



UNIVERSITÀ DI PARMA

**Safety Assessment of Plant-based Food:
Does the Shift to Alternative Dietary Pattern
Pose a Threat?**

Chiara Dall'Asta



HOW TO FIND US

MOTORWAYS

Autostrada del Sole (A1) "Parma" exit
Autostrada Parma/La Spezia (A15)
"Parma Ovest" exit

AIRPORTS

Parma airport "Giuseppe Verdi" is located a few minutes from the city.
Other international airports near by are Bologna, Genoa, Milan Malpensa, Milan Linate, Bergamo - Orio al Serio and Verona.

DISTANCES

Parma is located

120 km from Milan

100 km from Bologna

190 km from Florence

250 km from Venice

460 km from Rome



Parma is in the heart of northern Italy, close to many of the most important cities of the peninsula

The **river Po valley**, in which the city lies, is rich in **industries** and **business companies** well known at national and international level

These industrial and commercial giants often **get young graduates directly from the University**

Companies such as **Barilla, Chiesi, Dallara, Ferrari, Parmalat, Mutti** have their headquarters in this area



PARMA


About 200.000 inhabitants and **32.000 students**: a lively university city

Dynamic but not chaotic; tidy, beautiful, efficient, plenty of **things to do**

A city of art, open and modern, in the heart of the **“Food Valley”**

One of the best Italian cities for the highest levels of **quality of life**

OnFoods Foundation



Research and Innovation for Sustainable Food and Nutrition

We are a partnership for the Sustainable Development of Food and Nutrition,
funded under the Italian National Recovery and Resilience Plan (NRRP)

Development of an advanced interlaboratory analytical platform to ensure high quality occurrence data collection and crop monitoring

MycobEons

Integration of computational, biotechnological and toxicological methodologies to design innovative bio-based mitigation strategies



Delivering a comprehensive and intersectoral training to early and middle-career scientists

WP1

Advanced diagnostics for mycotoxin detection



WP2

Integrated platform for combined toxicity and safety assessment

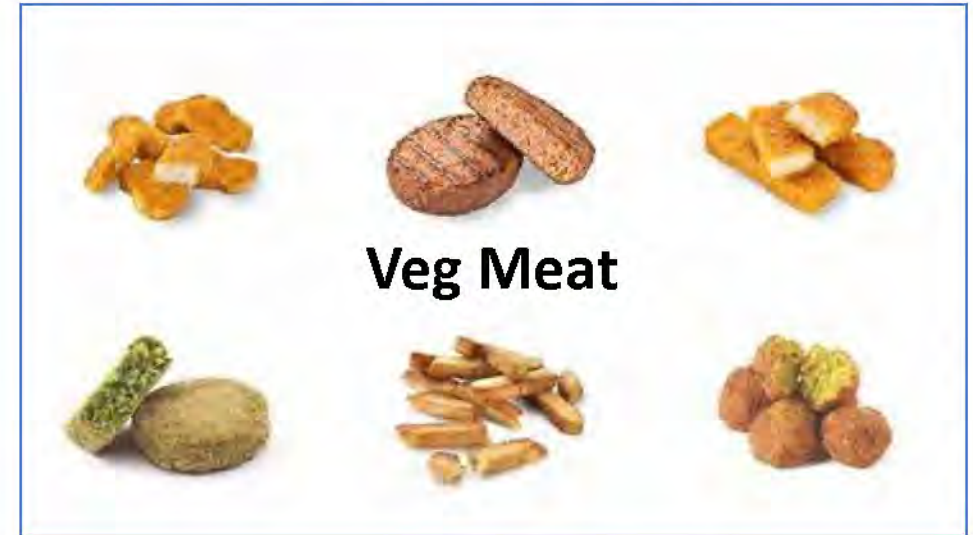
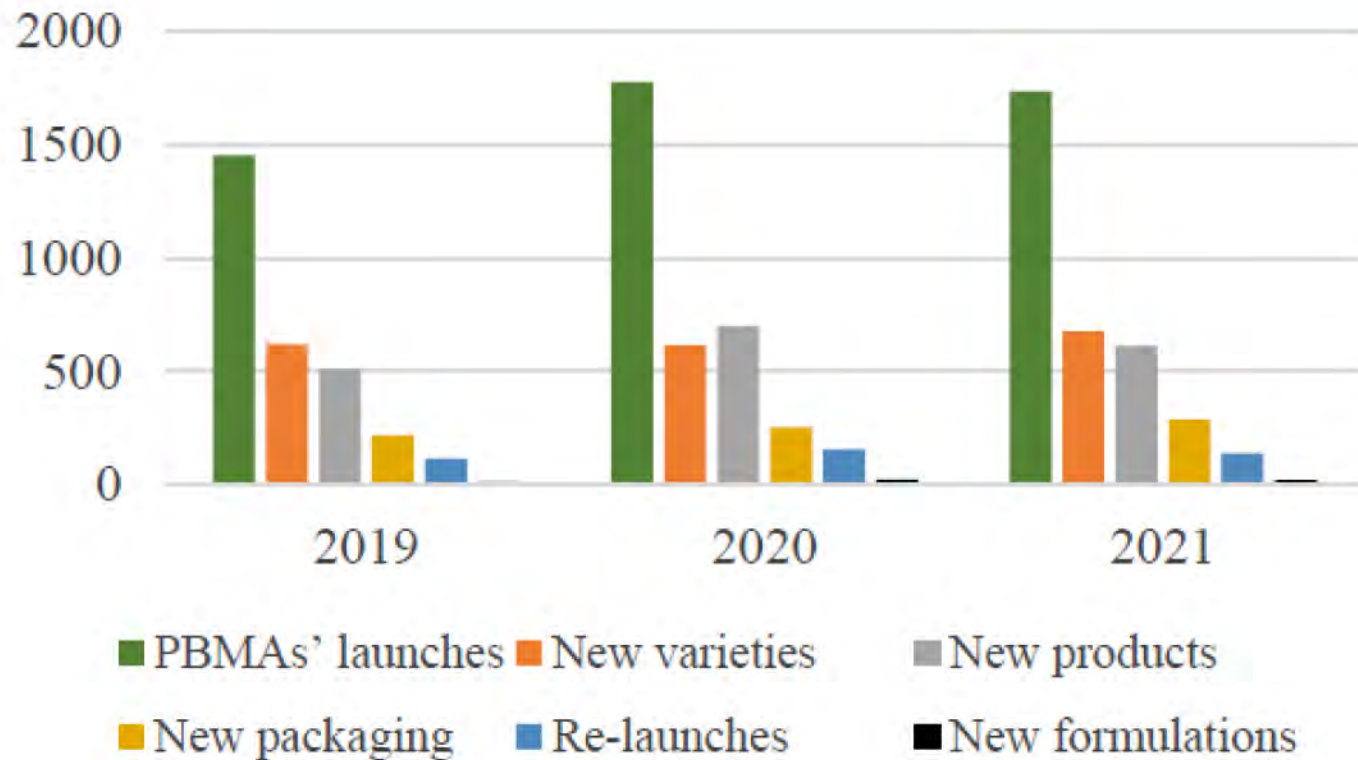


WP3

Biotechnological mitigation strategies

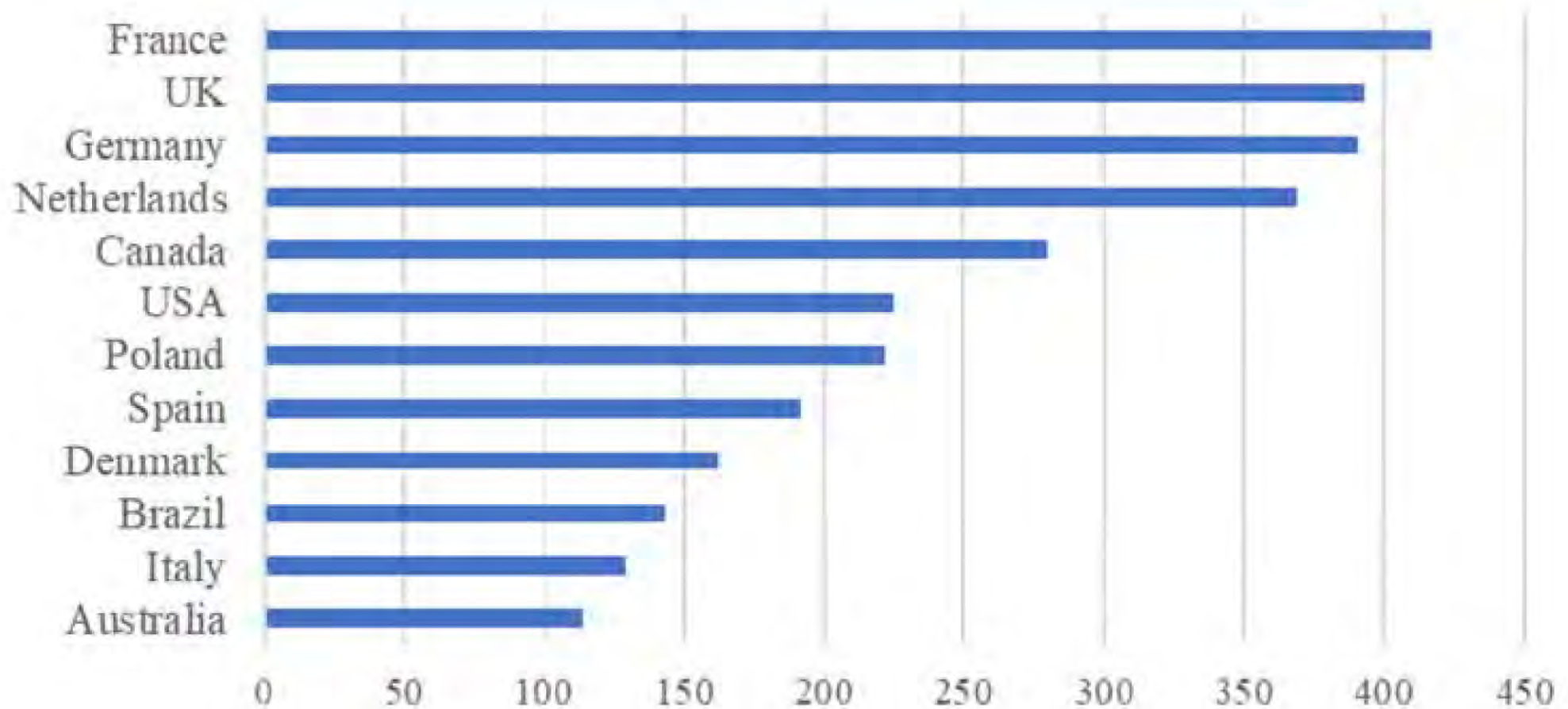


Sustainable diets – trends versus reality



Andreani et al. *Nutrients* **2023**, 15, 452.

Products launched in 2019 – 2021 by country



Andreani et al. *Nutrients* **2023**, 15, 452.

Front label claims on PBMA



Andreani et al. *Nutrients* 2023, 15, 452.

Plant-based meat alternatives – a multifaceted issue



Nutritional profile

	PLANT-BASED MEAT ANALOGUES	MEAT
Total fat	Comparable content	Comparable content
Saturated fatty acids	Lower content in saturated fatty acids	Higher content in saturated fatty acids
Total carbohydrates	Source of carbohydrates	Generally, it does not contain carbohydrates
Sugars	Discrete sugar content	Generally, it does not contain sugars
Protein	Wide variability, from a quite lower protein content to a comparable protein content (depending on the commercial category) Generally, lack of methionine	Good amount of proteins Proteins with a high biological value
Fibre	Source of fibres	Generally, it does not contain fibre
Salt	Wide variability, from higher salt content to a quite lower salt content (in the comparison between cured meats and their analogue)	Wide variability, from an almost zero content (in sliced meat) to a high salt content (in cured meats)
Vitamins	Lack of vitamin B12	Source of vitamin B12
Minerals	Source of calcium, phosphorus, and potassium	Source of iron and zinc

Nutritional profile – open issues

1. Presence of anti-trypsin inhibitors, that are usually thermally inactivated but, when still active, may affect the overall digestibility
2. Presence of anti-nutritional compounds (i.e. protease inhibitors, saponins, phytates, oxalates, etc.) that can lower the absorption of micronutrients
3. Allergenic proteins
4. Lack of proper regulation for natural toxins (i.e. mycotoxins)

ANALYTICAL ISSUES...

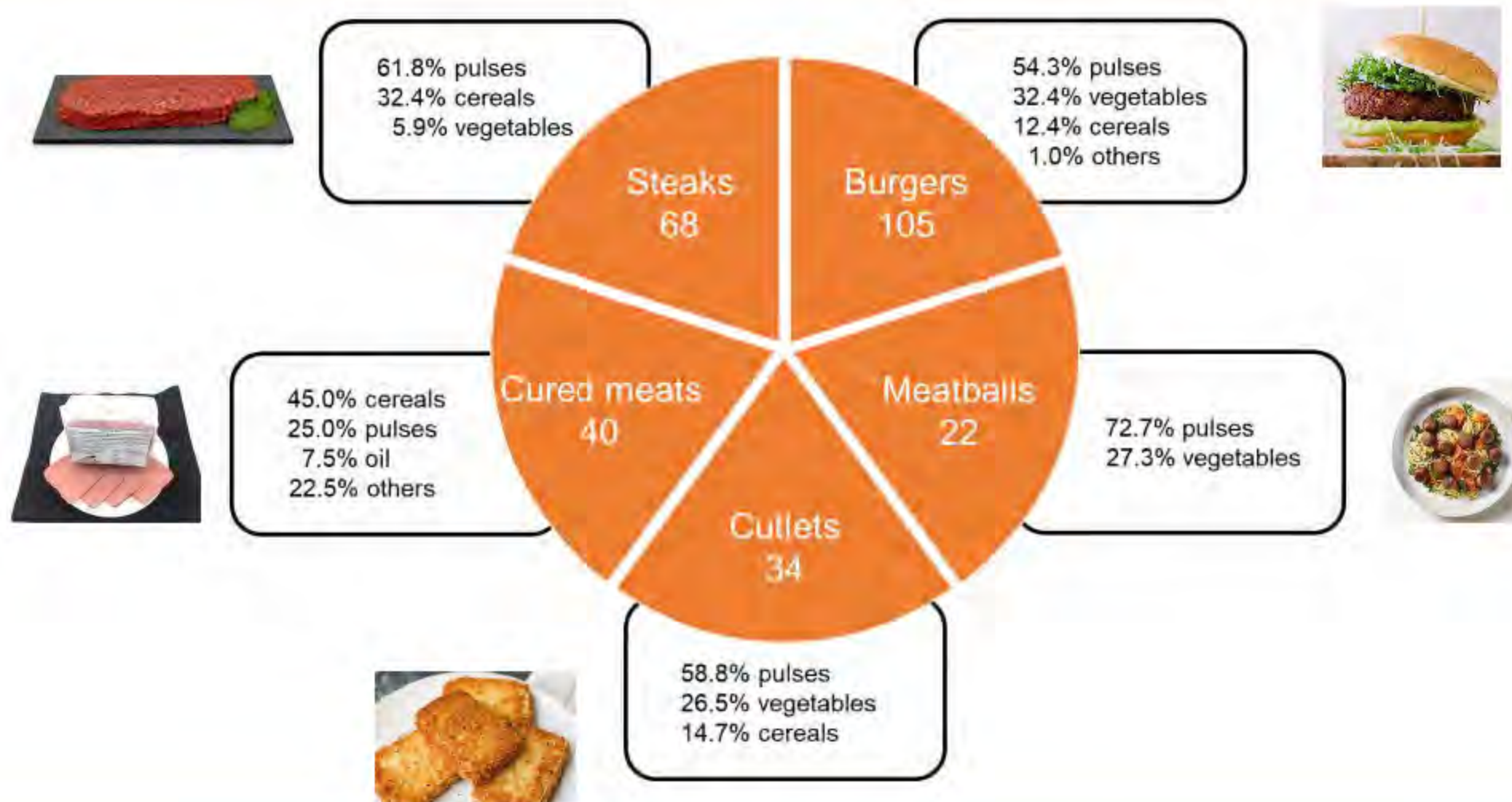


Low moisture extrusion



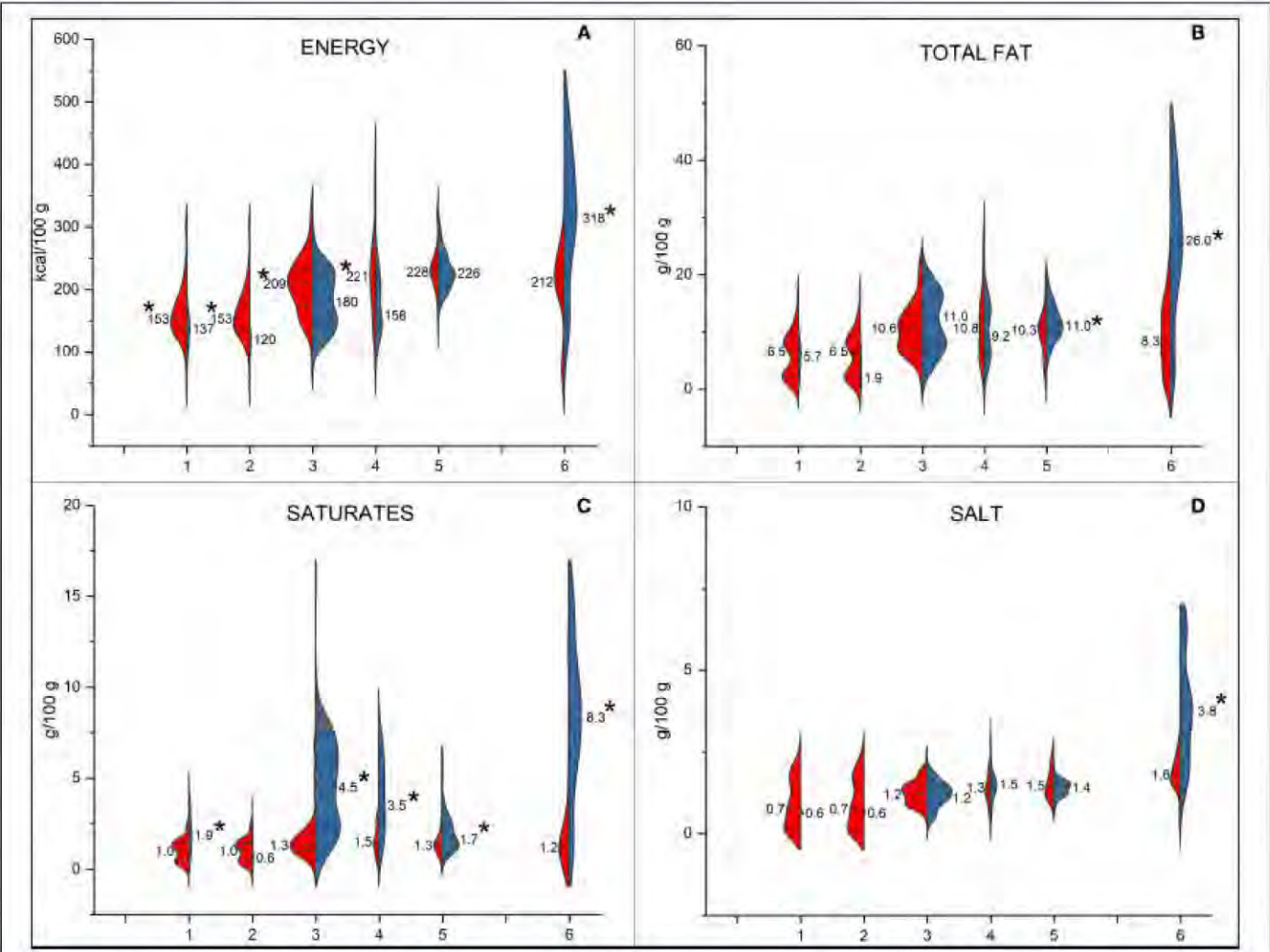
High moisture extrusion

Nutritional quality



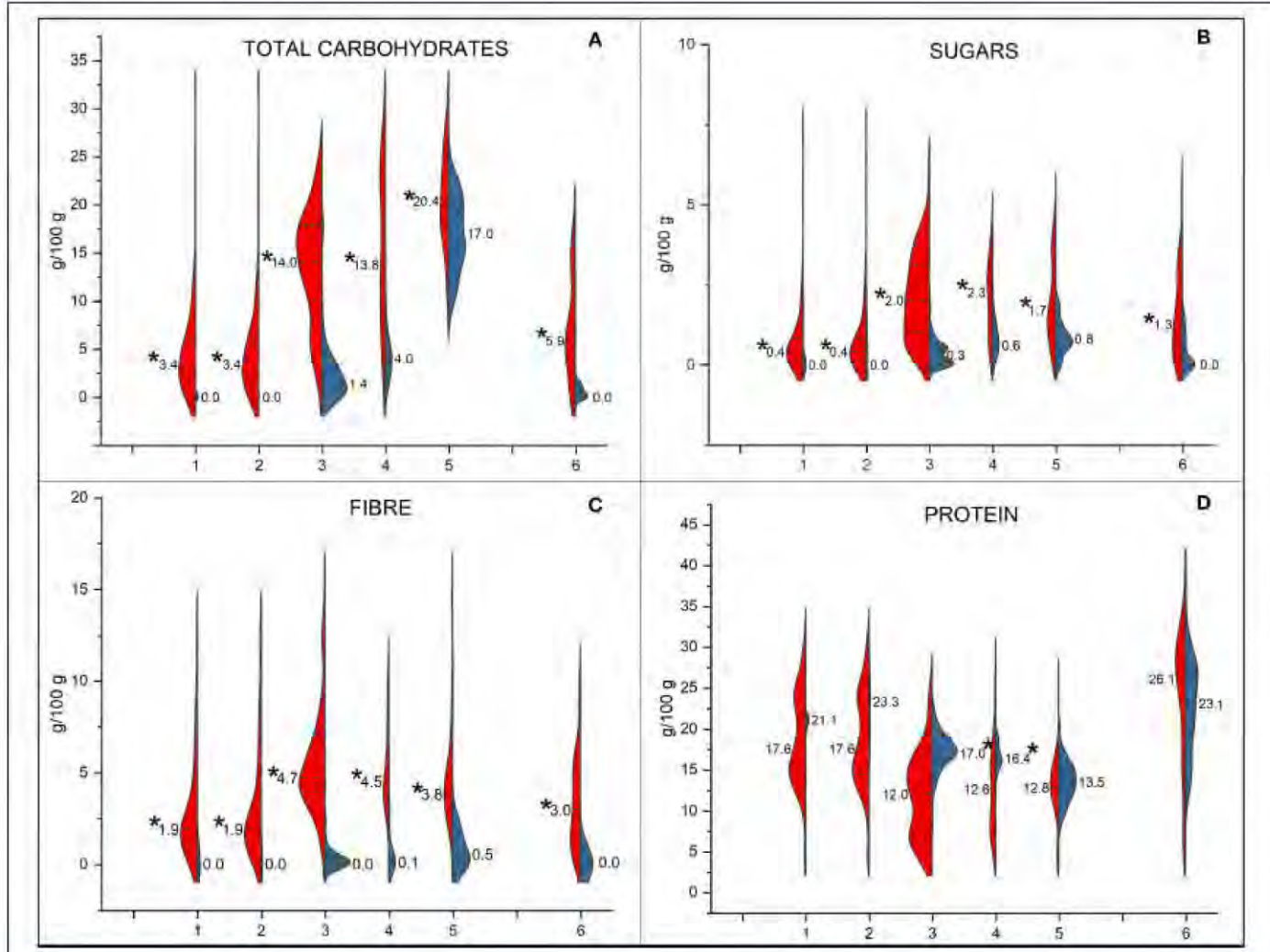
Nutritional quality - macroconstituents

- | | | |
|-----------------|---|---|
| | PB | meat |
| |  |  |
| 1. Red steack | |  |
| 2. White steack | |  |
| 3. burger | |  |
| 4. meatballs | |  |
| 5. cutlets | |  |
| 6. Cured meat | |  |



Nutritional quality - macroconstituents

- █ PB █ meat
1. Red steack 
 2. White steack 
 3. burger 
 4. meatballs 
 5. cutlets 
 6. Cured meat 



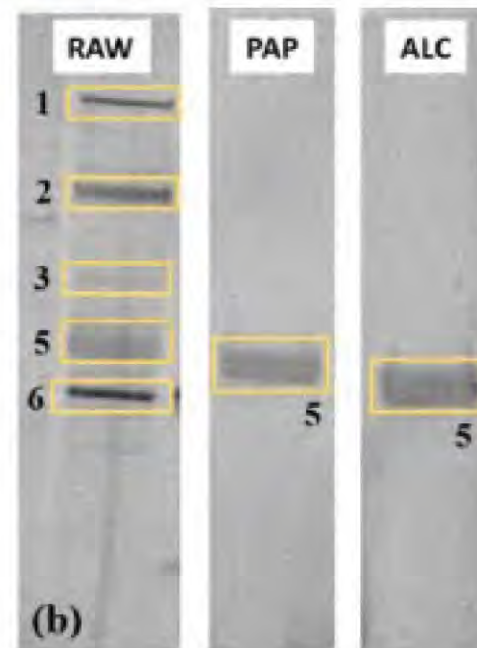
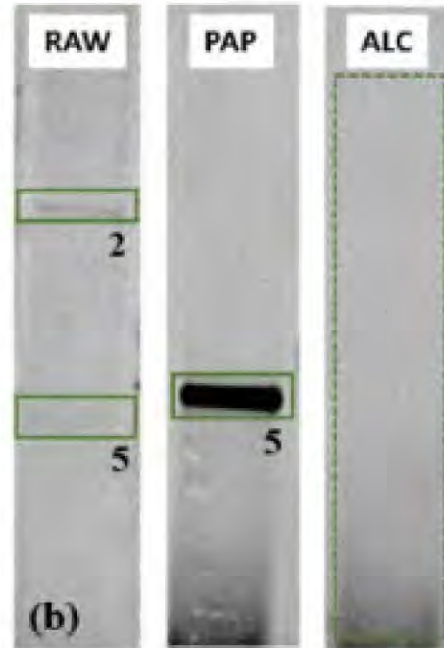
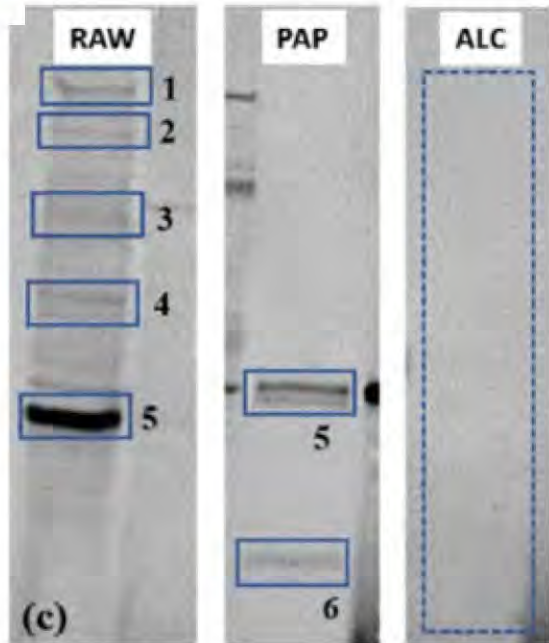
Allergenic potential



Patient code	Positive specific IgE	Positive in-vivo skin tests
A	Chickpea	None
B	Chickpea, green pea, white bean, soybean	Chickpea, green pea, leguminous mix
C	Chickpea, white bean	Chickpea, bean, soybean
D	Chickpea	None
E	Green pea, peanut	n.d
F	Green pea	n.d
G	Chickpea, green pea	Raw chickpea, cooked green pea
H	Green pea	None
I	n.d	Chickpea
J	Chickpea, white bean, soybean	Chickpea
K	Green pea, white bean, soybean	Green pea
L	Chickpea, green pea, white bean, soybean	Chickpea flour, cooked bean

Calcinai et al. Sci Rep **2022**, 12, 16902.

Allergenic potential - immunoblotting



RAW - protein extract

PAP - papain hydrolisates

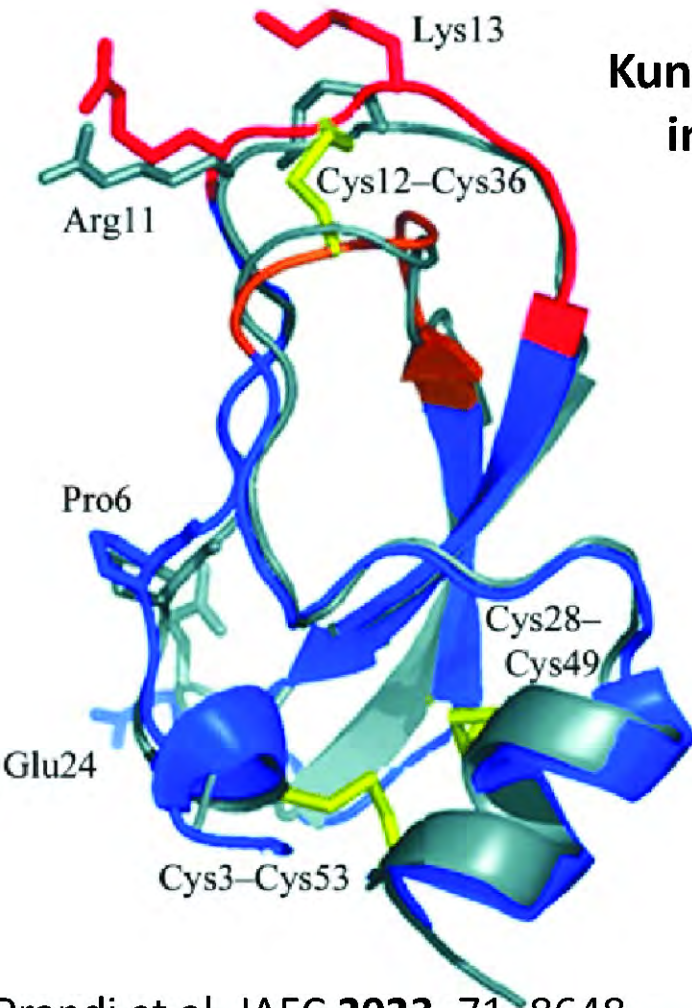
ALC - alcalase hydrolisates

Serum patient H
Positive specific IgE:
green pea

Calcinai et al. Sci Rep **2022**, 12, 16902.

Antinutritional factors

Kunitz-Type Trypsin inhibitor KT13



Prandi et al. JAFRC **2023**, 71, 8648.

sample	protein content (%)	KTI3 (mg/g) determined by LC-MS	KTI3 (mg per medium portion)
soybean (Energy)	39.3 ^b	1.69 ± 0.22 (CV 13%)	143 (85 g)
soybean (Namaste)	33.8 ^b	0.01 ± 0.00 (CV 0%)	0.49 (85 g)
soy drink	31.2 ^c	8.15 ± 3.83 (CV 47%)	191 (244 g)
proteic soy drink	40.5 ^c	79.14 ± 7.76 (CV 10%)	2387 (244 g)
Yofu	16.5 ^c	17.21 ± 1.08 (CV 6%)	791 (245 g)
tofu (raw)	45.9 ^c	14.84 ± 1.31 (CV 9%)	573 (124 g)
tofu (cooked)	48.0 ^d	6.21 ± 4.95 (CV 80%)	230 (124 g)
soy burger (raw)	39.9 ^c	9.00 ± 0.10 (CV 1%)	224 (71 g)
soy burger (cooked)	42.1 ^d	6.30 ± 0.06 (CV 1%)	149 (71 g)

Why a focus on natural contaminants?

>4,000 PBMA in the
last 6 years



Increased intake of
plant-based food

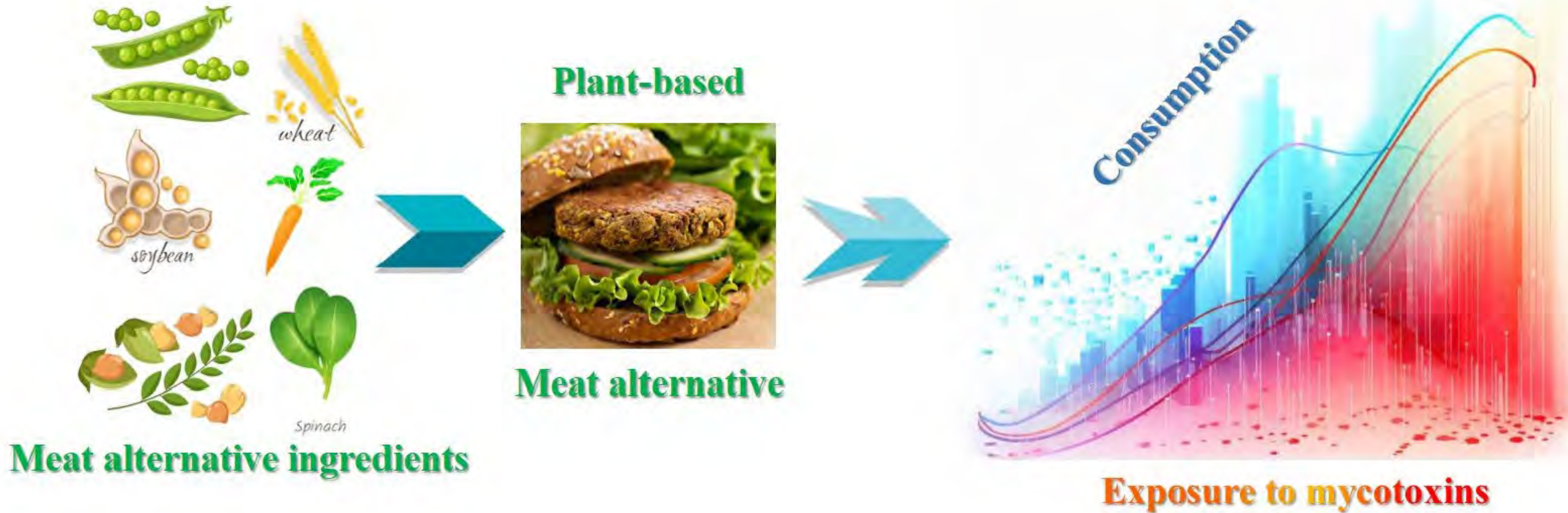
A likely change in the exposure of many
classes of contaminants



MYCOTOXINS MAY OCCUR IN ALL THE MAJOR
PLANT PROTEINS SOURCES, BUT WHILE WHEAT IS
FULLY REGULATED AND MONITORED,
BEANS ARE NOT REGULATED SO FAR IN THE EU

A CHANGE IN CONSUMPTION MAY LEAD TO A
SIGNIFICANT CHANGE IN EXPOSURE

A focus on natural contaminants?



Which data do we have by now?

RESEARCH ARTICLE

Molecular Nutrition
Food Research
www.mnf-journal.com

Mycotoxins in Serum and 24-h Urine of Vegans and Omnivores from the Risks and Benefits of a Vegan Diet (RBVD) Study

Katharina J. Penczynski,* Benedikt Cramer, Stefan Dietrich, Hans-Ulrich Humpf, Klaus Abraham, and Cornelia Weikert

Table 2. Mycotoxin levels in serum and 24-h urine samples of vegans and omnivores (quantified in at least 50% of the

	Vegans (n = 36)				Omnivores (n = 36)				
	<LOD	>LOQ	Median [IQR]	Min –Max	<LOD	>LOQ	Median [IQR]		
Serum [ng ml⁻¹]									
OTA	0%	100%	0.238 [0.165–0.335]	0.108 –0.733	0%	100%	0.120 [0.098–0.182]	0.071 –0.539	<0.0001
2'R-OTA	6%	83%	0.031 [0.017–0.096]	0.004 –0.347	6%	86%	0.068 [0.023–0.128]	0.004 –0.253	0.2
EnB	6%	92%	0.017 [0.013–0.031]	0.001 –0.104	11%	89%	0.024 [0.013–0.046]	0.001 –0.096	0.3
24-h Urine [µg 24 h⁻¹]									
DON-GlcA	25%	58%	8.05 [2.81–15.67]	0.43 –35.36	19%	56%	5.14 [2.31–18.75]	0.84 –48.37	>0.9

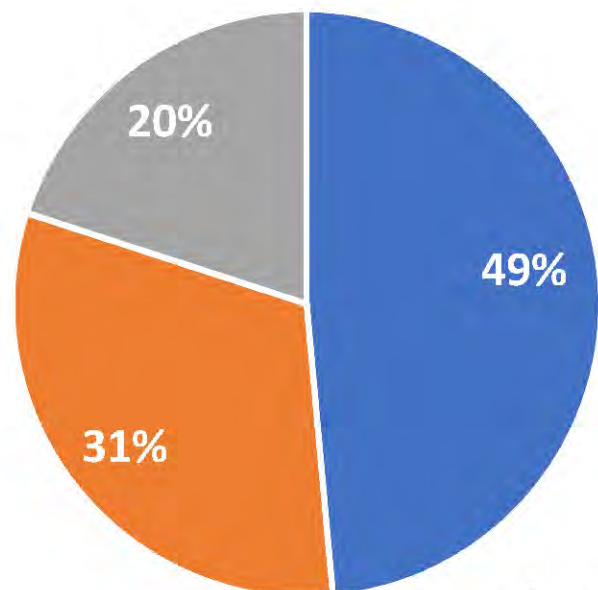
Values are based on middle bound substitution scenario: samples below LOD were set to LOD/2; samples between LOD and LOQ were set to LOQ/2. Medians [IQR], min and max were calculated including the substituted values. LOD and LOQ values reported in Table S1, Supporting Information. IQR indicates interquartile range. ^{a)} Mann–Whitney U test; significant differences are shown in bold.

Food Groups	Serum OTA	
	r	p
Vegan Products	0.50	<0.0001
Cereals & Cereal Products	0.20	0.11
Pasta & Rice	0.33	0.006
Muesli	0.26	0.031
Legumes	0.45	0.0001
Dried Fruits	0.23	0.053

"Vegan Products" summarized intakes of "plant-based milk," "plant-based milk products," "meat-alternatives," and "plant-based savory spreads." "Cereals & Cereal Products" summarized intakes of "whole-meal bread," "other bread," "pasta & rice," "muesli," "cornflakes," "cakes & cookies," and "pizza."

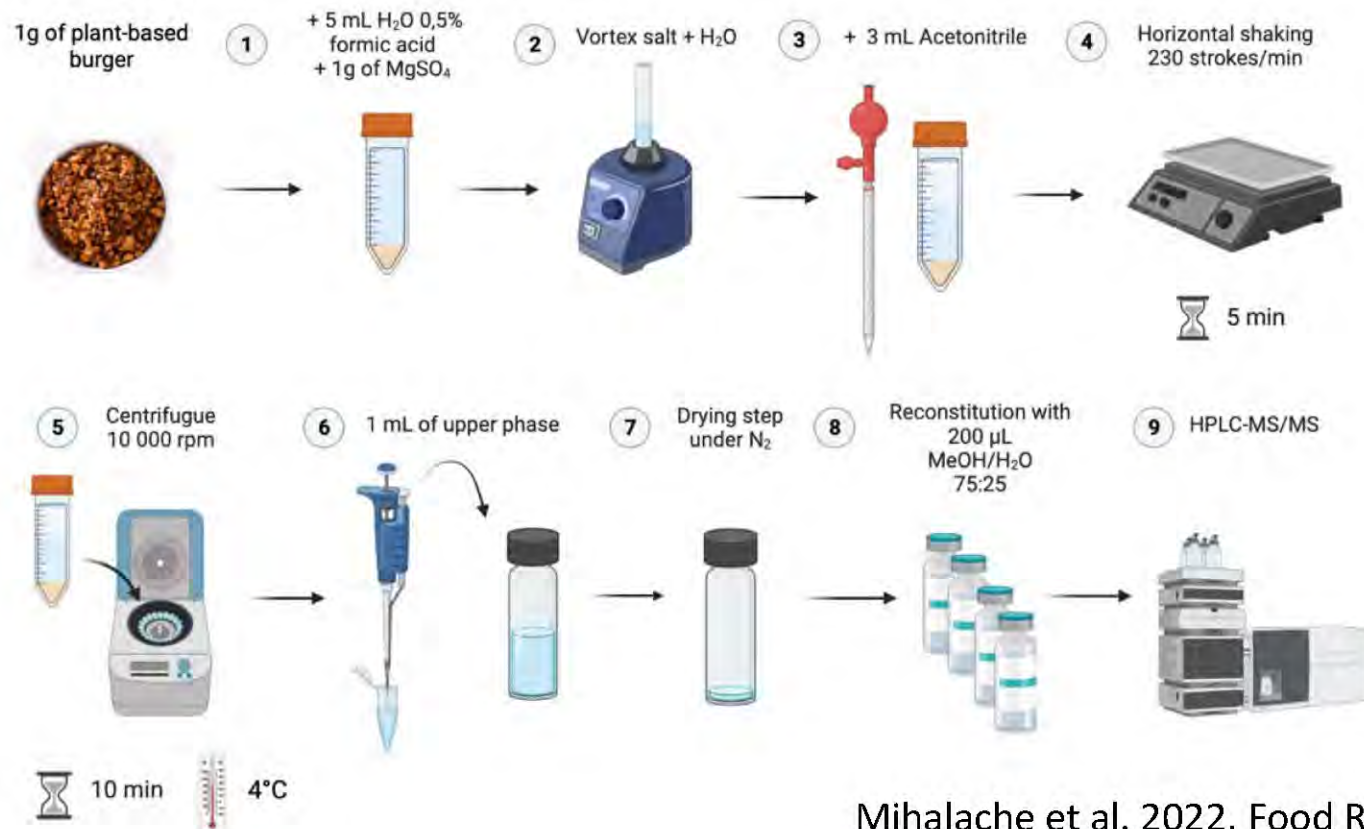


A survey on the Italian market for PBMA



- legume based
- wheat based
- veggie based

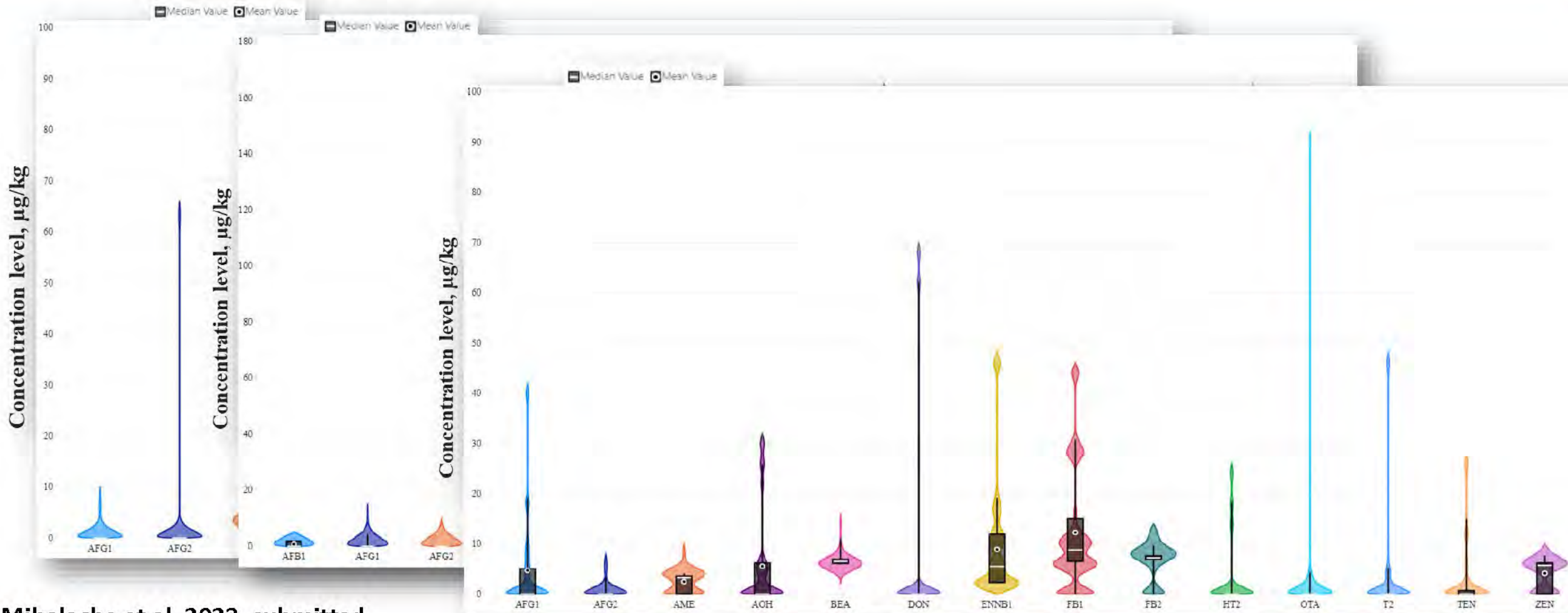
Sample prep development and multi-toxin QQQ detection In house validation



Mihalache et al. 2022, Food Res Int

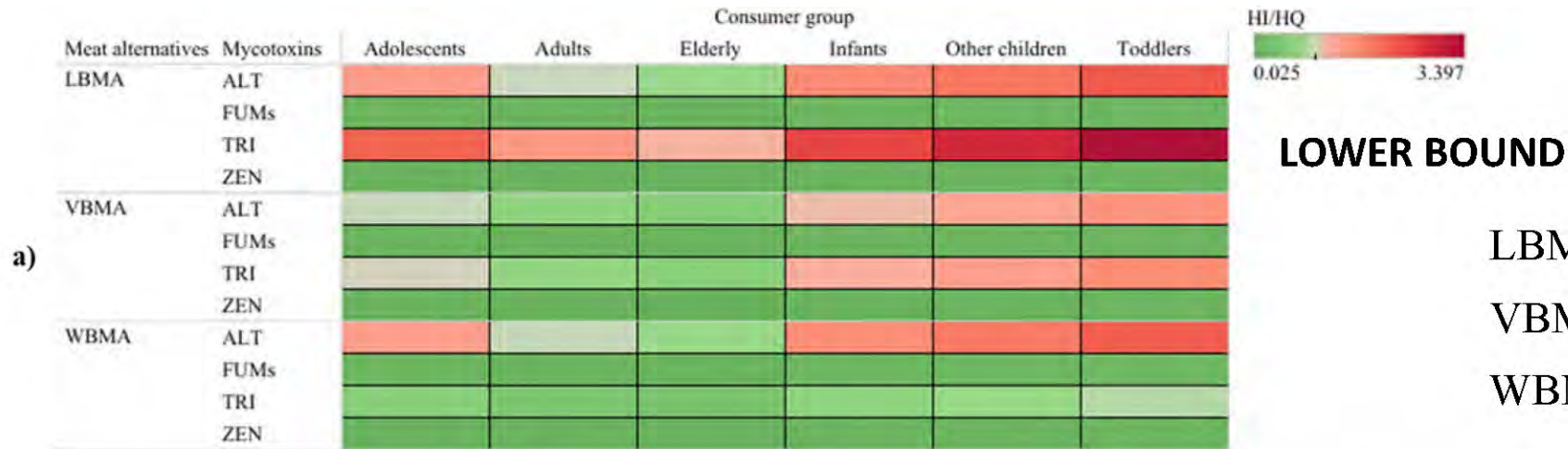
Mihalache et al. 2023, submitted

A survey on the Italian market



Mihalache et al. 2023, submitted

A simulated scenario of meat replaced with PBMA



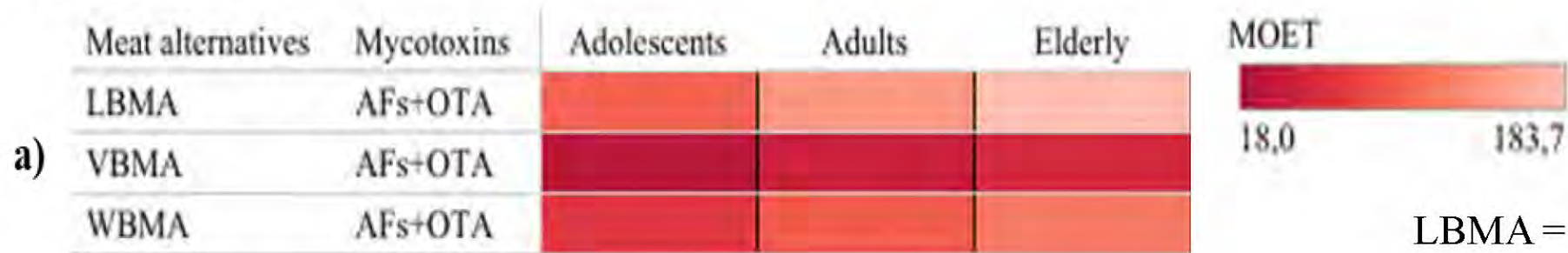
LBMA = legume-based meat alternatives;
 VBMA = vegetable-based meat alternatives;
 WBMA = wheat-based meat alternatives;



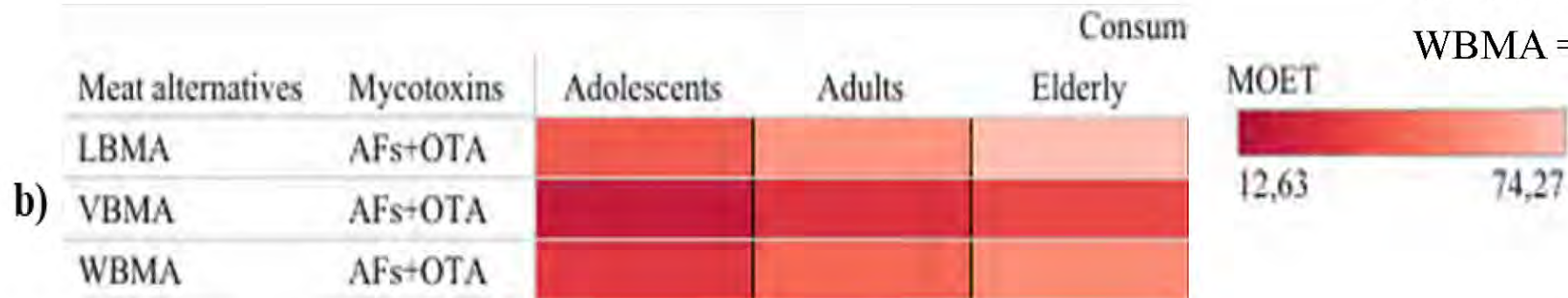
Mihalache et al. 2023, submitted

A simulated scenario of meat replaced with PBMA

LOWER BOUND



LBMA = legume-based meat alternatives;
 VBMA = vegetable-based meat alternatives;
 WBMA = wheat-based meat alternatives;



UPPER BOUND

Mihalache et al. 2023, submitted

WORST CASE SCENARIO

This is an extreme scenario based on a full replacement and data from the Italian survey

However, considering that the consumption will keep growing, some actions are advisable:

1. Implementation of monitoring plans for occurrence data
2. Update consumption data
3. Careful risk assessment and – when appropriate – a revised regulation

A survey on the Italian market for plant-based milk



Oat milk
Soy milk
Almond milk
Nut milk
Rice milk

Mycotoxin	Occurrence (%)	Median contamination level ($\mu\text{g L}^{-1}$) ($\pm\text{SD}$)	Range (min-max), $\mu\text{g L}^{-1}$
AFB ₁	0		
AFB ₂	0		
AFG ₁	0		
AFG ₂	0		
AOH	0		
TEN	3, (30%)	0.36 (0.10)	0.29-0.43
AME	3, (30%)	0.16 (0.02)	0.15-0.18
FB ₁	10, (100%)	0.20 (0.02)	0.18-0.24


Very preliminary survey:

1. Wide occurrence at very low concentration
2. Co-occurrence of multiple mycotoxins (up to 10)

ENN	8, (80%)	0.44 (0.12)	0.20-0.55
ENNB ₁	8, (80%)	0.21 (0.06)	0.14-0.30
ENNA	10, (100%)	<LOQ	
ENNA ₁	10, (100%)	<LOQ	
BEA	8, (80%)	0.36 (0.07)	0.22-0.39
DON	0		

Torrijos et al. 2023, submitted

TAKE HOME MESSAGE



Efforts are needed to decrease the allergenic potential and the presence of antinutritional factors

Combined efforts are necessary from the scientific community and the regulators to keep up with the quick shift towards plant-based dietary patterns.

Overall, available data are not enough to provide a reliable picture of mycotoxins occurrence in legume-based products, especially for pea/chickpea.

The increased consumption of meat alternatives should be accompanied by revised exposure scenarios and updated risk assessments to reflect consumers' current dietary habits and lifestyle.

WHAT NEXT?



Andreani et al. *Nutrients* 2023, 15, 452.

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