

**Yield and Quality of Pomegranate on Selected Geographical Areas in
Western Cape Province, South Africa**

BY

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DECLARATION

I, declare that the work done in this thesis is my own original work and that I have not previously in its entirety or in part submitted at any university for a degree. Title: Yield and Quality of Pomegranate on Selected Geographical Areas in Western Cape Province, South Africa

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Signature

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Date

Dedicated to my friend, colleague and late project initiator, Dr Claude Ron
Bekaardt (05 July 1970 – 21 June 2011) for believing in me.

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Abstract

The pomegranate fruit is one of the high valued crops, but there is insufficient information regarding the fruit properties in South Africa. The aim of the study was to evaluate the physico-chemical properties as well as total phenols, anthocyanin, antioxidant, organic sugars and acids of cultivar Wonderful on three locations of the Western Cape. This study was conducted on mature pomegranate fruits harvested in the 2012 and 2013 seasons. Fruit weight (g), length (mm), and width (mm), peel/aril colour and total arils weights (g) were measured. Fruits were also analysed for total soluble solids (TSS) or °Brix, titratable acidity (TA) and juice pH. Results of the study showed that there were significant differences in all measured factors with the exception of % aril yield between the three locations. Though varied per season, fruits produced at Bonnievalle had better physical and chemical properties than at the other localities. With the exception of Aril hue angle, all measured parameters had significant interaction effect regardless of locality ($P < 0.05$). Total soluble solids content varied from 16.0–17.3 (°Brix), pH values from 2.7–3.0, titratable acid content varied from 1.3–1.7 and maturity index from 9.7–13.4. The anthocyanin, total phenols and antioxidant were in order of 772–1134; 1611–1834 and 12.57–14.84. Organic acids (Citric and Malic) showed differences while Acetic acid was not significant in all areas and organic sugar (fructose, Glucose and Sucrose) all had significant differences. It can also be concluded that changes in colour of peel and arils of pomegranate (cv. Wonderful) was mostly as a result of seasonal variation as well as growing area as evident by the interaction between both main factors.

Keywords: Anthocyanin, Antioxidant, Aril colour, Chemical properties, Cultivar, Organic acids, Organic sugar, Peel colour, Physical properties, Pomegranate

CHAPTER 1

1. GENERAL INTRODUCTION

Growing of pomegranates (*Punica granatum L*) started in ancient period. It is estimated that pomegranate cultivation may have started somewhere during Neolithic age (Holland and Bar-Ya'akov, 2008). Cultivars of pomegranate which can be in excess of 500 have been named (IPGRI, 2001). Although pomegranate is an old fruit tree and spread over the world it has more synonyms and the same genotype can still be called with different names in different areas. Aril and husk colour can differ greatly when grown in various areas which result in more synonyms. The characteristic phenotypes used in identifying consumer preferences and the niche market are determined by husk colour (ranging from yellow to purple, with pink and red most common), aril colour (ranging from white to red), hardness of the seed, maturity, juice content, juice taste (ranging from sweet to astringent), acidity, as well as fruit size (IPGRI, 2001).

Healthy lifestyle may be the contributing factor to the increased demand of pomegranates all over the world with its health promoting effects. Modern scientific laboratory work strengthens the image of pomegranate fruit as an important medicinal fruit that contains valuable medically active compounds (Holland and Bar-Ya'akov, 2008). Analysis of bioactive compounds and phytochemicals produced by the pomegranate tree is just in an infant stage. Sterols and terpenoids in bark, leaves and seeds are some of the active phytochemical discovered in pomegranates, while alkaloids are also found in bark and leaves. Analysis of leaves, rind, fruit, bark and juice found some organic acids, flavonols, anthocyanin and anthocyanidins (Holland and Bar-Ya'akov, 2008). During the development of the tree and fruit maturation the amount of compounds in pomegranate tree or fruit differs due to environmental and cultivation practices as well as between cultivars (Holland and Bar-Ya'akov, 2008).

Mature pomegranate fruits are used in table arrangement because of their long life as well as their good appearance, this widespread usage is common in United States that they are bought for that sole purpose not for consumption (California Rare Fruit Growers, 1997). Selections of pomegranates were done mainly for external appearance, because

of its decorative value and not so much for its eating quality. Good colour and crown are very important characteristics of the fruit. Consumers in Israel do not distinguish among pomegranates according to names. Merchants know two groups: sweet and sour cultivars but the price is decided mainly by appearance (Blumenfeld *et al* 2000).

Although cultivar Wonderful was discovered in Florida and brought to California in 1896 (California Rare Fruit Growers, 1997), other countries such as Chile, Israel and Western Europe grow it (Sepulveda *et al* 2000). This is the primary cultivar of commerce in the United States. It is considered to have a good colour in both juice and husk, rich flavour, acidic and astringent to compare other fruits. It is ideal for juicing with high juice percentage, good taste and resistant to cracking (Karp, 2006).

In Southern Hemisphere South Africa is competing with countries such as Chile, Australia, Peru and Argentina, the growing export opportunity has encourage large scale of production allowing producer to fill the window period during spring and early summer months in Northern Hemisphere (Broadie, 2009). South African pomegranate production stands at 1,000 ha, and fruit export has increased from 70, 000 cartons (315 tonnes) in 2009/2010 to over 440, 000 cartons (198,000 tonnes) in 2011/2012 season (Citrogold, 2012).

Despite the fact that the industry is small or not fully established, farmers in the Western Cape have shown interest on the establishment of the crop in small scale. However, data on which pomegranate cultivar is adaptable to Western Cape condition is lacking, therefore there is a need to study specific cultivar Wonderful grown in particular regions in order to determine its adaptability to agro- climatic conditions of Western Cape. The aim of this research was to investigate the performance of cultivar Wonderful grown in three regions of the Western Cape Province, South Africa as an alternative agricultural commodity.

1.2 Problem statement

In South Africa, the pomegranate industry is a fast growing young industry with a lot of potential to become a competitive exporter in Southern Hemisphere. However, growth and performance of pomegranate has not been the subject of scientific investigation under South Africa conditions. In order to drive this profitable and medicinal crop to commercialization, scientific knowledge needs to be developed on production in South Africa. Successful cultivation of any crop requires the selection of suitable cultivars for a specific climatic condition. Cultivar performance can vary depending on the agro- climatic conditions or locations. Information on the varietal performance of pomegranate in South Africa is limited and no known systematic efforts have been made to assess the performance of available cultivars of pomegranate in the different provinces, with little available information on the physico-chemical properties of commercially grown cultivars. Some of the knowledge lacking in terms of pomegranates production in South Africa includes total varieties available in South Africa, production per each variety, chemical and antioxidants activity.

1.3 Motivation of the study

Currently there is growing interest in the pomegranate fruit because it is considered to be a functional product of great benefit in the human diet as it contains several groups of substances that are useful in disease risk reduction (Martinez *et al* 2006). Pomegranate is one of the few fruits that are still not evaluated even though it has a potential in the world market. However, data that describe the production, physico-chemical properties, antioxidant, acids and sugars in different cultivars is lacking in South Africa. Therefore, the current study will investigate the performance of cultivar Wonderful grown in Western Cape Province.

1.4 Hypothesis

H_0 = Pomegranate cultivar Wonderful fruits can produce quality fruits in terms of size, weight, colour, total antioxidants and total phenols.

H_a = Pomegranate cultivar Wonderful fruits cannot produce quality fruits in terms of size, weight, colour, total antioxidants and total phenols.

1.5 The specific objectives:

- To determine the effect of area on the physico-chemical properties of pomegranate cultivar Wonderful.
- To determine the influence of area on the colour, acids and sugars of pomegranate cultivar Wonderful.

CHAPTER 2

2. LITERATURE REVIEW

2.1 INTRODUCTION

Punica granatum is indigenous to the area of Iran up to the Himalayas in northern India (Meerts *et al* 2009) whilst Stover and Mercure (2007) state that it is a traditional fruit of the central Iranian plateau where it is thought to have originated. It is also one of the oldest fruit trees to be domesticated (Kumar, 1990). *Punica granatum* is known to have been grown in the Hanging Gardens of Babylon (Damania, 2005). A relatively less known species, *Punica protopunica* Balf related to *P. granatum*, originated in the Socotra Island of Yemen (Stover and Mercure, 2007; Holland and Bar-Ya'akov, 2008). The ability of *P. granatum* to adapt to wide range of temperature regimes has made it possible to grow in the Mediterranean regions of Asia, Africa, America and Europe (Kumar, 1990; Holland and Bar-Ya'akov, 2008).

Pomegranate fruit growth and maturity is characterized by different stages, each stage corresponds to an array of defined biochemical, physiological and physical as well as structural and textural attributes that result in changes in flavour, fruit respiration, size and colour which all make the fruit desirable for consumption (Ben-Arie *et al* 1984; Al-Maiman and Ahmad, 2002; Fawole and Opara, 2013a).

Pomegranate fruit quality can be assessed based on external properties such as shape, size and colour (Kader, 2006a; Holland *et al* 2009). However, fruit maturity for harvest cannot be assessed by skin colour only (Ben-Arie *et al* 1984), hence other factors are considered such as aril colour; total soluble solids and acidity to meet market standards (Ben-Arie *et al* 1984; Kader, 2006b; Holland *et al* 2009; Fawole and Opara, 2013a). Previous studies have reported the relationships among individual fruit physico-chemical attributes (at different fruit developmental stages) and their relevance in identifying fruit indices that could be used to predict fruit maturity (Ben-Arie *et al* 1984; Shwartz *et al* 2009; Al-Maiman and Ahmad, 2002; Fawole and Opara, 2013a). Fruit properties such as volume, weight and juice content are important in marketing because these parameters help in decision making for the consumer (Holland *et al* 2009).

2.2 POMEGRANATE MORPHOLOGY

Pomegranate tree height which usually reaches two to six meters is classified as a small deciduous tree (Morton, 1987). Although is classified as deciduous, other cultivars in certain areas retain their leaves throughout winter (Fig.1). The trunk is covered by a red-brown bark that later becomes grey. Branches are stiff, angular and often spiny (Stover and Mercure, 2007).



Fig 1. A Wonderful tree with matured fruits

In cooler area it is advisable to train the pomegranate tree to more than one trunk in order to reduce loss of tress (Stover and Mercure, 2007). Several suckers grow alongside the trunk and have to be removed frequently. The pomegranate bark has traditionally been used for the alkaloids it contains as well as in the trunk. The trunk of the pomegranate is round in shape and erect with alternate open branches, sometimes prickly at the apex. The tree itself varies in appearance from drooping to erect (Melgarejo, 2010). The trunk

bark usually contains 10 – 25% tannin and was used in the manufacturing production of leather in Morocco while the root bark have 28% tannin content and the leaves has 11% (Teixeira da Silva *et al* 2013).

The leaves in an early stage of growth or mixed clusters measure two and nine cm in length and one and three cm in width (Fig.1). The lateral buds are found on the axils of the leaves. The terminal bud occasionally becomes thorny and usually grows into a flower or clusters of flowers, or simply falls (Stover and Mercure, 2007). Since the plant does not possess real terminal buds, growth has to be from the lateral bud that is why pomegranate tree is for this reason included in the sympodial species (Melgarejo, 2010). The buds are smooth, opposed with no stipule although it becomes verticillate (forming a whorl), hairless, oblong, and deciduous and with short petioles. The reddish young leaves turn bright green when matured (Fig.1), the upper side becoming dark green while the reverse light green face, while the petiole maintains its reddish colour (Melgarejo, 2010).

The pomegranate flower colour ranges from red to red-orange and the shape is funnel with some ornamental collections having "double" and variegated flowers, this variety are not grown for fruit production. Pomegranate can be self-pollinated or cross-pollinated by insects (Morton, 1987). Flowers are mostly borne sub-terminally, mainly on short adjacent branches which are more than a year old (El-Kassas *et al* 1998), Flowers occur as single blossoms or in clusters of up to five. Pomegranate is considered as a monoecious species meaning that it has separate male and female flowers on the same plant and it also characterised by two types of flowers: hermaphroditic bisexual flowers and functionally male flower (Wetzstein *et al* 2011) which are generally, "bell-shaped"; and hermaphrodite flowers (fertile = perfect) with normal ovary developing to fruit, which are, in general, "vase-shaped" (Shulman *et al* 1984; Chaudhari and Desai, 1993). Male flowers, which their percentage is important in the fruit set, must be between 60 - 70% depending on cultivar and season. The male types drop and rarely set fruits leaving the hermaphrodite type to produce the majority of the crop.

Pomegranates are known to have flowers with styles of differing length and this is called heterostylous. Long-styled perfect flowers are larger with abundant ovaries and usually set more fruit than short style types. The percentage of these two flower types varies among cultivars and year to year (Martinez *et al* 2006). Cross-pollination increases the fruit set while wind pollination is reported to be insignificant (Chaudhari and Desai, 1993). Normal flowering of pomegranate varieties occurs between Oct-Dec. (South Africa, personal observation). It continues for up to 10 -12 weeks or more depending on variety and geographical situation. The period of full bloom lasts about one to two month, and it was observed that flowering and fruit set occurs in 3 or 4 distinct waves (Ben Arie *et al* 1984; El Sese, 1988; Hussein *et al* 1994). Bearing capacity and the percentage of perfect flowers was found to have positive correlation (El Sese, 1988; Chaudhari and Desai, 1993).

When marker gene were use on the pigmentation of bud flower and petiole base, the research confirmed that pomegranate cvs Ganesh and Kabul Yellow are self-pollinated and are very low in cross pollination which was found to be 13% (Jalikip and Sampath Kumar, 1990). The work reported by Karale *et al* (1993) on different cultivars showed that fruit set was 79% and 43.3%, respectively, intact open and self-pollinated flowers 26.4% and 66.2%. Pomegranate fruit is closely rounded and crowned at the base by the noticeable calyx and is essential character for any fruit crop. The tough leathery skin or rind is normally yellow covered with light or deep pink (Mir *et al* 2012). The inner is separated by membranous wall and white soft, bitter tissue into components packed with sacs filled with sweet acid, juicy, red, pink or whitish pulp or aril (Watson and Dallwitz, 1992, Mir *et al* 2012).The fruits are grown on short spurs arising from mature shoots (Stover and Mercure, 2007).

The aril juice sack is made up of many epidermal cells. According to cultivar, arils range from deep red to almost colourless, while the surrounded seed varies in content of sclerenchyma tissue, which affects seed softness. The number of locules and arils (and enclosed seeds) varies, but may be as high as 1300 per fruit (Levin, 2006; Stover and Mercure, 2007). The interior of the fruit is separated by membranous walls of white,

spongy, bitter tissue into sections packed with sacs filled with sweetly acid, juicy, red, pink or whitish pulp or aril (Fig.2) (Stover and Mercure, 2007).



Fig 2. Opened pomegranate fruit with red arils

The fruit has a noticeable calyx, which is maintained to maturity and is a common feature of the pomegranate fruit (Fig.3). The husk consist of two parts: the pericarp, which provides a cuticle layer and fibrous mat on the outside and the mesocarp (also known as the albedo), which is the spongy tissue and inner fruit wall where the arils attach (Morton, 1987; Teixeira da Silva *et al*/2013). There is an increasing interest in finding or developing cultivars that has more locules to fill the fruit interior with fewer sepal membranes which are easy to eat as well as thinner mesocarp (Stover and Mercure, 2007). Fruits usually

take 6 to 7 months to ripen after flowering (Morton, 1987) and are harvested when developed a distinctive colour and make a metallic sound when tapped (Teixeira da Silva *et al* 2013), and also deemed most suitable for expected market use (Morton, 1987). The fruits must be harvested just before over-matured, as they tend to crack open, especially when it rains severely. Unlike most horticultural fruits, inherent seed spreading is not realized through consumption of all or most of the fruit and seeds with associated spread. Rather, the pomegranate fruit structure has seemingly developed to ensure splitting of the leathery husk and exposure of the appealing arils and seeds to many happily willing birds and so serving as dispersal agents (Morton, 1987). Pomegranate fruit is regarded as non-climacteric (a stage in the ripening of some fruits such as apples when the rate of respiration increases) (Kader *et al* 1984). The fruits improve in storage, becoming juicier and more flavoursome (Morton, 1987). Harvest and storage factors affecting post-harvest quality of pomegranate have been recently reviewed and summarized by Kader, (2006).

2.3 CULTIVATION OF POMEGRANATES

Pomegranate seeds germinate easily without going through a rest period, but trees are not grown commercial from seed because seedlings do not come true to type. Such seedlings produce fruit of widely varying characteristics, large to small, juicy to woody, dark-red or purple to almost white and from sweet to sour (LaRue, 1980). Pomegranate cuttings root so easily that cuttings are sometimes placed directly into the orchard (Blumenfeld *et al* 2000) and this allows the retention of desirable characteristics (Morton, 1987).

Cuttings should be taken in winter from mature, one-year old wood. The leaves should be removed and the cuttings treated with rooting hormone and inserted about two-thirds their length into the soil or into some other warm rooting medium. Plants can also be air-layered but grafting is seldom successful. Efforts to graft pomegranate are reported not to be successful, but air-layering and root-sucker transplantation can be used for vegetative propagation (Morton, 1987). The best planting time is spring, specifically between February and March (northern hemisphere), with seedling of two years.

Traditionally 6x4 is the framework but new plantations tend to frame 5x3 meters. Once the field has been marked, the hole is dug to an approximate depth of 40cm, and the plant with bare root is placed in each hole.

For higher fruit yield, regular irrigation is required. Even though it hardly tolerates excessive water (Badizadegan, 1975; Kulenkamp et al 1985; Levin, 2006) and high soil moisture may lead to wilt disease in India (Sharma et al, 2006). Pomegranate water requirements are high: for example in Israel. Water application for pomegranate tree culture is around 5000 – 6000 m³/ha (Holland et al, 2009). More specifically, water use of control trees under no soil water limitations changed during the growing season from 0.23 to 5 mm/day under class 'A' pan evaporation values of 3.06 – 9.19 mm/day (Bhantana and Lazarovitch, 2010), although Sulochanamma et al. (2005) did not find any significant increase in fruit yield when water was applied at 0.6, 0.8 or 1.0 of pan evaporation

The fruit are widely consumed fresh or processed into juice, jams, syrup and sauce. The edible portion (aril) of the fruit is about 55 – 60% of the total fruit weight and consists of about 75 – 85% juice and 15 – 25% seeds (Al-Maiman and Ahmad, 2002). The fruits are ripe when they have developed a distinctive colour and make a metallic sound when tapped. The fruits must be picked before over-maturing, as they tend to crack open, particularly when rained on. The pomegranate is equal to the apple in having a long storage life. It is best maintained at a temperature of 32 to 41 °F (0 to 5 °C) and can be kept for a period of seven months within this temperature range and at 80 to 85% relative humidity without shrinking or spoiling. The fruits improve in storage, becoming juicier and more flavourful (Morton, 1987).

The fruit can be eaten out of hand by deeply scoring several times vertically and then breaking it apart (Fig.2). The clusters of juice sacs are then lifted out and eaten. The sacs also make an attractive garnish when sprinkled on various dishes. Pomegranate fruits are most often consumed as juice and juice can be made in several ways. The sacs can be removed and put through a basket press or the juice can be extracted by reaming the

halved fruits on an ordinary orange juice squeezer (Morton, 1987). The pomegranate is consumed mainly fresh, but the difficulty presented for peeling the fruit has limited its consumption. The commercialisation of fresh arils minimally processed and "ready-to-eat", could be a good alternative for the national market (Sepulveda *et al* 2000).

2.4 CONSTITUENTS OF FRUIT QUALITY

The most important quality traits on pomegranate are fruit size, husk colour (ranging from yellow to purple, with pink and red most common), aril colour (ranging from white to red), hardness of the seed, maturity, juice content, acidity, sweetness, and astringency (Stover and Mercure, 2007). Fruit quality is based on several dimensions, many of which may not be readily evaluated by the consumer prior to purchase. From a consumer perspective, major quality attributes of fruit include appearance, colour, texture, taste, flavour and nutritive value and safety (Reid, 2002). Growers scores high on characteristics such as yield, disease resistance, easy of harvest and transportation quality. Distributors and retailers, appearance together with firmness are important quality attributes and are concerned about the time-temperature profile during storage in order to keep the level of those attributes high (Aked, 2002). A number of pre-harvest cultural practices can influence postharvest quality and performance. There are also many handling practices during harvest, packing and distribution that influence quality (Crisosto *et al* 1995).

2.5 EXTERNAL FACTORS AFFECTING FRUIT QUALITY OF POMEGRANATE

2.5.1 Climatic condition

Pomegranate is more tolerate to light but reacts negatively to excessive shading (Chadha, 2005), although direct sun-light and considerable heating results in sun burns in fruit (Sharma *et al* 2006). Sunburn is physiological disorder resulting from high temperature, light and radiation and leads to losses in yield and quality (Schrader *et al* 2002). Fruits directly subjected to high sun light burns fruit surface and changes colour (Finkel and Holbrook, 2000). Thus, results in big economic losses. Schrader *et al.* (2002) observed that factors such as clouds, wind and precipitation caused rapid fluctuations of fruit surface temperature (FST). For example, appearance of a few clouds markedly

decreased solar radiation, and quickly decreased FST below the threshold temperature required to induce sunburn browning. However, best quality fruit are produced in arid regions having a long, hot and dry summer. It can easily withstand temperatures up to 45 – 48°C in combination with dry hot winds. It is well known that pomegranate is not frost resistant and cannot tolerate temperatures lower than –18°C. A humid climate adversely affects the formation of fruit (Levin, 1995). Pomegranates are cultivated today throughout the world in subtropical and tropical areas in many different microclimatic zones (Holland *et al* 2009). The ideal climatic growth conditions for pomegranate occur in Mediterranean like climates (Shwartz *et al* 2009). These include high exposure to sunlight; mild winters with minimal temperatures not lower than -12°C and dry hot summers without rain during the last stages of the fruit development (Levin, 1995; Shwartz *et al* 2009). Under this condition the fruit will develop to its best size and optimal colour and sugar accumulation without the danger of splitting.

2.5.2 Pruning

Light play an important role in pomegranate fruit bearing as well as fruiting quality. Summer pruning is required to remove suckers and new branches that appear constantly on the exposed trunks. The more light is intercepted by fruit and leaves, result in better quality such as SSC, colour, size and flavour. Fruit in the top of the tree; for example, always have better quality than fruit in the lower, shaded part of the canopy (Day *et al* 1992). Although fruit at a lower level can remain in the tree for a much more duration to reach maturity the differences is still significant.

2.5.3 Size

The fruit size is a very important character for any fruit crop. The shape of pomegranate fruit is nearly round and is crowned at the base by the prominent calyx. The strong leathery skin or rind is usually yellow overlaid with light or deep pink or rich red (Kays, 1999). Size of individual components of a product can considerably affect consumer appeal, handling practices, storage potential, market selection and end use (Kays, 1999). Physical properties of fruit such as weight, volume, and juice content are important from a marketing perspective because these qualities influence consumer liking (Holland *et al*

2009). Fruit size or weight is regarded as a varietal characteristic that may change depending on climatic and agricultural condition. Zaouay *et al* (2012) differentiated fruits into small (101), medium-sized (200 – 400) and big size fruits (>400g). Weerakkody *et al* (2010) reported that at fruit maturity the average yield of fruit juice was 37% of the total fruit weight on cultivar Wonderful grown in Australia while Shulman *et al* (1984) found that in immature fruit periods, fruit juice was 25% less but increased at harvest to between 35 – 40% on cultivar Mules Head and 40 – 45% on Wonderful cultivar.



Fig 3. Pomegranate fruit on the tree

2.5.4 Thinning

Fruit thinning is the most important practice in pomegranate growing for improving fruit quality. Since thinning can be done mechanically or chemically. Strength of thinning may

depend not only on the process used but also on the physiological condition of the trees and cultural practices employed (Link, 2000).

Fruit thinning usually result in increased fruit size while reducing total yield, a balance between yield and fruit size must be achieved (Day *et al* 1992). Generally, maximum profit does not occur at maximum marketable yield since larger fruit bring a higher market price (Crisosto *et al* 1995). Too many fruit on a tree not only reduces fruit size but also decreases their soluble solids content. Thus fruit quality can be sacrificed in several ways by incorrect thinning. Experience of the farmer is the best determinant of the optimum thinning level for each orchard and cultivar (Crisosto *et al* 1995).

2.5.5 Colour

While colour is used as a primary criterion to assess the general quality of many products, quality and colour do not necessarily correlate closely with each other. In pomegranates there is no correlation between the outer skin colour of the rind and the colour of the arils (Holland *et al* 2009). These colours could be very different or similar, depending on the variety (Fig.5). The external outer skin colour does not indicate the extent of ripening degree of the fruit or its readiness for consumption because it can attain its final colour long before the arils are fully ripened (Holland *et al* 2009). Colour of agrifood products such as fruit and vegetables is derived from natural pigments, many of which change as the plant proceeds through maturation and ripening. The primary pigments imparting colour quality are the fat-soluble chlorophylls (green), carotenoids (yellow, orange, and red), water-soluble anthocyanin's (red, blue), flavonoids (yellow) and betalains (red) (Barrett *et al* 2010). Colour is usually considered the most important attribute of any food's appearance (Francis and Clydesdale, 1975), especially if it is associated with other aspects of food quality, for example, the ripening of fruit or the visible deterioration which occurs when a food spoils.

The colour of pomegranate juice is influenced by variety of pre and postharvest factors, including growing conditions. Shulman *et al* (1984) reported that fruit grown under coastal plain areas developed stronger colour than those fruits grown in warmer valley areas in Israel. The research done by Borochoy-Neori *et al* (2009) revealed that the amount of the

fruit arils red colour was inversely related to heat units accumulated during fruit development and ripening. Visual appearance of the food manifested as its colour has a strong influence on a consumer's opinion about the food quality (Pereira *et al* 2009; Nisha *et al* 2011).

The Hunter Lab L^* , a^* , b^* and the modified CIE system called CIELAB colour scales were opponent-type systems commonly used in the food industry. The latter a^* , represent positive values for reddish colours and negative values for the greenish ones, while the b^* latter represent positive values for yellowish colours and negative values for the bluish ones. L^* , which is an approximate measurement of luminosity, is the property according to which each colour can be considered as equivalent to a member of the greyscale, between black and white (Granato and Masson, 2010).

Chroma (C^*), considered the quantitative attribute of colourfulness, is used to determine the degree of difference of a hue in comparison to a grey colour with the same lightness. The higher the chroma value, the higher is the colour intensity of samples perceived by humans. Hue angle (h^*), considered the qualitative attribute of colour, is the attribute according to which colours have been traditionally defined as reddish, greenish, etc., and it is used to define the difference of a certain colour with reference to grey colour with the same lightness. This attribute is related to the differences in absorbance at different wavelengths. A higher hue angle represents a lesser yellow character in the assays. A 0° or 360° degree represents red hue, whilst 90° , 180° and 270° degree represent yellow, green and blue hues, respectively. It has been widely used in the evaluation of colour parameters in green vegetables and fruits (Barreiro *et al* 1997) and meats (Lopez *et al* 1997).

2.5.6 Irrigation

The pomegranate enjoy heat and thrive in arid and semi-arid areas, they need regular irrigation throughout the dry season to reach optimal yield and fruit quality (Sulochanamma *et al* 2005; Levin, 2006). Trees will survive for years and when properly

irrigated, they grow vigorously and produce good crops, but the fruit tends to be soft and has poor shipping and storage quality (LaRue, 1980). Sulochanamma *et al* (2005) found that drip irrigation had positive effects on pomegranates growth parameters such as tree height, stem diameter and plant spread. Positive effect was also noted on fruit yield and fruit weight (Prasad *et al* 2003; Shailendra and Narendra, 2005). Adequate soil moisture must be maintained throughout the growing season, particularly as harvest approaches in late summer and early autumn, because it helps to reduce the number of split fruit (LaRue, 1980). It is especially important to avoid drought stress during initial fruit set (Still, 2006).

Commercial production in California is concentrated in dry summer climates, and pomegranate is extremely drought tolerant once established, but crops perform much better with more generous moisture. Pomegranate thrives on a wide variety of soils and has a high resistance to salinity (Melgarejo, 2003). Newly planted trees require adequate moisture for establishment. Most plantings are established in late winter to spring when the soil is wet from winter rain in California (California Rare Fruit Growers, 1997). Development of optimal dripping irrigation methods together with usage of recycled water make it possible to grow high yielding pomegranate trees in arid regions that are otherwise highly unsuitable for pomegranate cultivation. Most of the large commercial orchards in Israel, India, and the U.S.A. utilize drip irrigation methods (California Rare Fruit Growers, 1997). In certain experiments done in India and in Iran, drip irrigation saves up to 66% of water compared to surface irrigation (Chopade *et al* 2001). Some growers prefer to use sprinklers but they cause difficulties in weed control. In view of the global warming phenomenon and the increasing water shortages, water availability for irrigation is a major consideration in pomegranate cultivation areas (Holland and Bar-Ya'akov, 2008).

2.5.7 Fertilization

The available data on pomegranate fertilization is very limited. Pomegranate orchards benefit from 0.2 to 0.5 kg N per tree per year, applied once in autumn or winter, or a split application in late winter and in spring (LaRue, 1980). Excessive or late applications of

nitrogen may delay fruit maturity and colour. Some evidence indicates that excessive nitrogen applications cause increased vegetative growth and reduce fruit production and colour development (LaRue, 1980). In Israel, the recommended quantity for nitrogen is 200 kg/ha and for potassium (potassium oxide) is 300 kg/ha (Kosto *et al* 2007). About 60 kg/ha of phosphorus (Phosphorus pentoxide) is recommended.

Zinc (which plays a role in plant growth) is the only other nutrient recommended for application to pomegranate trees in California. When Zn deficiency (short internodes and a decrease in leaf size) is evident, sprays should be applied on foliage in spring and early summer (LaRue, 1980). There is no evidence to show that phosphorous (P) or potassium (K) will improve growth or fruit quality when used to fertilize pomegranate orchards. In Israeli conditions, K₂O is applied at rates similar to N (Blumenfeld *et al* 2000).

2.5.8 Mineral nutrient concentration

There is limited information on elemental changes in pomegranate fruit parts during maturation. Potassium (K) element was reported to be more than calcium and sodium on the work done by Al-Maiman and Ahmad (2002). Sodium content was found to be 6.6790.86 and 7.2590.72 mg/100 ml on six pomegranates ecotypes and juice had an equivalent composition of phosphorus and sodium to the peel. Potassium was the most abundant element in the fruit, with 210.86910.70 in peel and a highest value in juice of 271.94960.59 mg/100 ml (Elfalleh *et al* 2009) Report indicate that *P. granatum* has high levels of potassium than fresh orange (206.7 mg/100 ml), grapefruit (166.7 mg/100 ml) and apple juice (122.9 mg/100 ml) (Elfalleh *et al* 2009). The relationship between time and mineral nutrients build-up in fruit arils and peel of cultivar Mala Yazdi was reported by Mirdehghan and Rahemi (2007). The authors observed increases in the build-up of macronutrients at successive harvests throughout fruit development, whereas the concentration of all micronutrients (exception Boron) in both arils and peel decreased from a maximum value to a minimum value between 10 and 140 day after full bloom. The composition and concentration of mineral nutrients at fruit developmental stages have been implicated in cracking incidence in pomegranate fruit and this might be associated with B and Ca deficiency (Mir *et al* 2012). Mirdehghan and Rahemi (2007) highlighted the significant accumulation of calcium during early growth to the fruit structural properties of

cultivar Mala Yazdi. For Ruby cultivar, calcium concentration was higher in immature fruit than in fruit at commercial harvest (Fawole and Opara, 2013a).

2.6 POSTHARVEST FACTORS AFFECTING FRUIT QUALITY

2.6.1 Harvest maturity

The maturity of fruits at harvest will determine their ability to achieve high eating quality, their susceptibility to mechanical injuries, their postharvest performance and their potential postharvest life. Any maturity index should clearly separate fruit based on physiological maturity, and any legal standard should be independent of growing conditions or location (Kader and Mitchell, 1989; Crisosto, 1992).

Fruit harvested at too high a maturity will be incapable of withstanding the rigors of postharvest handling and distribution, may have increased susceptibility to invasion by fruit rotting organisms (Mitchell *et al* 1991). These fruit will result in a short postharvest life, and may develop undesirable off flavours and mealy texture.

Fruit harvested at too low maturity will be incapable of ripening to their potential flavour and texture qualities. They will also lose water more readily, and may be at increased risk of physiological deterioration, especially if susceptible to internal breakdown (Mir *et al* 2012). With experience, correct harvest maturity for pomegranate fruit can be achieved by tapping the fruit and listening for a metallic sound (Mir *et al* 2012). However, there is lack of scientific-based maturity index for pomegranate cultivars except that of cultivar Wonderful. In general for Wonderful cultivar, the acids should be lower than 1.85%, soluble sugar content greater than 15 – 17% and the sugar: acid ratio greater than 18.5 (Shulman *et al* 1984). For USA market red juice colour must be equal to or darker than Munsell colour 5 R 5/12 (Kader, 2002; 2006a). In Iran, it was reported that the acceptable harvesting time was when the soluble solids content reached 17.5% (Sherafatian, 1994).

2.6.2 Total soluble solid

Total soluble solid (TSS), which is mostly made of sugars, increased considerably during major fruit developmental stages. The work done by Fawole and Opara (2013a) indicate that during the fruit growth of Bhagwa grown in South Africa, total soluble solids content

increased by nearly 1.5-fold between 54 days after full bloom and commercial harvest at 165 day after full bloom. The TSS content increased from 10.30°Brix in unripe fruit at 20 days after fruit set to 19.56°Brix in fully ripe fruit at 140 day after fruit set (Zarei *et al* 2011). TSS content of Spanish clones 'ME5', 'ME17' and 'MO6' was found to be constant throughout the ripening stages (Legua *et al* 2000) and the sweet Mollar cultivar (Gil *et al* 1995). The results by Shulman *et al* (1984) indicated that on two cultivars Mule's Head and Wonderful, there were significant differences in TSS content which happened at the later stages of fruit ripening. The study on cultivar Wonderful by Ben-Arie *et al* (1984) grown in two different areas and over two seasons in Israel, and observed that TSS content improved during the first 4.5 months after flowering period, and continued fairly constant later. When the TSS level of 15% is reached the fruit were considered fully ripe in terms of eating quality.

Al-Maiman and Ahmad (2002) reported different form of TSS accumulation in pomegranate cultivar Taifi, where the content was 16.4°Brix in immature fruit, with very insignificant growth in TSS during the last stages of fruit development resulting in 16.9 °Brix at full-ripe maturity stage. Weerakkody *et al* (2010) observed different levels on TSS in Wonderful cultivar over two growing seasons. TSS content in the first season was reported to increase from 8% at 30 day after fruit set to 15.5% at harvest, whereas in the second season the fruit had a lower TSS content of only 12.2% at harvest. On the contrary, ripening season did not influence TSS level on four pomegranate accessions 'PG 128-29', 'PG 130-31', 'EG1' and 'EG 2' (Borochoy- Neori *et al* 2011). Positive correlation was reported by Shwartz *et al* (2009) between TSS content and sugar components in the '121-2' and '101- 2' accessions of the 'Wonderful' during fruit development. This finding was also reported by Kader *et al* (1984) on 'Wonderful' grown in California, USA.

2.6.3 pH

The pH value of pomegranate juice describes its acidic taste, the previous having a contrast correlation with the last (Zarei *et al* 2011). Factors that result in differences in pH are usually postharvest handling, maturity status and fruit variety (Opara *et al*. 2009).The

studied of pH juice on Taifi cultivar increased with maturity, attaining a maximum value of 3.57 at the full-ripe stage (Al-Maiman and Ahmad, 2002). Fawole and Opara (2013a) reported a decrease on the early immature to early half ripe stage (3.57 and 3.18) respectively on Bhagwa cultivar grown in South Africa. The work done by Borochoy-Neori *et al* (2009) found pH value on early and late cultivar in Israel during fruit development to be between 3.8 – 4.2 to 3.2 – 3.4, respectively. Gil *et al* (1996) however, found no significant differences in pH of 'Mollar' fruit harvested at different maturity stages. This is however different to the results by Zarei *et al* (2011) who reported significant increases in pH value during fruit ripening of Rabbab-e-Fars cultivar

2.6.4 Maturity index

The ratio, which is usually called maturity index, is calculated as the relation between TSS and TA (Mena *et al* 2011), influence the taste and flavour of pomegranate and is an important factors used to classify cultivars (Melgarejo *et al* 2000; Martinez *et al* 2006). The following classification has been established for Spanish varieties (Melgarejo, 1997): Sweet varieties: MI = 31 – 98, Sour-sweet varieties: MI = 17 – 24 and Sour varieties: MI = 5 – 7. Total soluble solid: acid ratio (TSS: TA), also called maturity index (MI) by Hernandez *et al* (1999) is usually used during development of fruit to describe the taste of pomegranate (Shwartz *et al* 2009). Ben-Arie *et al* (1984) reported that the ratio did not associate suitably with taste for cultivar Wonderful grown in Israeli. In South Africa study on pomegranate cultivar Bhagwa, the ratio of TSS/TA differed significantly from 16.68 at 54 day after full bloom to 39.19 at 140 day after full bloom, but also displayed no significant increase till 165 day after full bloom (Fawole and Opara, 2013a). TSS: TA value recorded for Wonderful cultivar grown in Israel varied between locations and growing seasons (Ben-Arie *et al* 1984).

2.6.5 Titratable acid

Titrateable acidity (TA) is an important quality feature of pomegranate juice (Shwartz *et al* 2009). Shulman *et al* (1984) used high acidity content in juice during fruit development of cultivar Wonderful to classify it as a late cultivar. TA in pomegranate juice declines with increasing fruit maturation but the degree of decline varies among cultivars and growing

area. TA content significantly decline in juice of accession '101 – 2' while a decrease in TA for accession '121 – 22' was insignificant as reported by Shwartz *et al* (2009). According to Varasteh *et al* (2008) the TA level increased at the beginning of fruit set and then later decreased until the end of growing season for Malas-e-Torsh-e- Saveh cultivar grown in Iran. TA level in pomegranate grown in warmer valley region was found to be lower than in those grown in the coastal plain region of Israel (Shulman *et al* 1984). Decline in TA during fruit growth and development was also reported in juice of cultivar Wonderful (Ben-Arie *et al* 1984, 'Taifi' (Al-Maiman and Ahmad, 2002), 'Codpa' (Labbé *et al* 2010) and 'Ganesh' (Kulkarni and Aradhya, 2005). Chace *et al* (1981) concluded after two seasons that titratable acidity content of 1.8% and soluble solid content of 17% could be used as the indicator of maturation in some pomegranate cultivars grown in California.

2.6.6 Antioxidant activity

Several authors reported that the consumption of pomegranates fruit to have many positive health benefits (Seeram *et al* 2007; Basu and Penugonda, 2009; Tehranifar *et al* 2010). Recent attention in the pomegranate has increased due to its nutritional and antioxidant activity. Al-Maiman and Ahmad (2002) has analyzed changes in physical and chemical properties during pomegranate fruit maturation. Fawole *et al* 2011 studied chemical, phytochemical and antioxidant properties of pomegranate cultivars grown in South Africa. The work done by (Holcroft *et al* 1998; Melgarejo *et al* 2000; Heshi *et al* 2001; Ozkan, 2002; Tehranifar *et al* 2010) showed that composition of pomegranate fruit is strongly dependent on the cultivar type, growing area, climate, maturity and cultural practices. Reports by (Melgarejo *et al* 2000; Al-Maiman and Ahmad, 2002; Ozgen *et al* 2008; Tezcan *et al* 2009; Tehranifar *et al* 2010) have shown significant differences in organic acids, phenolic compounds, sugar and water-soluble vitamins composition of pomegranates fruit over the years. These factors could be used to supply important information to consumers to make an independent assessment in terms of recognizing nutritional values of the fruit.

2.6.7 Total phenols

The main compounds responsible for most of the useful properties of many foods that also include pomegranate fruit, are phenolic compounds in their different forms (Viuda-Martos *et al* 2010). Total phenolic content is one of the main parameters for evaluating the description of pomegranates cultivar, with respect to their nutraceutical value and potential use for different purposes. Their activity is believed to be mainly because of irredox properties, which play a central role in adsorbing and neutralising free radicals (Zheng and Wang, 2001; Laranjinha *et al* 1995). The loss of astringency is one of the necessary changes that occur during maturation and ripening of many pomegranate cultivars and this is mainly due to the drop in phenolic compounds during fruit maturation (Al-Maiman and Ahmad, 2002).

A fifty percent decline in total phenolic concentration in the first growing period on Wonderful cultivar was reported by Weerakkody *et al* (2010) but during the second season, phenolic concentration increased for ten weeks after fruit set and then quickly declined. Borochoy-Neori *et al* (2011) reported that pomegranate cultivars grown in Israel that ripened in midwinter had the highest concentration of total phenolics compared to early summer, late summer and autumn ripened fruit (Bind *et al* 2014). Total decrease in phenolic concentration with advancing maturation was as due to concurrent reduction in total flavonoid and total gallotannin concentrations in cultivar Ruby and Bhagwa grown in South Africa (Fawole and Opara, 2013a). The influence of maturity stage and growing area on the phenolic concentration of Chilean grown Codpa cultivar was studied by Labbé *et al* (2010) and the results showed that the highest total phenolic concentration was found in fruit juice at green maturity stage, which significantly decreased with advancing maturity.

The higher the level of phenolic compounds the higher the total antioxidant activity of pomegranate fruit juice and its relative human health benefit (Aviram *et al* 2004; Gil *et al* 2000; Tzulker *et al* 2007). Although juice containing very high concentrations of phenolic compounds could be less desirable due to high astringency (Kader, 2006), several reports by (Borochoy-Neori *et al* 2009; Shwartz *et al* 2009; Labbé *et al* 2010; Weerakkody

et al 2010) have demonstrated that a considerable decrease in phenolic compounds in pomegranate corresponds with a sharp decline in juice antioxidant capacity during fruit development.

2.6.8 Anthocyanin

Anthocyanin accumulation in plants is sensitive to environmental conditions (Oren-Shamir, 2009). Low and high temperature are antagonistic to each other in terms of the enhancement of anthocyanin accumulation and concentration (Mori *et al* 2007; Tarara *et al* 2008; Borochoy-Neori *et al* 2011). Oren-Shamir (2009) states that reduction in concentration of anthocyanin may be as a result of decrease in synthesis rate and its accelerated degradation. Studies have shown that anthocyanin compounds are identical in many pomegranate cultivars irrespective of the growing area, but the relative amounts of individual anthocyanin types vary among cultivars (Gil *et al* 1995; Alighourchi *et al* 2008). Six anthocyanins constitute the profile of pomegranate juice namely, delphinidin 3-glucoside, delphinidin 3,5-diglucoside, pelargonidin 3-glucoside, pelargonidin 3,5-diglucoside, cyanidin 3-glucoside (5.78 – 30.38 mg/L), and cyanidin 3,5-diglucoside (Gil *et al* 1995; Hernandez *et al.* 1999; Seeram *et al.* 2006; Borochoy-Neori *et al* 2011). Hernandez *et al* (1999) in their work pointed out that anthocyanins concentration in the fruit increased with increase in fruit maturity.

2.6.9 Sugars

In matured pomegranate fruit the sugar content could range from 12 – 16%, consisting primarily of glucose and fructose (Gil *et al* 1996; Al-Maiman and Ahmad, 2002). According to Legua *et al* (2000) glucose was the major sugar than fructose. Fawole and Opara (2013a) reported substantial increases in the concentrations of glucose and fructose during fruit maturation, with proportions of glucose to fructose ranging between 0.67 – 0.85 and 0.72 – 0.86 in ‘Bhagwa’ and ‘Ruby’ grown in South African, respectively. Al-Maiman and Ahmad (2002) also reported that the concentration of glucose was higher than fructose in unripe, half-ripe and full-ripe stages of fruit development of ‘Taifi’ cultivar. Fructose and glucose concentration increased significantly during fruit maturation in ‘121 – 2’ and ‘101 – 2’ accessions of the ‘Wonderful’ pomegranate (Shwartz *et al* 2009).

According to the authors, the sweet accession '121 – 2' had comparable levels of glucose and fructose as those found in accession 101 – 2, which has a sweet-sour taste.

2.6.10 Acids

The palatability of fruits depend in many ways on its acidic content. Given that sourness is generally attributed to proton release from acids (Sweetman *et al* 2009). The composition and concentration of organic acids are important factors that determine consumer perceptions of both sweetness and sourness in pomegranate fruit cultivars (Holland *et al* 2009). Different organic acids have been reported in pomegranate fruit juice but the most important acid accounting for titratable acidity is citric acid (Melgarejo *et al.* 2000; Poyrazoglu *et al* 2002). The amount of different acids and total organic acids considerably varied among clones (Legua *et al* 2000). The study done on cultivar Wonderful grown under the same agro-climatic environments, citric acid was predominant in accession '101 – 2' and lowest in accession '121 – 2' related to other organic acids (Shwartz *et al* 2009). The work done in Turkey, pomegranate varieties, citric, oxalic and malic acids were the main organic acids (Poyrazoglu *et al* 2002). Fawole and Opara (2013a) reported on cultivar Ruby grown in South Africa, tartaric acid was the most abundant organic acid during immature stage and the concentration reduced but remained quantifiable with advancing maturity, whereas citric and malic acids became unquantifiable at advanced maturity stages.



Fig 4. Differences in colour on Wonderful arils

CHAPTER 3

3.1 MATERIAL AND METHODS

3.1.1 Study site

This study was conducted during the harvest seasons of 2011/2012 and 2012/2013, respectively with fruits of pomegranate cultivar Wonderful, randomly selected from trees located in Bonnievalle (33°55'39"S 20°6'2"E, 175m.asl), Ladismith (33°29'S 21°16'E, 544m.asl), and Calitzdorp (33°32'14.59"S 21°41'6.59"E, 279m.asl), farms of the Western Cape, South Africa (Table 1). Healthy plants with uniformity in trunk size were selected for the study. Hundred fruit samples were harvested from each growing location.

Table 1: Climatic conditions at three locations in the Western Cape, South Africa

2011/2012	Minimum Temperature (°C)	Maximum Temperature (°C)	Average Rainfall (mm)	Average Heat units
Bonnievalle	13.26	27.35	31.35	286.12
Calitzdorp	14.19	32.18	11.37	384.26
Ladismith	10.75	27.40	19.31	266.23
2012/2013				
Bonnievalle	12.93	27.33	18.99	273.10
Calitzdorp	13.36	31.08	16.29	347.15
Ladismith	10.99	27.70	26.26	268.15

3.1.2 Physical analysis

Fruit weight (g) was measured using an electric balance (Mettler, Toledo, Switzerland, 0.0001 g accuracy), while fruit length (mm) , fruit width (mm) were measured using a digital Vernier caliper (Mitutoyo, Kawasaki, Japan, ± 0.01 mm). After measuring the fruit properties, the arils were manually separated from the fruits and the total arils weights in grams were measured. Juice extraction from the arils was done using a Melware juicer. Results were expressed as means \pm S.E (n= 100).

3.1.3 Colour analysis

Fruit peel colour along the equatorial axis of each fruit at two opposite spots were recorded in CIE (L^* , a^* , b^*) using a Minolta Chroma Meter CR-400 (Minolta corp, Osaka, Japan) after calibration with white tile background. Duplicate colour measurements (L^* , a^* , b^*) were made on the arils placed in a colourless glass petri dish.

3.1.4 Chemical analysis

3.1.4.1 Total soluble solids

TSS or °Brix was measured using a refractometer (Atago, Tokyo, Japan) at room temperature. Juice pH was measured using pH meter, while the titratable acidity (TA), expressed as milligrams of malic acid per millimetre was measured by titration to an end point of pH 8.2. (CRISON TiCom V1.7, Spain). Only results of 2012 season were presented in this study. Results were expressed as means \pm S.E (n= 30).

3.1.4.2 Total anthocyanin

The method of Fuleki and Francis (1968) was followed with minor modifications. The juice sample (50 μ L) was pipetted in triplicate into the wells of a 96-well polystyrene microplate, followed by 150 μ L of a 0.55 mol L⁻¹ HCl solution. The plate was incubated at room temperature for 10 min and the absorbance measured at 515 and 700 nm. The absorbance at 700 nm was subtracted from that at 515 nm to correct for turbidity. The total anthocyanin content was calculated as mg cyanidin-3-O-glucoside equivalents L⁻¹ by using its extinction coefficient, $\epsilon = 18\,492$ L mol⁻¹ cm⁻¹, and molecular weight, $M_r = 449$ g mol⁻¹. The extinction coefficient of cyanidin-3-O-glucoside was calculated by submitting solutions in an appropriate concentration range to the same procedure as the juice samples.

3.1.4.3 Total polyphenols

The total polyphenol content of the juice sample were determined using the method described by Singleton and Rossi (1965) with minor modifications in accordance with Arthur *et al* (2011). Briefly, 100 μ l Folin-Ciocalteu reagent (diluted 1:10 with de-ionised water) and 80 μ l 7.5% (m/v) aqueous solution of sodium carbonate were added to 20 μ l

de-ionised water (blank), gallic acid standards (10 - 100 mg/l in de-ionised water) or appropriately diluted sample in a flat-bottomed polystyrene 96- well microplate. Absorbance values were read at 765 nm on a Bio-Tek SynergyHT microplate reader (Winooski, Vermont, USA) equipped with Gen5 Secure software. Results were expressed in mg gallic acid equivalents (GAE)/100 ml infusion.

3.1.4.4 Total antioxidants

Free radical scavenging activity of the infusions was determined in triplicate using the method of Rangkadilok *et al* (2007) with slight modifications. Briefly, 100 µM DPPH in methanol was prepared fresh daily and 270 µl added to 30 µl de-ionised water (blank), Trolox standards (1 mM stock solution in 10% ethanol diluted with de-ionised water to 50-400 µM) and appropriately diluted samples in a 96-well deep-well microplate. The plate was sealed with a silicon mat to prevent evaporation of methanol and incubated at room temperature for 2 h protected from light. After 2 h, 200 µl of the reaction mixture was pipetted into the corresponding wells of a flat-bottom polystyrene 96-well microplate and the absorbance measured at 515 nm using a BioTek SynergyHT microplate reader. Results were expressed as µmol Trolox/100 mL infusion.

3.1.4.5 Organic sugar and organic acids

Organic sugar and organic acid concentrations in pomegranate juice were analysed using high performance liquid chromatography (HPLC) (Agilent 1100 Series, Waldron, Germany) equipped with a diode array detector (DAD). A sample of ten microlitres of extracted juice sample was injected into the HPLC and optimal separation was performed in an isocratic mobile phase of 5mM H₂SO₄ (560 µl of H₂SO₄ in 2L) using an HPX 87H column (Aminex, 300 mm x 7.78 mm). A refraction index detector was utilized at 55°C at a flow rate of 0.5 mL/min with UV detection set at 210 nm. Sample preparation and chromatographic procedure were based on the method of Castellari *et al* (2000). Identification and quantification of sugar and organic acid composition were made by comparison of peak retention times, peak areas and spectra with those of external standards. Total sugar and organic acid composition was calculated by summation of

individual sugar and acids, respectively (Melgarejo *et al* 2000). Measurements were conducted in triplicate on pooled fruit samples.

3.2 Statistical Analysis

Two way analysis of variance (ANOVA) was performed using PROC General Linear Model (GLM) of SAS 9.2 (2002 – 2008). Significant differences between means were determined by the Student-t LSD (Least significant difference) test. Differences at $P < 0.05$ were considered statistically significant. Results were presented as mean values with the standard deviations in parenthesis.

CHAPTER 4

4.1 RESULTS AND DISCUSSIONS

4.1.1 Physical fruit properties

With the exception of aril yield %, all measured pomegranate physical properties were significantly affected by locality but responses differed between the different seasons ($p < 0.05$) (Table 2).

Table 2: Fruit characteristics of cultivar Wonderful grown in Western Cape, South Africa

Area(A)	Fw(g)	FL(mm)	Fw1(mm)	Arils/g	%Aril yield	%waste
Bonnievalle	508.87±53.87a	88.68±2.82a	98.32±2.77a	239.75±27.89a	46.78±2.24b	52.72±2.54a
Calitzdorp	423.80±72.91b	84.01±5.16b	93.93±5.21b	202.06±31.36b	48.17±3.64a	51.83±3.64b
Ladismith	406.17±65.62b	83.14±4.80b	92.20±4.65b	199.76±28.56b	49.40±3.11a	50.60±3.11b
Season(B)						
2012	418.49a	83.11b	92.61b	200.88b	48.60a	51.40a
2013	474.07b	87.45a	97.03a	226.83a	47.63a	52.03a
Probability value> F						
A	<.0001	<.0001	<.0001	<.0001	0.0261	0.0898
B	<.0001	<.0001	<.0001	<.0001	0.2130	0.4176
A * B	<.0001	<.0001	<.0001	0.0001	0.1080	0.0326

Means with different letters, in the same column, indicate significant differences at $p < 0.05$. Fw: Fruit weight, FL: Fruit Length, Fw1: Fruit width

Fruit weight

During 2012/2013 season fruit weight had significantly larger fruits (471.1 g) as compared to 2011/2012 (418.5 g) season. The difference between the largest and lowest was 55.6 g. However, Bonnievalle significantly had largest fruit weight (508.9 g) compared to Calitzdorp (402.8 g) and Ladismith (406.2 g). Zaouay *et al* (2012) classify fruit weight into small (101 g), medium-sized (200 – 400 g) and big sized fruits (>400 g). Based on this classification our fruits can be classified as big fruits in all the locations. Our findings are within range reported by Martinez *et al* (2012) and Al- Said *et al* (2009) with fruit weight ranging from 430 g to 535 g on six Moroccan cultivars and 390 g to 424.30 g on

pomegranate fruits cultivars grown in Northern Oman. In contrast, Tehranifar *et al* (2010) reported lower fruit weight of 196 g to 315 g in Turkey and Mellisho *et al* (2012) reported from 251.03 g to 315.56 g on cultivar Mollar de Elche in Spain. These differences might be attributed to cultivar types as well as agro climatic regions (Table 1), which have important impacts on fruit physical properties (Mditshwa *et al* 2013).

Fruit length

Values of fruit length were 87.45 mm and 83.11 mm irrespective of seasons. The difference in season being 4.34 mm. Amongst all the investigated locations, Bonnievalle had the highest fruit length (88.68mm) followed by Calitzdorp (84.01mm) and Ladismith (83.14mm). Zaouay and Mars (2011) reported that fruit length ranged from 49.25mm to 90.30mm. Zaouay *et al* (2012) reported fruit length that ranged from 51.54mm to 88.85mm on Tunisian cultivars while Tehranifar *et al* (2010) reported fruit length lower than our result which ranged from 69.49mm to 81.56 on twenty Iranian cultivars and also Al-Said *et al* (2009) reported fruit length which ranged from 78.99mm to 83.62mm on cultivar Jabal 1, Jabal 2, Jabal 3 and the wild in Oman.

Fruit width

The fruit width between the investigated two seasons, 2012/2013, had significantly highest fruit width (97.03mm) compared to 2011/2012 with (92.61) and with the difference of 4.41 mm. The widest fruit was found in Bonnievalle, with a significantly value of 98.32mm. However, there was no significant difference between Calitzdorp (93.93mm) and Ladismith (92.20mm). Results reported by Ferrara *at al* (2011) ranged from 79.0mm in 2008 to 89.3mm in 2009 while Italian cultivars ranged from 69.1mm to 95.8mm and Zaouay *et al* (2012) reported fruit width that ranged from 56.83mm to 101.33mm on Tunisian cultivars and classified fruit into small (up to 54.19mm), medium sized (70mm – 80mm) and large fruits (>80mm). The information on physical properties is particularly relevant in the design or selection of appropriate packaging of fruit handling and storage (Valero and Ruiz–Altisent, 2000).

Aril weight

Significant differences in aril weight per season were recorded, with 2012/2013 showing a high aril weight of (226.83g) and 2011/2012 showing the lowest weight of (200.88g). Bonnievalle significantly had largest aril weight (239.75g) compared to Calitzdorp (202.06g) and Ladismith (199.76g). Tehranifar *et al* (2010) reported lower aril weight ranging from 95 g to 170 g in Turkey. Durgac *et al* (2008) reported aril weight that ranged from 118 mg to 335 mg and Ferrara *et al* (2011) reported values between 277.4 mg to 519.1 mg these values were measured on only 100 arils weight while our results are based on total aril weight.

Aril yield percentage

The percentage aril yield over two seasons shows no significant difference, although varied across the different localities from 46.78% (Bonnievalle) to 49.40% (Ladismith). This might be attributed to climatic conditions which perhaps favour the development of the outer skin more than arils. Shulman *et al* (1984) reported that fruit arils constitute about 50% of the fruit weight in most stages of fruit development for Mules and Wonderful cultivar which is also agree with our results. Therefore, this information on aril weight and percentage yield is relevant for juice processors or industry.

4.1.2 Determination of colour attributes

Interaction effects on parameters recorded. With the exception of Aril hue angle, all measured parameters had significant interaction effect regardless of locality ($P < 0.05$) (Table 3). The attractive red colour of pomegranate is one of the parameters that are evaluated for the commercial classification of the product in relation to its quality, which influences consumer behaviour and can help in impulse purchases.

Table 3: Peel and aril colour of cultivar Wonderful grown in Western Cape, South Africa

Area A	Peel Colour				Aril Colour			
	L*	C*	h*	a*	L*	C*	h*	a*
Bonnievalle	48.31 ±2.38b	45.73 ±1.67a	30.20 ±2.42b	39.48 ±2.13a	7.73 ±1.60b	13.50 ±2.14c	25.48 ±1.25ab	12.17 ±1.87c
Calitzdorp	47.77 ±4.54b	44.76 ±1.7ab	29.58 ±4.03b	38.82 ±2.37a	8.73 ±8.73b	15.54 ±1.64b	24.82 ±1.79b	14.08 ±1.43b
Ladismith	50.23 ±2.27a	43.66 ±3.28b	35.97 ±3.66a	35.33 ±4.06b	12.67 ±3.07a	18.84 ±1.73a	26.02 ±1.82a	16.92 ±1.70a
Season B								
2012	47.67b	45.74a	30.72b	39.19a	8.87b	15.23b	25.51a	13.71b
2013	49.86a	43.69b	33.11a	36.56b	10.48a	16.70a	25.37a	15.07a
Probability value.> F								
A	0.0045	0.0039	<.0001	<.0001	<.0001	<.0001	0.0676	<.0001
B	0.0007	<.0001	0.0041	0.0002	0.0011	<.0001	0.7369	<.0001
A * B	<.0001	0.0004	0.0338	0.0238	0.0007	<.0001	0.0607	<.0001

Means with different letters, in the same column, indicate significant differences at $p < 0.05$ and L*: colour lightness; a*: colour redness; C: Chroma; H: hue angle

Peel colour, during 2012/2013 season, fruit L* value had significantly higher (49.86) compared to 2011/2012 (47.67) season. (Table 3). However, Ladismith significantly had higher fruit value of (50.23) compared to both Bonnievalle (48.31) and Calitzdorp (47.77). Fawole *et al* (2013a) reported similar results on Ruby and Bhagwa cultivars grown in Porterville South Africa; this could be attributed to climatic condition as they are grown under the same conditions. In contrast, Caliskan and Bayazit (2012) reported higher values of 51.1 to 75.2 on cultivars grown in Turkey, while Durgac *et al* (2008) reported lower values of 21.0 to 36.1 again trials conducted in Turkey.

Chroma(C*) value. Significant differences in the intensity of fruit colour per season were recorded, with 2011/2012 showing a high intensity of (45.74) and 2012/2013 showing the lower colour intensity of (43.69), however it significantly varied across the three localities from 45.73 (Bonnievalle) to 44.76 (Calitzdorp) and 43.99 (Ladismith). In contrast, the results reported by Durgac *et al* (2008) in Turkey and Mellisho *et al* (2012) in Italy was lower than our results with values that ranged from 9.2 to 19.9 and 40.6 to 42.5, respectively.

Hue angle (h*) value. The 2012/2013 season, had significantly higher hue angle (33.1°) compared to 2011/2012 (30.72°) season. However, Ladismith significantly had highest hue angle (35.39°) compared to Bonnievalle (30.20°) and Calitzdorp (29.58°). In Turkey, Durgac *et al* (2008) reported similar results which ranged from 17.7 to 70.1. Our results are within values reported by Caliskan and Bayazit (2012) ranging from (31.0 – 84.5) but lower than values reported by Mellisho *et al* (2012) ranging from (70.9 – 80.7).

a* value. The indices which indicate redness, showed a significant difference over the two seasons with (36.56 in 2012/2013 and 39.19 in 2011/2012) while areas varied from (39.48) in Bonnievalle compared to (38.82) Calitzdorp and (35.33) Ladismith. Similar results were reported of 35.57 in cultivar Jabal 1 in Oman by Al-Said *et al* (2009) and Caliskan and Bayazit (2012) reported values that ranged from 4.4 to 42.8 in Turkey. The significant variation in colour attributes of the pomegranate cultivars indicates the potential of utilizing these attributes as maturity indices for harvest management and segregation of harvest fruit into grades (Al-Said *et al* 2009).

Aril colour values of the pomegranates also showed significant seasonal and locality differences (Table 3). **L* value.** The 2012/2013 season, had significantly higher value (10.48) compared to 2011/2012 (8.87) season. The highest L* for the aril value was found in Ladismith, with a significantly higher value of (12.67). However, there was no significant difference between Calitzdorp (8.73) and Bonnievalle (7.73). In contrast, Al-Said *et al* (2009) reported results ranging from 90.77 to 103.136 in Oman cultivars; Mellisho *et al* (2012) found values that ranged between 13.2 to 24.5 in Spain and Caliskan and Bayazit

(2012) values ranging from 30.3 to 44.4 in Turkey, which are higher than our findings despite that the results were reported from different cultivars.

Aril Chroma(C*) value. Aril chroma in two seasons investigated, 2012/2013 had the higher chroma value of (16.70) than 2011/2012 (15.23). Amongst all the investigated locations, Ladismith had the highest value of (18.84) followed by Calitzdorp (15.54) and Bonnievalle (13.50). Similar results were reported by Caliskan and Bayazit (2012) in Turkey whose values ranged from 13.2 to 21.7. Mellisho *et al* (2012) in Spain reported higher values that ranged from 18.5 to 26.2 and also Mditshwa *et al* (2013) in South Africa reported values of 21.46 to 27.61.

Hue angle (h*) aril value. Hue angle arils show no significant difference over two seasons. However, Ladismith had significantly higher hue angle (26.02°) compared to Bonnievalle (25.48°) and Calitzdorp (24.82°). Similar results in Spain were reported by Mellisho *et al* (2012) with values that ranged from 19.7° – 44.3°. In contrast, Caliskan and Bayazit (2012) in Turkey; Fawole *et al* (2013a) in South Africa reported higher results of 41.1° – 63.5° and 40.18° – 54.17°, respectively.

a* values were 15.07 and 13.71 for 2012/2013 and 2011/2012, respectively. Ladismith had significantly higher a* value (16.92) compared to Calitzdorp (14.08) and Bonnievalle (12.17). Conversely, our results were higher than those of Al-Said *et al* (2009) who reported values of 1.88 – 3.01 on Oman cultivars. Mellisho *et al* (2012) and Caliskan and Bayazit. (2012) reported values of between 4.7 – 23.7 and 5.9 – 16.4, respectively. Mditshwa *et al* (2013) in South Africa reported higher values of 18.65 – 24.34, which might be attributed to cultivar differences.

4.1.3 Chemical properties

Total soluble solids

TSS showed a significant difference in three areas and varied from 17.33 (Bonnievalle) compared to 17.22 Calitzdorp and 16.01 (Ladismith) (Table 4).

Table 4: Total soluble solids, pH, titratable acidity and maturity index

Area	TSS	pH	TA	MI
Bonnievalle	17.33±0.30a	3.04±0.20a	1.32±0.12c	13.43±1.21a
Calitzdorp	17.22±0.45a	2.75±0.88b	1.56±0.16b	10.86±1.83b
Ladismith	16.01±0.26b	2.71±0.02b	1.70±0.14a	9.69±1.09b

Means with different letters, in the same column, indicate significant differences at $p < 0.05$

TSS which is mostly made of sugar was higher than those reported by Al- Said *et al.* (2009) with values ranging from 13.68 to 15.18 in Oman and Zaouay and Mars (2011) reported values between 13.08 and 15.87 in Tunisian cultivars. Similarly, our results concur with the results reported by Ferrara *et al.* (2011) on TSS that ranged from 14.7 to 18.0 in Italy and Martinez *et al.* (2012) who reported values ranging from 15.3 and 17.6 in Moroccan cultivars. Previous research has shown that rainfall impacts on fruit TSS (Mditshwa *et al.* 2013). Low rainfall at Bonnievalle and Calitzdorp could be responsible for high TSS value (Table 1). The low TSS for Ladismith fruit might reflect a negative effect of high rainfall. TSS for all the investigated locations ranged higher than the minimum threshold generally required for commercial use of 12% for Spanish cultivars (Martinez *et al.* 2006).

pH

The highest pH of fruit was found in Bonnievalle, with a significant value of 3.04. However, there was no significant difference between Calitzdorp (2.71) and Ladismith (2.71). Legua *et al.* (2012) reported higher pH of 3.94 to 4.07 on six Mollar group cultivars in Spain. In Saudi Arabia, Al-Maiman and Ahmad (2002) obtained a pH of 3.6 in pomegranate juice

for Taifi cultivar while Fadavi *et al* (2005) in Iran presented pH values comprised between 2.9 and 4.2.

Titrateable acid

The acidity of the fruit from the three areas shows significant differences and varied from 1.70% (Ladismith) compared to 1.56% (Calitzdorp) and 1.32% (Bonnievalle). Our results are within those reported by Shulman *et al* (1981) on cultivar Wonderful with values not less than 1.5% in coastal plain Bet Shean valley and also Chace *et al* (1981) on cultivar Wonderful reported value of around 1.8%. Titrateable acidity (TA) is an important quality attribute of pomegranate juice (Shwartz *et al.* 2009). High acidity content in juice of Wonderful cultivar during fruit development was used to classify the fruit as a late cultivar (Shulman *et al.* 1984).

Maturity index

MI was highest in Bonnievalle, with a significant value of (13.43) (Table 4). However, there was no significant difference between Calitzdorp (10.86) and Ladismith (9.69). Our results are within range reported by Ferrara *et al* (2011) in Italy with values ranging from 6.6 to 29.1 and also those of Tehranifar *et al* (2010) in Turkey values ranged from 5.04 to 46.31. In contrast, Legua *et al* (2012) reported higher value of 59.14 to 87.95 in Alicante province, Spain. According to Chace *et al* (1981) pomegranates are appropriate for the fresh market when their acidity content is lower than 1.8% and their MI between 7 and 12, when the MI ranges from 11 to 16, pomegranates are quite tasty. It can be suggested that our results fall to those which are good for consumption.

Anthocyanins

They are phenolic compounds that contribute to the red, blue, or purple coloration of many fruits and are well known for their antioxidant activity (Tehranifar *et al.* 2010). Anthocyanin showed a significant differences in three areas (Table 5) and varied from 1134 (Bonnievalle) compared to 1009 (Calitzdorp) and 772 (Ladismth). Zaouay *et al.* 2012 reported the total content of anthocyanin that varied from 50.5 mg L⁻¹ (CH8-2) to

490.4 mg L⁻¹ (JR1) while sour cultivars did not reveal high anthocyanin amounts compared to sweet ones.

Table 5: Anthocyanin, Phenolics and Antioxidant of cultivar Wonderful

	Anthocyanin	Phenolics	Antioxidant
Bonnievalle	1134a	1611.3b	12.57b
Calitzdorp	1009b	1834.6a	13.58a
Ladismith	772c	1814.8a	14.84a

Means with different letters, in the same column, indicate significant differences at p<0.05

Total phenolics

The phenolics were highest in both Calitzdorp (1834.6) and Ladismith (1814.8) while Bonnievalle had the lowest value of 1611.3. Our results were lower than those reported by other authors. Gil *et al* (1995) reported the total phenolics (TPs) of pomegranate juice (PJ) from fresh arils as 2117 ± 95 mg/L and for a commercial PJ as 2566 ± 131 mg/L. TPs of six pomegranate arils from Mediterranean region of Turkey were reported to be between 1245 and 2076 mg/L (Özgen *et al.* 2008), and of eight pomegranate cultivars widely grown in Turkey were between 2083 – 3436 mg/L (Çam *et al.* 2009).

Antioxidant

Ladismith and Calitzdorp showed no significant differences in antioxidant but had higher value of 14.84 and 13.58 respectively. Bonnievalle showed significant differences with the value of 12.57. Borochoy-Neori *et al* (2009) indicated that antioxidant and quality characteristics of pomegranate fruit are more dependent on cultivar and ripening date while fruit ripening later in the harvest season contained more soluble phenolics and exhibited a higher antioxidant activity.

Organic Sugars

The build-up of simple sugars is one of the processes occurring during the final developmental stages of fruit, resulting in increases in sweetness as fruit approach ripeness (Shwartz *et al.* 2009; Zarei *et al.* 2011). Fructose, glucose and sucrose were

individually analysed, as they play an important role in pomegranate quality. Glucose and fructose were the major soluble sugars found in all growing regions (Table 6).

Table 6: Organic acids and sugars of cultivar Wonderful

	Organic Acids			Organic Sugars		
	Acetic acid	Citric acid	Malic acid	Fructose	Glucose	Sucrose
Bonnievalle	0.048a	16.293b	0.620a	77.087a	68.160a	7.607a
Calitzdorp	0.030a	23.718a	0.388b	77.465a	68.403a	8.262a
Ladismith	0.051a	19.952ab	0.468b	63.448b	55.143b	6.188b

Means with different letters, in the same column, indicate significant differences at $p < 0.05$

This result is in agreement with previous studies on other pomegranate cultivars (Melgarejo *et al.* 2000; Al-Maiman and Ahmad, 2002; Shwartz *et al.* 2009; Tezcan *et al.* 2009; Mena *et al.* 2011). Bonnievalle and Calitzdorp showed the highest values of fructose (without significant differences), with 77.09mg/100ml and 77.47mg/100ml respectively, while Ladismith showed lowest value, 63.45mg/100ml. Glucose also showed no significant differences between Bonnievalle, 68.16mg/100ml and Calitzdorp, 68.40mg/100ml while Ladismith, 55.14mg/100ml showed a significantly lower value. Fructose and Glucose in particular serve as energy sources and contribute to sweetness. It is important to note that fructose is double as sweet as glucose (Nookaraju *et al.* 2010), and could be used as a measure of degree of juice sweetness during fruit ripening (Al-Maiman and Ahmad, 2002). Sucrose which was detected in small amount ranged from 8.26mg/100ml Calitzdorp, 7.61mg/100ml Bonnievalle and 6.19mg/ml Ladismith.

The fructose mean of (7.7 g/100 g) and glucose (6.8 g/100 g) contents of these accessions were higher than those reported for Spanish cultivars (6.6 and 6.1 g/100 g, respectively) (Melgarejo *et al.* 2000), and Turkish cultivars (6.4 and 6.8 g/100 g) (Ozgen *et al.* 2008) but were lower than those of Tunisian cultivars (9.1 and 7.3 g/100 g, respectively) (Hasnaoui *et al.* 2011). Our results also were quite similar to those obtained by Al-Maiman and Ahmad (2002) for cultivars from Saudi Arabia with fructose (~53% of total sugars) and glucose (~47% of total sugars) were found to be the dominant sugars

among the pomegranate accessions in the study, while sucrose contents were lower, but in contrast, the work done by Ozgen *et al* (2008) and Legua *et al* (2012) reported that glucose levels were higher than fructose levels in pomegranate fruits. This difference can be attributed to climatic conditions (Table 1), ripening times and cultivars (Legua *et al*. 2012). The low sucrose concentration in our results may be due to its conversion to invert sugars such as fructose and glucose during ripening (Hasnaoui *et al*. 2011).

Organic Acids

Previous study suggested that although several organic acids were found in pomegranate aril juices, the major acid accounting for titratable acidity is citric acid (Melgarejo *et al*. 2000). The result obtained in the current study show that this might also be the case since citric acid is found to be the major organic acid in this juice. Organic acid distribution of pomegranate was dominated by citric acid with Calitzdorp (23.72 mg/100ml), Ladismith (19.25 mg/100ml) and Bonnievalle (16.29mg/100ml). Citric acid contents of between 0.22g/100ml and 2.16g/100ml have been reported in pomegranates from Turkey and the United States of America, where it was found to be predominant acid. Citric acid levels of 40 Spanish pomegranate cultivars were reported by Melgarejo *et al* (2000) as between 0.142 – 2.317 g/100 g; by Poyrazog̃lu *et al* (2002) for 13 pomegranates from four different regions of Turkey, as between 0.033 – 0.896 g/100 ml, and by Ozgen *et al* (2008) for six pomegranate cultivars from Mediterranean region of Turkey, as between 0.20 and 3.20 g/100 mL. Malic acid was found to be the second dominant acid for the three areas with Bonnievalle (0.62mg/100ml), Ladismith (0.47mg/100ml) and Calitzdorp (0.39mg/100ml). The reported levels of malic acid in literature are 0.135 – 0.176 g/100 g by Melgarejo *et al* (2000); 0.056 and 0.686 g/100 mL by Poyrazog̃lu *et al* (2002), and 0.09 and 0.15 g/100 mL by Ozgen *et al* (2008). Acetic acid was also found in small amount and was not significantly different in all the areas.

CHAPTER 5

5.1 GENERAL DISCUSSION AND CONCLUSION

5.1.1 Introduction

The pomegranate grows from Iran to the Himalaya in northern India and was cultivated and naturalized over the whole Mediterranean region since ancient times. Wild relative species is *P. protopunica*, which is endemic to the island of Socotra (Yemen) in the Indian Ocean. The ability of pomegranate trees to adjust to variable climatic conditions is reflected in its wide distribution (Holland and Bar-Ya'akov, 2008).

Different studies have shown the effects of cultivar differences, growing region (Shwartz *et al.*, 2009) and maturity status on pomegranate fruit maturity indices (Al-Maiman and Ahmad, 2002). Authors such as (Ben-Arie *et al.* 1984; Shwartz *et al.* 2009; Al-Maiman and Ahmad, 2002) have explored individual fruit, physico-chemical parameters, and the relationships among these parameters to assist in identifying fruit indices for reliable prediction of fruit maturity indicators. The aim of this research was to investigate the performance of cultivar Wonderful grown in three regions of the Western Cape Province, South Africa as an alternative agricultural commodity.

5.1.2 General Discussion

Fruit characteristics in this study from three areas showed significant differences at $p < 0.05$ but when compared per season the percentage waste showed no significant differences. Peel and aril colour which are an important indicators in pomegranate fruit evaluation also showed significantly different results in all areas but when seasons were compared hue angle in arils had no significant differences.

Chemical properties (TSS, pH, TA and MI) of fruits in all areas had significant differences at $p < 0.05$ as well as anthocyanin, phenolics and antioxidant. Organic acids (Citric and Malic) showed differences while Acetic acid was not significant in all areas and organic sugar (fructose, Glucose and Sucrose) all had significant differences

5.1.3 General Conclusions

The study provides information on the physico-chemical and colour measurement of cultivar Wonderful fruit in all three pomegranate growing areas. The pomegranates grown on three farms showed significant differences in fruit weight, length and width, which can be useful in selection of superior desirable pomegranate as well as fruit sorting. The results indicate that Bonnievalle area could be the best area to grow cultivar Wonderful for better physical properties with more suitability for fresh consumption as well as to obtain higher yield percentage.

It can also be concluded that changes in colour of peel and arils of pomegranate (cv. Wonderful) was mostly as a result of seasonal variation as well as growing area as evident by the interaction between both main factors. These results showed that when assessing colour quality of pomegranate Wonderful cultivar, it is important to consider both growing season and location. This study provides information that could be used to assess cultivar Wonderful quality attributes grown in different agro-climatic regions in South Africa. The results from this study also supported other studies by Shulman *et al* (1981), Shwartz *et al* 2009 in Israel and Weerakkody *et al* 2010 in Australia with regards to chemical properties (TSS, pH, TA and MI). However, from this study Bonnievalle is the most promising area compared to the other evaluated areas.

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Appendix A. Fitted ANOVA models (2011/2012)

Fruit Weight

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	196033.1929	98016.5965	43.70	<.0001
Error	27	60558.8011	2242.9186		
Corrected Total	29	256591.9940			

Fruit Length

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	767.2494648	383.6247324	46.19	<.0001
Error	27	224.2513014	8.3056038		
Corrected Total	29	991.5007661			

Fruit Width

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	666.0359661	333.0179830	31.97	<.0001
Error	27	281.2299353	10.4159235		
Corrected Total	29	947.2659014			

Arils per gram

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	30571.20827	15285.60414	32.27	<.0001
Error	27	12788.16213	473.63563		
Corrected Total	29	43359.37041			

Percentage Yield

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	106.8998058	53.4499029	3.85	0.0339
Error	27	375.2404141	13.8977931		
Corrected Total	29	482.1402199			

Percentage Waste

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	106.8998058	53.4499029	3.85	0.0339
Error	27	375.2404141	13.8977931		
Corrected Total	29	482.1402199			

FruitCol L

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	171.1952571	85.5976285	11.50	0.0002
Error	27	200.9347092	7.4420263		
Corrected Total	29	372.1299662			

FruitCol C

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	7.07195556	3.53597778	1.08	0.3554
Error	27	88.78786103	3.28843930		
Corrected Total	29	95.85981660			

FruitCol H					
		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	309.3608024	154.6804012	15.86	<.0001
Error	27	263.3161538	9.7524501		
Corrected Total	29	572.6769563			

FruitCol a					
		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	27.9522837	13.9761419	2.52	0.0992
Error	27	149.7184105	5.5451263		
Corrected Total	29	177.6706942			

ArilsCol C					
		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	206.1420915	103.0710458	43.08	<.0001
Error	27	64.5932318	2.3923419		
Corrected Total	29	270.7353234			

ArilsCol H					
		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	17.03670267	8.51835134	3.48	0.0452
Error	27	66.09455287	2.44794640		
Corrected Total	29	83.13125554			

ArilsCol a

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	158.6331859	79.3165929	39.68	<.0001
Error	27	53.9671049	1.9987817		
Corrected Total	29	212.6002908			

ArilsCol L

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	55.7092127	27.8546063	6.27	0.0058
Error	27	119.8790122	4.4399634		
Corrected Total	29	175.5882249			

TSS

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	24.22498626	12.11249313	31.22	<.0001
Error	27	10.47408222	0.38792897		
Corrected Total	29	34.69906848			

Fitted ANOVA models (2012/201)

Fruit Weight

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	243850.3140	48770.0628	22.93	<.0001
Error	54	114858.1659	2127.0031		
Corrected Total	59	358708.4799			

Fruit Length

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	1044.973069	208.994614	27.98	<.0001
Error	54	403.321103	7.468909		
Corrected Total	59	1448.294172			

Fruit Width

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	967.805438	193.561088	20.82	<.0001
Error	54	502.135197	9.298800		
Corrected Total	59	1469.940635			

Arils per gram

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	41106.50546	8221.30109	15.84	<.0001
Error	54	28032.07923	519.11258		
Corrected Total	59	69138.58468			

Percentage Yield

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	123.7871197	24.7574239	2.81	0.0252
Error	54	476.1484829	8.8175645		
Corrected Total	59	599.9356026			

Percentage Waste

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	117.1786139	23.4357228	2.60	0.0352
Error	54	486.3349838	9.0062034		
Corrected Total	59	603.5135977			

FruitCol L

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	361.0914051	72.2182810	12.90	<.0001
Error	54	302.2935258	5.5980283		
Corrected Total	59	663.3849309			

FruitCol C

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	168.7197412	33.7439482	9.74	<.0001
Error	54	187.0747125	3.4643465		
Corrected Total	59	355.7944537			

FruitCol H

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	652.881684	130.576337	13.60	<.0001
Error	54	518.546148	9.602706		
Corrected Total	59	1171.427833			

FruitCol a

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	354.7785275	70.9557055	10.92	<.0001
Error	54	350.9560034	6.4991852		
Corrected Total	59	705.7345309			

ArilsCol C

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	386.5504493	77.3100899	42.13	<.0001
Error	54	99.0946986	1.8350870		
Corrected Total	59	485.6451479			

ArilsCol H

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	29.8971877	5.9794375	2.34	0.0541
Error	54	138.1341002	2.5580389		
Corrected Total	59	168.0312879			

ArilsCol a

	Sum of				
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	304.5041869	60.9008374	38.59	<.0001
Error	54	85.2210621	1.5781678		
Corrected Total	59	389.7252490			

ArilsCol L

	Sum of				
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	361.3059504	72.2611901	22.59	<.0001
Error	53	169.5149206	3.1983947		
Corrected Total	58	530.8208709			

TSS

	Sum of				
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	42.90730207	8.58146041	49.25	<.0001
Error	52	9.06001000	0.17423096		
Corrected Total	57	51.96731207			

pH

	Sum of				
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	0.62255064	0.31127532	19.93	<.0001
Error	26	0.40604632	0.01561717		
Corrected Total	28	1.02859697			

TA					
		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	0.71881831	0.35940916	17.91	<.0001
Error	25	0.50176840	0.02007074		
Corrected Total	27	1.22058671			

MI					
		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	69.7448550	34.8724275	25.40	<.0001
Error	25	34.3183359	1.3727334		
Corrected Total	27	104.0631909			

Anthocyanin content					
		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	8	3947299.776	493412.472	73.76	<.0001
Error	33	220759.867	6689.693		
Corrected Total	41	4168059.643			

Phenol content					
		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	8	2328094.202	291011.775	9.15	<.0001
Error	33	1049591.417	31805.801		
Corrected Total	41	3377685.619			

Antioxidant capacity

	Sum of				
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	8	135.6198602	16.9524825	15.64	<.0001
Error	33	35.7651017	1.0837910		
Corrected Total	41	171.3849619			

Citric Acid

	Sum of				
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	165.4036111	82.7018056	5.89	0.0130
Error	15	210.6653000	14.0443533		
Corrected Total	17	376.0689111			

Acetic Acid

	Sum of				
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	0.00163333	0.00081667	0.60	0.5637
Error	15	0.02056667	0.00137111		
Corrected Total	17	0.02220000			

L Malic Acid

	Sum of				
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	0.16614444	0.08307222	6.46	0.0094
Error	15	0.19276667	0.01285111		
Corrected Total	17	0.35891111			

D - Fructose

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	765.2284333	382.6142167	30.97	<.0001
Error	15	185.2889667	12.3525978		
Corrected Total	17	950.5174000			

D - Glucose

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	690.6408444	345.3204222	29.78	<.0001
Error	15	173.9520667	11.5968044		
Corrected Total	17	864.5929111			

Sucrose

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	13.47881111	6.73940556	7.39	0.0058
Error	15	13.67170000	0.91144667		
Corrected Total	17	27.15051111			

Appendix B. Interaction between season (A) and area (B).

		Degree of Freedom	Sum of Square	Mean Square	F value	Pr>F
Fruit weight	Season(A)	1	46339.825	46339.825	21.79	<.0001
	Farm(B)	2	120639.380	60319.690	28.36	<.0001
	AxB	2	76871.108	38435.554	18.07	<.0001
Fruit Length	Season(A)	1	282.690	282.690	37.85	<.0001
	Farm(B)	2	354.365	177.182	23.72	<.0001
	AxB	2	407.917	203.958	27.31	<.0001
Fruit width	Season(A)	1	293.187	293.187	31.53	<.0001
	Farm(B)	2	398.764	199.382	21.44	<.0001
	AxB	2	275.853	137.926	14.83	<.0001
Arils per gram	Season(A)	1	10099.895	10099.895	19.46	<.0001
	Farm(B)	2	20172.268	10086.134	19.43	<.0001
	AxB	2	10834.341	5417.170	10.44	0.0001
Percentage yield	Season(A)	1	14.006	14.006	1.59	0.213
	Farm(B)	2	68.866	34.433	3.91	0.026
	AxB	2	40.914	20.457	2.32	0.108
Percentage waste	Season(A)	1	6.010	6.010	0.67	0.418
	Farm(B)	2	45.416	22.708	2.52	0.090
	AxB	2	65.751	32.875	3.65	0.033
Fruit Col C	Season(A)	1	62.994	62.994	18.18	<.0001
	Farm(B)	2	42.736	21.368	6.17	0.0039
	AxB	2	62.988	31.494	9.09	0.0004

Fruit Col H	Season(A)	1	86.296	86.296	8.99	0.0041
	Farm(B)	2	497.288	248.644	25.89	<.0001
	AxB	2	69.296	34.648	3.61	0.0338
Fruit Col a	Season(A)	1	103.233	103.233	15.88	0.0002
	Farm(B)	2	199.413	99.706	15.34	<.0001
	AxB	2	52.131	26.065	4.01	0.0238
Fruit Col L	Season(A)	1	72.151	72.151	12.89	0.0007
	Farm(B)	2	66.933	33.463	5.98	0.0045
	AxB	2	222.006	111.003	19.83	<.0001
Arl Col C	Season(A)	1	33.111	33.111	18.04	<.0001
	Farm(B)	2	290.973	154.486	79.28	<.0001
	AxB	2	62.465	31.232	17.02	<.0001
Arl Col H	Season(A)	1	0.291	0.291	0.11	0.7369
	Farm(B)	2	14.496	7.248	2.83	0.0676
	AxB	2	15.108	7.554	2.95	0.0607
Arl Col a	Season(A)	1	28.007	28.007	17.75	<.0001
	Farm(B)	2	228.754	114.377	72.47	<.0001
	AxB	2	47.742	23.871	15.13	<.0001
Arl Col L	Season(A)	1	38.360	38.360	11.99	0.0011
	Farm(B)	2	269.637	134.818	42.15	<.0001
	AxB	2	53.307	26.653	8.33	0.0007

Appendix C: Turnitin report

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Appendix D: Abbreviation and acronyms used

FW	= Fruit weight
FL	=Fruit length
Fw1	=Fruit weight
L*	=Approximate measurement of luminosity
a*	=Positive value for reddish colour
C*	=Quantitative attributes or Chroma
h*	=Qualitative attributes or Hue angle
TSS	=Total soluble solids
pH	=A measure of acidity or basicity
TA	=Titratable acidity
TP	=Total phenols
