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CENTER OF EXCELLENCE FOR FOOD
CHAIN QUALITY, SAFETY & RISK ASSESSMENT

Quinolizidine alkaloids in food

Bram Miserez

Agenda

- LUPINEX and lupins
- Quinolizidine alkaloids
- Analysis of QA's
- Food
- Feed and transfer to meat products
- Risk assessment
- Processing: influence on QA presence
- Conclusions

LUPINEX

- "Exposure assessment to quinolizidine alkaloids and phomopsines by intake of lupin-containing food"
- Project funded by the FPS Public Health, Belgium
- Partners:
  - Ghent University: Prof Liesbeth Jacxsens, ir Sofie Schrijvers, Prof Mia Eeckhout, Chinaza Arinzechukwu, Sigrid Vonck
  - Primoris/Ciboris: Bram Miserez, Jet Van De Steene

Primoris/Ciboris

- Spin off from Ghent University since 2001

- Primoris laboratories (+/- 150 FTE)
  - Food and feed analysis
  - Belgium, Bulgaria, Costa Rica, Columbia
  - Commercial presence in more countries

- CKCert (+/- 30 FTE)
  - Certification of primary production in Belgium

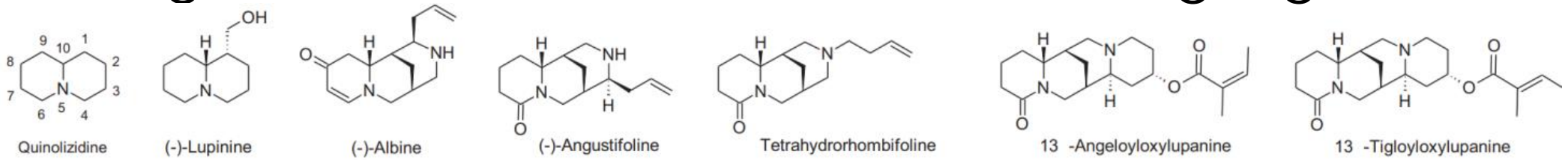
- Ciboris (3 FTE)
  - Centre of Excellence: research

Lupins?

- Protein-rich legume
  - meat-free, gluten-free, low CO_2 emission, etc
- Grown primarily in Australia
  - In EU: France, Germany, Netherlands, Poland, etc
- *Lupinus albus* (white lupin), *Lupinus angustifolius* (blue or narrow-leafed lupin), *Lupinus luteus* (yellow lupin) and *Lupinus mutabilis* (Andean lupin)
  - For agriculture
  - Over 400 species

Quinolizidine alkaloids (QA's)

- Secondary metabolites in lupins
- In wild lupins: total QA's over 10 000 mg/kg
  - In sweet lupins: TQA below 500 mg/kg
- Toxicity: mainly binding acetylcholinesterase receptors
  - Respiratory depression (and death)
  - Gastro-intestinal, cardiovascular, nervous system
- Legislation: Australia: feed limit of 200 mg/ kg TQA



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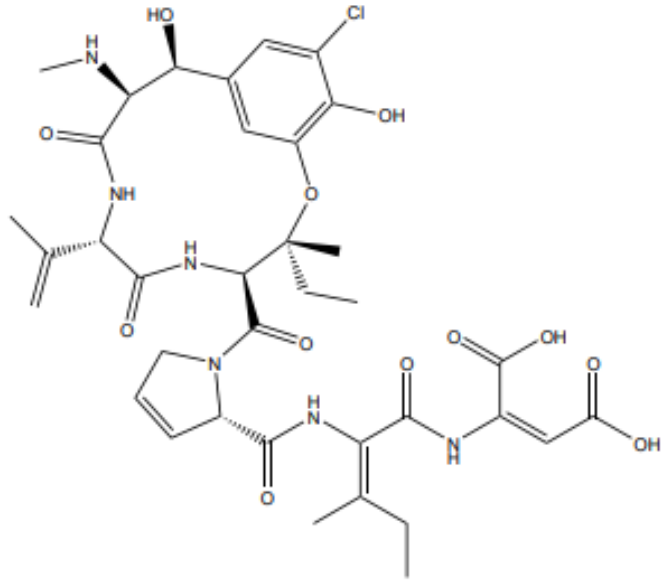
Phomopsins

- Mycotoxin family produced by *Diaporthe toxica*
- Macrocyclic hexapeptides
- Toxicity: liver, kidneys

- Analyzed in this project by LC-MS/MS
  - Only phomopsin A
  - Other phomopsins less important
- Low concentrations in feed
- Not detected in food
  - LOD: 1-5 ppb



Phomopsin A

Analysis of QAs: Targeted LC-MS/MS

- Limited number of analytes: lupinine, lupanine, sparteine, 13-hydroxylupanine, angustifoline, multiflorane, albine
- Extraction: 5g sample, 20 mL MeOH/Water 70/30, shake 15 min, centrifuge, filter
  - For high fat content: dSPE with C18 added
  - Dilutions needed for high QA content
- LC-MS/MS analysis
  - C18 RPLC with water/MeOH wth 0,1% formic acid
  - 10 min gradient, ESI +
- Validation in soy flour, dry biscuits, hummus, muscle, liver, compound feed (all lupin free)
  - LOQ: 10 ppb (except multiflorine: 50 ppb)

Analysis of QAs: HRMS

- Untargeted analysis
  - For any QAs not in the targeted list
- LC: as for targeted analysis
- HRMS: Q Exactive Orbitrap system
  - ESI +, MS: 70 000 resolution, MS2: 17 500 resolution
- Screened results for compounds known form literature
  - MS present → in silico MS2 prediction and similarity

- **Results: extra QA detected, but measured QA's are +/- 90% of TQA in tested samples**

Food analysis: market study

- Market study: 234 items identified on the Belgium market

Product categories	Number of product items found in this category	Share (%) of gluten free products
Dry lupin seeds	2	Not relevant
Lupin flour (meal)	5	100%
Jarred (or pickled) lupin	8	Not relevant
Bread and bakery products*	77	92%
Bread mixes	33	81%
Pasta and pasta-like products	0	Not relevant
Dough-based sweets, such as biscuits and cakes	68	87%
Plant-based meat alternatives	10	Not relevant
Dairy alternatives	15	Not relevant
Dips and spreads	6	Not relevant
Protein concentrates- and isolates	3	100%
Coffee imitates (coffee surrogates)	1	Not relevant
Miscellaneous	6	Not relevant

Food analysis: bread

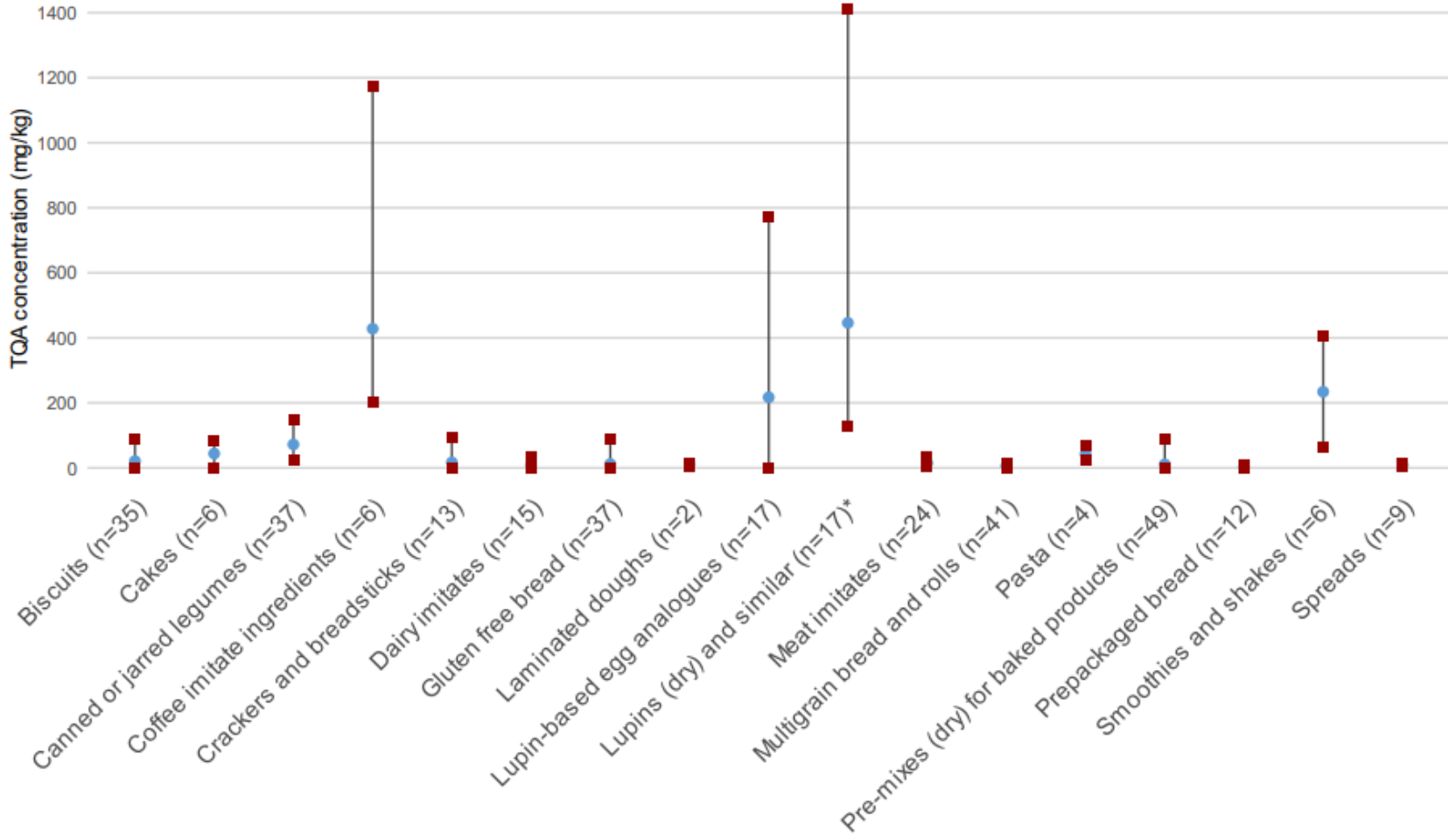
- Use of lupin (flour) in bread: limited use

Results market study: the share of contacted companies that use lupin in their products	
Mills	1/8
Manufacturers of raw materials	3/10
Industrial bakeries	2/10
Small-scale bakeries in the area of Ghent	1/9

	Proportion of lupin in bread
Spelt bread (lupin grits)	4 – 6%
Multigrain bread (lupin grits)	< 5%
Multigrain bread (lupin grits)	5 – 8%
Multigrain bread (lupin grits)	1.5 – 4.5%
Multigrain bread (lupin flour)	0.4%

Food analysis: results

- 335 samples analyzed
  - Some products re-sampled to cover variation



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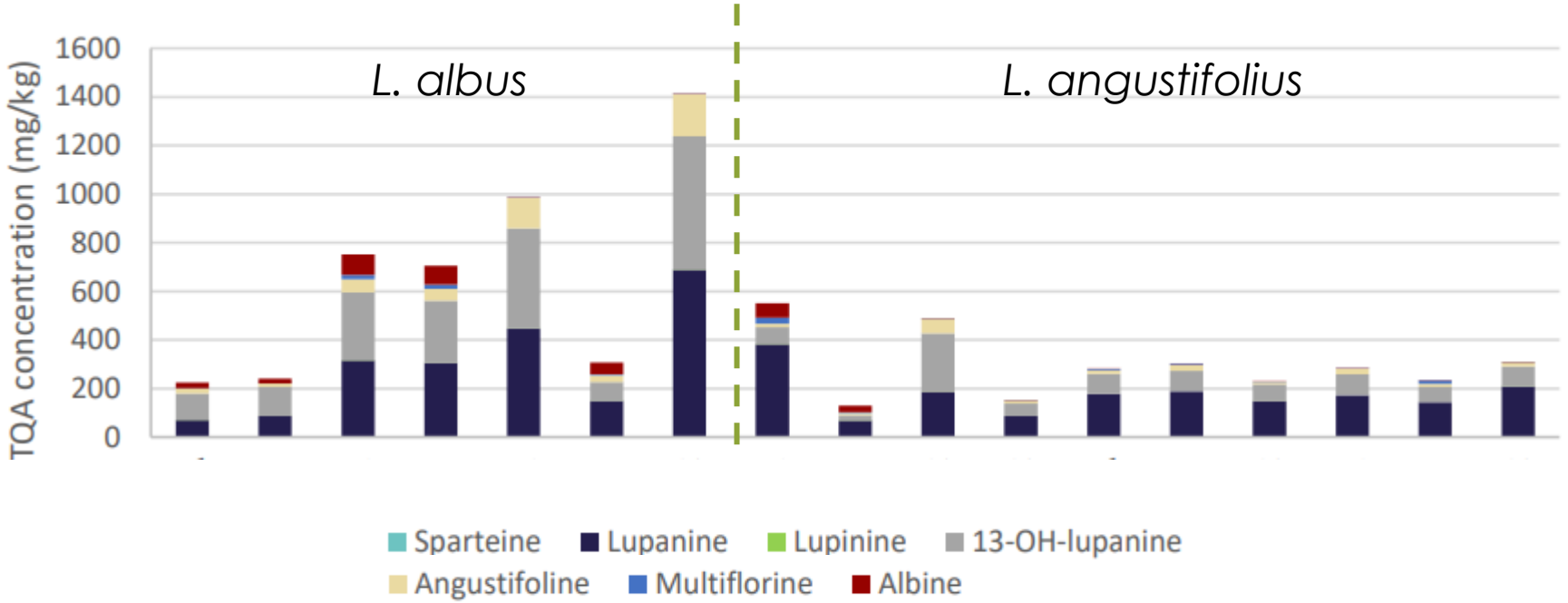
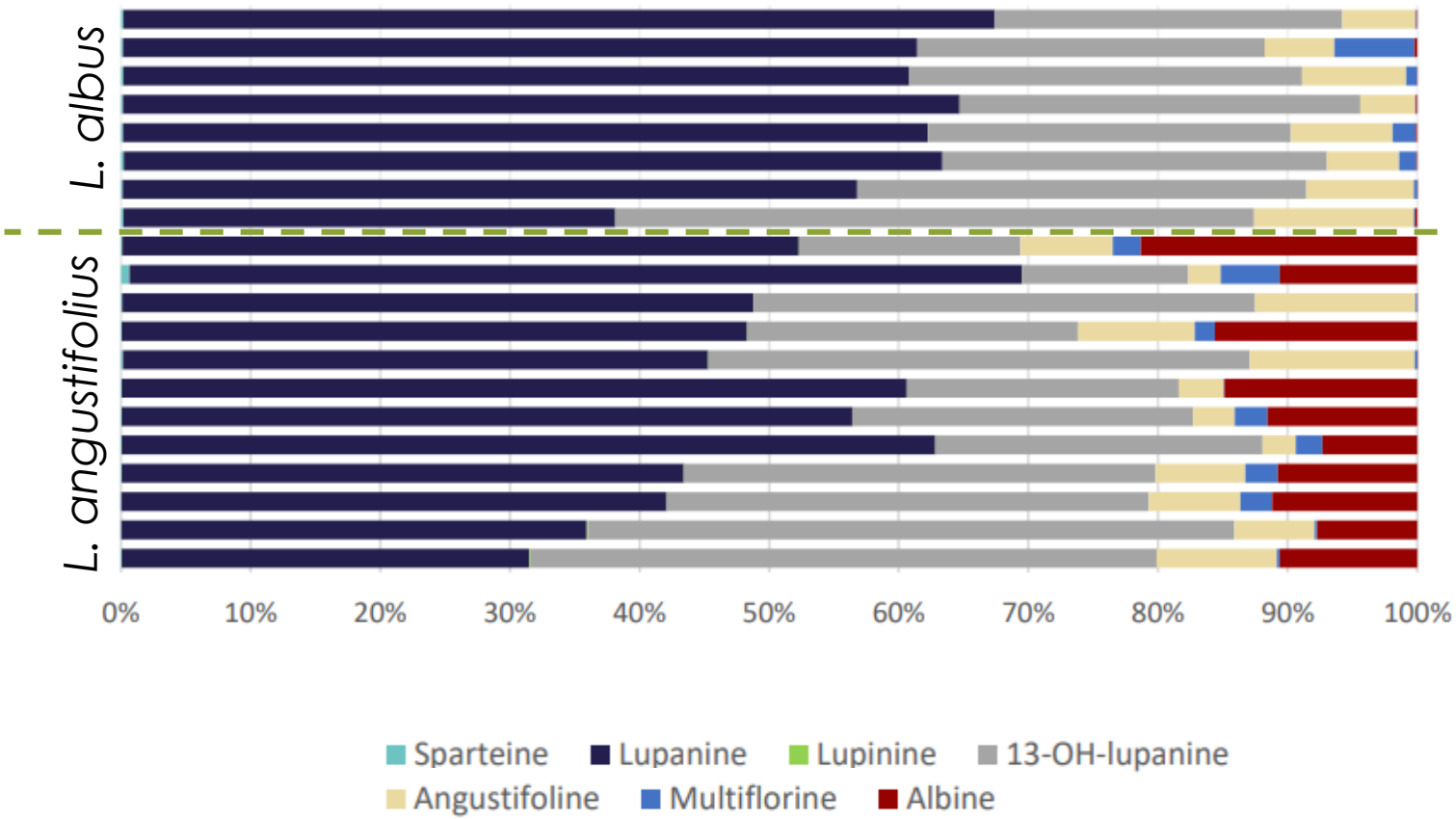
Food analysis: results (species)

- "Dry lupins" results



3 samples were left out: Egyptian lupins with TQA >20 000 mg/kg sold as sweet lupins

Food analysis: results (species)



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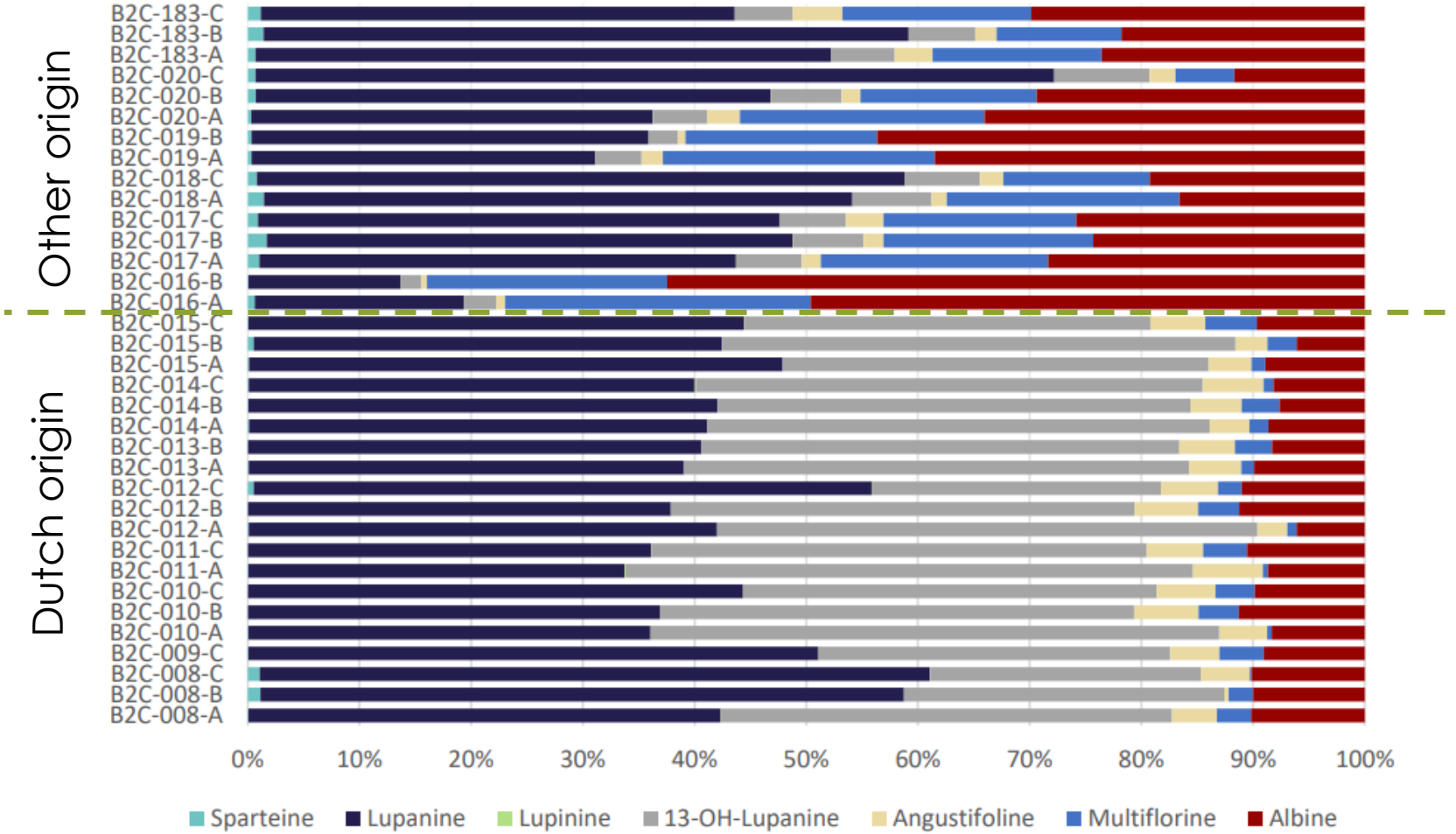
Food analysis: results (origin)

- Canned and jarred lupins



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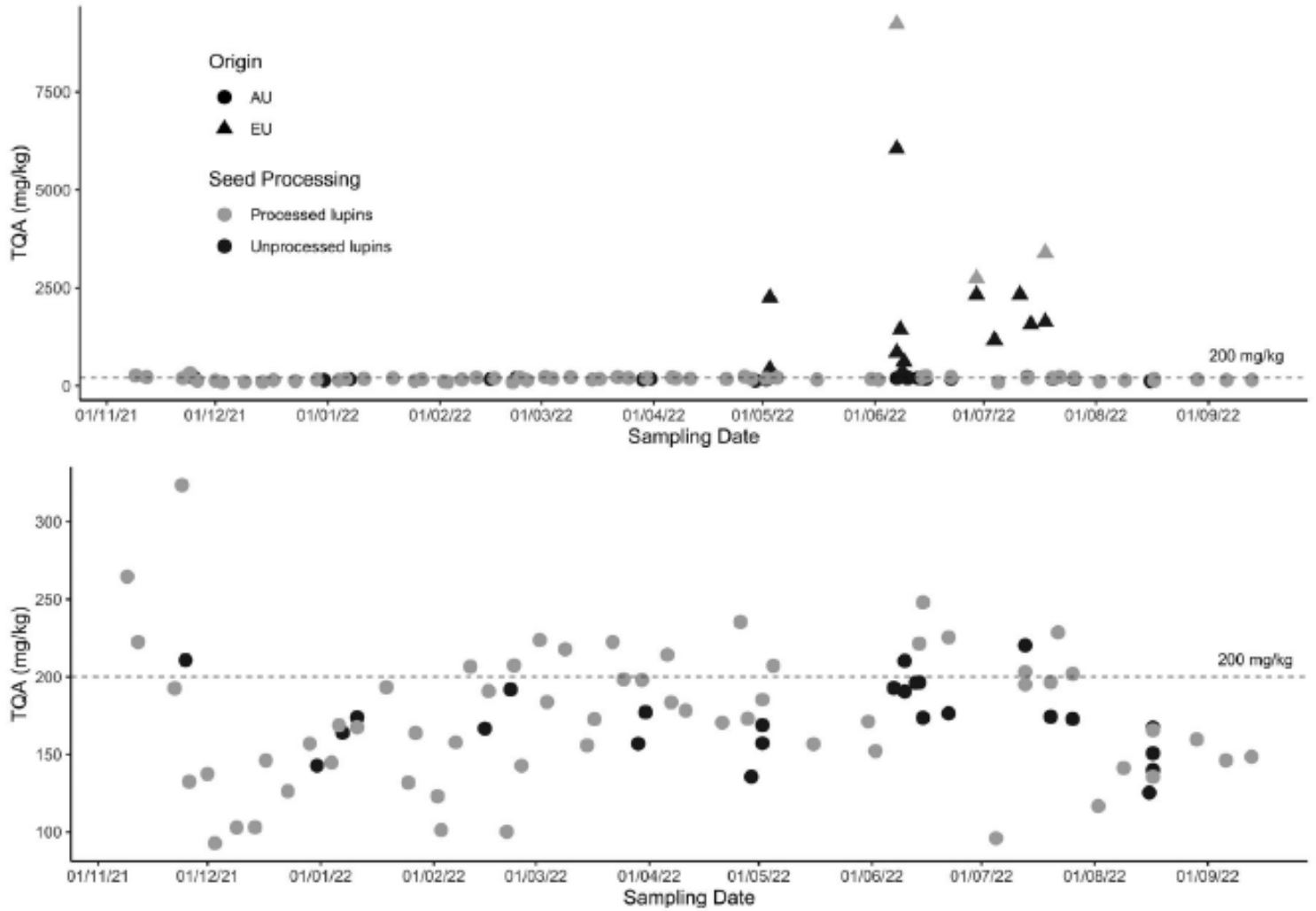
Feed and transfer to meat

- Belgian market
  - Lupins only in feed for calves
  - Calf sector: big integrators
    - Single company produces mixed feed, raises calves, slaughters, processes calves
    - Mixed feed includes processed lupins: roasted, flaked, etc
- 104 feed samples were taken
- 40 integrated samples were taken

	Composite feed	Liver	Muscle meat
Sample set description	5 stables x 4 months =20 x 1 kg	5 stables x 2 animals =10 x 1 kg	5 stables x 2 animals =10 x 1 kg
Nr. of samples	20 samples	10 samples	10 samples

Feed samples: results (EU vs AUS)



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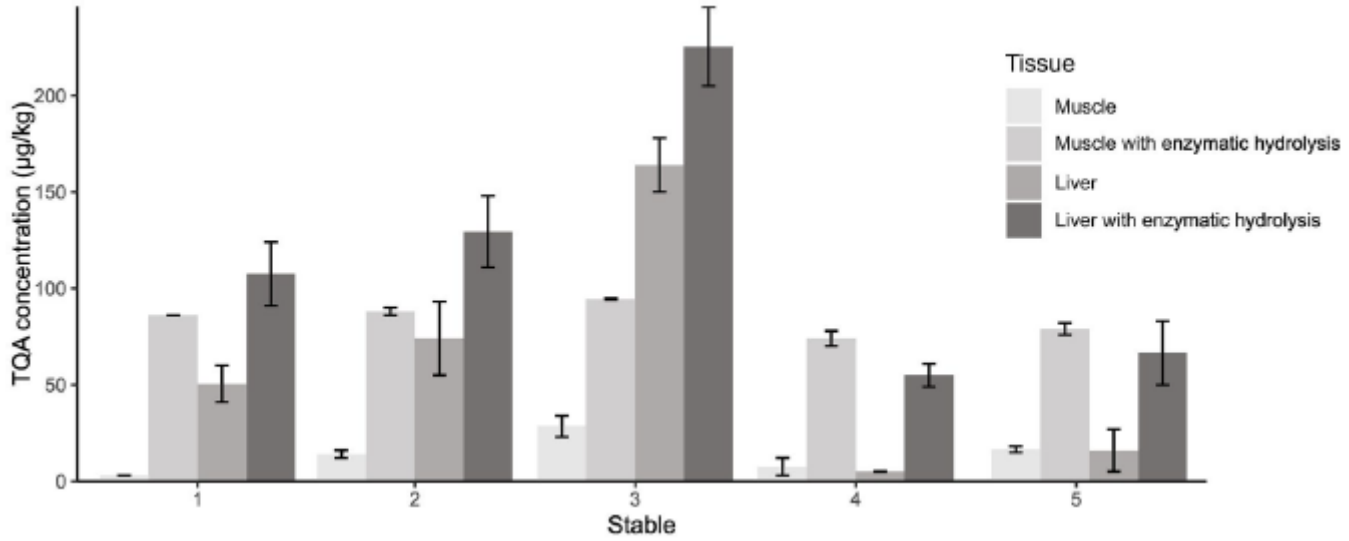
Feed: transfer to meat and liver

- Samples of muscle and liver analyzed
  - Without enzymtic hydrolysis
  - With enzymatic hydrolysis (de-GLU and de-SUL): higher results
- Weak link between TQA in feed and in animal
  - But in general +/- 1000 times lower



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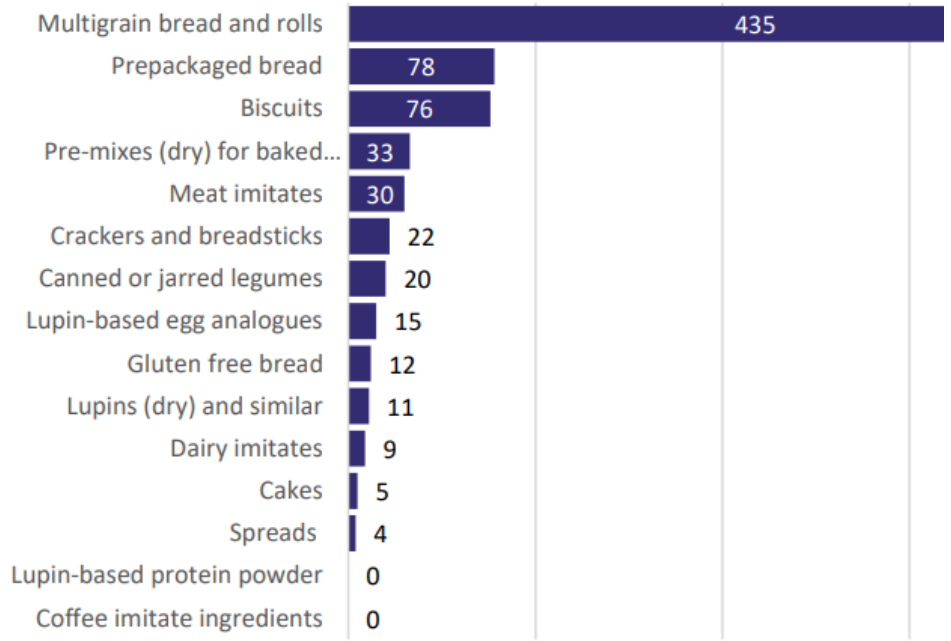
Exposure assessment

- Food Frequency Questionaire (FFQ)
- Convenience sample of 536 people
  - 18-80 years old
  - Geographical distribution

Characteristic	Percentage per category				
Sex	**Male**	**Female**			
	38.1%	61.9%			
Interview method	**Offline**	**Online**			
	38.4%	61.6%			
Region	**Brussels**	**Flanders**	**Wallonia**		
	12.9%	72.2%	14.9%		
Age	**<18**	**18-64**	**>64**		
	0%	92.0%	8.0%		
Diet characteristics	**Lactose free**	**Not lactose free**	**Gluten free**	**Not gluten free**	
	6.3%	93.7%	5.4%	94.2%	
	Flexitarian	**Vegetarian**	**Pescetarian**	**Vegan**	**None of the above**
	19%	5%	4%	3%	69%



FFQ

On food type level

Have you eaten lupin beans in a jar the past 12 months?

- ❑ Yes
- ❑ No

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FFQ

1. Which brand did you consume?

2. How often did you eat it the past 12 months?

Welke van deze merken heeft u gegeten?

* must provide value

☐ 1. Smaakt ☐ 2. Demeter ☐ 3. Your Organic Nature ☐ 4. Biona ☐ 5. Koro ☐ 6. Priméal ☐ 7. Biolân

	Meer dan één keer per week	één keer per week	één keer per twee weken	één keer per maand	Minder dan één keer per maand, maar minstens één keer per jaar	Ik weet het niet
Hoe vaak at u de voorbije 12 maanden lupinen in bokaal? * must provide value	○	○	○	○	○	○

reset

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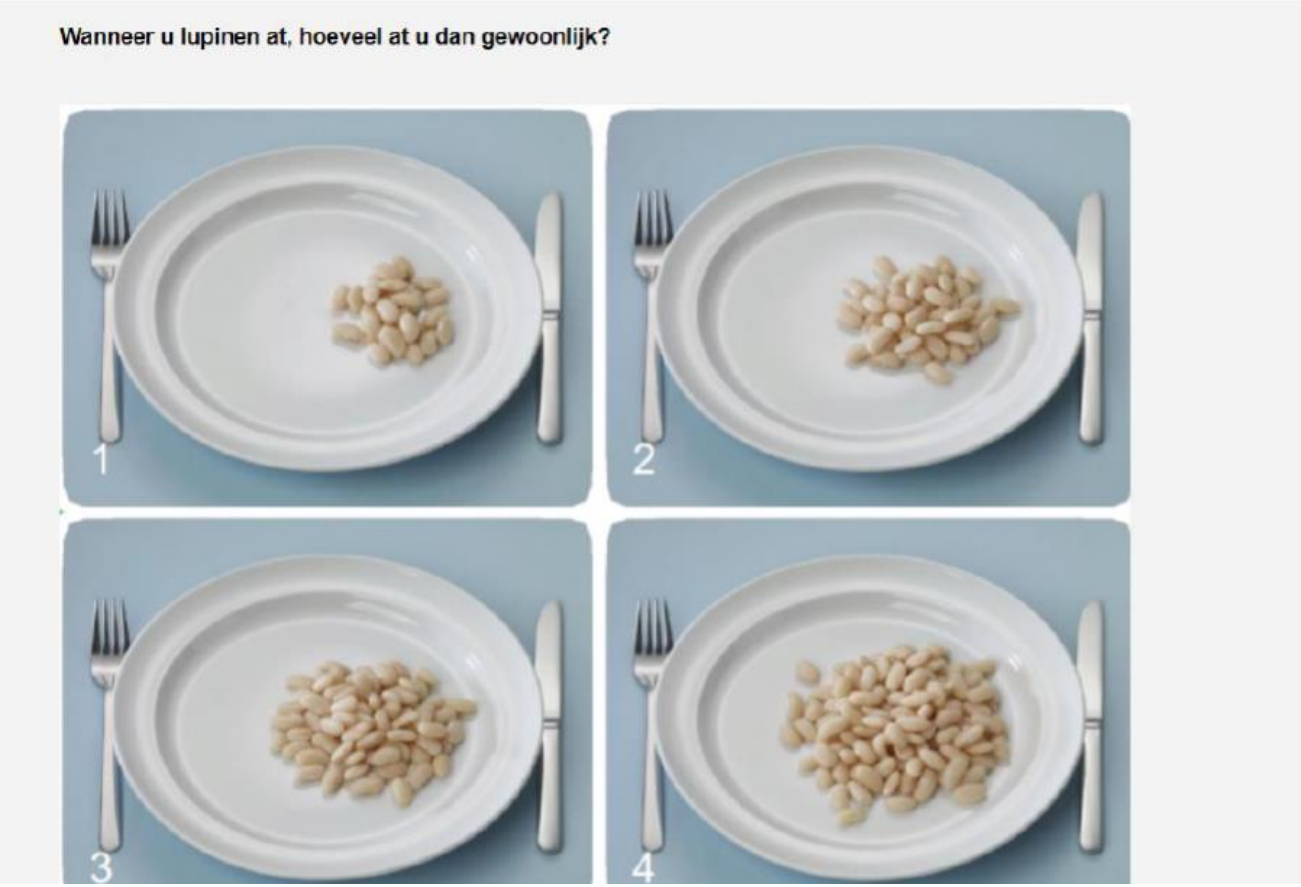
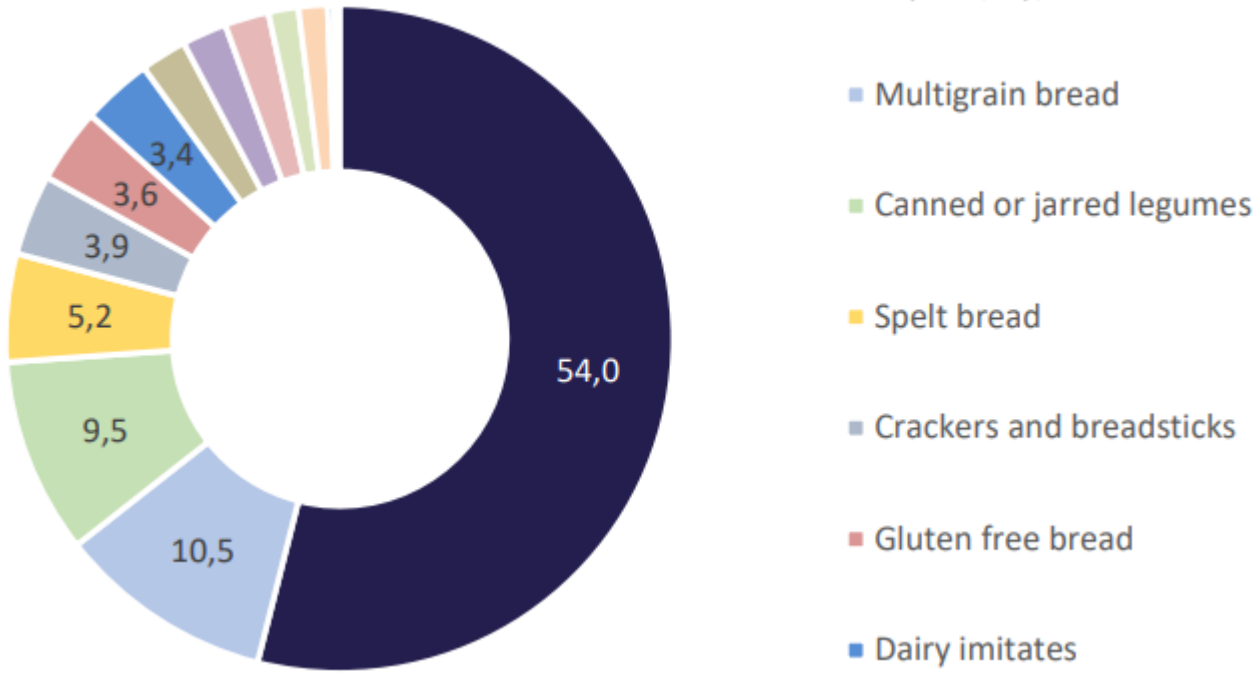
FFQ



IF YES

3. When you ate it, how much did you generally consume?

Risk characterization



Contributions of different food product categories to total average QA intake for the total population

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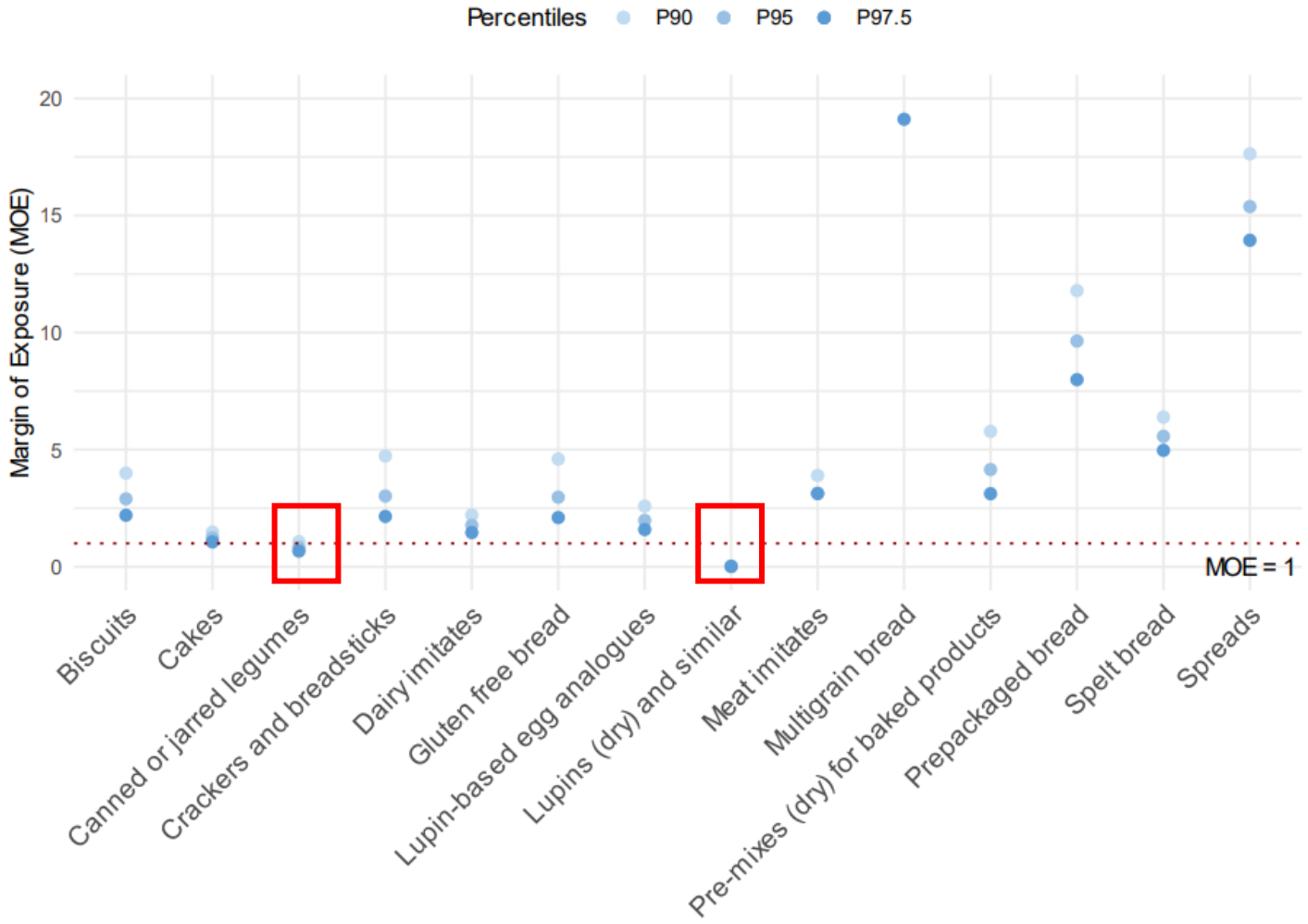
MOE calculation

- Margin of Exposure for TQA
- Based on 160 µg/kg BW day for sparteine (EFSA), and the same limit for all QAs



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QAs during processing

- Milling, toasting, dehulling, sterilization (in jar), oven baking (cookies), frying (chips), boiling in water (pasta)
- Analyzing before and after processing step
  - All processes in duplicate
- Starting material: 1 20 kg batch of high QA lupin beans

Effect of processing

Produced fraction/ food	Unit process	Dry matter content	Sparteine	Lupanine	Lupinine	13-OH-lupanine	Angusti-foline	Total QAs
Lupin hulls	Dehulling	0.882 ± 0.003	− 90%	− 87%	ND	− 95%	− 96%	− 88%
Dehulled lupin flour	Dehulling: dehulled lupin flour	0.924 ± 0.004	+ 21%	+ 31%	ND	+ 14%	+ 17%	+ 30%
Toasted Whole Lupin Flour - Autoclaved	Toasting	0.926 ± 0.005	− 23%	− 11%	ND	+ 15%	− 46%	− 11%
Toasted Whole Lupin Flour - Steam Cooked	Toasting	0.929 ± 0.002	− 29%	− 14%	ND	+ 21%	− 35%	− 13%
Toasted Dehulled Lupin Flour - Autoclaved	Toasting	0.931 ± 0.004	− 25%	− 24%	ND	+ 12%	− 43%	− 23%
	Toasting and dehulling	0.931 ± 0.004	−25%	− 32%	ND	− 1%	− 51%	− 32%
Toasted Dehulled Lupin Flour –Steam Cooked	Toasting	0.927 ± 0.004	− 17%	− 23%	ND	+ 2%	− 35%	− 22%
	Toasting and dehulling	0.927 ± 0.004	− 16%	− 15%	ND	+ 14%	− 24%	− 14%
Soaked lupin seeds	Soaking	0.352 ± 0.000	+ 3%	− 12%	ND	+ 103%	+ 21%	− 5%
Cooked lupin seeds (n = 6)	Cooking	0.332 ± 0.014	− 36%	− 27%	ND	− 26%	− 40%	− 28%
Sterilized jarred lupins (n = 6)	Auto-clavation	0.268 ± 0.003	− 31%	− 44%	ND	− 62%	− 64%	− 46%
	Whole production process	0.268 ± 0.003	− 55%	− 64%	ND	− 43%	− 74%	− 63%
Baked cookie	Baking	0.949 ± 0.003	+ 10%	− 15%	ND	− 3%	− 39%	− 15%
Lupin chips	Frying	0.972 ± 0.001	− 1%	− 19%	ND	− 25%	− 39%	− 19%
Cooked pasta	Boiling	0.329 ± 0.009	− 40%	− 53%	ND	− 50%	− 37%	− 52%

Conclusions

- Analysis: methods available (standards are a bigger issue)
- Food: high concentrations in dry lupins
  - Other: coffee and eggs imitates
- Feed: high QA levels in Eu lupins
  - Little transfer to meat
- Risk for humans: high consumption of dry lupins
- Processing: leaching out biggest impact

More information?

- Bram.miserez@ciboris.org

- Schryvers, S., Jacxsens, L., Croubels, S., Vonck, S., Miserez, B., Van De Steene, J., Necchi Rohers, G. and Eeckhout, M., 2024. Quinolizidine alkaloids and phomopsin A in animal feed containing lupins: co-occurrence and carry-over into veal products. *Food Additives & Contaminants: Part A*, pp.1-15.
- Schryvers, S., Arinzechukwu, C., Miserez, B., Eeckhout, M. and Jacxsens, L., 2023. The fate of quinolizidine alkaloids during the processing of lupins (Lupinus spp.) for human consumption. *Food Chemistry*, *429*, p.136847.

- More papers in preparation
  - Food analysis
  - Risk Assessment