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Report Series

Practical Guidance to Mitigation of Mycotoxins during Food Processing

Report

ILSI Europe
International Life Sciences Institute

Commissioned by the ILSI Europe Process-Related Compounds and Natural Toxins Task Force

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This publication is made possible by support of the ILSI Europe's Process-Related Compounds and Natural Toxins Task Force. Industry members of this task force, as well as the composition of the Board of Directors and the Scientific Advisory Committee are listed on the ILSI Europe website at http://www.ilsi.eu.

PRACTICAL GUIDANCE TO MITIGATION OF MYCOTOXINS DURING FOOD PROCESSING

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REPORT

COMMISSIONED BY THE PROCESS-RELATED COMPOUNDS AND NATURAL TOXINS TASK FORCE

August 2019

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Printed in Belgium
ISBN XXX

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FOREWORD

PRACTICAL GUIDANCE TO MITIGATION OF MYCOTOXINS DURING FOOD PROCESSING

Mycotoxin contamination of commodities is a significant challenge for food operators in both the developed and developing regions, and climate change is anticipated to alter and in some cases exacerbate the occurrence and concentration of mycotoxins (Battilani and others 2012). It has been estimated that currently around 500 million low income people, living in sub- Saharan Africa, Latin America, and Asia, whose diet are dependent mostly on cereals are exposed to aflatoxins and fumonisins (FAO/WHO 2003).

The aim of this document is to translate into a simplified guidance the findings of the Expert Group on 'Reactions and Potential Mitigation of Mycotoxins during Food Processing'; due to the high number of downloads from the journal Mycotoxin Research. (>16000 downloads as of August 2019). The original activity identified food processes that can significantly reduce the mycotoxin content. The report then summarized the impact of the different decontamination and detoxifying processes on various food commodities. Finally, the impact of modified or transformed mycotoxins leading to a lower mycotoxin concentration and a lower toxicity was illustrated and discussed.

This present Black & White Report has been updated since the original publication, taking into account scientific findings within the last 3 years. The intended audience is mainly industry wishing to understand and participate in the global mycotoxin mitigation. The data presented on occurrence, toxicity and mitigation strategies are based on recent scientific literature. Only high quality evidence has been used from internationally indexed, peer-reviewed and reputable scientific journals to help ensure the reliability of the data presented. A knowledge-based search by experts was used to ensure the suitable coverage of relevant literature on each topic presented. Specifically, data from the so defined "grey literature" (namely, those publications for whom the rigorous scientific process could not be taken for granted) were not considered. Similarly, any hypothesis/speculation concerning the effectiveness of the mitigation processes presented was discouraged whenever there was not reasonable support from available literature. The document is intended to help food operators and other stakeholders involved with mycotoxin food contamination, understand per commodity (for example cereals & derived products, cocoa, fruit juices, dairy products) the proven, easy to implement, and practical methods to mitigate mycotoxins.

In addition to the review of literature, as a part of the development of this text and in order to strengthen reliability, interviews were conducted with industry representatives for the commodities included with the following intention:

- To receive feedback on the adequateness of the production chart for each food chain;
- To understand the presence (or absence) of gaps in the analysis of the relevant processing steps together and;
- To further develop applied concrete action-strategies in terms of mycotoxin mitigation.

The mitigation steps included in this guide should be checked with local legislation before implementation.

It should be noted, that this guide could also be useful for official control agencies.
It is the intention that this publication will be regularly updated with the most recent scientific developments and periodically extended to other commodities or enriched with innovative processes every 3-4 years.

1. INTRODUCTION

The term "mycotoxins" refers to low-molecular-weight molecules of natural origin that may impair to various extents the health of humans and animals (Bennett and Klich 2003). They are bioactive compounds produced as secondary metabolites by ascomycetes (filamentous fungi) presumably to provide a competitive advantage on food sources compared to other microorganisms. They pose food safety concerns at a global scale, as they may contaminate a range of different food commodities (Karlovsky 2016) both in the field and during subsequent processing and storage. The contamination process may occur at multiple steps along the production chains posing potential concerns at all phases of food production; from raw materials to final products (Dellafiora and Dall'Asta 2017).

Plants intended for food and feed production are commonly regarded as the primary hosts of fungal growth resulting in mycotoxin contamination. However, food of animal origin (e.g. meat, eggs, milk and their derived products) may contain mycotoxins due to carryover when animals eat or are fed with contaminated products. In particular, mycotoxin contamination may occur pre-harvest during plant growth or post-harvest during processing, packaging, distribution and storage of food and intermediate products (Alshannaq and Yu 2017). The fungi species mostly involved in the production of mycotoxins of food concern belong to the genus Aspergillus, Claviceps Fusarium and Penicillium (Alshannaq and Yu 2017). The production and the relative abundance of mycotoxins in different foods strongly depend on environmental parameters such as temperature, humidity (figure 1 and 2) and damage to grains due to insect pests.

Figure 1 Decision tree for directing risk management decisions or actions based on environmental considerations and probability of fungal contamination in warm climates. Expected effects in susceptible animals are given for each group of mycotoxins (IARC 2012)

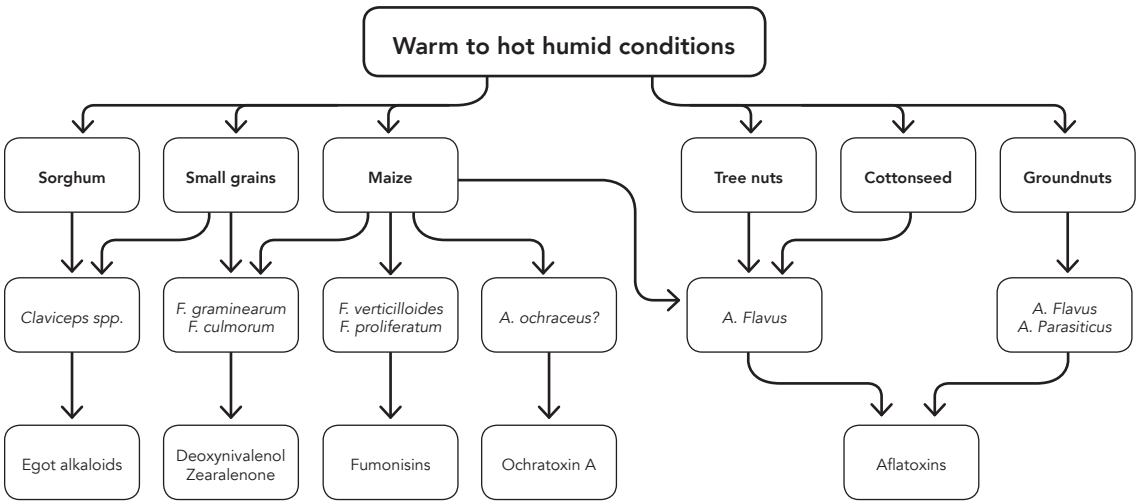
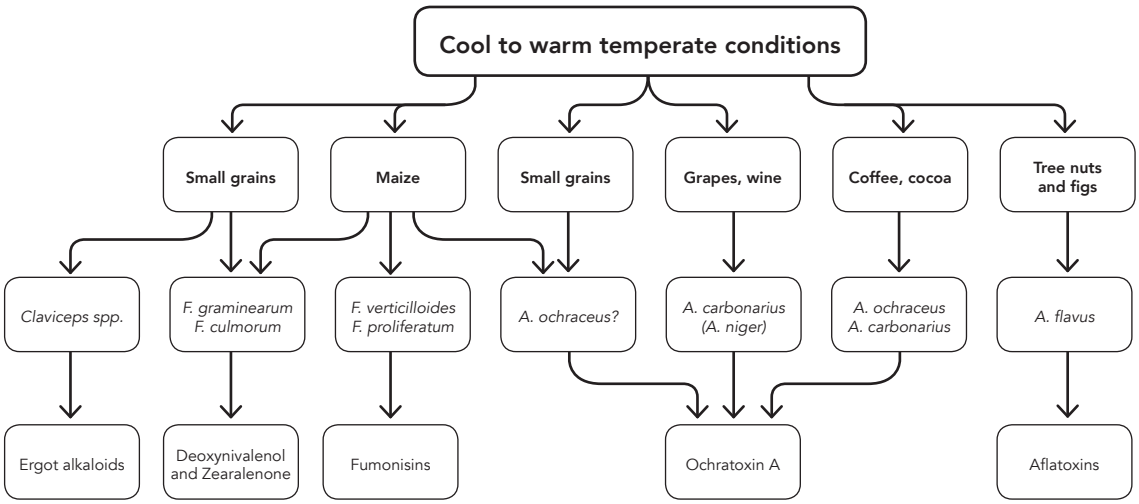


Figure 2 Decision tree for directing risk management decisions or actions based on environmental considerations and probability of fungal contamination in cool climates. Expected effects in susceptible animals are given for each group of mycotoxins (IARC 2012)



Regarding post-harvest contamination, crops including cereals that are improperly stored in conditions of temperature and humidity that promote mold growth may result in contamination by mycotoxins (Bennett and Klich 2003).

Many authorities at both national and international level, such as the US FDA, EFSA, FAO and WHO, are involved in managing mycotoxins in food and have proposed strict codes of practice and limits. In this regard, about 100 countries have set limits on the presence of well-known mycotoxins in food and feed (Lee and Ryu 2017). As an example, limits for aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FBs), zearalenone (ZEN), deoxynivalenol (DON) and patulin (PAT) have been set in a number of countries (Alshannaq and Yu 2017).

This report focuses on those mycotoxins have been well characterized in terms of both occurrence and toxicity, and for which regulations and/or recommendations have been proposed and/or adopted. The existence of already described mitigation strategies has been used as inclusion criteria. In this respect, mitigation strategies refer to processes and techniques applied along the production chain that may reduce the abundance of specific mycotoxins via biological, physical or enzymatic means (Hassan and Zhou 2018). However, selected emerging or modified mycotoxins of particular relevance (*vide infra*) are also discussed. This is despite their characterization in terms of occurrence, toxicity or mitigation fate not being adequately described.

2. MYCOTOXINS OF RELEVANT OCCURRENCE/ TOXICITY

2.1 Occurrence (general)

When contaminated by filamentous fungi, agricultural commodities can contain various amounts of mycotoxins. Their level of contamination varies with climatic conditions and fungal species. Foodstuffs may be contaminated with multiple strains of fungi and most fungal strains produce more than one type of mycotoxin leading to the presence of mixtures of mycotoxins. Table 1 and figure 3 describe, for different commodities, the mycotoxins with the highest reported occurrence as well as the main producing fungal species.

Table 1 Occurrence of the most relevant mycotoxin per each commodity

	Cereals	Apple Juice and Cider	Cocoa	Milk and Dairy	Vegetable Oils	Dried Fruits and Nuts	Spices	Coffee	Beers
Aflatoxins	x[1]	x[12]	x	x	x	x	x	X	
Ergot alkaloids	x[9]								
Ochratoxin A	x[1]		x	x		x	x[2]	X	x
Fumonisins	x[1]				x		x[3]	x[10]	x
Patulin		x							
Trichothecenes	x[1]				x	x[13]		x[10]	x
Zearalenone	x[1]				x	x[14]	x[4]		x
Alternaria toxins	x[11]	x[11]			x	x	x[5]		x[6]
Emerging *Fusarium* mycotoxins	x[15]		x[7]			x[7]	x[7]	x[10]	x[8]

[1] (Marin and others 2013); [2] (Ozbey and Kabak 2012); [3] (Waskiewicz and others 2013); [4] (Santos and others 2010); [5] (Dobson 2017); [6] (Siegel and others 2010); [7] (Prosperini and others 2017); [8] (Habler and others 2017); [9] (Belser-Ehrlich and others 2013); [10] (Garcia-Moraleja and others 2015); [11] (Fraeyman and others 2017); [12] (Fernández-Cruz and others 2010); [13] (Azaiez and others 2015); [14] (Scott 2008); [15] (Gruber-Dorninger and others 2017)

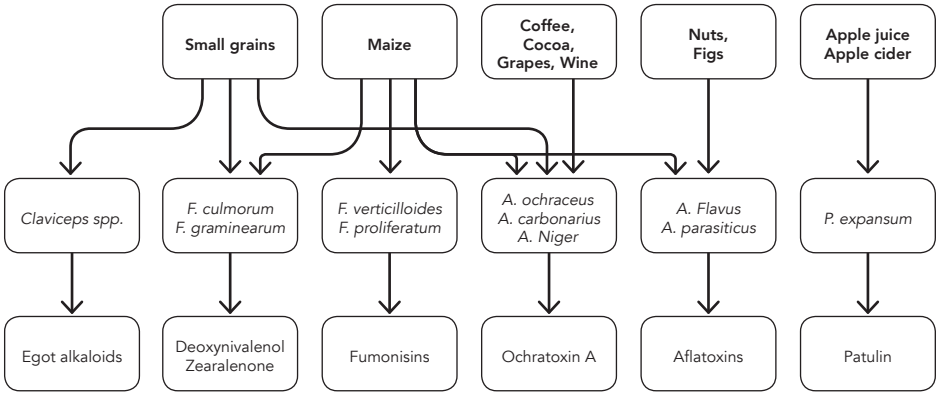


Figure 3
Decision tree for most relevant mycotoxin occurrence (IARC 2012)

2.2 Toxicity

Mycotoxins are bioactive compounds with a range of biological targets and as such exert a range of acute or chronic effects. Fortunately, contamination levels in food are usually not high enough to cause an acute toxicosis in humans. Acute effects are more often seen in farm animals following consumption of contaminated feed. Low levels of toxins are likely to result to unpredictable effects, as toxicity will depend on the toxin present, amount, duration of exposure and a variety of other factors, including age, nutrition and concurrent disease. In humans and livestock, exposure to mycotoxins induces various health problems including gastrointestinal pain, diarrhoea and liver cancer as well as retarded growth and development (Bryden 2007).

Mycotoxins can be genotoxic, immunotoxic, hepatotoxic, neurotoxic or nephrotoxic (table 2). Their mechanism of action can vary and depends on their capacity to inhibit protein, DNA or RNA synthesis, to induce lipid peroxidation or programmed cell death, and to alter membranes structure and function (Bondy and Pestka 2000; Dersjant-Li and others 2003; Lye and others 1995).

Table 2
Toxicity of mycotoxins

Mycotoxins	• Mechanisms of actions, deleterious effects on human and animal health	*Commodities commonly affected*
Aflatoxins (Marin and others 2013)	• Aflatoxins (AFs) are potent genotoxic carcinogens (Group 1) after metabolic conversion to 8,9-epoxides in the liver • AFs are also immunosuppressive and teratogenic • acute poisoning in humans leads to abdominal pain and vomiting, anorexia, pulmonary or cerebral oedema, necrosis, and fatty liver • chronic human exposure induces liver cancer, effects on the reproductive and immune systems, encephalopathy, fatty degeneration of viscera or pulmonary interstitial fibrosis • chronic exposure in animals leads to a decrease in productive parameters (weight gain, decreased egg or milk production), impairment of intestinal functions and increased susceptibility to infectious diseases	*maize,* *wheat,* *rice,* *peanut* *sorghum,* *pistachio* *almond,* *ground nuts* *tree nuts,* *figs* *cottonseed* *spices* *milk* *milk products*
Ergot alkaloids (Belser-Ehrlich and others 2013)	• Ergot alkaloids (EA) possess a high affinity for the receptors of different neurotransmitters (serotonin, dopamine, and adrenaline) • typical clinical symptoms of EA poisoning are vasoconstriction that may progress into gangrene, disruption of reproduction and abortion • neurotoxic signs of EA include feed refusal, dizziness and convulsions, agalactia and adverse effects to the cardiovascular system • in humans, exposure to high doses of EA can lead to death. Before, general symptoms are weakness, burning sensation, vomiting and diarrhea. The dry gangrene that can result in loss of one or more limbs and desquamation of the skin • livestock exposed to EA leads to cutaneous and gangrenous lesions of the tail and extremities, hyperthermia and production loss. Reproductive failure and convulsive or nervous symptoms are also observed.	*rye* *barley* *wheat* *oats* *triticale*
Fumonisins (Marin and others 2013)	• Fumonisins (FBs) inhibit sphingolipid biosynthesis leading to cardiovascular and possibly carcinogenic effects in humans • FBs are possibly carcinogenic to humans (Group 2B) • Human exposure to FBs has been associated with esophageal cancer in South Africa, liver cancer in China and neural tube defects in the Mexico–Texas border • in animals, liver and kidney are the major target organs • however, species-dependent differences exist: in horses, consumption of FBs-contaminated feeds targets the brain and induces a leukoencephalomalacia; in pigs FBs are cardiotoxic and cause pulmonary edema	*maize* *maize products* *sorghum asparagus*

Ochratoxin A (Marin and others 2013)	• Ochratoxin A (OTA), structurally similar to phenylalanine, inhibits enzymes, in particular the Phe-tRNA synthetase leading to inhibition of protein synthesis • OTA causes mitochondrial damage, oxidative burst, lipid peroxidation, and interferes with oxidative phosphorylation • OTA is immunotoxic, teratogenic, genotoxic and possibly carcinogenic to humans (Group 2B) associated with urothelial cancer of the upper urinary tract • as a potent toxin for kidney, OTA induces in this organ vascular lesions and hemorrhages • acute exposure to OTA is in humans, a causative agent of nephropathy • in animals, at high doses, OTA induces fibrin thrombi in different organs • chronic exposure in animals leads to a nephropathy • exerts adverse neurological effects and embryo toxicity	*cereals* *dried vine fruit* *wine* *grapes* *coffee* *cocoa* *cheese*
Patulin (Marin and others 2013)	• Patulin (PAT) has a strong affinity for sulfhydryl groups and inhibits the activity on many enzymes • acute effects of PAT include nausea, vomiting and other gastrointestinal symptoms, kidney damages and effects on the immune system • in long-term studies with animals, PAT is mutagenic, neurotoxic, immunotoxic, genotoxic, and may cause gastrointestinal effects • similar effects may occur in humans through chronic consumption of contaminated foods and beverages	*apples* *apple juice and concentrate*
Trichothecenes (Marin and others 2013)	• Trichothecenes (TCT) inhibit protein synthesis by binding to the ribosomal peptidyltransferase site, leading to activation of signaling mediators, the mitogen-activated protein kinases • Type A trichothecenes • acute effects of T-2 toxin are similar to high dose radiation (diarrhea, hemorrhage, hematotoxicity, and immune suppression) • extremely toxic on skin and mucous membranes • pig and poultry are the more sensitive farm species to T-2 and its metabolite HT-2 • Type B trichothecenes • Deoxynivalenol (DON) affects hematopoiesis and is immunosuppressive or immunostimulating (depending of the dose and duration of exposure) • chronic exposure of animals to DON causes weight loss, anorexia, and decreased nutritional efficiency due to neuro-endocrine effects • alters the intestinal barrier function • toxic effects of nivalenol (NIV) include immunotoxicity and hematotoxicity • DON and NIV are classified by the IARC in Group 3 carcinogens	*maize* *wheat* *barley* *oats*
Zearalenone (Marin and others 2013)	• ZEN and its metabolites interact with ?- and ?-estrogen receptors and are endocrine disruptors • acute toxicity of ZEN seems to be relatively low • toxicity is associated with reproductive problems in specific animal species and possibly in humans • ZEN is not classifiable regarding its carcinogenicity to humans (Group 3) but may have a role in the etiology of human breast cancer • in animals, the estrogenic activity of the modified forms of ZEN differs and targets the reproductive performances • susceptibility of pigs to ZEN has severe effects on animal reproduction.	*cereals* *cereal products,* *maize* *wheat* *barley*

Alternaria toxins (Fraeyman and others 2017)	• *Alternaria* mycotoxins such as alternariol (AOH), alternariol monomethyl ether (AME) cause DNA strand breakage and cell cycle arrest • *in vitro*, AOH and AME induce apoptotic cell death and, due to a structure similar to estradiol, exhibit an estrogenic response and interfere with steroidogenesis • *in vitro* studies suggest a low toxicity • *in vivo* studies on their effects on reproductive and developmental health are limited • Tenuazonic acid (TeA) inhibits the release of newly formed proteins from the ribosomes • however, *in vivo* TeA induces emesis, salivation, tachycardia, hemorrhages and hemorrhagic gastro-enteropathy in rats, mice, dogs and monkeys	*tomato products* *fruits* *wines* *dried figs* *olives* *Sunflower seeds and oils* *vegetable oil*
Emerging Fusarium mycotoxins (Gruber-Dorninger and others 2017)	• Information on the toxicological relevance of the emerging mycotoxins is still limited and mainly obtained from *in vitro* studies. • Enniatins (ENNs) and Beauvericin (BEA) possess lipophilic properties and are incorporated into lipid bilayers of cell membranes and express ionophoric characteristics related to their wide range of biological activity. • ENNs exhibit insecticidal, antifungal, antibacterial, and anthelmintic properties • in rodents, ENNs show low toxicity but cross the blood–brain barrier and demonstrate bioaccumulation in the lipophilic tissues • *in vitro*, ENNs induce cytotoxicity, oxidative stress, genotoxicity, estrogenic activity, impairment of cell cycle distribution or apoptosis • *in vivo*, BEA is not toxic to rodents and poultry but can accumulate in fat-rich tissues due to its lipophilic properties • *in vitro*, BEA induces cytotoxicity, oxidative stress, genotoxicity and apoptosis and acts as an enzyme inhibitor in liver microsomes • Moniliformin (MON) inhibits thiamin pyrophosphatase dependent enzymes, compromising the tricarboxylic acid cycle • MON toxicity on animal cells *in vitro* is dependent on the cell lines • MON does not inhibit the proliferation of human white blood cell progenitors or human platelet progenitors, but is cytotoxic to human red blood cell progenitors • MON shows more severe effects *in vivo*: depending on the species. MON induces damage to the heart muscle, muscular weakness, respiratory distress, decreased feed intake and body weight gain and impairs immune functions	*cereals* *cereals products*

3. CEREALS

3.1 Food Chain description

Farmers as primary producers play an important role for the quality characteristics of the grains they grow and harvest. Quality attributes are checked in particular as grains enter supply chains at the wholesale stage. Processors preserve, dry, prepare and pack the grains to allow the industrial use of batches in food, feed, or nonfood markets.

Wheats and oats are usually dry milled with the raw material coming mainly from intermediary collectors. Flake and other milled oats are produced mainly for human food (*e.g.* breakfast cereals, bread making) with by-products being used mainly for animal feed.
Semolina and flour may also be destined to human consumption (breakfast cereals, snacks, brewery industries, etc.). Co-products (bran, fodder flours and de-oiled germs) are sold into animal feed chains. Oil may be used by the cosmetics industry.

Starch production is also a primary processing industry. Starch, proteins, fibers and lipids of wheat and corn are extracted and used for human and animal nutrition as well as industrial applications (Intercereals 2014).

Milled oats are historically consumed heated with water or milk as porridge but are increasingly found in a wide range of processed and pre-packaged foods many of which are promoted on the basis of the nutritional benefits of oats.

3.2 Mycotoxins of relevant occurrence / toxicity

Cereal grains and their derivatives are susceptible to common mycotoxin producing fungi including *Aspergillus, Claviceps, Fusarium and Penicillium*. As a result, they may be contaminated by the mycotoxins, aflatoxins (AFs) and ochratoxin A (OTA). However, wwhen they are cultivated in cooler temperate regions, mycotoxins produced by *Fusarium* species as fumonisins (FBs), trichothecenes (TCT) as deoxynivalenol (DON), nivalenol (NIV), T-2 toxin and HT-2 toxin and zearalenone (ZEN), are the principal concern.

Infection of cereals and therefore potential production of mycotoxins in cereals are extremely variable. Even in climatic conditions that favor infection and growth, mycotoxin contamination can vary across a single field. Although all sampling regimes carry uncertainty in terms of their ability to detect hot spots of contamination, it is important that efforts are made to ensure that sampling is as representative as possible given the resources available. Concerning cases of exceedance of regulatory limits, these should be used as an indicator that corrective action is required, and should not be taken to infer a direct risk to health. Such corrective action could include confirmatory sampling to both confirm and bracket the point of contamination, and review of agronomical practices that may contribute to contamination or mitigate it. Recent periodic exceedances are reported for DON for Nordic oats and the sum of T-2 and HT-2 toxin for Scottish oats.

The predominant fungal species and therefore mycotoxin is influenced not just by climate, but also by the agronomic practices used. For example within Europe, oats are grown in cooler northern countries including the Nordic region and Scotland. Based on historical monitoring data, the principal concern with oats from the Nordic region is the presence of DON, whereas oats from the UK and in particular Scotland are more affected with T-2/HT-2 (Croucher 2018; Meyer and others 2018; Petterson and others 2018). Climatic and agronomical conditions lead to preferential growth for either *F. graminearum* or *F. langsethiae* respectively, and thus the predominant mycotoxin found (Martin and others 2018). Recently the presence of mycotoxins produced by *Alternaria* fungal species, in particular alternariol previously associated with fruits and vegetables, has been described in oat supply chains (Arcella and others 2016).

All these toxic metabolites can induce adverse effects in humans and animals and are of significant public health concerns (Bryden 2007).

The masked mycotoxins are biologically modified mycotoxins. For example, the metabolic activity of the plant, such as deoxynivalenol, can be found in the form of DON-3-glucoside. These chemical modifications can potentially affect both their toxicity (increased or decreased compared to the parent toxin molecule) and their analytical detectability.

Emerging toxins are defined as neither routinely determined, nor legislatively regulated. However, the evidence of their incidence is rapidly increasing. Commonly mentioned in this group are enniatins, beauvericin and moniliformin (Berthiller and others 2013).

Ergot alkaloids (EAs) are produced by *Claviceps* genus parasitic fungi. Detected in cereals and cereal products in Europe and North America, their occurrence has been increasing in the last few years and remains a source of concern (Malysheva and others 2014).

3.3 Health risk

The following mycotoxins explained in these production chains may pose the following health risk depending on the botanical origin of the cereal:

- Aflatoxins (AFs) are extremely potent toxins and genotoxic carcinogens (Group 1). Chronic exposure induces liver cancer and affects the reproductive, intestinal and immune functions.
- FBs are possibly carcinogenic to humans (Group 2B). It has been associated with esophageal and liver cancer as well as neural tube defects. In horses, FBs induces leukoencephalomalacia and in pigs, it causes pulmonary edema.
- Deoxynivalenol (DON) alters the intestinal barrier function, affects immunity and hematopoiesis. In animals, it causes weight loss and anorexia due to neuro-endocrine effects.
- ZEN and its metabolites interact with □- and □-estrogen receptors and are associated with reproductive issues.
- OTA is possibly carcinogenic to humans (Group 2B) and can lead to organ damage and immune suppression. The kidney is the main target organ for OTA.
- Ergot alkaloids (EA) possess a high affinity for receptors of the nervous system. General symptoms include weakness, burning sensation, vomiting and diarrhea. The dry gangrene can reach loss of one or more limbs and desquamation of the skin.

3.4 Legal limits

Regarding regulatory limits for human consumption, the EU enforces maximum limits for AFs, OTA, DON, ZEN, FBs. Furthermore, maximum levels are in the process of being finalized for the sum of T-2 and HT-2 (European-Commission 2013) and EA (European-Commission 2015). There are also official monitoring activities for *Alternaria* toxins and modified forms of DON, this being a usual prelude to regulatory action.

3.5 Mitigation Process

By carrying out preventive measures in the field, including appropriate crop rotation and harvesting practices, producers help to insure the sanitary quality of cereals produced and sold in connection with the collectors (FAO/WHO 2003).

The hull comprises at least 30% of the weight of the seed and this is where the majority of fungal biomass and therefore mycotoxin is located. When oats are dehulled before further processing and use in foods, a reduction of mycotoxin load of at least 60% has been observed (Peng and others 2018). Therefore, oat dehulling significantly reduces the total mycotoxin load of free and modified *Fusarium* mycotoxins (Ivanova and others 2016).

During milling process, short-term storage does not promote the development of mycotoxins. The cleaning steps (gravity separation, dehulling, optical sorting, grading and sieving) can reduce the grain mycotoxin content (Cheli and others 2013; Intercereals 2014; Saunders and others 2001; Scudamore and Patel 2000) and the milling process reduces mycotoxin levels in T45 to T65 flours (Tibola and others 2015). In general, milling does not affect the total amount of mycotoxins, but can cause a redistribution (Schaarschmidt and Fauhl-Hassek 2018). For example, dry milling can provoke mycotoxin concentration in bran (Kochiieru and others 2019) and a decrease in flour, as shown for DON. Conversely, it can reduce OTA concentration due to the removal of the surface layers where the mycotoxin tends to be concentrated (Mousavi Khaneghah and others 2018). Dry milling increases the concentration of *Alternaria* toxins in last break and milling flows and by-products (Hajnal and others 2019). During wet milling process, DON is solved in the used water. After wet milling the bran fraction, DON is found in isolated starch and destarched bran (Magallanes López and others 2019). There is limited information on the behavior of *Alternaria* toxins during processing of cereals.

Concerning starch production, mycotoxins present in grains at the entrance of the starch plant are distributed according to their chemical properties (hydrophilic or lipophilic) in the different fractions extracted. Therefore, batch selection, reception, storage, cleaning and milling performed all along the chain contribute to the mitigation in mycotoxin content (Intercereals 2014).

Furthermore, there are experimental demonstrations that thermal treatments (such as kilning baking, and toasting) have in general a positive effect in terms of mitigation of mycotoxins during cereal food processing (Alldrick and Hajšelová 2004; Bergamini and others 2010; Bretz and others 2005; Generotti and others 2015; Monaci and others 2011; Suman and others 2014; Suman and others 2012). Besides the effect of the thermal processing, mycotoxins might be affected by other accompanying factors such as additives, ingredients or fermentation (Schaarschmidt and Fauhl-Hassek 2018).

With the exception of oats, concerning chemical processes, only alkaline/ ammoniation treatments have been shown to provide a completely effective mitigation in specific cereal commodities (Müller 1983; Park and others 1988) while other chemical treatments (such as acid, oxidation, reduction, etc.) can be considered as partially effective (Aiko and others 2016; Ciegler and Peterson 1968; Dutton and Heathcote 1968). Furthermore, ozone as a strong oxidant is considered an eco-friendly and cost-effective food processing technique which impacts the composition (mycotoxins reduction) and physicochemical properties of components (*e.g.*, starch and protein) of different food grains (*e.g.*, wheat, rice and maize). It should be noted however that more studies are needed for a better optimization of processing and corresponding final product quality (Dwarakanath and others 1968; Maeba and others 1988).

3.6 Flow chart with traffic light system

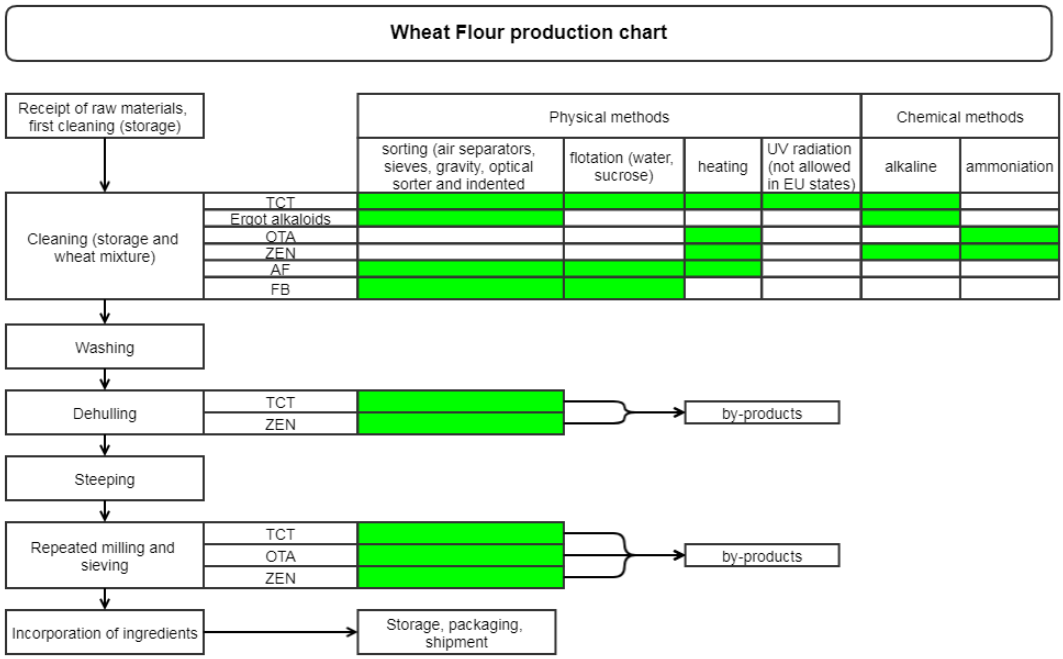


Figure 4 Wheat Flour production chart (Karlovsky and others 2016). The cells highlighted in green indicate that the process step has been reported to significantly reduce a given mycotoxin in the system.

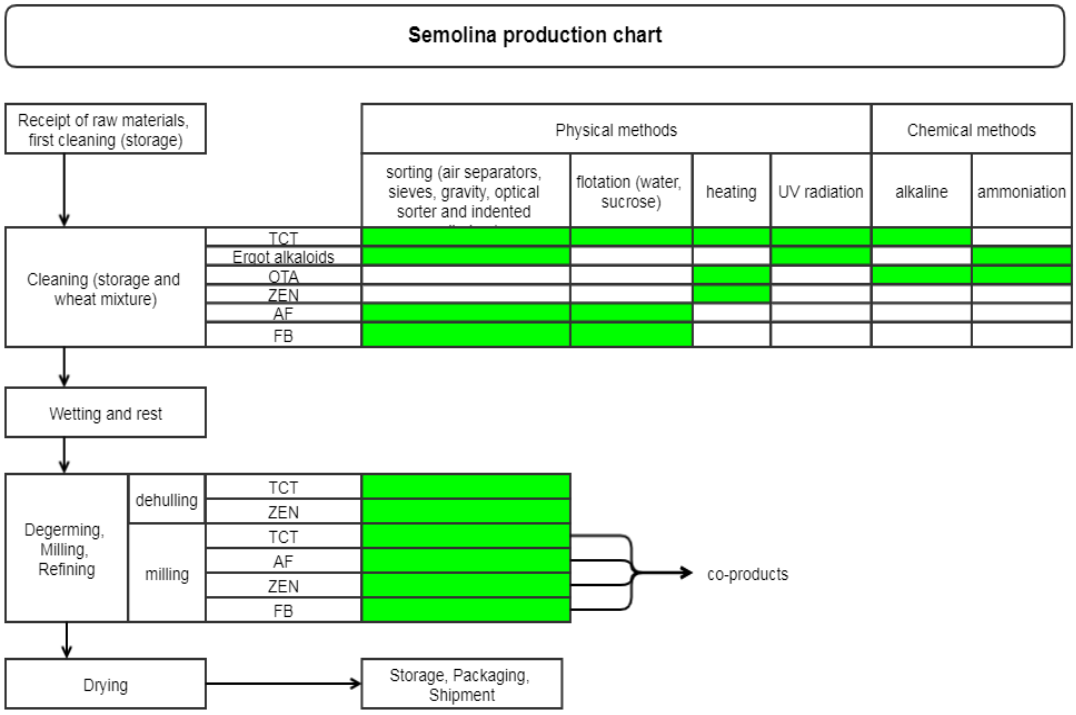


Figure 5 Semolina production chart (Karlovsky and others 2016). The cells highlighted in green indicate that the process step has been reported to significantly reduce a given mycotoxin in the system.

4. APPLE JUICE AND CIDER

4.1 Food Chain description

According to the Codex Alimentarius definition, unfermented palatable juice intended for direct consumption is the "liquid obtained by the mechanical processing of fresh or suitably treated sound ripe fruits, preserved exclusively by physical means." The quality of fruit juice is influenced by the methods used to harvest, store, wash, dry, peel, press, pasteurize, clarify, dry or concentrate, store and pack.

Washing steps aim to minimize physical and chemical contamination of fruits before they enter the juice extraction unit (Mushtaq 2018).

Depending on the apple cultivars used, it must first be milled to a pulp before pressing out the juice. The recovery of cellular liquid from solid fibrous material is performed using simple pressing and subsequent filtering the liquid part of the fruit (Mushtaq 2018). Clarification steps by mixing with gelatine and bentonite or using pectinases enzymes remove solid particles such as pectins or proteins from the juice solution (Diao and others 2018a).

Each step of extraction methodology influences the yield, flavor, quality, composition, shelf life and anticipated health benefits of the final product (Su and Wiley 1998). Whatever the extraction method or the conditions applied, the juice should retain necessary physical, chemical, organoleptic and nutritional characteristics of the fruit it comes from.

Presses are both the most common and the traditional method of juice extraction. Factors contributing to the efficiency of pressing include apple grinder, fruit maturity, viscosity of the juice, the resistance to deformation of the solid phase of the pulp, pulp porosity, and the applied pressure (Beveridge 1997).

Particular case of cider food chain:
For the cider food chain, the interval between milling and pressing takes usually only a few minutes but depends on when the subsequent brewing step is initiated. Once the juice is extracted, a preliminary sulfur dioxide treatment may be used, in order to reduce viable bacteria and undesirable yeasts. Subsequent brewing processes differ particularly between traditional and factory cider-makers(Le Quéré and others 2010; Coton and others 2016).

A ''clean'' and consistent fermentation through chemical, temperature control, and microflora reduction is unlikely to produce off-flavor compounds or other defects (Merwin and others 2008).

4.2 Mycotoxin of relevant occurrence / toxicity

Patulin (PAT) can contaminate various food products, in particular fruits such as apples, and this is reflected in various regulations in different countries (Cunha and others 2014; Harris and others 2009; Iha and Sabino 2008; Yuan and others 2010).

4.3 Health risk

When ingested at high doses, PAT induces nausea, vomiting and other gastrointestinal symptoms, kidney damages and effects on the immune system. PAT exhibits mutagenic, neurotoxic, immunotoxic and genotoxic properties and is classified by the IARC in Group 3 carcinogens.

4.4 Legal limits

Regarding human consumption, the EU determines the maximum levels for PAT in fruit juices as reconstituted and fruit nectars, spirit drinks, cider and other fermented drinks derived from apples or containing apple juice (50 µg/kg), solid apple products (25 µg/kg) and apple juice and solid apple products for infants and young children (10 µg/kg) (European-Commission 2006). The maximum level determined by the CODEX for PAT in apple juice and apple juice ingredients in other beverages is 50 µg/kg (CODEX 2003).

4.5 Mitigation Process

The degree of PAT contamination in a food product depends on the management of all steps in the food processing chain. Post-harvest and pre-processing conditions can have a large effect on the final quality of the fruits (Ioi and others 2017). Removal and detoxification of PAT can be performed using various methods. No single method is ideal but a combination of physical, chemical, and biological methods may provide effective solutions in removing or detoxifying PAT (Diao and others 2018a). The Food and Agriculture Organization (FAO) of the United Nations suggests storing fruits to less than 10°C or for less than 48 hours in order to prevent the risk of PAT contamination increasing. Sorting out any damaged fruits prior to processing and storage can help in decreasing the PAT content in the finished product (CODEX 2003). The diffusion of PAT in apples 1–2 cm away from the infected flesh must be taken into account (Rychlik and Schieberle 2001).

The use of a modified atmosphere (high carbon dioxide and/or nitrogen atmosphere with low oxygen content) can help control mold growth and rot in apples (Johnson and others 1993; Paster and others 1995). If needed, polyethylene packaging can provide a high degree of inhibition of fungal growth and PAT production.

Application of fungicides (either from natural or non-natural origin) can be a means of controlling fungal growth and PAT production in apples during storage (Ioi and others 2017).
The washing step aims to remove debris, including dirt, plant matter, bugs, and mold/fungi (Root and Barrett 2005). Additionally, a portion of the PAT content can be solubilized and removed (Ioi and others 2017). Washing can be performed through immersion or application of a high-pressure water stream. However, the efficacy of washing can vary, ranging from 10% to 100% PAT reductions, with duration of washing, temperature or water recycling impacting PAT removal efficiency (Jackson and others 2003).

Ascorbic acid is a potential mitigation solution to reduce PAT content in apple juice (Brackett and Marth 1979). This is because the lactone ring of PAT is susceptible to oxidizing agents such as ascorbic acid (Vitamin C) which can contribute to neutralize PAT toxicity in the presence of oxygen and free radicals (Alves and others 2000; Drusch and others 2007).

Ultraviolet radiation is an approved method for the preservation of fruit juices in some countries such as Canada and the United States. It can contribute to reduced PAT concentration in apple cider by about 40% (Dong and others 2010) but can destroy the nutritional and functional ingredients in treated foods (Diao and others 2018a).

Ozone, a strong oxidant, is capable of reacting with numerous chemical groups and is also able to detoxify PAT in a highly effective way. The degradation efficacy of PAT can vary depending on ozone concentration, duration of the treatment, initial PAT concentration, pH, and soluble solids content of apple juice. However, the color, malic acid, ascorbic acid, and total phenol of apple juice can also be significantly reduced. The critical nutritional properties of apple juice should be considered by processors prior to the application of ozone as a detoxification technique (Diao and others 2018b)

Alcoholic fermentation converts PAT into ascladiol, which is less toxic than PAT (Tannous and others 2017). During the process of yeast fermentation, sugars are converted into alcohols, gases, and/or acids. This process favors the reduction of PAT content (Burroughs 1977) as it results in both degradation by fermentation and adsorption to the yeast cells (Moss and Long 2002).

The aim of pasteurization treatment is to destroy detrimental microorganisms and to extend the shelf life and consequently, increase the safety of food. Contrasting results have been obtained in the effectiveness of pasteurization to partially detoxify PAT (Kadakal and Nas 2003; Welke and others 2009).

4.6 Flow chart apple juice-cider with traffic light system

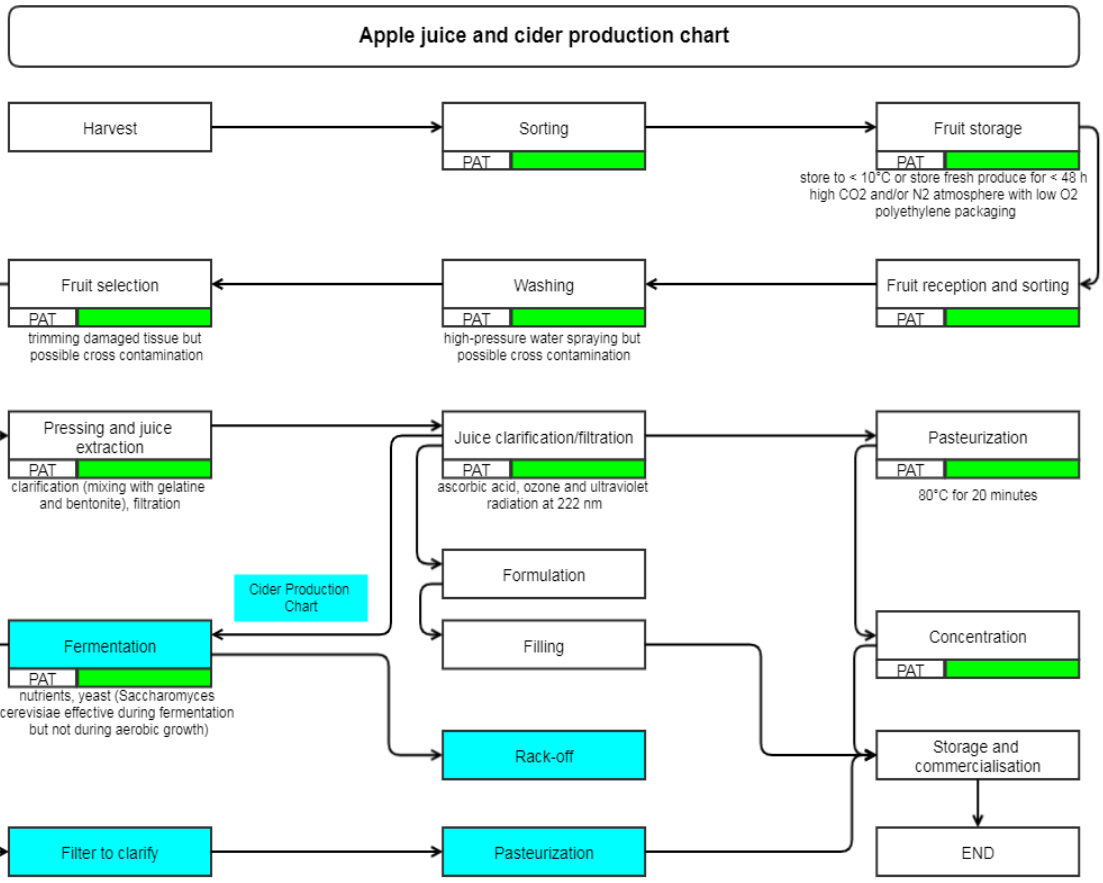


Figure 6
Apple juice and cider production chart (Karlovsky and others 2016). The cells highlighted in green indicate that the process step has been reported to significantly reduce a given mycotoxin in the system. Cells highlighted in blue are part of the cider production chart.

5. COCOA PRODUCTS

5.1 Food Chain description

The most critical steps wherein mycotoxin contamination may occur in cocoa and derived products are at the beginning of cocoa bean processing. This includes harvesting, fermentation, drying and storage of the fermented cocoa beans (FAO 2013). All the other processing steps variably reduce contamination. The fruit of cocoa derived from the cocoa tree, *Theobroma cacao L.*, is composed of the pericarp, a tissue that arises from the ripened ovary wall of a fruit, and the ovary itself. The main commercial use resides in the seeds, also known as cocoa beans. The cocoa bean is composed of an episperm or integument, embryo and cotyledon. The integument, the protective layer of the seed, is also called the shell when it is dried. During fermentation, the embryo dies and upon drying, the fat content of the cocoa bean ranges between 34% and 56%.

After the fermentation and drying processes, the cocoa beans are further processed industrially to produce various commercial cocoa products.

5.2 Mycotoxin of relevant occurrence / toxicity

Although a range of mycotoxins can be occasionally found in cocoa and derived products, scientific literature deals mainly with the occurrence of aflatoxins (AFB1 and AFB2) and Ochratoxin A (OTA).

5.3 Health risk

For cocoa products mainly storage mycotoxins as aflatoxins and ochratoxin A are impacting health in a negative sense as a result of poor storage conditions:

- OTA is a mycotoxin formed mainly by some species of *Aspergillus* and *Penicillium*; OTA has been shown to be carcinogenic, nephrotoxic, teratogenic, immunotoxic, and hepatotoxic in various experimental animal models, and the International Agency for Research on Cancer (IARC) has classified it as possibly carcinogenic to humans (group 2B) (WHO 1993).
- AFB1, AFB2, AFG1 and AFG2 are classified by the International Agency for Research on Cancer as group 1 carcinogens (WHO 2012). Additional health impacts of aflatoxins include teratogenicity, hepatotoxicity, cytotoxicity, and genotoxicity.

5.4 Legal limits

The European Commission (EC) has stated that it does not appear necessary to set a maximum level for OTA and AFB in cocoa and cocoa products (European-Commission 2010a; FAO 2013; European-Commission 2010b).

On the other hand, an important aspect of aflatoxin regulations is the fact that maximum levels are not based only on toxicological considerations to prevent health hazards but also based on technical feasibility. In this context, the most common levels for total aflatoxins (sum of AFB1, AFB2, AFG1 and AFG2) in cereal products have been set between 10 and 20 µg/kg. Although, some countries including Chile and members of the European Union, have established more restrictive tolerance limits (2–5 µg/kg).

Some member states as Germany and Denmark have implemented in their national legislation a generic category to limit the level of AFB1 to a maximum of 2 µg/kg and the sum of AFB1, AFB2, AFG1 and AFG2 to 4 µg/kg, which applies to all foodstuffs.

5.5 Mitigation Process

Since the cocoa beans are extracted from a fruit, contamination by microorganisms may occur and the development of OTA producing fungi could begin when conditions become appropriate for growth. Generally, the fermentation and drying processes could create this favorable condition if not adequately controlled.

It is important to emphasize that from the next manufacturing steps that involve removing shells, roasting (or vice versa), liquoring and refining; only the stage of shell removal can significantly reduce OTA levels. In the present document the focus remains on cocoa powder, as this is the main component for other chocolate-based products, where cleaning, drying, roasting and the alkalization processes are the most important processing steps regarding the mitigation of mycotoxins.

Cocoa beans surrounded by their pulp are traditionally fermented in heaps. Available data indicate the importance of fermentation length to avoid mycotoxin production, which should not exceed 7 days to minimize OTA contamination (FAO 2013). In this stage, also lowering the pH and/or the addition of mild organic acid (such as acetic acid) has been shown to reduce to some extent the accumulation of OTA due to the impairment of mycotoxigenic fungi growth (Copetti and others 2012a). Similar effects can be expected also for aflatoxin production. Concerning the drying step, the type of drying platforms used was not found to impact OTA concentration (Dano and others 2013). However, hygienic conditions should be carefully maintained to avoid the growth of mycotoxigenic fungi (Copetti and others 2010). In this regard, the positive effects against mycotoxin accumulation of good cleaning practices can be expected for both OTA and

aflatoxins, albeit the data collected so far concern only OTA. Notably, the use of wooden drying supports should be avoided due to difficulties in keeping them clean. The growth of fungi able to release mycotoxins can be impaired if during the drying process the moisture level achieves ≤ 8%. The efficacy of this control has been proven on OTA (Minifie 1999), but reduction can be reasonably expected also for aflatoxins.

The roasting process (generally conducted at 190 to 210°C for 20–30 min) may significantly reduce the content of both OTA and aflatoxins (Manda and others 2009; Mendez-Albores and others 2013) via thermal degradation. Nevertheless, the most part of OTA was found accumulated in bean shells and therefore, the improvement of shelling and winnowing processes was identified as a key element to reduce the carry-over of mycotoxins into the further processing steps (Amezqueta and others 2005; Copetti and others 2013). Some degrees of reduction can be expected also for aflatoxins as it has been shown that they also are preferentially located in bean shells (Copetti and others 2012b).

Concerning refined cocoa products, such as chocolate and chocolate powders, it has been proved that alkalization may have reducing effects on both aflatoxins and OTA content, with more significant effects on aflatoxins (Mendez-Albores and others 2013). Moreover, in chocolate, the diluting effects due to the addition of further ingredients to reach the final formulation will obviously reduce the overall mycotoxin concentration.

5.6 Flow chart with traffic light system

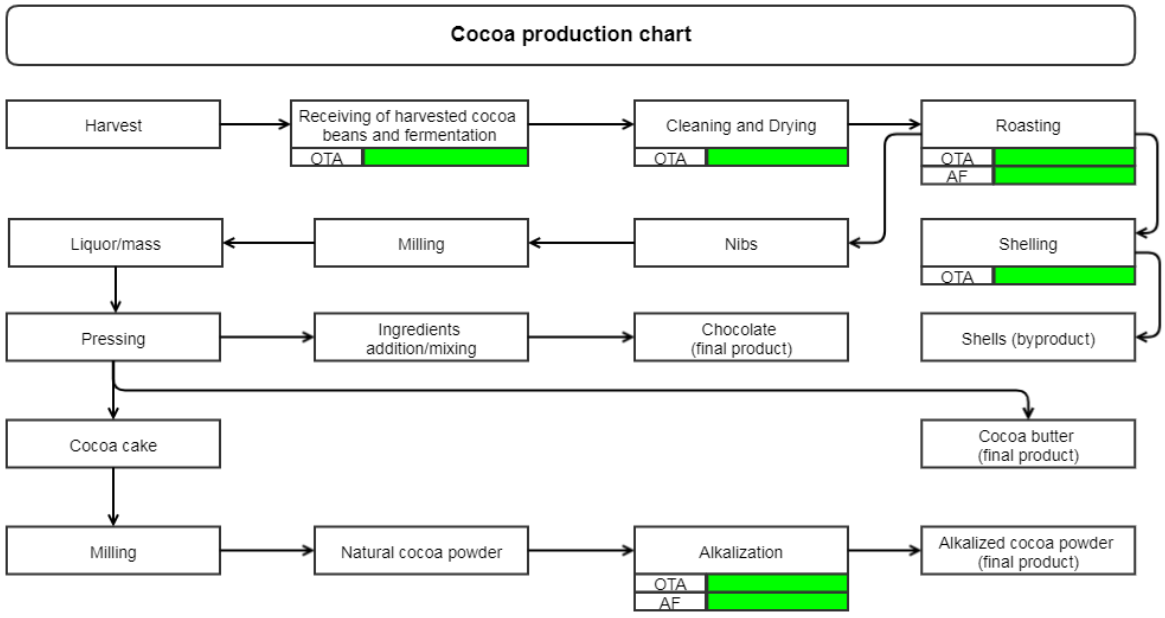


Figure 7
Cocoa production chart (Karlovsky and others 2016). The cells highlighted in green indicate that the process step has been reported to significantly reduce a given mycotoxin in the system.

6. MILK AND DAIRY

Available data indicate that the occurrence of mycotoxins in milk and dairy products present a range of possible contaminations, partly due to the complexity of dairy production chains. As an example, FB1, OTA, T-2, DON, ZEN and PAT have been described as being present in milk (Fink-Gremmels 2008) and their carryover in milk products is likely to occur (Becker-Algeri and others 2016). However, most of the scientific research dealing with the assessment of mitigation strategies has focused mainly on AFM1. The unique mycotoxin is regulated explicitly regarding milk and dairy products in many countries Sporadically, regulations on OTA have also been reported (Skrinjar and others 1996).

Therefore, on the basis of the data available so far, the report focuses on the mitigation of AFM1 or OTA in raw milk, fermented milk and processed milk products (such as yoghurt and cheese) (Ismail and others 2016).

6.2 Health risk

Many mycotoxins have been found potentially occurring in drinking milk (*e.g.* fumonisins, zearalenone and deoxynivalenol) (Fink-Gremmels 2008). However, AFM1 and OTA are the best described in terms of occurrence, toxicity and mitigation strategies:

- AFM1 may cause both acute and chronic toxicoses, mainly through ingestion of contaminated milk (WHO 1993). Long-term studies in different animal species proved the hepatotoxicity of AFM1 and demonstrated its carcinogenic effect, although lower by about one order of magnitude as compared to AFB1. Initially, AFM1 was categorized as group 2B human carcinogen by IARC (WHO 1993). However, more recent studies reclassified AFM1 as a group 1 human carcinogen (WHO 2002). Exposure to AFM1 via drinking milk may play an important causative role in the observed cases of aflatoxicosis, making the presence of AFM1 in raw milk intended for human consumption a health problem at a global scale (Giovati and others 2015).
- Concerning OTA, the chronic exposure in farm animals may produce nephropathy (Krogh 1976). However, human epidemiology has been inconclusive and on the basis of the data collected so far, it was concluded that the causality between the intake of OTA and human nephropathy cannot be established (Sorrenti and others 2013). Nevertheless, the International Agency for Research on Cancer (IARC) has classified OTA as possibly carcinogenic to humans (group 2B) (WHO 1993). In addition, the worldwide distribution of OTA contributes to consider it among the most serious dietary threatens to public health (Sorrenti and others 2013).

6.3 Legal limits

As milk and milk-based products vary from product, origin, storage and processing, a standard legal limit that generally applies for all dairy products cannot be fixed. Considering the health risks associated with AFM1, many countries have established legal limits for maximum allowed levels of AFM1 in milk. The Commission of the European Union has set a maximum residue level (MRL) value of 50 ng/kg in raw milk. The MRL for AFM1 set by Codex Alimentarius Commission, Southern Common Market (Mercosur) and US Food and Drug Administration is 500 ng/kg. To avoid carry-over, MRL for AFB1 in feed of lactating cows have also been set, ranging from 5 µg AFB1/kg of feed (European Union) to 10 µg/kg (China) and 20 µg/kg (USA) (Giovati and others 2015).

Concerning OTA, no limits have been explicitly set in milk. However, depending on countries, limits have been generally enforced for raw materials or intermediate products intended for feed production, such as in China (100 µg/kg in feeding corn) and Republic of Korea (200 µg/kg in all compound feed), possibly resulting in preventing the accumulation of OTA in milk. Conversely, in EU, recommendations exist on feed intended for companion and non-lactating animals (European-Commission 2016).

6.4 Products

6.4.1 Raw milk

6.4.1.1 Food Chain Description

The majority of raw milk is sold for food use only after processing while only a small amount is consumed without having been treated. Treatments may slightly vary depending on the production plant and on the type of final products, but some common steps can be defined (Figure 8).

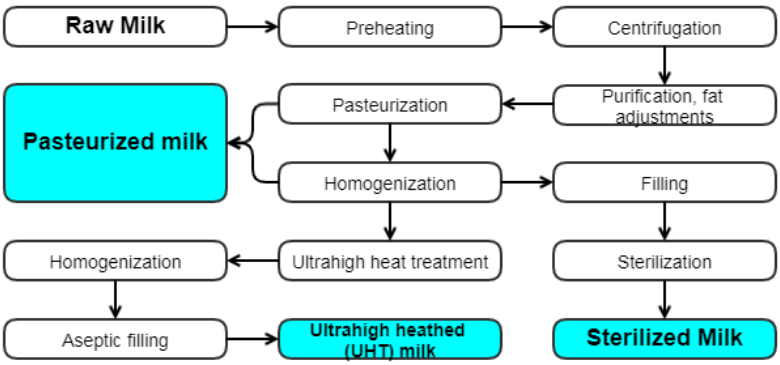


Figure 8
Schematic representation of milk treatments (Belitz and others 2009). The cells highlighted in blue are various types of final products that can be produced from raw milk

At the beginning of processing chains, milk is usually delivered in cooled tanks and is fed in to a clarifier (self-cleaning disk separator) via a de-aerating vessel. This step is usually followed by a creaming process where the milk is separated into cream and skimmed milk in a cream separator after being heated to about 40°C. Then, milk undergoes heat treatment aimed at killing pathogens and improving milk durability. Many treatments exist according to the length of treatment and temperature used. The most common types of heath treatments are:

- Pasteurization at high temperature in a short time (85°C for 2–3 s or 72–75 C for 15–30 s), or at low temperature in a long time (63–66°C for at least 30–32 min)
- Thermization, which uses milder heating conditions than those of pasteurization (57–68°C for 10-20 s)
- Ultrahigh temperature (UHT) treatment, which involves indirect heating by coils or plates (136–138°C for 5–8 s) or direct heating by steam injection (140–145°C for 2–4 s)
- Bactotherm process, which acts on pre-heated milk (65 to 70°C) and combines centrifugal sterilization using bactofugation and UHT heating (130-140°C, 3-4 s) of the separated sediment followed by recombination
- Sterilization, which involves heating process in autoclaves (107–115°C for 20–40 min, or 120–130°C for 8–12 min).

After being thermally treated, milk usually undergoes homogenization to stabilize the emulsion by reducing the size of the fat globules. Homogenization is achieved using high-pressure homogenizers (up to 35 MPa, 50–75°C) that press the milk through a valve reducing the size of fat globules.

According to the processing and thermal treatments, different milk types suitable to be consumed and/or used as ingredient in milk-based food production can be described:

- Raw milk, which undergoes no treatments and must comply with strict hygienic requirements
- Whole milk, which undergoes thermal treatments to kill disease-related bacteria and improve durability. It can be a standardized whole milk adjusted to a predetermined fat content (at least 3.5%), or unhomogenized to allow cream to rise to the top wherein the fat content may be more variable
- Low-fat milk, which undergoes thermal treatment and cream separation to reach a final fat content between 1.5 and 2%
- Skim milk, which is heat-treated with a fat content lower than 0.3%
- Reconstituted milk, which is made by emulsifying butter fat in a suspension of skim milk powder at 45°C. Then, the intermediate product with a fat content of 20–30% is subjected to two-stage homogenization (20 and 5 MPa, 55–60°C) and then diluted with the skim milk suspension
- Filled milk, wherein the butter fat is replaced with a vegetable fat
- Toned milk, which is a blend of a fat-rich fresh milk and reconstituted skim milk to concentrate the non-fat solids (Toning up). Conversely, the addition of water dilutes the non-fat solids (toning down).

6.4.1.2 Mitigation process

On the basis of the scientific data available so far, AFM1 is the main mycotoxin considered for mitigation purposes in milk and dairy products, while the mitigation of OTA has been much less investigated (Skrinjar and others 1996).

The contamination of milk by AFM1 is thought to be a direct consequence of mycotoxin carry-over when lactating animals are fed using AFB1-contaminated feed (Karlovsky and others 2016). Therefore, pre- and post-harvest interventions to counteract AFB1 accumulation in crops and raw material intended for feed production, along with the strict compliance with GAP (good agricultural practices) at both pre- and post-harvest phases, are currently considered the most effective strategies to reduce the accumulation of AFM1 in milk (Womak and others 2016). In particular, the biocontrol in field and during feed storage seems to be among the most effective strategies (Giovati and others 2015). In addition, the accumulation of AFM1 in milk can also be mitigated by reducing the gastrointestinal absorption of AFB1 by lactating animals administering through diet enterosorbents agents, such as dietary clay minerals and probiotics (Giovati and others 2015).

Conversely, thermal treatments of contaminated milk such as pasteurization and sterilization proved to be ineffective due to the thermal stability of AFM1. Nevertheless, some strategies supposed to reduce the contamination level have been studied over the years. Among them, physical and chemical methods such as filtrations, treatment with ozone, sorption using clay/bentonite polymers and heating in the presence of H_2O_2 proved to be effective in reducing AFM1 content. However, these methods are rarely applied due to alteration of food composition and palatability (Aman 1992; Carraro and others 2014; Higueraciapara and others 1995; Mohammadi and others 2017).

Mitigation strategies that may reduce OTA presence in milk would include good agricultural practices for the feed being used for the cattle. This can be done by avoiding pathogen contamination of feed or using adsorbent materials that may be able to minimize mycotoxin contamination in the feed (Assaf and others 2019; Turkoglu and Keyvan 2019).

6.4.2 Fermented milk

6.4.2.1 Food Chain Description

Fermented milk products include sour milk, kefir, taette (derived from cow milk) and kumis (derived from mare or goat milk).

Sour milk is obtained fermenting milk with mild thermal treatments (typically 20°C), wherein fermentation may occur either by spontaneous souring caused by various lactic-acid-producing bacteria or on addition of mesophilic microorganisms. The sour milk is manufactured from whole milk (at least 3.5% milk fat), low-fat milk (1.5–1.8% fat) or from skim milk (at most 0.3% fat), often by blending with skim milk powder.

6.4.2.2 Mitigation process

As previously mentioned, the most effective strategy to reduce the contamination of fermented milk by AFM1 and OTA relies in purchasing and processing milk with a low content of those mycotoxins. Nevertheless, some strategies have been reported effective in reducing the total amount of AFM1 and OTA. In particular, even though a consensus has not been reached yet, the use of lactic acid bacteria, some yeast strains and both probiotic and non-probiotic cultures were identified among the most effective and promising strategies to reduce the level of AFM1 and OTA (Ismail and others 2016). The shortage of data actually prevents the unambiguous identification of all-purpose effective microorganisms and/or strains, and the relative activities are thought to strongly depend on the fermentation conditions and production strains. However, it is clear that microorganisms may actively metabolize mycotoxins reducing their concentration and/or they may adsorb them on cell surface reducing the absorption in the gastrointestinal tract (Taheur and others 2017; Huang and others 2017). *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles* are given examples.

Streptococcus thermophilus (ST-36) proved to be effective in binding AFM1 (Arab and others 2012). It has also been reported that storage time correlates with the reduction of mycotoxins level, likely as a consequence of microbial metabolism rather than chemical sorption to bacterial wall or membrane (Arab and others 2012).

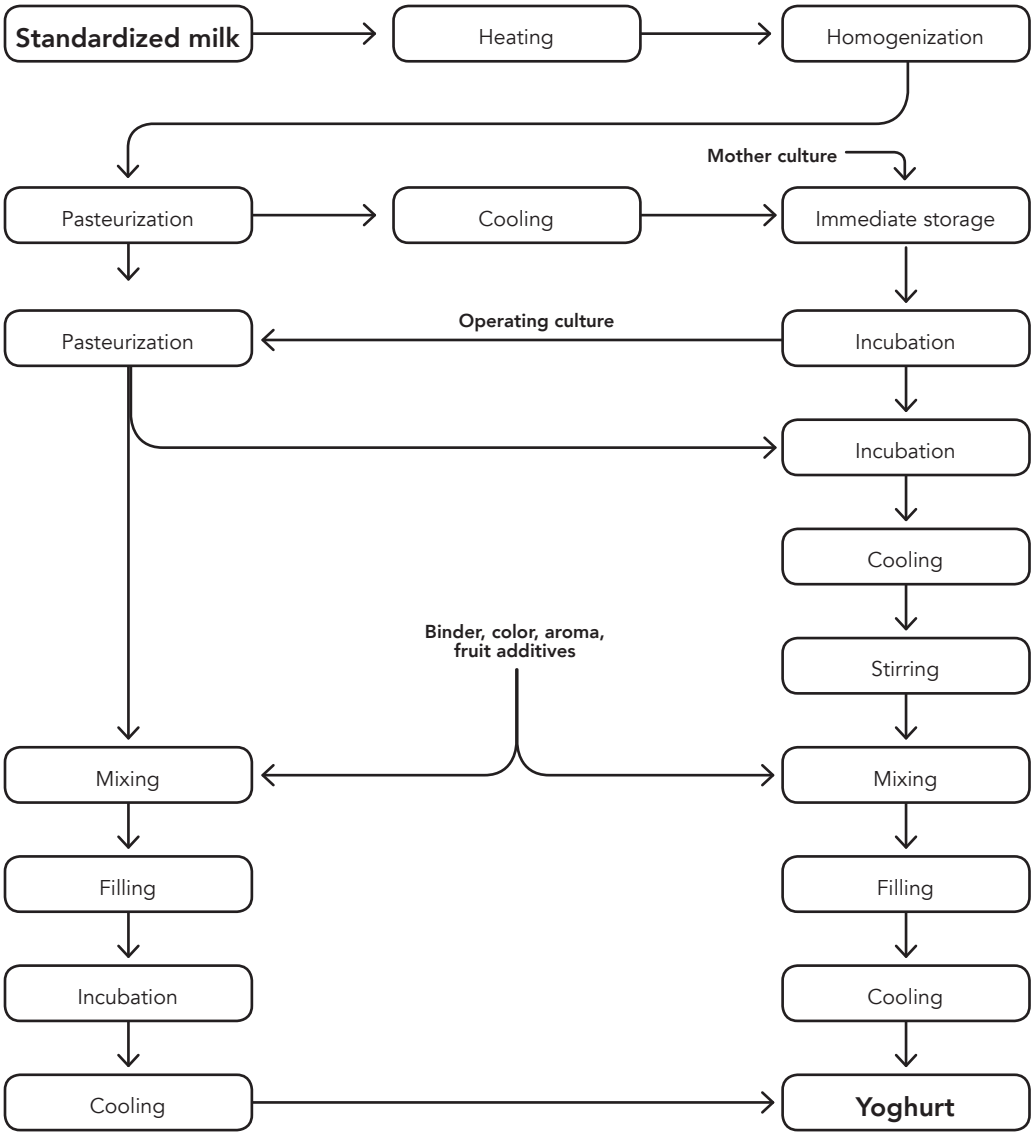
6.4.3 Processed milk products (yoghurt and cheese)

6.4.3.1 Food Chain Description

Yoghurt production starts from standardized milk batches and uses thermophilic acid bacteria. The processing steps of yoghurt production are reported in figure 9.

Yoghurt cultures consist of thermophilic lactic acid bacteria that live together symbiotically (*Streptococcus thermophilus* and *Lactobacillus bulgaricus).* Incubation is conducted on addition of 1.5–3% of the operating culture at 42–45°C for about 3 h. The final product has a pH value of about 4–4.2 and contains 0.7–1.1% of lactic acid. Functional foods include yoghurts which have been incubated with probiotics. Probiotics are defined, cultured strains of lactic acid bacteria, which have been isolated from human intestinal flora, *e.g.*, certain lactobacilli and bifidobacteria. On consumption, they are supposed to reach the large intestine and contribute to the formation of an optimal intestinal flora. The variety of products is increased by the addition of fruits and fruit pastes to yoghurt. The addition of fruit or fruit pastes and sugar yields special products (fruit yoghurts). An essential part of the specific yoghurt aroma comes from carbonyl compounds, predominantly acetaldehyde and diacetyl. In addition to 1-octen-3-one, 1-nonen-3-one has also been detected as an important odorant, which has an exceptionally low odor threshold. An autoxidation product of linoleic acid, (E)-2-nonenal is thought to be the precursor.

Figure 9
Schematic representation of processing steps in yoghurt production (Belitz and others 2009)



Cheese, is obtained from curdled milk by removal of whey and by curd ripening in the presence of special microflora. The great variety of cheeses (some thousands) in the world makes difficult to spread standardized processes in cheese making. Nevertheless, a schematic representation of key processing steps is reported in figure 10.

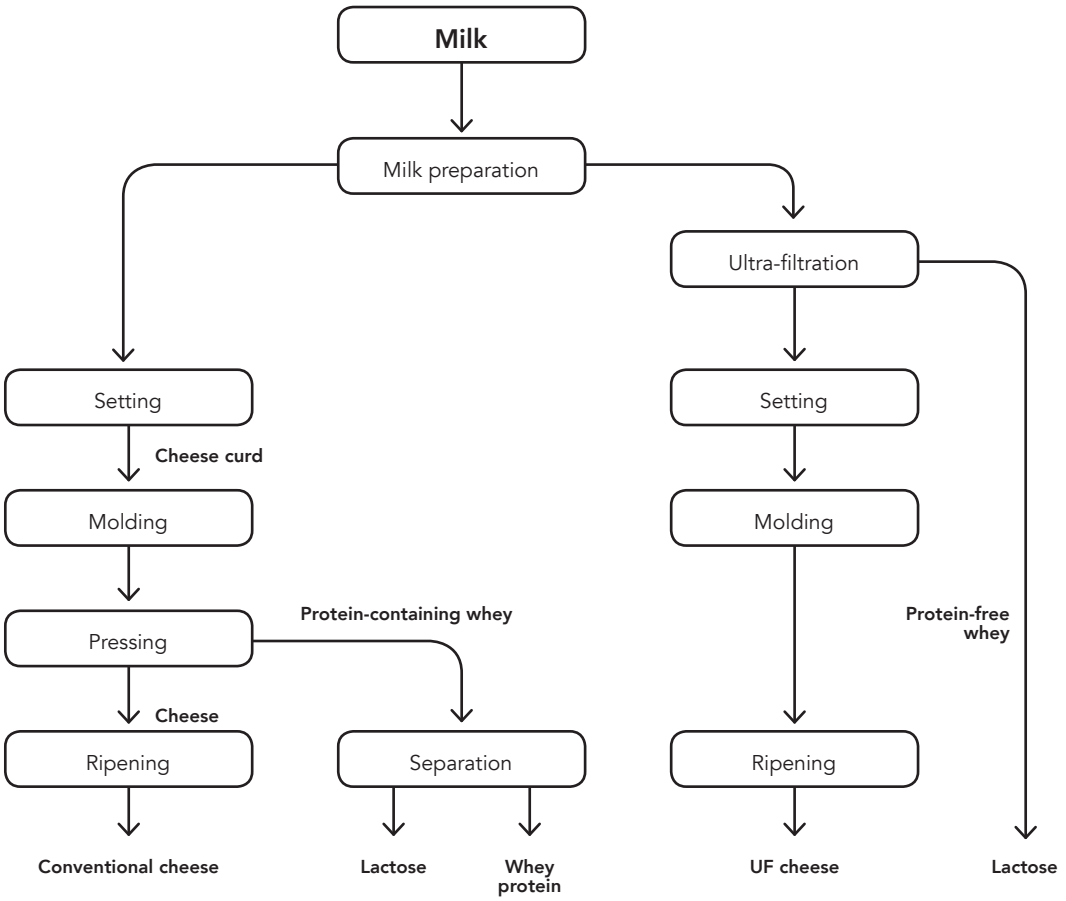


Figure 10
Schematic representation of processing steps in cheese production (Belitz and others 2009)

In cheese production, the curd formation is the main step and requires milk preparation in terms of fat and protein content adjustments. Some additives such are: (1) calcium salts which improve protein coagulation and cheese texture, (2) nitrates which to inhibit anaerobic spore-forming microflora, and (3) color pigments may be added. The prepared raw or pasteurized milk is mixed at 18–50°C with starter cultures mainly including lactic acid or propionic acid bacteria; molds or red- or yellow-smearing cultures. The curd may be formed after lactic acid fermentation, in the case of sour milk, or by addition of rennet, in the case of sweet milk cheese. Combinations of the two processes often exist. The whey is drained off while the retained curd is subjected to a firming process (syneresis) until the desired curd consistency is reached. Whey proteins may be included in the curd, *e.g.* upon ultra-filtration of whey and re-entering the collected whey protein into the curd. Ripening of ripened cheese usually requires salt bath for some time, dried, and then left to ripen in air-conditioned and temperature-controlled rooms (Belitz and others 2009).

Processed (melted) cheese is produced by shredding the cheese and melting it. In some productions, other ingredients can be added such as milk powder, cream, aromas, seasonings and vegetable and/or meat products.

6.4.3.2 Mitigation process

Even though the high complexity of processes taking place in the production of processed milk products, effective mitigating strategies are poorly described in the scientific literature. In addition, the efficacy of methods proved on a specific product is hard to be extrapolated to others due to the intrinsic diversity of processing steps among this diverse food group. However, as mentioned before, processing milk with a low content of mycotoxins is the best way to ensure the low contamination levels in the final processed milk products.

Nevertheless, storage time and acidification proved to reduce the content of AFM1 in milk and cheese (Arab and others 2012; Weidenbörner 2001). Specifically in yoghurt production, lowering the pH from 4.6 to 4.0 proved to slightly reduce the content of AFM1, while the use of lactic acid bacteria, as reported for fermented milk, proved to be the most effective strategy. In respect to cheese production, acidification during cottage cheese production proved to slightly reduce AFM1. Ripening in some cheese productions (*e.g.* brine solution at 6 and 18°C) also circumstantially proved to reduce AFM1 content (Motawee and McMahon 2009), but the high number of different cheese types and the variability in cheese making prevents to reach a consensus (Scott 1989).

6.5 Table chart with traffic light system

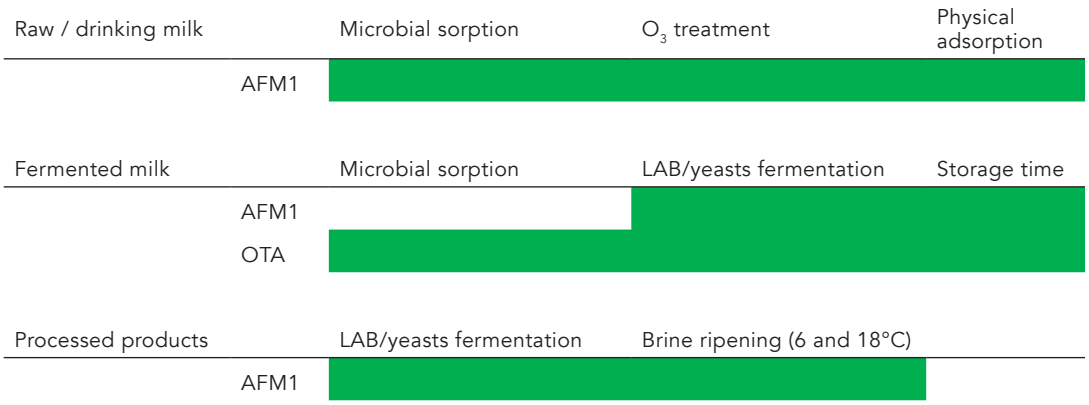


Table 3
Traffic light evaluation of mycotoxin mitigation in the milk production chart (Karlovsky and others 2016). The cells highlighted in green indicate that the process step has been reported to significantly reduce a given mycotoxin in the system.

7. VEGETABLE OILS

7.1 Food Chain description

Vegetable oils have a wide variety of food uses including direct consumption (salad oils). They also enter into many processed foods such as biscuits, bakeries, margarines, snacks and mayonnaise or indirectly as ingredients of foods. Furthermore, they can be heated to cook other food. Oils have become more popular when comparing to the consumption of fats. Specifically, highly unsaturated oils have risen in popularity compared to those containing more saturated fatty acids.

7.2 Mycotoxin of relevant occurrence / toxicity

Some of the most common oil yielding seeds is often colonized by toxigenic fungi, which can produce mycotoxins. Mycotoxins that were detected in oil seeds include aflatoxins (*e.g.* peanut, maize, soy beans, sun flower), ZEN (*e.g.* maize), DON (*e.g.* maize), FBs (*e.g.* maize) and OTA (*e.g.* maize, peanuts).

7.3 Health risk

The following mycotoxins have been found in vegetable oils and might be a source of health concern:

- Aflatoxins are extremely potent toxins and genotoxic carcinogens
- DON induces feed refusal, vomiting, and diarrhea
- ZEN and its metabolites interact with ?- and ?-estrogen receptors and endocrine disruptors
- FBs and OTA are classified as possibly carcinogenic to humans

7.4 Legal limits

The EU imposes maximum limits for AFB1 (2 µg/kg) and the sum of AFB1, AFB2, AFG1, AFG2 (4 µg/kg) in oil seeds and products pressed thereof (European-Commission 2010b). The maximum limits apply to crude vegetable oils. However, crude vegetable oils destined for refining and refined vegetable oils are exempt from the maximum limits. In refined maize oil a maximum limit of 400 µg/kg ZEN was established (European-Commission 2007).

7.5 Mitigation Process

Crude vegetable oil is extracted from the oil seed either by mechanical or solvent extraction. During extraction, mycotoxin content in the crude oil can be lowered compared with the concentration in the seed. During wet milling of maize, water soluble mycotoxins such as DON and FBs were found at higher concentration in the steeping liquor, but at low levels in the solid, which is used for further maize oil production (Karlovsky 2016). The inverse is true for ZEN, which has been reported in crude maize germ oil. A carryover of aflatoxins from peanuts to the crude oil in the range of 1–35 % has been reported (Shephard 2018).

The refining process can be physical and/or chemical. The chemical refining steps of oils are as follows: (1) Degumming, (2) Neutralisation through a caustic agent (Deacidification), (3) Winterisation, (4) Bleaching and (5) Deodorisation. The physical refining process on the other hand would only consist of bleaching (using absorbents) and deodorization (using steam under vacuum).

Refining of crude oil consists of a number of unit operations. In the first step, water or acidified solution is used to clean the oil from phospholipids (*e.g.* degumming). Thereafter, alkaline treatment is used to neutralize and remove free fatty acids and a number of contaminants. Alkaline treatment has been shown to reduce aflatoxins, trichothecenes, and zearalenone in vegetable oils (Kamimura and others 1986; Parker and Melnick 1966; Slope and others 2013). Pigments and other contaminants are subsequently removed by bleaching clay or other

absorbents. Bleaching clay was found to reduce aflatoxins and trichothecenes (Kamimura and others 1986; Parker and Melnick 1966). Finally, the oil is deodorized, by steam distillation of volatile components such as aldehydes and ketones. Deodorization was shown to result in the reduction of aflatoxins, trichothecenes and ZEN (Kamimura and others 1986).

7.6 Flow chart with traffic light system

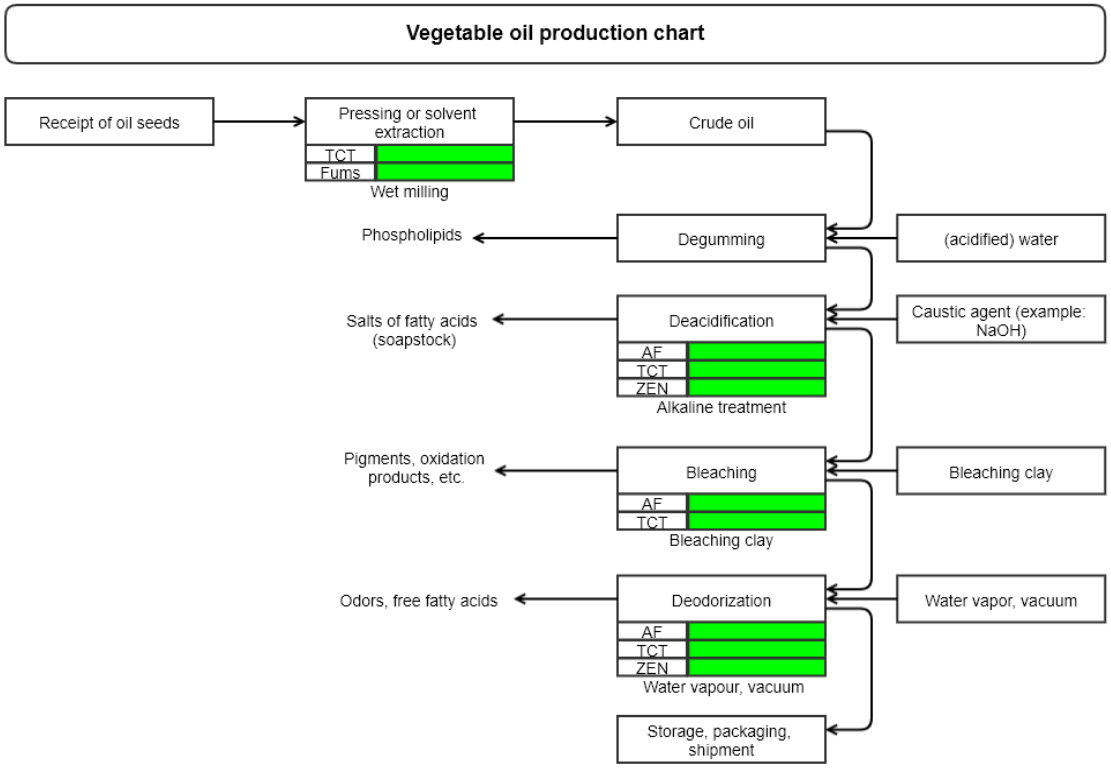


Figure 11:
Vegetable oil production chart (Karlovsky and others 2016). The cells highlighted in green indicate that the process step has been reported to significantly reduce a given mycotoxin in the system.

8. DRIED FRUITS AND NUTS

8.1 Food Chain description

Dried fruits and nuts are used in snacks, mueslis, chocolates and bakery products.

8.2 Mycotoxin of relevant occurrence / toxicity

Most of the dried fruits and nuts are produced in warm climates, which increase the risk of mycotoxin contamination, especially with aflatoxins and OTA particularly if processing conditions have not been adequately controlled.

8.3 Health risk

The following mycotoxins commonly found in dried fruits and nuts and require attention are the following:

- Aflatoxins are extremely potent toxins and genotoxic carcinogens.
- OTA is classified as possibly carcinogenic to humans and has been associated with kidney disease.

8.4 Legal limits

The EU imposes maximum limits for AFB1 and the sum of aflatoxins (AFB1, AFB2, AFG1, AFG2) for nuts and dried fruit (European-Commission 2012). Nuts that are subjected to sorting or other physical treatment are tolerated to have a maximum level of AFB1 between 5–12 µg/kg and the sum of aflatoxins of 10–15 µg/kg, depending on the type of nuts. In nuts intended for direct human consumption or as food ingredients, AFB1 and the sum of aflatoxins must not exceed 2–8 µg/kg and 4–10 µg/kg, respectively, depending on the type of nuts. The maximum level of AFB1 and the sum of aflatoxins in dried fruit subjected to sorting or other physical treatment is set with 5 and 10 µg/kg, respectively. For dried fruits intended for direct human consumption or as food ingredient, the maximum level for AFB1 and the sum of the aflatoxins is set with 2 and 4 µg/kg, respectively (European-Commission 2010b). Additionally, OTA is regulated in dried vine fruits (currants, raisins, sultanas) with 10 µg/kg.

8.5 Mitigation Process

The production of nuts often consists of the following unit operations: drying, sorting, shelling, roasting and grinding. Sorting and roasting have been shown to reduce aflatoxin levels through removal of 'hot spots' of contamination and thermal degradation respectively.
The drying step is particularly relevant and requires careful control of time, temperature and humidity conditions. Specifically, characteristically artisanal sun drying is intermittently a cause of mycotoxin concern compared to controlled industrial drying process (forced air).

Removing immature or damaged nuts lowers the aflatoxin concentration (Dorner 2008). As highly contaminated kernels are less dense, aflatoxin contamination can be lowered by gravity separation (Davidson and others 1981). Non-invasive sorting, aided by infrared (IR) and ultra-violet (UV) spectroscopy was shown to reduce aflatoxin levels (Durmuş and others 2017; Siciliano and others 2016). High temperatures, which are achieved during the roasting process, were shown to considerably degrade aflatoxins.

Dried fruits are often produced using the unit operations during washing/sorting, peeling/cutting, preservation, drying and final sorting. The use of sulfur dioxide, which is often used as preservation agent, was shown to be an effective mycotoxin mitigation strategy (Scott and Trucksess 2009). Removal of damaged fruits and sorting was shown to reduce aflatoxin levels. Sorting can be done manually or using an automated approach (gravity, density separators). The latter is often aided by IR or UV spectroscopy and becomes more effective in terms of prevention or mitigation when combined with proper ventilation.

Novel technologies such as ozonation and treatment with ionizing radiation were shown to reduced aflatoxins and OTA on different types of nuts and dried fruits (Pankaj and others 2018). However, for the application on food products, more studies are needed concerning the toxicology of the degradation products and the interaction with other food components.

8.6 Flow chart with traffic light system

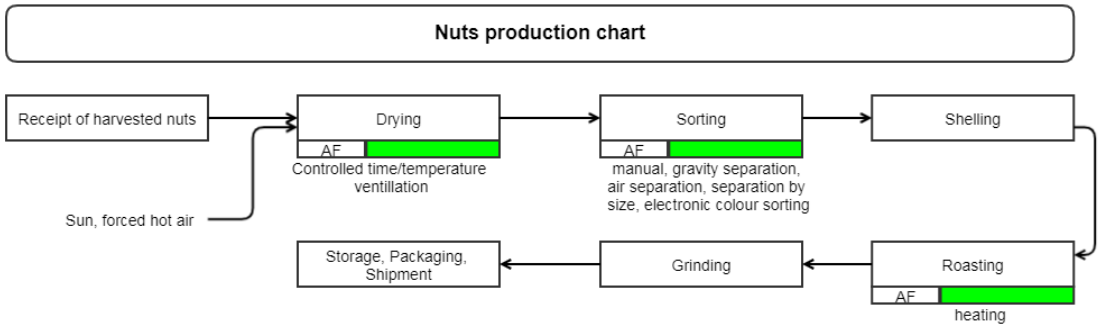


Figure 12
Nuts production chart (Karlovsky and others 2016). The cells highlighted in green indicate that the process step has been reported to significantly reduce a given mycotoxin in the system.

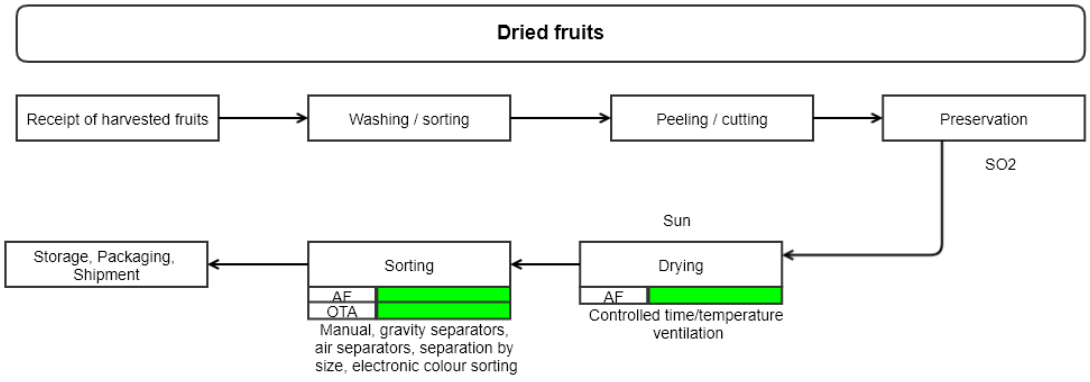


Figure 13
Dried fruits production chart (Karlovsky and others 2016). The cells highlighted in green indicate that the process step has been reported to significantly reduce a given mycotoxin in the system.

9. SPICES

Spice risk and mitigation

The term spice covers a wide range of fragments of plants used as ingredients, as illustrated in Table 4. These are distinct from herbs which are the fresh or dried soft parts of plants, generally the leaves. Spices are subject to minimal processing before use, and Table 4 also illustrates typical processing steps. Spices are often hand sorted and washed, and the principal steps that are likely to reduce mycotoxin contamination, specifically hand or optical sorting, are likely to remove fungal infestation (foci of high mycotoxin contamination). However, in some cases fumigation and steam treatment are used which may also be important in the reduction of mycotoxin load.

Seeds, rhizomes and fruits are at risk of field contamination. However, experience has demonstrated that a principal concern is downstream contamination due to the use of traditional preparation practices particularly the dehydration of fruits through prolonged sun drying of bell and red peppers (Sahar and others 2017). Another example is insufficient control of water activity during storage of bulk ground spices, particularly in tropical and subtropical regions. These concerns are mitigated by controlling drying processes and storage conditions. Notwithstanding, OTA and AFs remain a concern for spices originating from regions where traditional processing is commonplace and due to monitoring at ports of import into the EU. Due to established regulatory limits, some of these shipments, usually in the form of mixed spices, are prevented from entering the food chain (van Asselt and others 2018).

Although spices are generally used at low concentration in finished foods, they may be frequently present especially in certain cultural foods, and therefore exposure may be significant. Coupled with an 'ALARA approach' to regulatory limits (as opposed to being based on health risk), there are frequent alerts from control agencies (European-Commission. 2019). Although there are various new methods under evaluation for mitigation both at the commodity and finished product level (Chilaka and others 2018; Farawahida and others 2017), the most impactful methods to mitigate mycotoxins in spices are likely to remain the control of fruit dehydration at primary production, and appropriate storage of bulk ground spices particularly those originating from tropical and subtropical regions. In addition, it is likely to be important that downstream processing employs sorting and where appropriate washing.

Part of Plant	Temperate plant	Subtropical plant	Tropical plant	Usual Processing	Optional Processing
Seed or aril	Aniseed annatto celery seed coriander seed cumin seed fennel seed fenugreek seed mustard seed	Dill seed Nigella seed Poppy seed	Cardamom seeds Mace White pepper Sesame seed	Sieving Grinding	Hand sorting Fumigation Washing - drying Steam treatment
Seed without shell			nutmeg	Hand sorting Washing - drying Grinding	Fumigation Destoning Steam treatment
Bark or stem		wasabi	Cinnamon / cassia	Hand sorting Grinding	Washing – drying Steam treatment
Rhizome or Root or bulb	garlic onion lovage root liquorice	horseradish	Ginger turmeric	Hand sorting Washing - drying	Optical sorting Chopping
Flower bud & flower parts	saffron		cloves	Hand sorting Washing - drying	Grinding
Fruit	Caraway Juniper berries Paprika Star anise		Cardamom pods Chillies Black, green & red pepper Pimento vanilla	Hand sorting Washing – Drying Dehydration Ground / Crushed / Chopped	Fumigation Optical sorting Steam treatment

Table 4
Structural and geographic origin of spices (partly adapted from (ESA. 2019) and information provided by spice suppliers).

10. BEERS

10.1 Food Chain description

Beer is made from five main ingredients: barley, water, hops, yeast and adjuncts (*e.g.* maize, sugar syrup, unmalted cereals etc.). The quality of these commodities plays a decisive role in the creation of organoleptic characteristics of the final product. Keys in brewing are grains, usually barley (although sometimes wheat, rye, other cereals or other crude starch-rich sources are selected). Beer production process implies three main biochemical reactions: (1) enzyme activation in barley grain during germination, (2) starch degradation into fermentable sugars through the grain's enzymatic equipment and alcoholic fermentation realized by *Saccharomyces* yeasts with ethanol and carbon dioxide formation.

Malt is also often used which is prepared from barley. Malt is germinated cereal grains that have been dried in a process known as "malting". The grains are made to germinate by soaking in water and are then halted from germinating further by drying with hot air ("kilning").

The beer production process includes the following main steps: malting, milling, mashing, filtration (lautering), wort boiling, fermentation, maturation, another filtration, stabilization (*e.g.* clarification or pasteurization) and packaging which are schematically provided in Fig 14.

Following dry milling of malt and other starting materials, the grains go through a process known as mashing, in which they are steeped in hot, but not boiling, water for about an hour. This activates enzymes in the grains that cause it to break down and release its sugars. Once this is all done, the water from the mash is drained which is now full of sugar from the grains. This sticky, sweet liquid is called wort. The wort is boiled for about an hour while hops and other spices are added several times. Hops also act as a natural preservative, which is what they were initially used for.

After the boiled wort is cooled, strained and filtered, it is then put in a fermenting vessel and yeast is added. At this point, the brewing is complete and the fermentation begins. The beer is stored for a couple of weeks at room temperature (in the case of ales) or many weeks at cold temperatures (in the case of lagers) while the yeast facilitates the fermentation process. The yeast would consume the sugar in the wort and produces carbon dioxide and alcohol as products. Yeasts are removed from the beer volume by filtration and the product is transferred to aging tanks for more prolonged storage.

The result is alcoholic beer. However, it is still flat and uncarbonated. Additional processes are clarification and stabilization. The flat beer is bottled, at which time it is either artificially carbonated like a soda, or if it is going to be 'bottle conditioned' it is allowed to naturally carbonate via the carbon dioxide the yeast produces. After allowing it to age for anywhere from a few weeks to a few months the beer is ready for consumption.

10.2 Mycotoxin of relevant occurrence / toxicity

Surveys for occurrence of mycotoxins have been mainly focused on industrially produced beer. Currently, the beer industry is booming. This is mainly due to the steady rise of craft breweries worldwide. The presence of mycotoxins in beer relates to the adjuncts from different sources and the raw materials as barley, maize and malt. During the last years some reviews have been presented on the occurrence and dietary intake of mycotoxins through beer.

The occurrence of ochratoxin A, trichothecenes, fumonisins and aflatoxins in a sample of 106 beers produced in several European countries, was investigated (Bertuzzi and others 2011) Aflatoxins were not detected in any samples, whereas ochratoxin A, deoxynivalenol and fumonisins were found in a relatively high number of samples. Their presence was at low levels in all samples. However, some differences were observed between the European countries.

As regards ochratoxin A, beer samples from southern Europe showed levels always lower than 0.040 µg/L, while the samples from other European countries showed significantly higher values, up to 0.189 µg/L. For fumonisins, the levels of Italian beers were significantly higher compared to the samples from other countries.

The same researcher is summarizing the natural occurrence of mycotoxins in beer samples from about at least ten publications: OTA up to 0.5 µg/L and DON lower than 100 µg/L although some German wheat beer samples provided levels up to 570 µg/L (Bertuzzi and others 2011). For aflatoxins in beer, the level found over a broad set of samples and geographies was between 0.02 µg/L up to 0.23 µg/L. Several surveys provided for a widespread concentration of FB1 in beer but generally lower than 100 µg/L.

From 13 different European countries the occurrence of mycotoxins in 154 beer samples was investigated. A significant incidence of HT-2 toxin and DON were found in 9.1% and 59.7% of total samples, respectively (Rodriguez-Carrasco and others 2015). Wheat based beers showed the highest mycotoxin incidence for both DON (76 %) and HT-2 toxin (56%). Fourteen out of the 154 samples containing HT-2 toxin (9.1%) are belonging to this category. In addition, wheat-based beers also showed the highest mean of DON at 34 µg/L. It was shown that 78.3 % of the 46 analyzed wheat beers were contaminated by DON, with an average content of 18.4 µg/L and a maximum of 49.6 µg/L. It was demonstrated in 53 samples of craft beer from southern Brazil that pure malting barley beer can be contaminated with DON ranges from 127 µg/L to 501 µg/L. (mean : 201 µg/L) (Piacentini and others 2017).

Hundred samples of beer available on the Polish market have been analysed for the occurrence of nivalenol, deoxynivalenol and deoxynivalenol-3-glucoside (Bryła and others 2018). Fractions of positive beer samples were 56, 83 and 67% for nivalenol, deoxynivalenol and deoxynivalenol-3-glucoside, respectively. Mean concentrations of the analytes found in the beer samples were (all data in µg/L): 2.4 ± 1.9 (range 0.5–7.6), 9.0 ± 12.7 (range 1.0–73.6), 9.2 ± 7.5 (range 2.0–35.8) for nivalenol, deoxynivalenol and deoxynivalenol-3-glucoside, respectively. Higher concentrations of deoxynivalenol-3-glucoside than deoxynivalenol found in many beer samples reflect the activity of glucosyltransferase enzymes during the grain malting process when they assist secondary biosynthesis of deoxynivalenol-3-glucoside.

The occurrence of several mycotoxins, including ergot alkaloids, alternariol (AOH), DON, and zearalenone (ZEN) in beer (n = 44) from the German market was studied (Bauer and others 2016). All samples were positive for ZEN (0.35–2.0 µg/L, median 0.88 µg/L) and AOH (0.23–1.6 µg/L, median 0.45 µg/L). Most samples (93%) were positive for ergot alkaloids (0.07–0.47 µg/L, median 0.15 µg/L). Correlating toxin levels in beer with European Union tolerable daily intake (TDI) levels for DON (1 µg/kg b.w.), ZEN (0.25 µg/kg b.w.), and ergot alkaloids (0.6 µg/kg b.w.), beer does not represent a major source of intake of these toxins.

More than 1000 beers were collected from 47 countries, of which 60% were craft beers (Peters and others 2017). A selection of 1000 samples have been screened for the presence of aflatoxin B1, ochratoxin A (OTA), zearalenone (ZEN), fumonisins (FBs), T-2 and HT-2 toxins (T-2 and HT-2) and deoxynivalenol (DON) using a mycotoxin 6-plex immunoassay. The major mycotoxins detected were DON and its plant metabolite deoxynivalenol-3-□-D-glucopyranoside (D3G). The 6-plex immunoassay reported the sum of DON and D3G (DON+D3G) contaminations ranging from 10 to 475 µg/ L in 406 beers, of which 73% were craft beers. The popular craft beer style imperial stout had the highest percentage of samples suspected positive (83%) with 29% of all imperial stout beers having DON+D3G contaminations above 100 µg/L. LC-MS/MS analysis showed that industrial pale lagers from Italy and Spain, predominantly contained FBs (3 - 69 µg/L).

Besides FBs, African traditional beers also contained aflatoxins (0.1±1.2 µg/L). The presence of OTA, T-2, HT-2, ZEN, □-zearalenol, 3/15-acetyl-DON, nivalenol and the conjugated mycotoxin zearalenone 14-sulfate were confirmed in some beers.

10.3 Health risk

On a global level the health risk for beer is related to the botanical origin of the cereals, malt and the mycotoxins that are present in the adjuvants. Although beer is internationally widely consumed, the heavy drinkers will have the highest exposure to mycotoxins.

DON and D3G were mainly reported in European beers, while FBs were mainly reported in beers from Africa and Southern Europe. In general, very high contaminations for all mycotoxins, besides T-2 toxin (T-2) and HT-2 toxin (HT-2), were reported previously in African beers. Ochratoxin A (OTA) was mainly reported in European beers, while aflatoxins (AFs) were mainly reported in African and Asian beers. T-2 toxin (T-2), HT-2 toxin (HT-2) and zearalenone (ZEN) were rarely reported.

According to Brazil national regulations, adjuncts may replace malt up to 50 % and often maize is utilized to improve and accelerate the fermentation process. Maize is often contaminated with DON and FBs. The later might be found more frequently in Brazilian beers.

In general, wheat based beers have higher DON occurrence than barley and malt based beers. *F. graminearum and F. culmorum* are slightly more predominant in wheat than in barley and hence a greater mycotoxin contamination in wheat based products is to be expected. Also beers with higher alcohol content often have higher mycotoxin levels which are seemingly coming from the amount of malt that has been used.

Average consumers showed a probable daily intake lower than tolerable daily intake. No toxicological concern was associated to mycotoxins exposure for average beer consumers. Despite that, for heavy beer drinkers, the contribution of this commodity to the daily intake is not negligible, approaching or even exceeding the safety levels.

10.4 Legal limits

In Europe, maximum allowed mycotoxin levels are regulated by the Regulation (EC) 1881/2006 with subsequent amendments and updates. The limits for cereals and processed cereal products for direct human consumption or use as an ingredient in food will need to be met for beer. The applicable limits in cereals, barley and malt are set as follows: 750 µg/kg for DON and 100 µg/kg for ZEN. Beer is subjected to the legal limit in FBs content (maximum 400 µg/kg for the sum of FB1 and FB2) and 75 µg/kg for ZEN when maize is involved. For the storage mycotoxins as aflatoxins and OTA, one has 2 and 4 µg/kg for resp. AFB1 and the sum of AFs and 3 µg/kg for OTA would be chosen in absence of limits set for beer.

Barley, maize and malt are the largest contributors to the mycotoxin load in beers. To date no maximum limits are set although that depends on the geography and the used cereals different mycotoxins might be present. It is unclear in how far the mycotoxins in the starting materials end up in the final product and what the process can mitigate.

10.5 Mitigation Process

Besides barley, beer adjuncts can represent another source of mycotoxins, of particular note being maize (Marin and others 2013), which is proven to be susceptible mainly for Aspergillus section Flavi (aflatoxin producers), *F. proliferatum* and *F. verticillioides* (FBs producers) infestation.

Hops added during the boiling stage may also be subject to fungal invasion with subsequent mycotoxin accumulation. However, it has been studied that hops were a significant source of mycotoxins in brewing wort because of the relative low quantity added to the beer (Vaclavikova and others 2013).

Several stages of the production scheme are proved to decrease the initial mycotoxin contamination levels (Lancova and others 2008).

The complexity of these operations do not give to the brewer a complete control on chemical and biochemical reactions that take place in the batch, but the knowledge about mycotoxin properties can help in identifying the operations thereby decreasing their level in foodstuffs and in the development of mitigation strategies.

10.6 Flow chart with traffic light system

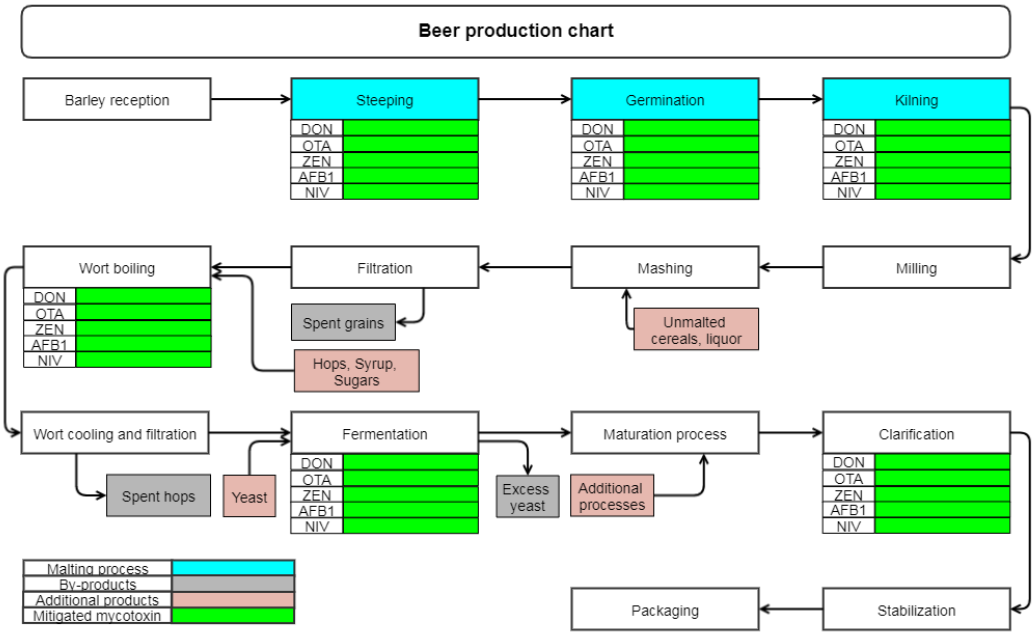


Figure 14
Beer production chart, modified from (Lewis and Young 1995; Pascari and others 2018). The cells highlighted in green indicate that the process step has been reported to significantly reduce a given mycotoxin in the system.

This chapter discusses available data about mycotoxin evolution during malting and brewing process. The operations that may lead to a decrease in mycotoxin load are found to be steeping, kilning, roasting, fermentation and stabilization operations applied over the process (*e.g.* clarification). Other general decontamination strategies used in the food industry such as hot water treatment or even the use of lactic acid bacteria starter cultures during malting or fermentation are considered.

Several works that studied the fate of naturally occurring or artificially added mycotoxins at various stages of the brewing process, have demonstrated that mycotoxins may be transmitted from contaminated grain into beer. As regards OTA, a fraction between 13 and 32% of the toxin content, present in the original grist, survived in the beer. For deoxynivalenol (DON), the transfer from malt grist in finished beer was between 80 and 93%. In the course of beer fermentation, ZEN was mainly converted to ß-zearalenol, which has lower estrogenic activity than that of ZEN. The average percentages of AFB1 and FB1 recovered in finished beer, referring to the amounts containing in raw materials, were 1.5 and 50.7%, respectively (Bertuzzi and others 2011).

Higher concentrations of deoxynivalenol-3-glucoside than deoxynivalenol found in many beer samples reflect the activity of glucosyltransferase enzymes during the grain malting process when they assist secondary biosynthesis of deoxynivalenol-3-glucoside(Lancova and others 2008).

A study of the DON, DON-3-G and ZEN concentrations change during malting was conducted (Pascari and others 2019). A significant washout effect on DON was observed by the end of the first water phase (between 22.4% and 34% reduction) with an even more pronounced reduction (up to 75% decrease) by the end of the steeping process. ZEN content remained almost unchanged (no significant difference between the initial and the final concentration). Germination was characterized by an increase in all the three toxins (ZEN, DON and DON-3-G) concentrations. However, it showed a decreasing trend in the last 24 hours of the stage compared to the first day of germination. Kilning lead to a significant reduction of DON in the naturally contaminated batch (46.6% and 78.8%), nevertheless an increase in all other toxins and contamination levels was observed.

In a study on the transfer from malt to boiled wort (Pascari and others 2019), it was demonstrated that DON increased up to 150% during mashing. Important reduction of DON (60%), ZEN (99%) and FBs (90%) after just 30 minutes of wort boiling is observed, with levels remaining constant until the end of boiling. DON and its metabolites were reduced to their initial level contained in the malt before mashing, or even lower, however in none of the samples they were completely eliminated. Zearalenone was not quantitated at the end of boiling, although there was a significant initial level of ZEN. □-Zearalenol remained unaltered during the process. Fumonisins were reduced by between 50 and 100 per cent during mashing and boiling.

Fermentation of wort is a process initiated by yeasts of *Saccharomyces* genus. Excess yeast is removed but is able to adsorb mycotoxins as demonstrated with beer fermentation residue (BFR). Very high ratio of adsorption were also observed in the case of ZEN (75.1%) as well as AFB1 (48.1%) and OTA (59.4%) (Campagnollo and others 2015). The reduction of DON did attain only 11.6%. The adsorption is due to the binding of the toxins (especially ZEN) to □-glucans from yeast cell wall. Barley is also known as containing high □-glucan content (2.5 to 3.5%). The interactions of combinations of deoxynivalenol, zearalenone and fumonisin B_1 on yeast have been studied. It was found that a synergistic interaction between deoxynivalenol and zearalenone exists, but only at very high concentrations (Boeira and others 2000).

The most studied mycotoxins in beer are DON and its derivatives, ZEN, FBs, HT-2 and T-2 toxins, AFs. The most important stages of beer production process having an inhibitory impact on mycotoxin levels are steeping, kilning, mashing, fermentation and clarification. During these stages, the mycotoxins are removed with drainage water, spent grains and fermentation residue, diluted or destroyed as a result of thermic treatment, or adsorbed on the surface. Germination does not actually impact DON levels in beer but promote its transformation into its glycosylated derivate (DON-3-Glc). During mashing, the enzymes stimulate the release of conjugated DON from protein structures but also decrease the initial toxin concentration due to dilution. This step can be a source of AFs and FBs contamination because of maize based unmalted adjuncts added to increase the amount of fermentable sugars. Hops added while boiling might be contaminated with mycotoxins, but the amount is too small to be considered significant for the final product. ZEN is mainly removed with the spent grains (approximately 60%).

Strategies of mycotoxin decontamination and prevention can be applied at all production stages: fungicide treatments on the field, ozonation of starting materials, hot water treatment of barley grains, lactic acid bacteria during malting and brewing with special yeast strains.

11. COFFEE

11.1 Food Chain description

Over the past 50 years, both production and consumption of coffee have increased considerably. Especially raising income in developing countries has caused an increased demand of coffee.

11.2 Mycotoxin of relevant occurrence / toxicity

Green coffee beans may be significantly contaminated with OTA. Furthermore, they may be contaminated with aflatoxins.

11.3 Health risk

For coffee, mainly storage myctoxins are of concern

- Aflatoxins are extremely potent toxins and genotoxic carcinogens
- OTA is classified as possibly carcinogenic to humans and has been associated with kidney disease

11.4 Legal limits

The EU imposes maximum limits for OTA in roasted coffee beans and ground roasted coffee of 5 µg/kg. Soluble coffee (instant coffee) must not contain more than 10 µg/kg OTA.

11.5 Mitigation Process

Once the cherries are harvested, the beans have to be removed by using either the dry or the wet method. The wet method is more expensive than the dry method, but the coffee it produces has better quality properties (FAO). The dry method (also called the natural method) is the oldest, simplest and requires little machinery. There are three basic steps, cleaning, drying and hulling. The key difference to the dry method is that, in the wet method, the pulp of the cherry is removed between the cleaning and drying stage. Subsequently, the mucilage is broken down by natural enzymes and can easily be washed away. As coffee beans are hygroscopic, proper drying to 11% moisture content is essential as they are otherwise vulnerable to contamination with fungi producing OTA during storage and transport.

Proper sorting of the coffee beans can reduce OTA and aflatoxin levels by removing damaged beans, which are more likely to be infested by toxigenic fungi. Thermal degradation during roasting has been shown to reduce OTA up to 84 % (Blanc and others 1998; Milanez and Leitao 1996).

High grade coffee beans in combination with proper sorting, and drying, followed by roasting is the best protection against the contamination of coffee with OTA (Heilmann and others 1999).

11.6 Flow chart with traffic light system

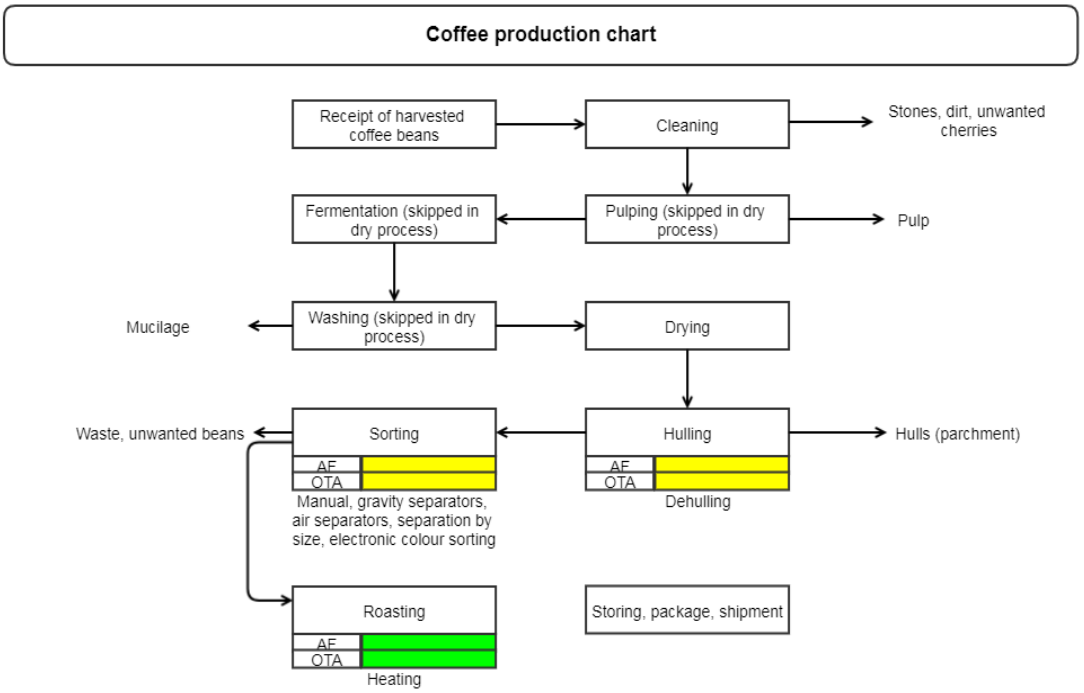


Figure 15
Coffee production chart with traffic light system (Karlovsky and others 2016). The cells highlighted in green indicate that the process step has been reported to significantly reduce a given mycotoxin in the system. Cells highlighted in yellow indicate that a reduction of mycotoxins may be possible but to a lesser extent.

12. CONCLUSIONS

A range of fungi produces bioactive mycotoxins therefore such natural contaminants are commonly found worldwide in foods. There is a wealth of evidence on their adverse health effects, especially from *in vitro* and *in vivo* experiments. However, in some cases the epidemiological consequences of the dietary exposure to mycotoxins other than aflatoxins still needs clarification. Nonetheless, the contamination commonly found in food consumed and traded in low-income countries in particular may be assumed to be responsible for serious health effects in those regions. Furthermore, the pervasive but potentially lower contamination present in the food chains of higher-income countries still presents significant concern with respect to chronic effects. As such, the adoption of strategies to mitigate as much as possible the contamination of foods presented to the consumer is strongly encouraged. This Black & White Report focuses on mitigation measures that can be applied post-harvest during the processing of foods.

Although most regulatory limits apply to commodities before they are processed, several effective strategies have been identified, that should be practically implementable in production chains for specific food categories. In many cases, the most effective mitigation strategies are in controlled sorting, storage and washing. However, this is not possible with liquid products such as milk without changing the characteristics of the commodity. Currently, feeding cows with compliant feed, which should be as uncontaminated as possible, remains the most effective way to reduce the final contamination of toxins in milk and dairy products.

It is important to highlight that the effective strategies identified so far account mainly for those mycotoxins that are recognised as problematic and therefore regulated in a given food. The assessment of mitigation of non-regulated mycotoxins that are likely to co-occur with the regulated ones is neglected in literature. As an example, the so defined "emerging mycotoxins" (*e.g. Alternaria* toxins, ENNs, BEA, and MON) may simultaneously co-occur in cereals and in many cereal-based products (including beer). Nevertheless, the efficacy of the already established strategies to reduce their level is still largely unexplored, although a degree of effect is reasonably expected. Therefore, a broader assessment of the efficacy of mitigation strategies accounting not only the regulated toxins, but also those likely to co-contaminate food is required, and it is intended to update this Report as information becomes available. The specificity of mitigation actions to reduce contamination and not nutrients needs to be assessed to avoid pauperization of food when mitigation strategies are designed or implemented. It is necessary to make a distinction between the physical methods to remove mycotoxins (*e.g.* cleaning and hulling) and the chemical transformations to degrade mycotoxins (*e.g.* via thermal treatments, microbial fermentation or enzymatic reactions). Mitigation strategies that use chemical degradation should account for the characterization of degraded products to avoid the possible formation of toxic by-products. Nevertheless, neither the chemical characterization of degraded products nor their toxicological assessment is routinely carried out posing a degree of uncertainty to the actual safety of final products. As a part of the development of mitigation methods, the assessment of by-product formation and their toxicity is encouraged to ensure the safety of food.

There is a gap in the current scientific literature between the mitigation strategies currently available and the number of mycotoxins that may contaminate food. In particular, the efficacy of strategies has been assessed only on few mycotoxins compared to the number potentially contaminating foods. In this respect, it is suggested that mitigation strategies should be investigated as to their impact on emerging mycotoxins prior to the future establishment of regulations. In addition, as climatic change will undoubtedly impact mycotoxins a change of paradigm is needed towards the implementation of novel and more effective multi-mycotoxin mitigation strategies.

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ANNEX: LIST OF ABBREVIATIONS

AFs	Aflatoxin
AME	Alternariol monoethyl ether
AOH	Alternariol
ALARA	As Low As Reasonably Achievable
BEA	Beauvericin
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
DON-3-G	Deoxynivalenol-3-glucoside
EA	Ergot alkaloids
EC	European Commission
EFSA	European Food Safety Authority
ENNs	Enniatin
FAO	Food and Agriculture Organization of the United Nations
FBs	Fumonisins
GAP	Good Agricultural Practices
IARC	International Agency for Research on Cancer
IR	Infrared
MON	Moniliformin
MRL	Maximum Residue Level
NIV	Nivalenol
OTA	Ochratoxin A
PAT	Patulin
RNA	Ribonucleic acid
TCT	Trichothecenes
TeA	Tenuazonic acid
UHT	Ultrahigh temperature
UV	Ultraviolet
US FDA	United States Food and Drug Administration
WHO	World Health Organization
ZEN	Zearalenone

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ISBN 9789078637455