Ripening and development of chilling injury in persimmon fruit: an electrical impedance study

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Abstract  Electrical impedance spectroscopy was used to follow ripening and chilling injury development in persimmon fruit (Diospyros kaki L. ‘Fuyu’). Tissue resistance and reactance were measured at frequencies between 50 Hz and 1 MHz, and then fitted to an electrical model. Fruit responses to both ripening at 20°C and storage in modified atmosphere at 7°C were distinct and easily detected using electrical impedance spectroscopy. Plots of reactance against resistance at each series of frequencies traced a semicircular arc. During ripening, the arcs dilated between Days 1 and 21, then contracted, until at Day 35 they were smaller than at Day 1. Electrical modelling indicated that the dilation occurred as a result of a 43, 115, and 17% increase in resistance $R_1$ (cell wall resistance), $R_2$ (cytoplasm resistance), and $R_4$ (vacuole resistance), respectively. After 35 days of ripening, $R_1$ was 39% lower and $C_3$ (membrane capacitance) was 19% higher than at Day 1. Chilling injury developed with increasing time at 7°C in modified atmosphere storage (MA), until severe symptoms were observed after 5 weeks. Chill-injured fruit differed from other fruit in that $R_2$ was significantly lower upon removal from storage, although it rapidly increased when fruit were transferred to 20°C for ripening. These results are discussed in relation to the physiological changes that occur during ripening and development of chilling injury in persimmon.

Keywords  persimmon; Diospyros kaki; fruit ripening; chilling injury; electrical impedance

INTRODUCTION
Measurements of electrical impedance have been used to determine physiological condition of vegetative and woody tissues, particularly in relation to cold acclimation and freezing injury (Greenham & Daday 1957; Stout 1988; Privé & Zhang 1996), dormancy induction (Colombo & Blumwald 1992), nutritional deficiency (Greenham et al. 1972), and heat injury (Zhang et al. 1993). In fruit, electrical impedance measurements have been used to determine internal condition and maturity (Weaver & Jackson 1966; Zachariah 1976; Furmanski & Buescher 1979; Lougheed et al. 1981). Generally, electrical impedance measurements detect rapid changes (within hours) associated with physiological dysfunction and membrane damage (e.g., freezing injury; Zhang & Willison 1992), or changes that occur over a longer period and are associated with a positive response of the plant (e.g., seasonal changes associated with cold acclimation; Privé & Zhang 1996). In contrast, when fruit are removed from low temperature storage they undergo a series of changes including: recovery of physiological function, initiation of ripening, senescence, and cell dysfunction (Brady 1987). These changes can occur within a short period, and thus might be expected to provide a unique biological system for demonstrating the interaction between physiological condition and electrical properties of plant tissues.

Electrical models are able to partition complex impedance into resistances and capacitances of cell compartments and membranes (Zhang et al. 1990; Zhang & Willison 1991; Repo & Zhang 1993). Using this approach Harker & Maindonald (1994) were able to simultaneously assess changes in the condition of the cell wall, cell membranes, and intracellular compartments during ripening of nectarine. They found that during normal ripening
the resistance of the cell wall and vacuole declined by 60 and 26% respectively, and capacitance of the membranes decreased by 9%. An additional resistance component, which declined by 63% during ripening, was required to model the impedance data. Harker & Maindonald (1994) speculated that this was the resistance of the membranes. A number of studies have demonstrated that electrical impedance measurements are able to differentiate between ripening of nectarines and peaches with and without chilling injury (Furmanski & Buescher 1979; von Mollendorff et al. 1992; Harker & Maindonald 1994).

This study aimed to further investigate the ability of electrical impedance measurements to provide useful and physiologically relevant insights into fruit ripening. Persimmon (*Diospyros kaki* L. 'Fuyu') were selected on the basis that they have markedly different ripening and textural characteristics to nectarine, although also developing chilling injury. Persimmon are generally divided into astringent and non-astringent cultivars depending on whether they have lost their astringency by the time of harvest (Kitagawa & Glucina 1984). Our study focuses on the non-astringent cultivar ‘Fuyu’ which has unique ripening characteristics, having a long shelf-life. ‘Fuyu’ can develop chilling injury when stored at temperatures <15°C (Beede 1983; Kader 1996). This injury can be alleviated by storage under modified atmosphere at 0°C (MacRae 1987). Chilling injury is initially expressed in the form of a gel developing within the flesh, and then later by the darkening of the fruit and increased skin transparency through which the characteristic gel may be seen (MacRae 1987).

**MATERIALS AND METHODS**

**Fruit**

Export quality, select grade persimmons (*Diospyros kaki* L. ‘Fuyu’) weighing c. 200 g, brix 14%, were harvested on 14 May 1994 from a commercial orchard in Pukekohe, Auckland, New Zealand. The fruit remained at ambient temperatures within the packhouse for 2 days and were then collected and held for a further day in the laboratory at 20°C, before being divided between two treatments. In one treatment, fruit were packed in commercial trays and ripened at 20°C for 35 days. In the other treatment, fruit were packed in commercial trays, each tray was placed into modified atmosphere low density polyethylene bags, 60 µm thickness and the bag was sealed using a heat sealer. Modified atmosphere packaging (MA) was used as a means of delaying the development of chilling injury at 7°C and thus providing an extended assessment period.

**Quality assessment**

At weekly intervals, fruit were removed from MA, and transferred to a 20°C ripening room to allow symptoms of chilling injury to develop. These fruit were evaluated after 1, 3, and 6 days, and scored for chilling injury using a scale of 0–5, where 0 indicates no chilling injury and 5 indicates severe chilling injury (MacRae 1987). Fruit that were stored at 20°C after harvest, were assessed at weekly intervals. At each assessment 10 fruit were examined. Fruit firmness was measured using a materials testing machine (Instron, model 4301, Canton, United States) to drive a 7.9 mm diameter probe into the flesh at a speed of 240 mm/min. A sample of tissue 5 x 10 x 50 mm was then removed from the flesh for impedance measurements. Five of the fruit were assessed for impedance characteristics using 14 frequencies ranging from 50 Hz to 1 MHz, whereas the remaining five fruit were examined at frequencies of 50 Hz and 300 KHz.

**Electrical impedance measurements**

Methods for measuring electrical impedance have been described previously (Harker & Dunlop 1994; Harker & Maindonald 1994). In brief, a block of tissue was excised from the flesh along the fruit equator, and placed on an array of five silver wire electrodes. Alternating current at frequencies between 50 Hz and 1 MHz were passed along the length of the tissue, and impedance was measured at interelectrode distances of 10, 20, and 30 mm. These measurements were then converted into tissue and electrode resistance and reactance using methods and formulae described by Zhang & Willison (1991). Only tissue resistance and reactance were considered further.

**Modelling of tissue impedance**

The values for tissue resistance and reactance were fitted to a series of mathematical models using S-PLUS functions described by Harker & Maindonald (1994). The primary model (Model A) was proposed by Zhang & Willison (1990), and is described by the circuit diagram shown in Fig. 1. Additional
models (Model B and Model C) were proposed by Hayden et al. (1969) and Zhang et al. (1990), respectively. Models A, B, and C were previously used to model ripening of nectarines (Harker & Maindonald 1994). Each model was used to separate tissue impedance into the resistive and reactive components associated with cell structures. For example, Model A (Fig. 1) provides values for cell wall resistance ($R_1$), cytoplasm resistance ($R_2$), vacuole resistance ($R_4$), plasma membrane capacitance ($C_3$), and tonoplast capacitance ($C_5$). Values for $C_3$ and $C_5$ were set as equal during modeling. Justification for this was indicated earlier (Harker & Maindonald 1994).

RESULTS

Ripening at 20°C
The fruit took 35 days to soften (Fig. 2) and did not develop chilling injury. Electrical impedance data was summarised by plotting tissue reactance against tissue resistance for each of the frequencies tested (Fig. 3). The resulting plot traced a semicircular arc which dilated during the first 21 days of ripening, and then contracted with further ripening, until after 35 days the arc was smaller than at harvest (Fig. 3). This pattern of development was confirmed by plots of average resistance at 50 Hz or 300 kHz against time for all 10 fruit examined at each sampling period (Fig. 4). Resistance at low frequencies (in this instance 50 Hz) reflects the properties of extracellular regions of the tissue, whereas resistance at high frequencies (in this instance 300 kHz) reflects the properties of all tissue compartments (Cole 1972). Fig. 4A indicates that the resistance at 50 Hz increases from 17 471 ohms at harvest, to 25 305 ohms after 21 days, and then declines to 12 558 ohms after 35 days ripening. In comparison, the resistance at 300 kHz varied between 164 and 230 ohms (Fig. 4C) and was not thought to indicate any marked physiological change.

Detailed impedance characteristics were collected for five fruit at each sampling time. This data was fitted to mathematical Models A, B, and C (Fig. 1). Decisions as to the most relevant model to use were based on the amount of data variation accounted for by the model (assessed using the sums of squares of the residuals) and the complexity of the model. The sum of squares of the residuals for each model were on average: 1550 for Model A, 3471 for Model B, and 1527 for Model C. Clearly, Models A and C were superior to Model B when fitting the data. Model C was a more elaborate version of Model A, and since it did not markedly
Fig. 2 Flesh firmness and severity of chilling injury in persimmon (*Diospyros kaki* L. 'Fuyu') fruit during storage and ripening. Values represent means ± SE for a sample of 10 fruit. Flesh firmness is presented for fruit ripened at 20°C for up to 35 days (C); and stored at 7°C in Modified Atmosphere (MA) packaging and then assessed after 1 day at 20°C (●, - - -), and ripened for a further 6 days at 20°C (○, - - -). Chilling injury is presented for fruit stored at 7°C in MA and then assessed after 3 days at 20°C (∆, - - -), and was assessed using a scale of 0–5, where 0 = no injury and 5 = severe injury. Fruit held at 20°C throughout the experiment did not develop any chilling injury.

Fig. 3 Plots of reactance against resistance for individual tissue blocks excised from persimmon (*Diospyros kaki* L. 'Fuyu') fruit after 1 (○), 21 (∆), and 35 (□) days ripening at 20°C; and in a tissue block that had been frozen and then thawed (■). The median of five curves was selected as typical of each specific ripening stage.

improve fitting, there was little reason to use it. Thus, Model A (Fig. 1) was used in all subsequent analysis. Table 1 indicates the changes in the resistances and capacitances of sub-cellular components at harvest, after 21 days ripening, and after 35 days ripening. R<sub>1</sub>, R<sub>2</sub>, and R<sub>4</sub> increased between Days 1 and 21, then declined between Days 21 and 35. C<sub>3</sub> increased throughout the 35-day ripening period. Although the results were significant according to Fishers' LSD (5% level; see Table 1), they were of marginal significance or not significant when Tukey's LSD was used. Thus, although a significant increase in tissue resistance was detected at 50 Hz (Fig 4A), the process of electrical modelling tended to reduce the significance of these changes. This may reflect an increase in variability introduced by the modelling process and/or the smaller number of fruit used for modelling. However, the transient increase in tissue resistance is clearly evident in the raw data.
Fig. 4  Changes in tissue resistance at: A and B, 50 Hz; or C and D, 300 kHz of persimmon (Diospyros kaki L. ‘Fuyu’) fruit ripened at 20°C for up to 35 days (■); stored at 7°C in Modified Atmosphere packaging and assessed after 1 day at 20°C (○, - - -), and ripened for a further 3 and 6 days (○, ——). Each point represents the mean ± SE for a sample of 10 fruit.

Table 1  Changes in cell wall resistance (R₁), cytoplasm resistance (R₂), vacuole resistance (R₄), and membrane capacitance (C₃) during storage (7°C and modified atmosphere (MA)) and ripening (20°C) of persimmon (Diospyros kaki L. ‘Fuyu’) fruit. Each value represents the mean ± SE of five fruit. Within a column, means marked with the same letter are not significantly different (5% level, Fishers’ LSD).

<table>
<thead>
<tr>
<th>Storage treatment</th>
<th>Ripening (days at 20 °C)</th>
<th>R₁, cell wall (ohms)</th>
<th>R₂, cytoplasm (ohms)</th>
<th>R₄, vacuole (ohms)</th>
<th>C₃, membrane (nF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td>16762 ± 416 b</td>
<td>7596 ± 556 bc</td>
<td>435 ± 18 bc</td>
<td>3.96 ± 0.15 abc</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>24032 ± 658 c</td>
<td>16356 ± 1060 d</td>
<td>494 ± 38 c</td>
<td>4.38 ± 0.16 cde</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>10221 ± 1645 a</td>
<td>7102 ± 1202 abc</td>
<td>377 ± 38 ab</td>
<td>4.71 ± 0.07 d</td>
</tr>
<tr>
<td>3 weeks MA at 7°C</td>
<td>1</td>
<td>15940 ± 2630 ab</td>
<td>6535 ± 3056 ab</td>
<td>325 ± 61 a</td>
<td>3.47 ± 0.18 ab</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>19024 ± 892 bc</td>
<td>12041 ± 1959 bc</td>
<td>436 ± 21 bc</td>
<td>4.84 ± 0.40 e</td>
</tr>
<tr>
<td>5 weeks MA at 7°C</td>
<td>1</td>
<td>16088 ± 1468 abc</td>
<td>3149 ± 226 a</td>
<td>338 ± 9 ab</td>
<td>3.41 ± 0.06 a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>14608 ± 662 ab</td>
<td>5174 ± 1657 ab</td>
<td>319 ± 21 a</td>
<td>4.08 ± 0.29 bcd</td>
</tr>
</tbody>
</table>
Storage at 7°C and subsequent ripening behaviour

During storage at 7°C, the atmospheres in the MA packaging reached 5.2 ± 0.1 kPa CO₂ and 4.8 ± 0.2 kPa O₂ after 1 week; and 7.5 ± 0.3 kPa CO₂ and 3.4 ± 0.3 kPa O₂ after 5 weeks (means ± SE). Fruit were removed from MA at weekly intervals and the subsequent changes in firmness are described in Fig. 2. Chilling injury became progressively worse during storage at 7°C in MA, until symptoms of severe injury were observed after 5 weeks (Fig. 2). During storage and the subsequent ripening period, the resistance at 50 Hz was relatively constant at c. 18 000 ohms, and did not show the dramatic changes observed when fruit were held at 20°C throughout ripening (Fig. 4). Resistance at 300 kHz showed no particular pattern of change during MA storage or subsequent ripening varying between 171 and 241 ohms (Fig. 4). However, modelling of the data, indicated that R₂ (cytoplasmic resistance) and C₃ declined during storage, but recovered when fruit were returned to 20°C (Table 1).

To investigate the effect of chilling injury symptoms on the modelled subcellular components R₁, R₂, R₄, and C₃, it was necessary to compare fruit of similar ripeness. This was achieved by matching the firmness of fruit with and without chilling injury. The comparisons included fruit of two firmness categories (firm–ripe: 37–54 N; and soft–ripe: 14–25 N) as is presented in Table 2. The only difference likely to be of physiological relevance, was R₂ which was always lower in chill-injured fruit, although this was only statistically significant in one out of the four comparisons (Table 2).

DISCUSSION

Cell changes during ripening at 20°C

As fruit ripened, the low frequency resistance (50 Hz) of the tissue gradually increased (Days 1, 7,
14), with a marked increase occurring between Days 14 and 21, before declining again (Fig. 4A). Resistance at 50 Hz generally reflects the mobility of ions in the apoplast (Stout 1988), however modelling of the data indicated that the phenomena observed at 21 days represented changes in $R_1$ (cell wall), $R_2$ (cytoplasm), and $R_4$ (vacuole; Table 1). Such increases in resistance may reflect decreases in the concentration of mobile charged species or an increase in concentration of compounds with insulator properties such as sugars.

An earlier study on nectarines found that resistance at 50 Hz was correlated to changes in fruit texture, particularly firmness and apparent juice content (Harker & Maindonald 1994). In the present study, it is clear that electrical and mechanical properties of the cell wall-extracellular solution can change independent of each other. Resistance (50 Hz) of persimmons in MA did not change although considerable fruit softening was observed, and when resistance of fruit ripening at 20°C increased between Days 14 and 21, firmness remained unchanged (Fig. 2 cf. Fig. 4). Resistance (50 Hz) was, however, correlated with firmness for persimmons during the final stages of softening at 20°C (Days 21–35; $Y = 245X + 13641$, $r^2 = 0.71$, $P < 0.001$).

Resistance at high frequencies (in this instance 300 kHz) reflects the properties of the combined extracellular and intracellular compartments (Stout 1988). In fruit cells, this resistance mainly reflects the ionic content of the vacuole, which represents 90% of the cell volume and has high concentrations of ions (Harker & Maindonald 1994). The break down of compartmentation (e.g., by freeze thawing) results in low frequency current having access across the entire tissue. Thus, low and high frequency resistance of freeze-thawed tissue are similar to high frequency resistance of undamaged tissue (Fig. 3). The resistance at 300 kHz was not markedly influenced by storage temperature, MA, or ripening (Fig. 4).

Reactance of a plant tissue is related to the presence of membranes and their function as capacitors. Capacitance of the plasma membrane and tonoplast increased during ripening at 20°C both with and without prior MA treatments at 7°C (Table 1). This suggests that either the area of membrane or the specific capacitance of the membranes increases during ripening. An apparent increase in membranes within the tissue blocks might be expected as a result of water loss during ripening (i.e., as cells shrink—the number of cells per fixed tissue volume increases). Alternatively, an increase in the specific capacitance of membranes may have occurred. More detailed studies are required to characterise the actual cause of this increase in capacitance.

**Chilling Injury**

Storage at 7°C in MA tended to maintain $R_1$ (cell wall resistance) at a level similar to those measured at harvest, and although $R_4$ (vacuole resistance) and $C_3$ (membrane capacitance) were lower than at harvest they remained relatively constant throughout storage (Table 1). The value for $R_2$ declined from 7600 ohms at harvest to 3100 ohms after 5 weeks of storage (Table 1). Indeed, this low value of $R_2$ was the only component that was specifically associated with development of severe chilling injury, which only occurred after 5 weeks of storage. $R_2$ is usually described as the resistance of the cytoplasm (Zhang et al. 1990; Zhang & Willison 1991; Fig. 1). However, Harker & Maindonald (1994) suggest that changes in $R_2$ may reflect alterations in fruit membrane function. They argue that the $R_2$ component in the Zhang & Willison model is located in a position superficially similar to the component for membrane resistance in the Hayden model; that a contraction of an impedance arc is often interpreted as a decrease in membrane resistance; and that changes in membrane permeability are well known in fruit whereas changes in the cytoplasm are not well documented (Harker & Maindonald 1994). Irrespective of identifying $R_2$, low values seem to indicate that chilling injury has occurred in nectarine (Harker & Maindonald 1994) and also in the present study on persimmon. $R_2$ increased when persimmon were transferred to 20°C (Table 1). This suggests that persimmon were able to recover physiologically, although this did not lead to a reduction in symptoms. It may be that low values of $R_2$ indicate a generalised rundown of metabolic and repair mechanisms rather than being specifically linked to induction of chilling-injury symptoms. After 5 weeks at 7°C in MA, persimmons had severe chilling injury and $R_2$ did not recover when fruit were transferred to 20°C (Table 1).

Gelling of the flesh is one of the more notable symptoms of chilling injury in persimmon (MacRae 1987), and is associated with rapid and extensive solubilisation of pectic polymers which possess a higher $M_r$ and are more viscous than those solubilised during normal ripening (Grant et al.

We might expect the resistance of gelled tissue to be higher than in non-gelled tissue. In stonefruit, chilling injury results in a dry texture (woolliness) and a higher resistance of the cell wall (often measured as resistance at 50 Hz; Furmanski & Buescher 1979; von Mollendorff et al. 1992; Harker & Maindonald 1994). It is suggested that this high resistance is the result of cations becoming bound with demethylated pectin and forming a rigid gel within the wall (Furmanski & Buescher 1979). To avoid confusing the processes of fruit softening and development of chilling injury, it was necessary to ensure persimmons were of similar firmness. When this was done, all the modelled electrical parameters for chill-injured fruit were similar or lower than the resistances and capacitances for uninjured fruit of similar firmness (Table 2). These lower values for $R_1$, $R_2$, $R_4$, and $C_3$ probably reflect structural and physiological damage that had occurred. Formation of a gel within either the intracellular or extracellular compartments was expected to result in higher resistance. Since no such increase in resistance was observed in chill-injured fruit, we concluded that formation of a gel did not affect the electrical properties of the tissue. In this way, the gel that develops in chill-injured persimmon seems to differ from that occurring in stonefruit.

This study demonstrated the complex physiological changes that occur during storage and ripening of persimmon fruit. Many of these changes have not been described previously and are only apparent in the present electrical impedance study. Perhaps the most interesting fruit response, was the rapid and transient increase in resistance that occurred at about Day 21 during ripening at 20°C. This phenomenon, could be explained by transient reductions in concentration of free ions in the tissue (perhaps by binding to cell structures or by some form of chelation process), or by a transient increase in concentration of compounds with insulatory properties such as sugars. Similar abrupt changes in physiological condition have been observed in magnetic resonance imaging studies of persimmon, when a sharp increase in $T_1$ relaxation was observed between 14 and 21 days of storage at 7°C in MA (Clark & MacFall 1996). Such an increase in $T_1$ might reflect an increase in water or the aqueous nature of the tissue, a dilution of compounds in solution, or a decrease in viscosity (Clark pers. comm.). The explanation common to both electrical impedance and magnetic resonance imaging studies is that a dilution of charged compounds has occurred abruptly. However, the nature and cause of this phenomenon is unknown, emphasising the paucity of knowledge on persimmon ripening.

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