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Aflatoxins, Ochratoxins, Trichothecenes, Patulin, Fumonisins and Beauvericin in Finished Products for Human Consumption

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Abstract

Mycotoxins are considered a serious threat for mankind health due to their ability to form toxic secondary metabolites. For this reason, many efforts are actually in progress to reduce their impact on food, feed and food chain by-products. In particular, a large part of the scientific investigation is focused on reducing the on field contamination using an *in situ* agronomic approach or specific biological tools. In this paper, the reduction of Aflatoxins (AFs), Ochratoxins (OTAs), Trichothecenes (TC), Patulin (PAT), Fumonisins (FBs) and Beauvericin (BEA) in industrial processes addressed to obtain finished products for human consumption will be explored and analyzed. In particular, performances of the main mycotoxins and corresponding reduction methodologies will be examined together with the processes and the process conditions used during the usual commodities handling and foodstuff manufacturing.

Keywords: Mycotoxins; Process; Foodstuff; Reduction; Health; Safety

Introduction

Mycotoxins are naturally occurring toxic fungal secondary metabolites that exhibit a toxic or potentially carcinogenic activity to animals and humans. The term mycotoxin was coined in 1962 after a veterinary crisis in England (Turkey X disease), during which approximately 100000 turkey poults died [1]. Mycotoxins are low molecular weight molecules produced as secondary metabolites by filamentous micro fungi mainly belonging to the genera *Fusarium, Penicillium* and *Aspergillus* [2]. These molecules have different chemical structure and are very stable, making it very difficult their elimination from food and feed matrixes [3]. The most relevant mycotoxins, in term of their presence in food and feed, are considered: aflatoxins, fumonisins, ochratoxin, trichothecenes, zearalenone, patulin [4]. Classifying mycotoxins is quite a difficult task; due to their different chemical structures and biosynthetic origins, their many biological effects, and their production by a wide number of different fungal species, classification schemes depend on the problem assessment. Clinicians often arrange them by the organ which they affect: mycotoxins can then be classified as hepatotoxins, nephrotoxins, neurotoxins, immunotoxins, etc. Cell biologist classify them as teratogens, mutagens, allergens, and carcinogens. Organic chemists organized mycotoxins according to their chemical structures (e.g., lactones, coumarins, etc.), while biochemists according to their biosynthetic origins (polyketides, coumarins, amino acid-derived, etc.). Mycologists propose a classification based on the fungi species which produce them (e.g., *Aspergillus* toxins, *Fusarium* toxins, *Penicillium* toxins, etc.). However, no classification can be considered entirely satisfactory from all the different existing perspectives.

Mycotoxins can commonly penetrate the food chain from the field until the post-harvest, where the contamination depends on storage conditions. Even excellent quality field crops can be contaminated if they are improperly stored. The different chemical structure of mycotoxins could explain their quite different toxicity. Figure 1 shows the chemical structure of relevant mycotoxins.

As an example, the aflatoxins chemical structure allows the formation of DNA adducts with guanine inducing liver cancer [5], while fumonisins can inhibit ceramide synthase inducing adverse effect on the sphinganine/sphingosine ratio [6]. This ratio could serve as an effective biomarker for consumption of fumonisine containing food/feeds. Following this biomarker value evolution could be used to check if fumonisins exposition is still present.

Most mycotoxins are resistant to technologic processes, and can persist through the entire food industrial processing of food and/or feed. Moreover, they are also resistant to pH, ions and water content, temperature, heating time and heating rate. The Food and Agriculture Organization (FAO) estimated that 25% of the world's agricultural commodities are contaminated by mycotoxins [7]. For this reason, the interest about the effects of the mycotoxin presence and quantity on food processing has increased during the last decade. In general, sorting can remove the greatest part of mycotoxins contaminated units, and their level in contaminated foodstuff can also be reduced by washing, wet and dry milling, roasting, baking, frying, nixtamalization and extrusion [8] nevertheless these secondary metabolites represent a serious threat for humans. The aim of this review is to analyze and discuss the efficacy of the main strategies currently adopted to reduce the most common mycotoxins occurrence in food during post-harvest.

Aflatoxins

Aflatoxins (AFs) are difuranocoumarin derivatives synthesized primarily by the fungi *Aspergillus flavus*, *Aspergillus parasiticus* and,

to a lesser extent, by the *Aspergillus nomius* [9,10]. In 1996 Goto et al. identified *Aspergillus ochraceoroseus* as another new AFs producer by isolating it from the Taï National Forest in Ivory Coast [11].

Williams et al. have estimated that 75% of the world's population is exposed to AFs [12]. Twenty different AFs have been identified, but only four of them, namely AFB1, AFB2, AFG1 and AFG2 are significant contaminants of a wide variety of foodstuff. AFB1 is the most common and the most toxic one in terms of both acute and chronic toxicity. In fact, since 1986 the International Agency for Research on Cancer (IARC) classified AFB1 in the group 1 carcinogen (i.e. carcinogenic to humans). Aflatoxins M1 and M2 (AFM1 and AFM2), which are the hydroxylation products of AFB1 and AFB2, have been identified in milk and dairy products [13].

The growth of fungi that produced AFs in stored commodities is strongly influenced by environmental conditions: in fact a high moisture content can increase the AFs amount of 10 fold in 3-day period [14]. As an example, sun drying is the common drying method for peanuts and this is considered one of the ways to contaminate them and consequently the subsequent steps in the food chain [15].

In temperate zones of the United States, commercial practice is to screen kernels under UV light, where bright fluorescence shows the AFs presence. Sorting of individual kernels, is not normally attempted on maize lots, although it is possible to do this with special machinery. The use of drying platforms can increase the efficacy of AFs reduction in grain such as, drying outside the field and drying on mats [14]. It has been reported that the roasting of naturally contaminated peanuts at 150°C for 30 minutes can reduce the AFs levels significantly [16]. Roasting pistachios at 90°C, 120°C and 150°C for 30-60 and 120 minutes, reduced the AFs contamination from 17% to 63%. The addition of 50 g/kg of sodium chloride (NaCl) during the roasting process, reduces of 48% the AFs level [17]. Nakajima et al. reported that the effect of roasting of green coffee beans on the level of AFs was approximately reduced of 50% [18]. The AFs reduction during coffee bean roasting however was dependent on the roasting method and temperature with reductions of about 42 to 56% [19]. Roasted coffee dried at 140°C for 40 min, reduced AFs levels by 59%, and if the coffee is dried at 150°C for 25 minutes, the reduction increases to 69% [20].

Maize is considered one of the most AFs contaminated crops. In Central America, a process known as "nixtamalization" (alkaline-cooking) is commonly used in the preparation of maize meals to make tortillas and similar foods. This process destroys AFs up to reach the food safety objectives [21]. Torres et al. [22] reported an elimination of 51.7%, 84.5% and 78.8% of the AFB1 content of tortilla, tortilla chips and maize chips, respectively, while Elias-Orozco et al. discuss a reduction by 94% [23]. Pérez-Flores et al. evaluated the effectiveness of maize detoxification achieved with a modified tortilla-making process in which maize grits are mixed with water and lime, cooked in a microwave oven, steeped, and then milled to obtain the fresh masa able to make tortillas. This method led until to 84% decrease in AFB1 content [24].

The fermentation process also can increase the safety of some foods contaminated with AFs. Fandohan et al. [25] evaluated a 93% reduction of AFs in fermented maize ("makume"). In this case, the authors identified sorting, winnowing, washing, crushing combined with dehulling of maize grains as the critical AFs reducing steps [26]. AFs levels can be reduced by up to 92% during the preparation of the Mexican drink "atole" by heating at 94°C for 10 minutes and then drying at 40°C for 48 hours. In the production of corn flakes the AFs level is reduced by heating up to 64%, while after roasting, both with and without added sugar, the reduction is 78-85%, respectively [27]. Extrusion is a process frequently used to produce breakfast cereals and snacks: in this case temperature higher than 150°C can reduce AFB1 contamination of 50-80%, while the addition of 0.7% ammonia, 1% hydroxide or 0.4% bicarbonate allows to reach a reduction up to 95% [28].

Rice is one of the most important food matrix and fungi able to produce AFs can easily colonies it; however when rice is cooked up to 89% of the lactone ring of the AFs can be hydrolyzed to the corresponding carboxylic acid [29].

The usual procedure of rice cooking even in AFB1 contaminated rice, showed an average reduction of 34%, and a further reduction (78-88%) was obtained with pressure cooking [30]. An applied pressure of 0.10 MPa during cooking can reduce then up to 88% versus a 34% of reduction obtained by using only boiling water [31].

During the parboiling step of the rice, a 2.2-4.4% of the AFB1 in a sample may be lost [30], while is sufficient to increase the soaking time of rice from four to six hours to reduce up to 82% the contamination of AFB1 [32].

Gummert et al. described the very positive effect dryers had on maintaining rice quality and reducing mycotoxin risk in Southeast Asia [33].

Gamma rays use ranging from 0 to 60 kGy of adsorbed radiation dose of ionizing radiation, were applied to black pepper with a maximum reduction at 60 kGy corresponding to 43%, 24%, 40% and 36% for AFB1, AFB2, AFG1 and AFG2, respectively. Gamma rays even at 60 kGy were not effective to destroy completely the AFs [34].

In fungi and chili fresh vegetal samples, a dose of only 6 kGy reduced the micro fungi contamination by ten thousand time, while the same gamma rays intensity has been able to reduce the AFs level only of about 100 times [35].

The problem of AFM1 in the milk was investigated by El-Deep et al. that found 9.5% and 26% reduction after heat treatment at 63°C for 30 min and at 121°C for 15 min respectively [36]. Choudhary et al. investigated the effect of various heat treatments on AFM1 stability of cow's milk and reported that sterilization of milk at 121°C for 15 min caused a 12.21% degradation of AFM1, while boiling the milk produced a reduction in the level of AFM1 of 14.50% [37].

Santini et al. studied AFM1 presence in 73 milk samples from different animal breeds and 24 dairy products samples from Sicily (Italy) [38]. AFM1 was detected in 48% and 42% of the milk and dairy samples in a concentration range of <5.0-16.0 and <5.0-18.0 ng/L, respectively. In dairy products, ultra-high temperature treated (UHT) milk, milk cream and cheese, the incidence was 42%. Out of this 42%, a percentage of 83% of the samples contained less than 5.0 ng/L and 17% contained 10.0–20.0 ng/L AFM1.

A recent study carried out by Şanli et al. showed that pasteurization during the yogurt production can degrade the AFM1 and this reduction is due to the simultaneous interactions of whey proteins with the casein by hindering the extraction of the complex casein-AFM1 and consequently by preventing heat destruction of the mycotoxin [39].

Ochratoxin A

Ochratoxin A (OTA) is to be considered one of the first fungal metabolites toxic to animals. This toxin is the most toxic member of the ochratoxins family. OTA is one of the most studied mycotoxins, based on the observed teratogenic, embryotoxic, genotoxic, neurotoxic, immunosuppressive, carcinogenic (IARC group 2B), and nephrotoxic effects (JECFA) [40].

OTA's resistance to thermal processes during processing of products such as coffee, cocoa, wine, beer, and cereals has been reported. However, the thermal processes can give different effects, according to the temperature reached. The roasting of cocoa to produce chocolate requires a heat treatment of the beans in the range 110-140°C for about 30 min [41,42] reported a reduction of the 93.6% of the OTA after thermal treatment. Moreover Amezqueta et al. has shown that more than 98% can be eliminated by an alkaline treatment of the pods [43].

Paterson et al. studied the roasting of the coffee as a function of the treatment time in the range between 2.5 and 10 min [44]. The differences in OTA reduction between the different roasting time and color differences (color varied from light medium to dark as a function of the roasting time) did not reach any statistical significance. Seven out of nine samples of coffee evidenced a quite large range of OTA reductions, in the range between 69 % and 96%. When the processing conditions are those applied to obtain a typical espresso coffee, OTA degradation is greater than 90%, in samples with both high and low levels of contamination [45,46]. Suarez-Quiroz et al. reported that OTA is masked by substrate interactions during roasting and depends on how the coffee beverage is prepared [47]. The coffee beverage making affects the OTA content, which could paradoxically be higher than that of the initial roasted coffee.

Bread is one of the most important sources of carbohydrates in the human diet [48], and the evidence that OTA is often associated with cereals is an important food safety issue.

Scudamore et al. studied the fate of OTA through initial cleaning and milling into whole meal wheat. Bread was baked from both whole meal flour and straight-run white flour [49]. The best step to remove up to 44% of OTA is the scouring because only a small further loss occurred in the bread-making process. A reduction of about 75% can be achieved in white bread by combining of cleaning scouring and removal of the bran and offal fractions. The maximum reduction in producing whole meal bread was about 40%. The baking of cookies reduces about the 65% of the OTA [46]. The extrusion processing, largely used in breakfast cereal production, can also reduce the levels of OTA. Scudamore et al. studied the stability of OTA during extrusion of contaminated whole meal wheat flour, observing that a higher temperature, a long contact time and high moisture content were related to a bigger OTA reduction with a maximum loss observed no greater than 40% of the initial amount [46,49]. In the case of rice, it has been observed that OTA contamination level, similarly to the AFs, can be reduced up to 86% if the rice is cooked using a large amount of water [28].

Procedures such as heating, ripening, drying, and storage have no effects on the degradation of the OTA in meat products and about 20% of reduction has been recorded in some pig products after frying [50].

As an alternative decontamination method, the use of radiation treatment has been reported as a suitable method for decontaminating foods from fungi and mycotoxins [51]. Mustapha et al. reported that gamma radiation effectively inhibited fungal growth and reduced the concentrations of OTA in millet flour samples [52]. In particular, the OTA levels were reduced approximately of 13% and 44% using 1 kGy and 3 kGy radiation doses, respectively. A greater reduction of OTA level (74%) was reached using 10 kGy, but it is noteworthy that the dose of 10 kGy is the maximum dose acceptable for cereals [53,54].

Trichothecenes

The group of the trichothecenes (TC) includes structurally related mycotoxins produced by *Fusarium spp*. with different levels of cytotoxic activity. This group includes deoxynivalenol (DON), nivalenol (NIV), T-2 toxin and diacetoxyscirpenol, which are commonly found in cereals e.g. wheat, corn, barley, oats and rye.

The TC produced by *Fusarium culmorum* and some related species that grow especially on wheat and on barley, are able to produce together to the DON, also the oestrogenic mycotoxin zearalenone (ZEA). Physical and mechanical processes, such as sorting, cleaning and milling may reduce DON and NIV levels since much of the toxin is accumulated in the germ [55]. The level of mycotoxins in cleaned wheat ranged from 7 to 63% for DON, from 7 to almost 100% for NIV, and from 7 to 40% for ZEA [56]. A reduction of 62% and 53% of T-2 and HT-2, respectively, has been reported by Pascale et al. [57]. The grains infected by *Fusarium spp*. may have lower relative physical density if compared with the healthy grains, so they may be removed efficiently by the use of gravity separators [58]. The distribution of mycotoxins in dry milled corn fractions has been deeply investigated in order to understand the effect of industrial processing. DON, ZEA as well as many other mycotoxins are usually concentrated in few fractions of the commodities, which are less likely to be used for human food production, e.g. germ and bran [46]. The wet milling of the corn is not adequate to destroy the TC like other mycotoxins that may be dissolved into the steep water or distributed among the transformation byproducts. As a result mycotoxins can be found in the steep water, in the gluten fiber and in the germ, while the starch, corresponding to most refined flour, is generally free from these contaminants [59].

The debranning of wheat by mechanical process allows to remove the outer layers of wheat grains prior to the milling process, commonly adopted in the industrial processing and it enhances the milling performance of wheat as well as the refinement degree of semolina and flour [60].

DON reduction after debranning is quite variable, ranging from 15 to 78% [61,62]. Israel-Roming and Avram evaluated the possibility of reducing the contamination level in wheat by milling and baking at different temperature [63]. Using naturally contaminated wheat, with 1.11 μg/g of DON, and spiked samples at 1.0 μg/g level, it has been observed a tantalising distribution of the DON. In particular, DON in flour was 29% lower for naturally contaminated samples if compared to 40% for artificially spiked samples.

All the samples were baked at 190°C for 30 minutes, at 200°C for 30 minutes and at 230°C for 20 minutes: the heat treatment reduced only minimally the DON level, between 1.7 and 4.1%. The baking at 230°C reduced DON level in the range 7.6-9.9%. Pacin et al. estimated the reduction of contamination levels in French bread and Vienna bread [64]. The average DON level reduction between flour and baked products was, for French bread 33% and for Vienna bread 58.5%.

It has been observed that in tortillas preparation process using contaminated maize, 72-88% of the DON was lost, but in some lots

DON may not be detected after baking. It has also been observed that a derivate or an isomer of the DON with unknown toxicity could still remain [65].

A recent study on rice evaluated eight lots per year from five different brands of parboiled rice collected in four different seasons (32 lots considered). The DON was naturally present in the 22% of the samples (the amount was variable from from 180 to 400 ppb), while ZEA was observed in the 19% of the samples and in a concentration range from 317 to 396 ppb [66]. In the case of parboiled rice, the optimal conditions to reduce the ZEA migration into the endosperm require a soaking time of 4 hours [32].

The production of the pasta has been well investigated and a significant degradation of DON was reported during each of the processing steps from uncleaned durum wheat to cooked spaghetti.

In particular, the average levels of DON were reduced of 77% in step of cleaning of the wheat, 37% to produce the semolina, 33% to transform the semolina in the spaghetti and another 20% was lost during the cooking of spaghetti [67].

Raiola et al. investigated the presence of DON in Italian pasta intended for children and average levels of contamination ranging between 73.80 μg/kg and 387 μg/kg were evidenced [68]. In cooked pasta, the amount was significantly reduced at levels between 10.27 μg/kg and 42.10 μg/kg.

For wheat decontamination, another strategy is to soak the wheat with sodium bisulfite: this approach reduces DON with formation of sulfonate salts which are stable under acidic conditions. Nevertheless, these adducts may break down resulting in free DON under alkaline conditions [69]. The addition of sodium bisulfite, L-cysteine or ammonium phosphate could reduce DON levels of more than 40% [58].

Nowicki et al. observed that the DON levels may be reduced during the processing in basic condition such as boiling of Chinese noodles containing Kansui, a commercial preparation of carbonate and phosphate salts of potassium and sodium [70].

These conditions were investigated by Farahany et al. that observed the effects of processing for two types of Asian noodles production (yellow alkaline and instant) on the DON levels [71]. The significant reductions of the initial DON levels, 43.2% and 66.6%, were found in yellow alkaline and instant noodles, respectively. In particular, the ingredients as alkaline salts appeared to be the main factor influencing the reduction in the two types of noodles explaining the significant reduction of the DON during cooking and frying of both yellow alkaline and instant noodles. The mechanism of reduction is probably due to a leaching of the DON out of the noodle into the cooking medium.

Samar et al. studied the effect of the frying process on naturally (1200 μg/kg) and artificially DON contaminated flour (260 μg/kg) [72]. Frying was performed at three temperatures (169°C, 205°C and 243°C) for different times. The reduction of the DON was greater in the artificially contaminated samples (>66% at 169°C, 43% at 205°C and 38% at 243°C) while for naturally contaminated samples, the average percentage of the reduction of the DON was 28% when the dough covers were fried at 169°C, 21% at 205 °C and 20% at 243°C. The baking process may reduce broadly the DON by 24-71% in bread and of the 35% in cookies preparation [73], while the extrusion if conducted at 150-180°C may cause loss more than 95% of the DON in maize. The extrusion of corn grits reduces ZEA levels by 77-83% at 120°C, by 74-83% at 140°C, and by 66-77% at 160°C.

Patulin

Patulin (PAT) is a toxic metabolite produced by several species of fungi belonging to the genera *Penicillium*, *Aspergillus* and *Byssochlamys*. The accumulation of PAT in the commodities appears to be independent from the use of fungicides before the storage step, while data support that the temperature of food prior of refrigeration is probably the most important variable in the production and accumulation of PAT [74,75].

In 2003, Ritieni investigated the presence of PAT in Italian apple products and the positive samples had a concentration ranging between 1.4 and 74.2 μg/L with a mean value of 26.7 μg/L [76].

The PAT concentration in unprocessed fruit stored indoors reached a value of 90 ng/g after 5 days, 400 ng/g after 15 days, and 2.200 ng/g after 33 days. These values decreased to 75 ng/g, 100 ng/g and 695 ng/g, respectively, after washing of fruits before their industrial processing.

The washing of the apples with water under high pressure reduced from 10 and up to 100% the initial concentration of PAT depending on the initial level of PAT contamination.

For heavily contaminated apples, i.e. 350 μg/L or more, even after washing, the PAT level did not go below 50 μg/L [77].

A refrigerated storage with a controlled atmosphere often does not guarantee the prevention of fungal growth, and PAT production, as well as additional treatments, including fungicides application.

Exposition to trans-2-hexenal vapour, an aromatic volatile compound produced by many fruits and vegetables, at a concentration of 12.5 μg/L can potentially control blue mold onset, can also minimize the PAT content and maintain the quality of apples of the variety Golden Delicious [78].

Finally, immersion of apples in a sodium hypochlorite 3% solution for five minutes at 25°C completely inhibited fungal growth and the related damages to the fruits [79].

PAT concentration in apple juice increases with the decomposition of the peel of the apple. In fact, the level of PAT found in the juice products prepared from healthy apples are all smallest than 50 ng/g, while the juices produced from apples where more than 30% of peel were decomposed, contained PAT at levels higher than 50 ng/g.

PAT can be removed from the juice using granular activated carbon, in particular apple juice can be filtered through activated charcoal (40-60 mesh) and PAT levels are reduced of about 98% with respect to unprocessed apple juice. Another approach used three grams of activated charcoal per liter added to apple juice containing 62 ng/g of PAT, followed by stirring for five minutes. In this case the PAT contamination was reduced of about the 50% [80].

Other carbon-based adsorbents have been studied and can efficiently reduce the PAT levels in apple juice, but these adjuvants also result in a loss of flavour and of the nutritional quality of the final products [81].

In a study of Gökmen et al., is shown how the clarification of apple juice with activated carbon unfortunately reduced the colour and the phenol content together with a 40% reduction of the PAT level [82].

It has been also reported that resins with pores with diameter pores <20 Å can retain PAT via chemo-absorption [83], and their use is advantageous in industrial processes since the resins can be regenerated several times and re used with an effective cost reduction. An interesting side effect of this approach is the formation of derivatives deriving from interaction between PAT and the resin. These derivatives are more easily degraded with either ammonia or with a volatile base, preferably generated *in situ* from a solution with high pH value. Thus, the level of the PAT in the juice is reduced by more than 85% with a minimal environmental impact, due to the regeneration of the resin that are inactivated by the interactions with the mycotoxin.

One of the most common approaches to reduce the level of the PAT in the apple juice is the use of pectins that are able to clarify the juice by filtration under vacuum. This this process reduces the level of the PAT of about 39%. Alternatively, a mixture of gelatin/bentonite clay, followed by ultra-filtration, is able to reduce the level of the PAT up to 25% [77].

Different conventional techniques also can reduce PAT levels in clarified apple juice, for example centrifugation is the most effective among the adopted industrial treatments, with a reduction of 21%.

The use of refined bentonite clay is the second efficient choice, and a PAT reduction of about 8.5% has been reported [84]. On the opposite, filtration with food grade diatomaceous earth and pectinase enzymatic treatment are much less effective, allowing to obtain reductions of 3% and 4.5%, respectively. Many studies have been realized combining different approaches, for example the centrifugation and the use of the refined bentonite are able to reduce the level of the PAT of 21%, while the combination of centrifugation and the treatment by pectinase reduces the level of the PAT of 17% [84].

Kadakal and Nas (2003) reported the treatment of apple juices at 90°C and 100°C for 20 min observing the reduction of PAT level of 19% and 26%, respectively [85].

Raiola et al. simulated an industrial thermal treatment in artificially spiked apple purees and apple juices, observing an average loss of 1.41% and 62.62% respectively [86]. In Turkey, is common an industrial process for apple juices that requires a treatment at 90°C for 10 seconds because the level of the PAT is reduced averagely of 13% [87]. This Turkish industrial protocol was compared to conventional pasteurization (90°C for 10 minutes) and with the high temperature short time method (HTST) (60°C, 70°C, 80°C and 90°C for 10 sec) which confirmed the best performance at 90°C for 10 sec with a maximum reduction of the 19%.

A possible risks related to this process is the possibility that the PAT is produced by heat-resistant fungal species during storage and the juices consequently are not safe for consumers.

Another process related aspect regards the vacuum distillation, needed to concentrate the juice to obtain a reduction of volume of about 24% with relevant advantages for transport and manipulation of puree and juices. During distillation however there is the possibility that PAT can be transformed in volatile substances that are distilled away. Kadakal and Nas reported that evaporation for 20 minutes at 70°C reduced the level of the PAT by 9.4% and using a temperature of 80°C the levels are reduced by 14% [85].

More recently, Janotová et al. evaluated the PAT degradation in apple samples spiked at four levels of concentration (539 µg/kg, 140 µg/kg, 23 µg/kg and <2 µg/kg) and then processed [88]. Janotovà has simulated the complete process to obtain apple juices with homogenization, pulping, pasteurization and the aseptic packaging to evaluating the contribution of the single steps to the reduction of the PAT.

The critical operation was pulping, where the level of the PAT was reduced from 29% to 80% with respect to the original content.

The PAT level also can be affected by chemical additions to juices and nectars, in fact PAT disappears rapidly at 25°C following the addition of 2% ascorbic acid or ascorbate/phosphate buffer. The addition of the thiamine hydrochloride, pyrodoxine hydrochloride and calcium pantothenate also can cause significant reduction of PAT levels and all these compounds are often added to apple juice during the industrial processing to improve their salutistic and nutritional quality. Also the sulfur dioxide if added to final product at level of 100 mg/L can reduce the level of the PAT of 50%. Interestingly, PAT is decomposed by the free radicals generated from the oxidation of dehydroascorbic acid, and, when all of the ascorbic acid is oxidized, is not possible a further PAT degradation.

Considering the low quantity of oxygen present in the headspace of juice packages resulting from industrial manifacturing, ascorbic acid addition prior to packaging is not a good strategy to reduce the level of the PAT in the juices.

A method based on UV radiations has been evaluated. This method can be easily implementable, is high-throughput and cost-effective. It offers simultaneous UV pasteurization of juices and reduction of PAT without severe alterations in the final product [89].

Recently Qingfang et al. studied the impact of exposure to germicidal UV radiation fresh apple cider [90]. UV exposure of 14.2 to 99.4 mJ/cm^2 resulted in a linear decrease of PAT levels by 9.4 up to 43.4%. The intrinsic advantage observed in this method resides in no correlation with any changes in the chemical composition or organoleptic properties. In apple puree naturally contaminated with 29 µg/kg of PAT, exposure to a pulsed light dose of 12 J/cm^2 provoked a 51% reduction in PAT concentration, while no residual contamination was detected for higher irradiation times [91].

Fumonisins

Fumonisins (FBs) are a group of secondary metabolites produced by micro fungi of the genus *Fusarium* and particularly by the *F. verticillioides* that is associated with maize and foods and feeds based on maize [92]. Out of the more than 15 FBs isomers that have been described so far, fumonisins B1 (FB1), B2 (FB2), and B3 (FB3) are the most abundant and frequent in the analyzed samples. The FB1 was classified by the International Agency for Research on Cancer as a Group 2B carcinogen, i.e. possibly carcinogenic to humans [93].

The cleaning process can remove FBs from the kernel pericarp and from the cracked, broken and damaged grains reducing its content to less than half that of uncleaned maize [94]. The decontamination process begins during the germ separation, which is carried out since its high oil content can reduce the shelf-life of the finished products due to the oxidation reactions causing rancidity. An indirect consequence is that the germs carries an higher FBs content than the whole kernel [95]. The introduction of European Commission Regulation (EC) No 1126/2007 [96], has stressed the necessity of controlling the FBs content in the whole chain from the unprocessed grains to the processed maize-based foods. The main fractions

intended for human consumption are derived from the milling of the endosperm and Vanara et al. reported that the amount of FBs in a kernel is re-distributed over the different industrial processing derived products [97].

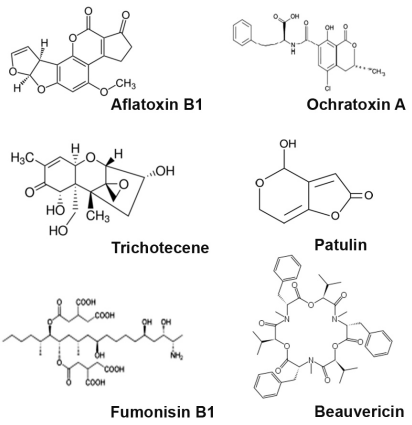

Figure 1: Chemical structure of relevant mycotoxins.

An inverse relationship between the particle size and the FB contents has been observed by th Authors when studying grain based meals. The toxin tends to concentrate in the bran and germ parts, while the endosperm is only partially contaminated. Van der Westhuizen et al. optimized the reduction of FBs in home-grown maize based on customary methods of a rural population under laboratory-controlled conditions [98].

The maize obtained from subsistence farmers was analyzed for the main FBs (FB1, FB2 and FB3) and in this experiment the hand-sorting of maize kernels was optimized by removing the visibly infected/damaged kernels. This approach reduced the average presence of FBs from 2.32 mg/kg to 0.68 mg/kg. The optimal reduction rate required however the hand washing of the selected maize kernels for a period of 10 min at 25°C.

The thermal processing below 150°C had little effect on the FBs concentrations, but extrusion, used extensively in the production of breakfast cereals and snack foods, substantially reduces the FBs levels, especially in presence of glucose [99]. Probably, the temperature adopted, the presence of reductive sugars as glucose, and the amino groups of the FBs are in favor to develop the ideal conditions to mask the FBs trough the Maillard reaction whose derivatives may clarify the FBs disappearance from the matrix.

De Girolamo et al. investigated the stability of FB1 and FB2 in the corn flakes industrial processing [100]. They have observed a reduction of the amount of the FBs from 60% to 70% during the whole process and only a 30% of the losses were attributed to the extrusion process, where the material was exposed to a temperature of 70 and up to 170°C for 2 to 5 min.

The process of nixtamalization removes almost all the FBs, as well as AFs, and as a result tortillas and maize based foods are substantially free from these mycotoxins [20].

Bullerman et al. studied the degradation of FB1 during the extrusion treatment: an uncontaminated corn grits, grits spiked with 30 µg/g FB1, and grits fermented with *Fusarium verticillioides* (40-50 µg/g FB1) were extruded in presence and in absence of glucose (10%, w/w) using a single-screw extruder [101]. The extrusion grits cooking caused significant reduction of FB1 in all treatments if compared with the not extruded used as control. The use of glucose resulted in a greater reduction (44.8–66.6%) compared to fructose (32.4–52.2%) or sucrose (26–42.7%) use.

Moreover a correlation between the reduction of level of FB1 in extruded grits and the speed of the screw has been observed in addition to the glucose concentration effect. In fact, the greater reduction of FB1, up to 92,7%, has been observed at the lower speed of the screw and when the higher concentration of glucose was used [102].

Another study reported that cooking extrusion and gelatinization in corn flakes may decrease FBs levels to 30–55%, while cooking the grits for flaking reduced the infection from 20% to 65%, while the roasting of the flakes reduced the FBs content from 6% to 35% [103]. Mohanlall et al. determined the thermo stability of FBs in contaminated maize, at different time/temperature combinations [104]. They used an initial FB1 concentration of 217 mg/g in the control, that was reduced to 184 mg/g after treatment at 100°C for 2 h. The temperature of oven was fixed at 220°C for 30 min and an extensive reduction of FB1 until to a concentration of 1.1 mg/g as compared to the concentration of 94 mg/g in the control sample was observed.

In muffins produced with maize spiked with 1.25 µg/g of FB1 and baked at 200°C for 20 min, was observed as result an average of FB1 loss of 70 %. Frying of maize chips spiked with 5 µg/g of FB1 at 190°C to 210°C for 5 to 10 min resulted in an average FB1 loss of 67% evidencing the effect of this cooking method on the mycotoxin level.

Beauvericin

Beauvericin (BEA) is an ionophore molecule with an uncommon chemical structure based on a cyclic oligomer depsipeptide which is able to transport the monovalent cations across membranes as a free carrier uncoupling oxidative phosphorylation [105].

In the same group having similar chemical structure it is be possible to include enniatins (ENs) that have same toxic potential. BEA is well known as a minor *Fusarium* produced mycotoxin and has a relevant toxicity on different biological systems such as bacteria and cells.

Juan et al. analyzed the natural presence of BEA and enniatins (ENs) in baby foods based on multicereals as main ingredient, which are available on the market from the great food distribution factories of Campania region (Italy) [106]. This surveillance study showed that the natural occurrence of BEA and ENs were below 68% and 74%, respectively. In a recent survey, the BEA was detected in the 1% of several categories of analyzed baby foods [107].

However, there is a deep lack in the scientific literature regarding the availability of data related to the thermal degradation of BEA during industrial production processes of foodstuff, while the surveillance data are growing. Some studies simulating the industrial conditions were carried out by Meca et al. [108] by investigating the BEA degradation at a 5 mg/kg concentration in a model solution considering different crispy breads produced with different flours typologies at three different temperatures, namely 160°C, 180°C and 200°C and five different incubation time: 3, 6, 10, 15 and 20 min.

The BEA concentration decreased in the experiment to 2.89 ± 0.13 mg/kg at 160°C for 3 min and it complete degradation was obtained at 200°C and 20 min of incubation time.

In the experiments carried out using the crispy breads, the percentage of the degradation of the BEA resulted variable from 20 to 90%.

The same authors reported a biotechnological approach based on bacteria that appear to be able to reduce the BEA concentration present in the medium [109]. In particular, part of the BEA was physically adsorbed by cell wall while the remaining part of the BEA was internalized in the cytoplasm. All the bacteria examined showed a significant ability to reduce the BEA during the fermentation process, in particular the average diminution varied from 66% to 83%. The influence of the fermentation processes and that of the formation of degradation byproducts of the BEA were evaluated by Meca et al. in beer and bread making processes [110]. In the first process, the degradation of the BEA ranged from 23 to the 82%, while during the bread making, the BEA reduction ranged from 75% to the 95%. Luciano et al. evaluated the reduction of the BEA at level of 25 mg/kg on kernels, added to a buffer solution of phosphate (PBS) at pH of 4, 7 and 10 after treatment with the phenyl isothiocyanate (PITC) and benzyl isothiocyanate (BITC) [111]. In solution, the BEA reduction ranged from 9% to 94% on a time-dependent fashion, and lower pH values resulted in higher BEA reduction. Cereal kernels and flours presented a BEA reduction from 9% to 97% and the data were dose-dependent. PITC caused the higher BEA reduction, and should be chosen as a fumigant to decrease the BEA levels in grains and flours.

Discussion and Conclusions

The mankind health risks associated with mycotoxin contaminated commodities can be reduced adopting an integrated prevention approach as well as a control management even if their complete elimination from food, without altering its nutritional and sensorial properties, is not possible at the moment. Table 1 reports relevant literature data relative to the reduction of mycotoxins through the most common post-harvest processing summarizing what has been discussed above. The most effective decontamination procedures include good on field cultural practices, the use of resistant crops, the biological control approach, the physical removal of damaged or incomplete kernels/seeds and the chemical inactivation e.g. nixtamalization. The cleaning removes broken and mouldy grain kernels, but it cannot ensure the complete risk elimination. The milling processes may dilute and distribute the mycotoxins into fractions that usually are addressed to become animal feed: this way the mycotoxins are eliminated from the direct human food chain.

Mycotoxin*	Process	Foodstuff	Conditions	Reduction (%)	Reference
AFB_1	roasting	peanuts coffee	90-150°C for 30-120 min 140°C for 40 min	17-63% 59%	[16,20]
	nixtamalization	tortillas	alkaline-cooking	51.7%-78.8%	[22]
	microwave-cooking and milling		presence of water and lime	84%	[24]
	fermentation	maize	solide state	93%	[25]
	extrusion	breakfast cereals and snacks	150°C	50-80%	[28]
	parboiling	rice	soaking for 4-5 h	82%	[32]
	gamma radiation	black pepper	0-60 kGy	43%	[34]
AFM_1	sterilization	milk	121°C for 15 min	12%	[37]
OTA	roasting	cocoa	110-140°C for 30 min	93.6%	[42]
			alkaline treatment	98%	[43]
		coffee	90-150°C for 2.5-10 min	69-96%	[44]
	cleaning and milling	white flour	room temperature	75%	[49]
	extrusion	breakfast cereals	150°C	40%	[46]
	baking	biscuits	200°C	66%	[46]
	Gamma radiation	Millet flour	1-3 kGy	13-44%	[52]
DON	Baking	wheat	190-230°C for 20-30 min	1.7-4.1%	[63]
	Boiling	pasta	100°C for 10 min	80%	[67]
				86-89%	[68]
		noodles	alkaline treatment	43.2-66.6%	[71]

	Frying	flour	169-242°C	38-66%	[72]
	Extrusion	maize	150-180°C	95%	[59]
ZEA	Extrusion	corn grits	120-160°C	77-83%	
PAT	Activated carbon	apple juice	40-60 mesh	98%	[80]
	Evaporation		70°-80°C for 20 min	9.4-14%	[80]
	Pasteurization		90-100°C for 20 min	19-26%	
	Thermal treatment		80°C for 20 min	62.62%	[88]
	Ascorbic acid addition		25°C	100%	[89]
	SO_2		SO2 level of 100 ppm	50%	
	Germicidal UV radiation	fresh apple cider	14.2-99.4 mJ/cm^2	9.4-43.4%	[90]
	Pulsed light	apple puree	12J/cm^2	51%	[91]
FB_1	Extrusion	corn flakes	70-170°C for 2-5 min	30%	[100]
		grits	in presence of glucose	44.8%-66.6%	[102]
	Baking	maize muffin	200°C for 20 min	70%	[104]
	Frying	maize chips	190-210°C for 5-10 min	67%	
BEA	Baking	Crispy breads	160-200°C for 3-20 min	20-90%	[108]
		Bread	200°C	75-95%	[109]
	Fermentation	Beer	4 days at 23°C in anaerobiosis	23-82%	
		Cereal kernels and flours	pH 4-10	9-97%	[111]

*AFB_1: Aflatoxin B_1; AFM_1: Aflatoxins M_1; OTA: Ochratoxin A; DON: Deoxynivalenol; ZEA: Zearalenone; PAT: Patulin; FB_1: Fumonisin B_1; BEA: Beauvericin.

Table 1: Reduction data for some relevant mycotoxins food contaminants obtained with widely adopted post-harvest processing.

One of the weak points of all treatments previously described is concerned with the efficiency of the different processing operations which depend on the mycotoxins structure, on the kind of matrices considered, and on the mycotoxins level in the starting material. Although most mycotoxins are moderately heat stable, a variable degree of reduction can be obtain with the use of high-temperature processing, but this parameter cannot be stressed too far. For example, fruits or other delicate foods which are sensitive to heat treatments cannot be treated with high temperatures. Furthermore, the level of degradation is highly linked to cooking conditions, to pH value, to the time of processing and to the use of additives and antioxidants. In general, the industrial food processing, combined with a postharvest control of the mycotoxins carried out through HACCP, can give the best efficacy in the amout reduction of the most dangerous ones. The best possible approach which is possible to advice as enucleated by the literature available data and by the industrial processing experience, is to share among all the actors of the food chain: farmers, food companies, carriers, cooks, consumers, the best on field protocols and the best storage and treatments of foods to obtain a shared information able to stimulate the best practices onset addressed to lower the possible contamination and reduce the risk of mycotoxin presence in foodstuff.

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