

Toxigenic Molds in Ripened Foods and Biocontrol Strategies

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Ripened foods of animal origin comprise meat products and dairy products, being transformed by the wild microbiota which populates the raw materials, generating highly appreciated products over the world. Together with this beneficial microbiota, toxigenic molds such as *Penicillium* spp. and *Aspergillus* spp., can contaminate these products and pose a risk for the consumers. Thus, effective strategies to hamper these hazards are required. Additionally, consumer demand for clean label products is increasing. Therefore, the manufacturing sector is seeking new efficient, natural, low-environmental impact and easy to apply strategies to counteract these microorganisms.

dry-cured meat

ripened cheese

molds

yeasts

lactic acid bacteria

essential oils

mycotoxins

1. Introduction

Some molds can cause a wide variety of human diseases such as allergic or invasive infections due to excessive inhalation of spores (mainly from *Aspergillus* spp.) or their transmission through infected wounds, as well as through the smoking of contaminated plants ^[1]. However, these infections are infrequent, and from the food safety view, the main problem associated with the mold contamination of ripened animal products is the production of mycotoxins, which are secondary metabolites with a wide range of toxic effects. The most important mycotoxins in dry-cured meat products are the ochratoxin A (OTA) and aflatoxins (AFs), due to their frequency and their toxicity, although other mycotoxins can be detected in these products, such as cyclopiazonic acid (CPA), sterigmatocystin (STG) and citrinin (CIT) ^{[2][3]}. Similarly, the abovementioned mycotoxins have also been described in cheeses as well as PR toxin, roquefortine C and patulin ^{[4][5]}. The main molds that produce mycotoxins in animal-origin ripening foods are described below.

2. Biocontrol Strategies against Ochratoxin A-Producing Molds

Ochratoxin A (OTA) can be produced by different species of *Penicillium* and *Aspergillus*, such as *Penicillium nordicum*, *Penicillium verrucosum*, *Aspergillus westerdijkiae* and *Aspergillus carbonarius*. Within these species, *P. nordicum* has been described as the main OTA producer in dry-cured meat products and cheeses ^[6]. This mycotoxin is nephrotoxic, hepatotoxic, teratogenic, immunotoxic and has been classified as a possible human

carcinogen (group 2B) by the International Agency for Research on Cancer (IARC) [7][8][9]. Preserved meats and cheeses are the main contributors to dietary exposure to OTA in several European countries [9].

The biocontrol of ochratoxigenic molds employed in dry-cured meat products includes the use of starter and protective cultures which contain lactic acid bacteria (LAB), gram-positive catalase-positive cocci (GCC+), yeasts and non-toxigenic molds, as displayed in **Table 1** [10][11][12][13][14][15][16][17][18]. Different strains from *Enterococcus faecium* were demonstrated to control OTA production via growing *P. nordicum* in a dry-cured fermented sausage based medium, although they did not affect the OTA produced by *P. verrucosum* [14]. GCC+, as *Staphylococcus xylosus*, successfully decreased the OTA content using different strains of *P. nordicum* (Pn15, Pn92 and Pn856) in a dry-cured ham-based medium, although no effect was detected in sausages inoculated with the same strain of *S. xylosus* and Pn15 [10][17]. Fermented extracts developed from the fermentation of a meat model system (BFS) by *L. plantarum* and *P. pentosaceus* were able to totally eliminate the presence of *P. nordicum* and *P. verrucosum* using different concentrations depending on the bacterium and the mold strain tested [19]. Meftah et al. [16] revealed the ability of the yeasts *Candida zeylanoides* and *Rhodotorula mucilaginosa* to reduce the OTA concentration produced by *P. nordicum* and *A. westerdijkiae* in three matrices (ham, and dry-cured sausages with industrial and traditional processing). Other yeasts, such as *Debaryomyces hansenii* and *Saccharomycopsis fibuligera*, were able to completely inhibit the OTA produced by *P. nordicum* and *Aspergillus ochraceus* in speck, a typical meat product in the European Alpine area [11]. *D. hansenii* has also been displayed as an effective BCA against *P. nordicum*, *P. verrucosum* and *A. westerdijkiae* tested in other studies in dry-cured meat products or meat-model systems [10][16][18][20][21]. Additionally, the strain of *D. hansenii* used in some of these studies did not negatively modify the sensorial quality of dry-cured fermented sausages which contained it [22]. The non-toxigenic mold *Penicillium chrysogenum*, producer of the antifungal protein PgAFP, was proposed as a BCA against *P. nordicum* in a dry-cured ham-based medium [15] and in a meat-model system [23], showing in both studies possible nutrient competition. Similarly, this strain of *P. chrysogenum* controlled the growth of potentially ochratoxigenic molds, reducing the OTA accumulation in dry-cured Iberian hams which had undergone industrial processing [24]. This strain was also proposed as a good protective culture with no technological drawbacks during the ripening of dry-cured fermented sausages [22]. The protective potential of a commercial starter culture of *Penicillium nalgiovense* was displayed by the decrease in the OTA concentration produced by *P. verrucosum* in the dry-cured fermented sausage “salchichón” [25].

Table 1. Main effects of biocontrol agents on toxigenic molds in ripening food matrices of animal origin.

Ochratoxin A (OTA) Producers				
Matrix	Mold	Biocontrol Agent	Effect on Growth or Mycotoxin Production	Reference
Dry-cured fermented sausage medium	<i>Penicillium nordicum</i>	<i>Enterococcus faecium</i>	↓ ¹ OTA ↓ Growth	[14]
Dry-cured ham based medium	<i>P. nordicum</i>	<i>Staphylococcus xylosus</i>	↓ OTA ↓ Growth	[17]

Ochratoxin A (OTA) Producers				
Matrix	Mold	Biocontrol Agent	Effect on Growth or Mycotoxin Production	Reference
Fermented meat model system	<i>P. nordicum</i> and <i>Penicillium verrucosum</i>	<i>Lactiplantibacillus plantarum</i> and <i>Pediococcus pentosaceus</i>	↓ Growth	[19]
Ham, industrial and traditional dry-cured sausages	<i>P. nordicum</i> and <i>Aspergillus westerdijkaiae</i>	<i>Candida zeylanoides</i> and <i>Rothia mucilaginosa</i>	↓ OTA ↓ Growth	[16]
Speck	<i>P. nordicum</i> and <i>Aspergillus ochraceus</i>	<i>Debaryomyces hansenii</i> and <i>Saccharomycopsis fibuligera</i>	↓ OTA ↓ Growth	[11]
Dry-cured fermented sausages	<i>P. nordicum</i>	<i>D. hansenii</i>	↓ OTA	[10][18][20]
Dry-cured meat model systems	<i>P. verrucosum</i> , <i>P. nordicum</i>	<i>D. hansenii</i>	↓ OTA ↓ Growth	[13][21]
Dry-cured fermented sausage medium	<i>A. westerdijkaiae</i>	<i>D. hansenii</i>	↓ OTA	[12]
Dry-cured ham based medium	<i>P. nordicum</i>	<i>Penicillium chrysogenum</i>	↓ OTA	[15]
Meat model system	<i>P. nordicum</i>	<i>P. chrysogenum</i>	↓ OTA ↓ Growth	[23]
Dry-cured Iberian ham	Wild mycobiota containing OTA producer strains	<i>P. chrysogenum</i>	↓ OTA	[24]
Dry-cured fermented sausage	<i>P. verrucosum</i>	<i>Penicillium nalgiovense</i>	↓ OTA	[25]
Dry-cured fermented sausage medium	<i>P. nordicum</i>	Rosemary and oregano leaves	↓ OTA	[22]
Dry-cured fermented sausage medium	<i>P. nordicum</i>	Smoked paprika "pimentón"	↓ OTA	[26]
Dry-cured fermented sausages	<i>P. nordicum</i>	Rosemary leaves	↓ OTA	[20]
Dry-cured fermented sausage medium	<i>P. nordicum</i>	Rosemary leaves and rosemary essential oil (EO)	↓ OTA	[27]
Cheeses	<i>P. nordicum</i>	<i>Lactobacillus buchneri</i> and	↓ OTA	[28]

Ochratoxin A (OTA) Producers				
Matrix	Mold	Biocontrol Agent	Effect on Growth or Mycotoxin Production	Reference
<i>Lactiseibacillus casei</i>				
Aflatoxins (AFs) producers				
Dry-cured ham based medium	<i>Aspergillus flavus</i>	<i>S. xylosum</i>	↓ AFB1 ↓ Growth	[17]
Dry-cured ham based medium	<i>Aspergillus parasiticus</i>	<i>S. xylosum</i>	↓ AFB1 and AFG1 ↓ Growth	[17]
Fermented meat model system	<i>A. flavus</i> and <i>A. parasiticus</i>	<i>L. plantarum</i> and <i>P. pentosaceus</i>	↓ Growth	[19]
Dry-cured fermented sausages slices	<i>A. parasiticus</i>	<i>D. hansenii</i> + PgAFP + <i>Pediococcus acidilactici</i>	↓ AFB1 and AFG1 ↓ Growth	[29]
Dry-cured fermented sausages; dry-cured ham	<i>A. parasiticus</i>	<i>D. hansenii</i>	↓ AFB1 and AFG1	[30]
Dry-cured meat model system	<i>A. parasiticus</i>	Smoked paprika“pimentón”	↓ AFB1 and AFG1	[26]
Cheese slices	<i>A. parasiticus</i>	<i>D. hansenii</i> + PgAFP + <i>P. acidilactici</i>	↓ AFG1 ↓ Growth	[29]
Minas cheese formulation	<i>A. flavus</i>	<i>Origanum vulgare</i> EO	↓ Spores germination	[31]
Cheese slices	<i>A. flavus</i>	Parsley EO	↓ Growth	[32]
Kashar cheese	<i>A. parasiticus</i>	Thymol	↓ Growth	[33]
Cyclopiazonic acid A (CPA) producers				
Dry-cured ham-based model	<i>Penicillium griseofulvum</i>	<i>S. xylosum</i>	↓ CPA	[17]
Fermented meat model system	<i>P. griseofulvum</i> and <i>Penicillium commune</i>	<i>Lc. plantarum</i> and <i>P. pentosaceus</i>	↓ Growth	[19]
Dry-fermented sausage-based medium; dry-fermented sausages	<i>P. griseofulvum</i>	PgAFP	↓ CPA	[34]

reducing pimentón” to a meat-based medium decreased the amount of OTA produced by *P. nordicum* [26][39]. Rosemary leaves were able to decrease the mycotoxin produced by *P. nordicum* during dry-cured sausage ripening and, together with their essential oil, the OTA produced by *A. westerdijkiae* in a dry-cured fermented sausage based medium [20][27]. However, the sensorial impact of these biocontrol agents (BCAs) was not checked, although the concentrations of herb leaves used were expected to have no negative influence on consumer perceptions [20][39]. Additionally, other

Ochratoxin A (OTA) Producers				
Matrix	Mold	Biocontrol Agent	Effect on Growth or Mycotoxin Production	Reference
Edam cheese	<i>P. commune</i>	Clove, thyme, red thyme, litsea, cumin and marjoram eOs	↓ Growth [5][28][42][43]	[35]
Citrinin (CIT) producer				
Arzúa-Ulloa cheese	<i>Penicillium citrinum</i>	Eugenol and thymol	↓ CIT	[36]
Iranian and mozzarella cheeses	<i>P. citrinum</i>	<i>Zataria multiflora</i> Boiss EO	↓ CIT	[37][38]

Certainly, *Penicillium commune* cheese, showed the ability of these LAB to decrease the growth of *P. nordicum* in MRS agar, suggesting a possible future use as preservative agents during cheese manufacturing [44]. The lack of studies about the biocontrol of ochratoxinogenic molds in cheese systems is a new field of study necessary to reduce the risk posed by OTA presence in these ripened products.

3. Biocontrol Strategies against Aflatoxin-Producing Molds

AFs are highly toxic secondary metabolites produced by molds such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* [45]. Although these fungi are more frequent in cereal crops, they can colonize the surface of ripening products of animal origin [46][47][48]. The most important AFs are B1, B2, G1 and G2. These mycotoxins are carcinogenic (Group 1) and mutagenic for animals and humans according to the IARC [7].

Similar to the strategies employed against ochratoxigenic molds, different LAB, GCC+ and yeasts have been studied as BCAs against AF producers in dry-cured meat products. *S. xylosus* Sx8 was able to control the growth and the AFB1 produced by *A. flavus* and AFB1 and AFG1 produced by *A. parasiticus* in a dry-cured ham-based medium at three different temperatures (15, 20 and 25 °C) [17]. The BFS extract from *L. plantarum* and *Pediococcus pentosaceus* reduced the growth of *A. flavus* and *A. parasiticus* in a meat model system by up to 50% using concentrations between 21 and 43 g L⁻¹ [19]. *D. hansenii* combined with the antifungal protein PgAFP and *Pediococcus acidilactici* on slices of dry-cured fermented sausages successfully diminished AFB1 and AFG1 amounts produced by *A. parasiticus* and the mold counts [29]. In another study, *D. hansenii* was tested against *A. parasiticus* and decreased the AFB1 in more than 53.85% and the AFG1 by up to 59.06% in dry-fermented sausages, while the AFG1 was below the limit of quantification in dry-cured ham [30].

Regarding the agents of plant origin, the smoked paprika “pimentón” reduced the AFB1 and AFG1 production by *A. parasiticus* in a dry-cured meat model system, although they did not decrease the mold’s growth [26].

The presence of AFs in cheeses has been described worldwide. In addition to the AFM1 that can be present in the milk used for cheese manufacturing, common aflatoxins have been detected due to the surface colonization of the product by aflatoxigenic molds [4][49][50][51]. Despite the risk that AFs pose in cheeses, there are only a few studies based on the biocontrol of AFs in this matrix. In cheese slices, the use of the protein PgAFP combined with *D.*

hansenii in the presence or absence of *P. acidilactici* decreased the *A. parasiticus* growth and its AFG1 production below the method's limit of detection [29]. On the other hand, the addition of *Oreganum vulgare* EO (0.02% v/v) to the Minas cheese formulation inhibited the germination of spores of *A. flavus* for up to 15 days of ripening, and the cheese flavor and taste were accepted by the panelists [31]. Moreover, Vitalini et al. [32] demonstrated that parsley EO applied on cheese slices was effective in preventing *A. flavus* growth. Tatlisu et al. [33] demonstrated the antifungal activity of thymol (main component of numerous EOs) and nanofibers with thymol applied to "kashar" cheese cube surfaces against *A. parasiticus*, although no sensory analyses were performed [33].

4. Biocontrol Strategies against Cyclopiazonic Acid-Producing Molds

CPA can be produced by different molds, such as *A. flavus*, *A. parasiticus* and *Penicillium griseofulvum*, in ripened meats, and mainly *Penicillium commune*, *Penicillium roqueforti* and *Penicillium camemberti* in cheeses [2][47][52]. Due to the little amount of toxicological data, the IARC has not declared an acceptable CPA toxicity level yet, but it is well known that it includes severe gastrointestinal and neurological disorders and organ necrosis [53]. Therefore, this lack of data and legal limits results in a shortage of studies about the biocontrol of molds that only produce CPA.

Concerning the biocontrol studies in dry-cured meat products, the bacterium *S. xylosus* Sx8 decreased CPA production using two strains of *P. griseofulvum* grown in a dry-cured ham-based medium after 30 days of incubation at 25 °C [17]. Concentrations of 85 g L⁻¹ of BFS extract from *L. plantarum* and between 21 and 85 g L⁻¹ from *P. pentosaceus* did not allow for the growth of *P. griseofulvum* and *P. commune* in a meat model system [19]. Moreover, Delgado et al. [34] showed that *P. chrysogenum*, producer of the antifungal protein PgAFP, was able to diminish CPA amounts produced by *P. griseofulvum* under the limit of detection on a dry-fermented sausage-based medium and more than 97% on dry-cured fermented sausages after 21 days following industrial ripening [34].

In Edam cheeses, the clove, thyme, red thyme and litsea EOs completely inhibited the growth of two CPA producer strains of *P. commune*, while cumin and marjoram EOs showed high antifungal activity, although they did not totally inhibit the growth of the molds [35]. In this research, the evaluators recognized some EOs in sensory evaluation via the triangle test, but they did not have a negative effect on the taste and smell of the treated cheeses [35].

5. Biocontrol Strategies against Sterigmatocystin-Producing Molds

STG is a precursor of AFB1, so the producing molds mainly include different *Aspergillus* species, although there are other ones such as *Eurotium*, *Fusarium* and *Podospora* spp., which demonstrate an ability to produce this mycotoxin [54]. Concerning its toxicity, it has been found that STG induces tumors in animals and humans [54]. In spite of this evidence, the IARC only classified STG into Group 2B (possible human carcinogen) [55].

Regarding the biocontrol strategies, the BCA against AF producers could be applied for the toxigenic molds which produce both mycotoxins (AFs and STG), but STG production deserves to be further studied. Within the ripened animal products, STG has been mainly described in a wide range of cheeses contaminated with *Aspergillus versicolor*, *A. flavus* or *A. parasiticus* [54][56]. However, there are no studies of ripened matrices of animal origin based on the biocontrol of molds that only produce STG, although EOs from tarragon, oregano and savory showed the inhibition of two producers of STG isolated from cheeses, *Aspergillus puulaauensis* and *Aspergillus jenseii* [57].

6. Biocontrol Strategies against Citrinin-Producing Molds

Citrinin (CIT) is produced by different species of *Penicillium* and *Aspergillus*, including *P. cambemberti*, *Penicillium expansum*, *P. verrucosum*, *Penicillium citrinum*, *Penicillium viridicatum* *Aspergillus carneus* and *Aspergillus niveus* [4]. Several studies have shown frequent cooccurrence of OTA and CIT in dry-cured meat products and cheeses [3][58]. CIT is nephrotoxic and hepatotoxic to humans and has been classified into Group 3 by the IARC [55] due to evidence of its in vivo carcinogenicity [59][60].

Given that some toxigenic strains can produce both OTA and CIT, the strategies to prevent OTA producers may also be effective for CIT accumulation. However, it must be considered that some molds can switch the production of OTA to CIT or vice versa to deal with different stressful environments [61][62]. Therefore, different strategies might be needed for reducing both mycotoxins. To our knowledge, there are no studies only focused on the biocontrol of this mycotoxin in dry-cured meat products. However, in cheeses a concentration of 150 $\mu\text{g mL}^{-1}$ of eugenol and thymol inhibited CIT production by *P. citrinum* in “Arzúa-Ulloa” cheese, while in “Cabreriro” cheese these antifungal agents did not affect the CIT amounts [36]. In other studies, the *Zataria multiflora* Boiss EO decreased the growth and CIT production by *P. citrinum* in Iranian cheese and mozzarella [37][38]. Despite the use of EOs and compounds with a strong flavor in the above-mentioned studies, only the organoleptic effect of *Zataria multiflora* Boiss EO was tested. Concentrations over 600 ppm, which were more effective against CIT production, were disliked by the consumers and, consequently, their applications were limited [38].

Despite the studies about the presence of mycotoxins in ripened products of animal origin, no notifications were made regarding the presence of mycotoxins in both meat and dairy products (RASFF).

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