Food Chemistry 138 (2013) 762–769

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

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

K.Y. Ee [a,*], P. Yates [b]

[a] E. H. Graham Centre for Agricultural Innovation and School of Agricultural and Wine Sciences, Charles Sturt University, Private Bag 588, Wagga Wagga NSW 2678, Australia
[b] Charles Darwin University, Ellengowan Drive, Darwin NT 0909, Australia

ARTICLE INFO

Article history:
Received 15 July 2012
Received in revised form 17 September 2012
Accepted 4 October 2012
Available online 10 November 2012

Keywords:
Acacia saligna seed
Food security
Proximate analysis
Antinutritional factors
Heat treatments

ABSTRACT

Raw and processed (soaked, soaked/boiled, roasted) wattle, *Acacia saligna* subspecies (subsp.) *saligna*, *pruinescens*, *stolonifera* and *lindleyi*, seeds were analysed for nutritional and antinutritional qualities. Whole wattle seeds mainly comprised proteins (27.6–32.6%) and carbohydrates (30.2–36.4%), which had approximately 12.0–14.0% fat and 13.0–15.0% crude fibre. Palmitic (9.6%), stearic (2.0%), oleic (20.0%) and linoleic (64.3%) acids were identified by gas chromatography (GC) analysis. Phenolic (~0.2%), oxalate (2.2–3.4%) and saponin (2.6–3.0%) contents were fairly high; phytate content was low. All untreated samples contained a high level of trypsin inhibitor (2474.3–3271.4 trypsin inhibitor units per gramme (TIU/g) of flour) and low level of α-chymotrypsin inhibitor (120.4–150.6 CIU/g). Soaking overnight following with 2-min boiling led to a significant reduction of protease inhibitor activity. Roasting at 2 min or longer was sufficient to reduce both trypsin and α-chymotrypsin inhibitors to negligible values, also to reduce phytate, oxalate and saponin contents, simultaneously enhanced the nutritional values of wattle seeds.

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, 1991; Rinaudo & Cunningham, 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

Abbreviations: SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; kDa, kilo Daltons; GC, gas chromatography; UV, ultraviolet; PI, protease inhibitor; TU, trypsin unit; TIU, trypsin inhibitor unit; CU, chymotrypsin unit; CIU, chymotrypsin inhibitor unit; TAME, *p* -toluenesulfonyl-L-arginine methyl ester; BTEE, *N*-benzoyl-L-tyrosine ethyl ester; ME, β-mercaptoethanol.

* Corresponding author. Tel.: +61 420670288; fax: +61 2 69332866.
E-mail address: e2kahyaw@hotmail.com (K.Y. Ee).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.10.085

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 antinutritonal 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*, *pruinescens*, *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 2147×*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 μl) were introduced into a high polarity Zebron™ 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 $CaCl_2$, 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 $CaCl_2$, 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 (A_{247}) 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:

$$\text{TIU/g seed flour} = \frac{(\text{T}\Delta A_{247}/\text{min} - \text{I}\Delta A_{247}/\text{min}) \times 3 \times 1000}{540 \times \text{seed flour (g)}}$$

where $\text{T}\Delta A_{247}$/min is the change in A_{247}/min in the absence of inhibitor (substrate and trypsin only), $\text{I}\Delta A_{247}$/min is the change in A_{247}/min in the presence of inhibitor. The factor 540 is the molar extinction coefficient at A_{247}, 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 $CaCl_2$, 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 (A_{256}) 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: