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Lupin seed coat as a promising food ingredient: Physicochemical, nutritional, antioxidant properties, and effect of genotype and environment

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Summary

The high proportion of seed coat of legume lupins results in big milling lose during kernel flour production, though the seed coat could be value-added as human food. The physicochemical, nutritional properties and antioxidant capacities of seed coats of six Australian sweet lupin cultivars grown at two locations were evaluated. Results showed that genotype, environment and their interaction were significant for seed coat percentage, proximate composition, dietary fibre content, polyphenols and antioxidant capacities. Strong correlations between seed coat lightness and polyphenol content were found. A comparison using multivariate analysis of the seed coat properties showed clear separation based on growing sites. This study indicates the enormous potential of Australian sweet lupin seed coat as an "antioxidant dietary fibre" food source. The results could also benefit to breed varieties with desirable levels of nutrients and phytochemicals.

Keywords: Australian sweet lupin; seed coat; environment; genotype; dietary fibre; physicochemical properties, antioxidant capacity

1. Introduction

The legume lupins play an essential role in Australian and European farming systems through wheat: lupin rotation. Narrow-leafed lupin or Australian sweet lupin (ASL, *Lupinus angustifolius*) is the most worldwide grown domesticated lupin, followed by white lupin (*L. albus*) and yellow lupin (*L. luteus*) (Gresta *et al.,* 2017). ASL cultivars have been continuously bred to improve ASL performance, such as yield, seed qualities, adaptation, disease resistant and herbicide tolerance (DPIRD 2018). Recently, the ASL varieties that have dominated Australian production are Mandelup (released in 2004), Coromup (2006), Jenabillup (2007), PBA Gunyidi (2010), PBA Barlock (2013), PBA Jurien (2015) and PBA Leeman (2017) (DPIRD 2018).

Lupin is a promising protein crop alternative to soybean and its use in human food is expanding rapidly. The high protein and high fibre lupin kernel flour have been widely used in bakery and meat products (Lucas *et al.,* 2015, Leonard *et al.,* 2019). However, the high proportion of lupin seed coat, which comprises 24% in average of the seed and hence results in high milling loss, complicates its economical usage in human food (Zhong *et al.,* 2018b). Recently, a renewed intention has been paid to value-add the by-product. For example, albus lupin seed coat has been commercially developed as an insoluble fibre ingredient (Vitafiber®, Avelup Ltd., Chile). Seed coat flour of ASL and yellow lupin (*L. luteus*) were incorporated into bakery foods as a source of dietary fibre (Wandersleben *et al.,* 2018, Tucek 2009), and used to produce cellulose nanofiber aerogel (Ciftci 2017). In contrast, only a few studies have investigated the nutritional and physicochemical properties of seed coats of several now redundant lupin varieties (Bailey *et al.,* 1974, Brillouet and Riochet 1983, Michalczyk *et al.,* 2006, Evans *et al.,* 1993, Lampart-Szczapa *et al.,* 2003a, Lush and Evans 1980, Mohamed and Rayas-Duarte 1995). Therefore, a systematic study was needed to investigate the properties of seed coats of the recent and agriculturally dominant ASL varieties.

ASL seed characteristics were reported to be significantly affected by genotypic and environmental factors (Winnicki *et al.,* 2019, Karnpanit *et al.,* 2016, Clements *et al.,* 2002). Likewise, our previous study demonstrated that individual polyphenols of ASL seed coats were genotype and environment-dependent (Zhong *et al.,* 2019b). Therefore, the purposes of this work are to (i) provide a new characterisation of nutritional and functional properties of seed coats of the current ASL varieties, (ii) investigate the genotypic and environmental effects on the properties. To this end, six recent ASL cultivars grown in 2015 at two locations in Western Australia (WA) were analysed for their physiochemical and compositional properties, as well as antioxidant capacities. Univariate and multivariate analyses were performed to evaluate the significance of genotype, environment and their interaction on these properties.

2. Materials and methods

2.1. Lupin seed coat samples

The milled seed coat of six currently produced ASL cultivars (PBA Jurienp, PBA Barlock, PBA Gunyidi, Mandelup, Jenabillup and Coromup) grown at Wongan Hills (WH) and Eradu (ER) Western Australia were obtained as previously described (Zhong *et al.,* 2019b). The climatic characteristics (monthly mean temperature and rainfall) of the two growing locations are presented in Fig S1. The seed coats were pre-dried at 50 °C overnight before milling then passed 100% through a 500 μm screen. The resulting flours were vacuum-packaged into two bags and stored separately at -20°C (only for polyphenol extraction) and 4°C.

2.2. Determination of physicochemical properties of lupin seed coat

Instrumental colour of whole seeds and the seed coat flour were determined by a HunterLab spectropolarimeter with the CIE L*, a* and b* colour space. Water-binding capacity (WBC), oil-binding capacity (OBC) and swelling capacity (SC) of the milled seed coat were evaluated in triplicate as described by Robertson *et al.,* (2000). Olive oil was used to determined OBC. WBC/OBC were expressed as g water/ oil retained in the pellet per g of dry sample. SC was calculated as volume/g original substrate dry weight. Solubility in water (SOL) was expressed as a percentage of lost weight during soaking (Fuentes-Alventosa *et al.,* 2009).

2.3. Determination of proximate composition and dietary fibre

Proximate composition analyses of the seed coat were carried out following standard methods (AOAC, 2011) in at least duplicate. Lipid content was evaluated by E-816 Soxhlet extraction unit (Buchi Labortechnik AG, Postfach, Switzerland). The Kjeldahl digestion distillation method with 5.40 as the conversion factor was used to determine protein content. Dietary fibre composition was determined using the Megazyme K-TDFR Kit (Megazyme International Ireland Ltd, Bray Ireland).

2.4. Determination of total polyphenol content and antioxidant capacities

Free and bound polyphenols were extracted as described previously (Zhong *et al.,* 2019b). For both free and bound fractions, the Folin-Ciocalteu assay was performed to investigate total polyphenol content (TPC), while DPPH and ABTS• + free radical scavenging activity assay and oxygen radical absorbance capacity (ORAC) assay were used to determine antioxidant capacities (Zhong *et al.,* 2018a). The results of TPC and antioxidant capacity assays were expressed as mg gallic acid equivalent per 100 g dry sample (mg GAE/100 g db) and mg Trolox equivalents per 100 g dry sample (mg GAE/100 g db), respectively.

2.5. Statistics

All the results were reported on dry basis and expressed as mean ± standard deviation (n≥2). Two-way ANOVA was used to investigate the main effects of genotype (G), environment (E) and their interaction (G×E). One-way ANOVA with Tukey post hoc test was performed to compare differences among varieties and locations on IBM SPSS V25.0 (SPSS Inc., Chicago, IL, USA). $p < 0.05$ indicated significant difference. R (Version 3.5.2, R Core Team, 2019) was employed to perform principal component analyses (PCA) using "FactoMineR" and "ggplot2" packages. Means of dependent variables were used for PCA and correlation analyses.

3. Results and discussion

3.1. ASL seed coat colour, seed weight and seed coat percentage

3.1.1. Seed coat colour

As shown in Figure 1, all the six ASL varieties had similar speckled beige seed coats, but the colour values varied considerably among individuals within and across the two sites (Table S1). Although location exhibited no significant effects on the L* values, genotypic and its interactive effects with the environment were significant (Table 1). In contrast, both location and genotype showed significant effects on the a* values, but the interaction was not significant. However, neither genotypic nor environmental factors showed significant effects on the b* value (Table 1).

Seed coat colour, which is genetically and environmentally determined, is a commercially important indicator of maturity and qualities of the lupin seeds (Moïse *et al.,* 2005). Flavonoids (including anthocyanins, and proanthocyanidins) were reported to be the main contributor to pulse seed coat colour (Moïse *et al.,* 2005). Black seed coat colour is controlled by several genes in the flavonoid pathway which is believed to be a mechanism to achieve fast ripening and drying by absorbing sunlight and heat efficiently (Oomah *et al.,* 2006). In addition, polyphenols play a key role as protectants adopted by plants to respond to various environmental stresses (Agati *et al.,* 2012). The speckling of ASL seed coat develops soon after the seed reaches physiological maturity (largely early November in WA) (Dracup and Kirby 1996).

3.1.2. Seed weight and seed coat percentage

As shown in Figure 1, the average seed size of the varieties was Coromup> Jenabillup> PBA Jurien> Mandelup> PBA Barlock> PBA Gunyidi, in agreement with the seed weight ranking within both two locations (Table 2). Both genotype and environment, as well as their interaction, showed significant effects on seed weight (Table 1). Across the two grown locations, seed weights of all the six ASL varieties from ER were significantly higher than those from WH. The lower temperature and rainfall during sowing (April to middle of May), and lower temperature during flowering (July) but consistently higher temperature (>30°C) during seed development (October) at WH may have reduced the accumulation of biomass and thus the weight of individual seeds (Dracup and Kirby 1996).

The seed coats of the six ASL varieties comprised 19.74 to 23.93% of total seed dry mass (Table 2). Clements *et al.,* (2002) reported a range from 21.8 to 26.2% of seed coat percentage in ASL old varieties. The authors also revealed a negative relationship between seed weight and seed coat. Similarly, a significant albeit weak negative correlation (r = -0.38, p = 0.022) between seed weight and seed coat was found in the current study. According to the univariate analyses, genotype,

166 environment and their interaction showed significant effects on seed coat percentage,
167 what was in line with those reported by Clements *et al.,* (2002) (Table 1).

168 **3.2. ASL seed coat physicochemical properties**

169 Results of water solubility (SOL), water binding capacity (WBC), oil binding
170 capacity (OBC) and swelling capacity (SC) are presented in Table 2. The univariate
171 analyses revealed that genotype was the determining factor for SOL, with the effects
172 of the environment being nonsignificant (Table 1). In contrast, genotypic,
173 environmental effect and their interaction were significant on WBC. Regarding OBC
174 and SC, the effects of all the investigated factors were not significant. Moreover, both
175 OBC and SC were relatively constant independent of varieties and locations.

176 In a previous study, SOL of Coromup lupin seed coat was reported to be 4.02%
177 db (Zhong *et al.,* 2019a). The result was lower than those of the current study, which
178 varied from 5.62% db to 8.06% db. The difference may be due to the different
179 dehulling and seed coat separation methods. However, the WBC values of this study
180 (3.39-3.80 g/g db) agreed well with this previous study (3.84 g/g db). Nevertheless,
181 WBC levels of ASL seed coat from both of the studies were lower than those reported
182 by Pfoertner and Fischer (2001) (7-8 g/g). The current WBC of lupin seed coat was
183 similar to those of lentil and mung bean seed coat but lower than field pea (4.0-7.1 g/g)
184 and chickpea (6.2 g/g) (Zhong *et al.,* 2018b). The variability in reported WBC may be
185 a result of differences in seed coat composition, particle size, and milling process
186 (Robertson *et al.,* 2000). The range of the OBC values was less than previously
187 reported data of ASL seed coat (1.5-1.6 g/g) and other legume seed coats (1.4-1.9 g/g)
188 (Zhong *et al.,* 2018b). As anticipated, the values of SC were low, ranging from 4.47 to
189 5.07 mL/g db, but were consistent with the existing data of other legume seed coats
190 (Zhong *et al.,* 2018b).

191 **3.3. Proximate composition**

192 The proximate composition of seed coats of some old ASL cultivars were found
193 to contain 9-11g/100g moisture, 3-6.6 g/100g protein, around 1.5 g/100g lipid, 2-3

194 g/100g ash and 80-90 g/100g non-starch polysaccharides (NSP) (Bailey *et al.,* 1974,
195 Evans *et al.,* 1993, Lampart-Szczapa *et al.,* 2003b). In the present study, the
196 proximate compositions of the six modern cultivars were relatively consistent (Table
197 3). Ash content varied from 2.81 to 3.01 g/100g db, in agreement with the reported
198 results above. However, the fat and protein content in this study, ranging from 1.62 to
199 2.42 g/100g db and 6.34 to 8.59 g/100g db (N×5.40) respectively. The protein results
200 here were slightly higher as compared to those reported (4.6% to 6.7% after adjusting
201 the N conversion factor from 6.25 to 5.40) (Lampart-Szczapa *et al.,* 2003b, Evans *et*
202 *al.,* 1993). As summarised in Table 1, genotypic, environmental and their interactive
203 effects on ash, fat and protein content in lupin seed coat were significant with PBA
204 Gunyidi and Mandelup showing higher ash, fat and protein level.

205 **3.4. Dietary fibre**

206 Dietary fibre (DF) is the major component of ASL seed coat (Zhong *et al.,*
207 2018b). As shown in Table 3, all the lupin seed coats had a high content of total
208 dietary fibre (TDF), accounting for 79.84 to 86.59 g/100 g db, which primarily
209 consisted of IDF. The results were in accordance with the previous studies, with SDF
210 comprising of less than 3.5% of TDF (Zhong *et al.,* 2019a). However, a higher IDF
211 were reported by Evans *et al.,* (1993), 88.4-90.9 g/ 100g db, and consequently higher
212 TDF, which could be explained by the different dehulling methods. From the results
213 of univariate analyses, both genotypic and environmental factors, together with their
214 interaction contributed to the observed differences in the levels of IDF and TDF
215 (Table 1). With respect to SDF, despite the
216 non-significant G × E interaction, genotype and environment were significant
217 determinates of SDF (Table 1). However, except PBA Jurien and PBA Gunyidi, IDF,
218 SDF and TDF of ASL seed coats from the two sites were similar ($p > 0.05$). In
219 contrast, high content of fibre was found in the seed coat of wild lupins and lupins
220 grown in dry conditions (Miao *et al.,* 2001).

3.5. Total polyphenol content and antioxidant capacity

3.5.1. Total polyphenol content (TPC)

Lupins have high levels of polyphenols (Khan *et al.,* 2015). As presented in Table 4, the range of total polyphenol content in free fraction (FTPC) varied from 57.24 mg gallic acid equivalents (GAE)/ 100g db in PBA Jurien to 93.52 mg GAE/ 100g db in PBA Barlock, both of which were from WH. Moreover, according to the combined analysis of variance analysis, significant effects of environment and genotype and their interaction on FTPC values were detected (Table 1). Seed coat FTPC values of seeds from WH were higher than those from ER. Besides, among the six varieties, PBA Jurien was consistently the lowest in FTPC across the two locations (Table 4).

Regarding bound polyphenols, the ASL varieties showed a range in total bound polyphenols (BTPC) from 16.71 mg GAE/100g db in PBA Jurien (ER) to 34.78 mg GAE/100g db in PBA Barlock (WH) (Table 4). BTPC accounted for up to 27.15% of the total phenolics in the lupin seed coats. By examining the results of the analysis of variance for BTPC, the main effects of genotype and location dominated the variation, but the G×E was also a significant albeit weak source (Table 1). Additionally, ANOVA analyses revealed that BTPC in seed coats of the seeds grown at ER was significantly lower than those from WH. Similar to the pattern in FTPC, the highest BTPC were found in PBA Gunyidi within both two locations while the values in PBA Jurien were the lowest (Table 4). Collectively, highly significant effects of the genotype, site and their interaction (G×E) were detected in explaining the variations among TPC (FTPC+BTPC) levels (Table 1). Besides, PBA Barlock (WH) contained the highest TPC among the varieties over the two sites, followed by PBA Gunyidi and Mandelup, with PBA Jurien being the lowest (Table 4).

In the previous study, considerable levels of free and bound individual polyphenols in lupin seed coats were identified and semi-quantified using HPLC-DAD-ESI-MS/MS (Zhong *et al.,* 2019b). The results showed distinctive polyphenol profiles in free and bound fractions, with main flavonoids being in the

free fraction while phenolic acid glucosides predominated in the bound fraction instead. Moreover, in line with this study, the study demonstrated that genotype, location and their interaction exhibited significant effects on both free and bound total individual polyphenol (Zhong *et al.,* 2019b). In this study, 68.34-127.99 mg GAE/ 100 g db of TPC was observed in the six ASL cultivars (Table 4). In contrast, a range of 18.4 mg caffeic acid equivalents /100g db (mg CE/ 100 g db) to 449 mg CE/ 100g fresh weight of TPC was previously reported in lupin seed coats (Lampart-Szczapa *et al.,* 2003b, Ranilla *et al.,* 2009). Apart from lupins, other legume seed coats were also demonstrated to have high levels of TPC, ranging 76 to 5798 mg GAE/ 100 g db (Gan *et al.,* 2016, Sreerama *et al.,* 2010). In fact, the majority of legume polyphenols were concentrated in their seed coats (Zhong *et al.,* 2018b, Gan *et al.,* 2016). In the current study, FTPC and BTPC were significantly and negatively correlated with L* values, reflecting that seeds with darker colour contained higher FTPC, BTPC and thus TPC (Table S2). As discussed earlier, polyphenols in the epidermal layer of the seed coat contribute to the seed coat colours (Moïse *et al.,* 2005). Polyphenols can serve as UV-B attenuators, inhibit oxidation and improve osmotic under exposure to thermal stress (Agati *et al.,* 2012).

3.5.2. Antioxidant capacity assays (AOXs)

The *in vitro* antioxidant activities of the lupin seed coats were investigated using three assays, i.e. DPPH, ABTS and ORAC. As presented in Table 4, the values of free DPPH varied from 23.96 to 36.59 mg TE/ 100g db. Previously, DPPH value of the seed coat of a Brazilian ASL cultivar was demonstrated to be 13 mg TE/100 g fresh weight, which equivalent to free DPPH in the current study (Ranilla *et al.,* 2009). The values of DPPH and ABTS assays were significantly affected by genotype, environment and G×E interaction. In contrast, G×E interaction was nonsignificant on ORAC levels despite the significant effects of genotype and environment (Table 1). Similar to the trend of TPC, seed coat of the lupin seeds from WH had significantly higher in both free and bound AOXs than those from ER. Legume seed coats have

shown high antioxidant capacities using multiple *in vitro* assays (Gan *et al.,* 2016). However, given the limitations in the *in vitro* antioxidant assays, *in vivo* studies (animal studies and human studies, in particular) are better in evaluating physiological antioxidant capacities.

3.6. Principal component analysis

In this study, 44 responses of 12 samples were analysed, consequently generated a large amount of data (528 data points). To reduce the dimensionality of the data, principal component analysis (PCA) was conducted. Fig S2 shows the PCA scores plot and factor loadings plot. The first two principal components (PC1, PC2) were detected to explain 46.96% and 15.83% of the total variation in the data set respectively. PC1 differentiates the seed coat samples based on contents of TPC (free, bound and total), levels of all the three antioxidant capacity assays (free, bound and total), L*, a*, SOL and seed weight (factor loadings > 0.60) (Granato *et al.,* 2018). In contrast, PC2 separates the samples according to contents of IDF, TDF, fat and protein. Taken together, samples harvested from ER were clearly separated from those originating from WH, implying the environmental effects. Notably, PBA Jurien from both sites were distanced from others and located at the left of the plot, which was determined by the low TPC and AOXs values but high IDF and TDF content in PBA Jurien.

Conclusions

This study examined physicochemical, nutritional properties and antioxidant capacities of seed coats of six new Australian sweet lupin (ASL) cultivars grown at two locations in Western Australia. Whereas great genotypic and environmental variations were found in those investigated properties, the results support that ASL seed coats can be value-added as a natural "antioxidant dietary fibre" in human food products, and suggest that variety and growing location-based utilisation must be of interest. However, it is noted that the weakness of this work is that it does not include seasonal factors (different years). In addition, the presence and amounts of

anti-nutritional factors such as saponins, phytic acid and oligosaccharides in the seed coat are yet unclear and merit further investigation. Whole-genome sequencing has been performed for ASL (Yang *et al.,* 2015), and other nine legumes, such as soybean, chickpea and common bean (Foyer *et al.,* 2016). By comparing genomics across legume species, candidate genes of ASL which are related to their seed and seed coat development, some key metabolic pathways (e.g. protein and polyphenols accumulations) could be identified to develop new genomics-assisted breeding strategies for ASL improvement.

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Availability statement

Research data are not shared.

Ethical guidelines

Ethics approval was not required for this research

Conflict of interest

The authors declare that they have no conflict of interest.

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458 Figure captions

459

460 **Figure 1** Six modern genotypes of Australian sweet lupin (ASL) seeds grown at two
461 locations and harvested in 2015 (Eradu, ER; Wongan Hills, WH)

1

2 Table 1 Effects of genotype, environment and their interaction (G×E) presented as p-values from analysis of variance (ANOVA)

Sees coat properties	p-value											
Physicochemical properties												
	Seed weight	% Seed coat	L*	a*	b*	SOL	WBC	OBC	SC			
Genotype	<0.0001	<0.0001	<0.0001	<0.001	0.339	<0.0001	0.025	0.999	0.182			
Environment	<0.0001	<0.0001	0.443	0.010	0.173	0.825	0.006	0.798	0.407			
G×E	0.011	<0.0001	<0.0001	0.235	0.963	0.038	<0.0001	0.820	0.070			
Proximate composition and dietary fibre												
	Ash	Fat	Protein	IDF	SDF	TDF						
Genotype	<0.0001	<0.0001	<0.0001	<0.0001	<0.01	<0.0001						
Environment	<0.001	<0.01	0.011	<0.0001	<0.01	<0.0001						
G×E	<0.01	<0.0001	<0.0001	<0.001	0.311	<0.01						
TPC and antioxidant capacity												
	FTPC†	BTPC‡	TTPC§	FDPPH†	BDPPH‡	TDPPH§	FABTS†	BABTS‡	TABTS§	FORAC†	BORAC‡	TORAC§
Genotype	<0.0001	<0.0001	<0.0001	<0.01	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Environment	<0.0001	<0.0001	<0.0001	<0.0001	<0.01	<0.0001	<0.0001	<0.0001	<0.0001	0.010	<0.01	<0.01

G×E	<0.001	0.023	<0.0001	0.031	0.473	0.013	<0.01	0.015	<0.01	0.341	0.450	0.278

SOL, water-solubility; WBC, water binding capacity; OBC, oil binding capacity; SC, Swelling capacity; IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre.

† total polyphenol content (TPC) and antioxidant capacities (DPPH, ABTS, ORAC) in free fractions; ‡ TPC and antioxidant capacities in bound fractions; § total TPC and antioxidant capacities.

Table 2 Seed weight (mg, db), seed coat percentage (%, db) and physicochemical properties of lupin seed coats

		PBA Jurien	Coromup	PBA Gunyidi	Mandelup	PBA Barlock	Jenabillup
Seed weight	ER	136.38±0.27^{cA}	144.88±1.18^{dA}	115.44±2.34^{aA}	130.48±3.17^{bA}	130.28±2.51^{bA}	142.53±1.72^{dA}
	WH	117.08±1.61^{bB}	120.78±1.74^{bB}	103.95±2.21^{aB}	112.33±4.51^{abB}	106.38±1.65^{aB}	119.41±2.43^{bB}
% Seed coat	ER	22.54±0.17^{aA}	23.04±0.22^{bA}	22.92±0.38^{eA}	23.92±0.01^{dA}	23.93±0.11^{dA}	23.27±0.14^{cA}
	WH	22.62±0.13^{aA}	19.74±0.02^{aB}	22.19±0.50^{dA}	22.45±0.26^{bcB}	22.41±0.14^{cdA}	21.94±0.35^{bA}
SOL	ER	6.06±0.01^{aA}	7.67±0.27^{bA}	6.92±0.03^{bA}	7.23±0.32^{bA}	7.28±0.12^{bA}	7.42±0.30^{bA}
	WH	5.62±0.06^{aB}	7.34±0.05^{bA}	7.40±0.18^{bA}	8.06±0.54^{bA}	7.25±0.01^{bA}	7.08±0.57^{bA}
WBC	ER	3.40±0.02^{aA}	3.74±0.04^{bdA}	3.63±0.04^{cdA}	3.59±0.00^{cA}	3.80±0.03^{bA}	3.74±0.05^{bdA}
	WH	3.72±0.05^{aB}	3.48±0.02^{bcB}	3.64±0.02^{acA}	3.71±0.11^{acA}	3.56±0.05^{abcB}	3.39±0.06^{bB}
OBC	ER	0.73±0.09^{aA}	0.74±0.06^{aA}	0.67±0.01^{aA}	0.68±0.00^{aA}	0.71±0.07^{aA}	0.69±0.01^{aA}
	WH	0.68±0.01^{aA}	0.68±0.03^{aA}	0.71±0.00^{aA}	0.70±0.16^{aA}	0.69±0.02^{aA}	0.71±0.01^{aA}
SC	ER	4.80±0.02^{aA}	4.92±0.10^{aA}	4.79±0.02^{aA}	4.81±0.23^{aA}	5.06±0.12^{aA}	5.05±0.13^{aA}
	WH	5.07±0.04^{aB}	4.88±0.01^{aA}	4.84±0.00^{aA}	4.75±0.03^{aA}	5.07±0.11^{aA}	4.47±0.49^{aA}

ER, Eradu; WH, Wongan Hills.

SOL, water solubility (% db); WBC, water binding capacity (g/g db); OBC, oil binding capacity (g/g db); SC, Swelling capacity (mL/g db).

5 Means assigned with different small letters in the same row and capital letters in the same column within each dependent variable indicate

6 significant differences ($p < 0.05$).

Table 3 Proximate compositions and the dietary fibre content (g/100 g db) of lupin seed coats

		PBA Jurien	Coromup	PBA Gunyidi	Mandelup	PBA Barlock	Jenabillup
Ash	ER	2.92± 0.02^{aA}	2.93± 0.02^{abA}	3.01± 0.01^{bA}	2.89± 0.03^{aA}	2.93± 0.02^{abA}	2.88± 0.02^{aA}
	WH	2.81± 0.01^{aB}	2.96± 0.03^{dA}	2.95± 0.01^{dB}	2.86± 0.01^{bcA}	2.90± 0.00^{cA}	2.85± 0.02^{abA}
Fat	ER	1.83±0.01^{aA}	2.13±0.04^{cA}	2.28±0.06^{dA}	1.85±0.00^{abA}	1.96±0.01^{bA}	1.94±0.01^{abA}
	WH	1.67±0.04^{aB}	2.41±0.08^{dB}	1.77±0.00^{abB}	2.15±0.02^{cB}	1.97±0.09^{bcA}	1.62±0.03^{aA}
Protein	ER	8.09± 0.12^{aA}	8.31± 0.07^{bA}	8.29± 0.00^{bA}	8.23± 0.04^{bA}	7.75± 0.06^{cA}	7.65± 0.05^{cA}
	WH	6.34± 0.01^{aB}	8.59± 0.01^{bB}	8.35± 0.08^{cA}	8.44± 0.18^{bcA}	8.06± 0.03^{bcB}	8.02± 0.01^{cB}
IDF	ER	80.36±0.59^{aA}	78.51±0.04^{abA}	78.64±0.13^{abA}	76.95±0.15^{bA}	79.32±0.75^{abA}	79.79±0.04^{abA}
	WH	83.30±0.01^{aB}	78.91±0.01^{bA}	81.13±0.02^{cB}	77.46±0.14^{dA}	79.72±0.19^{bcA}	80.68±0.44^{abA}
SDF	ER	2.97±0.31^{aA}	2.74±0.17^{aA}	2.79±0.23^{aA}	3.31±0.18^{aA}	3.17±0.29^{aA}	2.86±0.05^{aA}
	WH	3.22±0.24^{aA}	3.42±0.28^{aA}	2.96±0.04^{aA}	3.76±0.09^{aA}	3.25±0.19^{aA}	3.00±0.04^{aA}
TDF	ER	83.33±0.90^{aA}	81.24±0.21^{abA}	81.43±0.10^{abA}	80.26±0.33^{bA}	82.49±1.04^{abA}	82.65±0.01^{abA}
	WH	86.52±0.23^{aB}	82.33±0.27^{bcA}	84.09±0.02^{cB}	81.22±0.05^{bB}	82.97±0.37^{bcA}	83.67±0.47^{cA}

ER, Eradu; WH, Wongan Hills.

IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre.

Means assigned with different small letters in the same row and capital letters in the same column within each dependent variable indicate significant differences ($p < 0.05$).

1 Table 4 Total phenolics content (mg GAE/100g db) and antioxidant capacities (mg TE/100g db) of lupin seed coats

			PBA Jurien	Coromup	PBA Gunyidi	Mandelup	PBA Barlock	Jenabillup
TPC	Free	ER	60.10±1.13^{aA}	82.02±4.22^{bA}	82.17±2.17^{bA}	77.72±0.25^{bA}	74.23±3.40^{bA}	84.11±3.13^{bA}
		WH	57.24±0.20^{aA}	86.07±3.61^{bA}	93.24±1.98^{bB}	91.82±1.29^{bB}	93.52±0.79^{bB}	89.94±1.36^{bA}
	Bound	ER	8.23±1.54^{aA}	10.36±1.43^{abA}	21.76±1.08^{dA}	14.25±2.01^{abcA}	20.46±0.89^{cdA}	16.01±2.60^{bcdA}
		WH	16.71±2.23^{aB}	20.54±0.92^{aB}	34.75±0.46^{bB}	30.23±0.18^{bB}	34.78±1.50^{bB}	31.08±0.68^{bB}
	Total	ER	68.34±0.41^{aA}	92.38±2.79^{bA}	103.93±1.09^{bA}	91.97±2.26^{bA}	94.68±4.29^{bA}	100.13±5.73^{bA}
		WH	73.94±2.43^{aA}	106.61±2.69^{bB}	127.99±2.44^{cB}	122.04±1.11^{cB}	128.31±0.71^{cB}	121.02±0.67^{cB}
DPPH	Free	ER	23.96±0.35^{aA}	25.97±1.39^{abA}	27.29±0.02^{abA}	29.82±1.89^{bA}	27.16±0.92^{abA}	24.87±1.01^{aA}
		WH	25.04±0.79^{aA}	31.78±2.70^{abA}	36.59±2.33^{bB}	32.26±0.25^{abA}	34.58±1.03^{bB}	33.35±4.00^{abA}
	Bound	ER	9.01±0.21^{aA}	10.88±1.58^{abA}	13.71±0.82^{bA}	10.23±0.15^{aA}	11.08±0.24^{abA}	9.22±0.84^{aA}
		WH	9.06±0.40^{aA}	10.98±0.81^{abA}	14.50±0.84^{dA}	12.03±0.04^{bcB}	14.03±0.18^{cdB}	11.65±0.49^{bA}
	Total	ER	33.22±0.32^{aA}	36.85±0.19^{abA}	41.00±0.80^{cA}	40.05±1.74^{bcA}	38.24±1.17^{bcA}	34.09±0.16^{aA}
		WH	34.09±0.39^{aA}	42.76±3.52^{abA}	51.09±1.49^{bB}	44.29±0.29^{bA}	48.61±1.21^{bB}	45.00±4.49^{bA}
ABTS	Free	ER	28.14±1.19^{aA}	35.73±0.10^{bA}	37.65±3.07^{bA}	28.91±0.38^{aA}	37.48±1.24^{bA}	32.00±0.49^{abA}
		WH	33.98±0.85^{aB}	39.53±0.94^{abB}	52.13±0.49^{cB}	46.12±0.07^{bcB}	51.16±0.33^{cB}	37.60±3.11^{abA}
	Bound	ER	17.73±1.06^{abA}	15.69±1.71^{aA}	26.44±1.48^{dA}	20.57±1.53^{bcA}	24.29±0.68^{cdA}	21.07±0.52^{bcA}

		WH	18.64±0.67^{aA}	23.24±0.69^{bB}	28.58±1.52^{cA}	23.05±0.60^{bA}	25.80±1.32^{bcA}	22.42±1.00^{abA}
	Total	ER	45.37±2.07^{aA}	51.42±1.82^{aA}	64.10±4.56^{cA}	49.48±1.91^{aA}	61.78±0.55^{bcA}	53.06±1.01^{abA}
		WH	52.62±0.18^{aB}	62.78±0.24^{bcB}	80.71±2.00^{eB}	69.17±0.67^{cdB}	76.97±0.99^{deB}	60.03±4.67^{abA}
ORAC	Free	ER	354.48±13.33^{aA}	589.03±5.89^{bcA}	554.25±36.20^{bcA}	548.69±7.35^{bcA}	632.00±42.93^{cA}	508.16±26.87^{bA}
		WH	429.79±39.48^{aA}	600.42±36.11^{bcA}	586.44±15.43^{bcA}	549.14±21.43^{abA}	688.65±9.69^{cA}	577.67±46.57^{bcA}
	Bound	ER	114.73±2.63^{aA}	158.81±8.12^{abA}	225.69±4.56^{cA}	193.67±10.53^{bcA}	216.43±10.85^{bcA}	161.54±31.48^{abA}
		WH	131.83±3.42^{aB}	178.08±7.36^{abA}	224.29±15.43^{cdA}	211.83±3.98^{cdA}	262.15±21.72^{dA}	180.28±11.01^{abA}
	Total	ER	469.21±10.70^{aA}	747.84±2.23^{bcA}	779.94±40.76^{cdA}	742.36±17.88^{bcA}	848.43±32.08^{dA}	669.70±4.61^{bA}
		WH	561.62±36.06^{aA}	778.49±43.48^{bcA}	788.25±57.98^{bcA}	760.98±25.41^{bA}	950.79±31.41^{cA}	757.95±57.58^{bA}

ER, Eradu; WH, Wongan Hills.

Means assigned with different small letters in the same row and capital letters in the same column within each dependent variable indicate significant differences ($p < 0.05$).

Figure 1 Six modern genotypes of Australian sweet lupin (ASL) seeds grown at two locations and harvested in 2015 (Eradu, ER; Wongan Hills, WH)