Nutritional and antinutritional evaluation of raw and processed Australian wattle (Acacia saligna) seeds

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1. Introduction

Wattle (Acacia saligna) is a temperate species that originates in the south-west corner of Western Australia. It is fast growing and hardy, and has been widely used in Eastern Australia, the Mediterranean, North Africa, Chile, highland East Africa and Southern Africa (Adewusi, Falade, & Harwood, 2011). A. saligna has been planted in great numbers by the Tigray Regional State Government in Ethiopia since the 1980’s, in an attempt to reverse the serious soil erosion that has afflicted the region following severe deforestation. Tigray was home to an estimated 4.5 million people in 2008, around 80% of whom are rural. In recent years, poverty and hunger are widespread due to rapid population growth, declining rainfall, land fragmentation and declining soil fertility (Hagazi, 2011). A 2009 survey in Tigray found high levels of malnutrition in children <5 years of age, with 46.9% stunted, 33% underweight and 11.6% wasted, and showed a distinct worsening of nutrition status with increasing age (Mulugeta et al., 2010).

Australian acacias have been shown to have significant potential to reduce poverty and provide valuable environmental services in semi-arid regions of Africa (Adewusi et al., 2011; Harwood, 1984; Harwood, Rinaudo, & Adewusi, 2008; Yates, 2010). A. saligna in Tigray is recently recognised as a multi-purpose plant, benefiting communities through soil stabilisation, nitrogen fixation, mulch production, fuel wood production and building poles, and livestock fodder (Hagazi, 2011). A. saligna also produces good crops of seed, which have been reported to be edible (Maslin & McDonald, 2004; Maslin, Thomson, McDonald, & Hamilton-Brown, 1998). In addition, the seeds of Acacia species, such as prickly wattle (Acacia victoriae Bentham), have long been an important food source for the indigenous people of Australia and the prickly wattle has been recognised to have economic potential due to its high amounts of proteins and soluble carbohydrates (Agboola, Ee, Mallon, & Zhao, 2007; Ee, Zhao, Rehman, & Agboola, 2008; Ee, Zhao, Rehman, & Agboola, 2009). Heat processing is usually applied to the seeds before consumption to eliminate antinutritional factors, such as protease inhibitors, lectins, alkaloids, saponins and oxalates, which can interfere with the digestion and absorption of nutrients.

However, there is very little or no information available on the nutritional and antinutritional properties of A. saligna seed, as well as the processing conditions required for eliminating antinutrients. If this relatively high protein A. saligna seed proved to be suitable for human consumption, the regional food supply could be dramatically increased, since the species can grow and produce very well...
on steep mountain slopes and on severely degraded lands in Africa. Acacia seed, including *A. saligna* have been shown to be high in protein, and rich in the essential amino acid lysine, making it an excellent complement to the mostly cereal-based diet of poor Tigrains (Yates, 2010). It is the purpose of this study to assess the nutritional value and antinutritional content of *A. saligna* seed as a human food. The research sought to compare nutritional and antinutritional components of four subspecies of *A. saligna* in order to ascertain whether any subspecies should be preferred in any future plantings. A further component of the research was to compare the effects of basic village-available technologies on the levels of antinutritional factors, so as to be able to recommend the processing method most appropriate if the seed is to be used for human consumption.

2. Materials and methods

2.1. Material

The seeds of four subspecies (subsp.) of *A. saligna* (*saligna*, *prunesens*, *stolonifera* and *lindleyi*), approximately 500 gramme each subsp., were supplied by Department of Environment and Conservation and Future Farm Industries Cooperative Research Centre, WA, Australia. All other chemicals used were of analytical grade and purchased from Sigma–Aldrich, Castle Hill, NSW, Australia, and E. Merck, Darmstadt, Germany, unless stated otherwise.

2.2. Processing of wattle seeds

Whole wattle seeds (200 g) were soaked in deionised water overnight (18 h) and the water was drained. In a further treatment, wattle seeds (100 g) that were soaked overnight were heated at 100 °C in a water bath (Julabo Labortechnik GmbH, Germany) for varying periods of time (0.25, 0.50, 1, 2, 5 and 10 min). After that, the seeds were lyophilised using Christ-Alpha 2–4 LDplus freeze dryer (Biotech International, Germany). For roasting, whole wattle seeds (100 g) were spread out in an aluminium tray and roasted using a Premium Laboratory Oven (Thermoline Scientific, NSW, Australia). The tray was covered with aluminium foil to prevent the seeds popping out from the tray during roasting. The seeds were roasted at 200 °C for 2, 5 and 10 min, then cooled immediately at room temperature. All samples, including untreated, soaked, soaked and boiled, and roasted wattle seeds, were ground into a fine meal using an IKA M20 universal mill (IKA®Labortechnik, Staufen, Germany).

2.3. Proximate analysis and estimation of total carbohydrate

Moisture content of untreated and treated seed flours were determined by oven-drying at 102 °C for three hours using a Premium Laboratory Oven (Thermoline Scientific, NSW, Australia). Ash content was evaluated gravimetrically, based on the weight of the sample after burning at 550 °C for 24 h in a muffle furnace (Ceramic Engineering, Sydney, Australia). Total protein, crude fibre and fat were analysed using standard Association of Official Analytical Chemists methods (AOAC, 2005), while total carbohydrates were estimated by the difference.

2.4. Fatty acid profile

Lipid was extracted from 1 g of wattle seed flour using hexane in a final volume of 5 ml. The mixture was centrifuged (Eppendorf AG, Hamburg, Germany) at 21,475 × g for 10 min. The supernatant (3 ml) was removed and mixed with 0.5 ml of 0.2 M sodium methoxide (2.3 g sodium in 200 ml anhydrous methanol). The mixture was vortexed for 15 s and then left for 10 min at room temperature. About 2–3 drops of bromothymol blue were added and mixed. This was followed by adding 0.4 ml of 1 M HCl, or further acid drop wise, until it turned yellow. Then, about 0.6 ml of 1.5% (w/v) sodium carbonate in water was mixed, or added drop wise, until the colour changed to blue again. The hexane layer was brought to the top of the test tube by addition of distilled water, and then removed for analysis on a Perkin Elmer AutoSystem XL gas chromatograph (Perkin Elmer, Massachusetts, USA). Samples (1 ml) were introduced into a high polarity ZebronTM ZB-FFAP GC Column (Phenomenex, California, USA) of 30 m × 0.32 mm i.d., with a stationary phase thickness of 0.25 μm. The flame ionisation detector was set at 300 °C and the injector port was set at 250 °C. Separation was carried out after injection at 80 °C (hold for 2 min) in the column, by heating the sample to 220 °C at a rate of 30 °C/ min with a final holding at 220 °C for 5.5 min, and a total running time of 12.17 min. Nitrogen, hydrogen and air were used as the carrier gas at a linear velocity of 3.5 ml/s. Sample compounds were identified by comparing their retention times with standard ester derivatives of fatty acids. The relative level of each fatty acid was measured.

2.5. Extraction of protease inhibitors

Wattle seed flour was extracted with extraction buffer (0.023 M CaCl₂, 0.092 M Tris–HCl, pH 8.1) in a final concentration of 20 mg/ml. Samples were kept overnight at 4 °C before they were clarified by centrifugation at 10,000 × g for 2 min. The supernatant of each sample was assayed immediately for protease inhibitor activity or stored at −20 °C (Ee et al., 2008).

2.6. Protease inhibitor activity assays

Assays for bovine trypsin and α-chymotrypsin inhibitors were carried out according to the spectrophotometric method described in a previous study (Ee et al., 2009). Briefly, for trypsin inhibitor activity assay, 2–10 μl of wattle seed extract was mixed with 2.6 ml of assay buffer (10.3 mM CaCl₂, 41.4 mM Tris–HCl, pH 8.1) and 0.1 ml of bovine trypsin (20 μg/ml in 1 mM HCl) in a quartz cuvette, and incubated at room temperature (25 °C) for 6 min. An aliquot (0.3 ml) of 10 mM TAME substrate was then added and the absorbance (A₅₄₀) was recorded immediately and continuously for at least 3 min. The trypsin inhibitor activity was calculated in trypsin inhibitor units per gramme of seed flour as follows:

\[
\text{TIU/g seed flour} = \frac{(\Delta A_{540}/\text{min} - \text{IAA}_{540}/\text{min}) \times 3 \times 1000}{540 \times \text{seed flour (g)}}
\]

where ΔA₅₄₀/min is the change in A₅₄₀/min in the absence of inhibitor (substrate and trypsin only), IAA₅₄₀/min is the change in A₅₄₀/min in the presence of inhibitor. The factor 540 is the molar extinction coefficient at 247 nm, which was empirically estimated, given the assay buffer composition and light path length of 10 mm in the cuvette. A trypsin unit (TU) is defined as the amount of trypsin that catalyses the hydrolysis of 1 μmol of substrate per min and a TIU is the reduction in activity of trypsin by 1 TU.

For α-chymotrypsin inhibitor activity, 10–15 μl of each of the extract solutions was mixed with 1.4 ml assay buffer (0.1 M CaCl₂, 0.1 M Tris–HCl, pH 7.8) and 0.1 ml of bovine α-chymotrypsin (20 μg/ml in 1 mM HCl) in a quartz cuvette, and incubated at room temperature (25 °C) for 6 min. An aliquot (1.5 ml) of 1 mM BTEE substrate in 50% (w/w) aqueous methanol was then added and the absorbance (A₁₇₆) was recorded immediately and continuously for at least 3 min. The α-chymotrypsin inhibitor activity was calculated in chymotrypsin inhibitor units per gramme of seed flour as follows:
CIU/seed flour = \[
\frac{(C_\text{A}_{256}/\text{min} - 1A_\text{A}_{256}/\text{min}) \times 3 \times 1000}{964 \times \text{seed flour (g)}}
\]

where, \(C_\text{A}_{256}/\text{min}\) is the change in \(A_{256}/\text{min}\) in the absence of inhibitor (substrate and enzyme only), \(1A_\text{A}_{256}/\text{min}\) is the change in \(A_{256}/\text{min}\) in the presence of inhibitor. The factor 964 is the molar extinction coefficient at 256 (empirically estimated, given the assay buffer composition and light path length of 10 mm in the cuvette). A chymotrypsin unit (CU) is defined as the amount of chymotrypsin that catalyses the hydrolysis of 1 µmol of substrate per min and a CIU is the reduction in activity of chymotrypsin by 1 CU.

2.7. Polyacrylamide gel electrophoresis (PAGE)

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) (PhastGel gradient 10–15) and native-PAGE (PhastGel homogeneous 20) of all extracts were carried out with the Pharmacia Phast System (GE Healthcare Life Sciences, Uppsala, Sweden) by using the method outlined by Ee et al. (2009). SDS and β-mercaptoethanol (ME) were excluded from Laemmli’s buffer (Laemmli, 1970) for native-PAGE. Samples for SDS–PAGE were incubated for 10 min at 100 °C and samples for native-PAGE were incubated at room temperature for 30 min before resolving onto the gel. Wide range molecular mass marker (SigmaMarker™ Wide Range), ranging between 6.5 and 205 kDa, was employed. All gels were stained by PhastGel Blue R and analysed using a Gel Doc 2000 Visual Gel Documentation System (Bio-Rad Laboratories Pty. Ltd., Sydney, NSW, Australia).

2.8. Trypsin and \(\alpha\)-chymotrypsin inhibitor activity gels

Native-PAGE gels (PhastGel homogeneous 20) were stained for trypsin and \(\alpha\)-chymotrypsin inhibitor activities as described in a previous study (Ee et al., 2009). Briefly, the gels were rinsed with deionised water after separation, followed by incubation in assay buffer containing 2 mg/ml bovine trypsin or \(\alpha\)-chymotrypsin for 20–30 min at room temperature (25 °C). For each gel, 17.5 mg of N-acetyl-DL-phenylalanine \(\beta\)-naphthyl ester was dissolved in 5 ml of N,N-dimethylformamide and 25 mg of tetraozetised (zinc chloride complex) o-dianisidine (Fast blue B salt) was dissolved in 50 ml of assay buffer separately. These solutions were mixed before they were poured on the gel. The stained gels were rinsed in deionised water after 20–30 min staining. The inhibitor bands, on exposure to trypsin or \(\alpha\)-chymotrypsin, reacted with the respective enzyme and were depleted, showing up as clear bands on the gel with dark violet or pink background after staining.

2.9. Extraction and assay of phenolic compounds

The soluble phenolic compounds were extracted twice from the wattle seed flour with 10 volumes of 70% (v/v) aqueous acetone under constant stirring at room temperature (25 °C) for 2 h. The mixture was then centrifuged at 2147 × g for 10 min and the supernatant was filtered using Whatman No. 1 filter paper prior to evaporation under vacuum at 40 °C, followed immediately by lyophilisation (Ee, Agboola, Rehman, & Zhao, 2011).

Total phenolics in the acetone extracts were determined using the Folin–Ciocalteu procedure (Ee et al., 2011). Briefly, 5 ml of deionised water (D, O), 0.5 ml of the lyophilised extract containing 5–10 mg/ml of dry matter in D, O and 1.0 ml of Folin–Ciocalteu reagent were mixed in a 25 ml volumetric flask and allowed to stand for 5–8 min at room temperature (25 °C). After that, 10 ml of 7% (w/v) Na2CO3 solution was added, followed by D, O to the final volume of 25 ml. After standing at room temperature (25 °C) for 2 h, absorbance \((A_{276})\) of the mixture was measured. This assay was standardised against a calibration curve of gallic acid (0.5–3.0 mM) in 80% (v/v) aqueous ethanol. The results were expressed as gallic acid equivalents (GAE) per 100 grammes of flour in percentage.

2.10. Extraction and determination of phytate

Extraction of phytate from wattle seed was carried out following a modified procedure of Harland and Oberleas (1977). The principle of this method relies on a conversion of free phytic acid and a colourimetric measurement of the liberated organic phosphorus. Wattle seed flour (2.0 g) was extracted with 40 ml of 2.4% HCl (68.6 ml of 35% hydrochloric acid in total volume of 1 l of D, O) under constant shaking at room temperature (25 °C) for 3 h. All extracts were then filtered using Whatman No. 1 filter paper. The content of phytate was determined by using a spectrophotometric method, with an absorbance \((A)\) wavelength at 640 nm, outlined in AOAC (2005). The amount of phytic acid was calculated from the organic phosphorus by assuming that one molecule of phytic acid (containing six molecules of phosphorus \((P)\)) was digested as per the equation below (AOAC, 2005):

\[
\text{Phytate (mg/g seed flour)} = \frac{\text{Mean } "K" \times A \times 20}{0.282 \times 1000}
\]

where, \(A = \text{absorbance; } "K" = \text{standard } P (\mu g)/[A \text{volume (ml)}]; \text{phytate = 28.2% } P = 20 = \text{extract volume (ml)} \times 1 \text{ g seed flour}; 1000 = \text{conversion from } \mu g/\text{g to mg/g}. \) The results were reported in percentage of phytate in 100 grammes of flour.

2.11. Extraction and determination of oxalate

Oxalate was estimated using the method described by Falade, Owoyomi, Harwood, and Adewusi (2005). Wattle seed flour (2.0 g) was extracted with 190 ml of D, O and 10 ml of 6 M HCl (273.40 ml of 32% v/v HCl was mixed with 255 ml D, O) in boiling water bath for 2 h, and then made up to 250 ml with D, O. The extract was filtered through Whatman No. 1 filter paper. Ten ml of 6 M HCl was added to 50 ml of aliquot. Two drops of the methyl red indicator were added into the filtrate and was titrated against concentrated NH4OH until either the salmon pink colour of the methyl red indicator changed to a faint yellow or until about pH 7 by using a pH metre. The solution was heated to 90 °C and 10% of 5% (w/v) CaCl2 solution was added to precipitate the oxalate overnight without stirring. The solution was centrifuged at 2147 × g for 10 min and the precipitate was washed free of calcium with cold D, O by vigorously shaking. Centrifugation and decanting steps were repeated trice and the precipitate was drained completely. The precipitate was then washed into 100 ml conical flask with 10 ml of hot (about 90 °C) 25% (v/v) H2SO4 followed by 15 ml of D, O. The solution was heated to about 90 °C (not boiling) in a boiling water bath to promote dissolving. The final solution was then titrated against standardised 0.05 M KMnO4 solution until a faint purple colour of the solution persisted for 30 s. The oxalate was then calculated as the sodium oxalate equivalents.

2.12. Extraction and assay of total saponins

The extraction and determination of saponins were performed as described by Chen, Xie, and Gong (2007), with slight modification. Saponins were extracted from the ground wattle seeds with 20 volumes of 95% (v/v) aqueous ethanol under constant shaking overnight at room temperature (25 °C). All extracts were filtered using Whatman No. 1 filter paper prior to evaporation under vacuum at 40 °C, followed immediately by lyophilisation. To determine the total content of saponins, 0.2 ml of 2–20 mg/ml of dry matter in 95% (v/v) aqueous ethanol was transferred into a 10 ml test tube. After the solution was heated to evaporation in a water
bath, 0.2 ml freshly prepared 5% (w/v) vanillin-acetic acid solution and 1.2 ml perchloric acid were added, mixed and incubated at 70 °C for 15 min. After that, the test tubes were taken out and cooled in running water for 2 min, followed by adding ethyl acetate to the final volume of 5 ml. After being cooled to room temperature (25 °C), with a blank solution as reference, the absorbance of the mixture was scanned in the range of 200–700 nm. Our results showed that the maximum absorbance was at 550 nm, and was thus used for the measurement. This assay was standardised against a calibration curve of oleanolic acid (0–4 mg/ml) in 95% (v/v) aqueous ethanol. The results were expressed as oleanolic acid equivalents per 100 gramme of flour in percentage.

3. Results and discussion

3.1. Effect of roasting on the proximate composition of wattle seed

The proximate analysis of four subspecies (subsp.) of A. saligna (saligna, prunescens, stolonifera and lindleyi) before and after roasting at 200 °C for up to 10 min was carried out. As shown in Table 1, roasting caused significant losses in moisture from the seeds, as expected, with the initial 2 min being the period when most, up to 56.0%, of the moisture loss happened. Thereafter, moisture was lost gradually, with about 26.0–37.0% of the initial moisture remaining after 10 min. The levels of the non-moisture components, except total carbohydrate, showed a general increase after initial 2 min of roasting, which could partly be attributed to the concentration effect of the corresponding decrease in moisture. With further roasting, however, changes in the proximate compositions of all subspecies became more complicated. Generally, the crude fat and ash rose a little further after 10 min of roasting, but the level of protein decreased slightly. On the other hand, the crude fibre content of all subspecies increased significantly, especially at the final stage of roasting, rising from approximately 10.0% at 5 min to 22.0% at 10 min. Furthermore, the level of total carbohydrate in A. saligna subsp. saligna, subsp. stolonifera and subsp. lindleyi increased a little after the initial 2–5 min of roasting, but decreased gradually with further roasting. However, the level of carbohydrate was found to fluctuate in A. saligna subsp. prunescens.

A similar pattern of changes of the seed compositions for four A. saligna subspecies was found. Our results suggest the possible occurrence of browning reaction with prolonged roasting (Chukwumah, Walker, Vogler, & Verghese, 2007; Yusuf, Ayedun, & Sanni, 2008), whereas the Maillard reaction between amino groups and reducing sugars could happen with further roasting up to 10-min. Simultaneously, the reduction level of measured protein in dry matter basis was observed. It is possible; even likely, that the Maillard reaction did occur, although the loss of protein is not remarkably apparent. Similarly, decreased protein content as a result of roasting has been reported in similar studies on Sesamum indicum, Vigna subterraneana (Yusuf et al., 2008) and mango grove legume (Seena, Sridhar, Arun, & Young, 2006).

Roasting is a well-established food processing method for wattle seeds (Ee et al., 2011; Maslin & McDonald, 2004) mainly to develop characteristic nutty flavour and aroma before the seeds being used as an ingredient (Hegarty, Hegarty, & Wills, 2001). However, processing of food materials may cause a reduction in the quality of the constituents, especially proteins and carbohydrates. Our result, in general, appears to suggest that nutritive products were slightly reduced as a result of prolonged roasting, for up to 10 min, of A. saligna seeds at the high temperature.

3.2. Fatty acid analysis

Palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids from the untreated seed extracts of four A. saligna subspecies were indicated (Fig. 1B) by comparing retention time of fatty acid standards (Fig. 1A). Generally, omega-3 fatty acids, consisted exclusively of linolenic acid, were predominant in most bean varieties, while omega-6 fatty acids, mainly linoleic acid, predominated in the other types of legumes (Kalogeropoulos et al., 2010). Our result indicates that four subspecies of A. saligna have relatively high levels of essential fatty acid, linoleic acid (64.3 ± 8.1%), followed by oleic (20.0 ± 1.3%), palmitic (9.6 ± 1.5%) and stearic (2.0 ± 0.9%) acids. Wattle seed had a higher amount of linoleic acid compared to other legumes, such as broad beans (43.5%), chickpeas (54.1%),

<table>
<thead>
<tr>
<th>Subspecies</th>
<th>Roasting time (min)</th>
<th>Moisture</th>
<th>Proteinsb</th>
<th>Crude fat</th>
<th>Crude fibre</th>
<th>Ash</th>
<th>Total carbohydratesc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saligna</td>
<td>0</td>
<td>9.6 ± 0.8</td>
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<td>12.8 ± 0.0</td>
<td>12.9 ± 0.3d</td>
<td>4.2 ± 0.3d</td>
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<tr>
<td></td>
<td>2</td>
<td>6.4 ± 0.6</td>
<td>31.2 ± 0.2a</td>
<td>13.1 ± 0.1</td>
<td>12.9 ± 0.3d</td>
<td>4.2 ± 0.1d</td>
<td>33.2 ± 0.2</td>
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<tr>
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<td>32.3 ± 0.2d</td>
<td>13.5 ± 0.1</td>
<td>14.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
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</tr>
<tr>
<td></td>
<td>10</td>
<td>3.6 ± 0.2</td>
<td>31.9 ± 0.2</td>
<td>15.2 ± 0.1</td>
<td>16.0 ± 0.3</td>
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<tr>
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<td>15.0 ± 0.1</td>
<td>18.5 ± 0.4</td>
<td>4.6 ± 0.9</td>
<td>31.8 ± 0.2</td>
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</table>

a Based on 100 gramme wattle seed flour.

b N × 6.25 (gramme per 100 gramme flour), on a dry matter basis.

c Total carbohydrates were estimated by difference.

d Data in row with the same superscripts are not significantly different while data without superscripts are significantly different at 95% confidence level.
yellow split peas (45.8%), small lentils (30.4%) and black-eyed beans (33.7%) (Kalogeropoulos et al., 2010). A similar study on Canavalia cathartica seeds revealed that only oleic and stearic acids were found in the raw seeds, but more essential fatty acids were found after roasting and cooking (Seena, Sridhar, & Jung, 2005; Seena et al., 2006). The increase of crude fat content (Table 1) of A. saligna after roasting in our study was in agreement with Seena et al. (2006, 2005).

3.3. Molecular properties of soluble proteins extracted from raw and processed wattle seeds

Fig. 2A and B show the SDS–PAGE gel electrophoretograms of extracts from four subspecies of raw A. saligna seeds obtained under non-reducing and reducing conditions, respectively. For raw wattle seed extracts, more protein bands appeared under reducing conditions than under non-reducing conditions and protein molecules smaller than 36 kDa were broken down. Overall, the results suggest that water soluble proteins from raw wattle seeds were predominantly polypeptides with molecular weights lower than 66 kDa, which was in agreement with the findings reported in previous studies on a different species, A. victoriae Bentham (Agboola et al., 2007; Ee et al., 2009). After soaking overnight followed by boiling for various periods, proteins with molecular weight lower than 45 kDa were mostly degraded, and only three faint protein bands remained (Fig. 2, lane 6). Additionally, with 2 min and further roasting, no protein band was visible (result not shown), suggesting that the heat treatment had caused the soluble proteins in wattle seeds to breakdown into fragments at least smaller than 6.5 kDa. Such thermal breakdown of protein has also

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**Fig. 1.** Gas chromatography profiles of fatty acid standards (A) and typical A. saligna subspecies (B) fatty acids. Peaks: 1, palmitic acid (C16:0); 2, stearic acid (C18:0); 3, oleic acid (C18:1) and 4, linoleic acid (C18:2).

**Fig. 2.** SDS–PAGE profiles of A. saligna seed flour obtained under (A) non-reducing condition and (B) reducing condition with β-mercaptoethanol (ME). Lane 1: sigma wide-range molecular mass marker from 6.5 to 205 kDa as labelled; lane 2: A. saligna subsp. saligna; lanes 3: A. saligna subsp. prunescens; lane 4: A. saligna subsp. stolonifera; lane 5: A. saligna subsp. lindleyi; lane 6: typical soaked and boiled wattle seed extract. Arrow indicates direction of protein migration.
been suggested by previous studies (Ejigui, Savoie, Marin, & Desrosiers, 2005; Mugendi et al., 2010).

3.4. Protease inhibitor activity in raw wattle seeds

Protease inhibitor (PI) activity assays of whole wattle seed flour revealed varying trypsin and α-chymotrypsin inhibitor activity levels (Fig. 3A and B). A. saligna subspp. stolonifera (3271.4 TIU/g) had the highest level of trypsin inhibitor units per gramme of seed flour, followed by A. saligna subspp. pruinascens (3074.2 TIU/g), A. saligna subspp. saligna (3030.7 TIU/g), and the lowest level was found in A. saligna subspp. lindleyi (2474.3 TIU/g). Compared to the total trypsin inhibitor activity, all four A. saligna subspecies had a 20-fold lower chymotrypsin inhibitor units per gramme (CIU/g) of seed flour, in an approximate range of 120.4–150.6 CIU/g. To further verify the presence of PIs in A. saligna subspecies, a native-PAGE gel (Fig. 4A) was obtained from all raw samples, whereas its activity gel counterpart (Fig. 4B) was also prepared. Electrophoresis was performed on the two gels at the same time to obtain protein bands, which aligned with each other. A typical trypsin inhibitor activity gel (Fig. 4B, lane 1) revealed the presence of several inhibitor bands with various intensities of clear bands against the dark background in all of the A. saligna subspecies. Results for trypsin inhibitor activity were compatible with the spectrophotometric analysis of PIs, where the sample showed high intensity (clearer bands) in raw seeds (Fig. 4B, lane 1). Apparently, two most intense protein bands (Fig. 4A and B) predominate over the total trypsin inhibitor activity of wattle seeds. However, chymotrypsin inhibitor activity was not detected under the activity gel conditions.

Our results appear to suggest that trypsin inhibitor is the predominant type of PI in the seeds of A. saligna subspecies. This was in agreement with previous study (Ee et al., 2008) on A. victoriae Bentham seeds, where high trypsin inhibitor (239.5 TIU/g flour) and low α-chymotrypsin inhibitor activities (32.8 CIU/g flour) were reported. It has been stated that most trypsin inhibitors are found to be active against α-chymotrypsin at various degrees but the inhibition is not significant compared with trypsin (Ee et al., 2009). Therefore, our results suggest that the relatively low α-chymotrypsin inhibitor activity obtained with spectrophotometric analysis is the effect of unstable form of inhibitor-chymotrypsin complex. This complex probably dissociated during the incubation period of the activity gel and showed insignificant activity compared with the trypsin activity gel. Furthermore, it is well established that PIs stochiometrically inhibit trypsin and α-chymotrypsin in a 1:1 M ratio. Results indicated that both enzymes were most likely inhibited at an identical reactive site. Similar degrees of inhibition of α-chymotrypsin have been reported for a number of other trypsin inhibitors that have a single reactive site, such as seeds of A. victoriae Bentham (Ee et al., 2009), Acacia confusa (Wu & Lin, 1993) and Acacia elata (Kortt & Jermyn, 1981).

3.5. Effect of soaking and heat processing on antinutrients

Soaking whole wattle seeds overnight resulted in a significant decrease in both trypsin and α-chymotrypsin inhibitor activities (Fig. 3A and B). However, heating at 100 °C (boiling) for up to 10 min on soaked seeds did not effectively reduce TIU, while gradual reduction of CIU was recorded after boiling for up to 2 min and the CIU was completely eliminated thereafter. Results indicated that moist heat treatment was not an effective processing method to destroy trypsin inhibitor in the A. saligna subspecies. Even though a significant decrease of TIU occurred after soaking and a gradual decrease after heating, high density (clear) bands were observed in the trypsin activity gel (Fig. 4, lanes 2 and 3). The incompatibility of the spectrophotometric results with the trypsin activity gel explained the possible occurrence of other coexisted inhibitory factors, such as natural phenolic compounds (Goncalves, Soares, Mateus, & de Freitas, 2007), rather than only PIs in the crude extracts, especially when whole wattle seed flour was used for extraction. Our results, therefore, suggest that soaking overnight massively removed the soluble phenolic compounds, particularly from the seed coat. On the other hand, since the activity gel method can only pick up proteins and the fact that the inhibitors are also proteins, the trypsin inhibitor bands were therefore intensified in the activity gel. Further studies will be carried out in the future to elucidate the effects of soaking and soaking/boiling on the phenolic compounds in wattle seeds.

Other than moist heat treatment, roasted wattle seeds were also analysed for PI activity. Interestingly, roasting for even 2 min at 200 °C was adequate to destroy the PI activity (result not shown). Similarly, no protein band and inhibitor active band was indicated in the native-PAGE (Fig. 4, lane 4). Previous studies also have shown that elimination of PI activity was achieved after dry
heating of mucuna bean (Mugendi et al., 2010), peanuts and red kidney beans (Ejigu et al., 2005) as well as velvet bean (Sid-dhuraju, Vijayakumari, & Janardhanan, 1996). Our results appear to suggest that, although the protein content of the seed flour in dry matter basis decreased slightly, it is not likely correlated with PI activity. Despite the fact that PIs are also proteins, the extraction of inhibitors is not proportional to the overall amount of protein that is present in each sample. Apparently, roasting is a more effective method to eliminate PIs compared to boiling. Besides, under such conditions, the development of a commercially acceptable flavour profile is very encouraging.

Phenolic compounds (~0.2%) of the raw A. saligna subspecies were reported (Table 2). In comparison, raw A. saligna subspecies contain about double the amount of phenolic compounds in A. victoriae seed (Ee et al., 2011). Roasting in both A. saligna subspecies and A. victoriae (Ee et al., 2011) gradually increased the content of phenolic compounds with the increase of roasting time, and further (Table 2). It was very low or not detected in subsp. Lindleyi 0 0.2 ± 0.0 0.1 ± 0.0 N/D 3.4 ± 0.3 2.8 ± 0.1 f 5 0.6 ± 0.0 N/D 1.4 ± 0.2 1.8 ± 0.0 f 10 0.9 ± 0.0 N/D 1.7 ± 0.0 1.6 ± 0.0 f Lindleyi 0 0.2 ± 0.0 0.1 ± 0.0 2.2 ± 0.2 2.6 ± 0.1 f 2 0.4 ± 0.0 N/D 2.4 ± 0.2 2.4 ± 0.0 f 5 0.5 ± 0.0 N/D 2.7 ± 0.1 f 10 0.8 ± 0.0 N/D 2.5 ± 0.0 f Subspecies Roasting time (min) % Phenolics b % Phytatec % Oxalated % Saponinef

4. Conclusions

The results obtained in the present study showed that A. saligna subspecies seeds contained a fairly high amount of proteins compared to other legumes. Additionally, the relatively high levels of linoleic acid and carbohydrates in the seeds significantly increased the nutritional benefits from the seeds. Boiling up to 10 min did not completely eliminate trypsin inhibitor, although soaking significantly reduced the protease inhibitor activities. Roasting for 2 min or longer significantly destroyed protease inhibitors, partially decomposed phytates, oxalates and saponins, simultaneously enhanced the nutritional values of A. saligna seed. Further research is needed to rule out other toxic factors, such as cyanides, fluorooacetate and alkaloids.

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