JAQUELINE GOZZI BORDINI

EFEITO DA MOAGEM A SECO NA MINIMIZAÇÃO DE FUMONISINAS EM MILHO E DERIVADOS

Londrina
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Tese apresentada ao Programa de Doutorado em Biotecnologia da Universidade Estadual de Londrina como um dos requisitos para obtenção de título de Doutora em Biotecnologia.

Orientadora: Profa. Dra. Elisabete Yurie Sataque Ono

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BANCA EXAMINADORA

Profa. Dra. Elisabete Yurie Sataque Ono
Universidade Estadual de Londrina - UEL

Prof. Dr. Benedito Corrêa
Universidade de São Paulo – USP

Profa. Dra. Joice Sifuentes dos Santos
Universidade Norte do Paraná – UNOPAR

Profa. Dra. Margarida Masami Yamaguchi
Universidade Tecnológica Federal do Paraná – URFPR

Profa. Dra. Tereza Cristina Rocha Moreira de Oliveira
Universidade Estadual de Londrina – UEL

Londrina, 04 de agosto de 2017.

Dedico este trabalho aos meus pais, Antonio e Carmen e ao meu esposo João Paulo, pelo amor, incentivo e compreensão.

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RESUMO

As fumonisinas são um grupo de micotoxinas frequentemente detectadas em milho e estão relacionadas a diversos efeitos tóxicos em seres humanos e animais. A moagem a seco é um processamento industrial que separa o grão em três frações principais: o gérmen e o pericarpo, que são as frações externas, e o endosperma, a fração interna que é transformada em fubá e grits destinados à alimentação humana. Contudo, as fumonisinas não são destruídas durante esse processamento, podendo ser concentradas em certas frações. Portanto, o objetivo deste estudo foi avaliar a contaminação fúngica e a distribuição da fumonisina B_1 (FB_1) e fumonisina B_2 (FB_2) em milho convencional e transgênico e em suas frações obtidas por moagem a seco (gérmen, pericarpo, endosperma, fubá e grits), bem como avaliar o grau de exposição humana às fumonisinas no Brasil e em países importadores. Para esse propósito, quatro lotes de milho (dois lotes convencional e dois lotes transgênico) e de suas frações, (N = 480) foram coletadas em uma das maiores indústrias de processamento do Brasil, localizada no norte do Paraná. Em ambos os tipos de milho, as fumonisinas (B_1 + B_2) foram detectadas em maiores níveis no gérmen e no pericarpo e em menores níveis no endosperma, fubá e grits. As taxas de concentração de fumonisinas no gérmen e pericarpo corresponderam a 220% e 215% (transgênico) e a 317% e 226% (convencional), respectivamente, em relação ao milho íntegro. Por outro lado, houve uma redução da contaminação por fumonisinas no endosperma, fubá e grits em uma taxa de 86%, 90 e 91% obtidas de milho transgênico e de 70%, 76% e 89% de convencional, respectivamente. Não houve diferença significativa ($p > 0,05$) entre os níveis de fumonisinas (B_1 +B_2) de milho íntegro transgênico (média de 1030 μg/kg) e convencional (média de 918 μg/kg). Porém, houve diferença significava entre os níveis de fumonisinas no gérmen, endosperma e fubá, sendo maior nas frações internas provenientes de milho convencional ($p < 0,05$). Baseando-se na contaminação média de fumonisinas (B_1 + B_2) dos quatro lotes de milho íntegro, endosperma, fubá e grits e nos dados de ingestão destes alimentos, a Ingestão Diária Provável (IDP) de fumonisinas no Brasil e nos países importadores foi calculada. A IDP total de fumonisinas no Brasil foi de 98 ng/kg peso corpóreo por dia, o que representa 5% da ingestão diária máxima tolerada provável para fumonisinas (2000 ng/kg peso corpóreo/dia). Nos países importadores de milho brasileiro, a IDP foi maior em Angola (1710 ng/kg peso corpóreo/dia) do que nos países da Europa (de 40 a 192 ng/kg peso corpóreo/dia) e Malásia (680 ng/kg peso corpóreo/dia). A contagem fúngica total média dos quatro lotes de milho e de seus derivados variou de $1,6 \times 10^2$ UFC/g (fubá) a $1,9 \times 10^3$ UFC/g (milho), mais baixo que o limite máximo de 10^4 UFC/g recomendado pela *Food and Drug Administration* (FDA). Além disso, 92,5 e 87,5% das amostras de milho (transgênico e convencional, respectivamente) apresentaram níveis de fumonisinas (FB_1 + FB_2) abaixo do limite máximo tolerado pela Comissão Européia (2000 μg/kg). Portanto, o milho e suas frações obtidas por moagem industrial a seco apresentaram uma boa qualidade micológica e este processamento foi eficiente em minimizar a contaminação por fumoninisas em derivados de milho destinados à alimentação humana.

Palavras-chave: Micotoxinas. Processamento industrial. Grits. Fubá. Gérmen.

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ABSTRACT

Fumonisins are a group of mycotoxins frequently detected in corn and are associated to several toxic effects in humans and animals. Dry–milling is an industrial processing that separates the kernel in three main fractions: germ and pericarp, the outer fractions, and the endosperm, internal fraction which is transformed into cornmeal and grits intended for human consumption. However, fumonisins are not destroyed during this processing and can be concentrated in certain fractions. Therefore, the objective of this study was to evaluate the fungal contamination and the distribution of fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) in non-transgenic and transgenic corn and their fractions obtained by dry-milling, as well as to evaluate the degree of human exposure to fumonisins in Brazil and importing countries. For this purpose, corn samples of four lots (two of non-transgenic and two of transgenic) and their fractions, i.e., germ, pericarp, endosperm, cornmeal and grits (N = 480) were collected from one of the largest corn dry-milling processing industries from Brazil located in Northern Paraná State. In both corn types, fumonisins (B_1 + B_2) were detected at higher levels in the germ and pericarp and at lower levels in the endosperm, corn meal and grits. Fumonisins concentration in the germ and pericarp corresponded to 220% and 215% (transgenic) and 317% and 226% (non-transgenic), respectively, in relation to whole corn. On the other hand, there was a reduction of the fumonisins contamination in the endosperm, cornmeal and grits at a rate of 86%, 90 and 91% obtained from transgenic corn and 70%, 76% and 89% of non-transgenic corn, respectively. There was no significant difference ($p > 0.05$) between the fumonisins levels (B_1 + B_2) of transgenic corn (mean 1030 μg/kg) and non-transgenic corn (mean 918 μg/kg). However, there was a significant difference between the germ, endosperm and cornmeal, and the levels of fumonisins were higher in these fractions from non-transgenic corn ($p < 0.05$). Based on the mean fumonisin (B_1 + B_2) contamination of the four lots of whole corn, endosperm, cornmeal and grits and in the intake data of these foods, the Probable Daily Intake (PID) of fumonisins in Brazil and in importing countries was calculated. The total Probable Daily Intake (PDI) for fumonisins in Brazil was 98 ng/kg body weight/day, representing 5% of the provisional maximum tolerable daily intake (PMTDI) for fumonisins (2000 ng/kg body weight/day). In importing countries of Brazilian corn, PDI was higher in Angola (1710 ng/kg body weight/day) than in European countries (from 40 to 192 ng/kg body weight/day) and Malaysia (680 ng/kg body weight /day). Mean total fungal count from the four lots ranged from 1.6×10^2 CFU/g (cornmeal) to 1.9×10^3 CFU/g (whole corn) and it was lower than 10^4 CFU/g, the maximum limit recommended by the Food and Drug Administration (FDA). In addition, in most non-transgenic and transgenic corn samples (92.5 and 87.5%, respectively) FB_1 and FB_2 levels were below the maximum limit tolerated by the European Commission (2000 μg/kg). Corn and its fractions obtained by industrial dry-milling showed a good mycological quality and this processing was efficient for minimizing fumonisin contamination in corn derivatives intended for human consumption.

Key Words: Mycotoxins. Industrial processing. Grits. Cornmeal. Germ.

SUMÁRIO

1 INTRODUÇÃO

O Brasil é o terceiro maior produtor mundial de milho, com uma produção estimada em 93 milhões de toneladas para a safra de 2016/17 (CONAB, 2017). Além disso, é o segundo maior exportador mundial, com 35 milhões de toneladas (USDA, 2017). Porém, o clima tropical predominante no Brasil favorece o desenvolvimento de fungos toxigênicos, que causam redução da qualidade dos grãos, bem como a produção de micotoxinas (CAST, 2003).

Micotoxinas são metabólitos secundários tóxicos produzidos por fungos filamentosos que contaminam os produtos agrícolas e derivados. As fumonisinas são uma classe de micotoxinas produzidas principalmente por *Fusarium verticillioides* e *F. proliferatum*, prevalentes em culturas de milho, e estão relacionadas a diversas doenças em seres humanos e animais (RHEEDER, MARASAS, VISMER, 2002; THIEL et al., 1992). As fumonisinas causam leucoencefalomalácia em equinos (ELEM) (MARASAS et al., 1988), edema pulmonar em suínos (ROSS et al., 1990), redução no desenvolvimento e imunossupressão em aves (NAGARAJ; WU; VESCONDER, 1994; WEIBKING et al., 1994) e ação hepatotóxica e hepatocarcinogênica em ratos (GELDERBLOM et al., 1991). Em seres humanos, estudos epidemiológicos indicam a associação das fumonisinas com o câncer esofágico e hepático primário (RHEEDER et al., 1992; SYDENHAM et al., 1990; UENO et al., 1997), além de defeitos no tubo neural (MISSMER et al., 2006), sendo classificadas pela *International Agency for Research on Cancer* (IARC) como carcinógenos do grupo 2B, i.e., provavelmente carcinogênicos para seres humanos (IARC, 2002).

Existem, ao menos, 28 análogos de fumonisinas (RHEEDER; MARASAS; VISMER, 2002), sendo que os análogos da série B, incluindo as fumonisinas B_1 (FB_1) e B_2 (FB_2) são as mais tóxicas e de ocorrência natural (MARASAS, 1996). A alta frequência de fumonisinas em milho tem sido relatada no Brasil (HIROOKA et al., 1996; MORENO et al., 2009; OLIVEIRA et al., 2017; ONO et al., 2008) e, devido à estabilidade térmica, não são destruídas por processamentos industriais, podendo ser detectadas em produtos finais (BULLERMAN; BIANCHINI, 2007).

O processamento industrial a seco de milho é amplamente utilizado na fabricação de produtos derivados. Neste processo, o grão é degerminado e separado em três partes principais: pericarpo, gérmen e endosperma. O pericarpo é destinado à alimentação animal, no entanto, com processamento posterior pode ser utilizado em alguns alimentos especializados como fonte de fibras; o gérmen é uma matéria-prima para a produção do óleo de milho e também para a ração animal; o endosperma é utilizado na alimentação humana,

principalmente no preparo de pratos típicos do Brasil como a canjica ou pode ser convertido em farinhas, fubá e grits. O grits é a matéria-prima para a produção de flocos de milho, cerveja e *snacks* (CARDOSO et al., 2011; SAUNDERS; MERDITH; VOSS, 2001). Estima-se que 2.200 milhões de toneladas de milho por ano são destinadas a moagem a seco no Brasil (ABIMILHO, 2013). As micotoxinas não são destruídas durante esse processamento e podem ser redistribuídas e concentradas em certas frações, tendendo a se concentrar no gérmen e no pericarpo (BRERA et al., 2004; CASTELLS et al., 2008; KATTA et al., 1999). Contudo, a redistribuição de fumonisinas nas diferentes frações depende da variedade do milho utilizado e do tipo de processamento, que difere entre os países (SCUDAMORE et al., 2009).

A fim de minimizar os efeitos tóxicos, a ANVISA estabeleceu um limite máximo tolerado de 5.000 μg/kg para fumonisinas (B_1 + B_2) em milho, e de 1500 μg/kg em fubá, canjica, flocos e farinha de milho (BRASIL, 2011). Além disso, a *World Health Organization* (WHO) recomenda uma ingestão diária máxima provisória de 2.000 ng/kg de peso corpóreo/dia de fumonisinas (WHO, 2002), a qual pode ser utilizada como parâmetro para avaliar o grau de exposição humana às fumonisinas (JARDIM; CALDAS, 2012).

O grau de exposição é uma importante etapa da avaliação do risco, pois determina a ingestão provável de um contaminante em uma população, com base na contaminação e no consumo do alimento (IPCS, 2009). O risco à saúde existe se os níveis de fumonisinas extrapolarem os limites máximos recomendados e se a ingestão provável de fumonisinas for maior que a ingestão diária máxima provisória (JARDIM; CALDAS, 2012).

Portanto, a avaliação da distribuição das fumonisinas durante a moagem a seco do milho, bem como da qualidade micológica e do grau de exposição humana às fumonisinas são essenciais a fim de monitorar os riscos aos quais seres humanos e animais são expostos e minimizar as perdas econômicas aos produtores e processadores de grãos.

2 OBJETIVOS

2.1 Objetivos Gerais

Avaliar o efeito da moagem industrial de milho a seco na contaminação por fumonisinas;

Avaliar o grau de exposição humana às fumonisinas.

2.2 Objetivos Específicos

Avaliar a contaminação fúngica de milho e das frações obtidas por moagem a seco;

Determinar a ocorrência natural de FB_1 e FB_2 em milho e nas frações gérmen, pericarpo, endosperma, fubá e grits em milho convencional e transgênico;

Avaliar a distribuição das fumonisinas durante a moagem industrial de milho a seco convencional e transgênico;

Estimar o grau de exposição humana às fumonisinas no Brasil e em países importadores de milho brasileiro.

3 REVISÃO BIBLIOGRÁFICA

3.1 MILHO

Botanicamente, o milho é uma gramínea pertencente à família *Gramineace/Poaceae*, ao gênero *Zea* e à espécie *Zea mays L*, que provavelmente foi originada no México há aproximadamente 7.000 anos e foi a alimentação básica de várias civilizações ao longo dos séculos. Os Maias, Astecas e Incas reverenciavam o cereal na arte e religião e grande parte de suas atividades diárias estavam relacionadas ao seu cultivo. Com a descoberta da América e das grandes navegações do século XVI, a cultura do milho se expandiu para outras partes do mundo (ABIMILHO, 2011; FARNHAM; BENSON; PEARCE, 2003; MAGALHÃES et al., 2002).

Devido à fácil adaptação em diferentes ecossistemas e a diferentes formas de utilização, o milho é amplamente cultivado em todo o mundo e de grande importância econômica (ABIMILHO, 2011; PAES, 2006). A partir do milho obtêm-se centenas de derivados empregados em várias indústrias como, a alimentícia, química, farmacêutica e de rações. Em sua cadeia produtiva e tecnológica, utiliza-se desde alta tecnologia em grandes multinacionais ou a venda direta, sem processamento (CARDOSO et al., 2011).

Aproximadamente 70% da produção mundial de milho são destinadas à alimentação animal, principalmente para suínos e frangos de corte, e somente 15% é destinada à alimentação humana (ABIMILHO, 2015).

Os grãos de milho são classificados como uma cariopse, isto é, são secos (não carnosos) e, geralmente, apresentam coloração amarela ou branca, podendo variar de preta até vermelha. A composição média do milho em base seca é de 72% de amido, 9,5% de proteínas, 9% de fibras e 4% de óleo, sendo o grão constituído de três principais partes: gérmen, pericarpo e endosperma (Figura 1) (PAES, 2006; WATSON, 2003).

O endosperma representa aproximadamente 83% do peso seco do grão, consistindo principalmente de amido (88%), organizado na forma de grânulos. No endosperma estão também presentes as proteínas de reserva (8%) do tipo prolaminas, chamadas zeínas. Essas proteínas formam os corpos protéicos que compõem a matriz que envolve os grânulos de amido dentro das células no endosperma. Com base na distribuição dos grânulos de amido e da matriz de proteína, o endosperma é classificado em dois tipos: farináceo e vítreo. No primeiro, os grânulos de amido são arredondados e estão dispersos, não havendo matriz protéica circundando essas estruturas, o que resulta em espaços vagos durante

o processo de secagem do grão. Por outro lado, no endosperma vítreo, a matriz protéica é densa, com corpos protéicos estruturados, que circundam os grânulos de amido de formato poligonal, não permitindo espaços entre estas estruturas (PAES, 2006; WATSON, 2003).

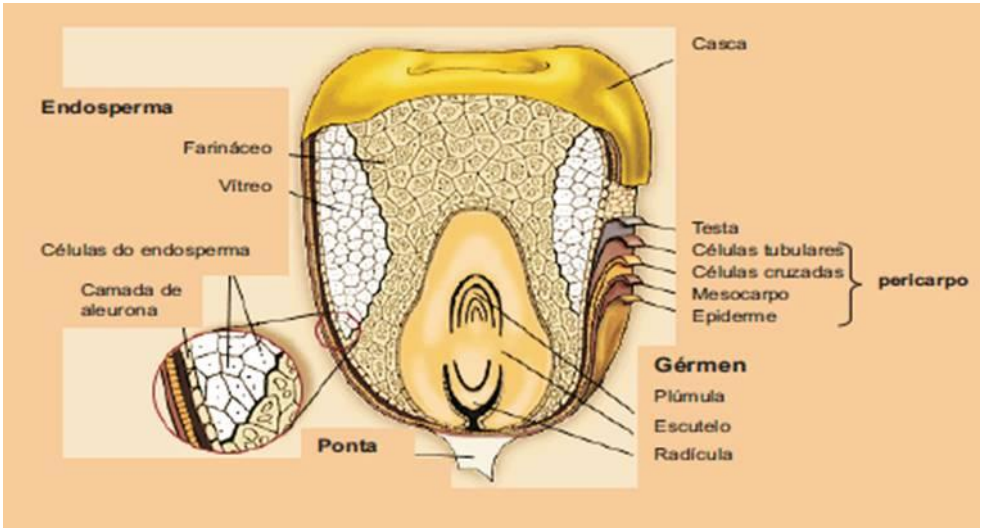


Figura 1. Anatomia e Morfologia do grão de milho. Fonte: Paes, 2006.

No endosperma, especificamente na camada de aleurona e no endosperma vítreo, estão presentes os carotenóides, substâncias lipídicas que conferem cor aos grãos. Zeaxantina, luteína, betacriptoxantina, alfa e beta-carotenos são os principais carotenóides nos grãos de milho (PAES, 2006).

O gérmen representa 11% do grão e concentra quase a totalidade dos lipídeos (óleo e vitamina E) (83%) e dos minerais (78%), além de conter quantidades importantes de proteínas (26%) e açúcares (70%) (PAES, 2006).

Para o consumo humano, o milho requer alguma transformação por meio de moagem. À exceção do consumo quando os grãos estão em estado leitoso, ou "verde", os grãos secos não podem ser consumidos diretamente pelos seres humanos (GARCIA et al., 2006).

3.1.1 Processamento Industrial do Milho

A moagem do milho é considerada a primeira etapa na elaboração de produtos derivados (ALEXANDER, 1987; CASTELLS et al., 2008; SCUDAMORE; PATEL, 2009). Pode ser realizado por via seca, um processo físico, que gera uma variedade maior de

produtos derivados, ou por via úmida, um processo químico, onde o principal produto é o amido (BRASIL, 2007; GERAGE et al., 1999).

Na moagem a seco, o grão de milho é dividido em três partes principais: gérmen, pericarpo e endosperma. Do endosperma resultam as canjicas, que podem ser destinadas à produção de cereal matinal ou com posterior processamento podem ser convertidos em grits, fubá, creme de milho e outros subprodutos secundários; e, do gérmen e pericarpo, o óleo e a ração animal (CARDOSO et al., 2011; GERAGE et al., 1999; BRASIL, 2007).

A Figura 2 esquematiza e indica de onde são originadas as principais frações do milho obtidas por moagem a seco. A camada externa do grão é o pericarpo (A), da qual o farelo é derivado, circundando o endosperma e relativamente o embrião. O endosperma é composto pela camada córnea, que contribui para a maioria dos grits de partículas maiores (B e C) e pela parte macia que são transformadas em farinha ou compostos mais finos (D, E e F). O embrião (G) contém alta quantidade de óleo e não contribui para o rendimento de grits, podendo ser destinado ao refino de óleo e à ração animal (SCUDAMORE; PATEL, 2009).

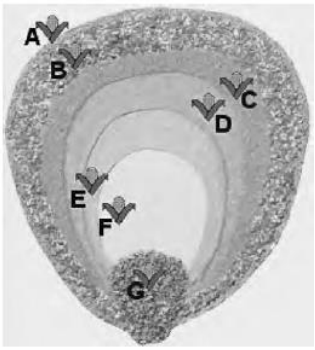


Figura 2. Esquema de uma semente de milho. Origem das diferentes frações obtidas por moagem: A – farelo; B e C – grits maiores; D – grits fino; E – polenta; F – farinha, G – gérmen. Fonte: Scudamore e Patel (2009)

A Figura 3 apresenta um esquema da moagem a seco. A primeira etapa consiste na limpeza dos grãos por meio de máquinas sopradoras e classificadoras que utilizando peneiras, dispositivos magnéticos e de sopramento, removem as impurezas (materiais metálicos, terra, pedra, palha, sabugo e milho quebrado) (BRERA et al., 2004; FANDOHAN, et al., 2005; CARDOSO et al., 2011).

Após o processo de limpeza, pode ser adicionada uma etapa de degerminação úmida do grão, por meio da adição de água a fim de aumentar a umidade do

milho para aproximadamente 20%. Com isso, é obtido um cereal mais macio e elástico, facilitando a degerminação e o processo de descamação (BRERA et al., 2004; PIETRI, ZANETTI, BERTUZZI, 2009).

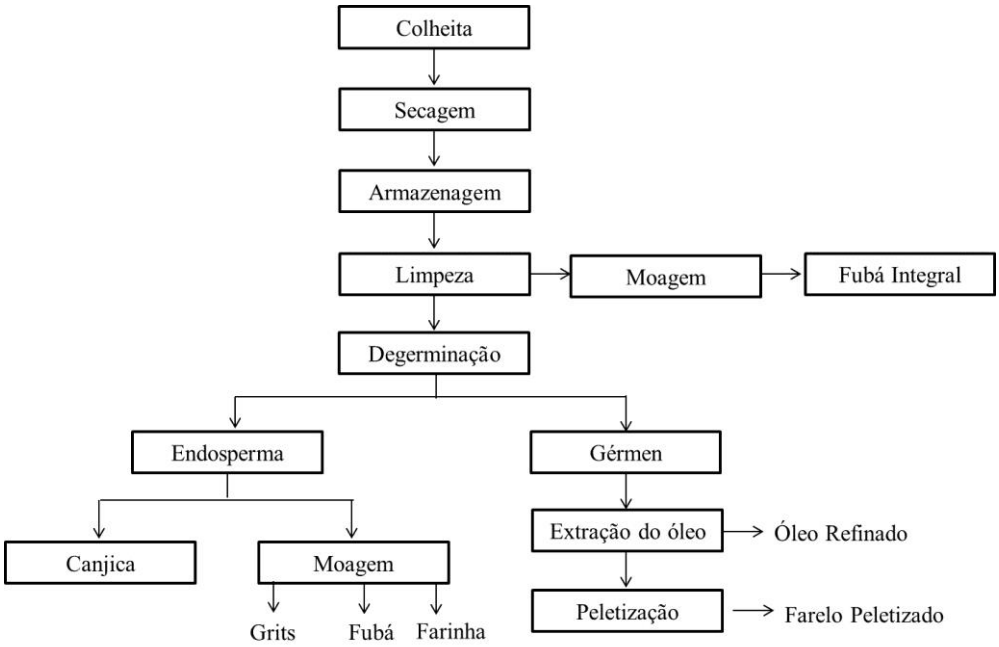


Figura 3. Esquema do processamento via seca do milho. Fonte: Adaptado de Cardoso et al. (2011).

O processo de degerminação é realizado por degerminadores ou canjiqueiras intermitentes ou contínuas (Figura 4) (BRERA et al., 2004; CARDOSO et al., 2011; PIETRI; ZANETTI; BERTUZZI, 2008). As canjiqueiras intermitentes são constituídas por um cilindro de chapa de ferro com fundo perfurado. Internamente, há um eixo constituído por facas cortantes, dispostas radialmente e opostas em posição alternada de 90°. A alimentação da máquina é realizada por moega com graduação variável, que regula a entrada do milho e o tempo para que as facas em rotação removam, por impacto e fricção, a casca e o gérmen (CARDOSO et al., 2011). Os degerminadores contínuos são utilizados somente para processamento em grande escala e são constituídos por cilindros cônicos que giram dentro de outro cilindro e podem deslocar longitunalmente, regulando e graduando a intensidade da trituração (CARDOSO et al., 2011). Um dispositivo de descarga conduz o milho degerminado para peneiras vibratórias que realizam a limpeza e separação do gérmen, pericarpo e endosperma. O milho degerminado pode ser designado como canjica (*flaking grits*) sendo caracterizado pelo endosperma em pedaços desprovido de pericarpo e gérmen (BRASIL, 2007; CARDOSO et al., 2011).

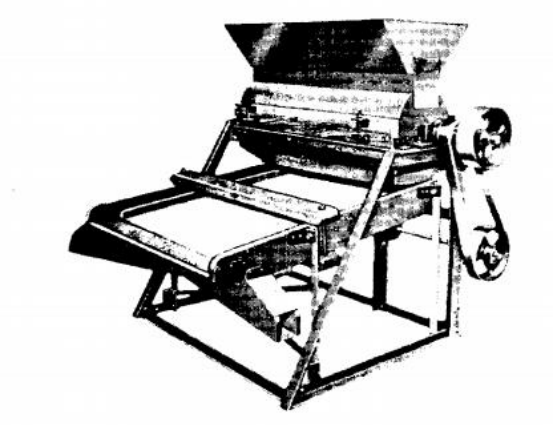


Figura 4. Canjiqueira ou degerminador de milho. Fonte: Cardoso et al. (2011)

O milho degerminado é então triturado e peneirado em diferentes tamises obtendo grits e fubás com granulometrias distintas (BRERA et al., 2004; CARDOSO et al., 2011; PIETRO; ZANETTI; BERTUZZI, 2008). A Tabela 1 mostra os principais tipos de grits e fubás obtidos da moagem do milho.

Tabela 1. Produtos obtidos da moagem do milho por via seca com diferentes granulometrias e aplicações industriais.

Frações do milho	Granulometria (mesh US)	Aplicação Industrial
Canjica de milho	Peneira 3,5 a 6	Produção de cereal matinal e canjica (destinada ao preparo de sobremesa)
Grits grosseiro	Peneira 10 a 20	Produção de cereais matinais e *snacks*
Grits regular	Peneira 15 a 30	Produção de cervejas, cereais matinais e *snacks*
Grits fino	Peneira 30 a 40	Produção de cerais matinais e *snaks*. Também utilizado como carboidrato fermentescível para a produção de bebidas alcoólicas
Fubá de milho	Peneira 40 a 60	Fabricação de produtos de panificação, massas e pratos regionais brasileiros
Farinha de milho	Peneira 60	Produção de pães e em misturas de farinhas

Fonte: Cardoso et al. (2011)

Da moagem úmida, o principal produto extraído é o amido, que é o maior componente do endosperma, seguido do concentrado protéico (60% de proteína), do farelo

(composto pelo pericarpo, fibras, resíduos de amido e 20% de proteínas) e do gérmen. A fração proteica e o farelo são direcionados às fábricas de rações para animais, e do gérmen se extrai o óleo que, após refinado, é comercializado como óleo comestível (BRASIL, 2007; CARDOSO et al., 2011; GERAGE et al., 1999).

Devido a sua alta qualidade nutricional, o milho está suscetível à contaminação por fungos produtores de micotoxinas, que são metabólitos secundários relacionados a diversos efeitos tóxicos em seres humanos e animais acarretando perdas econômicas (MUNKVOLD, 2003).

As micotoxinas são compostos estáveis e não são destruídas por muitos processos industriais, podendo ocorrer em alimentos à base de cerais (BULLERMAN; BIANCHINI, 2007). No processo de moagem por via seca do milho, as micotoxinas não são destruídas e os níveis de contaminação diferem entre as frações, sendo maiores em gérmen e pericarpo (BRERA et al., 2004; CASTELLS et al., 2008; SCUDAMORE; PATEL, 2009).

3.1.2 Milho Transgênico

Apesar de sua importância para a utilização industrial, o milho pode ser afetado por insetos, principalmente da ordem Lepdoptera, como a lagarta-do-cartucho (*Spodoptera frugiperda*), a lagarta-do-colmo (*Diatrea saccharalis*) e a lagarta-da-espiga (*Helicoverpa zea*) (CRUZ et al., 1995). Os danos causados pela infestação de insetos fornecem sítios de penetração de fungos no grão, além de serem vetores de fungos toxigênicos (MUNKVOLD; DESJARDINS, 1997; SOBEK; MUNKVOLD, 1999). O controle dessas pragas tem sido realizado, principalmente, com o cultivo de milho transgênico que possuem o gene de *Bacillus thuringiensis (Bt)* (VASCONCELOS; CARNEIRO; VALICENTE, 2011).

O Brasil é o segundo maior produtor de culturas biotecnológicas no mundo, sendo a área de milho transgênico plantada de 15,7 milhões de hectares (ISAAA, 2016). Do total da produção brasileira de milho, 83% são de milho transgênico (USDA, 2016).

Os híbridos de milho *Bt* possuem a resistência ao ataque de insetos por serem geneticamente modificados pela inserção do gene C*ry* de *Bacillus thuringiensis (Bt)*. Essa bactéria ocorre em diversos habitats como solo, grãos, água e é caracterizada por formar cristais proteicos durante a fase estacionária e/ou esporulação (HOFTE; WHITLEY, 1989).

Ao ser ingerida, a toxina protéica se dissolve no intestino médio do inseto e sofre ação de enzimas proteolíticas que liberam proteínas tóxicas menores chamadas de δ-endotoxinas, o que ocorre somente em pH elevado (> 9,5). As endotoxinas aderem a

receptores da membrana intestinal criando canais de cácio e poros, resultando no rompimento do intestino do inseto (BRODERICK; RAFFA: HANDELSMAN, 2006; LUTHY; EBERSOLD, 1981).

O híbrido *Bt*, portanto, tem sido efetivo na redução dos danos no grão causados por insetos e, consequentemente, no controle da infecção por fungos e da produção de micotoxinas (MUNKVOLD; HELLMICH; RICE 1999; BOWERS et al., 2013). Munkvold, Hellmich e Rice (1999) mostraram que os níveis de fumonisinas foram menores em milho transgênico com resistência a broca europeia (*Ostrinia nubilalis*), quando comparado com o milho convencional. Bakan et al. (2002) relataram que a biomassa fúngica em milho *Bt* foi de 4 a 18 vezes menor que em milho convencional. Além disso, os níveis de fumonisinas variaram de 50 a 300 µg/kg e de 400 a 9000 µg/kg em milho transgênico e convencional, respectivamente. Contudo, tem sido relatado que a redução de micotoxinas ocorre somente em certas condições, sendo o clima, a localização do plantio e a época de colheita, os fatores determinantes na produção de micotoxinas (BARROSO et al., 2017).

3.2 GÊNERO *Fusarium*

O principal fungo toxigênico contaminante da cultura do milho é o *Fusarium* sp, considerado um fitopatógeno, que causa doenças em todos os estágios de desenvolvimento da planta e de difícil controle (D'MELLO, PLACINTA; MACDONALD, 1999; MUNKVOLD, 2003; PLACINTA et al., 1999). Além disso, pode produzir fumonisinas, zearalenona e tricotecenos, micotoxinas de ocorrência mundial em cereais (CAST, 2003).

O Gênero *Fusarium* pertence ao Filo Ascomycota e inclui espécies que formam colônias com colorações diversas (branco, rosa pálido, roxo, alaranjado, púrpura), produzem macroconídios hialinos septados que podem ou não apresentar microconídios e clamidósporos (CAST, 2003). *Fusarium verticilioides* (Sacc.) Nirenberg (sinônimo *F. moniliforme* J. Sheld) pertence ao complexo de espécies *Gibberella fujikuroi* e é o principal produtor de fumonisinas (DESJARDINS, 2006). As espécies de *Fusarium* são os fungos toxigênicos mais comuns que contaminam o milho no campo em regiões temperadas e semi-tropicais (LOGRIECO et al., 2002). *Aspergillus* spp. e *Penicillium* spp. são descritos como fungos associados com a estocagem dos grãos (CAST, 2003).

Fusarium sp. causam, principalmente, a podridão de espigas de milho no mundo todo e são caracterizadas pela co-ocorrência ou sucessão de diferentes espécies

(BOTTALICO, 1997; LOGRIECO et al., 2002). As espécies de *Fusarium* são responsáveis por ao menos dois tipos de podridão de espiga, que podem ser diferenciadas em fusariose ou podridão rosada, causadas principalmente por *F. verticillioides*, *F. proliferatum* e *F. subglutinans*; ou podridão Gibberella ou podridão avermelhada, causada principalmente por *F. graminearum, F. culmorum e F. cerealis* (LOGRIECO et al., 2002; SHURTLEFF, 1980).

F. verticillioides, principal produtor de fumonisinas, está associado a doenças em todas as fases de desenvolvimento do milho, causando danos em plântulas e podridão de raiz, colmo e espiga, bem como danos em milho armazenado. Durante a colheita, o fungo pode ser encontrado tanto nas plantas como nos restos das culturas. Por outro lado, *F. verticillioides* pode também colonizar o milho de forma assintomática, e, neste caso, não causa doenças visíveis, relação esta denominada de endofítica (MUNKVOLD; DESJARDINS, 1997; MUNKVOLD; McGEE; CARLTON, 1997)

A contaminação de milho por *F. verticillioides* ocorre principalmente por meio da infecção dos estigmas por conídios carreados pelo ar ou água. No entanto, a doença pode se estabelecer via contaminação da semente chegando à espiga e grãos por meio da circulação sistêmica caulinar; pela infecção da raiz atingindo os grãos através do colmo e espiga; e via lesões causadas por insetos, os quais também podem atuar como vetores de inóculo (MUNKVOLD; McGEE; CARLTON, 1997; SORIANO; DRAGACCI, 2004).

Ambas as infecções, sintomáticas e assintomáticas, por *F. verticillioides* podem resultar na perda da qualidade do grão de milho devido à contaminação por fumonisinas, uma micotoxina associada a graves doenças em seres humanos e animais (GELDERBLOM et al., 1991; MARASAS et al., 1988; MISMER et al., 2006; ROSS et al., 1990; WEIBKING et al., 1994).

3.3 FUMONISINAS

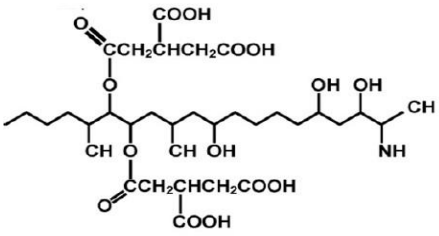
3.3.1 Estrutura Química

As fumonisinas foram isoladas pela primeira vez em 1988 de culturas de *F. verticillioides* MRC 826 por Gelderblom et al. (1988).

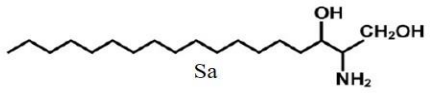
Desde 1988, ao menos 28 análogos de fumonisinas foram caracterizados e são divididos em quatro grupos principais, identificadas como fumonisinas das séries A, B, C e P (RHEEDER; MARASAS; VISMER, 2002). Os análogos da fumonisina B, incluindo as

fumonisinas B_1 (FB_1), B_2 (FB_2) e B_3 (FB_3), são os mais tóxicos e abundantes em milho, sendo que a fumonisna B_1 é a prevalente e detectada em maiores concentrações (MARASAS, 1996).

Estruturalmente, as fumonisinas consistem em uma cadeia linear com 19 ou 20 carbonos ramificados com grupos hidroxil, metil e ácidos tricarboxílicos em várias posições ao longo da cadeia (BENZUIDENHOUT et al., 1988). A FB_1 é descrita como um diéster de propano-1,2,3-ácido tricarboxílico e um 2-amino-12,16-dimetil, 3,5,10,14,15-pentahidroxicosano com grupos hidroxil esterificados com o grupo carboxiterminal dos ácidos em C-14 e C-15. A FB_2 e a FB_3 são os análogos que não possuem um grupo hidroxil na cadeia principal na posição C-10 e C-5, respectivamente (BENZUIDENHOUT et al., 1988; PLATTNER et al., 1992). As fumonisinas da série C são quimicamente similares às fumonisinas da serie B, exceto pelo fato de não haver um grupo metil terminal em C-1 (SEO; KIM; LEE, 1996), enquanto que as fumonisinas da série A e P diferem das fumonisinas da série B por possuírem uma N-acetilação no C-2 e um grupo 3-hidroxipiridina no C-2, respectivamente (BENZUIDENHOUT et al., 1988; MUSSER et al., 1996). Os efeitos tóxicos das fumonisinas estão relacionados à sua estrutura, que é similar aos esfingolipídios (Figura 5) (MERRIL et al., 2001).



FB_1



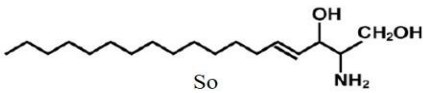


Figura 5. Estrutura química da fumonisina B_1 (FB_1), esfinganina (Sa) e esfingosina (So). Fonte: Voss; Smith; Haschek (2008).

As fumonisinas são moléculas polares e, ao contrário das outras micotoxinas contaminantes de alimentos, não possuem nenhuma estrutura aromática ou cromófora de fácil detecção analítica (MURPHY et al., 2006).

3.3.2 Biossíntese de Fumonisinas

As fumonisinas são sintetizadas pela via das policetidas durante o metabolismo secundário do fungo que se inicia, frequentemente, após o término da fase de crescimento (GRIFFIN, 1994).

A biossíntese dessas toxinas inicia-se com a formação da cadeia carbônica principal a partir da condensação de uma molécula de acetil-CoA, 8 moléculas de malonil-CoA e duas moléculas de metionina, sob forma de *S*-adenosil (Figura 6). O produto desta reação, catalisada por uma policetida sintase, é um policetídeo de 18 carbonos o qual é condensado ao aminoácido alanina (BOJJA et al., 2008; SWEENEY; DOBSON, 1999). Posteriormente, ocorrem subsequentes oxidações nas posições C-14 e C-15, catalisadas por oxigenases citocromo P450 dependentes; esterificações com propano-1,2,3-ácidos tricaboxílicos nos grupos hidroxilas em C-14 e C-15, catalisadas por uma aciltransferase, e hidroxilação no C-5 pela ação da dioxigenase 2-ceto-glutarato-dependente (BOJJA et al., 2004).

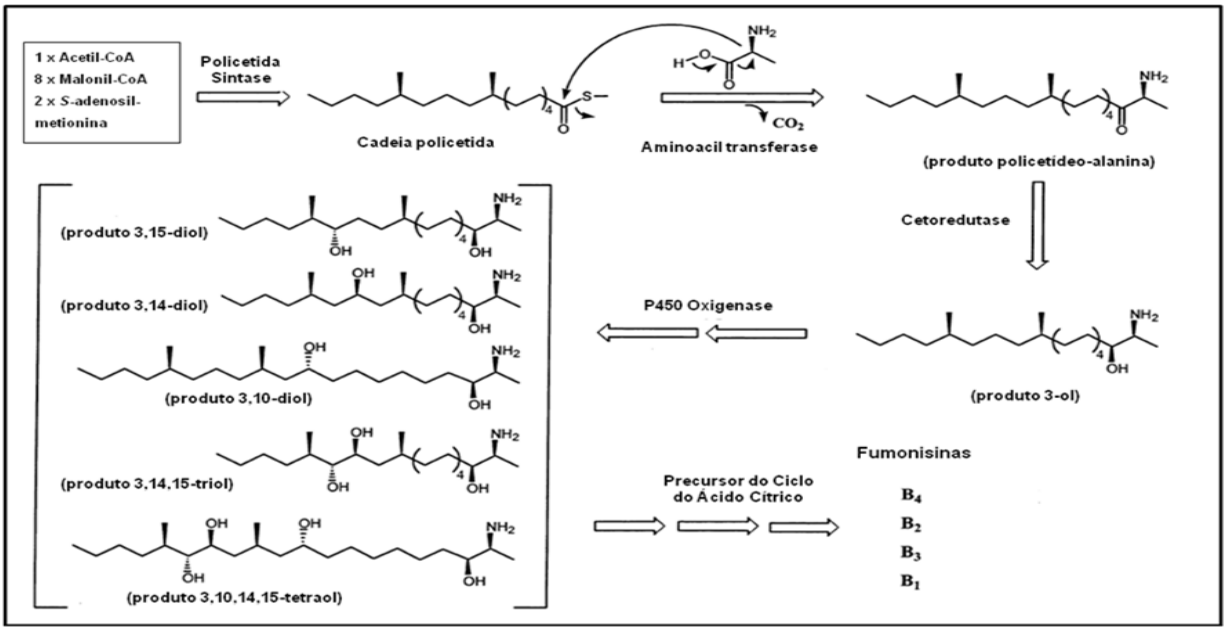


Figura 6. Via biossintética das fumonsinas. Fonte: Adaptado de Bojja et al. (2004).

Os componentes das moléculas de fumonisinas apresentam diferentes origens biogênicas. Os carbonos 3-20 são derivados do acetato, os grupos aminos em C-1 e C-2 da alanina (BLACKWELL et al., 1996; BRANHAM; PLATTNER, 1993), e os dois grupamentos metil nos carbonos 12 e 16 da metionina (PLATTNER; SHACKELFORD, 1992). O grupo hidroxila no C-3 é proveniente do grupo carbonila derivado do acetato, enquanto que os grupos hidroxila nos carbonos 5, 10, 14 e 15 são originados do oxigênio molecular (CALDAS et al., 1998). Os ácidos tricarboxílicos provavelmente são derivados do ácido glutâmico pela via do ciclo do ácido cítrico (ex. ácido aconítico) (BLACKWELL et al., 1996).

3.3.3 Biossíntese de Esfingolipídios e Mecanismo de Ação das Fumonisinas

A síntese *de novo* dos esfingolipídios inicia-se com a condensação da serina e do palmitoil-CoA catalisada pela enzima serina palmitoil transferase, formando a 3-cetodiidroesfingosina. A 3–cetodiidroesfingosina é subsequentemente reduzida para formar a diidroesfingosina (esfinganina), a qual é fosforilada originando a esfinganina-1-fosfato ou é *N*-acetilada pela ação da enzima ceramida sintase para produzir a diidroceramida, que posteriormente é dessaturada à ceramida. A ceramida pode ser transformada em esfingomielina pela adição de fosfocolina, a qual é a principal representante dos esfingofosfolipidios; em glicolipídios pela adição de oligossacarídeos; ou em esfingosina pela ação catalítica da ceramidase. A esfinganina-1-fosfato e a esfingosina-1-fosfato produzem etanolamina ou aldeídos graxos que podem ser convertidos em serina e palmitoil-CoA, que podem ser novamente utilizados para biossíntese de esfingolipídios (BARTKE; HANNUM, 2009; SORIANO; GONZÁLES; CATALÁ, 2005). Os membros da família dos esfingolipídios, incluindo a ceramida, esfingosina, esfingosina-1-fosfato e a ceramida 1-fosfato, participam de diversos processos celulares, como a regulação das vias de transdução de sinais, do crescimento celular, da diferenciação celular, da apoptose e da secreção de proteínas, além de mediarem as interações células-células ou células-substrato (BARTKE; HANNUM, 2009; MERRIL et al., 1997).

Devido à similaridade das fumonisinas com as bases esfingóides (esfingosina e esfinganina) (Figura 5), o mecanismo de ação das fumonisinas ocorre principalmente por alteração na biossíntese e no metabolismo dos esfingolipídios (MERRIL et al, 2001; RYLEI et al., 2001; VOSS; SMITH; HASCHEK, 2007).

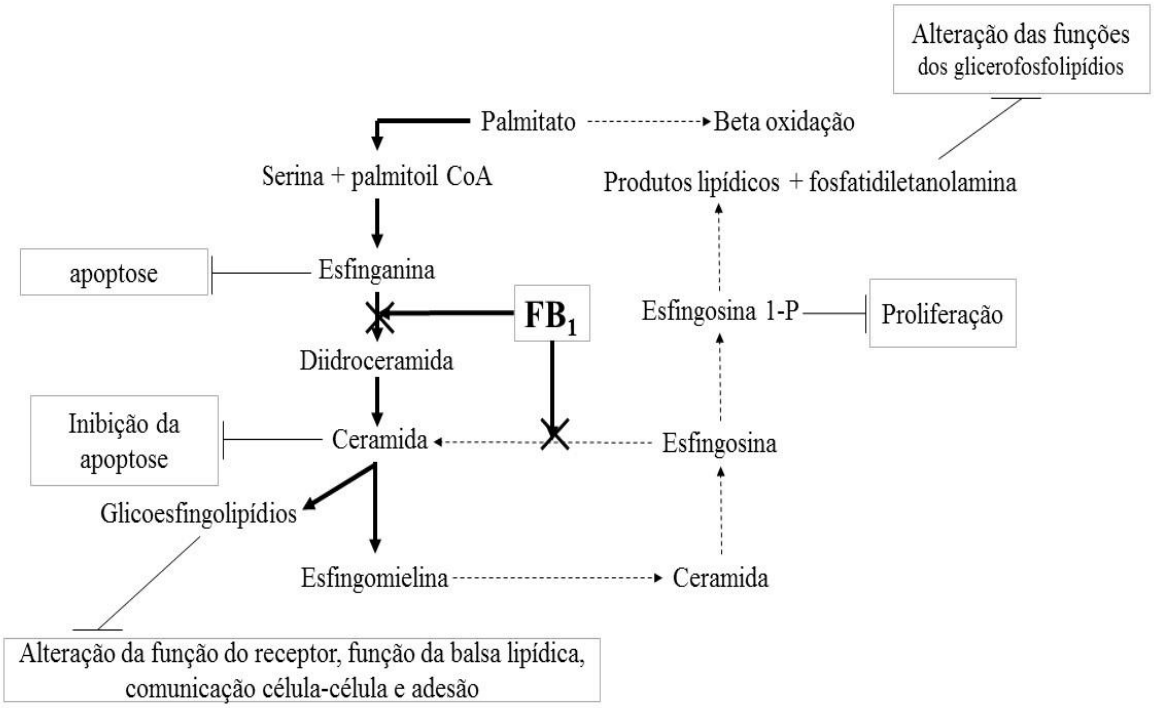


Figura 7. Biossíntese de esfingolipídios em células de mamíferos. O símbolo **X** indica a via enzimática inibida pela fumonisina B_1. Adaptado de IARC (2002).

As fumonisinas inibem competitivamente a enzima ceramida sintase, enzima chave no metabolismo dos esfingolipídios, a qual catalisa a acilação da esfinganina, esfingosina e de outras bases esfingóides, por meio de interações não covalentes entre a enzima e os grupos amino no C-2 e o ácido tricarboxílico das fumonisinas. Como consequência, os níveis celulares de esfinganina aumentam dramaticamente, bem como a proporção esfinganina/esfingosina. Além disso, ocorrem a redução da reacilação das esfingosinas derivadas do turnover dos esfingolipídios e a redução da degradação dos esfingolipídios da dieta (Figura 7) (MERRIL et al., 1993; MERRIL et al., 1996; RILEY et al, 1998; WANG et al., 1991). O aumento da esfinganina e da proporção esfinganina/esfingosina são utilizadas como biomarcadores para avaliar a exposição em algumas espécies animais (CAST, 2003).

Esse mecanismo de ação acarreta toxicidade da FB_1, caracterizada por efeitos na atividade da proteína quinase, no crescimento e diferenciação celular, morte celular (apoptose), carcinogenicidade e envolvimento na peroxidação de lipídios (SORIANO; GONZÁLES; CATALÁ, 2005).

3.3.4 Efeitos Tóxicos

Em aninais, os principais efeitos tóxicos incluem leucoencefalomalácia em equinos (MARASAS et al., 1988), edema pulmonar em suínos (ROSS et al., 1990), efeitos hepatotóxico e hepatocarcinogênicos em ratos (GELDERBLOM et al., 1991), efeitos nefrocarcinogênicos em ratos (HOWARD et al., 2001) e redução do desenvolvimento de aves (LEDOUX et al., 1992).

Os equinos são os animais mais sensíveis à toxicidade das fumonisinas sendo os órgãos alvos o sistema nervoso central, o coração e o fígado. A síndrome nomeada de leucoencefalomalácia (malacia= amolecimento; leuko= matriz branca) é caracterizada por uma necrose liquefativa da matriz branca do cérebro que acarreta a morte dos animais em poucas horas ou em uma semana (MUNKVOLD; DESJARDINS, 1997; VOSS; SMITH: HASCHEK, 2007). Estudos realizados em amostras de ração relacionadas a surtos de leucoencefalomalácia na América do Norte, América do Sul e África mostraram que a doença pode ocorrer com concentrações de FB_1 entre 0,2 a 126 µg/kg (MARASAS, 1995).

Em um estudo realizado por Harrison et al. (1990), suínos que receberam FB_1 (400 µg/kg de peso) em injeção intravenosa, morreram após 4 dias e apresentaram edema pulmonar e hidrotórax. O edema pulmonar é um efeito espécie-específico e não tem sido reportado em outras espécies. As mortes podem ocorrer dentro de poucas horas após a apresentação de dificuldades respiratórias ou sem sinais clínicos evidentes (VOSS, SMITH; HASCHEK, 2007).

A FB_1 também diminui a contratilidade cardíaca, a pressão sistêmica, frequência cardíaca e o débito cardíaco em suínos (CONSTABLE et al, 2000; 2003). Além disso, o edema pulmonar pode estar relacionado à insuficiência cardíaca (SMITH et al., 1999) devido à inibição dos canais de cálcio, causada pelo aumento nas concentrações da proporção esfingosina/esfinganina no coração (VOSS; SMITH; HASCHEK, 2007).

As aves são relativamente resistentes à toxicidade causada pelas fumonisinas (VOSS; SMITH; HASCHEK, 2007). Em um estudo realizado por Ledoux et al. (1992), 45 frangos de corte, machos, com 1 dia de idade, foram submetidos a dietas contendo 100, 200, 300 ou 400 mg de FB_1/kg de ração durante 21 dias. Após esse período, o peso das aves e a média de ganho de peso diária diminuíram significativamente com o aumento da dose de FB_1, enquanto que os pesos do fígado, pró-ventrículo e moela, e os níveis de cálcio, colesterol e das enzimas aspartato amino-transferases no soro, aumentaram. As aves também apresentaram diarréia, atrofia cortical do timo, necrose hepática multifocal e hiperplasia biliar.

Em estudo com 128 codornas japonesas poedeiras em início de postura, 7 semanas de idade, alimentadas com ração contendo 10, 50 e 250 mg de FB_1/kg, durante 28 dias, ocorreu diminuição no consumo de alimentos e do ganho de peso e aumento no peso relativo do fígado, nos animais tratados com as duas últimas concentrações. No entanto, histopatologicamente, os tecidos hepático, renal e do miocárdio não apresentaram diferenças em relação aos do grupo controle. No grupo exposto a 250 mg de FB_1/kg houve diminuição da produção média e do peso dos ovos (BUTKERAITIS, 2003).

Em seres humanos, estudos epidemiológicos indicam a associação das fumonisinas com o câncer esofágico na região de Transkei (África do Sul) e China e câncer hepático primário (RHEEDER et al., 1992; SYDENHAM et al., 1990; UENO et al., 1997), além de defeitos no tubo neural (CIFUENTES, 2002; MISSMER et al., 2006).

Em um estudo realizado em ratos LMBc para a indução de defeitos no tubo neural por FB_1 *in vivo,* foi demonstrado que o mecanismo do defeito no tubo neural ocorre por meio da diminuição da utilização de folato mediado pela alteração no metabolismo dos esfingolipídios, induzidas pelas fumonisinas (GELINEAU-VAN WAES, et al., 2003; MARASAS et al., 2004).

Devido aos efeitos tóxicos, limites máximos foram estabelecidos para as fumonsinas em alimentos e rações no mundo todo. Além disso, a *International Agency for Research on Cance*r (IARC) classificou as fumonisinas como carcinógenos do grupo 2B, i.e., provavelmente carcinogênicos para seres humanos (IARC, 2002).

3.3.5 Regulamentações das fumonisinas em alimentos e rações

Os limites regulatórios para micotoxinas em alimentos e rações estão cada vez mais rigorosos**.** A União Europeia estabeleceu um limite máximo tolerado para fumonisinas (FB_1 + FB_2) em milho e produtos derivados de milho destinados à alimentação animal de 60000 µg/Kg (EUROPEAN COMISSION, 2006). Para a alimentação humana, o limite máximo tolerado em milho não processado e alimentos à base de milho é de 1000 µg/kg, enquanto que em cereais matinais e em alimentos infantis à base de milho é de 800 e 200 µg/kg, respectivamente (EUROPEAN COMISSION, 2007).

No Brasil, a Agência Nacional de Vigilância Sanitária (ANVISA) estabeleceu um limite para fumonisinas de 1500 µg/Kg para vários produtos derivados de milho (farinha de milho, creme de milho, flocos, fubá, canjica e canjiquinha) e de 1000 µg/Kg

para o amido e outros produtos à base de milho destinado ao consumo humano (BRASIL, 2017). Para o milho em grãos, o limite máximo tolerado é de 5000 µg/Kg (BRASIL, 2011).

Não há uma legislação sobre os limites máximos tolerados de fumonisinas em ração animal no Brasil. No entanto, em 2006, foi criado o Grupo de Trabalho sobre Micotoxinas pelo Ministério da Agricultura (BRASIL, 2006) que recomendou um limite máximo tolerado de fumonisinas em milho em grãos ou seus subprodutos destinados à alimentação animal de 10000 µg/Kg. O limite recomendado para ração animal destinado a animais monográstricos é de 5000 µg/Kg.

Tabela 2 – Limites máximos tolerados (LMT) e recomendados para fumonisinas em alimentos (BRASIL, 2011, 2017) e rações (BRASIL, 2006), respectivamente.

Micotoxina	**Alimentos**	**Limite Máximo (µg/Kg)**
Fumonisinas ($B_1 + B_2$)	Milho de pipoca	2000
	Alimentos à base de milho para alimentação infantil	200
	Farinha de milho, creme de milho, fubá, flocos, canjica, canjiquinha	1500
	Amido de milho e outros produtos a base de milho	1000
	Milho em grãos para posterior processamento	5000
	Rações e concentrados para monogástricos, exceto aves domésticas	5000
	Rações e concentrados para aves domésticas	1000

3.3.6 Efeito do Processamento do Milho a Seco na Contaminação por Fumonisinas

Durante o processamento do milho, as micotoxinas podem ser concentradas em certas frações. No processamento por via seca, as micotoxinas não são destruídas e tendem a concentrar-se no gérmen e no pericarpo (BRERA et al., 2004; BROGGI et al., 2002; CASTELLS et al., 2008; PIETRO; ZANETTI; BERTUCCI, 2009).

Broggi et al. (2002) analisaram amostras de milho e suas frações obtidas por via seca (fubá, farinha, gérmen + pericarpo) quanto à contaminação por FB_1, FB_2 e FB_3, na Argentina. A FB_1 foi detectada em todas as amostras de milho e derivados; o gérmen e o

pericarpo apresentaram maior contaminação (média = 4220 µg/kg), seguida pelo milho integral (média = 1540 µg/kg) e pela farinha (média = 358 µg/kg). A contaminação no gérmen + pericarpo foi três vezes maior do que no milho integral. O fubá e o grits apresentaram menor contaminação, com uma concentração média de 148 e 135 µg FB_1/kg, respectivamente.

Brera et al. (2004) avaliaram a contaminação por FB_1 em dois lotes de milho e em cada fração obtida da moagem a seco associada a uma degerminação úmida, em uma indústria localizada na Itália. O milho mostrou uma contaminação de 4540 e 5090 µg/kg nos lotes 1 e 2, respectivamente. O gérmen, o pericarpo e a farinha destinada à ração animal estavam contaminados com uma concentração média de FB_1 de 8920 µg/kg (lote 1) e 9560 µg/kg (lote 2), 7080 µg/kg (lote 1) e 8.080 µg/kg (lote 2) e 9.360 µg/kg e 8860 µg/kg, respectivamente, enquanto que as concentrações em grits de maior e menor granulometria e em farinha destinada à preparação de polenta cozida apresentaram uma menor concentração média de FB_1, que variou de 390 a 1010 µg/kg.

Castells et al. (2008) analisaram o destino de fumonisinas (FB_1, FB_2 e FB_3) durante o processamento do milho a seco e o efeito do cozimento, em duas indústrias localizadas na Argentina. A contaminação por fumonisinas ocorreu em 100% das amostras de milho (n = 92), farinha de ração animal (n = 92), fubá (n = 90), farinha de milho (n = 90) e grits (n = 78), enquanto que somente em 13% e 21% das amostras de grits cozido e flocos de milho, respectivamente. A concentração de fumonisinas no milho inteiro variou de 337 a 10613 µg/kg (média = 2610 µg/kg), sendo que nas frações compostas por gérmen e pericarpo houve uma maior concentração de fumonisinas comparadas a frações compostas por endosperma (canjica). Na farinha destinada à ração animal, a concentração de fumonsinas variou de 333 a 19976 µg/kg (média = 8268 µg/kg), enquanto que na farinha de milho, variou de 892 a 6307 µg/kg (média = 2640 µg/kg). Em grits e fubá, os níveis de fumonisinas variaram de 73 a 1.053 (média = 366 µg/kg) e de 144 a 2.003 µg/kg (média = 761 µg/kg), respectivamente. A média da contaminação de fumonisinas em grits cozido e em flocos de milho foram de 140 e 42 µg/kg, respectivamente, indicando uma redução de 92% das fumonisinas durante o cozimento e a torragem.

Pietri, Zanneti e Betuzzi (2009) analisaram dois lotes de milho com diferentes concentrações de fumonisinas (FB_1 e FB_2) sobre o efeito da etapa de limpeza e do processamento por via seca na distribuição de fumonisinas, na Itália. O milho obtido após a etapa de limpeza apresentou menor contaminação por FB_1, sendo que a redução da

contaminação foi de 11 e 34% no 1° e 2° lote, respectivamente (correspondentes a 4770 µg/kg e 5862 µg/kg), quando comparado com o milho não processado (5379 µg/kg e 8841 µg/kg). Das frações obtidas por via seca, o farelo apresentou maior nível de contaminação em relação ao milho limpo (aumento de 50 e 167%, lote 1 e 2, respectivamente) e o grits grossos e finos destinados ao consumo humano apresentaram menor contaminação por FB_1 (redução de 90 e 88% para o lote 1, e de 90 e 73%, para o lote 2). A farinha com tamanho de partícula indesejada (< 2 mm) e destinada à produção de ração animal apresentou maior concentração de FB_1 (16011 µg/kg e 28712 µg/kg, respectivamente) e, além disso, cerca de 90% de FB_1 foi retida nessa fração.

Vanara, Reyneri e Blandino (2009) analisaram a redistribuição da FB_1 durante o processamento por via seca do milho em uma indústria na Itália, num total de 164 amostras entre o milho integral e suas frações (gérmen, farelo para ração animal, farinha e fubá). As frações que apresentaram maior contaminação foram o gérmen e o farelo para a ração animal, com concentrações de FB_1 de 3450 e 11400 µg/kg, respectivamente, em comparação com 4580 µg/kg do milho integral. A farinha de milho e o fubá apresentaram menor contaminação que o milho integral, com uma concentração média de 1690 e 499 µg/kg. Adicionalmente, foram analisados produtos derivados do milho destinados à alimentação humana, incluindo farinhas com diferentes granulometrias, farinha pré-cozida e grits para a produção de cerveja. Nessas amostras, houve um aumento na concentração de fumonisinas conforme a diminuição do tamanho da partícula da farinha.

Burger et al. (2013) analisaram o efeito da moagem a seco do milho branco na contaminação por fumonisinas na África do Sul. Primeiramente, o milho foi degerminado produzindo o milho degerminado e o gérmen. Em seguida, o milho degerminado foi moído em condições laboratoriais e quatro frações (fubá especial, fubá super, semolina e ração de canjica moída + gérmen) foram coletadas. O processo de degerminação reduziu o nível de fumonisinas (média = 141 µg/kg) entre 2,2 e 3,2 vezes em relação ao milho integral (média = 413 µg/kg). Contudo, houve uma concentração na ração de canjica moída entre 240 e 280% (média = 1157 µg/kg). Em relação às frações obtidas a partir do milho degerminado, a fração fubá especial continha maiores concentrações de fumonisinas (média = 338 µg/kg), seguida pela semolina (média 143 µg/kg) e pelo fubá super (61 µg/kg).

Geralmente, as micotoxinas concentram-se nas camadas mais externas do grão de milho e os produtos resultantes da moagem dessas frações como o gérmen, casca, pericarpo e ponta tendem a serem mais contaminados (BRERA et al., 2004; BURGER et al., 2013; KATTA et al., 1997; SCUDAMORE; PATEL, 2009). Isto ocorre devido à colonização

dos fungos nas camadas externas, e a contaminação por micotoxinas progride da parte externa para as camadas mais internas do grão (BRERA et al., 2004; CASTELLS et al., 2008). Além disso, o pericarpo age como uma barreira física contra a penetração do fungo no endosperma, acarretando reduzida transferência de micotoxinas para as partes mais internas do grão (SCUDAMORE; PATEL, 2009).

Diversos estudos demonstram que quando o tamanho da partícula do endosperma diminui, a concentração de micotoxinas aumenta (PIETRI; ZANETTI; BERTUCCI, 2009; SCUDAMORE; PATEL, 2009; VANARA; REYNERI; BLANDINO, 2009). Endospermas duros são menos suscetíveis à quebra na pós-colheita, reduzindo a contaminação por fungos e micotoxinas, e tendem a produzir frações mais grosseiras durante a moagem (STROCHINE et al., 1986). Além disso, endospermas mais duros e rígidos apresentaram menor contaminação por fumonisinas comparado com híbridos mais macios (BLANDINO; REYNERI, 2008), sendo a textura, portanto, um fator determinante na distribuição de fumonisinas no grão. Grãos mais densos, pericarpos mais compactos (COSTA et al., 2003) e ceras mais densas no grão estão relacionados a menores concentrações de fumonisinas (SAMPIETRO et al., 2009).

Ressalta-se ainda que existem variações na redistribuição de micotoxinas nas diferentes partes do grão de milho decorrente da estratégia de moagem utilizada, que varia entre os países, e do destino das frações moídas (BURGER et al., 2013; ESCOBAR et al., 2013; SCUDAMORE; PATEL, 2009).

3.4 AVALIAÇÃO DA EXPOSIÇÃO HUMANA A FUMONISINAS

Os alimentos contêm uma grande variedade de substâncias essenciais para a manutenção da saúde e outras potencialmente tóxicas, como micotoxinas, resíduos de pesticidas, aditivos e metais pesados. A falta de algum nutriente ou a presença excessiva de substâncias tóxicas podem representar um risco à saúde humana. Assim, a estimativa dos valores ingeridos é necessária para avaliar os riscos aos quais seres humanos e animais são expostos (JARDIM; CALDAS, 2009; KROES, 2002).

A avaliação de risco é um processo de quatro etapas que inclui: identificação do perigo, caracterização do perigo, avaliação da exposição e caracterização do risco. Essa última etapa integra as informações coletadas nas três etapas precedentes (KROES, 2002).

A identificação do perigo tem como objetivo identificar os potenciais efeitos adversos à saúde humana associados a uma substância química. A caracterização do perigo consiste na relação entre a exposição e a incidência de um efeito adverso, na qual, a relação dose-resposta é avaliada. Nessa etapa é estimada, para substâncias que apresentam limiar de dose para o efeito adverso (*threshold*), a dose em que não foi observado efeito adverso em animais (*Non-Observed-Adversed-Effects-Levels* – NOAEL) ou a menor dose na qual um efeito adverso foi observado (*Lowest-Observed-Adverse-Effect-Level* – LOAEL) (JARDIM; CALDAS, 2009; RINGOT; CHANGO, 2010).

A avaliação da exposição é uma etapa importante no processo de avaliação de risco, e consiste na determinação quantitativa da ingestão provável de contaminantes por seres humanos e animais por meio dos alimentos e rações, respectivamente. E, a última etapa, a caracterização do risco, pode ser definida como a estimativa qualitativa ou, sempre que possível, quantitativa, incluindo as incertezas esperadas, da probabilidade de ocorrência de um efeito adverso de um dado agente em um dado organismo ou população sob condições definidas de exposição (KROES, 2002).

O NOAEL e o LOAEL são utilizados no cálculo dos parâmetros seguros de ingestão crônica no homem e nos animais. Esses parâmetros são calculados dividindo-se o NOAEL ou o LOAEL por um fator de incerteza, ou de segurança, para extrapolar os resultados obtidos com animais para outras espécies ou para seres humanos. Dentre os parâmetros de ingestão crônica segura estimada estão a Ingestão Diária Aceitável (IDA), ingestão semanal tolerável (*Tolerable Weekly Intake* – TWI), a ingestão máxima diária tolerável provisória (*Provisional Maximum Tolerable Daily Intake* – PMTDI) e a ingestão semanal provisória (*Provisinal Tolerable Weekly Intake* – PTWI) (JARDIM; CALDAS, 2009). O risco existe se os valores de exposição diária ultrapassarem esses valores seguros pré-determinados (IPCS, 2009). Uma PMTDI para fumonisinas foi estimada em 2 μg/Kg de peso corpóreo/dia (WHO, 2011).

No Brasil, alguns estudos reportaram a ingestão de fumonisinas pela população brasileira (BORDIN et al., 2014; CALDAS; JARDIM, 2007; MARTINS et al., 2012; MORENO et al., 2009; SAVI et al., 2016). Bordin et al (2014) avaliaram a contaminação por FB_1 em amostras de fubá, farinha de milho, flocos de milho, milho enlatado e pipoca, coletados em residências de moradores de Pirassununga, São Paulo e calcularam o IDP de fumonisinas com base nos dados de consumo utilizando um questionário de frequência alimentar. A IDP foi de 63,3 ng/kg de peso corporal/dia, representando aproximadamente 3% do PMTDI para fumonisinas.

Caldas e Silva (2007) estimaram a ingestão de FB_1 + FB_2 pela população brasileira por meio do consumo de produtos de milho, incluindo fubá, cereal matinal e milho, utilizando dados de consumo de milho e peso corpóreo do Instituto Brasileiro de Geografia e Estatística (IBGE) do ano de 2002 e dados de contaminação de amostras coletadas no Distrito Federal e dos Estados de São Paulo, Santa Catarina e Pernambuco. A ingestão total de fumonisinas representou 24% do PMTDI para a população total.

Moreno et al. (2009) avaliaram a ingestão de fumonisinas (B_1 + B_2) baseando-se na contaminação do milho do norte do Estado do Paraná da safra de 2003 e 2004 e dos dados da ingestão de milho da população brasileira do IBGE de 2002. A IDP foi de 950 ng/Kg de peso corpóreo/dia.

Savi et al. (2016) estimaram a ingestão de fumonisinas por meio do consumo de milho, fubá, farinha de milho, grits, flocos de milho e pipoca do IBGE (2011) e da contaminação de amostras obtidas por moagem a seco sob condições laboratoriais, em Santa Catarina. O fubá foi o derivado de milho que mais contribuiu para a ingestão de fumonisinas (130 ng/Kg de peso corpóreo/dia) representando 6,7% do PMTDI.

Considerando que a contaminação por fungos e fumonisinas em milho constitui um risco para a saúde humana e animal, além de acarretar perdas econômicas para produtores e processadores de grãos, a avaliação da qualidade micológica e do destino das fumonisinas durante o processamento é essencial para determinar os riscos à saúde humana e animal.

4 MATERIAL E MÉTODOS

4.1 AMOSTRAGEM

As amostras de milho e dos derivados (gérmen, pericarpo, endosperma, grits e fubá) das safras de 2014 a 2016 foram coletadas em uma das maiores indústrias de processamento de moagem a seco do Brasil, localizada no Norte do Estado do Paraná. A indústria processa aproximadamente 12000 toneladas de milho por mês. O milho já havia sido limpo em cooperativas antes de chegar à indústria e foi coletado da peneira de separação do milho e da quirera. O milho limpo foi conduzido para a torre de moagem e a quirera, para ração animal. O milho inteiro foi submetido a uma degerminação úmida gerando três frações principais: pericarpo, gérmen "in natura" e canjica. O pericarpo e o gérmen foram coletados das tubulações que dirigiam as frações para *bags* a serem destinados à indústria de ração animal e de extração do óleo de milho, respectivamente. O milho degerminado, isto é, o endosperma, foi armazenado em silos e as coletas das amostras foram realizadas das tubulações que conduziam o endosperma para a moagem. A moagem do endosperma originou dois produtos: o fubá e o grits. Estes foram coletados das tubulações que alimentavam os *bags* de 1200 kg.

Foram analisados dois lotes de milho transgênico recém-colhido da safra de janeiro/2014 e janeiro/2016 e dois lotes de milho convencional colhido no mês de julho/2014 e julho/2015. Cada lote corresponde a uma moagem de 24 h de 500 toneladas de milho. A amostragem do milho integral e de cada fração foi realizada coletando aproximadamente 10 kg de cada amostra. Em seguida, as amostras foram homogeneizadas e aproximadamente 500 g foram coletados. Este processo foi realizado vinte vezes em intervalos regulares durante 1 h. Para cada lote foram coletados o milho integral (n = 20), pericarpo (n = 20), gérmen (n = 20), endosperma (n = 20), fubá (n = 20) e grits (n = 20), totalizando 480 amostras.

As amostras de 500 g foram trituradas a 50 mesh, homogeneizadas e 200g foram utilizadas para a análise de bolores e leveduras e de fumonisinas.

4.2 CONTAGEM FÚNGICA TOTAL

Uma alíquota de 10 g de milho e de suas frações (gérmen, pericarpo, endosperma, fubá e grits) (50 mesh) foi homogeneizada em 90 mL de água peptonada estéril 0,1% (v/v) e submetida a diluições seriadas em tubos contendo 9,0 mL do mesmo diluente até

fator 10^{-4}. Em seguida, 1 mL de cada diluição foi plaqueado, em duplicata, pelo método de *Pour Plate* utilizando ágar batata dextrosado (BDA - pH 4,0) adicionado de 50 µg/mL de cloranfenicol. As placas foram incubadas a 25ºC por seis dias e, submetidas à identificação de *Fusarium* spp. e à contagem fúngica total de acordo com os métodos preconizados por Nelson, Tousson e Marasas (1983) e Singh et al. (1991).

4.3 DETERMINAÇÃO DE FUMONISINAS

4.3.1 Extração de fumonisinas

Para extração de fumonisinas, uma alíquota de 10 g de milho e de suas frações foi adicionada de 30 mL de metanol: água (3:1, v/v), agitada 150 rpm por 30 minutos e, após, filtrada em papel filtro Whatman nº 1. O filtrado (1 mL) foi submetido à pré-limpeza em cartucho de troca aniônica Sep-Pak accell plus QMA (Waters Co., Ltda), previamente acondicionada com 5 mL de metanol seguido de 5 mL de metanol:água (3:1, v/v). Após lavagem da coluna com 6 mL de metanol:água (3:1, v/v) seguidos por 3 mL de metanol, as fumonisinas foram eluídas com 10 mL de ácido acético 0,5% em metanol. O eluato foi seco a 45 ºC em chapa aquecedora e o resíduo ressuspenso em 800 µL de metanol: água (3:1, v/v). Este foi fracionado em alíquotas de 200 µL, as quais foram secas sob fluxo de gás N_2 a 45 ºC, procedendo-se o acondicionamento em freezer (-20 ºC) para posterior análise de fumonisinas (UENO et al., 1993).

4.3.2 Determinação de fumonisinas

A determinação de fumonisinas (FB_1 e FB_2) foi realizada por cromatografia líquida de alta eficiência (CLAE) segundo o método de Shephard et al. (1990) modificado por Ueno et al. (1993). A alíquota de 200 µL foi ressuspensa em acetonitrila: água (1:1, v/v) e submetida à derivatização com 200 µL de o-ftaldialdeído (OPA) (40 mg de OPA, 1 mL de metanol, 5 mL de tetraborato de sódio a 0,1 M e 50 µL de 2-mercaptoetanol). A amostra foi injetada dentro de 1 minuto no sistema cromatográfico (isocrático de fase reversa) utilizando como fase móvel metanol: fosfato de sódio (CH_3OH: NaH_2PO_4) 0,1 mol/L (80:20, v/v) pH 3,3 ajustado com ácido orto-fosfórico e fluxo de 1 mL/min. O sistema consistiu de bomba Shimadzu LC-10 AD, detector de fluorescência RF-10A XL e coluna C18 (2) Luna 5µ 100Å (4,6 x 250 mm, Phenomenex). Os comprimentos de onda de excitação e emissão foram de

335 nm e 450 nm, respectivamente. O limite de detecção para FB_1 e FB_2 foi 27,5 and 35,3 μg/kg, respectivamente, definido como a quantidade mínima de toxina que pode gerar um pico cromatográfico três vezes acima da taxa sinal/ruído da linha de base. A recuperação média de FB_1 e FB_2 em milho adicionado de uma faixa de 100–400 μg/g de FB_1 e 250–450 μg/g de FB_2 foi de 103,4% e 92,6% (CV médio 12,4% e 12,7%), realizada em triplicata.

4.4 DETERMINAÇÃO DA ATIVIDADE DE ÁGUA

A determinação da atividade de água (aw) foi realizada em medidor Aqua Lab/Decagon CX-2 calibrado com água destilada a_w = 1,000±0,003 a 20 – 25°C.

4.5 ANÁLISE ESTATÍSTICA

As diferenças entre as médias da concentração de fumonisinas (B_1 + B_2) e da a_w entre o milho e suas frações obtidas por moagem a seco (gérmen, pericarpo, endosperma, fubá e grits), bem como diferença da contaminação na moagem do milho transgênico e convencional ($p < 0,05$) foram analisadas estatisticamente pelo teste-t. A diferença entre a contaminação por *Fusarium* sp. e contagem total de bolores e leveduras foi avaliada estatisticamente pela Análise de Variância seguida pelo teste de Tukey. A correlação entre a concentração de fumonisinas em milho e em suas frações processadas foi analisada pela correlação de Pearson ($p < 0,05$). As análises estatísticas foram realizadas pelo Statistica software, version 10.0 (Stat Soft, Inc., Tulsa, Ok, USA).

4.6 ESTIMATIVA DO GRAU DE EXPOSIÇÃO ÀS FUMONISINAS

A IDP foi calculada de acordo com a fórmula (IPCS, 2009):

$$\text{IDP (ng/Kg/dia)}: \frac{\text{consumo médio de milho ou derivado x concentração de fumonisinas}}{\text{Peso corpóreo}}$$

Os dados de peso corpóreo e consumo médio de milho, milho degerminado, fubá e grits foram obtidos da Pesquisa de Orçamento Familiar (POF) conduzida pelo IBGE

(2011) de maio de 2008 a maio de 2009 e a concentração de fumonisinas foi obtida dos quatro lotes analisados. Como a POF não especifica o consumo de endosperma e grits, foi considerada a soma do consumo de alimentos derivados dessas matérias primas: para o endosperma foram considerados os dados de farinha de milho, cereal matinal e creme de milho; para o grits foram obtidos os dados de flocos de milho e pão de milho (IBGE, 2011).

Para a determinação do grau de exposição em países importadores de milho brasileiro, foram utilizados dados de consumo em países da Europa da SCOOP Task (SCOOP, 2003) e na Malásia e Angola, da FAO (FAO, 2013), para os quais a indústria onde foram coletadas as amostras exporta.

Os níveis médios de fumonisinas (FB_1 + FB_2) foram calculados considerando que as amostras que apresentaram níveis de fumonisinas abaixo do limite de detecção (LD), continham ½ LD, de acordo com critério recomendado pelo IPCS/GEMS (1995), a fim de evitar subestimação do grau de exposição às fumonisinas. O critério recomenda o seguinte: primeiro, quando todas as observações estiverem acima do LD então a média verdadeira é calculada; segundo, quando a proporção de observações menores que LD é inferior ou igual a 60%, a média é calculada substituindo estas observações por ½ LD. Terceiro, quando a proporção de observações menores que o LD está acima de 60% e menor ou igual a 80%, a média é calculada substituindo primeiro as observações por 0 e segundo pelo LD. No presente estudo, menos de 60% das observações estavam abaixo do LD nas amostras de grits, portanto, a média foi calculada substituindo essas observações por ½ LD. Acima de 60% e abaixo de 80% das amostras de fubá apresentaram níveis de fumonisinas abaixo do LD, sendo a média calculada substituindo essas observações primeiro por 0 e depois pelo LD. Nas demais amostras, mais de 60% das amostras apresentaram contaminação por fumonisinas, portanto, a média verdadeira foi utilizada.

5 RESULTADOS E DISCUSSÃO

Os resultados e discussão foram redigidos sob a forma de artigos científicos a serem submetidos para publicação, os quais se encontram listados abaixo:

- Artigo 1 – Impact of Industrial dry-milling on fumonisin redistribution in non-transgenic corn in Brazil, publicado no periódico Food Chemistry, v. 220, p.438 – 443, 2017. doi: 10.1016/j.foodchem.2016.10.028.
- Artigo 2 – Comparison of the effect of industrial dry-milling process on fumonisin contamination in transgenic and non – transgenic corn, foi redigido de acordo com as normas do periódico Food Research International;
- Artigo 3 – Mycological quality of corn – based products and dietary exposure assessment of fumonisins, foi redigido de acordo com as normas do periódico Food Chemistry.

Impact of industrial dry-milling on fumonisin redistribution in non-transgenic corn in Brazil

Jaqueline Gozzi Bordini[a], Mario Augusto Ono[b], Glauco Tironi Garcia[c], Victor Hugo Meconi Fazani[a], Édio Vizoni[d], Karem Caroline Bonacin Rodrigues[c], Elisa Yoko Hirooka[e], Elisabete Yurie Sataque Ono[a]

[a]State University of Londrina, Department of Biochemistry and Biotechnology, P.O. box 10.011, Zip Code 86057-970, Londrina, Paraná, Brazil; [b]State University of Londrina, Department of Pathological Sciences, P.O. box 10.011, Zip Code 86057-970, Londrina, Paraná, Brazil; [c]Integrada Agroindustrial Cooperative, 86380-000, Andirá, Paraná, Brazil; [d]State University of Londrina, Department of Applied Mathematics, P.O. box 10.011, Zip Code 86057-970, Londrina, Paraná, Brazil; [e]State University of Londrina, Department of Food Science and Technology, P.O. box 10.011, Zip Code 86057-970, Londrina, Paraná, Brazil.

*Corresponding author: Elisabete Yurie Sataque Ono, State University of Londrina Department, of Biochemistry and Biotechnology, P.O. box 10.011, Zip Code 86057-970, Londrina, Paraná, Brazil - Fax: +55 (43) 3371-4054, Phone number: +55 (43) 3371-4270, E-mail: eysono@hotmail.com

Abstract

The aim of this study was to evaluate the fate of fumonisins B_1 (FB_1) and B_2 (FB_2) during industrial dry-milling in two lots from 2014 (n = 120) and 2015 (n = 120) of non-transgenic corn and their fractions (germ, pericarp, endosperm, cornmeal and grits), collected from one of the major Brazilian milling industries. Fumonisins were concentrated in the germ and pericarp at a rate of 322% and 188% (lot 1) and 311% and 263% (lot 2), respectively. In the endosperm, cornmeal and grits fumonisin levels decreased from 60 to 95%. Fumonisin levels in cornmeal and grits were below the maximum limit tolerated by the European Commission. Therefore, corn industrial dry – milling can contribute to reducing fumonisin levels in corn products intended for human consumption.

Key-words: germ, pericarp, cornmeal, grits, fumonisin, dry-milling

Comparison of the effect of industrial dry-milling process on fumonisin contamination in transgenic and non-transgenic corn

Abstract

The aim of this study was to compare the effect of industrial dry-milling on fumonisin (B_1 + B_2) distribution in transgenic and non-transgenic corn. For this purpose, whole corn samples and their fractions (germ, pericarp, endosperm, cornmeal and grits) were collected from one of the major Brazilian milling industries, totaling 480 samples. There was no significant difference ($p > 0.05$) between mean fumonisin (B_1 + B_2) levels in transgenic (1,030 μg/kg) and non-transgenic (918 μg/kg) whole corn. However, the inner fractions (endosperm and cornmeal) and the outer fraction (germ) obtained after corn dry-milling were more contaminated in non-transgenic corn than transgenic corn. Nevertheless, fumonisin (B_1 + B_2) levels in most whole corn samples and in cornmeal and grits were below the maximum limit tolerated by the European Commission in both corn type. Therefore, industrial corn dry-milling in both transgenic and non-transgenic is an efficient method for reduction of fumonisin contamination in corn-derived products intended for human consumption.

Key – words: grits, cornmeal, fumonisins, germ, pericarp

Introduction

Brazil is the third largest corn producer and accounted for 88 million tons, exporting 35 million tons in 2016 (CONAB, 2017). However, corn is susceptible to infection by *Fusarium verticillioides* and *F. proliferatum*, which are the main fumonisin producers, a group of toxic secondary metabolites (CAST, 2003). Fumonisin contamination in corn is of great impact due to their toxic effects in humans and animals, in addition to economic loss for grain producers and industrial processors. Fumonisins are thermostable and can be detected in final corn-based products (Bullerman & Bianchini, 2007, CAST, 2003).

Epidemiological studies have suggested the association of high fumonisin levels in corn with the occurrence of esophageal and primary liver cancer in South Africa (Rheeder et al., 1992) and China (Ueno et al., 1997) and neural tube defects in children (Missmer et al., 2006). In animals, fumonisins have been associated with leukoencephalomalacia in equines (Marasas et al., 1988), pulmonary edema in swine (Ross et al., 1990) and hepatocellular carcinoma in rats (Gelderblom, Abel, & Smuts, 2001). The International Agency for Research on Cancer (IARC) classified FB_1 as 2B group, i.e., possibly carcinogenic for humans (IARC, 2002).

Corn production chain includes dry-milling process which separates the grain into three main parts, i. e., the germ, pericarp and endosperm, which are raw materials for several corn- based products (Cardoso et al., 2011; Saunders et al., 2001). However, in the industrial corn dry-milling process, fumonisins are not destroyed and are redistributed among the fractions (Castells et al., 2008; Savi et al., 2016). In general, this process reduces fumonisin contamination in the inner fractions and concentrated in the outer fractions (Bordini et al., 2017; Brera et al., 2004; Castells et al., 2008), but it depends on the corn type and dry-milling process used, which are different among the countries (Scudamore & Patel, 2009).

Transgenic *Bt* (*Bacillus thuringiensis*) hybrid is known to accumulate lower fumonisin levels than non-transgenic corn by controlling the insect injuries (Bowers et al., 2013; Du et al., 2014; Munkvold & Hellmich, 1999). However, there is no information whether there is an influence of industrial dry - milling process in fumonisin redistribution between transgenic and non-transgenic corn. Therefore, the aim of this study was to compare the fumonisin redistribution during the industrial dry-milling in transgenic and non – transgenic corn from Brazil.

Material and Methods

Sampling

The samples were collected from one of the major Brazilian corn milling industry located in North Paraná State. The industry process approximately 12,000 tons of corn per month. Freshly harvested corn samples (transgenic and non-transgenic) from 2014 to 2016 were evaluated. For each corn type 240 samples were collected as follows: whole corn (n = 80) and their fractions germ (n = 80), pericarp (n = 80), endosperm (n = 80), corn meal (n = 80) and grits (n = 80), totaling 480 samples. Sampling was performed collecting 10 kg of each sample, homogenized and collected 500 g. This process was repeated twenty times for each sample type at five-minute intervals.

The corn dry-milling process occurs as following: the whole corn was cleaned in a sieve to remove broken corn, stalk, husks and other dust. The cleaned whole corn was conducted to a milling tower, which receive a heat water (160 °C) treatment to favor the degerming process. At these step, the corn was separated in three main fractions: germ and pericarp (the outer fractions) and endosperm (the inner fraction). In this industry, the germ is used for refined oil production; the pericarp is destined for animal feed and the endosperm can be exported to Europe for breakfast cereal production or can be stored for further milling to

produce cornmeal and grits. The cornmeal can be used for internal consumption to prepare the "polenta" and other common dishes in Brazil or exported to Africa. The grits is destined for brewing industry and snack production.

The whole corn was collected from sieves and the germ and pericarp from the bags filling pipes; the endosperm, from the pipes that conducted it to silos; the corn meal and grits from the bag filling (1,200 kg).

The samples were grounded to 50 mesh, homogenized and 200 g was stored to – 20 °C for FB_1 and FB_2 analysis.

Fumonisin determination

FB_1 and FB_2 were analyzed according to Shephard et al. (1990) with some modification (Ueno et al. 1993). The whole corn and their fractions (germ, pericarp, endosperm, corn meal and grits) (200g) were ground to 50-mesh in a laboratory mill and 10g of powder were mixed with 30 ml methanol/water (3:1, v/v). The suspension was shaken at 150 rpm for 30 min and centrifuged at 4500 g for 10min. The crude extract (1.0 ml) was applied to preconditioned SepPak Accell plus QMA cartridges (Waters, Milford, MA, USA). After washing the cartridge with methanol/water (3:1, v/v, 6 ml) followed by methanol (J.T. Baker, Phillipsburg, NJ, USA; 3 ml), fumonisins were eluted with 10 ml methanol containing 0.5% acetic acid. The eluate was evaporated to dryness under a stream of nitrogen at 45 °C; the residue was dissolved in methanol/water (3:1, v/v; 800 µl) and a 200 µl aliquot dried under nitrogen. After derivatization with 200 µl O-phthaldialdehyde (OPA; Sigma, St. Louis, MO, USA) reagent (40mg OPA, 1ml methanol, 5ml 0.1M sodium borate and 50 ml 2-mercaptoethanol), HPLC injections were made within 1min. Fumonisins were analyzed by a reversed-phase isocratic HPLC system (Shimadzu LC-10 AD pump and RF-10A XL fluorescence detector), using a C-18 Luna Phenomenex column (250 x 4.6 mm, 5 µm, Scharlau, Barcelona, Spain). Excitation and emission wavelengths were 335 and 450 nm,

respectively. The eluent was CH_3OH/0.1M NaH_2PO_4 (J.T. Baker; 80:20, v/v) adjusted to pH 3.3 with ortho-phosphoric acid (J.T. Baker). The flow-rate was 1 ml/min. The detection limits for FB_1 and FB_2 were 27.5 and 35.3 ng/g, respectively, defined as the minimum amount of toxin that could generate a chromatographic peak five times over the height/noise rate of the baseline. Recoveries of FB_1 and FB_2 from spiked corn in the range 100–400 ng/g FB_1 and 250–450 ng/g FB_2 averaged 95.6% (mean CV 8%) and 96.9% (mean CV 10%), respectively, based on duplicate spiking and triplicate analyses.

Water activity

The water activity (a_w) was determined by Aqua Lab/Decagon CX-2 using distilled water for calibration (a_w = 1,000±0,003) at 20 – 25°C.

Statistical analysis

Differences in mean fumonisin levels between whole corn samples and the fractions germ, pericarp, endosperm, corn meal and grits as well as between transgenic and non-transgenic corn were statistically evaluated using t-test ($p \leq 0.05$). Statistical analysis was performed by Statistica software, version 10.0 (Stat Soft, Inc., Tulsa, OK, USA).

Results and Discussion

Table 1 shows the aw, the relative frequency fumonisins (FB_1 + FB_2) and their levels in whole corn and in their fractions obtained by industrial dry-milling process in transgenic (n = 240) and non-transgenic corn (n = 240). Relative frequency of fumonisins (B_1 + B_2) were higher in whole corn and in outer fractions (germ and pericarp) in transgenic corn (100%), while in non-transgenic corn it was higher in whole corn and germ (98% and 100%, respectively), but lower in pericarp (88%). Concerning inner fractions, relative frequency of

fumonisin in endosperm was similar in both corn type (95 and 93%, respectively) and there was a reduction in grits and it was the fraction with lower fumoninin frequency (50 and 58%, respectively) in both corn type (Table 1).

A_w is a critical intrinsic factor in the food chain that influences fungal spoilage and mycotoxin production (Magan & Aldred, 2007). The mean a_w ranged from 0.53 (pericarp) to 0.69 (germ) in both corn type. In addition, the a_w values suggest that fumonisin production occurred at pre-harvest stage, i.e., in the field. It has been reported that poorly dried corn with 0.93 to 0.97 a_w represents conditions for naturally FB_1 and FB_2 production by *F. verticillioides* and *F. proliferatum* (Marin et al., 1995). Marín, Homedes, Sanchis, Ramos and Magan (1999) also showed that *Fusarium verticillioides* and *F. proliferatum* produced more FB_1 at 0.98 than 0.95 and 0.92 a_w at 15 and 30 °C.

Mean fumonisin (B_1 + B_2) initial levels in whole corn were 1,030 µg/kg (range 155 – 2,863 µg/kg) and 918 µg/kg (range 174 – 2,539 µg/kg) in transgenic and non - transgenic corn, respectively, and there was no significant difference ($p > 0.05$). These results are in accordance with Barroso et al. (2017) who reported that there was no statistical difference in fumonisin contamination between transgenic and non-transgenic whole corn from Brazil. On the other hand, other studies have shown that Bt corn technology reduce insect damage and fumonisin levels when compared with non-transgenic corn (Abbas, Bellaloui, & Bruns, 2016; Munkvold, Hellmich, & Rice, 1999). However, it has been shown that fumonisin contamination is more influenced by environmental factors as location, wheather, and crop season than the Bt itself (De la Campa, Hooker, Miller, Schaafsma, & Hammond, 2005).

Although fumonisin levels in transgenic and non-transgenic whole corn were similar, there was difference in germ contamination. After dry-milling process, fumonisins were concentrated in outer fractions of the grain in both corn types. Contamination in germ from non-transgenic (2,938 µg/kg) was significantly higher than transgenic corn (2,178 µg/kg) ($p <$

0.05) (Table 1). On the other hand, there was no significant difference between fumonisin levels in pericarp of the both corn type.

There was a reduction in fumonisin contamination in inner fractions (endosperm, cornmeal and grits) from both corn types. Fumonisin (B_1 + B_2) levels in endosperm and cornmeal fractions derived from non – transgenic corn was higher (251 and 186 µg/kg) than from transgenic corn (122 and 84.6 µg/kg, respectively) ($p < 0.05$) (Table 1). There is a lack of information about the differences on fumonisin redistribution during dry-milling in industrial scale between non – transgenic and transgenic corn. It has been reported that transgenic corn hybrids with Bt technology, i. e., the hybrids expressing *cry* genes from *Bacillus thuringiensis* can contain lower fumonisin levels than non-transgenic hybrids (Munkvold & Hellmich, 1999; Bakan et al., 2002). This occurs because the *cry* genes express toxic protein to common corn pests and the transgenic hybrids are less susceptible to insects attack (Du et al., 2014). Insect damage creates kernel wounds that allow fungal colonization and insects themselves act as vector of fungal spore (Munkvold & Hellmich, 1999; Du et al., 2014). Probably, this resistance to insect attack can contribute to decrease fumonisin levels in inner parts of corn kernel.

Fumonisin (B_1 + B_2) distribution rates were 220% and 317% in germ and 215% and 226% in pericarp from transgenic and non – transgenic corn, respectively, in relation to the contamination in whole corn. In endosperm, cornmeal and grits, distribution rates indicated that the reduction of fumonisin (B_1 + B_2) contamination in the inner fractions obtained by industrial dry – milling process was higher in transgenic corn (86 – 91%) than in non-transgenic corn (70 – 89%) (Table 1). These results are in line with other studies (Burguer et al., 2013; Castells et al., 2008). Burguer et al. (2013) also reported a fumonisin concentration ratio of 280% in total hominy feed (composed by germ, pericarp and some endosperm) and a reduction of 66% in degermed corn, during the corn dy-milling process. Castells et al. (2008)

showed a distribution ratio of 317% in hominy feed (germ + pericarp) and of 29% and 14% in cornmeal and flaking grits, respectively, showing a contamination reduction in inner fractions.

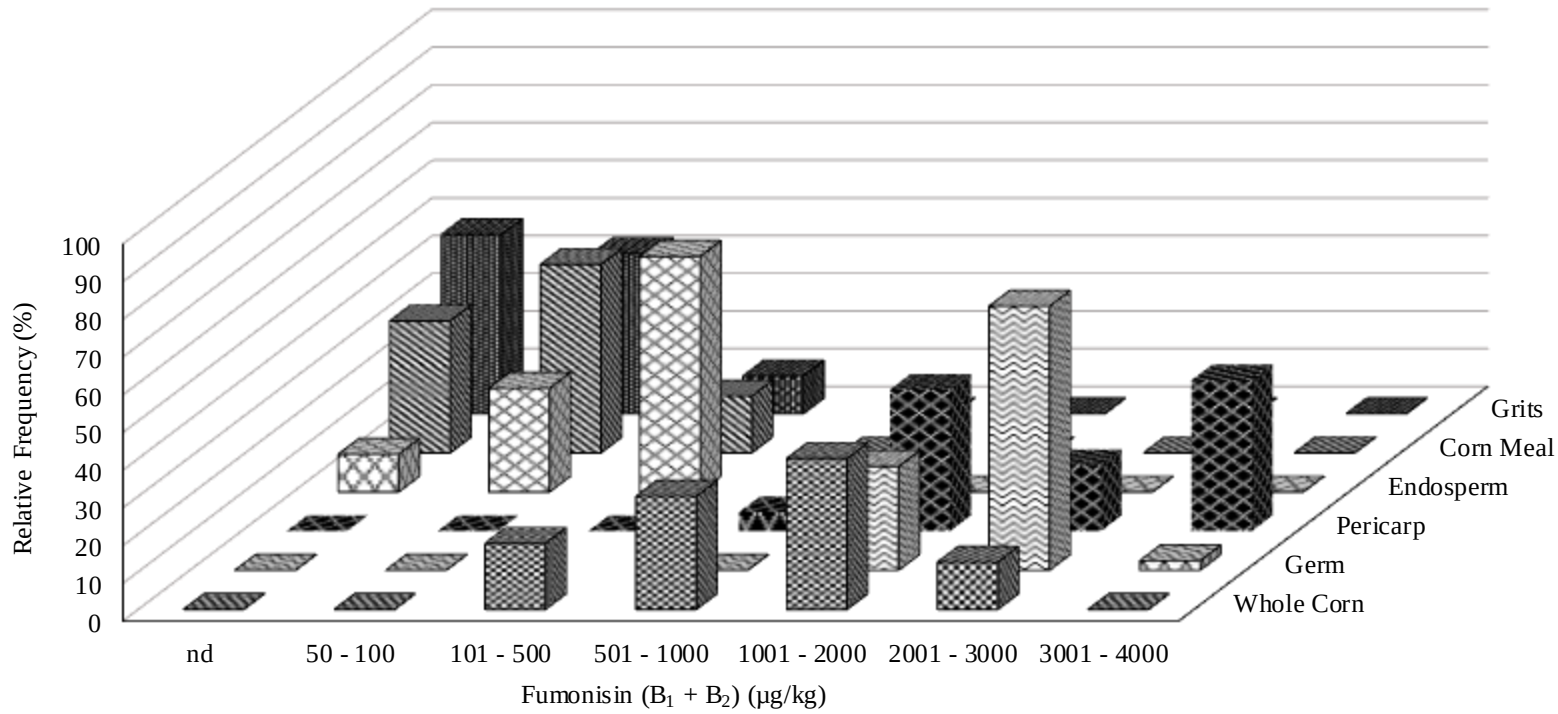
Figures 1 and 2 show the distribution of fumonisin (B_1 + B_2) levels in whole corn and their fractions, from transgenic and non – transgenic corn, respectively. In spite of high fumonisin (B_1 + B_2) frequency, 87.5% of the whole corn samples showed levels lower than the maximum levels (2000 µg/kg) allowed by the European Commission (2006) and all the samples showed levels lower than the maximum levels (5,000 µg/kg) allowed by the Brazilian Legislation (Brasil, 2011). Fumonisin levels in the endosperm, cornmeal and grits were below the maximum level of 1,000 µg/kg (EC, 2006).

In summary, transgenic and non-transgenic whole corn showed similar fumonisin (B_1 + B_2) levels, but there was an influence of industrial dry-milling process. Fumonisin levels in germ, as well as in endosperm and cornmeal from non – transgenic corn were higher than from transgenic corn. However, all the samples of transgenic and non-transgenic corn inner fractions destined for human consumption, i.e., endosperm, cornmeal and grits, showed low fumonisin levels suggesting that the industrial dry-milling is an efficient method for reducing fumonisin levels in transgenic and in non-transgenic in corn derived products.

Table 1. Water activity (a_w), mean fumonisin ($B_1 + B_2$) levels in transgenic and non - transgenic whole corn and their fractions obtained by industrial dry-milling in Brazil

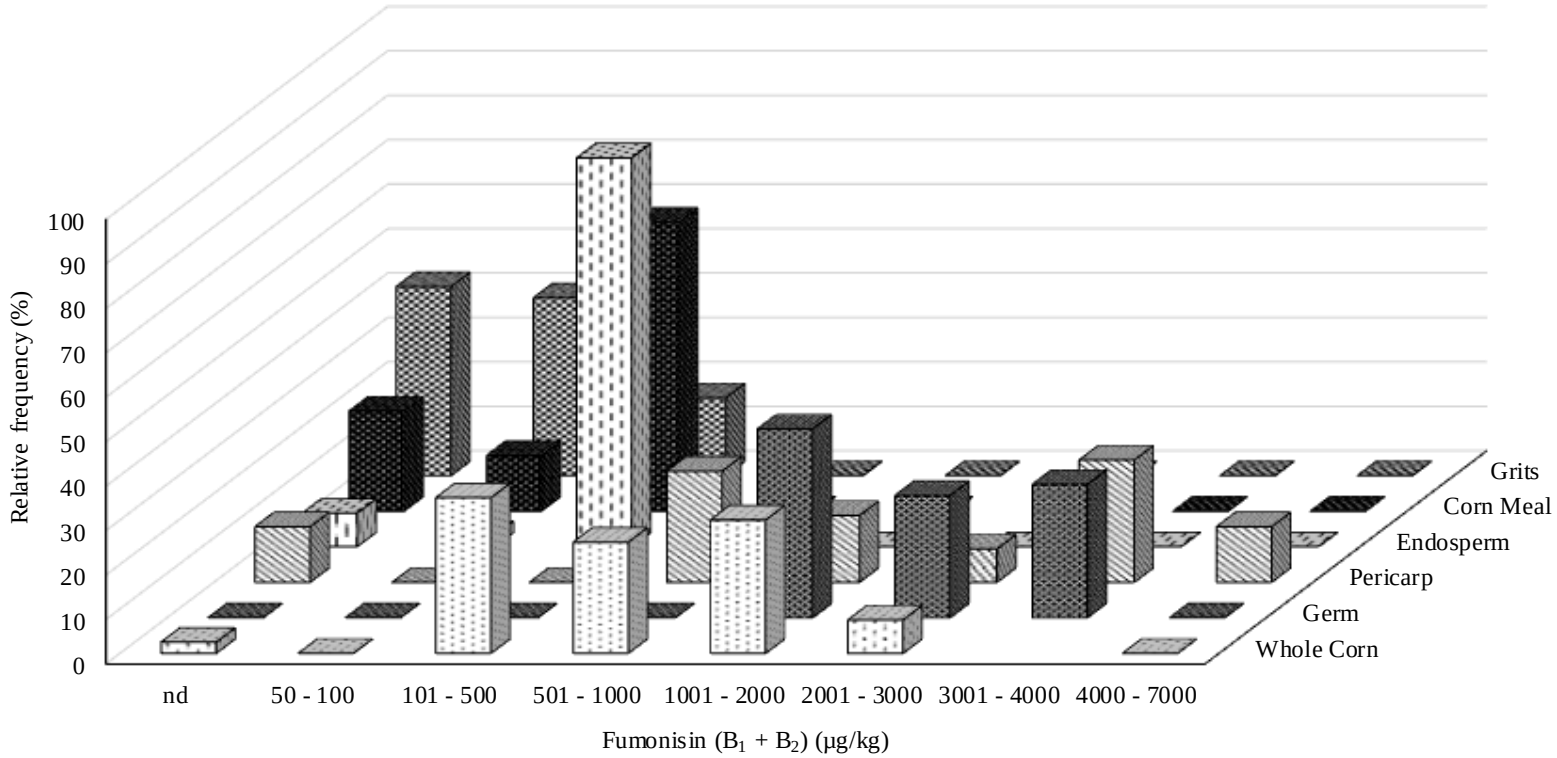
Corn Fraction	Transgenic corn						Non – transgenic corn					
	N	Positive samples (%)	aw	Mean fumonisin levels ($FB_1 + FB_2$) (µg/kg)	Range	Distribution factor (%)	N	Positive samples (%)	aw	Mean fumonisin ($FB_1 + FB_2$) levels (µg/kg)	Range	Distribution factor (%)
Whole corn	40	100	0.68^{aA}	$1,030^{aA}$	155 – 2,863	100	40	98	0.62^{aB}	918^{aA}	174 – 2,539	100
Germ	40	100	0.64^{bA}	$2,178^{bA}$	1,169 – 3,035	220	40	100	0.69^{bA}	$2,938^{bB}$	1233 - 4,887	317
Pericarp	40	100	0.53^{bA}	$2,419^{bA}$	981 – 3,953	215	40	88	0.65^{aB}	$2,352^{bA}$	649 – 6,352	226
Endosperm	40	95	0.64^{aA}	122^{bA}	33 – 260	14	40	93	0.64^{bA}	251^{bB}	99.1 - 422	30
Corn meal	40	65	0.63^{aA}	84.6^{bA}	41.1 – 175	10	40	78	0.63^{aA}	186^{bB}	61.1 – 389	24
Grits	40	50	0.64^{aA}	125^{bA}	30.1 – 112	9	40	58	0.61^{aA}	77.3^{bB}	30.8 – 208	11

Means between whole corn and their fractions followed by the same lowercase letters in the same column are not significantly different by the t- test ($p < 0.05$)
Means between whole corn and their fractions followed by the same uppercase letters in the same line are not significantly different by the t- test ($p < 0.05$)
Distribution factor was based on fumonisin ($B_1 + B_2$) levels in each fraction and the initial concentration in whole corn.
Detection limit: $FB_1 = 27.5$ µg/kg; $FB_2 = 35.3$ µg/kg



Nd = not detected

Figure 1. Distribution of fumonisin (B_1 + B_2) levels in transgenic whole corn and in their fractions obtained by industrial dry-milling process in Brazil from 2014 to 2016 (n = 240).



Nd = not detected

Figure 2. Distribution of fumonisin (B_1 + B_2) levels in non-transgenic whole corn and in their fractions obtained by industrial dry-milling process in Brazil from 2014 to 2016 (n = 240).

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G. Picariello, National Research Council of Italy (CNR), Avellino, Italy
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C. S. Ranadheera, Victoria University, Melbourne, Victoria, Australia
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Mycological quality of corn-based products and human exposure assessment to fumonisins

Abstract

Mycological quality and probable daily intake (PDI) of fumonisins (FB_1 + FB_2) was estimated for corn grain and its derivatives (endosperm, cornmeal and grits; n = 320) collected from one of the major corn processing industry from Brazil. Mean *Fusarium* sp. count ranged from 1.7 x 10^2 to 5.9 x 10^3 CFU/g and in most samples, total fungal count was lower than 10^4 CFU/g, the maximum recommended by the Food and Drug Administration (FDA). The total PDI for fumonisin in Brazil would be 98 ng/kg body weight/day, which represents 5% of the provisional maximum tolerable daily intake (PMTDI) of 2,000 ng/kg b.w./day for fumonisins. In importing countries of Brazilian corn, the total PDI was lower in European countries (from 40 to 192 ng/kg b.w./day) and higher in Angola (1,710 ng/kg b.w./day). These results indicated a good hygienic quality and low exposure to fumonisins through corn derivatives consumption from Brazilian industry.

Key-wods: estimated daily intake, mycotoxins, cornmeal, grits

1. Introduction

Corn is an economic important crop in Brazil and is a staple food in many developing countries due to its high nutritional value (ABIMILHO, 2016). Brazil is the third largest corn producer in the world and accounted for 85 millions tons in 2016/17 crop, and exported 35 million tons (CONAB, 2017). However, the tropical and subtropical climates in Brazil favor corn contamination by a variety of mould species, which can deteriorate grain causing loss in hygienic quality, in addition to mycotoxins production (CAST, 2003).

Fumonisins are a group of mycotoxins produced mainly by *Fusarium verticillioides* and *F. proliferatum*, primary corn pathogens that causes disease in all development stage of the plant, and are associated to several toxic effects in humans and animals (Munkvold & Desjardins, 1997). Although 28 fumonisins analogues have been characterized since 1988, fumonisins B_1 (FB_1) and B_2 (FB_2) are the most frequent in corn (CAST, 2003; Marasas, 1996; Rheeder, Marasas & Vismer, 2002).

Epidemiological studies have associated the human exposure to fumonisins with esophageal and primary liver cancer in South Africa (Rheeder et al., 1992) and China (Ueno et al., 1997) and neural tube defects in children along Texas – Mexico border (Missmer et al., 2006). In addition, the International Agency for Research on Cancer (2002) classified fumonisins in group 2B, i.e, possibly carcinogenic to humans. Due to their health risk, a PMTDI for fumonisins was estimated at 2,000 ng/kg b. w./day based on the No Observed Adversed Effect Level (NOAEL) of 0.2 mg/kg bw/day for renal toxicity and application of uncertainty factor of 100 (WHO, 2011).

Brazilian corn dry–milling processing originates the corn derivatives destined for exportation for human consumption. It includes the corn endosperm used for breakfast cereal production, or it can be converted in cornmeal and grits, used to cornflakes, snacks and beer production, respectively. However, fumonisins are thermostable compound, are not destroyed during this process and can be detected in corn products constituting a potential hazard to human health (Bullerman & Bianchini, 2007).

Corn has been considered the cereal that most contribute for total fumonisin intake (SCOOP, 2003). Dietary intake of fumonisins can be estimated by the exposure assessment tool, a step of the risk assessment and an essential parameter for quantifying the risk (Marasas, 1997; Marin, Cano-Sancho & Sanchis, 2013; IPCS, 2009). Exposure degree is

calculated based on food intake and naturally occurring levels of a mycotoxin and expressed as PDI used to compare to PMTDI (Marasas, 1997).

Previous studies have reported the hygienic quality and human exposure assessment of fumonisins through corn and corn-based product consumption (Bordin, et al., 2014; Caldas & Silva, 2007; Martins et al., 2012). But in most of the studies samples were collected from local market and evaluated the fumonisin exposure in specific population group. To the best of our knowledge, there is no information about human exposure degree to fumonisins based on corn products obtained from Brazilian processing industry destined for exportation. Therefore, the aim of this study was to evaluate the mycological quality and the human exposure degree of fumonisins in Brazil and in importing countries of Brazilian corn (Europe, Malaysia and Angola), through corn grain and corn-based products obtained by industrial process, i. e., endosperm, cornmeal, grits from Brazilian industry.

2. Material and Methods

2.1 Sampling

The samples were collected from one of the major Brazilian corn milling industries located in the North of Paraná State. The industry processes approximately 12,000 tons of corn per month and exports to Europe, Asia and Africa.

Four lots of freshly harvested corn grain samples and their derivatives (endosperm, cornmeal and grits) from the 2014 to 2016 crops were evaluated. One lot means dry-milling during 24 h and corresponds to approximately 500 tons of corn. Corn grain (n = 80) and its fractions after dry – milling process intended to human consumption, i. e., endosperm (n = 80), cornmeal (n = 80) and grits (n = 80) samples were collected, totaling 320 samples. Sampling was performed by collecting 10 kg of each sample, which were homogenized and

about 500 g were collected. This process was repeated twenty times for each sample type at five-minute intervals. The samples were ground to 50 mesh, homogenized and 200 g were used for total fungal count and for FB_1 and FB_2 determination. In this industry, the endosperm can be exported for breakfast cereal production or can be stored for further milling to produce cornmeal and grits. The cornmeal can be used for internal consumption to prepare "polenta" and other common dishes in Brazil. The grits are destined for corn flakes and snack production.

2.2 Total Fungal Count

A sub-sample (10 g) of ground corn and their fractions (endosperm, corn meal and grits) were blended with 90 ml of sterile 0.1% peptone water (v/v) and serial dilutions were carried out with 9.0 ml of the same diluent to 10^{-4}. One milliliter of each dilution was transferred into a Petri dish and pour-plated with potato dextrose agar (PDA, pH 4.0) with 50 µg/ml chloramphenicol and incubated at 25 °C for 7 days. After total fungal colony count, genera were identified according to Nelson, Tousson and Marasas (1983) and Sing, Frisvad, Thran, & Mathur (1991).

2.2 Fumonisin determination

FB_1 and FB_2 were analyzed according to Shephard, Sydenham, Thiel and Gelderblom (1990) with some modification (Ueno et al. 1993). The corn grain and its derivatives (endosperm, cornmeal and grits) (200 g) were ground to 50-mesh in a laboratory mill and 10 g of powder was mixed with 30 ml methanol: water (3:1, v/v). The suspension was shaken at 150 rpm for 30 min and the filtered crude extract (1.0 ml) was applied to preconditioned SepPak Accell plus QMA cartridges (Waters, Milford, MA, USA). After washing the

cartridge with methanol: water (3:1, v/v, 6 ml) followed by methanol (J.T. Baker, Phillipsburg, NJ, USA; 3 ml), fumonisins were eluted with 10 ml methanol containing 0.5% acetic acid. The eluate was evaporated to dryness under a stream of nitrogen at 45 °C. After derivatization with 200 µl O-phthaldialdehyde (OPA; Sigma, St. Louis, MO, USA) reagent, HPLC injections were made within 1 min. The fumonisins were analyzed by a reversed-phase isocratic HPLC system (Shimadzu LC-10 AD pump and RF-10A XL fluorescence detector), using a C-18 Luna Phenomenex column (250 x 4.6 mm, 5 µm, Scharlau, Barcelona, Spain). Excitation and emission wavelengths were 335 and 450 nm, respectively. The eluent was CH3OH: 0.1M NaH2PO4 (J.T. Baker; 80:20, v/v) adjusted to pH 3.3 with ortho-phosphoric acid (J.T. Baker). The flow-rate was 1 ml/min. The detection limits for FB_1 and FB_2 were 27.5 and 35.3 ng/g, respectively, defined as the minimum amount of toxin that could generate a chromatographic peak three times above the height/noise rate of the baseline. Recoveries of FB_1 and FB_2 from spiked corn grain and the endosperm in the range 100–400 ng/g for FB_1 and 250–450 ng/g for FB_2 averaged 103.4% and 92.6% (mean CV 12.4% and 12.7%) and 108.0% and 94.6% (mean CV 16.8% and 18.8%), respectively, based on triplicate analyses.

2.3 Food consumption data

The corn grain, endosperm, cornmeal and grits consumption data were obtained from a Household Budget Survey (HBS) conducted by the Brazilian Institute of Geography and Statistics (IBGE, 2011) from May 2008 to May 2009. As the HBS does not specify the consumption of endosperm and grits, the sum of the consumption of derived foods from these corn fractions was considered. For endosperm, the data from corn flour and breakfast cereal were considered; for grits, the corn flakes data intake was considered. National data were collected from the food purchase of 55.970 households during seven consecutive days from urban and rural areas of all Brazilian States. The food consumption data of each household

was divided by seven and then by the household size to generate daily *per capita* consumption (IBGE, 2011).

Taking into account that corn and its derivatives are exported to European countries, Malaysia and Angola, the PDI was calculated in these locations using the fumonisin contamination of this study and the corn consumption according to SCOOP (2003) and FAO (2013).

2.4 Estimation of fumonisin Probable Daily Intake

The PDI calculation was performed using mean fumonisin (FB_1 + FB_2) levels in corn grain and its derivatives of the four lots multiplied by the daily consumption of each product and divided by average body weight (70 kg) (IBGE, 2011), according to the formula:

$$\text{PDI (ng/kg/day)} = \frac{\text{mean corn or derivatives consumption} \times \text{mean fumonisin contamination}}{\text{Average body weight}}$$

The mean fumonisin level for PDI calculation was performed according to the recommendation of the IPCS/GEMS (1995) criteria adopted to estimate mycotoxin contamination when values lower than the LOD are observed, to avoid underestimation of fumonisin exposure. The criteria were as follows: first, when all observations were over the LOD then the true mean was calculated; second, when the proportion of observations less than LOD was lower than or equal to 60%, the mean was calculated replacing those observations by LOD/2. Third, when the proportion of results lower than the LOD was over 60% and lower than or equal to 80%, the mean was calculated replacing first those observations by 0 and second replacing them with the LOD. In the present study, less than 60% of grits samples showed FB_1 levels lower than LOD, and the mean was calculated replacing these observations by ½ LOD. Over 60% and lower than 80% of cornmeal samples showed FB_1 levels lower than LOD and the mean was calculated replacing those observations

first by 0 and then for LOD. Corn and endosperm showed contamination levels higher than LOD in more than 80% of the samples and the true mean was used for PDI calculation.

2.5 Statistical analysis

Differences in mean *Fusarium* sp. and total fungal count and mean fumonisin levels between corn grain samples and its derivatives (endosperm, cormeal and grits) were statistically evaluated using ANOVA and Tukey test ($p < 0.05$). Statistical analysis was performed by Statistica software, version 10.0 (Stat Soft, Inc., Tulsa, OK, USA).

3. Results and Discussion

Table 1 shows the relative frequency of *Fusarium* sp. and total fungal count in corn grain, endosperm, cornmeal and grits (n = 320) of the four lots from 2014 to 2016. *Fusarium* sp. was detected in 97.5% of corn grain, while in endosperm, cornmeal and grits it was detected at low relative frequency, which ranged from 5.0% (grits) to 41% (endosperm).

The high relative frequency of *Fusarium* sp. in corn is in accordance with Moreno et al. (2009) and Ono et al. (2008), but in cornmeal and grits it was lower than the data reported by Broggi, Resnik, Pacin, Gonzáles, Cano and Taglieri (2002). Moreno et al. (2009) reported that *Fusarium* sp. was the prevalent genera in Brazilian corn (100%) from 2003 (n = 150) and 2004 (n = 90) crops. Corn intended for industrial processing from Brazil was more frequently contaminated by *Fusarium* sp. (100 %) (Ono et al., 2008). Broggi et al. (2002) showed that *F. verticillioides* was detected in 80% of corn samples (n = 15), but in 50% and 40% in grits and cornmeal, respectively.

Mean *Fusarium* sp. contamination levels were higher in corn grain than endosperm cornmeal and grits (Table 1). In most corn grain sample (46%) *Fusarium* sp. count was ranged from 10^3 to 10^4 CFU/g. Katta et al. (1997) showed that *Fusarium* sp. was detected in

low levels (< 100 CFU/g) in flaking grits, however, in corn flour it ranged from < 100 to 10^3 CFU/g. In spite of the high frequency of fungi, mean total fungal count in corn grain and in corn derivatives (endosperm, cornmeal and corn grits) were 1.9 x 10^3 and 10^2 CFU/g, respectively. Most of corn grain samples (92%) showed total fungal count lower than 10^4 CFU/g, the maximum recommended for microbiological quality of corn by the Food and Drug Administration (2013) and all the corn derivatives showed good mycological quality.

Water activity (a_w) is one of the most critical factors in determining quality and safety of food. It has been shown that water availability (water activity, a_w) plays an important role in determining the fungal growth and extent of fumonisin production (Marín et al. 1999). The a_w values ranged from 0.51 (corn) to 0.70 (cornmeal) and there was no significant difference ($p > 0.05$) in the mean a_w values among all the sample types (Table 1). According to Samapundo et al. (2005), growth rates for *F. verticillioides* was higher at 0.969 a_w. Marín et al. (1999) evaluated FB_1 production on irradiated corn by *F. verticillioides* at 0.89 - 0.97 a_w and temperature (7-37^{o}C) and reported no FB_1 production at 0.89-0.91 a_w regardless of temperature. Therefore, the a_w values (Table 1) suggested that fungal and mycotoxin contamination occurred at the field, i. e., at pre-harvest step.

FB_1 and FB_2 levels in corn grain, endosperm, cornmeal and grits (n = 320) from 2014 to 2016 are shown in Table 2. FB_1 was detected in 99% of corn samples with levels ranging from 155 to 2,144 ng/g (mean 783 ng/g), while FB_2 was detected in 70% with levels ranging from 77 to 703 ng/g (mean 346 ng/g). In endosperm, cornmeal and corn grits, only FB_1 was detected, with levels ranging from 35.8 to 569 ng/g. Previous studies have shown high frequency of fumonisins in corn and derived-products from Brazil (Oliveira, Rocha, Sulyok, Krska & Mallmann, 2017; Ono et al., 2008; Martins et al., 2012). Oliveira et al. (2017) detected FB_1 and FB_2 in 100% of corn samples from Paraná State (n = 148) and the mean fumonisin (FB_1 + FB_2) levels were 3,153 ng/g (ranging from 63.8 to 66,272 ng/g). Ono et al.

(2008) analysed 870 freshly harvested corn samples at two point of the corn production chain and FB_1 was detected in 100% of samples, while FB_2 was detected from 57 to 73.7%. Fumonisin levels (FB_1 + FB_2) ranged from 20 to 18,780 ng/g (mean 1,460 to 2,870 ng/g) (Ono et al., 2008). Martins et al. (2012) reported that mean fumonisin (FB_1 + FB_2) levels were 297 and 129 ng/g in cornmeal (n = 29) and corn grits (n = 28), respectively, while in corn flakes (n = 11) and corn flour (n = 15) were 311 and 206 ng/g, respectively.

Figure 1 shows the distribution of fumonisin (FB_1 + FB_2) levels in corn grain and its derivatives from 2014 to 2016. All the positive corn grain samples were contaminated with fumonisin (B_1 + B_2) levels lower than the maximum levels allowed by the European Commission (2006) and Brazil (2011) (4,000 ng/g and 5,000 ng/g, respectively) in unprocessed corn. Taking into account that the maximum level allowed for fumonisins in corn grain intended for direct human consumption and corn-based products is 1,000 ng/g (EC, 2006), 56.3% of the corn grain and all endosperm, cornmeal and corn grits samples were safe for human consumption, concerning fumonisins.

The low fungal and fumonisin contamination in endosperm, cornmeal and grits are due to the industrial processing that remove the outer parts of corn grain (germ and pericarp) to produce corn-based products intended for human consumption from corn endosperm. It has been reported that germ and pericarp are the corn fractions more contaminated by mycotoxins (Bordini et al., 2017; Brera et al., 2004; Castells et al., 2008).

Dietary exposure degree is a step for risk assessment and was expressed as fumonisin PDI. The PDI estimation is an essential data for comparing with its health-based guidance value. When the exposure degree exceeds this value, the risk to human health may exist (Caldas & Jardim, 2012; IPCS, 2009). The PDI was estimated using the data of household *per capita* food purchase of corn grain and corn-based products from Brazil (Table 3) (IBGE, 2011), and the mean fumonisin levels of four corn lots and derivative fractions intended for

human consumption collected from 2014 to 2016, considering 70 kg as average individual body weight. The corn grain was the sample that most contributed for fumonisin intake by Brazilian population (86 ng/kg b.w. /day) representing 5% of the PMTDI (2,000 ng/kg body weight/day) (Table 3). Although the cornmeal is the most consumed corn-based product (6.63 g/person/day), the PDI for fumonisins was only 10.2 ng/kg b.w./day due to the low fumonisin contamination (108 ng/g) (Table 3). Considering the intake of corn grain and corn-based products, the total PDI would be 98 ng/kg b.w./day, which is lower than the fumonisin PDI for Brazilian population reported by Caldas and Silva (2007) and Martins et al. (2012), but higher than those reported by Bordin, Rosim, Neef, Rottinghaus and Oliveira (2014). Caldas and Silva (2007) estimated the total daily intake of fumonisins in Federal District and in Brazilian population through the contamination of corn-based products obtained at local retail stores. The total daily intake of fumonisins (B_1 + B_2) was 9.0% and 24.1% of the PMTDI in Federal District and Brazil for total population. Martins et al. (2012) reported low fumonisin exposure (120 ng/kg b.w./day, 6.0% of the PMTDI) by Brazilian population using fumonisin levels in corn-based products from markets of Paraná State, Brazil and the corn intake data from IBGE (2011). In Pirassununga, São Paulo State, the PDI was estimated at 60 ng/kg b.w./day (3.0 % of the PMTDI) based on FB_1 contamination of 120 corn-based products collected from 39 residences (Bordin et al., 2014).

Based on the fumonisin levels in corn and its derivatives in this study, the fumonisin PDI was estimated for importing countries of Brazilian corn, i. e., Europe, Malaysia and Angola (Table 3). In European countries, the fumonisin PDI ranged from 40 ng/kg b.w./day (France) to 192 ng/kg b.w./day (United Kingdom), wich was lower than the PMTDI of 2,000 ng/kg b.w./day. The European Commission estimated the dietary exposure of fumonisins for the population of the European Union member states and it ranged from 5 ng/kg b.w./day (in Norway) to 350 ng/kg b.w./day (among Italian consumers) (SCOOP, 2003). In Malaysia and

Angola, corn and corn products consumption are higher than in other countries evaluated (43.3 and 106 g/person/day, respectively) (FAO, 2013) and there is a lack of information about the comsumption of endosperm, cornmeal and grits products, which was a limitation for estimating the human exposure to fumonisins through intake of these corn fractions. Considering the fumonisin levels in corn grain, the fumonisin PDI in Malaysia and Angola population would be 680 and 1710 ng/kg b.w./day, respectively, which represents 34% and 85.5% of the PMTDI. However, considering the low fumonisin levels in corn derivatives (endosperm, cornmeal and grits), the fumonisin PDI would be between 70 and 290 ng/kg b.w./day in these locations. Corn consumption in Africa is one of the largest in the world and contributes for the high fumonisin exposure. In the former Transkei region of South Africa with high incidence of oesophageal cancer the PDI for fumonisins was 8,670 ng/kg b.w./day, which was higher than the region with low oesophageal cancer incidence (3,430 ng/kg b.w./day), where the corn consumption was lower (Shephard et al., 2007).

The total fungal count in corn grain and its derivatives (endosperm, cornmeal and grits) was lower than the maximum allowed by the FDA for corn, indicating a good hygienic quality. Taking into account the low fumonisin levels in corn derivatives obtained by industrial corn processing, dietary exposure to fumonisins in populations from Brazil and importing countries was low, even for high corn consumption regions. Nevertheless, a continuous monitoring of corn contamination and the PDI evaluation are essential to estimate human health risk. In addition, strategies to reduce the exposure to fumonisins should be considered by corn processing industries.

Table 1. Relative frequency of *Fusarium* sp. and fungal contamination distribution in corn, endosperm, cornmeal and grits from four lots collected from 2014 to 2016 in a corn industrial processing from Brazil.

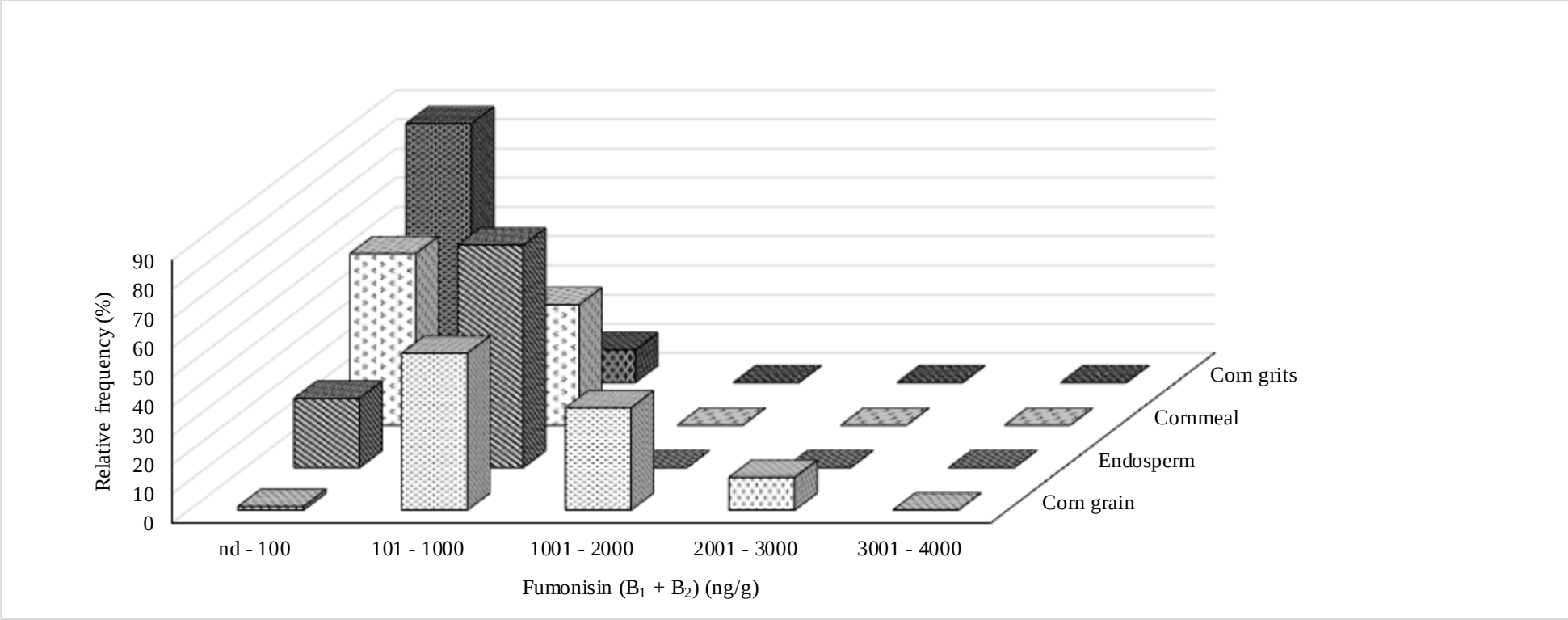
Fungal genera	Sample	N	Water activity (a_w)		RF (%) [y]	Mean [z] CFU/g	Percentage of samples with contamination			
			Mean[z]	Range			< 100	10^2 - $<10^3$	10^3 - $<10^4$	10^4 - < 10^5
Fusarium sp.	Corn grain	80	0.63[a]	0.51 – 0.69	97	5.9×10^{3a}	7.5	29	46	17
	Endosperm	80	0.64[a]	0.61 – 0.69	41	5.5×10^{2b}	91	10	1.3	-
	Cornmeal	80	0.63[a]	0.55 – 0.70	18	$1.7 \times 10^{2a,b}$	96	4	-	-
	Grits	80	0.61[a]	0.55 – 0.68	5	$1.5 \times 10^{3a,b}$	99	1	-	-
Total count [w]	Grain corn	80				1.2×10^{4a}	-	3.8	46	48
	Endosperm	80				4.9×10^{2b}	54	42	3.8	-
	Cornmeal	80				1.8×10^{2b}	54	31	-	-
	Grits	80				1.3×10^{2b}	90	10	-	-

[w] Total count = Total mould and yeast count
[y] RF* = Relative frequency
[z] Means followed by different superscript lowercase letters are significantly different by the Tukey test ($p < 0.05$) among corn grain, endosperm, cormeal and grits

Table 2. Fumonisin B_1, fumonisin B_2 and total fumonisin (B_1 + B_2) levels in corn grain, endosperm, cornmeal and corn grits collected from 2014 to 2016 from corn processing industry from Brazil.

Sample	N	Fumonisin B_1			Fumonisin B_2			Fumonisin B_1 + B_2	
		Positive (%)	Mean (ng/g)	Range (ng/g)	Positive (%)	Mean (ng/g)	Range (ng/g)	Mean (ng/g)	Range (ng/g)
Corn grain	80	99	783	155 – 2,144	70	346	77 – 703	1,026[a]	121 – 2,727
Endosperm	80	91	191	53.9 – 569	-	-	-	191[b]	53.9 – 569
Cornmeal	80	71	137	41.1 – 389	-	-	-	137[b]	41 – 389
Corn grits	80	50	70.6	35.8 – 256	-	-	-	70.6[b]	35.8 – 256

Means followed by the same lowercase letter in the same column are not significantly different by the Tukey test ($p < 0.05$)
Detection limit: FB_1 = 27.5 ng/g; FB_2 = 35.3 ng/g



nd = not detected

Figure 1. Distribution of fumonisin (B_1 +B_2) levels in corn grain, endosperm, cornmeal and corn grits collected from 2014 to 2016 from corn processing industry from Brazil.

Table 3. Mean fumonisin (B_1 + B_2) levels in corn and derived products, mean consumption and probably daily intake in Brazil and in corn importing countries.

Sample type	Mean Fumonisin levels[a] (ng/g)	Average consumption (g/person/day)								Probably daily intake (ng/kg bw/day)							
		Brazil[b]	Europe[c]					Malaysia[d]	Angola[d]	Brazil	Europe					Malaysia	Angola
			United Kingdon	France	German	Netherlands	Italy				United Kingdon	France	German	Netherlands	Italy		
Corn	1026	5.80	10.4	2.44	7.48	3.0	11.5	43.3	106	86	167	40	120	48	185	680	1710
Endosperm	192	0.59	1.20		18.4		1.5			1.62	3.30		50		4.13		
Cornmeal	99.9[a1] 107[a2]	6.63	14.9		3.99		1.0			9.46 10.3	3.00		10		2.99		
Grits	47.1	0.02			11.6					0.001			10				
Total		13.0								98	173	40	190	48	192	680	1710

a= average fumonisins (FB_1 + FB_2) calculated according to IPCS (1995) criteria.
a1= first average fumonisins (FB_1 + FB_2) calculated replacing non-detected samples by 0, according to IPCS (1995) criteria when the results lower than the LOD is over 60% and lower than or equal to 80%.
a^2= second average fumonisins (FB_1 + FB_2) calculated replacing non-detected samples by LOD, according to IPCS (1995) criteria when the results lower than the LOD is over 60% and lower than or equal to 80%.
b= average consumption according to the Household Budget Survey (HBS) conducted by the Brazilian Institute of Geography and Statistics (IBGE, 2011)
c= average consumption based on data from the EU SCOOP Task (SCOOP, 2003)
d= average consumption according to FAO Food Supply (FAO, 2013)

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A distribuição de fumonsinas B_1 e B_2 durante o processamento industrial de milho a seco mostrou uma concentração da contaminação maior no gérmen e pericarpo em relação à contaminação inicial do milho inteiro tanto na variedade transgênica quanto na convencional. Por outro lado, houve uma redução da contaminação nas frações internas (endosperma, fubá e grits) de milho após a remoção do pericarpo e do gérmen em ambas as variedades. Contudo, a redução da contaminação por fumonisinas foi maior nas frações internas de milho transgênico. Apesar da alta frequência, os níveis de fumonisinas estavam abaixo do limite máximo recomendado pela Comissão Europeia e ANVISA, contribuindo para um menor grau de exposição humana às fumonisinas no Brasil e nos países importadores de milho brasileiro. Portanto, estudos de monitoramento e do efeito do processamento na contaminação por fumonisinas na cadeia produtiva do milho são necessários para minimizar as perdas econômicas aos produtores e processadores de grãos, bem como os riscos à saúde humana e animal visando à segurança de alimentos.

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