et al*. (2008). However, Liu *et al.* (2009) reported only 4.63 - 5.18 mg.100g^{-1} in pomegranate seed oil (Table 2.5).

BETA-CAROTENE

Red, green, yellow and orange coloured fruits and vegetables contain carotenoid pigments. During the ripening process green chlorophyll pigments are broken down while more stable carotenoid pigments are produced (Kader & Barrett, 2005). Beta-carotene is an antioxidant compound commonly known as pro-vitamin A due to its high vitamin A activity compared to the other carotenoids. Two molecules of beta-carotene can be converted to one molecule retinol (vitamin A) in the body; however 12 µg beta-carotene is necessary to provide 1 µg retinol within the body (Whitney & Rolfes, 2005). Vitamin A plays an important part in promoting vision, biosynthesis of proteins and supports growth and reproduction (Whitney & Rolfes, 2005). Curl (1963) found traces of beta-carotene in pomegranate fruit (0.016 mg.100 g^{-1}) compared to Japanese persimmons (5.4 mg.100 g^{-1}). Nutritional analysis of Bangladesh fruits revealed higher β-carotene levels for pomegranates (97 mg. 100 g^{-1}) compared to the aforementioned studies (Paul & Shaba, 2004). Other Bangladesh fruit with similar β-carotene levels of pomegranates were olives (103 mg. 100 g^{-1}) and grape fruit (98 mg. 100 g^{-1}) according to Paul and Shaba (2004).

Table 2.5 Vitamin and pre-vitamin content (mg.100 g^{-1}) of different pomegranate cultivars from different countries

Cultivars	Vitamin C	Vitamin E	β-carotene	B vitamins	References
Iran	9.91 - 20.9				Teharanifar *et al*., 2010
Saudi-Arabia	0.18				Al-Maiman & Ahmad, 2002
		270*			Wiesman *et al.*, 2008
China		4.63 - 5.18*			Liu *et al*., 2009
USA			0.02		Curl, 1963
Bangladesh	15.0		97.0	0.06 Thiamine 0.10 Riboflavin	Paul & Shaba, 2004
South Africa	6.00	0.55	0.03	0.03 Thiamin 0.03 Riboflavin 0.30 Niacin 0.60 Vitamin B6 0.60 PA**	MRC, 2010
Iranian	8.68 - 15.1				Zarei *et al*., 2010
Egypt	0.70				El-Nemr *et al*., 1990
Iran	19.0				Nikniaz *et al*., 2009
Turkey	105 - 312				Dumlu & Gürkan, 2007
Cultivars from India, Egypt and Oman	52.8 - 72.0				Opara *et al*., 2009

*Seed oil was used to determine vitamin E (α-tocopherol)
** PA: Pantothenic acid

B VITAMINS

Thiamine, riboflavin, niacin, biotin, pantothenic acid, vitamin B6, folate and vitamin B12 are all collectively known as water soluble B vitamins (Whitney & Rolfes, 2005). Thiamine, riboflavin, niacin and pantothenic acid are part of co-enzymes that assist other enzymes to release energy from macronutrients (fat, protein and carbohydrates), while vitamin B6 helps to produce amino acids (Whitney & Rolfes, 2005). Pomegranates contain minute quantities of B vitamins (Table 2.5); levels of thiamine (0.06 mg. 100 g^{-1}) and riboflavin (0.10 mg. 100 g^{-1}) have been reported in Bangladesh pomegranates (Paul & Shaba, 2004). Fruits alone are generally not a good source of all B vitamins and should be consumed as part of a diet containing whole grains, dairy, nuts, eggs, meat fish and poultry (Whitney & Rolfes, 2005).

Pomegranates as a Functional Fruit

Cancer research focuses progressively more on improvement of cancer prevention in addition to sole treatment strategy (Bailar & Gornik, 1997). Awareness of various preventative diseases such as cancers, obesity and type II diabetes and osteoporosis have influenced our society to adopt a healthier lifestyle (Faria & Calhau, 2010). Through the course of history health practitioners has evolved in their way of treating diseases from a natural herbal approach to using medicinal synthetic drugs, and now modern science has returned to study natural products yet again (Dewick, 2009).

Pomegranate fruit are not only attractive but contain various phytochemical or bioactive compounds that benefit human health above normal nutrition. These constituents are "essential and non-essential compounds (e.g., vitamins and polyphenols) that occur in nature, are part of the food chain, and can be shown to have an effect on human health" (Biesalski *et al*., 2009). Phenolics, bioactive compounds, functional foods and antioxidants are some of the keywords that the consumer links to healthy foods. Pomegranate as a functional food has increased consumer interest due to the bioactive compounds present within the different parts of the tree (Viuda-Martos *et al.,* 2011). Kim *et al.* (2002) illustrated the phenolic compounds that are distributed in different parts of the pomegranate plant, which contributes to the total antioxidant activity and might play a role in cancer prevention and therapy (Fig. 2.2).

The various functional components of pomegranates could reduce the risk of many chronic diseases according to a review done by Viuda-Martos *et al.* (2011). Syed *et al*. (2007) reported that pomegranate fruit and its associated antioxidants may possess a strong potential as a chemo preventive and possibly a therapeutic agent against various human cancers such as skin, prostate, lung, and breast and colon cancers. Ellagic acid, caffeic acid, luteolin and punicic acid are important components of pomegranate fruit and individually they help to invade prostate cancer cells (Lansky *et al*., 2007). Many health promoting qualities have been found in different parts of the pomegranate plant (leaves, flowers, rind and arils). Pomegranate leaf extract fed to rats on a high fat diet has been reported to have an inhibitory effect on obesity (Lei *et al.,* 2007). Pomegranate leave extracts have antibacterial properties and is used by rural traditional healers in the Limpopo region of South Africa to treat diarrhoea (Mathabe *et al.*, 2006). Higher vitamin C content was reported in the peel of pomegranate fruit compared with the aril pulp (Opara *et al.*, 2009). Quercetin, kaempferol, luteolin and naringenin are non-steroidal estrogenic flavonoids present in the peel and fermented juice of pomegranates (Kim *et al.*, 2002). These flavonoids bind competively to the estrogen receptors resulting in an antiestrogenic effect (Kim *et al.*, 2002).

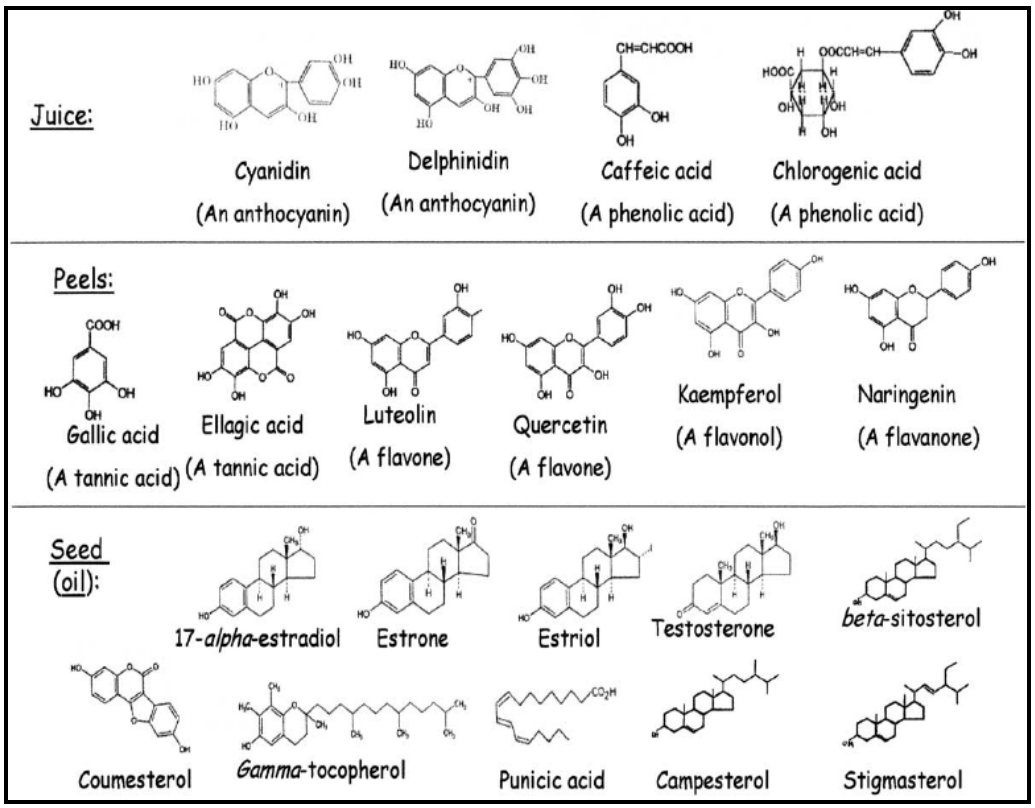

Figure 2.2 Phenolic compounds found in pomegranate (Kim *et al*., 2002)

Pomegranate juice have an antioxidant activity three times the amount of green tea or wine (Gil *et al.,* 2000) The polyphenolic phytochemicals present in pomegranate juice plays an important role in prevention of colon cancer (Adams *et al.*, 2006). Ignarro *et al*. (2006) reported that pomegranate juice protects nitric oxide against oxidative destruction, and could support inhibition of vascular smooth muscle cell proliferation. Even though Naveena *et al*. (2008) reported no effect of anthocyanidins (delphinidin, cyanidin and pelargonidin) on nitric oxide, an inhibitory effect to lipid peroxidation was shown. In another study polyphenolic compounds in pomegranate juice improved antioxidant function and reduced oxidative damage in elderly subjects better than apple juice (Guo *et al*., 2008).

Phenolic Content and Antioxidant Activity

Phenolic compounds as a group, within their natural juice form, work together to achieve a greater antioxidant activity than the purified phenolic compounds on their own (Adams *et al.,* 2006). This emphasizes the importance of consuming fruit in its raw or processed form instead of replacing them with supplements. According to Tzulker *et al*. (2007) phenolic content of pomegranate juices and homogenates was mainly responsible for the fruit's high antioxidant activity. The authors reported that the concentration of hydrolysable tannins (ellagic acid, punicalin, gallagic acid, punicalagin) in homogenates of the whole fruit was 20-

fold higher than the anthocyanins in juices from arils only. This contributed to the greater antioxidant activity in homogenates of whole fruits and peels compared to juices prepared from arils alone. Similar findings showed that commercial juice containing rind tannins exerted a greater antioxidant effect than hand-squeezed juice containing mainly anthocyanins and ellagic acid (Gil *et al*., 2000).

Anthocyanins: Colour and Antioxidant Activity

Anthocyanins are highly pigmented water-soluble flavonoids located in the vacuole of the fruit cell that exist bound (anthocyanins) or unbound (anthocyanidins) to sugars (McWilliams, 2008). According to this author the anthocyanins have a more intense red colour than anthocyanidins, but are collectively known as anthocyanins. Pelargonidin, cyanidin and delphinidin are the most prominent anthocyanidins found in pomegranate fruit juice and cause the red, blue and an intermediate colour respectively (Noda *et al.,* 2002). These anthocyanins (e.g. cyanidin, delphinidin) have also been linked to the antioxidant activity of pomegranate arils and juice (Noda *et al.,* 2002; Drogoudi & Constantinos, 2005; Tzulker *et al*., 2007). Darker coloured pomegranate arils were related to a higher antioxidant activity compared to lighter coloured arils (Tzulker *et al*., 2007). These authors suggest that other antioxidant rich phenolic compounds might play a role to intensify the colour of arils.

Anthocyanin components extracted from pomegranate juice include delphinidin-3,5-diglucoside, cyanidin-3,5-diglucoside, delphinidin-3-glucoside, cyanidin-3-glucoside, pelargonidin-3,5-diglucoside and pelargonidin-3-glucoside (Table 2.6). The three methods used to determine anthocyanins include pH differential method (Ozgen, 2008; Ayhan et al. 2009; Tehranifar, 2010; Zarei *et al.*, 2010; Fawole *et al.*, 2011), high-performance liquid chromatography (HPLC) method (Holcroft *et al*. 1998; Artés *et al*. 2000; López-Rubira *et al*. 2005; Alighourchi *et al.*, 2009) and liquid chromatography-mass spectrometry (LC-MS) method (Mirsaeedghazi *et al.*, 2011). According to Table 2.6 most prominent anthocyanidin found in most pomegranate cultivars is cyanidin-3,5-diglucoside, however López-Rubira *et al*. (2005) showed cyanidin-3-glucoside levels exceeded those of cyanidin-3,5-diglucoside. The pH differential method measures cyanidin-3-glucoside as an indication of total anthocyanins content (AOAC, 2005). Individual and total anthocyanin levels in pomegranate juice differ across countries and cultivars; total anthocyanin content ranging from 6.10-4400 $mg.L^{-1}$ (Table 2.6).

Many factors negatively influence anthocyanins like pH, excessive processing (jam making, drying), metallic ions (Fe, Cu, Al, Sn) and enzymes (anthocyanase, peroxidise, phenolases, glycosidase) according to McWilliams (2008). Even prolonged frozen storage at -25°C showed 11% reduction in total anthocyanin levels of pomegranate juice (Mirsaeedghazi *et*

Table 2.6 Anthocyanin (mg.L^{-1}) content of different pomegranate cultivars from different countries

Country of Origin	D-3,5-DG	C-3,5-DG	D-3-G	C-3-G	P-3,5-DG	P-3-G	Total Anthocyanins	Reference
LC-MS method								
India	-	165	101	101	68.3	17.5	453	Mirsaeedghazi *et al.*, 2011
HPLC method								
Spain	13.6 - 16.8	51.2 - 53.5	11.8 - 11.9	55.6 - 71.6	15.1 - 15.3	28.3 - 31.7	179 - 197	López-Rubira *et al*. 2005
Spain	10.6	41.3	7.00	15.2	2.20	5.5	81.8	Artés *et al*. 2000
Iran	43.8	102.9	15.0	16.9	5.68	6.55	191	Alighourchi *et al.*, 2009
USA	-	-	-	-	-	-	206	Holcroft *et al*. 1998
Turkey	-	-	-	-	-	-	2100 - 4400	Dumlu & Gürkan, 2007
pH Differential Method								
Turkey	-	-	-	-	-	-	311	Ayhan *et al*., 2009
South Africa	-	-	-	-	-	-	165 - 269	Fawole *et al.*, 2011
Turkey	-	-	-	-	-	-	6.10 – 219	Ozgen *et al.*, 2008
Iran	-	-	-	-	-	-	55.6 - 301*	Tehranifar *et al.*, 2010
Iran	-	-	-	-	-	-	79.3 - 277*	Zarei *et al.*, 2010
India	-	-	-	-	-	-	185	Mirsaeedghazi *et al.*, 2011

Delphinidin-3,5-diglucoside (D-3,5-DG) Cyanidin-3,5-diglucoside (C-3,5-DG) Delphinidin-3-glucoside (D-3-G) Cyanidin-3-glucoside (C-3-G) Pelargonidin-3,5-diglucoside (P-3,5-DG) Pelargonidin-3-glucoside (P-3G)
* Values were expressed per kilogram (g.kg^{-1})

al., 2011). CO_2 have also been reported to negatively influence colour and anthocyanin expression of strawberries (Mousavinejad *et al*., 2009). Anthocyanins are pH sensitive because the positively charged oxygen ion (oxonium ion) changes at different pH levels and cause a shift in colour from red anthocyanins (pH ≤ 3.0) to violet quinones (pH > 3.0) to blue quinone salts with an alkaline pH (McWilliams, 2008).

Jaiswal *et al*. (2010) reported that anthocyanins were relatively heat stable without oxygen, but reduced considerably (65%) in the combined presence of heat and oxygen. High temperatures proved to deactivate the enzyme polyphenol oxidase (PPO) in pomegranate arils when PPO levels dropped with 75%, while only 2.5% of total anthocyanins were lost. Oven-dried pomegranate arils (97.4 $\mu g.g^{-1}$) retained their total anthocyanin levels better than the sun-dried pomegranates (42.2 $\mu g.g^{-1}$), possibly because of the higher levels of residual PPO in sun-dried (356 $units.mL^{-1}$) compared with oven-dried (206 $units.mL^{-1}$) arils (Jaiswal *et al.,* 2010). Total anthocyanins might be preserved by boiling and oven-drying processes which deactivates the PPO enzyme (Jaiswal *et al.,* 2010).

Chemical Composition of Pomegranate

The flavour sensation experienced by the tongue are usually caused by non-polar, water-soluble and non-volatile compounds generating a characteristic sweetness, saltiness, bitterness, sourness and pungent, umami or astringent feeling in the mouth (Coultate, 2007). Organic acids and sugars are responsible for the sourness and sweetness of pomegranates, respectively (Melgarejo *et al*., 2000). Organic acid content may vary depending on country of cultivation and cultivars as seen in Table 2.7. Organic acids can be transformed into amino acids when proteins are required or into sugars during the ripening process (Kader & Barrett, 2005).The major organic acids in pomegranates are citric (0.03-3.20 g.100 mL^{-1}) and malic acid (0.03-0.69 g.100 mL^{-1}) (Table 2.7). Titratable acidity is expressed as g.100 mL^{-1} citric acid since citric acid is strongly correlated with titratable acidity of pomegranate aril juice (Dafny-Yalin *et al.*, 2010). Other organic acids reported in pomegranate are tartaric, oxalic acid, acetic and traces of fumaric, succinic and lactic acid (Melgarejo *et al*., 2000; Dafny-Yalin *et al.*, 2010). The sweetness of various pomegranates was caused by the following sugars: fructose, glucose and sucrose and traces of maltose (Melgarejo *et al*., 2000). Sour pomegranate cultivars have shown high citric acid and low sugar (fructose and glucose) levels while sweet pomegranate cultivars showed high sugar (fructose and glucose) and low citric acid levels (Melgarejo *et al*., 2000). Titratable acidity and citric acid of 29 pomegranate cultivars showed a greater contribution to the taste of the arils than total soluble solids and sugar content (Dafny-Yalin *et al.*, 2010). Contrary to the discussion above, higher total

soluble solids content was found in a few sour pomegranate cultivars, which suggest the total soluble solids: titratable acidity ratio is a clearer indication of flavour than titratable acidity and total soluble solids alone (Dafny-Yalin *et al.*, 2010).

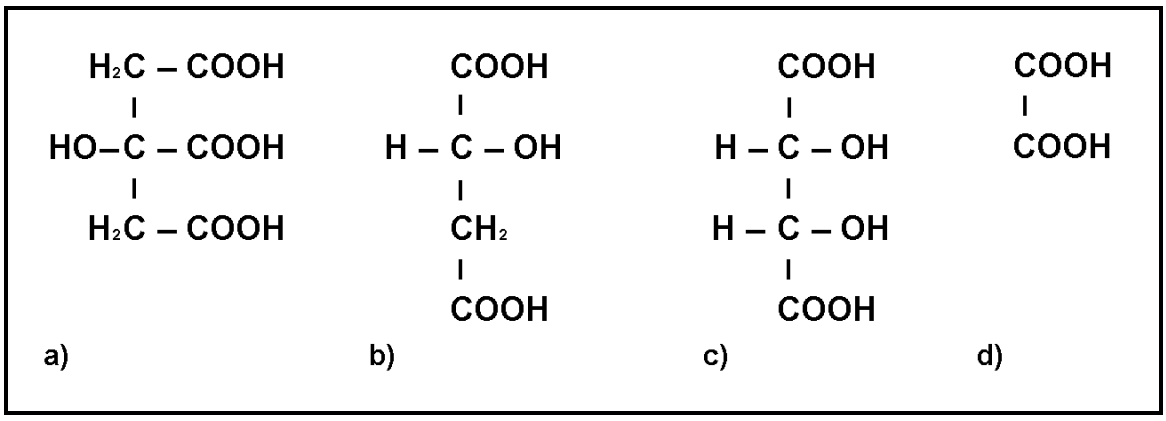

Figure 2.3 **Chemical structure organic acid present in pomegranate juice (a) citric acid, (b) malic acid, (c) tartaric acid and (d) oxalic acid.**

Table 2.7 **Organic acid content (g.100 mL^{-1}) of different pomegranate cultivars from different countries**

Cultivar	Malic acid	Citric acid	Tartaric acid	Total acids	Reference
Spain	0.14 - 0.18	0.14 - 2.32	0.00 - 0.05	0.22 - 2.92	Melgarejo *et al*., 2000
Turkey	0.03 - 0.41	0.39 - 1.31			Tezcan *et al*., 2009
Turkey	0.06 - 0.69	0.03 - 0.90			Poyrazoglu *et al*., 2002
Egypt		0.10			El-Nemr *et al*., 1990
Turkey	0.09 - 0.15	0.20 - 3.20			Ozgen *et al*., 2008

Soluble solids is an estimation of sugar content and determined in industry using a refractometer; this instrument measures the difference in density of distilled water and the juice sample by its refractive index and express the soluble solids as °Brix or percentage sucrose (Nielsen *et al.*, 1998). The sugar: acid ratio (TSS: TA) is used as a flavour indicator. The chemical composition of various pomegranate cultivars differ over a wide range from pH of 2.76-4.10, total soluble solids of 11.4-19.6°Brix and a TSS: TA ratio of 0.87-244 (Table 2.8).

Table 2.8 Chemical analyses of different pomegranate cultivars from different countries

Cultivar	pH	TSS	TA	TSS: TA	Reference
Iran	3.16-4.09	11.4-15.1	0.33-2.44	5.04-46.3	Teharanifar *et al.,* 2010
Spain	4.10	17.4	0.19	90.2	Artés *et al.,* 2000
USA	3.54	19.2	1.03	14.8	Holcroft *et al.,*1998
Saudi-Arabia	3.57	16.9	19.5	0.87	Al-Maiman & Ahmad, 2002
Turkey	3.30	15.0	3.39	4.42	Ayhan & Eştürk, 2009
Iran	3.06-3.74	15.8-19.6	0.51-1.35	31.0	Zarei *et al.,* 2010
Sultanate of Oman	2.76-4.03	13.7-15.2	0.06-0.48	31.6-244	Al-Said *et al.,* 2009
Spain	-	18.04	0.26	69.4	Gil *et al.*, 1996

Pre-harvest Factors Affecting Nutritional Quality

General guidelines are available to determine the ripeness and control the quality of pomegranate fruit since no formal maturity and quality indices have been established in South Africa yet. According to these guidelines pomegranates are ripe after 135-150 days of fruit set when the calyx starts closing, when the skin indents slightly and a metallic crack or 'hollow' sound is audible when tapping the fruit (Citrogold, 2011). Pomegranate quality is determined using skin appearance (colour, smoothness, free of cracks, cuts, bruises, sun scalding and decay), flavour (sugar/acid ratio), soluble solids content (>17%) and tannin content (<0.25%) (Citrogold, 2011).

The quality of fruit and vegetables is dependent of many pre-harvest factors i.e. biological, physiological, environmental, mechanical damage, extraneous matter and genetic factors (Kays, 1999). Entomological pests is a pre-harvest biological factor that affect the quality of South African pomegranates (Wohlfarter *et al*., 2010) The quality of pomegranate arils have been found to vary at different harvesting times, between cultivars and different regions of a country (Table 2.9). These factors will be discussed below.

Table 2.9 Pre-harvest factors affecting quality attributes of pomegranates

Pre-harvest factors	Country	References
Entomology	South Africa	Wohlfarter *et al.*, 2010
Region	Israel	Schwartz *et al.,* 2009
Cultivar	Iran	Mousavinejad *et al*., 2009
	Oman	Al-Said *et al*., 2009
	Israel	Tzulker *et al*., 2007
Cultivar and Season	Israel	Borochov-Neori *et al*., 2009;
Season	Spain	López-Rubira *et al*., 2005

Biological

Pomegranate cultivation is new in South Africa and no pre- and postharvest pesticides or fungicides have been registered to be used on the fruit yet (Brodie, 2009). Entomological pests threatening pomegranate production have been identified recently and include false codling moth, Thaumatotibia leucotreta, long tailed mealybug, Pseudococcus longispinus, various thrips species and small weevils (Wohlfarter *et al*., 2010). The targeted areas of destruction are young seedlings, leaves and fruit (Wohlfarter et al., 2010).

Region

Pomegranates are drought tolerant; favours mild winters (with temperatures above -12°C) and dry, hot summers (Levin, 2006). However pomegranates have been reported to grow in tropical, sub-tropical, Mediterranean, semi-arid and desert climates (Stover & Mercure, 2007; Ozgen *et al.*, 2008; Borochov-Neori *et al*, 2009; Schwartz *et al.*, 2009). Summer precipitation during the last ripening phase could result in undesirable peel-splitting that attract infection and pests to the arils (Levin, 2006). The South African is divided between the Western Cape which has a Mediterranean climate with winter precipitation and hot, dry summers while the rest of the country has a more semi-arid climate with summer afternoon showers and dry winters (Cowling *et al.*, 2009). South African pomegranates are mostly cultivated in Western Cape, Northern Cape, North West, Mpumalanga and Limpopo provinces (Wohlfarter *et al.*, 2010).

Pomegranate production is jointly dominated by India and Iran, followed in no specific order by USA, Turkey, Spain, Afghanistan and Israel (Brodie, 2009). However pomegranates have been grown and studied in, Australia, Argentina, Brazil, China, Greece, Russia, Saudi Arabia, South Africa and the Sultanate of Oman. Schwartz *et al*. (2009) showed that the quality and chemical composition of pomegranates grown in Israel was greatly influenced by different environmental regions (Mediterranean and desert region). Pomegranate arils from the Mediterranean region showed the higher antioxidant activity, total phenolics, total anthocyanins, TSS, glucose and fructose than the desert region. While the pomegranate peels from the desert region showed higher antioxidant activity and higher levels of total phenolics, especially punicalagin and punicalin than the Mediterranean region (Schwartz *et al*., 2009).

Seasonal

Spain is the main European pomegranate producer and harvest fruit from September to November (Gil *et al.,* 1996a) or even to December (Lopez-Rubira *et al*., 2005). Pomegranate

harvesting season in Israel start mid-July and ends in October (Borochov-Neori et al., 2009), while South African pomegranates are harvested between March and late May.

Harvesting fruit later during the season improved the sensory quality and antioxidant activity of Israeli pomegranates (Borochov-Neori *et al*., 2009). However when the arils are subjected to postharvest cold storage, López-Rubira *et al*. (2005) reported late-harvested Spanish pomegranate arils to have a shelf life of 10 days compared to early harvested fruits which had a prolonged shelf life of 14 days at 5°C and 95% relative humidity (RH). Higher respiration rate and higher initial yeast number of the late-harvested fruit was proposed as being a result of a more mature with higher sugar than an earlier harvested fruit, which could in turn have accelerated microbial growth and ultimately reduced the shelf life (López-Rubira *et al*., 2005). Pomegranate juice from late harvested pomegranate fruit showed higher total soluble solids, titratable acidity and red colour parameters (Dafny-Yalin *et al.* 2010).

Cultivar

Many cultivar pomegranates are available these days with various colours and sizes. In some cultivar the colour of the peel and the arils are not remotely the same. Physico-chemical characteristics of pomegranates that vary between cultivars include: fruit size, husk colour (yellow, purple, pink and red), aril colour (white, pink, red), hardness of the seed, maturity, juice content, acidity, sweetness, and astringency (Stover & Mercure, 2007) (Table 2.10).

Table 2.10 Summary of primary characteristics for some pomegranate cultivars (Stover & Mercure, 2007)

Cultivar	Traits	Origin
Early Wonderful	Deep red arils, medium-hard seeds, sweet/sour	USA, 2 weeks earlier than 'Wonderful'
Ganesh	Yellow-pink rind and pink-red arils, very soft seeds, sweet/sour	India
Granada	Deep-red arils, medium-hard seeds, sweet/sour	USA, redder, 1 month earlier than 'Wonderful'
Hicaznar	Dark-red skin, red arils, sweet/sour	Turkey
Kandhari (also called Arakta)	large fruit, deep-red rind, with deep-pink to blood-red arils, hard seeds, sweet/sour	India
Mollar de Elche	Deep-red arils with soft seeds, sweet, low acid	Spain
Mollar de Orihuela	Red-pink arils with soft seeds, sweet, low acid	Spain
Valenciana	Small, early but not top quality	Spain
Wonderful	Deep-red arils, medium-hard seeds, sweet/sour	USA

The nutritional quality, bioactive compounds and antioxidant activity may also vary in different cultivars. Stover & Mercure (2007) described 'Wonderful' as one of "the most deeply coloured of pomegranates in both husk and juice, with a rich flavour, good juice yield and both sprightly acidity and slight thirst quenching astringency similar to that of grapefruit and cranberries". But it all depends to which cultivar 'Wonderful' was compared to. The type of cultivar used will determine the sensory qualities, chemical composition and antioxidant activity of the fruit (Borochov-Neori *et al*., 2009). The most common grown cultivars in South Africa are: 'Arakta', 'Acco', 'Bahgwa', 'Ganesh', 'Herskawitz' 'Mollar de Che', 'Ruby' and 'Wonderful' (Brodie, 2009; Fawole *et al.*, 2011; Caleb *et al.*, 2012).

Postharvest Handling of Pomegranates

Pre-treatments of Pomegranates

Pomegranates are perishable products and various postharvest handling practices could influence their nutritional quality (Table 2.11). Chilling injury, husk scald and water dipping are some of the physiological disorders that occur when pomegranates are not stored at the correct temperature (D'Aquino *et al*., 2010). These authors reported that the combined use of a fungicide (fludioxonil) with film wrapping could reduce chilling injury. Nanda *et al.* (2001) reported that a sucrose polyester skin coating could extend the shelf life of 'Ganesh' fruits but shrink wrapping was most effective. You-lin & Run-guang (2008) reported that an acid peel coating reduced browning of the peel and could reduce chilling injury. Washing pomegranate arils with chlorine and then with antioxidant solution reduced browning of arils according to Gil *et al*. (1996b). The authors studied minimally processing of pomegranate arils and found the best quality arils were obtained after washing, packing inside with low levels of carbon dioxide and oxygen in polypropylene films and storing at 1°C.

Processing

A wide variety of pomegranate products are currently on the market, ranging from whole fruit and arils to pomegranate juice, fermented juice, wine, seed oil, vinegar, supplements, skin care products and the list continues to expand. Pomegranate juice consumption exceeds whole fruits not only by the time consuming process of peeling, but also by its perishable and seasonal nature (Gil *et al*., 1996b; Brodie, 2009). (Juven, 1984; Mirdehghan & Rahemi, 2007; Borochov-Neori *et al*., 2009). The chemical composition of various pomegranate products will differ by processing methods and the part of the fruit which was used (Kim *et al.,* 2002).

Storage of Pomegranates

Fruit can be classified into climacteric fruit (bananas) that continues to ripen after harvest and non-climacteric fruit (grapes) that should be harvested when ripe (McWilliams, 2008). During the ripening process fruits consume oxygen and produce carbon dioxide; this exchange is known as the respiration and is essential for the breakdown of macronutrients (carbohydrates, fats, protein) to useful end products and energy (Kader, 2002). Respiration continues past full maturation of the fruit into the senescence phase where energy reserves are depleted and undesirable metabolic changes occur (Kader, 2002). Non-climacteric fruit have a low respiration rate and ethylene production that stay relatively constant during ripening, while respiration rate and ethylene production is highest at the ripening stage in climacteric fruit (Kader, 2002). This classification is very important and influence the way we preserve fruit on a postharvest level. Literature classified whole pomegranates as non-climacteric fruit due to their low respiration rate (5-10 mg CO_2.kg^{-1}.hr^{-1} at 5°C) and very low ethylene production (<0.1 µL C_2H_4.kg^{-1}.hr^{-1} at 20°C) during storage (Kader, 2002).

During postharvest handling fruit can be preserved by manipulating the relative humidity, atmospheric composition and temperature of its storage environment Kader, 2002). When fruits transpire, water is lost by evaporation and result in nutritional, physical and aesthetic quality loses (Kader, 2002). Maintaining a high relative humidity during storage controls transpiration rate and preserves fruit from the aforementioned losses (Kader, 2002).

The basic gas composition air that we breathe which consists of nitrogen N_2 (78-79%), O_2 (20%), some CO_2 and other gases (<1%) can be modified passively or actively to preserve fruit (Hoehn *et al.*, 2009). Reducing the O_2 while increasing and/or decreasing CO_2 could delay the natural process of respiration and degradation (Hoehn *et al.*, 2009). Modified atmosphere packaging is a passive way to use films with different permeability that creates an atmosphere within the package that is different from the normal air outside the pack (Hoehn *et al.*, 2009). Controlled atmosphere (CA) is a continuous system where the level of O_2 and CO_2 are closely regulated and controlled (Hoehn *et al.*, 2009).

Modified Atmosphere Packaging (MAP) is a revolutionary way to extend the shelf life of pomegranate arils and is currently an important area of investigation worldwide (Gil *et al*., 1996a; Gil *et al.*, 1996b; Artés *et al*., 2000; Lopez-Rubira *et al*., 2005a; Lopez-Rubira *et al*., 2005b; Ayhan & Eştürk, 2009;). Pomegranate fruit stored at temperatures below 5°C are prone to distinctive browning and surface pitting, a condition known as chilling injury (Elyatem & Kader, 1984). Elevated storage temperature (25°C) of whole pomegranate fruit also showed detrimental effects on quality compared to lower temperature storage (8°C) in terms of sensory (marketable appearance), physical (weight, firmness) and nutritional

(moisture, sugar and vitamin C) attributes (Nanda *et al*., 2000). Elyatem & Kader (1984) found an increase in respiration rate of whole pomegranate fruit when stored at elevated temperatures. The rate of respiration is related to a fruit's perishability and is much higher in minimally processed compared to whole fruit (Kader, 2002).

The postharvest behaviour of the protected whole pomegranate fruit is different to exposed arils. Whole pomegranate fruit have a longer shelf life than arils and can be kept at low temperatures and high humidity for long periods of time (Table 2.11).

Table 2.11 Shelf life, temperature and packaging conditions of whole pomegranate fruit and arils

Cultivar	Arils/ Whole fruit	Shelf life duration	Temperature	Packaging conditions	Reference
'Mollar de Elche'	Whole fruit	13 weeks	Intermittent warming: 1 day at 20°C after 6 days at 2°C (95% RH)	Plastic boxes in gas-tight stainless steel containers	Artés *et al.*, 2000
'Ganesh'	Whole fruit	12 weeks	8°C (70-75% RH)	Shrink wrap film (25 µm) in ventilated corrugated fibre board	Nanda *et al.*, 2001
'Shlefy'	Whole fruit	16 weeks	5 and 7°C (85% RH)	Polyethylene packaging	Ghafir *et al*., 2010
'Mollar de Elche'	Arils	7 days	1°C	MAP: heat sealed pouches OPP*** film (40 µm)	Gil *et al.*, 1996b
Mollar de Elche'	Arils	10 days (late harvest) and 14 days (early harvest)	5°C	MAP: PP** baskets sealed with BOPP**** film (25 µm)	López-Rubira *et al.*, 2005
'Hicaznar'	Arils	18 days	5°C	PP** trays sealed with BOPP film (20 µm)	Ayhan & Eştürk, 2009
'Wonderful'	Arils	14 days	4°C	Perforated & semi-permeable film	Sepulveda *et al.*, 2000

*RH Relative Humidity **PP polypropylene; ***OPP Oriented Polypropylene; ****BOPP Biaxially oriented polypropylene

Minimally processed fruits should generally be stored at low temperature (0-5°C) with 95% relative humidity to reduce respiration rate, enzymatic processes and microbial activity (Gil *et al.*, 1996b; Kader, 2002; Nicola *et al*., 2009). Pomegranate arils stored at 4° and 8°C showed higher respiration rate and browning and lower pigmentation compared to 1°C (Gil *et al*., 1996b). Arils stored at temperatures between 1 and 5°C have a shelf life of between 7 and 14 days depending on postharvest treatments like packaging and storage temperature (Gil *et al*., 1996b; Lopez-Rubira *et al*., 2005; Ayhan & Eştürk, 2009; Ergun & Ergun, 2009).

There are various stages in the cold chain where temperature abuse might occur (Table 2.12). Therefore maintaining a constant storage temperature is essential to avoid fruit spoilage. Transportation and unloading of fruits from refrigerated vehicles to the retail supermarkets or distributor risk temperature fluctuation. Warm air from outside enters the refrigerated vehicles every time the doors are opened and might lead to an increase in product temperature especially those packed near the door entrance (Nicola *et al*., 2009).

Table 2.12 Stages and temperatures of the cold chain where temperature abuse might occur in fresh-cut products (Florkowski *et al*., 2009)

Stages in the Cold Chain	Air Temperature
At harvest	25°C*
Sorting and preparation	<12°C
Washing, cutting and packaging	4°C - 6°C
Shipping	> -0.5°C
Refrigerated transport vehicles (door open) Unloading at distributor or supermarkets	>10°C
Storage and display (retail refrigerators)	8°C - 10°C

*Room temperature

Packaging

With a well-managed cold chain, the packaging material and air composition surrounding the product could preserve the quality of fruit for longer (Nicola *et al*., 2009). With appropriate packaging material, the processed product would be safely contained and protected from physical damage when carrying the fruit to stores and the consumers' home. "It is also used, via labelling, to identify the product, provide a brand identity, and convey important information, such as use-by dating, preparation instructions, nutrient information, and storage instructions" (Schlimme, 1995). Pérez-Vicente *et al*., (2004) reported carton paperboard packaging material could deteriorate the colour compound anthocyanins in pomegranate juice due to its oxygen permeable properties.

Pomegranate arils are highly perishable and have a short shelf life. When pomegranate arils are not packaged, undesirable changes such as dehydration and shrivelling occurs (Gil *et al*., 1996b). Through modified atmosphere packaging, the ageing process of fruit can be slowed, delaying respiration rate of fruits by saturating the container with carbon dioxide and reducing oxygen levels to a bare minimum without causing undesirable metabolic changes or fermentation (Nicola *et al*., 2009). Modified atmosphere packaging in combination with other treatments and low storage temperature prolonged the shelf life of pomegranate arils (Gil *et al*., 1996b; López-Rubira *et al*., 2005; Ayhan & Eştürk, 2009). Gil *et al*. (1996b) reported that oriented polypropylene pouches with different gas permeabilities could be used to maintain

the fresh quality of minimally processed pomegranate seeds. This is not a substitute for cold chain management since respiration is greatly influenced by temperature. Juven *et al*. (1984) established that modified atmosphere packaged arils could last for three to four weeks at 1°C or 10-14 days at 5°C. The following spoilage microorganisms were found to be responsible for the off-flavour development: acetic acid bacteria (*Gluconobacter* and *Acetobacter*) and yeasts (*Hanseniaspora guilliermondii, Metschnikowia pulcherrima* and *Debaryomyces hansenii*) (Juven *et al.*1984). Furthermore, the increase of carbon dioxide during storage was sufficient to reduce bacterial and fungal counts between 15 and 25 days of storage.

Nanda *et al.* (2001) showed that peel thickness and freshness and firmness of the fruit were retained and weight loss greatly reduced by shrink wrapping. Holcroft *et al*. (1998) found that controlled atmosphere with elevated carbon dioxide levels reduced microbial growth on the whole pomegranate. The authors concluded that pomegranates stored at 10°C with carbon dioxide concentrations of 10kPa preserved the quality of the whole fruits as well as aril colour, and suggested the higher buffering capacity of pomegranate juice could preserve aril pigmentation, since carbon dioxide did not affect pH or TA. Whether pomegranates are sold as whole fruit, arils or as a juice, packaging is very important to maintain sensory quality, nutritional and chemical composition.

Conclusion

Various pomegranate cultivars are globally available and are distinguished by a diversity of characteristics such as peel colour, aril colour, size, weight, sweetness, acidity and hardness of the peel. Pomegranates have high antioxidant activity which is attributed to the contents of polyphenols, (anthocyanins and hydrolysable tannins), organic acids and vitamin C. Antioxidant activity of pomegranate peels are mainly derived from hydrolysable tannins and arils from anthocyanins. Polyphenols are broken down during fruit development to produce anthocyanins. The quality of pomegranate fruit is influenced by various pre-harvest (e.g. seasonal, cultivar, environmental conditions) and postharvest factors (e.g. processing, packaging, storage temperature and duration). Storage temperature, relative humidity and atmosphere affect respiration rate, shelf life and therefore fruit quality. Integrated modified atmosphere packaging and washing treatments could delay quality degradation and extend the shelf life of minimally processed fruit. There is a dearth of knowledge on the effects of postharvest handling on the nutritional quality of pomegranates grown in South Africa to support the development of relevant cold chain handling protocols.

References

Adams, L.S., Seeram, N.P., Aggarwal, B.B., Takada, Y., Sand, D. & Heber, D. (2006). Pomegranate Juice, Total Pomegranate Ellagitannins, and Punicalagin Suppress Inflammatory Cell signaling in Colon Cancer Cells. *Journal of Agricutural and Food Chemistry*, **54**, 980-985.

Al-Maiman S.A. & Ahmad, D. (2002). Changes in physical and chemical properties during pomegranate (*Punica granatum)* fruit maturation. *Food Chemistry*, **76**, 437-441.

Al-Said, F.A., Opara, L.U. & Al-Yahyai, R.A. (2009). Physico-chemical and textural quality attributes of pomegranate cultivars (*Punica granatum* L.) grown in the Sultanate of Oman. *Journal of Food Engineering*, **90**, 129-134.

American Association of Cereal Chemists (AACC) (2001) The definition of dietary fiber. *Cereal Foods World.* **46**, Pp. 112-126.

Artés, F., Villaescusa, R. & Tudela, J.A (2000). Modified Atmosphere Packaging of Pomegranate. *Journal of Food Science,* **65**, 1112-1116.

Association of Official Analytical Chemists International (AOAC). (2006). Chapter 37: Fruits and Fruit Products. In: *Official methods of analysis of AOAC International*. Gaithersburg, Md: AOAC International. Pp37-39.

Ayhan, Z. & Eştürk, O. (2009). Overall Quality and Shelf Life of Minimally Processed and Modified Atmosphere Packaged" Ready-to-Eat" Pomegranate Arils. *Journal of Food Science*, **74**, C399-C405.

Bailar, J.C. & Gornik, H.L. (1997) Cancer Undefeated. *The New England Journal of Medicine*, **336**, 1569-1574.

Bielsalski, H., Dragsted, L. O., Elmadfa, I., Grossklaus, R., Mulle, M., Schrenk, D., Walter, P. & Weber, P. (2009). Bioactive compounds: Definition and assessment of activity. *Nutrition*, **25**, 1202-1205.

Booth, S.L., Broe, K.E., Gagnon, D.R., Tucker, K.L., Hannan, M.T., McLean, R.R., Dawson-Hughes, B., Wilson, P.W.F., Cupples, L.A. & Kiel, D.P. (2003). Vitamin K intake and bone mineral density in women and men. *American Journal of Clinical Nutrition*, **77**, 512-516.

Borochov-Neori, H., Judeinstein, S., Tripler, E., Harari, M., Greenberg, A., Shomer, I & Holland, D. (2009). Seasonal and cultivar variations in antioxidant and sensory quality

of pomegranate (*Punica granatum L.*) fruit. *Journal of Food Composition and Analysis*, **22**, 189-195.

Brodie, L. (2009). Pomegranate Production in South Africa. *South African Fruit Journal,* **8**, 30-35.

Caleb, O.J., Mahajan, P.V., U.L. Opara and Witthuhn, C.R. (2012). Modelling the effect of time and temperature on respiration rate of pomegranate arils (cv. “Acco” and “Herskawitz”). *Journal of Food Science, **77**,* E80-E87.

Citrogold. (2011). Producing Pomegranates in South Africa. URL. http://www.citrogold.co.za/Producing%20Pomegranates%20in%20South%20Africa%20Citrogold%202011.pdf. Accessed 17/11/2012.

Coultate, T.P. (2007). *Food The Chemistry of Its Components*. Pp. 42, 62-63, 184, 221-237, 281-289. UK: RSC Publishing.

Cowling, R.M., Procheş, S. & Patridge, T.C. (2009). Explaining the uniqueness of the Cape flora: Incorporating geomorphic evolution as a factor for explaining its diversification. *Molecular Phylogenetics and Evolution*, **51**, 64-74

Curl, A.L. (1963). The carotenoids from several low carotenoid fruits. *Journal of Food Science*, **29**, 241-245.

Dafny-Yalin, M., Glazer, I., Bar-Ilan, I., Kerem, Z., Holland, D. & Amir, R. (2010). Colour, Sugars and Organic Acids Composition in Aril Juices and Peel Homogenates Prepared From Different Pomegranate Accessions. *Journal of Agricultural and Food Chemistry,* **58**, 4342-4352.

Dalimov, D.N., Dalimova, G.N. & Bhatt, M. (2003). Chemical Composition and Lignins of Tomato and Pomegranate Seeds. *Chemistry of Natural Compounds,* **39**, 37-40.

D’Aquino, S., Palma, A., Schirra, M., Continella, A., Tribulato, E. & La Malfa, S. (2010). Influence of film wrapping and fludioxonil application on quality of pomegranate fruit. *Postharvest Biology and Technology*, **55**, 121-128.

Defilippi, B.G., Whitaker, B.D., Hess-Pierce, B.M. & Kader, A.A. (2006). Development and control of scald on wonderful pomegranates during long-term storage. *Postharvest Biology and Technology*, **41**, 234-243.

Dewick, P.M. (2009). About this book and how to use it. In: *Medicinal Natural products: A biosynthetic approach.* 1st ed. p1. Chichester: Wiley.

Drogoudi, P.D. & Constantinos, T. (2005). Physical and Chemical Characteristics of Pomegranates. *HortScience*, **40**, 1200-1203.

Dumlu, M.U. & Gürkan, E. (2007). Elemental and Nutritional Analysis of *Punica granatum* from Turkey. *Journal of Medicinal Food*, **10,** 392-395.

Elyatem, S. M. & Kader, A. A. (1984). Post-harvest physiology and storage behaviour of pomegranate fruits. *Scientia Horticulturae*, **24**, 287-298.

Ergun, M. & Ergun, N. (2009). Maintaining quality of minimally processed pomegranate arils by honey treatments. *British Food Journal*, **111**, 396-406.

Fadavi, A. Barzegar, M. & Azizi, M.H. (2006). Determination of fatty acids and total lipid content in oil seed of 25 pomegranate varieties grown in Iran. *Journal of Food Composition and Analysis*, **19**, 676-680.

Faria, A. & Calhau, C. (2010). Pomegranate in Human Health: An Overview. In: *Bioactive Foods in Promoting Health: Fruits and Vegetables* (edited by R.R. Watson & V.R. Preedy). Pp. 551-563. Amsterdam: Academic Press.

Fawole, O.A., Opara, U.L. & Theron, K.I. (2011). Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa. *Food Bioprocess Technology*, DOI: 10.1007/s11947-011-0533-7.

Gil, M.I., Artés, F. & Tomás-Barberán, F.A. (1996a). Minimally Processing and Modified Atmosphere Packaging Effects on Pigmentation of Pomegranate Seeds. *Journal of Food Science*, **61**, 161-164.

Gil, M. I., Martínez, J. A. & Artés, F. (1996b). Minimally Processed Pomegranate Seeds. *Lebensmittel-Wissenschaft und-Technologie*, **29**, 708-713.

Gil, M. I., Tomás-Barberán, F.A., Hess-Pierce, B., Holcroft, D.M. & Kader, A.A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural Food Chemistry*, **48**, 4581-4589.

Guo, C., Wei, J., Yang, J., Xu, J., Pang, W. & Jiang, Y. (2008). Pomegranate juice is potentially better than apple juice in improving antioxidant function in elderly subjects. *Nutrition Research*, **28**, 72-77.

Heber, D. & Bowerman, S. (2009). Californian Pomegranates: The ancient fruit is new again. *Nutrition Today,* **44**, 180-184.

Hoehn, E., Prange, R.K. & Vigneault, C. (2009). Storage Technology and applications. In: *Modified and Controlled atmospheres for the storage, transportation and packaging of horticultural commodities.* (Edited by Yahia, E.M.) Pp. 1, 26. Boca Raton, FL: CRC Press.

Holcroft, D.M., Gil, M.I. & Kader, A.A. (1998). Changes in Anthocyanin Concentration, Phenylalanine Ammonia Lyase, and Glucosyltransferase in the Arils of Pomegranates Stored in Elevated Carbon Dioxide Atmospheres. *HortScience,* **32,** 496-497.

Ignarro, L. J., Byrns, R. E., Sumi, D., de Nigris, F. & Napoli, C. (2006). Pomegranate juice protects nitric oxide against oxidative destruction and enhances the biological actions of nitric oxide. *Nitric Oxide*, **15**, 93-102.

Jaiswal, V., DerMarderosian, A. & Porter, J.R. (2010). Anthocyanins and polyphenol oxidase from dried arils of pomegranate (*Punica granatum* L.). *Food Chemistry*, **118**, 11-16.

Juven, B. J., Gagel, S., Saguy, I. & Weisslowicz, H. (1984). Microbiology of spoilage of a perishable pomegranate product. *International Journal of Food Microbiology* **1**, 135-139.

Kader, A.A. & Barrett, D.M. (2005). Classification, Composition of Fruits, and Postharvest Maintenance of Quality. In: *Processing Fruits. Science and Technology* (edited by D.M Barrett, L. Somogyi & H. Ramaswamy). Pp. 3-22. Danvers: CRC Press LLC.

Kader, A.A. (2002). Postharvest Biology and Technology: An Overview. In: *Postharvest Technology of Horticultural Crops.* (edited by A. A. Kader). 3rd ed. Pp 39-48. California: University of California.

Kays, S. J. (1999). Preharvest factors affecting appearance. *Postharvest Biology and Technology*, **15**, 233-247.

Kim, N.D., Metha, R., Yu, W., Neeman, I., Livney, T., Amichay, A., Poirier, D., Nicholls, P., Kirby, A., Jiang, W., Mansel, R., Ramachandran, C., Rabi, T., Kaplan, B. & Lansky, E. (2002). Chemopreventive and adjuvant therapeutic potential of pomegranate (*Punica granatum*) for human breast cancer. *Breast Cancer Research and Treatment*, **71**, 203–217.

Kulkarni, A.P. & Aradhya, S.M. (2005). Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chemistry*, **93**, 319-324.

Lansky, E. P. & Newman, R. A. (2007). *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology,* **109**, 177-206.

Lei, F., Zhang, X.N., Wang, W., Xing, D.M., Xie, W.D., Su, H. & Du, L.J. (2007). Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice. *International Journal of Obesity*, **31**, 1023-1029.

Levin, G.M. (2006). *Pomegranate Roads: A Soviet Botanist's Exile from Eden.* Pp. 15-183. California: Floreant Press.

Liu, G., Xu, X., Hao, Q. & Gao Y. (2009). Supercritical CO_2 extraction optimisation of pomegranate (*Punica granatum*) seed oil using surface response surface methodology. *Food Science and Technology*, **42**, 1491-1495.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 74-185.

McWilliams, M. (2008). Overview of Carbohydrates. In: *Foods. Experimental Perspectives*. Pp. 131-142. Upper Saddle River: Pearson Prentice Hall.

Medical Research Council (MRC) (2010). Fruit. In: *Condensed Food Composition Tables for South Africa* (edited by Wolmarans, P., Danster, N., Dalton, A., Rossouw, K. & Schönfeldt, H.) 1st ed. p. 3-15. Cape Town: Medical Research Council.

Melgarejo, P., Salazar, D.M., Amorós, A. & Artés, F. (1995). Total lipids content and fatty acid composition of seed oils from six pomegranate cultivars. *Journal of the Science of Food and Agriculture*, **69**, 253-256.

Melgarejo, P., Salazar, D.M. & Artés, F. (2000). Organic acids and sugars composition of harvested pomegranate fruits. *European Food Research and Technology,* **211**, 185-190.

Mirdehghan, S. H. & Rahemi, M. (2007).Seasonal changes of mineral nutrients and phenolics in pomegranate (Punica granatum L.) fruit. *Scientia Horticulturae*, **111**, 120-127.

Mirsaeedghazi, H., Emam-Djomeh, Z. & Ahmadkhaniha, R. (2011). The effect of frozen storage on the anthocyanins and polyphenolic components of pomegranate juice. *Journal of Food Science and Technology,* (in press)

Mousavinejad, G., Emam-Djomeh, Z., Rezaei, K. & Khodaparast, M.H.H. (2009). Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. *Food Chemistry*, **115**, 1274-1278.

Nanda, S., Rao, D.V.S. & Krishnamurthy, S. (2001). Effects of shrink film wrapping and storage temperature on the shelf life and quality of pomegranate fruits cv. Ganesh. *Postharvest Biology and Technology*, **22**, 61-69.

Naveena, B. M., Sen, A. R., Vaithiyanathan, S., Babji, Y. & Kondaiah, N. (2008). Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. *Journal of Food Science and Technology.* **43**, 1807-1812.

Nielsen, S.S. (1998). Chapter 8: Moisture & Total Solids Analysis. In: *Food Analysis*. (edited by R.L. Bradley). 2nd ed. Pp. 134. Maryland: Aspen Publishers.

Nikniaz, Z., Mahdavi, R., Rafraf, M. & Jouyban, A. (2009). Total phenols and vitamin C contents of Iranian fruits. *Nutrition and Food Science*, **39**, 603-608.

Nicola, S. Tibaldi, G. & Fontana, E. (2009). Fresh-cut Produce Quality: Implications for a Systems Approach. In: *Postharvest Handling: A Systems Approach.* (edited by W.J. Florkowski, R.L. Shewfelt, B. Brueckner, & S.E. Prussia). Pp. 270-273. Oxford: Academic Press.

Noda, Y., Kaneyuki, T., Mori, A. & Packer, L. (2002). Antioxidant Activities of Pomegranate Fruit Extract and Its Anthocyanindins: Delphinidin, Cyanidin, and pelargonidin. *Journal of Agricultural and Food Chemistry*, **50**, 166-171.

Opara, L.U., Al-Ani, M.R. & Al-Shuaibi, Y.S. (2009). Physico-chemical Properties, Vitamin C Content and Antimicrobial Properties of Pomegranate Fruit (*Punica granatum* L.). *Food and Bioprocess Technology*, **2,** 315-321.

Patil, B.S., Jayaprakasha, G.K., Murthy, K.N. & Vikram, A. (2009). Bioactive Compounds: Historical Perspectives, Opportunities, and Challenges. *Journal of Agricultural Food Chemistry*, **57**, 8142-8160.

Paul, D.K. & Shaba, R.K. (2004). Nutrients, Vitamins and Nutrient Content in Common Citrus Fruits in the Northern Region of Bangladesh. *Pakistan Journal of Biological Sciences*, **7**, 238-242.

Pérez-Vicente, A., Serrano, P., Abellá, P. & García-Viguera, C. (2004). Influence of packaging material on pomegranate juice colour and bioactive compounds, during storage. *Journal of the Science of Food and* Agriculture, **50**, 639-644.

Ramulu, P. & Rao, P.U. (2003). Total, insoluble and soluble dietary fiber contents of Indian fruits. *Journal of Food Composition and Analysis*, **16**, 677-685.

Roy, S.K. & Waskar, D.P. (1997). Pomegranate. In: *Postharvest Physiology and Storage of Tropical and Subtropical Fruits* (edited by S.K. Mitra). Pp. 365-374. Oxford: CAB International.

Schwartz, E., Tzulker, R., Glazer, I., Bar-Ya'akov, I., Wiesman, Z., Tripler, E., Bar-Ilan, I., Fromm, H., Borochov-Neori, H., Holland, D. & Amir, R. (2009). Environmental Conditions Affect the Color, Taste, and Antioxidant Capacity of 11 Pomegranate Accessions' Fruits. *Journal of Agricultural and Food Chemistry*, **57**, 9197-9209.

Schlimme, D. (1995). Marketing Lightly Processed Fruits and Vegetables. *HortScience*, **30**, 15-17.

Sepúlveda, E., Galletti, l., Sáenz, C. & Tapia, M. (2000). Minimal Processing of pomegranate var. Wonderful. In: *Symposium on production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology*. CIHEAM – IAMZ p 237-242. Zaragoza, Spain.

Stover, E. & Mercure, E.W. (2007). The Pomegranate: A New Look at the Fruit of Paradise. *HortScience*, **42**, 1088-1092

Syed, D.N., Afaq, F. & Mukhtar, H. (2007). Invited article: Pomegranate derived products for cancer chemoprevention. *Seminars in Cancer Biology*, **17**, 377-385.

Tehranifar, A., Zarei, M., Nemati, Z., Esfandiyari, B. & Vazifeshenas, M.R. (2010). Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. *Scientia Horticulturae*, **126**, 180-185.

Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B. & Erim, F.B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry*, **115**, 873-877.

Türk, G., Sönmez, M., Aydin, M., Yüce, A., Gür, S., Yüksel, M., Aksu, E.H. & Aksoy, H. (2008). Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. *Clinical Nutrition*, **27**, 289-296.

Tzulker, R. Glazer, I., Bar-Ilan, I., Holland, D., Aviram, M. & Amir, R. (2007). Antioxidant Activity, Polyphenol Content, and Related Compounds in Different Fruit Juices and Homogenates Prepared from 29 Different Pomegranate Accessions. *Journal of Agricultural and Food Chemistry,* **55**, 9559-9570.

United States Department of Agriculture (USDA). (2010). National Nutrient Database for Standard Reference, Release 23, Food Group: Fruit and Fruit Juices. Pomegranates, raw [WWW document]. URL http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/SR23/reports/sr23fg09.pdf. Accessed 19/11/2010.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., Sendra, E., Sayas-Barberá, E. & Pérez-Álvarez, J.A. (2011). Antioxidant properties of pomegranate (*Punica granatum* L.) bagasses obtained as co-product in the juice extraction. *Food Research International*, **44**, 1-7.

Whitney, E. & Rolfes, S.R. (2005). *Understanding Nutrition*. Pp. 111,124, 125, 156-161, 323, 366-389. Belmont: Thomson Wadsworth.

Wiesman, Z., Kaufman, M., Wiessbein, I.D.S., Ram, A., Golan, R., Kogan, L., Berman, S. & Eshet, I. (2008). Negev Pomegranate Project with Emphasis on Seed Oil. *ISHS Acta Horticulturae*, **778**, 45-52.

Wohlfarter, M., Giliomee, J.H. & Venter, E. 2010. A Survey of the Arthropod Pests Associated with Commercial Pomegranates, Punica granatum (Lythraceae), in South Africa. *African Entomology*, **18**, 192-199.

You-lin, Z. & Run-guang, Z. (2008). Study on the Mechanism of Browning of Pomegranate (*Punica granatum* L. *cv.* Ganesh) Peel in Different Storage Conditions. *Agricultural Sciences in China*, **7**, 65-73.

Zarei, M., Azizi, M. & Bashiri-Sadr, Z. (2010). Studies on Physico-Chemical properties and bioactive compounds of Six Pomegranate Cultivars Grown in Iran. *Journal of Food Technology,* **8**, 112-117.

CHAPTER 3

EFFECTS OF STORAGE TEMPERATURE AND DURATION ON CHEMICAL PROPERTIES, PROXIMATE COMPOSITION AND SELECTED BIOACTIVE COMPONENTS OF POMEGRANATE (PUNICA GRANATUM) ARILS

Abstract

The effects of storage temperature on the nutritional composition of three pomegranate cultivars ('Arakta', 'Bahgwa' and 'Ruby') were investigated. Pomegranates were procured from Porterville, South Africa, hand-peeled and the arils stored at 1°, 4°, and 8°C at 95% RH for 14 days. Arils stored at 1°C was removed from cold storage after 14 days and kept at ambient conditions (~21°C) for an additional 15th day. Physico-chemical attributes, anthocyanins, ascorbic acid and proximate composition was measured on day 0, 7, 14 and 15. O_2 consumption and CO_2 production increased at elevated temperatures and throughout storage duration. The proximate composition of pomegranate arils was very similar between cultivars: 80% moisture, 0.52% Ash, 1.3-1.5% fat, 2.7-2.9% dietary fibre, 1.1-1.2% protein, 14-15% carbohydrate 310-320 kJ.100g^{-1} energy. 'Arakta' had higher of TA level (0.33±0.01 g.100 mL^{-1}) and ascorbic acid (25.6±1.25 mg.L^{-1}), 'Bahgwa' with highest level of anthocyanins (112.5±2.09 mg.L^{-1}) and 'Ruby' with lighter coloured arils with a lower hue angle (*L** 35.5±0.33; *H*° 17.3±0.42). Low β-carotene levels (1.71-3.54 mg.L^{-1}) were detected in all pomegranate arils. Nutritional composition of pomegranate arils was mostly unaffected when stored at 1° and 4°C for 14 days. Higher temperature storage did not affect the TSS but increased the TA and reduced the TSS/TA in all cultivars. Storage duration caused an increase in TA and a decrease in TSS/TA in 'Ruby' arils after 14 days. No visual detection of mould growth was seen in 'Arakta' and 'Bahgwa' arils stored at 1°C and 95% RH after 14 days. During this study higher storage temperature did affect the proximate composition, physico-chemical attributes and bioactive components negatively.

Introduction

Pomegranate (*Punica granatum* L.) fruit is known for its chemo preventative and anti-inflammatory potential due to its high antioxidant activity (Kim *et al.*, 2002; Lansky *et al*., 2007; Syed *et al*., 2007). Some bioactive components reported in pomegranate arils in

variable proportions are anthocyanins, ascorbic acid and β-carotene (Curl, 1963; Drogoudi & Constantinos, 2005; Dumlu & Gürkan, 2007; Tzulker *et al*. 2007). Anthocyanins are responsible for the attractive colour of pomegranate arils and some of the fruit's anti-oxidant activity (Borochov-Neori *et al*., 2009). Extracting arils is a time consuming process and therefore minimally processed pomegranate arils are sold as a convenience product (Gil *et al.*, 1996). However the storage duration of arils differ to whole fruit. Whole fruit may be stored for 3-4 months at temperatures below 10°C (Elyatem & Kader, 1984; Artés *et al.*, 2000; Nanda *et al.*, 2001; Ghafir *et al*., 2010) and arils for 1-2 weeks at temperatures below 5°C (Gil *et al.*, 1996; Artés *et al.*, 2000; Sepulveda *et al.*, 2000; López-Rubira *et al.*, 2005; Ayhan & Eştürk, 2009). Higher temperature storage (4°, 8°C) have shown higher respiration rate and browning and lower pigmentation in pomegranate arils (Gil *et al*., 1996b). Although many studies have been reported on pomegranates around the world, there is a dearth of information regarding the effect that storage temperatures have on the nutritional properties of South African grown pomegranate arils. This is particularly important since several researchers have shown that fruit quality of pomegranates differ significantly among growing regions (Schwartz *et al*., 2009). The objective of this investigation is to study the effect of storage temperature and duration on the nutritional composition, physico-chemical properties and bioactive components of minimally processed pomegranate fruit ('Arakta', Bahgwa and 'Ruby').

Material and Methods

Plant Material

Commercially ripe pomegranates ('Arakta', 'Bahgwa' and 'Ruby') were procured from Houtconstant farm in the Western Cape (33°01'00"S, 18°58'59"E). No formal maturity index are available for pomegranates, therefore the producer used general guidelines like the period after fruit set (135-150 days), appearance (skin colour and smoothness) and flavour (sugar/acid ratio) to determine commercial ripeness of the fruit **(Citrogold)**. Two hundred fruit of each cultivar was hand-picked and care was taken to pick fruit that was of good quality, which was not bruised or worm infested. The fruit was put into plastic open crates and cardboard boxes and transported in the boot of an air-conditioned car for ±120km the same day to the Postharvest Technology and Research Laboratory, Stellenbosch University. Pomegranate fruit was harvested on 6 April 2011 ('Ruby'), 20 April 2011 ('Arakta') and 4 May 2011 ('Bahgwa'), kept at ambient temperature (~21°C) overnight before peeling the next day.

Storage Condition and Packaging

Fruit was sorted before peeling to exclude any rotten or severely sunburnt fruit. Each fruit was rinsed with distilled water and peeled manually using a sharp knife and extra care was taken to avoid damaging the arils. Pomegranates were handled during peeling, sorting and packaging at ambient temperatures (~21°C). Healthy, firm, and bright red arils were considered good quality arils and used in this study whilst soft, brown, black, white (sunburnt), and ruptured or damaged arils were considered poor quality arils and discarded. Arils from each fruit was packaged into individual zip-lock bags and kept at 5°C until all the fruit of that cultivar was peeled. Arils were sorted at ambient temperature (~21°C) to ensure that all the arils peeled were of good quality before being mixed together to create a homogenous batch of all the fruit from the same cultivar. Freshly peeled arils of each cultivar were analysed in triplicate on day 0 for all analyses. The remaining arils were divided into three batches to fill (100 g arils) the punnets necessary to study each temperature treatments (1°, 4° and 8°C) for analysis days 7, 14 and 15. Arils from three punnets of the same temperature treatment were pooled and mixed together and analysed in triplicate on day 7 and 14. Only arils stored at 1°C was visibly free of mould growth after 14 days and therefore analysed for an additional day (day 15) at ambient conditions (~21°C). Figure 3.1 illustrates the type of packaging used during this study.

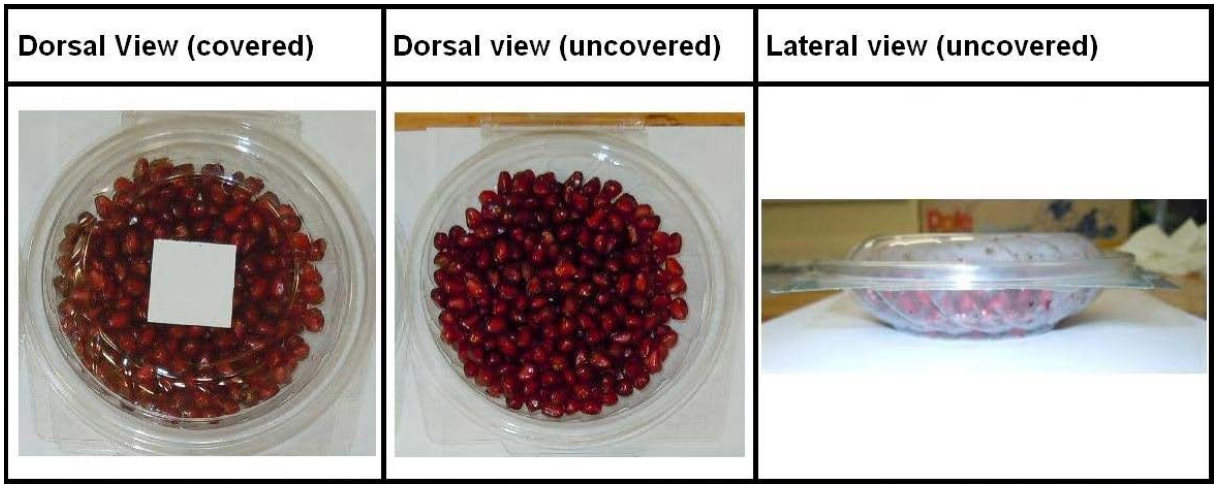

Figure 3.1 Depiction of type of packaging used during this study from the dorsal (covered and uncovered) and lateral views.

Arils were packaged into 330 mL, 300 micron 'clampshell' polyethylene terephthalate punnets (Zibo plastics, South Africa) and stored for 14 days in 3 sliding door refrigerators set at 1°, 4° and 8°C with a measured relative humidity of 95%. Currently, no packaging film or

gas flushing is used to package arils in South Africa yet. Therefore the arils were packed as is using the packaging material illustrated in Fig 3.1. After 14 days of cold storage, arils stored at 1°C from 'Arakta' and 'Bahgwa cultivars were taken out of cold storage and placed at ambient conditions (22°C, 68% RH) for the 15th day to simulate unrefrigerated transportation and/or storage conditions of the product.

Fruit Processing

From the single sample, triplicate samples of 60 g arils were hand pressed using cheesecloth and used for physico-chemical and bioactive component analyses (Ayhan & Eştürk, 2009). Of each freshly squeezed aril juice sample, 20 mL were used for physico-chemical analyses, stored at 5°C until required and 20 mL of the same aril juice were used for bioactive component analyses and stored at -80°C using an ultra-low temperature freezer (U725 Innova, New Brunswick Scientific, England). Proximate composition was determined using 40 g triplicate sample fresh arils which 5 g were used for moisture analysis, and 35 g arils were subjected to freeze drying (SP Scientific, USA) for 5 days, grounded with a handheld grinder to a powder and stored at -20°C for fat, protein, dietary fibre and ash analyses. The moisture loss due to freeze drying were noted for each sample and used to calculate the proximate composition of pomegranate arils on a wet weight basis (wwt%). Samples for the chemical and bioactive component analysis of 'Ruby' arils were not measured on the 15th day, because it was decided to measure a 15th day only after the 'Ruby' have already been packaged and rationed for 14 analyses days. The juice samples of 'Ruby' arils for the bioactive component analysis for day 14 was accidently discarded and could also not be measured during this study.

Headspace Gas Composition Analysis

Before opening the packages, the internal headspace gas composition (oxygen and carbon dioxide) was determined using a headspace gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Gas analysis was performed by inserting the needle attached to the gas analyser through the lid of each package. Results were expressed as O_2% and CO_2%, respectively. The atmospheric gas composition (20.2±0.14 O_2%; 0.1±0.00 CO_2%) was measured before measuring the headspace gas composition of the packages.

Visual Quality Analysis

Microbial analysis was not in the scope of this study and therefore a subjective visual inspection was performed to estimamte the visible mould growth of the arils inside the packages. The visual mould growth was estimated on a scale of 0-100% with 25%

intercepts. There was very little incidence of browning, therefore % browning was not reported during this study.

Physico-Chemical Analyses

The colour of triplicate sample of 15 mL aril juice was measured in a covered glass petri dish, swirling the juice between triplicate readings. The colour was measured in terms of Commission International del' Eclairage (CIE) *L**, *a**, *b** colour coordinates using a chroma meter (Model CR-400/410 Konica Minolta sensing Inc., Japan). The (CIE) colour coordinates (*L**, *a**, *b**) were measured in three readings from three triplicate samples and the mean of these nine measurements were determined for each temperature treatment. The instrument was calibrated against a white plate (*L* = 97.3, *a* = -0.27 *b* = 2.26). These CIE tristimulus colour coordinates (*L**, *a**, *b**) measure colour in terms of lightness or brightness (*L**), red/green (*a**) and blue/yellow (*b**) (Aligourchi & Barzegar, 2009). The hue angle (*H°*) calculated from $\tan^{-1}$ (*b**/*a**) represents the colour: 0°= red-purple; 90°=yellow; 180°bluish-green; 270°=blue (Schwartz *et al.* 2009). The chroma was calculated from $(a^{*2} + b^{*2})^{1/2}$ and represents the intensity or saturation of that colour (Aligourchi & Barzegar, 2009; Opara *et al.*, 2009). Difference in colour represents a measure of quality and was calculated from $[(L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$ (Aligourchi & Barzegar, 2009). Triplicate samples of fresh aril juice was used to measure colour, pH, total soluble solids (TSS) and titratable acidity (TA). A pH meter (pH-Meter BASIC 20+, Crison; Model 00924; Spain) was used to measure the pH after calibrating with pH buffers 4 and 7. The TSS level was measured using a hand refractometer (Atago PR-32 α, Japan) and expressed as °Brix. TA of 2 mL fresh aril juice diluted with 70 mL distilled water was measured based on the AOAC method 965.30 (AOAC, 2006) using an automated 862 Compact Titrosampler (Metrohm, Switzerland). The titrosampler potentiometrically titrated the diluted juice with 0.1N NaOH (Merck) to an end point of pH 8.1 and expressed TA as g. $100mL^{-1}$ citric acid. The total soluble solids: titratable acidity ratio of the pomegranate juice was calculated and expressed as TSS/TA.

Proximate Composition Analyses

Moisture content was measured using the AOAC method 925.45 by drying 5g of fresh arils from triplicate samples at 50°C for 4 days in a dry oven (Model nr. 072160, Prolab Instruments, Sep Sci, South Africa) to a constant weight (AOAC, 2005). The moisture content method was modified by drying the arils at a lower temperature for a longer duration instead of at 100°C for 3 hours. Protein, dietary fibre, ash and fat content were measured using the freeze dried samples. Protein content was determined using the Kjeldahl AOAC method 920.152 (AOAC, 2005), where 1g freeze dried sample was digested at 400°C for 1.5h in the presence of sulphuric acid (Merck) and a catalyst tablet (Merck) using a DKL

heating digester (Velp Scientifica, Italy). The digest was diluted with 45 mL distilled water before neutralisation with 35% sodium hydroxide through a four minute distillation process using the distillation unit (Model UDK129, Italy) The neutralised digest was distilled into boric acid solution (Merck) containing a methyl red (Merck) and bromocrescyl green indicator (Merck) solution that changed colour from acidic red to alkali green colour. The green boric acid solution was in turn titrated with 0.1N sulphuric acid until the solution turned red again. A conversion factor of 6.25 was used to calculate the protein% from the N%. Percentage ash content was determined using 2g freeze dried sample measured in porcelain dishes and incinerating the sample at 510°C for 13 hours in an electric muffle furnace using AOAC method 940.26 (Model LEF-203P, LabTech, Korea) (AOAC, 2005). Dietary fibre was determined using the non-enzymatic gravimetric AOAC method 993.21 for total dietary fibre in foods and food products with <2% starch (AOAC, 2005). Fat content was measured via the soxhlet extraction method 963.16 (AOAC, 2006) using the solvent extraction unit (Model nr. SER 148, Velp Scientifica, Italy). Carbohydrate content was calculated by difference; subtracting the sums of the percentage protein, total fat, dietary fibre, moisture and ash from 100. Total available carbohydrates%: 100 – (Protein% + Total Fat% + Dietary Fibre% + Moisture% + Ash%).

Analyses of Selected Bioactive Components

ANTHOCYANIN

Anthocyanin pigments are subject to change between pH 1.00 and pH 4.50. This colour change is caused by the change in structure of pigment itself. At pH 1.00 the anthocyanin pigment exists as the coloured oxonium or flaylium form and change to the carbinol at pH 4.50 (Lee *et al*, 2005). This change in colour was used to measure the total monomeric anthocyanin content of pomegranate juice using the pH differential method 2005.02 (AOAC, 2005). Pomegranate juice thawed at room temperature, diluted with 50% aqueous methanol (Kimix) and sonicated in cold water (Ultrasonic Cleaner DC400H, MRC Ltd. Holon, Israel) for 10 min to extract the anthocyanin pigments, before being centrifuged (Eppendorf Model nr. 5810 R, Merck, Hamburg, Germany) to obtain a clear solution of the extract. The extract was diluted with potassium chloride (pH 1.00 buffer, Sigma) until the absorbance of the solution was in linear range of the spectrophotometric absorbance at 510 nm using a UV-Vis Helios Omega spectrophotometer (Thermo Fisher Scientific, Madson, USA). The same dilution factor was used to dilute the extract with sodium acetate (Merck). The wavelength reading was performed at 510 and 700 nm after 10 min incubation in a dark cabinet. The triplicate aril juice samples were measured twice and 50% methanol was used as a blank. The difference in absorbance was calculated using the following equation: $A = (A_{510} - A_{700})_{pH\ 1.00} - (A_{510} - A_{700})_{pH\ 4.50}$ (AOAC, 2005). Total monomeric anthocyanin pigments (TMAP) were calculated

as follows: TMAP = [(A x MW x DF x 100) / (ε x L)] where A: Absorbance; MW: molecular weight of anthocyanin (449.2); DF: dilution factor; ε: molar absorptive coefficient (26900) and L: cell pathway (AOAC, 2005). Results were expressed as mg cyanidin-3-glucoside per L of juice (AOAC, 2005).

ASCORBIC ACID

Ascorbic acid is a reducing agent and was determined spectrophotometrically against a standard curve using 0.0025% 2,6-dichlorophenolindophenol (DCP) dye (Fluka) and 1% metaphosphoric acid (MPA) (Sigma) (Barros *et al.*, 2007). The combination of blue coloured DCP dye and colourless MPA resulted in a pink coloured solution, which was decolorised (reduced) in the presence of ascorbic acid. Ascorbic acid of unknown concentrations in pomegranate juice samples was quantified using a standard curve of known concentrations from a stock solution (1 $mg.mL^{-1}$) L-ascorbic acid (Sigma). Both the stock solution and the juice samples were diluted with a DCP-MPA solution and the absorbance measured at 515 nm wavelength using a UV-Vis Helios Omega spectrophotometer (Thermo Fisher Scientific, Madson, USA). The concentration ascorbic acid was inversely proportional to the absorbance values (Barros *et al.*, 2007). Pomegranate juice samples were thawed at room temperature, diluted with MPA, vortexed (Model nr. G560E, Scientific Industries, USA) and sonicated (Ultrasonic Cleaner DC400H, MRC Ltd. Holon, Israel) for 3 min in cold water to extract the ascorbic acid present in the juice. The extract was centrifuged at 12857 g at 4°C to obtain a clear homogenous solution, diluted with DCP dye and kept in a dark cabinet for 10 min. To correct for the natural pink colour of pomegranate juice, another set of centrifuged extract samples were taken and diluted with distilled water instead of MPA. The absorbance of the samples (MPA and water diluted extracts) and standard curve was read at 510 nm wavelength using a UV-Vis Helios Omega spectrophotometer (Thermo Fisher Scientific, Madson, USA). The triplicate aril juice samples were measured twice and MPA served as the blank (Barros *et al.*, 2007). Ascorbic acid values were extrapolated from a standard curve with R^2>0.90. Ascorbic acid content was expressed as mg ascorbic acid per L pomegranate juice

TOTAL CAROTENOIDS

Total content of carotenoids was determined colometrically against a β-carotene standard curve (Sigma) (Quiro's & Costa, 2006). Pomegranate juice was thawed and diluted with ethanol: hexane (1:1) (Servochem, Merck) with added butylhydroxy toluene (BHT) (Merck). The samples were vortexed (Model nr. G560E, Scientific Industries, USA), sonicated (Ultrasonic Cleaner DC400H, MRC Ltd. Holon, Israel) in cold water for 10 min and centrifuged at 6429 g, 4°C for 5 min. The absorbance of the samples as well as the standard

curve was measured at 470nm wavelength using a UV-Vis Helios Omega spectrophotometer (Thermo Fisher Scientific, Madson, USA). The triplicate aril juice samples were measured twice and ethanol: hexane (1:1) with added BHT was used as a blank. Results were extrapolated from a standard curve with R^2>0.90 and expressed as mg β-carotene per L pomegranate juice.

Statistical Analysis

Statistical analyses were carried out using Statistica software version 10 (Tulsa, USA), to evaluate the effects that different storage temperatures have on various quality attributes of individual pomegranate cultivars. Triplicate data was pooled and subjected to analyses of variance analysis (ANOVA) while significant difference of the mean values was determined using Fisher's least significant difference (L.S.D.) multiple comparison test at $P < 0.05$.

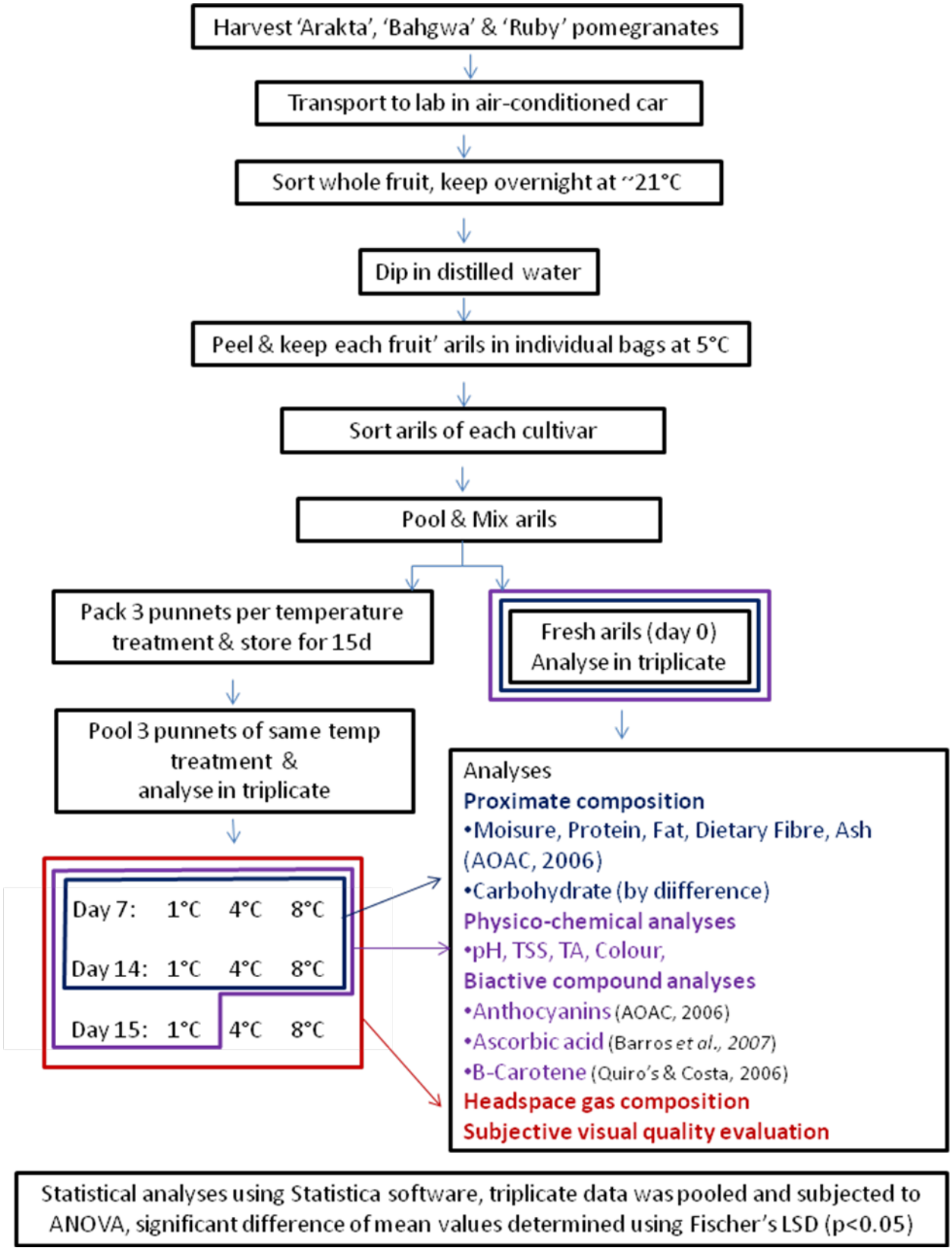

Figure 3.2 Experimental design

Results and Discussion

General Composition

The antioxidant activity, physico-chemical and phenolic attributes have been reported to vary in pomegranate fruit due to cultivar and geographical variation (Stover & Mercure, 2007; Ozgen *et al.*, 2008; Borochov-Neori *et al*, 2009; Schwartz *et al.*, 2009). The general composition of freshly harvested pomegranate arils of 'Arakta', 'Bahgwa' and 'Ruby' will be discussed in this section. The colour of arils differed slightly among pomegranate cultivars in terms of the uniform CIE *L** (+ light; - dark), *a** (+ red; - blue), *b** (+ yellow; - green), *H°* (Hue angle) and *C** (chroma) colour space. Ruby juice had a pinkish colour and showed slightly higher *L**, *a** and *C** levels, while 'Arakta' and 'Bahgwa' juice were darker red and showed slightly higher *H°* levels (Table 3.1). The TSS levels did not differ between the cultivars studied and ranged from 15.1-15.9°Brix. Similar TSS levels were found in pomegranates grown in the Sultanate of Oman (13.7-15.2°Brix) (Al-Said *et al.*, 2009) as well as Iranian pomegranates (11.4-15.1°Brix) (Teharanifar *et al.,* 2010). The TSS/TA was slightly higher in 'Ruby' (58.6±8.76) compared to 'Arakta' (45.3±1.87) and 'Bahgwa' (49.9±3.28). 'Arakta' cultivar had slightly higher TA (0.33±0.01 mg.100 mL^{-1}) and ascorbic acid content (25.6±1.25 $mg.L^{-1}$) compared to 'Bahgwa' and 'Ruby' (Table 3.1). The ascorbic acid level was higher than Egyptian pomegranate cultivar 7.0 $mg.L^{-1}$ (El-Nemr *et al.*, 1990). 'Bahgwa' showed higher anthocyanin level (112.5 $mg.L^{-1}$) compared to 'Arakta' (73.3 $mg.L^{-1}$) and 'Ruby' (34.1 $mg.L^{-1}$) (Table 3.1). The anthocyanin level agreed with Ozgen and others (2008) who reported a variation of 6.10-219 mg. L^{-1} between 6 pomegranate cultivars grown in Turkey. The β-carotene levels in pomegranates were very low (1.7-3.5 $mg.L^{-1}$) in agreement with Curl (1963). The proximate composition of pomegranate arils was similar between cultivars and the MRC's food composition table (Table 2.1, chapter 2): 80% moisture, 0.5% ash, 1.3-1.5% fat, 1.1-1.2% protein, 2.7-2.9% dietary fibre, 14-15% carbohydrates, with an energy value of 315 - 320 $kJ.100\ g^{-1}$ (Table 3.1). However the fat and dietary fibre percentage from this data was 10 fold and 3 fold greater than the MRC's food composition table respectively.

Table 3.1 Physico-chemical and nutritional information of fresh 'Arakta', 'Bahgwa' and 'Ruby' pomegranates

Characteristics	Arakta	Bahgwa	Ruby
Colour characteristics			
*L**	34.1±0.35^{b}	33.9±0.60^{b}	35.5±0.33^{a}
*a**	12.2±1.07^{ab}	11.2±1.33^{b}	12.8±0.88^{a}
*b**	4.83±0.44^{a}	4.35±0.57^{b}	3.90±0.30^{c}
Chroma	13.0±1.15^{ab}	11.9±1.43^{b}	13.3±0.92^{a}
H°	22.1±0.29^{a}	21.7±0.43^{b}	17.3±0.42^{c}
Chemical composition			
TA	0.33±0.01^{a}	0.29±0.01^{ab}	0.28±0.05^{b}
pH	2.71±0.04^{a}	2.84±0.11^{a}	2.80±0.05^{a}
TSS (°Brix)	15.1±0.10^{b}	14.6±0.52^{b}	15.9±0.21^{a}
TSS/TA	45.3±1.87^{b}	49.9±3.28^{ab}	58.6±8.76^{a}
Bioactive components			
Anthocyanins (mg.L^{-1})	73.3±5.71^{b}	112.5±2.09^{a}	34.1±1.25^{c}
Ascorbic Acid (mg.L^{-1})	25.6±1.25^{a}	13.7±1.81^{c}	21.0±2.14^{b}
β-Carotene (mg.L^{-1})	1.71±6.32^{b}	3.54±1.26^{a}	2.24±5.01^{b}
Proximate composition			
Moisture	79.6±0.31^{a}	79.7±0.55^{a}	79.4±0.15^{a}
Ash (% wwt)	0.54±0.00^{a}	0.52±0.03^{a}	0.53±0.00^{a}
Fat (%wwt)	1.50±0.03^{a}	1.51±0.36^{a}	1.26±0.18^{a}
Dietary fibre (%wwt)	2.78±0.22^{a}	2.92±0.10^{a}	2.67±0.18^{a}
Protein (%wwt)	1.20±0.06^{a}	1.18±0.10^{a}	1.13±0.03^{a}
Carbohydrates (%wwt)	14.4±0.26^{a}	14.1±0.88^{a}	15.0±0.45^{a}
Total energy (kJ.100g^{-1})	319.3±5.16^{a}	314.5±3.59^{a}	318.6±4.75^{a}

Different letters denote a significant difference according to the LSD multiple range test.

Moisture content (82.5% moisture) of different pomegranate cultivars agreed with literature findings (76-81% moisture) (Chapter 2, Table 2.2). Ash, moisture, protein and carbohydrate content measured for all cultivars were within range of literature findings of Al-Maiman & Ahmad (2002) and the MRC Food Composition Tables (2010). The fat content was four to five times the amount reported by literature (Al-Maiman & Ahmad, 2002; Paul & Shaba, 2009; MRC, 2010) and might be due to different method of analysis that was used. However higher lipid levels were reported in different pomegranate cultivars as dry weight basis in pomegranate seeds 2.41-3.37%, 9.74-14.8%, 27.2% by Dumlu & Gürkan (2007), Fadavi *et al.* (2006) and El-Nemr *et al.* (1990) respectively. Dietary fibre level in pomegranates was 4 to 5 times higher than reported literature findings (Paul & Shaba, 2009; MRC, 2010). Carbohydrate content was calculated by difference (14.1-15.0%) and according to literature is comprised of sugars (fructose and glucose) (Melgarejo *et al.*, 2000; Tezcan *et al.*, 2009; Dafny-Yalin *et al.*, 2010). Dafny-Yalin and others (2010) declared a strong correlation between TSS and soluble sugars level. Therefore TSS level (14.6-15.9°Brix) might be related to the carbohydrate content (14.1-15.0%) and reflect the sugar content of the three pomegranate cultivars.

Whole Fruit Storage Duration and Seasonal Variation

The chemical attributes of the same pomegranate cultivars were measured directly after harvest (freshly harvested) in 2010 by Fawole and others (2011). Therefore the seasonal variation in chemical attributes could be compared between the harvest season 2010 (Fawole *et al.*, 2011) and 2011 using data of chapter 3 (Table 3.2). Between chapter 3 and 4 whole pomegranates were subjected to cold storage at 7°C and 95% RH for a duration of 10 ('Bahgwa'), 12 ('Arakta') and 14 ('Ruby') weeks. The effects of this storage duration on the chemical attributes of the whole fruit were investigated using data of the initial day of analyses (day 0) for both chapters 3 and 4 (Table 3.2).

Table 3.2 Effects of season (2010 and 2011 chapter 3) as well as whole fruit storage duration (2011 chapter 3 and 4) on the chemical parameters of 'Arakta', 'Bahgwa' and 'Ruby' pomegranate arils measured after peeling (day 0).

Cultivar	Chemical parameters	2010	2011	
		Freshly harvested	Freshly harvested	Post-storage
		Fawole *et al.*, 2011	Chapter 3	Chapter 4
Arakta	TA	0.28±0.01	0.33±0.01	0.33±0.03
	pH	3.43±0.02	2.71±0.04	3.29±0.00
	TSS (°Brix)	15.1±0.12	15.1±0.10	15.4±0.47
	TSS/TA	53.4±1.91	45.3±1.87	47.6±5.73
Bahgwa	TA	0.32±0.01	0.29±0.01	0.28±0.01
	pH	3.32±0.03	2.84±0.11	3.24±0.01
	TSS (°Brix)	14.4±0.27	14.6±0.52	15.7±0.10
	TSS/TA	45.7±1.72	49.9±3.28	55.4±1.17
Ruby	TA	0.22±0.01	0.28±0.05	0.26±0.01
	pH	3.64±0.04	2.80±0.05	3.35±0.01
	TSS (°Brix)	14.1±0.28	15.9±0.21	15.8±0.10
	TSS/TA	63.1±2.73	58.6±8.76	60.0±1.36

Different letters denote a significant difference according to the LSD multiple range test

Considering the initial data (day 0) of chapter 3 and 4 it is clear that cold storage between these studies did not affect the TA or TSS/TA even though the intial TSS in 'Bahgwa' was slightly higher in chapter 4 compared to chapter 3 (Table 3.2). Very low pH values were obtained from freshly harvested fruit in chapter 3 (pH 2.7-2.8) compared to chapter 4 (pH 3.2-3.4) and 2010 (pH 3.4-3.6) (Table 3.2). The pH level of all cultivars might appear to increase after cold storage of whole fruit between chapter 3 and 4. However the mean pH values of 'Arakta' (pH 3.21±0.05) and 'Bahgwa' (pH 3.25±0.03) taken on day 7 during this study (Table 3.6a, b) correspond better with initial pH values of 'Arakta' (pH 3.29±0.00) and

'Bahgwa' (pH 3.24±0.01) of chapter 4 (Table 3.2). Therefore the effect of seasonal variability on pH will be better explained using pH values of chapter 4 instead of chapter 3. During 2011 the pH level of all cultivars was slightly lower compared to 2010 (Table 3.2).

The TSS/TA was unaffected by harvesting season and whole fruit storage duration in all cultivars (Table 3.2). The TA of 'Arakta' and 'Ruby' was slightly higher while TA of 'Bahgwa' was slightly lower during 2011 compared to 2010 (Table 3.2). The TSS of 'Arakta' was unaffected by both harvesting season and whole fruit storage duration. However the TSS of 'Ruby' was slightly higher in 2011 compared to 2010 (Table 3.2).

In summary, even though the TSS/TA was relatively unaffected by both harvesting season and whole fruit storage, it seems as though season did affect the pH, TA and TSS levels of different pomegranate cultivars. Whole fruit storage duration seem to cause an slight accumulation of TSS level in 'Bahgwa' arils.

Headspace Gas Composition Analysis

The headspace gas composition and a subjective quality evaluation for mould prevalence of packaged pomegranate arils stored in PET packages was measured on day 0, 7, 14 and 15 to investigate the effect that storage duration and temperature have on the quality and headspace gas composition of packaged 'Arakta' and 'Bahgwa' pomegranates arils. Pomegranate arils use oxygen for metabolic processes and release the by-products of these processes in the form of moisture, heat and carbon dioxide (Kader & Barrett, 2005) just like the respiratory system of human beings. During this study the headspace gas concentration was only measured for three days in an open system experiment with no gas flushing and close monitoring of oxygen consumption and carbon dioxide production. Therefore respiration rate was not measured, but the headspace gas concentration shows a clear picture of decreasing O_2 and increasing CO_2 over time and especially after 15th day at ambient conditions (~21°) in both 'Arakta' and 'Bahgwa' cultivars (Fig. 3.3a, b). If we assume that the decreasing O_2 and increasing CO_2 was an indication of oxygen consumption and carbon dioxide production, then these findings could be related to an increasing respiration rate over time. The CO_2 production and O_2 consumption of 'Arakta' and 'Bahgwa' arils increased at higher temperatures, although headspace gas composition of 'Arakta' did not differ significantly at both 1° and 4°C after 15th day (Fig. 3.3a).

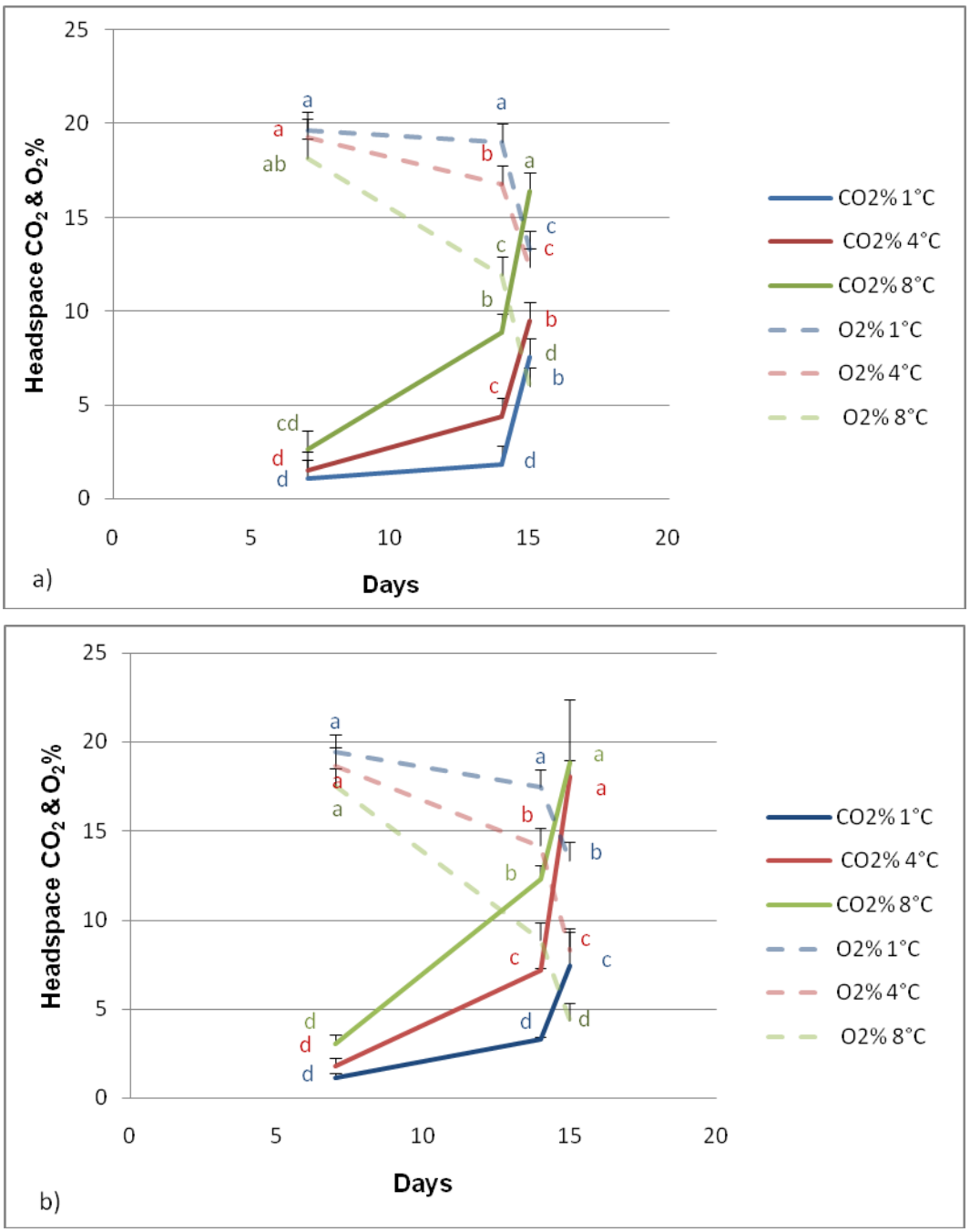

Figure 3.3 **Headspace CO_2 and O_2 of a) 'Arakta' and b) 'Bahgwa' arils stored at 1, 4 and 8°C for 14 days and an additional 15th day at ambient temperature (~21°). The 15th day at ambient conditions simulated temperature conditions that would occur after purchasing the product. Error bars denote standard error of the mean values.**

No visual mould growth was detected on 'Arakta' and 'Bahgwa' arils when stored at 1°C for 14 days (Table 3.3). However, mould growth was detected after 14 days on 'Bahgwa' arils stored at 4°C and even more so on both 'Arakta' and 'Bahgwa' arils stored at 8°C. When 'Arakta' and 'Bahgwa' pomegranate arils were stored for an additional day (up to 15th day) at ambient conditions (~21°C), only arils previously stored at 1°C showed no visble signs of

mould growth. The aforementioned samples were subjected to further physico-chemical and selected bioactive component analyses and those previously stored at 4° and 8°C were discarded.

Table 3.3 Effect of storage duration and temperature on the mould incidence of 'Arakta' and 'Bahgwa' pomegranate arils stored for 14 days at 1°, 4 and 8°C for 14 days with an additional 15th day at ambient temperature (~21°) to simulate possible temperature variation after purchase.

Cultivar	Temp	Visual mould estimation (%)			
		Day 0	Day 7	Day 14	Day 15
Arakta	1°C	0	0	0	0
	4°C	0	0	0	25-50%
	8°C	0	0	<25%	>50%
Bahgwa	1°C	0	0	0	0
	4°C	0	0	<25%	<25-50%
	8°C	0	<25%	<25%	50%

Some pomegranate cultivars might have the ability to handle higher storage temperatures due to their different gas exchange rates. 'Arakta' might be more acceptable at both 1° and 4°C and 'Bahgwa' at only at 1°C for 14 days. Storage temperature at 1°C for 14 days had little effect on CO_2 and O_2 levels in packaged pomegranate arils, but when the packaged arils were left at ambient conditions (~21°C) after the 15th day, significant changes did occur. Gil *et al.* (1996) used the lowest respiration rate as one of the measures to recommend 1°C temperature for best quality preservation of pomegranate arils. Pomegranates stored at 1°C for 14 days had the least effect on headspace gas composition and would be recommended as the best storage temperature of pomegranate arils, but 4°C could also be adequate especially in the industry where it is sometimes difficult to maintain a cold chain of 1°C.

Physico-Chemical Analysis

COLOUR ANALYSIS

The effect of storage duration and temperature on the CIE *L**, *a**, *b**, chroma and *H*° coordinates of 'Arakta', 'Bahgwa' and 'Ruby' is shown in Table 3.4a, b and c respectively.

Table 3.4a Effect of storage temperature on the physical parameter (colour) of 'Arakta' pomegranate arils

Colour	Temp	Day 0	Day 7	Day 14	Day 15
	1°C	34.2±0.35^{abc}	33.8±0.38^{de}	33.4±0.36^{e}	33.9±0.15^{bcd}
*L**	4°C	34.2±0.35^{abc}	34.2±0.48^{ab}	33.8±0.36^{cd}	-
	8°C	34.2±0.35^{abc}	34.4±0.42^{a}	33.8±0.42^{d}	-
	1°C	12.2±1.07^{ab}	10.3±0.87^{c}	10.0±0.91^{c}	11.8±0.45^{ab}
*a**	4°C	12.2±1.07^{ab}	12.2±1.26^{ab}	11.6±0.95^{b}	-
	8°C	12.2±1.07^{ab}	12.7±1.01^{a}	11.7±1.30^{b}	-
	1°C	4.38±0.44^{ab}	3.88±0.42^{c}	3.77±0.35^{c}	4.69±0.23^{b}
b*	4°C	4.38±0.44^{ab}	4.79±0.57^{ab}	4.62±0.43^{b}	-
	8°C	4.38±0.44^{ab}	5.12±0.52^{a}	4.81±0.64^{ab}	-
	1°C	13.0±1.15^{ab}	10.9±0.95^{c}	10.6±0.97^{c}	12.6±0.50^{ab}
Chroma	4°C	13.0±1.15^{ab}	12.9±1.37^{ab}	12.4±1.03^{b}	-
	8°C	13.0±1.15^{ab}	13.5±1.11^{a}	12.5±1.42^{b}	-
	1°C	22.1±0.29^{bc}	21.1±0.84^{d}	21.1±0.26^{d}	22.1±0.28^{bc}
H°	4°C	22.1±0.29^{bc}	22.0±0.44^{c}	22.2±0.22^{bc}	-
	8°C	22.1±0.29^{bc}	22.5±0.85^{ab}	22.9±0.50^{a}	-

Different letters denote a significant difference according to the LSD multiple range test.

Table 3.4b Effect of storage temperature on the physical parameter (colour) of 'Bahgwa' pomegranate arils

CIE Lab	Temp	Day 0	Day 7	Day 14	Day 15
	1°C	33.9±0.60^{ab}	33.1±0.39^{c}	34.3±1.37^{a}	34.2±0.57^{a}
*L**	4°C	33.9±0.60^{ab}	33.4±0.68^{bc}	33.9±0.27^{ab}	-
	8°C	33.9±0.60^{ab}	33.1±0.24^{c}	33.9±0.43^{ab}	-
	1°C	11.2±1.33^{ab}	9.67±1.37^{c}	12.4±3.04^{a}	12.4±1.16^{a}
*a**	4°C	11.2±1.33^{ab}	9.91±2.19^{bc}	11.5±0.74^{a}	-
	8°C	11.2±1.33^{ab}	9.31±0.66^{c}	12.1±0.87^{a}	-
	1°C	4.35±0.57^{b}	3.70±0.72^{c}	4.50±0.92^{ab}	4.83±0.38^{ab}
*b**	4°C	4.35±0.57^{b}	3.57±1.07^{c}	4.46±0.26^{ab}	-
	8°C	4.35±0.57^{b}	3.42±0.32^{c}	4.99±0.44^{a}	-
	1°C	11.9±1.43^{ab}	10.3±1.51^{c}	13.0±3.15^{a}	13.2±1.21^{a}
Chroma	4°C	11.9±1.43^{ab}	10.4±2.38^{bc}	12.2±0.77^{a}	-
	8°C	11.9±1.43^{ab}	9.83±0.72^{c}	13.0±0.96^{a}	-
	1°C	21.7±0.43^{b}	21.2±1.41^{bc}	20.6±1.20^{cd}	21.8±0.40^{b}
H°	4°C	21.7±0.43^{b}	19.9±1.58^{d}	21.8±0.34^{b}	-
	8°C	21.7±0.43^{b}	20.6±0.50^{cd}	22.9±0.38^{a}	-

Different letters denote a significant difference according to the LSD multiple range test.

Table 3.4c Effect of storage temperature on the physical parameter (colour) of 'Ruby' pomegranate arils

CIE Lab	Temp (°C)	Day 0	Day 7	Day 14
	1°C	35.5±0.33^{b}	36.4±1.18^{a}	36.7±0.46^{a}
*L**	4°C	35.5±0.33^{b}	36.7±0.77^{a}	36.2±0.33^{a}
	8°C	35.5±0.33^{b}	36.5±0.38^{a}	36.5±0.68^{a}
	1°C	12.8±0.88^{d}	13.0±0.86^{cd}	13.5±0.53^{bc}
*a**	4°C	12.8±0.88^{d}	14.0±0.69^{b}	12.9±0.28^{cd}
	8°C	12.8±0.88^{d}	14.8±0.48^{a}	13.7±0.82^{b}
	1°C	3.90±0.30^{c}	4.06±0.30^{c}	4.01±0.07^{c}
*b**	4°C	3.90±0.30^{c}	4.35±0.14^{b}	3.92±0.10^{c}
	8°C	3.90±0.30^{c}	5.02±0.13^{a}	4.26±0.2^{b}
	1°C	13.3±0.92^{d}	13.5±0.84^{cd}	14.0±0.53^{bc}
Chroma	4°C	13.3±0.92^{d}	14.5±0.68^{b}	13.4±0.26^{cd}
	8°C	13.3±0.92^{d}	15.5±0.48^{a}	14.3±0.82^{b}
	1°C	17.3±0.42^{bc}	17.8±1.56^{b}	16.9±0.41^{c}
H°	4°C	17.3±0.42^{bc}	17.6±0.71^{b}	17.3±0.59^{bc}
	8°C	17.3±0.42^{bc}	19.2±0.44^{a}	17.6±0.54^{b}

Different letters denote a significant difference according to the LSD multiple range test.

Lightness (*L**) was unaffected by temperature except in 'Ruby' which increased in lightness at 8°C on day 7 and 14 Table 3.4a. To simplify interpretation, redness (*a**), yellowness (*b**), colour intensity (chroma) and shade (*H°*) will be jointly referred to as 'individual colour attributes'. Storage temperature affected the individual colour attributes of 'Arakta' and 'Ruby' in the same way (Table 3.4a, c). The individual colour attributes increased with higher temperatures 1°C<4°C<8°C on day 14 compared to day 0. Individual colour attributes were mostly stable at 1° and 4°C on day 7 and 14 for all cultivars. On day 7 a general decrease was observed in individual colour attributes of 'Bahgwa' (Table 3.4b) irrespective of storage temperature and colour attributes were especially higher in 'Ruby' arils at 8°C (Table 3.4c). The individual colour attributes of 'Bahgwa' was unaffected by storage temperature except for the hue angle which increased with increasing temperatures (Table 3.4b). Studies have shown packaging arils could lighten the colour of 'Mollar' arils as opposed to unpacked arils, while higher temperature storage (8°C) caused increased browning and reduced pigmentation of these arils (Gil *et al*., 1996). It might be possible that the increased *H°* was related to browning in 'Bahgwa' arils over time.

A total colour difference (Δ*E**) of Δ*E**=3 in red wine has been reported to be visible with the human eye according to Martinez *et al*., (2001). Using the aforementioned criteria, a total colour difference greater than 3 was only visible to the human eye in 'Bahgwa' over the storage period of 15 days (Table 3.5). The Δ*E** of 'Bahgwa' was stable at 4°C throughout the

study, while ΔE^* >3 between day 7 and 14 at both 1° and 8°C, as well as between day 0-14 and day 14-15 at 1°C. A general increase in $H°$ at all temperatures between day 7 and 14 caused ΔE^* >3 in 'Bahgwa' arils. This colour difference did not differ significantly between temperatures and therefore could be attributed to the effect of storage duration rather than storage temperature. Even though the $H°$ decreased at 1°C between day 7 and day 14, the $H°$ was the same for day 0 and 15. This confirms the observation that colour was stable in all cultivars at 1° and 4°C.

Table 3.5 The effect of storage temperature on the total colour difference of 'Arakta', 'Bahgwa' and 'Ruby' cultivars.

Cultivar	Temp (°C)	ΔE^*_{d0-7}	ΔE^*_{d7-14}	ΔE^*_{d0-14}	ΔE^*_{d14-15}	ΔE^*_{d0-15}
Arakta	1°C	2.19±1.42^{a}	1.07±0.81^{a}	2.55±0.91^{a}	2.10±1.39	1.25±0.65
	4°C	1.38±0.61^{ab}	1.69±0.98^{a}	0.85±0.60^{b}	-	-
	8°C	1.17±0.54^{b}	1.26±0.53^{a}	1.14±0.95^{b}	-	-
Bahgwa	1°C	2.11±1.47^{a}	4.34±2.88^{a}	4.04±1.98^{a}	3.78±2.58	1.38±1.05
	4°C	1.74±1.46^{b}	2.37±1.70^{a}	1.07±0.84^{c}	-	-
	8°C	2.38±1.59^{a}	3.32±1.16^{a}	2.47±0.87^{b}	-	-
Ruby	1°C	1.71±0.75^{a}	1.52±1.11^{a}	1.60±0.39^{a}	-	-
	4°C	2.04±1.16^{a}	1.47±0.79^{a}	0.96±0.38^{b}	-	-
	8°C	2.58±0.76^{a}	1.59±0.49^{a}	1.56±0.65^{a}	-	-

Different letters denote a significant difference according to the LSD multiple range test

CHEMICAL ANALYSES

Chemical attributes like TA, TSS and TSS/TA is used to describe the taste (flavour) in terms of sourness or sweetness. TA and citric acid greatly contribute to the flavour of pomegranate aril juice and was reported to vary more often in cultivars of different taste profiles compared to TSS and sugars (Dafny-Yalin, 2010). The effect of storage duration on the chemical attributes of pomegranate arils stored at 1°C for 14 days and at ambient temperature (~21°C) for the 15th day can be seen in Table 3.6a b and c.

Table 3.6a Effect of storage temperature on the chemical properties of 'Arakta' pomegranate arils

Chemical Parameters	Temp (°C)	Day 0	Day 7	Day 14	Day 15
TA	1°C	0.33±0.01^{d}	0.35±0.01^{c}	0.33±0.01^{d}	0.33±0.01^{d}
	4°C	0.33±0.01^{d}	0.36±0.02^{bc}	0.33±0.01^{d}	
	8°C	0.33±0.01^{d}	0.38±0.02^{b}	0.40±0.01^{a}	
pH	1°C	2.71±0.04^{d}	3.24±0.05^{a}	3.23±0.02^{ab}	3.21±0.04^{ab}
	4°C	2.71±0.04^{d}	3.22±0.02^{ab}	3.17±0.01^{b}	
	8°C	2.71±0.04^{d}	3.17±0.06^{b}	3.06±0.03^{c}	
TSS (°Brix)	1°C	15.1±0.10^{b}	14.8±0.15^{bc}	15.7±0.15^{a}	15.7±0.06^{a}
	4°C	15.1±0.10^{b}	14.5±0.47^{c}	15.0±0.15^{a}	
	8°C	15.1±0.10^{b}	14.8±0.49^{bc}	15.6±0.15^{a}	
TSS/TA	1°C	45.3±1.87^{a}	42.0±1.04^{b}	47.6±1.25^{a}	47.5±1.46^{a}
	4°C	45.3±1.87^{a}	40.4±1.98^{bc}	47.7±0.43^{a}	
	8°C	45.3±1.87^{a}	39.2±0.67^{c}	39.3±0.75^{c}	

Different letters denote a significant difference according to the LSD multiple range test.

Table 3.6b Effect of storage temperature on the chemical properties of 'Bahgwa' pomegranate arils

Chemical Parameters	Temp (°C)	Day 0	Day 7	Day 14	Day 15
TA	1°C	0.29±0.01^{b}	0.31±0.00^{b}	0.31±0.01^{b}	0.30±0.01^{b}
	4°C	0.29±0.01^{b}	0.30±0.00^{b}	0.31±0.00^{b}	
	8°C	0.29±0.01^{b}	0.31±0.01^{b}	0.46±0.03^{a}	
pH	1°C	2.84±0.11^{c}	3.23±0.04^{ab}	3.31±0.02^{a}	3.32±0.03^{a}
	4°C	2.84±0.11^{c}	3.27±0.02^{a}	3.32±0.03^{a}	
	8°C	2.84±0.11^{c}	3.26±0.02^{a}	3.11±0.01^{b}	
TSS (°Brix)	1°C	14.6±0.52^{b}	14.5±0.98^{b}	15.4±0.21^{a}	15.2±0.26^{ab}
	4°C	14.6±0.52^{b}	15.5±0.15^{a}	15.3±0.12^{ab}	
	8°C	14.6±0.52^{b}	15.0±0.47^{ab}	14.9±0.06^{ab}	
TSS/TA	1°C	49.9±3.28^{ab}	46.8±3.18^{b}	49.8±0.97^{ab}	51.2±0.21^{a}
	4°C	49.9±3.28^{ab}	51.6±0.51^{a}	49.3±0.37^{ab}	
	8°C	49.9±3.28^{ab}	49.0±0.67^{ab}	32.3±2.15^{c}	

Different letters denote a significant difference according to the LSD multiple range test.

Table 3.6c Effect of storage temperature on the chemical properties of 'Ruby' pomegranate arils

Chemical Parameters	Temp (°C)	Day 0	Day 7	Day 14
TA	1°C	0.28±0.05^{de}	0.24±0.00^{e}	0.35±0.01^{bc}
	4°C	0.28±0.05^{de}	0.28±0.07^{de}	0.33±0.04^{cd}
	8°C	0.28±0.05^{de}	0.41±0.01^{ab}	0.47±0.00^{a}
pH	1°C	2.80±0.05^{c}	2.63±0.05^{d}	3.04±0.02^{a}
	4°C	2.80±0.05^{c}	2.69±0.02^{d}	2.99±0.00^{a}
	8°C	2.80±0.05^{c}	2.41±0.00^{e}	2.91±0.02^{b}
TSS (°Brix)	1°C	15.9±0.21^{a}	15.7±0.52^{ab}	15.9±0.00^{a}
	4°C	15.9±0.21^{a}	15.7±0.55^{ab}	16.0±0.06^{a}
	8°C	15.9±0.21^{a}	15.9±0.38^{a}	15.2±0.06^{b}
TSS/TA	1°C	58.6±8.76^{ab}	65.4±2.17^{a}	45.5±1.11^{c}
	4°C	58.6±8.76^{ab}	59.6±13.85^{ab}	49.1±6.21^{bc}
	8°C	58.6±8.76^{ab}	39.1±1.90^{cd}	32.8±0.12^{d}

Different letters denote a significant difference according to the LSD multiple range test.

The pH values were stable from day 7 to 15 in 'Arakta' and 'Bahgwa' stored at 1°C and 4°C cultivars, but decreased significantly on day 14 when arils were stored at 8°C. The pH value of 'Ruby' arils showed some fluctuation during the trial which was similar to reports from Ayhan & Eştürk (2009). The pH of 'Ruby' arils stored at 8°C was significantly lower compared to arils stored at 1°C and 4°C after 14 days.

TA increased in all cultivars when stored at 8°C over time. When pomegranate arils were stored at 1°C and 4°C, the TA was stable in 'Bahgwa' arils, in 'Arakta' arils the TA was stable at day 14 compared to day 0. TA in 'Ruby' arils stored at 1°C and 4°C also increased after 14 days. Increased TA after 7 storage days at 1°C was previously reported due to water loss in unpackaged arils (Gil *et al.*, 1996).

Higher storage temperature (8°C) caused a decreasing effect on TSS level in 'Ruby' arils, while lower storage temperatures (1°C) caused increased TSS level in both 'Arakta' and 'Bahgwa' arils after 14 days. This is in agreement with studies by Gil and others (1996) as well as Ayhan and Eştürk (2009) who reported declining TSS levels over time during modified atmosphere packaging (MAP) conditions.

The TSS/TA of all cultivars decreased when arils were stored at 8 °C for either 7 or 14 days. The decline in TSS/TA was attributed to the decline in TSS and increased TA when arils were stored at 8°C for 14 days. In 'Ruby' cultivar the TSS/TA decreased after 14 days in arils stored in arils stored at both 1° and 8°C.

Selected Bioactive Component Analysis

Table 3.7a, b and c shows the effect that storage duration and temperature have on anthocyanin, ascorbic acid and β-carotene levels in 'Arakta', 'Bahgwa' and 'Ruby' pomegranate arils respectively.

Table 3.7a Effect of storage temperature on the anthocyanin, ascorbic acid and β-carotene content of 'Arakta' pomegranate arils

Bioactive Components	Temp (°C)	Day 0	Day 7	Day 14	Day 15
Anthocyanins ($mg.L^{-1}$)	1°C	73.3±5.72^{ab}	76.0±2.35^{a}	71.1±0.80^{bcd}	72.8±2.07^{abc}
	4°C	73.3±5.72^{ab}	69.2±1.93^{cd}	71.9±1.88^{bcd}	
	8°C	73.3±5.72^{ab}	63.7±2.41^{e}	68.0±1.25^{d}	
Ascorbic Acid ($mg.L^{-1}$)	1°C	25.6±1.25^{e}	42.2±2.31^{a}	24.6±2.90^{e}	29.9±1.18^{d}
	4°C	25.6±1.25^{e}	39.8±2.89^{a}	37.0±1.48^{b}	
	8°C	25.6±1.25^{e}	34.1±3.02^{c}	30.3±1.90^{d}	
ß-Carotene ($mg.L^{-1}$)	1°C	1.71±0.63^{cde}	1.32±0.18^{e}	1.92±0.54^{bcd}	2.01±0.28^{bc}
	4°C	1.71±0.63^{cde}	2.61±0.41^{a}	1.39±0.36^{e}	
	8°C	1.71±0.63^{cde}	2.39±0.28^{ab}	1.43±0.14^{de}	

Different letters denote a significant difference according to the LSD multiple range test.

Table 3.7b Effect of storage temperature on the anthocyanin, ascorbic acid and β-carotene content of 'Bahgwa' pomegranate arils

Bioactive Components	Temp (°C)	Day 0	Day 7	Day 14	Day 15
Anthocyanins ($mg.L^{-1}$)	1°C	112.5±2.09^{a}	93.48±6.45^{b}	80.0±2.80^{de}	77.0±1.60^{ef}
	4°C	112.5±2.09^{a}	87.77±6.03^{c}	74.6±1.56^{f}	
	8°C	112.5±2.09^{a}	83.69±3.78^{cd}	97.2±6.85^{b}	
Ascorbic Acid ($mg.L^{-1}$)	1°C	13.7±1.81^{f}	38.6±2.74^{a}	22.9±1.82^{e}	15.4±2.78^{f}
	4°C	13.7±1.81^{f}	33.7±1.53^{b}	29.6±2.04^{cd}	
	8°C	13.7±1.81^{f}	31.3±1.76^{c}	28.4±1.68^{d}	
ß-Carotene ($mg.L^{-1}$)	1°C	3.54±1.26^{a}	2.26±0.25^{bc}	0.57±0.22^{d}	2.93±1.22^{ab}
	4°C	3.54±1.26^{a}	1.32±0.45^{cd}	1.49±0.08^{cd}	
	8°C	3.54±1.26^{a}	3.18±0.50^{ab}	1.56±0.14^{c}	

Different letters denote a significant difference according to the LSD multiple range test.

Table 3.7c Effect of storage temperature on the anthocyanin, ascorbic acid and β-carotene content of 'Ruby' pomegranate arils after 7 days of storage

Bioactive Components	Day 0	Day 7		
		1°C	4°C	8°C
Anthocyanins (mg.L^{-1})	34.1±1.25^{a}	32.1±1.96^{b}	33.3±1.10^{ab}	33.2±0.92^{ab}
Ascorbic Acid (mg.L^{-1})	21.0±2.14^{a}	5.27±2.98^{c}	7.69±3.75^{c}	12.8±1.84^{b}
ß-Carotene (mg.L^{-1})	2.24±0.50^{a}	1.88±0.40^{a}	1.93±0.24^{a}	1.93±0.46^{a}

Different letters denote a significant difference according to the LSD multiple range test.

ANTHOCYANIN ANALYSIS

Anthocyanins (e.g. cyanidin, delphinidin) are linked to pigmentation and antioxidant activity of pomegranate arils and juice (Drogoudi *et al.*, 2005; Tzulker *et al*., 2007). Tehranifar *et al*. (2010) reported a large variation of total anthocyanin levels in 20 pomegranate cultivars ranging from 5.56-30.11mg cyanidin-3-glucoside 100 g^{-1} juice. Pelargonidin, cyanidin and delphinidin are the most prominent anthocyanidins found in pomegranate fruit juice and cause the red, blue and an intermediate colour respectively (Noda *et al.,* 2002). Jaiswal *et al*., (2010) reported that anthocyanins were relatively heat stable without oxygen, but reduced considerably (65%) in the combined presence of heat and oxygen. These authors suggested that the enzyme polyphenol oxidase (PPO) might facilitate the destruction of total anthocyanins over time and that boiling and oven-drying arils could destroy PPO and preserve these anthocyanins.

The level of cyanidin-3-glucoside content in fresh 'Bahgwa' (112.49±2.09 mg.L^{-1}) was three times, and 'Arakta' (73.32±5.72 mg.L^{-1}) was twice that of 'Ruby' (34.06±1.25 mg.L^{-1}). Anthocyanin level was stable in 'Arakta' stored at 1°C but declined in proportion to higher temperature at 4°C and 8°C over time (Table 3.7a). There was no effect of storage duration and temperature on the anthocyanin level of 'Ruby' cultivar. The reduction in anthocyanin content might be attributed to the PPO enzyme as suggested by Jaiswal *et al*. (2010). A decrease in pigmentation was also reported in 'Mollar' pomegranate arils at 8°C (Gil *et al*., 1996). 'Ruby' samples were very limited and even though every precaution was taken to protect the samples for analyses, temperature abuse could have resulted in the low levels of some bioactive components.

ASCORBIC ACID ANALYSIS

'Arakta' (25.62±1.25 mg.100 mL^{-1}) contained the highest level of ascorbic acid followed by 'Ruby' (20.99±2.14 mg.100 mL^{-1}) and 'Bahgwa' (13.66±1.81 mg.100 mL^{-1}). Nikniaz *et al*., (2009) reported that Iranian pomegranates contained higher vitamin C content (19.4 mg.100 g^{-1}) than those of Pakistan (17.4 mg.100 g^{-1}). Opara *et al.* (2009) reported ascorbic acid to vary across 5 cultivars (52.8-72.0 mg.100 g^{-1}) and to be higher in peel than in arils.

Ascorbic acid levels first increased after 7 days of cold storage at 1°, 4° and 8°C before declining over time in 'Arakta' and 'Bahgwa' arils. Ascorbic acid reduced over time in all temperatures in 'Ruby' arils stored for 7 days. Ascorbic acid is very sensitive to the enzyme phenolase, temperature, pH, oxygen and light (Coultate, 2007) and therefore a reduction of ascorbic acid during storage was expected. Low levels of ascorbic acid in pomegranate fruit which declined over time were reported in pomegranates of 'Hicaz' (9.9 mg.100 mL^{-1}) (Küpper *et al.,* 1995) and 'Shlefy' (5.29-5.07 $mg.100^{-1}$ mL) (Ghafir *et al.,* 2010) cultivars.

Addition of ascorbic acid to pomegranate juice was attempted in one study to enhance the ascorbic acid content of the juice, but led to the degradation of anthocyanin pigments (Martí *et al.,* 2001; González-Molina *et al.*, 2009). Ascorbic acid level in pomegranate juice was sustained for 7 days when a natural source of ascorbic acid (lemon juice) was used; however, ascorbic acid was destroyed within 4 days of using a synthetic ascorbic acid (Martí *et al.,* 2001; González-Molina *et al.*, 2009).

Ghafir *et al.* (2010) reported declining ascorbic acid levels in 'Shlefy' cultivar at higher storage temperatures. Degradation of ascorbic acid seemed to slow down in 'Hicaz' cultivar at higher storage temperatures (Küpper *et al.*,1995). Higher storage temperature resulted in increased ascorbic acid levels in tomatoes, although this was attributed to the protective effect that total phenolics and ascorbic acid might have on each other (Toor *et al*., 2005).

CAROTENOID ANALYSIS

Carotenoid pigments are usually quite stable until extracted or heated (Coultate, 2007). There is a dearth of information regarding β-carotene content in pomegranates. Curl (1963) found traces of beta-carotene in pomegranate fruit (0.16 $mg.kg^{-1}$) compared to Japanese persimmons (54 $mg.kg^{-1}$). 'Bahgwa' contained the highest β-carotene level, 3.54±1.26 $mg.l^{-1}$, followed by 'Ruby' 2.24±0.50 $mg.L^{-1}$ and 'Arakta' 1.71±0.63 $mg.L^{-1}$. Storage duration did not affect the β-carotene levels in 'Arakta' arils when stored at 1°C between day 0 and 14. The β-carotene content of pomegranate arils declined during storage duration. A reduction in β-carotene of 'Bahgwa' was seen after 14 days of 54%, 58% and 84% at 8°, 4° and 1°C respectively. 'Bahgwa' arils stored at ambient temperature (~21°C) on day 15 increased to 5 times compare to day 14, but this value was similar to the initial value at day 0. 'Arakta' arils stored at higher temperatures (4°C and 8°C) had a slight enhancing effect on β-Carotene levels on day 7, but declined on day 14. Lycopene which is also part of the carotenoid family have been found to be stable at 4°C in tomatoes and increased at higher temperatures of 15° and 25°C (Toor *et al*., 2006). Processing adversely effects β-carotene as seen in jackfruit where higher drying temperatures caused a 73.1%, 85.2% and 98.7% degradation of β-carotene at 50°, 60° and 70°C (Saxena *et al.*, 2010).

Proximate Composition Analysis

There is a dearth of information about the effect that storage temperature have on the proximate composition of pomegranate arils. During fruit maturation Al-Maiman & Ahmad (2002) reported no difference in protein content. Weight loss and shrivelling was found to be greater in unpacked pomegranate arils compared to packed arils due to probable moisture loss, but different temperatures did not have a significant effect on weight loss of pomegranate arils (Gil *et al.*, 1996). The effect of temperature storage on the proximate composition of 'Arakta', 'Bahgwa' and 'Ruby' arils are depicted in Table 3.8a, b and c respectively.

Table 3.8a Effect of storage temperature on the proximate composition (wet basis) and total energy of freeze dried 'Arakta' pomegranate arils

Chemical Parameters	Temp (°C)	Day 0	Day 7	Day 14
Moisture (%wwt)	1°C	79.6±0.31^{b}	79.8±0.12^{ab}	79.8±0.18^{ab}
	4°C	79.6±0.31^{b}	79.9±0.02^{ab}	79.7±0.09^{ab}
	8°C	79.6±0.31^{b}	80.0±0.09^{a}	79.9±0.03^{ab}
Ash (%wwt)	1°C	0.54±0.00^{ab}	0.54±0.00^{ab}	0.52±0.06^{b}
	4°C	0.54±0.00^{ab}	0.55±0.01^{ab}	0.56±0.01^{a}
	8°C	0.54±0.00^{ab}	0.55±0.00^{ab}	0.55±0.03^{a}
Fat (%wwt)	1°C	1.50±0.03^{a}	1.36±0.03^{ab}	1.20±0.20^{c}
	4°C	1.50±0.03^{a}	1.42±0.12^{ab}	1.37±0.07^{ab}
	8°C	1.50±0.03^{a}	1.45±0.02^{a}	1.27±0.13^{bc}
Dietary fibre (%wwt)	1°C	2.78±0.22^{b}	3.10±0.34^{ab}	3.25±0.04^{ab}
	4°C	2.78±0.22^{b}	3.13±0.58^{ab}	3.36±0.39^{ab}
	8°C	2.78±0.22^{b}	3.02±0.27^{ab}	3.41±0.33^{ab}
Protein (%wwt)	1°C	1.20±0.06^{a}	1.13±0.04^{a}	1.18±0.03^{a}
	4°C	1.20±0.06^{a}	1.14±0.04^{a}	1.19±0.02^{a}
	8°C	1.20±0.06^{a}	1.16±0.01^{a}	1.16±0.01^{a}
Carbohydrates (%wwt)	1°C	14.4±0.26^{a}	14.1±0.30^{ab}	14.1±0.21^{ab}
	4°C	14.4±0.26^{a}	13.9±0.53^{b}	13.8±0.38^{b}
	8°C	14.4±0.26^{a}	13.8±0.16^{b}	13.7±0.32^{b}
Total Energy (kJ.100 g^{-1})	1°C	319.3±5.16^{a}	311.9±6.15^{b}	313.0±11.2^{b}
	4°C	319.3±5.16^{a}	307.5±3.07^{b}	309.9±4.39^{b}
	8°C	319.3±5.16^{a}	3.11.4±2.58^{b}	307.2±6.12^{b}

Different letters denote a significant difference according to the LSD multiple range test.

Table 3.8b Effect of storage temperature on the proximate composition (wet basis) and total energy of freeze dried 'Bahgwa' pomegranate arils

Chemical Parameters	Temp (°C)	Day 0	Day 7	Day 14
Moisture (%wwt)	1°C	79.7±0.55^{a}	79.5±0.34^{a}	79.5±0.95^{a}
	4°C	79.7±0.55^{a}	79.8±0.17^{a}	79.5±0.14^{a}
	8°C	79.7±0.55^{a}	79.6±0.18^{a}	79.6±0.45^{a}
Ash (%wwt)	1°C	0.52±0.03^{ab}	0.50±0.01^{b}	0.54±0.02^{a}
	4°C	0.52±0.03^{ab}	0.50±0.01^{b}	0.54±0.00^{a}
	8°C	0.52±0.03^{ab}	0.51±0.00^{ab}	0.55±0.01^{a}
Fat (%wwt)	1°C	1.51±0.36^{a}	1.36±0.12^{a}	1.57±0.07^{a}
	4°C	1.51±0.36^{a}	1.30±0.05^{a}	1.48±0.01^{a}
	8°C	1.51±0.36^{a}	1.33±0.07^{a}	1.44±0.09^{a}
Dietary fibre (%wwt)	1°C	2.92±0.10^{d}	3.10±0.22^{bcd}	3.34±0.36^{abc}
	4°C	2.92±0.10^{d}	3.03±0.08^{bc}	3.40±0.32^{ab}
	8°C	2.92±0.10^{d}	2.98±0.07^{d}	3.51±0.27^{a}
Protein (%wwt)	1°C	1.18±0.10^{a}	0.93±0.17^{b}	1.22±0.05^{a}
	4°C	1.18±0.10^{a}	1.08±0.04^{ab}	1.19±0.21^{a}
	8°C	1.18±0.10^{a}	1.11±0.03^{ab}	1.21±0.02^{a}
Carbohydrates (%wwt)	1°C	14.1±0.88^{a}	14.6±0.17^{a}	13.9±0.47^{a}
	4°C	14.1±0.88^{a}	14.3±0.03^{a}	13.9±0.11^{a}
	8°C	14.1±0.88^{a}	14.4±0.26^{a}	14.4±1.88^{a}
Total Energy (kJ.100 g^{-1})	1°C	314.5±3.59^{a}	312.4±1.43^{a}	301.6±4.62^{a}
	4°C	314.5±3.59^{a}	306.4±8.65^{a}	302.8±5.77^{a}
	8°C	314.5±3.59^{a}	300.7±1.02^{a}	287.1±8.60^{a}

Different letters denote a significant difference according to the LSD multiple range test.

Table 3.8c Effect of storage temperature on the proximate composition (wet basis) and total energy of freeze dried 'Ruby' pomegranate arils

Chemical Parameters	Temp (°C)	Day 0	Day 7	Day 14
Moisture (%wwt)	1°C	79.4±0.15^{bc}	79.4±0.22^{c}	79.4±0.18^{bc}
	4°C	79.4±0.15^{bc}	79.7±0.26^{b}	79.1±0.22^{c}
	8°C	79.4±0.15^{bc}	79.8±0.13^{b}	80.1±0.38^{a}
Ash (%wwt)	1°C	0.53±0.00^{a}	0.51±0.01^{a}	0.52±0.01^{a}
	4°C	0.53±0.00^{a}	0.51±0.01^{ab}	0.53±0.01^{a}
	8°C	0.53±0.00^{a}	0.52±0.00^{a}	0.49±0.04^{b}
Fat (%wwt)	1°C	1.26±0.18^{ab}	1.23±0.09^{ab}	1.17±0.07^{ab}
	4°C	1.26±0.18^{ab}	1.30±0.09^{ab}	1.08±0.12^{b}
	8°C	1.26±0.18^{ab}	1.30±0.02^{a}	1.10±0.12^{ab}
Dietary fibre (%wwt)	1°C	2.67±0.18^{c}	3.06±0.21^{b}	3.57±0.05^{a}
	4°C	2.67±0.18^{c}	3.13±0.22^{b}	3.66±0.09^{a}
	8°C	2.67±0.18^{c}	3.45±0.09^{a}	3.66±0.09^{a}
Protein (%wwt)	1°C	1.13±0.03^{c}	1.18±0.06^{bc}	1.25±0.01^{a}
	4°C	1.13±0.03^{c}	1.18±0.02^{bc}	1.24±0.01^{ab}
	8°C	1.13±0.03^{c}	1.14±0.04^{c}	1.20±0.04^{ab}
Carbohydrates (%wwt)	1°C	15.0±0.45^{a}	14.7±0.24^{ab}	14.1±0.11^{c}
	4°C	15.0±0.45^{a}	14.1±0.36^{bc}	14.4±0.13^{bc}
	8°C	15.0±0.45^{a}	13.8±0.01^{cd}	13.4±0.32^{d}
Total Energy (kJ.100 g^{-1})	1°C	318.6±4.75^{a}	301.9±6.09^{ab}	301.6±4.62^{c}
	4°C	318.6±4.75^{a}	303.4±9.11^{bc}	302.8±5.77^{c}
	8°C	318.6±4.75^{a}	305.6±3.86^{c}	287.1±8.60^{d}

Different letters denote a significant difference according to the LSD multiple range test.

Storage duration and temperatures of 1° and 4°C did not show any significant effect on proximate composition of 'Arakta', 'Bahgwa' and 'Ruby' after 14 days (Table3.8a, b, c). However higher temperature storage did cause a change in proximate composition after 14 days and is summarised in Table 3.9.

Table 3.9 Change of proximate composition after 14 days at 8°C in 'Arakta', 'Bahgwa' and 'Ruby' pomegranate arils

Proximate composition	Arakta	Bahgwa	Ruby
Ash			-7%
Fat	-15%		
Dietary Fibre		+20%	+27%
Protein			+10%
Carbohydrates	-5%		-10%
Total Energy	-7%		-11%

A 15% and 20% loss in fat content was found after 14 days in 'Arakata' arils stored at 8° and 1°C respectively. Carbohydrate seemed to decline after 14 days with 2-5% and 4-10% in 'Arakta' and 'Ruby' arils respectively. The reduction in carbohydrate content in both cultivars was greatest at 8°C storage. Energy content (kJ.100 g^{-1}) declined with 5-7% in 'Arakta' and 5-11% in 'Ruby' arils after 14 days, where storage at 8°C caused the greatest loss. Dietary fibre increased at all temperatures in 'Bahgwa' (14-20%) and 'Ruby' (25-27%), especially at 8°C. Protein was 22% lower in 'Bahgwa' arils stored at 1°C for 7 days, but this value was restored on day 14. An increase of 6-10% in protein was observed at all storage temperatures after 14 days in packaged 'Ruby' arils especially at 4° and 8°C. The ash% of packaged 'Bahgwa' arils were lower on day 7 compared to day 14, but did not differ significantly compared to fresh arils. The ash content in 'Ruby' arils was 7% lower after 14 days when stored at 8°C. Packaged 'ruby' arils stored at 8°C had 1% higher moisture content after 14 days compared to fresh arils.

Conclusion

This study confirms the importance of low temperature storage of pomegranate arils and provides nutritional information of three pomegranate cultivars grown in Porterville, South Africa. These results will reveal each cultivar's unique characteristics that will aid producers in the selection and marketable suitability of pomegranates. The macronutrients of three pomegranate cultivars were similar with high moisture content, low fat, protein and dietary fibre levels, while carbohydrates provided most of the arils' energy. The uniqueness of every cultivar was highlighted by its physico-chemical and selected bioactive components: 'Arakta' with highest levels of TA and ascorbic acid, 'Bahgwa' with highest level of anthocyanins and 'Ruby' with highest level of TSS level and lightest coloured arils.

During this study higher storage temperature did affect the proximate composition, physico-chemical attributes and bioactive components negatively. No visual detection of mould growth was seen in 'Arakta' and 'Bahgwa' arils stored at 1°C and 95% RH after 14 days and 4°C and 95% RH after 7 days. However microbial analysis is recommended to confirm the safety of pomegranate arils during cold storage. Therefore results agree with other researchers who suggested low (0-5°C) storage temperature and 95% RH for minimally processed pomegranate arils (Gil *et al.*, 1996; Kader, 2002; Nicola *et al*., 2009).

References

AOAC International. (2006). Official methods of analysis of AOAC International. Gaithersburg, Md: AOAC International.

Alighourchi, H. & Barzegar, M. (2009). Some physicochemical characteristics and degradation kinetic of anthocyanin of reconstituted pomegranate juice during storage. *Journal of Food Engineering,* **90,** 179-185.

Al-Maiman S.A. & Ahmad, D. (2002). Changes in physical and chemical properties during pomegranate (*Punica granatum)* fruit maturation. *Food Chemistry*, **76**, 437-441

Artés, F, Tudela, J.A. & Villaescusa, R. (2000). Thermal postharvest treatments for improving pomegranate quality and shelf life. *Postharvest Biology and* Technology, **18,** 245-251.

Ayhan, Z. & Eştürk, O. (2009). Overall Quality and Shelf Life of Minimally Processed and Modified atmosphere Packaged "Ready-to-Eat" Pomegranate Arils. *Journal of Food Science,* **74,** C399-C405.

Barros, L., Ferreira, M, Queiros, B., Ferreira, I.C.F.R. & Baptista, P. (2007). Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry* **103**, 413-419.

Borochov-Neori, H., Judeinstein, S., Tripler, E., Harari, M., Greenberg, A., Shomer, I & Holland, D. (2009). Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum L.*) fruit. *Journal of Food Composition and Analysis*, **22**, 189–195.

Calderón, M.M. (2010). Internal quality profile and influence of packaging conditions on fresh-cut pineapples. PhD Thesis. University of Lleida, Spain.

Coultate, T.P. (2007). *Food The Chemistry of Its Components*. Pp. 42, 62-63, 184, 281-289. UK: RSC Publishing.

Curl, A.L. (1963). The carotenoids from several low carotenoid fruits. *Journal of Food Science*, **29**, 241-245.

Dafny-Yalin, M., Glazer, I., Bar-Ilan, I., Kerem, Z., Holland, D. & Amir, R. (2010). Colour, Sugars and Organic Acids Composition in Aril Juices and Peel Homogenates Prepared From Different Pomegranate Accessions. *Journal of Agricultural and Food Chemistry,* **58**, 4342-4352.

Drogoudi, P.D., Tsipouridis, C. & Michailidis, Z. (2005). Physical and Chemical Characteristics of Pomegranates. *HortScience*, **40**, 1200-1203.

Dumlu, M.U. & Gürkan, E. (2007). Elemental and Nutritional Analysis of *Punica granatum* from Turkey. *Journal of Medicinal Food*, **10,** 392-395.

El-Nemr, W.E., Ismail, I.A. & Raǵab. (1990). Chemical Composition of juice and seeds of pomegranate fruit. *Die Nahrung*, **34**, 601-606

Elyatem, S. M. & Kader, A. A. (1984). Post-harvest physiology and storage behaviour of pomegranate fruits. *Scientia Horticulturae,* **24**, 287-298.

Fawole, O.A., Opara, U.L. & Theron, K.I. (2011). Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa. *Food Bioprocess Technology*, DOI: 10.1007/s11947-011-0533-7.

Ghafir, S.A.M., Ibrahim, I.Z. & Zaied, S.A. (2010). Response of Local Variety 'Shlefy' Pomegranate Fruits to Packaging and Cold Storage. *Acta Horticulturae*, **877** 427-432.

Gil, M.I., Martínez, J. A. & Artés, F. (1996). Minimally Processed Pomegranate Seeds. *Lebensmittel-Wissenschaft und-Technologie*, **29**, 708-713.

Giusti M.M. & Wrolstad R.E. (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: *Current Protocols in Food Analytical Chemistry.* (edited by R.E. Wrolstad). NY: John Wiley & Sons.

González-Molina, E., Moreno, D.A. & García-Viguera, C. A new drink rich in healthy bioactives combining lemon and pomegranate juices. *Food Chemistry*, **115**, 1364-1372.

Jaiswal, V., DerMarderosian, A. & Porter, J.R. (2010). Anthocyanins and polyphenol oxidase from dried arils of pomegranate (*Punica granatum* L.). *Food Chemistry*, **118**, 11-16.

Kader, A.A. (2002). Postharvest Biology and Technology: An Overview. In: *Postharvest Technology of Horticultural Crops.* (edited by A. A. Kader). 3rd ed. Pp 39-48. California: University of California.

Kader, A.A. & Barrett (2005). Classification, composition of fruits, and postharvest maintenance of quality. In: *Processing Fruits Science and Technology* (edited by D.M. Barett, L. Somogyi, & H. Ramaswamy). Pp 3-21, USA: CRC Press.

Kim, N.D., Metha, R., Yu, W., Neeman, I., Livney, T., Amichay, A., Poirier, D., Nicholls, P., Kirby, A., Jiang, W., Mansel, R., Ramachandran, C., Rabi, T., Kaplan, B. & Lansky, E.

(2002). Chemopreventive and adjuvant therapeutic potential of pomegranate (*Punica granatum*) for human breast cancer. *Breast Cancer Research and Treatment*, **71**, 203–217.

Küpper, W., Pekmezci, M. & Henze, J. (1995). Studies on CA-Storage of Pomegranate (*Punica granatum* L., cv. Hicaz). *Acta Horticulturae*, **398,** 101-108.

Lansky, E. P. & Newman, R. A. (2007). *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology,* **109**, 177-206.

Lee, J., Durst, R.W. & Wrolstad, R.E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International*, **88**, 1269-1278.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 74-185.

Martí, N. Pérez-Vicente, A. & García-Viguera, C. (2001). Influence of storage temperature and ascorbic acid addition on pomegranate juice. *Journal of the Science of Food and Agriculture,* **82**, 217-221.

Martinez, J.A., Melgosa, M., Perez, M.M., Hita, E. & Negueruela, A.I. (2001). Visual and instrumental color evaluation in red wines. *Food Science and Technology International*, **7,** 439-444.

Medical Research Council (MRC) (2010). Fruit. In: *Condensed Food Composition Tables for South Africa* (edited by Wolmarans, P., Danster, N., Dalton, A., Rossouw, K. & Schönfeldt, H.) 1st ed. p. 3-15. Cape Town: Medical Research Council.

Melgarejo, P., Salazar, D.M. & Artés, F. (2000). Organic acids and sugars composition of harvested pomegranate fruits. *European Food Research and Technology,* **211**, 185-190.

Mousavinejad, G., Emam-Djomeh, Z., Rezaei, K. & Khodaparast, M.H.H. (2009). Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. *Food Chemistry*, **115**, 1274-1278.

Nanda, S., Rao, D.V.S. & Krishnamurthy, S. (2001). Effects of shrink film wrapping and storage temperature on the shelf life and quality of pomegranate fruits cv. Ganesh. *Postharvest Biology and Technology*, **22**, 61-69.

Noda, Y., Kaneyuki, T., Mori, A. & Packer, L. (2002). Antioxidant Activities of Pomegranate Fruit Extract and Its Anthocyanindins: Delphinidin, Cyanidin, and pelargonidin. *Journal of Agricultural and Food Chemistry*, **50**, 166-171.

Nikniaz, Z., Mahdavi, R., Rafraf, M. & Jouyban, A. (2009). Total phenols and vitamin C contents of Iranian fruits. *Nutrition and Food Science*, **39**, 603-608.

Nicola, S. Tibaldi, G. & Fontana, E. (2009). Fresh-cut Produce Quality: Implications for a Systems Approach. In: *Postharvest Handling: A Systems Approach.* (edited by W.J. Florkowski, R.L. Shewfelt, B. Brueckner, & S.E. Prussia). Pp. 270-273. Oxford: Academic Press.

Opara, L.U., Al-Ani, M.R. & Al-Shuaibi, Y.S. (2009). Physico-chemical Properties, Vitamin C Content and Antimicrobial Properties of Pomegranate Fruit (*Punica granatum* L.). *Food and Bioprocess Technology*, **2,** 315-321.

Ozgen, M., Durgaç, C., Serçe, S. & Kaya, C. (2008). Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. *Food Chemistry*, **111**, 703-706.

Paul, D.K. & Shaba, R.K. (2004). Nutrients, Vitamins and Nutrient Content in Common Citrus Fruits in the Northern Region of Bangladesh. *Pakistan Journal of Biological Sciences*, **7**, 238-242.

Quiro's, A.R. & Costa, H.S. (2006). Analysis of carotenoids in vegetable and plasma samples: A review. *Journal of Food Composition and Analysis,* **19**, 97-111.

Schlimme, D.V. (1995). Marketing Lightly Processed Fruits and Vegetables. *HortScience*, **30**, 15-17.

Schwartz, E., Tzulker, R., Glazer, I., Bar-Ya' Akov, I., Wiesman, Z., Tripler, E., Bar-Ilan, I., Fromm, H., Borochov-Neori, H., Holland, D. & Amir, R. (2009). Environmental Conditions Affect the Colour, Taste, and Antioxidant Capacity of 11 Pomegranate Accessions' Fruits. *Journal of Agricultural and Food Chemistry*. **57,** 9197-9209.

Sepúlveda, E., Galletti, l., Sáenz, C. & Tapia, M. (2000). Minimal Processing of pomegranate var. Wonderful. In: *Symposium on production, processing and marketing of*

pomegranate in the Mediterranean region: Advances in research and technology. CIHEAM – IAMZ p 237-242. Zaragoza, Spain.

Stover, E. & Mercure, E.W. (2007). The Pomegranate: A New Look at the Fruit of Paradise. *HortScience*, **42**, 1088-1092

Syed, D.N., Afaq, F. & Mukhtar, H. (2007). Invited article: Pomegranate derived products for cancer chemoprevention. *Seminars in Cancer Biology*, **17**, 377-385.

Tehranifar, A., Zarei, M., Nemati, Z., Esfandiyari, B. & Vazifeshenas, M.R. (2010). Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. *Scientia Horticulturae*, **126**, 180-185.

Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B. & Erim, F.B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry*, **115**, 873-877.

Toor, R.K. & Savage, G.P. (2006). Changes in major antioxidant components of tomatoes during post-harvest storage. *Food Chemistry*, **99**, 724-727.

Tzulker, R. Glazer, I., Bar-Ilan, I., Holland, D., Aviram, M. & Amir, R. (2007). Antioxidant Activity, Polyphenol Content, and Related Compounds in Different Fruit Juices and Homogenates Prepared from 29 Different Pomegranate Accessions. *Journal of Agricultural and Food Chemistry,* **55**, 9559-9570.

CHAPTER 4

EFFECT OF PACKAGING ON THE PHYSICO-CHEMICAL PROPERTIES AND SELECTED BIOACTIVE COMPONENTS OF POMEGRANATE (PUNICA GRANATUM) ARILS

Abstract

Pomegranate (*Punica granatum* L.) has recaptured consumer interest worldwide due to its health promoting benefits (Heber & Bowerman, 2009). Packaging is especially important in pomegranate arils to preserve the quality of the fruit by reducing shrivelling, dehydration and weight loss. The physico-chemical properties and selected bioactive components (anthocyanins, ascorbic acid, β-carotene) of pomegranate arils ('Arakta', 'Bahgwa' and 'Ruby') packed in three punnets made of polyethylene terephthalate (PET1 - clampshell; PET2 - tub and lid) or polypropylene (PP - tub and lid) material were studied for a period of 14 days at 5°C 95% RH. Higher titratable acidity (TA) was observed in 'Arakta'. No visual mould growth was detected in 'Arakta' arils after 7 days irrespective of the type of packaging, while mould growth was detected in all 'Ruby' in all types of packaging. Packaging caused minimal changes in the physico-chemical and bioactive components, although PET2 had relatively stable headspace gas composition within the punnets. Storage duration caused a rise in pH and decline in TA irrespective of packaging type.

Introduction

It is hard to imagine the world without packaging since everything you purchase is signed, sealed and delivered using some kind of packaging. Science and technological advances are constantly improving the face of packaging, giving it a life of its own. The four main functions of packaging are containment, protection, convenience, and communication (Smith *et al.*, 2005). Packaging protects the fruit and provides structural support for convenient storage and transportation purposes. Retailers use printed information on packaging for recognition, stock control, tracing as well as marketing purposes. Packaging is especially important in pomegranate arils to preserve the quality of the fruit by reducing shrivelling, dehydration and weight loss (Gil *et al*., 1996; Nicola *et al*., 2009). Pomegranate (*Punica granatum* L.) has recaptured consumer interest worldwide due to its health promoting benefits (Heber & Bowerman, 2009). Most recent packaging studied for pomegranate arils include heat sealed pouches of oriented polypropylene film, rigid polystyrene vessels, perforated polyethelene bags, ethyl vinyl acetate films, polypropylene trays and bi-axially oriented polypropylene films

(Gil *et al.*, 1996; Sepúlveda *et al.*, 2000; Sepúlveda *et al.*, 2001; Ayhan & Eştürk, 2009). Polyethylene terephthalate material is mostly used for pomegranate aril packaging in South Africa and is available in various shapes and sizes. Modified atmosphere packaging has not been used in the South African pomegranate industry thus far, but the industry is moving in that direction. The aim of this study is to investigate the effects of South African commercial packaging material on physico-chemical properties and selected bioactive components (anthocyanins, ascorbic acid, β-carotene) of pomegranate arils ('Arakta', 'Bahgwa' and 'Ruby') during short term storage duration.

Material and Methods

Plant Material

Commercially ripe pomegranate fruit ('Arakta', 'Bahgwa', 'Ruby') were procured from Houtconstant farm as described in chapter 3. Whole pomegranate fruit 'Bahgwa', 'Arakta' and 'Ruby' were sorted, rinsed with distilled water and air-dried 5 hours after harvest date. Two storage periods will be discussed throughout this study: Short term storage (or cold storage) and long term storage duration. Long term storage duration refers to the storage period (10-14 weeks) of whole pomegranate fruits after harvest in a cold room at 7°C 95% RH. While short term storage or cold storage refers to the storage period (7-14 days) of extracted and packaged pomegranate arils in a refrigerator at 5°C, 95% RH. The whole pomegranate fruit was subjected to long term storage of 10 ('Arakta'), 12 ('Bahgwa') and 14 ('Ruby') weeks at 7°C 95% RH prior to the start of this study. Pomegranate fruit yielded 50% arils and whole fruit weight varied between 'Arakta' (265 g), 'Bahgwa' (235 g) and 'Ruby' (210 g). The arils were manually extracted and packaged at ambient conditions (22°C, 53% RH). The arils of each individual fruit were kept in individual plastic bags at 5°C until each cultivar was peeled, before being mixed together and packaged.

Packaging

The packaging used within this study will be referred to as individual punnets. Three different punnets of polyethylene terephthalate (PET1 and PET2) and polypropylene (PP) material were used as indicated by both Table 4.1 and Fig. 4.1.

Table 4.1 Description of punnets used during this study

Pack name	Description	Volume (mL)	Thickness (mm)	Material	Manufacturer
PET1	Clamp shell	330	0.30	Polyethylene terephthalate	Zibo Containers (Pty) Ltd South Africa
PET2	Rectangular Tub + Lid	180	0.35 + 0.35	Polyethylene terephthalate	Thermopac (Pty) Ltd South Africa
PP	Round Tub + Lid	320	0.70 + 0.40	Polypropylene	Zibo containers (Pty) Ltd South Africa

The packaging material used differed in volume, shape and size (Fig. 4.1) and were selected in agreement with the pomegranate producer who process and package pomegranates in the packaging plant on his farm (Olivier, F. 2009, Chief Executive Officer, Pomegranate Fruit SA, Porterville, South Africa, personal communication, 14 April). Packaging was selected based on what was previously (PET1) and currently (PET2) used in industry. Currently, no packaging film or gas flushing is used to package arils in South Africa yet. PET2 tub and lid structure was easier and quicker to close and therefore replaced the PET1 clampshell that occasionally flipped open during handling and transportation (Olivier, F. 2009, Chief Executive Officer, Pomegranate Fruit SA, Porterville, South Africa, personal communication, 14 April). PP was selected to investigate if the twofold thickness of its material would affect the chemical composition of pomegranate arils any differently compared to PET1 and PET2.

Arils were packaged according to the volume weight ratio as indicated by Fig. 4.1. Since PET2 is currently used in the market, its volume: mass ratio (2.2 $g.mL^{-1}$) was used as a guideline to fill PP and PET1 with arils (Fig. 4.1). The water holding capacity of each punnet was used to determine its volume. However the headspace area of PET1 is greater than PET2 or PET1 and this was not accounted for at the beginning of the study when the determining the volume: mass ratio of each pack. The volume: mass ratio of each punnet (including the headspace) was measured once more (2.1-2.6 $g.mL^{-1}$) and varied slightly between punnets: PET1 > PET2 > PP (Fig. 4.1).

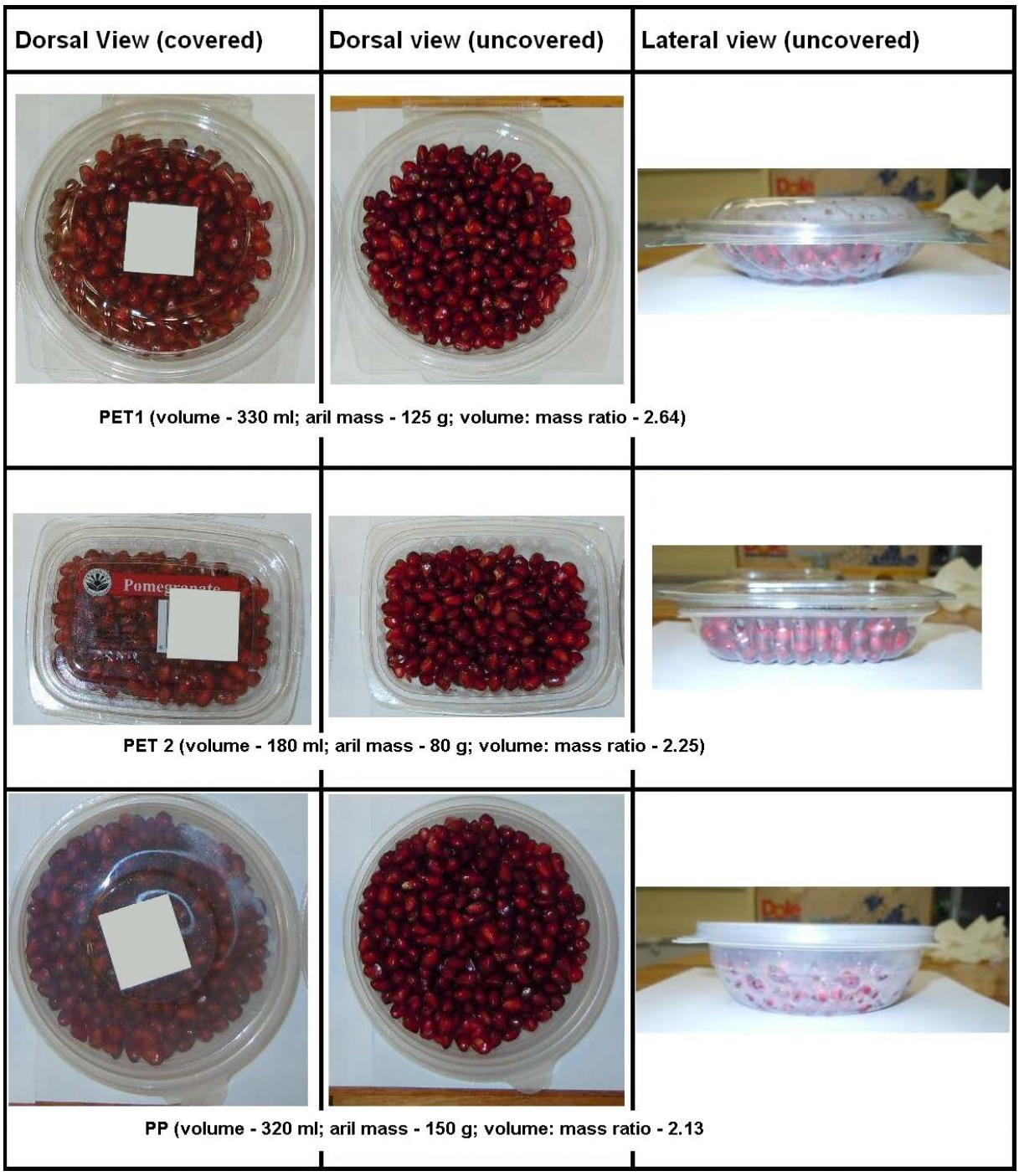

Figure 4.1 **Depiction of arils in different punnets (PET1, PET2, PP) from dorsal (covered and uncovered) and lateral views with volume, aril mass as well as the volume: mass ratio of the arils in each pack.**

Sample Preparation and Storage

After packaging, the arils were stored for 14 days at 5°C and 95% RH (short term storage). Even though chapter 3 recommended a storage temperature of 1°C at 95% RH for 14 days, it was decided to store the arils at 5°C 95% RH to reflect practical storage temperatures in

retail markets (López-Rubira et al., 2005). Freshly peeled arils of each cultivar were analysed in triplicate on day 0 for physico-chemical, proximate composition and selected bioactive component analyses. The remaining arils were divided into three batches to fill the three types of punnets according to the volume: mass ratio shown in Fig. 4.1 for analysis days 7 and 14. Arils from three punnets of the same punnet variety were pooled and mixed together and analysed in triplicate on day 7 and 14 for physico-chemical, proximate composition and selected bioactive component analyses.

Headspace Gas Composition Analysis

The headspace gas composition was measured as described in chapter 3.

Quality Analyses

Microbial analysis and not included in the scope of this study and therefore a subjective visual inspection was performed to estimamte the visible mould growth of the arils inside the packages. The punnets were divided in quadrants to aid in estimation of mould growth using a scale from 0, <25, 25, 25-50, 50, 50-75, 75, >75 and 100%. A robust estimate of browning was estimated on a scale from 1 to 5 and then converted to percentage browning incidence. Weight loss of arils was measured since a relative humidity of 95% was recommended to minimise weight loss in pomegranates (Elyatem & Kader, 1984). The punnets were weighed directly after packaging the arils and after 7 and 14 days to assess the percentage weight loss of arils over time. Juice leakage (mL.100 g^{-1}) was determined after removing all the arils from the punnets by tilting the punnets at a 20° angle for 5 min and measuring the liquid with a 5 mL syringe (Calderón, 2010).

Physico-chemical Analysis

Physico-chemical attributes such as colour (CIE *L*, a*, b*,* chroma and hue angle (*H°*)), pH, titratable acidity (TA), total soluble solids content (TSS) were measured using aril juice from fresh arils as described in chapter 3. These attributes were measured and the TSS/TA was calculated on day 0, 7 and 14. TSS level is closely related to soluble sugar content of pomegranate arils (Dafny-Yalin *et al.*, 2010). So TSS was measured as an indication of soluble sugars in pomegranate arils. During the first 7 days of storage, the use of texture analyses was considered as an additional way to differentiate between the punnet varieties. Therefore hardness and toughness of arils were not measured on day 0, but after 7 and 14 days. A random sample of 5 arils was tested individually for each punnet type. Measurements were taken using a texture analyser (TA-XT plus; Stable Micro Systems, UK). Hardness (N) was expressed as the force of compression required to break the aril, while toughness (N.mm^{-1}) was calculated using the area under the curve with force plotted over

displacement and expressed as the energy required to crush the sample completely (Al-Said *et al*, 2009).

Selected Bioactive Component Analyses

Anthocyanin compounds, ascorbic acid and carotenoid content were analysed according to methods described in the chapter 3 (Quiro's & Costa, 2006; AOAC, 2005; Barros *et al.*, 2007).

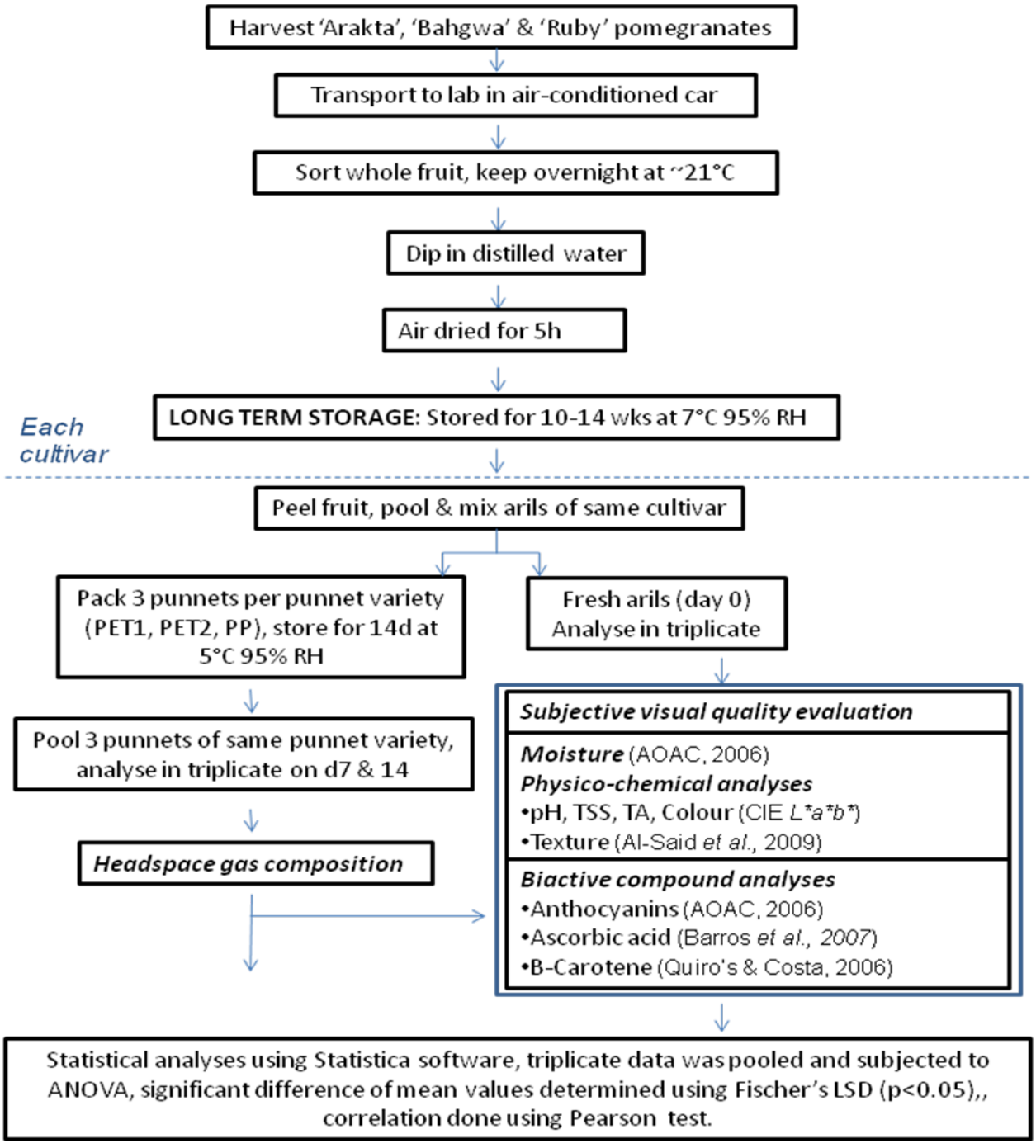

Figure 4.2 Experimental design

Statistical Analysis

Statistical analyses were carried out using Statistica software version 10 (Tulsa, USA), to evaluate the effects that packaging has on various quality attributes of individual pomegranate cultivars. Triplicate data was pooled and subjected to analyses of variance analysis (ANOVA) while significant difference of the mean values was determined using Fisher's least significant difference (L.S.D.) multiple comparison test at $P < 0.05$. The Pearson test was used to show correlation between visual mould growth incidence and measured headspace gas composition in different punnet varieties as well as the correlation between visual mould growth incidence and chemical attributes.

Results and Discussion

General Composition

The colour of pomegranate aril juice differed slightly but significantly ($p<0.05$) amongst cultivars where *L**, *a**, *b** and chroma was highest in 'Ruby' cultivar, followed by 'Arakta' and then 'Bahgwa' arils (Table 4.2). The *H°* was highest in 'Bahgwa', followed by 'Arakta' and 'Ruby' pomegranate cultivar arils (Table 4.2). The colour parameters of the pomegranate aril juice was comparable to *L**, *a**, *b** values reported by Ayhan and Eştürk (2009). The TA ranged from 0.26 to 0.33 mg.100 mL^{-1} and TSS/TA from 47.6 to 60.0 in pomegranate arils (Table 4.2) in agreement with other studies (Gil *et al.*, 1996; Al-Said *et al.*, 2009; Teharanifar *et al.*, 2010). The TSS (15.4-15.8°Brix) and pH levels (3.24-3.35) did not vary much between cultivars and was similar to pomegranates grown in Oman (Al-Said *et al.*, 2009) and Iran (Zarei *et al.*, 2010). Anthocyanin levels on day 0 was highest in 'Bahgwa' (112.5±2.09 $mg.L^{-1}$), followed by 'Arakta' (92.1±1.83) and 'Ruby' (73.8±2.82). Anthocyanin levels were half as much as the previous year's harvest (Fawole *et al.*, 2011) and similar to other studies (Artés *et al*. 2000; Ozgen *et al.*, 2008; Teharanifar *et al.*, 2010). Results confirmed Curl's (1963) notion that pomegranate aril juice is a low carotenoid (3.34-6.20 $mg.L^{-1}$ β-carotene, Table 4.2). However Paul and Shaba (2004) did report much higher β-carotene levels (970±1.2 $mg.L^{-1}$) in pomegranate arils. During the storage duration of 14 days ascorbic acid levels of pomegranate aril juice ranged between 12.0-56.5 $mg.L^{-1}$ (Table 4.10b) which was in agreement with other studies (Al-Maiman & Ahmad, 2002; El-Nemr *et al*., 1990).

Table 4.2 The colour, chemical, selected bioactive components and proximate composition of fresh 'Arakta', 'Bahgwa' and 'Ruby' pomegranate arils

Characteristics	Arakta	Bahgwa	Ruby
Physical parameters			
*L**	35.0±0.28^{b}	34.0±0.47^{c}	35.9±0.58^{a}
*a**	14.3±0.37^{b}	12.7±1.21^{c}	15.5±1.10^{a}
*b**	5.57±0.16^{b}	4.70±0.56^{c}	5.90±0.36^{a}
Chroma	15.4±0.40^{b}	13.5±1.33^{c}	16.6±1.16^{a}
H°	21.2±0.13^{a}	20.3±0.47^{c}	20.9±0.24^{b}
Chemical parameters			
TA	0.33±0.03^{a}	0.28±0.01^{b}	0.26±0.01^{c}
pH	3.29±0.00^{b}	3.24±0.01^{c}	3.35±0.01^{a}
TSS (°Brix)	15.4±0.47^{b}	15.7±0.10^{a}	15.8±0.10^{a}
TSS:TA ratio	47.6±5.73^{c}	55.4±1.17^{b}	60.0±1.36^{a}
Bioactive components			
Anthocyanins (mg.L^{-1})	92.1±1.83^{b}	112.5±2.09^{a}	73.8±2.82^{c}
Ascorbic Acid (mg.L^{-1})	24.8±2.92^{a}	24.2±1.94^{a}	20.5±1.21^{b}
β-Carotene (mg.L^{-1})	4.17±0.32^{b}	6.20±0.70^{a}	3.34±0.78^{c}

Different letters indicate significant difference (p<0.05) according to the multiple LSD test.

Headspace Gas Composition

The headspace gas composition of pomegranate arils showed an increased CO_2 levels and declining O_2 levels over 14 days of storage duration (Fig. 4.3), similar to findings from the chapter 3 and other authors (Sepúlveda *et al.*, 2000; López-Rubira *et al.,* 2005; Ayhan & Eştürk, 2009). If we assume that the decreasing O_2 and increasing CO_2 was an indication of oxygen consumption and carbon dioxide production, then these findings could be related to an increasing respiration rate over time. Pomegranates have shown to spoil faster with increasing respiration rate (Gil *et al.,* 1996; Kader & Barrett, 2005). After 14 days the O_2 and CO_2 headspace gas composition remained unchanged in PET2, while O_2 consumption and CO_2 production increased in PP and even more so in PET1. The tub-and-lid structure of PP and PET2 might have allowed air to escape more easily, while the slight accumulation of CO_2 in PET1 could be attributed to a slightly tighter fitting clamp-shell design. Every PET1 punnet was closed carefully during this experiment; however as mentioned previously, when these punnets were used in the industry the punnets were not closed properly due to hasty packaging of the arils, which resulted in some punnets flipping open. So even though these results suggest a tighter fitting punnet, it is important to note that this might not be reflective of what would happen if the punnets are not properly closed in industry.

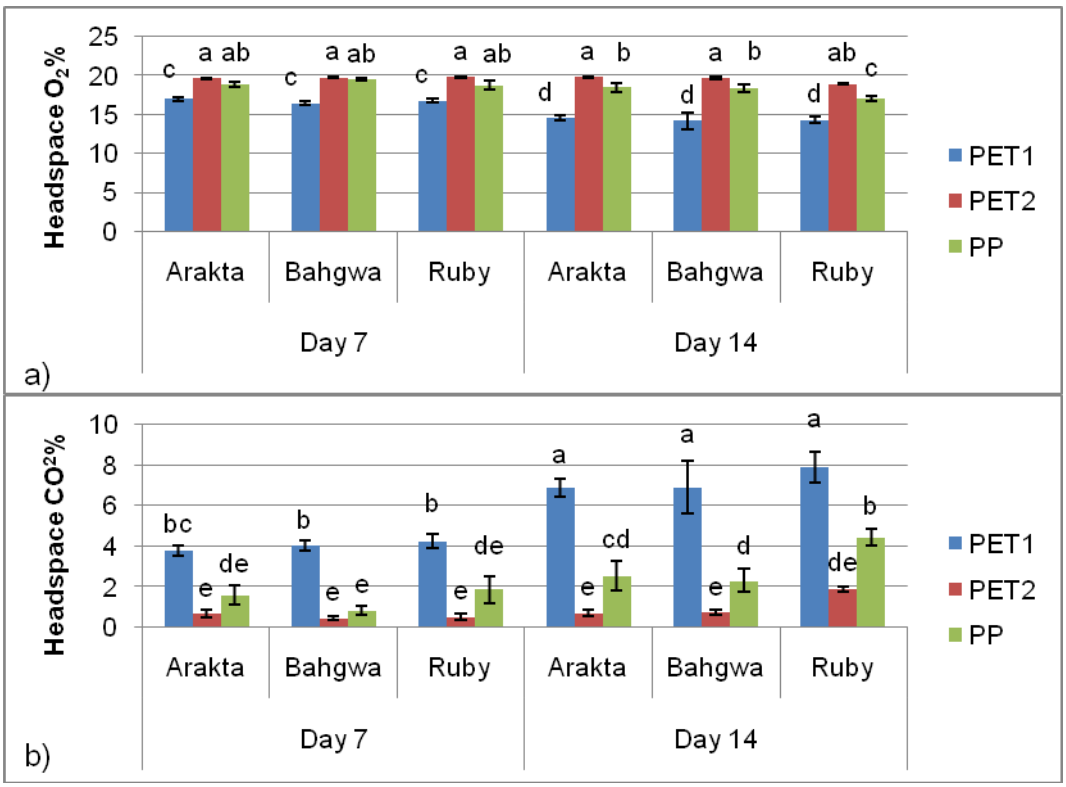

Figure 4.3 **The effect of packaging on headspace a) O_2 and b) CO_2 in 'Arakta', 'Bahgwa' and 'Ruby' pomegranate arils. Different letters indicate significant difference (p<0.05) according to the multiple LSD test.**

An accumulation of CO_2 was noted previously in EVA bags with lower oxygen permeability, while O_2 and CO_2 levels remained unchanged in polystyrene vessels and perforated polyethelene bags (Sepulveda *et al.*, 2001). Gil *et al.* (1996) recommended packaging material due to the lowest respiration rate of pomegranate arils. PET2 is currently used in the market and showed the most stable headspace gas composition between the punnet varieties during the storage period. However it should be noted that PET2 also showed earlier onset of mould growth compared to PET1 punnets (Table 4.3).

Qualitative Analyses

VISUAL QUALITY EVALUATION

An unexpected quality degradation due to mould growth occurred in the pomegranate arils during 7 days of cold storage. This quality degradation was quantified using a subjective visual assessment to determine mould growth incidence (%), juice leakage (mL.100 g^{-1}), weight loss (%) and browning incidence (%) as depicted by Table 4.3 and Fig. 4.4 a, b, c.

Table 4.3 Estimated mould incidence on pomegranate arils after 7 and 14 days in 'Arakta', 'Bahgwa' and 'Ruby' arils in three punnet varieties (PET1, PET2, PP).

Cultivar	Punnet	Visual mould estimation (%)		
		Day 0	Day 7	Day 14
Arakta	PET1	0	0	0-25%
	PP	0	0	25-50%
	PET2	0	0	50-75%
Bahgwa	PET1	0	0	<25%
	PP	0	0	0-25%
	PET2	0	<25%	25%
Ruby	PET1	0	<25%	0-25%
	PP	0	<25%	25-75%
	PET2	0	<25%	75%

High moisture content (80%), TSS level (15.4-15.8°Brix) and a low pH of 3.3 are all intrinsic factors of pomegranate arils which increase the susceptibility of pomegranate arils to spoilage organisms like yeast and moulds (Cloete, 2005). The microbial study of pomegranate arils was not part of the objectives set out for chapter 4, although the below mentioned results did lead to a baseline microbial investigation that will be discussed in more detail in chapter 5. Enzymatic browning in fruit occurs when *o*-diphenol compounds are oxidised to *o*-quinones (brown pigments) in the presence of the pH sensitive enzyme, polyphenolase oxidase (Coultate, 2007). Citric acid is strongly related to TA in pomegranates and is known to reduce enzymatic browning (Coultate, 2007; Dafny-Yalin *et al.*, 2010). Besides the variation of TA levels between cultivars, TA levels also declined in all cultivars throughout storage duration (Table 4.9a, b, c). This was concurrent to browning incidence (%) that was more prominent in 'Ruby' arils and in all cultivars after 14 days of cold storage (Fig. 4.4c).

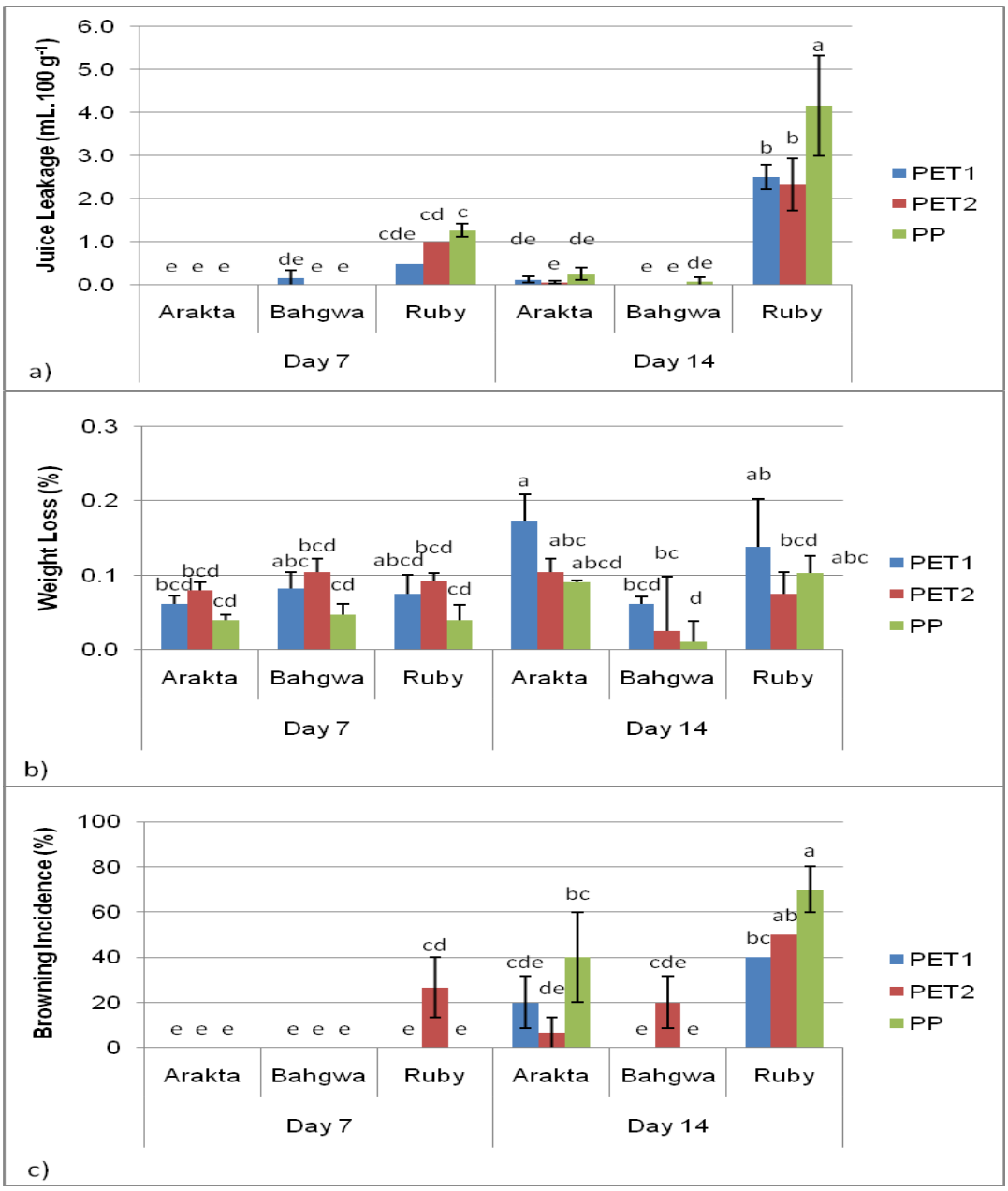

Figure 4.4 **Estimated a) juice leakage b) weight loss and c) browning incidence after 7 and 14 days in 'Arakta', 'Bahgwa' and 'Ruby' arils in three punnet varieties (PET1, PET2, PP). Different letters indicate a significant difference (p<0.05) according to Fischer's LSD multiple comparison test. Error bars denote standard error.**

The correlation test showed that browning incidence (%) was negatively correlated to TA, and positively correlated to TSS/TA and pH at a significant level (p<0.05) (Table 4.4). Jalikop *et al.* (2010) suggested browning to be more prevalent in light pink arils with higher TSS levels compared to vividly red coloured arils with lower TSS levels. TSS did not differ significantly between cultivars, but 'Ruby' was significantly (p<0.05) lighter than 'Arakta' and

'Baghwa' aril juice (Table 4.2). Browning incidence (%) was also positively correlated to lightness (p<0.05) in agreement with Jalikop *et al.* (2010).

Table 4.4 Correlation between browning incidence (%), lightness (*L) and chemical attributes of pomegranate arils.**

Variable	Browning Incidence (%)	TSS	TA	TSS/TA	pH	*L**
Browning Incidence (%)	1.00	0.11	-0.68*	0.73*	0.69*	0.63*
TSS	0.11	1.00	0.17	0.10	-0.29	-0.24
TA	-0.68*	0.17	1.00	-0.96*	-0.93*	-0.84*
TSS/TA	0.73*	0.10	-0.96*	1.00	0.86*	0.79*
pH	0.69*	-0.29	-0.93*	0.86*	1.00	0.95*
L*	0.63*	-0.24	-0.84*	0.79*	0.95*	1.00
H°	0.07	-0.82*	-0.26	0.06	0.48	0.51

*Correlations were significant at p<0.05 level

Juice leakage of arils was most prominent in 'Ruby' arils, especially in PP after 14 days of cold storage. Weight loss of pomegranate arils was less than 0.2% in all punnet varieties with highest weight loss occurring in PET1 after 14 days. The above mentioned weight loss was lower than weight loss (0.6-0.8%) recorded in arils packed in oriented polypropylene heat-sealed pouches stored at 4°C for 7 days (Gil *et al.*, 1996). The arils were stored at 95% relative humidity which could have played a role in minimal weight loss (Elyatem & Kader, 1984).

No mould growth was detected in 'Arakta' arils after 7 days, while mould growth was detected in PET2 packaged 'Bahgwa' arils and all 'Ruby' arils irrespective of punnet type. The quality of all cultivars deteriorated even further from day 7 to day 14 due to proliferated mould growth, especially in PET2 and PP punnets (Table 4.3). The CO_2 in PET1 was about 4% on day 7 and doubled to between 7-8% on day 14 in all cultivars (Fig. 4.3b). Cloete (2005) related a storage atmosphere of 10% CO_2 to a modified atmosphere and 15-20% CO_2 has previously been shown to reduce microbial growth in fruit (Gorny, 1997). The CO_2 levels of PET1 were adequate to allow mould growth but too low to inhibit mould growth. The correlation matrix (Table 4.5) showed a significant ($p \leq 0.01$) positive correlation between mould incidence, CO_2 production and O_2 consumption in all punnet varieties. This is also in agreement with Kader & Barrett (2005) who linked the rate of perishability of harvested commodities to a product's respiration rate.

Table 4.5 Correlation between percentage O_2, CO_2 and mould incidence in different punnet varieties

Punnet	Variable	CO_2 (%)	O_2 (%)	Mould Incidence (%)
	CO_2 (%)	1.00	-0.97*	0.75*
PET1	O_2 (%)	-0.97*	1.00	-0.66*
	Mould Incidence (%)	0.75*	-0.66*	1.00
	CO_2 (%)	1.00	-0.97*	0.74*
PET2	O_2 (%)	-0.97*	1.00	-0.61*
	Mould Incidence (%)	0.74	-0.61*	1.00
	CO_2 (%)	1.00	-0.99*	0.68*
PP	O_2 (%)	-0.99*	1.00	-0.59*
	Mould Incidence (%)	0.68*	-0.59*	1.00

*Correlations were significant at $p \leq 0.01$ level

After 14 days all pomegranate arils showed signs of visual mould growth (Table 4.3). However after 7 days, visual mould growth was prominent in all punnet varieties of 'Ruby', while 'Arakta' arils showed no visible signs of mould growth (Table 4.3). Furthermore the pH increased and the TA level decreased during short term storage in all cultivars (Table 4.9a, b, and c). This was contrary to Sepúlveda *et al*. (2000) who reported increasing TA level after 14 days in PET bags. The initial cultivar differences in chemical composition showed higher TA and lower TSS/TA in 'Arakta' compared to 'Ruby' arils (Table 4.2). As mentioned previously, citric acid is strongly correlated with TA and has antimicrobial properties according to Cloete (2005). A possible relationship between the pH, TA and the visual mould incidence of pomegranates were suspected and the correlation tested. Visual mould incidence in pomegranate arils was negatively related to TA and positively related to pH and TSS/TA at a significance level of $p<0.05$ (Table 4.6). Therefore microbial spoilage of 'Arakta' pomegranate arils might have been naturally delayed due to a higher initial TA.

Table 4.6 Correlation between mould growth incidence (%) and chemical attributes of pomegranate arils.

Variable	Mould Growth Incidence (%)	TSS/TA	pH	TA	TSS
Mould growth incidence (%)	1.00	0.31*	0.66*	-0.25*	0.20
TSS/TA	0.31*	1.00	0.71*	-0.99*	0.10
pH	0.66*	0.71*	1.00	-0.66*	-0.05
TA	-0.25*	-0.99*	-0.66*	1.00	-0.04
TSS	0.20	0.10	-0.05	-0.04	1.00

*Correlations were significant at $p<0.05$ level

The mould incidence in 'Arakta' and 'Bahgwa' arils was also studied in chapter 3 and appeared much earlier and was more aggressive during this study (chapter 4). Possible reasons for this occurrence are explained as follows:

a) The storage duration of whole fruits prior to extraction process might influence the quality of extracted pomegranate arils during the subsequent storage period of the arils. In chapter 3 pomegranates were peeled directly shortly after harvest, while the pomegranates used in chapter 4 were stored for a period of 10-14 weeks at 7°C 95% RH before being peeled. 'Arakta' fruit were subjected to the 10 weeks long term storage duration and was also the only cultivar that did not show any visible mould growth in arils after 7 days at 5°C (short term storage duration). 'Bahgwa' (12 weeks long term storage) arils showed visible mould growth in only PET2 punnet, while 'Ruby' (14 weeks long term storage) showed visible mould growth in all punnet varieties after 7 days (short term storage). This is only a rough estimate and microbial analyses would be necessary to confirm this notion. Artés *et al*. (2000) recorded the shelf life of extracted pomegranate arils to be 6 days at 15°C 75% RH after the whole pomegranate fruit was stored for a period of 12 weeks at 5°C 95% RH. Mr. F Olivier observed a reduction in shelf life of pomegranate arils after prolonged long term storage (5-8 days) compared to freshly harvested fruit (12-14 days) (Olivier, F. 2009, Chief Executive Officer, Pomegranate Fruit SA, Porterville, South Africa, personal communication, 14 April). The effects of long term storage of whole fruit on the shelf life (short term storage) of pomegranate arils have yet to be investigated.

b) Delays between harvesting and cooling or processing could negatively affect the quality of pomegranates (Kader & Barret, 2005). During this study pomegranates were extracted manually in the laboratory at 22°C, 53% RH. However the pomegranate industry uses an aril processing equipment (Juran Technologies, Israel) in a temperature controlled facility (12-15°C) to extract arils, after which the arils are stored at 0-2°C (Olivier, F. 2009, Chief Executive Officer, Pomegranate Fruit SA, Porterville, South Africa, personal communication, 14 April). The duration of aril extraction and handling is minimized by the aril processing equipment while the temperature is maintained at the minimum.

c) The whole pomegranate fruit was rinsed with water after harvest as mentioned by Paul and Shaba (2004) and left to air-dry before being stored for 7-12 weeks. The rinsing process was originally thought to remove dust particles which could introduce microbial contamination (Cloete, 2005). However pomegranates do not require any postharvest washing treatment and a 'light brushing' would have been sufficient according to Artés (1992). Pomegranate fruit might not have dried completely before

being placed in the cold room, resulting in favourable moist conditions for microbial growth (Cloete, 2005). Therefore this study strongly advises against rinsing whole pomegranate fruits before long term storage duration.

Factors that could have accelerated the deterioration of pomegranate arils include long term storage duration and peeling conditions of the whole fruit as well as washing treatments of arils.

(i) 'Ruby' cultivar had higher incidence of mould incidence and spoiled much faster compared to other cultivars in chapter 4 (Table 4.3). Possible reasons for this might be due to the prolonged long term storage duration (12 weeks) 'Ruby' was subjected to and the lower TA levels in 'Ruby' arils as discussed previously.

(ii) During this study the pomegranates were peeled at ambient conditions (22°C). Gil *et al.* (1996) peeled pomegranate arils in colder conditions of 13°C, while other studies did not specify the peeling temperature. The higher peeling temperature prior to the commencement of the study might have enhanced the microbial growth due to (a) and (b) mentioned above.

(iii) During this study the most natural state of the pomegranate arils were studied, therefore no antioxidant washing treatments were used. However, antioxidant oxidant washing of pomegranate arils is known to protect extracted arils that are exposed to air and vulnerable to external environmental factors (Gil *et al.,* 1996; Ergun & Ergun, 2009).

Physical Analyses

The effects of packaging on the chromatic parameters of 'Arakta' arils were very slight (Table 4.7a). The lightness of 'Arakta' aril juice was slightly higher when stored in PET1 and PET2 but remained the same in PP on day 14 compared to day 0 (Table 4.7a). Packaging did not affect the redness (*a**) of 'Arakta arils, even though slight changes occurred on day 7. The colour intensity (chroma) of 'Arakta' arils were unaffected by packaging throughout the study. The *H°* of 'Arakta' arils increased slightly in all punnets on day 14 (PET2>PET1>PP) (Table 4.7a).

Table 4.7a The effect of packaging on the colour of 'Arakta' pomegranate arils

Days	Punnet variety	*L**	*a**	*b**	Chroma	*H°*
0		35.0±0.28^{c}	14.3±0.37^{cd}	5.57±0.16^{c}	15.4±0.40^{c}	21.2±0.13^{e}
7	PET1	35.6±0.61^{ab}	15.5±0.81^{a}	6.13±0.32^{a}	16.7±0.87^{a}	21.6±0.11^{d}
	PET2	35.5±0.25^{ab}	15.1±0.56^{ab}	5.89±0.26^{ab}	16.2±0.62^{ab}	21.3±0.21^{e}
	PP	35.5±0.29^{ab}	14.9±0.61^{abc}	5.78±0.22^{bc}	16.0±0.64^{abc}	21.2±0.13^{e}
14	PET1	35.2±0.35^{bc}	14.2±0.87^{d}	5.8±0.37^{bc}	15.3±0.95^{c}	22.2±0.09^{c}
	PET2	35.6±0.51^{ab}	14.4±1.20^{bcd}	6.0±0.49^{ab}	15.7±1.29^{bc}	22.6±0.30^{a}
	PP	35.6±0.66^{a}	14.7±1.11^{bcd}	6.0±0.44^{ab}	15.9±1.20^{bc}	22.4±0.14^{b}

Different letters indicate significant difference (p<0.05) according to the multiple LSD test.

Packaging did not affect any colour attributes of 'Bahgwa' arils after 7 days of cold storage (Table 4.7b). After 14 days all the chromatic parameters (*L**, *a**, *b**, chroma, *H°*) was slightly higher in PET2 and PP punnets (Table 4.7b).

Table 4.7b The effect of packaging on the colour of 'Bahgwa' pomegranate arils

Days	Punnet variety	*L**	*a**	*b**	Chroma	*H°*
0		34.0±0.47^{bc}	12.7±1.21^{c}	4.70±0.56^{c}	13.5±1.33^{c}	20.3±0.47^{d}
7	PET1	34.0±0.16^{c}	12.8±0.44^{c}	4.76±0.22^{c}	13.7±0.49^{c}	20.4±0.29^{d}
	PET2	34.1±0.02^{bc}	13.0±0.04^{bc}	4.90±0.05^{bc}	13.9±0.04^{c}	20.6±0.20^{cd}
	PP	34.3±0.28^{bc}	13.4±0.75^{abc}	5.08±0.34^{bc}	14.3±0.82^{abc}	20.8±0.24^{c}
14	PET1	34.4±0.13^{b}	13.2±0.38^{abc}	5.17±0.17^{b}	14.2±0.42^{bc}	21.4±0.16^{b}
	PET2	35.1±0.65^{a}	14.0±1.34^{a}	5.77±0.60^{a}	15.2±1.46^{a}	22.3±0.27^{a}
	PP	34.8±0.24^{a}	13.9±0.60^{ab}	5.64±0.26^{a}	15.0±0.66^{ab}	22.1±0.18^{a}

Different letters indicate significant difference (p<0.05) according to the multiple LSD test.

After 14 days 'Ruby' arils were slightly lighter in PET1 and PET2 punnets and H° increased in all punnet varieties compared to day 0 (Table 4.7c). The chroma, *a** and *b** was slightly lower in 'Ruby' arils stored in PP punnets after 7 and 14 days (Table 4.7c).

Table 4.7c The effect of packaging on the colour of 'Ruby' pomegranate arils

Days	Punnet variety	*L**	*a**	*b**	Chroma	H°
0		35.9±0.58^{c}	15.5±1.10^{a}	5.90±0.36^{bc}	16.6±1.16^{a}	20.9±0.24^{c}
7	PET1	36.2±0.19^{bc}	14.7±0.28^{ab}	5.61±0.07^{cd}	15.8±0.28^{ab}	20.9±0.27^{c}
	PET2	36.2±0.24^{bc}	14.4±0.47^{b}	5.53±0.15^{d}	15.4±0.49^{b}	21.1±0.15^{c}
	PP	36.4±0.75^{bc}	14.2±0.93^{b}	5.42±0.23^{d}	15.2±0.95^{b}	20.9±0.49^{c}
14	PET1	36.6±0.16^{ab}	14.2±0.43^{b}	6.03±0.07^{ab}	15.4±0.39^{b}	23.1±0.79^{b}
	PET2	37.2±0.43^{a}	14.6±0.84^{b}	6.32±0.37^{a}	15.9±0.90^{ab}	23.4±0.71^{ab}
	PP	36.3±1.36^{bc}	12.8±1.41^{c}	5.64±0.76^{cd}	14.0±1.59^{c}	23.8±0.58^{a}

Different letters indicate significant difference (p<0.05) according to the multiple LSD test.

Packaging had no effect on the total colour difference (ΔE*) of pomegranate arils during the first 7 days (Fig. 4.5a), while most changes occurred between days 7-14. From 7-14 days the ΔE* was slightly higher in PET2 ('Bahgwa') and PP ('Ruby') punnets (Fig. 4.5b). The ΔE* of pomegranate arils between days 0 and 14 was highest in 'Ruby' arils stored in PP punnets. Even though the colour differences were significant, they were very small; PET1 had the least effect on total aril colour difference (ΔE*) throughout the 14 day storage period (Fig. 4.5b, c).

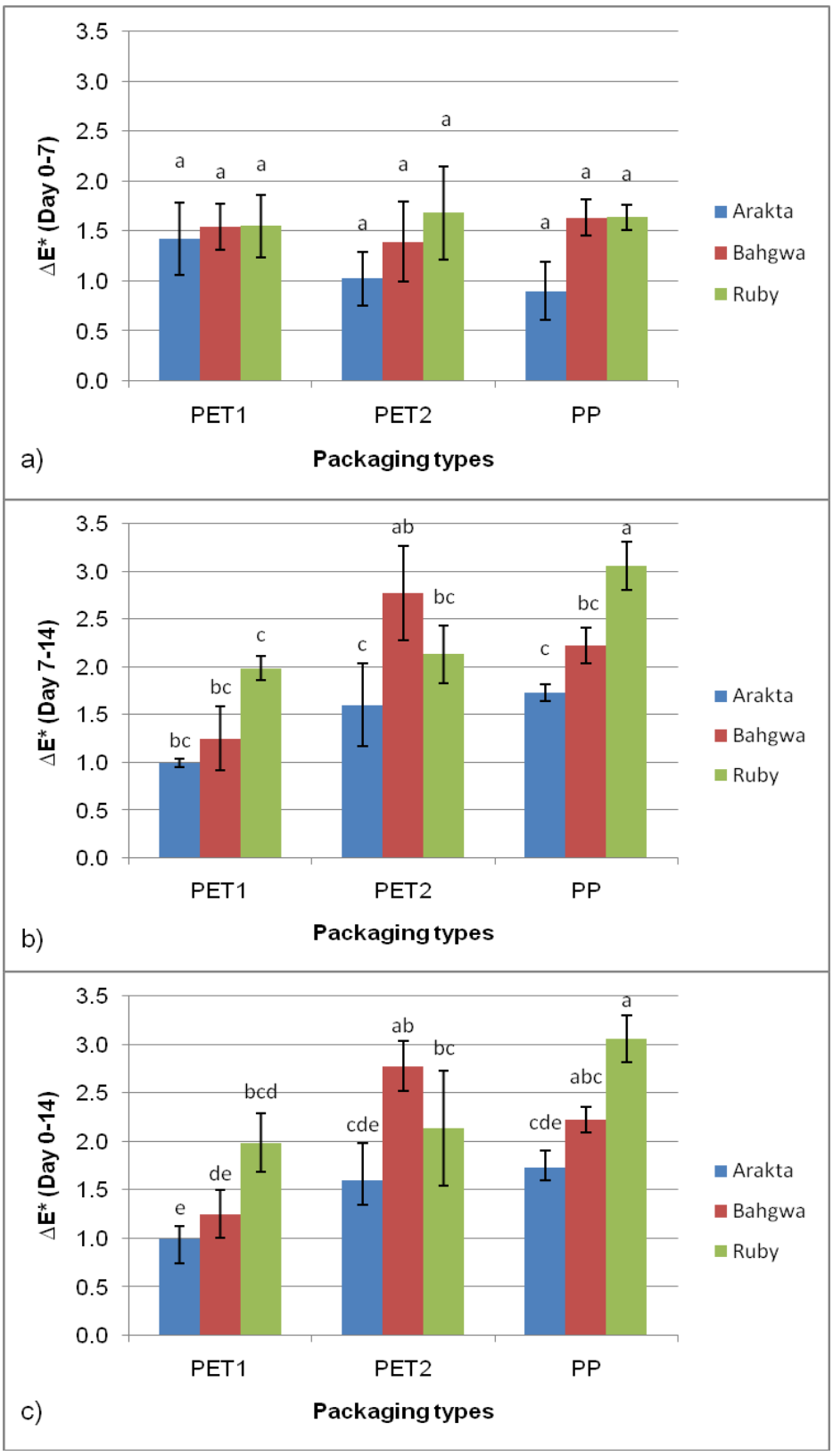

Figure 4.5 **The effect of packaging on the total difference (ΔE*) of *L**, *a** and *b** colour coordinates of 'Arakta', 'Bahgwa' and 'Ruby' cultivars between (a) day 0-7 (b) day 7-14 and (c) day 0-14. Different letters indicate significant difference ($p<0.05$) according to the LSD test. Error bars denote standard error**

TEXTURE

The effect of packaging and cultivar on textural properties of pomegranate arils was observed after 7 and 14 days of cold storage (Table 4.8). The hardness and toughness of pomegranate arils were cultivar dependent. The texture of 'Bahgwa' arils was unaffected by packaging, while 'Arakta' arils were harder and tougher in PET1 compared to PET2 punnets. Packaging did not affect the hardness of 'Ruby' arils, but the toughness was slightly higher in PET1 compared to PET2 and PP. 'Arakta' and 'Ruby' pomegranate arils were softer due to the lower hardness and toughness levels when packed in PET2 punnets. PET2 might therefore be desirable to use since consumers generally prefer 'softer' arils (Al-Said *et al.*, 2009). However Ayhan & Estürk (2009) reported that even though a significant difference in measured firmness of pomegranate arils stored for 15 days were found, the sensory panel did not detect this difference. A sensory evaluation would be necessary to confirm if the sensory panel perceive a difference in texture and if they enjoy the softer arils more. The effect of short term storage duration on the textural properties will not be discussed as the data from day 0 is missing.

Table 4.8 The effect of packaging on the textural qualities of 'Arakta', 'Bahgwa' and 'Ruby' pomegranate arils

Cultivar	Punnet variety	Hardness (N)	Toughness (N.mm^{-1})
Arakta	PET1	102.4 ± 12.2^{a}	132.3 ± 41.8^{a}
	PET2	96.1 ± 12.5^{b}	112.3 ± 17.9^{b}
	PP	101.2 ± 11.3^{ab}	123.6 ± 16.0^{ab}
Bahgwa	PET1	105.4 ± 9.80^{a}	130.2 ± 15.4^{a}
	PET2	103.8 ± 11.5^{a}	121.8 ± 18.8^{a}
	PP	100.7 ± 8.45^{a}	131.8 ± 27.2^{a}
Ruby	PET1	99.2 ± 13.7^{a}	126.9 ± 34.9^{a}
	PET2	97.6 ± 9.83^{a}	105.6 ± 17.4^{b}
	PP	94.9 ± 11.7^{a}	109.7 ± 15.8^{b}

Different letters indicate significant difference (p<0.05) according to the multiple LSD test.

Chemical Analyses

The TA, TSS and TSS/TA of 'Arakta' and 'Bahgwa' aril juice were unaffected by packaging and after 14 days of cold storage (Table 4.9a, b). In agreement with Sepúlveda *et al*. (2001) packaging had no effect on TA of three pomegranate cultivars during short term storage duration. Short term storage duration did however cause a significant (p<0.05) reduction in TA levels in 'Ruby' arils (Table 4.9c). The declining TA level could have been attributed to metabolic activities as suggested by Ayhan and Eştürk (2009). The TSS of 'Ruby' aril juice

remained relatively the same for all punnet varieties after 14 days, although lower levels were detected in PET2 (15.3±0.29°Brix) and PP (15.4±0.40 °Brix) after 7 days (Table 4.9c). The TSS/TA of 'Ruby' aril juice increased after 14 days, especially in PET1 (70.2±1.13) and PP (71.4±0.00) (Table 4.9c). Short term storage duration caused an increase in pH level after 14 days in all cultivars, especially in PET2 and PP punnet varieties (Table 4.9a, b, and c). An increasing pH during short term storage duration has been previously reported in pomegranate whole fruit (Elyatem & Kader, 1984; Artés *et al.*, 2000) and juice storage (Alighourchi & Barzegar, 2009). Moisture content was also unaffected by short term storage duration and packaging in the arils of all three cultivars after 14 days cold storage (Table 4.9a, b, c).

Table 4.9a The effect of packaging on the chemical attributes of 'Arakta' aril juice

Days	Punnet variety	TA	pH	TSS (°Brix)	TSS/TA	Moisture (%)
0		0.33±0.03^{a}	3.29±0.00^{d}	15.4±0.47^{abc}	47.6±5.73^{b}	80.3±1.47^{a}
7	PET1	0.28±0.02^{bc}	3.30±0.03^{d}	15.1±0.35^{c}	53.2±1.67^{ab}	79.6±0.22^{a}
	PET2	0.31±0.04^{abc}	3.37±0.01^{b}	15.5±0.12^{abc}	50.1±6.28^{ab}	79.6±0.15^{a}
	PP	0.28±0.01^{c}	3.38±0.02^{ab}	15.2±0.25^{bc}	55.1±0.68^{a}	79.5±0.12^{a}
14	PET1	0.32±0.01^{ab}	3.33±0.02^{c}	15.6±0.21^{abc}	48.9±2.03^{ab}	79.9±0.40^{a}
	PET2	0.31±0.00^{abc}	3.39±0.01^{ab}	15.9±0.15^{a}	51.2±0.49^{ab}	80.0±0.18^{a}
	PP	0.30±0.01^{abc}	3.40±0.01^{a}	15.8±0.26^{ab}	53.3±0.81^{ab}	79.8±0.05^{a}

Different letters indicate significant difference (p<0.05) according to the multiple LSD test.

Table 4.9b The effect of packaging on the chemical attributes of 'Bahgwa' aril juice

Days	Punnet variety	TA	pH	TSS (°Brix)	TSS/TA	Moisture (%)
0		0.28±0.01^{a}	3.24±0.01^{c}	15.70±0.10^{ab}	55.4±1.17^{ab}	82.3±5.91^{a}
7	PET1	0.28±0.01^{a}	3.25±0.01^{c}	15.93±0.21^{a}	56.3±1.17^{ab}	79.2±0.23^{a}
	PET2	0.28±0.00^{a}	3.25±0.02^{c}	15.70±0.17^{ab}	56.1±0.62^{ab}	79.6±0.07^{a}
	PP	0.28±0.00^{a}	3.26±0.01^{c}	15.57±0.25^{b}	55.6±0.90^{ab}	79.3±0.24^{a}
14	PET1	0.28±0.00^{a}	3.32±0.02^{b}	15.97±0.15^{a}	57.0±0.55^{a}	79.5±0.28^{a}
	PET2	0.28±0.00^{a}	3.36±0.00^{a}	15.83±0.21^{ab}	56.6±0.74^{ab}	79.7±0.15^{a}
	PP	0.29±0.01^{a}	3.35±0.02^{a}	15.80±0.17^{ab}	55.1±0.51^{b}	79.5±0.11^{a}

Different letters indicate significant difference (p<0.05) according to the multiple LSD test.

Table 4.9c The effect of packaging on the chemical attributes of 'Ruby' aril juice

Days	Punnet variety	TA	pH	TSS (°Brix)	TSS/TA	Moisture (%)
0		0.26±0.01^{a}	3.35±0.01^{d}	15.8±0.10^{a}	60.0±1.36^{e}	78.7±10.60^{a}
	PET1	0.23±0.01^{b}	3.43±0.01^{c}	15.5±0.15^{abc}	66.3±1.63^{d}	78.9±0.08^{a}
7	PET2	0.22±0.01^{c}	3.43±0.01^{c}	15.3±0.29^{c}	68.4±0.47^{bc}	78.7±0.24^{a}
	PP	0.22±0.01^{c}	3.47±0.02^{b}	15.4±0.40^{bc}	70.9±0.02^{a}	78.9±0.07^{a}
	PET1	0.22±0.01^{c}	3.55±0.01^{a}	15.7±0.25^{ab}	70.2±1.13^{ab}	79.3±0.17^{a}
14	PET2	0.23±0.01^{b}	3.56±0.02^{a}	15.7±0.10^{ab}	67.3±1.70^{cd}	79.1±0.20^{a}
	PP	0.22±0.00^{c}	3.57±0.01^{a}	15.7±0.00^{ab}	71.4±0.00^{a}	79.3±0.28^{a}

Different letters indicate significant difference (p<0.05) according to the multiple LSD test.

Selected Bioactive Component Analyses

Ascorbic acid and anthocyanin compounds are temperature and pH sensitive and have been shown to degrade over time (Küpper *et al.*, 1995; Coultate, 2007; Alighourchi & Barzegar, 2009; Ghafir *et al.*, 2010). Carotenoids are more labile when extracted from their relatively stable natural state (Coultate, 2007). A reduction of anthocyanin and ascorbic acid level was therefore expected after 14 days cold storage, but the contrary occurred: ascorbic acid and anthocyanin levels were lower on day 0 compared to day 7 and 14 (Table 4.10a, b). Pomegranate aril juice samples of day 0 was analysed on the same day and samples were thawed at room temperature (22°C), while the samples of day 7 and 14 were thawed at refrigerated temperatures of 5°C 95% RH. So the difference in thawing procedure could have resulted in lower initial values (Table 4.10a, b). Anthocyanin levels of 'Bahgwa' and 'Ruby' arils were slightly lower in PP after 7 and 14 days (Table 4.10a). Ascorbic acid levels declined between day 7 and 14 in all cultivars irrespective of punnet variety (Table 4.10b). This is in agreement with the declining levels of ascorbic acid during storage reported for 'Hicaz' (9.9 mg.100 mL^{-1}) (Küpper *et al.,* 1995) and 'Shlefy' (5.29-5.07 $mg.100^{-1}$ mL) (Ghafir *et al.,* 2010) pomegranate cultivars. The appearance of declining levels of anthocyanins and ascorbic acid could also be due to possible oxidation and condensation of these compounds (Choi *et al*., 2002). A very low ascorbic acid level of 'Arakta' arils in PET1 after 14 days could not be attributed to the effect of packaging, since 'Bahgwa' and 'Ruby' did not show the same effect. Very little β-carotene was found in pomegranate arils of 'Arakta' (4 $mg.L^{-1}$) 'Bahgwa' (6 $mg.L^{-1}$) and Ruby (3.3 $mg.L^{-1}$) cultivars and levels declined throughout the study. Curl (1963) reported traces of 0.16 $mg.kg^{-1}$ β-carotene in pomegranate fruit. After 7 days the β-carotene level of 'Arakta' arils in PP declined by 35% (Table 4.10c). While the β-carotene levels were reduced in all cultivars irrespective of punnet variety after 14 days. 'Bahgwa' showed less changes in β-carotene compared to 'Arakta' and 'Ruby' pomegranate aril juice (Table 4.10c).

Table 4.10a The effects of packaging on the anthocyanin (mg.L^{-1}) levels of 'Arakta', 'Bahgwa' and 'Ruby' pomegranate aril juice.

Days	Punnet varieties	Arakta	Bahgwa	Ruby
0		92.1±1.83^{e}	112.5±2.09^{d}	73.8±2.82^{abc}
7	PET1	98.2±3.36^{d}	132.5±2.64^{a}	70.6±1.64^{cd}
	PET2	99.2±1.54^{d}	127.3±1.54^{ab}	69.6±3.78^{d}
	PP	101.8±3.16^{d}	124.8±4.79^{b}	62.0±2.24^{e}
14	PET1	117.6±4.72^{b}	127.2±9.70^{ab}	75.9±5.72^{a}
	PET2	108.3±11.36^{c}	128.5±7.41^{ab}	74.4±4.26^{ab}
	PP	123.9±2.53^{a}	118.7±1.40^{c}	71.2±1.73^{bcd}

Different letters indicate significant difference (p<0.05) according to the multiple LSD test

Table 4.10b The effects of packaging on the ascorbic acid (mg.L^{-1}) levels of 'Arakta', 'Bahgwa' and 'Ruby' pomegranate aril juice.

Days	Punnet varieties	Arakta	Bahgwa	Ruby
0		24.8±2.92^{d}	24.2±1.94^{e}	20.5±1.21^{e}
7	PET1	40.8±3.66^{b}	47.2±2.38^{b}	48.8±0.98^{b}
	PET2	45.4±3.81^{a}	49.7±1.34^{a}	56.5±2.94^{a}
	PP	33.9±1.26^{c}	48.6±2.61^{ab}	56.8±2.07^{a}
14	PET1	12.0±1.50^{e}	34.5±1.90^{cd}	42.6±1.37^{d}
	PET2	39.5±0.28^{b}	33.1±2.29^{d}	45.5±0.86^{c}
	PP	38.7±1.85^{b}	35.7±1.13^{c}	44.8±0.99^{c}

Different letters indicate significant difference (p<0.05) according to the multiple LSD test

Table 4.10c The effects of packaging on the β-carotene (mg.L^{-1}) levels of 'Arakta', 'Bahgwa' and 'Ruby' pomegranate aril juice.

Days	Punnet varieties	Arakta	Bahgwa	Ruby
0		4.17±0.32^{a}	6.20±0.70^{a}	3.34±0.78^{a}
7	PET1	4.17±0.38^{a}	5.63±1.08^{ab}	0.63±0.68^{c}
	PET2	4.26±1.25^{a}	5.21±2.19^{ab}	2.76±2.15^{ab}
	PP	2.73±0.50^{b}	2.45±0.72^{d}	2.55±1.24^{ab}
14	PET1	0.65±0.49^{c}	4.03±0.79^{c}	0.91±0.35^{c}
	PET2	1.31±0.64^{c}	4.58±0.43^{bc}	1.66±0.38^{bc}
	PP	0.93±0.47^{c}	3.96±0.52^{c}	1.13±0.44^{c}

Different letters indicate significant difference (p<0.05) according to the multiple LSD test

Conclusions

This study shows that current commercial packaging used for pomegranate arils in South Africa had minimal effects on the physico-chemical and bioactive compounds of pomegranate arils. Of the three punnet varieties, PET2 showed the most stable headspace gas composition during the short term storage period. PET1 showed higher CO_2 levels and might have been attributed to the tighter fitting clampshell design. The overall quality of pomegranate arils deteriorated throughout the storage period. Postharvest rinsing prior to long term storage of the whole fruit could also have played a role in the quality of the arils, since this process was deemed unnecessary by Artés *et al.* (1992). Storage duration of whole pomegranates might result in the earlier onset of visible microbial growth of extracted pomegranate arils. 'Arakta' showed higher TA compared to the other cultivars and no mould growth was detected in 'Arakta' arils after 7 days irrespective of punnet variety. Visual mould growth was detected in extracted arils of 'Ruby' irrespective of punnet variety after 7 days at 5°C 95% RH after long term storage duration of 10-14 weeks at 7°C 95% RH. Pomegranate breeders, farmers and retailers will find this information very useful to aid in cultivar selection as well as improved postharvest handling practices.

References

Alighourchi, H. & Barzegar, M. (2009). Some physicochemical characteristics and degradation kinetic of anthocyanin of reconstituted pomegranate juice during storage. *Journal of Food Engineering,* **90,** 179-185.

AOAC International. (2006). Official methods of analysis of AOAC International. Gaithersburg, Md: AOAC International.

Al-Maiman S.A. & Ahmad, D. (2002). Changes in physical and chemical properties during pomegranate (*Punica granatum)* fruit maturation. *Food Chemistry*, **76**, 437-441

Al-Said, F.A., Opara, L.U. & Al-Yahyai, R.A. (2009). Physico-chemical and textural quality attributes of pomegranate cultivars (*Punica granatum* L.) grown in the Sultanate of Oman. *Journal of Food Engineering*, **90**, 129-134.

Artés, F. (1992). Factores de calidad y conservación frigorífica de la Granada. In: *II Jomadas Nacionales del Granado.* Universidad Politécnica de Valencia. (As cited by Artés & Tomás-Barberán, 2000)

Artés, F & Tomás-Barberán, F.A. (2000). Post-harvest technological treatments of pomegranate and preparation of derived products. In: *Symposium on production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology.* CIHEAM – IAMZ p. 199-204. Zaragoza, Spain.

Artés, F., Villaescusa, R. & Tudela, J.A (2000). Modified atmosphere Packaging of Pomegranate. *Journal of Food Science,* **65**, 1112-1116.

Ayhan, Z & Eştürk, O. (2009). Overall Quality and Shelf Life of Minimally Processed and Modified atmosphere Packaged “Ready-to-Eat” Pomegranate Arils. *Journal of Food Science,* **74,** C399-C405.

Barros, L., Ferreira, M., Queiros, B., Ferreira, I.C.F.R. & Baptista, P. (2007). Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry* **103**, 413-419.

Calderón, M.M. (2010). Internal quality profile and influence of packaging conditions on fresh-cut pineapples. PhD Thesis. University of Lleida, Spain.

Choi, M.H., Kim, G.H. & Lee, H.S. (2002). Effects of ascorbic acid retention on juice color and pigment stability in blood orange (Citrus sinensis) juice during refrigerated storage. *Food Research International* **35**, 753–759.

Cloete, T.E. (2005). Basic and applied microbiology. The Department of Microbiology and Plant Pathology, Pp. 168-195. South Africa: University of Pretoria.

Coultate, T.P. (2007). *Food The Chemistry of Its Components*. Pp. 42, 62-63, 184, 195, 281-289. UK: RSC Publishing.

Curl, A.L. (1963). The carotenoids from several low carotenoid fruits. *Journal of Food Science*, **29**, 241-245.

Dafny-Yalin, M., Glazer, I., Bar-Ilan, I., Kerem, Z., Holland, D. & Amir, R. (2010). Colour, Sugars and Organic Acids Composition in Aril Juices and Peel Homogenates Prepared From Different Pomegranate Accessions. *Journal of Agricultural and Food Chemistry,* **58**, 4342-4352.

El-Nemr, W.E., Ismail, I.A. & Raġab. (1990). Chemical Composition of juice and seeds of pomegranate fruit. *Die Nahrung*, **34**, 601-606

Elyatem, S. M. & Kader, A. A. (1984). Post-harvest physiology and storage behaviour of pomegranate fruits. *Scientia Horticulturae,* **24**, 287-298.

Ergun, M. & Ergun, N. (2009). Maintaining quality of minimally processed pomegranate arils by honey treatments. *British Food Journal*, **111**, 396-406.

Fawole, O.A., Opara, U.L. & Theron, K.I. (2011). Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa. *Food Bioprocess Technology*, DOI: 10.1007/s11947-011-0533-7.

Ghafir, S.A.M., Ibrahim, I.Z. & Zaied, S.A. (2010). Response of Local Variety 'Shlefy' Pomegranate Fruits to Packaging and Cold Storage. *Acta Horticulturae*, **877** 427-432.

Gil, M.I., Martínez, J. A. & Artés, F. (1996). Minimally Processed Pomegranate Seeds. *Lebensmittel-Wissenschaft und-Technologie*, **29**, 708-713.

Gorny, J. (1997). CA and MA requirements and recommendation for fresh-cut (minimally processed fruits and vegetables. In: *Fresh Cut Fruits and Vegetables and MAP Proceedings of 7th International Controlled Atmosphere Research Conference.* Pp. 19. July 1997. Davis, California, USA.

Jalikop, S.H., Venugopalan, R. & Kumar, R. (2010). Association of fruit traits and arils browning in pomegranate (*Punica granatum* L.). *Euphytica*, **174**, 137-141.

Kader, A.A. & Barrett (2005). Classification, composition of fruits, and postharvest maintenance of quality. In: *Processing Fruits Science and Technology* (edited by D.M. Barett, L. Somogyi, & H. Ramaswamy). Pp 3-21, USA: CRC Press.

Küpper, W., Pekmezci, M. & Henze, J. (1995). Studies on CA-Storage of Pomegranate (*Punica granatum* L., cv. Hicaz). *Acta Horticulturae*, **398,** 101-108.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 74-185.

Nicola, S. Tibaldi, G. & Fontana, E. (2009). Fresh-cut Produce Quality: Implications for a Systems Approach. In: *Postharvest Handling: A Systems Approach.* (edited by W.J. Florkowski, R.L. Shewfelt, B. Brueckner, & S.E. Prussia). Pp. 270-273. Oxford: Academic Press.

Ozgen, M., Durgaç, C., Serçe, S. & Kaya, C. (2008). Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. *Food Chemistry*, **111**, 703-706.

Paul, D.K. & Shaba, R.K. (2004). Nutrients, Vitamins and Nutrient Content in Common Citrus Fruits in the Northern Region of Bangladesh. *Pakistan Journal of Biological Sciences*, **7**, 238-242.

Quiro's, A.R. & Costa, H.S. (2006). Analysis of carotenoids in vegetable and plasma samples: A review. *Journal of Food Composition and Analysis,* **19**, 97-111.

Sepúlveda, E., Galletti, l., Sáenz, C. & Tapia, M. (2000). Minimal Processing of pomegranate var. Wonderful. In: Symposium on production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology. CIHEAM – IAMZ p 237-242. Zaragoza, Spain.

Sepúlveda, E., Sáenz, C. Berger, H., Galletti, l., Valladares, C. & Botti, C. (2001). Minimal Processing of pomegranate cv. Española: Effect of three package materials. *Acta Horticulturae*, **553**, 711-712.

Smith, J.P., Zagory, D. & Ramaswamy, H.S. (2005). Packaging of Fruits and Vegetables. In: *Processing Fruits* (edited by D.M. Barrett, L. Somogyi & H. Ramaswamy). 2nd ed. Pp 358, Florida: CRC Press.

Tehranifar, A., Zarei, M., Nemati, Z., Esfandiyari, B. & Vazifeshenas, M.R. (2010). Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. *Scientia Horticulturae*, **126**, 180-185.

Zarei, M., Azizi, M. & Bashiri-Sadr, Z. (2010). Studies on Physico-Chemical Properties and Bioactive Compounds of Six Pomegranate Cultivars Grown in Iran. *Journal of Food Technology,* **8**, 112-117.

CHAPTER 5

EFFECT OF PRE-STORAGE WATER DIPPING OF WHOLE POMEGRANATE FRUIT ON THE MICROBIAL QUALITY OF PROCESSED POMEGRANATE (PUNICA GRANATUM) ARILS DURING COLD STORAGE

Abstract

The early detection of visual mould growth in the previous chapter (chapter 4) lead to an investigation of the effect of pre-storage water dipping of whole fruit on the microbial quality of extracted pomegranate arils stored for 8 days at 5°C 95% RH. Freshly harvested 'Bahgwa' pomegranates were subjected to a pre-storage dipping in distilled water (dipped fruit) and air-dried at ambient conditions (~21°C) or stored as is (dry fruit) at 7°C 95% RH for 15 weeks. Subsequently, arils were extracted, packaged and further stored at 5°C 95±8.34% RH for 8 days. Total viable aerobic mesophillic bacteria as well as yeasts and moulds were enumerated to quantify spoilage microorganisms, while *Escherichia coli* and *Staphylococcus aureus* were enumerated for general hygiene purposes. After 8 storage days at 5°C 95% RH the microbial counts increased to 4.74 log cfu.g^{-1} yeast and moulds and 3.73 log cfu.g^{-1} total viable aerobic mesophillic bacteria count. Arils from 'dry fruit' were clear of any microbial growth on yeast and mould growth as well as total viable aerobic mesophillic bacteria for the whole duration of this study. Therefore it is advised to avoid pre-storage water dipping of whole pomegranate fruit otherwise the shelf life of the pomegranate arils will be reduced to 6 days at 5°C 95% RH according to South African microbiological standards pertaining to fruit juice.

Introduction

Pre-packaged pomegranate arils are a convenient way to consume the health benefits of arils without the time consuming process of peeling the whole fruit (Gil *et al.*, 1996; Artés *et al.*, 2000). The shelf life duration of minimally processed arils is limited 7-25 days while whole fruit could be stored for 12-16 weeks depending on the storage temperature and atmospheric conditions (Juven *et al.,* 1984; Gil *et al.*, 1996; Artés *et al.*, 2000; Sepúlveda *et al.* 2000; Nanda *et al.*, 2001; Ayhan & Eştürk, 2009; Ghafir *et al.*, 2010).

Pomegranate contains a physical protection of the arils by the outer layering (peel), while the chemical composition of both (peels and arils) provide antibacterial properties which makes the fruit a useful natural alternatives for the pharmaceutical and food preservative industries

(Tantipaibulvut *et al.*, 2011). Even though fruit has natural antimicrobial properties, its low pH and high sugar and moisture content makes them susceptible to microbial spoilage by bacteria and especially yeast and mould growth (Montville & Matthews, 2008). Pomegranate arils from various cultivars in different countries have shown a general low pH (2.76-4.10) and high sugar (11-23%) and moisture content (76-81%) (Artés *et al.,* 2000; Melgarejo *et al.,* 2000; Ramulu *et al.*, 2003; Al-Said *et al.,* 2009; Zarei *et al.,* 2010). The following microflora has been reported in minimally processed pomegranate arils: Yeasts (*Hanseniaspora guilliermondii, Metschinikowia pulcherrima, Debaryomyces hansenii*), mould, acetic acid bacteria (*Gluconobacter, Acetobacter*) aerobic mesophillic bacteria, aerobic psychrotrophic bacteria, micro-aerophillic lactic acid bacteria and *Enterobacteriaceae* (Juven *et al.,* 1984; Sepúlveda *et al.* 2000; Ayhan & Eştürk, 2009). Sensory evaluation conducted in conjunction with microbiological analyses showed yeasts and acetic acid bacteria (*Acetobacter, Gluconobacter*) are also responsible for off-flavour development in pomegranate arils during cold storage (Juven *et al.*, 1984). Extending the shelf life of both whole pomegranate fruit and arils has recently enjoyed global scientific interest (Sepúlveda *et al.*, 2000; López-Rubira *et al.*, 2005; Tedford *et al*., 2005; Ayhan & Estürk, 2009). Postharvest technology provides important tools to enhance the quality and shelf life of lightly processed fruit and vegetables (Schlimme, 1995). The importance of low temperature storage of minimally processed pomegranate arils has already been emphasized in chapter 3 and other literature (Gil *et al.*, 1996, Sepúlveda *et al.*, 2000, Ayhan & Estürk, 2009).

Visual mould growth was detected 7 days earlier in processed pomegranate arils of which the whole fruit was subjected to a long term postharvest storage duration (chapter 4) compared to fruits that were processed directly after harvest (chapter 3). Pomegranate fruit used in both chapter 3 and 4 were dipped in distilled water and air-dried. After the previously mentioned water dipping process, fruit used in chapter 3 was directly processed, while fruit used in chapter 4 was subjected to long term cold storage at 7°C 95% RH for 10-14 weeks before being processed. The initial intention of the water dipping process was to reduce the microbial load on fruit (Montville & Matthews, 2008). According to literature pomegranate fruit need only be brushed lightly without any further postharvest treatments such as washing, waxing or fungicides (Artés *et al*., 1992). Although a new postharvest fungicide (Scholar) is currently being used in the U.S. to successfully reduced gray mould infection by its active ingredient, fludioxonil (Tedford *et al.*, 2005). The gray mould infection is caused by the pathogen *Botrytis cinerea* and ensues during the blooming period of pomegranates and remains dormant until the flower part in the crown of a mature pomegranate fruit becomes wet (Tedford *et al.*, 2005). Long term cold storage conditions of 7°C 95% RH has also shown to promote the growth of gray mould spoilage (Tedford *et al.*, 2005). The early onset of microbial growth during chapter 4 was suspected to be caused by the prolonged storage

period (7°C 95% RH) of the whole fruit as well as the water dipping process used directly after harvesting the fruit. The aim of this study is to investigate the effect of pre-storage whole fruit dipping in distilled water on the microbial quality of extracted pomegranate arils stored for 8 days at 5°C 95% RH.

Material and Method

Fruit Procurement, Processing and Packaging Procedures

Pomegranate fruit (*Punica granatum*). 'Bahgwa' were procured from Houtconstant farm in Porterville, Western Cape. Fruit were sorted to exclude damaged, bruised or sunburnt fruit. One group pomegranates were previously dipped in distilled water (dipped fruit) and air-dried for 5 hours at room temperature (~21°C) directly after harvesting period, while the other group was never subjected to postharvest dipping (dry fruit); all fruit were stored at 7°C 95% RH for 15 weeks. During this time a few of the fruits from the 'dipped group' showed signs of visual mould growth and were discarded to avoid infection with other fruit within the same group. No visible signs of mould growth were detected in fruit from the 'dry group'. Four pomegranates without any visible mould growth were used from each group during this study. Aseptic handling of pomegranate fruit during peeling, mixing and packaging process was done at ambient temperature (~21°C). Pomegranate fruit were peeled manually by one individual using a sharp knife while cleaning the working surface area with 70% ethanol between each fruit. Firm, undamaged, bright red arils were considered good quality arils and used during this study. Arils from each fruit were packaged into individual sterile bags and kept at 5°C until all the fruit from both groups (dipped and dry fruit) were peeled. Arils from each group were mixed together to form a homogenous batch, packed in clampshell (PET1) packaging (Fig. 5) and stored at 5°C 95±8.34% RH for 8 days.

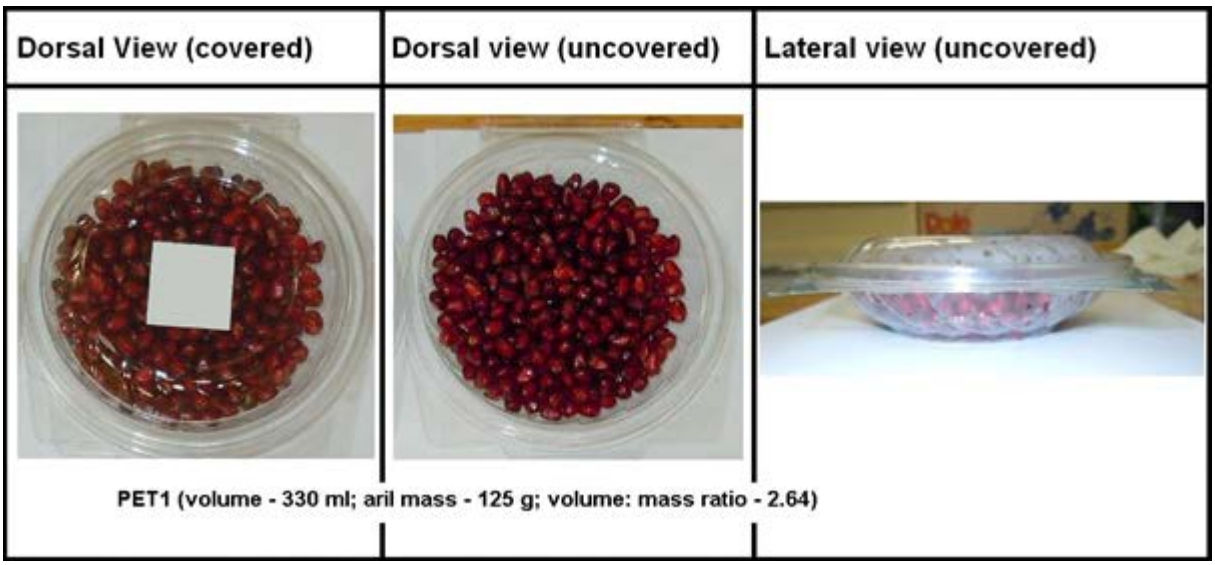

Figure 5.1 **Depiction of type of packaging used (clamp-shell, PET1) from dorsal (covered and uncovered) and lateral views with the volume, aril mass as well as the volume: mass ratio of the arils.**

Microbial Analysis

Sampling was performed on storage days 0, 3, 6 and 8. Two 10 g samples of pomegranate arils were weighed into sterilized tubs from each package. Arils were transferred to sterile stomacher bags with 90 mL peptone buffered water (Merck), homogenated for five min with a stomacher and serial dilutions were made between 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} (Ayhan & Eştürk, 2009).

Pour-plates of plate count agar (PCA, Merck) was used to enumerate mesophillic aerobic count using the colony count technique (SANS 4833, 2007) and inverted plates were incubated at 30°C for 72 h. Enumeration of yeasts and moulds (SANS 21527-1, 2009) were performed with spread-plates on potato dextrose agar (PDA, Merck) supplemented with 100 $\mu g.mL^{-1}$ chloramphenicol (Sigma) and incubated in upright position at 30°C for 72 h. *Staphylococcus aureus* were enumerated with spread-plates on Baird-Parker media (BP, Merck) supplemented with egg yolk tellurite emulsion using the Baird-Parker technique (SANS 6888-1, 1999) and inverted plates were incubated at 35°C for 48 hours. Pour-plates of violet red bile agar with added 4-methylumbelliferyl-β-D-glucoronide (VRB-MUG) (Merck) was used to enumerate *Escherichia coli* under fluorescent light (Feng & Hartman, 1982) and inverted plates were incubated at 35°C for 24 h. Microbial colonies were counted between 30 and 300 and results were expressed as 10 log $cfu.g^{-1}$ (SANS 4833, 2007).

Experimental Design

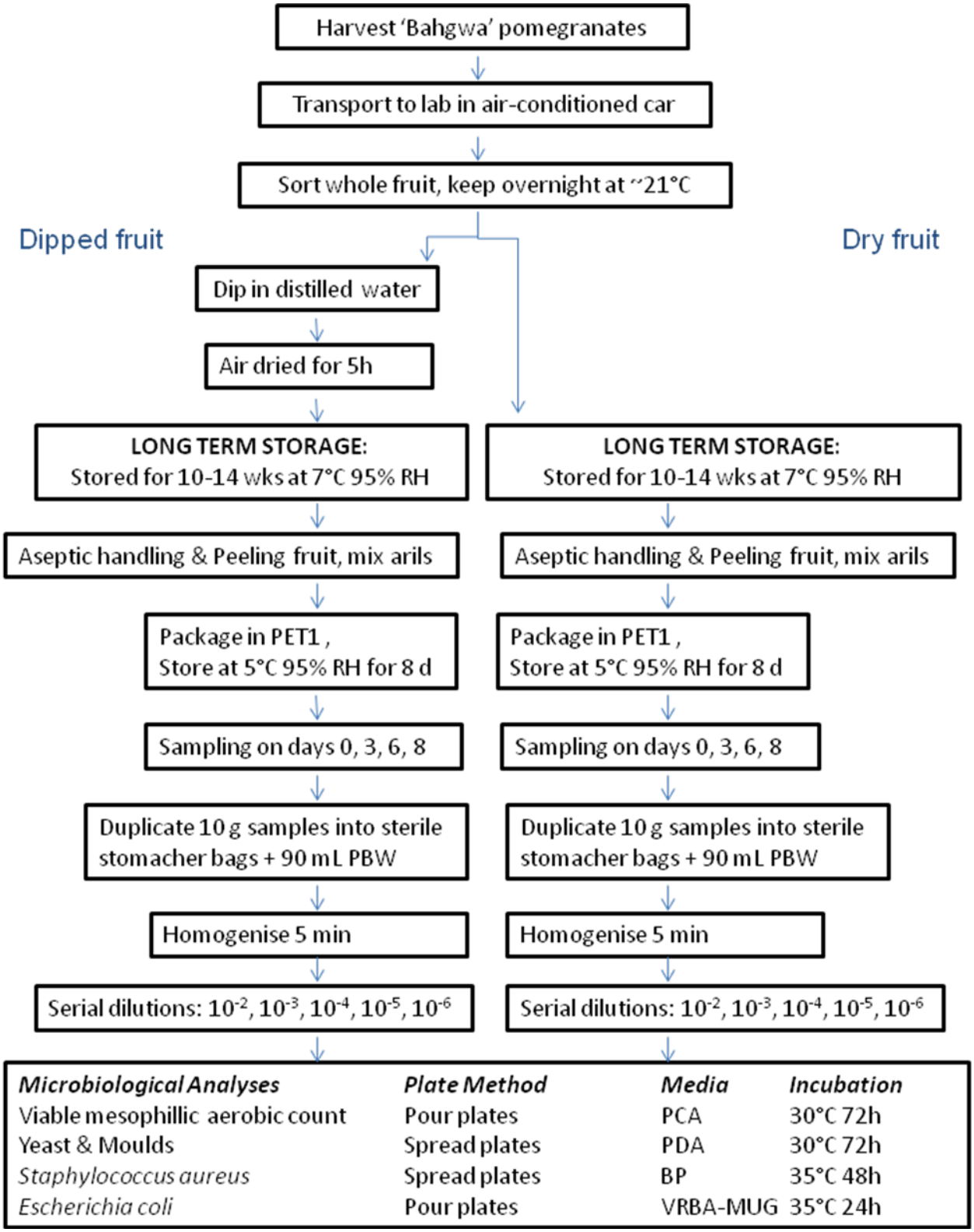

Figure 5.2 Experimental Design

Results and Discussion

No visual mould growth was observed on any of the processed pomegranate arils during cold storage at 5°C 95% RH. Total viable aerobic mesophillic bacteria as well as yeasts and moulds were enumerated to quantify spoilage microorganisms, while *Escherichia coli* and *Staphylococcus aureus* were enumerated for general hygiene purposes due to human handling during peeling (Madigan & Martinko, 2006). No *Staphylococcus aureus* or *Escherichia coli* colonies were detected on any of the arils throughout this study (Table 5).

Arils from 'dry fruit' were clear of any microbial growth on yeast and mould growth as well as total viable aerobic mesophillic bacteria for the whole duration of this study (Table 5). During the storage duration of 6 days at 5°C 95% RH no microbial growth was detected on arils from either 'dry' or 'dipped fruit' (Table 5), similar to findings of Ayhan & Eştürk (2009). Other studies showed higher initial microbial counts of yeast and moulds (2-5 log cfu.g^{-1}) as well as aerobic mesophillic bacteria (2-5 log cfu.g^{-1}) (Juven *et al.*, 1984; Sepúlveda *et al.*, 2000; López-Rubira *et al.*, 2005).

After 8 storage days at 5°C 95% RH the microbial counts increased to 4.74 log cfu.g^{-1} yeast and moulds and 3.73 log cfu.g^{-1} total viable aerobic mesophillic bacteria count (Table 5). Moulds and especially yeast are known to grow quicker than bacteria in processed fruit due to the availability of sugar and water (Montville & Matthews, 2008). Ayhan & Eştürk (2009) reported no development of yeast and mould growth on pomegranate arils for the entire duration of 18 days and no viable aerobic mesophillic bacteria for the first 9 days at modified atmospheric conditions. Viable mesophillic aerobic counts detected after 8 days (Table 5) was similar to those reported by Ayhan and Eştürk (2009) after 18 days, even though the marketable shelf life for those arils were set at 15 days due to the low oxygen atmosphere. During the previously mentioned study many precautions were used to prevent microbial growth like brushing the whole fruit with a chlorine solution, dipping the arils in combined chlorinated and citric acid solution and sanitizing the packaging material with hydrogen peroxide (Ayhan & Eştürk, 2009). None of these practices were used during this thesis to study pomegranate arils in its most natural minimally processed condition.

Even though air-drying at room temperature was used in previous studies the drying duration was not mentioned (Palou *et al.*, 2007). Incomplete drying of the whole pomegranate fruit could also lead to favourable moist conditions for mould growth during storage period, especially when the dipping solution do not exert any fungicidal properties. Postharvest decay was controlled most effectively when the whole pomegranate fruit was dipped in synthetic fungicide solution, but potassium sorbate dipping solution used in combination with

controlled atmosphere conditions was recommended as organic alternatives (Palou *et al.*, 2007).

Table 5 Microbial quality of pomegranate arils of dipped and dry whole fruit, results expressed as log colony-forming units.g^{-1}

Application	Storage Days	Yeasts & Moulds	Total viable aerobic mesophillic count	*Escherichia coli*	*Staphylococcus aureus*
Dipped fruit	0	N.D.	N.D.	N.D.	N.D.
	3	N.D.	N.D.	N.D.	N.D.
	6	N.D.	N.D.	N.D.	N.D.
	8	4.74	3.73	N.D.	N.D.
Dry fruit	0	N.D.	N.D.	N.D.	N.D.
	3	N.D.	N.D.	N.D.	N.D.
	6	N.D.	N.D.	N.D.	N.D.
	8	N.D.	N.D.	N.D.	N.D.

According to Spanish Legislation (BOE, 2001) and Debevere (1996) the maximum microbiological limits are 7 log cfu.g^{-1} for aerobic bacteria and 5 log cfu.g^{-1} for yeast and mould counts; these criteria for acceptable microbial quality were used by Lopéz-Rubira *et al.* (2005). All the samples analysed in this study (chapter 5) were still of acceptable quality according to Spanish guidelines of microbiological limits of acceptability. South African legislation governs the microbial standards for fruit juice but have yet to establish microbiological standards for fresh produce (Anonymous, 2005). According to South African legislation fruit juice may not be sold if the microbial limit for yeast and moulds exceeds 3 log cfu.mL^{-1}, the total viable count exceeds 4 log cfu.mL^{-1} or any trace of *E.coli* or *Salmonella* is detected (Anonymous, 2005). The yeast and mould count after arils were stored for 8 days at 5°C 95% RH was above the aforesaid South African microbial limits pertaining to fruit juice (Table 5). Therefore it is advised to keep pomegranate fruit dry, since pre-storage water dipping reduced the shelf life of pomegranate arils to 6 days at 5°C 95% RH according to South African microbiological standards pertaining to fruit juice.

Conclusion

This baseline information advise pomegranate producers, industry and researchers against pre-storage water dipping of whole pomegranate fruit and encourage further research on other novel postharvest handling techniques to extend the shelf life of whole fruit and processed arils.

References

Al-Said, F.A., Opara, L.U. & Al-Yahyai, R.A. (2009). Physico-chemical and textural quality attributes of pomegranate cultivars (*Punica granatum* L.) grown in the Sultanate of Oman. *Journal of Food Engineering*, **90**, 129-134.

Anonymous (2005). Foodstuffs, Cosmetics and Disinfectant Act and Regulations. Act no.54 of 1972, G.N.R. 1111/2005.Pretoria, South Africa.

Artés, F. (1992). Factores de calidad y conservación frigorífica de la Granada. In: *II Jomadas Nacionales del Granado.* Universidad Politécnica de Valencia. (As cited by Artés & Tomás-Barberán, 2000)

Artés, F & Tomás-Barberán, F.A. (2000). Post-harvest technological treatments of pomegranate and preparation of derived products. In: *Symposium on production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology.* CIHEAM – IAMZ p. 199-204. Zaragoza, Spain.

Ayhan, Z & Eştürk (2009). Overall Quality and Shelf Life of Minimally Processed and Modified atmosphere Packaged “Ready-to-Eat” Pomegranate Arils. *Journal of Food Science,* **74,** C399-C405.

BOE. (2001). *Boletin Oficial del Estado*. Normas de higiene para la elaboración, distribcion y comercio de comidas preparadas. Pp. 1435-1441. Madrid, Spain: Real Decreto (As cited by López-Rubira, 2005).

Debevere, J. (1996). Criteria en praktiesche methoden voor de bepaling van de houbaarheidsdatum in de etikettering. In: *Etikettering houdbaarheid en bewaring (voedingsmiddelen en recht 2).* Pp. 37-64. Brugge, Belgium: Die Keure (As cited by López-Rubira, 2005).

Feng, P.C. & Hartman, P.A. (1982). Fluorogenic assays for immediate confirmation of *Escherichia coli*. *Applied Environmental Microbiology* **43**, 1320-1329.

Ghafir, S.A.M., Ibrahim, I.Z. & Zaied, S.A. (2010). Response of Local Variety ‘Shlefy’ Pomegranate Fruits to Packaging and Cold Storage. *Acta Horticulturae*, **877** 427-432.

Gil, M.I., Martínez, J. A. & Artés, F. (1996). Minimally Processed Pomegranate Seeds. *Lebensmittel-Wissenschaft und-Technologie*, **29**, 708-713.

Juven, B. J., Gagel, S., Saguy, I. & Weisslowicz, H. (1984). Microbiology of spoilage of a perishable pomegranate product. *International Journal of Food Microbiology* **1**, 135-139.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 74-185.

Madigan, M.T. & Martinko, J.M. (2006). *Brock biology of microorganisms.* 11th Edition. Pp. 353, 680, 865, 908. Upper Saddle Valley NJ: Pearson Prentice Hall.

Melgarejo, P., Salazar, D.M. & Artés, F. (2000). Organic acids and sugars composition of harvested pomegranate fruits. *European Food Research and Technology,* **211**, 185-190.

Montville, T.J. & Matthews, K.R. (2008). Chapter 20 Spoilage Organisms In: *Food Microbiology An Introduction.* 2nd Edition. Pp. 295-296 USA: ASM Press.

Nanda, S., Rao, D.V.S. & Krishnamurthy, S. (2001). Effects of shrink film wrapping and storage temperature on the shelf life and quality of pomegranate fruits cv. Ganesh. *Postharvest Biology and Technology*, **22**, 61-69.

Madigan, M.T. & Martinko, J.M. (2006). *Brock biology of microorganisms.* 11th Edition. Pp. 353, 680, 865, 908. Upper Saddle Valley NJ: Pearson Prentice Hall.

Montville, T.J. & Matthews, K.R. (2008). Chapter 20 Spoilage Organisms In: *Food Microbiology An Introduction.* 2nd Edition. Pp. 295-296 USA: ASM Press

Palou, L., Crisosto, C.H. & Garner, D. (2007). Combination of postharvest antifungal chemical treatments and controlled atmosphere storage to control gray mold and improve storability of 'Wonderful' pomegranates. *Postharvest Biology and Technology,* **43**, 133-142.

Ramulu, P. & Rao, P.U. (2003). Total, insoluble and soluble dietary fiber contents of Indian fruits. *Journal of Food Composition and Analysis*, **16**, 677-685.

SANS. (2007). *South African National Standard.* Horizontal method for enumeration of microorganisms (method 4833). Pretoria: Standards South Africa.

SANS (1999). *South African National Standard*. Horizontal method for enumeration of coagulase positive staphylococci (method 6888-1). Pretoria: Standards South Africa.

SANS (2009). *South African National Standard*. Horizontal method for enumeration of yeasts and moulds. (method 21527-1). Pretoria: Standards South Africa.

Schlimme, D. (1995). Marketing Lightly Processed Fruits and Vegetables. *HortScience*, **30**, 15-17.

Sepúlveda, E., Sáenz, C. Berger, H., Galletti, I., Valladares, C. & Botti, C. (2001). Minimal Processing of pomegranate cv. Española: Effect of three package materials. *Acta Horticulturae*, **553**, 711-712.

Tantipaibulvut, S., Nuamsetti, T. & Dechayuenyong, P. (2011). Antibacterial activity of pomegranate fruit peels and arils. In: *37th Congress on Science and Technology of Thailand.* Pp. 1-5. October 2011. Bangkok, Thailand.

Tedford, E.C., Adaskaveg, J.E. & Ott, A.J. (2005). Impact of Scholar (A New Post-harvest Fungicide on the California Pomegranate Industry. *Plant Health Progress*. doi:10.1094/PHP-2005-0216-01-PS.

Zarei, M., Azizi, M. & Bashiri-Sadr, Z. (2010). Studies on Physico-Chemical properties and bioactive compounds of Six Pomegranate Cultivars Grown in Iran. *Journal of Food Technology,* **8**, 112-117.

CHAPTER 6

GENERAL DISCUSSION & CONCLUSIONS

Background

The ancient pomegranate (*Punica granatum* L.) fruit has recaptured consumer interest worldwide due to its health promoting benefits (Heber & Bowerman, 2009). Studies have shown the chemical attributes of pomegranates to be very useful to prevent cancer, combat inflammation and help control the HIV pandemic (Kim *et al.*, 2002; Neurath *et al.*, 2004; Lansky *et al*., 2007; Syed *et al*., 2007). The physico-chemical, physiological and phytochemical properties of pomegranates vary worldwide due to seasonal, agro-climatic and cultivar differences (Kays, 1999; Borochov-Neori *et al*., 2009; Opara *et al.*, 2009; Schwartz *et al*., 2009; Fawole *et al.*, 2011). Literature showed physiological, physico-chemical, phytochemical and microbial quality of pomegranate fruit are influenced by storage temperature, atmosphere conditions and packaging (Elyatem & Kader, 1984; Gil *et al*. 1996; Artés *et al.*, 2000; Nanda *et al.*, 2001; Lopez-Rubira *et al*., 2005; Ayhan & Eştürk, 2009; Ergun & Ergun, 2009; Ghafir *et al*., 2010). Therefore, correct management of postharvest handling practices like storage temperature and packaging material would lead to optimal preservation of these qualities that render the fruit so desirable. Pomegranate arils in South Africa are currently packaged using a wide range of polyethylene terephthalate punnets under normal air atmospheric conditions without using any sealable films (Olivier, F. 2009, Chief Executive Officer, Pomegranate Fruit SA, Porterville, South Africa, personal communication, 14 April). There is a dearth of information on the effects of postharvest handling practices on the nutritional quality attributes of pomegranate fruit grown in South Africa.

Nutritional quality of pomegranate arils

The nutritional composition of pomegranate arils has been studied globally and differs slightly amongst cultivars and countries (Al-Maiman & Ahmad, 2002; Paul & Shaba, 2004; Al Said *et al.*, 2009). The medical research council of South Africa (MRC) has published the nutritional composition of pomegranate fruit however these results do not differentiate between cultivars (MRC, 2010). The proximate composition of ‘Arakta’, ‘Bahgwa’ and ‘Ruby’ pomegranate arils was very similar to each other and data from the MRC food compositional table and amounted to 80% moisture, 0.52% Ash, 1.3-1.5% fat, 2.7-2.9% dietary fibre (using the AOAC 993.21 method), 1.1-1.2% protein, 14-15% carbohydrate (calculated by difference) and 310-320 kJ.100g^{-1} energy. Pomegranates analysed during this study showed 3 and 10 times higher dietary fibre and fat content than the MRC’s food composition tables, respectively.

Low β-carotene levels (1.71-3.54mg.L^{-1}) were detected in all pomegranate arils. Higher levels of Titratable acidity (TA) (0.33±0.01 g.100 mL^{-1}) and ascorbic acid (25.6±1.25 mg.L^{-1}) was detected in 'Arakta' arils, higher level of anthocyanins (112.5±2.09 mg.L^{-1}) in 'Bahgwa' and 'Ruby' arils were lighter in colour with a lower hue angle (*L** 35.5±0.33; *H*° 17.3±0.42). This information contribute to the nutritional knowledge of 'Arakta', 'Bahgwa' and 'Ruby' pomegranate fruit of South Africa and could be used for food compositional, labelling, cultivar selection, marketing and research purposes.

Storage Temperature of Pomegranate Arils

The importance of storage temperature during storage of pomegranate arils have been emphasised by many researchers who reported low temperatures (0-5°C) at modified atmosphere conditions and 95% relative humidity was effective to reduce respiration rate, enzymatic processes and microbial activity (Gil *et al.*, 1996b; Kader, 2002; Nicola *et al*., 2009). There is a dearth of information regarding the effect that storage temperatures have on the nutritional properties of South African grown pomegranate arils. Physico-chemical attributes, anthocyanins, ascorbic acid and proximate composition was measured on day 0, 7, 14 and 15. O_2 consumption and CO_2 production increased at elevated temperatures and throughout storage duration. Nutritional composition of pomegranate arils was mostly unaffected when stored at 1° and 4°C for 14 days, however higher temperatures increased the TA and reduced the Total Soluble Solids/Titratable acidity (TSS/TA) in all cultivars. Storage duration caused an increase in TA and a decrease in TSS/TA in 'Ruby' arils after 14 days. No visual detection of mould growth was seen in 'Arakta' and 'Bahgwa' arils stored at 1°C and 95% relative humidity (RH) after 14 days. During this study higher storage temperature affected the proximate composition, physico-chemical attributes and bioactive components negatively. This study agrees that with other researchers and advises pomegranate producers and retailers that the cold chain should be maintained at low (0-5°C) storage temperature and 95% RH for optimal quality of minimally processed pomegranate arils (Gil *et al.*, 1996; Kader, 2002; Nicola *et al*., 2009).

Packaging of Pomegranate Arils

Packaging is especially important in pomegranate arils to preserve the quality of the fruit by reducing shrivelling, dehydration and weight loss (Gil *et al*., 1996; Nicola *et al*., 2009). Most recent packaging studied for pomegranate arils include heat sealed pouches of oriented polypropylene film, rigid polystyrene vessels, perforated polyethelene bags, ethyl vinyl acetate films, polypropylene trays and bi-axially oriented polypropylene films (Gil *et al.*, 1996; Sepúlveda *et al.*, 2000; Sepúlveda *et al.*, 2001; Ayhan & Eştürk, 2009). Polyethylene terephthalate material is mostly used for pomegranate aril packaging in South Africa and is

available in various shapes and sizes. Modified atmosphere packaging using films has not been used in the South African pomegranate industry thus far, but the industry is moving toward that direction. The aim of this study was to investigate the effects of South African commercial packaging material on physico-chemical properties and selected bioactive components (anthocyanins, ascorbic acid, β-carotene) of pomegranate arils ('Arakta', 'Bahgwa' and 'Ruby') during short term storage duration. The results showed that current commercial packaging used for pomegranate arils in South Africa had minimal effects on the physico-chemical and bioactive compounds of pomegranate arils. Of all the punnet varieties, PET2 showed the most stable headspace gas composition during the short term storage period. Long and short term storage duration did however influence the overall quality of pomegranate arils. 'Arakta' showed higher TA than the other cultivars and did not show any mould growth after 7 days irrespective of punnet variety. Visual mould growth was detected in 'Ruby' arils after 7 days irrespective of punnet variety at 5°C 95% RH after long term storage duration of 10-14 weeks at 7°C 95% RH. Pomegranate breeders, farmers and retailers will find this information very useful to aid in cultivar selection as well as improved postharvest handling practices.

Pre-storage Water Dipping of Whole Pomegranate Fruit

The early onset of microbial growth during chapter 4 was suspected to be caused by the prolonged storage period (7°C 95% RH) of the whole fruit as well as the water dipping process used directly after harvesting the fruit. The initial intention of the water dipping process was to reduce the microbial load on fruit (Montville & Matthews, 2008). According to literature pomegranate fruit need only be brushed lightly without any further postharvest treatments such as washing, waxing or fungicides (Artés *et al*., 1992). Long term cold storage conditions of 7°C 95% RH has also shown to promote the growth of gray mould spoilage if no fungicide is used (Tedford *et al.*, 2005). The aim of this study was to investigate the effect of pre-storage whole fruit dipping in distilled water on the microbial quality of extracted pomegranate arils stored for 8 days at 5°C 95% RH. Pomegranate fruit that were not dipped in water before long term storage did not show any microbial growth the minimally processed arils after 8 days. While pre-storage water dipping of whole pomegranates increased the microbial load to 4.74 log cfu.g^{-1} yeast and moulds and 3.73 log cfu.g^{-1} total viable aerobic mesophillic bacteria count in pomegranate arils after 8 days. The increased yeast and mould counts were beyond the South African microbiological standards for fruit juice. And therefore this baseline information advise pomegranate producers, industry and researchers against pre-storage water dipping of whole pomegranate fruit and

encourage further research on other novel postharvest handling techniques to extend the shelf life of whole fruit and processed arils.

Recommendations and Future Research

While this study did not use sensory evaluation to determine the acceptability of pomegranate arils during storage, a shelf life study of minimally processed pomegranate arils using both a descriptive sensory panel and microbiological assays will be invaluable to the pomegranate industry as well as regulatory authorities in South Africa to establish quality criteria and safety limits for minimally processed pomegranate arils. The effect of fruit juice processing on the microbiological quality of fresh fruit could also be useful information in establishing South African microbiological standards. The effect of different pre-storage antifungal treatments on the shelf life of South African grown pomegranate fruit would benefit the global scientific community as well as local producers in the process of establishing a universal quality management programme. Another area of research could focus on the effect of pre-packaging washing treatments on both arils and packaging materials, such as chlorinated water or antioxidant solutions, to reduce mould and yeast development in arils of South African grown pomegranates. Due to the fast changing pace of packaging, an important area of research are the effects of modified atmosphere packaging on the shelf life of South African grown pomegranate arils.

References

Al-Maiman S.A. & Ahmad, D. (2002). Changes in physical and chemical properties during pomegranate (*Punica granatum)* fruit maturation. *Food Chemistry*, **76**, 437-441.

Al-Said, F.A., Opara, L.U. & Al-Yahyai, R.A. (2009). Physico-chemical and textural quality attributes of pomegranate cultivars (*Punica granatum* L.) grown in the Sultanate of Oman. *Journal of Food Engineering*, **90**, 129-134.

Artés, F. (1992). Factores de calidad y conservación frigorífica de la Granada. In: *II Jomadas Nacionales del Granado.* Universidad Politécnica de Valencia. (As cited by Artés & Tomás-Barberán, 2000)

Artés, F & Tomás-Barberán, F.A. (2000). Post-harvest technological treatments of pomegranate and preparation of derived products. In: *Symposium on production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology.* CIHEAM – IAMZ p. 199-204. Zaragoza, Spain.

Ayhan, Z & Eştürk, O. (2009). Overall Quality and Shelf Life of Minimally Processed and Modified atmosphere Packaged "Ready-to-Eat" Pomegranate Arils. *Journal of Food Science,* **74,** C399-C405.

Borochov-Neori, H., Judeinstein, S., Tripler, E., Harari, M., Greenberg, A., Shomer, I & Holland, D. (2009). Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum L.*) fruit. *Journal of Food Composition and Analysis*, **22**, 189–195.

Elyatem, S. M. & Kader, A. A. (1984). Post-harvest physiology and storage behaviour of pomegranate fruits. *Scientia Horticulturae,* **24**, 287-298.

Ergun, M. & Ergun, N. (2009). Maintaining quality of minimally processed pomegranate arils by honey treatments. *British Food Journal*, **111**, 396-406.

Fawole, O.A., Opara, U.L. & Theron, K.I. (2011). Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa. *Food Bioprocess Technology*, DOI: 10.1007/s11947-011-0533-7.

Ghafir, S.A.M., Ibrahim, I.Z. & Zaied, S.A. (2010). Response of Local Variety 'Shlefy' Pomegranate Fruits to Packaging and Cold Storage. *Acta Horticulturae*, **877** 427-432.

Gil, M.I., Martínez, J. A. & Artés, F. (1996). Minimally Processed Pomegranate Seeds. *Lebensmittel-Wissenschaft und-Technologie*, **29**, 708-713.

Heber, D. & Bowerman, S. (2009). Californian Pomegranates: The ancient fruit is new again. *Nutrition Today,* **44**, 180-184.

Kader, A.A. (2002). Postharvest Biology and Technology: An Overview. In: *Postharvest Technology of Horticultural Crops.* (edited by A. A. Kader). 3rd ed. Pp 39-48. California: University of California.

Kays, S. J. (1999). Preharvest factors affecting appearance. *Postharvest Biology and Technology*, **15**, 233-247.

Kim, N.D., Metha, R., Yu, W., Neeman, I., Livney, T., Amichay, A., Poirier, D., Nicholls, P., Kirby, A., Jiang, W., Mansel, R., Ramachandran, C., Rabi, T., Kaplan, B. & Lansky, E. (2002). Chemopreventive and adjuvant therapeutic potential of pomegranate (*Punica granatum*) for human breast cancer. *Breast Cancer Research and Treatment*, **71**, 203–217.

Lansky, E. P. & Newman, R. A. (2007). *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology,* **109**, 177-206.

López-Rubira, V., Conesa, A., Allende, A. & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, **37**, 74-185.

Medical Research Council (MRC) (2010). Fruit. In: *Condensed Food Composition Tables for South Africa* (edited by Wolmarans, P., Danster, N., Dalton, A., Rossouw, K. & Schönfeldt, H.) 1st ed. p. 3-15. Cape Town: Medical Research Council.

Montville, T.J. & Matthews, K.R. (2008). Chapter 20 Spoilage Organisms In: *Food Microbiology An Introduction.* 2nd Edition. Pp. 295-296 USA: ASM Press

Nanda, S., Rao, D.V.S. & Krishnamurthy, S. (2001). Effects of shrink film wrapping and storage temperature on the shelf life and quality of pomegranate fruits cv. Ganesh. *Postharvest Biology and Technology*, **22**, 61-69.

Neurath, A. R., Strick, N.,Li, Y. & Debnath, A.K. (2004). Punica granatum (Pomegranate) juice provides an HIV-I entry inhibitor and candidate topical microbicide. *BMC Infectious Diseases,* **4**,

Nicola, S. Tibaldi, G. & Fontana, E. (2009). Fresh-cut Produce Quality: Implications for a Systems Approach. In: *Postharvest Handling: A Systems Approach.* (edited by W.J.

Florkowski, R.L. Shewfelt, B. Brueckner, & S.E. Prussia). Pp. 270-273. Oxford: Academic Press.

Opara, L.U., Al-Ani, M.R. & Al-Shuaibi, Y.S. (2009). Physico-chemical Properties, Vitamin C Content and Antimicrobial Properties of Pomegranate Fruit (*Punica granatum* L.). *Food and Bioprocess Technology*, **2,** 315-321.

Paul, D.K. & Shaba, R.K. (2004). Nutrients, Vitamins and Nutrient Content in Common Citrus Fruits in the Northern Region of Bangladesh. *Pakistan Journal of Biological Sciences*, **7**, 238-242.

Schwartz, E., Tzulker, R., Glazer, I., Bar-Ya'akov, I., Wiesman, Z., Tripler, E., Bar-Ilan, I., Fromm, H., Borochov-Neori, H., Holland, D. & Amir, R. (2009). Environmental Conditions Affect the Color, Taste, and Antioxidant Capacity of 11 Pomegranate Accessions' Fruits. *Journal of Agricultural and Food Chemistry*, **57**, 9197-9209.

Sepúlveda, E., Galletti, l., Sáenz, C. & Tapia, M. (2000). Minimal Processing of pomegranate var. Wonderful. In: *Symposium on production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology*. CIHEAM – IAMZ p 237-242. Zaragoza, Spain.

Sepúlveda, E., Sáenz, C. Berger, H., Galletti, l., Valladares, C. & Botti, C. (2001). Minimal Processing of pomegranate cv. Española: Effect of three package materials. *Acta Horticulturae*, **553**, 711-712.

Syed, D.N., Afaq, F. & Mukhtar, H. (2007). Invited article: Pomegranate derived products for cancer chemoprevention. *Seminars in Cancer Biology*, **17**, 377-385.

Tedford, E.C., Adaskaveg, J.E. & Ott, A.J. (2005). Impact of Scholar (A New Post-harvest Fungicide on the California Pomegranate Industry. *Plant Health Progress*. doi:10.1094/PHP-2005-0216-01-PS.