Fact sheet
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Alkaloid analysis in lupins

Prerequisite for food production

Lupins are an interesting arable crop for cultivation. They provide a source of vegetable protein, can bind nitrogen in the soil as a legume, and have commercialisation potential thanks to their wide range of uses. However, lupins contain alkaloids, plant defence substances that can be toxic to humans and animals above a certain dose. The alkaloid content can vary depending on the variety and growing conditions, and should be determined after harvesting.

The fact sheet provides information and guidance on analysing and reducing alkaloids in lupins and is intended for producers, collection centres, processing companies and all interested parties in the field. Some sections of this fact sheet refer specifically to the Swiss context, for example, the Swiss food law.

The most important lupin species in Switzerlands arable farming are the white lupin (left) and the narrow-leaved "blue" lupin (right). Note that both species can have white as well as bluish or blue flowers.

The total alkaloid content of lupins depends on the lupin species, the lupin variety and environmental factors such as soil quality and climatic conditions. In recent years, increased alkaloid levels have been found in sweet lupins. There are probably several reasons for this, including changes in climate and defence mechanisms against diseases.

Only an alkaloid analysis by a suitable laboratory can provide precise information on the alkaloid content in the harvested grain.

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Potentials and risks in lupin cultivation

The lupin has been known in the Mediterranean region as a protein-rich "wolf bean" for human and animal nutrition and for soil improvement since Roman antiquity at the latest. Until the 20th century, they were bitter lupins that could only be eaten after the alkaloids they contained had been washed out. In the 20th century, the alkaloid content was reduced by breeding to such an extent that from then on they were known as sweet lupins and could be used without the debittering process.

Only a few of the world's native lupin species are used for cultivation. In Switzerland, these are mainly the white lupin *Lupinus albus* and the narrow-leaved or blue lupin *Lupinus angustifolius*. Yellow lupins and Andean lupins are also cultivated.

Positive characteristics

Society's demand for plant-based proteins is increasing. Grain legumes therefore have great potential as an alternative arable crop.

Compared to other grain legumes, lupins contain very high levels of proteins, unsaturated fatty acids and bioactive components such as tocopherol (antioxidant).

Depending on the lupin variety, the protein content is between 30 and 44 per cent of the dry grain mass.

Lupin crops have other advantages in cultivation:

- Nitrogen fixation, low fertiliser requirement in subsequent crops
- Active mobilisation of phosphorus
- Late and long flowering period, ideal for insects
- Widely branched and deep root system, thus improving the soil structure
- Frost tolerance, therefore early sowing possible

The challenges

The biggest obstacles to cultivation at present are anthracnose disease and often too high a content of alkaloids for feed and food processing.

Anthracnose is a lupin disease caused by the fungus *Colletotrichum lupini*. The fungus is transmitted via the seed and can lead to total crop failure in susceptible varieties. In contrast to the white lupin, the narrow-leaved lupin is relatively resistant to anthracnose. The white lupin varieties Frieda and Celina show good resistance, but often have elevated alkaloid levels.

In addition to anthracnose and alkaloid content, the following factors can make cultivation more difficult:

- Highly variable yields
- High weed pressure at the end of the growing season
- Partial lack of infrastructure, few collection points

Lupins are an attractive source of pollen for insects.

What are alkaloids?

Alkaloids are naturally occurring, nitrogen-containing organic compounds. They serve as defence substances for plants against predators and are usually toxic to the animal and human organism above a certain threshold. Well-known alkaloids are, for example, nicotine in tobacco, solanine in the potato plant, morphine in the opium poppy or caffeine in the coffee plant.

Lupin alkaloid

A total of around 170 lupin alkaloids are known. These include toxic and non-toxic alkaloids. This information sheet deals exclusively with the toxic quinolizidine alkaloids. The sum of the most frequently occurring quinolizidine alkaloids is relevant for alkaloid analysis in the lupin harvest.

Lupin alkaloids have a bitter taste. At high concentrations, the harvested product can therefore become inedible and even lead to poisoning. Typical symptoms of poisoning affect the nervous, circulatory and digestive systems, resulting for example in dizziness, tachycardia, nausea, loss of motor control and, in high doses, cardiac arrest and respiratory paralysis. Lupin varieties with a low alkaloid content are also known as sweet lupins, while lupins with a high alkaloid content are known as bitter lupins.

The Food Safety Authority of Australia and New Zealand (FSANZ) calculates an average content of 130 to 150 milligrams (0.013 to 0.015 percent) of alkaloids per kilo of sweet lupin seeds. In contrast, bitter lupins can have a total alkaloid content of 40,000 to 80,000 milligrams (4 to 8 percent) per kilo, depending on the variety.

Depending on the lupin variety, different alkaloid compositions are possible.

The following have an influence on the alkaloid content:

- Lupin species
- Lupin variety
- Environmental factors such as soil quality and climate

Table 1: The most common quinolizidine alkaloids in lupin crops

White lupin *Lupinus albus*	**Narrow-leaved lupin** *Lupinus angustifolius*	**Yellow lupin** *Lupinus luteus*	**Andean lupin** *Lupinus mutabilis*
• Lupanine • Albine • Multiflorine • 13-Hydroxylupanine	• Lupanine • Angustifoline • 13-Hydroxylupanine	• Lupinine • Sparteine	• Lupanine • 13-Hydroxylupanine, • 3-Hydroxylupanine, • Sparteine • Tetrahydrorhombifoline

Legal basis

Lupins contain a mixture of different alkaloids. The toxicity of not all of them is sufficiently known. The toxic effect of sparteine, probably the most toxic lupin alkaloid, has been best investigated. The available risk assessment for all alkaloids contained in lupins is therefore based on knowledge of sparteine. To ensure food safety, it is important to analyse lupins for their total content of quinolizidine alkaloids.

The legal regulations in European Countries are different. For example in Switzerland, there are no legally defined maximum levels for alkaloids in food or animal feed. The principle of self-regulation[1] applies here. This means that companies must ensure that only safe foodstuffs are placed on the market[2]. Food is considered unsafe if it can be assumed that it is either harmful to health or unsuitable for human consumption[3].

The German Federal Institute for Risk Assessment (BfR) has defined the following guideline threshold values, to ensure the protection of human and animal health:

- A total alkaloid content of less than 200 mg/kg (0.02 % of dry matter) applies to foodstuffs. The guideline value refers to the end product to be consumed.
- A total alkaloid content of less than 500 mg/kg (0.05 % of the dry matter) applies to animal feed. Only sweet lupins are authorised as animal feed in Switzerland and the EU[4].

Australia is the world's largest producer of lupins. There, the import and export standards of the industry organisation Pulse Australia stipulate a maximum alkaloid content of 0.02 %.

Recommendation on the procedure for an alkaloid analysis

When does the sampling take place?

The alkaloid content should be determined after the first rough pre-cleaning of the harvest. It is important to take a representative sample (p. 5 Figure 2).

Lupins must be cleaned immediately after threshing, otherwise moisture from weed seeds and other impurities will be transferred to the harvest. The harvests from different fields (batches) should be stored separately (e.g. in big bags) until the analysis results are available. In this way, contamination of good batches with bitter batches can be avoided. There is a conflict of objectives for the collection centres here, as lupins often have to be post-dried and this requires a certain minimum quantity. The individual collection centres provide information on the minimum quantity of lupins they can process.

If the alkaloid content exceeds the guideline value, the lupins can either be used as animal feed or debittered (p. 7). If necessary, when the alkaloid content exceeds the guideline value in the first analysis, a further analysis is necessary after the debittering process (p. 5 Figure 1).

The alkaloids should be analysed immediately after harvesting.

Referring to Swiss food law

1 Foodstuffs Act FSA, SR 817.0, Article 26 Lebensmittel- und Gebrauchsgegenständeverordnung LGV, SR 817.02, Art. 73–75
2 FSA, SR 817.0, Art. 7 para 1
3 FSA, SR 817.0, Art. 7 and LGV, SR 817.02, Art. 8
4 Futtermittelbuch-Verordnung FMBV SR 916.307.1, Annex 1.4

How is a representative sample taken?

Individual lupin seeds within a batch may have an increased alkaloid content. This means that the alkaloid content is unevenly distributed. Similar to mycotoxins in cereals, "alkaloid nests" or lupin seeds with particularly high concentrations can occur. Representative samples are taken in order to obtain meaningful measured values. They consist of several samples from a batch and can therefore represent an average alkaloid content of the entire lupin batch.

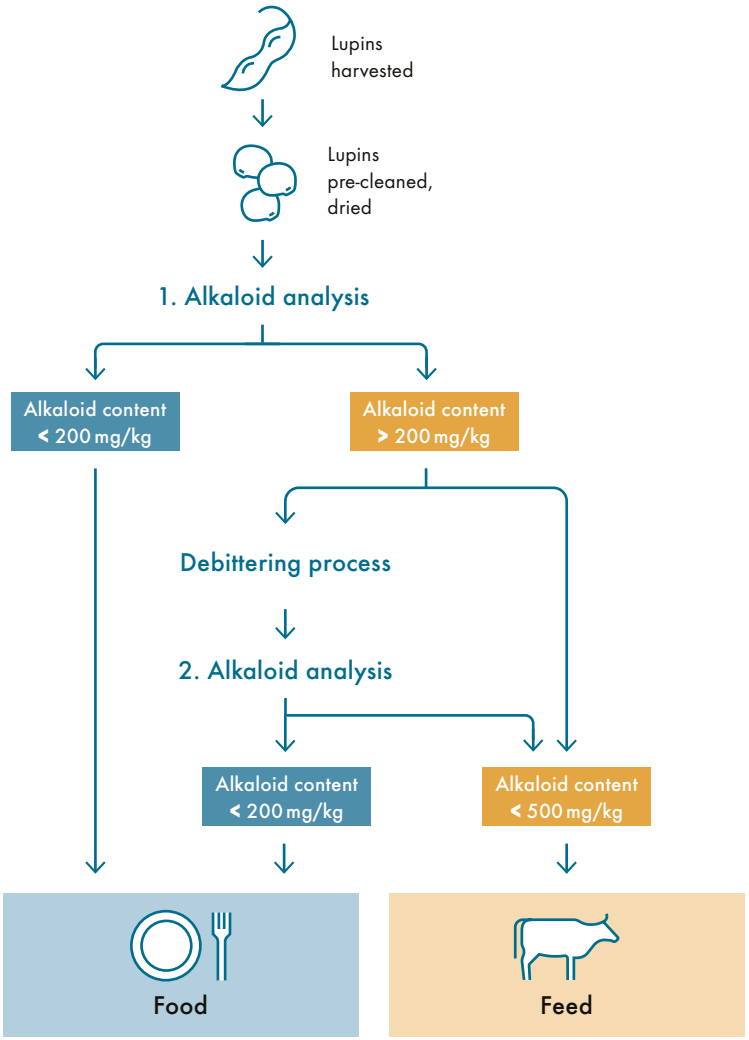
Set-up of a representative sampling
Individual samples: sample taken from a single point of the lupin batch
Aggregate samples: sample composed of different individual samples from the same lupin batch

The respective collection centres carry out the representative sampling professionally. If the harvest is not delivered to a collection centre, the recommended sampling procedure for checking the mycotoxin content of cereals or cereal products can provide guidance: Table 2 on page 6 builds on this and indicates how many individual samples and how many composite samples are required for representative sampling, depending on the weight of the lupin batch. The quantities given by the laboratories are exemplary. The exact size of the laboratory sample must be requested from the respective laboratory.

A sample calculation

For a batch of lupins weighing between 50 and 500 kilograms, five individual samples of 200 grams each are taken and mixed to form an aggregate sample of one kilo (Figure 2). The laboratory sample is then taken from this aggregate sample (images p. 6).

Figure 1: Decision tree alkaloid content



Samples are taken directly after harvesting or after pre-cleaning of the harvest. Depending on the result of the alkaloid analysis, a different use may be necessary. If the alkaloid content exceeds 500 mg/kg, further measures must be taken to reduce the alkaloid content.

Figure 2: Assembling the sample

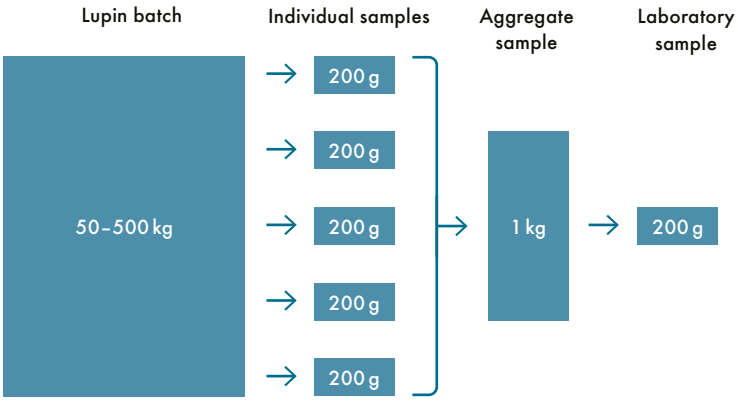


Table 2: Sampling methods for monitoring alkaloids in lupins

Lupin batch (kg)	Number of individual samples	Aggregate sample (kg)	Laboratory sample (quantity depends on the respective laboratory) (g)
≤ 50	3	1	1 × 200
> 50 to ≤ 500	5	1	1 × 200
> 500 to ≤ 1000	10	1	1 × 200

EU Regulation 401/2006, Section B.4 Table 2

Sampling procedure

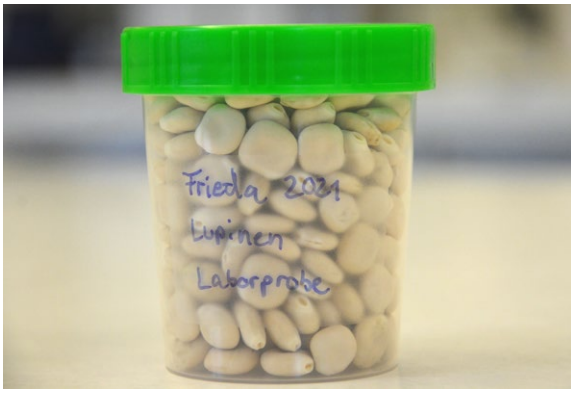
The individual samples should not only be taken from the surface of the container (trolley, sack, big bag) but if possible from all "layers" of the lupin batch. A bulk material collector or a sample spear is used for this purpose (image on the right).

If these devices are not available, it is better to take the samples during the work processes in which the grains are moving. This is the case during cleaning, for example. With a small container, individual samples can be collected at different times when the lupin seeds are discharged. The sum of the individual samples must be at least equal to the weight of the bulk sample.

In the final step, all individual samples are homogenised into a collective sample, i.e. the lupin seeds are mixed in a container (image bottom left). Approximately 200 grams of lupins are taken from this homogenised bulk sample and sent in as a laboratory sample.

The sample spear can also be used to take individual samples from the lower layers of a bag.

The samples from different locations of a batch are mixed to form a aggregate sample (well homogenised).



A laboratory sample is taken from the aggregate sample (left).

Where can the alkaloid content be analysed?

There are few laboratories that analyse lupin alkaloids. Table 3 lists the currently known laboratories in Germany and Switzerland. The following links can help in searching for possible future laboratories, as they arise:

- Switzerland: swisstestinglabs.ch > Labfinder
- Germany: vup.de > Service > Labor-finder (GER)

There are also different limits of quantification for different alkaloid contents. The total alkaloid content is often determined exactly up to 500 milligrams per kilo of lupins. If the sum of alkaloids exceeds this value, a second analysis must be carried out to determine the exact total alkaloid content of more than 500 milligrams of alkaloids. On request, the respective laboratories will provide information on the exact value up to which the total alkaloid content can be determined. It is important that the total content of quinolizidine alkaloids is analysed.

Table 3: Possible laboratories for analysing quinolizidine alkaloids in lupins

Eurofins Scientific AG	QSI GmbH	PiCA GmbH	JenaBios GmbH
CH-5012 Schönenwerd eurofins.ch info@eurofins.ch	DE-28199 Bremen qsi-q3.de sales@qsi-q3.de	DE-12489 Berlin pica-berlin.de sales@pica-berlin.de	DE-07749 Jena jenabios.de samplemanagement@ jenabios.de

Alkaloid reduction in food processing

If the total alkaloid content of a lupin batch exceeds the guideline value, it is possible to debitter the lupin seeds. The description of the following methods is based on the literature and international experience.

Reduction by means of soaking

Although alkaloids are heat-resistant, they are water-soluble. This means that alkaloids in lupins can be effectively reduced by the cooking process and soaking for several days. Peeling or roasting, on the other hand, does not reduce the alkaloid content, as the alkaloids are located inside the lupin seeds and are not destroyed by dry heat.

A debittering method described by various sources[5] includes the following steps:

- Add six parts cold water to one part lupins and soak for 24 hours
- Drain and rinse
- Add six parts water again and cook for about 10 minutes; drain and rinse
- Soak again for several days until the lupins no longer taste bitter; change the water two to three times a day

In the Mediterranean region, lupins have been debittered for centuries using this process.

Promote leaching

At low pH values between 2.2 and 2.4, alkaloids are even more soluble in water. Citric acid can be added for this purpose. The addition of common salt (NaCl) also favours the leaching of alkaloids.

Soaking is a proven method for alkaloid reduction in lupins.

5 BfR "Risk assessment of the alkaloid occurrence in lupin seeds"

Depending on the initial alkaloid content, the soaking time and the number of water changes can be adjusted. The alkaloids must be washed out until the lupins no longer taste bitter.

Reduction by means of fermentation

Lupin alkaloids are relatively stable and are not normally reduced by fermentation. However, there is one exception: in a scientific experiment, it was shown that fermentation with a special strain of the *Rhizopus oligosporus* fungus reduced the alkaloid content by more than half after 48 hours at 25 degrees Celsius and a pH value of 5.5. Researchers had specifically searched for a strain with this property.

The fungus is used in tempeh production and can be purchased commercially, but presumably not the specific strain that can break down lupin alkaloids. Further trials are therefore necessary for practical application.

Tempeh comes from Indonesia and is made from soya by fermentation. Other grain legumes can also form the basis of tempeh.

Tempeh is traditionally made from grain legumes by fermentation, the picture shows lupin tempeh.

References and information

Project LUPINNO SUISSE

fibl.org > Topics/Projects > Project database > LUPINNO SUISSE

swiss.legumehub.eu > Anbau > Lupinen (GER, FR)

bioaktuell.ch/ackerbau > Körnerleguminosen (GER, FR) > Biolupinen > LUPINNO SUISSE

Legal basis (Switzerland, EU)

Foodstuffs Act FSA SR 817.0 Article 26; FSA Art. 7; FSA Art. 8

Lebensmittel- und Gebrauchsgegenständeverordnung (GER, FR, IT) LGV SR 817.02 Art. 73-75

Futtermittelbuch-Verordnung FMBV 916.307.1, Annex 4.1 (GER, FR, IT)

Catalogue of feed materials: EU Regulation No. 68/2013

Sampling methods for checking the mycotoxin content of cereals and cereal products: EU Regulation 401/2006, Section B.4, Table 2

Risk assessment of alkaloid occurrence in lupin seeds

Federal Institute for Risk Assessment Germany (2017). Risk assessment of alkaloid occurrence in lupin seeds. Statement of 27 March 2017. DOI 10.17590/20170327-102936
bfr.bund.de > Publikationen > BfR Stellungnahmen > laufende Nummer 003, Jahrgang 2017 (GER)

Scientific foundations

EFSA Panel on Contaminants in the Food Chain (CONTAM) (2019). Scientific opinion on the risks for animal and human health related to the presence of quinolizidine alkaloids in feed and food, in particular in lupins and lupin-derived products. EFSA Journal of 25 September 2019. DOI: 10.2903/j.efsa.2019.5860

Estivi, L., Buratti, S., Fusi, D., Benedetti, S., Rodríguez, G., Brandolini, A., & Hidalgo, A. (2022). Alkaloid content and taste profile assessed by electronic tongue of Lupinus albus seeds debittered by different methods. Journal of Food Composition and Analysis No. 114 of July 2022, DOI:10.1016/j.jfca.2022.104810

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