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A Review of Post-harvest Approaches to Reduce Fungal and Mycotoxin Contamination of Foods

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Abstract: Contamination of agricultural and food products by some fungi species that produce mycotoxins can result in unsafe food and feed. Mycotoxins have been demonstrated to have disease-causing activities, including carcinogenicity, immune-toxicity, teratogenicity, neurotoxicity, nephrotoxicity and hepatotoxicity. Most of mycotoxins are heat stable and cannot be easily destroyed by conventional thermal food processing or domestic cooking methods. Post-harvest approaches to prevent growth of mycotoxin-producing fungi and detoxify mycotoxins from contaminated food are important topics in food safety research. Physical, chemical and biological methods have been applied to prevent fungal growth or mycotoxin production, or to reduce mycotoxin content in the post-harvest period and contribute towards mitigating against the effects of mycotoxins on human health. This literature review aims to evaluate post-harvest approaches that have been applied to control both fungi growth and mycotoxin content in food and discuss their potential for upscaling to industrial scale.

Key words: Mycotoxin; Fungi; Contamination; Post-harvest; Food Safety; Anti-Fungal, Reduction, Prevention or Mitigation approaches.

Words: 13131

1 Introduction

Agricultural and food products can be contaminated by fungi, most particularly during the post-harvest period. Some fungi can produce toxic metabolites, named mycotoxins, which have a negative impact on the safety of food and feed. Dietary exposure to mycotoxins cause health issues due to their biological activities which include carcinogenicity, immune-toxicity, teratogenicity, neurotoxicity, nephrotoxicity and hepatotoxicity (Dalié, Deschamps, & Richard-Forget, 2010; Jard, Liboz, Mathieu, Guyonvarc'h, & Lebrihi, 2011). Some of these toxicities can be acute (WHO, 2015, 2017), resulting in illness or death within a few days of exposure to heavily contaminated food. Meanwhile, mycotoxins can have cumulative effects at lower doses, resulting in chronic health effects that manifest over several months or years (Tola, Kebede, & Yildiz, 2016).

More than 100 fungi species have been found to produce over 400 poisonous metabolites (Jard, Liboz, Mathieu, Guyonvarc'h, & Lebrihi, 2011). The most common agricultural mycotoxins comprise aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂ and AM₁), fumonisins (FB₁, FB₂), ochratoxin A (OTA), the trichothecene mycotoxins (type A: T-2 and HT-2, type B: deoxynivalenol (DON), nivalenol (NIV)), and zearalenone (ZEN), patulin (PAT) and egot

42 alkaloid. Minor mycotoxins include cyclopiazonic acid, sterigmatocystin, gliotoxin, citrinin
43 and citreoviridin. Mycotoxins are produced primarily by *Aspergillus* sp., *Penicillium* sp.,
44 *Fusarium* sp. and *Claviceps* sp. (CAST, 2003; Hathout & Aly, 2014; Petruzzi et al., 2014;
45 Schaarschmidt & Fauhl-Hassek, 2018).

46 Mycotoxin producing fungi are prevalent worldwide. According to recent report published in
47 2011, more than a quarter of the world's agricultural products are contaminated by
48 mycotoxins at levels above the European Union (Table 1) and *Codex Alimentarius* limits (Jard,
49 Liboz, Mathieu, Guyonvarc'h, & Lebrihi, 2011). Moreover, a more recent report indicates that
50 mycotoxins are detected in 60–80% of agricultural products. The increase is likely due to a
51 combination of the improved sensitivity of analytical methods and impact of climate change
52 (Eskola et al., 2019). Moreover, more than 50% of food products are showing co-occurrence
53 of more than one mycotoxin (BIOMIN, 2015). Mycotoxin contamination may occur during
54 pre- and/or post-harvest periods. The occurrence of mycotoxins in different crop products is
55 shown in Table 1. It appears that cereals (such as wheat, maize, rice, barley and sorghum) are
56 the most commonly contaminated products, although mycotoxins can also be found in animal
57 products (meat, eggs and milk), pulses, oilseeds, dry fruits and nuts. The most important
58 agricultural pathogens are *Aspergillus*, *Fusarium* and *Penicillium* sp. *Aspergillus* sp. exists in
59 warm (25 to 42°C) environments, which can be humid or dry (even down to -35 MPa water
60 potential). These conditions are common in soil, food storage areas and manufacturing
61 facilities (Klich, 2007; Tola, Kebede, & Yildiz, 2016). In *Aspergillus* sp., the production of
62 aflatoxins is related to spore production (Klich, 2007; Tola, Kebede, & Yildiz, 2016). In
63 temperate regions *Aspergillus* sp. also contributes to OTA production. *Penicillium* sp. can
64 produce ochratoxins at temperatures as low as 5°C (Tola, Kebede, & Yildiz, 2016).

65
66 As some mycotoxins are highly toxic, maximum limit (MLs) standards have been established
67 to protect the consumers' health. In the early 21st century, approximately 100 countries in the
68 world (covering about 85% of inhabitants) have set MLs to regulate the maximum amount of
69 mycotoxins permitted in human and animal feed (van Egmond, Schothorst, & Jonker, 2007).
70 The MLs of main mycotoxins set by the European Commission are shown in Table 1.
71 However, these limits exert an impact on the agricultural export market, where least
72 contaminated crops are exported to generate income, while more contaminated foods may be
73 traded in the producing country, especially in low income countries where regulations are
74 poorly enforced. As a result, it is critical for the food and economic security of low income
75 countries to reduce fungal and mycotoxin contamination of foods.

76
77 Because of the different distinct hazards caused by fungi (microbiological) and toxins
78 (chemical), the risk control strategies should be addressed simultaneously and where possible
79 synergistically. Prevention of fungal growth is usually considered as an early step during
80 production and post-harvest storage. If fungal growth cannot be avoided, approaches to de-
81 contaminate the food of the toxin through processing must be considered.

82 This is a comprehensive review of post-harvest approaches that have been applied to reduce
83 fungal growth and mycotoxin contamination in foods. The review includes a comparative
84 evaluation of the efficacy of different approaches, including physical, chemical, biological
85 and their combination, on fungal growth and mycotoxin content. The review discusses the

86 feasibility of these different approaches to be upscaled from laboratory to industrial scales
87 within different food systems.

88

89 **2 Control of fungal growth and prevention of mycotoxin production**

90 The most effective way to reduce the mycotoxins in the food chain is to prevent the fungus
91 growing in the first place, and if fungi do happen to be present in the food, then to prevent
92 the toxin from being produced. A range of physical, chemical and biological approaches have
93 been applied, both at industrial and laboratory scale.

94 **2.1 Physical approaches**

95 **2.1.1 Temperature and humidity control**

96 Storage of crops causes a mini ecosystem containing the biotic factors (crops, microorganisms,
97 etc.) and abiotic factors (water, air, temperature, etc.) suitable to fungal growth (W. X. Peng,
98 Marchal, & van der Poel, 2018). Similar to other living organisms, fungi require water and
99 an optimal temperature to survive and thrive. Moisture content and storage temperature can
100 be controlled to be outside of the microorganisms' optimum to reduce metabolic activity and
101 decreased growth. Moreover, water content and temperature are one of the easiest factors to
102 control during food storage, at both industrial and domestic scale. Although both relative
103 humidity (RH) and moisture content (MC) are used to reflect the water content of food, it is
104 better to use equilibrium RH, because the impact of equilibrium RH on spoilage organisms is
105 consistent across different foods, regardless of their composition (Bradford et al., 2018).
106 When the equilibrium RH is below 65%, microorganisms stop growing, meaning food is safe
107 for at least one year of storage at ambient temperature. Storage temperature can also
108 contribute to crop longevity (Bradford et al., 2018). In stored rice, both raising temperature
109 (10 to 40°C) and RH (12 to 98%) significantly increased the growth of both *Aspergillus* sp.
110 and *Penicillium* sp. by about 4 to 6 log colony forming units per gram (CFU/g) from about
111 3.8 log CFU/g. According to the result of multiple linear regression analysis, changing one
112 unit of temperature resulted in stronger impacts on fungal populations than changing humidity
113 (Mannaa & Kim, 2018). Additionally, Choi et al. (2015) showed that both 21°C with 97%
114 humidity and 30°C with 85% humidity were associated with an increase of population of *A.*
115 *flavus* by about 3 log CFU/g and the production of aflatoxins during the 120-day storage
116 period, while when the rice was stored at 21°C with 85% humidity, *A. flavus* population could
117 be constant and no aflatoxins were produced. For *F. graminearum*, 97% humidity encouraged
118 fungal growth from 2.5 to 4 log CFU/g at 21°C. When the humidity was reduced to 85%, *F.*
119 *graminearum* and DON production could be controlled. Thus, dry (below 85% RH) and low
120 temperature (below 21°C) are good strategies for controlling fungal growth. However, these
121 conditions may be difficult to maintain in warm and humid countries where a refrigerated and
122 ventilated store may not be available. Moreover, vegetable foods tend to respire, causing
123 moisture and temperature rises during post-harvest storage, and thus the environmental
124 conditions must be regularly monitored.

125

126 **2.1.2 Modified atmosphere treatment**

127 Modified atmosphere (MA) approaches includes modification of the gas composition (e.g.
128 O₂, CO₂, N₂) considering temperature, RH, MC and competing microorganisms (Magan and
129 Olsen 2004), and is usually applied for fresh food preservation (Bouletis et al., 2016; de

130 Siqueira Mendes, Aguayo, de Oliveira Pessoa, Nastaro, & Kluge, 2019; Putnik et al., 2017).
131 Fungi have a different sensitivity to atmosphere compositions. In general, high CO₂ and low
132 O₂ content can contribute to inhibition of foodborne fungi. *P. roqueforti* and *A. flavus* could
133 not grow in both 40% and 60% CO₂ environments balanced with N₂ and less than 0.5% O₂,
134 but weakly grow (about 30mm in 30-day incubation) in 20% CO₂ (Taniwaki, Hocking, Pitt,
135 & Fleet, 2009). MA at a large scale can be expensive, and there may be issues in displacing
136 and replacing the gases during the storage period.

137 Modified atmosphere packaging (MAP) is a strategy that controls gas composition
138 immediately surrounding the food within gas-impermeable packaging. Wheat and rye bread
139 artificially inoculated with several fungi were packaged with 0%, 50%, 75% or 100% CO₂,
140 1%, 0.03% O₂ or in the presence of O₂-absorber, and balanced with N₂. Notably, the gas
141 composition would be changing during the storage of the bread. MAP was more effective
142 against fungal growth on rye bread, as fewer fungi grew with the increase of CO₂. But for *P.*
143 *roqueforti*, this main contaminant of rye bread was inhibited only in the presence of O₂-
144 absorber. For wheat bread, the most resistant to CO₂ was *P. commune* which could grow in
145 99% CO₂. *A. flavus* grew in the lowest O₂ concentration and 75% CO₂ (Suhr & Nielsen, 2006).
146 So far, MAP has become a widely used way of food preservation because of its efficiency,
147 convenience and safety. It is cheaper and easier than large scale MA as it is only necessary to
148 fill the packaging with modified gas. MAP requires suitable packaging which is generally
149 plastic-based, and will inevitably cause usage and disposal of a large number of plastic
150 packaging.

151 **2.1.3 Irradiation treatment**

152 Irradiation of food for safety is based on the utilization of ionizing energy to inactivate
153 microorganisms by changing their cellular structure or physiological functions, including
154 DNA strand breakage, cell membrane rupture/leakage, or mechanical damage of cell walls
155 (Calado, Venâncio, & Abrunhosa, 2014). The effectiveness of the irradiation method depends
156 on many factors, such as irradiation dose, the microbial attributes (e.g. morphological
157 structures, physiological stage) and the environmental condition of the irradiated materials
158 (e.g. temperature, pH) (Magan & Olsen, 2004).

159 Aziz, El-Far, Shahin, and Roushy (2007) treated wheat, maize and barley collected from
160 Cairo (Egypt) markets with gamma-irradiation and evaluated the occurrence of 4 *Fusariums*
161 strains and FB₁ production. At 5 kGy, both *Fusarium* sp. counts and FB₁ production on barley
162 samples were completely decontaminated. Same results could be observed on wheat and
163 maize under 7 kGy irradiation. When the dose was below 5 kGy for barley or 7 kGy for wheat
164 and maize, the growth of *Fusarium* sp. and production of FB₁ could be inhibited by up to 85%
165 and 97% respectively. Similar observations were later reported by Akueche et al. (2012) who
166 studied the effect of gamma-radiation treated on sesame grains sampled from Abuja (Nigeria)
167 markets. In this study, 135 fungal strains including *Aspergillus* sp., *Penicillium* sp., and
168 *Fusarium* sp. were isolated from non-irradiated sesame grains. But only 34 strains were found
169 on grains after 3 kGy gamma-irradiation, and no of fungal species was found on grains
170 irradiated between 6 to 15 kGy.

171 In an experiment aimed at preventing fungal infection in fruits and vegetables, fungi were
172 usually artificially inoculated on the fruits or vegetables, and then treated with various doses
173 of irradiation. In general, the results showed better fungal inhibition comparing to control

174 group with the increasing irradiation dose in peppers, oranges, broccoli, cabbage, tomato,
175 bean sprout and papaya (Bari et al., 2005; Cia, Pascholati, Benato, Camili, & Santos, 2007;
176 Jeong, Chu, Lee, Cho, & Park, 2016; Yoon et al., 2014). The irradiation could not only reduce
177 the fungi, but also affected the production of mycotoxins. For instance, the total fungi isolated
178 from packed hot peppers were 4.8×10^3 CFU/g, total *Aspergillus* count were 4.7×10^2 CFU/g
179 and aflatoxin level was 1.14 ppb on average. After 2, 4, 6 kGy irradiation treatment, over 90%
180 fungi could be reduced. But only a non-significant reduction of 6% on aflatoxin levels was
181 observed at 6 kGy gamma radiation (Iqbal, Amjad, Asi, & Arino, 2012). In addition, as the
182 fruits and vegetables tend to easily lose their sales value, the effect of irradiation treatment on
183 product qualities should be considered. In a study of Bari et al. (2005), appearance, texture,
184 color, taste and overall acceptability were used as sensory indicators to determine the quality
185 of broccoli, mungbean sprouts, cabbage and tomato in both untreated and treated groups. Of
186 all indicators, texture was the worst affected parameter after irradiation treatment in the four
187 tested vegetables, and the sensory evaluation of other indicators gradually got worse with
188 increasing dose (maximum 1.0 kGy). Despite this, with 1.0 kGy, less than 7 days storage was
189 acceptable for each vegetable at refrigeration temperature.

190 The primary advantages of irradiation are non-residual chemicals and high efficacy, so that it
191 can be considered as an environment friendly mycotoxin reduction approach. Nevertheless,
192 the nutrient loss, high costs and secondary products of uncertain safety in treatment are not
193 negligible as well (Calado, Venâncio, & Abrunhosa, 2014), added to the deterioration of
194 sensory quality that can be caused by irradiation.

195 **2.2 Chemical approaches**

196 **2.2.1 Control by chemical antifungal agents**

197 In general, many antifungal agents are low-molecular-weight organic acids and their salts
198 (Magan & Olsen, 2004), and some of them are applied as food additives (1333/2008, 2017).
199 Marín et al. (2000) revealed that *Penicillium* sp. had the highest sensitivity to both 0.5 and
200 1.0 g/kg propionates than *Aspergillus* sp. and *Fusarium* sp. at 25°C in culture medium. The
201 efficacy of propionates was higher at 7 days rather than at 14 to 21 days. In another study,
202 Valencia-Chamorro, Palou, Río, and Pérez-Gago (2008) screened 15 chemicals and their
203 mixtures in hydroxypropyl methylcellulose-lipid edible composite films on the effects of
204 fungal growth. Amongst the chemicals, sodium bicarbonate, potassium sorbate (2%), sodium
205 benzoate (2.5%), sodium salt of methyl paraben (1%/1.5%), sodium salt of ethyl paraben (1%)
206 and sodium salt of propyl paraben (1%) and the mixtures of potassium sorbate (1.5%) with
207 sodium propionate (0.5%), sodium benzoate (2%) with potassium sorbate (0.5%) and sodium
208 benzoate (2%) with sodium propionate (0.5%) displayed the inhibition on growth of *P.*
209 *digitatum* and *P. italicum* at all inoculation concentrations (10^3 , 10^4 and 10^5 spores/mL). The
210 inhibitory effects were dose dependent. Sodium salt of methyl paraben at the concentration
211 of 1.5% showed the best performance, while no synergistic effect could be found in the
212 mixtures of two antifungal agents. This edible coating displayed potential application
213 prospects. In a recent research, a novel material, zinc oxide slightly coated with silver
214 nanoparticles, was demonstrated to inhibit the growth of *A. niger* (Tornero et al., 2018).
215 Coating is one of the popular methods to preserve fresh fruits and vegetables. The coatings
216 inhibit respiration, delay softening and color changes via controlling the internal gas
217 composition and water vapor (Conforti & Totty, 2007; Mehvar, El Assi, Alsmairat, & Holley,

218 2014). When fungal inhibitors are added into the coatings, the counting of not only mycotoxin
219 producing fungi but also other spoilage microorganisms, can be significantly reduced. For
220 example, Salas-Méndez et al. (2019) compared antifungal effect of control group, edible
221 nanolaminate coating (synthesized by the aminolysis of polyethylene terephthalate) (NL) and
222 nanolaminate coating with added an extract from *Flourensia cernua*, a Mexican endemic
223 plant growing in arid and semiarid areas (NL+FcE). Fungi could be found from the beginning
224 of storage in control and NL group at 20°C, while NL+FcE coating could prevent the fungal
225 infection for 6 days. On the 15th day, the counting of fungi and yeasts in control group was
226 about 1000 times those in NL+FcE group, and was about 100 times those in NL group.
227 Nevertheless, in a report of Mehyar, El Assi, Alsmairat, and Holley (2014), the coating of
228 date palm cultivar with pea starch + carnauba wax and zein protein + carnauba wax could
229 only reduce fungi and yeast about 1 log CFU/g after 14 days, but the coatings lost their effect
230 in third week at 25°C. Antifungal agent treatment, with or without coating are low-cost and
231 easy-used control approaches, but the safety of the remaining fungicide residues in the treated
232 products is also a major concern and this has highlighted the necessity of using antimicrobial
233 compounds that are safe to humans and animals. Antifungal agents also tend to lose their
234 effectiveness over time, putting into question their application for large scale crop and food
235 storage.

236 **2.2.2 Photodynamic treatment**

237 Photodynamic treatment is a method that utilizes the interaction of a non-toxic photosensitizer
238 and a particular wavelength of visible light (Al-Asmari, Mereddy, & Sultanbawa, 2018). This
239 approach is mainly used in oncology, ophthalmology and dermatology (Preuß et al., 2014).
240 In recent years, the photodynamic treatment has been investigated for its antimicrobial
241 properties, as the photosensitizer, induced by light of specific wavelength, generates cytotoxic
242 substances that cause biochemical and functional disturbances of the cell membrane
243 component and leads the damage to microbial cells (Al-Asmari, Mereddy, & Sultanbawa,
244 2018; Temba, Fletcher, Fox, Harvey, & Sultanbawa, 2016). Curcumin is one of the most
245 common photosensitizers in photodynamic studies. In a study by Temba, Fletcher, Fox,
246 Harvey, and Sultanbawa (2016), about three log magnitudes of *A. flavus* spores counts were
247 reduced by 84 J/cm² irradiation at 420 nm with both 15 and 20 µM of curcumin. When 5 log
248 CFU/mL of spores were spiked into whole maize kernels, 1.9 log CFU/mL of spores were
249 decreased at 60 J/cm² light with both 25 and 45 µM of curcumin, while 2.8 log CFU/mL of
250 spores were reduced in milled kernels under same conditions. In another study by Temba et
251 al. (2019), the effect of pH and temperature on *A. flavus* elimination under the reaction
252 condition of 100 µM curcumin stock solution with irradiation at 420nm wavelength at
253 60J/cm² was investigated. Compared to the non-illuminated group, the *A. flavus* spores in the
254 illuminated group were about two magnitudes lower at pH from 1.5 to 9, and showed a sharp
255 decrease at pH in both groups. Similar pattern could be found on hyphae reduction. In the
256 temperature-depending assay, although the counts in non-illuminated group were still higher
257 than those in illuminated group, temperature (from 15 to 45°C) did not have significant
258 influence on *A. flavus* spores and hyphae. In addition, about 66.7% of produced AFB₁ was
259 not detected under the light treatment with curcumin stock solution. Njoki, Okoth, and
260 Wachira (2017) reported 6 plants extracts (*Solanum aculeastrum*, *Syzygium cordatum*, *Prunus*
261 *africana*, *Ocimum lamiifolium*, *Lippia kituiensis*, and *Spinacia oleracea*) could inhibit the

262 growth of colony of *A. flavus* 4 to 47 mm (up to 42%) at concentration of 450 and 600 mg/mL.
263 However, when *A. flavus* were treated with the increasing treatment dose and time of visible
264 light (420 nm), the fungi were inhibited up to 95% at same concentration of plants extracts.
265 Preuß et al. (2014) synthesized new photosensitizers and observed the prevention of growth
266 of *A. niger* and *P. purpurgenum*. Besides, the new synthesized photosensitizer inactivated
267 germination of conidia. As a novel method, the photodynamic treatment shows potential to
268 control mycotoxin producing fungi. However, current studies mainly focus on fundamental
269 research at laboratory scale, while the future research could consider the safety of
270 photosensitizers and photolysis products and the application of photodynamic treatment in
271 real and large scale food systems.

272 **2.2.3 Electrolyzed oxidizing water treatment**

273 Electrolyzed oxidizing water (EOW) is obtained from electrolyzed NaCl solution,
274 transforming water molecules and chloride ions into chlorine oxidants (Cl_2 , HOCl/ClO^-) that
275 are show antimicrobial properties. EWO contains two types of water: strongly acidic EOW
276 and neutral electrolyzed water (NEW). The antimicrobial effect mainly depends on the level
277 of $\cdot\text{OH}$. The radicals can break the normal morphological structure of spores, and is closely
278 related to the damage of conidium cell wall and membrane, which leads to the spores losing
279 their normal function. NEW is non-toxic and safe to humans, it can be applied to fungi
280 decontamination (Gómez-Espinosa et al., 2017; Guentzel, Lam, Callan, Emmons, & Dunham,
281 2010; Xiong, Liu, Liu, & Li, 2010). Okull and Laborde (2004) used EOW to inactivate 1 to
282 4 magnitudes of *P. expansum* spores depending on concentration and exposure time. In an
283 apple infection test, the apples were inoculated with 10^6 CFU/mL of spores and treated with
284 50% and 100% EOW for 5min, and stored at 25°C for 6d. In non-treated apples, once wounds
285 were infected by spores, the decay was in evitable (100% incidence). But when treatments
286 were applied, decay in apple were only 18.4% for 50% EOW and 10.2% for 100% EOW.
287 Therefore, the use of EOW can be considered as a potential method in an apple cleaning
288 system. Xiong, Liu, Liu, and Li (2010) compared the elimination of *A. flavus* by both EOW
289 and acidic EOW. The results illustrated the population of spore survival treated by acidic
290 EOW was 5.77 log conidia/mL, which was 1.48 log conidia/mL less than control group, and
291 no spores could be found in EOW group. This because that EOW showed a stronger signal
292 on $\cdot\text{OH}$ level than acidic EOW. With the addition of mannitol (a radical scavenger) in reaction
293 system, the survival population was increased, which also provided a strong evidence
294 that $\cdot\text{OH}$ played the most important role in the inhibition of *A. flavus* spores.

295 **2.2.4 Plasma treatment**

296 Plasma is an ionized gas, with zero net electrical charge, that can be induced in any neutral
297 gas at particular pressure and temperatures conditions. Examples of natural plasma are sun
298 and polar gases, whereas artificial plasma include dielectric barrier discharges plasma,
299 microwave plasma, inductively coupled plasma, radio-frequency and commercial ozone
300 (Misra, Yadav, Roopesh, & Jo, 2019). These plasmas could inactivate a variety of mycotoxin
301 producing fungi on a range of foods, including fruits, vegetables, herbs, spices, cereals, nuts
302 and meat products in seconds. However, the effect of plasma on food quality depends on the
303 type of plasma, the duration of treatment and plasma intensity (Misra, Yadav, Roopesh, & Jo,
304 2019).

305 Among the cold plasmas, ozone is one of the best documented plasma on antifungal activity.

306 This strong oxidant can progressively oxidise unsaturated lipids in the microbial membrane
307 or cellular proteins, leading to a leakage or rapid death of the cell (Freitas-Silva & Venancio,
308 2010). In addition, ozone can reduce conidia germination (Savi, Bittencourt, et al., 2015). *A.*
309 *flavus* artificially spiked on wheat was reduced by up to 96.6% with the 60 µmol/mol O₃
310 gas treatment for 120min and 100% with the same concentration for 180min (Savi, Souza, et
311 al., 2015), *P. citrinum* behaved in a similar way, while *F. graminearum* was more sensitive to
312 the same concentration of O₃ gas, inhibited by up to 96.81% in 30min and completely
313 inhibited in 180min (Savi, Bittencourt, et al., 2015). Naturally occurring *Aspergillus* sp. and
314 *Penicillium* sp. on rice could be reduced up to 70% in short-time treatment (30min) at 10
315 mg/L O₃ gas (Beber-Rodrigues, Savi, & Scussel, 2015). Moreover, O₃ treatment can decrease
316 also mycotoxin production. In one of above studies, produced AFB₁ degraded 69.5% and 72.2%
317 exposed under 40 and 60 µmol/mol of O₃ gas respectively for 180min (Savi, Souza, et al.,
318 2015). Similar findings were reported by Savi, Piacentini, and Scussel (2015).
319 Although O₃ showed the highly efficient inhibition of fungi, the oxidation could still result in
320 some negative effects on food quality. Savi, Souza, et al. (2015) reported that wheat could
321 still germinate normally after 60 µmol/mol of O₃ gas treated for 120min. However, seed
322 germination of wheat, maize and paddy rice was significantly affected (up to 67%) when the
323 seeds were exposed under 4.8 mg/L for 12h (S. Wang, Liu, Lin, & Cao, 2010). For the
324 unmilled productions, ozone did not show a large impact on the total phenol content,
325 antioxidant capacity and odor, but the colour of some grains could fade to somewhat white
326 color (Santos Alexandre et al., 2018; S. Wang, Liu, Lin, & Cao, 2010). In contrast, for flour
327 products, ozonation resulted in the degradation of starch in whole wheat flour decreasing
328 viscosity and swelling capacity and increasing the pasting temperature (Alexandre, Castanha,
329 Calori-Domingues, & Augusto, 2017; Alexandre et al., 2019). The ozonation process also
330 contributed to the peroxide value, and accelerated the oxidation of unsaturated fatty acids
331 (Alexandre et al., 2019). Plasma treatment has good potential as a strategy to control fungal
332 growth and aflatoxin production, but more research is needed to understand undesirable
333 effects, including potential production of toxic compounds.

334

335 **2.3 Biological approaches**

336 **2.3.1 Inhibition by microorganisms and their metabolites**

337 In nature, fungi often share habitats with plants and with other microorganisms, resulting in
338 competition for space and nutrients. Therefore, fungal propagation would be weakened if
339 outcompeted by other microorganisms (Abbas, Zablutowicz, Bruns, & Abel, 2007; Appell,
340 Kendra, & Trucksess, 2009; Cavaglieri, Andres, Ibanez, & Etcheverry, 2005).

341 This natural competition phenomenon has been exploited by researchers to control fungi and
342 their toxins though the direct use of certain antagonist microorganisms as biocontrol agents
343 (BCAs) or the use of microbial metabolites. Biological control of mycotoxin-producing fungi
344 has been largely covered by several reports (Bhat, Rai, & Karim, 2010; de Medeiros et al.,
345 2012; Kagot, Okoth, De Boevre, & De Saeger, 2019; Kong, 2017; Mannaa & Kim, 2016). It
346 appears that biological control using microbial antagonists such as bacteria, fungi and yeasts
347 could be a feasible substitute to reduce the use of antifungal chemicals. Great successes in
348 reducing aflatoxin contamination in fields of different crops by 70% to 90% have been
349 achieved by application of atoxigenic strains of *Aspergillus*. For the biocontrol of *Fusarium*

350 and its associated fusariotoxins, species of *Trichoderma*, *Bacillus* and atoxigenic *Fusarium*
351 have being tested as the most promising candidates. However, questions remain about the
352 ability of the atoxigenic fungi to produce other mycotoxins, or to potentially exchange genetic
353 material and become aflatoxigenic. The low efficacy of many antagonists in the field
354 conditions, despite showing high potential in the lab is another concern. Overall, it is
355 suggested that integrated management approaches should be considered, involving a
356 combination of multiple BCAs, with reduced fungicide application, in conjunction with good
357 agricultural practices, and coupled with good postharvest management. In this section, we
358 focus on the inhibition of fungal growth and toxin production by microbial and plant
359 metabolites.

360 Fungal growth and toxin production may be affected by metabolites produced by other
361 microorganisms. Some proteins and peptides inhibit the growth of microorganisms and are
362 therefore termed as antifungal proteins (AFPs) and antimicrobial peptides (AMPs). AFPs
363 from molds show a high stability to pH and proteolysis and exhibit a broad inhibition
364 spectrum against filamentous fungi, and thus have prospects to control hazardous molds in
365 fermented foods. An AFP isolated from *P. chrysogenum* (PgAFP) at 4.9 µg/mL significantly
366 reduced the growth of *A. flavus* with over 50% inhibition rate (Delgado et al., 2015).

367 A compilation of the antifungal peptides produced by molds by Delgado, Owens, Doyle,
368 Asensio, and Nunez (2016) showed 16 compounds, produced by *Aspergillus*, *Penicillium*,
369 *Fusarium*, *Monascus*, and *Neosartorya* sp., with molecular weights (MW) between 5773 and
370 10 000 Da. A peptide (MW 2500Da) isolated from *Bacillus* strain B-TL2 had strong inhibitory
371 activity against mycelial growth of *A. niger*, as well as *Bipolaris maydis*, *Alternaria brassicae*,
372 and *Cercospora personata*. Moreover, this peptide showed thermostability, which means the
373 peptide could keep 100% activity at 100°C (B. B. Zhang, Xie, & Yang, 2008). In addition,
374 four AMPs, namely PPD1 (FRLHF), 66-10 (FRLKFH), 77-3 (FRLKFHF) and D4E1
375 (FKLRAKIKVRLRAKIKL) at concentrations between 1 to 40 µg/mL reduced the aflatoxin
376 production by *A. flavus* and *A. parasiticus* in a dose-dependent manner. At near minimum
377 inhibitory concentrations (MIC) the AMPs inhibited aflatoxins, without hindering the growth
378 of the fungi. An almost 99% inhibition of aflatoxins produced by *A. parasiticus*. *Parasiticus*
379 was observed. *Conidiation* of the fungi was also negatively influenced by the peptides (Devi
380 & Sashidhar, 2019). A peptide purified from *Lactobacillus plantarum* with amino acidic
381 sequence SGADTTFLTK reduced by 73% the growth of *A. parasiticus* in liquid medium after
382 48 h incubation (Luz, Saladino, Luciano, Mañes, & Meca, 2017). Similarly, three newly
383 identified peptides from *Bacillus megaterium* (L-Asp-L-Orn (D1O), L-Asp-L-Asn (D1N) and
384 L-Asp-L-Asp-L-Asn (D2N)) at concentrations above at 0.32 mg/well significantly inhibit the
385 growth of *A. flavus*, but without any effect on spore germination. At concentrations ranging
386 between 0.04 and 0.64 mg/mL, the reduction of AFB₁ by the peptides was from 70% to 80%
387 (Chen, Kong, & Liang, 2019).

388 Efforts are being made to elucidate the mechanism of inhibition of AMPs. A single peptide is
389 often capable of more than one mode of action, depending on the target cell type, and the
390 antifungal activities of peptides cannot be inferred from studies on their antibacterial activities.
391 AMPs usually act via membrane permeabilization, while antifungal activity for these peptides
392 is generally more complex and often involves entry of the peptide into the cell (van der
393 Weerden, Bleackley, & Anderson, 2013). As evidenced by confocal microscopy and

394 quantitative RT-PCR (qRT-PCR), three peptides from *B. megaterium* D1O, D1N and D2N
395 could spontaneously enter into the hyphae of *A. flavus* and inhibited conidiation and aflatoxin
396 production, but did not inhibit hyphae vegetative growth and spore germination (Chen, Kong,
397 & Liang, 2019). A more detailed mechanism was proposed by Devi and Sashidhar (2019),
398 which shows that the AMPs, at concentrations near MIC, induced membrane permeabilisation,
399 without inducing cellular leakage. The AMPs also show antioxidant properties which interact
400 with oxidative stress and impair aflatoxin production. At molecular level, the AMPs were
401 responsible of down regulation of the aflatoxin gene cluster '*aflR*' (a regulatory gene for
402 aflatoxin biosynthesis), and the expression of downstream genes. Similarly, a decrease in the
403 expression of manganese-superoxide dismutase (Mn-SOD) has been shown to be correlated
404 to aflatoxin synthesis, was obtained in peptide-treated samples.

405 During food fermentation, some non-peptides metabolites have been shown to have
406 antifungal activities. These compounds produced by lactic acid bacteria included organic
407 acids, phenol compounds, hydroxy fatty acids, hydrogen peroxide and reuterin (Dalié,
408 Deschamps, & Richard-Forget, 2010). For example, acetic and phenyl lactic acids produced
409 by *L. plantarum* CRL 778, *L. uteri* CRL 1100, and *L. brevis* CRL 772 and CRL 796 displayed
410 antifungal activity on *A. niger* (<40%), *Penicillium* sp. (40%-70%) and *F. graminearum*
411 (>70%) isolated from contaminated bread. The effect of organic acids depends not only on
412 the type of acid, but also on their concentration, the type of matrix, and pH of the matrix
413 (Gerez, Torino, Rollán, & Font de Valdez, 2009). Selected *Lactobacillus* sp. (*L. fermentum*
414 M107 and *L. fermentum* 223) and yeasts (*Hanseniaspora opuntiae* H17 and *Saccharomyces*
415 *cerevisiae* H290) were used for to inhibit the growth of *A. flavus* S075, *P. citrinum* S005 and
416 *Gibberella. moniliformis* S003 in cocoa bean fermentation. On average, *Lactobacillus* sp. (63%
417 and 75% respectively) showed higher inhibition ability than yeast (25% and 31%), when they
418 were cultural individually. Glucose, fructose, and citric acid in medium were converted to
419 mannitol, acetic acid and lactic acid by *Lactobacillus* sp., whereas the glucose and fructose
420 was metabolized to ethanol during culture. In the co-culture of *Lactobacillus* and yeasts, *A.*
421 *flavus* S075 was inhibited completely after 10 to 14 days (Romanens et al., 2019). The
422 antifungal interaction between fungi growth/mycotoxin production and lactic acid bacteria or
423 yeasts was summarized by Hassan, Zhou, and Bullerman (2015) and Bourdichon et al. (2012).
424 The application of AMPs and AFPs, as well as fermentation metabolites seems promising
425 strategies for fungal and mycotoxin control. Further research is needed to elucidate the
426 mechanism of action and potential negative effects of the microbes or microbial metabolites.

427

428 **2.3.2 Inhibition by plant extracts**

429 Higher plants can produce a number of secondary metabolites that display wide biochemical
430 and physiological functions (Prakash, Kedia, Mishra, & Dubey, 2015). A volatile substance
431 containing secondary metabolites, obtained from distillation of plants is called an essential
432 oil (EO). EOs have been used for antimicrobial and insecticidal applications in the
433 pharmaceutical, cosmetic, agricultural and food industries (Bakkali, Averbeck, Averbeck, &
434 Idaomar, 2008). The major compounds of EO are phenylpropanoids, phenolics, terpenoids,
435 steroids, aromatic and alkaloids, whose content determine the properties of the EOs (Bakkali,
436 Averbeck, Averbeck, & Idaomar, 2008; Prakash, Kedia, Mishra, & Dubey, 2015). The
437 composition of EOs is highly variable and, depending on plant species, modes of extraction

438 and storage conditions. Different parts of one plant or even the same plant harvested from
439 diverse regions or at different harvest time can vary in antifungal ability. Table 2 summarises
440 and compares studies that studied the inhibition of mycotoxin-producing fungi by EOs. EOs
441 from diverse plant species in eight countries showed inhibition effect from 58% to 100%
442 under different concentrations. Leaf and aerial parts were the most common organs for
443 extraction of EOs. As the EOs are at preventing fungi contamination, the safety assessment
444 of EOs should be an important concern. In a number of studies, high LD₅₀ values have been
445 recorded, such as 11 mL/kg for *Ocimum gratissimum* (Prakash et al., 2011), 4 mL/kg for
446 *Cinnamomum glaucescens* (Prakash, Singh, Yadav, Singh, & Dubey, 2013), 4.5 mL/kg for
447 *Ocimum sanctum* L. (A. Kumar, Shukla, Singh, & Dubey, 2010), and 9 mL/kg for *Caesulia*
448 *axillaris* roxb. (Mishra, Shukla, Singh, Prakash, & Dubey, 2012). Besides, the EOs of
449 cinnamon, clove, lemon grass, oregano, thyme, nutmeg, and basil are confirmed as safe in
450 America. In European countries, EOs components carvacrol, carvone, cinnamaldehyde, citral,
451 p-cymene, eugenol, limonene, menthol, linalool, vanillin, and thymol are registered as flavour
452 additives in foods (Prakash, Kedia, Mishra, & Dubey, 2015).

453 Apart from EOs, some plant AFPs and AMPs have also been identified (S. C. Park et al., 2017;
454 Subramanyam et al., 2012; D. J. Yun et al., 1998). These components comprise defensins,
455 lectins, chitinases, glucanases and other proteins obtained from seeds, bulbs, leaves, tubers,
456 fruits, shoots, and roots (Yan et al., 2015). Both low molecular weight proteins and high
457 molecular weight proteins could show fungal inhibition capability. For example, a 5.4 kDa
458 highly homologous plant defensins peptide purified from *Phaseolus vulgaris* L. impeded the
459 growth of *F. oxysporum* around paper discs containing this peptide (Chan & Ng, 2013). In the
460 same way, a designated Chitinase A (Chit A) and Chitinase B (Chit B) of 28 kDa purified
461 from maize seeds totally inhibited *F. oxysporum* (Huynh et al., 1992). A 35.7 kDa and 65 kDa
462 lectin from seeds of *Archidendron jiringa* and *Pachira aquatic* respectively showed effective
463 effect on the growth of *F. oxysporum* (Paiva, Vasconcelos, & Oliveira, 2014).

464 Plant antifungal metabolites are not limited to Eos, AFPs and AMPs. Polyphenols, flavonoids
465 in particular, are a group of plant secondary metabolites that play important role on fungal
466 defence (Bouarab-Chibane et al., 2019). The butanol extract and oxime derivative of fresh
467 peppermint (*Mentha piperita*), which is rich in flavonoid, was found to inhibit the growth of
468 *F. moniliforme* by (Ilboudo, Bonzi, Tapsoba, Somda, & Bonzi-Coulibaly, 2016). Butanol
469 extract and oxime derivative at 5 mg/mL caused about 52% and 70% inhibition respectively.
470 Conidial germination was delayed by the butanol extract by 1h compared with the control
471 group, and less than 10% of spores germinated in total. Similarly, the oxime derivative group
472 had less than 10% germinated spores at 2h, after which the quantity declined. In other studies,
473 high-carotenoid content in maize could lead to low fumonisins and aflatoxins production by
474 *Fusarium* sp. (Diaz-Gomez, Marin, Nogareda, Sanchis, & Ramos, 2016) or *Aspergillus* sp.
475 (Diaz-Gomez, Marin, Nogareda, Sanchis, & Ramos, 2016; Suwarno et al., 2019) respectively.
476 In a review of Atanasova-Penichon, Barreau, and Richard-Forget (2016), phenolic acids and
477 tocopherols were mentioned as similarly active compounds.

478 The diversity of plant metabolites make plants promising sources of novel antifungal agents.
479 Some of these can be extracted from agricultural by-products, making them potentially
480 economically interesting. However, the variation in their composition may cause
481 inconsistency in their performance. Purified compounds or synthetic mimetics could provide

482 more reproducible alternatives.

483

484 **2.4 Combined approaches**

485 Most approaches have limitations in terms of specificity to fungi and food matrices. For this
486 reason, combinations of approaches have been tested by researchers, to offer an integrated
487 management strategy that can target multiple microorganisms in various matrices.

488 The combination of MAP and antifungal additives can prolong the shelf life of food. For
489 instance, pre-treatment with 3% potassium sorbate and 20% of ethanol solution decreased the
490 incidence of molds and yeasts on table grapes by approximately 11%-30% higher than those
491 only packed in MAP conditions (O₂:CO₂:N₂-6:5:89) at 4°C until the end of shelf life.
492 Meanwhile, 3000 ppm of citrus extract only caused a 9% decrease in the same system
493 (Cristina, Annalisa, Amalia, Francesco, & Del Nobile, 2013). Similarly, the population of
494 yeasts and molds on ready-to-cook poultry treated with 1.5% chitosan and 0.2% thyme extract
495 under MAP conditions (30% CO₂ and 70% N₂) was 2.2 log CFU/g lower than that under the
496 MAP only when stored at 4°C for 14d. The antifungal effect of chitosan and thyme was
497 greater when used in combination compared to each individually (Gitrakou, Nizimani, &
498 Savvaidis, 2010).

499 Yoon et al. (2014) and Jeong, Chu, Lee, Cho, and Park (2016) used irradiation in combination
500 with the chemical sodium dichloroisocyanurate (NaDCC) to reduce the activity of grey mold
501 (*Botrytis cinerea*) and green mold (*P. digitatum*). Their results indicated that the decrease of
502 quantity of grey mold relied on the increase of radiation (from 0.2 to 4 kGy) and increase in
503 NaDCC (from 5 to 50 ppm) dose, but the great reduction (<5%) of green mold only occurred
504 under the treatment of 0.4 kGy of gamma irradiation and 6 or 10 ppm of the NaDCC.
505 Combining physical with chemical approaches appears to be effective at preventing fungal
506 growth. More research is needed to understand the combined effects on a wider range of
507 microbes and food matrices.

508

509 **3 Reduction of mycotoxin content in contaminated food**

510 The scale of food production makes control of fungal growth challenging. It is not always
511 possible to prevent fungal growth or mycotoxin contamination. Therefore, approaches to
512 remove mycotoxins from the edible part of the food must be considered.

513 **3.1 Physical detoxification approaches**

514 **3.1.1 Cleaning, dehulling and milling**

515 Sorting and cleaning are the most common and cost-effective mycotoxin removal processes.
516 Matumba, Van Poucke, Njumbe Ediage, Jacobs, and De Saeger (2015) investigated the effect
517 of sorting, flotation/washing or dehulling on the levels of 11 mycotoxins in white maize
518 grown in Malawi. In general, hand sorting showed the greatest reduction of mycotoxins (more
519 than 90%), followed by dehulling (more than 70%, except DON and AcDON). When the
520 procedures were combined, less than 5% of mycotoxins could be detected. Similarly, it is
521 reported that the level of aflatoxins, fumonisins, DON, NIV, and ZEN in washed food samples
522 was lower than in original samples (Matumba et al., 2017; Tibola, Fernandes, & Guarienti,
523 2016; van der Westhuizen et al., 2011). According to Tibola, Fernandes, and Guarienti (2016),
524 the lowest mycotoxin level could be obtained in milled flour products. Notably, the milling
525 could cause a redistribution of mycotoxins in milling fractions. In general, lower mycotoxin

526 content are found in flour and semolina while the higher mycotoxins content are found in
527 brans and flour shorts screenings These fractions were generally used for animal feeding
528 (Cheli, Pinotti, Rossi, & Dell'Orto, 2013). An educational intervention trained women in
529 Gambia to recognize and remove moldy groundnuts by hand sorting. The intervention
530 resulted in a reduction of 42.9% AFB₁ (based on median AFB₁ levels at baseline and after
531 hand sorting), and a reduction of 96.7% (based on the total AFB₁ in moldy and clean
532 groundnuts), with a loss of only 2% of the groundnuts. By roasting the already clean sorted
533 groundnuts, AFB₁ reduction of 39.3% was achieved (based on median levels) (Y. A. Xu et al.,
534 2017). Due to the low cost and easy operation of sorting and cleaning, these procedures can
535 be used not only before crop storage, but also during other processing operations and before
536 food consumption. However, it is still necessary to consider the disposal of sorted
537 contaminated seeds and waste water containing mycotoxin.

538 **3.1.2 Heat treatment**

539 The majority of mycotoxins are heat stable. Aflatoxins and OTA could be partially destroyed
540 at the temperatures around 250°C and 200°C respectively. The complete degradation of
541 fumonisins takes place at over 180°C (Magan & Olsen, 2004; Vidal, Sanchis, Ramos, &
542 Marin, 2015), and for DON degradation takes place at 210°C (Milani & Maleki, 2014). In
543 general, mycotoxin destruction is dependent on both the temperature and duration of exposure.
544 For example AFB₁ and AFB₂, degradation in pistachio nuts was proportional to both
545 temperature (90 to 150°C) and treatment time (30 to 120min) during roasting processing,
546 although this degradation was more affected by temperature than by time (Yazdanpanah,
547 Mohammadi, Abouhossain, & Cheraghali, 2005). In ground corn, Dupuy, Le Bars, Boudra,
548 and Le Bars (1993) found a linear relationship between calculated half-lives of FB₁ and
549 temperature, which were at 75, 100, 125 and 150, and for 8h, 175, 38 and 10 min respectively.
550 For more efficient reduction of mycotoxins during food processing, high temperature can be
551 combined with high pressure. Extrusion cooking is a food procedure that uses high
552 temperature and high pressure to process foods in a short time, which usually applied in
553 relatively dry viscous material (moisture contents around 20%), such as cereal grains, grits,
554 and flours (Castells, Marín, Sanchis, & Ramos, 2006). In general, initial moisture content of
555 food materials, extruded temperature, processing duration, screw speed and mycotoxins type
556 are the main variables influencing the reduced efficiency of mycotoxins. In rice meal,
557 aflatoxins were reduced by 51% to 95% during extrusion cooking. Broadly speaking, the
558 longer the processing duration, the higher the reduction of aflatoxins content. In rice, 170°C
559 was the best temperature among three temperatures (140, 170, 200°C) for reducing AFB₁ and
560 AFB₂, and no significant difference on AFG₁ reduction between 170°C and 200°C was
561 observed, while AFG₂ was most reduced at 200°C (Castells, Marín, Sanchis, & Ramos, 2006).
562 For OTA in oat flakes, according to a central composite design analysis, the highest reduction
563 of 28% could be obtained at 162°C, 30% moisture and 221 rpm of screw speed (Lee et al.,
564 2017). Pleadin et al. (2019) compared the effects of different thermal treatment on reduction
565 of DON and ZEN in cereals. The results showed the content of DON and ZEN only reduced
566 by 11% in 30min by cooking at 96°C, while the DON and ZEN declined up to 40% and 46%
567 when the roast temperature was increased to 220°C. The highest degradation of DON and
568 ZEN were 75% and 80% by extrusion cooking (135-190°C).

569 **3.1.3 Irradiation treatment**

570 Ionizing radiation can not only inhibit growth and development of fungi, but also reduce some
571 mycotoxins, and the effect is dose-dependent. In general, there is a positive correlation
572 between irradiation dose and reduction effect in the same matrix (Herzallah, Alshawabkeh,
573 & Fataftah, 2008; Jalili, Jinap, & Noranizan, 2012; Kumar, Kunwar, Gautam, & Sharma,
574 2012). Meanwhile, irradiation also shows diverse performance on mycotoxin detoxification
575 between irradiation types. Kumar, Kunwar, Gautam, and Sharma (2012) obtained 93% of
576 OTA reduction in aqueous coffee bean by gamma irradiation at 5 kGy. Herzallah,
577 Alshawabkeh, and Fataftah (2008) achieved the destruction of about 30% of total aflatoxin
578 and AFB₁ by microwave treatment at 2450 MHz and 1.45 kW for 10min. Sunlight (solar
579 irradiation) reduced more than 60% aflatoxins under 30h exposure and about 40% aflatoxins
580 under 3h exposure (Herzallah, Alshawabkeh, & Fataftah, 2008). Recently, electron beam
581 irradiation (EBI) has been used for decontamination of ZEN and OTA in maize kernel and
582 maize flour. At the dose of 50 kGy, the degradations of ZEN were approximately 60% and
583 71% for maize flour and maize kernel respectively, and those of OTA were about 60% and
584 73% respectively (Luo et al., 2017). PAT was successfully reduced using UV radiation. In a
585 study on PAT degradation in apple juice or apple cider using UVC wavelengths, Zhu,
586 Koutchma, Warriner, and Zhou (2014) found that UV exposure at 19.6, 84.3, 55.0, and 36.6
587 mJ/cm² resulted in 90% reduction of the toxin, with the order of efficiency of the three
588 wavelength lamps were: far UVC (222 nm) > far UVC plus (282 nm) > UVC (254 nm). A
589 non-significant increase in the L* (lightness) value and decreases in a* (redness) and b*
590 (yellowness) values of the juices treated with 222 nm were obtained. The treatment also
591 resulted in 36.5% loss of juice ascorbic acid. Assatarakul, Churey, Manns, and Worobo (2012)
592 reported a reduction of PAT from 5.14% to 72.57% with UV exposure, ranging from 14.2
593 mJ/cm² (one pass) to 99.4 mJ/cm², respectively, from an initial PAT contamination of 1,000
594 ppb. The UV treatment did not significantly change titratable acidity and ascorbic acid of the
595 juice, but there was modification of the pH, the degrees Brix and in the sensory perception
596 for the finished apple juice. In a similar study, Kim, Shukla, Oh, Chung, and Kim (2018)
597 observed that in PAT-spiked apple juice samples that were UV-irradiated at a range of 200–
598 280 nm for different time intervals, PAT levels reduced from 94.11 µg/L to 69.28, 54.55, and
599 5.92 µg/L after 5, 10, and 30min, respectively. After 30 min of UV exposure, PAT was not
600 detected in spiked apple juice samples. However, UV irradiation reduced the yellowness (b*)
601 of the apple juice.

602 The matrix is another factor to reflect different detoxification efficiencies. With gamma
603 radiation dose of 10 kGy, OTA in methanolic suspension demonstrated 24% lower reduction
604 than same concentration of the toxin in water. OTA powder the lowest reduction effect by
605 gamma radiation (Kumar, Kunwar, Gautam, & Sharma, 2012). At gamma irradiation dose of
606 1 kGy, compared to practical degradation in distilled water, the degradation rate of PAT in 1%
607 organic acid solutions (malic acid, citric acid, lactic acid, acetic acid), 1% amino acid
608 solutions (aspartic acid, serine, threonine and glutamic acid, histidine), ascorbic acid and
609 ethanol ranged from 31% to 98%. Therefore, in irradiated apple juice, 33% of PAT retention
610 was due to its main elements of organic acid (5.68% of malic acid) and amino acid (0.08% of
611 serine and 0.06% of threonine) (H. Yun et al., 2008). In another study, however, the
612 detoxification of ZEN between distilled water and all orange juice, pineapple juice and tomato
613 juice had no significant difference. While, the optimized model analysed by response surface

614 methodology (RSM) concluded that the determinant factors of detoxification was both
615 irradiation dose and ZEN concentration in fruits juices. It was noted that irradiation-mediated
616 detoxified ZEN showed lower toxicity than non-irradiated ZEN in cell line models.
617 Furthermore, to assess the quality of fruit juices, the sensory profile, total phenolic content,
618 total flavonoid content, total antioxidant activity and acidity were taken into account. In three
619 fruit juices, the values of every parameter slightly decreased with increasing irradiation dose
620 of 2.5, 5 and 7.5 kGy, while 10 kGy of irradiation had significant deterioration on quality
621 parameters. Overall, irradiation with certain dose range could be used to reduce toxin content
622 of fruits juices (Kalagatur, Kamasani, & Mudili, 2018).

623 When the irradiation is applied on foods, the primary reaction is the ionization of water, which
624 decomposes the water molecules into positively charged water radicals and negatively
625 charged free solvated electrons. Next, the water radical is split into hydroxyl radicals and
626 hydrogen ions. The reaction ends until forming the final products of hydrated electrons,
627 hydroxyl radicals, hydrogen ions, and hydrogen atoms. The radicals can be added into double
628 bonds of mycotoxins, such as aromatic rings, heterocyclic rings and lactone rings, which leads
629 to lower the mutagenicity and toxicity of mycotoxins (Di Stefano, Pitonzo, Cicero, & D'Oca,
630 2014; Jalili, Jinap, & Noranizan, 2012). Irradiation looks a promising approach to reduce
631 mycotoxins content in fruit juices. Other matrices, including dried products, need to be
632 considered and the degradation products assessed for their toxicity. Further development to
633 prevent quality deterioration as a result of irradiation is necessary.

634

635 **3.2 Chemical detoxification approaches**

636 **3.2.1 Adsorption by chemical adsorbents**

637 Some chemicals form weak interactions with mycotoxins due to their characteristics
638 including polarity, solubility, molecular size, shape, surface area and, in the case of ionized
639 compounds, charge distribution and dissociation constants (Jard, Liboz, Mathieu,
640 Guyonvarc'h, & Lebrihi, 2011; Sabater-Vilar, Malekinejad, Selman, van der Doelen, & Fink-
641 Gremmels, 2007; Sun, Song, Wang, Wang, & Zheng, 2018), causing adsorption between
642 adsorbents and mycotoxins. Hydrated sodium calcium aluminosilicates (HSCASs) are one of
643 the most popular clay-based adsorbents, obtained from natural aeolite (Şişman, 2006). The
644 adsorption of pyrophyllite-type HSCAS, usually occurs in either or both octahedral and
645 tetrahedral layers (the structure can be found in El Gaidoumi et al. (2019)) causing weak
646 bonds of exchangeable cations in interlayer positions (Aly, Abdel-Galil, & Abdel-Wahhab,
647 2004). Apart from HSCAS, there are many other adsorbents, displaying diverse adsorption
648 ability, shown in Table 3. These adsorbents included clay, activated charcoal, esterified
649 glucomannan, cholestyramine and other modified polymers, showing 17% to 100%
650 adsorption of AFB₁, FB₁, DON, ZEN, OTA and T-2 in liquid environments. There is no doubt
651 that adsorbent adsorption is one of the most economical methods in mycotoxin reduction.
652 Nevertheless, the safety of the adsorbent materials, removal from foods and disposal of
653 adsorption chemicals and adsorbent-mycotoxin complex are still under question. Some
654 chemical adsorbents have been forbidden as detoxification materials in food industry by the
655 European Union (Jard, Liboz, Mathieu, Guyonvarc'h, & Lebrihi, 2011).

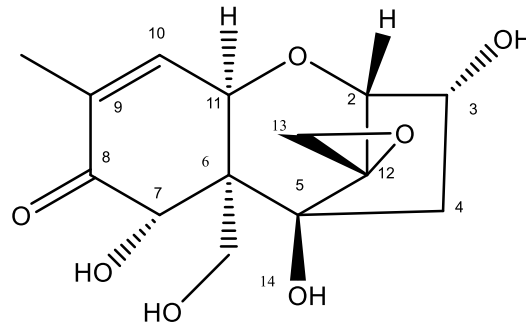
656 Chemical adsorbents find more practical applications in mycotoxins detoxification of animal
657 feeds. As a source of animal protein (milk, meat, eggs), the contaminated livestock products

658 can result in direct or indirect risk to human health (Halász, Lásztity, Abonyi, & Bata, 2009).
659 For example, AFB₁ can be converted into aflatoxin M₁ (AFM₁) in cattle bodies, which is
660 secreted in milk and consumed by humans, especially children (W. X. Peng, Marchal, & van
661 der Poel, 2018). Adding additives into fodder is a low-cost and user-friendly detoxification
662 method in animal feeding, and has been used in practical production. These additives mainly
663 include aluminosilicate clays (with or without organic acid) and montmorillonite (Table 3),
664 which are not always appropriate for human foods (Kolossova & Stroka, 2012). Although
665 some in vivo studies have shown that the feeding additives decreased the impact of
666 mycotoxins on growth and did not increase the toxicity in animals, adding additives could
667 still cause the loss of essential nutrients and decline in growth performance to some extent.
668 Thus, additives are not recommended for extensive use (Kolossova & Stroka, 2012).

669 **3.2.2 Alkaline/acid treatment**

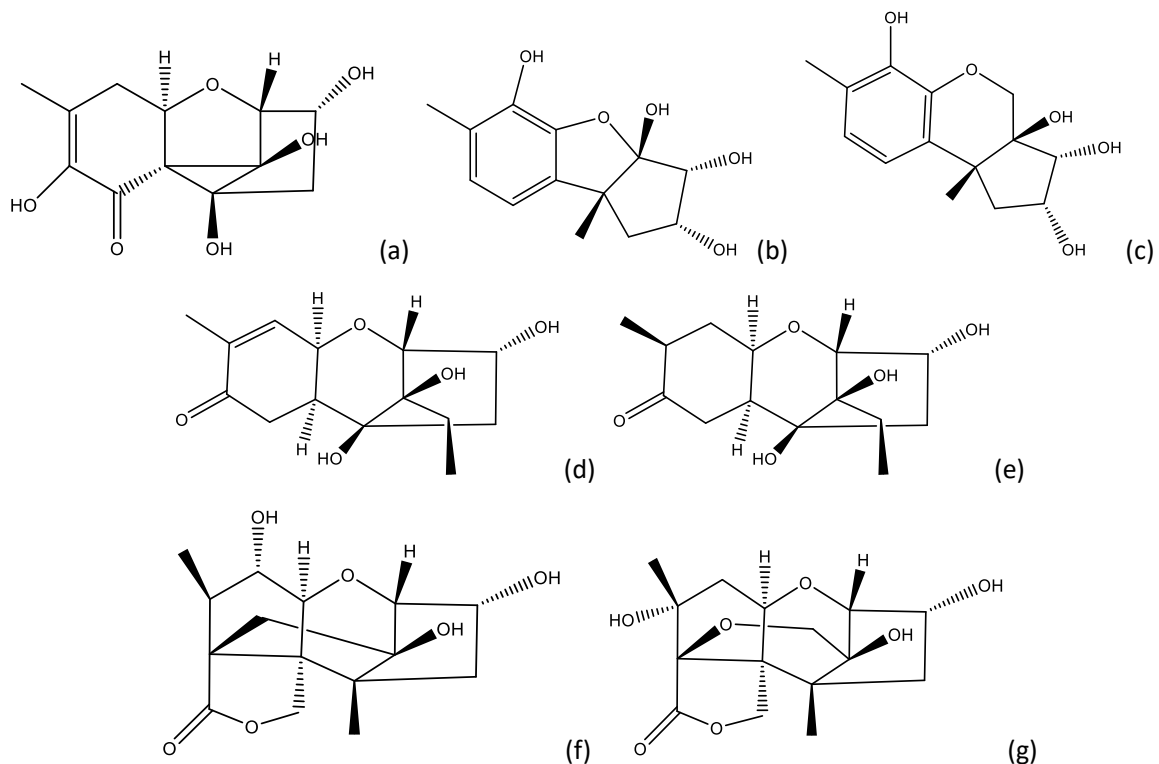
670 Many major toxins are unstable in alkaline environments. Researchers have worked on the
671 effect of common alkaline reagents on mycotoxin reduction. Ca(OH)₂, NaOH, KOH and
672 NaCO₃ were found to reduce DON, ZEN, aflatoxins, OTA (Jalili, Jinap, & Son, 2011). In
673 alkali treatment, ammoniation is one of the best documented methods of reducing toxins. So
674 far, it has been demonstrated that ammonia could reduce almost all aflatoxins (Brekke et al.,
675 1977), including 45% of FB₁ (Norred, Voss, Bacon, & Riley, 1991) and 64% of ZEN (Bennett,
676 Shotwell, & Hesseltine, 1980). This treatment was more widely used in animal feeding from
677 last century. In the 1970s, 1.5% ammonium hydroxide was added into aflatoxin-contaminated
678 maize basal diet of rainbow trout, which caused the detoxification of aflatoxin in diet and
679 decreased of hepatocarcinogenicity in rainbow trout (Brekke et al., 1977). Later, in the 1990s,
680 Bailey, Price, and Hendricks (1994) reported that ammoniated aflatoxin-contaminated
681 cottonseed, a kind of cattle feedstock, led to a 94% reduction in the content of AFB₁. When
682 the rainbow trout (*Oncorhynchus mykiss*) ate the dried milk from the cattle fed by treated
683 cottonseed meals, the incidence of hepatic tumors decreased by around 40%. Into the 21st
684 century, ammonia vapor was used in decontamination of broiler chick diet. Broilers fed diets
685 containing aflatoxin showed the high mortality rate (about 30% in 6 weeks) during the rearing
686 period. Chicks fed ammonia-treated maize did not show significant differences on mortality
687 rate, dietary intake, body weight gain, and feed conversion ratio of chicks (Allameh et al.,
688 2005). Ammonia treatment did not significantly affect the detoxification of FB₁ in maize meal
689 under air condition (Norred, Voss, Bacon, & Riley, 1991). This may be because ammonia
690 could directly attack the lactone ring of aflatoxins and retain the difuran moiety, but had no
691 direct reaction sites in FB₁ (Karlovsy et al., 2016; Norred, Voss, Bacon, & Riley, 1991;
692 Temba et al., 2016). Furthermore, DON (Fig. 1) has been found to be mainly degraded to
693 norDON A, norDON B, and norDON C (Fig. 2) in alkaline environments. These degraded
694 compounds could be isolated from NaOH solution (75°C, 60min) and other processed
695 samples, and have been shown to be less toxic than original DON. Other 4 new compounds,
696 norDON D, norDON E, norDON F and 9-hydroxymethyl DON lactone (Fig. 2) were
697 identified as degradation compounds (Bretz, Beyer, Cramer, Knecht, & Humpf, 2006). In
698 many reports, alkaline ammonia treatment was mainly reported in the 1990s, and primarily
699 used in animal feeding. This might be because the safety and applicability of alkaline
700 ammonia treatment could not completely used in the food industry. However, in recent reports,
701 baking soda has been shown to reduce the content of OTA in cereal-based foods. In a 85°C

702 direct steam injected process that exposes food to high temperature with high steam pressure,
 703 19.8% of OTA in oat-based infant cereals was lost. In contrast, OTA reduced by 36.1% and
 704 43.4% when 0.5% and 1% baking soda was added respectively (Lee, Gu, Ganjyal, & Ryu,
 705 2019). Peng reported that a small decline of OTA (6.73% to 9.63%) occurred in Chinese fried
 706 bread sticks containing 0.4% soda during processing (C. H. Peng et al., 2015).



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Fig. 1 Structure formula of DON (Wu, Kuca, Humpf, Klimova, & Cramer, 2017)



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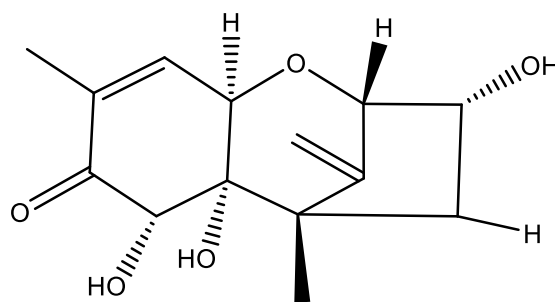
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Fig. 2 Structure formula of norDON A (a), norDON B (b), norDON C (c), norDON D (d), norDON E (e),
 714 norDON F (f), and 9-hydroxymethyl DON lactone (g) (Bretz, Beyer, Cramer, Knecht, & Humpf, 2006)

715

716 Although the majority of mycotoxins are resistant to weak acids (Karlovsky et al., 2016),
 717 some acids also influence the presence of mycotoxins. Sulfuric acid, chloridric acid,
 718 phosphoric acid, benzoic acid, citric acid and acetic acid all displayed less than 30% reduction
 719 of AFB₁, AFB₂, AFG₁, AFG₂ and OTA in black and white pepper during washing, which was
 720 generally less effective than that in alkaline solutions (Jalili, Jinap, & Son, 2011). In another
 721 study, these five toxins were treated by 2% sodium hydrosulphite (Na₂S₂O₄) with atmospheric
 722 pressure (low pressure) and 100°C for 30min or 1.5bar (high pressure) and 121°C for 15min.

723 Except for AFB₂, other four samples under low pressure lost 64.8% to 83% of the toxin, and
724 those under high pressure lost more than 96% (Jalili & Jinap, 2012). The use of 5% of both
725 citric acid and lactic acid reduced DON about 20% to 40% in feeds soaked for more than 5h.
726 Lactic acid showed the better performance than citric acid in this treatment (Humer et al.,
727 2016). The detoxification of DON is considered to be due to the opening of the C12, 13-epoxy
728 group (Fig. 1). In the extreme acidic environment (pH 1 to 2), DOM-1 (Fig. 3) might be
729 degraded from DON (Wu, Kuca, Humpf, Klimova, & Cramer, 2017).
730 Sometimes, a combination of chemicals can reduce the level of mycotoxins. In the report of
731 Rempe, Brezina, Kersten, and Danicke (2013), a mixture of methylamine, Ca(OH)₂ and
732 sodium metabisulphite (2+4+1) caused the recession of 91% of DON and 79% of ZEN in
733 naturally contaminated maize at 80°C. However, there is little evidence to show the safety of
734 new derivatives produced from mycotoxins after treatment.



735

736

Fig. 3 Structure formula of DOM-1 (Wu, Kuca, Humpf, Klimova, & Cramer, 2017)

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3.2.3 Plasma treatment

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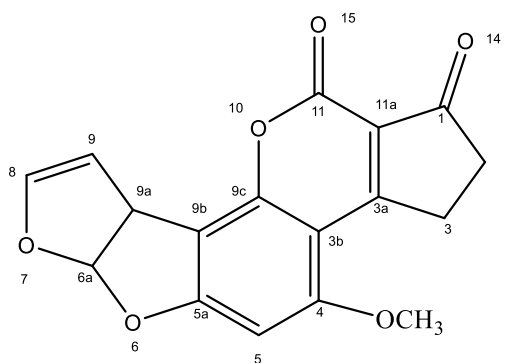
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Plasma is an ionized gas, with zero net electrical charge, that can be induced in any neutral gas, and able to induce by innovative physical equipment at pressure and temperatures conditions (Misra, Yadav, Roopesh, & Jo, 2019). Many studies have suggested the mycotoxin detoxification effect of plasma. For instance, the effect of cold atmospheric plasma on aflatoxin contamination in both solution matrix (liquid) and hazelnuts matrix (solid) was evaluated by Siciliano et al. (2016). In this study, gas composition (proportion of N₂ and O₂), power of the generator, exposure time and reaction matrix were factors tested on detoxification efficiency. Among them, the power and exposure time were inversely proportional to aflatoxin loss. Besides, gas mixture with more N₂ and liquid matrices were more conducting to aflatoxin degradation. When AFB₁, DON and NIV were exposed to self-designed microwave-induced argon plasma system, the decrease of these toxins was significantly time-dependent, with complete degradation in 5s (B. J. Park et al., 2007). Ozone has the ability to degrade mycotoxins as well. 15min treatment by ozone on wheat bran contaminated with DON and ZEN caused approximately 29% and 52% degradation respectively, and no significantly difference in longer treatment times. Notably, ozonisation protected the quality of wheat bran at the greatest extent by preserving total phenolic compound content and antioxidant activity (Santos Alexandre et al., 2018). It was mentioned in the review of Misra, Yadav, Roopesh, and Jo (2019) that the plasma degrades mycotoxins by direct interaction of free radicals (e.g. O•, OH•) of plasma with the mycotoxin structure. With AFB₁ (Fig. 4), the degradation is through epoxidation and oxidation by introducing water molecule, hydrogen atom, aldehyde group or hydroperoxyl radical (HO₂•) and leading the breakdown of C8 to C9 double bond of the dihydrofuran rings. Meanwhile, the toxicity and

760 carcinogenicity of AFB₁ would be reduced because of the loss of terminal furan ring.



761
762 **Fig. 4** Structure formula of AFB₁ (Luo et al., 2014)

763 Ozone was also used in the degradation of mycotoxins. For instance, highest reduction (48%
764 and 64.3% respectively) of total aflatoxins and DON in soft wheat grains occurred at a
765 concentration of 60mg/L for 300min (Trombete et al., 2017). ZEN in whole wheat powder
766 quickly reduced by 62.3% in first 20 min at the condition of 51 mg/L of ozone (Alexandre et
767 al., 2019). 15min-treatment by ozone on wheat bran contaminated with DON and ZEN caused
768 approximately 29% and 52% degradation respectively, and no significantly difference in the
769 longer treatment times. Notably, ozonisation protected the quality of wheat bran by keeping
770 total phenolic compound content and antioxidant activity (Santos Alexandre et al., 2018). The
771 effectiveness not only depends on exposure time and gas concentration, but also the physical
772 characteristic of samples, moisture content and processing method (Trombete et al., 2017). L.
773 Wang et al. (2016) showed that ozone treatment at same concentration was more effective in
774 the flour than whole wheat with increasing of ozone concentration (from 0 to 100 mg/L),
775 suggesting penetration into the kernels is not effective. Meanwhile, in the same study, higher
776 moisture content (20.1%) of both whole wheat flour and wheat kernels showed greater
777 degradation of DON (about 75% and 60% respectively), as high moisture content might
778 promote oxidation power of ozone and its penetration ability. Similar result could be found
779 in Alexandre, Castanha, Calori-Domingues, and Augusto (2017) study as well. S. Wang, Liu,
780 Lin, and Cao (2010). They compared the degradation of AFB₁ by a dry method which
781 involved delivering the O₃ gas to cereals, compared to an aqueous method which involved
782 soaking cereals into ozone solutions and a semi-wet method which involved pumping ozone-
783 rich steam into cereals. The results indicated that the ozonation reaction in aqueous or semi-
784 wet conditions showed better effect than the dry method. The most effective reduction method
785 for paddy rice and maize was semi-wet method, which reduced toxin content by about 92%
786 and 85% respectively, while the aqueous method displayed the best performance on AFB₁
787 degradation (about 93%) in wheat.

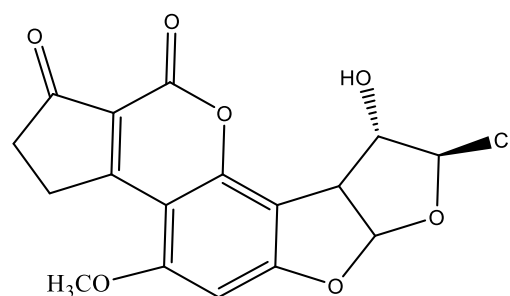
788 Ozone preferentially attacks the unsaturated compounds in an electrophilic attack mechanism
789 (Freitas-Silva & Venancio, 2010). The major mycotoxins including aflatoxins, FB₁, OTA,
790 ZEN, DON and PAT could be degraded rapidly, within minutes. After ozone treatment, none
791 of the by-products of OTA, ZEN and PAT could be detected by UV or fluorescence detector.
792 However, a larger fraction of polar compounds were formed from ozonized AFB₁, and FB₁.
793 For DON, ozone attacked at the C9 to C10 double bond (Fig. 1) with two additional atoms of
794 oxygen but kept the rest of molecule (McKenzie et al., 1997; Young, Zhu, & Zhou, 2006). It
795 was mentioned in the review of Misra, Yadav, Roopesh, and Jo (2019) that plasma interacts

796 with mycotoxins via free radicals (e.g. $O\cdot$, $OH\cdot$). With AFB₁ (Fig. 4) for example, the
797 degradation is through epoxidation and oxidation by introducing water molecule, hydrogen
798 atom, aldehyde group or hydroperoxyl radical ($HO_2\cdot$) and leading the breakdown of C8 to C9
799 double bond of the dihydrofuran rings. Meanwhile, the toxicity and carcinogenicity of AFB₁
800 would be reduced because of the loss of terminal furan ring. In bioassay, apart from FB₁, all
801 of treated aflatoxins, OTA, ZEN and PAT were not found to affect the activity of *Hydra*
802 *Attenuate*, but treated FB₁ still kept the toxicity (McKenzie et al., 1997). In the induced
803 toxicity assay of Caco-2 cells, ozone treatment weakened the cellular metabolic disorder by
804 DON derivatives, but no impact on latent inflammation and oxidative stress effects, which
805 shows some of the non-negligible toxicity of ozonised DON (Y. Xu et al., 2019). It is
806 noteworthy that low O₃ concentration (below 0.05 ppm) had an enjoyable odor, while, when
807 the concentration were above 0.05 ppm, O₃ affected human eyes and respiratory systems,
808 which might be related to premature death, heart attack, bronchitis, asthma, and other
809 cardiopulmonary problems (Jian, Jayas, & White, 2013). Therefore, when considering the
810 application of ozone in cereal storage, attention should be paid to the harm caused by ozone
811 to workers and the natural environment. Plasma, and in particular ozone, are effective at
812 reducing mycotoxin content in foods. However, the toxicity of degradation products and
813 impacts of ozone directly on human health need to be further considered.

814 **3.2.4 Neutral electrolyzed oxidizing water (EOW)**

815 Neutral electrolyzed oxidizing water is also an aflatoxin detoxifying substance. One view was
816 that aflatoxin detoxified by hypochlorous acid from EOW eliminated the toxicity of double
817 bond in the terminal furan ring and converted it to 8-chloro-9-hydroxy-aflatoxin B₁ (Fig. 5)
818 (Escobedo-González et al., 2016). The derivative was shown to have significantly lower
819 cytotoxicity and genotoxicity effects *in vitro* in a HepG2 cell model (Jardon-Xicotencatl,
820 Díaz-Torres, Marroquín-Cardona, Villarreal-Barajas, & Méndez-Albores, 2015; Sakudo,
821 Toyokawa, Misawa, & Imanishi, 2017). There was an ameliorative effect of EOW on the
822 health and performance of turkeys fed on de-contaminated feed (Gómez-Espinosa et al.,
823 2017).

824



825

826 **Fig. 5**Structure formula of 8-chloro-9-hydroxy-aflatoxin B₁ (Escobedo-González et al., 2016)

827 **3.3 Biological detoxification approaches**

828 **3.3.1 Metabolite degradation**

829 Biological enzymatic degradation reactions include acetylation, glucosylation, ring cleavage,
830 hydrolysis, deamination, and decarboxylation caused by extra - or intra-cellular enzymes
831 produced from bacteria and fungi (Hathout & Aly, 2014). In a report of Guan et al. (2008),
832 *Stenotrophomona smaltophilia* was isolated from a selective medium containing coumarin as

833 the only carbon source and displayed reducing ability towards AFB₁ (82.5%) at 37°C for 72h.
 834 After treatment of factors that could affect the enzymatic activity, reaction efficiency
 835 significantly drops, which indicated that reduced AFB₁ was produced by enzymatic
 836 degradation. Microbial species with similar functions were listed by Hathout and Aly (2014),
 837 including *Bacillus* sp., *Brevibacterium* sp., *Eubacterium* sp., *Flavobacterium aurantiacum*,
 838 *Mycobacterium fluoranthenivorans*, *Myxobacteria* sp., *Pseudomonas* sp., *Rhodococcus*
 839 *erythropolis*, *Trichosporon mycotoxinivorans*, *Aspergillus* sp., and *Rhizopus* sp.. The
 840 degraded toxins covered all major mycotoxins. Besides, some enzymes have been found in
 841 mushroom showing the detoxification ability. Manganese peroxidase (MnP) purified from the
 842 mushroom *Pleurotus ostreatus* detoxified AFB₁ by 6% at 0.1 U/mL enzyme activity for 8h,
 843 and by 90% at 1.5 U/mL enzyme activity for 48h (Sayed, 2014). In a review of Jard, Liboz,
 844 Mathieu, Guyonvarc'h, and Lebrihi (2011), the multiple degradation pathways of each major
 845 mycotoxin has been summarized. In simple terms, AFB₁ (Fig. 4) lactone ring or difuran ring
 846 could be opened resulting in loss of the toxicity. OTA was degraded to OT α and phenylalanine
 847 (Fig. 6); while ZEN could be transformed into oxidised, hydroxylated and methylated
 848 compounds, gluco- or sulfo-conjugates and hydrolysed compounds. Detoxification of DON
 849 was by opening the 12,13-epoxy ring (Fig. 1), and formed de-epoxidised DON and 3-keto-
 850 DON (Fig. 8). FB₁ has been found to be converted into polyolamine (Fig. 9) by extracellular
 851 carboxylesterase. Most degradation products showed no toxicity. However, the produced α -
 852 zearalanone (classified as hydroxyl compound) was more toxic than the original compound
 853 (ZEN) (Fig. 7).

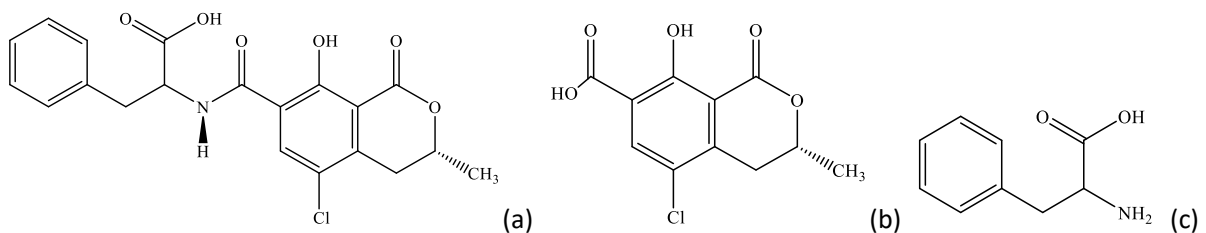
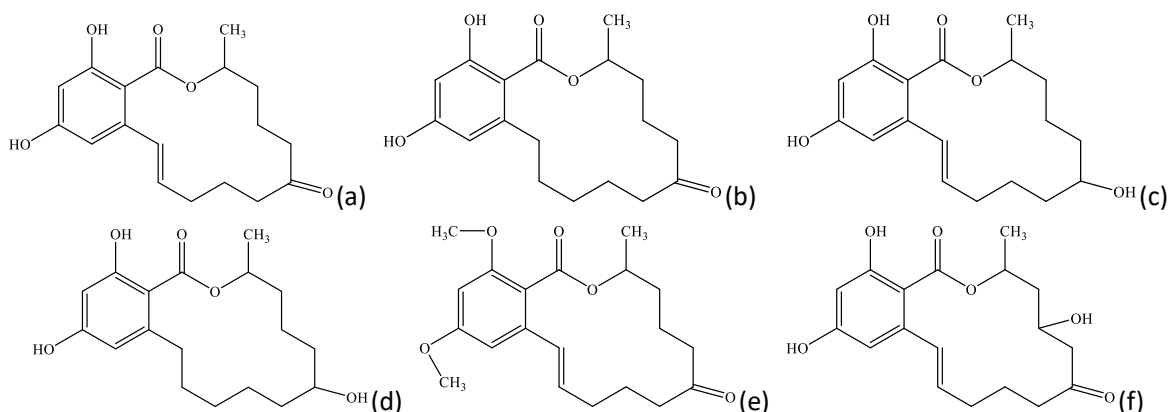
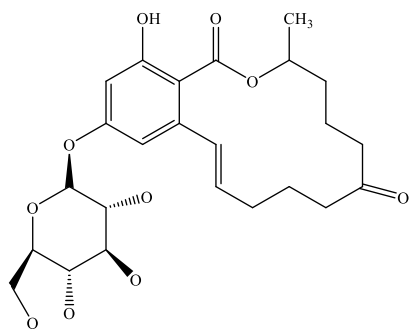


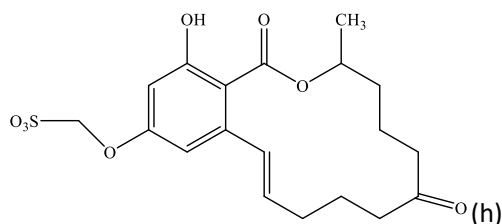
Fig. 6 Structure formula of OTA (a), OT α (b), and phenylalanine (c) (Jard, Liboz, Mathieu, Guyonvarc'h, & Lebrihi, 2011)



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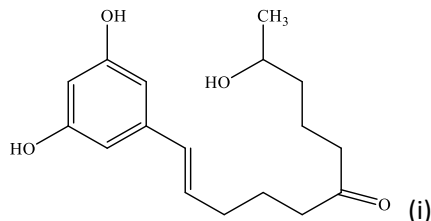


(g)

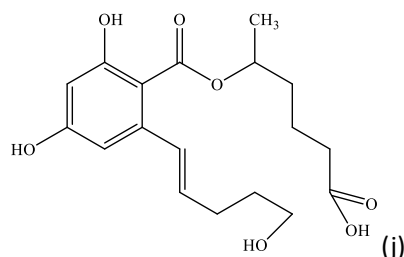


(h)

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(i)



(j)

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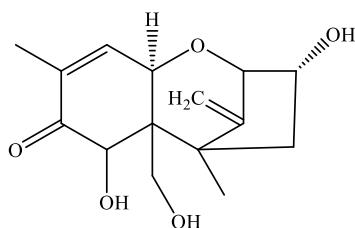
Fig. 7 Structure formula of ZEN (a), oxydised compounds: zearalanone (b), hydroxyleed and methyl compounds: a-β zearalenol (c), a-β zearalanol (d), methoxy-ZEN(e), hydroxy-ZEN(f), gluco- or sulfo-conjugates: ZEN-4- β-glucopyranoside (g), ZEN-4-sulfate (h), and hydrolysed compounds: decarboxylated ZEN (i), Hydroxylated ZEN(j) (Jard, Liboz, Mathieu, Guyonvarc'h, & Lebrihi, 2011)

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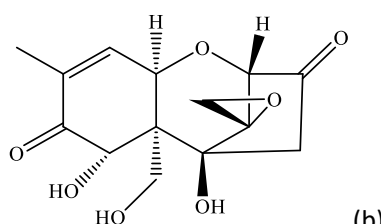
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(a)

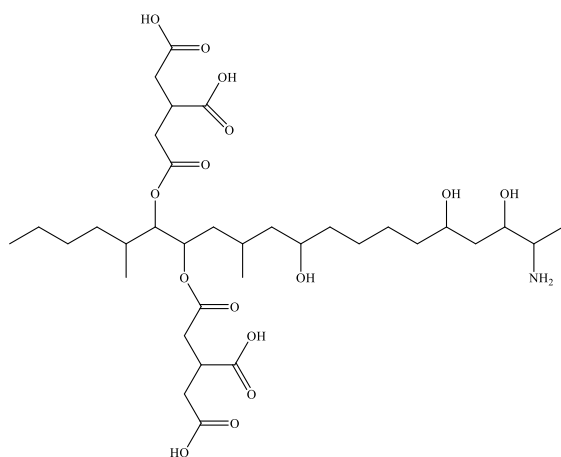


(b)

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Fig. 8 Structure formula of de-epoxy DON(a) andketonic compound(b) (Jard, Liboz, Mathieu, Guyonvarc'h, & Lebrihi, 2011)

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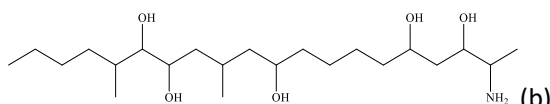
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(a)



(b)

Fig. 9 Structure formula of FB₁(a) andpolyolamine (b) (Jard, Liboz, Mathieu, Guyonvarc'h, & Lebrihi, 2011)

3.3.2 Adsorption by biological polymer

Microorganism could not only degrade mycotoxins, but also remove them by adsorption to their cell walls. Most gram-positive bacteria and yeasts have demonstrated the adsorption capability. Recent studies are presented in Table 4. It could be observed that microorganism could adsorb 20% to 90% mycotoxins in different liquid food system or even body

876 environment. It has been suggested that heat-treated microorganisms (inactive
877 microorganisms) showed similar or even higher mycotoxin adsorption capability when
878 compared to live microorganisms in aqueous solution (El-Nezami, Polychronaki, Salminen,
879 & Mykkanen, 2002; Turbic, Ahokas, & Haskard, 2002; Vosough, Sani, Mehraban, &
880 Karazhyan, 2014). It was suggested that the adsorption was through physical adsorption
881 rather than through a biological degradation mechanism. Thus, mycotoxins would not have
882 the chemical reaction with binder during adsorption. This interaction usually occurs with the
883 cell walls of microorganisms. For bacterial, cell walls or peptidoglycans (purified from cell
884 walls) were isolated from lactic acid bacteria (Sreekumar & Hosono, 1998; Zhao et al., 2013;
885 Zou et al., 2012), and were found to bind more toxins than cell pellets after removing the cell
886 walls. The peptidoglycan played an important role in adsorption, and chemical methods can
887 increase the number of adsorption sites and adsorption efficiency (e.g. acid-treatment, heat-
888 treatment) (Zou et al., 2012). Haskard, Binnion, and Ahokas (2000) suggested that the
889 addition of urea (an anti-hydrophobic agent) or organic solvent destroyed the cell wall-toxin
890 complex, proving hydrophobic effect between the adsorption. For yeast, there were two layers
891 in its cell wall, an inner layer of β -1,3-glucan and chitin and an outer layer of β -1,6-glucan
892 with heavily glycosylated mannoproteins (Petruzzi et al., 2014). At the pH range of wine,
893 mannoproteins had negative charges, and OTA carried a positive charge of the amine function
894 (NH_3^+), so that cell wall and toxin could partially establish electrostatic and ionic interactions.
895 Moreover, as a less polar mycotoxin, OTA could bind with hydrophobic surfaces of yeast cell
896 wall through the phenol group and via interactions of two- π -electron orbital (Caridi, Galvano,
897 Tafuri, & Ritieni, 2006). However, the adsorption was relatively weak, because toxin-microbe
898 complexes would release toxins about 25-40% after washing with PBS buffer (Fernandez Juri,
899 Dalcero, & Magnoli, 2015; Zou et al., 2012). This might indicate that the adsorption hardly
900 occurs in nonpolar circumstances.

901 Most microorganisms with adsorption property belong to fermentation microorganisms, thus
902 biological adsorption usually occurs in the process of fermentation in practical production.
903 Food fermentation is a process of decomposing carbohydrates to alcohol or organic acids by
904 microorganisms in aerobic or anaerobic environments, used in the production of fermented
905 dairy products, wine, vinegar and bread-making. The raw food materials that are commonly
906 used for fermentation cover most food groups including dairy, meat, fish, vegetables, fruits,
907 legumes and cereals (Bourdichon et al., 2012), which could be contaminated by
908 mycotoxigenic fungi or metabolic mycotoxins. Therefore, the mycotoxins are generally
909 present in the fermentation process. The adsorption of mycotoxins by microorganisms during
910 fermentation was summarized in Table 4.

911 Recently, more attention has been focused on the animal polysaccharides. Chitin, from shrimp
912 shells, was investigated for its ability to bind with AFM_1 . Assaf, El Khoury, Atoui, Louka,
913 and Chokr (2018) demonstrated that chitin bound to 17% to 54% AFM_1 in PBS buffer,
914 depending on concentration of both chitin and toxin and incubation time. High adsorption
915 efficiency relied on high chitin concentration and long incubation. 0.15 g/mL of ground
916 shrimp shell or 0.25g/mL of unground shrimp shell showed more than 90% of the adsorption
917 rates when the incubation was up to 24h. By contrast, both ground and intact shrimp shell had
918 lower adsorption rates than extracted chitin at same concentration and incubation time.
919 However, the adsorption was not stable. After three times washing with buffer,

920 AFB₁adsorption rate decreased about 15% to 45% in different groups, which suggested the
921 implication of electrostatic bounds (e.g. hydrogen bonds, Van der Waals interactions) in
922 adsorption process.

923 Due to the presumed environmental and health friendliness of natural products (e.g. enzyme,
924 microorganism cell wall), these approaches have attracted attention. However, biological
925 control has shown lower effectiveness compared to other methods, and is also generally
926 constrained to liquid media. Biological control tends to be more costly than physical and
927 chemical approaches, and there is currently little evidence of the toxicity of enzymolysis
928 products. However, reduction of mycotoxins during production of fermented products would
929 permit the use of somewhat contaminated raw materials in their production.

930

931 **3.4 Combined approaches**

932 Pérez-Flores, Moreno-Martinez, and Méndez-Albores (2011) showed that the level of AFB₁
933 and AFB₂ in tortillas (a Mexican food) decreased 68% to 84%, according to different original
934 concentration, by microwave treatment (1650 W, 2450 MHz, 5.5min) with added Ca(OH)₂
935 (0.5%). Kim, Shukla, Oh, Chung, and Kim (2018) reported that among food-grade additives
936 (sodium bicarbonate, vinegar, mixture of sodium bicarbonate and vinegar, citric acid and
937 baking powder), sodium bicarbonate yielded significantly higher PAT reduction in apple juice
938 (from 94.11 to 7.55 µg/L), which was comparatively similar to 30min of UV irradiation. The
939 authors suggested that since irradiation requires a special UV-irradiation apparatus and energy
940 consumption, a food-grade additive sodium bicarbonate might be a useful alternative to UV
941 radiation for reducing PAT content in apple juice samples. However, sodium bicarbonate
942 treatment affected quality attributes including soluble solids, pH, and colour of apple juice.
943 Nevertheless, the colour and door of juice treated with sodium bicarbonate could be recovered
944 via addition of citric acid. The usage of some additives could contribute to the mycotoxin
945 destruction in high-temperature processing. Sugars had a positive effect on FB₁ reduction of
946 maize muffins baked at 200°C for 30min. In this processing, the influence of glucose (40%)
947 on the decrease of FB₁ was greater than that of fructose (27%) and sucrose (28%), and the
948 effect of glucose concentration was more significant, from 40% reduction for 0.075g
949 glucose/g maize meal to 52% reduction for 0.3 g glucose/g maize meal (Castelo, Jackson,
950 Hanna, Reynolds, & Bullerman, 2001). Castelo, Jackson, Hanna, Reynolds, and Bullerman
951 (2001) showed that when grits with added sugars of different concentration (2.5% and 5%)
952 were extruded at a screw speed of 80, 100 or 120 rpm, the amounts of FB₁ remaining were
953 around 40% to 80% at 140°C. 10% glucose with 40 rpm of extrusion at 160°C led to about
954 90% reduction of FB₁, which was about 20% higher than the FB₁ treated without glucose
955 (Voss et al., 2011). In another extrusion study with conditions of sample moisture (15% or
956 30%), screw speed (120 rpm), temperature (150°C or 180°C) with or without 1% sodium
957 metabisulphite addition, DON was significantly reduced (>95%) in maize flour treated under
958 every condition, but AFB₁ content was not greatly affected (10% to 25%). Compared to
959 glucose, sodium metabisulphite did not show a significant contribution to the reduction of
960 both DON and AFB₁ (Cazzaniga, Basílico, González, Torres, & De Greef, 2001). With the
961 addition of 30 mL of lemon juice and 6 g of citric acid, AFB₁ decreased up to 93.1% in 50 g
962 pistachio nuts. When lemon juice and citric acid reduced to 15 mL and 2.25 g respectively,
963 only 49.3% of AFB₁ could be detected (Rastegar et al., 2017). Furthermore, adding baking

964 soda under twin-screw extrusion could contribute to the reduction of OTA in oat-based food,
965 and the degree of content reduction improved from about 40% to 65% with the increase of
966 added soda from 0 to 1%. On the contrary, the baking soda did reduce OTA by a modest 10%
967 in rice-based food (Ryu, Kowalski, Ganjyal, & Lee, 2019). The degradation of PAT with
968 added ascorbic acid was predicted by nonlinear Weibull model to be higher than that without
969 ascorbic acid, and the degradation increased with the raising of temperature. This might
970 because the oxidized ascorbic acid formed free radicals, so that could attack the lactone
971 structure of PAT (Kokkinidou, Floros, & LaBorde, 2014).
972 The exploration of combined treatments is to pursue higher removal efficiency, taking
973 advantage of the additional effect of integrated management. This is becoming a trend
974 gradually. The combined treatments do reduce toxin contamination to a greater extent, and
975 they can be better adapted to different food matrices.

976

977 **4 Evaluation of the feasibility of the approaches to be applied to food production**

978 Several reports have shown the potential of various methods for preventing and reducing
979 fungi or mycotoxins in foods. Here we perform a comparative evaluation of all the methods
980 discussed in this review, on the basis of their technical advantages and disadvantages, the
981 food matrices for which each method is suitable, the safety concern of a method, and the
982 economical implication of large scale application. Using all these parameters, the potential
983 for upscaling each method is then estimated as high, medium or low (Table 5).

984 In general, physical approaches, which include temperature and humidity control, MA
985 treatment, irradiation treatment, cleaning, milling and sorting, and heat treatment, showed
986 medium to high potential for using at a large scale. The advantages include versatility to use
987 in various matrices, safety, and few changes to the nutritional and sensory properties of foods.
988 However, the high cost of equipment and high energy required to operate over long times
989 may limit the industrial deployment of these methods. Among the four methods, temperature
990 and humidity control appears to have the highest upscaling potential.

991 Chemical approaches include photodynamic treatment, plasma treatment or ozonisation,
992 EOW, chemical antifungal/anti-mycotoxins agents, and chemical removal of mycotoxin.
993 Application of these methods in large scale also showed a medium to high potential. This can
994 be justified by their high efficiency and their suitability for a wide range of food matrices.
995 The limitations are mainly due to the negative impact on the quality and safety of foods. The
996 use of EOW showed the highest potential for large scale application with low level of safety
997 concern, once the cost of EOW production equipment and energy can be lowered.

998 Biological approaches include methods such as the use of biocontrol agents, use of antifungal
999 plant metabolites and biological removal of mycotoxins. These approaches showed low to
1000 medium potential for upscaling. They are claimed to be environmental friendly, they have a
1001 high efficiency (although the replication of lab performance of biocontrol agents in the field
1002 remains a challenge), and they can be applied to various foods of plant and animal origin.
1003 However, these methods may actually deteriorate food quality; the binders are difficult to
1004 remove from food and feed, the potential toxicity can be high, and the cost of production of
1005 the biological or plant agent is also high. Among the three methods, the biological removal
1006 of mycotoxins, which is already largely used in feed industry, shows a great prospect.

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1008 **5 Conclusion and perspectives**

1009 In order to reduce the contamination of foods by mycotoxins and minimize their negative
1010 effects on consumers health, 15 of strategies have been reviewed, which can be classified into
1011 physical, chemical and biological approaches, singly or in combination. These strategies are
1012 mainly focused on control of fungi growth in raw food materials and removal of mycotoxins
1013 from foods. Some new and efficient methods, such as plasma and EOW treatment show great
1014 potential but currently remain limited to laboratory applications. Currently physical
1015 approaches can be adapted into a wider range of food matrixes, including dry or liquid, raw
1016 or cooked foods. Physical approaches can be applied at large scale (e.g. crop storage) and
1017 small scale (MAP). Chemical and biological approaches are usually applied high humidity
1018 conditions (e.g. coating of fruits and vegetables) or liquid environment (e. g. mycotoxin
1019 binders in wine). However, so far no single approach is universal for all matrices or 100%
1020 effective at removing the risk of aflatoxin contamination. With the increasing demands in
1021 food safety and advances in technology, the mycotoxin reduction strategy has become to
1022 multi-dimensional, including a combination of multiple control methods as an integrated
1023 management strategy. Food safety concerns as a result of these treatments remain. It is critical
1024 when developing or applying a method, to test the toxicity of the applied agents and the
1025 derived secondary products. Nutrient loss and deterioration of sensory properties of foods by
1026 methods such as irradiation and plasma treatment must be tackled. For biological control
1027 methods, the efficacy of the biocontrol agents in field condition must be proven, and their
1028 short and long term toxicity be monitored. In methods involving plant-extracted metabolites,
1029 large amount of plant materials are needed to obtain sufficient amount of metabolites.
1030 Valorisation of plant waste can offer an alternative low cost source of the plant metabolites.
1031 On the other hand, the high cost of equipment and running, limits the industrial application
1032 of most of the methods, while low cost and easy operations such as sorting and cleaning can
1033 be upscaled if they are mechanized. This underlines the need of multidisciplinary
1034 collaboration involving engineering, physical and biological sciences in the fight against
1035 mycotoxins. Moreover, environmental aspects must be considered during the disposal of
1036 toxin-contaminated sorted seeds, waste water or binders.
1037 Research usually remains at the laboratory level with little consideration for upscale
1038 applications. Physical approaches have shown the highest potential for upscaling, followed
1039 by chemical approaches, while biological approaches necessitate further improvements.
1040 Finally, in addition to developing mycotoxin reduction methods, educating producers and
1041 consumers on the toxicity of mycotoxins, improving the diversity of food choices (to prevent
1042 acute doses from single sources such as maize or rice) and guiding to change the food
1043 preferences (towards foods that are less prone to mycotoxin contamination) can also reduce
1044 the harmful effect of mycotoxins from human health.

1045 **Table 1.** The occurrence of main mycotoxins in raw and processed products and regulation of mycotoxins in European Communities

Mycotoxins	Products	European Communities standards (ng/g)	Reference	
Aflatoxins	Raw products	Almonds, pistachios and apricot kernels,	10 (for human direct consumption)	de Medeiros et al. (2012)
		Oilseeds	15 (for oil production)	Milani and Maleki (2014)
		Cereals	10 (for processing)	Calado, Venâncio, and Abrunhosa (2014)
		Spices	10 (for human direct consumption)	Eskola et al. (2019)
		Milk	Cannot be detected	Cinar and Onbaşı (2019)
	Processed products	Dried fruits, copra	4 (for human direct consumption)	EU (2006)
		Maize grits	Cannot be detected	
	Cheese	Cannot be detected		
Fumonisin	Raw products	Germ, bran, rice, sorghum, legumes, cowpea seeds, triticale,	4000	de Medeiros et al. (2012)
		Maize	1000 (for human direct consumption)	Milani and Maleki (2014)
		Asparagus	NM	Calado, Venâncio, and Abrunhosa (2014)
		Milk	NM	Eskola et al. (2019)
	Processed products	Grits, maize-based products, wheat flour	800 (for adult direct consumption)	Cinar and Onbaşı (2019)
		Beer	200 (for human direct consumption)	EU (2006)
Deoxynivalenol	Raw products	Cereals	750 (for human direct consumption)	de Medeiros et al. (2012)
	Processed products	Wheat flour	750 (for human direct consumption)	Milani and Maleki (2014)
		Bread, pasta, pretzel, cookie	500	Eskola et al. (2019) Cinar and Onbaşı (2019) EU (2006)
Ochratoxin A	Raw products	Cereals, legumes, coffee beans, nuts, pulses, sesame seeds,	5 (for processing)	Larsen, Svendsen, and Smedsgaard (2001)
		Spices	15 (for human direct consumption)	Varga and Kozakiewicz (2006)
		Apples, peaches, strawberries, pears, oranges, figs, mangoes,	NM	Eskola et al. (2019)
		tomatoes, watermelons, yam, potatoes, garlic, onions,		Cinar and Onbaşı (2019)
		Milk, eggs, meat	NM	EU (2006)

	Processed products	Grape juices, wine vinegar	2.0 (for human direct consumption)	
		Breakfast cereals & snacks	3.0 (for human direct consumption)	
		Infant cereals	0.5 (for infant direct consumption)	
		Bread, pasta	0.5 (for human direct consumption)	
		Flour	0.5 (for human direct consumption)	
		Cocoa	5	
		Dried vine fruits	10	
		Sausage	NM	
		Cheese, milk-based products	0.5 (for human direct consumption)	
		Bottled water, plant food supplement, food colouring agent	NM	
Zearalenone	Raw products	Maize	350 (for processing)	de Medeiros et al. (2012)
		Cereals, sesame, soy beans, nuts	75 (for human direct consumption)	Calado, Venâncio, and Abrunhosa (2014)
	Processed products	Cereal-based products	50 (for adult direct consumption)	Eskola et al. (2019)
				Cinar and Onbaşı (2019)
				EU (2006)
Patulin	Raw products	Wheat straw residue	NM	CAST (2003)
	Processed products	Fruit juice	25 (for adult direct consumption)	EU (2006)

1046 NM: Not mentioned

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1056 **Table 2.** Examples of mycotoxin-producing fungi and their inhibition by plant essential oils

Fungal species	Plant species	Organ	Region	Concentration ($\mu\text{L}/\text{mL}$)	Main components	Inhibition (%)	Reference
<i>A. flavus</i>	<i>Hedychium sp.</i>	Leaf	USA	40000	NM	100	Rajasekaran, Sakhanokho, and Tabanca (2012)
	<i>Ocimum gratissimum</i>	Leaf	India	0.6	Methyl cinnamate; γ -Terpinene	91	Prakash et al. (2011)
	<i>Origanum majorana</i>	NM	India	3	NM	100	Prakash, Singh, Kedia, and Dubey (2012)
	<i>Coriandrum sativum</i>	NM	India	2.5	NM	100	
	<i>Hedychium spicatum</i>	NM	India	2.5	NM	100	
	<i>Arachis hypogaea</i>	Seed	India	1	NM	82	Prakash et al. (2012)
	<i>Arachis hypogaea</i>	Leaf	India	1	NM	62.5	
	<i>Cinnamomum glaucescens</i>	Berry	India	1	1,8-Cineole	58	Prakash, Singh, Yadav, Singh, and Dubey (2013)
	<i>Salvia officinalis</i>	Aerial parts	Jordan	5	1,8-Cineole	100	Abu-Darwish et al. (2013)
	<i>Artemisia herba-alba</i>	Aerial parts	Jordan	5000	Predominant; α - and β -Thujones	100	Abu-Darwish et al. (2015)
	<i>Caesulia axillaris</i>	Aerial parts	India	1	DL -Limonene; Eucasarone	100	Mishra, Shukla, Singh, Prakash, and Dubey (2012)
	Jamrosa	Leaf	India	0.4	Z-citral; Linalyl acetate	100	Mishra et al. (2012)
	<i>Lippia rugosa</i>	Leaf	Cameroon	1000	Geraniol; Nerol; Geranial	100	Tatsadjieu et al. (2009)
	<i>Coleus aromaticus</i>	Leaf	India	1	Z-citral; Precocenel	100	Jaya, Prakash, and Dubey (2011)
	<i>Hyptis suaveolens</i>	Leaf	India	1	Precocene I	93.8	
	<i>Ageratum conyzoides</i>	Leaf	India	1	Germacrene-D; Trans-caryophyllene	100	
	<i>Ageratum conyzoides</i>	Leaf	Brazil	1	Precocenel; Precocenell	63	Nogueira et al. (2010)
	<i>Lavandula multifida</i>	Aerial parts	Portugal	0.64	Carvacrol; <i>cis</i> - β -Ocimene	100	Zuzarte et al. (2012)
	<i>Citrus sinensis</i> var. Valencia	Orange peel	Mexico	16000	NM	100	Velázquez-Nuñez, Avila-Sosa, Palou, and López-Malo (2013)
<i>A. niger</i>	<i>Ocimum gratissimum</i>	Leaf	India	0.6	Methyl cinnamate; γ -Terpinene	85	Prakash et al. (2011)
	<i>Origanum majorana</i>	NM	India	3.5	NM	100	Prakash, Singh, Kedia, and Dubey (2012)
	<i>Coriandrum sativum</i>	NM	India	3	NM	100	
	<i>Hedychium spicatum</i>	NM	India	2.5	NM	100	
	<i>Commiphora myrrha</i>	NM	India	3.5	NM	100	

	<i>Cananga odorata</i>	NM	India	2	NM	100	
	<i>Cinnamomum glaucescens</i>	Berry	India	1	1,8-Cineole	63	Prakash, Singh, Yadav, Singh, and Dubey (2013)
	<i>Salvia officinalis</i>	Aerial parts	Jordan	5	1,8-Cineole	100	Abu-Darwish et al. (2013)
	<i>Artemisia herba-alba</i>	Aerial parts	Jordan	1250	Predominant; α -and β -Thujones	100	Abu-Darwish et al. (2015)
	<i>Caesulia axillaris</i>	Aerial parts	India	1	DL-Limonene; Eusarone	100	Mishra, Shukla, Singh, Prakash, and Dubey (2012)
	<i>Lavandula multifida</i>	Aerial parts	Portugal	0.32	Carvacrol; <i>cis</i> - β -Ocimene	100	Zuzarte et al. (2012)
<i>A. fumigatus</i>	<i>Cinnamomum glaucescens</i>	Berry	India	1	1,8-Cineole	70	Prakash, Singh, Yadav, Singh, and Dubey (2013)
	<i>Salvia officinalis</i>	Aerial parts	Jordan	5	1,8-Cineole	100	Abu-Darwish et al. (2013)
	<i>Artemisia herba-alba</i>	Aerial parts	Jordan	2500	Predominant; α -and β -Thujones	100	Abu-Darwish et al. (2015)
	<i>Caesulia axillaris</i>	Aerial parts	India	1.25	DL-Limonene; Eusarone	100	Mishra, Shukla, Singh, Prakash, and Dubey (2012)
	<i>Lavandula multifida</i>	Aerial parts	Portugal	0.32	Carvacrol; <i>cis</i> - β -Ocimene	100	Zuzarte et al. (2012)
<i>A. terreus</i>	<i>Caesulia axillaris</i>	Aerial parts	India	1	DL-Limonene; Eusarone	100	Mishra, Shukla, Singh, Prakash, and Dubey (2012)
<i>F. verticillioides</i>	<i>Hedychium</i> sp.	Leaf	USA	40000	NM	100	Rajasekaran, Sakhanokho, and Tabanca (2012)
<i>F. nivale</i>	<i>Ocimum gratissimum</i>	Leaf	India	0.6	Methyl cinnamate; γ -Terpinene	100	Prakash et al. (2011)
	<i>Origanum majorana</i>	NM	India	2.75	NM	100	Prakash, Singh, Kedia, and Dubey (2012)
	<i>Coriandrum sativum</i>	NM	India	2	NM	100	
	<i>Hedychium spicatum</i>	NM	India	2.25	NM	100	
	<i>Commiphora myrrha</i>	NM	India	2.5	NM	100	
	<i>Cananga odorata</i>	NM	India	1.5	NM	100	
<i>F. oxysporum</i>	<i>Caesulia axillaris</i>	Aerial parts	India	0.75	DL-Limonene; Eusarone	100	Mishra, Shukla, Singh, Prakash, and Dubey (2012)
<i>P. italicum</i>	<i>Ocimum gratissimum</i>	Leaf	India	0.6	Methyl cinnamate; γ -terpinene	100	Prakash et al. (2011)
	<i>Origanum majorana</i>	NM	India	2.5	NM	100	Prakash, Singh, Kedia, and Dubey (2012)
	<i>Coriandrum sativum</i>	NM	India	2.25	NM	100	
	<i>Hedychium spicatum</i>	NM	India	2.5	NM	100	
	<i>Commiphora myrrha</i>	NM	India	2.5	NM	100	
	<i>Cananga odorata</i>	NM	India	1.5	NM	100	
	<i>Caesulia axillaris</i>	Aerial parts	India	1	DL-Limonene; Eusarone	100	Mishra, Shukla, Singh, Prakash, and Dubey (2012)

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1060 **Table 3.** Mycotoxins detoxification by chemical adsorption to different matrices

Sorbents			Mycotoxin	Concentration (mg/mL)/(mg/mg)	Effects	Time	Matrix	Reference
Hydrated aluminosilicates (HSCASs)	sodium calcium	AFB ₁	40	>97% Adsorption	30min	Malt suspension	Aly, Abdel-Galil, and Abdel-Wahhab (2004)	
			0.01	Retard of the decline in the total number of offspring	30d	<i>In vivo</i> (<i>Drosophilamelanogaster</i>)	Şişman (2006)	
			2	No significant decrease on body weight gain; Retention on crude protein; Decrease on crude fat;	42d	Broilers basal maize-soybean meal	Y. L. Liu et al. (2011)	
			3	No significant decrease on calcium; Retention on the proportion of breast muscle, thigh muscle and abdominal fat; Improvement of growth performance, digestibility, and immune function; Reduction of deleterious effects and tissue residues caused by AFB ₁	21d	Broilers maize meal	N. Liu, Wang, Deng, Gu, and Wang (2018)	
			FB ₁	40	>84% Adsorption	30min	Malt suspension	Aly, Abdel-Galil, and Abdel-Wahhab (2004)
			OTA	2	No significant decrease on body weight gain; Retention on crude protein; Decrease on crude fat; No significant decrease on calcium; Retention on the proportion of breast muscle, thigh muscle and abdominal fat	42d	Broilers basal maize-soybean meal	Y. L. Liu et al. (2011)
			ZEN	10	50% Adsorption	90min	Acetate/or citrate buffer	Yiannikouris, Kettunen, Apajalahti, Pennala, and Moran (2013)
			5	Reestablishment of haematological parameters, levels of serum biochemical	48h	<i>In vivo</i> (mice)	Abbès et al. (2006)	

			enzyme activities and histological pictures of both liver and kidney				
	T-2	2	No significant decrease on body weight gain; Retention on crude protein; Decrease on crude fat	42d	Broilers basal maize-soybean meal		Y. L. Liu et al. (2011)
Hydrated sodium aluminosilicate	AFB ₁	5	No effect on hepatic lesions	1 year	Rainbow trout diet meal		Arana et al. (2011)
Activated charcoal	FB ₁	2	100% Adsorption	1h	Aqueous solution		Galvano et al. (1997)
	OTA	0.4	>95% Adsorption	1h	Aqueous solution		Galvano et al. (1998)
	DON	2	>90% Adsorption	1h	Aqueous solution		Galvano et al. (1998)
		1	67% Adsorption	90min	Phosphate buffer		Cavret, Laurent, Videmann, Mazallon, and Lecoeur (2010)
	ZEN	1	100% Adsorption	90min	Phosphate buffer		Cavret, Laurent, Videmann, Mazallon, and Lecoeur (2010)
Clay	AFB ₁	0.002	Reduction of effects by mycotoxins on immune system and the liver; Improve pig growth	42d	Pig meal		Weaver et al. (2013)
	OTA	0.002	Reduction of effects by mycotoxins on immune system and the liver; Improve pig growth	42d	Pig meal		Weaver et al. (2013)
Egyptian montmorillonite	AFB ₁	40	>97% Adsorption	30min	Malt suspension		Aly, Abdel-Galil, and Abdel-Wahhab (2004)
	FB ₁	40	>80% Adsorption	30min	Malt suspension		Aly, Abdel-Galil, and Abdel-Wahhab (2004)
Esterified glucomannan	AFB ₁	0.5	No significant decrease on body weight gain; Increase on crude protein; No significant decrease on calcium; Retention on the proportion of breast muscle, thigh muscle and abdominal fat	42d	Broilers maize-soybean meal		Y. L. Liu et al. (2011)
	OTA	0.5	No significant decrease on body weight gain; Increase on crude protein; No	42d	Broilers maize-soybean meal		Y. L. Liu et al. (2011)

	T-2	0.5	significant decrease on calcium; Retention on the proportion of breast muscle, thigh muscle and abdominal fat No significant decrease on body weight gain; Increase on crude protein; No significant decrease on calcium; Retention on the proportion of breast muscle, thigh muscle and abdominal fat	42d	Broilers meal	maize-soybean	Y. L. Liu et al. (2011)
Cholestyramine	DON	0.82	10% Adsorption	4h	Phosphate-citrate buffer	Döll, Dänicke, Valenta, and Flachowsky (2004) Cavret, Laurent, Videmann, Mazallon, and Lecoecur (2010)	
		1	65% Adsorption	90min	Phosphate buffer		
	ZEN	0.82	94% Adsorption	4h	Phosphate-citrate buffer	Döll, Dänicke, Valenta, and Flachowsky (2004)	
Modified aluminosilicate	DON	0.82	17% Adsorption	4h	Phosphate-citrate buffer	Döll, Dänicke, Valenta, and Flachowsky (2004)	
	ZEN	0.82	81% Adsorption	4h	Phosphate-citrate buffer	Döll, Dänicke, Valenta, and Flachowsky (2004)	
PVP-DEGMA-TAIC	FB ₁	0.005	86% Adsorption	24h	Wine-like model solution	Carrasco-Sanchez, Kreitman, Folch-Cano, Elias, and Laurie (2017)	
	FB ₂	0.005	94% Adsorption	24h	Wine-like model solution	Carrasco-Sanchez, Kreitman, Folch-Cano, Elias, and Laurie (2017)	
Poly(acrylamide-co-ethyleneglycol-methacrylate)	FB ₁	0.005	82% Adsorption	24h	Wine-like model solution	Carrasco-Sanchez, Kreitman, Folch-Cano, Elias, and Laurie (2017)	
	FB ₂	0.005	100% Adsorption	24h	Wine-like model solution	Carrasco-Sanchez, Kreitman, Folch-Cano, Elias, and Laurie (2017)	
Trimethylstearylammmonium bromide	AFB ₁	5.8	89% Adsorption	1h	Phosphate buffer	Sun, Song, Wang, Wang, and Zheng (2018)	
	ZEN	5.9	86% Adsorption	1h	Phosphate buffer	Sun, Song, Wang, Wang, and Zheng (2018)	

1061 PVP-DEGMA-TAIC:Resins of copolymerization of N-vinyl-2-pyrrolidinone with ethylene glycol dimethacrylate and triallyl isocyanurate

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1063 **Table 4.** Mycotoxins detoxification by bacteria and fungi through adsorption in different matrixes

Micro-	Mycotoxin	Genus	Strains	Effects	Time	Matrix	Reference
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organism							
Bacteria	AFB ₁	<i>Lactobacillus</i>	<i>L. fermentum</i>	61% Adsorption	48h	PBS Buffer	Fazeli et al. (2009)
			<i>L. plantarum</i>	56% Adsorption	48h	PBS Buffer	
			<i>L. casei</i>	48% Adsorption	48h	PBS Buffer	
			<i>L. paracasei</i> LOCK 0920	Decreased the extent of DNA damage	14d	<i>In vivo</i> (Chicken Fodder)	Slizewska, Nowak, Libudzisz, and Blasiak (2010)
			<i>L. brevis</i> LOCK 0944	Decreased the extent of DNA damage	14d	<i>In vivo</i> (Chicken Fodder)	
			<i>L. plantarum</i> LOCK 0945 (mixed)	Decreased the extent of DNA damage	14d	<i>In vivo</i> (Chicken Fodder)	
			<i>L. rhamnosus</i> strain GG	70% Adsorption	24h	PBS Buffer	Vosough, Sani, Mehraban, and Karazhyan (2014)
		<i>Bifidobacterium</i>	<i>B. bifidum</i>	55% Adsorption	72h	PBS Buffer	Hamad, Zahran, and Hafez (2017)
			<i>B. lactis</i> CSCC 5094	35% Adsorption	24h	PBS Buffer	Peltonen, El-Nezami, Haskard, Ahokas, and Salminen (2001)
			<i>B. longum</i> CSCC 5304	38% Adsorption	24h	PBS Buffer	
			<i>B. animalis</i> CSCC 1941	46% Adsorption	24h	PBS Buffer	
			<i>B. lactis</i> CSCC 1906	49% Adsorption	24h	PBS Buffer	
		<i>Enterococcus</i>	<i>E. faecium</i> MF4	23% Adsorption	24h	PBS Buffer	Fernandez Juri, Dalcerro, and Magnoli (2014)
			<i>E. faecium</i> GJ40	21% Adsorption	24h	PBS Buffer	
			<i>E. faecium</i> M74	19.3-30.5 % Adsorption	48h	PBS Buffer	Topcu, Bulat, Wishah, and Boyacı (2010)
			<i>E. faecium</i> EF031	23.4-37.5% Adsorption	48h	PBS Buffer	
	AFB ₂	<i>Streptococcus</i>	<i>P. freudenreichii</i> spp. <i>shermanii</i> JS (mixed)	83% Adsorption	4 weeks	<i>In vivo</i> (human)	El-Nezami et al. (2000)
	AFM ₁	<i>Lactobacillus</i>	<i>L. rhamnosus</i> GAF01	95% Adsorption	24h	PBS Buffer/Milk	Abbes et al. (2013)
<i>L. plantarum</i> MON03			77% Adsorption	24h	PBS Buffer /Milk		
<i>L. plantarum</i> MON03			16% Adsorption	14d	<i>In vivo</i> (mice)		
<i>L. bulgaricus</i>			58.5% Adsorption	6h	Yogurt	El Khoury, Atoui, and Yaghi (2011)	
<i>L. bulgaricus</i>			55% Adsorption	6h	PBS Buffer		

DON	<i>Lactobacillus</i>	<i>L. plantarum</i> strain 102	20% Adsorption	24h	PBS Buffer	Zou et al. (2012)	
		<i>L. rhamnosus</i> GGATCC 53103	54% Adsorption	24h	MRS Medium	Niderkorn, Boudra, and Morgavi (2006)	
		<i>L. delbruekiisp. Bulgaricus</i> R0149	55% Adsorption	24h	MRS Medium		
OTA	<i>Lactobacillus</i>	<i>L. rhamnosus</i> strain GG	47% Adsorption	2h	PBS Buffer	Turbic, Ahokas, and Haskard (2002)	
		<i>L. rhamnosus</i> strain LC-705	36% Adsorption	2h	PBS Buffer		
ZEN	<i>Lactobacillus</i>	<i>L. rhamnosus</i> GG	55% Adsorption	24h	MRS Medium	El-Nezami, Polychronaki, Salminen, and	
		<i>L. rhamnosus</i> LC705	55% Adsorption	24h	MRS Medium	Mykkanen (2002)	
FB	<i>Lactobacillus</i>	<i>L. rhamnosus</i> GG ATCC 53103	54% Adsorption	24h	MRS Medium	Niderkorn, Boudra, and Morgavi (2006)	
		<i>L. plantarum</i> R1039	40% Adsorption	24h	MRS Medium		
		<i>L. plantarum</i> R0011	30% Adsorption	24h	MRS Medium		
		<i>L. brevis</i> R0002	32% Adsorption	24h	MRS Medium		
		<i>L. acidophilis</i> R0052	34% Adsorption	24h	MRS Medium		
		<i>L. delbruekii ssp. bulgaricus</i> R0149	55% Adsorption	24h	MRS Medium		
		<i>L. casei</i> ssp. <i>casei</i> C3	36% Adsorption	24h	MRS Medium		
		<i>Streptococcus</i>	<i>Strep. thermophilus</i> B5	31% Adsorption	24h	MRS Medium	Niderkorn, Boudra, and Morgavi (2006)
		<i>Lactococcus</i>	<i>L. lactis</i> CS 43	23% Adsorption	24h	MRS Medium	Niderkorn, Boudra, and Morgavi (2006)
			<i>L. lactis</i> CS 202	40% Adsorption	24h	MRS Medium	
<i>L. lactis</i> CS 197	23% Adsorption		24h	MRS Medium			
<i>Leuconostoc</i>	<i>L. mesenteroides</i> R1107	46% Adsorption	24h	MRS Medium	Niderkorn, Boudra, and Morgavi (2006)		
<i>Lactobacillus</i>	<i>L. rhamnosus</i> 6149	51.1-52.0% Adsorption	24h	Physiological saline solution (0.85%, w/v)	S. Hatab, T. Yue, and O. Mohamad (2012)		
<i>Bifidobacterium</i>	<i>B. bifidum</i> 6071	52.9-54.1% Adsorption	24h	Physiological saline solution (0.85%, w/v)	S. Hatab, T. Yue, and O. Mohamad (2012)		
<i>Enterococcus</i>	<i>E. faecium</i> 21605	64.5% Adsorption	24h	Apple juice	S. Hatab, T. Yue, and O. Mohamad		
	<i>E. faecium</i> M74	15.8-41.6% Adsorption	48h	PBS Buffer	(2012)		
	<i>E. faecium</i> EF031	9.5-45.3% Adsorption	48h	PBS Buffer	Topcu, Bulat, Wishah, and Boyacı (2010)		
Fungi	AFB ₁	<i>Saccharomyces</i>	<i>S. cerevisiae</i>	48% Adsorption	1h	PBS Buffer	Campagnollo et al. (2015)

DON	<i>Saccharomyces</i>	<i>S. cerevisiae</i>	12% Adsorption	1h	PBS Buffer	Campagnollo et al. (2015)
PAT	<i>Saccharomyces</i>	<i>S. cerevisiae</i> strain YS3(laboratory-prepared)	70% Adsorption	24h	Apple Juice	Guo, Yue, Hatab, and Yuan (2011)
		<i>S. cerevisiae</i> strain YS3(commercial)	76% Adsorption	24h	Apple Juice	Yue, Dong, Guo, and Worobo (2011)
		<i>S. cerevisiae</i> YS1-YS10	50-7% Adsorption	24h	Apple Juice	Guo, Yue, Hatab, and Yuan (2012)
		<i>S. cerevisiae</i> YS3	100% Adsorption	36h	Apple Juice	Coelho et al. (2008)
		<i>S. cerevisiae</i>	90-96% Adsorption	143h	Apple Juice	
OTA	<i>Saccharomyces</i>	<i>S. cerevisiae</i> var. <i>boulardii</i> ATCC MYA-796	39% Adsorption	1h	PBS Buffer	Petruzzi, Corbo, Sinigaglia, and Bevilacqua (2016)
		<i>S. cerevisiae</i> BM45	39% Adsorption	1h	PBS Buffer	
		<i>S. cerevisiae</i> W13	39% Adsorption	1h	PBS Buffer	
		<i>S. cerevisiae</i> W28	39% Adsorption	1h	PBS Buffer	
		<i>S. cerevisiae</i> W47	42% Adsorption	4d	YPG Medium with Ethanol	
		<i>S. cerevisiae</i> Y28	37% Adsorption	4d	YPG Medium with Ethanol	Petruzzi, Sinigaglia, Corbo, Beneduce, and Bevilacqua (2012)
		<i>S. cerevisiae</i> Malaga LOCK 0173	85% Adsorption	10d	Grape/Blackcurrant Juice	
		<i>S. cerevisiae</i> Syrena LOCK 0201	83% Adsorption	10d	Grape/Blackcurrant Juice	
		<i>S. cerevisiae</i> bakery BS strain	64% Adsorption	10d	Grape/Blackcurrant Juice	Piotrowska, Nowak, and Czynowska (2013)
		<i>S. cerevisiae</i> RC008	57% Adsorption	1h	PBS Buffer	
		<i>S. cerevisiae</i> RC009	67% Adsorption	1h	PBS Buffer	
		<i>S. cerevisiae</i> RC012	71% Adsorption	1h	PBS Buffer	
		<i>S. cerevisiae</i> RC016	74% Adsorption	1h	PBS Buffer	Armando et al. (2012)
		<i>S. cerevisiae</i>	76% Adsorption	90d	White Wine	
		<i>S. cerevisiae</i>	86% Adsorption	90d	Red Wine	
		<i>S. cerevisiae</i>	90% Adsorption	90d	Rose Wine	
<i>S. cerevisiae</i>	59% Adsorption	1h	PBS Buffer	Csutorás et al. (2013)		
<i>S. cerevisiae</i>	30% Adsorption	1h	Dough Fermentation			
					Campagnollo et al. (2015)	
					Valle-Algarra et al. (2009)	
ZEN	<i>Saccharomyces</i>	<i>S. cerevisiae</i> RC008	21% Adsorption	1h	PBS Buffer	Armando et al. (2012)

<i>S. cerevisiae</i> RC009	33% Adsorption	1h	PBS Buffer	
<i>S. cerevisiae</i> RC012	29% Adsorption	1h	PBS Buffer	
<i>S. cerevisiae</i> RC016	34% Adsorption	1h	PBS Buffer	
<i>S. cerevisiae</i>	75% Adsorption	1h	PBS Buffer	Campagnollo et al. (2015)

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1084 **Table 5.** Evaluation of fungi/mycotoxin decontamination approaches

Classification	Treatments	Technical advantages	Technical disadvantages	Suitable food matrix	Safety	Economical concerns in large	Potential for using in	Reference
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						scale production	large scale			
Physical approaches	Temperature and humidity control	Easy-operated; less colour, odor and nutrition changes; shelf life extension	Inconvenient transportation	Almost all food types	No reported toxic substance induced and formed	Cost of temperature and humidity equipment; high energy consumption	High	-		
	Modified atmosphere treatment	Less colour, odor and nutrition changes; shelf life extension	Large consumption of packaging material	Packed foods	No reported toxic substance induced and formed	Cost of gas generator equipment and food packaging material	Medium	-		
	Irradiation treatment	High efficiency; environmental friendly; agrees with the legislations of food application in 55 countries	Food quality change (e.g. colour, odor); nutrition loss (e.g. oxidization of vitamin) at high dose	Packed foods; frozen foods; liquid foods; cereals; fruits and vegetables	No residual irradiation; may cause mutations to fungi	Low consumption of water and electrical energy (exception of electron beam and X-ray); high cost of food irradiation facilities	High		Calado, Venâncio, and Abrunhosa (2014)	
	Cleaning, milling and sorting	Easy-operated; effective with water-soluble mycotoxins	Less effective with organic-soluble mycotoxins	Raw food materials	No other introduced chemicals and new mycotoxin-derivative	High consumption of water	High		Temba et al. (2016)	
	Heat treatment	Already a necessary processing method in food production	Change of the desired physical properties of food	Cooked foods (e.g. roasted foods); sterilized food	Lack of studies on transformation mechanisms	Cost of heating equipment; high energy consumption	Medium		Rastegar et al. (2017)	

Chemical approaches	Photodynamic treatment	Environmental friendly; biochemically stable; photosensitizer adequately activated by using easy-available visible light	Limited light penetration	Cereals; fruits; sea foods; animal feeds	Food grade photosensitizer (e.g. curcumin); lack of studies on safety after treatment	Cost of light generator and photosensitizer; relative cost-effective		Njoki, Okoth, and Wachira (2017) Al-Asmari, Mereddy, and Sultanbawa (2018) Temba et al. (2019)
	Plasma treatment (Ozonisation)	High efficiency; rapid; no significant change of nutritional components to whole cereals	May cause the loss of nutrition in other foods; change of colour; production of undesirable odor	Cereals; meat; fruits and vegetables; herbs and spices; animal feeds	Lack of studies on safety of degraded residue	Low energy consumption; high cost of cold plasma production equipment; less maintenance and dust cleaning	Medium	Savi, Bittencourt, et al. (2015) Temba et al. (2016) Misra, Yadav, Roopesh, and Jo (2019) Alexandre, Castanha, Calori-Domingues, and Augusto (2017)
	Electrolyzed oxidizing water	High efficiency; environmental friendly; easy-operated	Loss of antifungal activity without continuous electrolysis; Cl ₂ production; possibility of metal corrosion	Fruits and vegetables; meat products; cereals	Safe to degrade mycotoxins; no not corrosive to skin, mucous membrane and organic material	High cost of EOW production equipment, electrical and water consumption; low cost of each litre	High	Okull and Laborde (2004) Q. Zhang, Xiong, Tatsumi, Li, and Liu (2012) Huang, Hung, Hsu, Huang, and Hwang (2008)
	Chemical antifungal/anti-mycotoxins agents	Effective; easy-operated	Unpleasant chemical residue	Coating; animal feeds; specific food conforming to food additives (e.g. soda in Chinese baking)	Lack of studies on transformation mechanisms; toxicity of induced chemicals at high concentration to human and	Cost of agents	Medium	Bretz, Beyer, Cramer, Knecht, and Humpf (2006) Temba et al. (2019)

environment

	Chemical removal of mycotoxin	Effective; easy-operated; some commercial clay materials enhance nutrition and digestibility of animal feeds	Difficult removal of mycotoxin-binder complex; need to be in aqueous environment	Animal feeds; clay capsules for human (potential)	Toxicity of released mycotoxins from mycotoxin-binder complex	Cost effective	Medium	Di Gregorio et al. (2014)
Biological approaches	Biological control agents/mycotox in degradation	High efficiency in lab experiments; easy-operated (e.g. soak, spray); environmental friendly	Less evidence on the correlation between laboratory inhibition assay and field performance; need to be under strict conditions (e.g. pH, solution, temperature)	Cereals; fruits and vegetables; fermented foods	Less toxicity shown on degraded residue	Cost of bacterial high density culture; used as antagonist solution cost of materials and equipment for production of the biocontrol agent	Low	Jard, Liboz, Mathieu, Guyonvarc'h, and Lebrihi (2011) de Medeiros et al. (2012)
	Antifungal plant metabolite	High efficiency; a wide range of sources	Change of colour and odor; mainly used in aqueous environment	Meat products; dairy products; vegetable and fruits; cereals	Potential toxicity (e.g. carcinogenicity) at high concentration	Large amount of plant materials needed; high cost of production equipment and energy consumption	Low	Burt (2004) Bakkali, Averbeck, Averbeck, and Idaomar (2008)
	Biological removal of mycotoxin	High efficiency; from food source; environmental friendly	Difficult removal of mycotoxin-binder complex; need to be in aqueous environment	Fermented foods; animal feeds	Toxicity of released mycotoxins from mycotoxin-	Cost of bacterial high density culture	Medium	Hathout and Aly (2014)

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bacteria complex

1086 **Data Availability**

1087 The data supporting the conclusions of this manuscript will be made available by the authors,
1088 without undue reservation, to any qualified researcher.

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1090 **Conflict of Interest Statement**

1091 The authors declare no potential conflict of interest.

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1097 **Author contributions**

1098 YL performed the literature review and evaluation of literature data. YL drafted the
1099 manuscript. All authors contributed ideas to the design and organisation of the work. All
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1102 **References**

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