DOI: 10.16210/j.cnki.1007-7561.2021.06.004.en
PAULA A, JONATHAN B, JOHAN De M, et al. Mitigation of mycotoxins during food processing: sharing experience among Europe and South East Asia[J]. Science and Technology of Cereals, Oils and Foods, 2021, 29(6): 59-70.

Mitigation of Mycotoxins during Food Processing: Sharing Experience among Europe and South East Asia (英文原文)

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Abstract: Worldwide the issue of mycotoxins results in economic losses estimated at billions of dollars and toxicological risk for both humans and animals. Preventive measures also include decontamination and mitigation actions that can be carried out through food processing. Several proposals have been tested and illustrated also in scientific papers during the last decades, however clear, easy to implement, practical suggestions and guidelines for process adaptation are much more needed. Europe and South East Asia can find synergies and complementarities moving from processing to analysis, from risk assessment to reduction strategies, from gap-analysis to communication roadmaps. Stakeholders from both Europe and Southeast Asia must then ensure that there is a way to ease and harmonize the regulation in the food supply chain in order to ensure food safety and at the same time facilitate trade in both regions, taking into account of the various landscapes, agrosystems and also different consumer preferences within the countries themselves. Concerning the example of cereals, processing steps cover primary processing (cleaning and milling operations) and secondary processing procedures (such as fermentation and thermal treatments during baking), special attention is devoted to the production of baked goods and to the estimation of

Received Date: 2021-09-03
Supported by: FCT/MCTES for the financial support to CESAM, Centre for Environmental and Marine Studies, University of Aveiro, (UIDP/50017/2020+UIDB/50017/2020), through national funds (Portugal).
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processing factors for DON in wheat bread production especially in Europe. With reference instead to the specific context of Asian producers, a case-study focuses on ochratoxin A in coffee in Southeast Asia region, combining the expertise of the farming community, coffee industry and science researchers. The strategy to reduce mycotoxins in a farm setting poses several challenges to coffee farmers: it needs to be analyzed in the context of good agricultural practices, socioeconomic and behavioral factors of both coffee producers and consumers. As the world becomes more globalized, food and feed supply chains also become more complexed and hence, a more comprehensive strategy to ensure food contaminants mitigation is needed.

Key words: mitigation; food processing; mycotoxins; cereals; coffee; Europe; South East Asia

Chinese Library Classification Number: TS201.1

Documentary Identification Code: A **Article ID:** 1007-7561(2021)06-0059-12

Published time on CNKI: 2021-11-03 09:36:14

Published address on CNKI: https://kns.cnki.net/kcms/detail/11.3863.TS.20211102.1027.010.html

1. INTRODUCTION

There are more than 300 known mycotoxins which are suspected to have widely differing modes of action, however formal toxicological evaluation and comprehensive risk assessment in Europe has been only conducted for about ten of best known or major mycotoxins[1]. Contrary to the free or parent forms, very little is known about the incidence and levels of contamination and the potential toxicological and biological effects of unregulated mycotoxins including modified and emerging mycotoxins as well as combination of mycotoxins and other food contaminants[2-4].

Face to this multiplicity of different forms mycotoxins and its potential effects on health, it is easily concluded that the challenge posed by mycotoxin risk, globally, is extremely complex and requires a joint effort by all players in the food supply chain. This is clearly stated in the last report-document from the International Agency for Research on Cancer (IARC) on the impact of climate change on mycotoxin proliferation where it is estimated that every day around 500 million low income people basing their diet on corn and cereals and living in sub-Saharan Africa, Latin America, and Asia are exposed to aflatoxins and fumonisins[5].

ASEAN principles and criteria for the establishment of maximum level (ML) for contaminants and toxins in food and feed (including mycotoxins contamination level) were finalized in 2016 and is gradually setting up. Although the ASEAN policy on mycotoxins is not fully completed yet, an example of risk assessment report on total aflatoxins and aflatoxin B_1 through the consumption of peanut and corn was endorsed in early 2019 by the ASEAN Risk Assessment Centre for Food Safety (ARAC).

The ASEAN Food Reference Laboratory (AFRL) for Mycotoxins (Singapore) has been organizing annual proficiency tests, providing support to strengthen the competency of national food testing labs with mycotoxin analysis capabilities and specifically addressing several commodities, such as cereal grains, nuts, oilseeds and spices (in which some of the leading producers are from the ASEAN countries)[6].

Moving now on food processing scenario, mycotoxins and their modified forms can be consequently influenced: mechanical or thermal energy during the transformation process may cause modification, inducing reactions with macromolecular components such as sugars, proteins or lipids as well as release of the parent compounds[7].

Preventative measures also include decontamination and mitigation actions that can be carried out through food processing and several proposals have been tested and illustrated in scientific papers during the last decades[8].

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) and problems depending on the geographical regions and sensitive population groups need to be taken into account.

In 2010, the International Life Sciences Institute Europe (ILSI Europe) Process-related Compounds and Natural Toxins Task Force dedicated a project to evaluate the agronomic practices for mitigation of natural toxins including a section dedicated to mycotoxins[9]. Later, in 2014—2016, the same Task Force dedicated a project to understand the possibility of mitigating mycotoxins, corresponding in improved safety of the food commodities. The main task defined was to critically review the state of the art about mycotoxin reduction through food processing: it summarized the impact of the different decontamination/ detoxifying processes on various food commodities; finally, the impact (work stimulated a corresponding new effort from 2017—2019 as a follow-up activity to translate the scientific findings of the Expert Group on 'Reactions and Potential Mitigation of Mycotoxins during Food Processing' to concrete guidance for industry. In 2019, the Practical Guidance became available[10] and takes into account a traffic light system approach to determine which processes in a given food product would significantly mitigate mycotoxins: it will now help international food producers (related to various commodities, such as cereals & derived products, cocoa, fruit juices, dairy products) with clear, easy to implement, practical suggestions and guidelines for process adaptation aiming to mitigate mycotoxins.

The present paper was born as a follow-up of a dedicated session[11] jointly organized by ILSI Europe's Task Force Process-Related Compounds and Natural Toxins & ILSI SEA Region-Singapore at the last WMFmeetsASIA2020 in Bangkok. It is therefore devoted to illustrate the aspects illustrated above under the light of Europe and South East Asia different and complementary experiences: (i) comparing food processing effects and indicating analytical tools available for their impact, (ii) updating toxicological assessment of processed-related mycotoxins, (iii) looking for synergies on regulatory frameworks, (iv) considering perspectives and implications on both large and small-medium enterprises within the farm to fork approach, and (v) identifying which are the remaining gaps and challenges faced by Southeast Asian producers in mitigating mycotoxins. With reference to this specific context of Asian producers, a case-study example was explored focusing on ochratoxin A mitigation in coffee in Southeast Asia region, combining the expertise of the farming community, coffee industry and science researchers.

2. COMPARING FOOD PROCESSING EFFECTS AND IDENTIFYING ANALYTICAL TOOLS AVAILABLE FOR THEIR IMPACT

Food processing is the transformation of agricultural products into food. Starting roughly 30 years ago, extensive research was carried out to investigate whether food processing can reduce the toxicological impact of mycotoxins[8]. Among the most investigated mycotoxins during food processing are (i) trichothecenes, in particular deoxynivalenol and T-2 and HT-2 toxins, during wheat processing (e.g. milling and baking)[12], (ii) fumonisins and aflatoxins during the production of corn-based products, such as tortillas and cornflakes[13], iii) mycotoxins in maize-based foods[14] and (iv) aflatoxins during sorting of nuts and drying of fruits[8].

Structural modifications of the parent mycotoxin that can occur during food processing include isomerization, decarboxylation, rearrangements and the reaction with other small molecules[15]. Food processing can lead to the formation of covalent adducts of the parent mycotoxin with matrix components, such as proteins or starch. This was observed for OTA during coffee roasting[16] and is thought to occur to fumonisins during the production of corn-based products such as cornflakes[17].

Priority should be given to the identification and isolation of the degradation products formed and the study of their toxicity[15]. The degradation products that are formed during food processing can be elucidated by targeted or untargeted analysis. The targeted approach requires reference standards for the identification and quantification of the analytes. However, two important limitations include the fact that the predetermined set of compounds may differ from the degradation products that are actually formed during food processing and the complexity of the food matrix may lead to the formation of degradation products that are not within the set of the predetermined targeted analytes[18]. To overcome this difficulty, the use of stable isotopically labelled tracer compounds used to fortify the matrix to be processed in combination with LC-HRMS offers great potential for the untargeted screening of mycotoxin degradation products in complex food and feed samples[19]. Although untargeted analysis allows for the elucidation of the complete spectrum of degradation products, only one such study is available so far[15]. According to the same authors, more studies need to be published that characterize the formed degradation products, collect data on their toxicity and thereby complete the knowledge about the mycotoxin mitigating effect during food processing.

3. TOXICOLOGICAL ASSESSMENT OF PROCESSED-RELATED MYCOTOXINS

3.1 Regulated and unregulated mycotoxins

Concerning free or parent mycotoxins (unchanged forms), national and international public health and government bodies have established legal regulatory limits in different food commodities in order to protect consumer´s health and facilitate international food trade[20-21]. In EU particularly, the Commission Regulation (EC) No 1881/2006 and in its amendments stipulated maximum limits (ML) in various foods. These include aflatoxins (AFs), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEA), fumonisins (FBs), patulin (PAT), and T-2 and HT-2 toxins[22].

Within the human risk assessment process, the Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (EFSA) reviewed the available published data on the toxicokinetics and the *in vivo* and *in vitro* toxicity data on mycotoxins in experimental animals, humans, and farm and companion animals. Based on the assessed data, the CONTAM Panel established health-based guidance values (HBGVs) – such as the tolerable daily intake (TDI) to characterise chronic health risks or the acute reference dose (ARfD) to characterise acute health risks[23].

Currently concern about risk and food safety has been extended to other potential mycotoxin contamination, such as modified and emerging mycotoxins, and the co-occurrence of several mycotoxins as well as combinations of mycotoxins and other food contaminants[2-4].

Modified mycotoxins (often called “masked”) could be hydrolysed into the parent compounds or released from the matrix during digestion, leading to potential adverse health effects[24]. Deoxynivalenol (DON) is one of the most frequently occurring mycotoxin in wheat crops worldwide. So, it is very important to investigate its stability during food processing. Recent outcomes of DON stability during milling, fermentation, and baking show some opportunities to reduce DON. Moreover, DON-3-glucoside (DON-3G) seems to increase during the breadmaking process, thus actions to prevent this occurring are required[25].

Emerging mycotoxins include other toxic secondary metabolites produced by *Fusarium* (fusaproliferin, enniatins ENN, beauvericin BEA, and moniliformin, among others), *Aspergillus* (sterigmatocystin STE), *Penicillium* (mycophenolic acid) and *Alternaria* (alternariol, alternariol monomethyl ether, and tenuazonic acid)[26]. No ML has been set for these emerging mycotoxins, probably due to their late recognition and therefore their limited data available on toxicity, concentration levels and occurrence[27]. The CONTAM Panel concluded that acute exposure to ENN and BEA do not indicate concern for human health but also highlighted the need for long-term studies to assess potential chronic toxic effects *in vivo* [28], available occurrence data are too limited to carry out a reliable human and animal dietary exposure assessment for STE[29] and, the use of the threshold of toxicological concern (TTC) approach is recommended to assess the relative level of concern for dietary exposure of humans to *Alternaria* toxins[30], as reported at Table 1.

The combination of mycotoxins with other food contaminants constitutes a rising concern, especially because health effects resulting from multiple mycotoxins exposure could lead to different output toxicity and carcinogenicity than exposure to single mycotoxin[3,31-32]. As an example, in cereals and derived cereal product samples, 127 mycotoxin combinations were described in the literature, with AFs+FUM, DON+ZEA, AFs+OTA, and FUM+ZEA being the most commonly observed[4].

3.2 Risk assessment of mycotoxins and health-based guidance values

The CONTAM Panel considered appropriate to assess human exposure to modified forms of the various toxins in addition to the parent compounds, because many modified forms are hydrolysed into the parent compounds or released from the matrix during digestion. The modified mycotoxins can occur simultaneously with the free mycotoxin, and, in some cases, the concentration of modified mycotoxins may exceed the level of free mycotoxin in processed foods. For modified forms of ZEA, nivalenol, T-2 and HT-2 toxins and FUM, 100%, 30%, 10% and 60% were added, respectively based on reports on the relative contribution of modified forms. Literature data showed that modified forms of mycotoxins may add substantially to the overall mycotoxin levels, in particular for FUM and ZEA[24]. Recently modified forms of mycotoxins have been included in the EFSA risk assessments[23]. Table 1 presents the most recent scientific opinions addressed by international bodies concerning the free parent forms, modified and emerging mycotoxins and the health-based guidance values (HBGVs) used in the human risk assessment.

Table 1 Most recent scientific opinions addressed by international bodies including the health-based guidance values (HBGVs) for mycotoxins used in the human risk assessments (adapted from[23]).

Mycotoxin	HBMGV for human risk assessment	Year of publication
Patulin	PMTDI of 0.4 μg/kg bw	2000
Alternaria toxins	TTC approach applied	2011
Ergot alkaloids	group ARfD 1 μg/kg bw group TDI 0.6 μg/kg bw	2012
Citrinin	other approach, see opinion	2012
Phomopsins	not established	2012
Sterigmatocystin	other approach, see opinion	2013
Beauvericin and enniatins	other approach, see opinion	2014
Zearalenone + modified forms	group TDI 0.25 μg/kg bw	2016
T-2 and HT-2 toxins + modified forms	group ARfD 0.3 μg/kg bw group TDI 0.02 μg/kg bw	2017
Nivalenol + modified forms	group ARfD 14 μg/kg bw group TDI 1.2 μg/kg bw	2017
Deoxynivalenol and acetylated + modified forms	group ARfD 8 μg/kg bw per eating occasion group TDI 1 μg/kg bw	2017
Fumonisins	group TDI 1 μg/kg bw	2018
OTA	BMDL10 4.73 μg/kg bw per day (non-neoplastic) BMDL10 14.5 μg/kg bw per day (neoplastic)	2020
Aflatoxins	BMDL10 0.4 μg/kg bw per day	2020

ARfD = acute reference dose; BMDL10 = Benchmark dose lower confidence limit for an extra cancer risk of 10%; bw = body weight; PMTDI = provisional maximum tolerable daily intake; TDI = tolerable daily intake; TTC = threshold of toxicological concern.

Concerning the routinely screened mycotoxins, the current regulations were established on toxicological data from studies taking into account only one mycotoxin exposure at a time, and do not consider the combined effects of mycotoxins. It is therefore of the utmost importance to evaluate the toxicological impact of mycotoxin combinations to better reflect feed and food contamination and their associated animal and human health risks[3,23,31,33].

EFSA had also delivered a guidance document on risk assessment of combined exposure to multiple chemicals describing the harmonised application of risk assessment (RA) methods for combined exposure to multiple chemicals to all relevant areas within European Food Safety Authority’s remit, i.e. human health, animal health and ecological areas[34].

4. LOOKING FOR SYNERGIES ON REGULATORY FRAMEWORKS

As the world becomes more globalized, food and feed supply chains also become more complex and hence, a more comprehensive strategy to ensure food contaminant mitigation is needed. Mycotoxin mitigation at industrial level is not an easy task, especially if inter-regional and inter-continental trade regulations are to be taken into account. Importers and exporters alike must comply with regulations within these countries in order to penetrate their markets. Stakeholders from both Europe and Southeast Asia must then ensure that there is a way to ease and harmonize the regulation in the food supply chain in order to ensure food safety and at the same time facilitate trade in both regions.

With a market of 447 million consumers, the European Union single market ranks globally as the world's largest single market covering 27 nations that trade freely on its products. Such harmonized integration boosts standing influence and increases global market share. However, with such advantages, the EU continues to identify factors that may disrupt this single market. Harmonisation at an EU-wide level is therefore a priority in order to ensure that there is a basis for all members that acts as a compliance guide[35].

Stakeholders from the European Union began the conduct risk assessment on undesirable substances in animal feed. This includes the opinion provided by the EFSA covering more than 30 risk assessments on food contaminants in the food and feed supply chain within Europe. This led EFSA to release various guiding documents that cover risk assessment on mycotoxins, tolerable daily intakes, toxicity data and determination of group health-based guidance values in several types of mycotoxins[36].

These guidance documents are used by regulatory institutions in the EU in order to create a harmonized set of regulations for the producers. These institutions also provide separate recommendations which are used to structure policies relating to mycotoxin mitigation. In 2011 for example, the Joint Research Centre published the Mycotoxin factsheet which provided stakeholders a list of the regulated mycotoxins within the region as well as information on toxicity levels, occurrence data and various analytical methods[37].

Regulation-wise, the European Commission has through EUR-Lex (https://eur-lex.europa.eu) a database that compiles regulations, directives, decisions and reports that pertain to policies as well as recommendations for producers and consumers. This includes the Commission Regulation (EC) No. 401/2006 which discusses the prescribed methods of sampling and analysis for the official control of the levels of mycotoxins in food[38]. This regulation is pursuant to the European Commission Regulation (EC) No 882/2004 that prescribes the official controls performed to ensure food and feed law compliance in terms of food contaminants[39]. One of the most recent EU-related publications is a review by the Joint Research Centre together with various research organizations, both from within the EU and outside, on the developments of mycotoxin analysis from 2017—2018 which includes updated and collated information on sampling, the use of LC-MS/MS multi-mycotoxin method to determine co-occurrence of these contaminants in cereals and a brief classification of different types of mycotoxins present in various types of foodstuff[40].

From the period of 2016 onwards, there has been a wide range of initiatives that aim to better inform the food industry of these regulations through reports, guidelines and a multi-stakeholder platform. In various food commodities, mycotoxin mitigation is commonly implemented early at the farm level through monitoring, prevention and control. Since farming conditions in Southeast Asia and Europe are auspicious to fungal growth, and various agricultural products may serve as suitable fungal substrates, farming practices often include various physical or chemical processes during post-harvest processing to eliminate or minimize fungal growth and prevent mycotoxin production.

5. IMPACT OF OTA ON COFFEE PRODUCTION IN SOUTHEAST ASIA

Coffee is among the most commonly consumed beverages globally, appealing to a wide range of consumers due to its distinct taste and flavor, and health effects. The International Coffee Organization revealed that in Southeast Asia, Indonesia and Vietnam were the main contributors to global coffee production. Recently, Philippines and Thailand are slowly gaining momentum as coffee producers.

Climatic conditions in Southeast Asian countries are favourable to coffee production, but poor post-harvest processing practices contribute to fungal growth and mycotoxigenesis in green coffee beans produced in these countries. Khaneghah[41] reported that high annual rainfall and poor harvest processing conditions contribute to high ochratoxin A (OTA) contamination in coffee. Ochratoxin A is a nephrotoxic, carcinogenic, teratogenic and immunosuppressive mycotoxin[42] produced by *Aspergillus* and *Penicillium* species[43-44]. Ochratoxigenic *Penicillium* species do not penetrate deep into a coffee bean[45], explaining the predominance of *Aspergillus* as the main cause of OTA contamination in coffee.

In Southeast Asia, the most popular coffee varieties are Arabica (*Coffea arabica*) and Robusta (*Coffea canephora* var. *Robusta*), contributing to the bulk of coffee production globally[46]. In the Philippines, Excelsa (*Coffea excelsa*) and Liberica (*Coffea liberica*) species are also cultivated. The type of coffee processing method generally depends on the coffee species grown. Arabica coffee, a specie which thrives well in cool areas, is processed using the ‘wet’ method while coffee species which are grown in warmer areas such as Robusta, Excelsa and Liberica are commonly processed using the ‘dry’ method. In the ‘wet’ method, mucilage and coffee pulp removal are done using fermentation. After removal of coffee pulp, sun-drying reduces the moisture content of parchment coffee. This process also facilitates additional fermentation[47], which is facilitated by microflora such as bacteria and fungi[48]. The composition of fungal flora is influenced by fermentation and drying using the ‘wet’ method[45,47].

The ‘dry’ method involves sun-drying of unsorted whole coffee cherries right after harvest. Dried coffee pulp (husk) is removed when green coffee beans are going to be sold. A speedy drying process is essential in ‘dry’ method to reduce OTA contamination. There is a greater opportunity for fungal growth when coffee is processed using the 'dry' method, unless the recommended duration per step and recommended agricultural practices are strictly followed[49].

The successful implementation of the two coffee processing methods in Southeast Asia may be limited by the type of coffee farm, amount of harvested coffee, and microclimate variations particularly during drying. These conditions also contribute to the incidence of fungal and OTA contamination in coffee, regardless of the processing method. Coffee farms in Vietnam range from 0.1 to 11.2 ha while the size of coffee farms in Indonesia is 0.3 to 8 ha[50]. Implementing the 'dry' method will require a greater cost if the farm size is larger, but income returns are expected to be higher. Small, backyard coffee farms on the other hand, introduce variations to processing methods based on farmers' skills and preference. The ILSI guidelines have provided a practical guidance using a traffic light system to determine which post-harvest step can be targeted to reduce mycotoxin contamination of final products. In coffee, the highlighted steps were hulling, sorting, and roasting[10].

Coffee husks are potential sources of OTA especially if toxigenesis occurred during drying[45]. As rich nutrient sources, coffee pulps serve may serve as suitable substrates for ochratoxigenic fungi. In the 'dry' process, dried coffee pulps and husks are typically removed after drying. When fungal growth and toxigenesis occurred in the drying yard, OTA from the pulp and husk may be transferred to roasted coffee beans. Coffee beans processed using the 'wet' process, in contrast, were reported to have lower fungal contamination[49].

Sorting of defective coffee beans decreases the likelihood of OTA contamination in coffee[51], although a standardized sorting method needs to be established in the Southeast Asian regions. Defective coffee beans negatively affect the over-all taste of coffee brews[47] and OTA contamination of coffee further reinforces the need to sort defective coffee beans to ensure good cupping quality and lower OTA exposure. Moldy beans, in particular, may reintroduce fungal contaminants to uncontaminated green coffee beans, especially if the beans are stored temporarily prior to selling or roasting. In the 'wet' process, sorting of defective coffee cherries can also be done early via flotation. The 'dry' process, however, involves sorting of defective coffee beans after drying, implying the need to manually identify defective coffee beans using morphological characteristics.

Reduction of OTA in coffee through thermal degradation in has been documented. Generally, as roasting temperature increases, the degradation of OTA in coffee also increases[52]. At a temperature as low as120 ℃, OTA can be converted to 2'*R*-ochratoxin A (2'*R*-OTA) while a longer roasting time and temperature above 240 ℃ results to fast degradation of OTA and 2'*R*-OTA[53]. In a recent study, coffee consumption was associated with the detection of 2'*R*-OTA in serum samples of coffee drinkers[54]. Ochratoxin A, however, exhibited higher affinity to human serum albumin compared to 2'*R*-OTA[43].

The roasting temperature for medium to dark roast of coffee only ranges from 215 ℃ to 225 ℃[55]. Since fast racemization of OTA occurs in medium to dark roasting parameters[53], it is suggested that roasted coffee should be tested for derivatives of OTA along with the changes in flavor parameters and their market acceptability since higher roasting time and temperature will increase the bitterness and produce a less desirable aroma[56]. Furthermore, a high roasting temperature and longer roasting time also causes polyphenol degradation but increases coffee melanoidins[57,58]. Light and medium roasting seems to preserve more phenolic compounds[58] but may not be effective in degrading OTA.

Overall, the reduction of mycotoxins in a farm setting poses several challenges to coffee farmers in Southeast Asia. Mycotoxin mitigation strategies need to be analyzed in the context of socioeconomic and behavioral factors of both coffee producers and consumers in Southeast Asia as compliance to good agricultural practices during post-harvest processing will still depend on the availability of materials and facilities, farmers' expertise, and consumer preference. Other challenges such as the extent of knowledge of farmers on mycotoxin contamination of coffee and the health risks associated with the consumption of OTA-contaminated coffee may also affect the compliance to good agricultural practices.

6. CEREAL PRODUCERS IN EUROPE: MITIGATION PROCEDURES TO REDUCE *FUSARIUM* TOXINS

Grain collectors and traders intervene after the grain is harvested. Crops are monitored and mould control using fungicides is carried out as needed.

Traders collect and analyse the mycotoxin levels in their lots at collection. Collectors dry (if necessary), clean and protect the crops from insect infestations to ensure compliance to regulatory and commercial requirements at EU and national level on food and feed safety. If MLs for food and feed are not met, the batch is diverted toward non-food applications as biogas production.

Dry milling operations from corn and wheat are providing for semolina, flours with different extraction yields, hominy to produce flakes, and co-products are moving mainly to animal feed. The starch industry is using corn and wheat in almost equal amounts for the production of starch, corn germs, wheat gluten for human consumption. Corn gluten fiber and meal, wheat bran, (wheat germs) are animal feed materials.

Harvested grains must be dried to < 14.5% moisture and stored at a relative humidity of about 70% with as low as possible temperatures swings. Despite efforts to control, mitigate and reduce fungal and mycotoxin contamination, postharvest decontamination approaches can offer a last resort to mitigate unavoidable and unpredictable contamination.

Cleaning and sorting results in good reduction as mycotoxins are routinely at a higher in level in dust, debris, damaged and shriveled grains. Milling leads to distribution of mycotoxins as highest levels are usually in the outer layers, so more in bran and less in flour. Cooking and baking normally have a negligible effect on mycotoxins except the dilution effect from other ingredients.

Schwake-Anduschus[59] have milled different batches of wheat using a Bühler MLU-202 mill into eight fractions and have studied the distribution of DON, DON-3G, ADON, ZEA and ZEA-14S. Interestingly, DON and DON-3G were found to be present to similar amounts in all fractions. In bran, the levels were only slightly higher than in the endosperm. By contrast, for ZEA and ZEA-14S a significantly higher amount of toxin is located in the fiber rich fractions. The relative mass proportion of DON-3G comprises for only between 2.9% and 11.2% of the free DON, while the relative mass proportion of ZEA-14S is estimated to exceed even the amount of free ZEA in certain fractions. Experimental results show that a significant reduction of the ZEA and ZEA-14S level in wheat flour is feasible by applying milling technology strategies. The almost even distribution of DON and DON-3G in all fractions does not allow for the technological removal of significant toxin amounts. The relative share of masked forms was higher for ZEA derivatives than for the DON conjugates in the investigated wheat lots.

Cereal crops are most susceptible to *Fusarium* species at flowering and the probability of infection rises with high moisture and humidity at flowering. A two-year observational study in the UK identified seasonal differences in the distribution of DON within the grain[60]. Thammawong[61] showed that fungal growth was largely restricted to the outer layers of the grain, but that mycotoxins diffused into the endosperm. The level of diffusion into the grain was independent of the level of fungal invasion, implying that environmental conditions post-infection will have a role in determining mycotoxin levels in milling fractions. In support of this conclusion, DON concentration was higher in white flour than in bran obtained from UK wheat samples in 2004, when high post-anthesis rainfall was recorded[62]. DON is highly water-soluble and can be translocated within host plants and it was proposed that the high pre-harvest rainfall in UK in 2004 caused the movement of DON within grain.

Legislative limits for *Fusarium* mycotoxins in the European Union decrease from unprocessed cereals to processed products. For wheat and wheat processed products maximum limits apply to DON, ZEA, AFs and OTA. A comprehensive overview on the different mycotoxins and their legal limits and on how processing of wheat affects contamination from raw material to highly processed final products, based on relevant scientific studies is published in the literature[12]. Of the four mycotoxins regulated in wheat-based foods in the EU, most data are available for DON, whereas aflatoxins were rarely studied in the processing of wheat. In summary, comparison of 27 cleaning studies on DON (22), OTA (3), NIV (3), ZEA (1), (H)T-2 (3) provides reductions up to 80% while comparing 51 milling studies mainly on DON, provides increase up to 20% as also decrease to 100% in wheat flour. Accordingly, an increase in bran was observed: for DON and ZEA concentration up to 300% while for T-2 and HT-2 toxin up to 500%. The processing steps cover primary processing (cleaning and milling operations) and secondary processing procedures (such as fermentation and thermal treatments during baking).

Corn is processed using either wet milling or dry milling. Dry milling is the physical process of removing the envelope of the grain to obtain part of the endosperm, yielding products such as corn grits, germ, and flour. Corn wet milling is a two-step process: after steeping during 30~36 hours at 50 ℃, the steep water is separated from the corn kernels. The swollen kernels are in a number of milling, sieving and centrifugation steps separated into corn germs, corn white fiber, corn gluten and corn starch. The white fiber is mixed with the steep water to provide for corn fiber.

These milling processes lead the production of corn by-products, which contain unevenly distributed mycotoxins after fractionation. The low levels of mycotoxins that may enter corn wet-milling plants are removed from food ingredient products through the normal processing steps used in their manufacture. Wet-milling is an effective process for removing mycotoxins like aflatoxins and fumonisins from corn starch, corn-derived sweeteners and corn oil[63].

Steeping of corn grains results in reduction of mycotoxins which depends on the solubility and partition properties of the individual mycotoxins. About 40% to 70% of the initial contamination end up in the steep water. Zearalenone is quite hydrophobic and about 50% of the initial load will remain in the corn gluten meal. Corn germs will independent from the type of mycotoxins take up about 10% of the mycotoxins. After removal of germs, the white fiber and the gluten, the crude starch is washed to reduce the soluble proteins – this washing process will usually reduce the mycotoxin load to a few percent of the load in the starting material[8]. Based on these observations for *Fusarium* mycotoxins over a period of three years, the European Commission provided the starch industry an exemption for the maximum limits for unprocessed corn that applies to corn which is intended for use in a wet milling process. Scientific data have shown that regardless the levels of *Fusarium* toxins present in unprocessed corn, *Fusarium* toxins were not detected or only at very low levels in starch produced from corn. Due to mixing of steep water with white fiber one gets fumonisins mainly in corn gluten feed[64,8].

Through the milling of wheat into wheat flour and wheat bran, most of the mycotoxins resides in the bran and the shorts. Wet wheat flour separations end up in the production of wheat gluten, wheat starch and wheat solubles. Wheat solubles are used as feed material or starting material for the production of ethanol.

7. THE SOUTHEAST ASIAN PERSPECTIVE AND REMAINING GAPS PRESENT ON MYCOTOXIN MITIGATION IN THE REGION

Regulatory policies with regards to mycotoxin mitigation are not harmonized in the Southeast Asian region. However, recommendations for standardization and the set-up of coordinating research and monitoring programme has been jointly proposed as early as 1987 by national governments, FAO, WHO and the UN Environment programme[65]. Below are the key factors discussed on the current situation on how the Association of South-East Asian Nations (ASEAN) is dealing with mycotoxin mitigation and regulation and which factors are considered as bottlenecks for the region.

7.1 Regulatory factors

The ASEAN has taken steps to optimize the trade between ASEAN member states. The region has established the ASEAN Risk Assessment Centre for Food Safety (ARAC) which is composed of scientific experts across ASEAN and provides scientific opinion in guiding the public by developing evidence-based data on food safety and quality. ARAC has enabled an ASEAN Consultative Committee on Standards and Quality Prepared Foodstuff and Products which proposed harmonized maximum levels for contaminants and toxins in food and feed. These levels were based on the CODEX STAN 193-1995 (Codex General Standard for Contaminants and Toxins in Foods) and aim to prevent unnecessary regulatory bottlenecks within the ASEAN region by recommending the harmonized maximum levels for contaminants in food and feed to be used by other relevant sectoral bodies in ASEAN[66]. However, monitoring these non-binding recommendations present difficulties with some SMEs, which account for up to 97% and provides up to 80% of the total employment in the ASEAN Region[67].

7.2 Environmental factors

As with other regions, the ASEAN region is also composed of various landscapes and agrosystems not just amongst the member states but within the countries themselves. Figure 1 shows the climactic variation in the region in terms of temperature and humidity.

These variations lead to higher prevalence of certain mycotoxins in one part of the region than the other. Climate change and its increasing unpredictability will also result in a change in the fungal diversity of

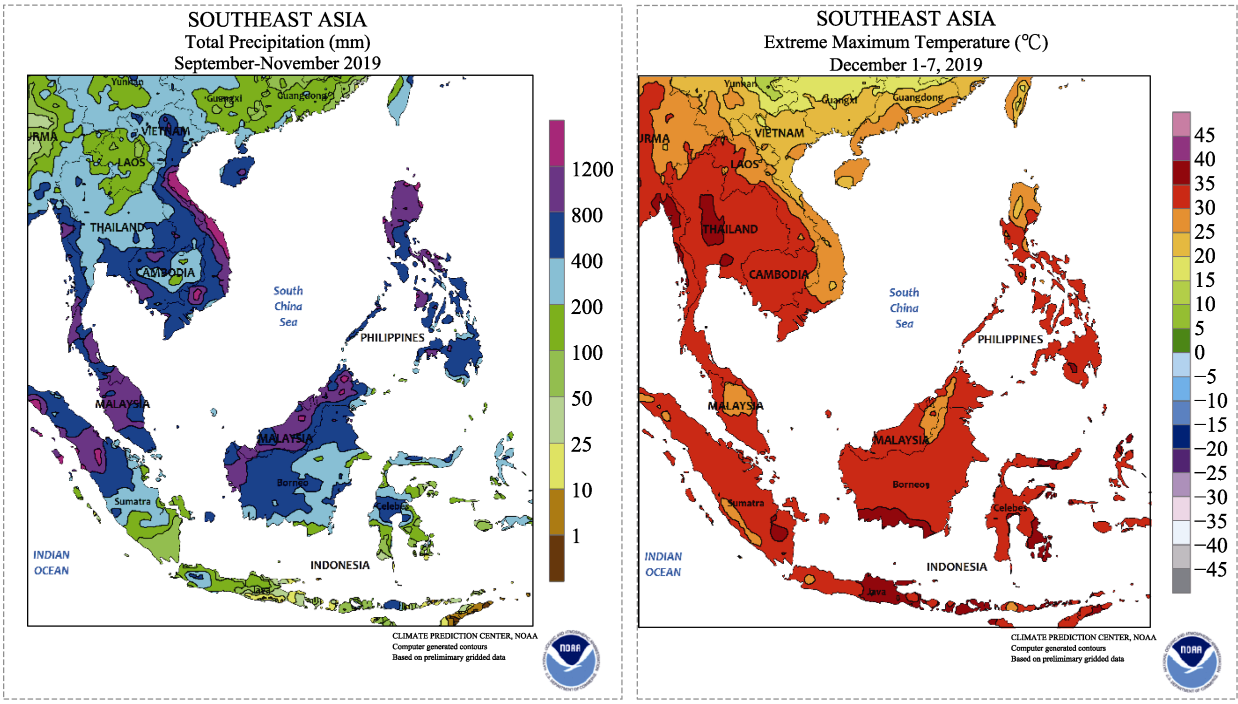

Fig.1 Variation in precipitation (left) and temperature (right) within the Southeast Asian region

crops common in the ASEAN region such a rice and corn. This may result to a compromised crop yield and quality[68].

These effects are also observed in other non-ASEAN countries in Asia. For example, high variability in aflatoxin contamination has been observed in Bangladesh, and the contamination is higher in developing countries where rice constitutes the major nutritional source of the diet, than in developed countries[69]. In the Punjab region (India), due to the cold season during August to March combined with the high humidity level, high fungal growth and consequently, high occurrences of contamination levels of AFs in rice have been also observed[70].

In terms of ASEAN monitoring for toxicity and occurrence, this varies from one member state to the other. In Thailand for example, two ministries (Agriculture and Public Health) are tasked to prevent mycotoxin growth within the fields through monitoring and providing evidence for the Thai policy-makers to evaluate. On the other hand, the Thai Food and Drug Administration (FDA) has established the maximum levels and conducts risk assessment in mycotoxins to ensure that the maximum level of a commodity are safe for consumers[71].

7.3 Economic factors

An increase in free trade policies and bilateral trade agreements may result in two sets of standards for internal and export products. For example, the EU and Vietnam agreed in February 2020 on a bilateral free trade agreement which aims to boost trade and investment between the two economies. With the approval of this trade deal however, the European Commission identified technical barriers to trade that Vietnam is asked to address. This includes the involvement of standardizing bodies in the preparation of international standards and the use of relevant international standards as the basis of domestic standards as well as the review of national and regional standards to avoid overlapping information and reduce regulatory bottlenecks hindering trade[72-73]. Other ASEAN member states who also wish to engage on free trade agreements with the EU would also be expected to follow these recommendations in the future. Lastly, as the consumer preference varies in each ASEAN member states, the regulation set by each national bodies also differ. There would be a need to harmonize these policies and more comprehensive record-keeping is needed in order to facilitate the success of these free trade agreements.

8. CONCLUSIONS AND OUTLOOK

From the comparison of the European and South East Asia scenario some conclusions and outlook can be inferred and are reported here below; they can also be included in a more general framework that involves other parts of the World.

Many international agencies are trying to achieve universal standardization of regulatory limits for mycotoxins. To date, more than 100 countries have mycotoxin regulations for raw materials, food and feed products. From the several hundred mycotoxins that have been described only a limited but increasing number are addressed in the regulations. It is implied that any regulations regarding these toxins will be in co-ordinance with any other countries with which a trade agreement exists. However, one must take note that scientific risk assessment is commonly influenced by culture and politics, which, in turn, will affect trade regulations of mycotoxins.

Often several commodities and processed products contain different mycotoxins which require better analytical strategies. More mycotoxins must be measured at the same time since food products are contaminated with more than one mycotoxin above levels of concern – in recent years multiple mycotoxins and the levels of conjugated forms have been better characterized.

Fusarium toxins are produced by over 50 species of *Fusarium* and have a history of infecting the grain of developing cereals such as wheat and corn during the flowering stage with favorable conditions. More information is becoming available on the levels of corresponding modified forms with respect to the parent mycotoxins, which depending on the mycotoxins can be as low as 10% (such as for the sum of T-2 and HT-2 toxin) but also as high as 100% (such as for zearalenone and related compounds).

Pre-harvest rainfalls have an effect as DON has a better mobility than ZEA due to the difference in solubility and partition coefficient. There is still contradictory data in the literature on the fate of DON during wheat and wheat-based products processing due to differences in processing, such as temperature, additives, processing time and loaf size, in addition to the occurrence of modified (masked) forms of DON. Therefore, further research must be carried out aiming to reveal the formation and occurrence of modified forms of DON. But this also applies to other less well studied mycotoxins and their conjugated forms.

How to manage the different mycotoxins in cereals and the cereals-based food production chain is clearly dependent upon the process that is chosen. Going from the unprocessed cereal to the processed cereal products it is possible to observe reductions depending upon the processes that are used. Legislative limits for *Fusarium* mycotoxins decrease from unprocessed wheat to processed products.

Redistribution of mycotoxins in dry milling operations often results in increased levels in bran and shorts, while in baking flour and second grade flours lower levels are obtained compared to the starting raw materials. The unit operations that provide the biggest reductions in mycotoxin levels are found in the starch industry. Washing the crude starch results in reductions of up to 99 % which has the drawback that mycotoxins are redistributed in the co-products that end up in animal feed. Mycotoxins in wheat gluten can be reduced when fresh water is used, but a limiting factor in modern starch processing is the aim of minimal water use and maximum re-use of water.

A number of differences become evident when comparing the EU and SEA situation. Climatologically SEA is in a zone that will favor production of mycotoxins in a number of crops. If we look at the quarterly overviews that companies like Biomin and Cargill (https://notox-online.com/) are providing one cannot deny that SEA and Asia provides the highest incidence in aflatoxins, fumonisins, zearalenone, ochratoxins and emerging mycotoxins which is a huge challenge for competent authorities to keep their population safe with respect to the exposure through food consumption.

The SEA region is more apt to allow the production of major crops such as rice and coffee which are not produced in Europe. Rice is the most important staple food in the SEA region but scientific literature is highlighting the number and levels of mycotoxins found in it. The impact of OTA in coffee production was discussed and showed that for some dark coffee, a post-harvest solution is available to provide for a safe final product.

As better analytical tools become available and through collaboration between food manufacturers and competent authorities who are refining and expanding their regulatory schemes to impose compliance to protect the population, there will be a better future when more appropriate risk assessments can be made by checking the impact of multiple mycotoxins by a simple check of biological samples.

As the negative effects of the different mycotoxins are better assessed, better risk assessments can be made, more restrictions will be imposed by the regulatory instruments of each independent country which will be weighed against trade agreements to allow circulation of major crops. Safer foodstuffs are produced by deploying a number of production steps impacting the final level of mycotoxins but the effect of climatological change cannot be denied. This will be a challenge which is common for both Europe and South East Asia.

ACKNOWLEDGEMENTS

This work is the proceeding of the session organized by ILSI Europe and ILSI South-East Asia at the WMF meets Asia and builds on the previous publications of the Process-Related Compounds Task Force of ILSI Europe. This proceeding was not conducted by ILSI Europe. The opinions expressed herein and the conclusions of this publication are those of the authors and do not necessarily represent the views of ILSI Europe nor those of its member companies, nor any regulatory authority. The authors warmly thanks Prof. Armando Venancio (University of Minho, Portugal), Dr. Michelangelo Pascale (Institute of Sciences of Food Production National Research Council, Italy), Dr. Gloria Pellegrino & Dr. Manuela Rosanna Ruosi (Lavazza, Italy) Dr. Natalie Thatcher (Mondelēz International, United Kingdom) and Dr. Gerrit Speijers (Consultant) for their fruitful suggestions and revisions.

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See in its Chinese version P56-P58.

· 信息窗 ·

《2021 年世界粮食安全和营养状况》中文版全文发布：疫情肆虐，全球饥饿人数激增

2020 年世界饥饿状况急剧恶化，新冠疫情的冲击可能是造成这一状况的主要原因。虽然疫情影响还有待于进一步评估[1]，但由多家联合国机构联合撰写的《2021 年世界粮食安全和营养状况》报告估计，去年全球大约十分之一的人口（8.11 亿人）面临食物不足的困境。这一数字表明，各国亟需付出巨大努力，立即采取行动，推动农业粮食体系转型，才能实现在 2030 年之前消除饥饿的承诺。

《2021 年世界粮食安全和营养状况》中文全本报告正式发布。©粮农组织

《2021 年世界粮食安全和营养状况》英文版于今年 7 月 12 日全球发布，其中文版于 10 月 11 日正式上线。本年度的《世界粮食安全和营养状况》是疫情暴发后发布的第一份此类全球评估报告。报告由联合国粮食及农业组织（粮农组织）、国际农业发展基金（农发基金）、联合国儿童基金会（儿基会）、联合国世界粮食计划署（粮食署）和世界卫生组织（世卫组织）联合发布。

《世界粮食安全和营养状况》往期报告一直强调，包括儿童在内的亿万民众面临着粮食不安全风险。五家联合国机构的负责人[2]在今年报告的前言中写道："不幸的是，疫情仍在继续暴露我们粮食体系中存在的问题，这些都威胁着世界各地人民的生命和生计。"

尽管对加强外交领域的势头寄予厚望，但五位负责人仍然提醒说，世界处于"紧要关头"：即将召开的联合国营养促增长峰会以及有关气候变化的第 26 次缔约方大会在今年带来了宝贵机遇，让我们能够通过农业粮食体系转型推进在粮食安全和营养方面取得进展。他们补充说，"这些会议的成果"必将继续推动'联合国营养行动十年'下半程的发展"。目前，这项全球政策承诺尚未进入大踏步落实阶段。

（来源：联合国粮农组织微信公众号，2021 年 10 月 11 日）