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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 (REF_{flat}) was captured of the white background with no sample, and a dark reference image (REF_{dark}) 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 ($\sigma = 3$) was applied to all reference and sample images prior to further processing. Pixel intensities of each sample image (I_n) were compared to those of the flat field image through the following pixel-wise calculation: $I_{ratio} = (I_n - REF_{dark})/(REF_{flat} - REF_{dark})$. Pixel color-intensity values for each channel (red, green, and blue) in a sample image were first translated by the REF_{dark} values ($I_n - REF_{dark}$) 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 I_{ratio} (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 = 1,800).

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 matter 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 Corporation, Kyoto, Japan). The chlorophyll content in the supernatant was calculated using the following equations and converted to mg/100 g for reporting results:

$$\text{Chlorophyll A}\,(\text{nMoles/ml}) = (14.18 \times \text{Abs}_{663} \times 2.91 \times \text{Abs}_{645}) - 0.22 \times \text{Abs}_{626},$$

$$\text{Chlorophyll B}\,(\text{nMoles/ml}) = 26.01 \times \text{Abs}_{645} - 4.66 \times \text{Abs}_{663} - 0.36 \times \text{Abs}_{626},$$

where

Abs_{626} = absorbance at 626 nm − absorbance at 700 nm;

Abs_{645} = absorbance at 645 nm − absorbance at 700 nm;

Abs_{663} = absorbance at 663 nm − absorbance at 700 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

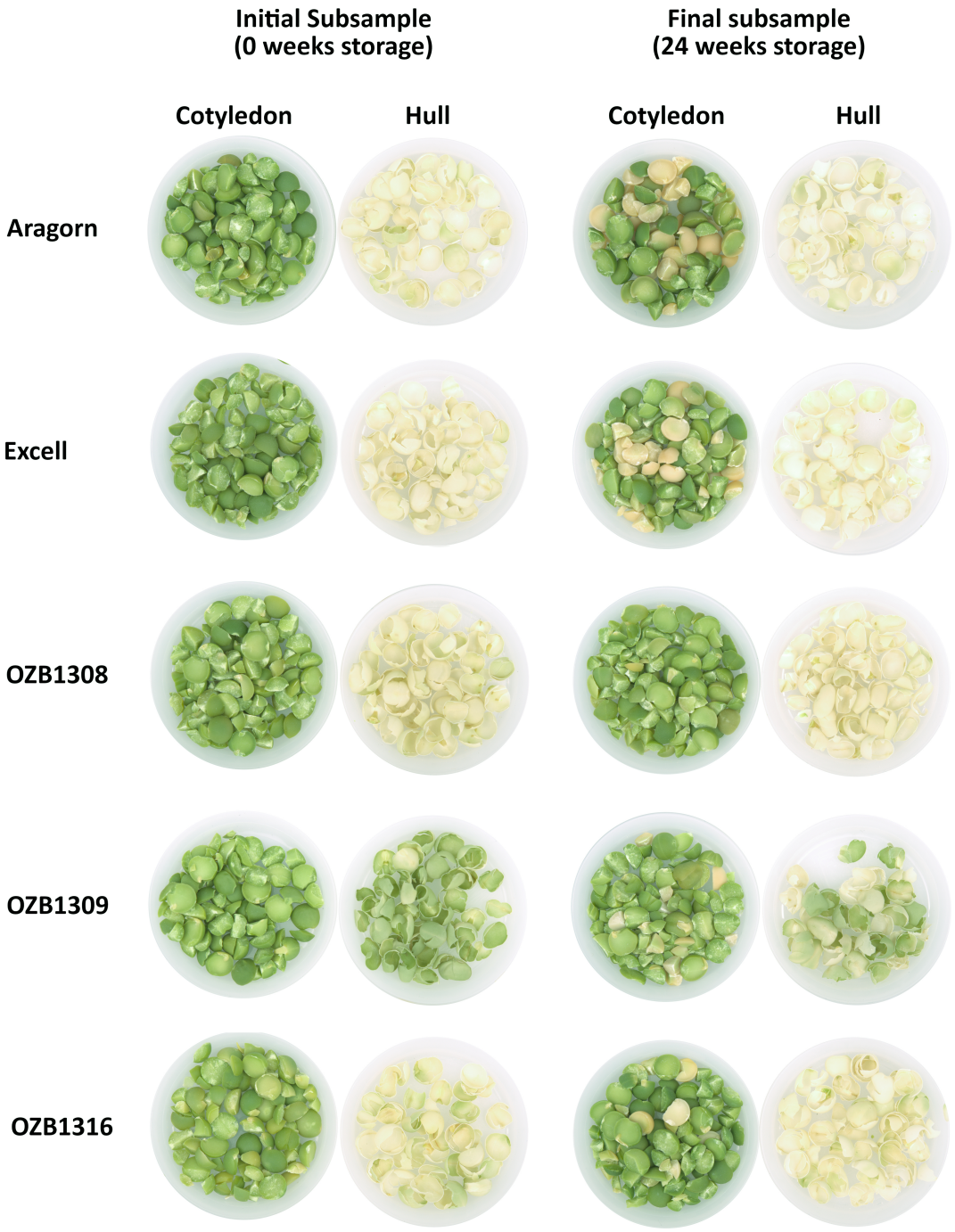

FIGURE 1 Bleaching color extremities observed within each genotype. Images of cotyledon and hull for each of the five green pea genotypes from an initial subsample prior to storage (left) and a final subsample after 24weeks of storage under light conditions (right)

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

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 gradient 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

					Total chlorophyll		
	Genotype	*N*	Chlorophyll A Mean ± SE	Chlorophyll B Mean ± SE	Mean ± SE	Range (max–min)	Ratio A/B Mean ± SE
Cotyledon	Aragorn	29	11.1 ± 0.5[a]	5.5 ± 0.1[a]	16.6 ± 0.7[a]	15.5	2.0 ± 0.1[a]
	Excell	30	5.3 ± 0.4[c]	3.7 ± 0.2[c]	9.0 ± 0.5[c]	11.4	1.4 ± 0.1[b]
	OZB1308	30	4.7 ± 0.2[c]	3.3 ± 0.1[c]	7.9 ± 0.3[c]	5.9	1.4 ± 0.0[b]
	OZB1309	30	8.5 ± 0.3[b]	4.6 ± 0.1[b]	13.1 ± 0.4[b]	10.0	1.9 ± 0.0[a]
	OZB1316	30	9.0 ± 0.3[b]	4.5 ± 0.1[b]	13.5 ± 0.4[b]	8.5	2.0 ± 0.1[a]
Hull	Aragorn	29	n.d.	n.d.	n.d.	n.d.	–
	Excell	30	n.d.	n.d.	n.d.	n.d.	–
	OZB1308	30	n.d.	n.d.	n.d.	n.d.	–
	OZB1309	30	n.d.	1.0 ± 0.1	1.0 ± 0.1	1.3	–
	OZB1316	28	n.d.	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

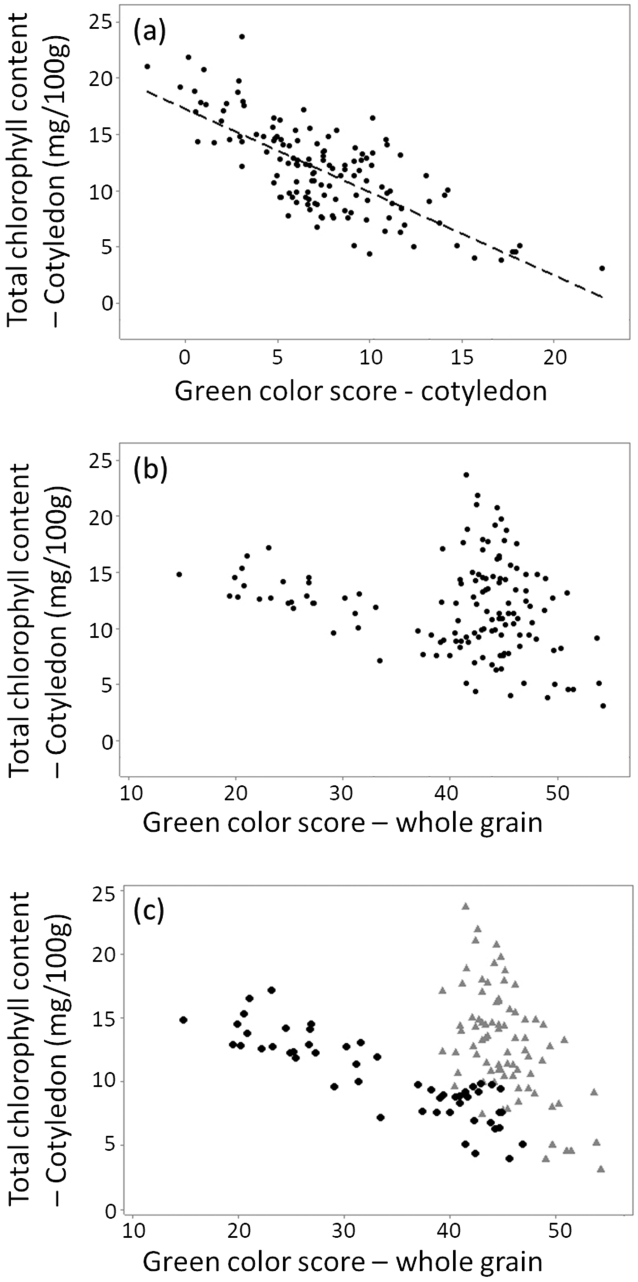

FIGURE 2 Relationship between cotyledon chlorophyll content and (a) cotyledon color score, (b) whole grain color score, and (c) whole grain color score grouped by genotypes with higher mean phenolic acid content, that is, >100 mg/kg, (black dots) and with lower mean hull phenolic content, that is, <100 mg/kg, (grey triangles)

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 *o*-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, *o*-coumaroyl malic acid, *p*-coumaroyl malic acid, and feruloyl malic acid accounted for approximaately 90% of all phenolic acids for OZB 1309 (Figure 4). These compounds were also observed within a previous study as the phenolic acids occurring in the greatest concentrations in white pea, which have similar opaque hulls to green pea (Maharjan et al., 2019).

Phenolic acids in the field pea hulls were typically in higher concentrations than those observed in the cotyledons. The *p*-coumaroyl malic acid content varied widely between genotypes but was the most prevalent phenolic compound overall, measured at concentrations between 0.1 and 17.4 mg/kg for cotyledon and 0.0 to 144.4 mg/kg for hull. The concentrations of phenolic acids observed in the hulls of all green pea genotypes was comparable to other studies, although there is a large variation in the concentration of phenolic compounds in field peas reported within the literature, due largely to differences between market classes (e.g., dun pea compared with white pea) (Dueñas et al., 2004; Magalhães et al., 2017; Maharjan et al., 2019; Marles, Warkentin, & Bett, 2013; Padhi, Liu, Hernandez, Tsao, & Ramdath, 2017; Parikh & Patel, 2018; B. Singh et al., 2017).

The concentration of phenolic acids in the hulls was significantly higher (p < 0.05) for OZB1308 and OZB1309 (Table 3); however, in the same two genotypes, there was also the greatest depletion of phenolic acids within the hull as the whole grain color score increased, that is, the phenolic content of the hull decreased as the grain bleached (Figure 5b). Phenolic acid content in the cotyledon showed no significant correlation, within each genotype, to the whole grain color (Figure 5a) and was significantly higher (p < 0.05) for OZB1308 than all other genotypes in both mean value and range (Table 3). OZB1316 contained the lowest concentration of phenolic acid content in both hull and cotyledon. The total mean phenolic acid concentrations in OZB1316 were only of the order of 2.7 to 21.4% of the concentrations observed in the other genotypes.

Ubayasena et al. (2013) reported ratio of phenolic compounds in the hull to chlorophyll content in the cotyledon may be related to bleaching-resistance within green field pea genotypes since the antioxidant activity of carotenoids and phenolic compounds in the hull may result in retarding the depletion of chlorophyll in the cotyledon. In the present study, there were significant differences between genotypes in the phenolic acid/chlorophyll ratio (Figure 3a); however, there was no clear trend observed between the phenolic acid/chlorophyll ratio and bleaching-resistance of each genotype

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

	Genotype	*N*	*o*-Coumaroyl malic acid Mean ± SE	*p*-Coumaroyl malic acid Mean ± SE	Feruloyl malic acid Mean ± SE	Sum of main phenolic acids 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$^{b, c}$
	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^{b}	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^{e}

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.

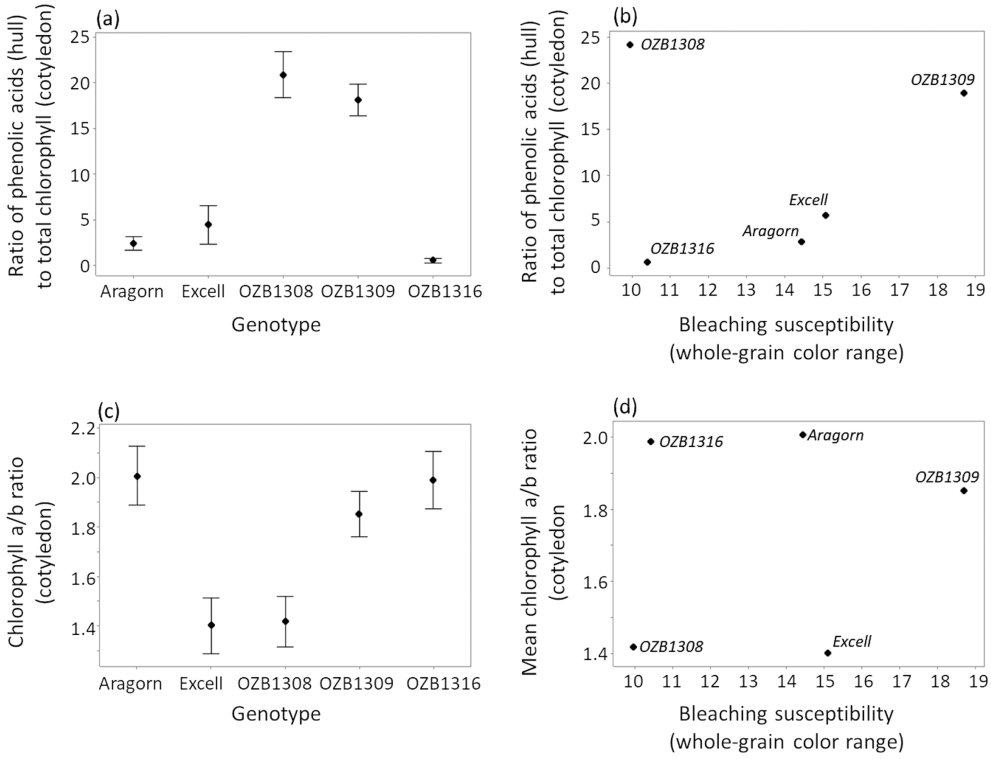

FIGURE 3 Genotypic differences in the ratio of phenolic content in pea hulls to the chlorophyll content of pea cotyledon; (a) interval plot of phenolic acid to chlorophyll ratio values grouped by genotype, (b) relation between phenolic acid to chlorophyll ratio values and the genotypic bleaching susceptibility, (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

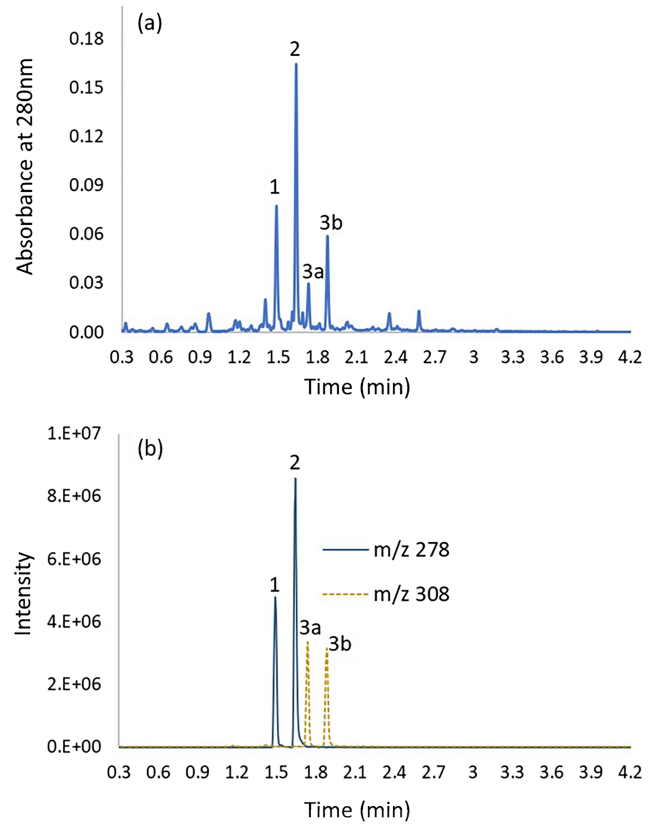

FIGURE 4 Chromatogram of phenolic extracts from hulls of OZB1309 (storage time = 0 weeks). (a) PDA chromatogram at 280 nm and (b) QDa chromatogram at *m/z* of 278 and 308. Peak identity: 1 = *o*-coumaroyl malic acid; 2 = *p*-coumaroyl malic acid; 3a and 3b = *cis* and *trans* isomers of feruloyl malic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

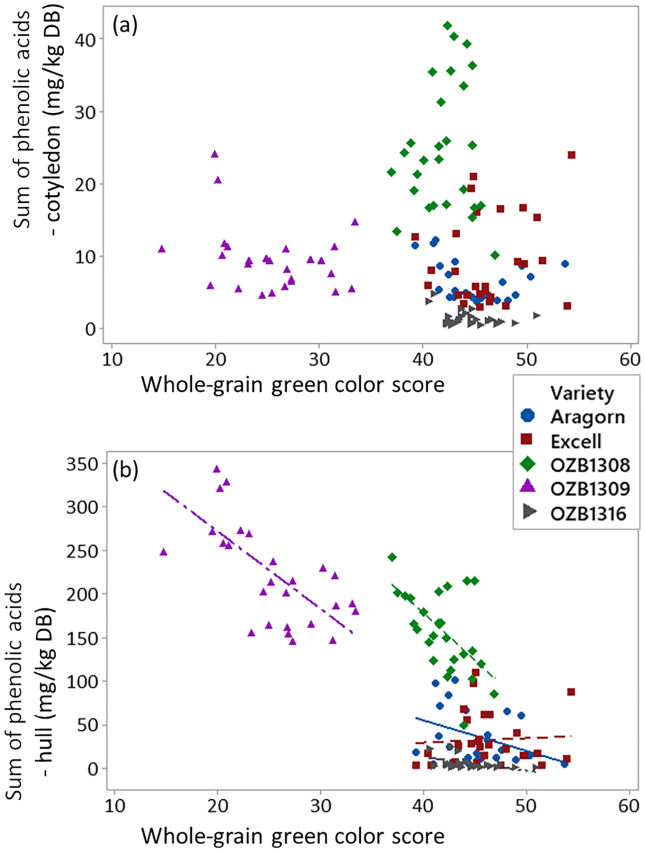

FIGURE 5 Genotypic differences in phenolic acid content. Interval plots, grouped by genotype, for phenolic acid content of (a) cotyledon and (b) hull samples. Changes in phenolic acid concentration in (c) cotyledon and (d) hull for each genotype as whole grain color scores (i.e., bleaching scores) increased

(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 postharvest 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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REFERENCES

Amarowicz, R., Estrella, I., Hernández, T., Robredo, S., Troszyńska, A., Kosińska, A., & Pegg, R. B. (2010). Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*). *Food Chemistry*, *121*(3), 705–711. https://doi.org/10.1016/j.foodchem.2010.01.009

Brand, J. (2016). Expanding the use of pulses in the southern region: 2015 Results Summary. 55–56. https://grdc.com.au/research/reports/report?id = 6,752.

Browning, T. H., & George, R. A. T. (1981). The effects of mother plant nitrogen and phosphorus nutrition on hollow heart and bleaching of pea (*Pisum sativum* L.) seed. *Journal of Experimental Botany*, *32*(5), 1,085–1,090. https://doi.org/10.1093/jxb/32.5.1085

Canadian Grain Commission. (2018). *Official grain grading guide vol. 2018*. Canadian Grain Commission.

Cheng, M., McPhee, K. E., & Baik, B. K. (2004). Bleaching of green peas and changes in enzyme activities of seeds under simulated climatic conditions. *Journal of Food Science*, *69*(7), 511–518. https://doi.org/10.1111/j.1365-2621.2004.tb13644.x

Dahl, W. J., Foster, L. M., & Tyler, R. T. (2012). Review of the health benefits of peas (*Pisum sativum* L.). *British Journal of Nutrition*, *108*(S1), S3–S10. https://doi.org/10.1017/S0007114512000852

Dueñas, M., Estrella, I., & Hernández, T. (2004). Occurrence of phenolic compounds in the seed coat and the cotyledon of peas (*Pisum sativum* L.). *European Food Research and Technology = Zeitschrift für Lebensmittel-Untersuchung und -Forschung. A*, *219*(2), 116–123. https://doi.org/10.1007/s00217-004-0938-x

Environment Response Team. (1994). Chlorophyll determination SOP#: 2030. Retrieved from https://clu-in.org/download/ert/2030-R00.pdf

Grain Trade Australia. (2018). Australian Pulse Standards 2018. from http://www.graintrade.org.au/commodity_standards

Gubbels, G. H., & Ali-Khan, S. T. (1990). Screening green field pea genotypes for resistance to color loss. *Canadian Journal of Plant Science*, *70*(1), 45–49. https://doi.org/10.4141/cjps90-005

Khan, T. N., & Croser, J. S. (2004). PEA|Overview. In C. Wrigley (Ed.), *Encyclopedia of grain science* (pp. 418–427). Elsevier.

Magalhães, S. C. Q., Taveira, M., Cabrita, A. R. J., Fonseca, A. J. M., Valentão, P., & Andrade, P. B. (2017). European marketable grain legume seeds: Further insight into phenolic compounds profiles. *Food Chemistry*, *215*, 177–184. https://doi.org/10.1016/j.foodchem.2016.07.152

Maguire, J., Kropf, J., & Steen, K. (1973). Pea seed viability in relation to bleaching. *Proceedings of the Association of Official Seed Analysts*, *63*, 51–58.

Maharjan, P., Penny, J., Partington, D. L., & Panozzo, J. F. (2019). Genotype and environment effects on the chemical composition and rheological properties of field peas. *Journal of the Science of Food and Agriculture*, *99*(12), 5,409–5,416. https://doi.org/10.1002/jsfa.9801

Marles, M. S., Warkentin, T. D., & Bett, K. E. (2013). Genotypic abundance of carotenoids and polyphenolics in the hull of field pea (*Pisum sativum* L.). *Journal of the Science of Food and Agriculture*, *93*(3), 463–470. https://doi.org/10.1002/jsfa.5782

Mazza, G., & Oomah, B. D. (1994). Color evaluation and chlorophyll content in dry green peas. *Journal of Food Quality*, *17*(5), 381–392. https://doi.org/10.1111/j.1745-4557.1994.tb00159.x

McCallum, J., Timmerman-Vaughan, G., Frew, T., & Russell, A. (1997). Biochemical and genetic linkage analysis of green seed color in field pea. *Journal of the American Society for Horticultural Science*, *122*(2), 218–225. https://doi.org/10.21273/JASHS.122.2.218

McDonald, L. S., Panozzo, J. F., Salisbury, P. A., & Ford, R. (2016). Discriminant analysis of defective and non-defective field pea (*Pisum sativum* L.) into broad market grades based on digital image features. *PLoS ONE*, *11*(5), e0155523. https://doi.org/10.1371/journal.pone.0155523

McDonald, L. S., Salisbury, P. A., Ford, R., & Panozzo, J. F. (2019). Quantifying the colour loss of green field pea (*Pisum sativum* L.) due to bleaching. *PLoS ONE*, *14*(8), e0221523. https://doi.org/10.1371/journal.pone.0221523

Mirali, M., Purves, R. W., & Vandenberg, A. (2016). Phenolic profiling of green lentil (*Lens culinaris* Medic.) seeds subjected to long-term storage. *European Food Research and Technology*, *242*(12), 2,161–2,170. https://doi.org/10.1007/s00217-016-2,713-1

Padhi, E. M. T., Liu, R., Hernandez, M., Tsao, R., & Ramdath, D. D. (2017). Total polyphenol content, carotenoid, tocopherol and fatty acid composition of commonly consumed Canadian pulses and their contribution to antioxidant activity. *Journal of Functional Foods*, *38*, 602–611. https://doi.org/10.1016/j.jff.2016.11.006

Parikh, B., & Patel, V. H. (2018). Total phenolic content and total antioxidant capacity of common Indian pulses and split pulses. *Journal of Food Science and Technology*, *55*(4), 1,499–1,507. https://doi.org/10.1007/s13197-018-3,066-5

Phelps, S. (2015). Bleaching in Green Pea. from https://saskpulse.com/files/general/151027_Bleaching_in_Green_Pea.pdf

Singh, B., Singh, J. P., Shevkani, K., Singh, N., & Kaur, A. (2017). Bioactive constituents in pulses and their health benefits. *Journal of Food Science and Technology*, *54*(4), 858–870. https://doi.org/10.1007/s13197-016-2,391-9

Singh, N., & Pratap, A. (2016). Food legumes for nutritional security and health benefits. In U. Singh, C. S. Praharaj, S. S. Singh, & N. P. Singh (Eds.), *Biofortification of Food Crops* (pp. 41–50). Springer India.

Steet, J. A., & Tong, C. H. (1996). Degradation kinetics of green color and chlorophylls in peas by colorimetry and HPLC. *Journal of Food Science*, *61*(5), 924–928. https://doi.org/10.1111/j.1365-2621.1996.tb10903.x

Ubayasena, L., Vijayan, P., Bett, K. E., Gray, G. R., Küster, H., & Warkentin, T. D. (2013). Gene expression profiles of seed coats and biochemical properties of seed coats and cotyledons of two field pea (*Pisum sativum*) cultivars contrasting in green cotyledon bleaching resistance. *Euphytica*, *193*(1), 49–65. https://doi.org/10.1007/s10681-013-0914-2

Williams, P. C., & Singh, U. (1988). Quality screening and evaluation in pulse breeding. In R. J. Summerfield (Ed.), *World crops: Cool season food legumes: A global perspective of the problems and prospects for crop improvement in pea, lentil, faba bean and chickpea* (pp. 445–457). Springer Netherlands.

Yamauchi, N., Funamoto, Y., & Shigyo, M. (2004). Peroxidase-mediated chlorophyll degradation in horticultural crops. *Phytochemistry Reviews*, *3*(1), 221–228. https://doi.org/10.1023/B:PHYT.0000047796.98784.06

Zhang, B., Deng, Z., Ramdath, D. D., Tang, Y., Chen, P. X., Liu, R., … Tsao, R. (2015). Phenolic profiles of 20 Canadian lentil cultivars and their contribution to antioxidant activity and inhibitory effects on α-glucosidase and pancreatic lipase. *Food Chemistry*, *172*, 862–872. https://doi.org/10.1016/j.foodchem.2014.09.144

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