# Effect of preharvest UV-C treatment of tomatoes (*Solanum lycopersicon* Mill.) on ripening and pathogen resistance.

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## Abstract

Treatment with UV-C of tomato fruit on the vine was conducted using a mobile unit that was designed to be conveyed between the rows of tomato plants in a commercial glasshouse. Trusses of fruit both at the ripe and mature green phase were treated with UV-C doses of 3 and 8 kJ/m$^2$. Ripe fruit were picked 8 hours after treatment and kept at room temperature for periods of up to 16 days during which colour development and texture were monitored and compared to untreated controls. Mature green fruit treated on the vine with UV-C doses of 3 or 8 kJ/m$^2$ showed only a slight loss in green pigmentation in contrast to the tomato colour index (TCI) of the control fruit which increased sharply 5 days after treatment. The TCI of ripe fruit treated with UV-C at a dose of 8 kJ/m$^2$ showed a lag of 10 days before increasing to a final value that was comparable to that of untreated fruit. Fruit treated with a dose of 3 kJ/m$^2$ did not display a lag but the increase in TCI occurred at a lower rate than for the controls. Firmness remained higher in fruit treated with the highest UV-C dose compared to fruit treated with the lower UV-C dose and controls. Fruit covered with UV impermeable film on the same plants as those that had received a UV-C dose of 3 kJ/m$^2$ had become ripe by day 6 in a manner similar to that of the controls. By contrast, fruit from trusses adjacent to

those that had been treated with a UV-C dose of 8 kJ/m$^2$ remained green over the same period of time. Ripe fruit treated as described above were inoculated with spores of *Penicillium digitatum* after UV-C treatment and their firmness monitored over 12 days. A dose response effect was noted with the fruit treated at the highest dose remaining firmer than those treated at the lower dose and the controls.

Keywords: preharvest UV-C treatment; tomatoes; ripening; pathogen resistance

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## 1. Introduction

Treatment of tomatoes with short wavelength ultraviolet light or, 'UV-C', has been shown to have a number of benefits. These include delayed senescence, as manifested by the maintenance of both firm texture and green pigmentation, and induction of resistance against phytopathogens such as *Rhizopus stolonifer* and *Botrytis cinerea* (Liu et al., 1993; Maharaj et al., 1999; Barka et al., 2000; Stevens et al., 2004). UV-C treatment as used in the studies mentioned above is often referred to as 'hormetic' – that is, intended to induce in the fruit a metabolic response that arises as a result of the perceived abiotic stress and that, furthermore, is systemic. In previous studies this has been achieved by the application of relatively low UV-C doses i.e. typically less than 10 $kJ/m^2$. Hormetic UV-C treatment must be distinguished from what is commonly referred to as 'germicidal treatment' where the objective is primarily to inactivate micro-organisms that are present at, or near, the surface of a fruit, or indeed, any other horticultural commodity. Whilst the physiological responses to UV-C of a number of fruits and vegetables has been well characterised and described (Shama and Alderson, 2005), it cannot yet be claimed that the identity of all the phytochemicals induced by UV-C treatment has been achieved. Notwithstanding, it is known that in tomatoes, the response includes the synthesis of the glycoalkaloid tomatine (Stevens et al., 1998), the polyamine putrescine (Maharaj et al., 1999), pathogenesis-related proteins (Charles et al., 2009) and the carotenoid lycopene (Liu et al., 2009). In addition to the potential commercial benefits of treating fruit with UV-C are benefits to human health, as consumption of fresh foods having elevated levels of tomatine and lycopene have been implicated in the alleviation of a number of chronic health conditions (Friedman, 2002; Lindshield et al., 2007).

Despite the benefits, there has been an apparent reluctance to implement such hormetic UV-C treatment in the horticulture sector. The factors that need to be considered in achieving this

were discussed by Shama (2007). All previous applications of UV-C treatment were made postharvest. Moreover, the strategy that has been adopted by the majority of previous workers has been to ensure that, as much as possible, the entire surface of the fruit receives exposure to UV-C. In laboratory studies this has been achieved by manually rotating the fruit whilst it is situated within the UV-C field (Liu et al., 1993; Maharaj et al., 1999). Naturally this would not be viable on a commercial scale and therefore some mechanical device for rolling or rotating the fruit so that it accumulates the requisite UV dose would be required. Devices of this type have been described (Michaloski, 1999; Brandt and Klebaum, 2000) and could well be integrated into existing packing lines, subject to space availability and consideration of the potential impact of any physical damage to the fruit.

One possibility that has not received previous investigation in this particular context is treatment of the fruit whilst it is still on the vine, i.e. preharvest treatment. There is relatively little work on the effects of UV-C on growing plants. This may be to some extent because UV-C has been claimed not to be 'physiologically relevant' for plants growing in the sun (Stapleton, 1992). Notwithstanding, sources emitting a variety of UV wavelengths, including some UV-C, were used by Del Corso and Lercari (1997) to condition tomato seedlings grown in glasshouses for outdoor transplantation. Whilst Bacci et al., (1999) attempting to simulate the effects of further depletions of the ozone layer, found that treatment of tomato plants with UV-B on a daily basis resulted in early ripening of fruits and a reduction in the size of fruits.

In the work described here tomatoes growing on the vine in a commercial greenhouse were treated with UV-C after which their firmness and colour were measured. These two factors are according to Schouten et al. (2007) the two most important quality attributes affecting the market value of the fruit. UV-C treated fruit were left on the vine and also had their colour measured post treatment. In addition, fruit from trusses that had not been directly treated with UV-C but which were on the same plant as trusses that had were also monitored. The ability

of UV-C treated red tomatoes to prevent the growth of the phytopathogen *Penicillium digitatum* when inoculated into the flesh is also reported here.

## 2. Materials and methods

### *2.1 Fruit*

The tomato fruit (*Solanum lycopersicon* Mill. var. Mecano) used in this study were grown in a commercial greenhouse in N.E. England. The mean temperature and relative humidity inside the glasshouses were 19 º C and 80 % respectively.

### *2.2 UV-C Equipment*

Postharvest UV-C treatment was applied to fruit using a specifically designed UV treatment chamber that permitted the treatment of up to 10 fruit simultaneously and was similar to that described by Obande and Shama, 2010. The chamber comprised a low pressure amalgam source of length 1000 mm and diameter 19 mm (GPHHA 1000 T6L/4P, LightTech Lamp Technology Ltd., Dunakeszi, Hungary) emitting principally at 254 nm and suspended over two rollers. The height of the UV burner could be adjusted so as to vary the intensity of UV at the position of the rollers. The intensity was measured using a radiometer (UVP Instruments, Cambridge) fitted with a probe with peak absorptivity at 254 nm.

Preharvest UV-C treatment was applied to trusses of tomato fruit whilst they were still on the vine using a purpose-built piece of equipment. This was designed to be conveyed along the hot water pipes which are used to maintain temperature in the glasshouse and which are situated just above floor level. The unit was equipped with two low pressure mercury sources of length 580 mm and diameter 15 mm (UVI 12OU2G11 CP15/469, UV-Technik Speziallampen GmbH., Wümbach, Germany) with principal emission at 254 nm. The sources were U-shaped, and therefore the effective length of each source, as quoted by the

manufacturer, was 1180 mm. The sources were housed in parabolic reflectors fabricated from anodised aluminium sheet which has a high UV reflectivity. The UV source housings were mounted on adjustable steel members so that they could be positioned a fixed distance away from trusses that were to be exposed to UV. Prior to commencing UV treatment the sources were switched on for 30 mins in order to achieve a constant emission. Furthermore, the sources were left on continuously throughout the experiments to maintain the emission constant; whilst the unit was not actually in use the sources were covered with UV-impermeable shields that slotted over the front of the parabolic housings to prevent unwanted irradiation of either plants or fruit trusses and also, for safety purposes.

*2.3 UV-C Treatment of Fruit*

Fruit were harvested at the mature green stage, collected directly from the producer, transported to the laboratory and treated with UV-C on the same day. Samples were then held at 16$^{o}$C in the dark for 20 days. Fruit were placed on the rollers within the chamber and rotated at a speed of 15 rpm. The intensity of the UV-C was maintained at 1000 µW/cm$^2$. The dosage applied was varied by altering the time of exposure 2.5, 5 and 10 mins to provide doses of 1.5, 3.0 and 6.0 kJ/m$^2$ respectively.

For treatment of tomatoes on the vine, the sources were positioned 10 cm from fruit trusses. Experiments were conducted at two UV-C doses, 3 and 8 kJ/m$^2$, which were achieved by exposure of trusses for 150 and 400 sec respectively. Both ripe (i.e. red) and mature green tomatoes were treated in this way. Treated fruit from both stages of development were picked 8 hours after UV-C treatment and monitored for colour, texture and elicitation of anti-fungal compounds (see below) in the laboratory under storage at room temperature (*circa* 16 ºC) and away from direct sunlight. The total delay between treatment and the initial measurement of the properties of the fruit was approximately 12 hours. Fruit from certain trusses after UV-C

treatment were left on the vine and monitored for changes in colour. Also monitored on the vine were trusses of fruit that were located on the same plant as trusses that had received direct UV-C exposure but which were themselves completely enveloped in plastic bags that prevented the transmission of UV-C. The treatment with UV-C was delivered in the glasshouses during the night to prevent any potential photoreversal.

*2.4 Colour Measurement*

Fruit colour was measured using a CR-200 Chroma Meter (Minolta (UK) Ltd., Milton Keynes, UK) set in the 'L*a*b*' mode (see below) after the instrument had been calibrated for use with a standard white calibration plate (CR-A47).

The instrument measures colour based on the Hunter colour scale which has an L* a* and b* axis. Three readings were taken at random positions from each fruit and converted into Tomato Colour Index (TCI) readings using the formula shown below (Hobson; 1987).

$$TCI = \frac{2000 * a}{\sqrt{L * (a^2 + b^2)}} \quad (1)$$

Colour measurements were made on fruit sample sizes of 10 or 5 for postharvest and preharvest treatments respectively.

*2.5 Firmness Measurement*

Measurements of the firmness of fruit were performed using a Digital Texture Analyser (TA.XT Plus, Stable Micro Systems Ltd., Haslemere, Surrey, UK). The instrument was set in compression mode. The maximum force (in g) required to compress the fruit by 4mm was recorded and monitored. Measurements were made at 4 randomly chosen points on the fruit. Two of these were in the equatorial regions of the fruit and two were at the polar regions. All firmness measurements were made on a fruit sample size of 5.

154 *2.6 Production of Fungal Spores and Inoculation of Fruit*

155 *Penicillium digitatum* sacc. (CBS 101026) was obtained from the Centraalbureau voor
156 Schimmelcultures (CBS), Utrecht, The Netherlands. This was stored frozen on beads at – 80°
157 C. To prepare spore stock a single bead was placed in Potato Carrot Broth (prepared
158 according to the recipe provided by the CBS) and cultured on a shaking incubator at 20° C
159 and 200 rpm for 24 h. Aliquots (100 µL) were spread onto the surface of Potato Dextrose
160 Agar (Oxoid Ltd., Basingstoke, Hants, UK) plates and incubated at 20°C for 4 days. The
161 spores were then harvested using Ringers solution and stored at 4°C until needed. The spore
162 concentration as determined using a haemocytometer was $3 \times 10^6$ spores per ml.

163 Tomatoes to be inoculated were first wiped clean with a paper tissue after which a cylindrical
164 cavity (Length, 5 mm; Diameter, 5mm) was created in each fruit using a flame-sterilised cork
165 borer having a diameter of 6 mm. Into this cavity was pipetted 10 µL of spore suspension
166 whereupon the tomato 'core' was carefully replaced. Inoculations with spores were made 12
167 h after UV-C treatment of the fruit and spore-inoculated fruit were stored at 20°C in an
168 incubator until required for sectioning. This was done using a scalpel, and digital images of
169 the cut fruit surfaces were immediately taken. The diameters of fungal lesions were obtained
170 using specialised software ('Screen Calipers', Iconico Ltd., New York, USA). These
171 measurements were made on a sample size of 3.

172

173 *2.7 Statistical Analysis*

174 Two way ANOVA tests were conducted on all the data obtained using SigmaPlot version 10
175 (Systat Software Inc., San Jose, USA). 'Significance' as referred to in the text below is taken
176 to mean $p \leq 0.05$.

177

## 3 Results and discussion

Selection of the UV-C doses employed in this work was made with reference to previous studies conducted with tomatoes in which the fruit had been treated postharvest. Liu et al. (1993) obtained optimal effects at doses between 2.4 and 4.8 kJ/m$^2$. A number of previous workers (Stevens et al., 1998; Maharaj et al., 1999; Barka et al., 2000; Charles et al., 2009) had treated tomatoes with doses of either 3.6 or 3.7 kJ/m$^2$. Whilst Liu et al. (2009) had found that daily treatments at doses of 13.7 kJ/m$^2$ yielded beneficial effects. At the upper end of the dose range, both Liu et al. (1993) and Maharaj et al. (1999) had observed browning of tomatoes at 20 kJ/m$^2$.

In the first instance the effect of a postharvest UV-C treatment on colour development of green tomato fruit was examined. Colour was measured at 3 day intervals. The results are shown in Figure 1. Fruit started with a TCI of about -18 indicating a green coloration. Control fruit developed the red coloration over a period of 9-10 days. This colour development was retarded by UV treatment and this was statistically significant for all three treatments. Similar results were obtained for fruit held at room temperature (data not shown). This served to show that these tomato fruit were responding to a postharvest UV-C treatment in a similar manner to that previously reported (Stevens et al., 1998; Maharaj et al., 1999; Barka et al., 2000; Charles et al., 2009).

In Figure 2a colour development of tomatoes at the red stage of development treated on the vine and subsequently picked, and control fruit, both stored at room temperature, are compared. The TCI of the control fruit increases sharply over the first 8 days post treatment indicating an intensification of red pigmentation after which colour development remains relatively constant over the remaining period for which measurements were taken. Fruit exposed to the lower UV-C dose of 3 kJ/m$^2$ show a similar trend, although the initial rise occurs at a lower rate than for the control fruit. By contrast, the fruit treated with a dose of 8

203 kJ/m$^2$ show a lag of just under 10 days before the TCI increases to a final value not
204 significantly different from the other two groups of fruit.

205 The firmness of fruit is depicted in Figure 2b. The firmness of all the fruit declines steadily
206 over 12 days but the firmness of the fruit treated at the higher UV-C dose decreased less than
207 that of the control fruit and those treated at the lower dose of 3 kJ/m$^2$. The firmness of fruit
208 treated with a UV-C dose of 8 kJ/m$^2$ at day 12 was significantly different to the two other
209 groups of fruit. The results obtained here are in general agreement with those presented by
210 both Liu et al. (1993) and Stevens et al. (2004) who treated fruit postharvest at various stages
211 of maturity and at a number of UV-C doses, including 3.6 and 7.5 kJ/m$^2$, which are close to
212 those used in this work.

213 Colour development of mature-green tomatoes treated and left on the vine is shown in Figure
214 3a. In this case tomatoes treated with both high and low doses of UV-C show only a very
215 slight loss in green pigmentation over 6 days and there are no statistically significant
216 differences between all three groups of fruit over this period. The control fruit initially follow
217 a very similar trend, but at day 5 the TCI rises sharply and within one day the fruit have
218 become red. The delay in senescence observed here for fruit treated and left on the vine is
219 similar to that previously reported for postharvest treatment of mature green fruit as reported
220 by Liu et al., (1993), Stevens et al. (1999) and Liu et al. (2009).

221 As mentioned in the introduction, a consensus seems to have developed for treating tomatoes;
222 this is that the entire surface of the fruit needs to be exposed to UV-C to obtain the benefits of
223 the hormetic effect. The conditions under which fruit were treated here, i.e. whilst still in
224 trusses on the vine, precluded exposure of the entire surfaces of the fruit to UV-C however,
225 seem nonetheless to have induced all the attributes associated with delayed senescence.
226 Stevens et al. (2005) first challenged this orthodoxy by demonstrating that for peaches, apples

and tangerines it was possible to apply the entire UV-C dose entirely at the stem end of the fruit and still obtain the maximum hormetic response.

Colour development of fruit from trusses that had not been directly exposed to UV-C but which were from the same plant as trusses which had, is shown in Figure 3b. These fruit were monitored whilst still on the vine. Differences in colour between the two UV-C treatments and the controls are not significant over the first 4 days. The untreated group rapidly turn red within a further two days and reach TCI values by day 6 that are comparable to those of picked mature green fruit (Figure 3a). The 3 $kJ/m^2$ treated fruit follow a similar trend but do not attain the same final TCI value. At the higher dose of 8 $kJ/m^2$ the TCI of the fruit remains negative at day 6 indicating that the fruit are still green. These findings are completely novel and suggest that application of an abiotic stress to a truss of fruit on a particular plant induces metabolic responses that are transmitted throughout the plant and have measurable effects on other trusses. This may constitute a form of chemical signalling. Encouragingly, no signs of leaf damage resulting from UV-C treatment were observed at the doses employed here.

The firmness of fruit inoculated with *P. digitatum* is shown in Figure 4. The control fruit shows a biphasic pattern of loss of firmness; softening occurs very rapidly over the first 4 days after which the fruit continues to soften at a lower rate. The fruit treated at 3 $kJ/m^2$ shows a more uniform rate of softening over this entire period. The rate of softening seen by the fruit treated with the higher dose of 8$kJ/m^2$ is similar to that for fruit treated at the lower dose but the fruit remains significantly firmer than either the control or 3 $kJ/m^2$ treated fruit from day 3 onwards.

A direct indication of the growth of the fungus after inoculation of fruit is given by the measurement of the diameter of fungal lesion (Figure 5). The fungus appears to grow at similar rates in the control fruit and in fruit treated at the lower UV-C dose, although the

diameter of the fungal lesion by day 10 for the control fruit is higher than that for the fruit treated at 3 kJ/$m^2$. The increase in lesion diameter occurs most slowly for fruit treated with a dose of 8kJ/$m^2$ and by day 10 the diameter is considerably smaller than that of the two other groups of fruit.

*P. digitatum* is not a natural phytopathogen of tomatoes, and its use in this work may appear unusual. However, in preliminary studies, this particular strain of *P. digitatum* and 4 strains of *Botrytis cinerea* along with one strain of *Colletotrichum gloeosporioides* were evaluated for their suitability as 'biosensors' in providing the greatest measurable response to the effects of UV-C; *P. digitatum* emerged as the most sensitive fungus of those tested and therefore it was selected on this basis. The pattern of lesion development of *P. digitatum* is markedly different from that of *R. stolonifer* as observed by Stevens et al. (2004); whilst the growth of *R. stolonifer* was slower in fruits treated with a UV-C dose of 3.6 kJ/$m^2$ for the first 72 hours of treatment, after a further 24 hours the lesion diameter in treated fruit was actually greater than that of the control. This was to some extent also mirrored in polygalacturonase activity. This is in contrast to the results obtained here with *P. digitatum* where the lesion produced by the fungus showed continued increase in diameter in the control group of fruit and in those treated with a UV-C dose of 3 kJ/$m^2$ whilst the lesions in fruit treated at the higher dose did not show a significant increase in the diameter of the lesion after day 6.

## Conclusions

UV-C treatment of tomatoes on the vine could constitute an alternative to postharvest treatment. The results obtained here suggest that it may be possible to apply a generalised treatment to plants rather than having to treat individually every truss on a particular plant. Further work needs to be undertaken to determine both the optimal dose and timing of this form of treatment. In addition, it would also be worthwhile examining other patterns of

delivering the UV-C dose, e.g. by fractionating the dose and delivering reduced doses at fixed intervals of time. Preharvest treatment of fruit such as strawberries which are not subjected to any postharvest treatments but simply packed into punnets may be the only way of treating such physically fragile fruit. Stevens et al. (1998) had found that exposure of fruit to UV-C followed by immediate exposure to white light, as emitted by ordinary fluorescent tubes, was capable of completely counteracting the hormetic effect through the phenomenon that has become known as 'photoreversal'. Treatment of tomato fruit at night appeared successful in avoiding such phenomena. Nocturnal UV-C treatment may also hold another benefit; the commercial glasshouses where the studies reported here were conducted contained beehives. Bees' compound eyes contain UV receptors in addition to those for green and blue light (Menzel and Greggers, 1985). Whilst the UV sources employed here emitted primarily at a wavelength 254 nm, they also emit at longer UV wavelengths that could serve to attract bees. The UV-C portion of the emission would be damaging to the bees, however, because treatment was conducted at night whilst the bees were in the hive this potential hazard was avoided.

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**Figure Captions**

Figure1: Tomato Colour Index (TCI) development of Mature Green Tomatoes Stored at 16 °C for 20 days following Postharvest UV-C Treatment.

• Control ■ UV-C dose of 1.5 kJ/$m^2$ ▲ UV-C dose of 3.0 kJ/$m^2$ ◆ UV-C dose of 6.0 kJ/$m^2$

Figure 2a: Tomato Colour Index (TCI) development of Picked Red Tomatoes Stored at 16 °C for 16 days following Pretharvest UV-C Treatment.

• Control ■ UV-C dose of 3 kJ/$m^2$ ▲ UV-C dose of 8 kJ/$m^2$

Figure 2b: Texture of Picked Red Tomatoes following Preharvest UV-C Treatment.

• Control ■ UV-C dose of 3 kJ/$m^2$ ▲ UV-C dose of 8 kJ/$m^2$

Figure 3a: Tomato Colour Index (TCI) development of Mature Green Tomatoes Monitored on the Vine following Preharvest UV-C Treatment.

• Control ■ UV-C dose of 3 kJ/$m^2$ ▲ UV-C dose of 8 kJ/$m^2$

Figure 3b: Tomato Colour Index (TCI) development of Mature Green Tomatoes not Directly Exposed to UV and Monitored on the Vine.

• Control ■ UV-C dose of 3 kJ/$m^2$ ▲ UV-C dose of 8 kJ/$m^2$

Figure 4: Effect of Preharvest UV-C Treatment on Texture of Picked Red Tomatoes Inoculated with *Penicillium digitatum* and stored at 20 °C.

• Control ■ UV-C dose of 3 kJ/$m^2$ ▲ UV-C dose of 8 kJ/$m^2$

Figure 5: Effect of Preharvest UV-C Treatment on Lesion Diameter of Picked Red Tomatoes Inoculated with *P. digitatum* and stored at 20 °C.

388 ● Control ■ UV-C dose of 3 kJ/$m^2$ ▲ UV-C dose of 8 kJ/$m^2$

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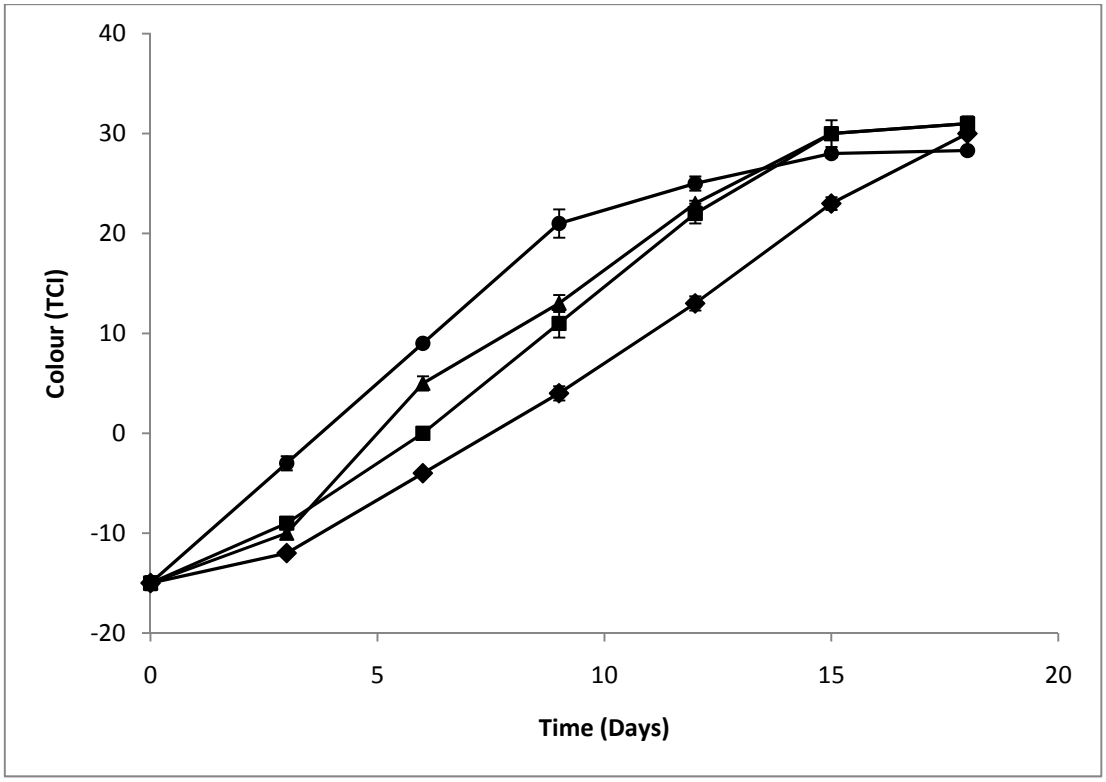
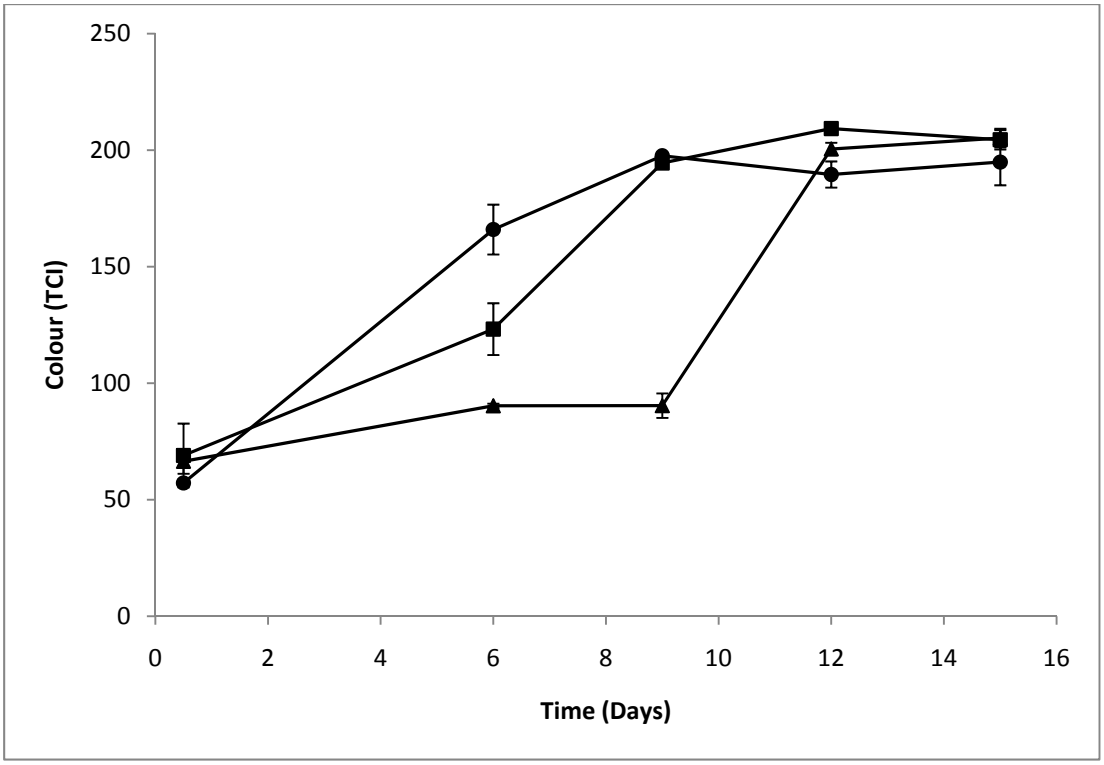



Figure 1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Colour (TCI)
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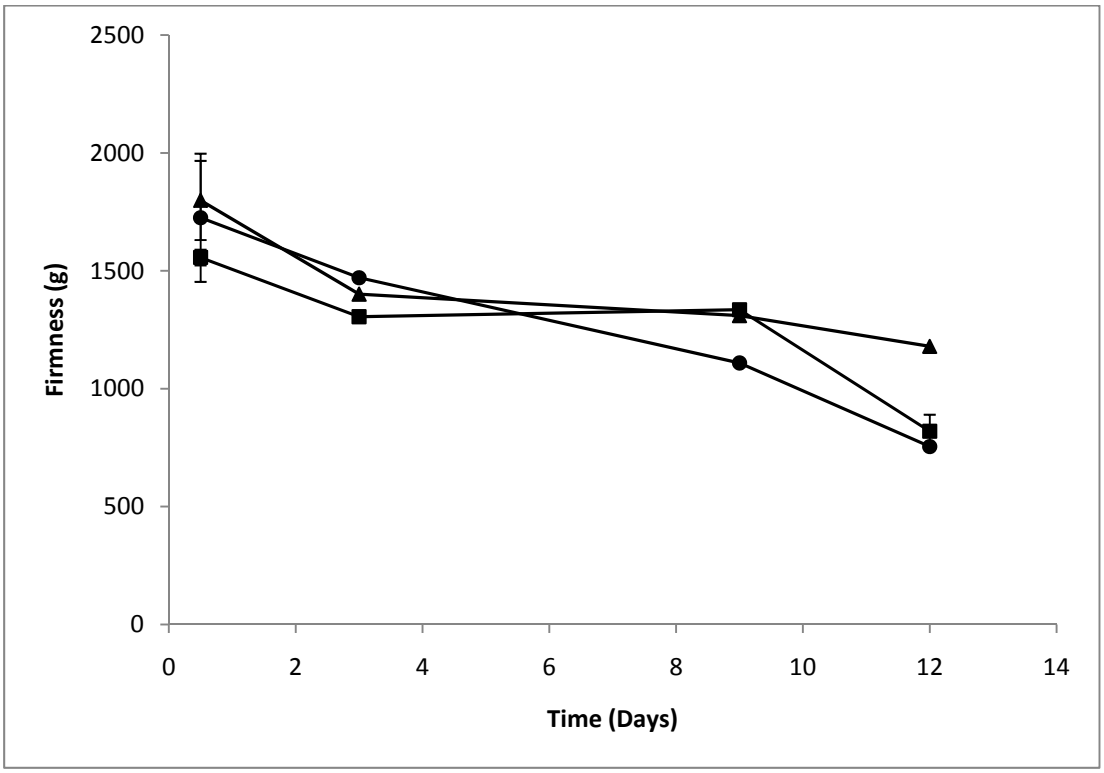
Time (Days)

(a)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Firmness (g)
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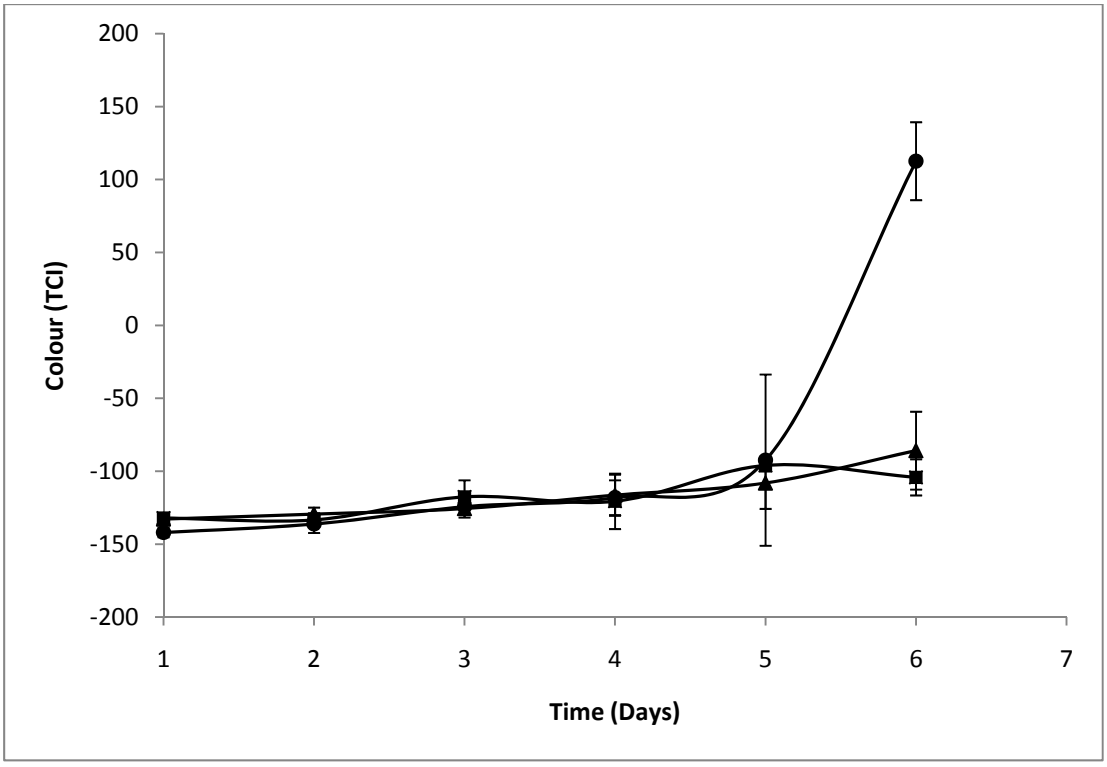
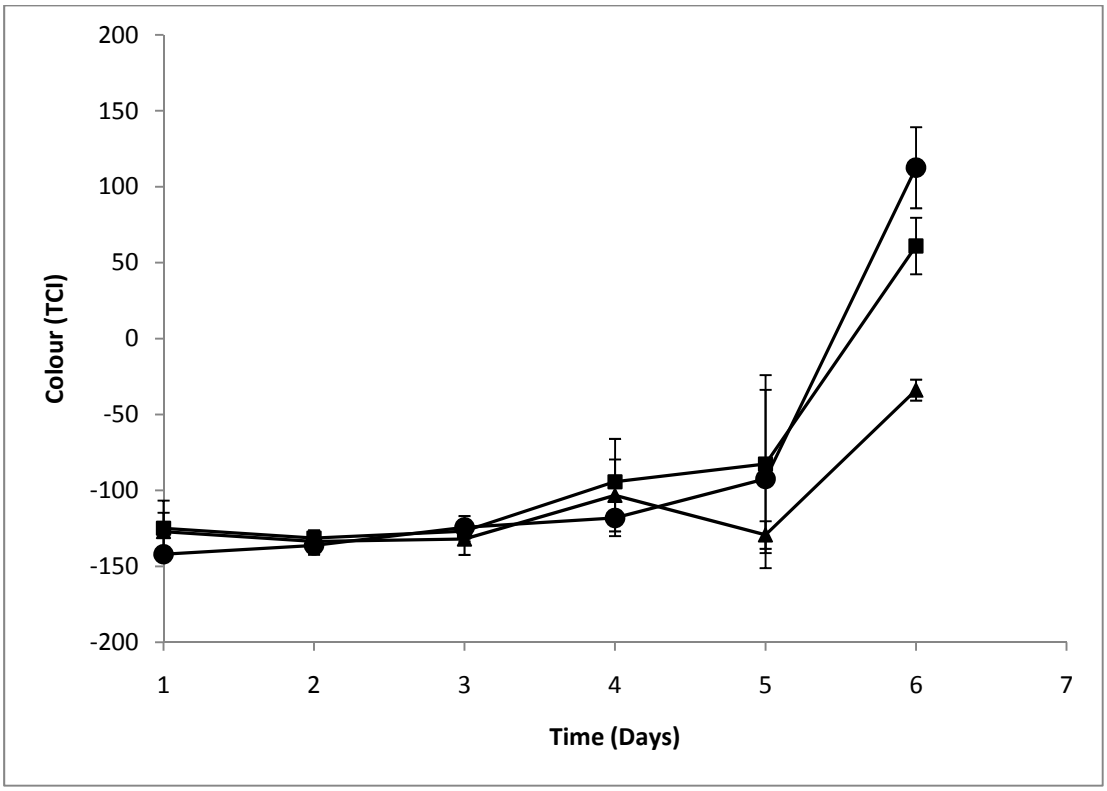
Time (Days)

(b)

Figure 2


200
150
100
50
0
-50
-100
-150
-200
Colour (TCI)
1
2
3
4
5
6
7
Time (Days)

(a)


200
150
100
50
0
-50
-100
-150
-200
Colour (TCI)
1
2
3
4
5
6
7
Time (Days)

(b)

Figure 3

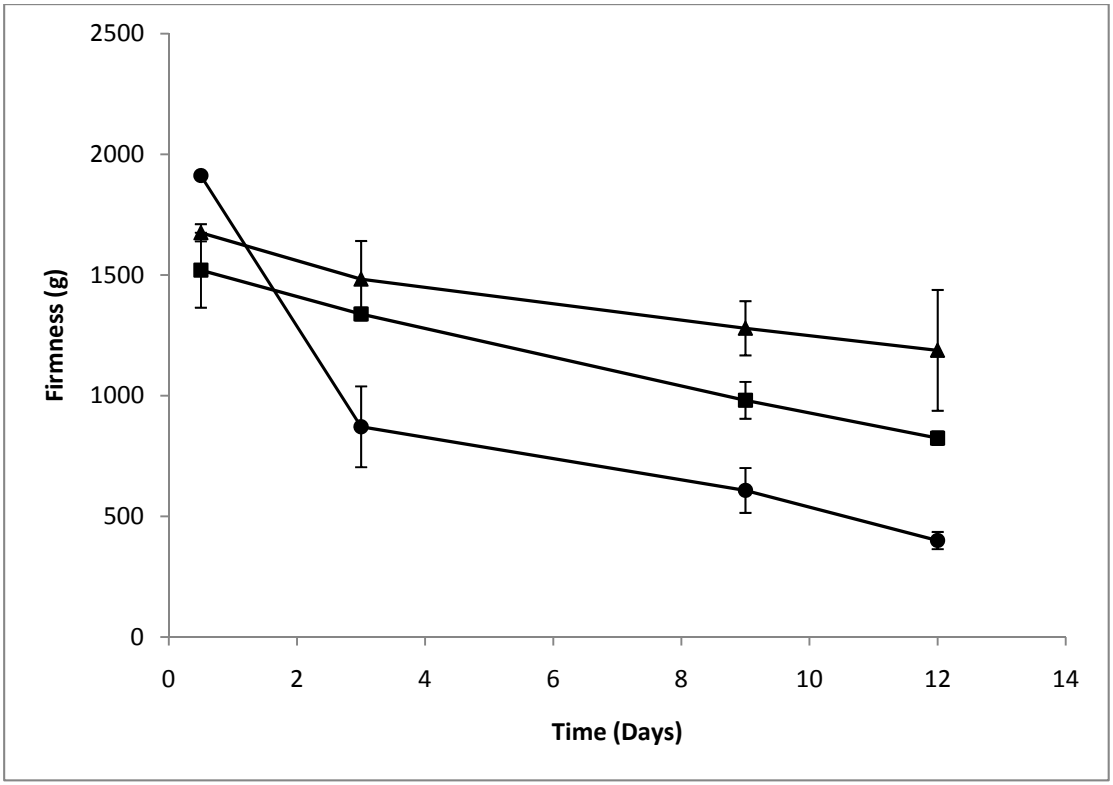



Figure 4

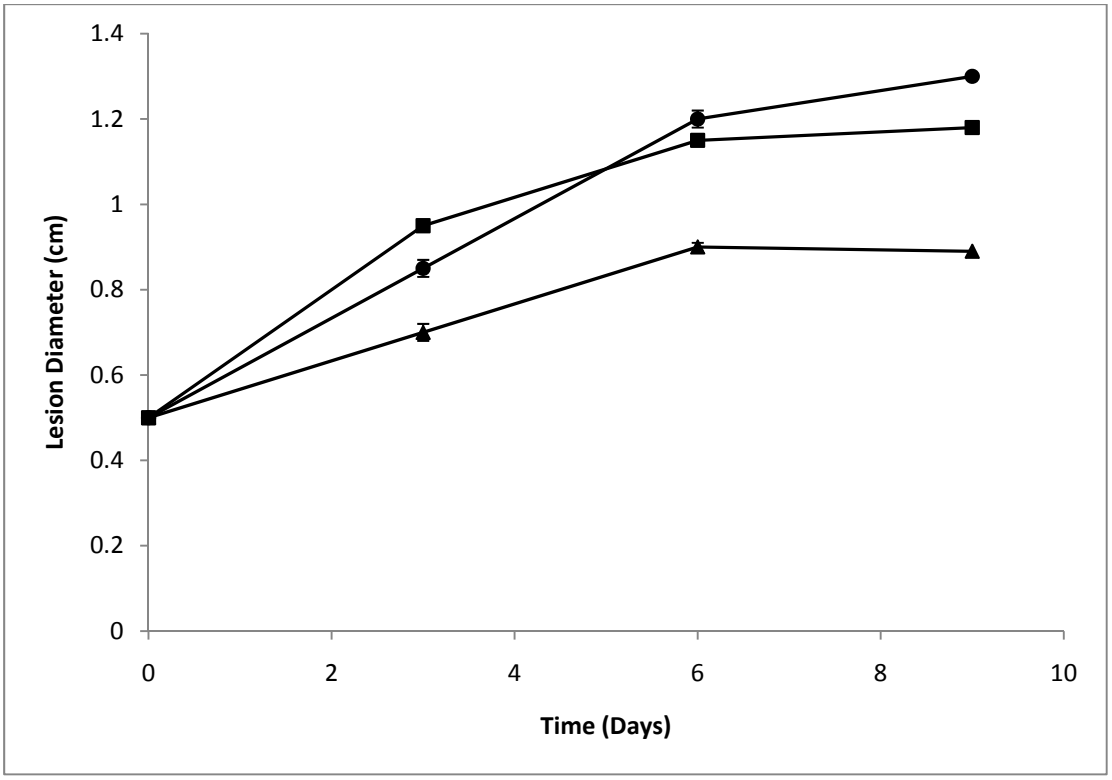



Figure 5