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The effect of maturity, sunburn and the application of sunscreens on the internal and external qualities of pomegranate fruit grown in Australia

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ABSTRACT

Consumer interest in pomegranate fruit (*Punica granatum* L.) is increasing in Australia as a result of its unique external and internal qualities. This work looked at the effect of applying sunscreen treatments to pomegranate fruit on the degree of sunburn damage and the effect of maturity and sunburn on the internal antioxidant concentration of the juice. The pomegranates, cultivar 'Wonderful' were grown in Condobolin, New South Wales, Australia.

The Kaolin based sunscreens Surround® and Parasol® significantly reduced the severity of sunburn damage but treatment with Anti-stress-500® did not. There was no significant affect of the sunscreen treatments on the total phenolic content or total antioxidant activity of the juice. However sunburn damage did significantly reduce both bioactive parameters in the juice. The results indicate that the sunburnt pomegranate fruit did not have the capacity to prevent oxidative stress as indicated by the visual damage and the reduced pool of soluble antioxidants in the juice. Total phenol content and the total antioxidant activity of the juice also decreased significantly during fruit growth and maturity.

More work is needed to determine the affect of sunscreen treatments on the internal quality of pomegranate juice grown under milder conditions. This work highlights how the physiological response of fruit to the environment impacts fruit quality both externally and internally.

1. Introduction

The consumption of pomegranate fruit (*Punica granatum* L.) juice has been reported to have many positive health benefits (Balasundram et al., 2006; Mertens-Talcott et al., 2006; Syed et al., 2007; Basu and Penugonda, 2009). The benefits are largely reported to be the result of the high level of antioxidant capacity of the juice (Ricci et al., 2006). The principle antioxidant polyphenols in pomegranate juice include the ellagitannins and anthocyanins. The concentration of these compounds in the juice has been reported to vary depending on the method of juice extraction, the cultivar and the stage of maturation and ripening (Basu and Penugonda, 2009; Mousavinejad et al., 2009; Shwartz et al., 2009).

Pomegranates are a new crop in Australia. The new industry will provide both fresh fruit and fruit processed for juice. Around the world pomegranates are grown in Mediterranean climates often with very warm summers. In this work the pomegranates were grown in Condobolin which is located in Western New South Wales where summer temperatures can be over 40 °C for several days at a time (http://www.bom.gov.au/climate/dwo/IDCJDW2032.latest.shtml). These high temperatures can cause sunburn damage to the outside skin of the fruit making then unsaleable. Melgarejo et al. (2004) have shown that the use of Kaolin sunscreen treatments can significantly reduce sunburn damage. Similar results have been reported for apples (Wand et al., 2006; Gindaba and Wand, 2007).

Our work looked at the effect of applying sunscreen treatments to pomegranate fruit on the degree of sunburn damage. We also looked at the effect of sunburn on the internal antioxidant concentration of the juice. Both factors are important in terms of fruit quality.

High temperature stress in plants results in the production of reactive oxygen species (ROS) which cause oxidative stress (Ma et al., 2008). Plants protect themselves from the cytotoxic effects of the active oxygen species by antioxidant enzymes or metabolites such as glutathione, ascorbic acid and carotenoids which may scavenge reactive oxygen (Sairam et al., 2000; Ma et al., 2008). In pomegranate fruit protective antioxidant metabolites include

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polyphenols such as ellagitannins, tannins, anthocyanins and flavonoids (Mertens-Talcott et al., 2006; Mousavinejad et al., 2009). The level of the antioxidant metabolites in plants changes in response to abiotic stress. For example, heating apple leaves from 28 to 40 °C increased the content of total ascorbate, ascorbic acid, total glutathione and glutathione initially but after a high temperature exposure of more than 2 h the contents of these antioxidant compounds declined (Ma et al., 2008). After 4 h at 40 °C the antioxidant content decreased to below that of the control leaves kept at 28 °C. Furthermore, in sunburnt apple peel an up-regulation of the antioxidant system in response to the increased reactive oxygen species generated by the high light and high temperature exposure was shown (Chen et al., 2008). However, the researchers concluded that the up-regulation in response to photooxidative stress was not enough to protect against the photooxidative damage and hence the resulting visual sunburn damage.

The aim of this work was to evaluate the effectiveness of three commercial sunscreen treatments Parasol® (Crop Care, Australia), Surround® (Ag Nova Tech., Australia) and Anti-stress-500® (EnviroShield Products Co., USA) for preventing sunburn damage of pomegranate fruit grown in Condobolin, New South Wales, Australia. The experiment also looked at the effect of maturity and external sunburn on the antioxidant content of the fruit juice.

2. Materials and methods

The experiments were carried out on a commercial orchard at Condobolin, New South Wales, Australia over 2 seasons. In both seasons the fruit was harvested from 3-year-old trees of the pomegranate cultivar 'Wonderful' at 2 m × 4 m spacing and managed using best commercial practice. In the 2008/09 season two north–south oriented rows were used for two different experiments. During the summer the mean daytime temperature ranged from 20 to 45 °C in both seasons. Hence, fruit growing in the outer canopy were exposed to temperatures above 35 °C temperatures for at least a few hours per day during week 4–18 after fruit set (http://www.bom.gov.au/climate/dwo/IDCJDW2032.latest.shtml).

2.1. Fruit growth and quality trials

In the first season 200 fruits were harvested on 24 March 2008 and brought back to the laboratory at the University of Sydney overnight and were subsequently separated into two maturity groups, mature and immature, based on fruit background colour and size. In the 2008/09 season data fruit was collected at regular intervals from November 2008 to harvest 8 April 2009. In this trial 6 blocks of 12 trees were used. Fruits were tagged 2 weeks after fruit set and these fruits were subsampled by harvesting 10 fruits per block every 2 weeks from 2 to 23 weeks after fruit set and these fruits were brought back to the laboratory at the University of Sydney overnight.

The assessment of fruit growth and quality was the same for 2008 and 2009. In the laboratory all the fruits were hand peeled and the husks where carefully cut at the equatorial zone with a sharp knife. Then the arils were manually extracted, weighed and processed with a commercial blender (Woolworths®, Australia). Then the blended extract was manually squeezed through two layers of gauze to extract the juice. For all fruit diameter, mass, mass of arils and volume of juice were recorded.

A subsample of juice of 5 fruits per maturity were weighed and put into individual polypropylene conical tubes with capacity for 50 mL (Falcon®). These samples were used for the measurement of total soluble solids (%TSS), antioxidant capacity and total phenolic content.

The %TSS was determined using a hand held refractometer (N 63124, Atago Co., Japan) and circumference was measured with a plastic 30 cm-tape measure and mass determined using an electronic balance (PM1200, Mettler-Toledo GmbH, Giessen, Germany).

The juice samples were frozen at −20 °C prior to analysis of antioxidant activity and total phenolic content.

2.2. Total phenolic content and antioxidant activity assays

The total phenolic content was analysed using the Folin–Ciocalteu method with gallic acid as the standard (Velioglu et al., 1998). The antioxidant activity of fruit juice was determined as the Ferric Reducing Ability of Plasma (FRAP) at low pH, based on the intense blue colour formation when Fe^{3+}–TPTZ complex is reduced to the ferrous form (Benzie and Strain, 1996). For the determination of the level of the total phenolic content and FRAP in the 2007/08 season a microplate spectrophotometer (Multiskan RC; Pathtech, Preston, Vic., Australia) was used and in the 2008/09 season a multiwell photometric plate reader (Bioscreen C, Labsystems Corp., Helsinki, Finland) was used in both cases the readings were taken at 600 nm.

2.3. Sunscreen trial

Three sunscreen treatments were evaluated in the 2008/09 season. In this trial, laid out as a completely randomized bock design, there were 3 replicates of 12 trees per treatment. The sunscreen treatments were hand sprayed using a backpack sprayer to run-off to eight plants in the middle of each plot, keeping a two-plant wide buffer between plots.

The sunscreen treatments Parasol® (40 mL L^{-1}, active ingredient $CaCO_3$; Crop Care, Australia), Surround® (60 g L^{-1}, active ingredient Kaolinite; Ag Nova Tech., Australia) and Anti-stress-500® (14 mL L^{-1}, Acrylic copolymer, EnviroShield Products Co., USA) were applied at rates recommended by the manufacturers. Parasol® and Surround® left a white powdery residue on the fruit and foliage after each application while the Anti-stress-500® treated trees had no visible residue.

At harvest for the sunscreen trial all fruits on the trees were harvested and rated for the level of external damage due to sunburn. Each fruit was visually rated into three groups depending on the area of the fruit surface that was damaged due to sunburn. The groups were <10% of the area affected by sunburn (minimum), 10–50% of the fruit surface area affected by sunburn (mild) and >50% of the fruit surface area affected by sunburn (severe). In addition five fruits were taken from each treatment replicate and another five fruits from each damage category from the untreated control replicated plots. The juice from these fruit was extracted as described previously for the total phenolic content and total antioxidant activity.

2.4. Statistical analysis

For all the parameters measured, either a regression analysis or an analysis of variance (ANOVA) was performed and least significant differences (5%) calculated using the general analysis of variance procedure in GenStat® statistical software (10th edition, version 10.1.0707, Lawes Agricultural Trust, supplied by VSN International Ltd.). In all cases data was checked for normality, transformed where required before analysis and back transformed for presentation.

3. Results and discussion

3.1. Fruit growth and quality

Fruit growth in 2008/09 in Condobolin showed that the increase in fruit mass was linear with the fruit reaching an average