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Review

From bitter toxins to bioactive assets: redefining quinolizidine alkaloids in *Lupinus* spp.

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ABSTRACT

Lupins (*Lupinus* spp.) are climate-resistant grain legumes gaining attention as sustainable protein sources. However, their use in the human diet is limited by quinolizidine alkaloids (QAs), a class of nitrogenous secondary metabolites. QAs represent a nutritional paradox: They are central to plant defence mechanisms, yet confer toxicity and bitterness that compromise food safety and consumer acceptance. Recent findings suggest that at sub-toxic concentrations, certain QAs may exert bioactivities that could be important for metabolic regulation. Thus, the sustainable integration of lupins into food systems depends on whether QAs are considered as undesirable contaminants or redefined as bioactive phytochemicals with dose-dependent functional potential. Significant methodological progress has been made over the last five years. Advances in high-resolution techniques and metabolomics have expanded the structural catalogue of QAs, facilitated species- and genotype-specific chemotyping and allowed first conclusions on biosynthesis. At the technological level, novel processing methods have improved the efficiency of QA removal while maintaining protein quality and, in some cases, have enabled the selective extraction of alkaloids for potential valorisation. This review critically synthesises recent advances in QA chemistry, biosynthesis, analytical methodologies, toxicology, processing strategies and emerging bioactivities. Despite this progress, major challenges remain, including the lack of standardised analytical protocols, insufficiently defined sensory thresholds, fragmented regulatory frameworks and the absence of clinical validation. Future research should shift from exclusive alkaloid elimination towards controlled modulation and selective valorisation to establish lupins as safe, multifunctional crops contributing to food security, human health and sustainable agriculture.

1. Introduction

Lupins (*Lupinus* spp.) are increasingly regarded as strategic crops in the global protein transition, as their seeds provide high-quality protein, dietary fibre and favourable lipid profiles (Akremi et al., 2025; Al-Amrousi et al., 2022; Munialo et al., 2025). Their agronomic attributes, biological nitrogen fixation and resilience to marginal environments, further enhance their sustainability value (Dourmap et al., 2025; Hama & Strobel, 2020; Hernández-López et al., 2022; Pereira et al., 2022; Szczepański et al., 2022). These characteristics justify the growing interest in *L. albus, L. angustifolius, L. luteus* and *L. mutabilis* as sustainable alternatives to traditional protein crops (Abraham et al., 2019; Estivi et al., 2023; Spina et al., 2022). Yet, despite their nutritional and agronomic potential, the large-scale use of lupins in food systems remains constrained by quinolizidine alkaloids (QAs), a diverse group of lysine-derived metabolites responsible for the characteristic bitterness and toxicity of lupin seeds (Boschin et al., 2022; Schryvers et al., 2023). This contrast between nutritional promise and metabolic risk constitutes the central paradox of lupins: compounds essential for plant defence simultaneously impose sensory and toxicological barriers to human and animal consumption (Abraham et al., 2019; Engel et al., 2022).

Historically, attempts to resolve this paradox have focused on genetic and technological suppression of QAs. Breeding programmes have successfully produced “sweet” cultivars with seed alkaloid contents below 0.02% dry weight (Pszczółkowski et al., 2025; Vishnyakova et al., 2020), marking a decisive advance in lupin domestication and enabling their incorporation into food and feed chains (Iqbal et al., 2020). However, the stability of this low-alkaloid phenotype remains a major concern. QA accumulation shows strong genotype × environment interactions, with drought, heat or pest pressure capable of inducing substantial increases even in certified “sweet” varieties (Khedr et al., 2024; Valente et al., 2023). This environmental plasticity complicates

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breeding, threatens regulatory compliance and undermines consumer confidence. Moreover, the traditional focus on QA elimination is increasingly challenged by emerging mechanistic evidence. In plants, QAs contribute to defence chemistry and stress-response pathways, while in humans certain congeners appear to influence metabolic and neurophysiological processes through specific molecular interactions. These insights weaken the rationale for complete alkaloid suppression and instead support a more balanced approach based on controlled modulation and selective valorisation.

The need for refined QA management is reinforced by advances in analytical chemistry. Classical methods such as TLC and HPLC-UV have been superseded by LC–MS/MS, UPLC, NMR-based quantification and untargeted metabolomics (Eugelio et al., 2023; Hellal et al., 2021; Hwang et al., 2020; Khedr et al., 2023; Lee et al., 2020; Namdar et al., 2024), offering unparalleled sensitivity and structural resolution. These techniques have expanded the known QA repertoire to more than 170 congeners and enabled detailed species- and genotype-specific alkaloid profiling (Cely-Velosa et al., 2023; Eldin et al., 2023). However, substantial methodological heterogeneity persists. Variability in extraction protocols, the limited availability of certified reference standards and inconsistent quantification strategies continue to hinder cross-study comparability and the establishment of harmonised benchmarks for breeding and regulatory assessment. In parallel, notable advances in post-harvest processing technologies have reshaped current approaches to quinolizidine alkaloid (QA) management (Yuan et al., 2025; Aguilar-Acosta et al., 2020). While conventional debittering based on soaking and boiling remains widely applied (Castañeta et al., 2024; Chamone et al., 2023), emerging strategies, including enzymatic detoxification, membrane-based separation, resin adsorption and supercritical CO_2 extraction, provide increasing selectivity for alkaloid removal while largely preserving protein integrity. By contrast, evidence supporting efficient QA reduction through microbial fermentation remains limited and mechanistically unresolved. Bacterial metabolism can contribute to QA depletion, as demonstrated by the utilisation of lupanine and related alkaloids as carbon sources in *Lupinus albus* aqueous extracts (Santana et al., 2002). Only a few studies demonstrate compound-specific microbial biotransformation under controlled conditions, whereas many reported decreases are more plausibly attributable to diffusion or leaching during aqueous processing rather than confirmed metabolic degradation. This uncertainty represents a critical knowledge gap requiring targeted biochemical elucidation and process-scale validation in future detoxification strategies. Importantly, several advanced processing approaches also enable the selective recovery of individual alkaloids, creating new opportunities for nutraceutical and pharmaceutical valorisation (Kumar et al., 2023). Collectively, these developments support the emerging concept of lupin-based biorefineries, in which both protein fractions and secondary metabolites contribute to integrated circular bioeconomy value chains (Rebolledo-Leiva, Almeida-García, et al., 2022).(;)

Despite technological and analytical advances, toxicological and sensory challenges remain the main constraints to lupin utilisation. QA concentrations vary widely among species and environments, and even low-alkaloid cultivars may exceed safe limits under stress conditions (Khedr et al., 2024). Regulatory frameworks remain fragmented, with no harmonised limits or evidence-based reference doses for chronic exposure (Keuth et al., 2023). Sensory thresholds are even more restrictive: bitterness is detected at QA concentrations far below toxicological thresholds, limiting consumer acceptance even when products meet regulatory standards (Soares et al., 2020). Although interactions with TAS2R receptors provide mechanistic insight, structure–perception relationships for individual congeners remain poorly characterised (Karolkowski et al., 2023). Further complicating this risk-oriented perspective, emerging evidence suggests that certain QAs exert antidiabetic, neuroprotective, cardiometabolic and anticancer effects (Atnaf et al., 2020; Mazumder et al., 2024), consistent with hormetic dose–response behaviour. While clinical validation is lacking, this duality highlights the need to consider QAs as dose-dependent phytochemicals positioned at the interface between risk and potential benefit.

In light of these challenges and opportunities, this review provides an integrative and critical reassessment of QAs in Lupinus spp., synthesising advances made over the past five years. We analyse recent progress in QA chemistry, biosynthesis and analytical profiling; evaluate developments in detoxification and valorisation technologies; and critically examine toxicological, sensory and regulatory dimensions that determine QA management. By identifying persistent methodological and knowledge gaps, we propose research priorities needed to transition from a paradigm of complete alkaloid removal to one focused on strategic modulation and sustainable valorisation. Ultimately, this perspective seeks to reposition QAs from strict antinutritional constraints to dose-dependent phytochemicals whose informed management is essential for food safety, nutritional innovation and the development of circular lupin-based value chains.

2. Nutritional, functional and sustainability relevance of *Lupinus* spp.

Lupins are increasingly recognised as multipurpose crops that combine nutritional, technological and environmental values (Buonvino et al., 2023; Caramona et al., 2024; Lo et al., 2021; Prusinski, 2017; Rebolledo-Leiva, Almeida-Garcia, et al., 2022) (Fig. 1). Their seeds contain 35–40% high quality protein, considerable dietary fibre and a favourable lipid profile enriched with unsaturated fatty acids, while they are naturally gluten-free and low in starch (Chukwuejim & Aluko, 2025; Estivi et al., 2023; Mavromatis et al., 2023; Taberner-Pibernat, Ribas-Agustí, et al., 2025). These compositional characteristics support the increasing use of lupins in plant-based formulations, including gluten-free baked products, pasta, dairy substitutes and protein isolates (Grossmann & Weiss, 2021; Mostafa et al., 2025; Munialo et al., 2025).

Beyond basic nutrition, emerging evidence indicates that lupin-derived macromolecules can influence host physiology. Isolated white lupin proteins have been reported to modulate the gut microbiota of rats, suggesting potential prebiotic effects (Rubio & Chiesa, 2025),and complementary studies highlight the broader capacity of lupin fractions to affect host-microbe interactions (Zaworska-Zakrzewska et al., 2020; Zuo et al., 2024). In livestock production, lupins provide a sustainable protein source for monogastric animals and aquaculture, reducing dependence on imported soya and contributing to regional protein self-sufficiency (Abraham et al., 2019; Maia et al., 2023; Šufliarský et al., 2024).

From a technological perspective, lupin proteins exhibit strong emulsifying, foaming and gelling properties, enabling their incorporation into various food matrices with minimal chemical modification (Etzbach et al., 2025; Chukwuejim et al., 2025; Nathia-Neves et al., 2025). Parallel research points to potential health-promoting properties, with protein isolates and enriched flours associated with improved glycaemic control, lipid metabolism and satiety. Although alkaloid fractions raise toxicological concerns (Alharbi et al., 2024), several QAs nevertheless display antimicrobial, anthelmintic and neuroactive activities (Alkanad et al., 2024; Chukwuejim et al., 2024; Dubois et al., 2019; Romeo et al., 2018). This emerging duality reflects a broader reconsideration of lupin bioactives: compounds that historically limited edibility may, if properly characterised and dosed, serve as leads for nutraceutical or pharmaceutical innovation (Alharbi et al., 2024; Bryant et al., 2022).

Equally important is the contribution of lupins to sustainability goals (Amoah et al., 2023; Rebolledo-Leiva, Almeida-Garcia, et al., 2022; Rebolledo-Leiva, Almeida-García, et al., 2022). As nitrogen-fixing legumes adapted to marginal or drought-prone environments, they improve soil fertility and support climate-resilient agricultural systems (Akchaya et al., 2025; Shrestha et al., 2021; Villacrés & Rosell, 2021). These nutritional, functional and environmental attributes position lupins among the strategically important crops in the global protein

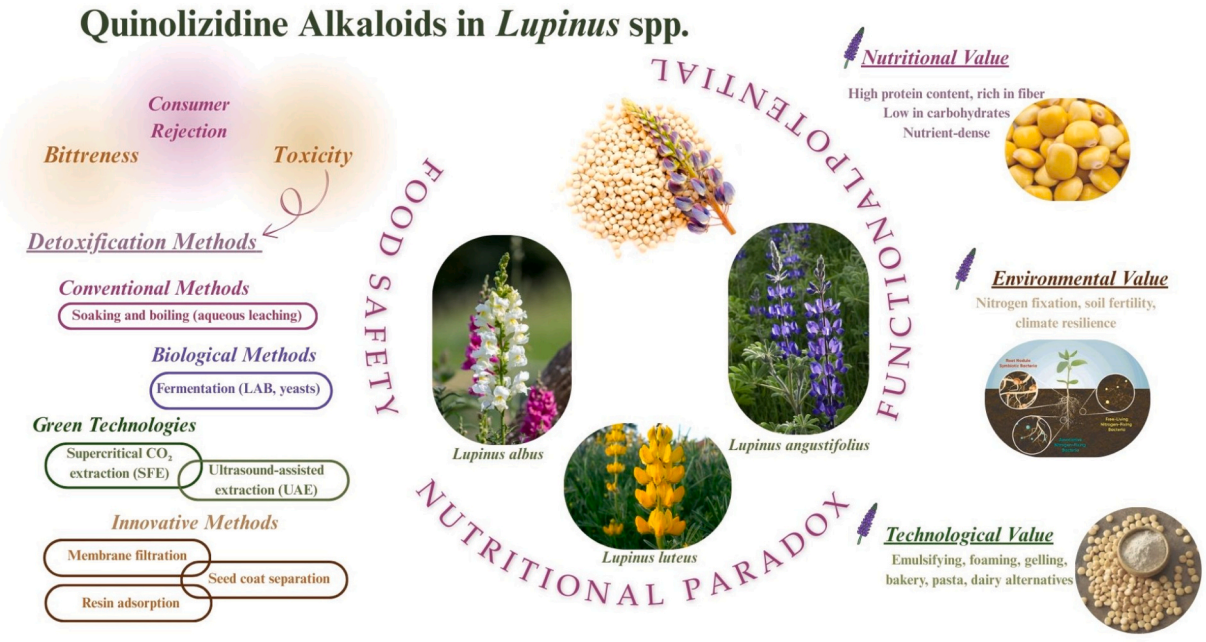

Fig. 1. Unlocking the potential of lupins: Balancing benefits with alkaloid management.

transition. Nevertheless, their broader utilisation in food and feed systems ultimately depends on the development of robust and scalable strategies for the reduction or valorisation of quinolizidine alkaloids, thereby ensuring consumer safety alongside industrial feasibility (Caramona et al., 2024).

Because QA levels, toxicity and sensory attributes arise from their underlying chemical structures and biosynthetic pathways, an in-depth understanding of these mechanisms is central to improving food safety and processing outcomes.

3. Chemistry and biosynthesis of quinolizidine alkaloids

QAs are lysine-derived secondary metabolites characteristic of *Lupinus* and related legumes, occurring as structurally diverse bi-, tri- and tetracyclic derivatives (Cely-Veloza et al., 2023). More than 170 alkaloids have been described, yet unlike glucosinolates or flavonoids, no harmonised classification system exists, a gap that complicates cross-study comparisons and risk assessment (Martínez-Hernández et al., 2023). Pronounced inter- and intra-specific variation is evident: *Lupinus albus* is dominated by lupanine-type alkaloids, *Lupinus angustifolius* by angustifoline and albine in comparatively lower amounts, while *Lupinus mutabilis* has the greatest diversity and abundance, a characteristic that offers opportunities for valorisation but raises serious food safety concerns (Halime et al., 2025; Mancinotti et al., 2023; Valente et al., 2024). Importantly, even 'sweet' cultivars exhibit environmental and developmental plasticity in QA content, complicating toxicological evaluation and destabilising breeding efforts (Cabrita et al., 2024; Khedr et al., 2025; Osorio & Till, 2022; Rodés-Bachs & Van der Fels-Klerx, 2023).

At the biosynthetic level, the pathway begins with the decarboxylation of lysine to cadaverine, followed by oxidative deamination and intramolecular cyclisation leading to piperideine intermediates, which then assemble to form the quinolizidine backbone (Mancinotti et al., 2022; Wink, 2019). However, recent advances in transcriptomics and genomics suggest that this metabolic pathway is both more complex and more tightly regulated than previously thought (Bulut et al., 2023; Patyi et al., 2025). In *Lupinus angustifolius*, an AP2/ERF transcription factor (RAP2-7) is genetically linked to the *iucundus* locus, creating a defined regulatory hub for alkaloid accumulation (Kroc et al., 2019). Similarly, re-examination of the *pauper* locus in *Lupinus albus* has revealed molecular markers for breeding varieties with low alkaloid content (Mancinotti et al., 2022; Patyi et al., 2025; Rychel & Książkiewicz, 2019). Comparative analyses of Mexican lupins also show that QA biosynthesis is evolutionarily plastic, with species-specific chemotypes underpinned by different candidate gene repertoires (Ramírez-Betancourt et al., 2021). In parallel, stereochemical reconstructions of tetracyclic homologues such as sparteine have revealed the necessity of multienzyme cascades and highlighted the biochemical sophistication of the pathway (Kroc et al., 2019; Ramírez-Betancourt et al., 2021). Recent spatial studies show that QAs preferentially accumulate in epidermal tissues, suggesting compartmentalised biosynthesis and transport processes that remain largely unexplored (Frick et al., 2025; Otterbach et al., 2019).

Taken together, current evidence reveals a persistent paradox: although the structural diversity of QAs is now catalogued in remarkable detail, the biochemical and regulatory architecture generating this diversity remains only partially resolved. Several core enzymatic steps remain hypothetical, regulatory networks are fragmentary, and the cellular compartmentalisation of biosynthesis, transport and storage is poorly defined. This disconnect has produced a field rich in descriptive chemistry but limited in mechanistic insight. Meaningful progress will require systems-level integration, multi-omics anchored in functional genetics, spatially explicit metabolic mapping, and ecological context to convert scattered observations into a coherent and predictive biosynthetic model. Without such advances, breeding strategies, food safety assessment and valorisation efforts will continue to rest on an incomplete mechanistic foundation, constraining both scientific understanding and practical application. Because QA-related safety, breeding and processing decisions ultimately depend on accurate structural and quantitative data, the development of advanced analytical platforms has become essential. Recent innovations have transformed QA profiling and now underpin modern risk assessment and processing optimisation.

4. Advances in analytical and metabolomic approaches for quinolizidine alkaloids

The understanding of the chemistry and biosynthesis of QAs has been greatly accelerated by advances in analytical methodologies. Traditional approaches such as thin-layer chromatography or simple HPLC were not

sufficient to resolve the structural diversity of QAs, recognise low-abundance congeners or ensure reproducible quantification in different matrices. However, over the last five years, significant advances have been made in sample preparation, high-resolution mass spectrometry and integrative workflows that not only allow accurate quantification, but also inferences about biosynthesis and regulatory monitoring (Table 1).

4.1. Extraction and clean-up

The accurate quantification of QAs is critically dependent on robust extraction and clean-up procedures given their structural similarity, broad polarity spectrum and occurrence in widely varying concentrations in different lupin species and tissues (Chukwuejim et al., 2024). Moreover, the extraction efficiency of quinolizidine alkaloids is fundamentally governed by their pKa-dependent protonation state, which modulates solvent partitioning behaviour, matrix binding affinity and susceptibility to ion-suppression during the detection subsequent LC–MS detection (Namdar et al., 2024). First-generation methods relied on acid-base-liquid-liquid extraction using solvents such as chloroform or dichloromethane, which provided only approximate estimates of total alkaloids and were compromised by low selectivity, co-extraction of matrix components and high solvent requirements (Khedr et al., 2023; Kushnareva et al., 2020). Although these methods were important in the past, they lacked the reproducibility and sensitivity required for food safety and breeding applications. Today's approaches have shifted to more selective and environmentally friendly solvent systems (Aguilar-Acosta et al., 2020; Nathia-Neves et al., 2025). Aqueous alcohols, especially methanol-water mixtures (typically 60:40, v/v), are now commonly used due to their ability to solubilise polar alkaloids such as angustifoline and more lipophilic alkaloids such as multiflorine simultaneously (Cely-Veloza et al., 2023; Spange et al., 2022). After extraction, solid phase extraction (SPE) has become the dominant clean-up strategy (Eugelio et al., 2023; Shi et al., 2025). Conventional C18 cartridges, while effective in reducing matrix loading, often show limited retention of less polar alkaloids (Zhao et al., 2020). Polymeric sorbents such as Strata-XL have shown superior performance by combining hydrophobic, hydrogen bonding and π-π interactions, improving recovery and reducing co-extracted interferences (Badawy et al., 2022; Eugelio et al., 2023; Fontanals et al., 2020). In parallel, modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction protocols, based on salting-out partitioning followed by dispersive solid-phase clean-up, have recently emerged as rapid and solvent-efficient alternatives capable of minimising matrix effects while improving the recovery of targeted quinolizidine alkaloids in lupin matrices, although their broader applicability across diverse alkaloid spectra, concentration ranges and tissue types still requires systematic validation (Khedr et al., 2023).

Recent innovations have expanded the extraction toolbox. Resin-based protocols allow the selective extraction of important alkaloids such as lupanine and provide extracts compatible with quantitative nuclear magnetic resonance (qNMR) analysis (Madelou et al., 2024). Molecularly imprinted polymers enable highly specific binding and purification of individual derivatives from complex lupin matrices, including industrial side streams such as wastewater (Esteves et al., 2022). Porous sorbents such as TiO_2 have also been explored for miniaturised SPE, which has shown promise for the simultaneous enrichment of alkaloids and other co-occurring xenobiotics (Shi et al., 2025). Despite these advances, methodological heterogeneity persists (Yu et al., 2025). Different laboratories use different solvent systems, cleaning materials and alkaloid panels, which makes comparability between studies difficult and limits the establishment of harmonised reference values (Schryvers et al., 2023; Święcicki et al., 2019). Furthermore, current validated workflows disproportionately target a narrow group of commonly occurring alkaloids (e.g. lupanine, sparteine, multiflorine), while the extraction efficiency, recovery dynamics and analytical signatures of lesser alkaloids remain largely uncharacterised despite their potential toxicological or sensory relevance. Looking to the future, the development of standardised, solvent-efficient and selective extraction procedures, ideally validated by inter-laboratory trials, remains a prerequisite for the generation of reproducible QA datasets in breeding, toxicological and regulatory contexts. Only with harmonised extraction and clean-up protocols can a reliable cross-comparison of alkaloid data support risk assessment, the definition of regulatory standards and the

Table 1
Extraction and clean-up strategies for quinolizidine alkaloids (QAs) in lupins: evolution, advantages and limitations.

Extraction method	Solvent system/clean-up	Analytical coupling	Advantages	Limitations	Typical applications	References
Supercritical fluid extraction (SFE)	CO_2 with ethanol cosolvent; can be combined with aqueous debittering	HPLC-UV, LC-MS	Short extraction time; reduced use of organic solvents; potential for scale-up; preservation of nutritional value	Inhomogeneous extraction in a large scale; high operating costs; limited industrial use	Debittering and QA reduction; valorisation of alkaloids from processing streams	Rosas-Quina et al., 2021
Acid-base liquid-liquid extraction (LLE)	Chloroform, dichloromethane; acidic back-extraction	GC-FID, GC-MS	Historically simple; provides estimates of total QA content	Unselective; high solvent consumption; poor reproducibility; unsuitable for food safety; environmental concerns	Early QA surveys; rough quantification of *bitter* vs. *sweet* accessions	Kushnareva et al., 2020 Lee et al., 2020
Methanol-water extraction	MeOH:H_2O (normally 60:40, v/v); no clean-up or simple filtration	LC-MS/MS	Efficient recovery of polar and moderately lipophilic alkaloids; more environmentally friendly than chlorinated solvents	Matrix effects if no cleaning is performed; variability between laboratories	Targeted routine analysis; food safety assessments	Namdar et al., 2024
Ultrasound-assisted extraction (UAE)	Aqueous or alkaline solvents; often combined with protein extraction	LC-MS/MS	Enhanced mass transfer; shorter extraction time; reduced solvent consumption; simultaneous protein recovery	Scale-up challenges; heterogeneity of extraction efficiency	Co-extraction of proteins and alkaloids; improved nutritional/functional isolates	Aguilar-Acosta, 2020
Solid-phase extraction (SPE)	Polymeric sorbents (Strata-XL, Oasis HLB) or C18 cartridges	LC-MS/MS, UHPLC-HRMS	Strong reduction of matrix interference; improved sensitivity and reproducibility; reduced ion suppression	Higher costs for consumables; requires careful optimisation of the method	Regulation-orientated quantification; validated protocols for food/feed compliance	Eugelio et al., 2023
Hybrid/green emerging methods	Fermentation, membrane separation, molecularly imprinted polymers (MIPs)	LC-MS/MS, UHPLC-HRMS	Potential for selective QA removal or modulation; compatible with food-grade processing; in line with the circular economy	Still experimental; validation and scalability not yet proven	Future debittering strategies; selective retention/removal for valorisation	Ortega and Rodríguez, 2013 Esteves et al., 2022

valorisation of lupin-derived foods.

4.2. From screening to high-resolution profiling

Technological innovations have been central to the reorganisation of QA research. Traditional chromatographic methods such as TLC and HPLC-UV have been replaced by LC-MS/MS, UPLC, NMR-based quantification and untargeted metabolomics, which offer unprecedented sensitivity, structural resolution and biological context. Classical titrimetric methods, such as the Gross assay and its subsequent modifications, have long been used to estimate total QA content (Villacrés, Álvarez, & Rosell, 2020). These methods are based on solvent extraction followed by acid-base titration and provide results in lupanine equivalents, reflecting the dominance of this alkaloid in *Lupinus mutabilis*. Their attractiveness lies in their simplicity, low cost and suitability for rapid screening in breeding or processing studies. However, they lack specificity, they cannot resolve individual alkaloid congeners and they may misrepresent the total QA content in species where lupanine is not the major component. Compared to chromatographic or spectrometric techniques, titrimetric approaches provide only approximate values and are increasingly seen as complementary rather than definitive tools for alkaloid assessment. High-resolution metabolite profiling has extended QA analysis beyond validated targeted workflows. Techniques such as LC-MS/MS, growing spectral libraries and complementary GC-MS confirmations have significantly expanded the accessible chemical space (Khedr et al., 2023; Shi et al., 2025; Zareena et al., 2021). A notable example is the characterisation of 31 wild populations of *Lupinus pilosus* and *Lupinus palaestinus*, where 15 alkaloids were quantified against authentic standards and additional metabolites were verified by GC-MS (Namdar et al., 2024). The two-platform strategy resulted in species-specific alkaloid fingerprints and uncovered rare, low-abundance compounds that may represent previously overlooked biosynthetic intermediates. These advances expand the catalogue of QA diversity and generated new hypotheses about the evolution and regulation of metabolic pathways. Importantly, correlation analyses between lysine precursor pools and alkaloid subclasses provided new evidence on the partitioning of metabolic fluxes, suggesting that precursor availability plays a more important regulatory role than previously thought (Huang et al., 2022).

While such innovations highlight the potential of analytical chemistry for modelling biosynthesis, they also highlight ongoing limitations. Coverage remains incomplete, with large parts of the quinolizidine metabolome remain unresolved due to the lack of authentic standards and the semi-quantitative nature of many MS-based annotations. Comparability between different studies is limited by differences in extraction methods, ionisation techniques and reference libraries, leading to inconsistent metabolite identification. Furthermore, while correlation analyses provide insights into regulatory principles, they remain inferential and cannot replace mechanistic validation at the genetic or enzymatic level. Therefore, high-resolution profiling is an indispensable but incomplete step. It refines descriptive inventories and points to a dynamic reconstruction of metabolic pathways, but alone cannot resolve the genetic and biochemical complexity of QA biosynthesis (Eugelio et al., 2024). Future progress will depend on integrating advanced metabolomics with isotope labelling, reverse genetics and spatially resolved analyses to transform statistical correlations into mechanistic understanding.

4.3. Integrative metabolomics and spatial resolution

Integrative omics has begun to transform QA research by moving beyond descriptive profiling to predictive genotype-phenotype frameworks (Martinez-Hernandez et al., 2025; Schwertfirm et al., 2024). The chromosome-level genome of *Lupinus luteus* illustrates this potential: it has not only resolved structural organisation, but also revealed expansions of gene families associated with terpene metabolism, stress responses and conglutins, as well as enzymes involved in alkaloid biosynthesis and transport (Martinez-Hernandez et al., 2025). Crucially, these data establish the link between genome architecture and metabolite accumulation, suggesting that QA biosynthesis is not an isolated metabolic pathway but is embedded in broader networks of defence and protein metabolism. However, these associations remain largely predictive. Only a few candidate genes have been functionally validated, so the causal architecture of QA regulation remains unclear. In *Lupinus albus*, Patyi et al. (2025) provided more applied evidence by associating alkaloid phenotypes with genetic loci. Their confirmation of *pauper* and the discovery of a new quantitative trait locus (QTL) on chromosome 5 emphasise two important points: (i) breeding based on a single determinant such as *pauper* is inherently unstable given the strong genotype × environment interactions, and (ii) allele stacking can drive alkaloid content to exceptionally low levels (< 25 ppm), raising the possibility of redefining thresholds for 'sweet' lupins. Yet even this advance exposes a paradox: ultra-low alkaloid chemotypes may enhance food safety but could compromise plant defence, creating new vulnerabilities that have not been systematically assessed (Czepiel et al., 2021). Moreover, the deployment of these QTLs into breeding pipelines remains slow, hindered by the lack of standardised phenotyping methods and the difficulty of translating controlled experimental results into field stability (Zafeiriou et al., 2021).

Parallel advances in spatially resolved techniques are beginning to address a long-standing blind spot in QA research: the precise sites of biosynthesis and accumulation. Imaging mass spectrometry (MS) and laser microdissection-MS now permit alkaloid localization at the tissue level, providing anatomical resolution that was previously unattainable (Frick et al., 2025;). Yet, applications in lupins remain scarce, and current models of QA distribution still rely largely on indirect inference from bulk extractions or whole-organ profiling. By contrast, recent work in *Gelsemium elegans* demonstrated how mass spectrometry imaging can resolve the spatiotemporal dynamics of multiple alkaloids within distinct tissues, uncovering developmental gradients and compartmentalization patterns invisible to conventional LC-MS approaches (Wu et al., 2022). The absence of comparable studies in lupins represents a critical gap: without spatially resolved evidence, key questions regarding the compartmentalization of QA biosynthesis, their mobilisation to seeds, and the regulation of transport remain hypothetical. Adapting MSI to lupins is therefore not simply a methodological refinement but a prerequisite for moving from descriptive inventories to mechanistic models of alkaloid biology.

Collectively, these methodological innovations have elevated QA research toward quantitative, systems-level biology. High-resolution profiling refines our understanding of pathway diversity and metabolic flux; multi-omics approaches begin to chart genetic regulators; and spatial analyses uncover tissue-specific accumulation. Yet critical barriers persist: heterogeneous extraction protocols, lack of authentic reference standards for most of the > 170 QAs described and limited validation between laboratories. Overcoming these gaps requires standardised workflows, collaborative ring trials and extended reference libraries. Such harmonisation is important not only to advance biosynthetic research, but also to enable the safe use of lupins in food. Reproducible and validated QA data is a prerequisite for consumer protection, risk assessment and market acceptance for both regulatory authorities and industry.

5. Toxicological profile and food safety assessment of quinolizidine alkaloids

QAs have long been recognised as antinutritional factors in lupins, with acute symptoms of poisoning having shaped both consumer perceptions and breeding priorities in the past (Alharbi et al., 2024; Ozkaya et al., 2021; Schrenk et al., 2019). Insufficiently debittered seeds of *Lupinus albus* or *Lupinus mutabilis* have been associated with nausea, vomiting, tachycardia and neurological disorders within hours,

occasionally leading to respiratory paralysis and, in rare cases, death (Akinboye et al., 2023; Lagrange et al., 2024). Experimental studies confirm that high doses of QAs have neurotoxic effects by impairing cholinergic neurotransmission, particularly by modulating nicotinic acetylcholine receptors (nAChRs) (Boschin et al., 2022; Rajput et al., 2022).

The toxicological profile of QAs is determined by absorption, distribution and metabolism. QAs are rapidly absorbed in the gastrointestinal tract, and lipophilic congeners can cross the blood-brain barrier, which explains their neurological manifestations (Bloothooft et al., 2025). Their metabolism is primarily mediated by cytochrome P450 enzymes, in particular CYP2D6, which leads to considerable inter-individual variability. Individuals with a poor metabolic phenotype exhibit slower clearance and higher susceptibility to toxic effects (Schrenk et al., 2019; Schreiber et al., 2025; Zaworska-Zakrzewska et al., 2021). Despite these mechanistic findings, there are still few systematic toxicokinetic data for humans, leaving critical uncertainties regarding chronic exposure at low levels, sensitive subgroups (e.g. children, pregnant women) and potential interactions with the gut microbiome or xenobiotics ingested with food.

The framework for risk assessment remains precautionary rather than evidence-based. Although the widely cited threshold of 200 mg QA kg^{-1} dry weight is frequently used in food risk assessment, it is neither derived from systematic dose-response studies nor legally binding in the EU. Instead, it has been adopted inconsistently, with Food Standards Australia New Zealand (FSANZ) enforcing it for lupin flour, while some national authorities such as Germany have applied limits in the past (Schrenk et al., 2019). The EFSA Panel on Contaminants in the Food Chain (Schrenk et al., 2019) explicitly concluded that neither an Acceptable Daily Intake (ADI) nor an Acute Reference Dose (ARfD) can be established due to insufficient data for humans and animals. Instead, EFSA applied the Margin of Exposure (MOE) approach, using sparteine as a surrogate, and identified a single lowest effective oral dose of 0.16 mg kg^{-1} body weight as the reference point for acute neurological and cardiovascular effects. While this approach is pragmatic, it assumes that sparteine is representative of all QAs, which is a problematic simplification given their structural diversity and potential differences in toxicodynamics.

This reliance on a single well-characterised congener reveals a deeper weakness, namely the almost complete lack of congener-specific toxicological data. The pharmacological and toxicological effects of sparteine are well documented, while other abundant QAs such as lupanine and multiflorine have been poorly studied despite clear structural differences that may affect bioavailability, receptor affinity and metabolic pathways. The extrapolation of toxicity estimates from sparteine to the entire QA class harbours the risk of over- or underestimating the hazard potential. Furthermore, EFSA acknowledged that the data situation does not allow for a meaningful assessment of chronic exposure, cumulative risk and vulnerability of vulnerable groups.

All in all, the current framework for risk assessment of QAs reflects regulatory pragmatism rather than mechanistic toxicology. The reliance on a standardised, non-binding threshold of 200 mg kg^{-1} masks significant uncertainties and fails to address the chemical heterogeneity of the QA family. In the absence of homologue-specific toxicokinetic and toxicodynamic data, particularly for chronic dietary exposure, the safety margins on which regulation is based remain fragile. This regulatory ambiguity not only undermines consumer protection, but also restricts the breeding, processing and international trade of lupin-based foods. To address these weaknesses, robust human toxicokinetic studies, systematic dose-response studies and the development of certified reference materials to support biomonitoring and exposure assessment are required. It is crucial that future regulatory frameworks move beyond the paradigm of elimination and adopt a dose-dependent dual role, balancing toxicological risks with the possibility of functional or nutraceutical benefits (Witt et al., 2025). Only through such an integrated risk-benefit assessment can lupin be positioned both safely and competitively in the protein and functional food market.

6. Detoxification strategies and processing challenges for quinolizidine alkaloids

Ensuring the safe consumption of lupins depends on the effective management of QAs, which remain the main challenge to their large-scale introduction. Detoxification is therefore both a prerequisite and a paradox: while essential for food safety, near-complete removal risks the loss of that may have functional or pharmacological value (Schryvers et al., 2023). Table 2 summarises the current detoxification strategies for QAs in lupins, highlighting their mechanisms, efficacy, nutritional impact and scalability.

Traditional household methods, such as soaking, boiling and germination usually achieve 70–90% reduction through leaching and partial thermal degradation (Ayalew et al., 2025; Baltacıoğlu & Tarım, 2024). Despite their effectiveness, traditional methods are still inefficient. They require an excessive amount of labour and water, cause nutrient losses and provide very different results depending on the variety, which affects reproducibility and severely limits their transfer to industrial practise (Estivi et al., 2022; Villacrés, Álvarez, & Rosell, 2020; Villacrés, Quelal, et al., 2020). Recent advances have shifted to biological and technical strategies, each with their own limitations. Fermentation has been explored as a bioprocessing route to mitigate bitterness/toxicity associated with quinolizidine alkaloid; however, evidence remains limited and highly process-dependent. For example, fungal fermentation of lupin substrates with Rhizopus oligosporus produced pH-dependent QA degradation, reaching ~63% reduction within 48 h under optimal conditions, whereas broader generalisation across lupin species, QA spectra, and fermentation consortia remains premature (Ortega-David & Rodríguez-Stouvenel, 2013). In contrast, the nutritional benefits of fermentation (e.g., improved protein digestibility and generation of bioactive peptides) are well documented for plant matrices (Kårlund et al., 2020), but these effects should not be interpreted as evidence for QA removal unless alkaloids are directly quantified in the same workflow.Membrane separation and resin adsorption represent promising selective detoxification strategies, capable of achieving > 90–95 % quinolizidine alkaloid removal with minimal protein loss and substantially lower water requirements than traditional aqueous debittering. Nevertheless, their practical deployment remains limited to pilot-scale applications, as high capital and operational costs, fouling-related performance decline, sorbent regeneration constraints and uncertain regulatory and consumer acceptance continue to restrict large-scale adoption. Green technologies such as supercritical CO_2 extraction achieve 80–98% removal and enable selective recovery of alkaloid fractions (Yu et al., 2025; Domínguez-Valencia et al., 2024; Rosas-Quina & Mejía-Nova, 2021). However, this approach is limited by expensive infrastructure, high energy requirements and the simultaneous extraction of lipids, which raises both economic and nutritional concerns. Similarly, separation of the seed coat with subsequent solvent extraction preserves the protein-rich kernels and valorises the phenolic-rich fractions (Mazumder et al., 2024), but is currently limited to laboratory scale, solvent-intensive and variety-dependent in its efficiency. Ultrasound-assisted hydration accelerates leaching and reduces water consumption (Baltacıoğlu & Tarım, 2024; Yaver & Bilgiçli, 2021), but scalability and uniformity across different seed lots remains untested, so industrial feasibility is uncertain (Cortés-Avendaño et al., 2020).

These approaches illustrate the paradox of QA processing: safety requirements demand almost complete removal of alkaloids, but methodological limitations restrict industrial application and functional opportunities are lost. Future research must therefore move beyond a narrow zero-residue strategy to controlled modulation in which detoxification strategies are coupled with genomic selection for low-alkaloid genotypes, metabolomic monitoring of residual alkaloid profiles and systematic assessment of functional effects at sub-toxic concentrations. An integrated systems-level strategy combining advanced breeding,

Table 2
Processing and detoxification approaches to reduce quinolizidine alkaloids (QAs) in lupins.

Method/ approach	Mechanism	QA reduction (%)	Effects on proteins/ nutrition	Functional/analytical outcomes	Scalability/ application	References
Soaking + boiling	Leaching of alkaloids into water; partial thermal degradation	70–90	Minimal loss of proteins; some leaching of soluble nutrients	Improves palatability and reduces bitterness	Household and small scale; traditional processing	Schryvers et al., 2023
Fermentation (LAB, yeast)	Microbial metabolism and enzymatic hydrolysis of alkaloids	60 – 95 (strain-dependent)	Can improve digestibility; can increase bioactive peptides	Fermented flours show improved antioxidant and sensory properties	Scalable; use in traditional and industrial food fermentation	Santana et al.,2002; Ortega and Rodríguez, 2013 Villacrés & Rosell, 2021
Membrane filtration	Physical separation of alkaloids from protein fractions	> 95	High preservation of protein quality and essential amino acids	Enables selective recovery of alkaloid-rich fractions	Pilot to industrial potential	Carmali et al., 2010
Supercritical CO_2 extraction	Solubilisation of alkaloids with CO_2 + ethanol as co-solvent under pressure	80 – 98	Proteins are largely preserved; possible co-extraction of lipids	Potential for selective alkaloid extraction; shorter processing time	Expensive; currently at pilot scale	Domínguez-Valencia et al., 2024; Rosas-Quina et al., 2021
Resin adsorption	Selective binding of alkaloids to functionalized resins	> 90	Low water consumption; minimal impact on proteins	Promising detoxification method with low water consumption	Innovative; pilot scale only	Madelou et al., 2024
Seed coat separation + solvent extraction	Physical dehulling; extraction of alkaloids and phenolics from the seed coat (e.g. with methanol)	40 – 90 (variety-dependent)	Protein-rich kernel is retained; valorisation of polyphenol-rich fractions	Extracts provide metabolomic fingerprints (LC-QTOF MS) and functional assays (antioxidant, emulsifying, foaming)	Laboratory scale; utilisation of by-products	Mazumder et al., 2024

innovative processing technologies and chemomics analyses is required to reconcile the dual objectives of food safety and functional valorisation of lupins.

7. Impact of quinolizidine alkaloids on food quality and sensory properties

The sensory profile of lupin-based foods is primarily determined by their QA content, with bitterness serving as both an evolutionary defence mechanism and a major barrier to consumer acceptance (Chen & Lin, 2019; Frick et al., 2017). Importantly, consumer rejection often occurs at QA concentrations well below established toxic thresholds, emphasising that compliance with food safety standards is no guarantee of marketability (Mieczkowska & Smulikowska, 2005; Tan et al., 2024). This leads to a critical trade-off: while traces of QAs are sufficient to trigger perceptible bitterness, intensive debittering strategies run the risk of compromising the nutritional quality and techno-functional properties of lupin proteins (Villacrés, Quelal, et al., 2020).

Estivi et al. (2022) used electronic tongue analysis to demonstrate that the perception of bitterness is determined by the composition of the residual homologues and not by the mass concentrations. At the molecular level, bitterness perception is triggered by QA interactions with human bitter taste receptors (Li et al., 2023; Tuzim & Korolczuk, 2021). Although the dynamics of receptor and ligand are not yet fully understood, evidence from structurally related alkaloids such as caffeine and quinine suggests broad activation of TAS2R receptors (Wooding et al., 2021). This suggests that the bitterness of lupin is due to a diffuse response of multiple receptors rather than a single binding event, complicating efforts to decouple sensory properties from alkaloid content. However, receptor interactions are only one part of the sensory mechanism (Karolkowski et al., 2023). Components of the food matrix such as proteins, polysaccharides and lipids can bind or mask QAs and thus attenuate the perception of bitterness (Delompré et al., 2025; Huang & Xu, 2021; Wang et al., 2024). Consequently, flours enriched with free QA fractions generally elicit a stronger bitterness than protein isolates or emulsified systems where dispersion reduces bioaccessibility.

The complexity at the molecular and matrix level leads directly to technological challenges. While conventional aqueous debittering methods are effective in reducing QAs, they also remove proteins and micronutrients, while more selective approaches such as resin adsorption or microbial fermentation preserve nutritional quality but can unpredictably alter flavour profiles (Villacrés, Quelal, et al., 2020). The trade-off between detoxification and preservation of protein functionality (e.g. emulsification, foaming, digestibility) therefore remains unresolved. Progress is also hampered by the lack of systematic sensory research on QAs. In contrast to glucosinolates and polyphenols, which are well characterised, the bitterness thresholds, inter-individual variability and congener-specific contributions of QAs remain poorly defined. For example, lupanine, sparteine, and multiflorine likely differ in their bitterness intensity, but most studies only report on total alkaloid content (Eugelio et al., 2023).

To fill these knowledge gaps, analytical chemistry and sensory analysis need to be integrated. A combined approach linking LC-MS/MS profiling, TAS2R assays and controlled consumer panels would provide mechanistic insights into bitterness perception and define quantitative acceptability measures. Such data are crucial not only for guiding breeding strategies aimed at developing varieties with low alkaloid content, but also for optimising processing methods that ensure safety without compromising sensory quality. In the longer term, an important open question is whether low-dose QAs could be tolerated in certain product categories or even upgraded as functional flavour markers. Until such multidisciplinary evidence is available, bitterness remains a key bottleneck limiting the wider use of lupin-based foods.

While bitterness and sensory rejection remain major constraints, recent evidence suggests that QAs may also exert biological activities at sub-toxic doses, reframing them as potentially functional phytochemicals rather than merely sensory defects. This emerging perspective is explored in the following section.

8. Emerging bioactivities and potential health effects of quinolizidine alkaloids

Although QAs are predominantly studied in the context of toxicity and food safety, increasing evidence suggests they may also have bioactive effects with potential health impacts (Cely-Veloza et al., 2023; Khedr et al., 2024). *In vitro* and animal studies report anti-cancer effects, with selected alkaloids inducing apoptosis, modulating cell cycle progression and suppressing proliferative pathways (Chaudhry et al., 2022; Khan et al., 2022). Others show neuroprotective potential by inhibiting cholinesterase and improving cognitive performance, paralleling

alkaloid-based drugs currently used in neurodegenerative diseases (Utpal et al., 2025). In the metabolic field, QAs have been associated with antidiabetic and cardioprotective functions, including modulation of glycaemic control, lipid metabolism and vascular tone (Guerra-Ávila et al., 2023; Mazumder et al., 2024). More recently, cross-species evidence has revealed interactions with host microbiome dynamics. Alkaloids from *Ramulus mori* have been shown to alter gut microbiome composition and metabolic performance in obese mice (Liu et al., 2023), raising the possibility that lupin-derived QAs could affect microbial ecology similarly. However, to date no comparable studies exist for *Lupinus* spp., making this an intriguing yet speculative hypothesis that highlights a major blind spot.

An emerging consensus in the literature supports a hormetic paradigm, indicating that quinolizidine alkaloids may confer protective or regulatory biological effects at low, sub-toxic concentrations, whereas elevated exposure shifts the response toward toxicity and functional impairment. (Zhang et al., 2023). This dose-dependent duality illustrates the dual nutritional role of lupin, where metabolites long considered undesirable due to their toxicological and sensory properties can simultaneously have functional or pharmacological potential if their concentrations are adequately controlled (Dubois et al., 2019). However, current knowledge remains fragmented and largely limited to preclinical studies, restricting the translation of these observations into evidence-based nutritional or therapeutic recommendations. This gap underlines the need for systematic and integrative research that combines toxicological, nutritional and pharmacological perspectives. Most studies are limited to cell cultures or rodent models, while clinical data from humans are virtually non-existent. Furthermore, the specific contribution of individual alkaloids (e.g., lupanine, sparteine, multiflorine) is rarely dissected, so it remains unclear whether the reported effects are due to single molecules, alkaloid mixtures or interfering metabolites. Without standardised extraction methods, validated quantification protocols and rigorous dose-response studies, claims about the bioactivity of QAs remain tentative. Further progress will depend on interdisciplinary research that combines analytical precision with mechanistic pharmacology and controlled human studies. Only under these conditions can QAs be credibly transformed from antinutrients to functional bioactives. Until then, they must be viewed primarily as a food safety issue, with their putative health effects representing a provocative but unproven hypothesis for future research.

As research continues to clarify the risk-benefit balance of QAs, opportunities are emerging for their controlled use in high-value applications. Leveraging these compounds will require innovative processing, formulation and biorefinery approaches, which are discussed in the next section.

9. Innovative applications and valorisation strategies

Innovative applications of QAs are becoming a frontier area in lupin research, with the focus shifting from simple detoxification to strategies that combine safety and valorisation (Fig. 2). While conventional breeding and processing has focused on reducing QAs to meet food safety standards, recent advances show the potential of these metabolites as value-adding compounds. Controlled extraction and selective removal technologies are now opening avenues for their recovery as bioactive ingredients, with applications ranging from dietary supplements and pharmaceuticals to biocontrol agents and functional food formulations. Positioning QAs within the circular bioeconomy broadens this perspective, where alkaloid fractions are no longer treated as waste but as by-products that can contribute to the economic and environmental sustainability of lupin-based value chains (Maia et al., 2023).

One promising avenue is the application of nanotechnological delivery systems, including liposomes, nanoemulsions and biopolymer-based carriers, to encapsulate purified QAs (Mohammadian et al., 2020). These systems can mask bitterness, protect alkaloid stability and enable targeted release in the gastrointestinal tract, thereby increasing bioefficacy and minimising systemic toxicity. Direct studies on the encapsulation of QA are still limited, but analogous work with other plant alkaloids and polyphenols such as caffeine and tea catechins demonstrate the feasibility of controlled release platforms in the development of functional foods (Oskan et al., 2024). Beyond encapsulation, QAs show potential in nutraceuticals and functional foods. For example, lupanine has been reported to enhance glucose-stimulated insulin release via inhibition of pancreatic K_ATP channels, suggesting a role in the regulation of blood glucose levels. Animal studies have also shown synergistic effects when lupanine is combined with lupin-γ-conglutin, resulting in improved glucose homeostasis and lipid profiles (Wiedemann et al., 2015). These results emphasise the possibility of developing QA-enriched formulations for metabolic health, provided the dosage is carefully defined and clinically validated. Encapsulation in food matrices could facilitate controlled exposure and convert the bitter alkaloids into health-promoting properties rather than raising safety concerns.

With the concept of lupin biorefineries aiming to utilise the two main

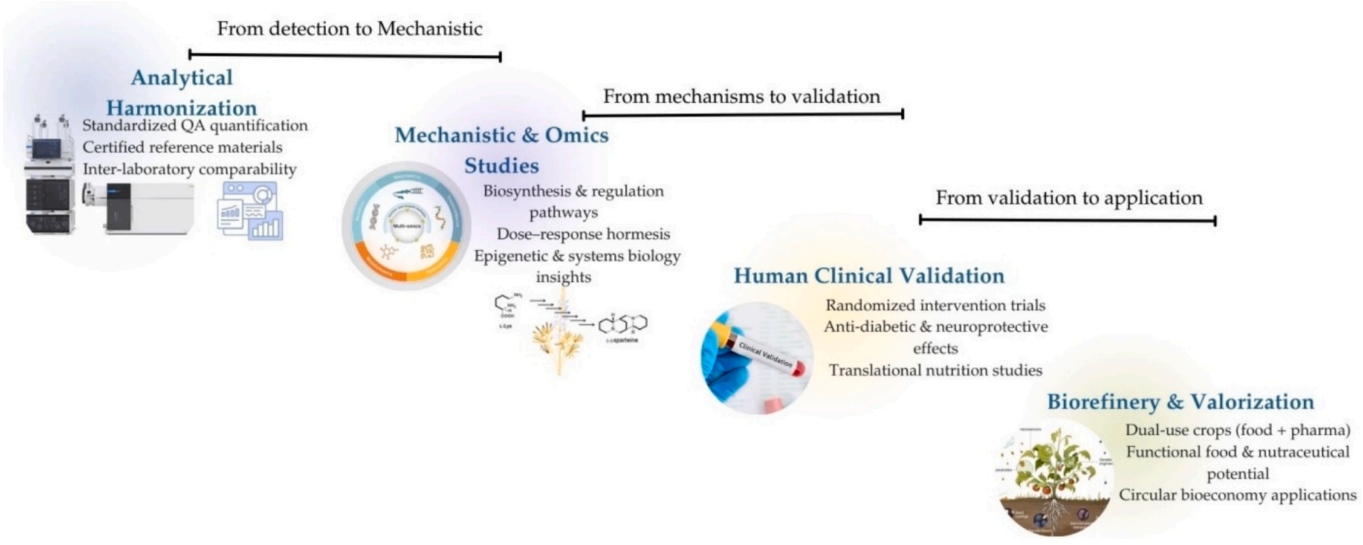

Fig. 2. Important lines of research for the valorisation of quinolizidine alkaloids (QAs) in lupins, which include advances in metabolic engineering, food safety, pharmacological applications, and sustainable crop improvement.

fractions of the seeds, a broader industrial vision emerges. In conventional detoxification, the bitter lupins are discarded to obtain protein isolates with low QA, neglecting the potential value of the alkaloid-rich fraction. A biorefinery approach would instead aim at a dual utilisation, namely the extraction of high-quality protein for food and of purified QAs for pharmaceutical, cosmetic or biopesticidal applications. The historical use of sparteine as an antiarrhythmic agent and the emerging antidiabetic properties of lupanine are examples of the possibility of reusing QAs as pharmacological scaffolds (Frick et al., 2025; Villalpando-Vargas & Medina-Ceja, 2016). Such co-valorisation would improve the economic sustainability of lupin cultivation and promote innovation in high-value applications.

These perspectives are consistent with circular bioeconomy frameworks that reposition lupin as a multipurpose and resource-efficient crop. Bitter genotypes, historically underutilised in human nutrition, offer opportunities for dual-value exploitation through the recovery of safe protein fractions for food applications together with alkaloid- and phenolic-enriched side streams suitable for functional ingredients, antioxidant systems and specialty biochemical products. Emerging evidence further indicates that processing strategies, particularly protein extraction conditions, critically regulate the co-recovery of structural proteins and associated bioactive metabolites from lupin matrices, thereby shaping pathways for waste valorisation and subsequent nutraceutical or pharmaceutical utilisation in line with sustainability and climate-driven resource efficiency. (Halime et al., 2025; Jančaitienė et al., 2025; Taberner-Pibernat, Bou, et al., 2025).

Despite these opportunities, significant challenges remain before QAs can be repositioned as strategic assets. First and foremost is the lack of harmonised toxicological thresholds, which makes acceptance by authorities difficult and complicates formulation strategies. The analytical and process pipelines for encapsulation and purification need to be further optimised to ensure reproducibility and scalability. Perhaps most importantly, human clinical trials are needed to confirm the bioactivity and safety of QAs in functional contexts, bridging the gap between promising preclinical evidence and translational nutrition. In the absence of such evidence, the regulatory framework will continue to favour detoxification over enhancement. Nonetheless, the paradigm shift from elimination to management and valorisation of lupin provides a powerful new framework for doing so. By integrating nanotechnology, nutraceutical innovation, biorefinery design and circular economy principles, QAs can be transformed from barriers to utilisation to drivers of food and health innovation, improving both the sustainability and economic value of this underutilised grain legume.

10. Toward an integrated framework for quinolizidine alkaloid research

Despite substantial progress, QA research remains constrained by methodological heterogeneity, insufficient mechanistic depth, and the absence of a unified framework linking biosynthesis, toxicology, sensory properties and valorisation potential (Fig. 3). Future advances will require a shift from descriptive inventories to predictive and integrative models.

10.1. Genetic and biosynthetic elucidation

Large gaps persist in the genetic and biochemical architecture of QA biosynthesis. Several core enzymes remain unidentified, regulatory nodes are only partially characterised and the functional roles of most candidate genes have not been validated. Although loci such as *iucundus* (RAP2-7) and pauper provide valuable entry points for molecular breeding, their downstream networks and metabolic consequences remain largely unresolved. Breeding strategies must therefore pursue dual objectives: (i) reducing QA levels and (ii) ensuring the stability of "sweetness" under abiotic and biotic stresses. Achieving this will require the integration of QTL mapping, genomic selection, multi-omics

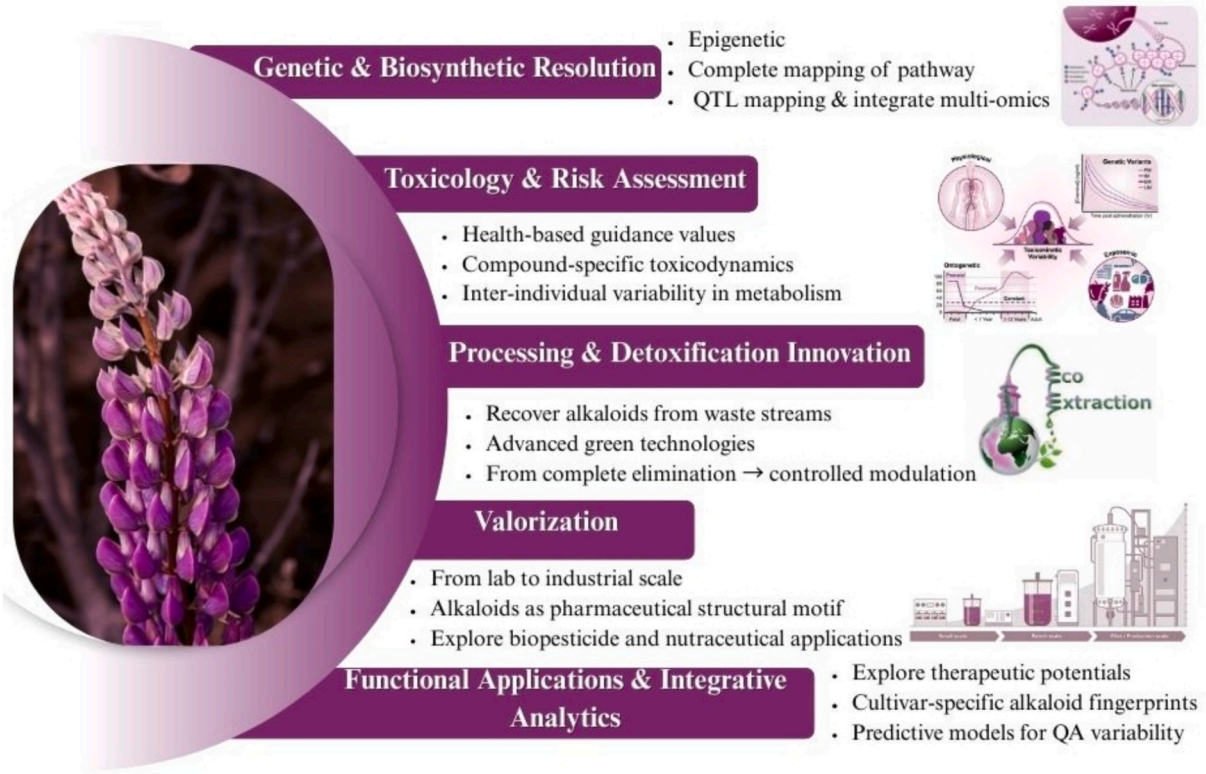

Fig. 3. Future perspectives for the research of quinolizidine alkaloids (QAs) in lupins with a focus on the integration of omics, breeding, biorefinery and safety for a balanced valorisation.

analyses and reverse genetics, combined with systematic mapping of metabolite fluxes and their environmental modulation. To translate these insights into breeding pipelines, rapid and cost-effective screening tools (e.g., NIRS, portable colourimetric assays, and miniaturised sensors) must be developed to enable high-throughput screening of large germplasm collections prior to confirmatory chromatographic analyses.

10.2. Toxicology and risk assessment

Toxicological evaluation remains hindered by the absence of harmonised health-based guidance values, including Acceptable Daily Intakes or Acute Reference Doses. Current thresholds are precautionary, based on total QA content rather than congener-specific toxicodynamics or inter-individual variability in metabolism. Robust human toxicokinetic data, particularly on chronic low-dose exposure, population variability and transfer into animal-derived foods (e.g., milk), are urgently required. Standardised regulatory limits across food and feed systems should become a priority, analogous to frameworks established for potato glycoalkaloids. Congener-specific toxicokinetic and toxicodynamic studies will be essential to refine risk assessment and reduce the current reliance on extrapolation from sparteine.

10.3. Processing innovation and controlled modulation

Innovation in processing has been limited by the long-standing assumption that QAs must be removed almost completely. Moving from total elimination to controlled modulation will require coupling advanced processing technologies (e.g., fermentation, membrane separation, molecularly imprinted polymers, SFE, ultrasound-assisted leaching) with breeding strategies targeting reduced, rather than absent, QA levels. The optimisation and scale-up of green debittering methods remain a key research frontier. In parallel, lupin biorefineries must progress from proof-of-concept to industrial applicability. Recovering and purifying QAs from process streams (e.g., wastewater, seed coats) could provide renewable sources of pharmacological scaffolds, biopesticides or nutraceutical leads.

10.4. Exploring functional and therapeutic potential

QAs hold untapped potential as functional bioactives, with emerging evidence for antimicrobial, anthelmintic, neuroactive and metabolic effects. Realising this dual-use potential requires rigorous interdisciplinary research, including standardised extraction and quantification, congener-specific dose-response studies, mechanistic pharmacology and controlled human clinical trials. Only under these conditions can QAs be reliably repositioned within a food–pharma continuum.

10.5. Data integration and predictive modelling

Chemomics, an emerging integrative approach that combines comprehensive chemical fingerprinting with multivariate and machine-learning analytics, provides a framework for linking complex metabolite patterns to genetic, environmental and functional traits. Within this framework, tools such as hierarchical clustering, Partial Least Squares Discriminant Analysis (PLS-DA) and advanced metabolomics pipelines enable the delineation of varietal alkaloid fingerprints, product authentication and the detection of ecological or geographical signatures. Yet, while these chemometric strategies refine descriptive inventories, they cannot by themselves resolve the regulatory logic of QA biosynthesis. Meaningful progress will require the integration of metabolomics with isotope labelling, fluxomics, functional genomics and spatial metabolite imaging to build predictive models capable of explaining and forecasting QA variability across environments and production systems. However, descriptive profiles alone cannot resolve pathway regulation. Integrating metabolomics with isotope labelling, fluxomics, functional genomics and spatial metabolite imaging will be essential for developing predictive models of QA variability across environments and production systems. Such models will be critical for strengthening food safety, improving quality assurance and enabling the rational valorisation of lupin-derived ingredients throughout the value chain.

11. Conclusions

Quinolizidine alkaloids in lupins exemplify the dual nature of plant bioactives: compounds that present toxicological risks at high doses yet demonstrate promising biological activities at controlled exposures. Historically regarded primarily as food toxins, QAs have driven breeding and processing strategies aimed at their complete removal. However, growing evidence indicates that certain QAs possess potentially beneficial properties, from glycaemic regulation to neuroprotection, positioning them as candidates for functional foods and nutraceutical applications. This dual identity highlights the need to reassess QAs not only as constraints but also as emerging opportunities for innovation.

The past five years have seen major advances in QA chemistry, analytical methods and toxicology. Developments in LC-MS/MS, qNMR and metabolomics now allow more precise quantification and profiling, while biotechnological and processing innovations enhance safety management. However, fundamental gaps remain: the lack of harmonised nomenclature, certified reference standards, and toxicological reference doses continues to undermine comparability, regulation and consumer confidence. The sensory dimension, particularly the bitterness that leads to consumer rejection, also remains poorly understood and under-researched. The greatest challenge for the future is to transform QAs from an obstacle into a benefit. This requires (i) the creation of internationally harmonised analytical and regulatory frameworks, (ii) mechanistic studies to clarify dose-response relationships and inter-individual variability, (iii) the integration of sensory science with biochemical profiling, and (iv) translational research, including human clinical trials to validate functional potential. Equally important is the development of lupin biorefineries and circular economy strategies that utilise proteins and alkaloids together rather than treating them as mutually exclusive fractions.

By bridging food safety and functional innovation, QAs can be repositioned within a food-pharma continuum, supporting the global shift towards sustainable protein sources and health-promoting diets. The future of QAs will therefore not be determined by their toxicity alone, but by our ability to measure, modulate and strategically utilise their complex bioactivity. In this sense, lupins should be viewed not as crops limited by bitter toxins, but as multifunctional resources at the intersection of nutrition, health and sustainability.

CRediT authorship contribution statement

Hajer Ben Ammar: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Barbara Pipan:** Writing – original draft, Supervision, Resources, Conceptualization. **Lovro Sinkovič:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have influenced the work in this article.

Data availability

No data was used for the research described in the article.

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