PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ
ESCOLA DE CIÊNCIAS DA VIDA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

KARLA CAROLINA PAIVA BOCATE

COMPOSTOS NATURAIS COMO AGENTES ANTIFÚNGICOS E DESCONTAMINANTES DE MICOTOXINAS EM MILHO ESTOCADO

(Natural compounds as antifungals and decontaminants of mycotoxins in stored corn)

CURITIBA
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Tese apresentada ao Programa de Pós-Graduação em Ciência Animal, área de concentração Saúde, Tecnologia e Produção Animal, da Escola de Ciências da Vida da Pontifícia Universidade Católica do Paraná, para obtenção do título de Doutor em Saúde, Tecnologia e Produção Animal Integrada.

Orientador(a): Prof. Dr. Fernando Bittencour Luciano

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ATA Nº 021 E PARECER FINAL DA DEFESA DE TESE DE DOUTORADO EM CIÊNCIA ANIMAL DA ALUNA KARLA CAROLINA PAIVA BOCATE

Aos dezenove dias do mês de agosto do ano de dois mil e vinte, às 13:30 horas, realizou-se a sessão pública de defesa da tese da doutoranda Karla Carolina Paiva Bocate, intitulada: **"Compostos Naturais como Agentes Antifúngicos e Descontaminantes de Micotoxinas em Milho Estocado"**. A doutoranda concluiu os créditos exigidos para obtenção do título de Doutora em Ciência Animal, segundo os registros constantes na secretaria do Programa. Os trabalhos foram conduzidos pelo Professor Orientador e Presidente da banca, Dr. Fernando Bittencourt Luciano (PUCPR), auxiliada pelos Professores Doutores Leandro Batista Costa (PUCPR), Claudia Turra Pimpão (PUCPR), Luciano Panagio (UEL) e Taiana Denardi de Souza (FURG). Procedeu-se à exposição da tese, seguida de sua arguição pública e defesa. Encerrada a fase, os examinadores expediram o parecer final sobre a tese, que foi considerada **APROVADA.**

MEMBROS	**ASSINATURA**
Prof Dr Fernando Bittencourt Luciano (Presidente)	
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Proclamado o resultado, a Presidente da Banca Examinadora encerrou os trabalhos, e para que tudo conste, eu Aline Francielle Bueno Rétzlaff, confiro e assino a presente ata juntamente com os membros da Banca Examinadora.

Curitiba, 19 de agosto de 2020.

Aline Francielle Bueno Rétzlaff
Secretária do Programa de Pós-Graduação em Ciência Animal

Profa. Dra. Renata Ernlund Freitas de Macedo
Coordenadora do Programa de Pós-Graduação em Ciência Animal

Dedicatória

Dedico esta tese aos meus pais e amigos que sempre me apoiaram,
sem eles eu não teria chego até aqui.

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FORMATO DA TESE

A presente tese é composta por capítulos. O capítulo 1 apresenta uma introdução geral, a contextualização do tema, justificativa e os objetivos de estudo. O capítulo 2 trata-se de artigo científico completo, contendo referências, e formatado nas normas da revista para o qual foi submetido. Os capítulos 3 e 4 tratam de artigos científicos completos, contendo referências, e formatados nas normas das revistas para os quais serão submetidos. O capítulo 5 finaliza esta tese com conclusões gerais e impacto e perspectivas do trabalho. As referências do capítulo 1 encontram-se ao final da tese.

RESUMO GERAL

A contaminação dos alimentos e grãos por micotoxinas gera perdas econômicas e nutricionais de grande magnitude, pois são substâncias tóxicas a saúde humana e animal. Sendo assim, métodos eficazes de descontaminação e prevenção são importantes e necessários. O objetivo deste estudo foi avaliar a capacidade antifúngica e antimicotoxigênica do isotiocianato de alila (ITCA), cinamaldeído (CIN) e óleo essencial de alho (OEA), avaliando a interação entre ITCA e CIN. Foi também avaliado o uso destes compostos como agentes fumigantes em grãos de milho estocados por 30 dias, com a finalidade de inibir ou reduzir o crescimento fúngico e a produção de micotoxinas. A capacidade do OEA em reduzir *in vitro* as micotoxinas e a bioacessibilidade das mesmas após processo de digestão simulada de suínos foi também analisada. Determinou-se a concentração inibitória mínima (CIM) dos compostos em microdiluição e a interação entre ITCA e CIN, como também a atividade em fase gasosa, utilizando a metodologia de avaliação de inibição radial de crescimento fúngico e a inibição do crescimento e produção de aflatoxinas (AFs), fumonisina (FB) e zearalenona (ZEA) em grãos de milho. Com o OEA foi também avaliado a capacidade de reação direta das micotoxinas (AFs, FB e ZEA) com o óleo e a capacidade de redução das mesmas quando realizado o processo de digestão simulada *in vitro* de suínos. Todos os compostos foram capazes de inibir todas as cepas toxigênicas quando testados em fase líquida. Para a determinação da CIM com relação ao ITCA e CIN, foram encontradas concentrações de 0,076, 0,605 e 0,303 mM de ITCA e 0,246, 1,964 e 0,982 mM de CIN para *A. parasiticus*, *F. verticillioides* e *G. zeae*, respectivamente. Quando utilizados em conjunto (ITCA + CIN), foram necessários 0,038 mM de ITCA e 0,061 mM de CIN para inibir *A. parasiticus*; 0,038 mM de ITCA e 1,964 mM de CIN para *F. verticillioides*; e 0,076 mM e 0,246 mM para inibir *G. zeae*. Na fase gasosa, combinações de 0,62, 0,47 e 0,23 µL / L (proporção 1:5 ITCA:CIN) foram capazes de reduzir significativamente o crescimento de *A. parasiticus, G. zeae* e *F. verticillioides* em placas de Petri, respectivamente, com inibição total em 1,25, 0,94 e 0,94 µL/L. A mesma razão combinatória (1:5) foi utilizada para avaliar o efeito dos óleos nos grãos de milho, nas doses de 30 e 300 µL/L. Uma redução significativa na população de fungos foi encontrada em 30 µL/L para todas as

espécies. Além disso, 300 µL/L reduziram a população de todas as espécies abaixo do limite de detecção (LD = 1,22 Log UFC/g). A produção total de aflatoxina também foi reduzida, na qual o grupo controle apresentou 32,25 ± 5,86 µg/kg de AFs totais, 30 µL/L apresentaram 2,18 ± 1,72 µg/kg e 300 µL/L apresentaram 2,61 ± 1,38 µg/kg. O OEA apresentou CIM de 7.8, 15.62, 62.5 e 7.8 µL/L para *A. parasiticus*, *A. flavus*, *F. verticillioides* e *G. zeae*, respectivamente. Com relação a concentração fungicida mínima CFM foram encontrados valores 4x maiores para *A. parasiticus*, *F. verticillioides* e *G. zeae* e 32x maior para *A. flavus.* O crescimento radial fúngico foi completamente reduzido com uma faixa de 2 - 10 µL/L de OEA. A população de *A. parasiticus* e *G. zeae* foi reduzida abaixo do limite de detecção e *F. verticillioides* a população diminuiu em 2,33 Log UFC/g após 30 d de armazenamento quando utilizado 1000 µL/L de OEA. Os testes de reação direta e digestão *in vitro* utilizando OEA não evidenciaram redução das micotoxinas. Em relação ao OEA contra *A. flavus*, uma redução significativa ($p<0,01$ ou 5) na população do fungo foi encontrada em todas as concentrações testadas, além disso, 300 µL/L reduziram a população fúngica abaixo do LD (1,22 Log UFC/g). A produção das aflatoxinas B1 e B2 foram significativamente reduzidas ($p<0,01$ ou 5) utilizando 300 µL/L de OEA. O presente estudo apresentou a eficácia do ITCA + CIN e do OEA em diminuir o crescimento fúngico e a produção de micotoxinas em grãos de milhos estocados. A dose de 300 µL/L de ITCA + CIN apresentou elevada eficácia em reduzir a contaminação fúngica e das aflatoxinas analisadas. Com relação ao OEA concentrações de 1000 µL/L se mostraram eficazes, porém para uma importante cepa toxigênica como *A. flavus*, a concentração de 300 µL/L foi capaz de reduzir a população fúngica abaixo do limite de detecção e a produção das aflatoxinas B1 e B2 reduzida após 15d de armazenamento nessa concentração. Dessa forma, estes compostos podem ser utilizados como novos agentes fumigantes para evitar a contaminação por fungos e suas micotoxinas em milho estocado.

Palavras-chave: Óleos essenciais; Grãos; Fungos; Contaminante.

ABSTRACT

The contamination of food and grains by mycotoxins generates economic and nutritional losses of great magnitude, as they are toxic to human and animal health. Therefore, effective methods of decontamination and prevention are important and necessary. The objective of this study was to evaluate the antifungal and antimicotoxygenic capacity of allyl isothiocyanate (AITC), cinnamaldehyde (CIN) and garlic essential oil (GEO), evaluating the interaction between AITC and CIN. The use of these compounds as fumigating agents in stored corn kernels for 30 days was also evaluated, in order to inhibit or reduce the fungal growth and mycotoxin production. The ability of the GEO to reduce mycotoxins *in vitro* and their bioaccessibility after a simulated swine digestion process was also analyzed. The minimum inhibitory concentration (MIC) of the compounds in microdilution and the interaction between AITC and CIN were determined, as well as the gas phase activity, using the methodology of radial inhibition of fungal growth, and the inhibition of growth and production of aflatoxins (AFs), fumonisin (FB) and zearalenone (ZEA) in corn kernels. With the GEO, the ability of mycotoxins (AFs, FB and ZEA) to react directly with oil was also evaluated, as well as their ability to reduce them when the simulated *in vitro* digestion process of swine was carried out. All compounds were able to inhibit all toxigenic strains when tested in the liquid phase. For the determination of MIC in relation to AITC and CIN, concentrations of 0.076, 0.605 and 0.303 mM of AITC and 0.246, 1.964 and 0.982 mM of CIN were found for *A. parasiticus*, *F. verticillioides* and *G. zeae*, respectively. When used together (AITC + CIN), 0.038 mM AITC and 0.061 mM CIN were needed to inhibit *A. parasiticus*; 0.038 mM AITC and 1,964 mM CIN for *F. verticillioides*; and 0.076 mM and 0.246 mM to inhibit *G. zeae*. In the gas phase, combinations of 0.62, 0.47 and 0.23 µL / L (ratio 1: 5 AITC: CIN) were able to significantly reduce the growth of *A. parasiticus*, *G. zeae* and *F. verticillioides* in plates petri, respectively, with total inhibition at 1.25, 0.94 and 0.94 µL / L. The same combinatorial ratio (1: 5) was used to evaluate the effect of oils on corn kernels, at doses of 30 and 300 µL / L. A significant reduction in the population of fungi was found by 30 µL / L for all species. In addition, 300 µL / L reduced the population of all species below the detection limit (LD = 1.22 Log UFC / g). Total

aflatoxin production was also reduced, in which the control group had 32.25 ± 5.86 µg / kg of total AFs, 30 µL / L showed 2.18 ± 1.72 µg / kg and 300 µL / L showed 2.61 ± 1.38 µg / kg. The GEO presented MICs of 7.8, 15.62, 62.5 and 7.8 µL / L for *A. parasiticus*, *A. flavus*, *F. verticillioides* and *G. zeae*, respectively. Regarding the minimum fungicidal concentration (CFM) values 4x higher for *A. parasiticus*, *F. verticillioides* and *G. zeae* and 32x higher for *A. flavus* were found. The fungal radial growth was completely reduced with a range of 2 - 10 µL / L of GEO. The population of *A. parasiticus* and *G. zeae* decreased below the detection limit and *F. verticillioides* decreased the population by 2.33 Log CFU / g after 30 days of storage when 1000 µL / L of GEO was used. Direct reaction tests and *in vitro* digestion using GEO did not show a reduction in mycotoxins. In relation to GEO against *A. flavus*, a significant reduction ($p < 0.01$ or 5) in the population of the fungus was found in all tested concentrations, in addition, 300 µL / L reduced the fungal population below the detection limit (LD = 1.22 Log UFC / g). The production of aflatoxins B1 and B2 was significantly reduced ($p < 0.01$ or 5) using 300 µL / L of GEO. The present study showed the effectiveness of AITC + CIN and GEO in decreasing fungal growth and mycotoxin production in stored corn kernels. The 300 µL / L dose of AITC + CIN was highly effective in reducing fungal contamination and the aflatoxins analyzed. With regard to GEO, concentrations of 1000 µL / L have been shown to be effective, but for an important toxigenic strain such as *A. flavus*, the concentration of 300 µL / L was able to reduce the fungal population below the detection limit and the production of aflatoxins B1 and B2 were reduced after 15d of storage at that concentration. Thus, these compounds can be used as new fumigants to prevent contamination by fungi and their mycotoxins in stored corn.

Keywords: Essential oils; Grains; Fungi; Contaminant.

CAPÍTULO 1

1. INTRODUÇÃO E CONTEXTUALIZAÇÃO

A contaminação dos alimentos por fungos pode ocorrer durante várias etapas de produção, sendo o mais agravante durante sua armazenagem, resultando em perdas econômicas, perda da qualidade dos alimentos, redução na disponibilidade de nutrientes e na geração de micotoxinas (Garcia et al., 2019).

Os principais gêneros de fungos formadores de micotoxinas em alimentos e rações são *Aspergillus, Penicillium, Fusarium* e *Alternaria*, podendo causar intoxicações em animais e seres humanos (Pitt e Hocking, 2009). As micotoxinas são estruturas de baixo peso molecular, agrupadas de acordo com o grau de toxicidade ao ser humano e outros animais, principalmente vertebrados. Segundo a *Internacional Agency for Research on Cancer* (IARC, 1993; 2002; 2012), as micotoxinas são consideradas carcinogênicas, nefrotóxicas, hepatotóxicas, genotóxicas, citotóxicas e imunotóxicas a vertebrados. As principais toxinas encontradas em alimentos são aflatoxinas, fumonisinas, ocratoxina A, desoxinivalenol, zearalenona, patulina, nivalenol, toxinas T-2 e HT-2.

O Brasil é um dos maiores países produtores e exportadores de grãos no mundo. A produção de milho na safra 2018-2019 mostrou que os Estados Unidos era o principal produtor com 366,6 milhões de toneladas, seguido pela China com 257,7 milhões de toneladas e o Brasil com 94,5 milhões de toneladas (Statista, 2019). No estado do Paraná, a produção de grãos é uma das principais atividades econômicas, sendo o estado responsável pela produção de 24,1 milhões de toneladas de milho na safra 2019/2020 (DERAL, 2020).

O processamento inadequado, estocagens e empacotamento dos grãos, alteram negativamente sua qualidade (Pereira et al., 2014). Métodos físicos, químicos e biológicos têm o objetivo de reduzir a concentração de micotoxinas nos grãos, além disso, o manejo correto no armazenamento dos grãos é recomendado (Varga et al., 2010).

Devido a grande contaminação de grãos com micotoxinas e a baixa efetividade dos compostos comumente utilizados, novas tecnologias vêm sendo pesquisadas. Uma delas é o uso de compostos naturais, como os óleos essenciais,

devido a sua segurança e aceitação dos consumidores pois possuem um status de *GRAS* (*Generally Recognized as Safe*) (Chen et al., 2014).

Nosso grupo de pesquisa demonstrou inibição microbiana utilizando o isotiocianato de alila (ITCA), principalmente a fumigação do composto, com significativa redução de fungos toxigênicos (Nazareth et al., 2016). Outros compostos de origem vegetal também têm demonstrado potencial antifúngico contra cepas produtoras de micotoxinas, como o cinamaldeído (CIN), componente principal do óleo essencial de canela (Dal Pozzo, 2011). A alicina, composto majoritário do óleo essencial de alho, apresenta atividade antimicrobiana relatada em diversos estudos na literatura (Wallock – Richards et al., 2014).

Diante da escassez de técnicas que promovam uma descontaminação dos grãos de maneira direta ou profilática, esta tese teve como objetivo analisar a atividade antifúngica do óleo essencial de alho (OEA), isotiocianato de alila (ITCA) e cinamaldeído (CIN), avaliando a interação entre ITCA e CIN. Além disso, o presente estudo propôs a utilização inédita do OEA, contendo 60% de alicina em sua composição, e o uso combinado de ITCA e CIN como agente fumigante em grãos de milho, com a finalidade de inibir ou reduzir o crescimento de fungos toxigênicos e suas micotoxinas. A capacidade do OEA em reduzir *in vitro* as micotoxinas e a bioacessibildade das mesmas após processo de digestão simulada também foi avaliada.

1.1. CONTAMINAÇÃO DO MILHO POR FUNGOS TOXIGÊNICOS

O milho é um grão importante devido à sua versatilidade de uso, sendo utilizado no consumo humano em sua forma bruta ou em produtos à base de milho, como ingrediente principal nas formulações de ração animal, principalmente para as indústrias avícola, suinícola e pecuária leiteira (Ageitec, 2014). Segundo o DERAL (Departamento de Economia Rural), a safra paranaense 2019/20 poderá produzir 24,1 milhões de toneladas de milho, em uma área de 6 milhões de hectares plantados. O Paraná possui uma posição geográfica favorável para o plantio deste grão, devido ao clima e qualidade do solo propício para o cultivo. Em contrapartida, o clima úmido e com temperaturas médias entre 20 e 30 ^{o}C é um fator chave ao

crescimento fúngico tanto no plantio quanto na estocagem.

O milho é um substrato perfeito para contaminação fúngica, uma vez que o amido é o componente principal do grão (Bankole e Adebanjo, 2003). A contaminação pode ocorrer em vários pontos da linha de produção, desde a pré-colheita dos grãos, até o processamento, armazenamento e o empacotamento (Pereira et al., 2014). Geralmente, os cereais são armazenados erroneamente, em locais de alta temperatura e umidade, onde propiciam o crescimento de fungos e produção de micotoxinas. Além disso, se não forem tomadas medidas específicas durante o transporte, pode ocorrer um estresse oxidativo aos microrganismos produtores de toxina, potencializando a sua produção (Bennett e Klick, 2003, Prakash et al., 2015). Produtos farináceos e grãos, quando contaminados com micotoxinas, perdem características nutricionais, o que reduz seu valor de mercado. Dentre as principais culturas, o milho é o mais suscetível à contaminação por micotoxinas (Chulze, 2010).

Os gêneros *Fusarium* e *Aspergillus* são os mais prevalentes como contaminantes em milho, sendo que a espécie *Fusarium graminearum* ocorre com maior frequência (Casa et al., 2007). Além disso, *Aspergillus flavus e Penicillum sp* são frequentemente encontrados durante a estocagem de milho (Henning et al., 2011).

1.1.1. Características do gênero *Aspergillus*

O gênero *Aspergillus,* descrito pela primeira vez em 1729, é caracterizado pela alta diversidade de espécies e por estar presente em habitats diversos. O gênero apresenta aplicações significativas em relação a produção de antibióticos, medicamentos diversos e enzimas de interesse biotecnológico (Samson, 2014).

1.1.1.1. *Aspergillus flavus* e *Aspergillus parasiticus*

A. flavus é um fungo presente em solos e contamina sementes na pré e principalmente na pós-colheita, produzindo as aflatoxinas B_1 e B_2 (Amaike e Keller, 2011). *A. flavus* é a principal espécie do gênero *Aspergillus* contaminante de milho e,

subsequentemente, produtor de aflatoxinas nestes grãos (Dadzie et al., 2019). *A. parasiticus*, é um organismo saprófito, com alta capacidade de degradação de polímeros como celulose, pectina e lignina. A espécie é relatada em diversos biomas (ex. florestas, desertos), podendo incidir em regiões tropicais, subtropicais, e em locais de clima temperado (Sebők et al., 2016). É uma espécie produtora de aflatoxinas B_1, B_2, G_1 e G_2, diferentemente da espécie *A. flavus* que produz apenas as micotoxinas B_1 e B_2 (Copetti et al., 2011*).* Em milho, *A. flavus* e *A. parasiticus* causam uma doença conhecida como podridão de orelha (Muller and Wise, 2014).

1.1.2. Características do gênero *Fusarium*

Fusarium é um gênero que causa doenças em plantas e produz diversas micotoxinas (Summerell, 2010). Pode ser encontrado em locais com climas tropicais e subtropicais, podendo sobreviver por longos períodos no solo, além de colonizar ramos, flores, inflorescências e frutos. As espécies de *Fusarium* são comumente relacionadas à contaminação pré-colheita das culturas de cereais, como o milho, sendo responsáveis pela morte de mudas, aborto e prodridão de sementes, ferrugem, clorose, murcha vascular e redução do crescimento em uma variedade de plantas hospedeiras (Nagaraja et al., 2016).

Espécies de *Fusarium* podem produzir micotoxinas como fumonisinas (FBs), zearalenona (ZEA), desoxinivalenol (DON), nivalenol (NIV), toxina T-2 (T-2) e outros menores como enniatinas (ENNs), beauvericina (BEA), moniliformina (MON) e fusaproliferina (FUS) (Smith et al., 2016).

1.1.2.1. *Fusarium graminearum*

F. graminearum é o patógeno primário que causa a podridão da orelha ou podridão do caule no milho (Ali et al., 2005; Yang, Balint-Kurt e Xu, 2017). Esse fungo é mais comum em áreas frias e úmidas e é visto como um mofo rosa avermelhado. A ingestão de milhos infectados por *F. graminearum* é prejudicial para os seres humanos e para o gado, devido à produção de diversas micotoxinas potentes, incluindo deoxinivalenol (DON) e zearalenona (ZEA) (Vigier et al., 2001; Duan et al., 2016).

1.1.2.2. *Fusarium verticillioides*

F. verticillioides também é uma espécie causadora de doenças em plantas, resultando em danos na produção de cereais e segurança alimentar, sendo conhecida, popularmente, com o agente causador da podridão da orelha do milho (Presello et al., 2008). Condições ambientais potencializam a ação do fungo, como a disponibilidade hídrica e de nutrientes no solo (Ramos et al., 2014). Esta espécie é produtora de fumonisinas principalmente em milhos no período pré e pós-colheita (Bacon, 2008).

1.2. MICOTOXINAS EM MILHO

As micotoxinas são metabólitos secundários fúngicos oriundos de um processo de seleção natural, conferindo uma vantagem seletiva ao microrganismo. A produção está relacionada a partir de alterações ambientais, geralmente desencadeada por condições de estresse (Magan e Aldred, 2007). As micotoxinas são encontradas em uma grande variedade de produtos alimentícios destinados ao consumo humano e de outros animais. Cerca de 400 micotoxinas foram identificadas, sendo a grande maioria estáveis a fatores físicos, como o calor (Berthiller et al., 2007). Diversos fatores físicos, biológicos e químicos favorecem a produção de micotoxinas, sendo demonstradas na Tabela 1.

Tabela 1: Principais características para a produção de micotoxinas fúngicas

	Ótimo para produção
Substrato	Elevado teor de carboidrato, principalmente em sementes oleaginosas.
Umidade	Umidade relativa acima de 80%.
Temperatura	Climas tropicais e subtropicais favorecem o crescimento.
Atmosfera	Maioria aeróbios.

Interação microbiana	Presença de outros organismos estimula a produção.

Fonte: (Taniwaki et al., 2013).

Cerca de 25% dos grãos em todo o mundo são afetados significativamente pelo crescimento fúngico e micotoxinas, incluindo amendoim, milho, feijão, arroz e trigo (Eskola, 2019). Cinco grupos principais de micotoxinas ocorrem nos alimentos: deoxinivalenol/nivalenol, zearalenona, ocratoxina, fumonisinas e aflatoxinas (Oliveira, 1997; Kempken e Rohlfs, 2010). As principais micotoxinas encontradas no milho são: aflatoxinas (AFs), fumonisinas (FUM), tricotecenos tipo A e B, zearalenona (ZEA), zearalenol, moniliformina e beauvericina (Tarazona et al., 2020). A produção de FUM e ZEA no milho é atribuída principalmente a *F. verticillioides* (FUM), *F. proliferatum* (FUM), *F. solani* (FUM) e *F. graminearum* (DON e ZEA) (Leslie e Summerell, 2006; Schollenberger et al., 2012). As AFs são produzidas principalmente por cepas de *A. flavus* (AFB1 e AFB2) e *A. parasiticus* (AFB1, AFB2, AFG1 e AFG2) (Tarazona et al., 2020).

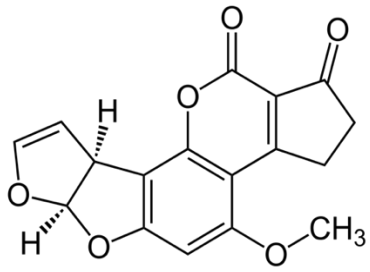
Muitos países estabeleceram níveis máximos toleráveis de micotoxinas em grãos de milho e produtos de grãos de milho. No Brasil, o limite tolerável de aflatoxinas e fumonisinas nos grãos de milho varia de um a 20 µg kg-1 e 200 a 5000 µg kg-1, respectivamente (ANVISA, 2011). Em 2006, a Comissão da Comunidade Europeia estabeleceu os níveis de aflatoxinas variando de cinco a 10 µg kg-1 para AFs e 200 a 2000 µg kg-1 para FMs em milho (EC, 2006). Por outro lado, os EUA permitem um limite de 20 µg kg-1 para AFB1 e um limite que varia de 2000 a 4000 µg kg-1 para FMs em milho (FDA, 2011).

1.2.1. Aflatoxinas em milho

As aflatoxinas são produzidas por cerca de 10 espécies do gênero, *Aspergillus* sendo *A. flavus* e *A. parasiticus* os maiores produtores (Pitt et al., 2012). Essas toxinas são altamente incidentes em alimentos, na qual as aflatoxinas B_1, B_2, G_1 e G_2 são consideradas as mais importantes na indústria de alimentos e na área clínica (Jay, 2005). Uma grande variedade de culturas, incluindo milho (*Zea mays L.)* são suscetíveis à infecção por fungos produtores de aflatoxina e subsequente

contaminação por aflatoxina no campo e após a colheita (Cotty e Jaime-Garcia, 2007). Outras toxinas são oriundas da modificação estrutural das aflatoxinas B. As aflatoxinas M_1 e M_2 são derivadas das toxinas B_1 e B_2, respectivamente, e sofrem alterações estruturais durante o processo de digestão no trato gastrintestinal dos animais ruminantes, na qual são detectadas no leite, secreções urinárias, intestinais e tecidos (Imamura et al., 2015). As aflatoxinas são inodoras e incolores, quimicamente estáveis em alimentos, termorresistentes, se mantendo estáveis a temperaturas acima de 150 ºC, sendo extremamente difíceis de serem removidas (Urrego, 2006). Segundo a *Internacional Agency for Research on Cancer* (IARC), as aflatoxinas são classificadas como grupo 1, ou seja, são potencialmente carcinogênicas. A aflatoxina B_1 (Figura 1) é um agente carcinogênico conhecido como hepatocarcinógeno mais potente em mamíferos. A aflatoxina G_1 é a segunda aflatoxina mais tóxica, estando ligada a relatos de câncer no pulmão e esôfago (Gonçalves, 2017).

Estudos realizados por Oliveira et al. (2017) mostrou que todas os milhos analisados coletados na região sul do Brasil estão contaminados com AFB1 e AFG1, sendo também encontrado em níveis acima do limite máximo tolerável que é de 20 µg/kg.



Fonte: Wikipedia

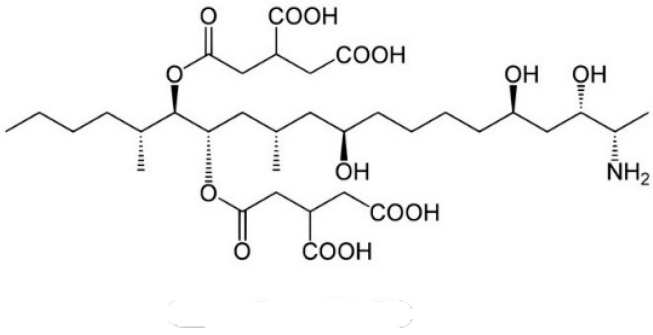
Figura 1: Estrutura molecular da aflatoxina B1.

1.2.2. Fumonisinas em milho

As fumonisinas (FBs) são micotoxinas produzidas por fungos do gênero *Fusarium*, especialmente *F. verticillioides e F. proliferatum,* e estão presentes no milho durante todo o ciclo de crescimento da cultura (Nesic et al., 2014) e

sintetizadas por essas espécies de fungos principalmente no estágio pré-colheita, caracterizado por condições de alta umidade (Munkvold, 2003). A temperatura ideal para a biossíntese da fumonisina está entre 25 e 30 °C (Marin et al., 1999). Entre as fumonisinas, a FB_1, FB_2 e FB_3 são as de maior importância toxicológica e frequência em alimentos (Marasas, 1996). A FB_1 (Figura 2) é o composto mais tóxico deste grupo, e responsável por 70% da contaminação de alimentos por FBs no mundo (Scott, 2012).

A FB_1 é encontrada frequentemente em milho e seus derivados, e também em arroz e trigo. A FB_1 está associada com leucoencefalomalácia equina (LEM) (Marasas et al., 1988), edema pulmonar em suínos (Osweiler et al., 1992), diminuição do ganho de peso em frangos e aumento de peso de órgãos como o fígado, proventrículo e moela (Ledoux et al., 1992). Em humanos o consumo de alimentos contaminados com FB_1 está relacionado à incidência de câncer esofágico, deficiências no tubo neural e nefropatia endêmica (Marasas 2001; Missmer et al. 2006). A IARC classificou a FB_1 como pertencente à classe 2B, ou seja, como possivelmente carcinogênico para humanos (IARC, 1993). Estudos realizados pela Biomin (2020), mostram que na América do Sul o milho está contaminado com fumonisina em 84% das amostras, com produção média de 3122 partes por bilhão (ppb).



Fonte: (Perincherry, Lalak-Kánkzugowskza e Stepien, 2019).

Figura 2: Estrutura química da fumonisina B1.

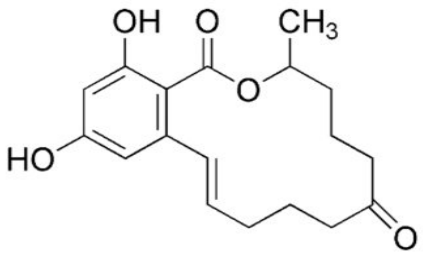
1.2.3. Zearalenona em milho

A zearalenona (ZEA) (Figura 3) é uma micotoxina estrogênica não-esteroidal

biossintetizada por alguns fungos do gênero *Fusarium,* especialmente *F. graminearum*. Os fungos produtores de ZEA contaminam, em sua grande maioria, o milho, mas também são encontrados em cevada, aveia, trigo, sorgo e arroz (Warth et al., 2013). ZEA é classificada segundo a IARC como pertencente ao grupo 3, ou seja, não-carcinogênica, mas é capaz de estimular o crescimento de células do câncer de mama em humanos (Ahamed et al., 2001), sendo também imunotóxica, genotóxica, capaz de induzir a formação de ligações químicas no DNA de culturas *in vitro* de linfócitos bovinos e fragmentação do DNA em células DOK, Vero e Caco-2 (Borchers et al., 2010; Ashiq et al., 2014).

ZEA e seus metabólitos por serem estrogênicos podem levar ao hiperestrogenismo, causando danos ao sistema reprodutivo de mamíferos. Os suínos são os animais mais afetados pela exposição a ZEA, por converterem mais facilmente ZEA a α-ZOL, aumentando a toxicidade (European Food Safety Authority – EFSA -, 2017). Os principais sintomas do hiperestrogenismo em suínos fêmeas são o aumento do útero e das glândulas mamárias, inchaço e vermelhidão da vulva, e em machos, atrofia testicular e redução do número de espermatozoides (Binder et al., 2017; Chen et al., 2015). Aves de corte e bovinos são muito mais resistentes a ação estrogênica de ZEA quando comparado aos suínos (EFSA, 2017).

A Autoridade Europeia para a Segurança dos Alimentos (EFSA) publicou um parecer científico sobre os riscos para a saúde pública em 2011 para a ZEA e propôs um IDT (ingestão diária tolerável) de 0,25 µg/kg massa corporal/dia baseando-se recentemente em dados sobre suínos, mas levando em consideração as comparações entre porcos e humanos (EFSA, 2011).



Fonte: (Nones e Scussel, 2013).

Figura 3: Estrutura química da zearalenona.

1.3. PROCESSOS DE DESCONTAMINAÇÃO

Os métodos propostos para o controle de micotoxinas são normalmente preventivos, incluindo boas práticas de manejo na agricultura e secagem adequada dos grãos após colheita, podendo também ser utilizado métodos químicos, físicos e biológicos. Entretanto, após a toxina estar presente no alimento, a sua descontaminação é muito difícil (Varga et al., 2010). A utilização de raios gama, X e ultravioleta são exemplos de métodos físicos. Os adsorventes de micotoxinas (argilas e bentonitas) são as substâncias mais comuns utilizadas para a descontaminação adicionadas à alimentação animal. Estes compostos são capazes de se ligar às toxinas no trato gastrintestinal e impedir a sua absorção sistêmica (Ramos et al., 1996; Magnoli et al., 2008). Apesar disso, até o momento, não há um método disponível que elimine as micotoxinas sem causar alterações no produto, e o uso desses adsorventes não é uma solução definitiva para o problema, pois possuem uma baixa eficiência dependendo do nível de contaminação e do tipo de micotoxina contaminante. Com relação a descontaminação química temos a amonização, na qual se utiliza amônia para detoxificação de milho contaminado e a ozonização, onde o gás ozônio age sobre um grande número de microrganismos (Varga et al., 2010).

Segundo a FAO/ONU (1997), os únicos compostos utilizados na indústria para inibir o crescimento fúngico em grãos são os ácidos propiônico, e sórbico (e seus sais), processo chamado acidificação. Contudo, esses compostos não são voláteis e sua aplicação é cara (necessita de altas doses) e dificultada em grandes volumes (homogeneidade e dispersão). A descontaminação biológica tem sido uma boa alternativa, onde microrganismos são capazes de degradar as micotoxinas em compostos não tóxicos (Wu, 2009).

1.4. COMPOSTOS NATURAIS

Recentemente há um grande interesse no uso de antimicrobianos naturais para o controle de patógenos em alimentos devido a sua aceitação e segurança para os consumidores, fazendo que muitos desses compostos recebam o status de GRAS (Chen et al., 2014). É de grande conhecimento que a maioria dos óleos

essenciais apresentam propriedades antimicrobianas atribuídas principalmente aos compostos bioativos presentes, sendo exercida, principalmente, por compostos terpenóides (Boyle, 1995; Mahmoud et al., 2002, Burt, 2004). Os óleos essenciais são oriundos do metabolismo secundário de plantas, e podem ser produzidos vários compostos biologicamente ativos, com ação fungicida, inseticida, citotóxica, antiviral, tranquilizante, analgésica, dentre outras. Esses óleos podem ser produzidos em diversas regiões da planta como: galhos, flores, frutos, caule, sementes, ficando armazenados em cavidades ou células epidérmicas, sendo liberados quando a planta sofre algum tipo de injúria, agindo como um mecanismo de defesa (Bajpai, 2012).

Um dos principais mecanismos de ação dos compostos voláteis, é devido a lipofilicidade, promovendo danos na membrana celular, tornando-a mais permeável, alterando o pH intracelular, desequilibrando o fluxo de elétrons e a homeostase celular, levando assim ao extravasamento do conteúdo citoplasmático e, consequentemente, a morte do agente toxigênico (Silveira et al., 2012).

1.5. COMPOSTOS NATURAIS CONTRA FUNGOS TOXIGÊNICOS

Diversas técnicas são utilizadas para a inibição do crescimento fúngico e a produção das toxinas. Nazareth et al. (2018) relatou redução significativa da população de fungos usando 50 µL/L de isotiocianato de alila (ITCA), após 180 dias em grãos de milho, diminuindo 3,17 log (UFC/g) e 3,9 log (UFC/g) de *A. parasiticus* CECT 2681 e *F. verticillioides* CECT 2983, respectivamente. Quiles et al. (2019) demonstrou redução significativa da população de *A. flavus* em milho tratado com ITCA; após 1 e 30 dias de tratamento a população reduziu 1,5 e 4,4 log (UFC/g), respectivamente.

A redução da produção de micotoxinas utilizando compostos naturais, principalmente os agentes voláteis, apresenta aplicação promissora. Lopes e colaboradores (2017) inocularam suspensões fúngicas de *Aspergillus parasiticus* em castanhas do Pará, o que reduziu o crescimento fúngico e a produção de aflatoxinas quando utilizado 2.5 µL/mL de ITCA em fumigação. Quiles et al., (2019), determinou a produção de micotoxinas após 30 dias de armazenamento em milho, *A. flavus*

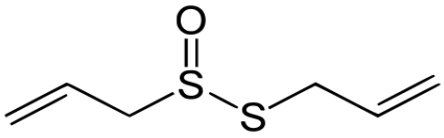
produziu no grupo controle 8.07 µg/kg de AFB1 no dia 30, com relação ao milho tratado com ITCA, o milho apresentou produção 0.12 µg/kg de AFB1 representando uma redução de 98,51%.

Bordin e colaboradores (2017) demonstraram a reação direta de zearalenona (ZEA) e α-zearalenol (α-ZEA) em diferentes tempos (0, 4, 8, 24 e 48 horas) com o ITCA, quando utilizado em diferentes concentrações (2, 20, 100 e 200 mM). O ITCA demonstrou ser mais reativo com ZEA do que com α-ZEA. A 20 mM pH 4, o ITCA reduziu mais da metade da concentração inicial de ZEA, enquanto α-ZEA reduziu apenas 28%. Outro ponto a ser destacado é que imediatamente após a reação com 100 mM de AITC, houve redução de 74% em pH 4 e 77% em pH 7 com ZEA, já para α-ZEA houve redução de 74% em pH 4 e 67% em pH 7. O AITC demonstrou promissora atividade na redução *in vitro* de micotoxinas, visto sua capacidade redutora de até 68% em concentrações de 20 mM.

1.6. CARACTERÍSTICAS E ATIVIDADE ANTIMICROBINANA DO ÓLEO ESSENCIAL DE ALHO

O alho é utilizado como tempero e medicamento desde o período do império egípcio. Ao longo da história, o alho tinha a função de tratar problemas respiratórios, dores, diarreia, e até mesmo tumores (Marchese, 2016). O alho contém aproximadamente 65% de água, 28% de carboidratos, 2,3% de compostos organossulfurados, 2% de proteínas, 1,2% de aminoácidos livres e 1,5% de fibras (Santhosha et al., 2013). Dziri e colaboradores (2014) analisaram a composição do óleo essencial de alho e observaram a composição majoritária de dois grupos, sendo o primeiro rico em enxofre e o segundo de terpenos. Vários componentes sulfurados do alho proporcionam propriedades benéficas a saúde, porém, o composto mais ativo é a alicina (dialil-tiosulfinato), representando, aproximadamente, 70% dos compostos sulfurados existentes no alho (Amagase, 2001; Arnault, 2003).

Cavalito e Bailey (1944) demonstraram que a atividade antimicrobiana do alho é devida à alicina, um composto volátil que apresenta enxofre em sua estrutura (Figura 4), e é responsável pelo odor característico de alho recém cortado.



Fonte: medchemexpress

Figura 4: Estrutura molecular da alicina.

As células presentes no alho possuem os componentes alina (s-ali-L-cisteína sulfóxido), um precursor não proteinogênico, e alinase, uma enzima, que são separados pela membrana celular (Omar e Al-wabel, 2010). Em uma injúria ou qualquer dano tecidual, as membranas se rompem, fazendo com que a alinase age sobre a aliina, produzindo ácido sulfúrico intermediário, que, a partir de uma condensação enzimática, forma a alicina (Miron, 2004). A alicina é altamente reativa, com uma ótima atividade antioxidante e excelente permeabilidade a membrana, que permite sua rápida penetração em diferentes regiões celulares (Omar e Al-wabel, 2010).

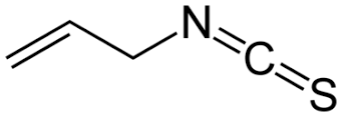
O óleo de alho apresenta atividade *in vitro* e *in vivo* contra várias espécies fúngicas como *Botrytis cinerea*, *Alternaria brassicicola* e *Magnaporthe grisea,* conhecidas por serem fitopatógenos de plantas economicamente importantes (Curtis, 2004). Benkeblia (2004) testou diferentes concentrações de extrato de alho (50, 100, 200, 300 e 500 ml/L) e observou a redução do crescimento bacteriano de *Staphylococcus aureus, Salmonella enteritidis* e fúngico com cepas de *Aspergillus niger, Penicillium cyclopium* e *Fusarium oxysporum.* Utilizando as propriedades voláteis de óleos essenciais, Seydim e Sarikus (2006) impregnaram o óleo essencial de alho em filmes comestíveis com a finalidade de liberar os compostos em embalagens. Em seus testes obtiveram resultados significativos na redução do crescimento das bactérias *Escherichia coli* O157:H7 (ATCC 35218), *Staphylococcus aureus* (ATCC 43300), *Salmonella enteritidis* (ATCC 13076), *Listeria monocytogenes* (NCTC 2167) e *Lactobacilus plantarum* (DSM 20174).

1.7. CARACTERÍSTICAS E ATIVIDADE ANTIMICROBINANA DO ISOTIOCIANATO DE ALILA

Os isotiocianatos (ITC) são compostos extraídos de plantas da família *Brassicacea*, como brócolis, couve, couve-flor, nabos e mostarda. O isotiocianato de alila (ITCA) (Figura 5) é extraído principalmente do óleo essencial da mostarda negra ou marrom, sendo formado a partir da transformação enzimática do glucosinolato sinigrina. Os glucosinolatos estão presentes no vacúolo das células vegetais, sendo liberados quando a planta sofre algum tipo de injúria (Nielsen e Rios, 2000). Uma vez liberados no citoplasma, esses compostos são clivados por uma reação de hidrólise pela enzima mirosinase formando compostos biologicamente ativos como os isotiocianatos, que apresentam atividade anti-fúngica, anti-carcinogênica, anti-parasitária e antibacteriana (D'Antuono et al., 2009).

O mecanismo de ação do isotiocianato de alila ainda é pouco elucidado, mas sabe-se que a estrutura química tem uma grande influência na sua ação, uma vez que o carbono eletrofílico central do grupamento isotiocianato (N=C=S) é capaz de reagir prontamente com grupamentos tiol, hidroxila e amina, podendo assim inibir enzimas e alterar proteínas através da quebra oxidativa das ligações de dissulfetos (Luciano e Holley, 2009). Além disso, esse composto é capaz de alterar a permeabilidade da membrana plasmática resultando em extravasamento do material citoplasmático (Ahn et al., 2001; Lin et al., 2000).

O isotiocianato de alila apresenta ação antibacteriana contra várias bactérias deteriorantes e patogênicas encontradas em alimentos, tanto em seu estado líquido quanto em estado de gasoso, incluindo *E. coli* O157:H7 (Luciano e Holley, 2009). O ITCA demonstrou excelente atividade antifúngica e na redução da produção de micotoxinas, através do método de fumigação (Nazareth et al., 2016; Saladino et al., 2017; Tracz et al., 2017).



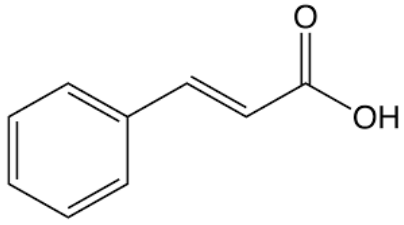
Fonte: wikidata

Figura 5: Estrutura molecular do Isotiocianato de alila.

1.8. CARACTERÍSTICAS E ATIVIDADE ANTIMICROBINANA DO CINAMALDEÍDO

Cinamaldeído (CIN) (Figura 6) é o principal constituinte do óleo de canela extraído de *Cinnamomum* spp., reconhecido como seguro (GRAS) e legalmente aprovado pela Organização das Nações Unidas para a Alimentação e Agricultura / Organização Mundial da Saúde (FAO / OMS). CIN é utilizado há muito tempo como agente aromatizante em gomas de mascar, sorvetes, bebidas e doces, assim como, para dar sabor e odor de canela a produtos médicos, cosméticos e perfumes (Cai Land, 1996; Uchida et al., 1996; Krishna e Banerjee, 1999; Nielsen e Rios, 2000; Hooty et al., 2004). CIN apresenta excelentes atividades biológicas, como inseticidas (Huang e Ho, 1988), antimicrobianas (Kwon, 2003; Sahdna et al., 2010), antifúngica (Xie et al., 2004; Namurol e Nirundorn, 2007), anti-inflamatória (Liao et al., 2008), anticarcinogênica (Imai et al., 2002) e anti-angiogênica (Kwon et al., 1997). Induz também a morte celular (Shih-Hua, Yerra e Yen-Min, 2004; Wu et al., 2005) e inibe o crescimento de tumores (Cabello et al., 2009).

Há relatos que CIN reduz a viabilidade fúngica em *Saccharomyces cerevisiae* através de dano celular e perda citoplasmática (Smid et al., 1996), que agora é considerado como um importante mecanismo antifúngico pelo qual os óleos essenciais, incluindo CIN, podem inibir o crescimento de vários fungos, incluindo *A. ochraceus, A. flavus, P. expansum e F. verticillioides* (Xing et al., 2014; Sun et al., 2016; Wang et al., 2018; Wang et al., 2018). Portanto, CIN pode ser usado como um substituto promissor para conservantes sintéticos, podendo ser aplicado para evitar a deterioração dos alimentos devido à sua atividade anti-séptica segura e satisfatória (Atarés e Chiralt, 2016; Gómez et al., 2018).



Fonte: wikipedia

Figura 6: Estrutura molecular do Cinamaldeído.

CAPÍTULO 2

Artigo científico submetido para publicação no periódico Food Bioscience

Combination of allyl isothiocyanate and cinnamaldehyde against the growth of mycotoxigenic fungi and aflatoxin production in corn

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Abstract

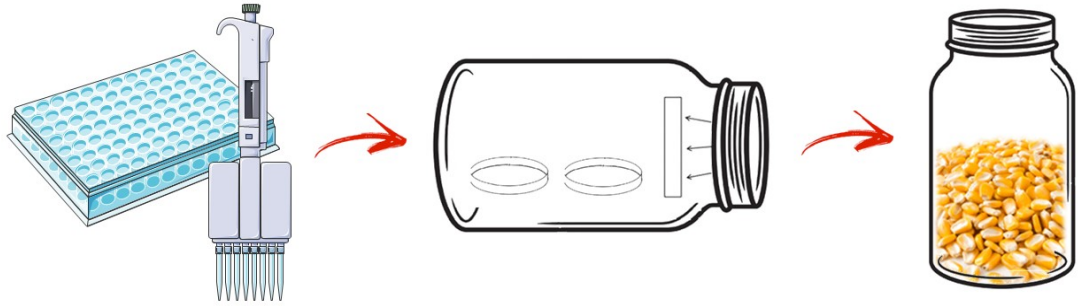
Fungal and mycotoxin contamination are some of the major causes of economic losses in grain production, and essential oils are an option for their control. We evaluate the use of cinnamaldehyde (CIN) and allyl isothiocyanate (AITC) isolated and in interaction against *Aspergillus parasiticus*, *Fusarium verticillioides* and *Gibberella zeae*, and aflatoxin production by *A. parasiticus*. The minimum inhibitory concentrations (MIC) and the interaction between compounds (Fractional inhibitory concentration) were determined. Inhibition in Petri dishes and in corn kernels were also performed, and the aflatoxin yield in corn kernels. Concentrations of 0.076, 0.605, and 0.303 mM of AITC, and 0.246, 1.964, and 0.982 mM of CIN were found of MIC for *A. parasiticus*, *F. verticillioides*, and *G. zeae*, respectively. When used together, 0.038 mM of AITC and 0.061 mM of CIN were required to inhibit *A. parasiticus*; 0.038 mM of AITC and 1.964 mM of CIN to *F. verticillioides*; and 0.076 mM and 0.246 mM to *G. zeae*. In gaseous phase, 0.62, 0.47, and 0.23 µL/L (1:5 ratio AITC:CIN) were able to inhibit *A. parasiticus*, *G. zeae*, and *F. verticillioides* in Petri dishes, respectively. In corn kernels, a reduction in fungal population was found at 30 µL/L (1:5). 300 µL/L reduced the population of all species below the detection limit (1.22 log (CFU g^{-1})). Aflatoxin production was also inhibited; the control group presented 32.25$\pm$5.86 µg/kg of AFs, 30 µL/L had 2.18$\pm$3.72 µg/kg and 300 µL/L had 2.61$\pm$1.38 µg/kg. These results confirm that AITC and CIN may be an alternative to the traditional chemicals used in corn to avoid fungi and mycotoxins.

Keywords: Natural compounds; Essential oils; Mycotoxins.

Highlights

- AITC and CIN may be used against contamination of mycotoxigenic fungi.
- AITC and CIN have the potential to inhibit mycotoxin production.
- Compounds can be used to reduce production losses.

Graphical abstract



1. MIC and FIC determination against mycotoxigenic fungi

2. Inhibitory activity of gaseous AITC and CIN towards fungal growth

3. Growth inhibition in corn kernels

4. Measurement of aflatoxins production in corn kernels treated

The combined use of AITC and CIN at concentrations of 30 and 300 µL/L was able to reduce the fungal growth, as well as to avoid the production of aflatoxins B1, G1, and G2.

1. Introduction

Corn is one of the most produced cereals worldwide (Yang, Balint-Kurti, & Xu, 2017), and the three largest producers are the United States, China, and Brazil, with a combined production estimated in 563 to 717 million tons yearly (Ranum, Peña-Rosas, & Garcia-Casal, 2014). Although production has been extensively technified, problems in cultivation, such as the use of broken seeds, use of untreated water in irrigation, expansion of cultivated areas and environmental issues, such as climate change and planting in high humidity regions, may be predisposing factors for fungal contamination.

Some of the most important pathogenic fungi during plant growth are those of the *Fusarium* genus, which can potentially produce mycotoxins such as fumonisins and zearalenone (ZEA). In addition, inappropriate storage conditions (i.e. environments without humidity and temperature control) may favor the growth of *Aspergillus* species, which can produce other mycotoxins, such as aflatoxins (Mahuku et al., 2019; Sarrocco & Vannacci, 2018; Silva et al., 2017; Yoo, Kerry, Ingram, Ortiz, & Scully, 2018).

Mycotoxins are compounds produced by fungi as a result of their secondary metabolism. Their biological function is the elimination of competitors that would make fungal growth unviable (Garcia-Cela, Verheecke-Vaessen, Magan, & Medina, 2018). Likewise, mycotoxins can be detrimental for human or animal health. According to the International Agency for Research on Cancer (IARC), aflatoxins belong to group 1 of carcinogens, meaning that these toxins were demonstrably carcinogenic. In addition, fumonisins are classified in the group 2B (potentially carcinogenic) and ZEA as part of group 3 (non-carcinogenic) (IARC, 1993; 2012; 2002). These mycotoxins are related, in addition to carcinogenesis, with liver and kidney damage, suppression of the immune system, release of pro-inflammatory cytokines and reproductive problems. In animal production, this is reflected in the increase in costs to the producer, due to the decrease in zootechnical indices, such as daily weight gain, feed conversion, pregnancy rate, mortality, among others (Cimbalo, Alonso-Garrido, Font, & Manyes, 2020; Magnoli, Poloni, & Cavaglieri, 2019).

Methods to avoid the development of mycotoxigenic fungi in grains are necessary to ensure food quality and safety of products intended for both human and animal consumption (Mohapatra, Kumar, Kotwaliwale, & Singh, 2017). Synthetic chemical compounds are used for this purpose. Although they guarantee the health of the grains, they can compromise food safety. The link between the use of chemical defensives with different types of cancer, diabetes, neurologic and reproductive disorders, and teratogenicity is reported (Machado & Martins, 2018). Thus, natural bioactive compounds may be safer and more viable alternatives for this use.

Allyl isothiocyanate (AITC) is an isolated compound of the essential oil extracted from plants from the *Brassicaceae* family. This substance is produced from the hydrolysis of the glucosinolate sinigrin by the enzyme myrosinase when the plant suffers any type of injury (Nazareth et al., 2016). AITC has already been described as an antifungal and antimycotoxigenic compound (Nazareth et al., 2016; Saladino et al., 2017; Bruno Ludvig Tracz et al., 2017). However, with pungent taste and odor, methods are necessary to enable its application in food and raw materials. Cinnamaldehyde (CIN) is a compound isolated from the essential oil of plants belonging to the *Cinnamomum* genus, which possess proven antimicrobial properties (Bravo Cadena et al., 2018; Muller, Casado Quesada, González-Martínez, & Chiralt, 2017; Wang, Yuan, Li, Li, & Jiang, 2016). With pleasant sensory acceptance to animals and humans, it may be a viable alternative for joint application with AITC. The objective of this work is to evaluate the antifungal activity of AITC and CIN combined against the growth of mycotoxigenic fungi, and to evaluate their activity against the production of aflatoxins.

2. Materials and Methods

2.1 Fungal Strains

Aspergillus parasiticus CECT 2681, *Fusarium verticillioides* CECT 2983 and *Gibberella zeae* (Anamorph: *Fusarium graminearum*) CECT 2150 were obtained from the Colección Española de Cultivos Tipo (CECT). Spores were kept in a 1:1 solution of culture medium and glycerol and stored in an ultra-freezer at -80 °C until

use. Reactivation was carried out in tryptone of soybean broth (TSB) at 25 ºC for 5 days, with posterior inoculation in potato dextrose agar (PDA) for maintenance.

2.2 Chemicals and microbiological media

Analytical standards for aflatoxins B1, B2, G1 and G2 (≥ 98% purity), AITC (95,1% purity) and CIN (98,9% purity), and the culture media TSB and PDA were purchased from Sigma-Aldrich (St. Louis, MO). Bacteriological peptone (BP) was obtained from Kasvi (Curitiba, Brazil). Methanol and acetonitrile (both HPLC grade) were purchased from Merck (Darmstadt, Germany).

2.3 Minimum inhibitory concentration (MIC) and fractional inhibitory concentration index (FIC_{index})

Minimum inhibitory concentration was obtained using the microdilution method (Clinical and Laboratory Standards Institute, 2017). Fungi grew on PDA plates at 25 °C until sporulation, and the spores were collected in BP 1%. Treatments were evaluated in 96-well plates. Each well contained 100 µL of TSB + AITC or CIN (4.844 mM, 2-fold diluted to 0.009 mM of AITC or 3.929 mM to 0.008 mM of CIN) and 100 µL of fungal inoculum ($2x10^4$ spores/mL), which were tested individually. Control groups received only TSB (negative control) and TSB + fungi (positive control), with a final volume of 200 µL. Dimethyl sulfoxide (DMSO) 2% was used to homogenize the essential oil compounds in the liquid medium. Plates were sealed with silicone sealants, closed and wrapped in laboratory film (Parafilm®, Bemis, USA) to avoid volatilization of the compounds to the external environment or between wells with different concentrations. Then, they were incubated for 48 h at 25 °C, followed by visual reading. The MIC was measured as > 50% inhibition visible fungal growth as described by CLSI (2017). The experiment was conducted in quadruplicate with three repetitions.

For determination of FIC_{index} was used checkerboard methodology according to Palaniappan & Holley (2010). Fungi grew on PDA plates at 25 °C until sporulation, and the spores were collected using BP 1%. Treatments were evaluated in 96-well plates, with 100 µL of TSB + AITC and CIN (4.844 mM, 2-fold diluted to 0.009 mM of

AITC, and 3.929 mM to 0.031 mM of CIN combined) and 100 µL of fungi ($2x10^4$ spores/mL), which were tested individually. Control groups received only TSB (negative control) and TSB + fungi (positive control), with a final volume of 200 µL. DMSO 2% was used to homogenize the essential oil compounds in the liquid medium. The plates were sealed with silicone sealants, incubated and fungal growth was measured as described above. With the determination of the combined inhibitory concentration, the following formula was used to determine FIC_{index}: *FIC of AITC = Concentration of AITC in combination / MIC of AITC alone*; *FIC of CIN = Concentration of CIN in combination / MIC of CIN alone*; FIC_{index} *= FIC of AITC + FIC of CIN*. Synergy was defined as a $FIC_{index} \leq 0.5$. When the FIC_{index} was $0.5 >$ and ≤ 4.0 it indicated there was no interaction between the agents. A $FIC_{index} > 4.0$ would indicate there was antagonism between the two agents. The experiment was carried out with three replications.

2.4 Inhibitory activity of gaseous AITC and CIN towards fungal growth

The methodology proposed by Tracz et al. (2017) with modifications was used. The fungi grew in PDA plates at 25 °C until sporulation, and the spores were collected using 1% BP. Solutions were diluted to 10^4 spores/mL in BP 1% and 10 µL aliquots were inoculated in the center of Petri dishes containing PDA. The plates were placed in 0.8 L glass jars and were added with 0.0097 - 5 µL/L AITC and CIN (1:5 ratio of each oil compound, respectively) for *A. parasiticus*, 0,12 - 60 µL/L for *F. verticillioides* and 0,058 - 30 µL/L for *G. zeae*. These concentrations are applied in paper filters that were glued to the jars' lids, allowing the volatilization of the compounds. Lids were hermetically closed, and jars were incubated at 25 °C for 5 d. Fungal radial growth were measured and compared after the incubation period. Treatments were compared to the positive control, in which the essential oil compounds were not added. The experiment was carried out in duplicate, with three repetitions.

2.5 Growth inhibition in corn kernels

Fungi were grown in PDA plates at 25 °C until sporulation, and the spores were collected in BP 1%. Solutions were made with 10^7 spores/mL in BP 1%. Then, 300 g

of sterilized corn were packed in hermetically sealed glass jars inoculated with 1 mL of the fungal spore solution. Corn kernels were treated with 30 µL/L or 300 µL/L of AITC and CIN (1:5 ratio). The control group only received BP 1%. Microbiological analyses were performed on days 0, 15 and 30 to examine the fungal population. In each checkpoint, 25 g of corn were homogenized in 225 mL of BP 0.1%. After homogenization, serial dilutions were prepared and inoculated into PDA plates. Plates were incubated for 5 d at 25 °C and fungal population was counted as log (CFU g^{-1}) (Nazareth et al., 2018).

2.6 Measurement of aflatoxins production in corn kernels treated with AITC and CIN

Corn kernels were also used to evaluate the reduction in aflatoxin production caused by the presence of AITC+CIN. This assay was only performed at the end of the experiment (30 d). Aflatoxin extraction was carried out using the method described by Saladino et al. (2017) with adaptations. In this method, 5 g of the samples were taken in 50 mL Falcon-type tubes with 25 mL of methanol, and then homogenized for 3 min by Ultra Ika T18 basic Ultra-turrax® (Staufen, Germany) at 10000 rpm. The extracts were centrifugated at 4000 rpm for 5 min at 4 °C, and the supernatant was transferred and evaporated using a Büchi Rotavapor R-200® (Postfach, Switzerland). The obtained residue was resuspended in 2 mL of methanol, filtered through a 0.22 µm syringe filter, transferred to a glass vial and injected into an LC-MS/MS system.

Aflatoxins concentration was determined using the liquid-chromatography system consisted of an LC-20AD pump coupled to a 3200QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA) using an ESI interface in positive ion mode. The mycotoxins were separated on a Gemini NX C18 column (150 × 2.0 mm I.D, 3.0 mm, Phenomenex, Palo Alto, CA, USA). The mobile phases were 5 mM ammonium formate and 0.1% formic acid in water (solvent A), and 5 mM ammonium formate and 0.1% formic acid in methanol (Solvent B) at a flow rate of 0.25 mL/min. The elution was carried out using a linear gradient from 0 to 14 min. The injection volume set was of 20 µL, the nebulizer, the auxiliary and the auxiliary gas were set at 55, 50, and 15 psi respectively. The capillary temperature and the ion spray voltage were of 550 ºC and 5500 V, respectively. The ions transitions used for

the mycotoxin identification and quantification were m/z 313.1/241.3 and 284.9 for AFB1, m/z 315.1/259.0 and 286.9 for AFB2, m/z 329.0/243.1 and 311.1 for AFG1, and m/z 331.1/313.1 and 245.1 for AFG2.

To validate the analytical method, the parameters of linearity, recovery, repeatability, reproducibility, limits of detection (LOD) and quantification (LOQ), and the matrix effect (ME) for each aflatoxin analyzed were carried out (Table 1). All aflatoxins showed good linearity, with resolution determination coefficients (R^2) greater than 0.9983.

Linearity was evaluated using paired matrix calibrations in triplicate at concentrations between 5 and 250 µg/kg. To calculate the matrix effect, the calibration slope from the matrix calibration curve was divided by the slope of the standard calibration curve and multiplied by 100. The value of the recovery was carried out in triplicate for three consecutive days using three addition levels: LOQ, 2 × LOQ, and 10 × LOQ.

The results were between 70.4% and 75.6% and the relative standard deviation was less than 17%. The values for intraday repeatability (n = 3), expressed as the relative standard deviation of the repeatability, varied from 7.5% to 11.6%; and the reproducibility between days (n = 5), expressed as the relative standard deviation of the reproducibility, varied from 8.2% to 17.3% for the same linearity addition values. LODs and LOQs were calculated by analyzing blank samples enriched with the standard mycotoxins; these parameters have been assessed as the lowest concentration of the molecules studied that showed a chromatographic peak at a signal-to-noise ratio (S/N) of 3 and 10 for LOD and LOQ, respectively.

2.7 Statistical analysis

Results are presented as mean ± standard deviation. GraphPad® Prism 7.0 (San Diego, CA) was used for statistical analysis. D'Agostino-Pearson normality test was applied. Variance analysis were performed by one-way ANOVA, followed by Tukey HSD for multiple comparisons. The significance level used was $p \leq 0.05$.

3. Results and discussion

3.1 MIC of AITC or CIN towards mycotoxigenic fungi and the determination FIC_{Index} of their combination

The individual action of both compounds against mycotoxigenic fungi was observed in low doses. AITC showed lower MIC in comparison to CIN for all fungal strains. The difference between compounds may be due to the different mechanisms of action. While CIN acts mainly on the cytoplasmic membrane, decreasing its integrity and changing its polarization, AITC acts on important enzymes of cellular metabolism, inhibiting the function of acetate kinase, thioredoxin reductase and cytochrome c reductase (Hyldgaard, Mygind, & Meyer, 2012; Kojima & M., 1971; Luciano & Holley, 2009; Okulicz, 2010). *F. verticillioides* CECT 2983 and *G. zeae* CECT 2150 required higher contractions of both compounds in comparison to *A. parasiticus* CECT 2681 (Table 2).

The AITC action against fungi found in this paper corroborates with the research of several authors (Lopes et al., 2018; Nazareth et al., 2018; Quiles, Manyes, Luciano, Mañes, & Meca, 2015; Saladino et al., 2017). Saladino et al. (2017) determined the concentration of 0.194 mM of AITC as the MIC for *A. parasiticus*, 2.5 times higher than this study. Studies whose results are higher than those presented in this study do not report the use of techniques to prevent the volatilization of compounds, which may interfere with their results.

The authors of the present study did not find in the literature the use of CIN in microdilution against the fungal species used. Hossain et al. (2016) evaluated the use of cinnamon essential oil in microdilution against *A. parasiticus*, demonstrating antifungal action at a concentration of 2.5 mL/L. Using cinnamon essential oil (85% CIN), Xing et al. (2014) obtained MIC of 60 µL/L against *F. verticillioides* when applying the agar dilution method. The difference observed between the results obtained in the present study and those described by the literature may be caused by the difference of using CIN and not cinnamon essential oil. The essential oil has different concentrations of CIN according to several factors including the technique of oil extraction, degree of maturation of the plant, and region of the plant used for extraction (Koketsu, Gonçalves, Godoy, Lopes, & Morsbach, 1997). In addition,

Hossain et al. (2016) have not reported the use of methods to prevent compound volatilization, creating an unknown variation in test concentrations.

When CIN (≥ 99% purity) was used against *F. verticillioides* strains through agar dilution method, Xing et al. (2014) demonstrated inhibition at concentrations of 0.27 to 0.35 mM, approximately 6.3 times lower than that obtained in this work. This difference may be due to the use of different methods. The preparation of the inoculum and the medium used can interfere with the results (Balouiri, Sadiki, & Ibnsouda, 2016), in addition to the use of different strains, with varying degrees of individual resistance.

Results of FIC_{index} showed that there is no interaction between AITC and CIN against *A. parasiticus* and *F.* verticillioides, and synergistic effect against *G. zeae* (Table 3). It was observed reduction in the AITC concentration in combination, and it is important for the development of an antimicrobial formula since this compound has a rather pungent taste and odor. CIN has more pleasant sensory aspects, which in addition to reducing the concentration of AITC, helps to mask its taste/odor.

No data was found in the literature describing the combined use of AITC and CIN against fungal species. These results show that AITC+CIN were effective to inhibit fungal growth at low doses in liquid media, opening the possibility for gas-phase research. Both compounds are volatile and could be used to fumigate grains and avoid fungal contamination. It was observed an action approximately 3.5 times greater of the AITC in relation to the CIN. Thus, a formulation of AITC+CIN with a 1:5 ratio was established for further testing based on sensory aspects of the combination (odor).

3.2 Gaseous AITC+CIN against fungal growth in PDA

The use of gaseous AITC+CIN promoted a greater inhibition in the growth of all three fungal species when compared to the MIC levels (Table 4). Concentrations between 0.94 and 1.25 µL/L were capable to totally inhibit the fungal growth in PDA when compared to the control group. This may have been caused due to the fact that the essential oil compounds may diffuse more freely in the headspace of the glass jars than in the liquid media. These compounds have a low affinity with water and other polar constituents of the broth and 2% DMSO was used for better dispersion. However, micelle formation may not be uniform, impairing the action of the

compounds. Nielsen and Rios (2000) showed the effectiveness of volatile compounds from spices and herbs such as garlic essential oil, vanilla, clove, oregano, cinnamon oleoresin, and mustard essential oil against spoilage fungi of bread. At this study, AITC was effective at the gas phase showing fungicidal effect with at least 3.5 µg/mL of AITC against all the strains tested. Cinnamon, garlic and clove were all potential inhibitors but less than AITC. Our data corroborate with this study, showing that the compounds when used in the gas phase are more effective than in liquid phase.

In corn kernels, an effective reduction was observed in the fungal population using higher concentrations, considering the absorption of the compounds by the matrix and its protective effect against the fungal population. As shown in Table 5, at the concentration of 30 µL/L the fungal population of *A. parasiticus* and *G. zeae* were decreased by 1.62 and 1 log (CFU g^{-1}) after 30 d, respectively, when compared to the control group. The population of *F. verticillioides* at the same concentration did not show significant reduction. For all the strains tested the concentration of 300 µL/L decreased the fungal population below the limits of detection after 15d.

Other studies corroborate the effectiveness of gas-phase AITC and CIN as an antifungal in corn kernel when these compounds were tested isolated. Mateo et al. (2017) showed that films containing 0.22-0.23 mg/plate of CIN were capable to reduce the growth in 90% (ED_{90}) of *Aspergillus flavus* and *A. parasiticus*. Nazareth et al. (2018) reported significant reduction of fungal population using 50 µL/L of AITC after 180 d in corn kernels, decreasing 3.17 log (CFU g^{-1}) and 3.9 log (CFU g^{-1}) of *A. parasiticus* CECT 2681 and *F. verticillioides* CECT 2983, respectively. In this study, we highlight the reduction of the fungal population below the detection limit for the three species when using a concentration of 300 µL/L of the compounds in combination after 15d of storage.

3.3 Reduction of aflatoxins production in corn by AITC+CIN

Significant reduction in the production of aflatoxins B1, G1, and G2 was observed, reaching <LOQ levels in the treatment of 300 µL/L for aflatoxins G1 and G2 (Table 6). The mycotoxins fumonisin B1 and zearalenone were also measured in our study but were <LOQ, even in the control groups (data not shown).

This study corroborate with Tracz et al. (2017), who found a significant reduction in aflatoxin production in corn kernels when using concentrations of 50 µL/L and above of AITC. In this study, with the association with CIN, significant reduction of aflatoxins B1, G1 and G2 were observed at 30 µL/L of AITC+CIN at 1:5 ratio. In this proportion we have only 5 µL/L of AITC, a low concentration that can be beneficial in the sensory quality of the grains that can be later destined for human consumption or animal feed. Sun et al. (2015) demonstrated a reduction to undetectable levels of aflatoxin B1 production by *A. flavus* in culture medium from CIN concentrations of 105.72 µL/L. Other matrices may also receive compound addition to prevent mycotoxin contamination, as demonstrated by Quiles et al. (2015), in which the complete inhibition of aflatoxin production was observed at concentrations from 10 µL/L of AITC on pizza crust.

The Food and Drug Organization sets a limit of 20 µg/kg of aflatoxins in corn kernels, while the European Commission sets a limit of 10 µg/kg (FDA, 2011; EC, 2007). From the results, it is observed that the combination of AITC and CIN in concentrations of 30 µL/L to 300 µL/L can be effective to avoid economic losses due to aflatoxin contamination.

Research is needed to determine whether joint use of the compounds, besides inhibiting fungal growth and mycotoxin production, has the potential for detoxification of the toxin already produced through the formation of less toxic products (Azaiez, Meca, Manyes, & Fernández-Franzón, 2013; Meca, Luciano, Zhou, Tsao, & Mañes, 2012). Studies show that corn has great potential to absorb AITC and cause long-term release of this compound (Tracz et al., 2018). This makes it possible to use the AITC+CIN in silos, increasing time of antimicrobial activity of the compound on mycotoxigenic fungi. The combination of AITC and CIN at gas phase also showed good effectiveness against other important agri-food fungi, with a significant reduction in the *Penicillium notatum* growth when used 2.1 µmol/L of AITC and 3.1 µmol/L of CIN with an total amount of 5.2 µmol/L (Tunc, Chollet, Chalier, Preziosi-Belloy, & Gontard, 2007), highlighting the possibility of using these compounds as substitutes for conventional chemicals in the conservation of products.

4. Conclusion

The combined use of AITC and CIN at concentrations of 30 and 300 µL/L was able to reduce the fungal growth of the 3 mycotoxigenic species, as well as to avoid the production of aflatoxins B1, G1, and G2. Therefore, these compounds may be used for the sanitary control of corn kernels during storage.

The dose of 300 µL/L was found to be a more viable alternative to keep the grains free from the analyzed fungi, whereas the dose of 30 µL/L showed similar efficacy when compared with 300 µL/L to reduce the concentration of aflatoxins. Based on this, there is the possibility of optimizing the dose, to obtain similar results with doses lower than 300 µL/L. Further studies should be conducted demonstrating the potential of these compounds when used to treat grains on a larger scale such as in silos or silo-bags.

Author contributions

A.G.E. and **K.C.P.B.:** Investigation, Formal analysis, and Writing - Original Draft. **G.M.:** Resources. **F.B.L.:** Investigation, Formal analysis, Resources, Writing - Review & Editing, Supervision, Project administration, and Funding acquisition.

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Competing interests Statement

None.

REFERENCES

Azaiez, I., Meca, G., Manyes, L., & Fernández-Franzón, M. (2013). Antifungal activity of gaseous allyl, benzyl and phenyl isothiocyanate in vitro and their use for fumonisins reduction in bread. *Food Control*, *32*(2), 428–434. https://doi.org/10.1016/j.foodcont.2013.01.020

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Bravo Cadena, M., Preston, G. M., Van der Hoorn, R. A. L., Flanagan, N. A., Townley, H. E., & Thompson, I. P. (2018). Enhancing cinnamon essential oil activity by nanoparticle encapsulation to control seed pathogens. *Industrial Crops and Products*, *124*, 755–764. https://doi.org/10.1016/j.indcrop.2018.08.043

Cimbalo, A., Alonso-Garrido, M., Font, G., & Manyes, L. (2020). Toxicity of mycotoxins in vivo on vertebrate organisms: A review. *Food and Chemical Toxicology*, *137*, 111161. https://doi.org/10.1016/j.fct.2020.111161

Clinical and Laboratory Standards Institute, C. (2017). *CLSI M100-ED27:2017 Performance Standards for Antifungal Susceptibility Testing for Yeasts* (1st ed.). Wayne, PA: CLSI.

Garcia-Cela, E., Verheecke-Vaessen, C., Magan, N., & Medina, A. (2018). The ``-omics'' contributions to the understanding of mycotoxin production under diverse environmental conditions. *Current Opinion in Food Science*, *23*, 97–104. https://doi.org/10.1016/j.cofs.2018.08.005

Hossain, F., Follett, P., Dang Vu, K., Harich, M., Salmieri, S., & Lacroix, M. (2016). Evidence for synergistic activity of plant-derived essential oils against fungal pathogens of food. *Food Microbiology*, *53*, 24–30. https://doi.org/10.1016/j.fm.2015.08.006

Hyldgaard, M., Mygind, T., & Meyer, R. L. (2012). Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology*, *3*(JAN). https://doi.org/10.3389/fmicb.2012.00012

IARC. (1993). Some naturally occurring substances: food items and constituents,

heterocyclic aromatic amines and mycotoxins. *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans*, Vol. 56, pp. 1–521. https://doi.org/10.1002/food.19940380335

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2012). Chemical agents and related occupations. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans / World Health Organization, International Agency for Research on Cancer*, *100*(Pt F), 9–562.

International Agency for Research on Cancer. (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans / World Health Organization, International Agency for Research on Cancer*, *82*, 1–556.

Kojima, & M. (1971). Studies on the effects of isothiocyanates and their analogues on microorganisms. I. Effect of isothiocyanates on the oxygen uptake of yeasts. *J. Ferment. Technol.*, *49*, 740–746. Retrieved from http://ci.nii.ac.jp/naid/10024489653/en/

Koketsu, M., Gonçalves, S. L., Godoy, R. L. de O., Lopes, D., & Morsbach, N. (1997). Óleos essenciais de cascas e folhas de canela (Cinnamomum verum Presl) cultivada no Paraná. *Ciência e Tecnologia de Alimentos*, *17*(3). https://doi.org/10.1590/s0101-20611997000300017

Lopes, L. F., Bordin, K., de Lara, G. H. C., Saladino, F., Quiles, J. M., Meca, G., & Luciano, F. B. (2018). Fumigation of Brazil nuts with allyl isothiocyanate to inhibit the growth of Aspergillus parasiticus and aflatoxin production. *Journal of the Science of Food and Agriculture*, *98*(2), 792–798. https://doi.org/10.1002/jsfa.8527

Luciano, F. B., & Holley, R. A. (2009). Enzymatic inhibition by allyl isothiocyanate and factors affecting its antimicrobial action against Escherichia coli O157:H7. *International Journal of Food Microbiology*, *131*(2–3), 240–245. https://doi.org/10.1016/j.ijfoodmicro.2009.03.005

Machado, S. C., & Martins, I. (2018). Risk assessment of occupational pesticide exposure: Use of endpoints and surrogates. *Regulatory Toxicology and Pharmacology*, *98*, 276–283. https://doi.org/10.1016/j.yrtph.2018.08.008

Magnoli, A. P., Poloni, V. L., & Cavaglieri, L. (2019). Impact of mycotoxin contamination in the animal feed industry. *Current Opinion in Food Science*, *29*, 99–108. https://doi.org/10.1016/j.cofs.2019.08.009

Mahuku, G., Nzioki, H. S., Mutegi, C., Kanampiu, F., Narrod, C., & Makumbi, D. (2019). Pre-harvest management is a critical practice for minimizing aflatoxin contamination of maize. *Food Control*, *96*, 219–226. https://doi.org/10.1016/j.foodcont.2018.08.032

Meca, G., Luciano, F. B., Zhou, T., Tsao, R., & Mañes, J. (2012). Chemical reduction of the mycotoxin beauvericin using allyl isothiocyanate. *Food and Chemical Toxicology*, *50*(5), 1755–1762. https://doi.org/10.1016/j.fct.2012.02.070

Mohapatra, D., Kumar, S., Kotwaliwale, N., & Singh, K. K. (2017). Critical factors responsible for fungi growth in stored food grains and non-Chemical approaches for their control. *Industrial Crops and Products*, *108*, 162–182. https://doi.org/10.1016/j.indcrop.2017.06.039

Muller, J., Casado Quesada, A., González-Martínez, C., & Chiralt, A. (2017). Antimicrobial properties and release of cinnamaldehyde in bilayer films based on polylactic acid (PLA) and starch. *European Polymer Journal*, *96*, 316–325. https://doi.org/10.1016/j.eurpolymj.2017.09.009

Nazareth, T. M., Bordin, K., Manyes, L., Meca, G., Mañes, J., & Luciano, F. B. (2016). Gaseous allyl isothiocyanate to inhibit the production of aflatoxins, beauvericin and enniatins by Aspergillus parasiticus and Fusarium poae in wheat flour. *Food Control*, *62*, 317–321. https://doi.org/10.1016/j.foodcont.2015.11.003

Nazareth, T. M., Corrêa, J. A. F., Pinto, A. C. S. M., Palma, J. B., Meca, G., Bordin, K., & Luciano, F. B. (2018). Evaluation of gaseous allyl isothiocyanate against the growth of mycotoxigenic fungi and mycotoxin production in corn stored for 6 months. *Journal of the Science of Food and Agriculture*, *98*(14), 5235–5241. https://doi.org/10.1002/jsfa.9061

Nielsen, P. V, & Rios, R. (2000). Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. *International Journal*

of Food Microbiology, *60*(2–3), 219–229. https://doi.org/10.1016/S0168-1605(00)00343-3

Okulicz, M. (2010). Multidirectional Time-Dependent Effect of Sinigrin and Allyl Isothiocyanate on Metabolic Parameters in Rats. *Plant Foods for Human Nutrition*, *65*(3), 217–224. https://doi.org/10.1007/s11130-010-0183-3

Palaniappan, K., & Holley, R. A. (2010). Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. *International Journal of Food Microbiology*, *140*(2–3), 164–168. https://doi.org/10.1016/j.ijfoodmicro.2010.04.001

Quiles, J. M., Manyes, L., Luciano, F., Mañes, J., & Meca, G. (2015). Influence of the antimicrobial compound allyl isothiocyanate against the Aspergillus parasiticus growth and its aflatoxins production in pizza crust. *Food and Chemical Toxicology*, *83*, 222–228. https://doi.org/10.1016/j.fct.2015.06.017

Ranum, P., Peña-Rosas, J. P., & Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences*, *1312*(1), 105–112. https://doi.org/10.1111/nyas.12396

Saladino, F., Quiles, J. M., Luciano, F. B., Mañes, J., Fernández-Franzón, M., & Meca, G. (2017). Shelf life improvement of the loaf bread using allyl, phenyl and benzyl isothiocyanates against Aspergillus parasiticus. *LWT - Food Science and Technology*, *78*, 208–214. https://doi.org/10.1016/j.lwt.2016.12.049

Sarrocco, S., & Vannacci, G. (2018). Preharvest application of beneficial fungi as a strategy to prevent postharvest mycotoxin contamination: A review. *Crop Protection*, *110*, 160–170. https://doi.org/10.1016/j.cropro.2017.11.013

Silva, J. J., Viaro, H. P., Ferranti, L. S., Oliveira, A. L. M., Ferreira, J. M., Ruas, C. F., … Fungaro, M. H. P. (2017). Genetic structure of Fusarium verticillioides populations and occurrence of fumonisins in maize grown in Southern Brazil. *Crop Protection*, *99*, 160–167. https://doi.org/10.1016/j.cropro.2017.05.020

Sun, Q., Wang, L., Lu, Z., & Liu, Y. (2015). In vitro anti-aflatoxigenic effect and mode of action of cinnamaldehyde against aflatoxin B1. *International Biodeterioration and Biodegradation*, *104*, 419–425. https://doi.org/10.1016/j.ibiod.2015.07.009

Tracz, B. L., Bordin, K., Bocate, K. C. P., Hara, R. V., Luz, C., Macedo, R. E. F., …

Luciano, F. B. (2018). Devices containing allyl isothiocyanate against the growth of spoilage and mycotoxigenic fungi in mozzarella cheese. *Journal of Food Processing and Preservation*, *42*(11), e13779. https://doi.org/10.1111/jfpp.13779

Tracz, Bruno Ludvig, Bordin, K., Nazareth, T. de M., Costa, L. B., Macedo, R. E. F. de, Meca, G., & Luciano, F. B. (2017). Assessment of allyl isothiocyanate as a fumigant to avoid mycotoxin production during corn storage. *LWT - Food Science and Technology*, *75*, 692–696. https://doi.org/10.1016/j.lwt.2016.10.030

Tunc, S., Chollet, E., Chalier, P., Preziosi-Belloy, L., & Gontard, N. (2007). Combined effect of volatile antimicrobial agents on the growth of Penicillium notatum. *International Journal of Food Microbiology*, *113*(3), 263–270. https://doi.org/10.1016/j.ijfoodmicro.2006.07.004

Wang, H., Yuan, H., Li, S., Li, Z., & Jiang, M. (2016). Synthesis, antimicrobial activity of Schiff base compounds of cinnamaldehyde and amino acids. *Bioorganic and Medicinal Chemistry Letters*, *26*(3), 809–813. https://doi.org/10.1016/j.bmcl.2015.12.089

Xing, F., Hua, H., Selvaraj, J. N., Zhao, Y., Zhou, L., Liu, X., & Liu, Y. (2014). Growth inhibition and morphological alterations of Fusarium verticillioides by cinnamon oil and cinnamaldehyde. *Food Control*, *46*, 343–350. https://doi.org/10.1016/j.foodcont.2014.04.037

Yang, Q., Balint-Kurti, P., & Xu, M. (2017). Quantitative Disease Resistance: Dissection and Adoption in Maize. *Molecular Plant*, *10*(3), 402–413. https://doi.org/10.1016/j.molp.2017.02.004

Yoo, E. H., Kerry, R., Ingram, B., Ortiz, B., & Scully, B. (2018). Defining and characterizing Aflatoxin contamination risk areas for corn in Georgia, USA: Adjusting for collinearity and spatial correlation. *Spatial Statistics*, *28*, 84–104. https://doi.org/10.1016/j.spasta.2018.06.003

Table 1. Limits of detection (LODs) and quantification (LOQs), recovery, and matrix effect (ME) for AFB1, AFB2, AFG1, and AFG2 in corn.

Mycotoxin	**LOD (µg/Kg)**	**LOQ (µg/Kg)**	**Recovery (%)**	**ME (%)**
AFB1	0.08	0.27	70.4%	78.2%
AFB2	0.08	0.27	64.2%	76.5
AFG1	0.16	0.53	62.8%	65.3
AFG2	0.30	1.00	66.1%	60.9

Table 2. Minimum inhibitory concentration (MIC) of allyl isothiocyanate (AITC) and cinnamaldehyde (CIN) when applied to mycotoxigenic fungi.

Strain	Concentration (mM)	
	AITC	**CIN**
Aspergillus parasiticus CECT 2681	0.076	0.246
Fusarium verticillioides CECT 2983	0.605	1.964
Gibberella zeae CECT 2150	0.303	0.982

Table 3. Determination of fractional inhibitory concentration index (FIC_{index}) of allyl isothiocyanate (AITC) in combination with cinnamaldehyde (CIN) against different fungal species.

Strain	Concentration in combination (mM)		
	AITC	**CIN**	**FIC_{index}***
Aspergillus parasiticus CECT 2681	0.038	0.061	0.75
Fusarium verticillioides CECT 2983	0.038	1.964	1.06
Gibberella zeae CECT 2150	0.076	0.246	0.50

*FIC_{index} ≤ 0.5 indicate synergy; FIC_{index} 0.5 to 4.0 indicate no interaction; FIC_{index} > 4.0 indicate antagonism.

Table 4. Radial inhibition of fungal growth caused by the combination of allyl isothiocyanate (AITC) with cinnamaldehyde (CIN) at different doses using a 1:5 ratio. Reduction was measured in comparison to a control group that did not receive any treatment.

***Aspergillus parasiticus* CECT 2681**	
Concentration (µL/L)	**Inhibition (%)**
≥1.25	100[a]
0.62	59.26±12.22[a]
0.31	12.35±1.74[b]
0.16	11.11±3.49[b]
0.08	8.64±6.99[b]
0.04	6.17±0.00[b]
0.02	1.23±0.00[b]
0.01	1.23±0.00[b]
0.005	ND
***Gibberella zeae* CECT 2150**	
Concentration (µL/L)	**Inhibition (%)**
≥0.94	100[a]
0.47	85.30±0.00[a]
0.23	50.00±4.16[b]
0.12	20.58±4.16[b]
0.06	13.23±2.08[b]
0.03	ND
***Fusarium verticillioides* CECT 2983**	
Concentration (µL/L)	**Inhibition (%)**
≥0.94	100[a]
0.47	79.66±4.79[a]
0.23	67.79±2.40[a]
0.12	ND

Different lowercase letters (a,b) in the same strain-group indicate statistical difference ($p \leq 0.05$). ND = No inhibition detected. (Mean± SD)

Table 5. Population of mycotoxigenic fungi when exposed to concentrations of allyl isothiocyanate (AITC) and cinnamaldehyde (CIN) in corn kernels (1:5 ratio) for 30 d in hermetically closed glass jars. **Population (log (CFU g^{-1}))**

***Aspergillus parasiticus* CECT 2681**			
Days	**Control**	**30 µL/L**	**300 µL/L**
0	3.91 ± 0.24^{Aa}	3.91 ± 0.24^{Aa}	3.91 ± 0.24^{Aa}
15	3.81 ± 0.09^{Aa}	3.57 ± 0.07^{Ab}	$< DL^{*Bc}$
30	6.08 ± 0.11^{Ba}	4.46 ± 0.36^{Bb}	$< DL^{*Bc}$
***Giberella zeae* CECT 2150**			
Days	**Control**	**30 µL/L**	**300 µL/L**
0	3.82 ± 0.14^{Aa}	3.82 ± 0.14^{Aa}	3.82 ± 0.14^{Aa}
15	3.97 ± 0.14^{Aa}	3.64 ± 0.33^{Ab}	$< DL^{*Bc}$
30	3.49 ± 0.20^{Ba}	2.49 ± 0.32^{Bb}	$< DL^{*Bc}$
***Fusarium verticillioides* CECT 2983**			
Days	**Control**	**30 µL/L**	**300 µL/L**
0	3.70 ± 0.33^{Aa}	3.70 ± 0.33^{Aa}	3.70 ± 0.33^{Aa}
15	4.26 ± 0.39^{Ba}	4.21 ± 0.71^{Ba}	$< DL^{*Bb}$
30	4.22 ± 0.71^{ABa}	3.68 ± 0.34^{Aa}	$< DL^{*Bb}$

Different uppercase letters (A,B) on the same column indicate statistical difference ($p \leq 0,05$).
Different lowercase letters (a,b,c) on the same line indicate statistical difference ($p \leq 0,05$).
*DL (Detection limit) = 1,22 log (CFU g^{-1}). (Mean± SD)

Table 6. Aflatoxin production by *Aspergillus parasiticus* CECT 2681 when exposed to concentrations of allyl isothiocyanate (AITC) and cinnamaldehyde (CIN) in corn kernels (1:5 ratio) for 30 d in hermetically closed glass jars.

Aflatoxin	Concentration (µg/kg)		
	Control	**30 µL/L**	**300 µL/L**
AFB1	13.87±3.30[a]	0.85±1.50[b]	2.28±1.40[b]
AFB2	1.76±1.80[a]	0.13±0.20[a]	0.24±0.20[a]
AFG1	12.49±4.20[a]	1.13±2.30[b]	<LOQ[b]
AFG2	3.25±2.30[a]	0.06±0.40[b]	<LOQ[b]
Total aflatoxins	**32.25±5.86[a]**	**2.18±3.72[b]**	**2.61±1.38[b]**

Different lowercase letters (a,b) in the same line indicate statistical difference ($p \leq 0.05$).

(Mean± SD)

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CAPÍTULO 3

Artigo científico a ser submetido para publicação no periódico Food Control

Garlic essential oil as an antifungal and anti-mycotoxin agent in stored corn

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Abstract

Corn kernels are susceptible to fungal growth and mycotoxins production, which are secondary metabolites produced by some filamentous fungi, such as *Aspergillus*, *Fusarium* and *Penicillium*. Natural antimicrobials have been used in food preservation due to the safe status of these compounds. The present study verified the effectiveness of garlic essential oil (GEO) to inhibit the growth and mycotoxin production of *Aspergillus parasiticus*, *Fusarium verticillioides* and *Gibberella zeae*, producers of aflatoxin B1 (AFB1), fumonisin B1 (FB1) and zearalenone (ZEA), respectively, in stored corn kernels. The potencial of the oil to react with those mycotoxins and the bioaccessibility after the simulated digestion was also evaluated. The methodologies applied were 1) the minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), gaseous activity of the oil on the fungal growth in vitro; 2) effect of the GEO on stored contaminated corn and production of mycotoxins; 3) in vitro reaction of mycotoxins with GEO in test tubes and during swine simulated digestion. GEO showed MIC for *A. parasiticus*, *G. zeae* and *F. verticillioides* of 7.8, 7.8 and 62.5 µL/L, respectively. MFCs were found at four-fold greater concentration when compared to MIC for all fungi strains. Fungal growth was reduced completely by gaseous GEO with a range of 2-10 µL/L. The population of *A. parasiticus* and *G. zeae* was decreased below the limit of detection and *F. verticillioides* decreased the population in 2.33 Log UFC/g after 30d of storage when treated with 1000 µL/L of the oil. GEO was not able to directly react with either AFB1, FB1 or ZEA in phosphate buffer or during simulated digestion. Those results of our study confirmed that GEO have antifungal activity but did not show the ability to chemically react with mycotoxins at the concentrations tested. Therefore, GEO may be used as a fumigant improving safety in corn kernels.

Keywords: Natural antimicrobials; Fumigant; Fungi; Contaminant.

Highlights

- GEO have the potential to reduce the growth of *A. parasiticus*, *F. verticillioides* and *G. zeae* in corn kernels.
- GEO have higher effectiveness when used in gas phase than at liquid phase.
- GEO at concentrations of ≥1000 µL/L can be used as a new fumigant to avoid spoilage in corn.

1. Introduction

Mycotoxins are structures with low molecular weight, grouped according to toxicity degree to humans and other animals. The International Agency for Research on Cancer (IARC, 1993) presents some mycotoxins as carcinogenic, nephrotoxic and immunotoxic to vertebrates, especially aflatoxins, zearalenone and fumonisins. The main genera of toxigenic fungi found in foods and animal feed are *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria*. These microorganisms contaminate mainly grains and can cause health issues for animals and humans (Bocate et al., 2019).

Corn production in the 2018-2019 harvest season showed that the United States was the main producer in the world with 366.6 million metric tons, followed by China with 257.7 million metric tons and Brazil with 94.5 million metric tons (Statista, 2019). Corn is commonly affected by mycotoxins, being a multifaceted problem and causing economic losses. Recurrent problems due to the presence of mycotoxins in corn include lower plant development and product value, reduced animal performance, and health problems to both humans and animals (Wind et al., 2012).

Mitigatory measures are used to reduce the effect of mycotoxins in animal production (Varga, Kocsubé, Péteri, Vágvögyi, & Toth, 2010; Carão et al., 2014). The use of adsorbents such as clay and bentonite are considered the most traditional in animal feed. These compounds are capable of binding to some toxins in the digestive tract, preventing their absorption (Ramos, Hernández, Plá-Delfina, & Merino, 1996; Magnoll et al., 2008). However, due to the high contamination of grains with mycotoxins and the low effectiveness of commonly used compounds, new technologies have been examined, such as the use of natural compounds (Chen et al., 2014).

Alternative measures are proposed to reduce the growth of toxigenic fungi and toxin production, especially with natural compounds that are safe and well accepted. One of the proposals is the use of essential oils from leaves, bark, seeds, twigs and epidermal flowers (Bajpai, Baek, & Kang, 2012). Essential oils present a wide array of bioactive compounds with reported antimicrobial activity (Cabral, Pinto, & Patriarca, 2013). Allyl isothiocyanate (AITC) demonstrates activity in mycotoxin reduction and fungal development when used as a liquid or gas (Nazareth et al., 2016). The chemical structure of AITC and allicin is similar, therefore, garlic essential

oil with allicin in its composition can be highly effective against fungi and mycotoxins, such as AITC.

Garlic essential oil has allicin as the major compound, which presents a highly reactive organosulfur moiety with excellent cell membrane permeability (Omar & Al-wabel, 2010). When garlic cells are injured, aliin, a non-proteinogenic amino acid, is exposed to alinase and converted to allicin (Miron, Bercovici, Rabinkov, Wilchek, & Mirelman, 2004). Allicin has shown antifungal activity against clinical isolates (*Candida albicans* and *Aspergillus fumigatus*) and phytopathogens (*Botrytis cinerea*), as well as against gram-positive and gram-negative bacteria (Marchese et al., 2016).

Thus, the objective of this work was to evaluate the antifungal activity of garlic essential oil against mycotoxigenic fungi strains in liquid and gas phase; evaluate the efficacy of the oil to reduce the fungal growth and mycotoxins production in corn; evaluate the potential of the GEO to react with mycotoxins and analyze the bioaccessibility of fumonisin B1, aflatoxin B1 and zearalenone after simulated digestion.

2. Materials and Methods

2.1. Microorganisms and culture conditions

The strains of *Aspergillus parasiticus* CECT 2681, *Fusarium verticillioides* CECT 2983 and *Gibberella zeae* CECT 2150 used in this study were obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain). These microorganisms were stored in sterile glycerol at - 80 °C before use. Then the microorganisms were recovered in Potato Dextrose Broth (PDB, Sigma Aldrich®, St Louis, MO, USA) at 25 °C after growth were inoculated in Potato Dextrose Agar (Sigma Aldrich®, St Louis, MO, USA) petri dishes.

2.2. Chemicals

Analytical standards for aflatoxin B1, fumonisin B1, zearalenone, the culture media potato dextrose broth (PDB), potato dextrose agar (PDA), o-phthalaldehyde and 2-mercaptoethanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Peptone water was obtained from Kasvi (Italy). HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Trifluoroacetic acid, N-hexane were also purchased from Merck (Darmstadt, DE). Garlic essential oil (GEO) containing 60% of allicin was obtained from Sigma Zengzou, China.

2.3. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined in quadruplicate from broth microdilutions in a 96-well plate according to the document M38-A2 of the Clinical Laboratory Standard Institute, with adaptations (CLSI, 2008). The fungus was initially grown in PDA and incubated at 25 °C for seven days. The fungal suspension was prepared by harvesting the spores from the surface of the plates with peptone water 0,1%. After counting the number of spores using a Neubauer chamber, the inoculum was adjusted to $2x10^4$ spores/mL in PDB medium (Sigma Aldrich®, St Louis, MO, USA). Then, 100 µL aliquots of fungal suspension were added to each well. Different concentrations of GEO (0,97 µL/L to 500 µL/L) was added by microdiluition, the final volume of the microplate was 200 µL/ well. The plate was incubated for 48h at 25 °C before a visual reading was performed.

2.4. Determination of minimum fungicidal concentration (MFC)

The minimum fungicidal concentration was determined according to the protocol described by Espinel-Ingroff et al. (2002). For this method, 10 µL of each well presenting complete inhibition of fungal growth was withdrawn and cultured in PDA plates for 72 h at 25 °C. The MFC was defined as the lowest GEO dilution that yielded fewer than three colonies or complete absence of growth.

2.5. Inhibition of fungal growth by gaseous GEO

The methodology proposed by Tracz et al. (2017) with modifications was used. The fungi were grown in PDA plates at 25 °C and the suspension was prepared in peptone water 0,1%. After counting the number of fungal spores using the Neubauer chamber, the inoculum was adjusted to 10^4 spores/mL in peptone water and 10 µL was inoculated in the center of Petri dishes containing PDA. Plates were placed in hermetically sealed glass jars that received concentrations of 0.125 to 2 µL/L GEO for *A. parasiticus*, and 0,31 to 10 µL/L for *F. verticillioides* and *G. zeae*. These concentrations were adjusted based on preliminary tests and applied in paper filters glued to the jar lid, allowing volatilization of the compound. Jars were incubated at 25 °C for 5 d. Then, the radial growth of the fungal colonies was measured and compared to the positive control.

2.6. Antifungal activity of garlic essential oil in corn kernels

The antifungal activity of garlic essential oil in corn kernels was carried out using the methodology described by Tracz et al. (2017). Corn kernels (*Zea mays*) were weighted (300 g) in 800 mL glass jars and sterilized at 121 °C for 20 min. After, corn kernels were contaminated with 10^4 spores/g of either *A. parasiticus* CECT 2681, *F. verticilliodes* CECT 2983 or *G. zeae* CECT 2150. Different concentrations of garlic essential oil (GEO) (10, 100 and 1000 µL/L) were added in filter papers and adhered in the jar lids. Jars were hermetically closed and incubated for 30 days at 25 °C. During incubation, corn samples were randomly drawn on days 0, 15 and 30 to analyze the fungal population of fungi on potato dextrose agar (PDA). At the same time-points, samples were also aliquoted and used for mycotoxins analysis. This experiment was carried out in triplicates.

2.7. Determination of the fungal population

After the incubation time, 25 g of each sample were added to 225 mL of sterile peptone water 0,1 % (Kasvi, Italy) and poured inside sterile mortars (500 mL) and the kernels were subsequently crushed with a sterile pestle for 5 min. The suspensions were serially diluted in sterile plastic tubes containing 0.1% of peptone water. After that, aliquots of 0.1 mL were plated on Petri dishes containing PDA and the plates were incubated at 25 ◦C for 7 d before microbial counting. The results were expressed in logs of colony-forming unit/g of corn kernels (log CFU/g). All analyses were conducted in triplicate.

2.8. Mycotoxins extraction

The mycotoxins extraction was carried out using the method described by Nazareth et al. (2018) with adaptations. Briefly, 5g of the all samples were taken in 50 mL-Falcon tubes, added with 25 mL of methanol and homogenized for 3 min using an Ultraturrax (Tecnal, São Paulo, Brazil) at 10000 rpm. The extracts were centrifuged at 4000 rpm for 5 min at 4°C. Then, to analyze aflatoxins, the supernatants were evaporated under airflow and derivatized adding 200 µL of n-hexane and 200 µL of trifluoroacetic acid (TFA). The reaction were homogenized, kept at 40 ◦C during 10 min, evaporated with airflow and resuspended with 1 mL of methanol:water (50:50 v/v). Thereafter, the extracts were filtered through a 0.22 µm nylon filter and 20 µL was injected in a high-performance liquid chromatography (HPLC) equipment with a fluorescence detector at excitation and emission wavelengths of 365 and 440 nm, respectively. Isocratic mobile phase was acetonitrile: methanol (50:50 v/v, phase B): water (phase A), at proportion of 40 B: 60 A at a flow rate of 0.8 mL/min.

For fumonisins, prior to derivatization O-phthalaldehyde (OPA) reagent was prepared dissolving OPA (0,02 g) in methanol (0.5 ml) and adding 2.5 ml of 0.1 M sodium borate and 25 µL of 2-mercaptoethanol. Derivatization was carried out manually prior to the HPLC analysis by suspending the extract (100 µL) with OPA reagent (200 µL). Then, 20 µL was injected in the HPLC system with a fluorescence

detector at excitation and emission wavelengths of 335 and 440 nm, respectively. Gradient mobile phase was acetonitrile (B) and water + acetic acid 1% (A) at a flow rate of 1 mL min^{-1}. Mobile phase was 30% B at 0 - 8.5 min, increasing to 60% B at 18 min, hold for 4 min and increasing to 95% B at 22.5 min, which was kept until 24 min, decreasing to 30% B at 25 min.

For zearalenone the extracts were filtered through a 0.22 µm nylon filter and 20 µL was injected in HPLC with a fluorescence detector. The isocratic mobile phase was consisted of water/acetonitrile (55:45, v/v) with a flow rate of 1 mL/min. All samples were analyzed using a high-performance liquid chromatography (HPLC) equipment (1220 Infinity, Agilent, Santa Clara, USA) with a fluorescence detector (1260 Infinity, Agilent, Santa Clara, USA) and a C18 column (Phenomenex, Torrance, USA) 4.6 x 150mm, 3µm particle size was used as stationary phase. Agilent OpenLAB CDS ChemStation Editor was used for data analysis.

The limits of detection (LOD) and quantification (LOQ) were calculated based on signal: noise ratio of 3:1 and 10:1, respectively for the three mycotoxins. Linearity was evaluated by calibration curve using six concentrations in triplicate. Matrix effects were investigated by comparing the slopes of standards diluted in solvent with the slopes of matrix extract spiked with standards.

The LOD were 0.6, 0.3, 1.0 and 0.4 µg kg^{-1} of AFB_1 AFB_2 AFG_1 AFG_2. And LOQ were 2.0, 1.0, 3.4 and 1.4 µg kg^{-1}. For FBs the LOD were 37.5 µg kg^{-1} and 41.2 µg kg^{-1} to FB1 and FB2, respectively. However, the LOQ was 125.0 for FB_1 and 138.8 for FB_2.

2.9. *In vitro* reaction of mycotoxins with garlic essential oil

Mycotoxins (200 ppb of AFB1, 5 ppm of ZEA and 30 ppm of FB1) were added in 2 mL of ultrapure water at pH 2 or 7 in the presence of 1000 ppm of garlic essential oil. The tubes were shaken at 200 rpm and incubated at 39 °C (simulating swine body temperature). Aliquots of the reaction solution were taken at 0, 4, 8 and 24h and analyzed by HPLC-FLD for mycotoxin quantification following the methodology described in 2.8. The experiment was carried out in triplicates.

2.10. Simulated swine digestion

The methodology of simulated swine digestion was based as previously described by Boisen and Fernández 1997, with modifications and carried out in triplicates. Mycotoxins (200 ppb of AFB1, 5 ppm of ZEA and 30 ppm of FB1) and garlic essential oil (1000 ppm) were added to the diet (2g of feed) and subjected to the *in vitro* digestion process, simulating the enzymatic conditions of pH and temperature of the digestive system of pigs (stomach and intestine). For the stomach phase was added 20 mL of phosphate buffer solution (0.1 M, pH = 6), 8 mL of HCl solution (0.2 M), 100 µL of pepsin solution (50 mg of pepsin + 1 mL phosphate buffer solution (0.1 M, pH = 6) and incubated for 2h at 39°C with agitation. For the intestinal phase was added 7 mL of phosphate buffer solution (0.2 M, pH = 6.8), 8 mL of NaOH solution (0.6 M), 1 mL of pancreatin solution (80 mg of pancreatin + 1 mL phosphate buffer solution (0.2 M, pH = 6.8) and incubated for 4h at 39°C with agitation. Samples of 10 mL of digestion were collected at 0 h (without digestive action), 2 h (after simulation of stomach acid-enzymatic digestion), 3.5h (period referring to the beginning of the intestinal phase) and 6h (consistent with the end of the digestive process). After collections, mycotoxins were extracted by immunoaffinity column, where the collected samples passed. The toxins present were retained by their reaction with the antibodies present in the column, forming an immunocomplex. This immunocomplex was removed by the addition of methanol that passed through the column denaturing the antibodies and releasing the toxins, which were collected in tubes at the end of extraction. After extraction, HPLC-FLD analysis was performed for residual evaluation of each mycotoxin.

2.11. Statistical analyzes

The results obtained are expressed as mean ± standard deviation. Analysis of variance (ANOVA) was followed by Tukey's test using the software GraphPad Prism 5 (San Diego, CA). Statistical differences were considered significant if $p \leq 0.05$.

3. Results and discussion

3.1. Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The minimum inhibitory concentration of garlic essential oil was determined against *A. parasiticus*, *F. verticillioides* and *G. zeae* using the methodology of broth microdilution. Results are shown in Table 1. The MIC for *A. parasiticus*, *F. verticillioides* and *G. zeae* were 0.0086, 0.069 and 0.0086 mg/mL, respectively. Results of MFC are presented in Table 1. All strains required a four-fold greater concentration of garlic essential oil for the fungicidal effect in comparison to the MIC.

Previous reports have shown a MIC using allicin, the major compound of garlic essential oil. Yoshida et al. (1999) demonstrated that allicin showed a MIC of 0.005 mg/mL and 0.01 mg/mL for strains of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, respectively. An et al. (2009) determined MIC for 40 isolates of *Candida albicans* with MIC50 ranged from 0.032 to 0.08 mg/mL. Another study was conducted by Kim et al. (2012) against *C. albicans* showing effect when treated with allicin in a concentration of 0.001 to 0.128 mg/mL. Jin et al. (2019) demonstrated that allicin inhibit 50 and 90 % of the mycelial growth of *Botrytis cinerea* with 0.014 and 0.084 mg/mL of allicin, respectively, when exposed during 3 days at 25 °C. Comparing our data with the studies cited above, we found higher and lower values. The differences between these results and those reported in the current study may be due to the different used species also to the heterogenic composition of our essential oil that contain 60% of allicin in the composition and other chemical compounds that may interfere with tested fungi. Furthermore, the studies cited does not report the purity of the allicin used. Despite this, our data corroborates with the antifungal activity of GEO and allicin.

3.2. Inhibition of fungal growth using gaseous GEO

The halo inhibition of garlic essential oil in gas phase against *G. zeae* and *F. verticillioides* during incubation for 5 d at 25 °C. was shown in Table 2. For *G. zeae,* a

concentration of 10 µL/L totally inhibited the fungal growth and 5 µL/L showed 89% of inhibition. On other hand, *F. verticillioides* was totally inhibited by 5 µL/L of GEO and 2.5 µL/L was capable to inhibit 72% of the growth. Table 3 shows the halo inhibition of GEO against *A. parasiticus* using the same conditions as the other strains. Fungal growth was completely inhibited at 2 µL/L of the essential oil and 1 µL/L inhibited 58% of the radial fungal growth.

Garlic essential oil when used in the gas phase was able to inhibit the fungal growth at concentrations that were 3 times lower than the MIC in liquid media for *A. parasiticus* and *G. zeae* and 30-times lower for *F. verticillioides*.

These results demonstrated that *A. parasiticus* was the most sensible strain using this method. These results show that the GEO when used in gas phase have higher effectiveness when compared with the findings in liquid phase, which can be very promising to be used used in grains. This may be explained by a better homogeneity of the essential oil in the gas phase than in the liquid media.

Curtis et al. (2004) showed that allicin is volatile compound and can kill bacteria via gas phase. This fact can be another explanation about the high effectiveness of the oil when used as fumigant against fungi. The authors of the present study did not find in the literature the use of GEO or allicin using this methodology. Based on the studies of our research group using other natural antimicrobials, such as allyl isothiocyanate (AITC) and the high and proven efficiency of this compound at gas phase, our results will be compared to AITC. Furthermore, allicin the major compound of GEO and AITC have similar chemical structure, both have reactive electrophilic carbons. Manyes et al. (2015) evaluated the capacity of AITC in a solution to avoid the micellar growth of *A. parasiticus* and *Penicillium expansum*. In that study, the mycelial growth was not observed when 25 µL/L and 50 µL/L of a solution of AITC was deposited in the center of the petri dishes inoculated with the mycotoxigenic fungi. These doses are far higher than the ones found for GEO, but the authors do not mention if concentrations < 25 µL/L were tested.

3.3. Determination of the fungal population and mycotoxins production in corn

The use of natural compounds to prevent the fungal growth in foods is increasing. Table 4 showed the residual fungal population of the 3 mycotoxigenic

fungi when treated with 10, 100 and 1000 µL/L of GEO. Treatment with 10 µL/L did not show effect during the 30 d when compared to the control group for all the strains. However, the treatment with 100 µL/L decreased the fungal population in 1.5 Log CFU/g for *F. verticillioides* and *A. parasiticus*, in relation to *G. zeae* this concentration did not show effect. Furthermore, the treatment with 1000 µL/L was the most effectiveness against all the strains, decreasing the population below the limit of detection for A*. parasiticus* and *G. zeae* and reducing 2.33 Log CFU/g for *F. verticillioides* after 30 d. The authors of the present study did not find in the literature the use of GEO or allicin using this methodology, so our results will be compared to other compounds.

Other studies have also shown the use of volatile natural compounds as antifungals. Nazareth et al. (2018) reported the reduction below of the limits of detection of fungal population of *A. parasiticus* CECT 2681 and *F. verticillioides* CECT 2983 using 50 µL/L of allyl isothiocyanate (AITC) during 150 d in corn kernels. Our data shows that higher concentration of garlic oil to obtain similar or equal results for the same fungal strains. This difference can be justified because our analyzes were realized with 30d of incubation and not 180d as reported by Nazareth and his colleagues. Lopes et al. (2018) and Tracz et al. (2017) showed the inhibition of *A. parasiticus* growth at levels such as 2.5 and 500 µL/L of gaseous AITC in Brazil nuts and corn, respectively. Quiles et al. (2019) demonstrated the reduction of *Aspergillus flavus* population in 1.5 log CFU/g at day 1 and 4.4 log CFU/g at day 30 using AITC on a gel dispositive at the concentration of 500 µL/L in corn. Our study required greater concentrations than those reported in these studies, what can be justified by the different compound used and also differences in the treatment methodologies applied. Despite these facts, these results showed that natural compounds, such as AITC or GEO, used in the gas phase have high effectiveness against mycotoxigenic strains of fungi in different types of grains.

Mycotoxins production was also analyzed but no data was shown because the strains were not capable to produce the toxins even in the control group.

3.4. In vitro reaction of mycotoxins with garlic essential oil

The reaction between garlic essential oil (GEO) and mycotoxins (AFB1, FB1 and ZEA) *in vitro* was monitored at different incubation times (0, 4, 8 and 24h) and pH levels (2 and 7). As shown in table 5, in general, there was no significant difference between the pH levels investigated or incubation times. For all the mycotoxins tested GEO was not effective to react and reduce the concentration of the toxins. Furthermore, we can notice that the reduction is not stable, increasing the concentration or unable to decrease the mycotoxins as the incubation time increases. These results can be partially explained by the fact that allicin, which is one of the main compounds of the GEO, is highly reactive and can become another molecule of which we are not aware of. Allyl isothiocyanate (AITC) has molecular structure similar with allicin. Studies using AITC reacting with mycotoxins have been performed. Bordin et al. (2017), showed the reaction between α-ZOL/ZEA (78 µM) and AITC (2, 20, 100 or 200 mM) at pH levels 4 and 7 and incubation times (0,4,8, 24 and 48h). The reduction ranged from 0 to 89.5 ± 1.2% for a-ZOL and 0.2 ± 0.3 to 96.9 ± 2.4% for ZEA, and as showed at our study the pH did not influence the results. Meca et al. (2012) evaluated the reduction of beauvericin (BEA) by AITC, that was able to reduce totally this mycotoxin after 48h of incubation.

3.5. Simulated swine digestion

After the digestion it is possible to know the bioaccessible fraction of one amount ingested and available for absorption (Versantovoort et al., 2005). In this study, the bioaccessibility of AFB1, FB1 and ZEA after the simulated in vitro digestion when swine diet was contaminated with mycotoxins and treated with 1000 µL/L of GEO is shown in Table 6. The results showed that bioaccessibility does not change for all mycotoxins and phases such as stomach and intestinal, showing no significant difference when treated or not. For AFB1 the gastric phase did not show difference in the control group and when treated (25.06±0.15% and 20.05 ± 0.05%, respectively), the same happened to the intestinal phase (15.03± 0.01% and 25.06±0.14%, respectively). Gastric and intestinal bioaccessibility of FB1 and FB1 - GEO and ZEA

and ZEA-GEO were also similar, showing no effect to in reduce the bioaccessibility and sometimes when treated with GEO the concentration of mycotoxins increases. This fact may occur because the digestion process has a lot of factors that can cause interference as enzymes, temperature, different pHs and some unknown reaction between the oil, enzymes and toxins can also occur.

4. Conclusion

The results obtained in this study showed the antifungal effectiveness of GEO as antifungal agent at a concentration of 1000 µL/L being able to decrease the fungal growth of the 3 mycotoxigenic species in corn kernels. The use of 100 µL/L of GEO also had a good effect against *A. parasiticus* and *F. verticillioides*, demonstrating that intermediary doses of the oil can be used in further studies, this may be a solution to decrease the high odor of the oil. The use of GEO did not show efficacy in the direct reaction with mycotoxins and also did not show a reduction in the bioaccessibility of mycotoxins after simulated swine digestion. In conclusion, GEO can be used as a natural fumigant agent to avoid fungi growth and spoilage in corn kernels.

Author contributions

K.P.B.: Investigation, Formal analysis, and Writing - Original Draft. **A.G.E.:** Investigation, Formal analysis. **F.B.L.:** Investigation, Formal analysis, Resources, Writing - Review & Editing, Supervision, Project administration, and Funding acquisition.

Competing interests Statement

None.

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Table captions

Table 1. Minimum Inhibitory Concentration (MIC) and minimum fungicidal concentration (MFC) obtained by the use of garlic essential oil (GEO), against *Aspergillus parasiticus* CECT 2681, *Fusarium verticillioides* CECT 2983 and *Gibberella zeae* CECT 2150.

Table 2. Inhibition of fungal radial growth in PDA plates using gaseous garlic against *Giberella zeae* CECT 2150 and *Fusarium verticillioides* CECT 2983 fungi.

Table 3. Inhibition of fungal radial growth in PDA plates using gaseous garlic essential oil against *Aspergillus parasiticus* CECT 2681 fungi.

Table 4. Effect of fumigation of garlic essential oil (0, 100 e 1000 µL/L) in corn kernels contaminated with 3 mycotoxigenic fungi and stored at 30d in hermetically glass jars. Fungal population was analyzed during 30d and expressed in Log CFU/g.

Table 5. Reduction of mycotoxins through in vitro reaction with 1000 µL/L of garlic essential oil (GEO) at different pH and incubation time.

Table 6. Mycotoxin bioaccessibility (%) in initial swine ration treated with 1000 of garlic essential oil (GEO) after simulated swine digestion.

REFERENCES

An, M., Shen, H., Cao, Y., Zhang, J., Cai, Y., Wang, R., & Jiang, Y. (2009). Allicin enhances the oxidative damage effect of amphotericin B against *Candida albicans*. *International Journal of Antimicrobial Agents*, *33*, 258-263.

Bajpai, V.K., Baek, K., & Kang, S.C. (2012) Control of *Salmonella* in foods by using essencial oils: A review. *Food Research International, 45*(2), 722-734.

Bocate, K. P., Reis, G.F., Souza, P.C., Oliveira Junior, A. G., Dúran, N., Nakazato, G., Furlaneto, M.C., Almeida, R.S., & Panagio, L.A. (2019). Antifungal activity of silver nanoparticles and simvastatin against toxigenic species of *Aspergillus*. *International journal of food microbiology, 291*, 79-86.

Boisen, S. & Fernández, J. A. (1997). Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. *Animal Feed Science and Technology, 68*, 277-286.

Bordin, K., Saladino, F., Fernández-Blanco, C., Ruiz, M.J., Mañes, J., Fernández-Franzón, M., Meca, G., Luciano, F.B. (2017). Reaction of zearalenone and α-zearalenol with allyl isothiocyanate, characterization of reaction products, their bioaccessibility and bioavailability in vitro. *Food chemistry*, 217, 648-54.

Cabral, L. C., Pinto, F., & Patriarca, A. (2013). Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods. *International Journal of Food Microbiology*, 166, 1-14.

Carão, A.C.P., Barbarelli, M.F.C., Polycarpo, G.V., Santos, A.R., Albuquerque, R., & Oliveira, C.A.F. (2014). Métodos físicos e químicos de detoxificação de aflatoxinas e redução da contaminação fúngica na cadeia produtiva avícola. *Ciência Rural, 44*,699-705.

Chen, H., Davidson, P. M., Zhong, Q. (2014). Impacts of sample preparation methods on solubility and antilisterial characteristics of essential oil components in milk. *Applied and Environmental Microbiology*, 80, 907-916.

Clinical and Laboratory Standards Institute (CLSI). Reference Method for Broth Dilution Tests for Determination of Sensitivity to Antifungal Filamentous Fungus Therapy: Approved Standard: Approved Standard M38-A2. Wayne, PA, USA, 2008.

Curtis, H., Noll, U., Störmann, J., Slusarenko, A.J. (2004). Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. *Physiological and Molecular Plant Pathology*, 65, 79-89.

Espinel-Ingroff, A., Fothergill, A., Peter, J., Rinaldi, M. G., & Walsh, T. J. (2002). Testing conditions for determination of minimum fungicidal concentrations of new and established antifungal agents for Aspergillus spp.: NCCLS collaborative study. *Journal of Clinical Microbiology, 40*(9), 3204-3208.

INTERNATIONAL AGENCY ON RESEARCH IN CANCER (IARC) (1993). Some Naturally Occuring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins In: Monographs on the Evaluation of Carcinogenic Risks to Humans. Monografia p.56

Ji, X., Li, J., Meng, Z., Dong, S., Zang, S., & Qiao, K. (2019). Inhibitory effect of allicin against *Meloidogyne incognita* and *Botrytis cinerea* in tomato. *Scientia Horticulturae, 253*, 203-208.

Kim, Y.S., Kim, K. S., Han, I., Kim, M.H., Jung, M. H., & Park, H.K. (2012). Quantitative and qualitative analysis of the antifungal activity of allicin alone and in combination with antifungal drugs. *PLoS One, 7*(6), e38242.

Lopes, L. F., Bordin, K., Lara, G.H.C., Saladino, F., Quiles, J.M., Meca, G. & Luciano, F.B. (2018). Fumigation of Brazil nuts with allyl isothiocyanate to inhibit the growth of *Aspergillus parasiticus* and aflatoxin production. *Journal of the Science of Food and Agriculture, 98*, 792-798.

Magnoll, A., Tallone, L., Rosa, C.A.R., Dalcero, A.M., Chiacchiera,S.M., & Sanchez, R.M.T. (2008). Comercial bentonites as detoxifier of broiler feed contaminater with aflatoxin. *Applied Clay Science, 40* (1-4), 63-71.

Manyes, L., Luciano, F.B., Mañes, J. & Meca, G. (2015) In vitro antifungal activity of allyl isothiocyanate (AITC) against *Aspergillus parasiticus* and *Penicillium expansum* and evaluation of the AITC estimated daily intake. *Food and Chemical Toxicology, 83*, 293-299.

Marchese, A., Barbieri, R., Sanches-Silva, A., Daglia, M., Nabavi, S.F., Jafari, N.J., Izadi, M., Ajami, M., Nabavi, S.M. (2016). Antifungal and antibacterial activities of allicin: a review. *Trends in Food Science & Technology*, *52*, 49-56. doi: 10.1016/j.tifs.2016.03.010.

Meca, G., Luciano, F. B., Zhou, T., Tsao, R., & Mañes, J. (2012). Chemical reduction of the mycotoxin beauvericin using allyl isothiocyanate. *Food and Chemical Toxicology*, 55, 1755–1762.

Miron, T., Bercovici, T., Rabinkov, A., Wilchek, M., & Mirelman, D. (2004). Allicin: preparation and applications**.** *Analytical Biochemistry*, *331*(2),364-369.

Nazareth, T. M., Bordin, K., Manyes, L., Meca, G., Mañes, J., & Luciano, F. B. (2016). Gaseous allyl isothiocyanate to inhibit the production of aflatoxins, beauvericin and enniatins by *Aspergillus parasiticus* and *Fusarium poae* in wheat flour. *Food control, 62*, 317-321.

Nazareth, T.M., Corrêa, J.A.F., Pinto, A.C.S.M., Palma, J.B., Meca, G., Bordin, K., Luciano, F.B. (2018). Evaluation of gaseous allyl isothiocyanate against the growth of mycotoxigenic fungi and mycotoxin production in corn stored for 6 months. *Journal of the Science of Food and Agriculture, 98*, 5235–5241. https://doi.org/10.1002/jsfa.9061

Omar, S., & Al-Wabel N. (2010). Organosulfur compounds and possible mechanism of garlic in cancer. *Saudi Pharmaceutical Journal, 18*(1): 51-58.

Quiles, J. M., Nazareth, T. D. M., Luz, C., Luciano, F. B., Mañes, J., & Meca, G. (2019). Development of an Antifungal and Antimycotoxigenic Device Containing Allyl Isothiocyanate for Silo Fumigation. *Toxins, 11*(3), 137.

Ramos, A.J., Hernández, E., Plá-Delfina, J.M., & Merino, M. (1996). Intestinal adsorption of zearalenona and in vitro study of non-nutritive sorbent materials. *International Journal of Pharmaceutics, 128*, 129-137.

STATISTA: https://www.statista.com/statistics/254292/global-corn-production-by-country/. Acessed: 16 Dec 2019.

Tracz, B.L., Bordin, K., Nazareth, T. de M., Costa, L.B., Macedo, R.E.F. de, Meca, G., Luciano, F.B. (2017). Assessment of allyl isothiocyanate as a fumigant to avoid mycotoxin production during corn storage. *LWT- Food Science and Technology*, *75,* 692–696. https://doi.org/10.1016/j.lwt.2016.10.030

Varga, J., Kocsubé, S., Péteri, Z., Vágvölgyi, C., & Tóth, B. (2010) Chemical, physical and biological approaches to prevent ochratoxin Induced toxicoses in humans and animals. *Toxins, 2*, 1718–1750.

Yoshida, H., Katsuzaki, H., Ohta, R., Ishikawa, K., Fukuda, H., Fujino, T., & Suzuki, A. (1999). Antimicrobial activity of the thiosulfinates isolated from oil-macerated garlic extract. *Bioscience, Biotechnology, and Biochemistry, 63*, 591-594.

Tables

Table 1. Minimum Inhibitory Concentration (MIC) and minimum fungicidal concentration (MFC) obtained by the use of garlic essential oil (GEO) against *Aspergillus parasiticus* CECT 2681, *Fusarium verticillioides* CECT 2983 and *Gibberella zeae* CECT 2150.

Fungal Specie	MIC/MFC (mg/mL)
Aspergillus parasiticus CECT 2681	0.0086/0.0340
Fusarium verticillioides CECT 2983	0.0690/0.2760
Gibberella zeae CECT 2150	0.0086/0.0340

Table 2. Inhibition of fungal radial growth in PDA plates using gaseous garlic against *Giberella zeae* CECT 2150 and *Fusarium verticillioides* CECT 2983 fungi.

Halo Inhibition of fungal growth (%)		
Concentration (µL/L)	Species	
	Gibberella zeae	*Fusarium verticillioides*
10	100.00[b]	100.00o[b]
5	89.55±0.36[b]	100.00[b]
2.5	27.00±2.20[a]	72.75±0.72[b]
1.25	26.78±0.68[a]	30.85±2.18[a]
0.625	15.25±1.43[a]	15.34±1.05[a]
0.3125	12.42±0.93[a]	7.81±1.77[a]
Control	ND	ND

Different lowercases letters in the same strain group (a, b) indicate statistical difference ($p \leq 0.05$). ND = No inhibition detected
(Mean± SD);

Table 3. Inhibition of fungal radial growth in PDA plates using gaseous garlic essential oil against *Aspergillus parasiticus* CECT 2681 fungi.

Concentration (µL/L)	Halo Inhibition of fungal growth (%)
2	100.00[b]
1	58.34±2.21[b]
0.5	30.70±1.76[a]
0.25	29.51±0.67[a]
0.125	2.39±1.86[a]
Control	ND

Different lowercases letters in the same strain group (a, b) indicate statistical difference ($p \leq 0.05$). ND = No inhibition detected
(Mean± SD);

Table 4. Effect of fumigation of garlic essential oil (0, 100 e 1000 µL/L) in corn kernels contaminated with 3 mycotoxigenic fungi and stored at 30d in hermetically glass jars. Fungal population was analyzed during 30d and expressed in Log CFU/g.

***Aspergillus parasiticus* CECT 2681**				
Days	(Mean ± SD)			
	Control	**10 µL/L**	**100 µL/L**	**1000 µL/L**
0	3.21 ± 0.25^{Aa}	3.21±0.25^{Ba}	3.21±0.25^{Ca}	3.21±0.25^{Ba}
15	3.66± 0.48^{Ac}	2.79±0.30^{Ab}	2.52±0.26^{Bb}	≤ LD*^{Aa}
30	3.44± 0.36^{Ac}	3.44±0.34^{Bc}	1.94±0.62^{Ab}	≤ LD*^{Aa}
***Fusarium verticillioides* CECT 2985**				
Days	(Mean ± SD)			
	Control	**10 µL/L**	**100 µL/L**	**1000 µL/L**
0	3.96±0.44^{Aa}	3.96±0.44^{Aa}	3.96±0.44^{Ba}	3.96±0.44^{Ca}
15	3.69±0.62^{Aab}	4.87±0.98^{Ab}	3.74±1.81^{Ba}	2.94±0.30^{Ba}
30	4.32±2.17^{Ab}	3.63±0.53^{Ab}	2.79±0.12^{Ab}	1.99±1.19^{Aa}
***Gibberella zeae* CECT 2150**				
Days	(Mean ± SD)			
	Control	**10 µL/L**	**100 µL/L**	**1000 µL/L**
0	3.41±0.14^{Aa}	3.41±0.14^{Aa}	3.41±0.14^{Aa}	3.41±0.14^{Ca}
15	5.48±1.43^{Bc}	5.13±0.93^{Bc}	3,57±0.59^{Ab}	2.75±0.73^{Ba}
30	3.26±0.38^{Ac}	3.61±0.26^{Ab}	3.01±1.32^{Ab}	≤ LD^{Aa}

Different capital letter (A,B,C) on the same column indicate statistical difference ($p \leq 0,05$).
Different lower case letters (a,b,c) on the same line indicate statistical difference ($p \leq 0,05$).

Table 5. Reduction of mycotoxins through in vitro reaction with 1000 µL/L of garlic essential oil (GEO) at different pH and incubation time.

Incubation Time (h)	**Reduction (%) ± SD * of AFB1, FB1 and ZEA when reacted with 1000 µL/L of GEO**	
	pH 2	**pH 7**
AFB1		
0	5.23±1.50	2.89±2.17
4	7.64±0.36	6.25±1.40
8	NE	3.85±1.30
24	NE	NE
FB1		
0	15.67±0.10	4.76±0.55
4	NE	NE
8	9.68±0.10	NE
24	NE	12.55±0.15
ZEA		
0	10.34±0.13	11.67±0.13
4	NE	5.71±0.53
8	12.47±0.47	NE
24	NE	NE

Percentage of reduction was calculated based on a blank prepared for each assay.
NE= No effect observed in mycotoxins levels.
*Statistical analysis was performed but the groups did not show statistical difference between them.

Table 6. Mycotoxin bioaccessibility (%) in initial swine ration treated with 1000 of garlic essential oil (GEO) after simulated swine digestion.

	Bioaccessibility in vitro AFB1 (%) (Mean ± SD) *	
	AFB1[1]	**AFB1 - GEO**
Time		
0	15.03 ±0.09	20.05 ±0.05
2	25.06 ±0.15	20.05 ±0.13
3.5	25.06 ±0.21	15.03 ±0.12
6	10.00 ±0.01	25.06 ±0.14
	Bioaccessibility in vitro FB1 (%) (Mean ± SD) *	
	FB1[2]	**FB1-GEO**
Time		
0	5.34 ±0.50	7.01 ±1.00
2	3.67 ±0.80	1.00 ±0.10
3.5	2.34 ±0.50	1.67 ±0.10
6	1.34 ±0.10	1.67 ±0.10
	Bioaccessibility in vitro ZEA (%) (Mean ± SD) *	
	ZEA[3]	**ZEA-GEO**
Time		
0	19.70 ±46.30	15.60 ±2.50
2	13.20 ±12.01	14.90 ±12.30
3.5	15.30 ±27.20	18.04 ±41.00
6	18.80 ±12.50	20.10 ±11.00

[1]200 ppb of AFB1; [2]30 ppm of FB1; [3]5 ppm of ZEA in 1000 µL/L of GEO.
T0 – time of incubation without the process of digestion; T2: after 2 hours of incubation also called as gastric phase; T3.5 and T6: after 3.5 and 6 hours of incubation, respectively, equivalent a duodenal phase.
* Statistical analysis was performed but the groups did not show statistical difference between them.

CAPÍTULO 4

Artigo científico a ser submetido para publicação no periódico International Journal of Food Microbiology

Garlic essential oil against *Aspergillus flavus* growth and aflatoxin production in stored corn kernels

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Abstract

Corn is most produced grain worldwide and is largely used in foods and animal feed. This crop is susceptible to contamination by fungi and, consequently, mycotoxins. Natural antimicrobials have been proposed as safe alternatives to control mycotoxin contamination in grains. The objective of this study was to evaluate the effect of garlic essential oil (GEO) in liquid and gas phase against the growth of *Aspergillus flavus* and its aflatoxin production in stored corn kernels. The minimum inhibitory concentration (MIC) of GEO was determined by microdilution, with subsequent determination of the minimum fungicidal concentration (MFC). Evaluation of growth inhibition in corn kernels was also performed using GEO in the gaseous phase in the concentration of 100, 200 and 300 µL/L. The production of aflatoxins in corn kernels during 30 d of contamination was also evaluated. GEO showed MIC and MFC of 0.0173 and 0.55 mg/mL, respectively. A significant reduction in fungus population was found in all concentrations tested after 30 days of incubation. Moreover, 300 µL/L reduced the fungal population below the detection limit (DL = 1.22 Log UFC/g). Aflatoxins B1 and B2 production were significantly reduced with 300 µL/L in 15d of incubation. These results confirm the antifungal and antimycotoxigenic action of GEO, which may be applied in stored corn to avoid fungal contamination and mycotoxin production.

Keywords: Natural compounds; Mycotoxins; Contamination.

Highlights

- GEO have the potential to reduce the growth of *A. flavus* at concentrations of 100, 200 and 300 µL/L after 30d of incubation at gas phase in corn kernels.
- GEO reduced the aflatoxins B1 and B2 production in 15 days of incubation at the concentration of 300 µL/L.
- GEO at concentrations ≥300 µL/L can be used to reduce production losses.

1. Introduction

Corn (*Zea mays L*.) is the most cultivated grain in the world and is traditionally used for the production of food and animal feed (Oliveira et al., 2016). This crop is highly susceptible to fungal contamination during cultivation, harvesting and storage, led mostly by inadequate environmental conditions as high temperature and humidity (Moses, Jayas and Alagusundaram, 2015).

Mycotoxins are secondary metabolites of fungi, mainly produced by species of the genus *Aspergillus*, *Penicillium* and *Fusarium* (Njobeh et al., 2010; Terzi et al., 2014; Anfossi et al., 2016). *Aspergillus flavus* is one of the most prevalent toxigenic species and causes wastage of several crops around the world through spoilage or mycotoxin production (Jedidi et al., 2018). This specie it is known for the ability to produce aflatoxins (AFs) B1 and B2. AFB1 is the most toxic. According to the IARC (International Agency for Research on Cancer, 2010), AFs are classified as group 1 carcinogens. Exposure to aflatoxins can cause chronic liver damage, immunosuppression, reduce animal performance and malnutrition in both humans and animals (Rushing and Selim 2019).

Several methods have been used to prevent fungal growth and mycotoxin production in grains. Pre-harvest examples are the development of genetically modified cultivars and use of crop rotation. Pos harvest measures such as grain drying, control of environmental storage conditions and the use of preservatives, which reduce the amount of contamination (Rushing and Selim, 2019). The use of adsorbents such as clay and bentonite are traditional in animal feed. They bind some toxins in the digestive tract, preventing their absorption (Ramos et al., 1996; MagnolI et al., 2008). However, due to the high contamination of grains with mycotoxins and the low effectiveness of commonly used compounds, new technologies are being researched.

The use of essential oils has been investigated with a great interest from the food industry due to their broad spectrum of antimicrobial activity and safety for consumption (Chen et al., 2014). *Allium sativum L*., commonly known as garlic, is a highly used as a food ingredient and for medicinal purposes (Zhu et al., 2018). Many studies demonstrated the biological functions of garlic, such as antioxidant, cardiovascular protection, anticancer, anti-inflammatory, immunomodulatory, anti-

diabetic, anti-obesity and antimicrobial properties (Boonpeng et al., 2014; Lee et al., 2012; Hayat et al., 2016; Percival, 2016; Lee, 2016; Seckiner et al., 2014; Yun et al., 2014).

Garlic essential oil (GEO) has antibacterial activity against Gram-positive and negative bacteria, including antibiotic-resistant strains, as well as anti-fungal activity (Leontiev et al., 2018; Putnik et al., 2019). The major bioactive compound responsible for most of GEO functional properties is allicin, which is a highly reactive organosulfurous molecule with excellent cell membrane permeability (El-Sayed et al., 2017).

The aim of this study was to evaluate the antifungal activity of GEO against a mycotoxigenic strain of *A. flavus* and the consequent reduction of fungal growth and aflatoxins production in corn kernels. These data may support a new strategy to preserve corn kernels during storage and avoid mycotoxin contamination.

2. Material and methods

2.1. Microorganism and culture conditions

The strain of *Aspergillus flavus* ISPA 8111 used in this study was obtained from the Institute of Food Production Science (ISPA, Italy). This microorganism was stored in sterile glycerol at - 80 °C. Prior to use, the microorganism was recovered in potato dextrose broth (PDB, Liofilchem, Italy) at 25 °C for 72h, and then transferred to potato dextrose agar (PDA, Liofilchem, Italy) plates.

2.2. Chemicals

Analytical standards for aflatoxin B1, B2, G1, G2 and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and acetonitrile were obtained by Fisher Scientific (Hudson, NH, USA). Potato dextrose broth (PDB), potato dextrose agar (PDA) and peptone water were purchased from Liofilchem

(Italy). Garlic essential oil (GEO) containing 60% of allicin was obtained from Sigma Zengzou, China.

2.3. Minimum inhibitory concentration (MIC) of GEO against *A. flavus*

The minimum inhibitory concentration (MIC) was determined in quadruplicate from broth microdilutions in a 96-well plate according to the M38-A2 procedure of the Clinical Laboratory Standard Institute, with adaptations (CLSI, 2008). Different concentrations of garlic essential oil (0,97 µL/L to 500 µL/L) were added. The fungus was initially grown in PDA and incubated at 25 °C for 7 d. The fungal suspension was prepared in peptone water 0,1%. Fungal spores were counted using the Neubauer chamber and the inoculum was adjusted to $2x10^4$ spores/mL in PDB medium (Liofilchem, Italy). Then, 100 µL aliquots of fungal suspension were added to each well. Plates were incubated for 48h at 25 °C before visual reading was performed.

2.4. Determination of minimum fungicidal concentration (MFC) of GEO against *A. flavus*

The minimum fungicidal concentration was determined according to the protocol described by Espinel-Ingroff et al., (2002). In this test, 10 µL of each well with complete growth inhibition was withdrawn and cultured in PDA plates for 48 h at 25 °C. The MFC was defined as the lowest GEO dilution that yielded fewer than three colonies or complete absence of growth.

2.5. Antifungal activity of garlic essential oil applied in stored corn kernels

The antifungal activity of garlic essential oil in corn kernels was carried out using the methodology described by Tracz et al., 2017. Corn kernels (*Zea mays*) were weighed (300 g) in 1L glass jars and sterilized at 121 °C for 20 min. Then, kernels were contaminated with 10^4 spores/g of corn with *A. flavus* ISPA 8111.

Different concentrations of GEO (100, 200 and 300 µL/L) were added in filter papers and adhered on the jar lids. Jars were hermetically closed and incubated for 30 d at room temperature. After incubation, the residual fungal population was analyzed at days 0, 15 and 30. Flasks were autoclavated and the corn was used for mycotoxins analysis after the aliquots used for microbiological analyses were sampled. The experiment was carried out in triplicates.

2.6. Determination of the fungal population

After the incubation time, 20 g of each sample was transferred to a sterile stomacher bag containing 180 mL of sterile peptone water 0,1% (Liofilchem, Italy) and homogenized with a stomacher (IUL, Barcelona, Spain) during 60 s. The suspensions formed were serially diluted in sterile plastic tubes containing 0.1% of peptone water. Then, aliquots of 0.1 mL were plated on Petri dishes containing PDA and the plates were incubated at 25 °C for 7d before microbial counting. Results were expressed in log CFU/g. The experiment and all analyses were conducted in triplicate.

2.7. Aflatoxins Extraction

The aflatoxins extraction was carried out using the method described by Saladino et al. (2017) with adaptations. Briefly, 5g of corn were added to 50-mL Falcon tubes containing 25 mL of methanol, which were homogenized for 3 min using an Ultra Ika T18 basic Ultraturrax (Staufen, Germany) at 10000 rpm. The extracts were centrifuged at 4000 x g for 5 min at 4°C and the supernatant was transferred and evaporated using a Büchi Rotavapor R-200 (Postfach, Switzerland). The residue obtained was resuspended in 2 mL of methanol, filtered through a 0.22 µm syringe filter, transferred to a glass vial and injected into an LC-MS/MS system.

2.8. LC-MS/MS Analysis

The liquid-chromatography system consisted of an LC-20AD pump coupled to a 3200QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA) using an ESI interface in positive ion mode. The mycotoxins were separated on a Gemini NX C18 column (150 × 2.0 mm I.D, 3.0 mm, Phenomenex, Palo Alto, CA, USA). The mobile phases were the solvent A (5 mM ammonium formate and 0.1% formic acid in water) and solvent B (5 mM ammonium formate and 0.1% formic acid in methanol) at a flow rate of 0.25 mL/min. The elution was carried out using a linear gradient from 0 to 14 min. The injection volume set was of 20 µL, the nebulizer, the auxiliary and the auxiliary gas were set at 55, 50, and 15 psi respectively. The capillary temperature and the ion spray voltage were of 550 ºC and 5500 V, respectively. The ions transitions used for the mycotoxin identification and quantification were m/z 313.1/241.3 and 284.9 for AFB1, m/z 315.1/259.0 and 286.9 for AFB2, m/z 329.0/243.1 and 311.1 for AFG1, and m/z 331.1/313.1 and 245.1 for AFG2.

2.9. Statistical analyses

The results obtained are expressed as mean ± standard deviation. Analysis of variance (ANOVA) was followed by Tukey's test using the software GraphPad Prism 5 (San Diego, CA). Statistical differences were considered significant if $p \leq 0.05$.

3. Results and discussion

3.1. Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The MIC and MFC of GEO was determined against *A. flavus* using broth microdilution and their values were 0.0173 and 0.55 mg/mL, respectively. Comparing the results, GEO required 32-fold greater quantity to have fungicidal effect comparing

to MIC. Other studies using GEO or allicin have been carried out. Arasu et al. (2019) evaluated the antifungal activity of essential oil from *A. sativum* (garlic) against *Aspergillus niger*, *A. flavus*, *P. notatum* and *R. microsporus*. The MIC value ranged from 0.0097 ± 2.8 to 0.027 ± 2.8 mg/mL and the MFC values ranged from 0.0194 ± 2.3 to 0.058 ± 1.2 mg/mL. For *A. flavus* the MIC and MFC were 0.0065 ± 1.8 and 0.014 ± 2.1 mg/mL, respectively; these results are lower than the one found in ours and this difference may be caused by the oil used, which can have more compounds that have antifungal effect. Yoshida et al. (1999) demonstrated a MIC of 0.005 µg/mL and 0,01 mg/mL for strains of *Schizosaccharomyces pombe* and *Saccharomyces cere* using allicin. Ji et al. (2019), demonstrated inhibition of 50 and 90% of the mycelial growth of *Botrytis cinerea* with 0.014 and 0.084 mg/mL off allicin, respectively, when exposed during 3 days at 25 °C. Comparing our data with the studies cited above, we found higher and lower values. These differences can be explained because of the species used are different and also that in our study we used the essential oil containing 60% of allicin in the composition and not only allicin. Furthermore, the studies cited does not report the purity of the allicin used.

3.2. Fungal population and reduction of aflatoxins production

The use of natural compounds had been increased to prevent microorganism growth in foods (Luciano and Holey, 2009) and animal feed. The population of *A. flavus* when treated with 100, 200 and 300 µL/L of GEO during 30 d was evaluated (Table 1). Treatment with 100 µL/L was effective in decreasing 3 Log CFU/g the population when compared to the control group after 30 d. In addition, the treatment with 200 µL/L decreased the fungal population in 3.7 and 4 Log CFU/g in 15 and 30d, respectively. The concentration of 300 µL/L decreased the fungal population below its limit of detection after 15d, showing that the oil works in a dose-dependent manner.

Regarding the production of aflatoxins (Table 2), it was observed that the concentration of 300 µL/L of GEO was able to significantly reduce the production of AFB1 after 15d of incubation. For AFB2 a significant reduce was observed only using 300 µL/L of GEO at day 15. It is also important to highlight that aflatoxin

concentration tend to reduce at higher levels of GEO, as shown by the average results. However, there was heterogenicity among samples, especially for the control group and lower doses of GEO, where some samples presented high levels of AFs and other showed the opposite. This fact resulted, for some cases, in no significant difference among groups, but the treatment with 300 µL/L was the only one that presented very low levels of AFs consistently.

Abd El-Aziz et al. (2015), evaluated the effect of GEO in a concentration of 4% to reduce the aflatoxins B production by *A. flavus,* using SMKY liquid medium (sucrose, 20g; magnesium sulfate, 0.5g; potassium nitrate, 3 g; yeast extract, 7 g; and distilled water, 1000 mL) with the oil and after inoculating disk of 6 mm diameter of the fungi at 25 °C for 7 days. Abd El-Aziz and colleagues showed that aflatoxins B decreased from 28.5 to 62.8%.

The authors of the present study affirm that there are no similar studies in the bibliography literature to compare, and our research group has developed many studies using natural compounds as allyl isothiocyanate (AITC) against different species of toxigenic fungi in corn and other food matrices (Tracz et al., 2017; Nazareth et al., 2016; Nazareth et al., 2018; Lopes et al., 2017). AITC and allicin have similar chemical structure, both present electrophilic carbons and high reactivity with other molecules. For this reason, our data will be compared to studies that used AITC as fumigant in food matrices.

Nazareth et al. (2018), showed that gaseous AITC at concentrations of 10 and 50 µL / L avoided the production of fumonisin B1 by *Fusarium verticillioides* CECT 2983 in corn kernels for 180d. Quiles et al. (2019) determined the production of mycotoxins produced by *Aspergillus flavus* ISPA 8111 and *Penicillium verrucosum* D-01847 VTT in corn, barley and wheat after 30d of storage when treated with AITC. *A. flavus* produced 8,07 µg/kg of AFB1 in corn after the storage period, whereas the production of AFB1 was 0,12 µg/kg when corn was treated with 500 µL/L of AITC, showing a reduction of 98,51%. Tracz et al. (2017), evaluated the effect of gaseous AITC at 50, 100 e 500 µL/L in the production of mycotoxins in corn kernels contaminated with *Aspergillus parasiticus* CECT 2947, *F. verticillioides* CECT 2983, *F. tricinctum* CECT 20150, *Alternaria alternata* CECT 2662 and *Gibberella zeae* CECT 2150 and stored during 30d. This study showed that all treatments avoided

mycotoxins production, in relation of aflatoxins, was related a significant reduction when used concentrations of 50 µL/L and above of AITC.

These studies confirm that natural compounds when used in gas phase have effectiveness against the fungal growth and mycotoxins production. Our results show that GEO was effective to reduce *A. flavus* population and aflatoxins production.

4. Conclusion

The effectiveness of GEO to decrease the fungal growth of *A. flavus* in all the concentrations tested in stored corn kernels was shown. The concentration of 300 µL/L was able to reduce the fungal growth below the limit of detection after 15 days of incubation. Moreover, the aflatoxins B1 and B2 were also reduced using the concentration of 300 µL/L of GEO. In conclusion, this compound may be used as a new fumigant of corn kernels during storage. Further studies should be conducted demonstrating the potential effect of this compound when used to treat other grains or toxins.

Author contributions

K.P.B.: Investigation, Formal analysis, and Writing - Original Draft. **J.M.Q:** Investigation and Formal analysis. **J.M:** Resources. **G.M.:** Investigation, Formal analysis, Resources, and Supervision. **F.B.L.:** Investigation, Formal analysis, Resources, Writing - Review & Editing, Supervision, Project administration, and Funding acquisition.

Competing interests Statement

None.

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Table captions

Table 1. Population of *Aspergillus flavus* ISPA 8111 in stored corn treated or not with 100, 200 e 300 µL/L of garlic essential oil.

Table 2. Determination of AFB1 and AFB2 in stored corn kernels contaminated with *Aspergillus flavus* ISPA 8111 with or without the application of 100, 200 e 300 µL/L of garlic essential oil.

REFERENCES

Anfossi, L., Giovannoli, C., Baggiani, C., 2016. Mycotoxin detection. Curr Opin Biotech. 37, 120-126.

Abd El Aziz A.R.M., Mahmoud M.A., Al-Othman M.R., Al-Gahtani M.F., 2015. Use of Selected Essential Oils to Control Aflatoxin Contaminated Stored Cashew and Detection of Aflatoxin Biosynthesis Gene. Sci. World J.

Arasu, M.V., Viayaraghavan, P., Ilavenil, S., Al-Dhabi, N.A., Choi, K.C., 2019. Essential oil of four medicinal plants and protective properties in plum fruits against the spoilage bacteria and fungi. Ind Crop Prod. 133, 54-62.

Boonpeng, S., Siripongvutikorn, S., Sae-Wong, C., Sutthirak, P., 2014. The antioxidant and anti-cadmium toxicity properties of garlic extracts. Food Sci. Nutr. 2, 792–801.

Chen, H., Davidson, P. M., Zhong, Q., 2014. Impacts of sample preparation methods on solubility and antilisterial characteristics of essential oil components in milk. Appl. Environ. Microbiol. 80, 907-916.

Clinical and Laboratory Standards Institute (CLSI), 2008. Reference Method for Broth Dilution Tests for Determination of Sensitivity to Antifungal Filamentous Fungus Therapy: Approved Standard: Approved Standard M38-A2. Wayne, PA, USA. doi.org/10.1016/j.foodcont.2016.07.033

Curtis, H., Noll, U., Störmann, J., Slusarenko, A.J., 2004. Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. Physiol Mol Plant P. 65, 79-89.

El-Sayed, H.S., Chizzola, R., Ramadan, A.A., Edris, A.E., 2017. Chemical composition and antimicrobial activity of garlic essential oils evaluated in organic solvent, emulsi- fying, and self-microemulsifying water based delivery systems. Food Chem. 221, 196–204.

Espinel-Ingroff, A., Fothergill, A., Peter, J., Rinaldi, M. G., & Walsh, T. J., 2002. Testing conditions for determination of minimum fungicidal concentrations of new and established antifungal agents for *Aspergillus* spp.: NCCLS collaborative study. J. Clin. Microbiol. 40, 3204-3208.

Hayat, S., Cheng, Z., Ahmad, H., Ali, M., Chen, X., Wang, M., 2016. Garlic, from remedy to stimulant: Evaluation of antifungal potential reveals diversity in phytoalexin allicin content among garlic cultivars; allicin containing aqueous garlic extracts trigger antioxidants. Front. Plant Sci. 7, 1235.

Jedidi, I., Soldevilla, C., Lahouar, A., Marín, P., González-Jaén, M.T., Said, S., 2018. Mycoflora isolation and molecular characterization of *Aspergillus* and *Fusarium* species in Tunisian cereals. Saudi J. Biol. Sci. 25, 868–874. https://doi.org/10.1016/j.sjbs.2017.11.050

Ji, X., Li, J., Meng, Z., Dong, S., Zang, S., Qiao, K., 2019. Inhibitory effect of allicin against *Meloidogyne incognita* and *Botrytis cinerea* in tomato. Sci Hortic. 253, 203-208

Lee, D.Y., Li, H., Lim, H.J., Lee, H.J., Jeon, R., Ryu, J.H., 2012. Anti-inflammatory activity of sulfur-containing compounds from garlic. J. Med. Food. 15, 992–999.

Lee, H.S., Lim, W.C., Lee, S.J., Lee, S.H., Lee, J.H., Cho, H.Y., 2016. Antiobesity effect of garlic extract fermented by *Lactobacillus plantarum* bl2 in diet-induced obese mice. J. Med. Food. 19, 823–829.

Leontiev, R., Hohaus, N., Jacob, C., Gruhlke, M.C.H., Slusarenko, A.J.A., 2018. Comparison of the Antibacterial and Antifungal Activities of Thiosulfinate Analogues of Allicin. Sci. Rep. 8, 6763. doi:10.1038/s41598-018-25154-9

Lopes, L. F., Bordin, K., Lara, G.H.C., Saladino, F., Quiles, J.M., Meca, G., Luciano, F.B., 2018. Fumigation of Brazil nuts with allyl isothiocyanate to inhibit the growth of *Aspergillus parasiticus* and aflatoxin production. J Sci Food Agri. 98, 792-798.

Luciano, F.B., Holley, R.A., 2009. Enzymatic inhibition by allyl isothiocyanate and factors affecting its antimicrobial action against *Escherichia coli* O157:H7. Int J Food Microbiol. 131, 240–245.

Magnoll, A., Tallone, L., Rosa, C.A.R., Dalcero, A.M., Chiacchiera, S.M., Sanchez, R.M.T., 2008. Comercial bentonites as detoxifier of broiler feed contaminater with aflatoxin. Appl. Clay Sci. 40, 63-71.

Moses, J. A., Jayas, D. S., Alagusundaram, K., 2015. Climate change and its implications on stored food grains. Agric Res. 4, 21-30.

Nazareth, T.M., Bordin, K., Manyes, L., Meca, G., Mañes, J., Luciano, F.B., 2016. Gaseous allylisothiocyanate to inhibit the production of aflatoxins, beauvericin and enniatins by *Aspergillus parasiticus* and *Fusarium poae* in wheat flour. Food Control. 62, 317-21.

Nazareth, T.M., Corrêa, J.A.F., Pinto, A.C.S.M., Palma, J.B., Meca, G., Bordin, K., Luciano, F.B., 2018. Evaluation of gaseous allyl isothiocyanate against the growth of mycotoxigenic fungi and mycotoxin production in corn stored for 6 months. J Sci Food Agri. 98, 5235-5241.

Njobeh, P. B., Dutton, M. F., Koch, S. H., Chuturgoon, A. A., Stoev, S. D., Mosonik, J. S., 2010. Simultaneous occurrence of mycotoxins in human food commodities from Cameroon. Mycotoxin Res. 26, 47-57.

Oliveira, M.S., Rocha, A., Sulyok, M., Krska, R., Mallmann, C.A., 2016. Natural mycotoxin contamination of maize (*Zea mays L.*) in the South region of Brazil. Food Control. 73, 127-132.

Percival, S.S., 2016. Aged garlic extract modifies human immunity. J. Nutr. 146, 433S–436S.

Putnik, P., Gabrić, D., Roohinejad, S., Barba, F. J., Granato, D., Mallikarjunan, K., Bursać Kovačević, D., 2019. An overview of organosulfur compounds from *Allium* spp.: From processing and preservation to evaluation of their bioavailability, antimicrobial, and anti-inflammatory properties. Food Chem. 276, 680-691. doi:10.1016/j.foodchem.2018.10.068

Quiles, J. M., Nazareth, T. D. M., Luz, C., Luciano, F. B., Mañes, J., Meca, G., 2019. Development of an Antifungal and Antimycotoxigenic Device Containing Allyl Isothiocyanate for Silo Fumigation. Toxins, 11, 137.

Ramos, A.J., Hernández, E., Plá-Delfina, J.M., Merino, M., 1996. Intestinal adsorption of zearalenona and in vitro study of non-nutritive sorbent materials. Int. J. Pharm. 128, 129-137.

Rushing, B. R., Selim, M. I., 2019. Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. Food Chem Toxicol. 124, 81-100.

Saladino, F., Quiles, J. M., Mañes, J., Fernández-Franzón, M., Luciano, F. B., Meca, G., 2017. Dietary exposure to mycotoxins through the consumption of commercial bread loaf in Valencia, Spain. LWT, 75, 697-701.

Seckiner, I., Bayrak, O., Can, M., Mungan, A.G., Mungan, N.A., 2014. Garlic supplemented diet attenuates gentamicin nephrotoxicity in rats. Int. Braz. J. Urol, 40, 562–567.

Terzi, V., Tumino, G., Stanca, A. M., Morcia, C., 2014. Reducing the incidence of cereal head infection and mycotoxins in small grain cereal species. J. Cereal Sci. 59, 284-293.

Tracz, B. L., Bordin, K., de Melo Nazareth, T., Costa, L. B., de Macedo, R. E. F., Meca, G., Luciano, F. B., 2017. Assessment of allyl isothiocyanate as a fumigant to avoid mycotoxin production during corn storage. LWT, 75, 692-696.

Yoshida, H., Katsuzaki, H., Ohta, R., Ishikawa, K., Fukuda, H., Fujino, T., Suzuki, A., 1999. Antimicrobial activity of the thiosulfinates isolated from oil-macerated garlic extract. Biosci Biotech Bioch. 63, 591-594.

Yun, H.M., Ban, J.O., Park, K.R., Lee, C.K., Jeong, H.S., Han, S.B., Hong, J.T., 2014. Potential therapeutic effects of functionally active compounds isolated from garlic. Pharmacol Ther. 142, 183–195.

Zhu, Y., Anand, R., Geng, X., Ding, Y., 2018. A mini review: garlic extract and vascular diseases. Neurol. Res. 1–5.

Tables

Table 1. Population of *Aspergillus flavus* ISPA 8111 in stored corn treated or not with 100, 200 e 300 µL/L of garlic essential oil.

Population of *Aspergillus flavus* ISPA 8111(Log CFU/g)				
Days	Control	100µL/L	200µL/L	300 µL/L
0	4.74 ± 0.16^{Aa}	4.74 ± 0.16^{Ba}	4.74 ± 0.16^{Ca}	4.74 ± 0.16^{Ba}
15	7.33 ± 0.23^{Cc}	6.86 ± 0.57^{Cc}	3.63 ± 0.49^{Bb}	$\leq LD^{Aa}$
30	5.65 ± 0.35^{Bd}	2.5 ± 2.93^{Ac}	1.56 ± 1.23^{Ab}	$\leq LD^{Aa}$

Different capital letter (A,B,C) in the same column indicate statistical difference ($p \leq 0,05$).
Different lower case letters (a,b,c,d) on the same line indicate statistical difference ($p \leq 0,05$).
*Detection limit = 1,22 Log CFU/g.
(Mean± SD)

Table 2. Determination of AFB1 and AFB2 in stored corn kernels contaminated with *Aspergillus flavus* ISPA 8111 with or without the application of 100, 200 e 300 µL/L of garlic essential oil.

Determination of **AFB1** in corn kernels (µg/Kg)				
Days	(Mean ± SD)			
	Control	**100 µL/L**	**200 µL/L**	**300 µL/L**
0	1.45± 1.05^{Aa}	1.45± 1.05^{Aa}	1.45± 1.05^{Aa}	1.45± 1.05^{Aa}
15	988.10±381.50^{Cc}	443.10±385.80^{Cb}	439.70± 126.60^{Cb}	17.20±10.81^{Aa}
30	362.20±538.00^{Bb}	243.20±352.50^{Bb}	181.10 ±169.50^{Bab}	31.01± 32.95^{Aa}
Determination of **AFB2** in corn kernels (µg/Kg)				
Days	(Mean ± SD)			
	Control	**100 µL/L**	**200 µL/L**	**300 µL/L**
0	9.41± 5.52^{Aa}	9.41± 5.52^{Aa}	9.41± 5.52^{Aa}	9.41± 5.52^{Aa}
15	145.10±124.40^{Bb}	35.81±42.65^{Aab}	59.38 ±82.67^{Aab}	8.28±3.43^{Aa}
30	68.94 ±57.71^{Ba}	25.48±16.15^{Aa}	26.95±35.58^{Aa}	35.53±29.21^{Aa}

Different capital letter (A, B, C), on the same column indicate statistical difference ($p \leq 0,05$).
Different lower case letters (a,b,c) on the same line indicate statistical difference ($p \leq 0,05$).

CAPÍTULO 5

CONCLUSÃO/ IMPACTO E PERSPECTIVAS FUTURAS

Os três compostos utilizados apresentaram atividade antifúngica, podendo ser utilizados como forma alternativa ao tratamento de grãos e outros alimentos, assim como podendo evitar a produção de micotoxinas. O uso combinado de ITCA com CIN nas concentrações de 30 e 300 µL/L foi capaz de reduzir o crescimento de *A. parasiticitus* CECT 2681, *F. verticillioides* CECT 2985 e *G. zeae* CECT 2150, assim como reduzir a produção das aflatoxinas B1, G1 e G2. Entretanto a dose de 300 µL/L foi a que apresentou maior potencial antifúngico.

O uso do ITCA + CIN na proporção 1:5 reduziu a concentração de ITCA o que é importante pois esse composto possui sabor e odor bastante forte quando utilizado sozinho. CIN apresenta odor e sabor mais agradável, sendo assim, essa formulação combinada viabiliza o desenvolvimento de um produto com melhores aspectos sensoriais.

O OEA mostrou efetividade como agente antifúngico na concentração de 1000 µL/L, sendo capaz de reduzir o crescimento fúngico de *A. parasiticus* CECT 2681, *F. verticillioides* CECT 2985 e *G. zeae* CECT 2150 em grãos de milho, porém esse óleo não apresenta capacidade de reduzir diretamente *in vitro* a concentração de micotoxinas e a bioacessibilidade das mesmas após processo de digestão simulada. Em concentrações menores de 100, 200 e 300 µL/L o OEA foi capaz de reduzir o crescimento fúngico de *A. flavus* ISPA 8111 em grãos de milho após 30 d de incubação, onde 300 µL/L foi capaz de reduzir a população fúngica abaixo do limite de detecção. As aflatoxinas B1 e B2 foram também reduzidas significativamente após 15 d de incubação utilizando 300 µL/L de OEA. Esses resultados mostram que AITC combinado com CIN e o OEA podem ser utilizados como agentes fumigantes para evitar a deterioração fúngica e a produção de micotoxinas em grãos de milho.

Entretanto para estudos futuros, sugere-se o uso do OEA combinado com outros compostos, pois este óleo possui odor e sabor muito pronunciado, podendo assim mascarar esse fator sensorial negativo. Os compostos podem ser utilizados em concentrações intermediárias as mostradas neste estudo e também em outros

grãos, como trigo, arroz e soja que são muito acometidos a contaminações por fungos micotoxigênicos e ficam longos períodos em estocagem. Dessa forma, propomos futuramente o desenvolvimento de um produto natural e volátil, que possa ser utilizado alternativamente a compostos químicos já em uso, sendo assim, um novo agente fumigante promovendo a segurança alimentar.

É de grande importância destacar que o OEA apesar de apresentar atividade antifúngica e antimicotoxogênica em concentrações entre 100 e 1000 µL/L como já mencionado em nosso estudo, apresenta um valor muito alto, sendo também de difícil obtenção. Além disso, o composto apresenta odor bastante pronunciado, o que pode inviabilizar o uso do mesmo para tratamento de grãos como o milho. Outras aplicabilidades como em produtos com maiores valores agregados, como sementes oleaginosas podem ser futuramente testados. Também é sugerido o seu uso em combinação com outros óleos essenciais para que possa se mascarar o seu odor pronunciado e, potencialmente, reduzir as doses utilizadas.

ANEXO 1 – Normas do periódico Food Bioscience

FOOD BIOSCIENCE

AUTHOR INFORMATION PACK

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ISSN: 2212-4292

DESCRIPTION

Food Bioscience is a peer-reviewed journal that aims to provide a forum for recent developments in the field of **bio-related food** research. The journal focuses on both fundamental and applied research worldwide, with special attention to **ethnic** and **cultural** aspects of food bioresearch. Topics covered in the journal include but are not limited to:
Biochemical, biophysical and biological **properties of foods**, ingredients, and components Mechanism of **functional foods** and ingredients including both novel and traditional fermented foods Genetic, and cellular and molecular biology germane to **food production** and **processing Foodomics**: comprehensive studies involving genomics, proteomics, metabolomics, nutrigenomics and chemogenomics of foods and their interactions with humans **Biomaterials** for food-related systems such as food packaging, food analysis, and delivery of nutraceuticals and functional food additives Application of novel **technology** to foods.

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ABSTRACTING AND INDEXING

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GUIDE FOR AUTHORS

INTRODUCTION

Description

Food Bioscience is a peer-reviewed academic journal publishing original research articles, reviews, and commentaries concerning the latest development in multidisciplinary areas in food science, with an emphasis on the mechanistic studies of food quality and stability at the molecular and cellular levels. Manuscripts with innovative ideas and/or approaches that bring together different fields will receive special priority. In addition, we also address up-to-date research highlights, news and views, and commentaries covering research policies and funding trends. All research and review articles are subject to strict peer review organized by the journal, and final acceptance or rejection decision resides with the Editor-in-Chief of *Food Bioscience*.

Aims and scope

Food Bioscience is a peer-reviewed journal that aims to provide a forum for recent developments in the field of bio-related food research. The journal focuses on both fundamental and applied research worldwide, with special attention to ethnic and cultural aspects of food bioresearch. Topics covered in the journal include but are not limited to:
(1) Biochemical, biophysical and biological properties of foods, ingredients, and components
(2) Mechanism of functional foods and ingredients including both novel and traditional fermented foods
(3) Genetic, and cellular and molecular biology germane to food production and processing
(4) Foodomics: comprehensive studies involving genomics, proteomics, metabolomics, nutrigenomics and chemogenomics of foods and their interactions with humans
(5) Biomaterials for food-related systems such as food packaging, food analysis, and delivery of nutraceuticals an d functional food additives
(6) Application of novel technology to foods. Articles relating only to structural identification and characterization of bioactive compounds without biofunctional data will not be published in *Food Bioscience*.
Articles reporting the following will not be published in Food Bioscience:
o Structural identification and characterization of bioactive compounds without biofunctional data
o Direct medical claims and/or clinical studies: therapeutic application of food compounds/isolates for treatment, cure or prevention of human diseases
o Processing/engineering without any chemistry
o Pharmaceutical, herbal, and traditional or folk medicines that are not consumed as foods
o Survey/surveillance data.

Article types

Submissions of the following types of articles are invited: short communications, mini-reviews, reviews (after discussion with the editors), and research articles. In addition, the journal will also present up-to-date research highlights, news and views, and commentaries covering food research and policy.

(1) Research Articles are a contribution describing original research, including theoretical expositions, extensive data and in-depth critical evaluation, and are peer reviewed. The total length of a manuscript excluding the abstract, acknowledgements, figures, tables and references must not exceed 6000 words.

(2) Review Articles and Mini-reviews are encouraged for giving an in-depth overview of a specific topic. The format and length of review papers are more flexible than for a full paper. There is a 6,000 word limit for Mini-reviews and a 10,000 word limit for Review Articles under normal circumstances. Authors may make a case to the editor if they believe there is justification for a longer length for these submissions. All review papers will be fully peer reviewed.

(3) Short Communications are for concise, but independent reports representing a significant contribution to food science and engineering, not as mechanism to publish preliminary results. Only if these results are of exceptional interest and are particularly topical and relevant will they be considered for publication. A Short Communication should be no more than 3000 words, and could include up to 4 figures or tables. It should have at least 8 references. Short communications will be fully peer reviewed.

Submission checklist
You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the working corresponding author with contact details (please see the discussion of the title page):
• E-mail address
• Full postal address
• Phone

All necessary files have been uploaded:
Manuscript:
• Include keywords
• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable). Please see the "Manuscript Preparation and Submission Guideline" for placement.
Supplemental files (where applicable). Please see the "Manuscript Preparation and Submission Guideline" for placement.

Further considerations
• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• A conflict of interest statement is provided in the manuscript before the acknowledgments, even if the authors have no competing interests to declare.
• This summary statement will ultimately be published if the article is accepted. Please see the "Manuscript Preparation and Submission Guideline" for suggested statement and Declaration of Interest for what is covered. Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Submission declaration and verification
Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked for originality using Ithenticate. The program has been set for Food Bioscience to not consider references, quotes, and phrases of less than 5 words. Crossref Similarity Check.

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Author contributions

For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example

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Language (usage and editing services)

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Authors must provide and use an email address unique to themselves and not shared with another author registered in Editorial Submission system, nor a department.

Referees

Please submit the names and institutional e-mail addresses of several potential referees. For more details, visit our Support site. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

It is expected that authors who publish in *Food Bioscience* will be asked to review future manuscripts submitted to the journal.

Review process

A peer review system involving two or three reviewers is used to ensure high quality of manuscripts accepted for publication. The Editor-in-Chief and Editors have the right to decline formal review of the manuscript when it is deemed that the manuscript is 1) on a topic outside the scope of the Journal, 2) lacking technical merit, 3) focused on foods or processes that are of narrow regional scope and significance, 4) fragmentary and provides marginally incremental results, or 5) is poorly written.

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. See more information on types of peer review.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Manuscript preparation and submission Guideline

General requirements
Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication elsewhere; that its offer for publication has been approved by all co-authors. The author warrants that his/her contribution is original and that he/she has full power to offer the manuscript. The publisher will not be held legally responsible should there be any claims for compensation. The manuscript should be a complete and authoritative accounts of work which have special significance, general interest and which are presented clearly and concisely. The review articles should give not only comprehensive and authoritative descriptions of one specific subject within the journal's scope, but also the specific recommendations for future research directions.

The following components are required for a complete manuscript: Title, Author(s), Author affiliation(s), Corresponding author, Abstract, Keywords, Main text (including Introduction, Materials and Methods, Results, and Discussion, Conclusion), References, Conflict of Interest, Acknowledgements, References, Tables, Figure Legend, and Figures. The length of the main text for Short Communication should not exceed 3000 words (as counted by a word processing program), and the total number of tables and figures should be no more than 4. The length of the main text for Original Research Articles and Mini-Reviews should not exceed the equivalent of 6000 words, and there is a 10,000 word limit for Review Articles. Exceptions should be discussed with the editor. See below for "Optional Components."

Contact details for submission
Submission of all types of manuscripts to *Food Bioscience* proceeds totally online. Via the Elsevier Editorial System (EVISE) website for this journal (https://www.evise.com/profile/api/navigate/EVISE_FBIO) you will be guided step-by-step through the creation and uploading of the various files.

Manuscripts for review articles
Reviews give a general overview of a particular field, providing the reader with an appreciation of the importance of the work, historical context, a summary of recent developments, and a starting point for delving further into the literature. Manuscripts should be divided into appropriate sections, with an extensive list of references. In addition to undergoing the same rigorous level of technical peer-review as Research papers, Review articles will be critiqued based on the general impact of the field being reviewed, the relevance of the field to current interest, preexisting reviews of the field, and acknowledgement of the contributing author as an important scientist in the field, although reviews based on the literature review for an advanced degree will be given consideration. Therefore, it is strongly recommended that authors interested in submitting a Review article correspond with the Editor prior to submission. General formatting of text, illustrations, and references are the same as outlined for research papers.

Manuscripts for research papers
Manuscripts should be prepared using Word. The following components are required for a complete manuscript: Cover letter, Title, Author(s), Author affiliation(s), Abstract, Keywords, Main text (including Introduction, Materials and Methods, Results and Discussion, Conclusion), References, Acknowledgements, Tables, Figure legend and Figures. Include page numbers on the document, beginning with the title page as number 1. Continuous line numbering on the left is also required. Please use the standard 12-point Times New Roman fonts.

BEFORE YOU START: INTRODUCTION AND SUGGESTIONS ABOUT STYLE

I. Goal of a scientific manuscript

The purpose of a scientific manuscript is to provide information to the reader. So, please focus on the reader. Ideally, think of a first or second year graduate student trying to read a number of papers to begin to understand the field in which she/he will be working, particularly far away geographically from where you are. And they might even be asked to duplicate the work. A general reader should not have to go to other documents to understand the paper and the student trying to duplicate the work should have enough information about the raw material and its initial handling to be able to duplicate the raw material and the work itself.

II. Consistency of presentation is critical

We are allowing you some flexibility to make certain choices in terms of style. However, you must stay consistent with your choices throughout the entire manuscript.

Towards the end of this guideline, there will be two discussions of general importance: the proper use of significant figures, and a list of words and suggested replacements to improve the manuscript. Science writing is supposed to be objective; do not use "emotional" words to describe things. Scientists are also supposed to be modest. Note that for publication in a peer-reviewed journal, the work needs to be innovative and/or novel. You do not need to tell us that in the text.

When you move from citing the literature to your work, the specification "in this study" may be helpful. Otherwise, it is simply redundant. Please look for other redundancies and extra words – "results obtained" can often be simply "results."

III. English tenses: What is past tense and what is present tense

Your work and the work reported in the literature are generally presented in the past tense. Conclusions can be past or present tense, although Food Bioscience generally prefers past tense. Figures and tables in the paper are referred to in the present tense as they are part of the paper, but are based on work done in the past, e.g., "Figure 1 shows that X is significantly higher ($p < 0.05$) than Y." But: "X was significantly higher ($p < 0.05$) than Y." Definition of terms in an equation are also present tense, i.e., A is absorbance.

IV. Wordiness

Shorter and tighter writing is easier to read. Expressions like "in this study", "the results?" and the listing of samples should not be repeated. Consider expressions like "ultrasonic procedure (method, technique, process)." Does it need the extra word or could it just be expressed as "ultrasound"? Leaving those words out often improves the readability. The goal is to help the reader. It is easier for readers if the same words are used throughout. Scientific writing is about good communications not great literature.

Highlights and/or Visual Highlights (Optional)

These both go before the title page.

Elements of a manuscript
Cover letter.

A cover letter must accompany each submission. It must include the following information:

(1) The brief explanation of the significance of the work presented in the manuscript

(2) The names and contact information for three potential referees

Title page.

Title: Try to keep the title short (<20 words) and not try to tell the whole story in one sentence. Start strong by not using words like "A study of the effects of". Rework so the first thing a reader sees are words related to the topic.

Running Title: This should be under 80 characters including spaces. Shorter is better.

Authors: All authors should have made a SIGNIFICANT contribution to the paper. Others involved in the work should be thanked in the acknowledgment. This is a controversial subject so it also needs to be consistent with the requirements of the country (countries) where the work was done.

Author Affiliation: This does not require full mailing address. The city, state/province, and country along with a postal code are appropriate.

Corresponding Author: This is the person submitting the paper. It should be one person. It is the person doing the actual work of staying in touch with Food Bioscience. However, some countries, including China, require that the head of each laboratory claiming credit for the work needs to be a corresponding author. We will accept this, but please make clear the corresponding author with whom we should be contacting for correspondence. If not noted, we will assume that the first author listed is the corresponding author. (Note: If your institution requires that the head of laboratory or department must be listed as corresponding author, note that he or she might not necessarily need to be listed as an author.) Please include the complete postal mailing address, phone, fax and email for the corresponding author.

Abstract.

The abstract should be an independent story that can stand alone: What you did, why you did it, how you did it and what the results are. Abbreviations are created only if used in the abstract and ideally should be minimized. Any abbreviation used without definition should be widely known, even by graduate students entering the field. The abstract does not usually have any references. Note that abstracts may be circulated without the paper itself. The abbreviations used in the abstract do NOT carry over to the rest of the manuscript.

No small "s" at the end of an abbreviation in the abstract or the text, e.g., CFUs.

It is assumed that any results in the abstract are statistically significant.

The abstract starts on a new page and should be <250 words.

Keywords.

Since keywords must be strong search terms, focus on words that will NOT give thousands of hits. The key materials are a good place to start (including their Latin name and common names as separate keywords). Methods are generally not good search terms unless you are developing them.

The keywords appear on the same page as the abstract.

List of Abbreviations (Optional)

If a list of abbreviations is included, it goes after the keywords. The list of terms should be alphabetized.

Most abbreviations are all capital letters (with no small "s" at the end); but when defining them, the words themselves are often not capitalized. E.g., differential scanning calorimetry (DSC).

Introduction.
The introduction starts a new page. The introduction only includes the material that is necessary for understanding the paper: why the food material you are studying is worth studying and a little about it. What is the background for the research questions you are asking? By the time you get to the objectives (the last part of the introduction), it should almost be obvious from the introduction. The introduction is NOT a review paper.

If you have a really good review in your thesis/report, then consider preparing it as a "review" paper. Food Bioscience is willing to consider review papers. This style guide should still be used even though the organization of such a paper is different and a few aspects of this guideline are not relevant.

References in the Text

References in the text should use "et al."for 3 or more authors. If the authors are in the sentence the format should be: "Jones et al. (2018) found..."[et al. can be italicisized if you like.] If the whole reference is in parentheses, the comma may or may not be added after "al.", but consistency is required within the paper, i.e., (Jones et al. 2018) or (Jones et al., 2018) for every reference. Other examples: (Zhang, 2012) or (Zhang 2012) and (Jones and Zhang, 2015), (Jones and Zhang 2015), (Jones & Zhang, 2015) or (Jones &Zhang 2015). The comma before the year can be used or not used as long as you are consistent with all references.

The ampersand (&) can only be used within parentheses, but not in the text, so Jones and Zhang (2015) or (Jones & Zhang, 2015) are acceptable in the same manuscript. You have choices but consistency remains the challenge.

In a list of multiple references in parentheses, please alphabetize by the first author's last name and then chronologically, i.e., Allan, 1996; 1999; Allan and Jones, 1995. Please use a semi-colon (;) between references.

Materials and methods

Although often thought of as the most boring section of a scientific paper, in many ways this is the most important section. This section is a critical component of science which is that the work is reproducible. So it should be very clear and relatively comprehensive. In this section, provide sufficient detail to allow the work to be reproduced by a relatively new researcher to the field in a country far away. Methods already published should be indicated by a reference and a very brief description. All relevant modifications should be described.

One of the hardest things to do is to describe your raw material. The more information offered about the raw materials, the more chance the data can be generalized from the specific experiments carried out in the paper and the more likely that the work is repeatable (which is a requirement for good science). So, details on the biological raw materials are particularly important. Please note that biological materials, including animals, have seasonal changes along with age, sex, and nutritional changes. Therefore, it is important to indicate specifically when samples were collected and how often. The more details offered the better. Generalizing from one or two samples in one small geographic area should be done with caution.

How was the identity of the material validated?

How did the material get from where you started to the actual start of the experiments, i.e., transport and initial storage, including both conditions and duration of such storage?

All materials and methods

A list of materials (chemicals, filter paper, etc.) might be introduced in one paragraph with their source or sources as they are used in the text. Otherwise, they should be identified properly at the time they are first listed. Either way, authors should be consistent in using one method or the other for chemicals and disposables.

Storage of materials

All materials that are stored for more than one day should include something like the following: "for a maximum of XX wk" (or what the storage time actually is).

Sourcing of materials: What information is needed All equipment and chemicals used should be identified clearly. Any non-routine reagents must be sourced along with all equipment used. The model information goes before the company information. The company's full legal name (generally capitalizing the significant words but not every letter in the company name) must be given and then its location, but the location is only given the first time the company is mentioned. Location should almost always be put in parentheses. If the product is obtained through a distributor but is clearly from a different company, the original company is the company of record. Each instrument needs to be identified only once unless more than one similar piece of equipment was used, e.g., two different centrifuges. (Abbreviations: Co. = Company; Corp. = Corporation, Inc. = Incorporated, Lab. = Laboratory.) This should include a city, state/province and country (and not a street address). For the USA, a state is mandatory and for other countries the state/province information is encouraged, especially for Canada and China. There are official two letter abbreviations for each US state and two and three letter abbreviations for each Canadian province (please stay consistent), and you are encouraged to use these (e.g., Ithaca, NY, USA, Toronto, ONT (or ON), Canada). You may write out the state's name- again, if you do so consistently. Note the preference for USA and US and not U.S.A. and U.S., with USA preferred over US. If the same company is cited with multiple locations, after the first time only the city should be mentioned.

For example, First time: (Sigma Aldrich Co., St. Louis, MO, USA) or Sigma Aldrich Co. (St. Louis, MO, USA). Thereafter (Sigma Aldrich). First time: (Model XYZ, Jiangsu Model Cars Ltd., Wuxi, Jiangsu, China) or Model XYZ (Jiangsu Model Cars Ltd., Wuxi, Jiangsu, China). Thereafter: (Jiangsu Model Cars). Note: Do not use all capital letters in a company name unless the company does so itself. For example, use Titan Corp. and not TITAN Corp.

As this is a Chinese-based journal, we require the use of the Chinese province information. This will help readers become more familiar with China. If the same equipment, chemical or material is used with more than one method, the sourcing is done only the first time. E.g., "A spectrophotometer (XX Corp., Wuxi, Jiangsu, China) was used to?.". And subsequently, "The spectrophotometer was used to?".

Standard method versus most actual methods Except for standard methods that are accepted transnationally (with the method number identified), all methods should be described in sufficient detail that a person can follow what was done without having to go on a literature search. Standard methods can be described briefly and the actual equipment used can be noted. The discussion does not have to be in all of the detail needed to reproduce the data if these are covered in the reference for the method or are standard laboratory practices. For example, one can say "brought to 25 mL with distilled water" rather than "added to a volumetric flask and distilled water added to bring it to the 25 mL mark." A reader should not have to read another paper to understand what was done.

In describing methods, it is not necessary to indicate every sample subjected to the method. That information will appear in the results (including tables and figures). Rather than "The FTIR was measured for XX, YY, ZZ and AA using?", use "The FTIR was measured using?"

If a good description of a method is already available, then it can be used if done properly with quotes and proper attribution. Example: The method of Zhang et al. (2012) was used and was briefly described by Liu et al. (2016) as "DESCRIPTION". The key is that quotation marks are used to show it is copied! Note also that the introduction to methods might best be introduced by starting the sentence with "The method of Zhang et al. (2012) was used." Again, this is a standardized format that helps the reader.

Protein

The Kjeldahl/Dumas methods measure total nitrogen, therefore the conversion factor must be identified and this is to be identified as crude protein. If a method is based on using bovine serum albumen (BSA) as a standard then it should identified as BSA equivalents. If purity was not determined, please add the phrase "assuming it were 100% pure" for BSA.

Please give each method at least one paragraph.

Kits

If you are using a kit, you still need to briefly describe the principle used and the actual method along with how calibration was done, including the units to report results. Please also report on any equipment used that was not part of the kit, e.g., the centrifuge or spectrophotometer.

Room or ambient temperature

Room temperature or ambient temperature should be identified. There are often multiple temperatures within a range as most laboratories are not the same temperature all the time. Use either "room" or "ambient" consistently, not both. The actual temperature needs to only be reported at the first mention.

Units Units need to be expressed consistently. If using mL, uL, and L, the whole paper should use the capital L but ml, ul and l are also acceptable. Temperature can be 25^{o}C or 25 ^{o}C, again consistently. Please use Celsius and no

t Kelvin except for an equation that requires Kelvin. If the equipment is actually built to English units, these may be put in parentheses, but the values in the text must be in metric units.

The "U" for units of enzyme or antibiotic activity units should be defined.

The preference is for "a 40 ml flask" rather than "a 40-ml flask," but if the latter is used consistently, it will be accepted.

Except as the first item in a sentence, the preference is for the numbers to be stated before the material, e.g., "Sample (50 mL) was added to 6 mL of 6% ethanol" rather than "Sample (50 mL) was added to ethanol (6 mL of 6%).

Please use v/w, w/w, and v/v rather than V/W, W/W and V/V. The same holds for bw (body weight), dw (dry weight), ww (wet weight) and fw (fresh weight). These abbreviations do not have to be defined.

Please put a space between the number and M, N, mM, uM, etc.

Percentages have the symbol next to the number, e.g., 40% with no space.

In a sequence with the same units, only the last number has the units attached.

Mixtures When describing mixtures with a colon, e.g., alcohol:water at a 3:5 ratio, there is no space before or after the colon.

Note that there is no space for the ratio symbol even with words.

Manufacturer's information Information obtained from the manufacturer should be identified as "according to the manufacturer." In particular, standards need to be fully described. Ideally, the number of points in the calibration curve and the regression equation that shows it is a linear function should be provided along with the regression value. Extrapolation versus interpolation needs to be considered. With extrapolation you might be going outside of the linear response region.

If the manufacturer gives a specific cut-off, e.g., "this dialysis tubing has a 3,500 Da cut-off," I suggest adding the word "nominal" to show you recognize that it may not be that accurate, e.g., "has a nominal cut-off of 3,500 Da." (Special note on dialysis tubing: There are many different ways to prepare dialysis tubing, so please share how you prepared the tubing with the readers.)

Equations

Equations may be numbered. Normally the numbering is done as (4) on the same line as the equation to the far right. But in the text it should be referred to as Equation 4.

When writing equations, a space before and after the = sign is preferred (e.g., R = 0.2) but if you do not want to do this, then both sides of the = sign should not have a space (e.g., R=0.2).

E.g.

Using the following equation:

A = a b c (1)

where A = absorbance, a = the absorbance coefficient, b = cuvette pathlength and c = concentration. Note that equation 1 assumes a linearity of response.

Chemical Symbols

The use of standard symbols for atoms is encouraged. These do not have to be defined (e.g., Ca, Fe, NaCl, HCl, NaOH?). But please try to use these consistently. More complex compounds can be described using words.

Software

Information obtained from software should be identified as such including which software (ideally including a version number or the year of purchase) with full company information. Software that is an integral component of the instrument but does more than provide the "raw" data should be identified, e.g., "using the software that came with the instrument."

Peak areas

If the assumption is that all peak areas are the same for equal amounts of all materials, this should be indicated as a critical assumption. And one needs to determine if this response is for equal weight or equal number of molecules. Therefore, the handling of the data needs to be described. Are peak areas for any one peak linear as a function of concentration? Are you assuming that the peak area of a peak represents the same amount of material as the peak area of the standard?

Centrifugation

Centrifugation should always indicate the "g force" at the bottom of the tube (maximum) and the time and temperature. Ideally tube size is also mentioned on the first use of that rotor. The "rpm" may be put in parentheses.

For example, the first time: the samples were centrifuged at 3,000 x g (1500 rpm in a M2 rotor, Model T centrifuge, Regenstein Centrifuge Co., New York, NY, USA) at 30oC for 20 min. Thereafter with different speed: samples were centrifuged at 2,000 x g (1000 rpm) at room temperature (22 to 25^{o}C) for 1 hr. [Notes: the room temperature is given as a range the first time it is mentioned. If you use a different centrifuge or different rotor you start over and then you do have to indicate thereafter which centrifuge was used.]

Microbes and other biological names After the first mention with their full *Genus species* name subsequent uses can be as *G. species*. This does not have to be defined as an abbreviation.

Important note: Averaging of CFU must be done prior to transforming to log. Log numbers cannot be averaged.

Animals

Please indicate the official approval received for the protocols to use any animals. The feed used should provide more information than "a pelleted feed was used," including either major ingredients or proximate composition.

Sensory Evaluation

If one is using hedonic measurements to evaluate products/samples, the panel should be a "consumer panel" of sufficient size to be meaningful. As a matter of interpretation, a 9 point scale gives the most sensitivity. A value of 7 out of 9 (78%) is probably the minimum that any consumer would give to a product that they would actually consider purchasing. Five and 7 points scales should probably also consider a ~75% score as the minimum for purchase or use. Another way to look at hedonic data is to indicate the perc ent of consumers who gave scores of 75% or above. With demographic data this can help determine the potential target audience for the product. The assumption that hedonics and other sensory scales are linear, i.e., can be handled like any data, is questionable. Using a trained or semi–trained panel and then asking about overall acceptability is also questionable. Other systems of evaluating products, i.e., with traits specified, are not hedonic scales.

Statistical Analysis

This should be the last section of the methods and materials. It should clearly indicate ALL of the statistics used. One or two way ANOVA should be clearly indicated along with how the means were separated statistically. It should also indicate the software used - treated like any other equipment/ chemicals.

Statistical acceptance level (p/P, lower case "p" is preferred) The acceptance level should be indicated. If you are going to use more than one, that should be clearly stated here. And the spacing of the statistical standard should be consistent. Any of the following spacing formats is acceptable as long as the same format is used throughout the paper: (P<0.05); (P <0.05); (P < 0.05); (p<0.05); (p <0.05); or (p< 0.05). I prefer that there be no spaces, but will ccept any of the above spacings for statistical significance as long as it is used consistently.

Notes on statistical issues

In the text with regular numbers the < and > signs should be directly attached to the number with no space in-between.

P=0.05 is possible so one of the two directions, i.e., P<0.05 or P>0.05 should be P<0.05 or P>0.05.

Properly speaking one should do everything with one significance level. However, to be realistic, other levels are used. Besides 0.05, one may sometimes want to use 0.01 and 0.001. In the text one only indicates one of these numbers - the actual "P" value can be given in a table if important (Please see the section on significant figures). In that case a wording like this might be appropriate: "Although a P<0.05 was generally used, the authors have also chosen to use 0.01 (and/or 0.001) for some of the data to indicate the greater significance of the differences."

Correlations One set of data can be correlated "WITH" another set of data either positively or negatively. Generally, a linear curve fitting should be done, showing the equation and the value of the correlation coefficient or other statistical evaluation.

Results

The actual numbers in Tables and Figures should normally NOT be re–reported in the text. Trends need to be statistically significant. An occasional key number might be mentioned. The order of presentation should come in the most logical order – not necessarily as the research was originally done. If one has a single data set like proximate composition of the starting material – it can go in the text and does not need a special table. If one puts data only in the text, then the standard deviation is needed in the text.

Please do not put methods in the results section.

Please do not feel that every sample has to be mentioned in full detail. Once you have established the "framework," the text can be simplified and that actually makes it clearer. You do not have to start each section by reminding everyone of the samples being studied. You also do not need to review the method again.

Please do not constantly say "In this study,""As can be seen," "The results showed" and similar terms unless you are going from the discussion of another paper and returning to your paper and it is not clear that you are making that transition back to your work. In reporting other studies, it generally is simpler to list the reference: "Chen et al. (2005) showed" and then talk about the actual results instead of "and it was reported that ? (Chen et al., 2016)".

References to the Tables and the Figures (or Fig.) should be capitalized. If more than one figure, use the word Figures only once (e.g., Figures 1 and 4).

Please use "these results" rather than "this result."

Discussion

The discussion should focus on the significant changes observed and why they are important. Generally, each experiment should be discussed and then the multiple methods brought together. Suggestion: If the work is applied work, do not try to go into mechanistic claims – it is not relevant, and confuses and devalues the actual work.

It should explore the significance of the results of the work, not repeat them. It should integrate your findings in a comprehensive picture and place them in the context of the existing literature. A combined Results and Discussion section can be appropriate. Avoid extensive citations and discussion of published literature.

Conclusion

This is not a summary. It should focus qualitatively on the key results, why they may be important, and what are the limitations of these results. Suggestions for further work are also appropriate. This should generally be kept to < 250 words.

Conflict of Interest
Conflict of Interest

Please use the following statement (if true) or a similar statement. Otherwise please bring to our attention any special issues that we need to be aware of. "The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript." Note that any consulting or business ties with any company that might benefit from your research needs to be reported.

Acknowledgements

Please acknowledge all funding (including project numbers where possible) and also thank all those who have contributed to the work who are NOT authors including those involved with the writing. (Even if someone is "paid" to do something, they should be recognized here.)

Contributions of Authors (Optional)

This can be included at this point in the manuscript.

Tables

The title should clearly define the content. Footnotes should deal with abbreviations and the statistics. Be careful to identify what is covered by the statistics, e.g., by rows or by columns. In many cases the statistics should be done in both directions. Think about the direction of the table - what is horizontal versus what is vertical. The horizontal should usually be fewer entries than the vertical. Generally, the horizontal shows the "methods" and the vertical shows the samples. Tables can be done single spaced.

Figures and tables are shown one/page.

Authors should take notice of the limitations set by the size and layout of the journal. Large tables should be avoided. Reversing columns and rows will often reduce the dimensions of a table. If a large amount of data needs to be presented, an attempt should be made to divide the data over two or more tables.

Table requirements

(1) Supply units of measure at the heads of the columns. Abbreviations that are used only in a table should be defined in the footnotes to that table.

(2) Should always use rows and columns to correlate two variables. Tables should be submitted single-spaced with appropriate open space in Word. Do not embed tables as graphic files, document objects, or pictures.

(3) Tables should have three “major” horizontal lines: one under the legend, one under the column heads, and one below the body. Vertical lines are generally not used.

(4) Label each table at the top with a Roman numeral followed by the table title. Insert explanatory material and footnotes below the table. Designate footnotes using lowercase superscript letters (a, b, c) reading horizontally across the table.

(5) Unless needed, the first letter of words within the tables should be capitalized.

(6) Must be sequentially numbered and referred to at least once in the text.

Figure legends

The written material for all figures should appear here with full details. The figure legend goes BEFORE the actual figures. If there are any supplemental figures, they also require a figure legend page. Only the figure number should be shown with the actual figure. The figure legend page should be double spaced like the rest of the text.

Figures

Graphs should be practically self-explanatory. Readers should be able to understand them at a glance. Dimensional drawings and diagrams should include only the essential details and as little lettering as possible. They should present more of a picture than a working drawing. If there is a need to present a construction drawing, please consult with the editor ahead of time.

Figure requirements

(1) Numbering and title: number all figures (graphs, charts, photographs, and illustrations) in the order of their citation in the text and cited as, e.g. Figure 1 (writing out the word "Figure"). Use (a), (b), (c)... to give titles for subfigures if there are any.

(2) Figure quality: should be sharp, noise-free, and of good contrast. All lettering should be large enough to permit legible reduction.

(3) Color of figures: unless necessary, it is best to use black and white for line-drawings; and a grayscale for images.

Many figures still need statistics

The figures that present data still need proper statistics, for example, plots of lines should have statistics both in terms of changes along the X axis for a single line and differences between lines at the same value of X in addition to the error bars, especially if there are statistically significant differences.

Bar graphs and similar presentations almost always need a proper statistical analysis.

Putting data into a figure (line drawings) is not an excuse to not do the proper statistical analysis. Please be sure to explain the statistics used in the figure legends.

Note that many people start a paper by looking at the figures before deciding if they will read the whole paper, so it is okay to not use abbreviations. However, details about methods are not appropriate, including details of the statistical tests used.

These should only appear with their Figure number. Please do not duplicate the figure legends on the pages with figures.

The use of color

Please think carefully about the use of color. We are still a print journal and there is a significant supplemental cost for printing color, which is born by the authors. Consider how it would look in black and white?

Abbreviations

Do not use abbreviations in the title or abstract and limit their use in the text. Expand all abbreviations at first mention in the text. The Journal's website will have a list of abbreviations that do NOT require writing out even the first time.

An abbreviations can only be used after it has been defined after its first use. It can be written out again in the Tables and Figures if you wish. Abbreviations can be used both as singular and plural. So there is no need for a little "s" at the end of any abbreviation. Since the abstract stands alone, it

requires the same guidelines for the use of abbreviation that apply to the paper itself. Abbreviations do not carry–over to the rest of the document where the abbreviations need to be re-established. If you use a list of abbreviations (a nice idea), please alphabetize and try to include all abbreviations used in the paper. Please place this immediately after the keywords.

Please use v for volume, w for weight, d for dry, f for fresh, b for basis, and rpm for revolutions/min as units for measurements. [Note that all are lower case.]

Time Abbreviations

s or sec, min, h or hr, d or day, wk, yr

(This is an example of where you have choices – but stick with your choice!)

Abbreviations for "Company"

Co., Corp., Ltd. (Note that the period is used in these cases.)

Molecular weight abbreviations

Please use MW for molecular weight as Mw could be used for weight average molecular weight.

"That is" and "for example" abbreviations

"I.e., and, e.g., each have a comma before and after.

Less-than, equal and more-than abbreviations, especially for statistical significance

The symbols >, <, ≥ and ≤ go directly with a number, e.g., <2.0. Note that in the case of p/P for statistical significance that $p<0.05$ and $p\geq 0.05$ must be used; the equal case should not be forgotten. One could also use $p\leq 0.05$ and $p>0.05$. The spacing of this information should be consistent throughout the text.

Specific requirements

Temperature: The temperature in Celcius is written as $10^{o}C$, with no space between the number and the degree sign.

Percentage is written with no space between the number and the symbol: It was 10% of the...

Molarity and normality are written with no space between the number and the symbol: The solution was 10M NaCl and could also be called 10N NaCl.

Chemical compounds: the chemical symbols can be used without prior definition so NaCl is preferred over salt, and other simple compounds should be listed using their chemical formula.

Significant Figures

All numbers in science can be expressed as 1.2345 x 10n. The number shown is 5 significant figures. For biological materials, where the variability is great and one has such a limited sample, even with measurements of high precision, it is probably not justifiable to have more than three significant figures, e.g., 1.23 x 10n. This is the rule for Food Bioscience, i.e., no more than 3 significant figures for results even if reporting the results from other authors.

Remember this is only the precision of the measurement and says nothing about the accuracy or the ability to use that number to generalize for the materials being studied.

Exceptions: IR data can be 4 significant figures, i.e., 3725 cm-1. Statistical results may also be 4 significant figures. Weight may also be more significant figures, but remember that routine weighings have an inherent error. Molecular weight and time using a mass spectrometer may also justify more significant figures, but, no more than 2 places after the decimal point.

Not even three significant figures can be justified in some cases as the standard deviation is simply too great. Note that the zero (0) in certain positions is not significant while in other places it is significant, e.g., for 350 the zero is not significant, but for 350.0 both zeroes are significant.

Note: Significant figures

Please referGeneral Language and Formatting

SI units

There are seven, dimensionally independent, base SI-units and two supplementary units. All other units can be derived from the base ones. Below, you can find the list of the base SI units as well as the list of the derived units.
E.g., 1 revolutions per minute is equal to 0.0167 hertz
(Food Bioscience prefers that you retain the term rpm.) Concentration: mol/l

SI base units

UnitSymbolQuantitymeter (metre)mLengthkilogramkgMasssecondsTimeampereAElectric currentkelvinKThermodynamic temperaturemolemolAmount of substancecandelacdLuminous intensity**SI Supplementary Units**radianradPlane angle (2D angle)steradiansrSolid angle (3D angle)

SI derived units

1**UnitSymbolIn SI unitsQuantityMechanics**pascalPakg m^{-1} s^{-2}Pressure, StressjouleJkg m^2 s^{-2}Energy, Work, HeatwattWkg m^2 s^{-3}PowernewtonNkg m s^{-2}Force, Weight**Electromagnetism**teslaTkg s^{-2} A^{-1}Magnetic FieldhenryHkg m^2 s^{-2} A^{-2}InductancecoulombCA sElectric ChargevoltVkg m^2 s^{-3} A^{-1} VoltagefaradFkg $^{-1}$ m^{-2} s^4 A^2Electric CapacitancesiemensSkg^{-1} m^{-2} s^3 A^2 Electrical ConductanceweberWbkg m^2 s^{-2} A^{-1}Magnetic FluxohmΩkg m^2 s^{-3} A^{-3}Electric Resistance**Optics** luxlxcd sr m^{-2}Illuminancelumenlmcd sr Luminous Flux**Radioactivity**becquerelBqs^{-1}RadioactivitygrayGym^2 s^{-1}Absorbed Dose sievertSvm^2 s^{-1}Equivalent Dose **Other**hertzHzs^{-1}Frequencykatalkatmol s^{-1}Catalytic Activity

Database linking and Accession numbers
Elsevier aims at connecting online articles with external databases which are useful in their respective research communities. If your article contains relevant unique identifiers or accession numbers (bioinformatics) linking to information on entities (genes, proteins, diseases, etc.) or structures deposited in public databases, then please indicate those entities according to the standard explained below.

Authors should explicitly mention the *database abbreviation (as mentioned below) together with the actual database number,* bearing in mind that an error in a letter or number can result in a dead link in the online version of the article.

Please use the following format: **Database ID: xxxx**

Links can be provided in your online article to the following databases (examples of citations are given in parentheses): •GenBank: Genetic sequence database at the National Center for Biotechnical Information (NCBI) (GenBank ID: BA123456) •PDB: Worldwide Protein Data Bank (PDB ID: 1TUP) •CCDC: Cambridge Crystallographic Data Centre (CCDC ID: AI631510) •TAIR: The Arabidopsis Information Resource database (TAIR ID: AT1G01020) •NCT: ClinicalTrials.gov (NCT ID: NCT00222573) •OMIM: Online Mendelian Inheritance in Man (OMIM ID: 601240) •MINT: Molecular INTeractions database (MINT ID: 6166710) •MI: EMBL-EBI OLS Molecular Interaction Ontology (MI ID: 0218) •UniProt: Universal Protein Resource Knowledgebase (UniProt ID: Q9H0H5)

Highlights

Highlights are optional yet highly encouraged for this journal, as they increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Artwork

Electronic artwork

General points

- Make sure you use uniform lettering and sizing of your original artwork.
- Embed the used fonts if the application provides that option.
- Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Provide captions to illustrations separately.
- Size the illustrations close to the desired dimensions of the published version.
- Submit each illustration as a separate file.
- Ensure that color images are accessible to all, including those with impaired color vision.

A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.

Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings, embed all used fonts.

TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

References

General format of Reference
References

Please use regular double space with the same type face. **General format of references, a true test of consistency** For journal articles make sure that all authors are listed (no et al.), and that the abbreviation of authors' surnames has consistent spacing. The use of commas should be consistent, and the final author should be preceded by an "and" or an "&" or may just continue the list. The date should come next. It may be in parentheses () or not. The journal article title should be lower case for all but the first word, and proper nouns and abbreviations that are capitalized. If there is a colon (:) or dash (also has a space before and after the dash) in the title then, the next letter is capitalized.

Book titles should be capitalized for each main word. They may or may not be italicized, but this needs to be consistent. For citations to a chapter in a book, the page numbers must be given. The name and location of the publisher needs to be given. The custom is to use major world cities alone, e.g., Beijing. Only the first city is given. Other cities need to be identified more fully (e.g., Boca Raton, FL, USA). Note that New York is the city. NY is the state. So one can write "New York, NY, USA" or New York but must write "Ithaca, NY, USA." Either the publisher or publisher location can be listed first as long as there is consistency for all references.

For example:

Cheeke, P.R. (1999). Applied Animal Nutrition (pp. 525). Upper Saddle River, NJ, USA: Prentice Hall.
Choi, J.S., Lee, C.K., Jeon, Y.J., Byun, H.G., & Kim, S.K. (1999). Properties of the ceramic composites and glass ceramics prepared by using the natural hydroxyapatite derived from tuna bone. Journal of the Korean Industrial Engineering Chemistry, 10, 394-399.

Waagb?, R. (2006). Feeding and disease resistance in fish. In R. Mosenthin, J. Zenek, & T. Zebrowska (Eds). Biology of Nutrition in Growing Animals (pp. 387-415). London, UK: Elsevier Health Sciences.

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ANEXO 2 – Normas do periódico Food Control

FOOD CONTROL

An official scientific journal of the European Federation of Food Science and Technology (EFFoST) and the International Union of Food Science and Technology (IUFoST).

AUTHOR INFORMATION PACK

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Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

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Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

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Engle, E.K., Cash, T.F., & Jarry, J.L. (2009, November). The Body Image Behaviours Inventory-3: Development and validation of the Body Image Compulsive Actions and Body Image Avoidance Scales. Poster session presentation at the meeting of the Association for Behavioural and Cognitive Therapies, New York, NY.

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ANEXO 3 – Normas do periódico International Journal of Food Microbiology

INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY

An official journal of the International Committee on Food Microbiology and Hygiene (ICFMH) of the IUMS

AUTHOR INFORMATION PACK

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ISSN: 0168-1605

DESCRIPTION

The *International Journal of Food Microbiology* publishes papers dealing with all aspects of food microbiology. Articles must present information that is novel, has high impact and interest, and is of high scientific quality. They should provide scientific or technological advancement in the specific field of interest of the journal and enhance its strong international reputation. Preliminary or confirmatory results as well as contributions not strictly related to **food microbiology** will not be considered for publication.

Full-length original research papers, review articles and book reviews in the fields of **bacteriology**, **mycology**, **virology**, **parasitology**, and **immunology** as they relate to the production, processing, service and consumption of foods and beverages are welcomed. Within this scope, topics of specific interest include: (1) incidence and types of food and beverage **microorganisms**, microbial interactions, **microbial ecology** of foods, intrinsic and extrinsic factors affecting microbial survival and growth in foods, and **food spoilage**; (2) microorganisms involved in food and beverage fermentations (including probiotics and starter cultures); (3) **food safety**, indices of the sanitary quality of foods, microbiological **quality assurance**, biocontrol, microbiological aspects of **food preservation** and novel preservation techniques, predictive microbiology and **microbial risk assessment**; (4) foodborne microorganisms of **public health** significance, and microbiological aspects of foodborne diseases of microbial origin; (5) methods for microbiological and immunological examinations of foods, as well as rapid, automated and molecular methods when validated in food systems; and (6) the biochemistry, physiology and molecular biology of microorganisms as they directly relate to **food spoilage**, **foodborne disease** and food **fermentations**.

Papers that do not have a direct food or beverage connection will not be considered for publication. The following examples provide some guide as to the type of papers that will not be admitted to the formal review process (for a more extensive list please refer to the journal's Guide for Authors:
Studies in animal models that determine the responses of probiotic microorganisms in the gastrointestinal tract; Fundamental physiology and gene expression studies of food/ beverage microorganisms, unless they directly relate to the food/ beverage ecosystem; The isolation and characterization of antimicrobial substances such as essential oils, bacteriocins etc, unless their efficacy is tested and validated in the food/beverage ecosystem; Development of new methods for the analysis of microorganisms, unless the method is tested and validated in the food/beverage ecosystem.

This journal also publishes special issues of selected, peer-reviewed papers from suitable meetings, workshops, conferences, etc, related to the field of food microbiology.

AUDIENCE

Industrial and food Microbiologists, Bacteriologists, Immunologists, Mycologists, Parasitologists, Virologists, Food Hygienists.

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GUIDE FOR AUTHORS

INTRODUCTION

Types of paper

• Full-length Research Articles are complete reports of original, scientifically sound research. They must contribute new knowledge and be organized as described in this Guide. Manuscripts should not exceed 8000 words. Please follow carefully the organization of the sections described in "Preparation of text files" (see below).
• Reviews are papers which provide an analysis of a scientific or applied field, which include all important findings and bring together reports from a number of sources. Manuscripts should not exceed 12,000 words (excluding references). Review articles may be invited by the Editor or the Editorial Board. Alternatively, potential authors considering the preparation of a Review article should contact the Editor to suggest the topic and its scope, providing an outline in the form of major headings and a summary statement. In any case, such articles are subject to the normal processes of peer review and revision.

Subjects not considered for publication

• Development of methods if not validated in situ. To be suitable for publication in IJFM, new methods for the detection and/or quantification of target microorganisms must be validated in artificially and naturally contaminated foods. Such papers focusing on method development without application in the food matrix should be submitted to journals dealing with microbiological methods or applied microbiology.
• Natural and safe antimicrobial substances: since an extended literature is available on this subject, IJFM publishes only relevant and innovative papers. More specifically:
- in the case of essential oils, spices and chemical compounds: the antimicrobial activity should be tested in real food systems to validate their efficacy, testing in vitro only would not be sufficient for publication in IJFM. Moreover a detailed chemical analysis of the natural extract should be presented with indication of which compounds are exerting the antimicrobial activity;
- for bacteriocins, surveys of bacteriocin-producing strains in food products would not be considered unless the genes responsible for production were genetically characterized to show originality of such genes. IJFM gives priority to papers describing new bacteriocins (as determined by genetic approaches, N-terminal sequencing or results on antimicrobial spectrum and mechanisms) and application of bacteriocinogenic strains in situ, other than surveys of bacteriocin-producing strains in food products.
• Surveys focusing on the detection and quantification of toxins and microbial metabolites (mycotoxins, bacterial toxins, biogenic amines) and papers presenting new methods for detection and quantification of toxins and microbial metabolites will not be published in IJFM, unless they do contain correlated microbiological data of food safety significance. Papers presenting analytical data only should be sent to toxicology or food control journals.
• Gut microbiology and probiotic-targeted papers will have to present relevant direct links to food microbiology/safety. Animal models or studies in which the host is the main target of investigation should be submitted to appropriate journals and not to IJFM.
• Microbiological aspects of the production of ingredients should be submitted to suitable biotechnology journals. However, papers that consider the use of microorganisms to enhance the level of specific vitamins, amino acids, flavours, colours, polysaccharides etc in foods/beverages will be considered by IJFM.
• Papers where the microbiology is only focused on primary production, without a clear connection to quality and safety of foods, should be sent to journals related to primary production.
• Microbiology specifically related to human health without a clear focus on its relation to foods/ beverages should be submitted to medical journals or similar.
• Repetition of studies conducted in other countries and locations, e.g. work on patterns of antibiotic resistance, prevalence of specific pathogens etc., will not be considered unless new scientific information has been achieved and clearly documented in the manuscript.

Furthermore:

• the word "probiotic" should only be used for organisms where real health effects are shown;
• "prediction and validation" should only be used if prediction and validation are really carried out with new independent data;
• for papers on modelling, parameter values should always be presented also with a measure of their confidence (confidence interval, standard error).

• the number of significant digits should be in relation to the accuracy of the data measured. It should be noted that significant number are NOT digits after the decimal point. Both 34 and 0.00025 have two significant figures and 34.0 and 0.000252 have three significant figures. Figures like 234.573 generally have largely overdone accuracy. Log numbers of microbial concentrations generally have only one digit after the decimal point.

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REFERÊNCIAS

Ahamed S, Foster JS, Bukoysky A, Wimalasena J. Signal transduction through the ras/Erk pathway is essential for the mycoestrogen zearalenone-induced cell-cycle progression in MCF-7cells. Mol Carcinog. 2001; 30(2):88-98.

Ahn ES, Kim YS, Shin DH. Observation of Bactericidal Effect of Allyl Isothiocyanate on *Listeria monocytogenes*. Food Sci. Biotechnol. 2001; 10:31–35.

Ali ML, Taylor JH, Jie L, Sun G, William M, Kasha KJ, Reid LM, Pauls KP. Molecular mapping of QTLs for resistance to *Gibberella* ear rot, in corn, caused by *Fusarium graminearum.* Genome. 2005; 48:521-533.

Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura YY. Intake of garlic and its bioactive components. J Nutri. 2001; 131(3):955S-9662S.

Amaike S, Keller NP. *Aspergillus flavus.* Annu Revi Phytopathol**.** 2011; 49:107-33.
ANVISA, Agência Nacional de Vigilância Sanitária. Regulamento técnico sobre os limites máximos tolerados (LMT) para alimentos em micotoxinas -Resolução No7, de 18 de fevereiro de 2011. http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2011/res0007_18_02_2011_rep.html [22 June 2020].

Arnault I, Christidès JP, Mandon N, Haffner T, Kahane R, Auger J. High-performance ion-pair chromatography method for simultaneous analysis of alliin, deoxyalliin, allicin and dipeptide precursors in garlic products using multiple mass spectrometry and UV detection. J Chromatogr A. 2003; 991(1):69-75.

Ashiq S, Hussain M, Ahmad, B. Natural occurrence of mycotoxins in medicinal plants: a review. Fungal Genet Biol. 2014,66:1-10.

Atarés L, Chiralt A. Essential oils as additives in biodegradable films and coatings for active food packaging. Trends in Food Science and Technology. 2016: 48: 51-62.

Bacon CW, Glenn AE, Yates IE. Fusarium verticillioides: Managing the endophytic association with maize for reduced fumonisins accumulation.Toxins Reviews. 2008; 27(3): 411-446.

Bajpai VK, Baek K, Kang SC. Control of *Salmonella* in foods by using essencial oils: A review. Food Res Int. 2012; 45(2):722-34.

Bankole SA, Adebanjo A. Mycotoxins in food in West Africa: current situation and possibilities of controlling it. African Journal of Biotechnology. 2003; 2(9):254-263.

Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). LWT- Food Sci Technol. 2004; 37(2):263-68.

Bennet JW, Klich M. Mycotoxins. Clin Microbiol Rev. 2003; 16(3):497-516**.**

Berthiller F, Sulyok M, Krska R, Schuhmacher R. Chromatographic methods for the simultaneous determination of mycotoxins and their conjugates in cereals. Int J Food Microbiol. 2007; 119(1-2): 33.-7.

Binder SB, Schwartz-Zimmerman HE, Varga E, Bichl G, Michlmayr H, Adam G e Berthiller F. Metabolism of zearalenone and its major modified forms in pigs. Toxins. 2017; 9(2) :1-15.

Borchers A, Teuber SS, Keen CL, Gershwin ME. Food Safety. Clin Rev Aller Immu. 2010; 39(2):95-141.

Bordin K, Saladino F, Fernández-Blanco C, Ruiz MJ, Mañes J, Fernández-Franzón M, Meca G, Luciano FB. Reaction of zearalenone and α-zearalenol with allyl isothiocyanate, characterization of reaction products, their bioaccessibility and bioavailability in vitro. Food chemistry. 2017; 217:648-54.

Boyle W. Spices and essential oils as preservatives. Am Perfum Essent Oil Rev.1995; 66:25-28.

Burt S. Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol.2004; 94(3):223-53.

Cabello CM, Bair WB, Lamore SD, Ley S, Bause AS, Azimian S, Wondrak GT. The cinnamon-derived Michael acceptor cinnamic aldehyde impairs melanoma cell proliferation, invasiveness, and tumor growth. Free Radic Biol Med. 2009; 15(46):220-31.

Cai Land CDW. Compounds from Syzgiiun aromaticum possessing growth inhibitory activity against oral pathogens. J Nat Prod. 1996;59:987-90.

Casa RT, Moreira EM, Bogo A, Sangoi L. Incidência de podridões do colmo, grãos ardidos e rendimento de grãos em híbridos de milho submetidos ao aumento na densidade de plantas. Summa Phytopathol. 2007; 33(4):353-57.

Cavallito C, Bailey J. Allicin the antibacterial principle of *Allium sativum.L.* Isolation, physical properties and antibacterial action. J Am Chem Soc. 1944; 66(11):1950-51.

Chen H, Davidson, PM, Zhong Q. Impacts of sample preparation methods on solubility and antilisterial characteristics of essential oil components in milk. Appl Environ Microbiol. 2014; 80:907-16.

Chen XX, Yang CW, Huang LB, Niu QS, Jiang SZ e Chi F. Zearalenone Altered the Serum Hormones, Morphologic and Apoptotic Measurements of Genital Organs in Post-weaning Gilts. Asian- Australasian journal of animal sciences. 2015; 28(2): 171-179.

Chulze SN. Strategies to reduce mycotoxin levels in maize during storage: A review. Food Addit Contam A. 2010; 27(5): 651-57.

Copetti MV, Iamanaka BT, Pereira JL, Fungaro MH, Taniwaki MH. Aflatoxigenic fungi and aflatoxin in cocoa. Int J Food Microbiol. 2011; 148(2): 141-44.

Cotty PJ, Jaime-Garcia R. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. Int. J. Food Microbiol. 2007; 119:109-115.

Curtis H, Noll U, Störmann J, Slusarenko AJ. Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. Physiol Mol Plant P. 2004; 65(2):79-89.

D'Antuono LF, Elementi S, Neri R. Exploring new potential health-promoting vegetables: glucosinolates and sensory attributes of rocket salads and related Diplotaxis and Eruca species. J. Sci. Food Agric. 2009; 89:713–722.

Dadzie MA, Oppong A, Ofori K, Eleblu JS, Ifie EB, Blay E, Warburton, M. L. Distribution of Aspergillus flavus and aflatoxin accumulation in stored maize grains across three agro-ecologies in Ghana. Food Control. 2019; 104: 91-98. doi:10.1016/j.foodcont.2019.04.035

Dal pozzo M, Viégas J, Santurio DF, Rossatto L, Soares IL, Alves SH, Costa MM. Atividade antimicrobiana de óleos essenciais de condimentos frente a *Staphylococcus* spp. Isolados de mastite caprina. Ciência Rural. 2011; 11: 54-56.

DERAL. Departamento de economia rural. [Internet]. Valor bruto de produção agropecuária em 2019. [acesso 5 de maio de 2020]
Disponível em: http://www.agricultura.pr.gov.br/.

Duan C, Qin Z, Yang Z, Li W, Sun S, Zhu Z, Wang X. Identification of pathogenic *Fusarium* spp. causing maize ear rot and potential mycotoxin production in China. Toxins. 2016; 8:186.

Dziri S, Casabianca H, Hanchi B, Hosni K. Composition of garlic essential oil (*Allium sativum L.*) as influenced by drying method. J Essent Oil Res. 2014; 26(2):91-96.

EC no. 1881/2006, European Comission - Amending regulation. Setting maximum levels for certain contaminants in foodstuffs. Off J Eur Commun . 2006; 255: 14-17.

EFSA, P. on C. in the F. C. Scientific Opinion on the risks for public health related to the presence of zearalenone in food. *EFSA Journal*, 2011; *9*(6:2197): 1-124.

Eskola M, Kos G, Elliott CT, Hajšlová J, Mayar S & Krska R. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%. Crit Rev Food Sci Nutr. 2019; Sep 3:1-17.

European Commission. COMMISSION REGULATION (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards Fusarium toxins in maize and maize products. *Official Journal of the European Union*, *L255*, 2007, 14-17.

FAO. Food and Agriculture Organization. Worldwide regulations for mycotoxins in food and feed in 2003. Food and Nutrition Paper: Roma, Itália; 2004. ISSN 0254-4725.

FDA, Food and Drug Organization. Regulatory Guidance for Mycotoxin. A guide for grain elevators, feed manufacturers, grain processors and exporters. National Grain Association: Washington, 2011.

Garcia MV, Bregao AS, Parussolo G, Bernardi, AO, Stefanello A, & Copetti MV. Incidence of spoilage fungi in the air of bakeries with different hygienic status. Int J Food Microbiol. 2019; 290: 254-61.

Gómez JV, Tarazona A, Mateo-Castro R, Gimeno-Adelantado JV, Jiménez M, Mateo EM. Selected plant essential oils and their main active components, a promising approach to inhibit aflatoxigenic fungi and aflatoxin production in food. Food Additives & Contaminants Part A. 2018; 35(8):1581-95.

Gonçalves B. Micotoxinas: Uma revisão sobre as principais doenças desencadeadas no organismo humano e animal. Revista de Saúde. 2017; 4(1).

Henning FA, Jacob Junior EA, Mertz LM, Peske ST. Qualidade sanitária de sementes de milho em diferentes estádios de maturação. Revista Brasileira de Sementes. 2011; 33(2):316-21.

Hooth MJ, Sills RC, Burka LT, Haseman JK, Witt KL, Orezech DP, Graves S, Bucher JR. Toxicology and carcinogenesis studies of microencapsulated trans-cinnamaldehyde in rats and mice. Food Chem Toxicol. 2004; 42:1757-68.

Huang Y, Ho SH. Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects, Tribolium castaneum (Herbst) and Sitophilus zeamais Motsch. J Stored Prod Res. 1988; 34:11-17.

IARC. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. 1993; 56:1-521. https://doi.org/10.1002/food.19940380335

IARC*. International Agency for Research on Cancer*. (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans / World Health Organization. 2002

IARC. Working Group on the Evaluation of Carcinogenic Risks to Humans. (2012). Chemical agents and related occupations. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans / World Health Organization, International Agency for Research on Cancer. 2012; *100*(Pt F): 9–562.

IGC. *Internacional grains council.* Indice de grãos e oleaginosas. [acessado 5 de maio de 2020] Disponível em: http://www.igc.int/en/Default.aspx

Imamura KB, Toni JCV, Bocche MAL, Souza DA, Giannoni JA. Ocorrência de aflatoxinas no amendoim (*Arachis hypogaea L*) cru em casca da região da alta

paulista – SP, durante o período de 2011 a 2012. Rev. Inst Adolfo Lutz. 2014; 73(2): 178-87.

Jay JM. Micotoxinas. In. Microbiologia de alimentos. 6° Ed, Porto Alegre: Artmed, 2005; 711.

Kempken F, Rohlfs M. Fungal secondary metabolite biosynthesis – a chemical defence strategy against antagonistic animals.Fungal Ecology.2010; 3(3): 107-14.

Krishna MDA, Banerjee AB. Antimicrobial screening of some Indian spices. Phytother Res. 1999; 13:616-18.

Kwon BM, Lee SH, Cho YK, Bok SH, So SH, Youn MR. Synthesis and biological activity of cinnamaldehydes as angiogenesis inhibitors. Bioorg Med Chem Lett. 1997;7: 2473-6.

Kwon JA, Yu CB, Park HD. Bactericidal effects and inhibition of cell separation of cinnamic aldehyde on Bacillus cereus. Lett Appl Microbiol. 2003; 37:61-65.

Ledoux DR, Brown TP, Weibking TS, Rottinghaus GE. Fumonisin toxicity in broiler chicks. Journal of Veterinary Diagnostic Investigation. 1992; 4(3):330-3.

Leslie JF, Summerell BA. The Fusarium Laboratory Manual. 2006; 1: 1 st sd Hoboken, NJ: Wiley-Blackwell.

Liao BC, Hsieh CW, Liu YC, Tzeng TT, Sun YW, Wung BS. Cinnamaldehyde inhibits the tumor necrosis factor-α-induced expression of cell adhesion molecules in endothelial cells by suppressing NF-κB activation: Effects upon IκB and Nrf2. Toxicol Appl Phar. 2008; 229:161-71.

Lin CM, Preston JF, Wei CI. Antibacterial mechanism of allyl isothiocyanate. J. Food Prot. 2000; 63:727–734.

Lopes LF, Bordin K, De Lara GHC, Saladino F, Quiles JM, Meca G, Lucinao FB. Fumigation of Brazil nuts with allyl isothiocyanate to inhibit the growth of Aspergillus parasiticus and aflatoxin production. J Sci Food and Agric. 2018; 98(2):792-98.

Luciano FB, Holley RA. Enzymatic inhibition by allyl isothiocyanate and factors affecting its antimicrobial action against *Escherichia coli* O157:H7. Int. J. Food Microbiol. 2009; 131:240–245.

Magan N, Aldred, D. Why do fungi produce mycotoxins? Food Mycology - A multifaceted approach to fungi and food. 2007; 121–134.

Magnoli AP, Tallone L, Rosa CAR, Dalcero AM, Chiacchiera SM, Sanchez RMT. Comercial bentonites as detoxifier of broiler feed contaminater with aflatoxin. Appl. Clay Sci. 2008; 40(1-2):63-71.

Mahmoud SS, Croteau RB. Strategies for transgenic manipulation of monoterpene biosynthesis in plants. Trends Plant Sci. 2002;7(8):366-73.

Marasas WF, Kellerman TS, Gelderblom WC, Coetzer JA, Thiel PG, Van der Lugt JJ. Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from *Fusarium moniliforme*. J Vet Res. 1988; 55(4):197-203.

Marasas WF. Discovery and occurrence of the fumonisins: a historical perspective. Environ Health Perspect. 2001; 109(2): 239-243.

Marasas WF. Fumonisins: history, worldwide occurrence and impact. Adv Exp Med Biol. 1996; 392(1):1-17.

Marchese, A, Barbieri R, Sanches-Silva A, Daglia M, Nabavi SF, Jafari NJ, Izadi M, Ajami M, Nabavi SM. Antifungal and antibacterial activities of allicin: A review. Trends Food Sci Technol. 2016; 52:49-56.

Marin S, Magan N, Belli N, Ramos AJ, Canela R, Sanchis V.Two-dimensional profiles of fumonisin B1 production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modelling toxin formation in maize grain. Int. J. Food Microbiol. 1999; 51:159-167.

Miron T, Bercovici T, Rabinkov A, Wilchek M, Mirelman D. [3H] Allicin: preparation and applications**.** Anal Biochem. 2004; 331(2):364-9.

Missmer SA. Suarez L, Felkner M, Wang E, Merrill AH, Rothman KJ, Hendricks KA. Exposure to Fumonisins and the Occurrence of Neural Tube Defects along the Texas-Mexico Border. Environ Health Perspect. 2006; 114(2): 237-41.

Muller D., Wise K. Corn Disease Loss Estimates from the United States and Ontario, Canada from 2012 to 2015. Plant Health Progress. 2016; 17(3).

Munkvold GP. Cultural and genetic approaches to managing mycotoxins in maize. Annual Review of Phytopathology. 2003; 41: 99-116.

Nagaraja H, Chennappa G, Poorna Chandra Rao K, Mahadev Prasad G, Sreenivasa MY. Diversity of toxic and phytopathogenic Fusarium species occurring on cereals grown in Karnataka state, India. Biotech. 2016; 6:1-8.

Narumol M, Nirundorn M. Effect of combined cinnamon and clove oil against major moulds identified from rubberwood (Heveabrasiliensis) Walailak. J Sci & Tech. 2007; 4:165-74.

Nazareth TM, Bordin K, Manyes L, Meca G, Mañes J, Luciano FB. Gaseous allylisothiocyanate to inhibit the production of aflatoxins, beauvericin and enniatins by *Aspergillus parasiticus* and *Fusarium poae* in wheat flour**.** Food Control**.** 2016; 62: 317-21.

Nazareth TM, Corrêa JAF, Pinto ACSM, Palma JB, Meca G, Bordin K, Luciano FB. Evaluation of gaseous allyl isothiocyanate against the growth of mycotoxigenic fungi

and mycotoxin production in corn stored for 6 months. Journal of the Science of Food and Agriculture. 2018; 98(14):5235-5241.

Nesic K, Ivanovic S, Nesic V. Fusarial toxins: Secondary metabolites of Fusarium fungi. Reviews of environmental contamination and toxicology. 2014; 228:101-120.

Nielsen PV, Rios R. Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. Int. J. Food Microbiol. 2000;60: 219–229.

Nones J, Scussel VD. Zearalenona, metabólitos, e seus efeitos no desempenho da reprodução suína: uma revisão. Pubvet. 2013; 7(9):707-775.

Oliveira CAS, Germano PML. Aflatoxinas: Conceitos sobre mecanismos de toxicidade e seu envolvimento na etiologia do câncer hepático celular. Revista de Saúde Pública. 1997; 31(4):417-24.

Oliveira MS, Rocha A, Sulyok M, Krska R, Mallmann CA. Natural mycotoxin contamination of maize (Zea mays L.) in the South region of Brazil. Food Control. 2017; 73:127-132.

Omar S, Al-Wabel N. Organosulfur compounds and possible mechanism of garlic in cancer. Saudi Pharm J. 2010; 18(1): 51-58.

Osweiler GD, Ross PF, Wilson TM, Nelson PE, Witte ST, Carson TL, Rice LG, e Nelson HA. Characterization of an epizootic of pulmonary edema in swine associated with fumonisin in corn screenings. J Vet Diagn Invest. 1992; 4:53-59.

Pereira VL, Fernandes JO, Cunha SC. Mycotoxins in cereals and related foodstuffs: A review on occurrence and recent methods of analysis. Trends Food Sci Technol. 2014; 36(2):96-136.

Perincherry L, Lalak-Kánkzugowskza J, Stepien L. *Fusarium*-Produced Mycotoxins in Plant-Pathogen Interactions. Toxins. 2019; 11(11):664.

Pitt JI, Hocking AD. Fungi and Food Spoilage. Springer Science Business Media. 2009; 3. Ed.

Pitt, J. I. et al. Improving public health through mycotoxin control. International Agency for Research on Cancer, 2012.

Prakash B, Kedia A, Mishra PK, Dubey N. K. Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities–Potentials and challenges. Food Control. 2015; 47:381-91.

Presello, DA, Botta G, Iglesias J, Eyhérabide GH. Effect of disease severity on yield and grain fumonisin concentration of maize hybrids inoculated with Fusarium verticillioides. Crop Protection. 2008; 27(4-5): 572–576.

Quiles, J. M., Nazareth, T. D. M., Luz, C., Luciano, F. B., Mañes, J., & Meca, G. Development of an Antifungal and Antimycotoxigenic Device Containing Allyl Isothiocyanate for Silo Fumigation. Toxins. 2019; 11(3), 137.

Ramos AJ, Hernández E, Pla-Delfiná JM, Merino M. Intestinal adsorption of zearalenona and in vitro study of non-nutritive sorbent materials. Int J Pharm**.** 1996; 128: 129-37.

Ramos DP, Barbosa RM, Vieira BGTL, Panizzi RC, Vieira RD. Infecção por Fusarium graminearum e Fusarium verticillioides em sementes de milho. Pesquisa Agropecuária Tropical. 2014; 44(1) 24-31.

Sadhna RS, Libin RS, Javier Z, Bibiana RG, Lynn L, Mendel F. Carvacrol and cinnamaldehyde inactivate antibiotic-resistant Salmonella enterica in buffer and on celery and oysters. J Food Protect. 2010; 73:234-40.

Saladino F, Quiles JM, Mañes J, Fernández-Franzón M, Luciano FB, Meca G. Dietary exposure to mycotoxins through the consumption of commercial bread loaf in Valencia, Spain. LWT. 2017; 75: 697-701.

Samson RA, Visagie CM, Houbraken J, Hong SB, Hubka V, Klaassen CHW, Perrone G, Seifert KA, Susca A, Tanney JB, Varga J, Kocsubé S, Szigeti G, Yaguchi T, Frisvad JC. Phylogeny identification and nomenclature of the genus Aspergillus. Stud Mycol. 2014; 78: 141-73.

Santos MC, Souza RB, Oliveira SEM, Lima KSC, Lima ALS. Micotoxinas e seu Potencial como Agentes de Guerra. Rev Virtual Quim. 2014; 6(3):761-78.

Schollenberger M, Müller HM, Ernst K, Sondermann S, Liebscher M, Schlecker C, Wisher G, Drochner W, Hartung K, Piepho, HP. Occurrence and distribution of 13 trichothecene toxins in naturally contaminated maize plants in Germany. Toxins, 2012; 4(10):778– 787.

Scott PM. Recent research on fumonisins: a review. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2012; 29(2): 242-48.

Sebők F, Dobolyi C, Zágoni D, Risa A, Krifaton C, Hartman M, Cserháti M, Szoboszlay S, Kriszt B. Aflatoxigenic Aspergillus flavus and Aspergillus parasiticus strains in Hungarian maize fields. Acta microbiologica et immunologica Hungarica. 2016; 63(4): 491-502.

Seydim AC, Sarikus G. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. Food Res Int. 2006; 39(5):639-44.

Shih-Hua F, Yerra KR, Yew-Min T. Cytotoxic Effect of trans-cinnamaldehyde from Cinnamomum osmophloeum leaves on human cancer cell lines. Int J Appl Sci Eng. 2004; 2:136-47.

Silveira SMD. Chemical composition and antimicrobial activity of essential oils from selected herbs cultivated in the South of Brazil against food spoilage and foodborne pathogens. Cienc. Rural. 2012; 42(7):1300-1306.

Smid EJ, Koeken JGP, Gorris LGM. Fungicidal and fungistatic action of the secondary plant metabolites cinnarnaldehyde and carvone. In H. Lyr, P. E. Russell & H. D. Sisler (Eds.), 'Modern Fungicides and Antifungal Compounds'. 1996; 173-80.

Smith MC, Madec S, Coton E, Hymery N. Natural Co-occurrence of mycotoxins in foods and feeds and their in vitro combined toxicological effects. Toxins (Basel). 2016; 8. https://doi.org/10.3390/toxins8040094

STATISTA. [acessado em 16 de dezembro de 2020] Disponível em https://www.statista.com/statistics/254292/global-corn-production-by-country/.

Summerell BA, Laurence MH, Liew ECY, Leslie JL. Biogeography and phylogeography of Fusarium: a review. Fungal Divers. 2010; 44(1) 3-13.

Sun Q, Shang B, Wang L, Lu Z, Liu Y. (2016). Cinnamaldehyde inhibits fungal growth and aflatoxin B_1 biosynthesis by modulating the oxidative stress response of *Aspergillus flavus*. Applied Microbiology and Biotechnology. 2006;100(3):1355-1364.

Taniwaki MH, Iamanaka BT, Silva N. Fungos deterioradores de alimentos: Ocorrência e detecção. Campinas - São Paulo: Instituto de Tecnologia de Alimentos (ITAL), 2013.

Tarazona A, Gómez JV, Jiménez M, Mateo F, Romera D, Mateo EM. Study on mycotoxin contamination of maize kernels in Spain. Food Control. 2020; 107:370 *107370.*

Tracz BL, Bordin K, Nazareth TM, Costa LB, Macedo REF, Meca G, Luciano, FB. Assessment of allyl isothiocyanate as a fumigant to avoid mycotoxin production during corn storage. LWT. 2017; 75: 692–696.

Uchida M, Nakajin S, Toyoshima S, Shinoda M. Antioxidative effect of sesamol and related compounds on lipid peroxidation. Biol Pharm Bull. 1996;19:623-26.

Urrego Novoa JR, Diaz GJ. Aflatoxinas: mecanismos de toxicidad en la etiología de cáncer hepático celular. Revista de la Facultad de Medicina. 2006; 54(2): 108-16.

Varga J, Kocsubé S, Péterl Z, Vágvölgyi C, Tóth B. Chemical, physical and biological approaches to prevent ochratoxin Induced toxicoses in humans and animals**.** Toxins. 2010; 2: 1718-50.

Vigier B, Reid LM, Dwyer LM, Stewart DW, Sinha RC, Arnason JT, Butler G. Maize resistance to gibberella ear rot: symptoms, deoxynivalenol, and yield. J. Plant Pathol.2001; 23(1):99-105.

Wallock-Richards D, Doherty CJ, Doherty L, Clarke DJ, Place M, Govan JRW, Campopiano DJ. Garlic revisited: Antimicrobial activity of allicin-containing garlic extracts against *Burkholderia cepacia* complex. Plos One*.* 2014; *9*:112726

Wang L, Jin J, Liu X, Wang Y, Liu Y, Zhao Y, Xing F. Effect of cinnamaldehyde on morphological alterations of *Aspergillus ochraceus* and expression of key genes involved in ochratoxin a biosynthesis. Toxins. 2018; 10(9): 340.

Wang Y, Feng K, Yang H, Yuan Y, Yue T. Antifungal mechanism of cinnamaldehyde and citral combination against *Penicillium expansum* based on FT-IR fingerprint, plasma membrane, oxidative stress and volatile profile. RSC Advances. 2018; 8(11):5806-5815.

Warth B, Sulyok M, Berthiller F, Schuhmacher R, Krska R. New insights into the human metabolism of the *Fusarium* mycotoxins deoxynivalenol and zearalenone. Toxicol Lett. 2013; 220(1): 88-94.

Wu Q, Jezkova A, Yuan Z, Pavlikova L, Dohnal V, Kuca K. Biological degradation of aflatoxins. Drug Metab Rev. 2009; 41(1):1-7.

Wu SJ, Ng LT, Lin CC. Cinnamaldehyde-induced apoptosis in human PLC/PRF/5 cells through activation of the proapoptotic Bcl-2 family proteins and MAPK pathway. Life Sci. 2005; 77:938-51.

Xie XM, Fang JR, Xu Y. Study of antifungal effect of cinnamaldehyde and citral on Aspergillus flavus. Food Science. 2004; 25:32-34.

Xing F, Hua H, Selvaraj JN, ZhaoY, Zhou L, Liu X, Liu Y. Growth inhibition and morphological alterations of *Fusarium verticillioides* by cinnamon oil and cinnamaldehyde. Food Control. 2014; 46, 343-350.

Yang Q, Balint-Kurti P, Xu M.Quantitative disease resistance: dissection and adoption in maize. Mol. Plant. 2017;10: 402-413.