ORIGINAL RESEARCH ARTICLE

Profiling the varietal antioxidative contents and macrochemical composition in Australian faba beans (Vicia faba L.)

Joel B. Johnson1, Tania Collins1, Daniel Skylas2, Ken Quail2, Christopher Blanchard3, Mani Naiker1

1School of Health, Medical and Applied Sciences, Central Queensland University, Rockhampton, Queensland, Australia
2Australian Export Grains Innovation Centre, North Ryde, New South Wales, Australia
3School of Biomedical Sciences, Faculty of Science, Charles Sturt University, Wagga, New South Wales, Australia

Correspondence
Joel B. Johnson, School of Health, Medical and Applied Sciences, Central Queensland University, Rockhampton, QLD 4701, Australia.
Email: joel.johnson@cqumail.com

Abstract
There is growing interest in pulses such as faba bean for the development of foods with enhanced nutrition, functionality, and health benefits. In this study, seed material from 10 faba bean varieties, grown in replicated field trials in South Australia over consecutive seasons (2016 and 2017), were analysed for ferric reducing antioxidant potential, total phenolics, and total monomeric anthocyanins. Differences in the macrochemical composition of varieties was investigated using attenuated total reflectance mid-infrared spectroscopy. The mean ferric reducing antioxidant potential of the varieties ranged from 237 to 531 mg trolox equivalents 100 g−1; the total phenolics from 258 to 571 mg gallic acid equivalents 100 g−1; and the total monomeric anthocyanins from 12.7 to 21.0 mg cyanidin-3-glucoside equivalents 100 g−1. Statistically significant variances in all three measures were found between varieties. Attenuated total reflectance Fourier transformed mid-infrared spectroscopy was found to provide a rapid assessment of the phytochemical composition of the samples. Partial least squares discriminant analysis was able to classify samples by growing year with reasonable accuracy (>87%). There is significant variation in the antioxidant, phenolic, and anthocyanin contents between Australian faba bean varieties. Mid-infrared spectroscopy may prove to be a valuable screening tool for breeders and researchers in the future.

KEYWORDS
anthocyanin, attenuated total reflectance mid-infrared (ATR-MIR) spectroscopy, Fourier transformed infrared (FTIR) spectroscopy, total phenolics

1 INTRODUCTION

Increased consumption of pulses worldwide is driven in part by growing consumer demand for new foods with enhanced nutrition and health benefits. Pulses such as faba bean (Vicia faba L.) have significant potential in the development of value-added foods and ingredients (López-Barrios, Gutiérrez-Uribe, & Serna-Saldívar, 2014; Vioque, Alaz, & Girón-Calle, 2012). Domestically, this crop is grown in South Australia, Victoria, Western Australia, New South Wales, and southern Queensland (Siddique et al., 2000). With annual production of around 300,000 tonnes, faba beans comprise 10–15% of total pulse production in Australia (Australian Export Grains Innovation Centre [AEGIC], 2017). Australia is one of the top five producers in the world and the largest exporter, responsible for one third of international trade for
this crop (AEGIC, 2017). Primary importers include the Middle East, particularly Egypt, and South East Asian countries (AEGIC, 2017).

Faba beans are reported to meet dietary requirements for all essential minerals except calcium (AEGIC, 2017). Furthermore, they contain relatively high levels of antioxidant and phenolic compounds, which may have beneficial effects including protection against radical species, antihypertensive, and anticancer activity (Siah, Konczak, Agboola, Wood, & Blanchard, 2012; Turco, Ferretti, & Bacchetti, 2016).

Over the past 35 years, pulse breeders have focussed on improving Australian faba bean varieties, primarily selecting for disease resistance and elevated yield (Siddique et al., 2000). However, new varieties specifically selected for these characteristics alone may display a concomitant, albeit unintentional, alteration in their nutritional and antioxidative properties as observed in other crops (Wrigley, Matakovsky, Melnik, Pascual, & Romanov, 2019). Recent work highlighted variation in nutritional and antinutritional properties in commercial Australian faba bean varieties (Skylas et al., 2019). However, similar profiling on phytochemical properties such as antioxidant compounds is lacking. Siah, Wood, Agboola, Konczak, and Blanchard (2014) investigated the effects of processing on antioxidant and phenolic content in five faba bean varieties. Similarly, Nasar-Abbas et al. (2009); Siah, Konczak, Wood, Agboola, and Blanchard (2014); and Siah et al. (2012) investigated only one, two, and three varieties, respectively. On the other hand, a number of studies have profiled the variation in antioxidant constituents of faba beans varieties in Europe (Valente et al., 2018; Valente et al., 2019), Africa (Chaieb, González, López-Mesas, Bouslama, & Valiente, 2011), and South America (Baginsky et al., 2013).

In this study, we analysed the antioxidative, phenolic, and anthocyanin content of 10 faba bean varieties, grown in replicated field trials, at two sites in South Australia and over two consecutive growing seasons. We also explored the use of mid-infrared (MIR) spectroscopy for non-invasive analysis of faba beans, as this technology has shown promise in other crops such as wheat and mungbeans (Johnson et al., 2019; Johnson, Collins, Skylas, & Naiker, 2019).

2 | METHODS

2.1 | Faba bean samples

Seed material of 10 faba bean varieties included in this study are listed in Table 1 and are described further in Skylas et al. (2019). These varieties constitute the bulk of domestic production for this crop (Pulse Australia, 2016). Varieties were grown at two locations in South Australia (Charlick and Freeling), over consecutive seasons (2016 and 2017). The four environments are designated herein as 16Char, 17Char, 16Free, and 17Free. Growing details, including rainfall conditions and trial yield data have been previously reported (Skylas et al., 2019). Two field replicates were analysed (in duplicate) for each of the 2016 sites, and three field replicates were analysed (in duplicate) for each of the 2017 sites. Seed samples were impact milled to produce whole seed flour (falling No. grinder, 0.8 mm screen), and moisture content were determined as previously described (Skylas et al., 2019).

2.2 | Reagents

All reagents used were of analytical grade. Methanol was purchased from Fisher Scientific Australia. Hydrochloric acid and sodium carbonate were purchased from Chem Supply. All other reagents were purchased from Sigma-Aldrich Australia. Unless otherwise specified, all dilutions and assay preparations were made using Milli-Q water. All solutions were stored at 4°C until usage.

2.3 | Extraction

Extracts were prepared in duplicate by combining approximately 0.5 g of faba bean flour with 8 ml of 90% v/v aqueous methanol, vortexing for 10 s, and mixing for 60 min using an end-over-end shaker (Ratek RM4) operating at 50 rpm. After centrifugation at 1,000 g for 10 min (Heraeus Multifuge; Thermo Fisher Scientific), the supernatant was collected. To extract any remaining phytochemicals, the extraction process was repeated with another 8 ml of 90% methanol added to the pellet and end-over-end mixing for 20 min. The combined supernatant was made up to 20 ml volume with 90% methanol. Extracts were stored in the dark at 4°C until required for analysis.

2.4 | Total phenolics

Total phenolics (TP) were determined through a modification of the Folin–Ciocalteu method developed by Singleton and Rossi (1965). First, 2 ml of a 1:10 aqueous dilution of Folin–Ciocalteu reagent was combined with 400 μl of sample extract. The samples were incubated at room temperature in darkness for 10 min before 2 ml of 7.5% w/v aqueous sodium carbonate was added. They were then vortexed for 10 s, incubated at 40°C for 30 min in a covered water bath, and vortexed for another 10 s. From the absorbance at 760 nm, the TP concentration was derived as a function of the equivalent absorbance of gallic acid in the range 20 to 120 mg L⁻¹ (R² = .9968). Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of oven dry sample weight (mg GAE/100 g).

2.5 | Ferric reducing antioxidant power

As a measure of total antioxidant capacity, the ferric reducing antioxidant power (FRAP) assay developed by Benzie and Strain (1996) was performed on the samples. FRAP reagent was prepared by combining 300-mM acetate buffer at pH 3.56, 20-mM aqueous ferric chloride, and 10-mM TPTZ (made in 40-mM hydrochloric acid) in the ratio...
The FRAP reagent, ferric chloride, and TPTZ solutions were prepared fresh each day. First, 3 ml of FRAP reagent, pre-equilibrated at 37°C, was combined with 100 μl of pre-equilibrated sample and vortexed for 10 s. The samples were incubated in a covered water bath at 37°C for 4 min, vortexed for 10 s, and their absorbances read at 593 nm. The FRAP derived was a function of the equivalent absorbance of trolox in ethanol solution in the range 10-175 mg L⁻¹ ($R^2 = .9999$). Results were expressed as milligrams of trolox equivalents per 100 g of oven dry sample weight (mg TXE/100 g).

### 2.6 | Total monomeric anthocyanins

The total monomeric anthocyanins were determined using a minor modification of the pH differential method described by Giusti and Wrolstad (2001). Buffer solutions consisting of 0.025-M aqueous potassium chloride and 0.4-M aqueous sodium acetate were prepared and adjusted to pH 1 and 4.5, respectively, using 32% hydrochloric acid.

In a cuvette, 400 μL of sample extract and 1.6 ml of pH 1 buffer were combined and mixed by inversion. After equilibration in darkness at room temperature for 15 min, its absorbance was read at 510 and 700 nm. This procedure was repeated on the same sample using the pH 4.5 buffer. The monomeric anthocyanin concentration was calculated using the following formula (Giusti & Wrolstad, 2001):

$$A = (pH_1: \text{Absorbance}_{510 \text{ nm}} - \text{Absorbance}_{700 \text{ nm}}) - (pH_{4.5}: \text{Absorbance}_{510 \text{ nm}} - \text{Absorbance}_{700 \text{ nm}})$$

Anthocyanin content (mg cyd-3-glu L⁻¹) = ($A$ × 449.38 × Dilution Factor x 1,000)/(26,900 x 1)

The molecular weight (449.38 g mol⁻¹) and molar extinction coefficient (26,900 M⁻¹ cm⁻¹) used were that of cyanidin-3-glucoside, the most abundant anthocyanin in nature (Markakis, 1989). Results were expressed as milligrams of cyanidin-3-glucoside (cyd-3-glu) per 100 g of oven dried sample weight (mg cyd-3-glu/100 g).

### 2.7 | Attenuated total reflectance MIR spectroscopy

A Bruker Alpha Fourier transformed infrared spectrophotometer (Bruker Optics GmbH, Ettlingen, Germany) fitted with a platinum diamond attenuated total reflectance (ATR) single reflection module was used for the MIR analysis. Homogenous faba bean flour was used to cover the reflection module and pressure applied to achieve uniform contact between the ATR interface and flour. Air was used as a reference background; the background measurement was performed every 10 samples. Cross contamination of samples was minimised by cleaning and drying the platform with isopropyl alcohol and laboratory Kimwipes® between samples (Gordon et al., 2019).

MIR spectra between 4,000 and 400 cm⁻¹ were recorded using the OPUS software version 7.5 (Bruker Optics GmbH, Ettlingen, Germany) as the average of 24 scans at a resolution of 4 cm⁻¹. Five replicates were performed on each sample.

### 2.8 | Statistical analysis

Statistical tests were performed in IBM SPSS. As a relatively high number of replicates were included in each test and all data were reasonably normally distributed, parametric testing was used throughout.

MIR spectra were analysed with The Unscrambler X software version 10.5 (Camo ASA, Oslo, Norway). Following previous work on barley (Gordon et al., 2019) and mungbeans (Johnson, Collins, Power, et al., 2019), the spectra were preprocessed to the second derivative using a Savitzky–Golay algorithm at a polynomial number of 2 and a smoothing window of 41 points (Savitzky & Golay, 1964). Using the second derivative removes spectral variations in the baseline and slope (Savitzky & Golay, 1964), minimising differences due to non-compositional variables such as the pressure and contact with the reflection module. Principal component analysis (PCA) and partial least

| Variety     | Release | Seed size | Germplasm origin          | Asc(f) | Asc(s) | CS | Rust | PSbMV | BLRV |
|-------------|---------|-----------|---------------------------|--------|--------|----|------|-------|------|
| Fiord       | 1980    | Small     | Greece                    | mS     | mS     | vS | S    | S     | -    |
| Fiesta VF   | 1998    | Medium    | Spain                     | mS/mR  | mR/R   | S  | S    | S     | -    |
| Farah       | 2003    | Medium    | Spain                     | mR/R   | mR/R   | S  | S    | S     | -    |
| Nura        | 2005    | Medium    | Ecuador and Greece        | mR/R   | mR/R   | S  | mS   | vS    | -    |
| Doza        | 2008    | Medium    | Ethiopia and Sudan        | vS     | vS     | mS | mR/R | -     | -    |
| PBA Rana    | 2011    | Large     | Ecuador and Lebanon       | R      | R      | mS | mS/mR| mR/R  | -    |
| PBA Warda   | 2012    | Large     | Ecuador and Greece        | vS/S   | vS/S   | mS | mR/R | -     | mT   |
| PBA Samira  | 2014    | Medium    | Lebanon, UK, Spain, Ecuador, and Greece | R | R | mS | mS | -     |     |
| PBA Zahra   | 2015    | Large     | Morocco and Spain         | R      | -      | mS | mS   | S     | -    |
| PBA Nasma   | 2015    | Large     | China, Sudan, and Italy   | vS/S   | vS/S   | mS | mS   | -     | mT   |

Note. A dash (-) indicates resistance is unknown. References: Pulse Australia (2016), Skylas et al. (2019). Abbreviations: Asc, ascochyta blight; BLRV, bean leafroll virus; CS, chocolate spot; f = foliage; m, moderately; PSbMV, pea seed-borne mosaic virus; R = resistant; s, seed; S, susceptible; T, tolerant; v, very.
squares (PLS) regression were performed in The Unscrambler X on the second derivative of the MIR spectra.

3 | RESULTS AND DISCUSSION

3.1 | Ferric reducing antioxidant potential

The average ferric reducing antioxidant potential determined for the varieties is shown in Table 2. There was a significant positive correlation between FRAP and the protein content of the samples ($r_{100} = 0.346, p < .001$). Although the Charlick site had slightly higher average FRAP values, independent samples t testing showed no significant difference between either site ($t_{99} = -0.757, p > .05$) or year ($t_{99} = 0.722, p > .05$). However, there was a significant variance in the FRAP values when considered by variety (one-way analysis of variance [ANOVA]; $F_{9,90} = 54.935, p < .001$).

Whereas most varieties displayed similar FRAP levels, those for Nura and PBA Samira were somewhat higher than most remaining varieties. PBA Rana had the highest FRAP values by far, being approximately double that of most other varieties. Although further exploration into the specific compounds present is required, it is possible that this variety may provide the greatest benefits towards antioxidative health effects.

3.2 | TP contents

The TP contents followed a similar trend to the FRAP values (Table 3). There was a linear correlation between FRAP values and TP contents ($r_{100} = 0.917; p < .001$), as previously observed for faba beans (Chaieb et al., 2011) and other crops (Chen et al., 2018; Hung & Morita, 2008; Zhang et al., 2013; Žilić, Serpen, Akkoğlu, Janković, & Gökmen, 2012). There was also a significant linear correlation between protein content and TPs ($r_{100} = 0.302, p < .01$), but there was no correlation between FRAP or TPs and moisture content ($p > .05$). However, there was no significant difference in TP values across sites (independent t test; $t_{98} = -0.558, p > .05$) or years (independent t test; $t_{98} = -0.919, p > .05$). TP levels varied significantly between varieties (one-way ANOVA; $F_{9,90} = 72.109, p < .001$). PBA Rana contained almost double the TP content of most varieties, whereas PBA Samira contained less phenolics than PBA Rana but more than all other varieties. The remaining eight varieties contained similar levels of phenolics (258–294 mg GAE 100 g$^{-1}$ dry weight).

3.3 | Total monomeric anthocyanin contents

There was no correlation with the mean total monomeric anthocyanin content of samples (Table 4) and FRAP or TP contents ($p > .05$ for both), although there was a negative correlation with moisture content ($r_{100} = -0.202, p < .05$).

There was a significant variation in the mean anthocyanin content when analysed by variety (one-way ANOVA; $F_{9,90} = 3.726, p = .001$). Statistical differences are shown from annotations reported in Table 4. The variety Fiord contained the highest levels of anthocyanins, followed by PBA Warda. The anthocyanin concentrations in Fiord were statistically higher than the three varieties containing the lowest anthocyanin levels (PBA Samira, PBA Zahra, and Doza) but not statistically different to the six remaining varieties.

Although no difference in anthocyanin concentrations was found between the two sites (independent t test; $t_{99} = 0.517, p > .05$), those from 2016 displayed a higher anthocyanin content than the 2017 samples (independent t test; $t_{98} = 4.033, p < .001$).

3.4 | Principal component analysis of moisture, antioxidant, phenolic, and anthocyanin contents

In order to further explore the variation in the moisture, antioxidant, phenolics, and anthocyanin contents, a principal component analysis

### TABLE 2 Average ferric reducing antioxidant potential (mg TXE 100 g$^{-1}$ DW) of the 10 faba bean varieties

| Variety | 16Char | 16Free | 17Char | 17Free | Mean ± SD |
|---------|--------|--------|--------|--------|-----------|
| Fiord   | 255    | 223    | 261    | 231    | 243 ± 24$^a$ |
| Fiesta VF | 259  | 222    | 237    | 233    | 237 ± 23$^a$ |
| Farah   | 253    | 250    | 249    | 231    | 245 ± 25$^a$ |
| Nura    | 320    | 307    | 326    | 261    | 301 ± 33$^{bc}$ |
| Doza    | 274    | 250    | 275    | 287    | 273 ± 27$^{ab,cd}$ |
| PBA Rana | 456   | 471    | 542    | 610    | 531 ± 90$^d$ |
| PBA Warda | 265  | 244    | 269    | 259    | 260 ± 16$^{ab}$ |
| PBA Samira | 346   | 302    | 321    | 324    | 323 ± 25$^c$ |
| PBA Zahra | 284   | 264    | 299    | 262    | 278 ± 23$^{ab,cd}$ |
| PBA Nasma | 262   | 236    | 272    | 272    | 249 ± 27$^{ab}$ |
| Mean ± SD | 297 ± 62 | 277 ± 74 | 305 ± 91 | 297 ± 114 |

Note. Samples with the same letter in the last column were not statistically different at $\alpha = .05$ according to post hoc Tukey testing.

Abbreviations: DW, dry weight; TXE, trolox equivalents.

The different letters correspond to different statistical groups.
was conducted on the data for these three measurements. As these parameters varied considerably in terms of their absolute values, each datapoint was weighted by dividing itself by the overall standard deviation for that measurement.

The first two principal components (PCs) explained 78% of the total variation observed. Across PC1, broad separation was observed between PBA Rana and the remainder of the varieties (Figure 1). Examination of the loadings associated with this PC indicated that PC1 scores were positively correlated with increased FRAP and TPs, confirming that levels of these compounds in PBA Rana were noticeably elevated compared with other varieties. PBA Samira also had higher scores along PC1 than the remainder of samples, indicating that its FRAP and TP levels were higher, albeit not as distinct as those of PBA Rana.

Separation along the PC2 was generally less clear. Both PBA Rana and Fiord were largely associated with positive scores along this axis, indicating above average anthocyanin levels and lower moisture contents. The negative PC2 scores observed for PBA Zahra indicated higher moisture contents and lower anthocyanin levels, as previously observed (Table 4). PBA Samira, which had the lowest mean anthocyanin levels of all faba bean varieties (Table 4), also was largely associated with negative PC2 values.

To further visualise the general relationship between the chemical composition of the varieties obtained through PCA, a cluster analysis was performed on the mean moisture, antioxidant, phenolic, and anthocyanin contents (Figure 2). This confirmed that the composition of PBA Rana was highly distinct from the remaining varieties, whereas the composition of PBA Samira was moderately different. The hierarchical cluster analysis also suggested that based on their chemical composition, two general groups could be made of the remaining eight varieties, one comprising Nura, Doza, and PBA Zahra and the other comprising Fiord, Fiesta VF, PBA Nasma, PBA Warda, and Farah.

### TABLE 3  Average total phenolic content (mg GAE 100 g\(^{-1}\) DW) of faba bean varieties

| Variety  | 16Char | 16Free | 17Char | 17Free | Mean ± SD |
|----------|--------|--------|--------|--------|-----------|
| Fiord    | 240    | 259    | 273    | 255    | 258 ± 22\(^a\) |
| Fiesta VF| 263    | 252    | 259    | 286    | 266 ± 29\(^a\) |
| Farah    | 254    | 320    | 286    | 289    | 287 ± 38\(^a\) |
| Nura     | 269    | 292    | 278    | 243    | 268 ± 27\(^a\) |
| Doza     | 257    | 270    | 302    | 322    | 293 ± 33\(^a\) |
| PBA Rana | 519    | 546    | 588    | 604    | 571 ± 59\(^a\) |
| PBA Warda| 247    | 280    | 273    | 289    | 274 ± 21\(^a\) |
| PBA Samira| 351   | 356    | 350    | 374    | 358 ± 32\(^a\) |
| PBA Zahra| 288    | 290    | 330    | 264    | 294 ± 34\(^a\) |
| PBA Nasma| 247    | 306    | 284    | 317    | 277 ± 39\(^a\) |
| Average  | 294 ± 86 | 317 ± 89 | 322 ± 96 | 324 ± 107 |

Note. Samples with the same letter in the last column were not statistically different at \(\alpha = .05\) according to post hoc Tukey testing.

Abbreviations: DW, dry weight; GAE, gallic acid equivalents.
The different letters correspond to different statistical groups.

### TABLE 4  Average total monomeric anthocyanin content (mg cyd-3-glu equivalents 100 g\(^{-1}\) DW) of faba bean varieties

| Variety  | 16Char | 16Free | 17Char | 17Free | Average |
|----------|--------|--------|--------|--------|---------|
| Fiord    | 18.9   | 18.6   | 19.2   | 18.0   | 18.6 ± 1.2\(^c\) |
| Fiesta VF| 19.8   | 20.3   | 16.5   | 12.4   | 16.7 ± 3.8\(^a\) |
| Farah    | 19.3   | 17.8   | 14.8   | 11.6   | 15.3 ± 3.7\(^a\) |
| Nura     | 21.1   | 16.5   | 15.2   | 17.0   | 17.2 ± 2.9\(^c\) |
| Doza     | 14.3   | 17.9   | 13.4   | 11.2   | 13.8 ± 3.6\(^b\) |
| PBA Rana | 14.7   | 16.9   | 17.2   | 15.3   | 16.1 ± 2.6\(^b\) |
| PBA Warda| 19.6   | 19.3   | 18.5   | 15.1   | 17.8 ± 2.8\(^c\) |
| PBA Samira| 12.2  | 10.6   | 12.8   | 14.4   | 12.7 ± 3.4\(^c\) |
| PBA Zahra| 17.1   | 13.5   | 11.3   | 13.2   | 13.5 ± 2.9\(^b\) |
| PBA Nasma| 21.7   | 20.2   | 15.8   | 12.1   | 21.0 ± 4.3\(^b\) |
| Average  | 17.9 ± 3.7 | 17.2 ± 3.6 | 15.5 ± 3.1 | 14.0 ± 3.2 |

Note. Samples with the same letter in the last column were not statistically different at \(\alpha = 0.05\) according to post-hoc Tukey testing.

Abbreviation: DW, dry weight.
The different letters correspond to different statistical groups.
There was a visible difference in the amplitude of the MIR absorbance between 2016 and 2017 samples, with the 2017 samples showing greater absorbance overall. However, the location of spectral peaks was virtually identical between the 2 years. There was little visible difference in the average spectra between the Charlick and Freeling sites, although slightly larger peak at 2,990–2,950 cm$^{-1}$ was observed in the Freeling samples. There was also little visible difference in the average spectra of the 10 faba bean varieties, although varietal differences in the size of the 2,990–2,950 cm$^{-1}$ peak were noted (Figure 3). The main spectral peaks observed were attributed to a range of constituent compounds, including water, compositional polysaccharides, and protein (Table S1).

Prior to further analysis, the individual spectra were preprocessed to the second derivative to remove any differences in the absorbance amplitudes resulting from variation in the level of contact between the sample and the reflection module. This successfully removed any baseline amplitude variation while amplifying the differences in peak positions, shapes, and relative amplitudes.

To further explore the spectral variation, principal component analysis was conducted on the second derivative of the MIR spectra. The first two principal components of the PCA explained 75% of the observed variation. Additional principal components did not improve this figure significantly. There was some incomplete separation when coloured by growing year (Figure 4a) and no clear separation when coloured by site (Figure 4b). Most faba bean varieties were spread across the PCA (Figure 4c) again with no clear separation. However, some varieties, such as Fiesta VF, appeared to show a larger amount of intra-varietal spectral variation, being distributed much further across both PC1 and PC2 than other varieties, such as Fiord.
PLS discriminant analysis (PLS-DA) has previously been highlighted as a powerful tool for the discrimination and authentication of grains of different origins (Gordon et al., 2019). Hence, PLS-DA was performed on the second derivative of the faba bean spectra. The growing year was able to be correctly classified in 87.4% of the samples (Table S2), indicating that this technique may be suitable for some authentication purposes when applied to faba bean flour. However, the successful classification rate by growing site was much lower, with an average of 60.2% of samples correctly assigned to their growing site (Table S2). Further refinement of the PLS regression, perhaps through the isolation and selection of the most relevant waveband, is required before this technique can be utilised for authentication of growing site in faba bean flour.

Overall, the MIR analysis was found to provide valuable information on the chemical composition of the faba bean varieties. The preliminary results presented here are quite promising, indicating that
agronomic aspects such as the year of growth can be determined from the MIR spectra with reasonable certainty. With the development of more sophisticated methods of data analysis, the usefulness of this technology can only increase.

4 | CONCLUSION

The 10 varieties of Australian faba beans tested show considerable variation in their anthocyanin, phenolic, and antioxidant contents. In particular, PBA Rana contains much higher content of total phenolics and antioxidants than all other varieties tested. Varietal differences in chemical composition were highlighted through principal component analysis and hierarchical cluster analysis. MIR spectra obtained from the faba bean flour provided insight into the phytochemical composition and variation between the varieties. Classification using PLS-DA allowed the growing year to be successfully predicted from the MIR over 87% of the time; however, prediction of growing location was less successful (mean accuracy 59%).

CONFLICT OF INTEREST

All authors declare no conflict of interest exists.

AVAILABILITY OF DATA

Due to technical limitations, the full data set is unable to be published at this time; however, it is available upon request from the authors.

ORCID

Joel B. Johnson https://orcid.org/0000-0002-9172-8587
Daniel Skylas https://orcid.org/0000-0003-4640-1531
Christopher Blanchard https://orcid.org/0000-0001-5800-4678
Mani Naiker https://orcid.org/0000-0002-6844-8325

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Johnson JB, Collins T, Skylas D, Quail K, Blanchard C, Naiker M. Profiling the varietal antioxidative contents and macrochemical composition in Australian faba beans (Vicia faba L.). Legume Science. 2020; e28. https://doi.org/10.1002/leg3.28