Bleaching color-loss of green field pea: An investigation on inference of genotypic-resistance based on chlorophyll and phenolic acid content

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Abstract
Color-loss of green field pea, commonly referred to as bleaching, can occur prior to harvest once the grain has ripened or during grain storage and is thought to be the result of chlorophyll depletion in the cotyledon. However, the mechanisms of bleaching-resistance, exhibited within some genotypes, are not well understood. The antioxidant activity of phenolic compounds can inhibit chlorophyll degradation, and therefore, the presence of phenolic compounds may improve bleaching-resistance. In this study, five green pea genotypes, differing in resistance to bleaching, were assessed for grain-color as well as content of chlorophyll and major phenolic acids, in both the hull and cotyledon. For each genotype, resistance to bleaching was inferred by the range of color scores observed. Chlorophyll and phenolic acid contents both decreased overall as the whole-grain color became lighter (bleached); however, there was no direct relation found between these compounds and the known resistance of each genotype. Chlorophyll content in the cotyledon was correlated ($r = -0.735$, $p < 0.001$) with cotyledon color but not with whole-grain color ($r = -0.212$, $p = 0.01$). Furthermore, the ratio of cotyledon chlorophyll A/B and the ratio of phenolic acid content (hulls) to total chlorophyll content (cotyledon) were not directly correlated with resistance. Although the total chlorophyll and phenolic acid contents were both depleted as the extent of bleaching increased, neither could fully explain differences in bleaching-resistance between genotypes. Furthermore, the green color and color-loss of whole-grain samples could not be fully attributed to chlorophyll pigments. Therefore, it is likely that other phenolic compounds contribute to both the grain color and the resistance to bleaching.

KEYWORDS
bleaching, bleaching-resistance, chlorophyll, green field pea, phenolic acids
1 | INTRODUCTION

Dry field pea (*Pisum sativum* L.) is an important agricultural commodity, cultivated globally both for human and stock consumption. It is a good source of plant-based protein, carbohydrates, minerals, and phenolic compounds (Dahl, Foster, & Tyler, 2012; Khan & Croser, 2004; B. Singh, Singh, Shevkani, Singh, & Kaur, 2017; N. Singh & Pratap, 2016). Green pea is one of the most significant broad classes of dry field pea and is largely marketed based on visual appeal (Khan & Croser, 2004; McDonald, Panozzo, Salisbury, & Ford, 2016; Ubayasena et al., 2013). Generally, green field pea have green cotyledons and opaque hulls (McDonald et al., 2016), although some genotypes have green hulls. Color uniformity is an important marketable trait for green pea and is regulated independently between countries (Cheng, McPhee, & Baik, 2004; Khan & Croser, 2004; McCallum, Timmerman-Vaughan, Frew, & Russell, 1997; McDonald, Salisbury, Ford, & Panozzo, 2019; Williams & Singh, 1988). Globally, Canada is the largest producer of green pea, and the Canadian Grain Commission specify a maximum allowance of 1%, 2%, and 3% of pea grains of "other color" to qualify for the market grades of "No. 1 Canada," "No. 2 Canada," and "No. 3 Canada," respectively (Canadian Grain Commission, 2018; Khan & Croser, 2004). Similarly, Australian Pulse Standards allow a maximum of 1% (by weight) off-color hull or kernel to qualify for the market class of "No. 1 grade" (Grain Trade Australia, 2018). Higher graded field peas are marketed for human consumption and attract premium prices compared with the lower grades, which are used as livestock feed.

Chlorophyll is the main pigment contributing to the color of green field pea, and it is reported to be depleted during cooking and processing of the grains (Cheng et al., 2004; Steet & Tong, 1996). However, within many green pea genotypes, there is also a susceptibility toward degradation of chlorophyll content in unprocessed grains resulting in the loss of green color. This discoloration is known as bleaching and can occur preharvest due to adverse environmental conditions or postharvest due to storage conditions (Gubbels & Ali-Khan, 1990). It is generally understood to be a cosmetic defect affecting marketability rather than functionality (Phelps, 2015); however, bleaching has been linked to loss of seed vigor and increased seed coat (hull) permeability for sugars, amino acids, and organic acids (Browning & George, 1981; Maguire, Kropf, & Steen, 1973). Chlorophyll degradation during bleaching has been attributed mainly to chlorophyllase activity; however, the activity of this enzyme was not found to be significantly different between bleaching-resistant and bleaching-susceptible green-pea genotypes (Cheng et al., 2004).

Phenolic compounds within the hulls of pulse grains are also closely linked to the expressed color (Williams & Singh, 1988). These compounds are understood to form a protective layer over the cotyledon due to their antioxidant and antimicrobial properties which can act to inhibit oxidative enzyme activity, effectively slowing the degradation of chlorophylls (Amarowicz et al., 2010; Dueñas, Estrella, & Hernández, 2004; Yamauchi, Funamoto, & Shigyo, 2004; Zhang et al., 2015). Dry field pea classes with darker colored hulls, such as dun-type peas, generally have much higher concentrations of phenolic compounds compared with the opaque hulls which are characteristic of most green pea genotypes (Magalhães et al., 2017; Maharjan, Penny, Partington, & Panozzo, 2019; Williams & Singh, 1988).

Despite the comparatively low concentration of phenolic compounds in the hulls of green pea compared with other field pea types, some green pea genotypes exhibit a level of resistance to bleaching. It has been suggested that the ratio of phenolic compounds to chlorophyll content may be related to genotypic resistance because significant differences in this ratio were observed between a resistant and susceptible genotype (Ubayasena et al., 2013).

Understanding the relation between genotypic resistance to bleaching and grain composition would be a valuable tool within field pea breeding programs in developing new cultivars with improved color stability and market acceptance. Therefore, the aim of the present study was to observe changes in chlorophyll and phenolic acid content of green peas during postharvest bleaching and determine whether a relationship exists between the presence of these compounds and the known resistance to bleaching of these genotypes. Five genotypes were included in the study, and the resistance of each to bleaching was assessed by the range of whole-grain colors observed. Color scores, chlorophyll content, and phenolic acid content were analyzed separately for hull and cotyledon to account for the role of each component.

2 | MATERIALS AND METHODS

2.1 | Samples

Green-pea samples were sourced from a field trial grown at Rupanyup in western Victoria, Australia, and stored in dark conditions immediately after harvest. There were 15 green pea samples, consisting of five genotypes (Aragorn, Excell, OZB1308, OZB1309, and OZB1316) replicated three times within the field trial. From previous studies, Excell was known to be susceptible to bleaching, and OZB1308 was known to exhibit some resistance (Brand, 2016; McDonald et al., 2019). In the following sections, the methods for producing bleached grains, assessing color, and quantifying composition constituents (chlorophyll and phenolic acids) are outlined.

2.2 | Bleaching green peas

The pea samples were first sieved to obtain uniform grain size (6–7 mm) and to remove any dust or contaminants. There was no visible sign of mold or microbial activity. Samples were then sorted into visibly uniform grain color within each genotype-replicate to obtain a homogeneous sample of 150 to 200 grains. Each color-sorted sample was divided evenly between two Petri dishes and stored according to the method outlined in McDonald et al. (2019); one Petri dish was wrapped in aluminum foil to block out light (and avoid bleaching), and
the other Petri dish was left unwrapped (to stimulate bleaching). All samples were stored at room temperature (22°C and 40% RH) and exposed to light intensity of 9,000 lux.

The loss of color occurred over a storage period of 24 weeks and subsampling (15 grains) was undertaken at 6-week intervals from the beginning of the storage period to capture grain exhibiting various degrees of bleaching. The sampled grain was placed in an airtight bag then wrapped in foil and stored at −18°C until the completion of the 24 weeks.

2.3 Image capture

Following the 24-week storage period all subsampled grain was imaged on a matte-white background using a Nikon D7200 camera fixed to a copy stand (macro lens, f8 aperture, 1/200 s shutter speed, and low 1.0 ISO), and the images were stored in Nikon raw format (NEF). A flat-field reference image (REFflat) was captured of the white background with no sample, and a dark reference image (REFdark) was captured with the lens cap on. Following the capture of whole-grain images, each sample was dehulled and imaged again under the same conditions with the hull and cotyledon separated (Figure 1).

2.4 Image processing and color analysis

Images were processed and analyzed within the Matlab R2019a programming environment with the image processing toolbox (The MathWorks). A Gaussian smoothing kernel (σ = 3) was applied to all reference and sample images prior to further processing. Pixel intensities of each sample image (In) were compared to those of the flat field image through the following pixel-wise calculation:

\[ I_{\text{ratio}} = \frac{I_n - \text{REF}_{\text{dark}}}{\text{REF}_{\text{flat}} - \text{REF}_{\text{dark}}} \]

Pixel color-intensity values for each channel (red, green, and blue) in a sample image were first translated by the REFdark values (In − REFdark) then divided by a correction factor. Each correction factor was determined by the location of the maximum peak in the pixel-intensity histogram of the relevant color channel in In (to match intensities in the common background pixels).

Color-standardized images were segmented to isolate the grains. Images were then transformed into the XYZ color space through the inbuilt Matlab function rgb2xyz. Mean pixel values were collected from each color channel in the grain regions of the XYZ images. These mean pixel values were used as the independent variables in a linear regression model with interactions to determine a green color score for each sample. The color scores (dependent variable) were based on the scale presented by McDonald et al. (2019), where low scores (close to zero) represent dark green and high scores (close to 100) indicate completely bleached grains. Development of the regression model was based on spectrophotometric color assessment (Konica Minolta CM5) and image analysis of an independent dataset of whole grain samples (n = 1,600).

2.5 Moisture analysis

The moisture content of each hull and cotyledon sample was determined by the Karl Fischer method using the automated Metrohm Karl Fischer system (Metrohm AG, Herisau, Switzerland). Ground samples (50 mg) were placed in sealed vials and incubated within the Metrohm oven at 180°C with and air flow of 40 ml/min. The resulting gas was collected within the reaction vessel containing Hydranal Methanol Rapid (Honeywell Fluka, North Carolina, USA) and titrated using Hydranal Composite 5 (Honeywell Fluka). The composition of chlorophyll and phenolic acids was reported on a dry mater basis (DB).

2.6 Analysis of chlorophyll content

The method for chlorophyll determination was adapted from Mazza and Oomah (1994) and US EPA SOP # 2030 (Environment Response Team, 1994). Briefly, ground samples were weighed (500 mg for cotyledon and 100 mg for hull) into a 24-well titer plate (SPEX® SamplePrep, Metuchen, NJ, USA). Stainless steel grinding balls (4 mm) and 4 ml of 80% acetone with 0.005% ammonium hydroxide were added to the sample. The plates were subsequently sealed with rubber-sealing cap-mats and locked into a 2010 Geno/Grinder® (SPEX® SamplePrep, USA). Chlorophylls were extracted into the solvent by homogenization for 3 min at 1,000 strokes/min. The homogenate was centrifuged at 3220× g for 5 min. The supernatant was transferred into a labelled tube, and the remnant chlorophyll was extracted again with a fresh 4 ml of extraction buffer. The absorbance of pooled supernatant from the two extractions measured at 626, 645, 663, and 700 nm were recorded in quartz cuvettes (1 cm path length) using UV-1800 spectrophotometer (Shimadzu Corpora- tion, Kyoto, Japan). The chlorophyll content in the supernatant was calculated using the following equations and converted to mg/100 g for reporting results:

\[
\text{Chlorophyll A (nMoles/ml)} = (14.18 \times \text{Abs}_{663} \times 2.91 \times \text{Abs}_{645}) \times 1.8 \times \text{Abs}_{626},
\]

\[
\text{Chlorophyll B (nMoles/ml)} = 26.01 \times \text{Abs}_{663} - 4.66 \times \text{Abs}_{663} \times 36 \times \text{Abs}_{626},
\]

where

\[
\text{Abs}_{626} = \text{absorbance at } 626 \text{ nm} - \text{absorbance at } 700 \text{ nm};
\]

\[
\text{Abs}_{645} = \text{absorbance at } 645 \text{ nm} - \text{absorbance at } 700 \text{ nm};
\]

\[
\text{Abs}_{663} = \text{absorbance at } 663 \text{ nm} - \text{absorbance at } 700 \text{ nm}.
\]

2.7 Analysis of phenolic profiles

The method for determining phenolic acid profiles was adapted from Mirali, Purves, and Vandenberg (2016). The cotyledon and hull
samples were ground to a fine powder using a Laboratory Mill 3303 (Perten Instruments, Hägersten, Sweden) and weighed into 2 ml Eppendorf tubes (100 mg for hulls and 250 mg for cotyledons). Acetone (1 ml of 70%) was added to the samples and then vortexed followed by sonication for 5 min. The extraction step was continued on the Thermo-Shaker PCMT (Grant Instruments Ltd, Shepreth, Cambridgeshire, UK) at 1,200 rpm at 25°C for 60 min. The extract was centrifuged at 12,000×g for 5 min. The supernatant was collected in a new tube, and the extraction process repeated. The pooled supernatant was vortexed, and an aliquot (1 ml) of the supernatant was dried at 40°C under a nitrogen blanket. The residue was redissolved in 1 ml of 10% methanol and filtered through a 0.22 μm syringe filter. The polyphenol contents in the extract were determined using Waters UPLC system (Waters Corporation, Milford, MA, USA), as described by Maharjan et al. (2019). The phenolic acids were identified using 3D UV spectrum from PDA, mass spectroscopy from QDa, and retention time of the peaks. The quantity of phenolic acids was calculated from the peak area obtained from the selected ion

**FIGURE 1**  Bleaching color extremities observed within each genotype. Images of cotyledon and hull for each of the five green pea genotypes from an initial subsample prior to storage (left) and a final subsample after 24 weeks of storage under light conditions (right).
recording (SIR) acquisition, that is, for o-coumaroyl malic acid and p-
coumaroyl malic acid the SIR of 278 Da and for cis- and trans-feruloyl
malic acids the SIR of 308 Da. Briefly, the UPLC system comprised
UPLC Binary Solvent Manager (BSM; H10UPB941A), UPLC Sample
Manager (G10UPA77M) and UPLC Photodiode Array Detector (PDA;
D10UPD 707A), and ACQUITY QDa Mass Detector. The column used
for polyphenol analysis was an ACQUITY BEH C18 column
(2.1 × 50 mm, 1.8 μm) (Waters Corporation, USA). Column
temperature was 45°C, and sample temperature was 25°C. The
mobile phases were acetonitrile with 0.1% acetic acid (A1) and Milli-Q
water with 0.1% acetic acid (B1). The solvent gradients were run as
follows (mm:ss): 00:00–0:30 isocratic flow of 1.0% A1 and 99.0% B1;
00:30–04:00 linear gradient to 30% A1 and 70% B1; 04:00–05:30
linear gradient to 95% A1 and 5% B1, 05:30–06:30 min: linear gradi-
et to 1% A1 and 99% B1: 06:30–08:00 isocratic flow of 1% A1 and
99% B1. The flow rate was kept constant at 0.8 ml/min throughout
the analysis. Empower 3 Software (Waters Corporation, USA) was
used for collection and analysis of chromatographic data. Phenolic
compound concentrations were recorded in mg/kg.

2.8 | Statistical analysis

A one-way ANOVA with the post hoc Tukey test was used to identify
significant differences between genotypes for green color scores and
chlorophyll concentrations and phenolic acid concentrations. The

| TABLE 1 | Descriptive statistics of color scores for whole grain, cotyledon, and hull |
|----------|-------------------------------------------------------------------------|
| Genotype | N  | Green color score (mean) | 95% CI mean | Variance | Range (max–min) |
|----------|----|--------------------------|-------------|----------|-----------------|
| Whole grain|
| Aragorn  | 30 | 44.6 a                   | (43.4, 45.9) | 10.7     | 14.4            |
| Excell   | 30 | 46.1 a                   | (44.9, 47.4) | 13.0     | 15.1            |
| OZB1308  | 30 | 41.6 b                   | (40.3, 42.8) | 7.7      | 9.9             |
| OZB1309  | 28 | 25.0 c                   | (23.7, 26.3) | 23.2     | 18.7            |
| OZB1316  | 30 | 44.2 a                   | (43.0, 45.5) | 5.9      | 10.4            |
| Cotyledon|
| Aragorn  | 30 | 3.0 c                    | (1.8, 4.2)  | 9.0      | 12.0            |
| Excell   | 30 | 9.7 a                    | (8.5, 10.9) | 22.8     | 17.9            |
| OZB1308  | 30 | 8.5 a                    | (7.4, 9.7)  | 7.2      | 10.1            |
| OZB1309  | 30 | 8.5 a                    | (7.3, 9.7)  | 7.7      | 9.3             |
| OZB1316  | 30 | 5.5 b                    | (4.4, 6.7)  | 7.0      | 10.9            |
| Hull     |
| Aragorn  | 30 | 65.4 a                   | (63.5, 67.3) | 12.8     | 14.0            |
| Excell   | 30 | 64.7 a                   | (62.8, 66.6) | 23.3     | 19.4            |
| OZB1308  | 30 | 56.8 b                   | (54.9, 58.7) | 32.7     | 18.3            |
| OZB1309  | 30 | 20.5 c                   | (18.6, 22.4) | 54.8     | 24.0            |
| OZB1316  | 30 | 60.2 b                   | (58.3, 62.1) | 14.0     | 14.1            |

Note. The whole-grain color score range for each genotype was used as the relative measure of bleaching-resistance. Smaller range values indicated greater bleaching-resistance. Significant differences of means calculated by using the Tukey method and 95% confidence. Groups are labelled in descending order of mean value.

| TABLE 2 | Descriptive statistics for the concentration (mg/100 g DB) of chlorophyll in green pea cotyledon and hull samples |
|----------|---------------------------------------------------------------------------------------------------------------|
| Genotype | N  | Chlorophyll A | Chlorophyll B | Total chlorophyll | Ratio A/B |
|          |    | Mean ± SE    | Mean ± SE     | Mean ± SE         | Mean ± SE |
| Cotyledon|
| Aragorn  | 29 | 11.1 ± 0.5 a | 5.5 ± 0.1 a   | 16.6 ± 0.7 a      | 2.0 ± 0.1 a |
| Excell   | 30 | 5.3 ± 0.4 c  | 3.7 ± 0.2 c   | 9.0 ± 0.5 c       | 1.4 ± 0.1 b |
| OZB1308  | 30 | 4.7 ± 0.2 c  | 3.3 ± 0.1 c   | 7.9 ± 0.3 c       | 1.4 ± 0.0 a |
| OZB1309  | 30 | 8.5 ± 0.3 b  | 4.6 ± 0.1 b   | 13.1 ± 0.4 b      | 1.9 ± 0.0 a |
| OZB1316  | 30 | 9.0 ± 0.3 b  | 4.5 ± 0.1 b   | 13.5 ± 0.4 b      | 2.0 ± 0.1 a |
| Hull     |
| Aragorn  | 29 | n.d.         | n.d.          | n.d.              | –         |
| Excell   | 30 | n.d.         | n.d.          | n.d.              | –         |
| OZB1308  | 30 | n.d.         | n.d.          | n.d.              | –         |
| OZB1309  | 30 | 1.0 ± 0.1    | 1.0 ± 0.1     | 1.3               | –         |
| OZB1316  | 28 | n.d.         | n.d.          | n.d.              | –         |

Note. Significant differences of means calculated by using the Tukey method and 95% confidence. Groups are labelled in descending order of mean value. Abbreviation: n.d., not detected.
relation between chlorophyll content and grain color scores was computed by a Pearson correlation. All statistical analyses were conducted at a significance level of $\alpha = 0.05$ and computed with the use of the Minitab 19 (State College, PA: Minitab, Inc.) software package.

3 | RESULTS AND DISCUSSION

3.1 | Grain color

Color scores for whole grain, cotyledon, and hull samples were derived through image processing and multiple linear regression (MLR). Low scores corresponded to dark green samples and higher scores to lighter green. Table 1 summarizes the sample color scores measured across the five genotypes. Because the model for assessing color was developed on whole grain samples, some negative values were computed for cotyledon color, as the cotyledons were significantly darker than the whole grains. In the context of this study, only whole grain color scores were used for quantifying the extent of, and resistance to bleaching, but the cotyledon color values were still considered appropriate for observing trends and differences.

Color scores varied between genotypes independent of any bleaching, as some genotypes are naturally darker than others. However, greater variability in color scores within a genotype indicated a greater extent of bleaching and therefore, the range of color scores (measured on whole grain samples) within each genotype was taken as a relative score for susceptibility to bleaching. In order of increasing susceptibility (i.e., decreasing resistance), the genotypes in this study were ranked as follows: OZB1308, OZB1316, Aragorn, Excell and OZB1309 (Table 1). These results support previous reports wherein Excell and OZB1308 were observed to be relatively susceptible and resistant respectively (Brand, 2016; McDonald et al., 2019).

Resistance to bleaching was not strongly related to the mean color of the whole-grain, hull, or cotyledon samples (Table 1). The least resistant genotype (i.e., the genotype with the greatest range of whole grain color scores) had the darkest mean whole-grain color and was also significantly darker than all other genotypes in the mean hull-color. However, there was no relation overall between the grain color and resistance to bleaching. For cotyledon-color scores, the least resistant (OZB1309) was statistically indifferent from the most resistant genotype (OZB1308) but significantly lighter than Aragorn, which ranked third in resistance (Table 1).

Although grain was sampled at regular intervals, the rate of bleaching over the storage period was not calculated. This is because bleaching does not occur uniformly within green pea genotypes (McDonald et al., 2019), and therefore, the subsamples would be too small to make an assumption of uniformity for that purpose. Measured concentrations of phenolic acids and chlorophyll were related to grain color (i.e., extent of bleaching) rather than to the time spent in storage.

3.2 | Chlorophyll content

Typically, the whole-grain color of green field pea is attributed to the chlorophyll content in the cotyledon because concentrations of chlorophyll in the hull are very low and often below the levels of detection (Table 2). Only one of the five genotypes, OZB1309, had colored hulls (Figure 1) and contained detectable concentrations of chlorophyll in the hull (Table 2). The chlorophyll content within the hull of OZB1309 was predominantly composed of chlorophyll B and was much lower in concentration than in the cotyledon.

Total chlorophyll concentration measured in the cotyledon samples ranged from 3.1 to 23.8 mg/100 g and was predominantly composed of chlorophyll A (Table 2). The concentration of total chlorophyll (cotyledon) was significantly higher ($p < 0.05$) for Aragorn...
than any other genotype, and the lowest chlorophyll concentrations were observed in Excell and OZB1308.

In agreement with the results of Cheng et al. (2004), the total chlorophyll concentration (cotyledon) was correlated \( r = -0.735, p < 0.001 \) with the cotyledon color (Figure 2a). However, the chlorophyll concentration was not highly correlated \( r = -0.212, p = 0.01 \) with the whole-grain color (Figure 2b) and therefore could not account fully for the differences in green color between genotypes or for bleaching within a genotype. Cheng et al. (2004) reported significant differences between a susceptible and resistant green pea cultivar in both the chlorophyll content and chlorophyll A/B ratio. Although the results of the present study do show that there are significant differences in both chlorophyll content and the chlorophyll A/B ratio between genotypes, there was no correlation of these values to resistance of the genotype (Table 3, Figure 3c,d). The chlorophyll A/B ratio for Excell, which is susceptible to bleaching, was not significantly different from that of the most resistant genotype (Figure 3c).

### 3.3 Phenolic acid content

The phenolic acid compounds detected in the highest concentrations were \( \beta \)-coumaroyl malic acid, \( p \)-coumaroyl malic acid, and feruloyl malic acid, where feruloyl malic acid is reported as the sum of its cis and trans isomers (Table 3). The sum of the three main phenolic acids was taken to be representative of the total phenolic acid content because the total peak area of these phenolic acids, within the 280 nm PDA chromatograms, constituted the majority of all phenolic acids present. For example, \( \beta \)-coumaroyl malic acid, \( p \)-coumaroyl malic acid, and feruloyl malic acid accounted for approximately 90% of all phenolic acids for OZB1309 (Figure 4). These compounds were also observed within a previous study as the phenolic acids occurring in the hull to chlorophyll ratio and bleaching-resistance of each genotype (Table 3, Figure 3c,d). The chlorophyll A/B ratio for Excell, which is susceptible to bleaching, was not significantly different from that of the most resistant genotype (Figure 3c).

### TABLE 3 Concentration (mg/kg DB) of the main phenolic acids detected in the green pea cotyledon and hull samples

| Genotype   | N  | \( \beta \)-Coumaroyl malic acid | \( p \)-Coumaroyl malic acid | Feruloyl malic acid | Sum of main phenolic acids |
|------------|----|---------------------------------|-----------------------------|-------------------|---------------------------|
|            |    | Mean ± SE                       | Mean ± SE                   | Mean ± SE         | Mean ± SE                 |
| Cotyledon  |    |                                 |                             |                   |                           |
| Aragorn    | 29 | 0.6 ± 0.1^c                     | 3.4 ± 0.3^c                 | 2.7 ± 0.2^b       | 6.7 ± 0.5^c               |
| Excell     | 30 | 2.7 ± 0.4^b                     | 3.7 ± 0.4^c                 | 2.5 ± 0.4^b       | 8.9 ± 1.1^bc              |
| OZB1308    | 30 | 7.9 ± 0.5^a                     | 10.4 ± 0.5^a                | 6.5 ± 0.5^a       | 24.8 ± 1.6^a              |
| OZB1309    | 30 | 2.5 ± 0.3^b                     | 6.2 ± 0.6^d                 | 2.3 ± 0.2^b       | 11 ± 1.1^b                |
| OZB1316    | 30 | 0.7 ± 0.1^c                     | 0.5 ± 0.1^d                 | 0.3 ± 0.1^c       | 1.4 ± 0.2^d               |
| Hull       |    |                                 |                             |                   |                           |
| Aragorn    | 29 | 4.5 ± 0.9^c                     | 15.4 ± 2.9^c,d              | 19 ± 3.1^c        | 38.9 ± 6.4^c              |
| Excell     | 30 | 9.2 ± 1.9^c                     | 15.1 ± 2.3^c                | 8.6 ± 1.6^c,d     | 33 ± 5.4^c,d              |
| OZB1308    | 30 | 49 ± 2.9^b                      | 67.6 ± 3.9^b                | 44.1 ± 3^b        | 160.8 ± 8.6^b             |
| OZB1309    | 30 | 69.6 ± 2.7^a                    | 101.9 ± 5.3^a               | 64.3 ± 5.3^a      | 235.8 ± 12.4^a            |
| OZB1316    | 28 | 2.8 ± 0.9^c                     | 1.7 ± 0.5^d                 | 1.9 ± 0.2^d       | 6.4 ± 1.5^a               |

Note. Significant differences of means calculated using the Tukey method and 95% confidence. Groups are labelled in order of descending mean value. Feruloyl malic acid is reported as the sum of the cis and trans isomers.
**FIGURE 3** Genotypic differences in the ratio of phenolic content in pea hulls to the chlorophyll content of pea cotyledon; (a) interval plot of phenolic acid to chlorophyll ratio values grouped by genotype, (b) relation between phenolic acid to chlorophyll ratio values and the genotypic bleaching susceptibility, (c) interval plot of cotyledon chlorophyll A/B ratio grouped by genotype, and (d) relation between cotyledon chlorophyll A/B ratio and the genotypic bleaching susceptibility.

**FIGURE 4** Chromatogram of phenolic extracts from hulls of OZB1309 (storage time = 0 weeks). (a) PDA chromatogram at 280 nm and (b) QDa chromatogram at m/z of 278 and 308. Peak identity: 1 = o-coumaroyl malic acid; 2 = p-coumaroyl malic acid; 3a and 3b = cis and trans isomers of feruloyl malic acid.

**FIGURE 5** Genotypic differences in phenolic acid content. Interval plots, grouped by genotype, for phenolic acid content of (a) cotyledon and (b) hull samples. Changes in phenolic acid concentration in (c) cotyledon and (d) hull for each genotype as whole grain color scores (i.e., bleaching scores) increased.
(Figure 3b). However, the genotypes which had a higher total concentration of phenolic compounds in the hull (>100 mg/kg) tended to also have a slower degradation of chlorophyll content in the cotyledon as bleaching color scores increased (Figure 2c). Nevertheless, the color of the whole grains still bleached even with the reduced degradation of chlorophyll, which supports the earlier finding that color changes in the wholegrains may also be attributed to more than chlorophyll degradation in the cotyledon. Although OZB1309 had the greatest phenolic acid content (hulls), it was also the least resistant to bleaching, and although bleaching in this genotype is due partially to the depletion of chlorophyll in the cotyledon and hull, it is likely that other compounds in the hull also contribute to the grain color and degradation thereof.

4 | CONCLUSIONS

Green field pea genotypes can vary widely in color, composition, and resistant to bleaching. This study has shown that, within genotypes, there is an association between the extent of bleaching (whole-grain color) and the concentration of phenolic acids and chlorophylls in the hull and cotyledon. The concentrations of total chlorophyll and phenolic acids decreased as bleaching increased. However, degradation of total chlorophyll in the cotyledon did not account fully for the observed changes in whole-grain color.

The concentrations of chlorophylls and total phenolic acids as well as ratios between these constituents could not account fully for anecdotal observations of resistance to bleaching of green pea genotypes. Therefore, it is possible that there are genotypic differences in the concentration of minor phenolic acids or other compounds which regulate color stability in green peas. Furthermore, it is not understood whether structural properties of the grain, such as hull adherence to cotyledon, could impact the bleaching response or whether preharvest conditions can impact the resistance of grain to post-harvest bleaching. Therefore, further examination of several green pea genotypes harvested across differing environments would be a valuable contribution to the study of bleaching resistance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Linda McDonald: Conceptualization, data curation, formal analysis, investigation, methodology, software, validation, visualization, writing—original draft and writing-review/editing; Dr. Pankaj Maharjan: Data curation, methodology, writing—original draft, and writing-review/editing; Drew Portman: Data curation; Dr. Joe Panozzo: Conceptualization, funding acquisition, methodology, project administration, resources, supervision, and writing-review/editing.

DATA AVAILABILITY STATEMENT

Data are available in Figshare: https://doi.org/10.6084/m9.figshare.12925868.

ETHICS STATEMENT

I, Linda McDonald, senior author and principal investigator, declare that this study did not involve human or animal studies.

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