



UNIVERSITÀ DI PARMA

**Caratterizzazione di sicurezza del riso rosso
fermentato: presenza di micotossine e profilo in
monacoline**

Chiara Dall'Asta

OUTLINE OF THE TALK

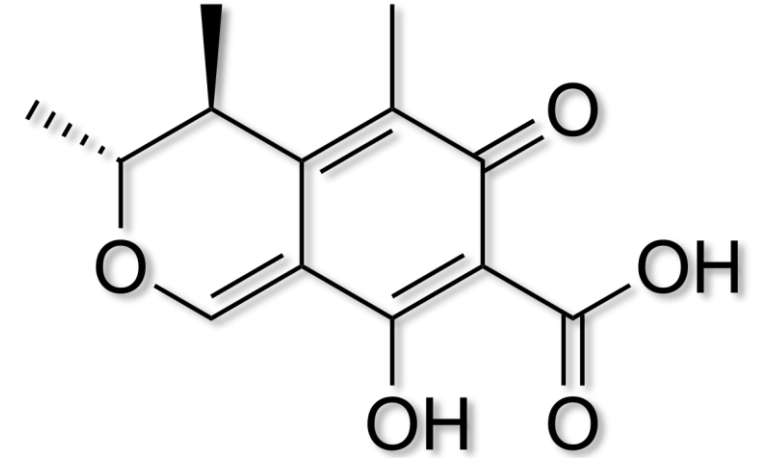
1. Background on Citrinin and RYR
2. Occurrence of CIT in RYR from the market
3. A tentative exposure assessment
4. Other quality parameters of RYR
5. Wrap up



CITRININ

CIT was isolated for the first time in 1831 from a *Penicillium citrinum* culture. It can be found in cereals, fruits, olives, nuts, seeds, and fermented products in general.

It can be synthesised by *Monascus* fungi (i.e. *M. purpureus* and *M. ruber*), often used for the production of supplements (i.e. red yeast rice, RYR)



TK studies reported on a quick absorption in humans (up to 40%) followed by liver and kidney distribution, and urine excretion as isocitrinone.

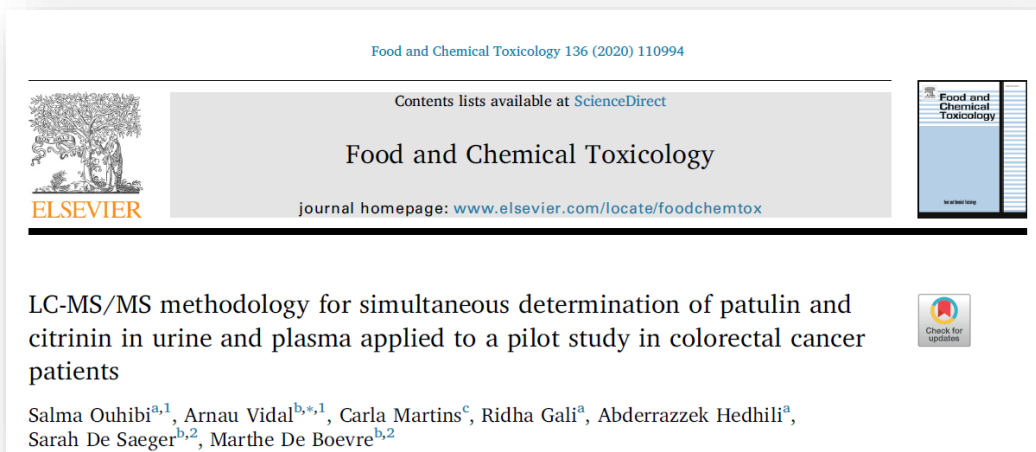
CIT may act synergistically with PAT and OTA, co-occurring also in the same foods.

However, due to thermal degradation, its occurrence in food is of less relevance than other mycotoxins

It has been observed that CIT

- affects the kidney function in different species, degenerating processes of the renal tubules
- induces micronuclei in HepG2 cells at levels of $\geq 10 \mu\text{M}$
- decreases the percentage of binucleated cells in a dose-dependent way

➔ POSSIBLE GENOTOXICITY IN HUMANS AND ANIMALS



High percentage prevalence of CIT in biological fluids
(38% in plasma, 72% in urine)

According to EFSA, the major contribution to dietary exposure is due to cereals
BUT low levels found in cereals.

RED RICE— often reported as *red fermented rice* (RFR), *red mold rice* (RMR) o *red yeast rice* (RYR), it is traditionally obtained by fermentation with *Monascus* spp. fungal starters, mainly *M. purpureus*, *M. pilosus*, or *M. ruber*.



Fermentation leads to the species-dependent accumulation of monakolins, pigments, and CIT



RYR is traditionally used as natural supplement due to its content of monacolin K (MK), a fungal polyketide that competitively inhibit the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key enzyme in the biosynthesis of endogenous cholesterol.

Hypocholesterolemic activity in RYR

2011 EFSA HEALTH CLAIM

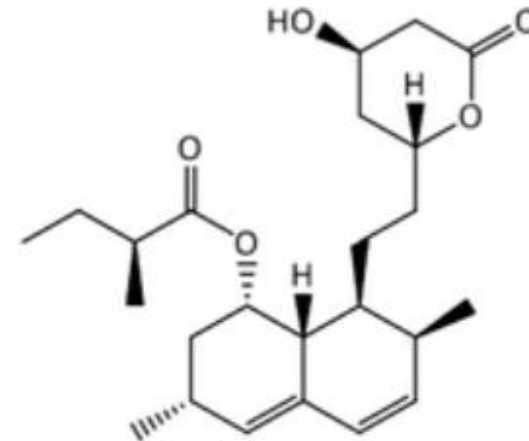
“Monacolin K from RYR contributes to the maintenance of normal blood cholesterol concentrations”

10 mg/die of MK required

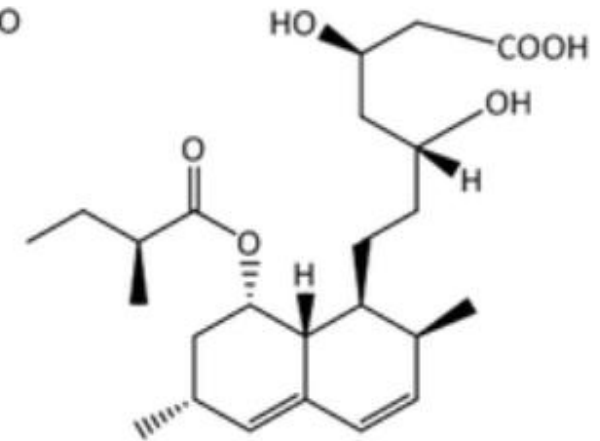


The total amount required is expressed as MKA + MK

MK is prevalent in RYR due to the pH conditions, and than converted to MKA in the gut



Monacolin K
(MK, lovastatin)
Inactive



Monacolin K β-hydroxy acid
(MKA)
Lipid-lowering active metabolite

SCIENTIFIC OPINION

Scientific Opinion on the risks for public and animal health related to the presence of citrinin in food and feed¹

EFSA Panel on Contaminants in the Food Chain (CONTAM)^{2,3}

Given the limitations and uncertainties in the current database on citrinin, the CONTAM Panel concluded that the derivation of a health based guidance value was not appropriate. For compounds that may be genotoxic and carcinogenic, EFSA recommends the use of a margin of exposure (MOE) approach for risk characterisation. However, due to the lack of data on human dietary exposure, no MOE could be calculated. In order to give risk managers a tool to evaluate the risk of citrinin in food and feed, the Panel decided to characterise the risk of citrinin on the available data on nephrotoxicity and determined therefore a level of no concern for nephrotoxicity. Applying a default uncertainty factor of 100 to the NOAEL of 20 µg/kg b.w. per day, accounting for inter-species variation and for inter-individual variation, the CONTAM Panel concluded that there would be no concern for nephrotoxicity in humans at an exposure level of 0.2 µg/kg b.w. per day. Based on the available data, a concern for genotoxicity and carcinogenicity could not be excluded at the level of no concern for nephrotoxicity.

7.3.2014

EN

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COMMISSION REGULATION (EU) No 212/2014

of 6 March 2014

amending Regulation (EC) No 1881/2006 as regards maximum levels of the contaminant citrinin in food supplements based on rice fermented with red yeast *Monascus purpureus*

(Text with EEA relevance)

Foodstuffs (1)		Maximum levels (µg/kg)
2.8	Citrinin	
2.8.1	Food supplements based on rice fermented with red yeast <i>Monascus purpureus</i>	2 000 (*)

(*) The maximum level is to be reviewed before 1 January 2016 in the light of information on exposure to citrinin from other foodstuffs and updated information on the toxicity of citrinin in particular as regards carcinogenicity and genotoxicity.

Generation of occurrence data on citrinin in food

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Terenzio Bertuzzi^c, Andrzej Starski^d, Jacek Postupolski^d, Massimo Castellari^e, Maria Hortós^e

According to the 2017 EFSA data collection, CIT occurrence in food is generally low with the only exception of RYR

Table 15: Average, median and 95th percentiles of citrinin concentration ($\mu\text{g}/\text{kg}$) in different food categories

Food category	Average ^(a)			Median ^(a)			95 th percentile ^(a,b)		
	LB	MB	UB	LB	MB	UB	LB	MB	UB
Industry cereals	1.2	1.4	1.6	0.0	0.3	0.5	1.2	1.2	1.2
Wheat	0.7	1.0	1.2	0.0	0.3	0.5	0.1	0.3	0.6
Barley	2.1	2.3	2.6	0.0	0.3	0.5	(1.8)	(2.0)	(2.6)
Rye	3.2	3.4	3.6	0.0	0.3	0.5	5.1	5.1	5.1
Rice	0.3	0.5	0.7	0.0	0.3	0.5	(1.0)	(1.0)	(1.3)
Cereal-based products	0.1	0.3	0.5	0.0	0.3	0.5	0.4	0.4	0.9
Flour	0.3	0.4	0.7	0.0	0.3	0.5	1.5	1.5	1.5
Rice retail	0.0	0.3	0.5	0.0	0.3	0.5	0.4	0.5	1.0
Bread and bread rolls	0.0	0.3	0.5	0.0	0.3	0.5	0.0	0.3	0.5
Pasta (dry)	0.0	0.3	0.5	0.0	0.3	0.5	0.3	0.4	0.7
Breakfast cereals (flakes&muesli)	0.1	0.3	0.5	0.0	0.3	0.5	0.2	0.3	0.7
RYR	162	164	166	0.0	2.5	5.0	830	830	830
ALL	12.9	13.2	13.6	0.0	0.3	0.5	2.0	2.5	5.0

LB: lower bound; MB: middle bound; RYR: red yeast rice; UB: upper bound.

(a): Concentration values were corrected for recovery.

(b): when $N < 60$ then the calculated 95th percentile is in between brackets and should be considered as an indicative value only due to the limited number of data (EFSA, 2011).

COMMISSION REGULATION (EU) No 212/2014

of 6 March 2014

amending Regulation (EC) No 1881/2006 as regards maximum levels of the contaminant citrinin in food supplements based on rice fermented with red yeast *Monascus purpureus*

(Text with EEA relevance)



Foodstuffs ⁽¹⁾		Maximum levels (µg/kg)
2.8	Citrinin	
2.8.1	Food supplements based on rice fermented with red yeast <i>Monascus purpureus</i>	2 000 (*)

(*) The maximum level is to be reviewed before 1 January 2016 in the light of information on exposure to citrinin from other foodstuffs and updated information on the toxicity of citrinin in particular as regards carcinogenicity and genotoxicity.

REGULATIONS

COMMISSION REGULATION (EU) 2019/1901

of 7 November 2019

amending Regulation (EC) No 1881/2006 as regards maximum levels of citrinin in food supplements based on rice fermented with red yeast *Monascus purpureus*

(Text with EEA relevance)



Foodstuffs ⁽¹⁾		Maximum levels (µg/kg)
2.8	Citrinin	
2.8.1	Food supplements based on rice fermented with red yeast <i>Monascus purpureus</i>	100'

Enforced by April 1st, 2020

OUR STUDY (Sept 2020 – Feb 2021)

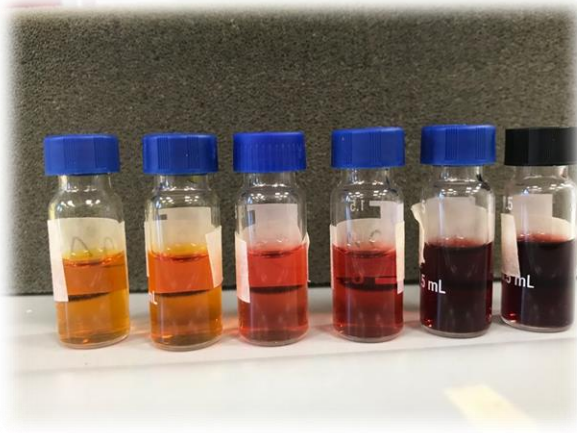
37 red fermented rice supplements
(8 RYR + 29 multi-ingredients)

24 purchased from the web + 13 purchased from shops
18 capsule + 15 tablets + 4 soft capsule

All the products declared the MKA content
4 products were labeled as «citrinin free»



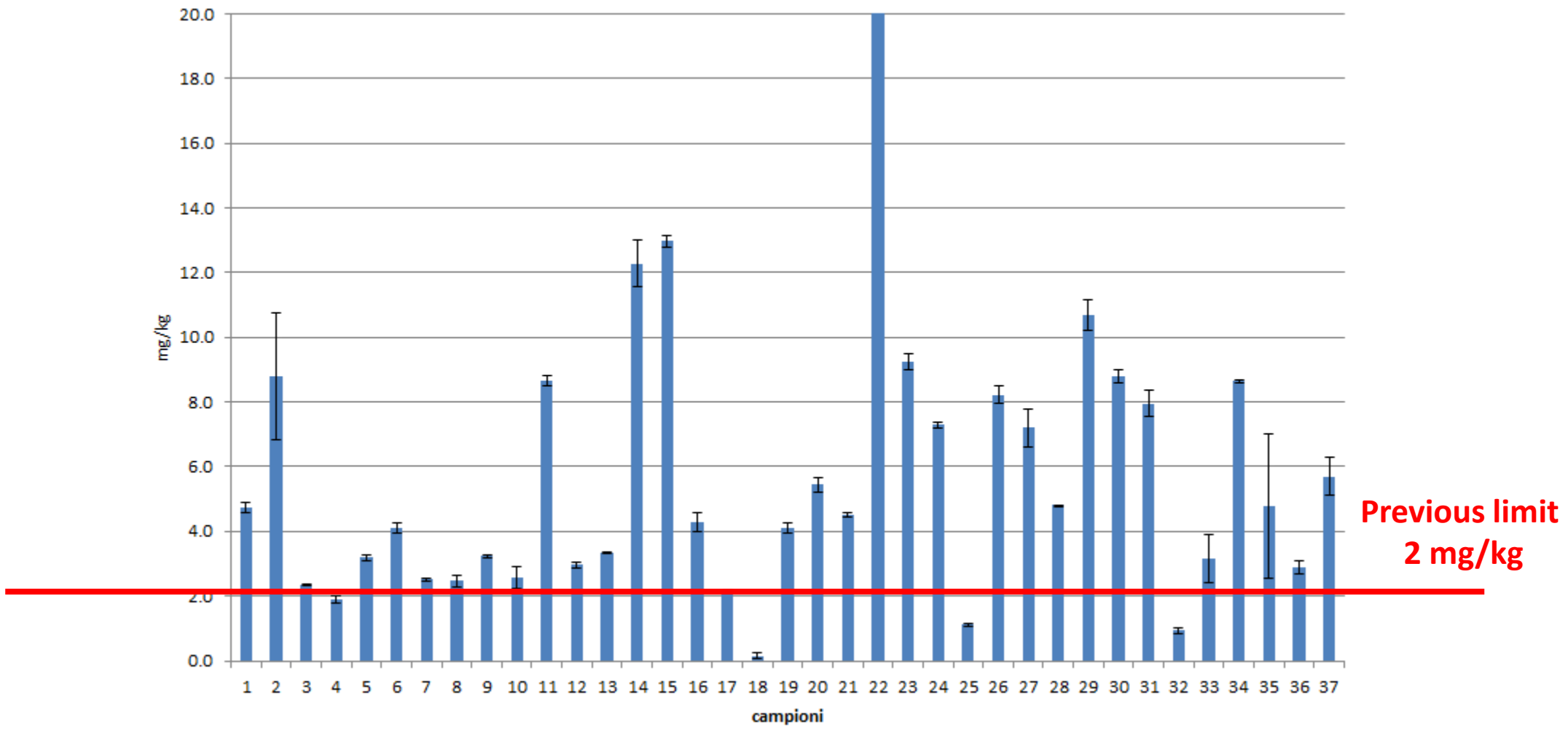
INGREDIENTI: Riso rosso fermentato (*Monascus purpureus*) estratto secco tit. 5% Monacolina K Citrinin-free; *beris aristata* D.C. corteccia estratto secco tit. 65% Berberina cloridrato; Agente di carica: Cellulosa microcristallina; Coenzima Q10; Antiagglomerante: Sali di magnesio de



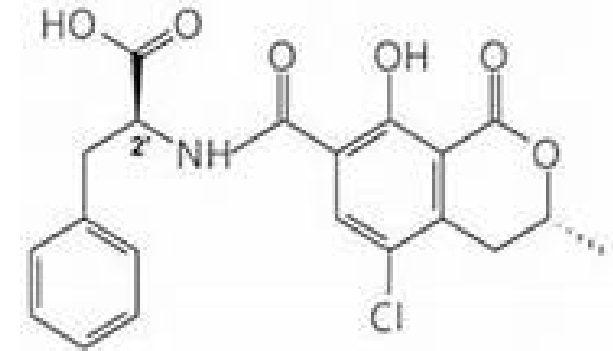
CIT + OTA
Monacolin K
Monacolin profile
Pigment profile

CITRININ OCCURRENCE

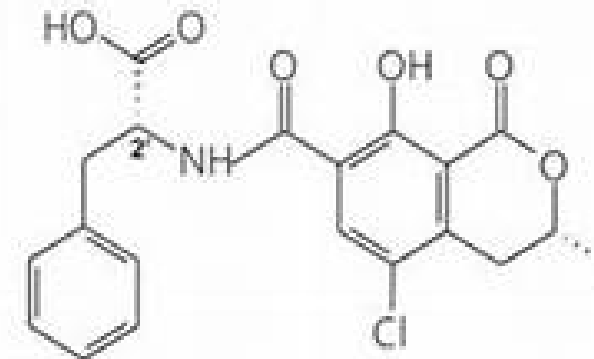
(38 out of 39 > 0.1 mg/kg)



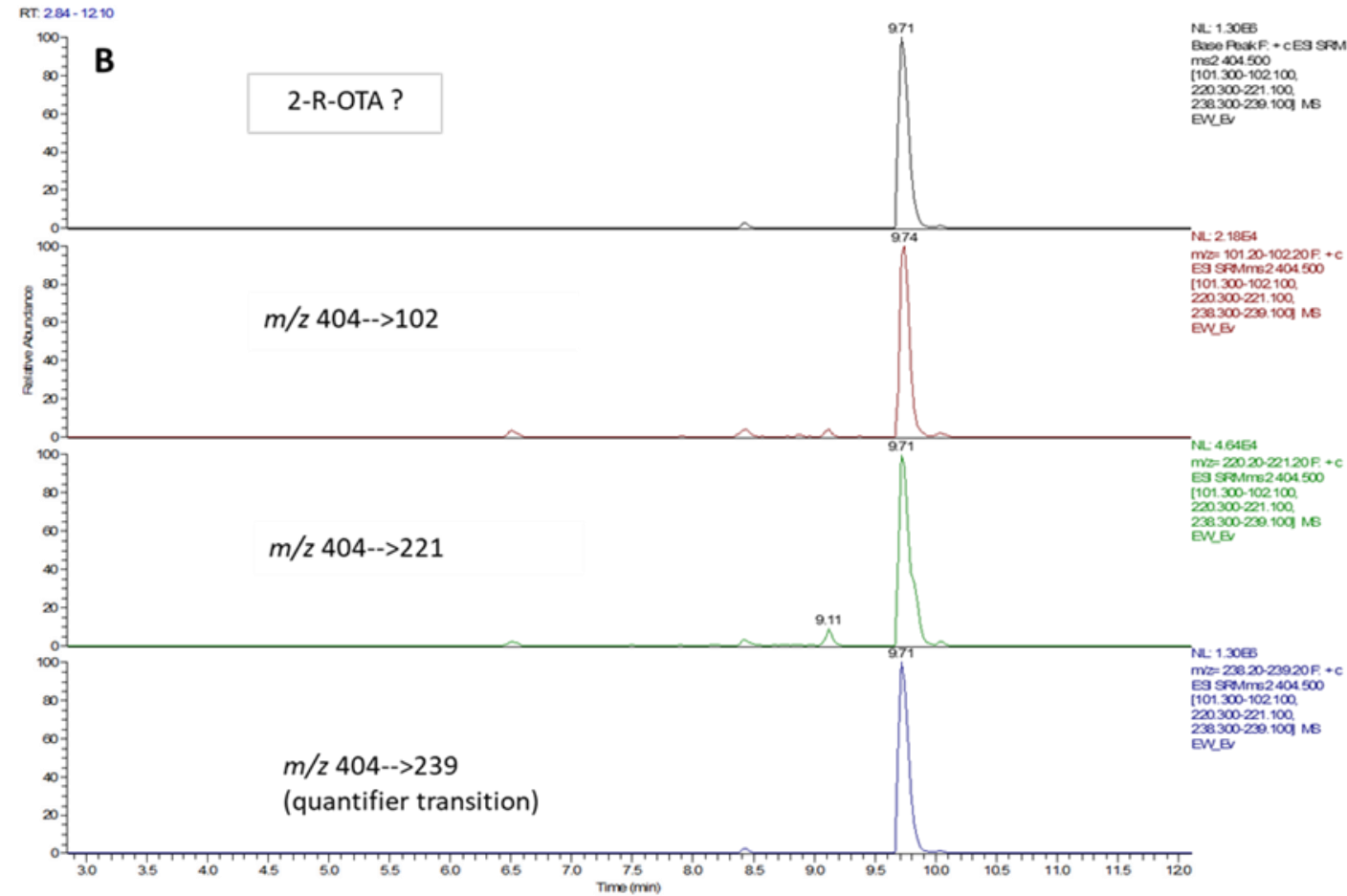
...POSSIBLE OCCURRENCE OF MODIFIED OTA?

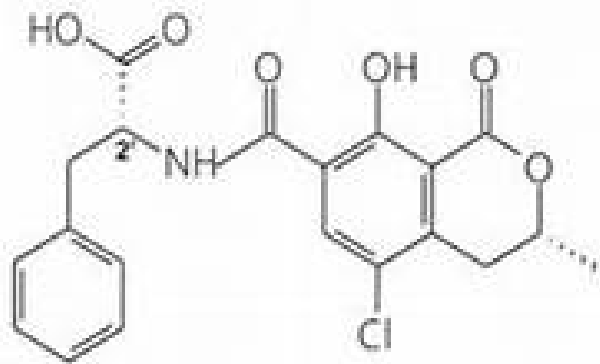


ochratoxin A (OTA)



2'R-ochratoxin A (2'R-OTA)





2'R-ochratoxin A (2'R-OTA)



According to previous studies, it is less toxic than OTA but it can competitively bind to HSA, thus affecting OTA TK








Perhaps from fermentation?

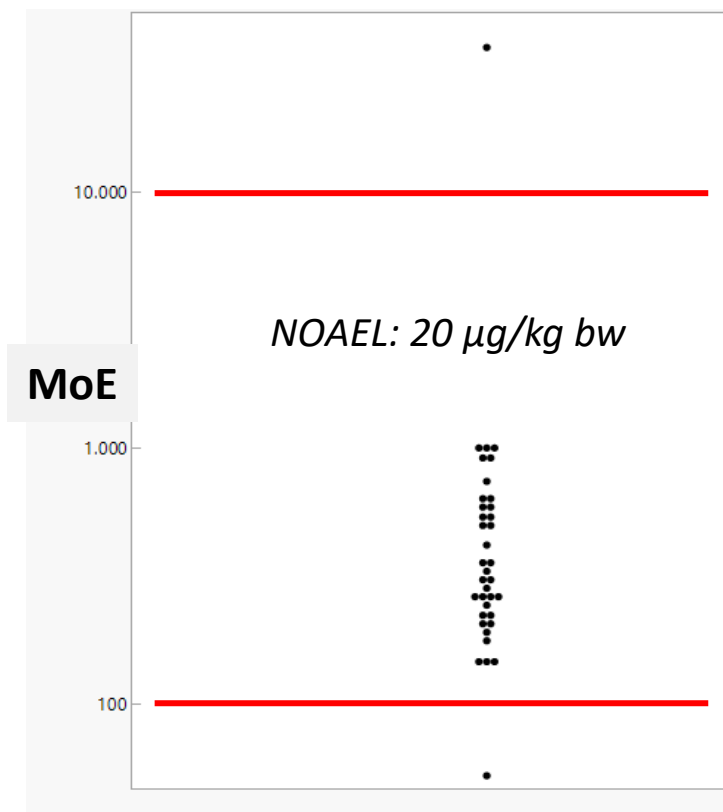
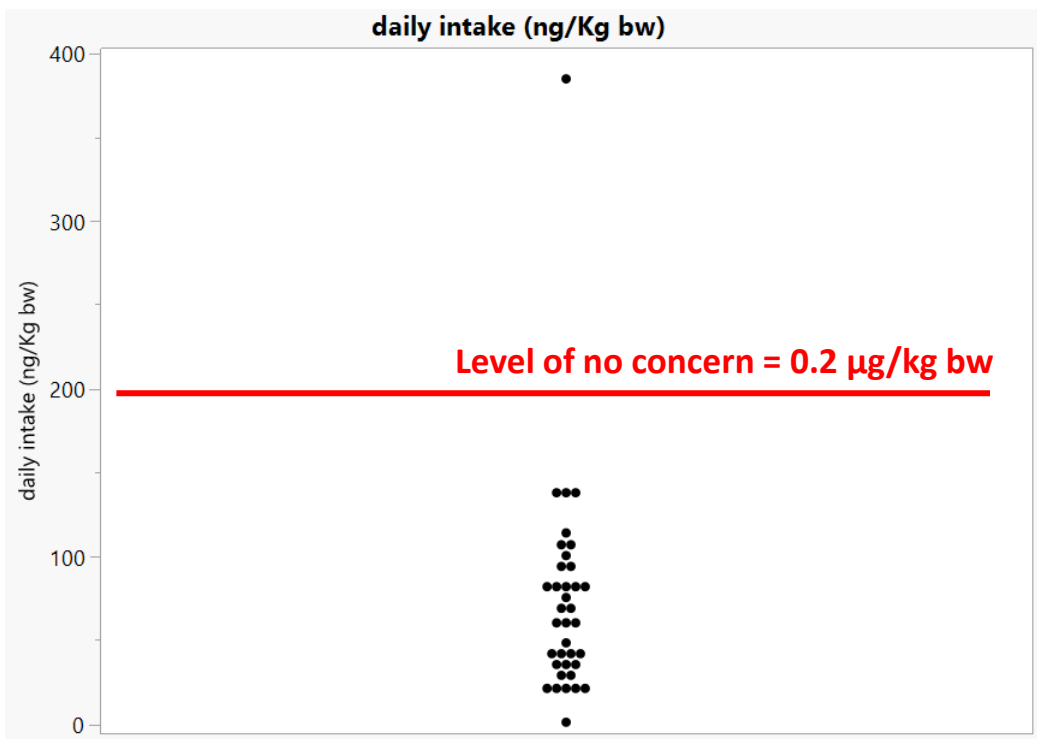
OTA is actually one of the major mycotoxins in rice. It could have been modified by *Monascus*

Article

Interaction of 2'R-ochratoxin A with Serum Albumins: Binding Site, Effects of Site Markers, Thermodynamics, Species Differences of Albumin-binding, and Influence of Albumin on Its Toxicity in MDCK Cells

Zelma Faisal ^{1,2}, Diána Derdák ^{2,3,4}, Beáta Lemli ^{2,3,4} , Sándor Kunsági-Máté ^{2,3,4}, Mónika Bálint ⁵, Csaba Hetényi ⁵, Rita Csepregi ^{2,6} , Tamás Kőszegi ^{2,6} , Franziska Sueck ⁷, Benedikt Cramer ⁷ , Hans-Ulrich Humpf ⁷ , and Miklós Poór ^{1,2,*}

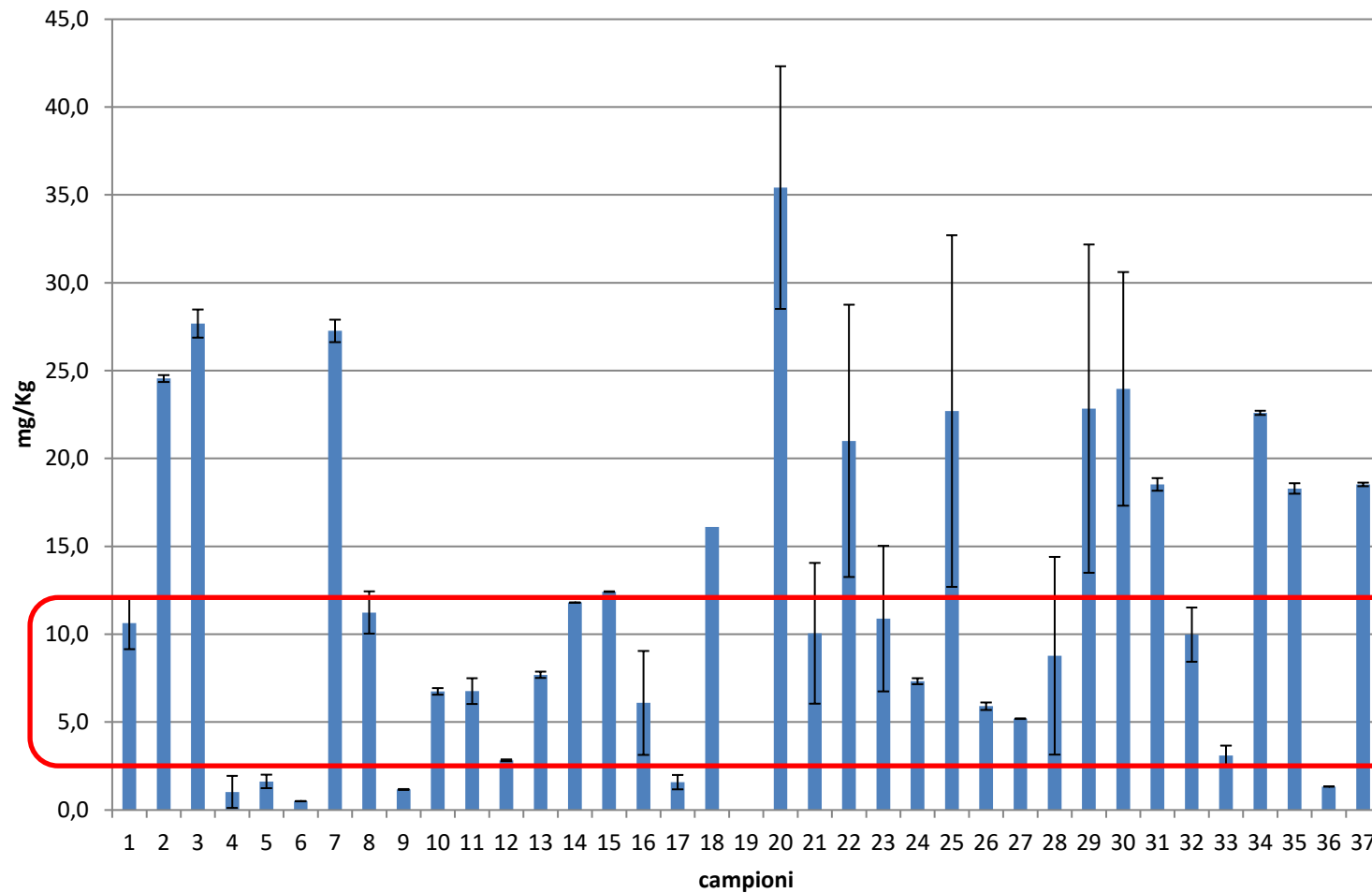
RYR CONTRIBUTION TO CIT DIETARY EXPOSURE



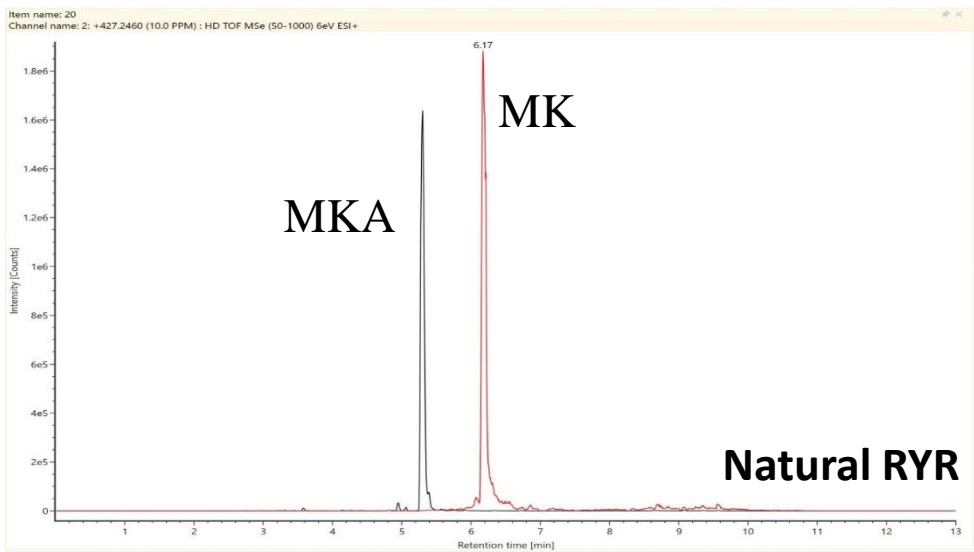
MKA + MK CONTENT IN RYR SUPPLEMENTS

The actual MKA+MK value was highly variable.

Only 5 out of 39 supplements provide a daily dose comparable with the EFSA requirements.



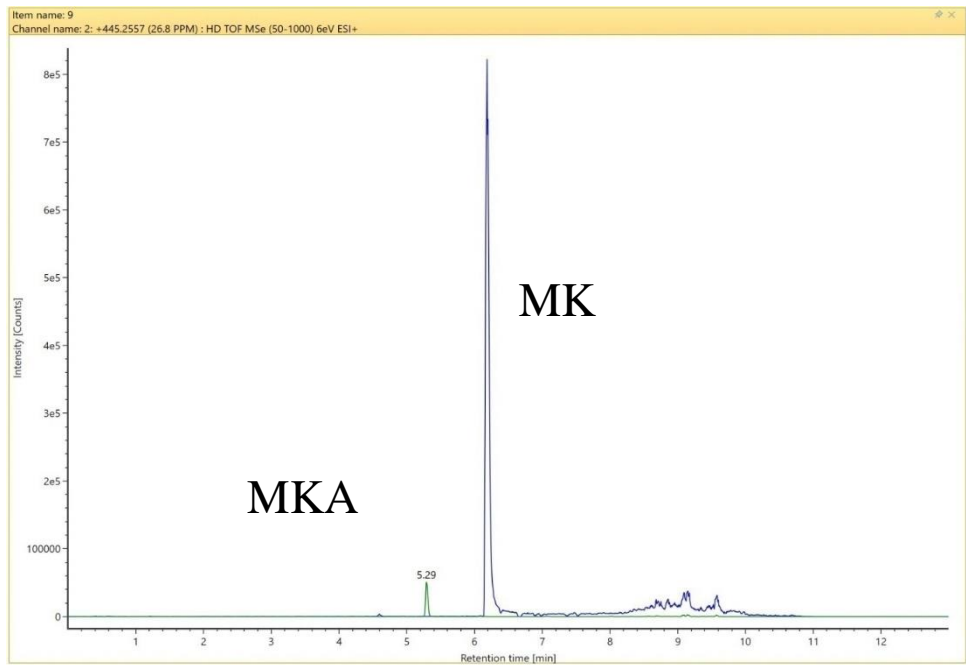
**MKA+MK RANGE
ON THE LABELS**



ANY POSSIBLE ADULTERATION?

During the fermentation process, the form responsible for the inhibition of cholesterol synthesis (MKA) prevails, with a content of approximately 80% on average. The final, drying step in RYR production induces a partial cyclization of MKA. Therefore, in the finished product, the acid: lactone ratio usually varies from 6: 4 to 4:6.

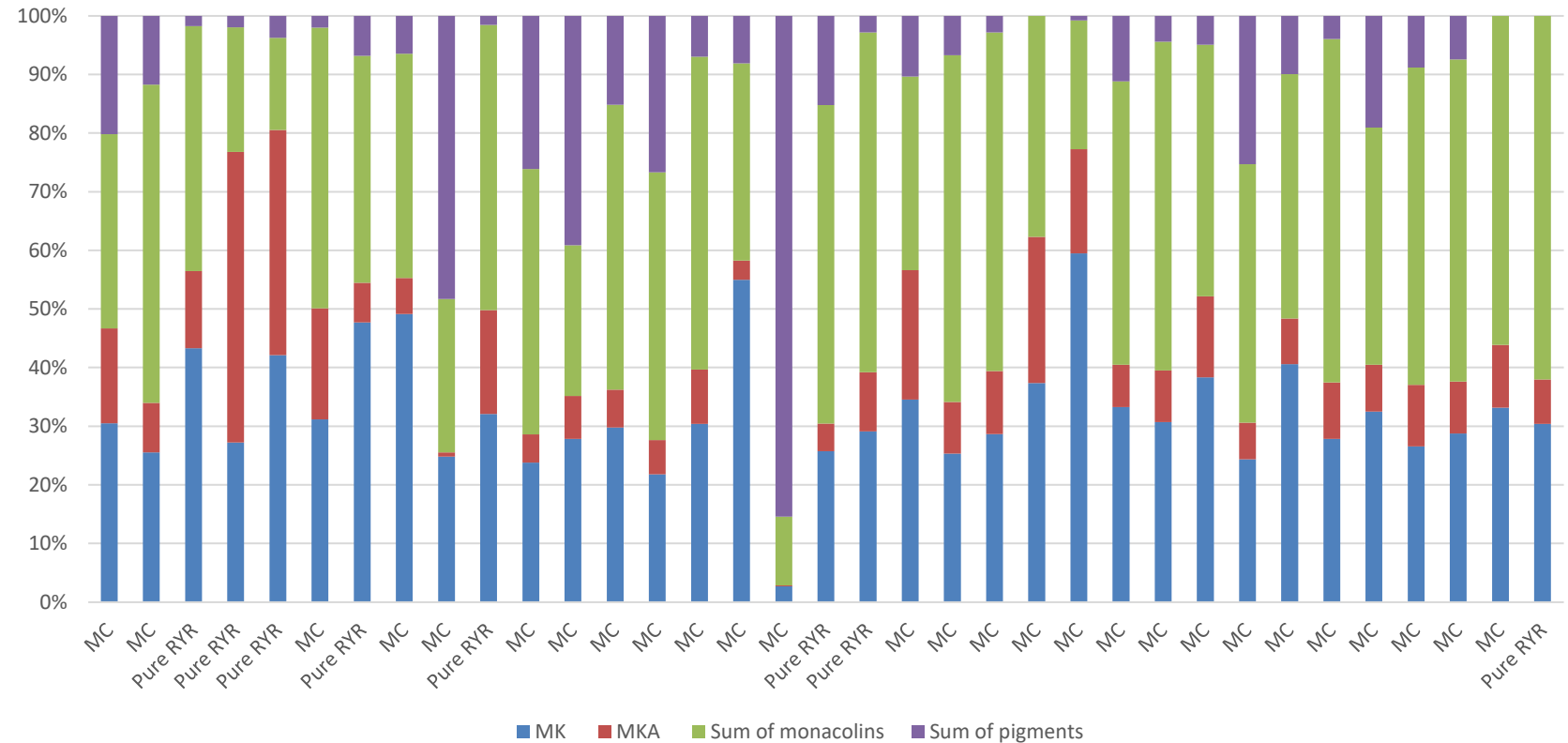
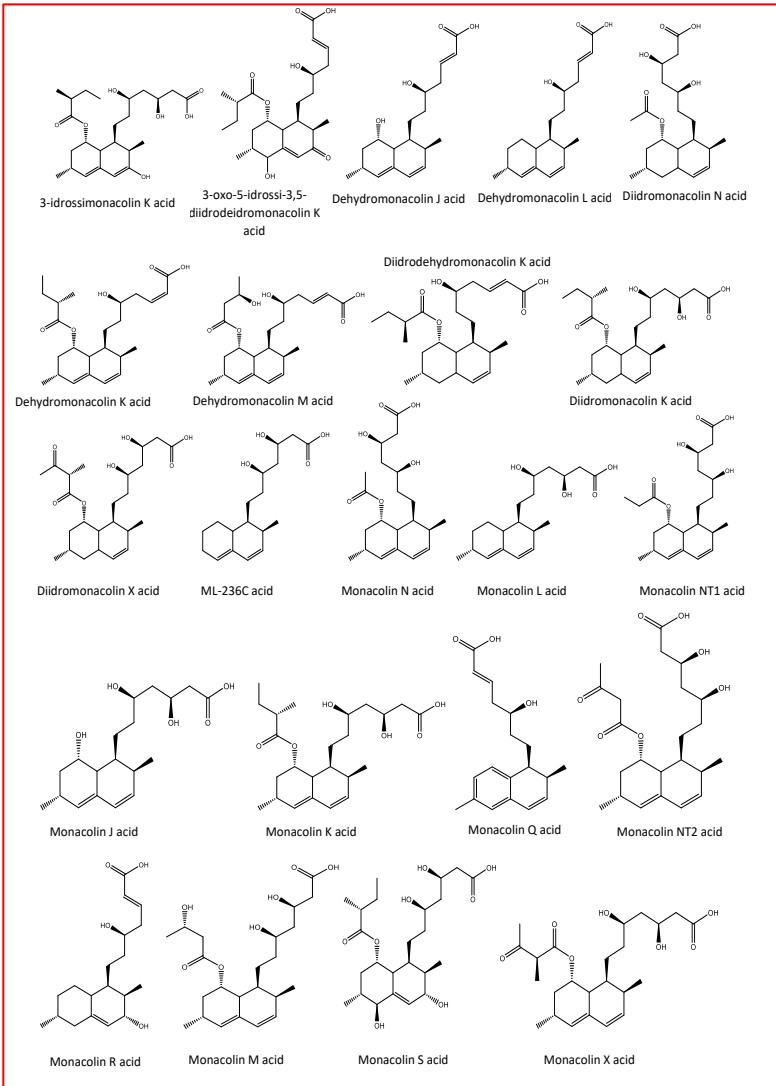
$$\frac{\text{MKA}}{\text{MK} + \text{MKA}} > 0.30$$



80% of the supplements in our study showed a ratio < 0.3 suggesting the potential addition of MK from external sources

OCCURRENCE OF MINOR MONACOLINS AND MONASCUS PIGMENTS

The HR-IMS fingerprint revealed an extreme variability among supplements



Dellafiora et al. 2021, in preparation

TO SUM UP 1/2

RYR supplements are highly variable in terms of MKs composition (→ pharmaceutical GMP?)
Minor MKs may exert some (antagonistic/synergistic) biological activity → need to better understand their role towards HMG-CoA inhibition

Concerning CIT occurrence, although largely cautionary, the MOE approach clearly shows that the exposure to citrinin from RYR supplements may pose a risk for the consumers.

It must be noticed that, compared to food the intake of RYR supplements is less affected by variability and a consequence of their pre-dosed form.

Users, often prompted by health-related issues, take supplements on a regular base according to the suggested dosage, and often choose the same brand over time.

While the exposure assessment to contaminants may vary a lot between mean and high-consumers of a certain food, in the case of RYR supplements the variability – and therefore the associated uncertainty – is strongly reduced.

TO SUM UP

Besides arguments related to public health and regulatory measures, our outcome is even more relevant in terms of communication, when considering that a “citrinin-free” claim was reported on the label of four batches samples in this study.

The halo of naturality of RYR supplements together with the associated “claims”, may prompt the consumers to subjectively increase the daily dosage without any perception of possible risk. Therefore, such unsolicited claims should be strongly mistrusted and carefully regulated

Supplement Facts		
Serving Size: 2 Capsules		
Servings Per Container: 60		
	Amount Per Serving	%DV
Red Yeast Rice powder <i>(Fermentum rubrum)(seeds)</i>	1200 mg	†
† Daily Value (DV) not established		
Other ingredients: Vegetable capsule (hypromellose), rice flour, stearic acid and silica.		
CITRININ FREE - Stringently screened using HPLC laboratory test method to under 1ppm (part per million).		

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