Postharvest changes in pigment concentrations in ‘Fuji’ apples with ‘Fuji’ stain

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A B S T R A C T

‘Fuji’ apples (Malus domestica Borkh cv. ‘Fuji’) sometimes develop a discolouration in the peel during cold storage, typically in the periphery of sunburned peel. We refer to this particular postharvest disorder as ‘Fuji’ stain as we have not observed it in any cultivar other than ‘Fuji’ and the discolouration looks like a stain on the peel. Because peel discolouration occurs, peel pigments are thought to be involved. Hence the concentrations of anthocyanins, epicatechin, quercetin glycosides, chlorogenic acid, chlorophylls, and carotenoids in peel disks taken from areas exhibiting ‘Fuji’ stain were compared to concentrations observed in peel disks not exhibiting ‘Fuji’ stain. In 2005, peel from sunburned apples exhibiting ‘Fuji’ stain was compared to peel from three areas of sunburned apples not exhibiting stain [i.e. sunburned peel, the area around the sunburned peel (halo), and the area around the halo (OH)]. Additionally, stained peel was compared to the sun-exposed side of non-stained non-sunburned apples (NSNB). The second year, 2006, we compared stained peel to NSNB peel and the area outside the stained area (OS) on those fruit with stain. The concentrations of idaein, epicatechin, and quercetin glycosides were consistently low in the stained peel both years. This is in contrast to our earlier studies in which sunburned ‘Fuji’ apples had high concentrations of quercetin glycosides and epicatechin and low concentrations of idaein, and non-sunburned apples had low concentrations of quercetin glycosides and epicatechin and high concentrations of idaein. The consistent and unique characteristic of stained peel reported here indicates an association of these compounds with the incidence of stain. In 2005, chlorogenic acid concentrations in the stain peel were lower than other peel types, but in 2006 they were higher. Differences in chlorophyll and carotenoid concentrations were observed among many of the peel types. However, there is not a clear association between stain development and changes in chlorophyll and carotenoid concentrations due to significant differences not being observed in both years. While our research provides insight into pigment changes associated with ‘Fuji’ stain formation, more work is needed to help clarify the inconsistencies observed between the 2 years.

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1. Introduction

One of the main factors affecting marketability of apples is their appearance; any blemishes or discolourations reduce their value. One such discolouration that occurs on ‘Fuji’ apples is stain (hereinafter referred to as ‘Fuji’ stain). The colour of ‘Fuji’ stain is usually greenish grey, dark brown, purple or muddy (Fig. 1). ‘Fuji’ stain is a postharvest storage disorder that appears on apples with sunburn damage, but whose origin and incidence are poorly understood. It appears to be unique to ‘Fuji’, as we have not observed this disorder in several other cultivars stored under the same conditions.

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This disorder was first reported by Warner (1994). She reported that researchers were confounded by a strange browning of ‘Fuji’ skin in much of Washington State’s 1993 crop. Mattheis (1996) reported that this disorder, referred to as stain, usually develops 1–2 months after harvest. Controlled atmosphere storage and/or bagging apples before harvest slowed down development of the disorder, but neither treatment eliminated the stain. Diphenylamine (DPA) had no effect on stain development. Later, Fan and Mattheis (1998) reported a greenish, dark discolouration on ‘Fuji’ apples that develops during cold storage and is typically on the portion of the fruit that had been exposed to the sun. This disorder usually has a well-defined border and often occurs at the periphery of sunburn. Non-bagged fruit and a small percentage of the fruit from which bags were removed 20 days before harvest developed stain during cold storage, while fruit bagged until harvest did not. These findings implicated sunlight as a factor in stain development.

Warner (2001) reported that Mattheis could induce something that looked very similar to staining by exposing ‘Fuji’ apples to ultraviolet-B (UV-B) light in the lab. He found that cold fruit were
The incidence of 'Fuji' stain was reduced \((P < 0.01)\) by more than 50\% compared to non-sunburned lots and then stored in regular atmosphere cold storage at 0.5 °C. After 4 months of storage, apples were sorted into those with and those without stain. From those apples without stain, 10 sunburned apples and 10 non-sunburned apples were selected and peel disks (16 mm diameter, 1 mm thick) of the SB-2 (sunburn browning degree of 2 on a 0–4 scale; Felicetti and Schrader, 2008, 2009a), halo, outside the halo (OH), and non-stained non-sunburned (NSNB) areas were taken as described previously (Felicetti and Schrader, 2008, 2009a). There was not enough stained peel on many of the apples with stain to collect the required four peel disks from each apple. Hence, 20 apples with stain were used and two disks from each of 10 stained apples were used for chlorophyll and carotenoid analyses, and two disks from each of the other 10 stained apples were used for the phenolic analyses.

Due to the low incidence of stain in the orchard used in 2005 and the general unpredictability of stain incidence, the experimental design was changed for the 2006 season. In 2006, the experiment was conducted on 'Fuji' apples with and without stain. These apples were collected after 2 months of regular atmosphere cold storage at 0.5 °C from bins stored at a local commercial storage facility. Samples consisted of peel disks (12 mm diameter, 1 mm thick) taken from the stained area of stained apples, the area outside the stained area (OS), and the sun-exposed side of non-stained non-sunburned apples (NSNB). Twenty apples were sampled with two peel disks from each apple. Four disks from four different apples were pooled to make one sample, making sure that one disk from each apple was analyzed for chlorophyll and carotenoids and the other disk was analyzed for phenolics. All peel disks were flash frozen in liquid nitrogen immediately after removal from the apple and stored at -80 °C until analyzed.

The extraction and pigment analysis methods used were as described previously (Felicetti and Schrader, 2008).

Peel colour analyses were performed in 2006 only. The analyses were performed prior to taking peel disk samples as described earlier (Felicetti and Schrader, 2009a).

These analyses were conducted as previously described (Felicetti and Schrader, 2009a).
Fig. 2. (A and B) Idaean, chlorogenic acid, and epicatechin concentrations in mg g\(^{-1}\) fresh wt. for 'Fuji' SB-2, halo, OH, NSNB, and stain peel types in 2005 (A). Idaean, chlorogenic acid, and epicatechin concentrations in mg g\(^{-1}\) fresh wt. for 'Fuji' NSNB, OS, and stain peel types in 2006 (B). SB-2, sunburn browning degree of 2 on a 0–4 scale; halo, area immediately surrounding sunburn area; OH, area outside the halo; NSNB, no stain and no sunburn apple; stain, stained peel; OS, area outside the stained area. Mean comparison tests were performed on each pigment in each experiment. For each pigment, bars with the same letter are not significantly different (LSD; \(P \leq 0.05\)).

3. Results

3.1. Phenolic analyses

In 2005, the idaein concentration of the stained peel was the lowest of all peel types. It was significantly lower than the halo, OH, and NSNB, but not SB-2 peel (Fig. 2A). In 2006, the idaein concentration of the stained peel was significantly lower than OS peel and NSNB peel (Fig. 2B).

The chlorogenic acid concentration in the SB-2 peel in 2005 was significantly higher than that found in the halo, OH, and stain peels (Fig. 2A). In 2006, chlorogenic acid concentrations were significantly higher in the stained peel than in the NSNB and OS peels (Fig. 2B).

In 2005, the stained peel had the lowest epicatechin concentrations of all peel types; however the concentrations were not significantly lower than that found in the OH peel (Fig. 2A). In 2006 the epicatechin concentration of the stained area was again the lowest of the peel types but was not significantly lower than NSNB (Fig. 2B).

In 2005, concentrations of quercetin glycosides generally decreased from SB-2 to halo to OH (Fig. 3A), and concentrations in the stained area were comparable to the concentrations observed in the NSNB and OH peels (Fig. 3A). In 2006, the stained peel had lower concentrations of the individual quercetin glycosides as compared to the NSNB and OS peels (Fig. 3B). The differences between the stained peel and the NSNB peel were always significant, but the differences between the stained peel and the OS peel were not (Fig. 3B).

3.2. Chlorophyll analyses

In 2005, the stain peel had significantly higher chl a and chl b concentrations than the other four peel types (Fig. 4A). In 2006, chl a concentrations did not differ among peel types, but chl b concentrations of the stained peel were significantly higher than in the NSNB peel (Fig. 4B). In 2005, the chlorophyll a/b ratios (chl a/b) of SB-2 and halo were higher than in OH and NSNB (Table 1). The ratio in stained peel was intermediate, but not different from other peel types. In 2006, the chl a/b of the stained peel was lower than the NSNB peel but was not different from the OS peel (Table 1).

3.3. Carotenoid analyses

In 2005, \(\beta\)-carotene concentrations in the SB-2 and halo peels were higher than in OH peel (Fig. 5A). The \(\beta\)-carotene concentration of stained peel was intermediate, but not different from other peel types (Fig. 5A). In 2006, the \(\beta\)-carotene concentration was higher in the stained peel than in the OS or NSNB peels (Fig. 5B).

In 2005, the concentration of lutein + zeaxanthin (L + Z, lutein and zeaxanthin coeluted) in the stained peel was significantly higher than all peel types except NSNB (Fig. 5A). The concentration of violaxanthin (V) was significantly higher in the stain peel than in SB-2. The concentration of antheraxanthin (A) in the stain peel was higher than in SB-2. The concentration of zeaxanthin (Z) was significantly lower in the stain peel than in SB-2.
Fig. 4. (A and B) Chlorophyll a and chlorophyll b concentrations in μg·g⁻¹ fresh wt. for ‘Fuji’ SB-2, halo, OH, NSNB, and stain peel types in 2005 (A). Chlorophyll a and chlorophyll b concentrations in μg·g⁻¹ fresh wt. for ‘Fuji’ NSNB, OS, and stain peel types in 2006 (B). SB-2, sunburn browning degree of 2 on a 0–4 scale; halo, area immediately surrounding sunburn area; OH, area outside the halo; NSNB, no stain and no sunburn apple; stain, stained peel; OS, area outside the stained area. Mean comparison tests were performed on each pigment in each experiment. For each pigment, bars with the same letter are not significantly different (LSD; P ≤ 0.05).

significantly higher than in SB-2, halo, and OH. In 2006, very few differences in L + Z, V, and A concentrations were observed among the different peel types (Fig. 5B).

3.4. Colourimetric data

The colourimetric data indicate that the L* of the stain and NSNB peels were not different from each other, but both were significantly lower than that of the OS peel (Table 2). The h° decreased significantly from stain to OS to NSNB while the C* increased significantly.

4. Discussion

We believe the type of stain reported here is unique to ‘Fuji’ as we have not observed this disorder in other apple cultivars stored under similar conditions. Noro et al. (1996, 1998) reported that the cultivar ‘Hokuto’ develops a disorder that is also called “stain”. They suggested that stain in ‘Hokuto’ apples might be related to apple scald as they were able to induce stain in ‘Hokuto’ and scald in ‘Mutsu’ apples using trans-2-hexenal. To our knowledge, the induction of ‘Fuji’ stain using trans-2-hexenal has not been reported. Stain observed in ‘Fuji’ apples is associated with sunburn and found

Table 1

<table>
<thead>
<tr>
<th>Year</th>
<th>Chl a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td></td>
</tr>
<tr>
<td>SB-2</td>
<td>2.5 (a)</td>
</tr>
<tr>
<td>Halo</td>
<td>2.5 (a)</td>
</tr>
<tr>
<td>OH</td>
<td>1.9 (b)</td>
</tr>
<tr>
<td>NSNB</td>
<td>2.0 (b)</td>
</tr>
<tr>
<td>Stain</td>
<td>2.3 (ab)</td>
</tr>
<tr>
<td>2006</td>
<td></td>
</tr>
<tr>
<td>NSNB</td>
<td>2.7 (a)</td>
</tr>
<tr>
<td>OS</td>
<td>2.5 (b)</td>
</tr>
<tr>
<td>Stain</td>
<td>2.5 (b)</td>
</tr>
</tbody>
</table>

SB-2, sunburn browning degree of 2 on a 0–4 scale; halo, area immediately surrounding sunburn area; OH, area outside the halo; NSNB, no stain and no sunburn apple; OS, outside stained area; stain, stained peel.

a Within experiments, means with the same letter in parenthesis are not significantly different (LSD; P ≤ 0.05).

Table 2
2006 colourimetric data.

<table>
<thead>
<tr>
<th></th>
<th>L°</th>
<th>h°</th>
<th>C°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stain</td>
<td>44.1 (b)</td>
<td>64.7 (a)</td>
<td>13.7 (c)</td>
</tr>
<tr>
<td>OS</td>
<td>49.7 (a)</td>
<td>49.6 (b)</td>
<td>19.4 (b)</td>
</tr>
<tr>
<td>NSNB</td>
<td>43.9 (b)</td>
<td>30.9 (c)</td>
<td>29.3 (b)</td>
</tr>
</tbody>
</table>

L°, lightness factor; h°, hue angle; C°, chroma; NSNB, no stain and no sunburn apple; OS, outside stained area; stain, stained peel.

a Within column, means with the same letter in parenthesis are not significantly different (LSD; P ≤ 0.05).
on the sun-exposed side of the apple, while scald is associated with the shaded side of susceptible apple cultivars. Because ‘Hokuto’ stain is linked to scald development, which develops on the shaded side of apples, whereas ‘Fuji’ stain is associated with sunburn, and develops on the sun-exposed side of apples, we believe the two disorders are different. ‘Fuji’ stain was the focus of this study and the intent was to determine if the discoloration associated with ‘Fuji’ stain was due to different concentrations of various pigments in the cells of the affected tissue. In 2005, we compared the stained peel of stained apples with different types of peel from non-stained sunburned apples (i.e. SB-2, halo, OH) as well as non-stained non-sunburned (i.e. NSNB) peel from non-stained non-sunburned apples. The premise was that stain is typically associated with sunburn and often appears outside the sunburned area (i.e. in the halo or OH areas). Hence, a comparison between the stained peel and the OH and halo peels should be valid, as they would have experienced the most similar environmental conditions. The premise behind the 2006 experiment was much simpler. We compared the pigment concentrations of two different coloured peel types that are side by side on the apple (e.g. stain and OS). The results from both years show that there are indeed differences between the stained peel and the other peel types. What does this mean?

The results from both years showed that in comparison to the other peel types the stain peel had low concentrations of both idaein and quercetin glycosides. Although their concentrations were not always significantly lower than all other peel types, the relatively low concentrations of idaein and quercetin glycosides found in concert has not been observed in the sun-exposed peel of ‘Fuji’ apples. Our previous work on apple sunburn indicated an inverse relationship between idaein and the quercetin glycosides. Non-sunburned ‘Fuji’ apples have relatively high concentrations of idaein and low concentrations of quercetin glycosides, and sunburned ‘Fuji’ apples have low idaein concentrations and high quercetin glycoside concentrations (Felicetti and Schrader, 2008, 2009b). The relatively low concentrations of both idaein and the quercetin glycosides are a characteristic of stained peel that distinguishes it from other sun-exposed peel types and are not changes that have been associated with sunburn. This is not to say that sunburn is not related to the pigment changes in stained tissue, but that the effects are secondary at best.

Because epicatechin and chlorogenic acid do not absorb visible light, they are not visible to the human eye: hence changes in their concentrations cannot be directly linked to the discoloration of stained peel. However, changes in their concentrations provide some helpful information because they share many biosynthetic steps with idaein and the quercetin glycosides. Idaein, quercetin glycosides, epicatechin, and chlorogenic acid are formed via a common pathway. All are formed from p-Coumaroyl-CoA, which is formed via the phenylpropanoid pathway (shikimic acid pathway). However, idaein, quercetin glycosides, and epicatechin require the additional step of fusing orsellinic acid (product from the polyketide pathway) to p-Coumaroyl-CoA (product of the phenylpropanoid pathway) to make a chalcone.

The concentration of epicatechin in the stain peel was low in both years while the chlorogenic acid concentrations were inconsistent between the 2 years. The fact that idaein, quercetin glycosides, and epicatechin were found in lower concentrations while chlorogenic acid was not suggests a reduction in the portion of the biosynthetic pathway that is not shared by chlorogenic acid. It is also possible that there is selective degradation of idaein, the quercetin glycosides, and epicatechin.

The 2005 results show significantly higher chl a and chl b concentrations in the stained peel indicating that chlorophylls may be related to the stain discoloration. However, in 2006 no significant differences in chlorophyll a concentrations were found. Additionally, the chl a/b ratio of the stained peel in the 2005 experiment was not different from the ratio for NSNB, but in the 2006 experiment the chl a/b ratio of the stained peel was significantly lower than that of the NSNB peel. The inconsistency between the 2 years makes it difficult to conclude how, or if, chlorophylls change in relation to stain. The apples in the two experiments were from different orchards and different growing season; thus many factors were different. Differences in nutrition and environments between the orchards could have directly caused the differences in chlorophyll concentrations or could have resulted in the differential expression of stain in each orchard. Risk of staining can be reduced by controlling the vigor of the trees and maximizing calcium levels in the fruit (Warner, 1998). We have observed the highest incidence of ‘Fuji’ stain in orchards that are highly vigorous as a result of excessive N fertilization (unpublished data). Others have reported high magnesium and low calcium concentrations in the affected tissues (Warner, 1994). Both nitrogen and magnesium are constituents of chlorophylls, and could affect their concentrations. Thus, despite being inconsistent, changes in chlorophylls cannot be ruled out as a characteristic of ‘Fuji’ stain.

In 2005, β-carotene concentrations in the stained area were not significantly greater than in the OH peel. In 2006, higher β-carotene concentrations in the stained area suggested that this pigment might play a role. As was discussed with regard to the chlorophylls, the inconsistency between the 2 years may be the result of differential expression of ‘Fuji’ stain in different orchards and growing seasons.

Changes in L + Z concentration might be related to the discoloration. However, the lack of significance among the peel types in 2006 makes it difficult to discern whether L + Z is associated with stain development. Violaxanthin is likely not related to stain development as it was found in higher concentrations in only the SB-2 peel type. Antheraxanthin may be related to stain development, as concentrations in the stained peel were higher than in the SB-2, halo, and OH peel types in 2005, and the OS peel type in 2006. However, antheraxanthin’s relationship to stain is clotted by the lack of significant differences between the stain peel and the NSNB peel, in both years.

The colour of ‘Fuji’ stain is usually referred to as greenish grey, dark brown, purple or muddy. Using the L*, h°, and C* values objectively describes the colour of the ‘Fuji’ stain in this study and allows readers to reproduce the colour (i.e. on a computer monitor). Using the values for stain given in Table 2, a muddy brownish grey colour is achieved. These values can also be used to objectively define colour changes between the different peels. Doing so shows that the h° increased from NSNB to OS to stain which indicates decreased redness and is consistent with the reduced idaein (i.e. red anthocyanin) concentrations.

5. Conclusions

In conclusion, the simultaneously low concentrations of idaein, quercetin glycosides, and epicatechin were not observed in our earlier research on sun-exposed apple peel. This appears to be a defining characteristic of ‘Fuji’ stain. Because significant differences in chlorophyll and carotenoid concentrations were not observed among the peel types over both years it is difficult to determine their role in stain development. However, their presence does affect the overall appearance and final colour of the stained area. Their continued presence along with reduced concentrations of idaein accounts for the higher h° of stained peel, which not only indicates that the stained peel is less red but also more yellow and green. While our research provides insight into pigment changes associated with ‘Fuji’ stain formation more work is needed to help clarify the inconsistencies observed between the 2 years.
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References