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Maturity, storage and ripening effects on anti-fungal compounds in the skin of 'Hass' avocado fruit

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ABSTRACT

Fruit rots are a major risk to the quality of avocado fruit in international trade. For New Zealand-grown 'Hass' fruit, rots become increasingly problematic later in the harvest season. The presence of antifungal compounds, and associated compounds, in avocado fruit skin has been long documented, with much of the research done on fruit of the cultivar 'Fuerte'. In this paper, results are presented from an investigation of the effect of fruit maturity, storage and ripening on the concentration of the antifungal compound persin and compounds associated with its metabolism in the fruit skin of New Zealand grown 'Hass' avocado fruit. The persin concentration decreased markedly during maturation, with a ~ 30% decrease in total persin (the combined persin and persenone-A), from ~ 600 mg kg^{-1} at the early harvest to ~ 400 mg kg^{-1} at the late harvest, approximately 4 months later. The concentration also decreased during storage and ripening, although the degree of change was dependent on the concentration present at harvest. Irrespective of harvest maturity or storage, the concentration of persin in the skin of ripe fruit did not decline below ~ 200 mg kg^{-1}. The epicatechin concentration declined by ~ 17% and catechin by ~ 50% between the early and late harvests. The total (*epi*)-catechin (the combined epicatechin and catechin monomers, epicatechin dimer B2, and (*epi*)-catechin oligomers) concentration declined by ~ 20% between the early and late harvests, from ~15.4 g kg^{-1} to 12.5 g kg^{-1}. The changes with storage and / or ripening were of a smaller magnitude than the change with maturation. Also, the total (*epi*)-catechins concentration tended to increase slightly during storage. The findings are discussed in the context of the risk of rots in late season New Zealand-grown 'Hass' fruit based on previously described associations between changes in skin composition with fruit rot development.

1. Introduction

The New Zealand avocado industry is based on producing fruit of the 'Hass' cultivar for export. In comparison with some growing regions in the world, New Zealand fruit are slow to accumulate oil, meaning that fruit may take 10–12 months to reach an acceptable oil content for harvest and may remain on the tree for up to 16 months for marketing purposes. This contrasts with other producer regions where fruit may be ready for harvest after 7–9 months. Fruit rots are a major quality risk for New Zealand fruit, in particular in late season fruit harvested more than 12 months after flowering (Dixon et al., 2004; Burdon et al., 2013). While the high rot incidence may be in part due to the time fruit are on the tree, the humid temperate environment in New Zealand may also play a role.

For New Zealand-grown 'Hass' fruit, rots generally occur from three fungal genera: *Colletotrichum*, *Botryosphaeria* and *Phomopsis* (Hartill, 1991). These rots occur either at the harvest scar, where the fruit was cut or snapped from the tree, or through the skin on the body of the fruit. The inoculum sources and infection pathways of these two categories of rot have been investigated (Hartill and Everett, 2002). While the pathogens may be present on or in the fruit at harvest, rots tend to only express once the fruit ripens.

The germination and growth of latent rots of *Colletotrichum gloeosporioides* has been associated with the lack of antifungal compounds in the avocado fruit skin (Prusky et al., 1982, 1991a). A range of antifungal compounds have been identified from avocado skin, the most active of which has been identified as (*Z,Z*)-1-acetoxy-2-hydroxy-4-oxoheneicosa-12, 15-diene (Prusky et al., 1982; Adikaram et al., 1993). This compound is referred to as an anti-fungal diene and is also known as persin. The concentration of persin in the fruit skin has been shown to decline with ripening, coincident with the quiescent fungal infections becoming active and the development of *C. gloeosporioides* rots (Prusky

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et al., 1983). It has therefore been suggested that the latency of *C. gloeosporioides* rots in avocado may be mediated by the concentration of persin.

The decrease in persin that occurs during ripening has been suggested to be the result of lipoxygenase activity (Prusky et al., 1983). The activity of lipoxygenase was shown to be mediated by the presence of epicatechin which inhibits avocado lipoxygenase (Prusky et al., 1985; Karni et al., 1989; Prusky et al., 1990; Leikin-Frenkel and Prusky, 1998; Ardi et al., 1998). The epicatechin concentration in the skin declines during ripening (Prusky and Keen, 1993), thereby reducing the inhibitory effect on the lipoxygenase that breaks down persin.

The overall model is therefore one of persin preventing latent infections from progressing until the concentration of persin has declined through lipoxygenase activity, which can only occur if there is insufficient inhibitor of lipoxygenase (epicatechin) present. Thus a high concentration of epicatechin is needed to maintain the persin concentration in the skin (Ardi et al., 1998).

Much of the anti-fungal diene research has been conducted on fruit of the cultivar 'Fuerte'. However, in a comparison of mature fruit of a range of cultivars, diene concentrations were shown to differ among the cultivars (Prusky et al., 1982). The diene concentration in the skin of fruit of 'Fuerte' was 925 mg kg^{-1} whereas 'Hass' was 594 mg kg^{-1}, both calculated on a fresh weight basis (Prusky et al., 1982). These concentrations are intermediate for the cultivars examined, with extremes of 203 mg kg^{-1} for 'Nabal' and 4412 mg kg^{-1} for 'McArthur' (Prusky et al., 1982).

In 'Fuerte', a decrease in persin was reported from 1820 mg kg^{-1} in early harvested fruit to 950 mg kg^{-1} in late harvested fruit (Wang et al., 2006), although no definition of early or late was provided. However, it does demonstrate that the persin concentration in the fruit skin may not be static within a single cultivar and may change with fruit maturation.

In this paper, results are presented from an investigation of the effect of fruit maturity, storage and ripening on the concentrations of persin, epicatechin and related compounds in the fruit skin of 'Hass' avocado fruit. The findings are discussed in the context of the risk of rots in 'Hass' fruit.

2. Materials and methods

2.1. Fruit

Fruit were sourced from three mature (~30 years old) trees of 'Hass' on 'Duke 7′ rootstocks growing at the Te Puke Research Orchard of Plant & Food Research in the Bay of Plenty, New Zealand. Fruit were harvested at approximately 11, 12 and 14 months post-flowering, between early October 2015 and mid-January 2016. These three harvests (designated Harvest 1, Harvest 2, and Harvest 3) are termed early, mid and late harvests with the fruit having average dry matters of 28.9%, 30.6% and 36.9%, respectively. At each harvest, 16 fruit were taken from each of three trees and transported immediately to the Plant & Food Research laboratories in Auckland.

2.2. Treatments

On receipt, ~ 4–5 h after harvest, fruit were divided into four lots, each of 12 fruit, maintaining the tree identity. Each lot of fruit was allocated to one of four treatments designated as unripe (UR) or ripe (R), without or with storage. The non-stored UR sample was assessed immediately, whilst the R sample was left to ripen at 20 °C and fruit were assessed when ripe according to the feel of the fruit in the hand (equivalent to ~15 N by flat plate compression test). The stored UR and R samples were placed into commercial packaging and stored at 5 °C for 28 d. At the end of storage, the UR sample was assessed immediately out of store whilst the R sample fruit were left to ripen at 20 °C before assessment.

2.3. Assessment methodologies

Fruit firmness was measured using a Fruit Texture Analyser (GÜSS, model GS14, South Africa) fitted with a flat plate. The Fruit Texture Analyser settings were: approach speed (forward) 5 mm s^{-1}; trigger force 30 gf; measure speed 5 mm s^{-1}; measure distance 2 mm; return speed 40 mm s^{-1}. Firmness was measured twice at the equator of each fruit, with the two measurements taken at 90° to each other, with the values averaged to give a mean fruit firmness value. Firmness was measured as kgf and converted to N, where 1 kgf = 9.81 N.

Fruit were assessed for skin colour immediately after removal from storage and also when ripe using a 0–100 scale, where 0 = bright glossy green, 30 = olive green, 60 = wood brown, 80 = purple brown and 100 = dull black (New Zealand Avocado Industry Council Fruit Assessment Manual; Dixon, 2003).

Flesh dry matter content was determined on two 18 mm diameter core samples taken at 90° to each other from the widest part of the fruit. The skin, seed coat and seed were removed and discarded and the four pieces of fruit flesh sliced into ~1 mm discs, dried at 65 °C for 24 h and then re-weighed. The dry matter is expressed as the percentage mass remaining after 24 h drying.

2.4. Chemical analysis

2.4.1. Sample extraction

Fruit were sampled individually, with the skin removed with a potato peeler from unripe fruit, or by peeling ripe fruit, and chopped and frozen immediately in liquid nitrogen. Only sound skin free from defects and rots was sampled. The frozen tissue was ground and stored at −80 °C until further analysis. A representative subsample (1 g) was extracted with 95% ethanol (5 mL) by shaking (20 min) followed by sonification (20 min). The sample was centrifuged (1000 × g, 15 min, 20 °C) and the ethanol layer transferred to a pre-weighed centrifuge tube. The extraction was repeated and the ethanol layers were combined and their total weight recorded. For quantitative analysis of common plant flavonoids and phenolic acids, an aliquot (0.5 mL) of the ethanolic solution was evaporated to dryness under nitrogen and the sample reconstituted in 10:90 methanol/water (1 mL). Following centrifugation (13,400 × g, 10 min, 4 °C), the supernatant was transferred to an autosampler vial for ultra-high performance liquid chromatography (UHPLC) analysis.

For quantitative analysis of persin and its related triene (*E,Z,Z*-1-acetoxy-2-hydroxy-4-oxoheneicosa-5,12,15-triene; persenone-A; Domergue et al., 2000), the remaining combined ethanolic extraction solution was reweighed, then evaporated to just dry under nitrogen. The residue was solubilised in a solution of acetonitrile/$salts_{(aq)}$ (magnesium sulphate 1.3 g, sodium chloride 0.3 g) 7:4 v/v mL by shaking (20 min). The mixture was centrifuged, an aliquot (1.5 mL) of the acetonitrile layer was evaporated to dryness under nitrogen and the residue reconstituted in 95% ethanol (1 mL). Following centrifugation (13,400 × g, 10 min, 4 °C), the supernatant was transferred to an autosampler vial for UHPLC analysis. Recorded weights (skin tissue, extraction solution) were used to accurately calculate the amount of sample for each analysis.

2.4.2. Ultra-high performance liquid chromatography analysis and analyte quantification

Persin and persenone-A levels were determined using a Dionex UltiMate 3000 Series UHPLC (ThermoFisher Scientific, San Jose, CA, USA) with photodiode array (PDA) detection at 205 nm, bandwidth 2 nm. Compound separation was achieved using an Accucore C18 2.6 µ column, 2.1 x 150 mm (Thermo Scientific, Waltham, Massachusetts USA), maintained at 35 °C. Solvents were (A) 5:95 acetonitrile:water + 0.1% formic acid v/v and (B) acetonitrile + 0.1% formic acid and the flow rate was 0.42 mL min^{-1}. The initial mobile phase, 32% A was held for 11 min, followed by a column flush at 80% B before resetting to the original conditions. Sample injection volume was 2 µL. Persin was