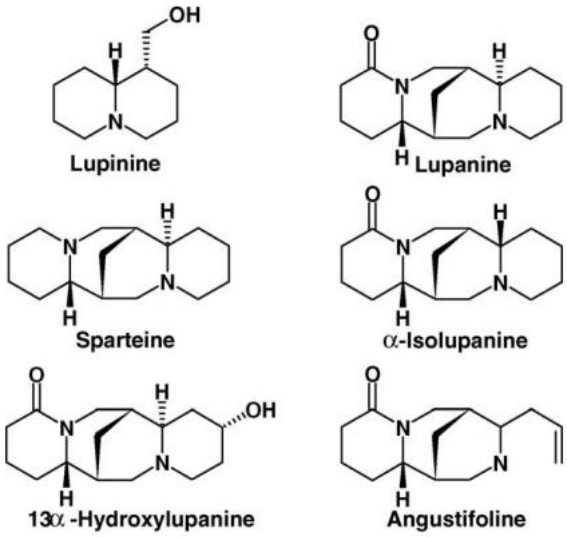
Lupin alkaloids – a scientific review

Alkaloids are low molecular weight nitrogen containing metabolites produced by plants to defend against insects and herbivores. Many have a range of bioactive and pharmacological properties but can be toxic at high concentrations. Examples of alkaloids include morphine, codeine, quinine, atropine, and caffeine.

Quinolizidine alkaloids (QAs) are a class of L-lysine derived alkaloids with over 170 chemical structures occurring predominantly within the legume family and is especially well-documented among the lupin (*Lupinus*) species and impart a very bitter taste. All lupin species produce QAs with each lupin species producing a different profile and proportion of different alkaloid molecules. Some lupin species also produce the indole alkaloid gramine (1).

Lupinus angustifolius produces mainly lupanine (42-59%), 13-hydroxylupanine (24-45%), angustifoline (7-15%), α-isolupanine (1-2%) and traces of other alkaloids (1,2) but even wider variation in the relative proportion of these alkaloids in *L. angustifolius* has been reported (3).



Molecular structure of some quinolizidine alkaloids that occur in lupin species

Lupin alkaloids are synthesized in the leaves and stem tissues and are translocated through the phloem into the developing pods and seeds (4).

Development of low alkaloid (sweet) lupin varieties

In their wild form, lupin seeds can contain as high as 10,000 – 40,000 mg/kg alkaloids and are referred to as 'bitter types'.

In the 20th Century plant breeders identified "sweet" varieties of *L. angustifolius* carrying a gene *iucundus* which confers seed alkaloid levels ranging from 20 – 600 mg/kg. Australian varieties of *L. angustifolius* typically have a seed content less than 200mg/kg which led to their commercially adopted name of the "Australian Sweet Lupin" (1).

Sweet varieties of *L. angustifolius* (known in Europe as the 'blue lupin' or 'narrow-leafed lupin') have also been bred in Poland, Germany and Russia. These European bred sweet *L. angustifolius* varieties tend to produce higher alkaloid content than those that have been bred in Australia (3).

Sweet varieties of *L. albus* (European White Lupin) and *L. luteus* (Yellow Lupin) have also been bred in Europe and Australia.

Seasonal growing conditions and geographic location can somewhat increase or decrease the alkaloid content of the harvested seed in the sweet lupin varieties in Western Australia. Larger seed tends to have a slightly higher alkaloid content (5).

In Europe, higher yielding Mediterranean climates result in higher QA levels in the case of *L. albus* and *L. angustifolius*, as compared to sub continental climatic conditions (44).

All the environmental factors that contribute to this variation in alkaloid level are not fully understood but the influence of light (photoperiod), temperature and drought stress are discussed in a recent review (45). However, it has been demonstrated that potassium deficiency of the lupin crop will increase the alkaloid levels in the harvested seed (6,7).

The Australian industry alkaloid standard for the Australian Sweet Lupin is a seed content less than 200mg/kg. This figure is also accepted by human health authorities in Australia and Europe based on toxicology data for humans and animals (8).

The Australian lupin breeding program released some varieties that consistently produce seed with alkaloid levels in the range 10-80mg/kg. Unfortunately, these varieties are very susceptible to aphid attack and are not favoured by growers as they often require insecticide application to protect the crop (9).

Lupin researchers aim to breed varieties that produce sufficient alkaloid in the vegetative tissue to protect against insect and herbivore damage but has low alkaloids in the seed (10).

Toxicology

Human alkaloid intoxication from consuming lupin seeds is infrequent and rarely leads to a fatal outcome. Reports of intoxication have been associated with inadequate debittering of *Lupinus albus* (lupini) seeds by consumers. No case of poisoning has been identified which can be attributed to consumption of commercially packaged lupin food (11).

Studies on the toxicology of lupin alkaloids have been mainly undertaken on laboratory animals and livestock.

Appetite depression in pigs can occur if the alkaloid content of the complete diet reaches 0.03% (15,16).

Knowledge on effects in humans is based on evidence from intake for medicinal purposes (sparteine), studies in healthy volunteers, or from case reports on accidental intake of bitter lupins leading to intoxication. Lethal poisoning cases have occurred where the estimated lupin alkaloid intakes range from about 10 - 50 mg/kg body weight (11).

Intoxication is always acute as lupin alkaloids are excreted rapidly in the urine of humans unchanged or as oxidised metabolites (46).

Symptoms of intoxication with lupin alkaloids in humans include vertigo, confusion, tachycardia, nausea, xerostomia, loss of motor control, and at high doses intoxication, cardiac arrest and respiratory paralysis (11).

Sparteine is the most toxic QA with an intraperitoneal administration LD50 value in mice of 36 mg/kg body weight, compared to an LD50 for lupanine of 175 mg/kg body weight (12). Sparteine is rarely detected or recorded at very low levels in *L. angustifolius* (1,13).

The effects of long term lupin alkaloid consumption was reviewed by Culvenor and Petterson (1986) who found that in rats and pigs the only attributable effect was reduced appetite or feed rejection with resultant depression of growth rate (14).

Australian research found eight generations of pigs and a total of more than 1100 litters had been fed grower or finisher diets containing from 10–40 % of *L. angustifolius* seeds, and sow diets containing 10–30 %. Alkaloid levels in the diets were estimated to range from 10–80 mg per kg. No indications of teratological effects or significant lesions were found in slaughtered sows. The production data were comparable to production data from the commercial pig industry that did not use lupin diets (1).

The only QA that is known to be tetratogenic is anagyrine that occurs in some North American species of lupin (1). Anagyrine does not occur in *L. angustifolius* (1,2).

Unlike indole and quinoline alkaloids, no binding or intercalation with DNA was identified for several QAs (including lupanine, 13-OH-lupanine, angustifoline, lupinine, and sparteine (17). Furthermore, lupanine, sparteine and cystine were unable to induce apoptosis as identified by DNA fragmentation, in HL60 cells (18). Also, lupanine did not induce gene mutations in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA1535, TA1538 in the presence or absence of exogenous metabolic activation (19).

Food Safety Authority Conclusions

In 2001, the Australia New Zealand Food Authority (ANZFA), published a human risk assessment on lupin alkaloids in food (8). It concluded that the no-observed-effect level (NOEL) of 90 - 105 mg/kg body weight per day for lupin alkaloids of *L. angustifolius* obtained in subchronic rat studies (20,21,22) is about three times higher than the lowest lethal dose of about 30 mg/kg body weight seen in human poisoning cases with lupin alkaloids (where sparteine was the major alkaloid). This suggested that the rat is not appropriate to derive tolerable exposure levels in humans1. Consequently, ANZFA derived a provisional tolerable daily intake (PTDI) of 35 µg/kg body weight per day based on reports of lupin consumption which indicate that in humans daily doses of 0.35 mg lupin alkaloids/kg body weight do not cause adverse effects (through reports of traditional use of bitter lupin seeds in Europe) and adding an uncertainty factor of 10 to account for individual variations (23).

1 The rat may also be an unsuitable model for chronic toxicity, potentially being more susceptible as they do not excrete alkaloids as quickly and completely as humans (22,46).

Based on dietary exposure assumptions ANZFA (now FSANZ) recommend a maximum permitted concentration of alkaloids of 200mg/kg (0.02%) in lupin food products (8).

A European Food Safety Authority Panel concluded that anticholinergic effects and the electrical conductivity of the heart following acute exposure to QAs are the most critical effects for the human hazard characterisation. The mode of action of sparteine and its pharmacological profile as an anticholinergic substance are considered similar to those of other main alkaloids in lupin seeds, including lupanine and 13-OH-lupanine. Based on the findings of studies on acute toxicity in experimental animals, sparteine is assumed to have a higher potency than other QAs from lupin seeds. The Panel decided to base the hazard characterisation on sparteine as a conservative approach. (Although sparteine is only described as one of the major alkaloids in the seeds of *L. luteus* and *L. mutabilis*). The available data were considered insufficient to propose HBGVs and instead an MOE approach using the lowest single oral antiarrhythmic dose of 0.16 mg sparteine/kg body weight was selected as a reference point to characterise the risk following acute exposure. No reference point could be identified to characterise the risk following chronic exposure (11).

Analytical Methods

Colorimetric and Thin Layer Chromatography

Dragendorff and Wagner reagents have a long history as a colorimetric assay for alkaloids including for QAs from lupin. Lupin crop breeders in Australia still use a rapid Dragendorff test in the field to eliminate very high alkaloid lines from their programs.

Wagner reagent has been reported to detect alkaloids in seed down to a level of 500mg/kg seed (24). However, the total alkaloid content in lupin seeds has been estimated by titration with p-toluenesulfonic acid and a pH colour indicator to have a limit of quantification of around 100 mg/kg seeds (25).

Thin-layer chromatography (TLC) on silica gel plates can provide qualitative information on the QA composition of lupin seeds with visualisation accomplished with Dragendorff's reagent and ultraviolet (UV) light (26). A quantitative 2 dimensional TLC method has also been described (27).

Gas Chromatography

Analytical methods based on capillary high resolution gas chromatography (GC) have been in widespread use since the early 1980s with a variety of extraction and clean-up steps described (28).

Finely ground lupin flour extracted with ethanol-water (70:30), defatted with diethyl ether and re-extracted with dichloromethane was separated by capillary column fitted with a flame ionisation detector (FID), confirming identity by mass spectroscopy (MS) of 10 major *L. angustifolius* QAs with sensitivity down to microgram levels (28). The Chemistry Centre of Western Australia has further refined the extraction procedures to enhance the sensitivity of the test (29).

The research group of Michael Wink has published a large number of papers on the analysis of lupin seeds and other plant parts using GC methods, culminating in the overview paper in which the spectral data and QA composition for 56 different lupin species (2).

GC-FID and GC-MS is still widely used to quantify QAs in lupin products (30).

Capillary electrophoresis (CE)

Wink's laboratory went on to develop a rapid capillary electrophoresis method for the analysis of QAs in lupins which enabled the separation of sparteine, lupanine, angustifoline and 13-hydroxylupanine in less than 10 minutes. The CE method was validated for linearity, sensitivity, accuracy and precision, and assessed seeds of seven different Lupinus species for their alkaloid content. Lupanine was present in all of them within a range from 0.02% to 1.47% (31).

High Performance Liquid Chromatography

More recently, a number of liquid chromatography with tandem mass spectroscopy (LC-MS and LC-MS/MS) methods have been published to quantify QAs from lupin seeds and from food products. Relatively simple extraction and clean-up procedures compared to GC-MS protocols are described (13,32,33) with detection of individual QA molecules in the range of 1 – 25 µg/kg (34).

Analytical standards.

The availability of commercial QA reference standards is limited. However, lupanine, lupinine, angustifoline and sparteine are available from different suppliers in the UK, EU, China and Australia.

Surveys of lupin alkaloids in food products

Concerningly, a recent survey of lupin flour and food products in Germany (species of lupins not specified) found that 70% of samples exceeded the 200mg/kg level (range 113 – 527 mg/kg) (35). This contrasts with an earlier European survey that found all 27 lupin food products tested were below 200mg/kg (36).

There have been no reports of lupin alkaloids exceeding 200mg/kg in food products in Australia.

Pharmacological uses of lupin alkaloids

Sparteine has demonstrated numerous pharmacological properties both in humans and animal models. In the cardiovascular system, sparteine has been found to exhibit antiarrhythmic activity (it is a class 1a anti-arrhythmic agent), to reduce the incidences of ventricular tachycardia and fibrillation, and to reduce heart rate and blood pressure (37).

Sparteine induces pancreatic insulin and glucagon secretion and exhibits a hypoglycemic effect and has also demonstrated protective activity against diabetes-associated DNA damage. Additionally, sparteine has been used to induce uterine contractions and has been demonstrated to exhibit diuretic and anti-inflammatory activities (38).

A more recent report indicates sparteine exhibits anti-convulsant effects in situations of acute seizure (39).

Lupanine has been reported as a positive modulator of insulin release and increased the expression of the Ins-1 gene based on in vitro cell studies and feeding to diabetic rats. Oral administration of lupanine did not induce hypoglycemia. By contrast, lupanine improved glycemic control in response to an oral glucose tolerance test in streptozotocin-diabetic rats (40).

Processing lupins to remove alkaloids

For thousands of years to the current day, the bitter European White Lupin (*L. albus*) and Andean Pearl Lupin (*L. mutabilis*) have been leached with large volumes of water over a period of days to remove the bitter alkaloids to make them palatable as a human food.

Unlike lupanine and 13-hydroxylupanine, sparteine is not be removed completely by aqueous debittering lupin beans because of its hydrophobicity (12). This is a potential issue for *L. mutabilis* and *L. luteus*, but not *L. angustifolius*.

Carvajal-Larenas et al. (2016) report debittering *L. mutabilis* seeds. At a commercial scale, 93% of the alkaloid was removed after treating lupin seeds with warm water (40°C) for 90 hours while 95% of the alkaloid was removed by cooking for 1/2 h and extraction with cold water for 72 h. Also, debittering with alkaline solutions is described as are biological processes can also significantly reduce the concentration of alkaloids in lupin seeds by as much as 90% (41).

There is a report that instantaneous controlled pressure drop (DIC) technology applied to moist dehulled seeds of *L. albus* and *L. mutabilis* decreasing alkaloid content by 60–70% (42).

Lupin protein isolates produced at a commercial scale via wet extraction and purification processes have been shown to have much reduced alkaloid levels (23).

De-caffeinated coffee is commercially manufactured by removing the alkaloid by treating the green coffee beans with either hot water, solvent (typically methylene chloride) or supercritical carbon dioxide (43). There are no reports of supercritical carbon dioxide being applied to reduce alkaloids in lupins.

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