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Non-protein amino acids in Australian acacia seed: Implications for food security and recommended processing methods to reduce djenkolic acid

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ABSTRACT

Seed of Australian acacia species, *Acacia colei*, *Acacia elecantha*, *Acacia torulosa*, *Acacia turmida* and *Acacia saligna*, were analysed for the presence of toxic non-protein amino acids and the levels of essential amino acids. Amines were derivatised with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate before analysis using liquid chromatography electrospray ionisation triple quadrupole mass spectrometry (LC-ESI-QQQ-MS). Multiple reaction monitoring (MRM) with optimised transitions and collision energies for each analyte were employed. The known nephrotoxic compound djenkolic acid was found to be present at elevated levels in all species tested. The lowest levels were in *A. colei* (0.49% w/w) and the highest in *A. saligna* (1.85% w/w). Observed levels of djenkolic acid are comparable to measured and reported levels found in the djenkol bean. Subsequent testing of seed processing methods showed djenkolic acid levels can be significantly reduced by over 90% by dry roasting at 180 °C rendering the seed safe for human consumption.

1. Introduction

The seed of certain species of Australian acacia have been shown to have significant potential to improve rural livelihoods and reduce malnutrition in semi-arid regions of Africa. Acacias provide a range of benefits including environmental services, such as nitrogen fixation and wind speed reduction; they produce valuable fuelwood and building materials and they produce edible seed (Adewusi, Falade, Oyedapo, Rinaudo, & Harwood, 2006; Cunningham et al., 2008; Harwood, Rinaudo, & Adewusi, 1999; Rinaudo, 2001; Rinaudo & Cunningham, 2007; Rinaudo, Patel, & Thomson, 2002; Thompson, Harwood, & Rinaudo, 1996; Yates, 2010).

The use of acacia seed as a food source is not new, with up to forty species known to have been eaten regularly by Aboriginal people in Australia for thousands of years (Devitt, 1992; Latz & Green, 1995; Lister, Holford, Haigh, & Morrison, 1996; Midgley,

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Brand, Harwood, Annison, & Richardson, 1991; O'Connell et al., 1983; Orr & Hiddens, 1987). At the present time, acacia seed is produced and marketed as 'wattleseed' by a small, but growing industry in Australia. Australian acacia seed of a number of species have been analysed by several groups, and shown to contain crude protein at between 22% and 27%, carbohydrate between 21% and 57%, and fats between 7% and 15% (Yates, 2014). Acacia seed is rich in the amino acid lysine, making it an excellent complement to the predominantly cereal based diets of semi-arid Africa (Adewusi, Falade, & Harwood, 2003; Yates, 2010). The seed of *Acacia colei* was the subject of a human volunteer trial in Niger in 1995 (Adewusi et al., 2006), and has been consumed regularly in the Maradi district since that time.

Acacia seed is known to contain several antinutritional factors, which if not removed through appropriate processing or cooking could reduce absorption of nutrients. The presence of trypsin inhibitors, oxalate, phytate, saponins and tannins are reported by Adewusi, Falade, Harwood (2011), Adewusi et al. (2003) and Ee and Yates (2013), though not in concentrations likely to be injurious to human health. Non-protein amino acids (NPAAs) also occur in acacia seed and may have potentially antinutritional or toxic effects (Bell, 2003). For example, Falade, Adewusi, and Harwood (2012) show that (S)-carboxyethyl cysteine in acacia seed interferes with the absorption of methionine in rats.

This study screened and quantified the levels of a variety of NPAAs in five species of acacia seed in order to assess the health risk that these compounds may pose if the seeds become a regular part of the human diet. Amino acids, NPAAs, biogenic amines and many other amines can be selectively derivatised and quantified using 6-aminoquinolyl-*N*-hydroxysuccinimidylcarbamate (Aqc) (Boughton et al., 2011). The initial screen comprised the NPAAs: (S)-carboxymethylcysteine (CMC), lanthionine, djenkolic acid (DJK), mimosine and canavanine.

In the first part of the study NPAAs were measured in the seeds of five species: *A. colei*, *Acacia elacantha*, *Acacia torulosa*, *Acacia tumida* and *Acacia saligna*. The *A. saligna* seed was tested in two preparations, as a raw seed and as a roasted seed. A sample of soybean was also tested as a negative control for comparative purposes. When concerning levels of djenkolic acid were found, commercially sourced Djenkol bean was also tested as a positive control, then a second part of the study sought to determine the most effective means of processing for reduction of this compound in three of the five species of acacia and the effect of processing methods on essential amino acids content.

The species selected for testing are all under investigation for their potential contribution to agricultural systems in semi-arid regions of Africa. *A. colei* has been used as a human food in Niger for over 15 years. *A. torulosa*, *A. elacantha* and *A. tumida* have performed well in agronomy trials and show potential for good seed yields in Niger and other parts of the Sahel (Cunningham & Abasse, 2005). *A. saligna* has been planted widely in the dry highlands of Tigray, in northern Ethiopia, with seed production representing a significant potential food resource (Hagazi, 2011; Yates, 2010). The species trialled in the Sahel could be expected to perform similarly in the Ethiopian lowlands, where many of the livelihood and land degradation problems seen in Niger are also evident.

2. Materials and methods

2.1. General

All chemicals and solvents used for analysis were purchased from Sigma Aldrich (Australia) and were either of analytical or mass spectrometric grades. The Aqc reagent was synthesized accordingly (Cohen & Michaud, 1993) and deionised water (18.2 MΩ) was produced using a Synergy UV Millipore System (Millipore) and was used throughout. An Eppendorf 5415R refrigerated mircrocentrifuge (Eppendorf AG, Hamburg, Germany) cooled to 0 °C was used for centrifugation. External calibration curves within the concentration range of 100 nM to 100 μM were prepared from a combined solution containing all standards.

Acacia seed lots from *A. colei*, *A. elacantha*, *A. torulosa*, *A. tumida* were sourced from trial plots growing at Danja, Niger. *A. saligna* seed lots were source from naturalised stands growing in Tigray, Ethiopia. Roasted *A. saligna* seeds were prepared by the Tigray Agricultural Research Institute, Ethiopia and were subject to 10 min pan-roasting over a charcoal fire. Djenkol beans and soybean were sourced from a commercial retail supplier.

2.2. Acacia seed pre-treatments

2.2.1. Dehusking

Raw seed was deshuked by cracking the seed coat then manually peeling the seed coat away, hardened seed coat from roasted seeds was removed by crushing the seed then removing as much seed coat as possible. Dehusking and separation of raw meal from *A. colei* seed proved difficult due to the small size of the seed.

2.2.2. Roasting of raw seeds

For initial roasting samples of *A. colei*, *A. saligna* and *A. torulosa* seed were pan roasted for 10 min at 180 °C prior to homogenisation. For time course roasting experiments lots of ten seeds from *A. colei*, *A. saligna* and *A. torulosa* were roasted in aluminium foil boats for 2, 4, 6, 8 and 10 min at 180 °C in a conventional fan forced oven. Each roasted seed lot was then split into two groups where one group was homogenized whole and the other dehusked then homogenized.

2.2.3. Germination of seeds

Seeds from *A. colei*, *A. saligna* and *A. torulosa* were germinated by pouring enough boiling water to submerge seeds placed on sterilised large filter paper in a petri dish. Seeds were soaked overnight and excess water removed. Samples were germinated on the laboratory bench and a separate lot in the dark for three days. Seedlings were snap frozen in liquid nitrogen and freeze dried.

2.2.4. Soaking of seed

Lots of ten seeds from *A. colei*, *A. saligna* and *A. torulosa* were placed in 15 ml Falcon tubes then soaked in deionised water (500 μl) for 24 h at 23 °C, the water was removed then frozen and stored at −20 °C. Seeds were snap frozen in liquid nitrogen then freeze dried.

2.3. Homogenisation of acacia seeds, djenkol bean and soybean

Frozen djenkol beans were hand crushed using a mortar and pestle to a course powder prior to homogenisation.

Raw or pre-treated acacia seeds (500 mg), raw soybean (500 mg) and course djenkol bean powder (500 mg) were homogenised in an IKA Yellowline A-10 water cooled mill grinder (IKA-Werke GmbH & Co. KG, Stuafen, Germany) for 30–60 s prior to extraction.

Freeze dried germinated acacia seedlings were homogenized at −20 °C in cryo-tubes (Precellys, Bertin Technologies, Montigny-le-Bretonneux, France) containing 1.4 mm ceramic beads and methanol (500 μl) containing internal standard ($^{13}C_5$,^{15}N-valine, 25 μM) using a Cryomill (Precellys 24, Bertin Technologies, Montigny-le-Bretonneux, France) with cryo attachment using 3 × 30 s program at 6000 rpm with 45 s delay intervals.