Author/s:
Johnson, J;Collins, T;Power, A;Chandra, S;Skylas, D;Portman, D;Panozzo, J;Blanchard, C;Naiker, M

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Antioxidative properties and macrochemical composition of five commercial mungbean varieties in Australia

Joel Johnson[1] | Tania Collins[1] | Aoife Power[1] | Shaneel Chandra[1] | Daniel Skylas[2] | Drew Portman[3,4] | Joe Panozzo[3] | Christopher Blanchard[4] | Mani Naiker[1]

[1]School of Health, Medical and Applied Sciences, Central Queensland University, RockhamptonQLD, Australia

[2]Australian Export Grains Innovation Centre, North Ryde, NSW, Australia

[3]Department of Economic Development, Jobs, Transport and Resources, Horsham, VIC, Australia

[4]School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia

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

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Abstract

Mungbeans are growing in popularity among Australian consumers, driven by their beneficial nutritional and phytochemical composition. However, data on the antioxidative, mineral, and phytochemical content of Australian mungbeans at the point of consumer purchase remains scarce. Here, five commercial mungbean samples were analysed for total antioxidant capacity, total phenolics, and total monomeric anthocyanins. Attenuated total reflectance midinfrared spectroscopy was utilised as a rapid and reliable method of obtaining information about the macrochemical composition of the mungbean hulls. Total antioxidant capacity ranged from 170 to 570 mg Trolox equivalents per 100 g, total phenolic content from 130 to 240 mg gallic acid equivalents per 100 g, and anthocyanin content from 10 to 40 mg cyanidin-3-glucoside equivalents per 100 g. There was a significant difference between varieties in all measures of antioxidant, phenolic and anthocyanin contents. Using principal component analysis, the midinfrared spectra for the five mungbean varieties could be isolated, highlighting the differences in their phytochemical composition. In general, whole Australian mungbeans appear to have the highest antioxidant, phenolic, and anthocyanin contents. Midinfrared spectroscopy appears to be a valuable method of obtaining and comparing the macrochemical composition of mungbeans. This technology is likely to be of increasing use in the future.

KEYWORDS

attenuated total reflectance midinfrared spectroscopy, Fourier transform infrared spectroscopy, mungbeans (*Vigna radiata*), principal component analysis, total antioxidant capacity, total monomeric anthocyanins, total phenolics

1 | INTRODUCTION

Currently, mungbeans (*Vigna radiata* L.) comprise around 5% of the total Australian pulse crop (Australian Export Grains Innovation Centre, 2017). However, driven by the development of new varieties featuring stronger resistance to disease, higher output yields, and improved nutritional quality, the market share of mungbeans is growing rapidly (Australian Mungbean Association, 2017; Siddique & Sykes, 1997). The 5-year average annual market value between 2011 and 2016 was $86 million, with a record

production level of over 150,000 t in 2016 (Australian Mungbean Association, 2017).

For the consumer, mungbeans provide a food source high in protein, carbohydrates, and fibre and is low in fat (Dahiya et al., 2015; Skylas, Blanchard, & Quail, 2017). They contain high levels of essential micronutrients including iron, potassium, and calcium (Dahiya et al., 2015). Mungbeans have also been found to contain high levels of antioxidant compounds (Anwar, Latif, Przybylski, Sultana, & Ashraf, 2007; Ganesan & Xu, 2018), which are known to have numerous beneficial effects on cardiovascular and general health (Lobo, Patil, Phatak, & Chandra, 2010; Thompson, 1994). Furthermore, mungbean extracts have been found to possess some level of antimicrobial, antifungal, anti-inflammatory, antidiabetic, antihypertensive, and antiproliferative (antitumour) properties (Tang, Dong, Ren, Li, & He, 2014).

Several studies have been conducted on the nutritional qualities of various mungbean varieties in China (Liu, Liu, Yan, Cheng, & Kang, 2015; Shi, Yao, Zhu, & Ren, 2016; Zhang, Shang, Qin, Zhou, Gao, Huang et al., 2013), India (Dahiya, Linnemann, Nout, Van Boekel, & Grewal, 2013), Myanmar (Kywe, Finckh, & Buerkert, 2007), and Pakistan (Ullah, Ullah, Al-Deyab, Adnan, & Tariq, 2014). These included the quantification of amylose, starch, protein, fibre, ash, carbohydrate, and minerals and the characterisation of constituent amino acids and fatty acids. Varietal influences on the antioxidant properties of mungbeans have also been studied in China (Shi et al., 2016; Zhang et al., 2013) and Myanmar (Ullah et al., 2014). However, information on the phytochemical or antioxidant composition of Australian mungbeans remains very limited. Moreover, all previously cited antioxidant studies, except for the Chinese study by Zhang et al. (2013) and Mamilla and Mishra (2017), who studied a single, unidentified variety, have used mungbeans obtained from farms or agricultural/government agencies, rather than from the point of supply to the consumer. Due to postharvest influences including handling, packaging, storage times, splitting, and other processing methods, the chemical composition of freshly harvested mungbeans does not necessarily represent the composition when they are consumed (Dahiya et al., 2015). Therefore, this study aimed to characterise some of the variation in the nutritional and antioxidant composition of mungbean varieties available to Australian consumers at the point of purchase. It is hoped that this work will highlight the potential of important phytochemical compounds, such as antioxidants, to further differentiate and substantiate the quality of mungbean samples in a manner beyond that afforded by traditional visual and physical attributes.

2 | MATERIALS AND METHODS

2.1 | Mungbean samples

Five commercial mungbean samples were purchased from two local supermarkets, as available to consumers. Table 1 gives the brand details and country of origin for each sample.

Mungbean samples were ground to a homogenous flour using a Retsch (Sydney, Australia) ZM1000 centrifugal grinding mill with a 1.0-mm mesh. This flour was used for all subsequent analyses except for midinfrared spectroscopy attenuated total reflectance (MIR-ATR).

2.2 | Reagents

All reagents used were analytical-grade. Methanol was purchased from Fisher Scientific Australia (Sydney, Australia). Hydrochloric acid and sodium carbonate were purchased from Chem Supply (Adelaide, Australia). All other reagents were purchased from Sigma-Aldrich Australia (Sydney, Australia). Unless otherwise specified, all dilutions and assay preparations were made using Milli-Q® water (Merck Millipore; Sydney, Australia). All solutions were stored in the refrigerator at 4°C until usage.

2.3 | Extraction of antioxidant compounds

Following protocols recently developed in our laboratory (Johnson, Collins, Skylas, & Naiker, 2019), duplicate extracts were prepared from around 2.5 g of sample and 45 ml of 90% v/v aqueous methanol. After vortexing briefly, the extracts were mixed for 60 min using an end-over-end shaker (Ratek RM4; Melbourne, Australia) operating at 50 rpm. The samples were then centrifuged at 1000 g for 10 min using a Heraeus Multifuge (Thermo Fisher Scientific; Sydney, Australia) prior to collecting the supernatant. The extraction was repeated on the pellet with another 45 ml of 90% methanol and end-over-end mixing for 20 min to extract any remaining antioxidative compounds. The combined supernatants were vacuum filtered using a 0.45-μm Advantec® filter paper (Toyo Roshi Kaisha; Tokyo, Japan) and volumetrically made up to 100 ml with 90% methanol. Extracts were stored in the dark at 4°C until required for further analyses.

2.4 | Cupric reducing antioxidant capacity

The total antioxidant capacity was determined using a modification of the cupric reducing antioxidant capacity (CUPRAC) method developed by Apak et al. (2013). To perform the CUPRAC analysis, 1 ml of 10-mM aqueous copper (II) chloride, 1 ml of 1-M aqueous ammonium acetate, 1 ml of Milli-Q® water, and 1 ml of freshly prepared 7.5-mM neocuproine ethanol solution were combined with 100 μL of the sample extract. After vortexing for 30 s, the samples were incubated in a covered water bath at 50°C for 30 min. The resulting absorbances were read at 450 nm using an ultraviolet spectrophotometer (Thermo Scientific Genesys 10S UV-Vis; Sydney, Australia) blanked with Milli-Q® water. The CUPRAC was derived as a function of the equivalent absorbance of Trolox (6-hydroxy-2,-5,7,8-tetramethyl-chromane-2-carboxylic acid), a vitamin E analogue (Scott, Cort, Harley, Parrish, & Saucy, 1974) in ethanol solution in the range of 50 to 600 mg L^{-1} (R^2 = .99).

TABLE 1 Details of the five commercial mungbean varieties used in this study

Mungbean variety	Description from packaging	Country of origin
Food Sing Trading	Mung bean whole	Not stated
Pattu	Mung bean Celera	Australia
Pattu	Mung split (with skin)	Myanmar
Katoomba	Australian mung bean (small)	Australia
Cock Brand	Unpeeled split mung bean (*Phaseolus aureus* Roxb.)	Thailand

2.5 | Ferric reducing antioxidant power

As it is recommended to use at least two methods for measuring total antioxidant capacity (Apak et al., 2013; Bartosińska, Buszewska-Forajta, & Siluk, 2016), the ferric reducing antioxidant power (FRAP) assay developed by Benzie and Strain (1996) was also performed on the sample extracts.

FRAP reagent was freshly prepared by combining 300-mM acetate buffer (pH 3.56), 20-mM aqueous ferric chloride, and 10-mM 2,4,6-Tris(2-pyridyl)-*s*-triazine (made up in 40-mM HCl) in the ratio 10:1:1 respectively. FRAP reagent (3 ml), pre-equilibrated at 37°C, was combined with 100 μL of pre-equilibrated sample and vortexed briefly. After incubation in a covered water bath at 37°C for 4 min, the resulting absorbances were read at 593 nm. The FRAP was derived as a function of the equivalent absorbance of Trolox in ethanol solution in the range 10 to 175 mg L^{-1} (R^2 = .99).

2.6 | Total phenolics

Total phenolics were determined through a modification of the Folin-Ciocalteu method developed by Singleton and Rossi (1965). To perform the total phenolic assay, 2 ml of a 1:10 aqueous Folin-Ciocalteu reagent dilution was combined with 400 μL of sample extract, followed by incubation in darkness at room temperature for 10 min. After adding 2 ml of 7.5% w/v aqueous sodium carbonate solution and vortexing for 30 s, the samples were incubated in a covered water bath at 40°C for 30 min. Their absorbance at 760 nm was then measured. The total phenolic concentration was derived as a function of the equivalent absorbance of gallic acid in the range 20 to 120 mg L^{-1} (R^2 = .99).

2.7 | Total monomeric anthocyanins

Total monomeric anthocyanins were determined using a modification of the pH differential method described by Cheng and Breen (1991). Aqueous buffer solutions at pH 1 and 4.5 were prepared with 0.025-M KCl and 0.4-M sodium acetate, respectively. Sample extract (500 μL) was combined with 2 ml of buffer and mixed. After equilibrating at room temperature in the darkness for 15 min, the absorbance at 510 and 700 nm was then measured.

The monomeric anthocyanin concentration was calculated using the following formula (Cheng & Breen, 1991):

Milligrams of cyanidin-3-glucoside per liter = ($A \times 449.38 \times$ Dilution Factor $\times$ 1000)/(26900 $\times$ 1),where A = [($Abs_{510} - Abs_{700}$) pH 1.0] − [($Abs_{510} - Abs_{700}$) pH 4.5].

The molecular weight (449.38 g mol^{-1}) and molar extinction coefficient (26,900 M^{-1} cm^{-1}) of cyanidin-3-glucoside were used, it being the most abundant anthocyanin in nature (Markakis, 1989) and also the most abundant anthocyanin present in mungbeans (Yao et al., 2013).

Results from all antioxidant tests were expressed as milligrams of the equivalent standard per 100 g of oven-dried mungbean sample.

2.8 | Carbon, nitrogen, and protein content analysis

Nitrogen and carbon contents were determined using a LECO TruMac Series Carbon and Nitrogen Analyser (Sydney, Australia). Following previous work on mungbeans (Skylas et al., 2017), protein content was estimated by multiplying the nitrogen content by a conversion factor of 6.25. Results were expressed on an oven-dry weight basis.

2.9 | Minerals and trace element analysis

Approximately 1.0 g of each mungbean sample was microwave digested (Biobase BMD-2; Jinan, China) in duplicate with 5 ml of 70% nitric acid. At a pressure of 400 PSI, the temperature was ramped to 90°C over 5 min followed by a 5-min hold time. The temperature was then ramped to 170°C over another 5 min then held for a final 10 min. After cooling, digests were made up to 50 ml with Milli-Q® water. Trace elements and minerals contents were determined through inductively coupled plasma-mass spectroscopy on a Shimadzu inductively coupled plasma-mass spectrometer 2030 (Sydney, Australia). Each duplicate was analysed in triplicate on the inductively coupled plasma-mass spectrometer.

2.10 | Midinfrared spectroscopy attenuated total reflectance analysis

Both nearinfrared (NIR) spectroscopy and midinfrared (MIR) spectroscopy have been used in the analysis of a broad range of grain quality characteristics, ranging from insect infestation status to starch content (Cozzolino, Roumeliotis, & Eglinton, 2013; Johnson, 2020). A Bruker Alpha Fourier transform infrared spectrophotometer (Bruker Optics Gmbh, Ettlingen, Germany) fitted with a platinum diamond ATR single reflection module was used for MIR analysis in this study. Whole mungbeans were placed on the platform and pressure applied to achieve uniform contact between the ATR interface and the mungbean seed coat. Air was used as a reference background; the background measurement was performed prior to analysis. Cross-contamination of samples was minimised by cleaning and drying the platform with isopropyl alcohol and laboratory Kimwipes® (Kimberly Clark; Sydney, Australia) 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}. Three replicates were performed on each mungbean variety.

2.11 | Statistical analysis

Statistical tests were performed using IBM SPSS (New York, USA). All error bars show ±2 standard deviations.

MIR spectra were analysed with the Unscrambler X software Version 10.5 (Camo ASA, Oslo, Norway). Based on previous work on barley seed (Gordon 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 noncompositional variables such as the pressure and contact with the reflection module. Principal component analysis was performed on the second derivative of the MIR spectra in the Unscrambler X, using full cross-validation.

3 | RESULTS AND DISCUSSION

3.1 | Carbon, nitrogen, and protein contents

The protein contents of the mungbean samples ranged from 25.8 to 28.2% (Table 2), similar to those found by Skylas et al. (2017) for Australian-grown mungbeans. There was minimal variation in the carbon contents between varieties.

3.2 | Minerals and trace elements

Table 3 gives the minerals and trace element concentrations in the various mungbean samples. There was a particularly large amount of

TABLE 2 Carbon, nitrogen and estimated protein contents of the mungbean samples

Mungbean variety	Carbon content (%)	Nitrogen content (%)	Estimated protein (% dry basis)
Food Sing (whole)	45.35	4.37	27.3
Pattu (whole)	45.30	4.17	25.9
Katoomba (whole)	45.54	4.51	28.2
Food Sing (whole)	45.51	4.48	28.0
Cock Brand (split)	45.40	4.13	25.8

All results are expressed on an oven-dry weight basis.

TABLE 3 Trace element and mineral concentrations in the mungbeans

Element	Food Sing (whole)	Pattu (whole)	Katoomba (whole)	Pattu (split)	Cock Brand (split)
Ca	698 ± 46^{a}	962 ± 48^{c}	928 ± 139^{c}	791 ± 38^{a,b}	886 ± 12^{b,c}
Cu	6.4 ± 0.3^{b}	5.5 ± 0.1^{a}	6.1 ± 0.9^{a,b}	6.1 ± 0.4^{a,b}	6.0 ± 0.2^{a,b}
Fe	41.0 ± 3.0^{c}	53.4 ± 1.3^{d}	40.5 ± 3.5^{b,c}	32.7 ± 0.8^{a}	36.8 ± 0.8^{b}
K	13740 ± 1070^{a}	15450 ± 1710^{b}	14530 ± 870^{a,b}	14900 ± 420^{a,b}	13820 ± 120^{a,b}
Mg	1620 ± 69^{a}	1925 ± 79^{b}	1738 ± 237^{a,b}	1806 ± 76^{a,b}	1848 ± 16^{b}
Mn	8.6 ± 0.6^{a}	11.5 ± 0.2^{d}	10.5 ± 1.0^{b,c}	9.7 ± 0.2^{b}	11.3 ± 0.1^{c,d}
Na	7.3 ± 1.2^{c}	7.0 ± 0.4^{c}	3.3 ± 1.1^{b}	1.3 ± 0.4^{a}	0.4 ± 0.4^{a}
P	3921 ± 262^{a,b}	3920 ± 129^{a,b}	3703 ± 411^{a}	4159 ± 183^{b}	4273 ± 39^{b}
S	2285 ± 162^{a,b}	2031 ± 122^{a}	2201 ± 429^{a,b}	1980 ± 110^{a}	2477 ± 43^{b}
Zn	19.7 ± 1.5^{a,b}	24.1 ± 5.6^{b}	16.8 ± 1.5^{a}	19.3 ± 0.6^{a,b}	22.7 ± 2.7^{b}

All values are given as mean concentration (mg/kg) ± SD (n = 3 replicates from 2 digests for each). Samples with different letters in the same row were significantly different according to a one-way ANOVA with post-hoc Tukey testing at α = 0.05.

variation in sodium, iron, and copper contents. Other elements, such as zinc, showed minimal differences in concentration between varieties. Copper and zinc values were comparable to those found by Anwar et al. (2007). The values of some other elements, in particular calcium, sodium and magnesium, varied considerably from that reported by Anwar et al. (2007). However, this may be due to Anwar et al. (2007) using only hull-less mungbean seeds, suggesting that these elements may be concentrated in the hull.

3.3 | Total antioxidant capacity (CUPRAC and FRAP)

The total antioxidant capacity, measured by both the CUPRAC and FRAP methods, is shown in Figures 1 and 2, respectively. There was a significant difference in the mean CUPRAC and mean FRAP of the samples ($P < .001$ for both). The Pattu whole variety showed the highest antioxidant capacity followed by Katoomba. The FRAP of the mungbeans tested here was lower than that of acetone–water extracts from Chinese mungbeans (Zhang et al., 2013) and ethanol–HCl extracts from South Korean mungbeans (Lee et al., 2011). This may be due to the varying polarity and extraction efficiency of the solvents used. No previous measurements of CUPRAC in mungbeans were found in the literature.

3.4 | Total phenolic content

There was a significant difference in phenolic contents between varieties (Figure 3; $P < .001$). The total phenolic content followed the same general trend as total antioxidant capacity, with the whole Pattu and Katoomba samples containing the highest phenolic levels. Overall, the five mungbean varieties analysed here showed much higher total phenolic levels than those reported for methanolic extracts of mungbeans grown in China but slightly lower than total phenolic levels from acetone–water extracts from the same country (Zhang et al., 2013).

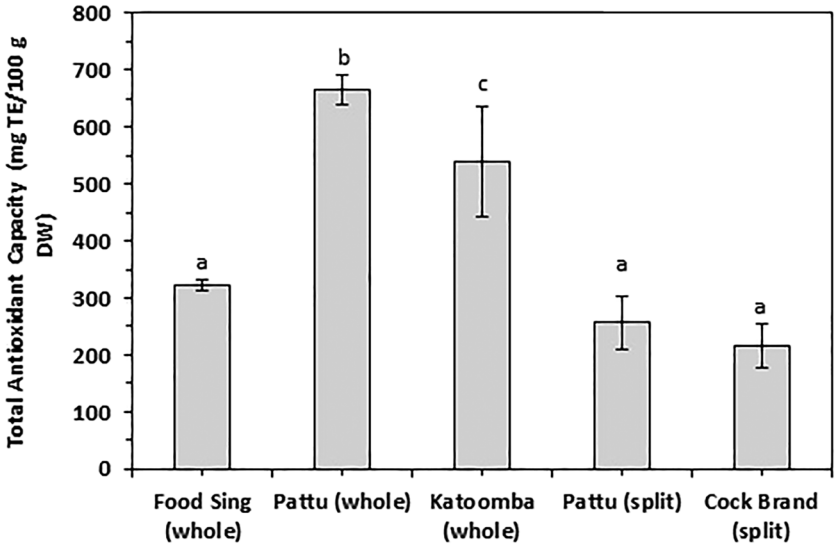

FIGURE 1 Average total antioxidant capacity of the five mungbean varieties, obtained using the CUPRAC assay. Samples with different letters were significantly different according to a one-way analysis of variance with post hoc Tukey testing at $\alpha = 0.05$. DW, dry weight; TE, Trolox equivalent

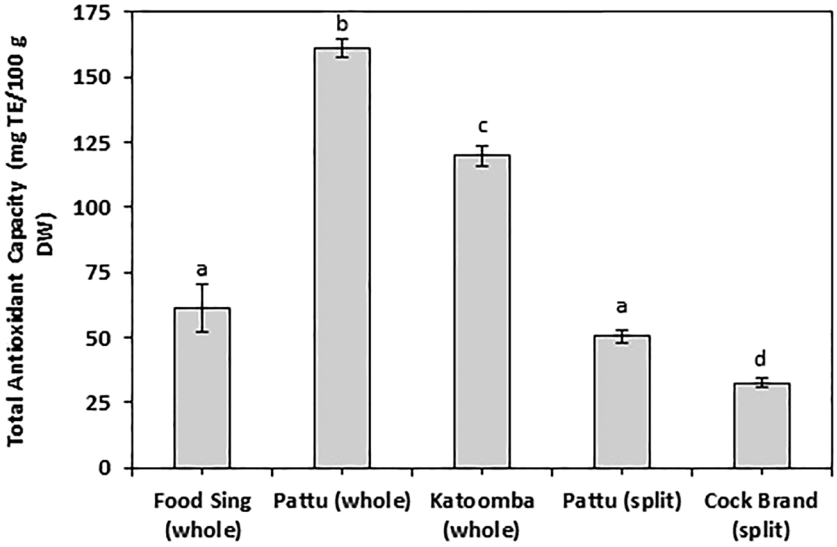

FIGURE 2 Average total antioxidant capacity of the five mungbean varieties, obtained using the FRAP assay. Samples with different letters were significantly different according to a one-way analysis of variance with post hoc Tukey testing at $\alpha = 0.05$. DW, dry weight, TE, Trolox equivalent

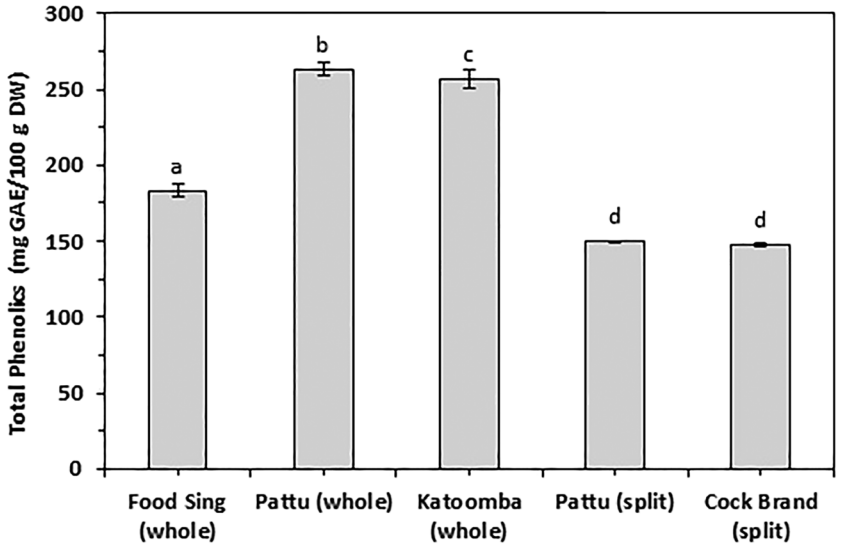

FIGURE 3 Average total phenolic concentrations of the five mungbean varieties. Samples with different letters were significantly different according to a one-way analysis of variance with post hoc Tukey testing at $\alpha = 0.05$. DW, dry weight; GAE, gallic acid equivalent

The phenolic levels were equal to or lower than those found by Shi et al. (2016) using 70% ethanol extracts from 20 Chinese mungbeans. concentrations of 5.19–5.70 mg per 100 g (Kan et al., 2018), somewhat lower than those found in this study.

3.5 | Total monomeric anthocyanin content

There was a significant difference in the average total monomeric anthocyanin content of the varieties analysed (Figure 4; $P < .001$). The anthocyanin content did not display any statistically significantly correlation with the total antioxidant capacity or total phenolic content of the variety ($P > .05$). The Cock Brand mungbeans had the highest anthocyanin concentration, followed by the Food Sing variety. Although anthocyanins have been previously detected (Ganesan & Xu, 2018; Ishikura, Iwata, & Miyazaki, 1981) and identified in mungbeans (Kan, Nie, Hu, Wang, Bai, Wang et al., 2018; Yao et al., 2013), only a handful of authors have reported the actual anthocyanin concentration in green mungbeans. Ultra-high performance liquid chromatography applied to Chinese mungbeans found anthocyanin

3.6 | Varietal differences in antioxidant levels

Although there appear to be few studies in the literature reporting the effect of splitting mungbeans on their antioxidant levels, it can be theorised that split mungbeans should have lower antioxidant levels. This would largely be due to the majority of the mungbean hull being fragmented and lost through the splitting process, with further loss of the hull during storage and packaging. Given that the vast majority of antioxidant compounds are found in the hull (Duh, Yen, Du, & Yen, 1997; Luo, Cai, Wu, & Xu, 2016), this would reduce the average antioxidant capacity of the entire sample. It is unlikely that increased light exposure to the endosperm of split mungbeans plays any significant role in the loss of antioxidant compounds. Indeed, Xiao et al. (2014) found that exposing antioxidant-rich radish seedlings to

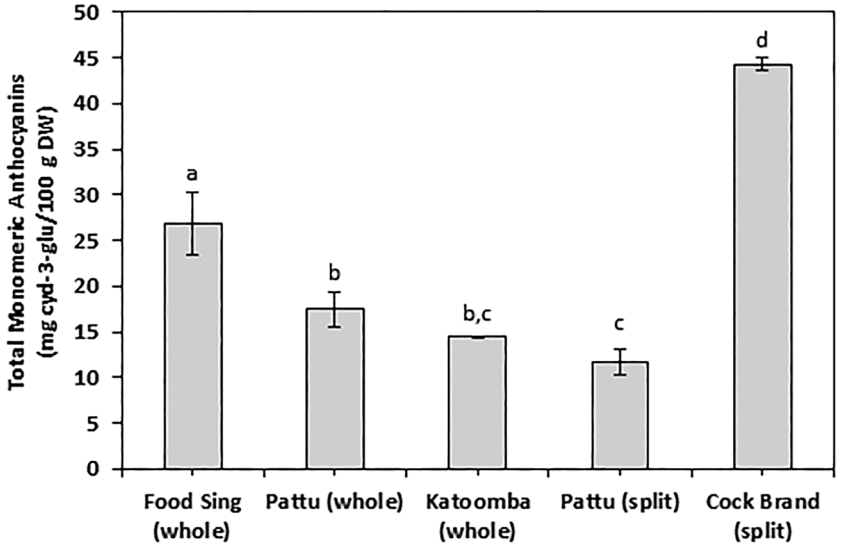

FIGURE 4 Average total monomeric anthocyanins of the five mungbean varieties. Samples with different letters were significantly different according to a one-way analysis of variance with post hoc Tukey testing at $\alpha = 0.05$. cyd-3-glu, cyanidin-3-glucoside; DW, dry weight

light had no effect on the total antioxidant capacity, total phenolic, or α-tocopherol concentration. This hull-loss hypothesis is also supported by Dahiya et al. (2014), who found that compared to dhal made from whole mungbeans, the polyphenol concentrations in split dhal were only 2% lower, but they were 33% lower in dehulled split dhal. Although this analysis was undertaken on a prepared food product, the general trend of antioxidant levels may hold true for raw mungbeans.

Incidentally, the whole Pattu and Katoomba mungbeans were of Australian origin, whereas the split Pattu and Cock Brand were grown in Myanmar and Thailand, respectively. The origin of the Food Sing mungbeans was not specified. Without a larger sample size, it is not possible to determine whether commercially available mungbeans grown in Australia have higher total antioxidant and phenolic levels than imported mungbeans. However, a previous study on barley has shown that prolonged storage typically decreases antioxidant levels (Do, Cozzolino, Muhlhausler, Box, & Able, 2015). Imported mungbeans would likely have greater storage times than Australian-grown mungbeans, due to transport time and different growing seasons in the Northern Hemisphere. Thus, it is possible that this could potentially lower their antioxidant contents.

3.7 | MIR spectral analysis

The MIR spectra of the mungbeans before processing are shown in Figure 5. In the unprocessed spectra, there were four major peaks (Figure 5), viz 3,600–3,020 cm^{-1}, 1,680–1,560 cm^{-1}, 1,450–1,350 cm^{-1}, and 1,120–940 cm^{-1}. In addition to several minor peaks and shoulders observed throughout the spectra, two smaller peaks at 2,935–2,910 cm^{-1} and 1,280–1,200 cm^{-1} can be found respectively.

The wide band between 3,600 and 3,020 cm^{-1} is mainly due to the O—H stretching vibrations of water molecules (Cozzolino, Roumeliotis, & Eglinton, 2014; Gnanasambandam & Proctor, 2000; Gordon et al., 2019; Lai, Wen, Li, Wu, & Li, 2010). The smaller 2,935–2,910 cm^{-1} peak is due to the antisymmetrical stretching of the C—H bond, from lipids or other compounds (Gnanasambandam & Proctor, 2000; Lai et al., 2010). Indeed, Cozzolino et al. (2014) reported that spectral patterns between 3,000 and 2,720 cm^{-1} are related to the unsaturated lipid content of the germ of cereals. There was some visible variance between samples in the band of 2,990–2,840 cm^{-1}, a slightly narrower peak width than that reported by Cozzolino et al. (2014) for barley.

The only obvious feature between 2,840 and 1,770 cm^{-1} was a tiny but distinguishable peak at 2,370–2,320 cm^{-1}, likely from aliphatic C—H bonds (Lai et al., 2010).

The small shoulder between 1,750 and 1,720 cm^{-1}, due to ester carbonyl (C═O) bonds, (Gnanasambandam & Proctor, 2000; Lai et al., 2010), is indicative of low levels of esterification with aromatic esters (Barron & Rouau, 2008). The major peak between 1,680 and 1,560 cm^{-1} is reported to be due to carboxylate (COO^-) stretching bonds, which show a peak absorbance at around 1,625 cm^{-1} (Gnanasambandam & Proctor, 2000; Lai et al., 2010). Some of the absorbance may also be from the C═C alkene bond, which absorbs between 1,680 and 1,600 cm^{-1} (Pavia, Lampman, & Kriz, 2001).

The peak between 1,450 and 1,350 cm^{-1} appears to be from the symmetric stretching of carboxylate groups (COO^-) (Gnanasambandam & Proctor, 2000; Lai et al., 2010). Some of the absorbance may also correspond to the CH_3^- bend, which absorbs at 1,450 and 1,375 cm^{-1} (Pavia et al., 2001). The smaller 1,280–1,200 cm^{-1} peak could be due to the ester O—C bond in acylglycerols, which absorbs over 1,300–1,000 cm^{-1} (Barron & Rouau, 2008; Cozzolino et al., 2014). This range (1,300–1,000 cm^{-1}) has been

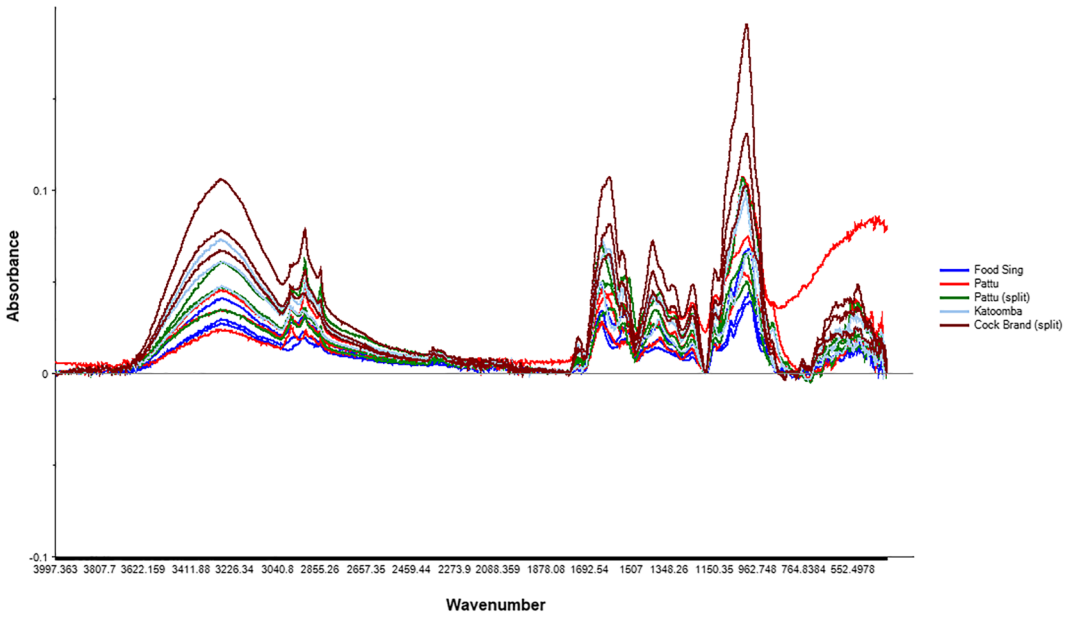

FIGURE 5 Unprocessed midinfrared spectra of five commercially sourced mungbean varieties

reported by previous workers to represent a "fingerprint region" for fatty acids and lipids (Barron & Rouau, 2008; Cozzolino et al., 2014).

The largest peak, between 1,130 and 940 cm^{-1}, has been attributed to O—C stretch vibrations in glucose (Cozzolino et al., 2014) or other pyranose rings (Lai et al., 2010). These rings could be constituents of glycosides, starch, cellulose, or other polysaccharides (Barron & Rouau, 2008; Cozzolino et al., 2014). The C—O—C and C—OH link bonds could also contribute to absorbance in this band (Lai et al., 2010).

There was a reasonable amount of visible differences between mungbean varieties in the band of 1,670–900 cm^{-1}, very similar to the band of 1,600–800 cm^{-1} that Cozzolino et al. (2014) reported to display spectral variation between barley varieties. The spectra band on the rightmost side of the graph, between 400 and 800 cm^{-1}, corresponds to the fingerprint region of the spectra and is difficult to attribute to specific bonds (Gordon et al., 2019; Lai et al., 2010).

Overall, the MIR spectra of the mungbean hulls was very similar to that obtained by Lai et al. (2010) using purified water-soluble polysaccharide fractions extracted from mungbean hulls. Although the amplitude of the peaks obtained here was much lower than that of Lai et al. (2010), the sensitivity of the MIR-ATR instrument used allowed all peaks above 1,000 cm^{-1} recorded by Lai et al. (2010) to be reliably distinguished in this study. This has an important bearing on future research, as it suggests that particularly for spectral peaks above 1,000 cm^{-1}, the macrochemical composition of mungbeans can be determined rapidly and reliably using reflectance spectra of the hull rather than performing the laborious process of extracting, isolating, and purifying the desired polysaccharides and other water-soluble constituents before MIR analysis.

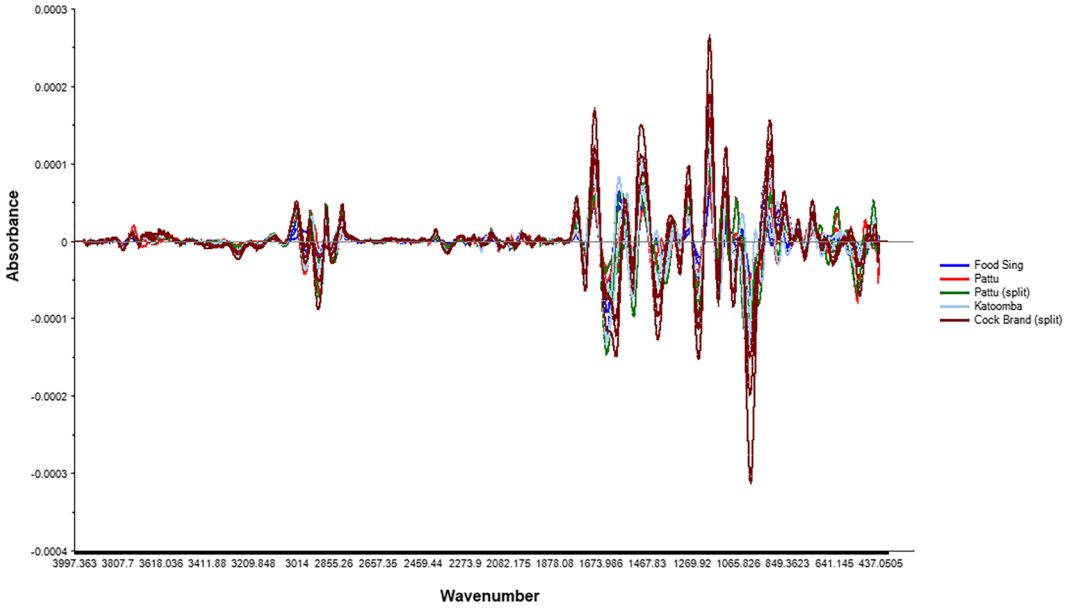

FIGURE 6 Second derivative of the midinfrared spectra of five commercial mungbean varieties

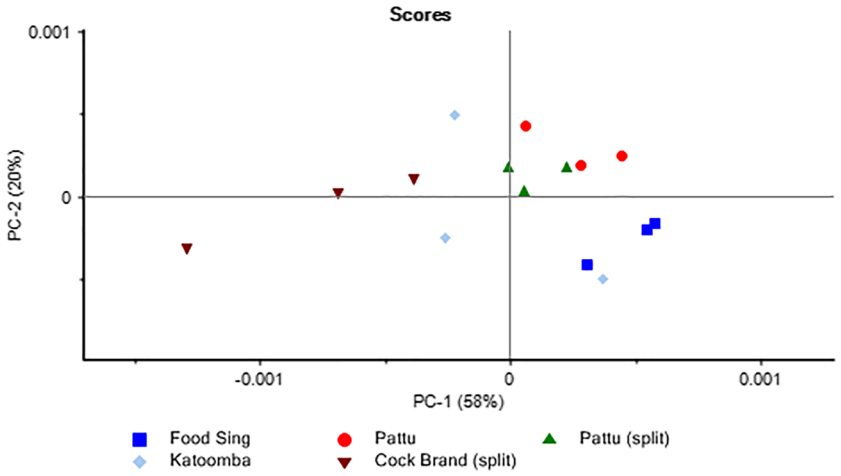

FIGURE 7 Scores plot showing the principal component analysis of the five mungbean spectra. PC, principal component

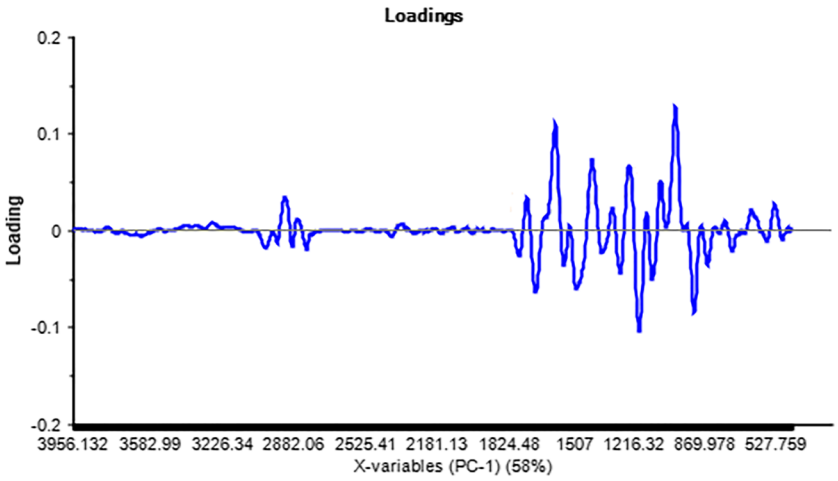

FIGURE 8 Spectral loadings of first component (Principal Component 1) in the principal component analysis. PC, principal component

3.8 | Processing of MIR spectra and principal component analysis

As can be seen in Figure 6, the second derivative successfully removed the variations in baseline absorbance and peak height variations, leaving only the relative differences in peak sizes, widths and positions.

Principal component analysis gave a reasonable separation of the five mungbean varieties (Figure 7), primarily along Component 1. The first component explained 58% of the variance in spectra, the second component 20%, and the third component 14%. The most widely scattered varieties were Katoomba and Cock Brand, possibly indicating higher levels of variance in the phytochemical composition of these varieties.

The loading plot for Principal Component 1 showed that most of the separation was due to spectral differences in the 800–1,600 cm^{-1} range (Figure 8), similar to the range found by Cozzolino et al. (2014) for barley.

4 | CONCLUSION

This study found significant variation in the antioxidant composition of commercially available mungbean varieties in Australia. Of the five varieties tested, whole mungbeans grown in Australia had the highest total antioxidant capacity and phenolics concentrations, whereas imported split mungbeans had the lowest. Total monomeric anthocyanins did not appear to correlate with total antioxidant capacity or phenolic levels. MIR spectra obtained using ATR spectroscopy supported the variation found in antioxidant levels, indicating fundamental differences in the phytochemical composition of the different mungbean varieties. Further studies are required to confirm the effects of country of origin and splitting mungbeans on their antioxidant and nutritional compositional values. However, whole Australian mungbeans may be the healthiest option for consumers.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

J.J., T.C., and M.N. conceptualised and conducted the study. J.J. prepared the original manuscript draft; all authors contributed to the review and editing of the manuscript.

DATA AVAILABILITY STATEMENT

Due to technical limitations, the full dataset is unable to be published at this time. However, it is available upon request from the authors.

ORCID

Joel Johnson https://orcid.org/0000-0002-9172-8587
Aoife Power https://orcid.org/0000-0002-7119-8486
Shaneel Chandra https://orcid.org/0000-0002-4257-5860
Daniel Skylas https://orcid.org/0000-0003-4640-1531
Drew Portman https://orcid.org/0000-0001-7373-0424
Joe Panozzo https://orcid.org/0000-0003-3011-9953
Christopher Blanchard https://orcid.org/0000-0001-5800-4678
Mani Naiker https://orcid.org/0000-0002-6844-8325

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