International Association for Food Protection®

WEBINAR

Presents: Mycotoxin Prevention and Control:
Food Processing Mitigation Strategies

ILSI Europe – IAFP webinar on the ‘Mycotoxin Prevention and Control: Food Processing Mitigation Strategies’

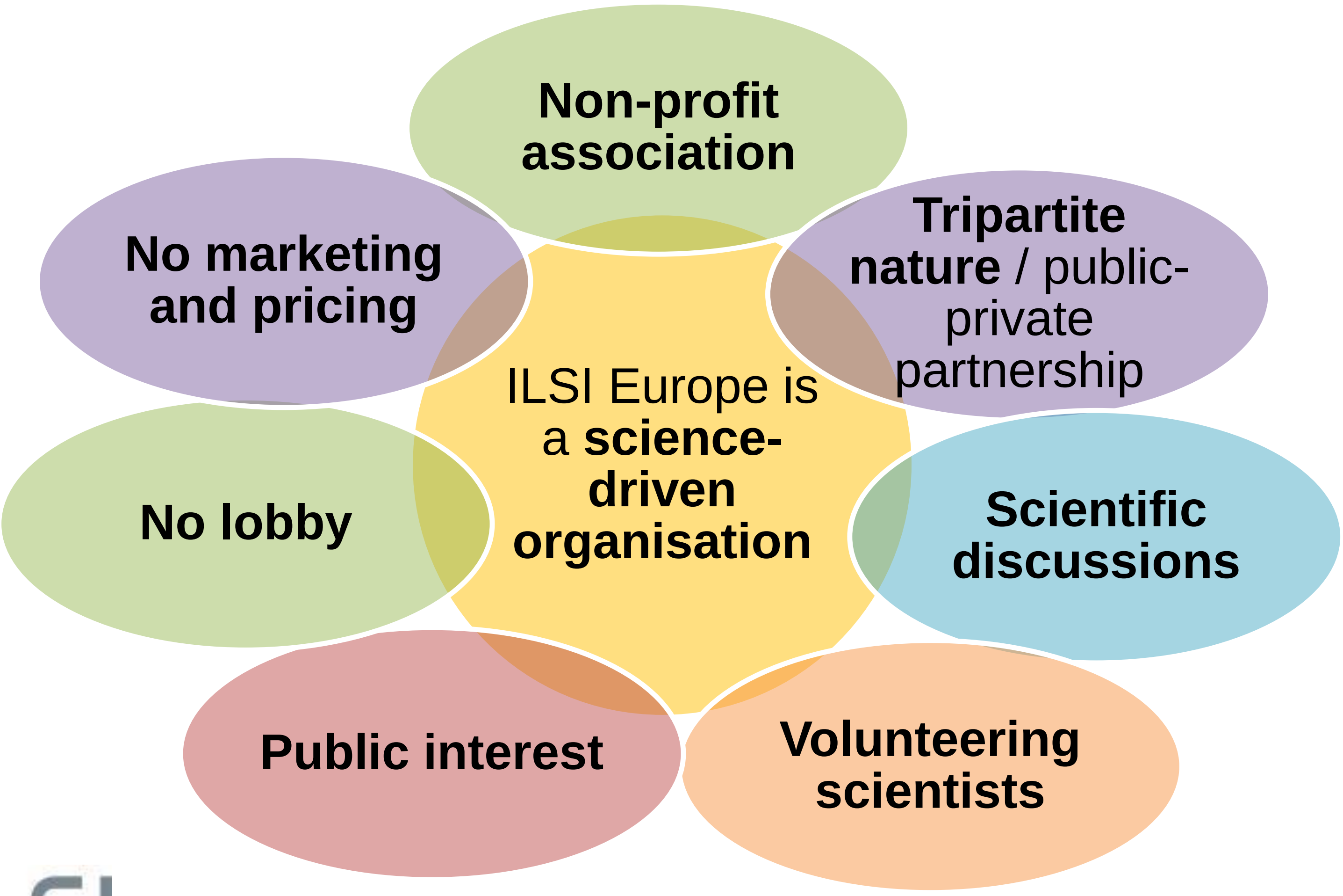
All opinions and statements are those of the individual making the presentation and not necessarily the opinions or views of ILSI Europe or IAFP

ILSI Europe – Vision

We build multi-stakeholder science-based solutions for a sustainable and healthier world.

ILSI Europe

ILSI Key principles



Process-Related Compounds and Natural Toxins Task Force

"We improve scientific knowledge on exposure and mitigation of contaminants in food and help ensure safer food products."

ILSI Europe
International Life Sciences Institute

Process-Related Compounds and Natural Toxins Task Force

Objectives

- Maintain and **improve public health** by advancing the **scientific understanding** of such substances and the magnitude of their **impact on potential risks/benefit to human health**.

- Main areas of focus include consideration of **toxicity**, **exposure, mitigation impact and analytical aspects**, providing a neutral forum for exchange of information and debate.

Process-Related Compounds and Natural Toxins Task Force

Impact

- Developed a **scientific framework on** the risk assessment of **acrylamide** formed during high temperature cooking processes.

- Proactive organisation of two **workshops** in 2009 and 2011 on risk assessment of **MCPD and glycidol esters** involving a wide range of stakeholders. In 2009, indirect and direct methods were developed rapidly. In 2011, analytical methods recently developed were reviewed. Several analytical issues were resolved, allowing for a better understanding of MCPD's impact on metabolism (B.D. Craft, *et al.* 2013 and C. Crews, *et al.* 2013).

- The **manuscript on masked mycotoxins** in food commodities was one of the most accessed in 2013 in *Molecular Nutrition and Food Research*.

Process-Related Compounds and Natural Toxins Task Force

Topics and Activities

Masked Mycotoxins	• Masked Mycotoxins: A Review
MCPD Esters	• Analytical Approaches for MCPD Esters and Glycidyl Esters in Food and Biological Samples – A Review and Future Perspectives
Mycotoxin Mitigation	• Reactions and Potential Mitigation of Mycotoxins during Food Processing
Biomarkers of Contaminant Exposure	• New Approaches to Exposure Assessment of Process-Related Contaminants in Food by Biomarker Monitoring

Expert group activities result in peer-reviewed publications

Reactions and Potential Mitigation of Mycotoxins during Food Processing

Franz	Berthiller	University of Natural Resources and Life Sciences - Vienna
Johan	De Meester	Cargill
Gerhard	Eisenbrand	University of Kaiserslautern
Petr	Karlovsky	University of Gottingen
Irène	Perrin	Nestlé
Isabelle	Oswald	INRA
Gerrit	Speijers	GETS
Michele	Suman	Barilla

International Association for Food Protection (IAFP)

International Association for Food Protection®

- >4,000 **food safety professionals**
- Committed to ***Advancing Food Safety Worldwide®***

International Association for Food Protection (IAFP)

Programme

Moderator: Dr Pierre Dussort (ILSI Europe, BE)

15.00 Introduction
Dr Pierre Dussort (ILSI Europe, BE)

15.05 Impact of Processing Techniques on Mycotoxin Occurrence in Food
Dr Michele Suman (Barilla Advanced Laboratory Research, IT)

15.30 Promising Detoxification Strategies to Mitigate Mycotoxins
Dr Isabelle Oswald (INRA, FR)

15.55 Management of Food Industrial Technologies Reducing Mycotoxins While Keeping the Quality of Finished Products
Dr Johan De Meester (Cargill, BE)

16.20 Q&A

16.30 Closure

IMPACT OF PROCESSING TECHNIQUES ON MYCOTOXINS OCCURRENCE IN FOOD

Dr Michele Suman

Barilla Advanced Laboratory Research

Food Research Labs

Barilla

International Life Sciences Institute

Where our "adventure" started 2 years ago…

Previous Episode

2014

HOW TO REDUCE MYCOTOXIN RISKS THROUGH FOOD PROCESSING?:

AN ILSI EUROPE PERSPECTIVE

What we are talking about today…

FATE OF MYCOTOXINS ALONG INDUSTRIAL PROCESSING… after 2 years of hard work… the picture is now more clear!

ILSI-Europe Process-related Compounds & Natural Toxins Task Force

30th anniversary ILSI Europe

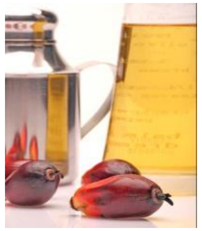
BACKGROUND AND OBJECTIVES

- Although its initial focus was on acrylamide, since few years it has a broader scope covering furan, MCPD (monochloropropane-1,2-diol) esters, mycotoxins and other process-related compounds.
- Process-related compounds and natural toxins may enter the food chain through plant and animal products we eat, either inherently present or generated as result of infection or during preparation and processing.
- Consumers are exposed to naturally occurring contaminants and process-related compounds. Therefore the Task Force designs and implements programs that help to understand how these compounds are formed, improve how we detect and measure them and assess their safety implications.
- Risk/benefit approaches to determine how these compounds may affect human health. Main areas of focus include consideration of toxicity, exposure and mitigation impact, providing a neutral forum for exchange of information/debate

MISSION

We improve scientific knowledge on exposure and mitigation of contaminants in food and help ensure safer food products

Process-Related Compounds and Natural Toxins Task Force

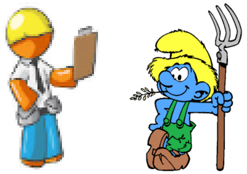


ILSI-EU Expert Group “Reactions and Potential Mitigation of Mycotoxins during Food Processing”

The Challenge/Objectives

- **Fate of mycotoxins during food and feed processing and perspectives of mitigation**; minimize food/feed losses while maximizing the safe use of crops: this is the point.

- The proposed research started from the premise that Good Agricultural Practices (**GAP**) and Good Manufacturing Practices (**GMP**) have been **already optimized**.

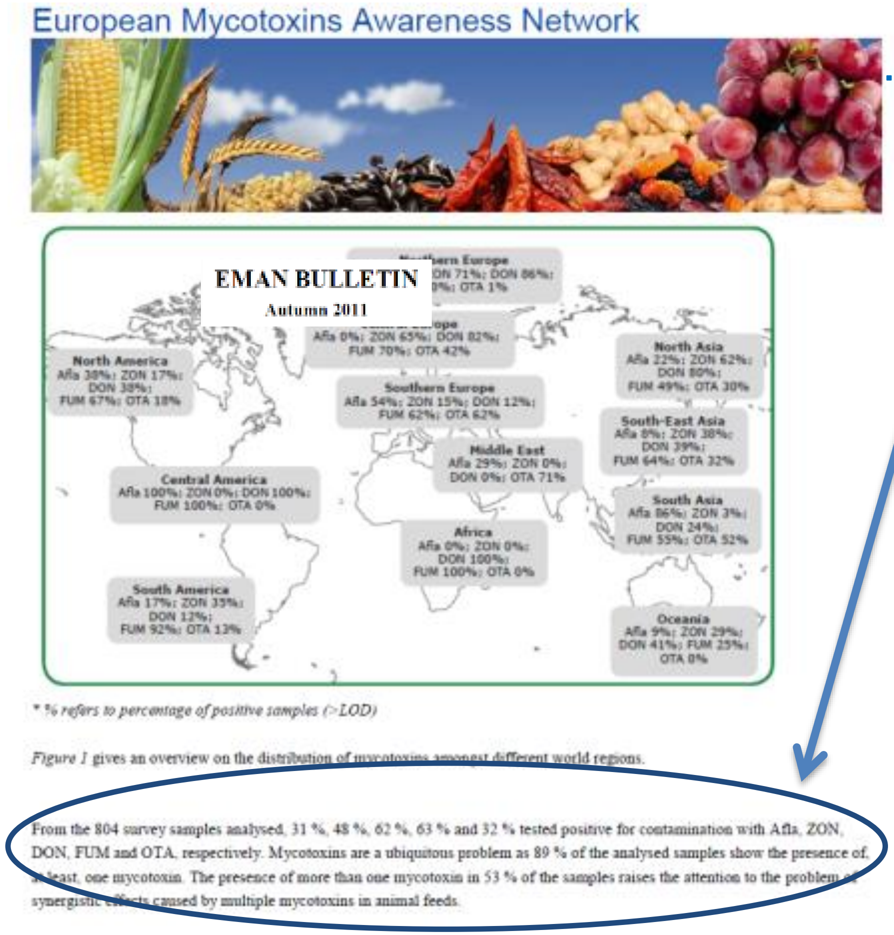


- Reviewing of the **evidence/data on the impact of food processing techniques on mycotoxin decontamination**, moving from raw materials, ingredients to finished products.
  - *Physical (sorting, milling, steeping, extrusion)*
  - *Chemical (acidic, alkaline conditions + high temp)*
  - *Enzymatic/Microbial*
  - *Commodity/process combinations*

- Appropriate management of industrial technologies for **not inducing unfavorable secondary effects in food** (transformation of mycotoxins into other compounds with safety implications or adverse changes in nutrient profiles)

- Different problems depending on the geographical regions taken into account, following an international approach, leaving **open the door to not only EU legislation**. Outlook, **potential new approaches** to mitigation, watch outs

… in a changing scenario …


European Mycotoxins Awareness Network

EMAN BULLETIN
Autumn 2011

** % refers to percentage of positive samples (>LOD)*

Figure 1 gives an overview on the distribution of mycotoxins amongest different world regions.

From the 804 survey samples analysed, 31 %, 48 %, 62 %, 63 % and 32 % tested positive for contamination with Afla, ZON, DON, FUM and OTA, respectively. Mycotoxins are a ubiquitous problem as 89 % of the analysed samples show the presence of, at least, one mycotoxin. The presence of more than one mycotoxin in 53 % of the samples raises the attention to the problem of synergistic effects caused by multiple mycotoxins in animal feeds.

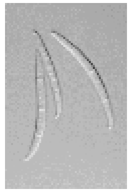
…**more than one** single mycotoxin involved…

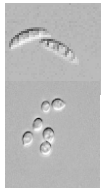
…**more than one** single form involved…

- **MASKED/MODIFIED MYCOTOXINS:** *plants are able to **partially convert mycotoxins** in polar derivatives **via conjugation** with sugars, amino acids or sulphate groups, to compartmentalize in vacuoles*

…**more than** already **known** involved…

- **"EMERGING" MYCOTOXINS:**
  - ✓ ***High contamination** levels in Europe;*
  - ✓ *Enniatins, Beauvericin, Alternaria Toxins,…*
  - ✓ ***Possible synergies** with other mycotoxins;*
  - ✓ *Toxicological role **not completed understood/investigated**;*
  - ✓ *No maximum limits setting*





Our “Vocabulary” about mitigation treatments…

- ❖ ***Transformation:*** *modification of the chemical structure of the molecule*

- ❖ ***Detoxification:*** *transformation which reduced the toxicity*

- ❖ ***Decontamination:*** *removal (from raw materials and/or finished products) or detoxification/inactivation)*

IMPORTANT:

- *Mitigation actions should be irreversible*
- *The processing procedures, agents and microorganisms must be adequate for use in food.*
- *Modified forms of mycotoxins should be affected together with parent compounds*
- *Products should be non-toxic*
- *Food should retain its nutritive value.*

(see also Milani and Maleki 2014 – J Food Sci and Agric 94:2372-2375)

Chemical structures of major mycotoxins modification due to food processing.

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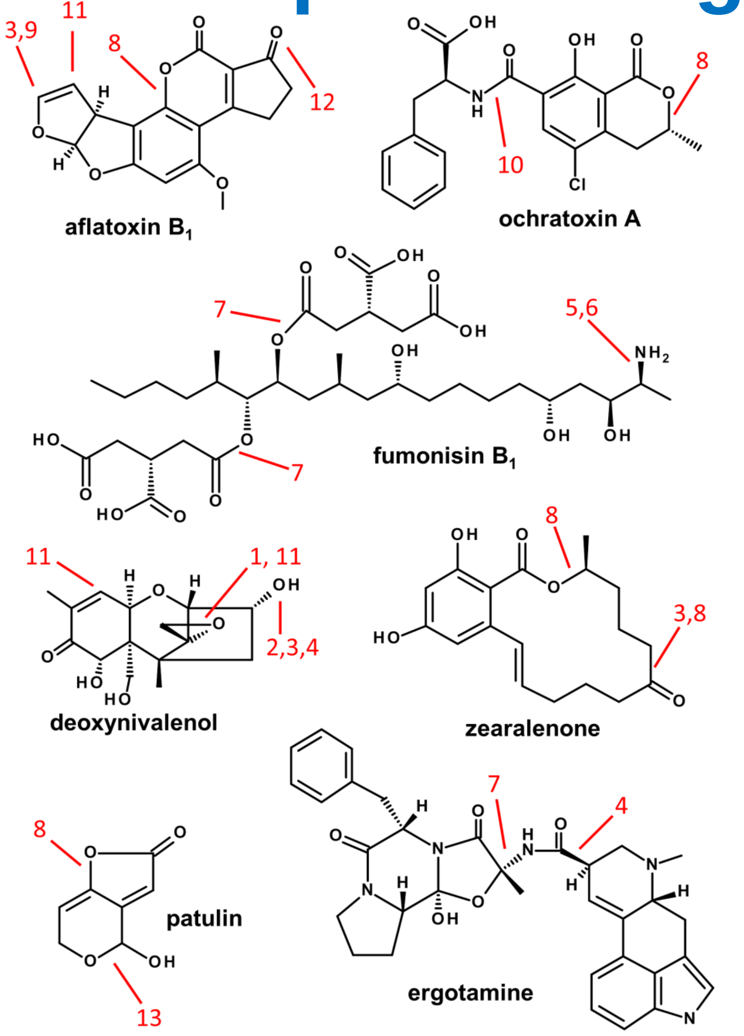

1) deepoxidation,
2) acetylation,
3) oxidation,
4) epimerisation,
5) deamination,
6) glucosylation,
7) hydrolysis,
8) lactone cleavage (hydrolysis),
9) hydroxylation,
10) peptide cleavage,
11) sulfonation,
12) reduction,
13) ether cleavage.

PHYSICAL PROCESSING METHODS examples

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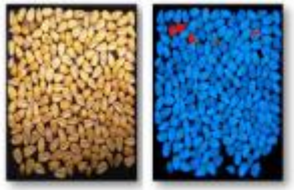
Sorting

Grain sorting *using UV light illumination for* ***aflatoxin reduction*** *is common.*



Sieving-cleaning

Removing kernels *with extensive mold growth, broken kernels and fine materials:. Example:* ***removal of ergot*** *from wheat grains by sieving.* ***Efficiency*** *in reduction demonstrated also* ***for T-2 and HT-2*** *toxins (Schwake-Anduschus et al. 2010).*

Drying

The ***OTA level*** *significantly increases in cocoa beans during transition from fermentation to drying (Dano et al. 2013). Drying must be* ***conducted as rapidly as possible****.*

Washing

Washing barley and corn three times in distilled water reduces the DON content by 65-69%, while ZEN by 2-61%. 1 M ***sodium carbonate*** *solution* ***reduces DON*** *by 72-75%* ***and ZEN*** *by 80-87% (Trenholm et al. 1992).*

Dehulling/Debranning

Dehulling of maize can ***remove up to 93% of aflatoxins*** *(Siwela et al. 2005).* ***DON reduction through debranning*** *also achievable. The* ***concept was extended to*** *masked mycotoxins such as* ***DON-3-glucoside*** *(Kostelanska et al. 2011).*

PHYSICAL PROCESSING METHODS examples

Steeping

Soaking maize for 36-50 hours at 50°C in water containing 0.1 to 0.2% SO_2 to facilitate germ separation and breaking down of protein matrix. ***Fumonisins migrate into steeping water*** *(Canela et al.1996).*

Heating

One of the most important interventions by which industrial processing can act.
Roasting can reduce OTA *in coffee beans by up to 97% (Oliveira et al. 2013).*
Ordinary cooking of rice *contaminated with* ***AFLA results in a reduction*** *of 34%.*
Wholegrain rusks *industrial production: increase in* ***time/temperature reduced DON and D3G*** *content by up to 30% (Generotti et al. 2015).*



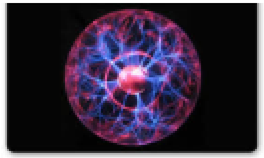

Irradiation

UV light *is very effective in* ***removal of PAT*** *in apple juice and cider.*

IRRADIATED FOODS

Cold Plasma

Selcuk et al (2008) used ***cold plasma to decontaminate grains as well as legumes infected*** *with Aspergillus spp. and Penicillum spp.*
Reduction of up to 99.9% of fungi was observed after 20 min of treatment.



Binders

Mycotoxin binders are a physical technique used for ***feed decontamination*** *(Jans et al. 2014) that principally can also be used in* ***human intervention****.*
However, ***very limited data*** *on the use in food are available.*

CHEMICAL PROCESSING METHODS examples

The use of ***chemicals in combination with physical*** *treatments may* ***increases the efficacy*** *of mycotoxin degradation.*

Chemical treatment *for the purpose of detoxification or decontamination is* ***not authorised within the EU*** *for commodities destined for human food.*

Dedicated mitigation treatments would ***require prior regulatory approval****.*

Common ***food processing*** *technologies may however reduce mycotoxin content as a* ***side effect of accompanying chemical processes.***

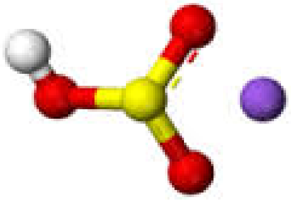
CHEMICAL PROCESSING METHODS examples

Treatment with bases

- ***Ammoniation decreases AFLA in maize*** *by more than 75% (Burgos-Hernandez et al. 2002) & completely* ***decomposes OTA*** *in maize-wheat-barley (Chelkowski et al. 1981).*
- ***Legally approved*** *in USA, Mexico, France, Senegal, Sudan, Brazil.*
- *Ammoniation* ***reduces FB1 in wheat*** *by 79% (Park et al. 1992).*

Use of oxidizing agents

Ozone degradation *has been shown to be effective* ***against DON*** *(Young, 1986) and moniliformin (Zhang and Li 1994).*



Treatment with reducing agents

Sodium bisulfite *(NaHSO3) was shown to* ***drastically reduce mycotoxins****, primarily AFB1 in maize (Doyle et al.1982)*

ENZYMATIC/MICROBIAL DETOXIFICATION examples

- *A **distinguishing feature** of enzymatic detoxification as compared to chemical and physical degradation is its **specificity**.*
- *Notable **exceptions are the activities of laccases and peroxidases**, though they modify a wide range of substrates and **may thus destroy valuable food components**.*
- ***No allergic reaction** to currrent food enzymes has been **reported so far**, indicating a limited concern regarding food allergies (Bindslev-Jensen et al. 2006).*
- *Like other proteins, enzymes added to food are **denatured and hydrolyzed during digestion.***
- *Although enzymes are **commonly used in food production** worldwide, **no enzyme has so far been authorized in EU to reduce mycotoxin** contamination **in food**.*

ENZYMATIC/MICROBIAL DETOXIFICATION examples

Malting & Brewing

*Adding **enzymes detoxifying DON** to amylases, glucanases, amyloglucosidase, proteases and other enzymes used **in beer production** (Aastrup and Olsen, 2008) is compatible with current brewing technology.*

Juices

***Production of juices** involves treatment with pectinases/arabanases, cellulases, glucoamylases and other enzymes. **Enzymatic activities degrading PAT** have been found in many species of bacteria and yeast (Zhu et al. 2015): degradation*

Dairy

*Manufacturing of many **dairy** products involves fermentation with lactic acid bacteria. **Detoxification of aflatoxins by lactic acid bacteria** has been studied for three decades*

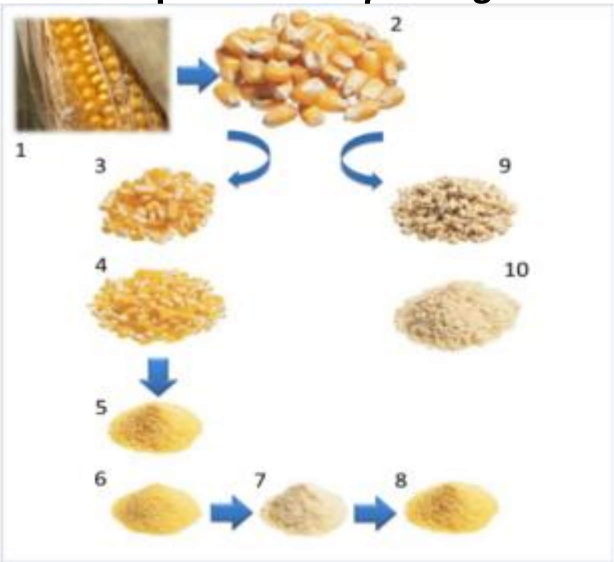
Bakery

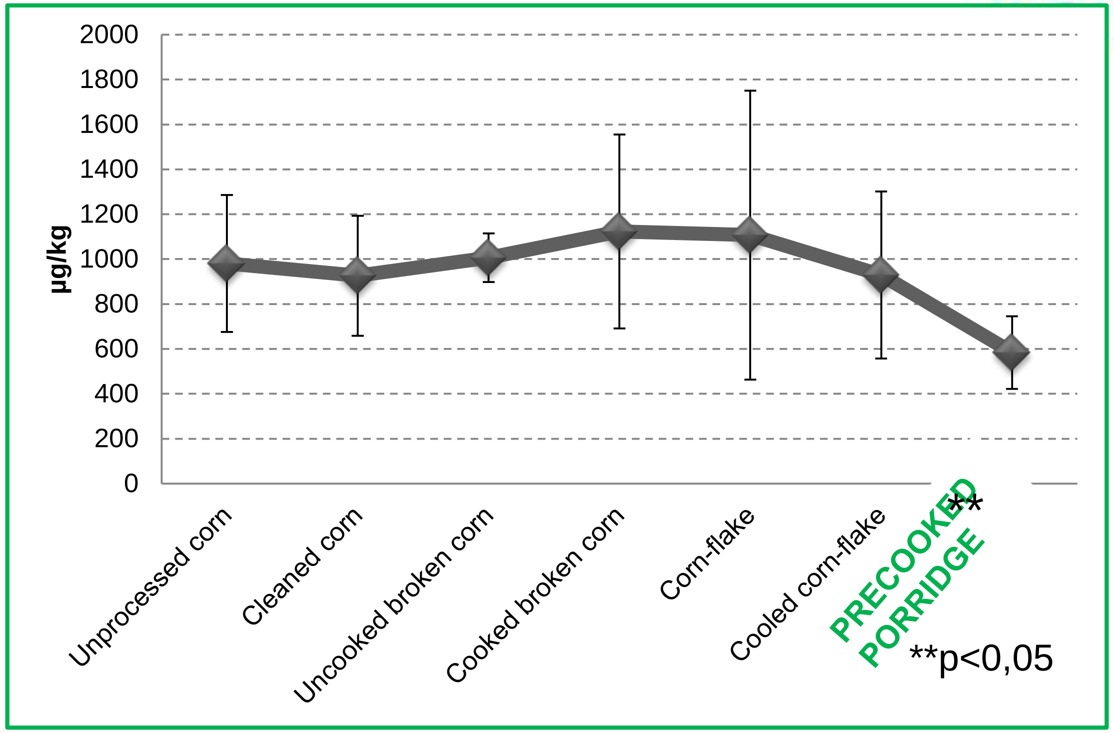
***Protease & xylanase released additional DON during kneading and fermentation** of dough (Simsek et al. 2012).*

Parallel aspects – byproducts
About cornmeal processing…

FUMONISINS mitigation

Cornmeal processing from caryopsis to pre-cooked *porridge*



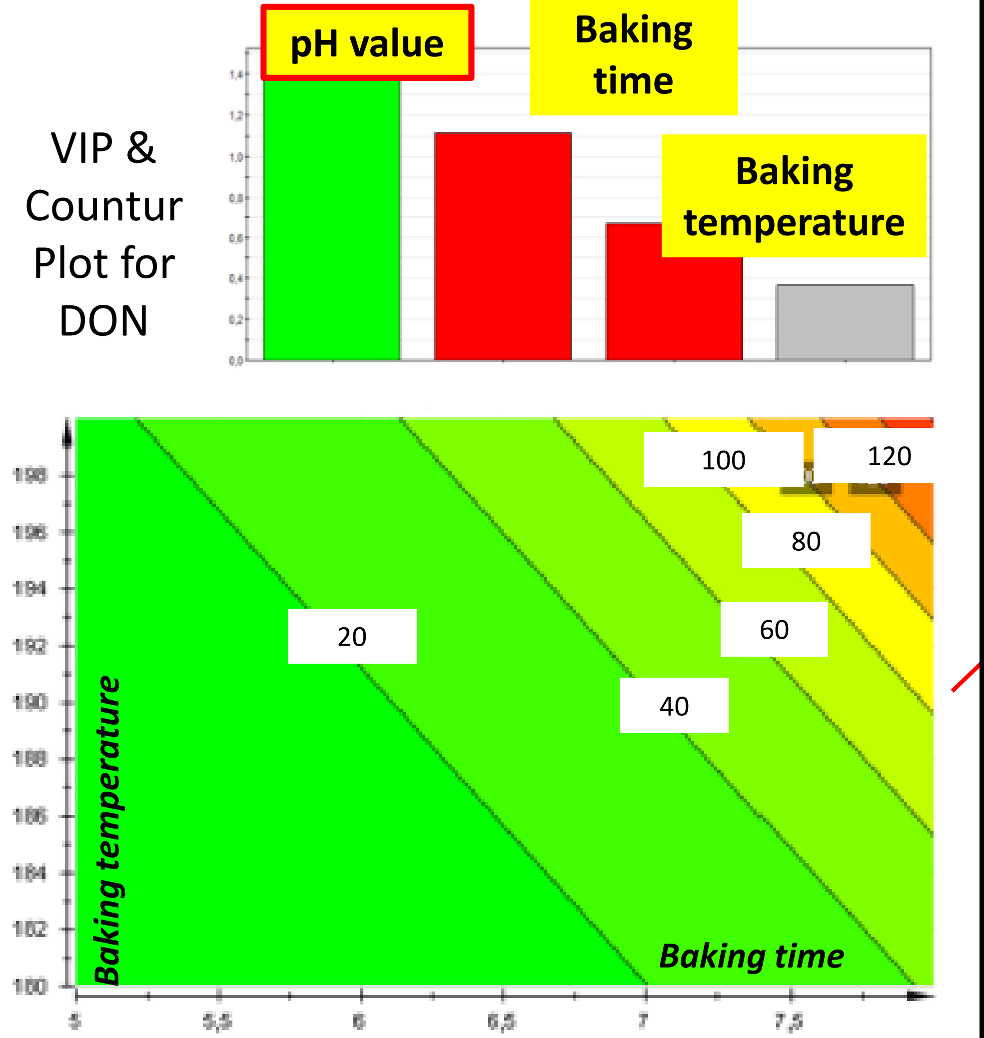


MIDDLINGS Contamination is 6 times higher than raw material


Up to 40% Reduction in PORRIDGE (vs starting contamination) in the finished product


Parallel aspects - processing contaminants
About biscuits processing…

DON mitigation



JUNE 2015

ACRYLAMIDE IN FOOD

Finished product 500 ppb

Minimum Acrylamide level: 137 ppb
Maximum Acrylamide level: 215 ppb

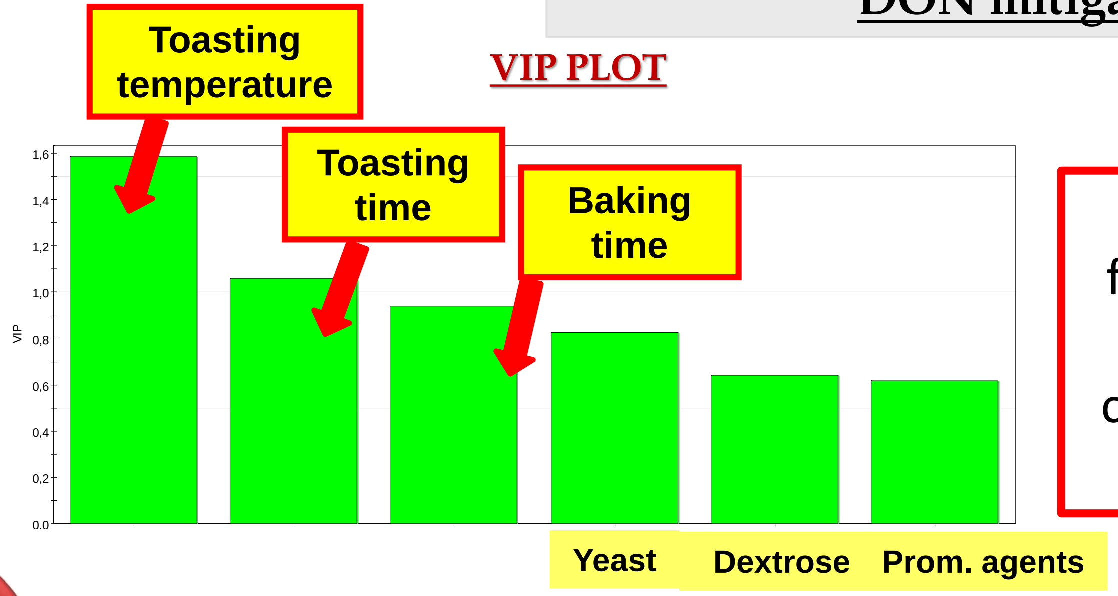
BAKING TIME/TEMPERATURE*, *within a potential 40% DON mitigation range, influence in a negligible way the acrylamide content

Suman et al. – Results under submission for publication

Parallel aspects - processing contaminants
About rusks processing…

DON mitigation

VIP PLOT



Not reliable as finished product to be commercialized : **overcooked**

RUSKS

Step	DON REDUCTION %	
	Reliable	Maximum
Total process	23	76
Baking step	5	30
Toasting step	19	30

About rusks processing…

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DON mitigation

RUSKS

Toasting temperature

VIP PLOT

Toasting time

Baking

Not reliable as finished product to be commercialized : overcooked

THERMAL OVERALL EFFECT ON RUSK PROCESSING

A **potential reduction** up to **30/35%** may be still within an organoleptical **acceptable range**

Not reliable finished prod… to be commercializ… uncooked

		…TION %	
		…m	Maximum
			76
Baking step	5	8	30

Generotti, S.; Cirlini, M.; Malachova, A.; Sulyok, M.; Berthiller F.; Dall'Asta, C.; Suman, M.
"Deoxynivalenol & Deoxynivalenol-3-Glucoside Mitigation through Bakery Strategies: Effective Experimental Design within Industrial Rusk-Making"
Toxins **2015**, 7, pp. 2773-2790

IMPLICATIONS & OUTLOOK

- ***Food processing can reduce mycotoxin exposure*** *by destroying, by transforming into less toxic derivatives, by adsorbing to solid surfaces or by reducing bioavailability due to chemical attachment to food matrix structures*

- ***Analytical tools*** *for mycotoxins transformed by processing (structural modification, binding to food matrix) need* ***to be further developed***

- ***Complete elimination*** *of mycotoxins from food product by processing can* ***rarely be achieved***

- *Several processing techniques of* ***proven value (mostly physical treatments)*** *have been in use for a long time. These are the only mycotoxin mitigation methods currently* ***applicable to human food***

- *While physical techniques currently offer the most efficient post-harvest reduction of mycotoxin content in food,* ***biotechnology possesses the largest potential*** *for future developments*

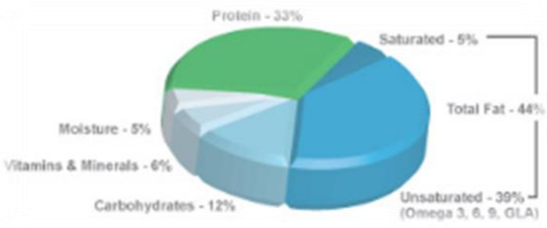
- *Few* ***chemical/biotechnological techniques*** *have been approved for animal feed but many promising strategies remain* ***at an experimental stage****, especially looking onto the human side*

IMPLICATIONS & OUTLOOK

- *Novel physical/chemical treatments (e.g. cold plasma,..) and novel detoxification agents (e.g. microbes, enzymes,..) have to undergo **regulatory approval, which implies a complete risk analysis**. EU regulation 1881/2006 provides direction*

- *In the absence of adequate toxicological data, mycotoxin forms generated during processing must be assumed to have **same toxicity/bioavailability/carcinogenic potency as parent compounds**.*

- ***Impact** of mycotoxin mitigation processes on the **nutritional composition and organoleptic quality** and influence on **other contaminants** (e.g. acrylamide, MCPDs…) have to be assessed.*



- *The large number of combinations of processing/commodities/mycotoxins calls for **prioritisation**: Criteria should be **consumption** - staple foods and commodities consumed by sensitive population groups like young children, **occurrence** in such commodities & unfavourable **toxicological profiles***

- ***Different geographical regions and target groups require different prioritizations**.*

ILSI-EU Expert Group “Reactions and Potential Mitigation of Mycotoxins during Food Processing”

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Petr Karlovsky[1], Michele Suman[2], Franz Berthiller[3], Johan De Meester[4], Gerhard Eisenbrand[5], Irène Perrin[6], Isabelle P. Oswald[7, 8], Gerrit Speijers[9], Alessandro Chiodini[10], Tobias Recker[10], Pierre Dussort[10]

Impact of food processing and detoxification treatments on mycotoxin contamination

ILSI Europe

Process-related Compounds and Natural Toxins

The Group

Expert Group – New
Reactions and Potential Mitigation of Mycotoxins during Food Processing

OPEN ACCESS

Mycotoxin Res
DOI 10.1007/s12550-016-0257-7

REVIEW

Impact of food processing and detoxification treatments on mycotoxin contamination

Petr Karlovsky[1] · Michele Suman[2] · Franz Berthiller[3] · Johan De Meester[4] · Gerhard Eisenbrand[5] · Irène Perrin[6] · Isabelle P. Oswald[7,8] · Gerrit Speijers[9] · Alessandro Chiodini[10] · Tobias Recker[10] · Pierre Dussort[10]

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Thanks for your kind attention

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International Life Sciences Institute

Promising detoxification strategies to mitigate mycotoxins

Dr Isabelle OSWALD

ToxAlim – Research Center in Food Toxicology

Mycotoxins: a global threat

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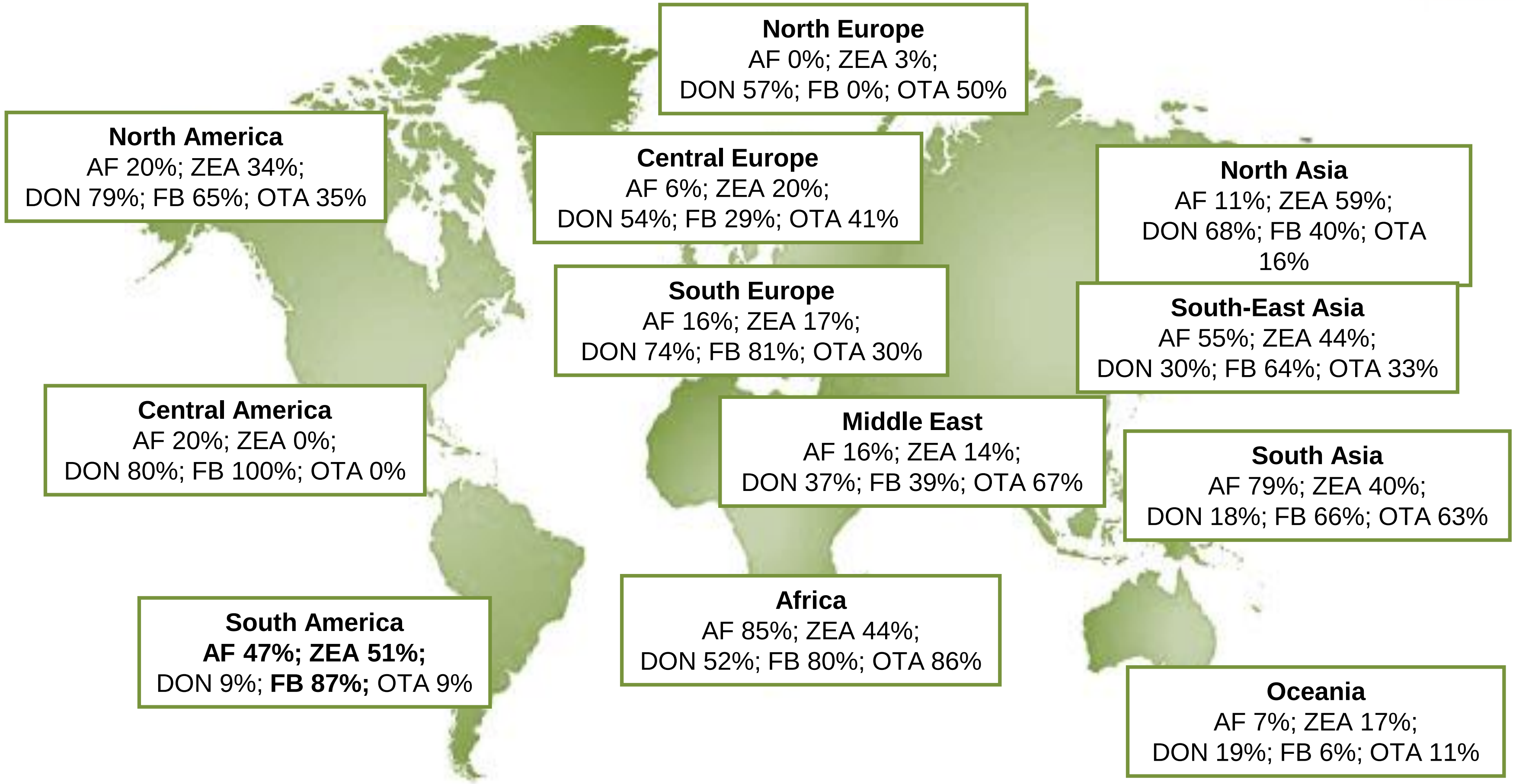


Need for strategies to mitigate mycotoxins

Overview

- **Mitigation by processing:** Dr Suman presentation
- **Two new aspects**
  - *Natural products to limit mycotoxin synthesis*
  - *Toxicity of metabolites formed during detoxification*

Part 1: Use of natural products to inhibit mycotoxin synthesis

Examples of eugenol and other natural products

Mitigation of mycotoxins

Prevention

Limiting fungal development

- Agricultural practice
  - *Plowing*
  - *Storage*
- Fungicides
- Bio-control: non-toxic strains

Post-contamination

Decontamination

- Physical elimination
- Mycotoxin detoxifying agent
  - *Reduced absorption*
  - *Biotransformation*

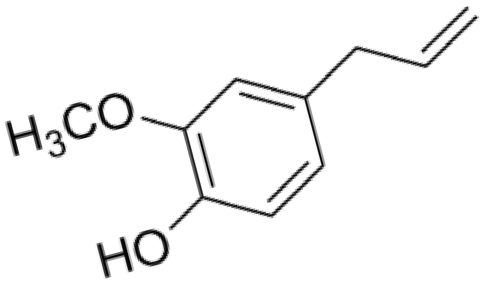
Natural extracts

Alternative strategies to mitigate mycotoxin

Screening for natural products to reduce aflatoxin contamination

Eugenol, a natural product

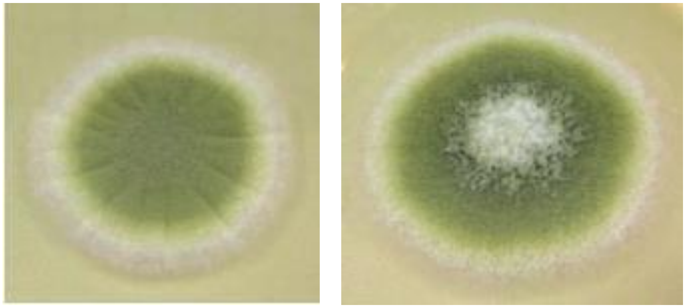
Eugenol

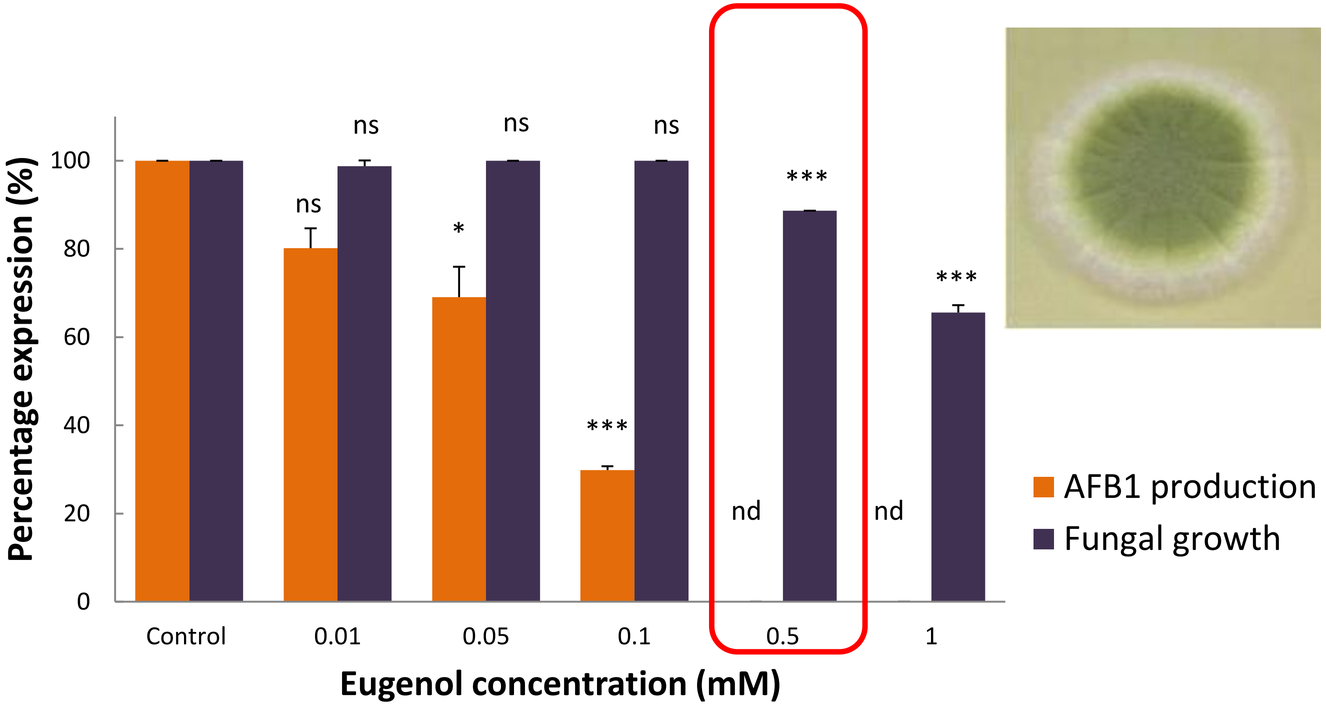


- Eugenol (4-allyl-2-methoxyphenol)
- Source: clove, cinnamon, chili peppers, basilic
- AFB1 Inhibition (*Hitokoto et al*. 1980, *Shan et al.* 2005, *Liang et al*. 2015, *Jahanshiri et al*. 2015)
- Mechanism of action yet to be elucidated

Effect on fungal growth and AFB1 production

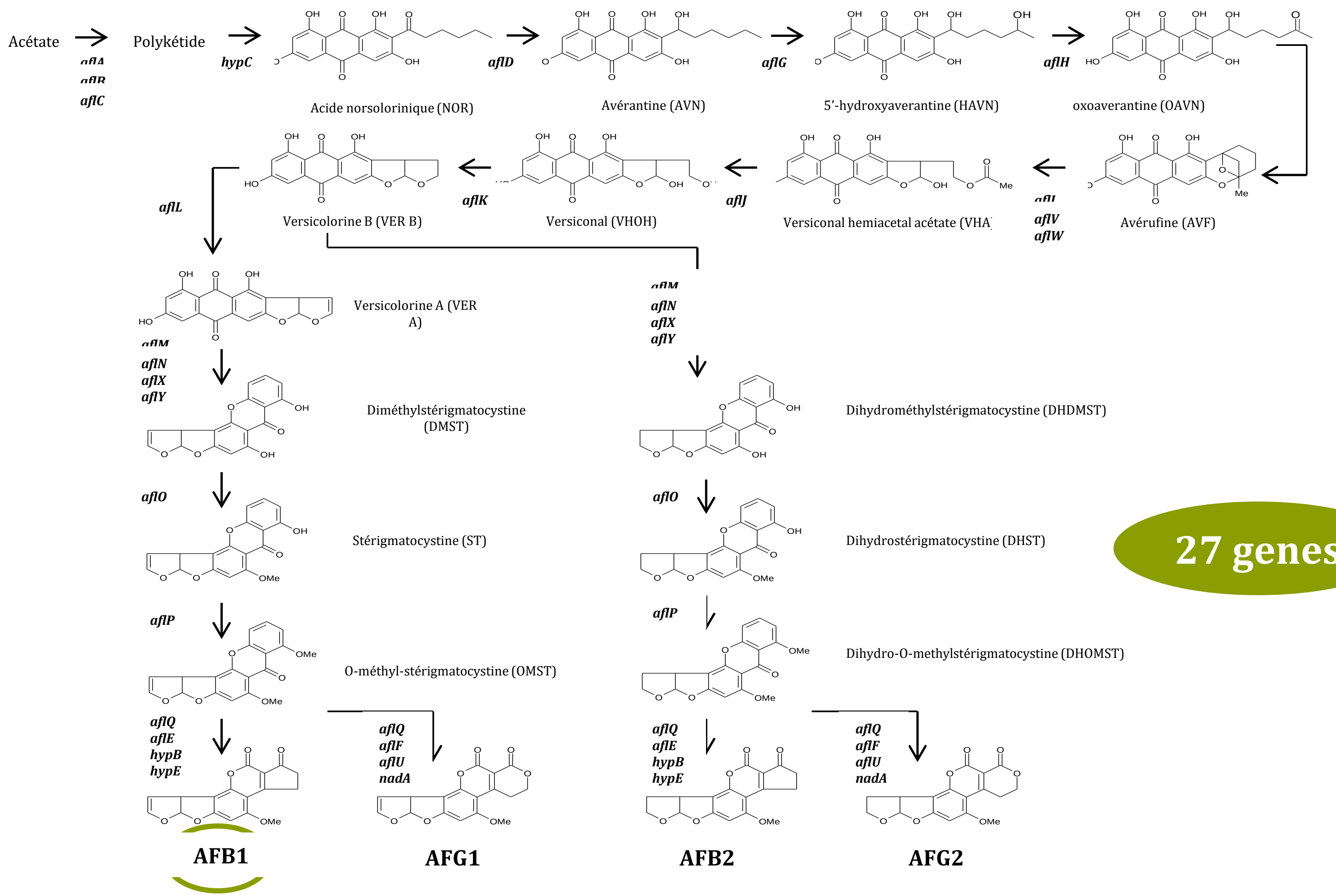
Aspergillus flavus NRRL 62477





Eugenol concentration reducing mycotoxin production while not affecting fungal growth.
If the humidity/temperature conditions are favorable for fungal growth, fungi will grow.

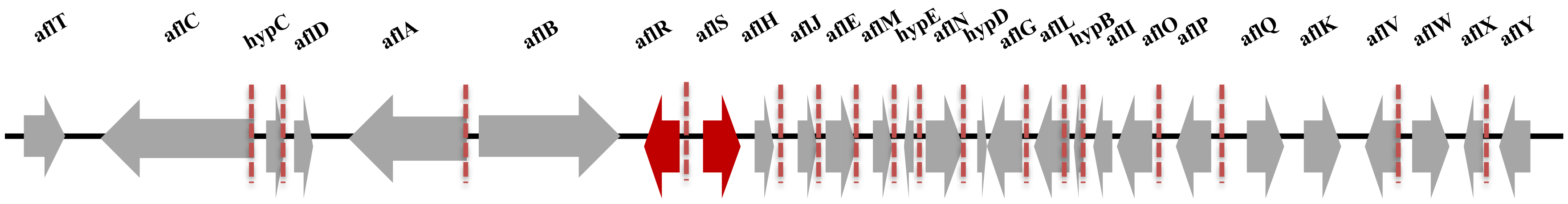
The Aflatoxin biosynthetic pathway



27 genes

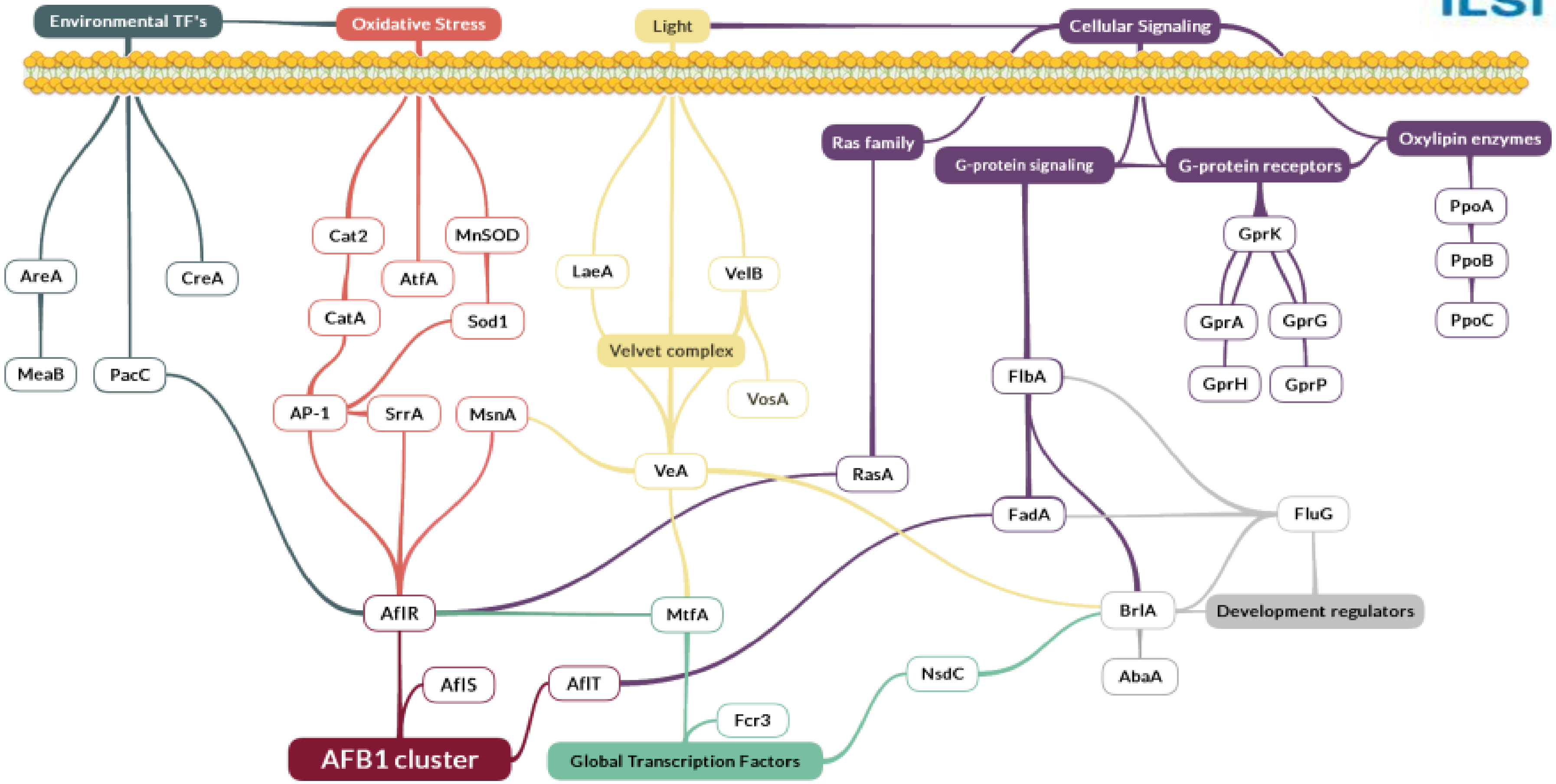
The Aflatoxin biosynthetic pathway

- A well known genome: 29 genes cluster within 2 intern regulators AflR and AflS



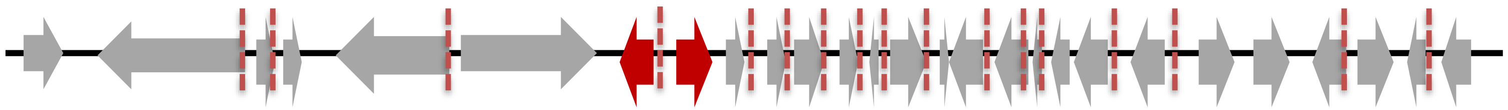
- Other external regulatory factors belonging to different families: fungal development, secondary metabolism regulators, transcription factors, environmental changes, oxidative stress, cellular signalization, etc.

Regulation of Aflatoxin biosynthesis



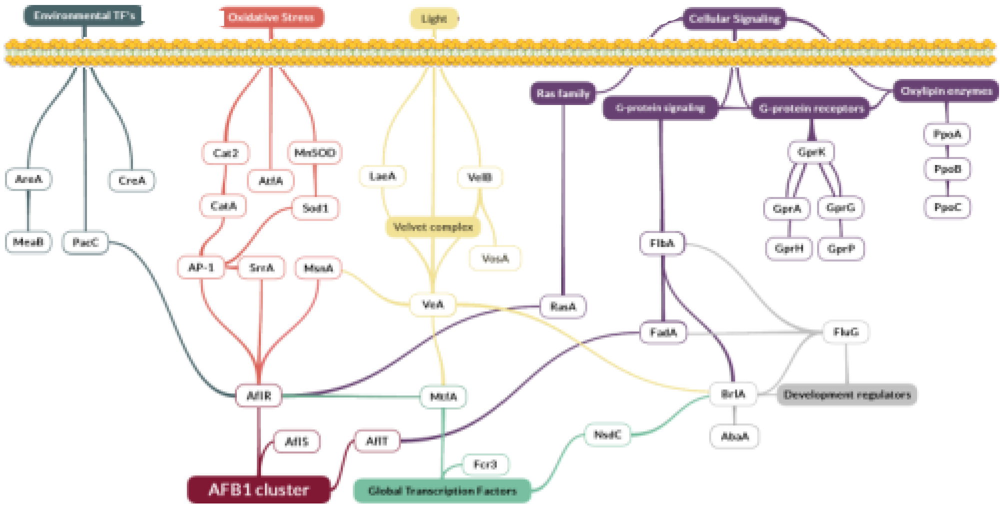
Construction of a q-PCR tool

Development of a large scale q-PCR molecular tool to investigate the mode of action of natural **60 genes**

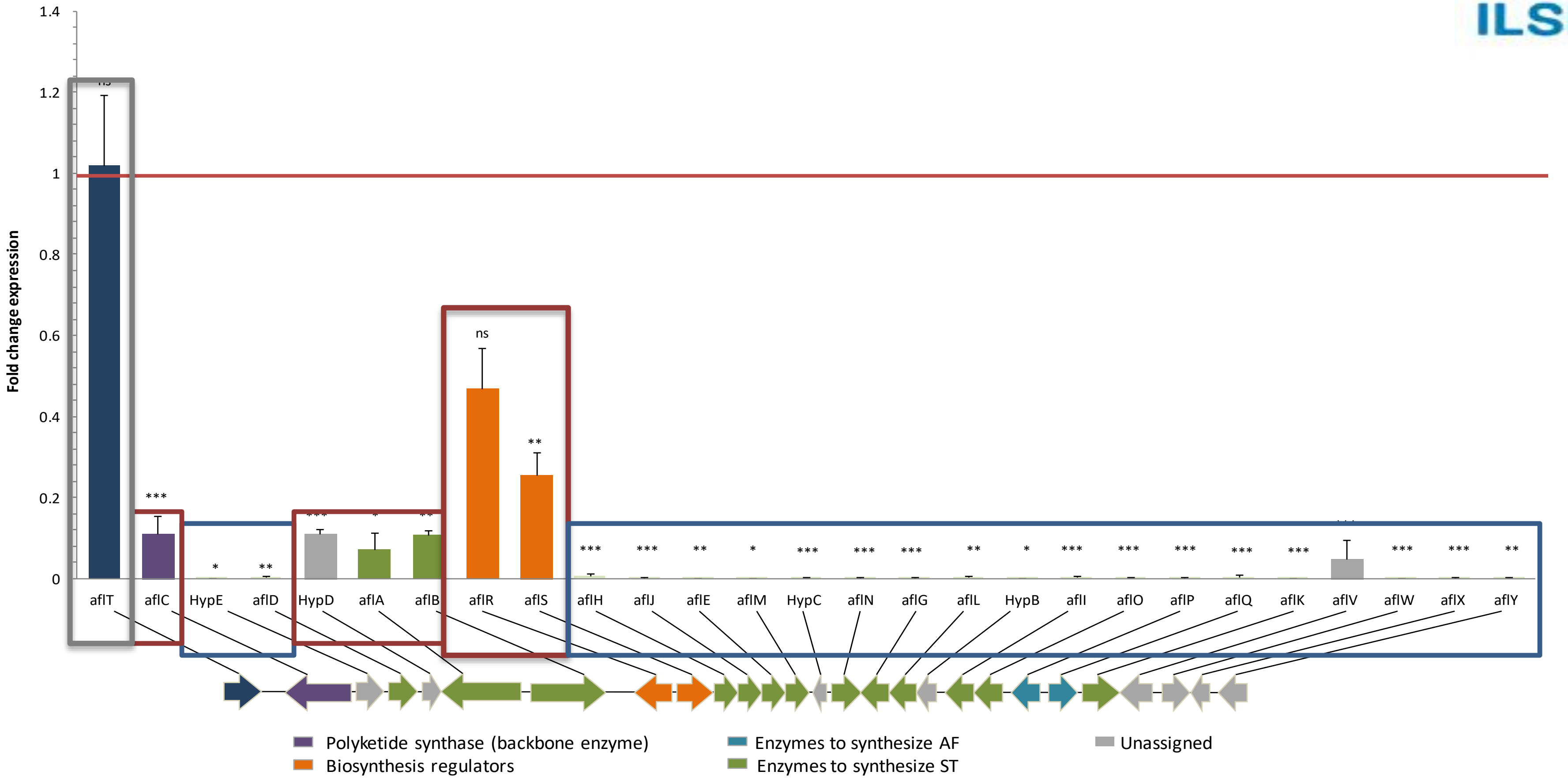


27 genes
AFB1 cluster

33 genes
involved in AFB1 regulation (fungal development, secondary metabolism regulators, transcription factors, environmental changes, oxidative stress, cellular signalisation)

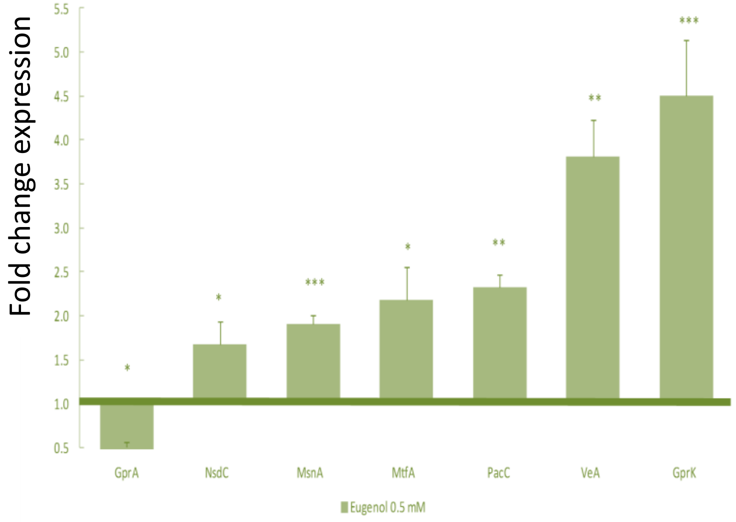


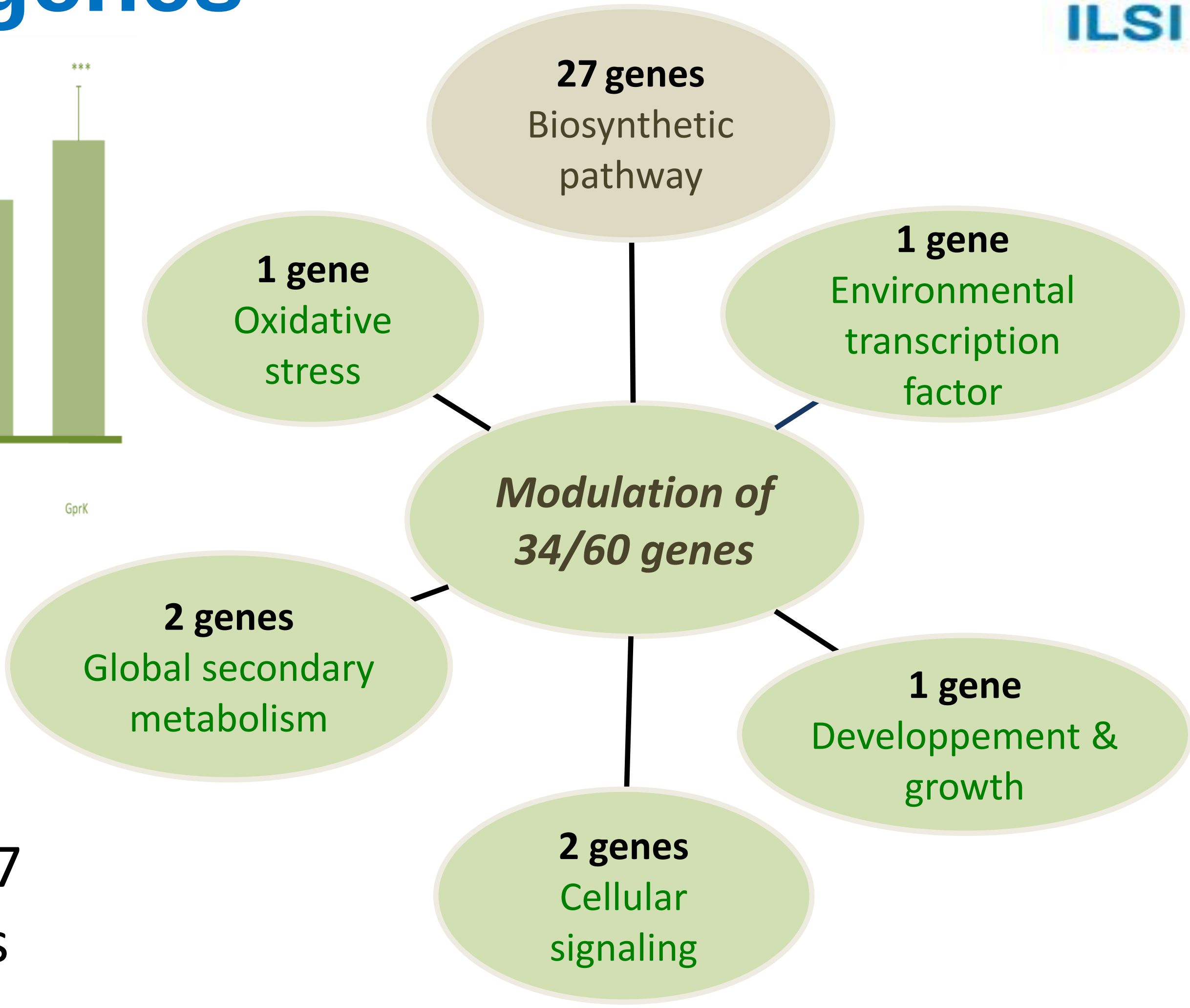
Effect of eugenol on the Aflatoxin cluster



Eugenol inhibits 27 genes of the aflatoxin cluster

Effect of eugenol on the regulatory genes





Eugenol inhibits 7 regulatory genes

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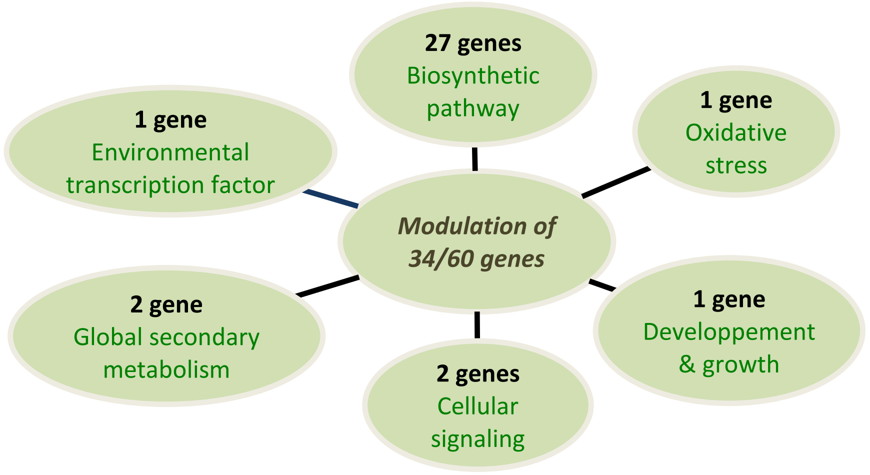
Eugenol: conclusion

- Eugenol inhibit Aflatoxin production while not affecting fungal growth

- It acts at the transcriptomic level, blocking the biosynthetic pathway



- Blockade of the biosynthetic pathway occurs at a very early stage; indicating that eugenol will not induce any aflatoxin metabolite production

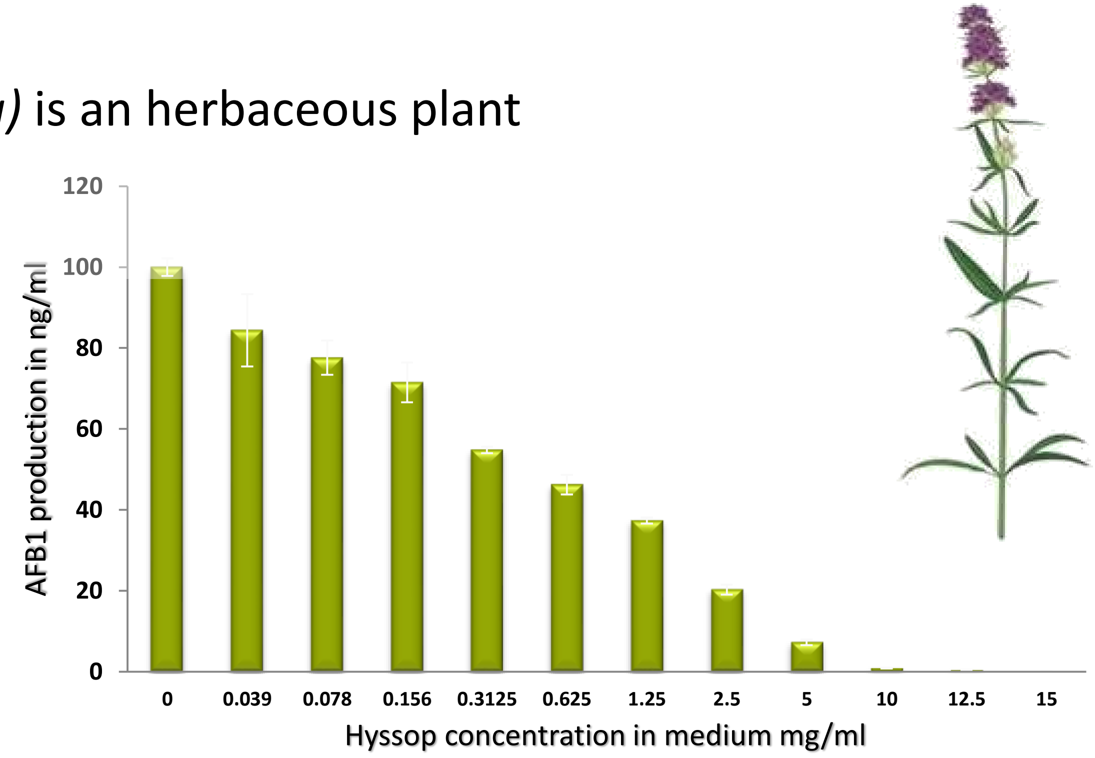
Caceres *et al.*, 2016

Screening for natural product inhibiting AFB1 production

- Screening of 20 medicinal Mediterrenean plant extracts

- Among them, Hyssop was the most potent in decreasing Aflatoxin production, while not affecting fungal growth

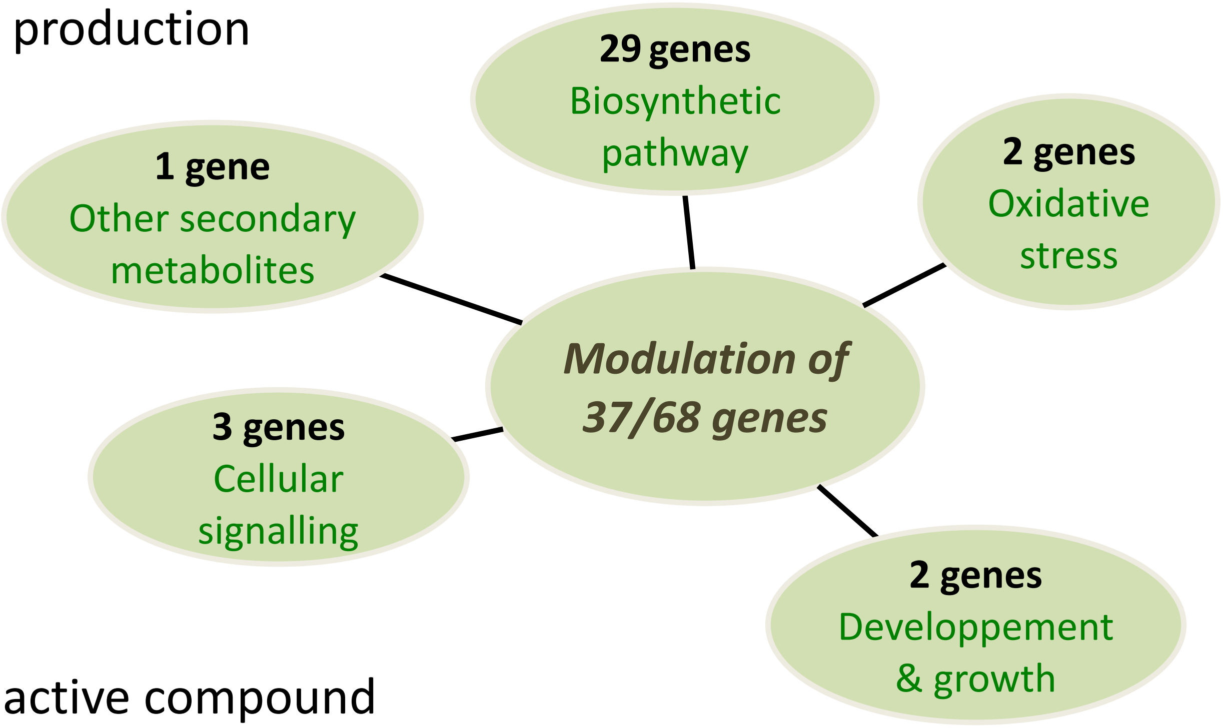
- Hyssop *(Micromeria graeca)* is an herbaceous plant
  - *Mediterranean region*
  - *Medicinal properties*:
    Expectorant, coughs, inflammations, asthmas, antiseptic, digestive, antiviral, healing, hypotensive, diuretic



Hysop extract: mechanism of action

- The mechanism of action for the Hysop inhibition of AFB1 was investigated using the large scale PCR tool

- Hysop acts at the transcriptomic level. The blockade at a very early stage suggest no metabolite production



- Next step: purify the active compound

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Natural products: conclusion

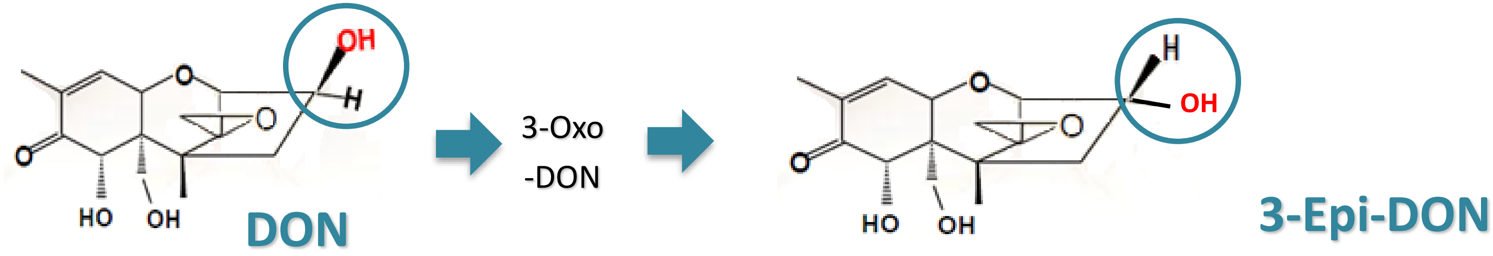
- Natural products limiting mycotoxin production is a promising strategy to mitigate mycotoxin.
- In this line we screened for products reducing mycotoxin production while not affecting fungal growth. Indeed if the humidity/temperature conditions are favorable for fungal growth, fungi will grow.
- Both eugenol and Hysop extract were inhibit AFB1 production, while not affecting fungal growth.
- A large scale PCR was develop to analyze the mode of action of these natural product. It includes the 29 genes of the aflatoxin biosynthetic pathway and 33 regulatory genes.
- This tool indicates us that both eugenol and Hysop act at the transcriptional level.
- The blockade occurs at a very early stage suggesting no metabolite production.

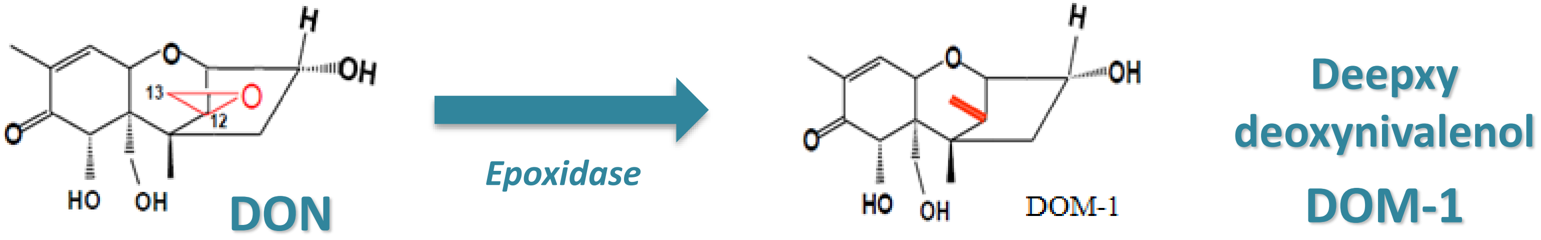
Part 2: Toxicity of metabolites formed during detoxification

Example of intestinal toxicity of deepoxy-DON (DOM-1) and 3-epi-DON

Bacterial detoxification of Mycotoxins

Microorganisim can metabolise mycotoxins other metabolites: example of DON





Need to test the toxicity of these metabolites

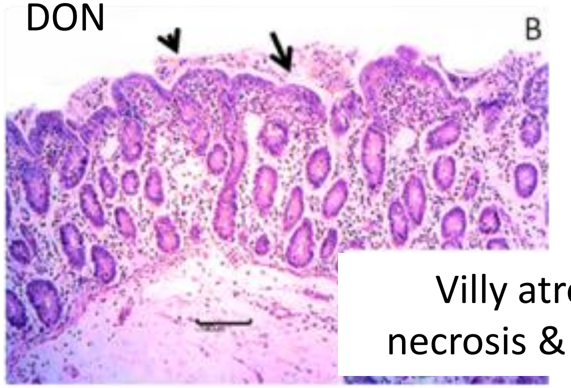
Effects on intestinal histology

- *Intestinal explants exposed to Control, DON, DOM-1 & 3-epi-DON*

Control

No damage

DON



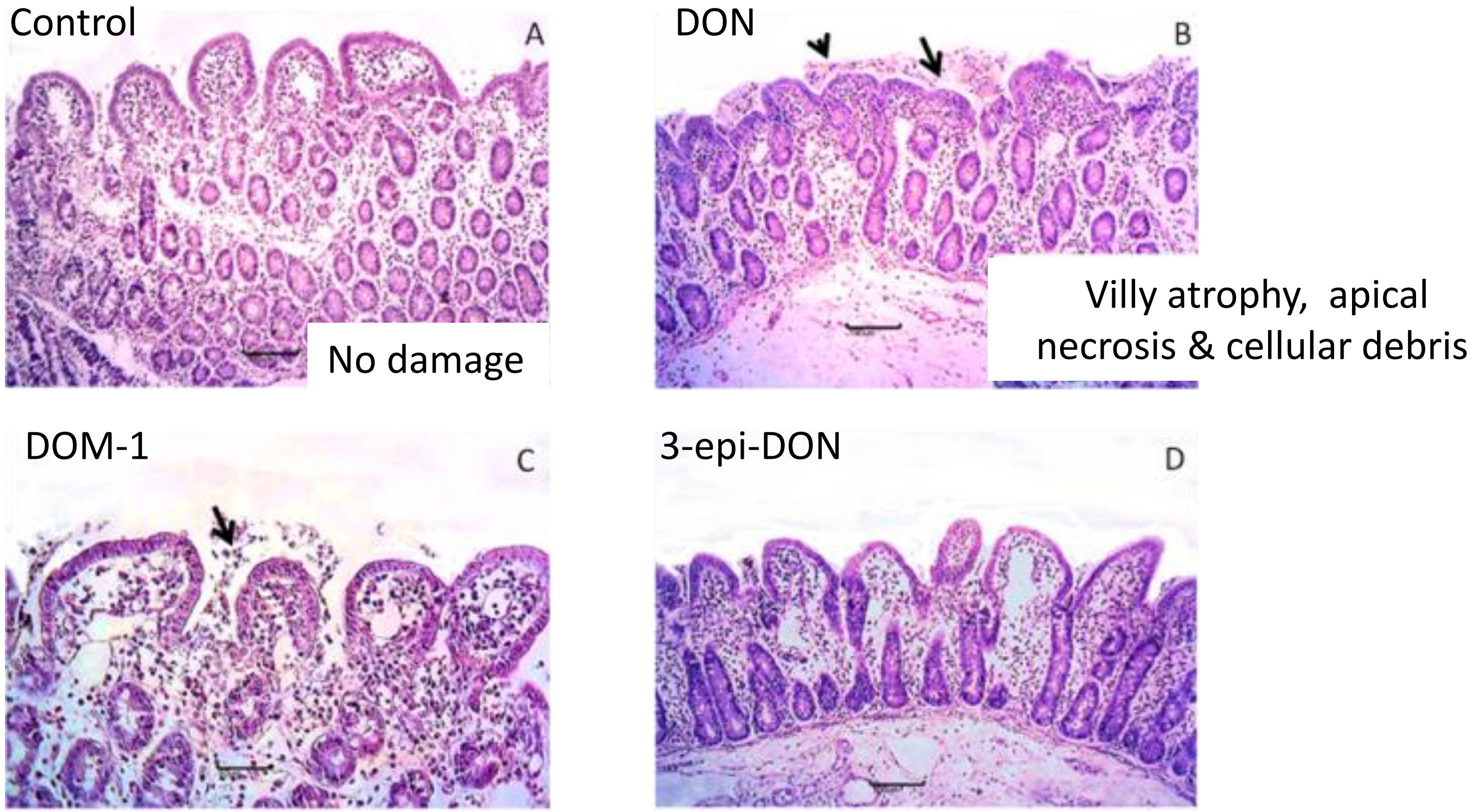
Villy atrophy, apical necrosis & cellular debris

DOM-1

3-epi-DON

Effects on intestinal histology

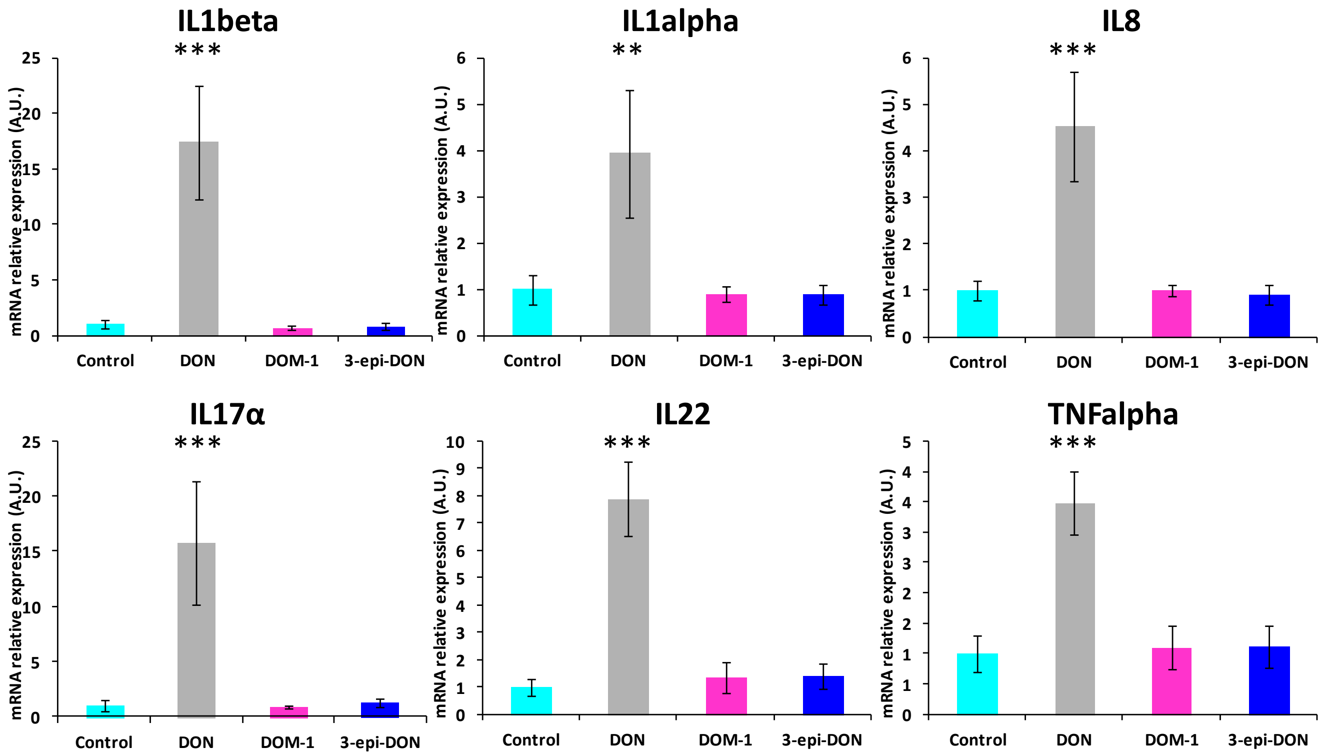
- *Intestinal explants exposed to Control, DON, DOM-1 & 3-epi-DON*



DOM-1 and 3-epi-DON don't induces histological damage

Effects on the immune response

- *Transcriptomic qPCR on inflammatory cytokines :*



DOM-1 and 3-epi-DON do not induce intestinal inflammation

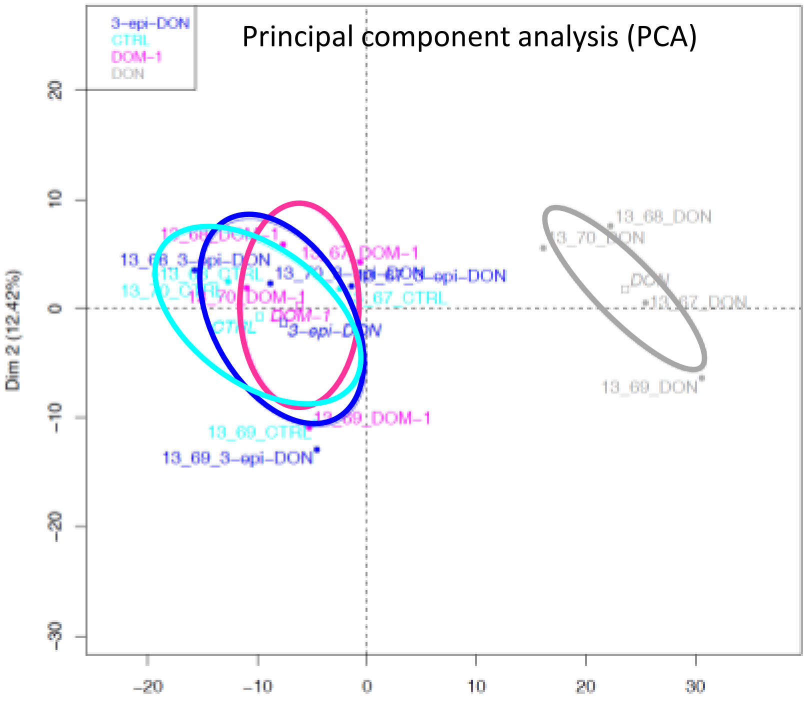
Transcriptomic analysis

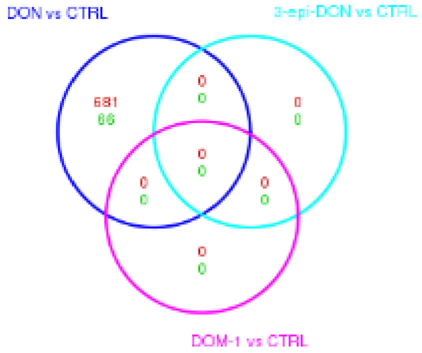
Microarrays « Agilent Porcinet 60K »

34775 selected probes Expressed in this tissue

***747** probes differentially expressed (DE)*

Lima order R Bioconductor packages
ajusted p-value (BH)<0.05





DOM-1 and 3-epi-DON don't change gene expression in the jejunum

Transcriptomic analysis

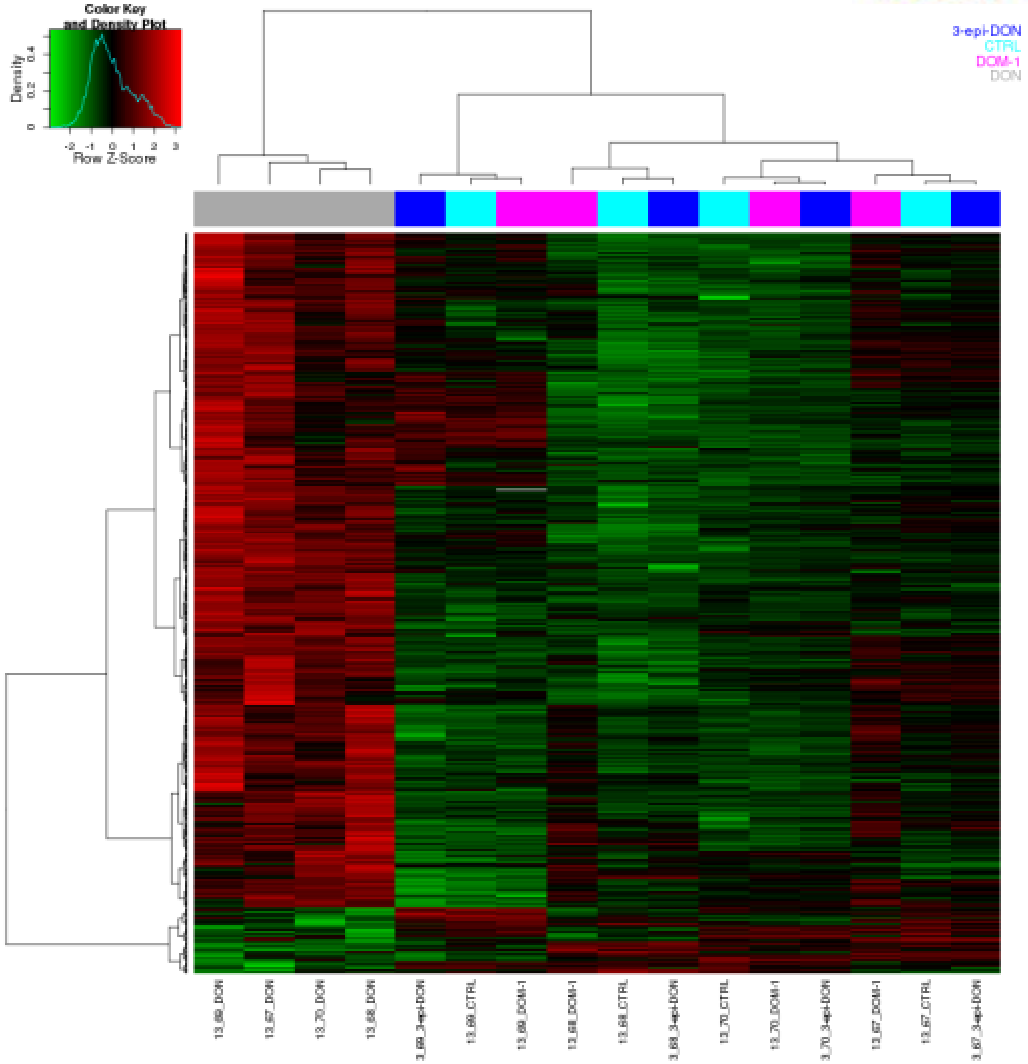
Microarrays « Agilent Porcinet 60K »

34775 selected probes Expressed in this tissue

***747** probes differentially expressed (DE)*

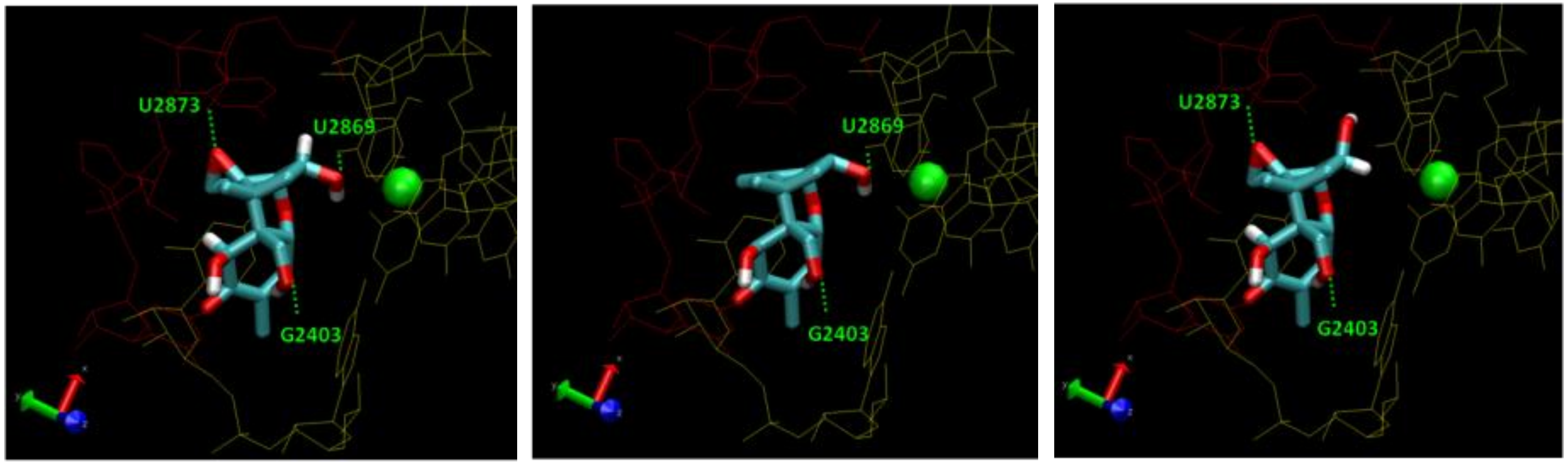
Lima order R Bioconductor packages
ajusted p-value (BH)<0.05

DOM-1 and 3-epi-DON don't change gene expression in the jejunum



in silico analysis

❑ Modelisation of DOM-1 and 3-epi-DON into the PTC of ribosome



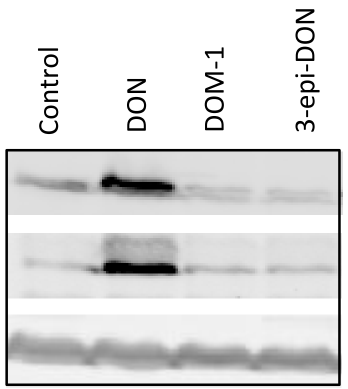
DON DOM-1 3-epi-DON

❑ Both 3-epi-DON & DOM-1 fit into the A-site but don’t form the three hydrogen bounds required for activation

Suggest an absence of «overall» toxicity of DOM-1 and 3-epi-DON

Effects on MAPKs

- Phosphorylation of MAPKs on cells



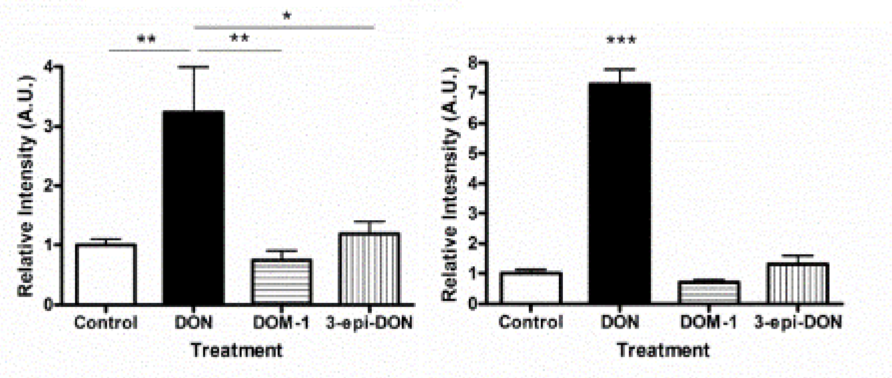
Phosphorylated P38

Phosphorylated Sapkjunk

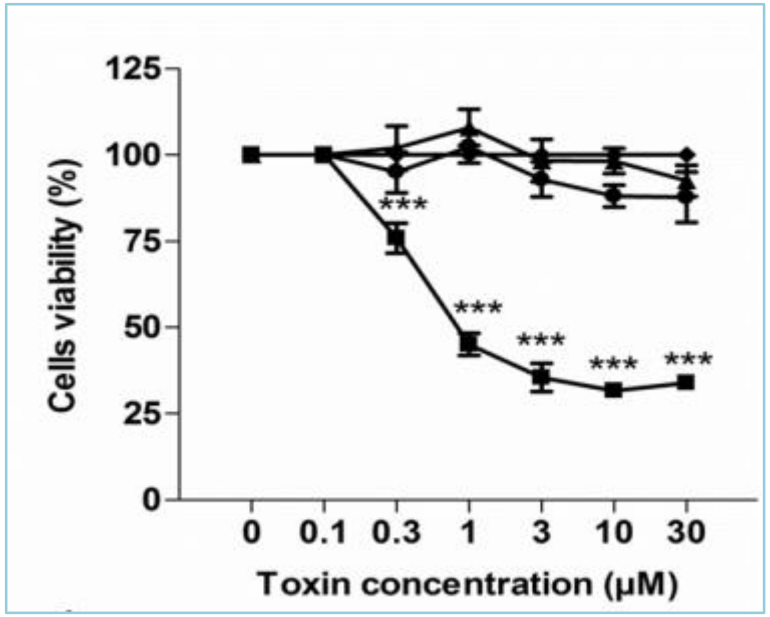
GAPDH

Phosphorylated P38

Phosphorylated SapkJNK



- Toxicity on human intestinal epithelial cells



DOM-1 and 3-epi-DON don't induces ribotoxic stress and toxicity

Toxicity of DOM-1 and 3-epi DON: conclusion

	DON toxicity	DOM-1 toxicity	3-epi-DON toxicity
❑ *In silico* analysis			
➢ Fit into the ribosome pocket	+	+	+
➢ Stable binding (3 hydrogen bonds)	+	−	−
❑ Biochemical analysis			
➢ MAPKs activation	+	−	−
❑ Histological analysis			
➢ Histological alteration	+	−	−
❑ *Transcriptome analysis*			
➢ Change on gene expression (inflammatory and pangenic analysis)	+	−	−

DOM-1 and 3-epi-DON are not toxic, and thus represent good mycotoxin mitigation strategies

Take home message

- Natural products limiting mycotoxin production is a promising strategy to mitigate mycotoxin

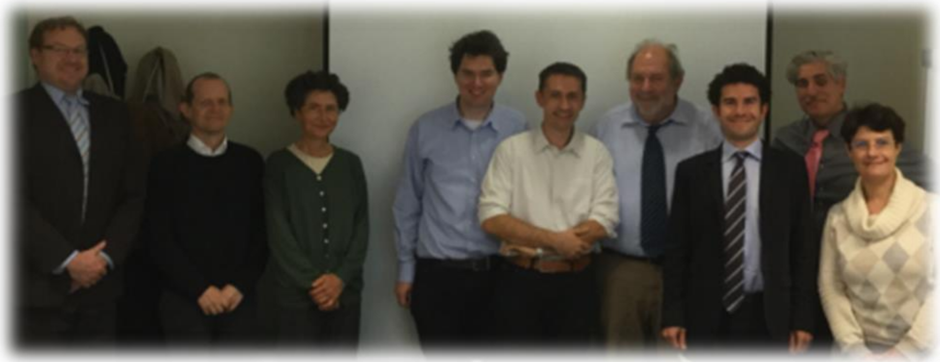
  In this line we selected products reducing mycotoxin production while not affecting fungal growth. Indeed if the humidity/temperature conditions are favorable for fungal growth, fungi will grow; it is thus important to limit mycotoxin

- Biotransformation can be used to mitigate mycotoxin

  Need to identify the metabolites formed during biotransformation
  Need to verify that these metabolites are non-toxic

  In this line *in silico* and omic technologies are very helpful tools

Acknowledgments



"Reactions and potential mitigation of mycotoxins during food processing" Expert Group

ILSI Europe

"Biosynthesis and toxicity of mycotoxins" team

Toxalim - Research center in Food Toxicology, Toulouse, France

Dr. T. Zhou, Agriculture & Agrifood Canada
Drs. D. Moll & G Schatzmayr, BIOMIN, Austria

Thank you for your attention

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ILSI Europe

International Life Sciences Institute

Management of food industrial technologies reducing mycotoxins while keeping the quality of finished products

Dr Johan De Meester
Cargill

ILSI Expert Group : review article

30th anniversary ILSI Europe

2014

2016

Process-related Compounds and Natural Toxins

The Group

EXPERT GROUP – NEW
REACTIONS AND POTENTIAL MITIGATION OF MYCOTOXINS DURING FOOD PROCESSING

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Petr	Karlovsky	University of Gottingen
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Michele	Suman	Barilla

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Introduction and outline

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- Intended and targeted chemical detoxification leads to potential toxic metabolites of mycotoxins, reduction in nutritional value, and changes to food product
- In a number of today's applied food processes on maize and oilseeds chemicals are added as processing aids in order to provide for highly refined food ingredients and/or high quality foods that result in lower mycotoxins levels compared to the starting material
- The ultimate goal of mycotoxin mitigation is to prevent adverse health effects caused by foodborne exposure to mycotoxins while preserving nutritional and organoleptic quality of food.
- Examples that are selected are :
  - Nixtamalization of maize to provide for masa and tortilla production
  - Native maize starch from corn wet milling
  - Refined vegetable oils by oilseeds processing

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Eu

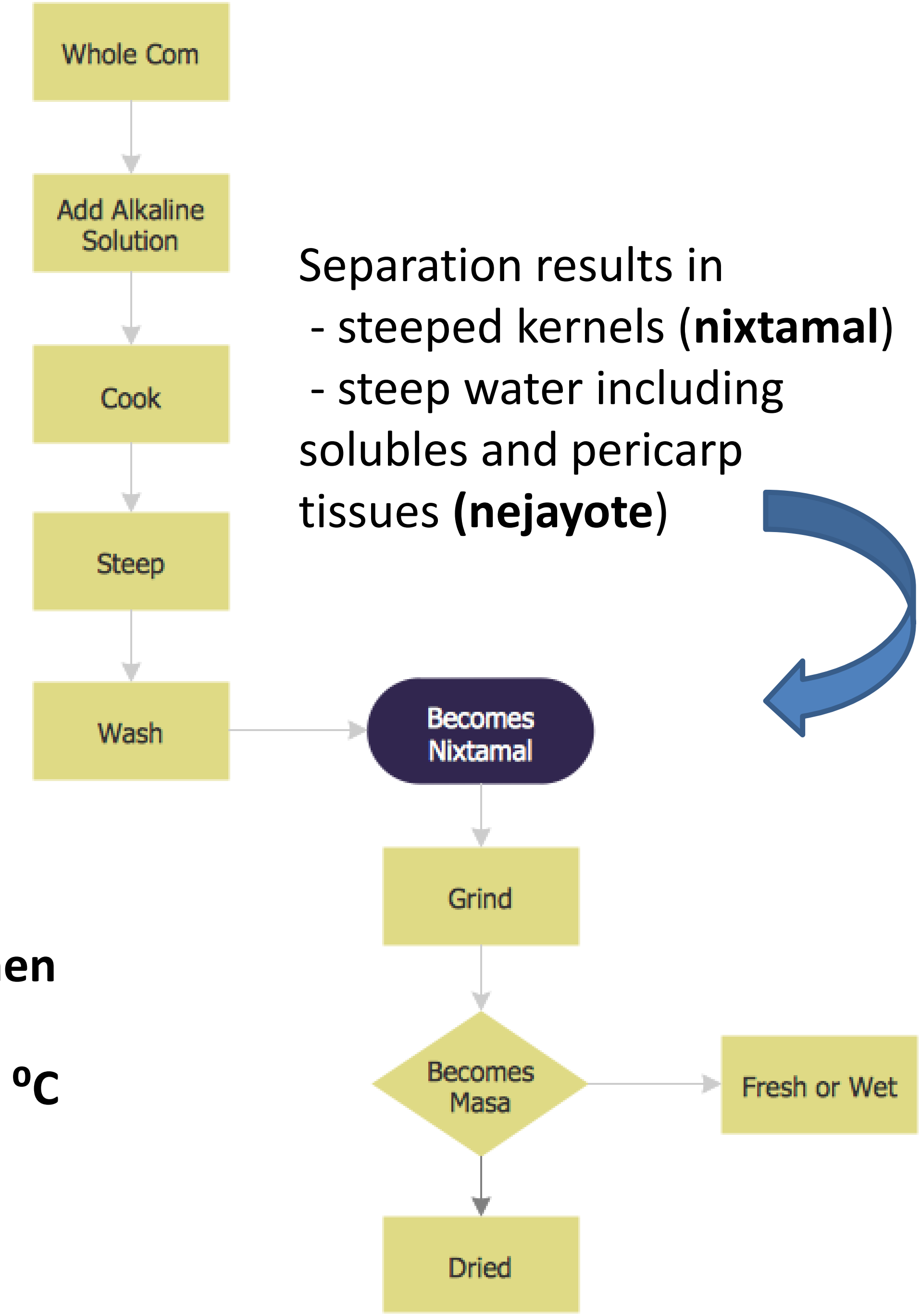
Maize foods from Benin

Mean percentage reduction during processing		Aflatoxins	Fumonisins
Raw maize -> mawe	SSF dough	≥ 91	≥ 87
Raw maize -> mawe -> makume	fermented wet meal (thick paste)	≥ 93	≥ 87
Raw maize -> ogi	precooking/steeping/milling /sieving (gruel)	80	29
Raw maize -> ogi -> akassa	additional fermentation	92	48
Raw maize -> lifin	maize meal		
Raw maize -> lifin -> owo	cooked maize meal	40	48

P. Fandohan, et al., *Int. J. Food Microbiology* **98** (2005), 249

Nixtamalization of maize using lime

Nixtamalization



Separation results in
- steeped kernels (**nixtamal**)
- steep water including solubles and pericarp tissues **(nejayote)**

Typical conditions are :

- **120-300 % of water,**
- **0.1 to 5.0 % lime based on corn,**
- **0.25 to 3 hours at 80-100 ^{o}C and then**
- **steeping during 24 hrs at 40 to 100 ^{o}C**

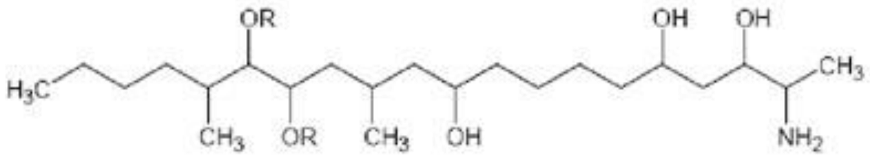
Cooking and steeping

Nixtamal and nejayote

Grinding

Alkaline effects on fumonisins

- Treatment with ammonia at higher temperatures and pressures resulted in 80 % reduction of FB1
- Ground maize treated with 0.1 M calcium hydroxide results in 70-80 % FB1 in the aqueous fraction and 10-25 % remains in the maize fraction, however when pericarp was removed only 5 % remains in the kernels
- Three forms of fumonisins are found : fumonisins (FBs), partially hydrolyzed fumonisins (PHFBs) and hydrolyzed fumonisins (HFBs)
- Alkaline substances hydrolyzes and reduce the level of fumonisins and their toxicity but did not eliminate them completely
- Control-cooking enhanced FBs and PHFBs reduction, due to the solubility of fumonisins in water during the steeping process, but did not form HFBs



R = $COCH_2CH(COOH)CH_2COOH$

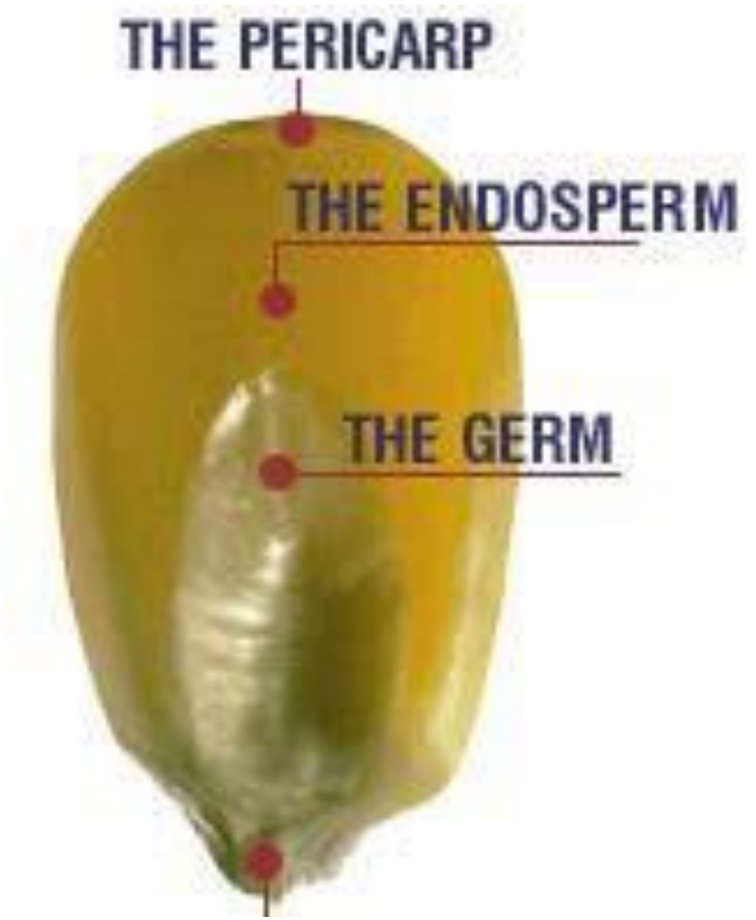
Fumonisin B_1

Distribution of mycotoxins in corn wet milling

ILSI Europe

Corn wet milling

- Wet milling of corn is already more than one century used to separate the constituents of the corn kernel into : germs and starch, but also corn gluten and corn fiber
- Key critical process to provide maximum yield is the **steeping process**

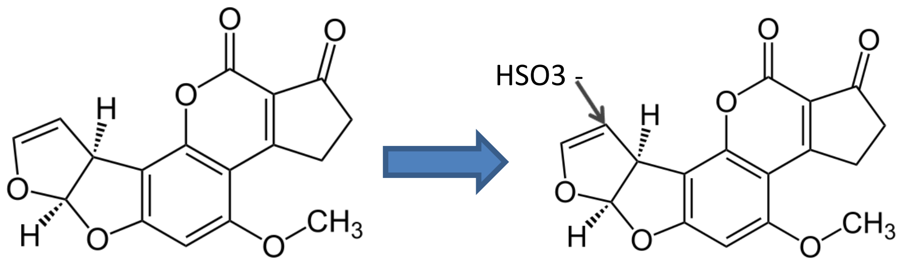


Steeping of corn

- “Physical treatment to reduce aflatoxin contamination” means any treatment, not involving chemical substances,…according EU guidance on contaminants
- Well controlled conditions of temperature, time, SO_2, and circulation of steep water. Typical conditions are :
    - **Temperature : ~ 50 ° C**
    - **Time : 20-40 hours (preferably 36 hours)**
    - **SO_2 : 1200 to 2000 ppm – effect on processing (nowadays typical value is 1000 ppm)**
- Corn is soaked in stainless steel or concrete tanks.
- Steeping process over 6-10 steep tanks using counter current principle : corn / steep water

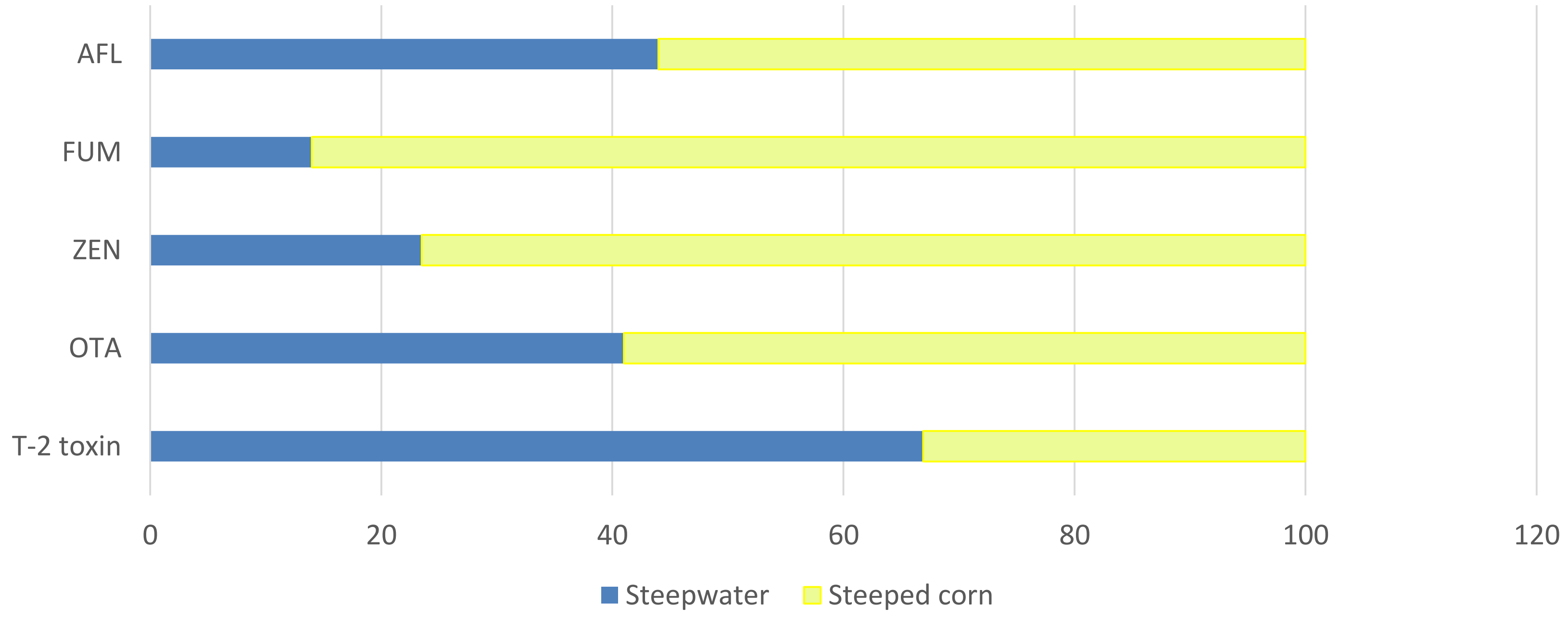
Steeping of corn

- Function of SO_2 :
  - Reducing agent : breaking disulfide bounds in proteins
  - Favors Lactobacillus producing lactic acid
  - Anticipated reaction of bisulfite with aflatoxin B_1 and G_1 (resp. AFL B_1S & AFL G_1S) :

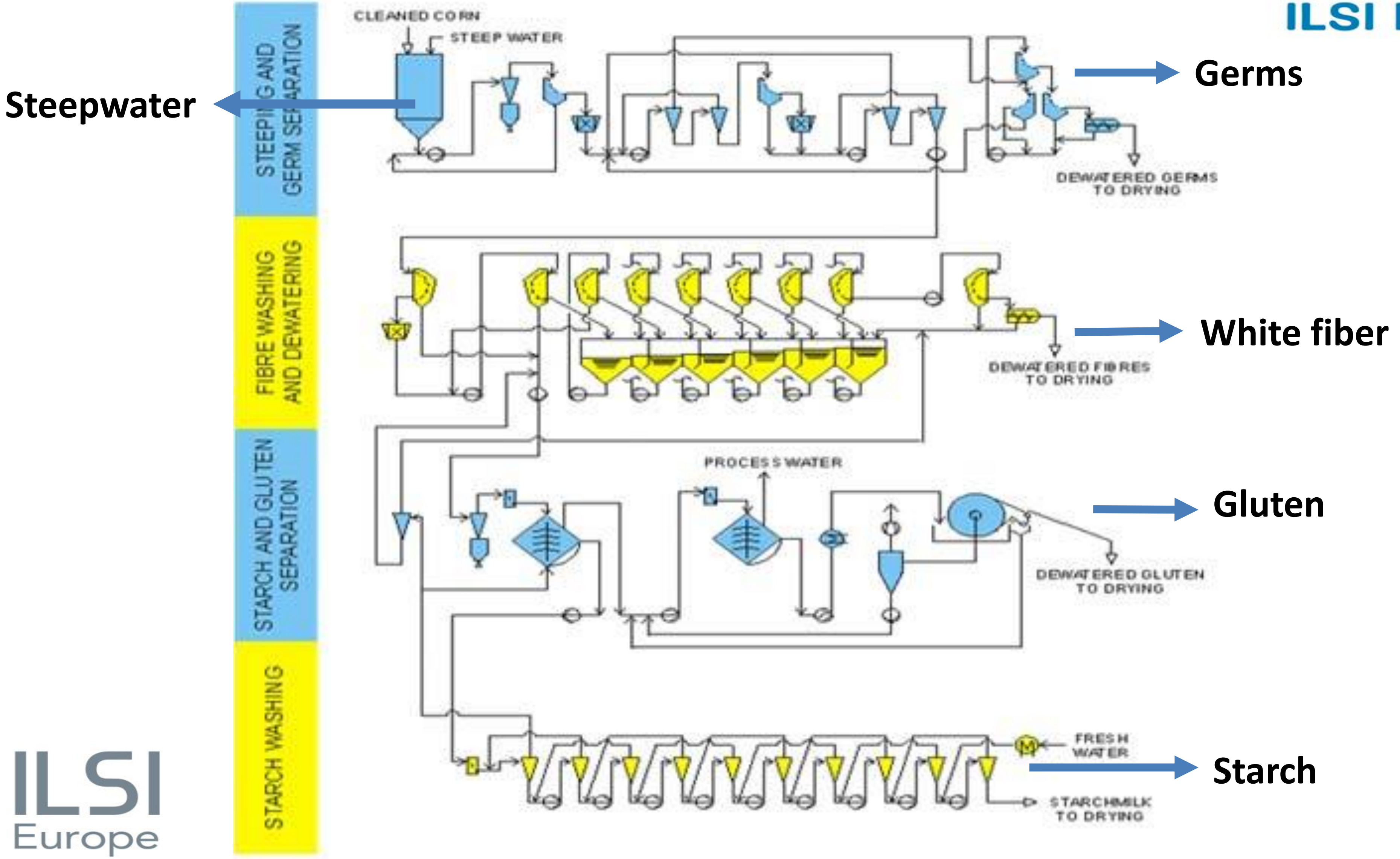


- Final result :
  - Steeped corn contain 45 % of moisture starting from 15 %
  - Light steep water contains solubles, but also e.g. mycotoxins present in the starting raw material

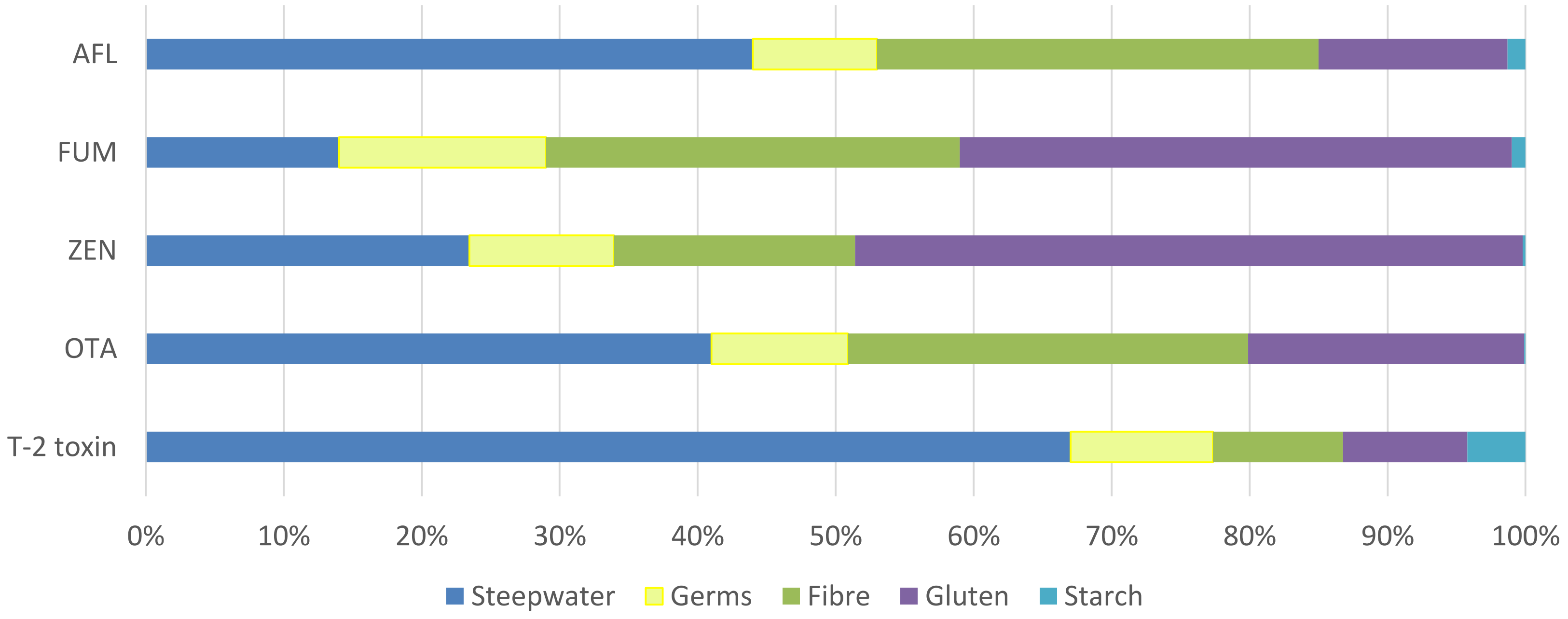
Distribution of mycotoxins



Corn wet milling process



Distribution of mycotoxins



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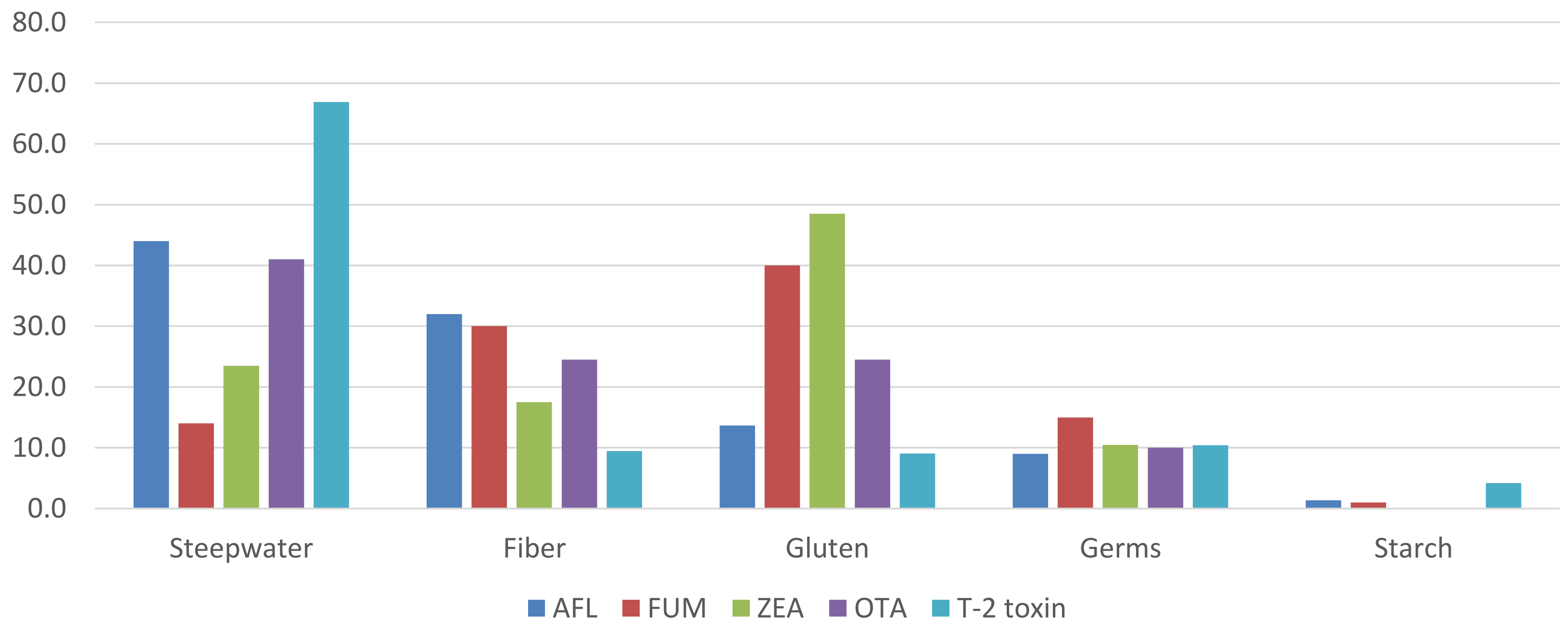
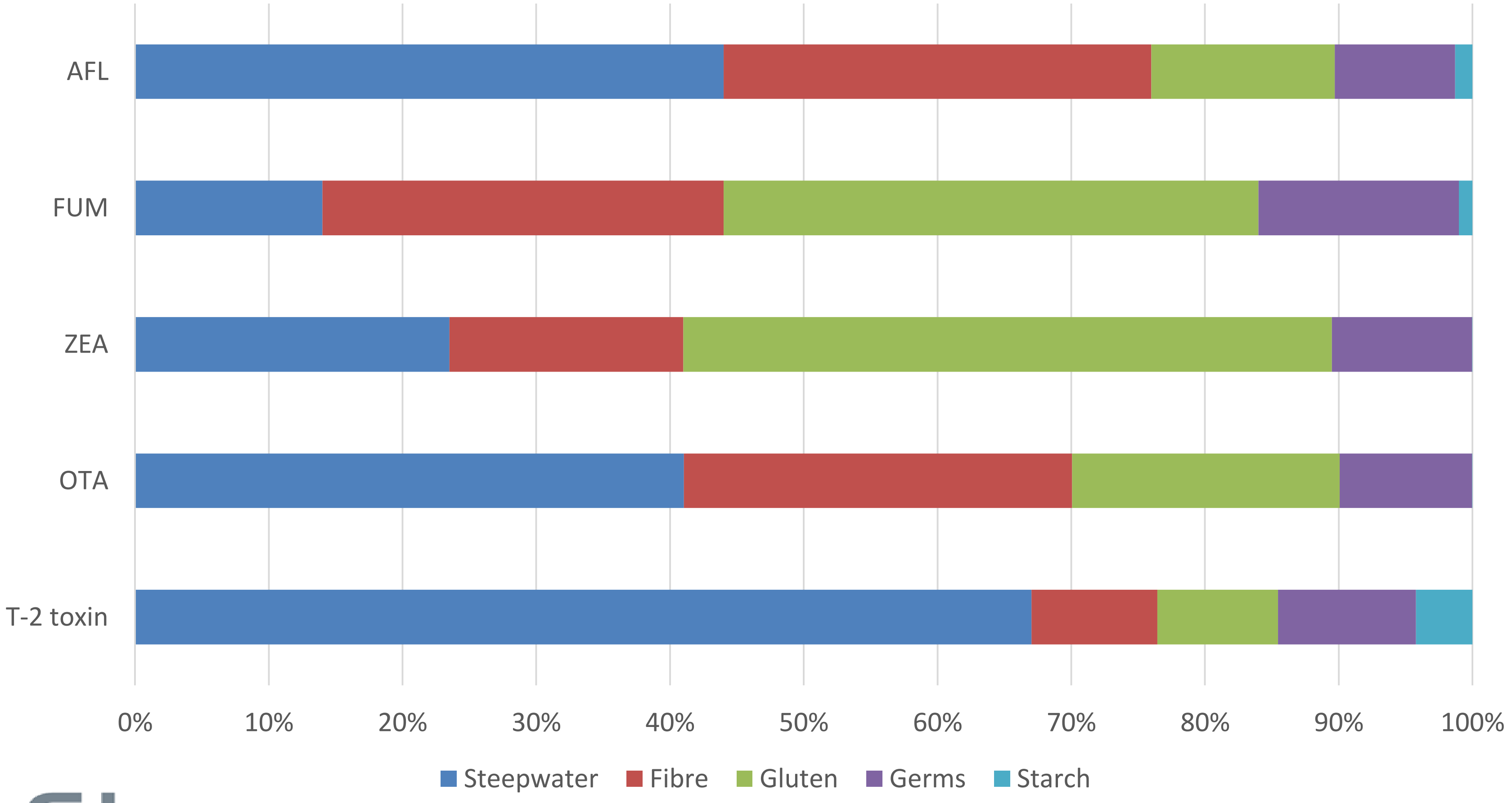
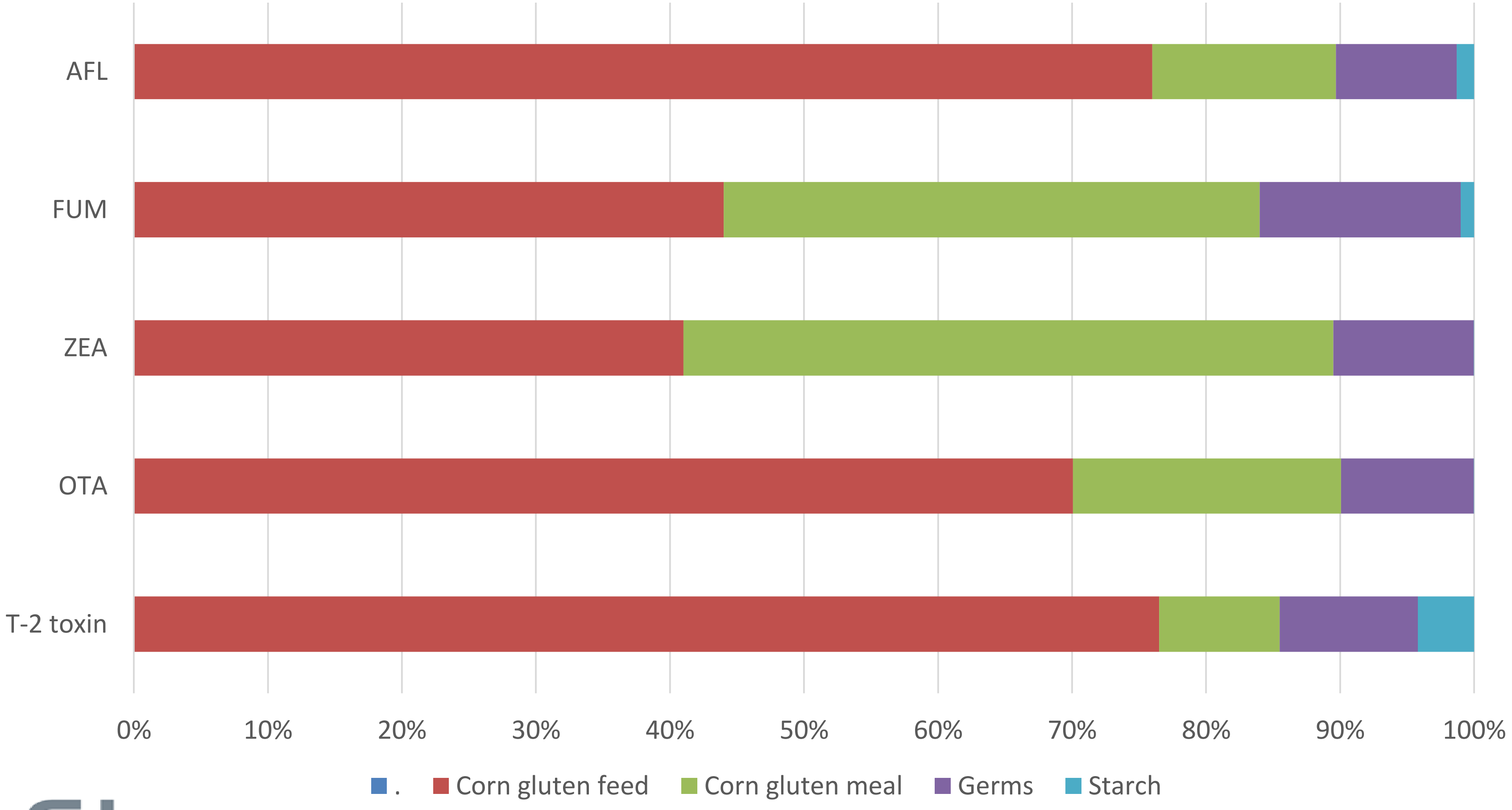
Distribution of mycotoxins in corn wet milling process streams



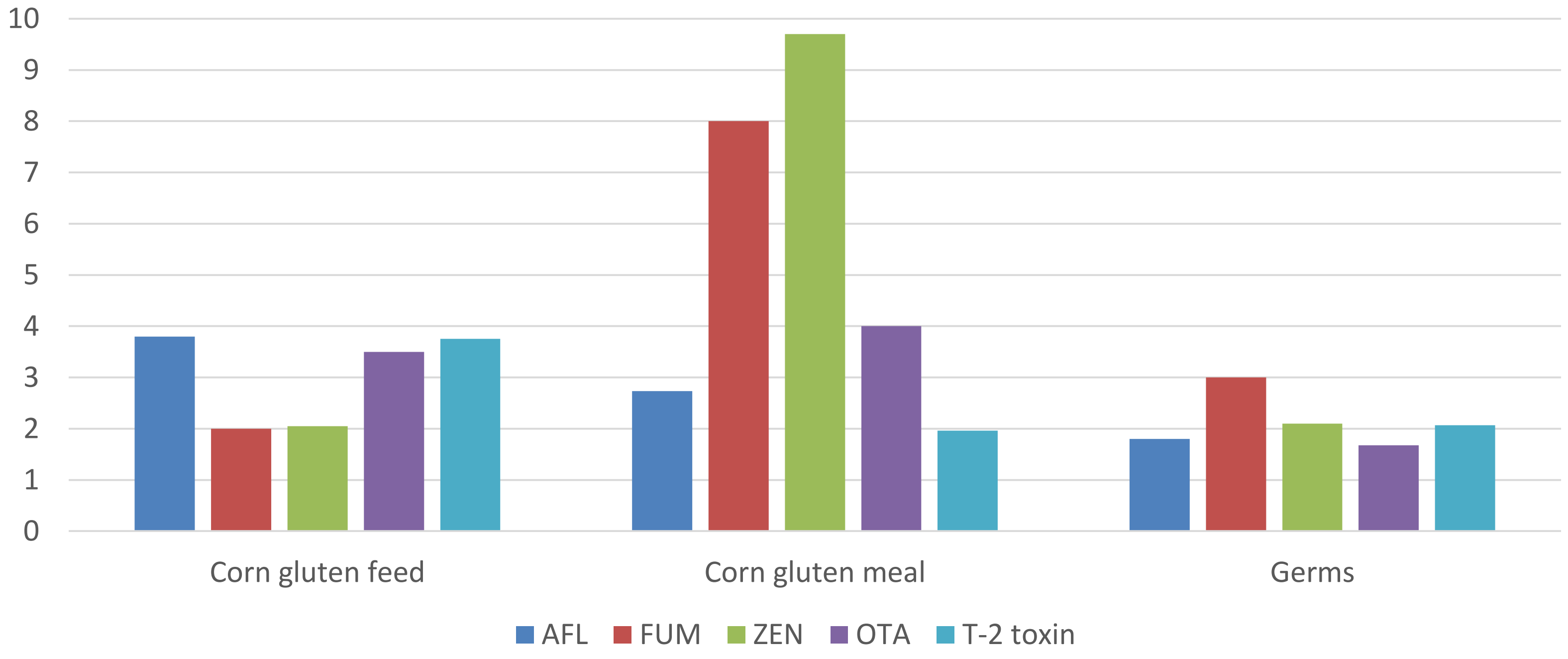
From process streams…



…to products of corn wet mill



Concentration factors in end products of corn wet mill



Refining of crude vegetable oils

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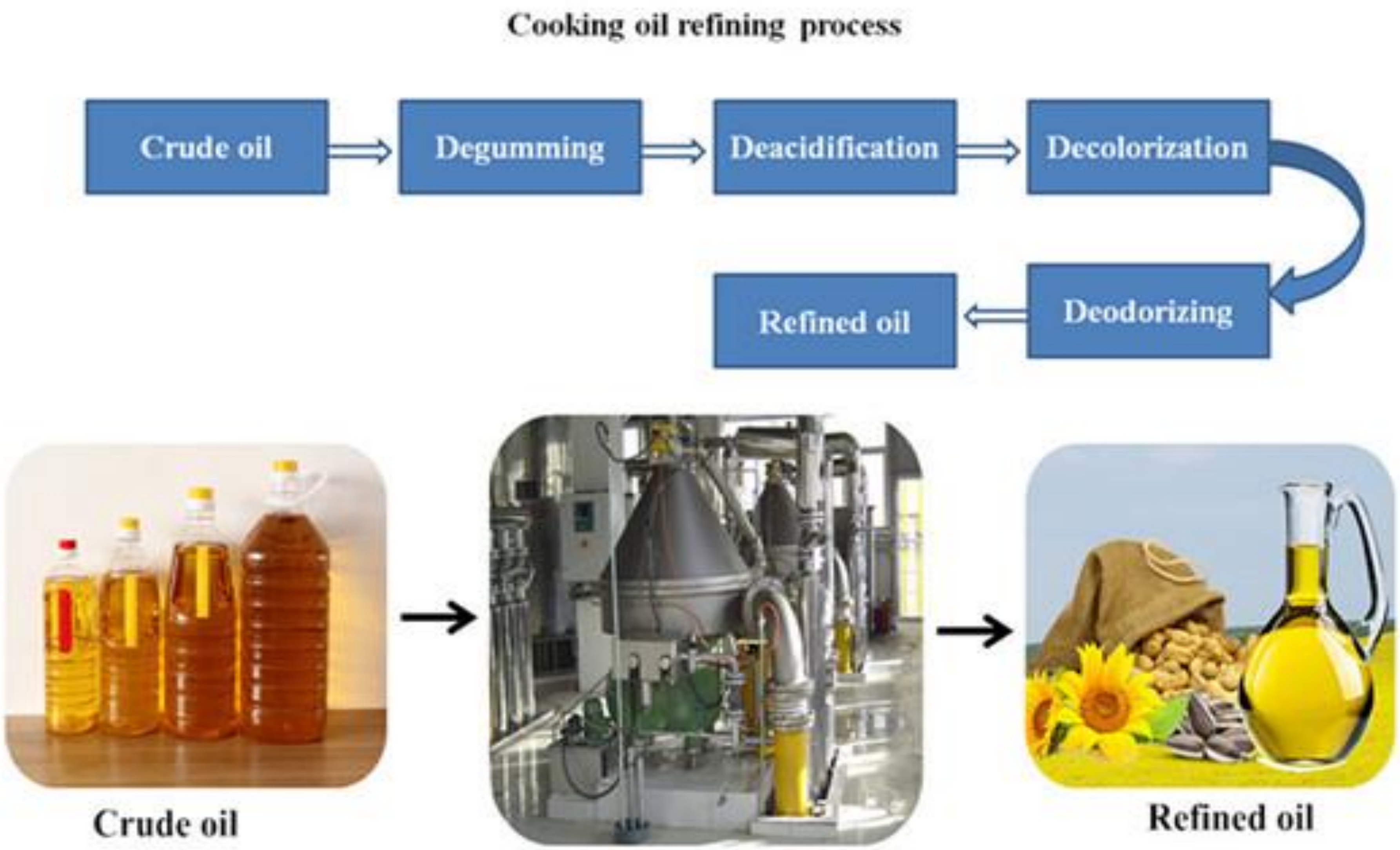
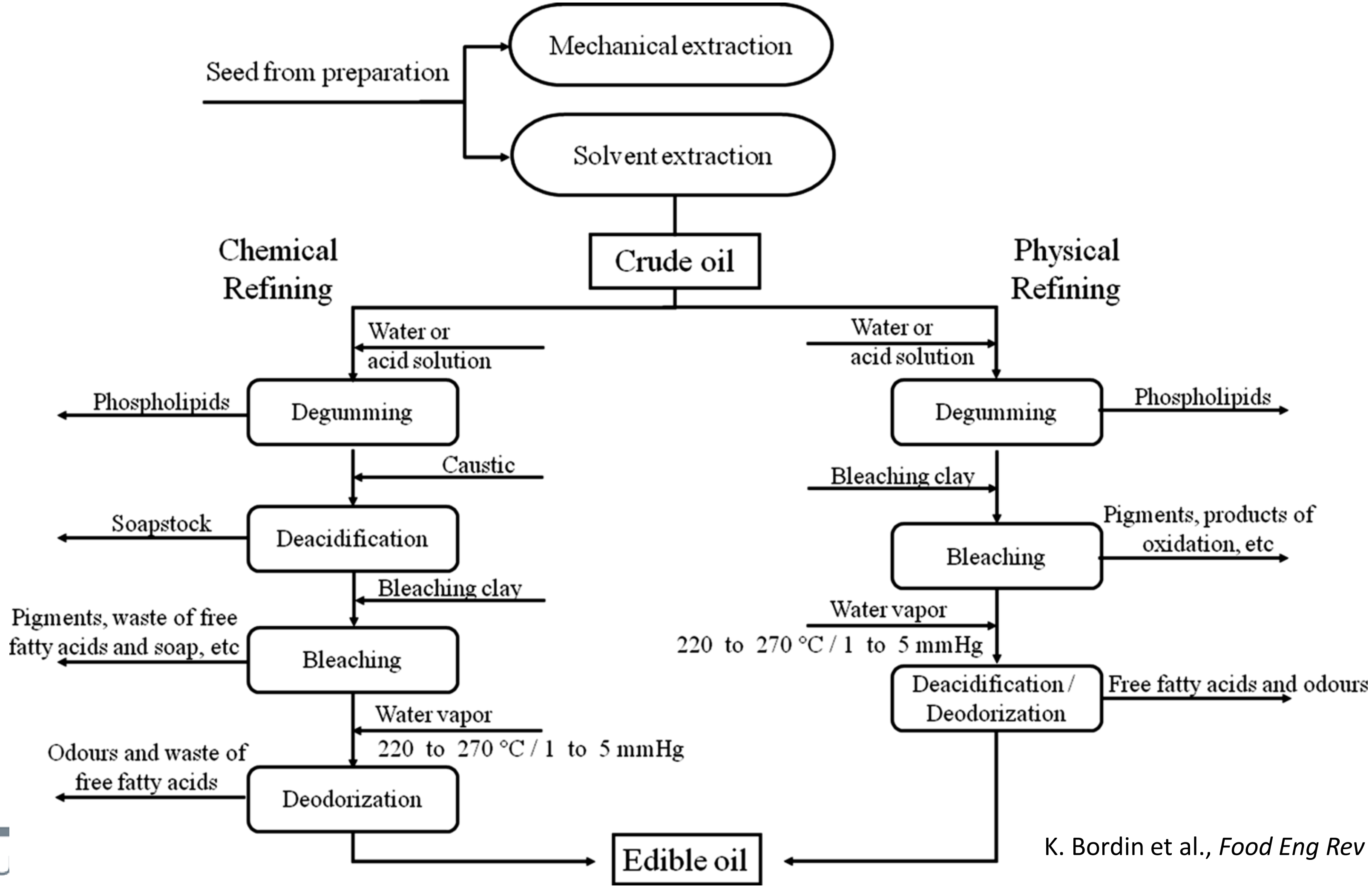
Refining of crude vegetable oils



Processing steps



K. Bordin et al., *Food Eng Rev* (2014) 6:20–28

Aflatoxin removal by pressing and extraction

Peanuts			AFL (ppb)	Oil (%)	Yield (%)
Pressing		Starting	5500	41.4	100
		Meal	7500	18.1	75.8
		Crude oil	812	~ 100	24.2
Extraction	Hexane	Starting	5500	41.4	100
		Meal	11000	0.9	49.5
		Crude oil	120	~ 100	50.5

Parker W.A. and Melnick D., *J Am Oil Chem Soc*, (1966), 43(11):635-8

Aflatoxin removal in corn germs by extraction

Corn germs			AFL (ppb)	Oil (%)	Yield (%)
Extraction	Chloroform	Starting	425	53.7	
		Meal	625	0.7	59.2
		Crude oil	135	~ 100	40.8
Extraction	Hexane	Starting	425	53.7	
		Meal	750	1.4	56.2
		Crude oil	8	~ 100	43.8

Parker W.A. and Melnick D., *J Am Oil Chem Soc*, (1966), 43(11):635-8

Effects of deacidification

- Removal of free fatty acids is required to refine the product – sodium hydroxide solution is added (5-15 % depending on FFA level)
- Aflatoxins are cleaved by alkaline agents resulting in hydrolysis of the lactone ring. Despite this observation Kamimura et al. (1986) concluded that sodium hydroxide provides for :
- - reduction of AFL B_1 and AFL B_2 by 50 % in 2 minutes ;
- - complete elimination of AFL G_1 and AFL G_2 within 10 minutes ;
- - zearalenone not reduced substantially ;
- - tricothecenes were reduced with 50 % within 8 minutes ;
- Parker and Melnick (1966) determined that 92 to 98 % of the aflatoxins in the crude oil is reduced by the effect of sodium hydroxide

Kamimura L.M.,*Food Hyg Soc of Japan* (1986) 27:59–63
Parker W.A. and Melnick D., *J Am Oil Chem Soc*, (1966), 43(11):635-8

Effects of bleaching and deodorization

- **Bleaching** using bleaching earth or clay results in brighter and more blend oil
- - at concentration of 2 %, elimination of aflatoxins and tricothecenes after 15 minutes
- - contact period of 30 minutes, leaves 82 % of initial amount of zearalenone
- - Parker et al. demonstrated for peanuts and corn germ oils < 1 ppb AFL

- **Deodorization** is desorption using water vapor at 220-270 °C and low pressure (1-5 mm Hg) to eliminate free fatty acids, aldehydes and ketones that are responsible for off odours and flavors
- Kamimura et al. (1986) provided by spiking solutions that by 120 minute treatment
- - 86 % of AFL B_1, 80 % of AFL B_2, 72 % of AFL G_1 and 70 % of AFL G_2 remained
- - 45 % of tricothecenes and only 7 % of zearalenone
- After 150 minutes operation all tricothecenes were completely eliminated

Kamimura L.M.,*Food Hyg Soc of Japan* (1986) 27:59–63
Parker W.A. and Melnick D., *J Am Oil Chem Soc*, (1966), 43(11):635-8

Summary unit operations

Reduction during processing			AFL	ZEN	Tricothecenes
Pressing	Crude oil		50 - 85 %		
Extraction	Crude oil		68 - 98 %		
Degumming	Degummed oil		N/A		
Deacidification	Soapstocks		2 -10 min (50 - 100 %)	not	50%
Bleaching	Bleaching oil		15 min (100 %)	18%	15 min (100 %)
Deodorization	Refined oil	AFL B1	14%	> 90 %	55% in 120 min
		AFL B2	20%		100 % in 150 min
		AFL G1	28%		
		AFL G2	30%		
Refining	Chemical			> 80 %	
Refining	Physical			70 - 80 %	

Take aways

- Nixtamalization can reduce fumonisins to a large extent (>90 %)
- Corn starch produced in corn wet mills has levels of mycotoxins which are ~ 1 % of the starting materials
- Refining crude vegetable oils in crude form will results in food grade products meeting levels below concern

Johan De Meester*, Isabelle P. Oswald, Michele Suman, Peter Karlovsky, Franz Berthiller, Gerhard Eisenbrand, Irène Perrin, Gerrit Speijers, Tobias Recker, Pierre Dussort

ILSI Europe Process-related Compounds and Natural Toxins Task Force,
Expert Group “Reactions and Potential Mitigation of Mycotoxins During Food Processing”
Brussels, Belgium

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We appreciate your feedback.

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515-276-3344

This webinar will be available at www.foodprotection.org
within the next 2 business days.