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CCQA

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FUNGOS E AFLATOXINAS NO ARROZ: OCORRÊNCIA E SIGNIFICADO NA SAÚDE PÚBLICA

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RESUMO

O arroz é um dos cereais mais consumidos no Brasil e no mundo. O país destaca-se como nono maior produtor mundial. O arroz é um ótimo substrato para a produção de micotoxinas e quando os fungos toxigênicos encontram condições de crescer e produzir toxinas, estes produzem em grandes quantidades. Embora não seja comum a presença de micotoxinas no arroz, alguns relatos sobre a ocorrência de aflatoxinas e fungos aflatoxigênicos no arroz têm sido relatado, contudo mais estudos são necessários para compreender a origem destas toxinas no arroz. Nestas condições, na presente dissertação são apresentados os resultados sobre a micobiota e ocorrência de fungos produtores de aflatoxinas, utilizando metodologias adequadas para seu isolamento e identificação, e produção de toxinas nas amostras de solo, arroz do campo e arroz do processamento de dois sistemas de produção representados pelos estados do Maranhão (arroz de sequeiro) e Rio Grande do Sul (arroz irrigado) e nas amostras de arroz comercial dos estados do Maranhão, Rio Grande do Sul e São Paulo. Além disso, são apresentados a presença de aflatoxinas no arroz, o efeito do cozimento do arroz na redução de aflatoxina B, a estimativa de ingestão diária de aflatoxina B considerando a redução durante o cozimento e a margem de exposição (MOE). Houve predominância de *Aspergillus* section *Flavi* nas amostras do campo do sistema irrigado, enquanto que no arroz do sequeiro esta predominância foi maior nas amostras do processamento e do solo. A distribuição fúngica nas amostras comerciais foi homogênea nos três estados, com presença de *A. candidus*, *A. penicillioides*, *A. restrictus*, fungos dematiáceos, *E. chevalieri*, *E. rubrum*, *Fusarium* spp. e *P. citrinum.* A ocorrência de fungos aflatoxigênicos e aflatoxinas no arroz foi baixa, porém duas amostras comerciais de arroz vermelho apresentaram níveis muito acima do estabelecido pela ANVISA (5µg/kg), uma amostra apresentou nível de cerca de 5 vezes superior e outra apresentou nível 14 vezes maior. O cozimento apresentou uma redução média de 77,55% de aflatoxina B, a ingestão diária de aflatoxina B apresentou uma faixa de 0,016 a 4,862 ng/kg peso corpóreo/dia e o MOE uma faixa de 346.939-10.625.000, o que indica um baixo risco de exposição da população brasileira à aflatoxina pelo consumo do arroz.

Palavras-chave: arroz, micobiota, aflatoxinas, redução, cozimento, avaliação do risco de exposição.

ABSTRACT

Rice is one of the most consumed cereals in Brazil and around the world. Brazil is ranked as the ninth biggest rice producer. Rice is an excellent substrate for fungal growth and mycotoxin production, and when toxigenic fungi find conditions to grow and produce toxins, they are produced in large quantities. Although the presence of mycotoxins is not common, there are some reports about the occurrence of aflatoxins and aflatoxigenic fungi in rice; however, more studies are necessary to understand the origin of these toxins. Thus, the present dissertation shows results about the mycobiota and occurrence of aflatoxigenic fungi, using appropriate methodologies for isolation and identification, and production of toxins from samples of soil, field and processed rice from two different production systems in the states of Maranhão (dryland system) and Rio Grande do Sul (wetland system), and in market samples from the states of Maranhão, Rio Grande do Sul and São Paulo, as well as the presence of aflatoxins in rice, the effect of cooking in aflatoxin B reduction, the estimation of the daily intake of aflatoxin B considering the reduction during the cooking and margin of exposure (MOE). There was a predominance of *Aspergillus* section *Flavi* in field samples from wetland, while in dryland this predominance was higher in processing and soil sample. The fungi distribution and occurrence in market samples were more homogeneous in the three states with presence of *A. candidus*, *A. penicillioides*, *A. restrictus*, dematiaceus fungi, *E. chevalieri*, *E. rubrum*, *Fusarium* spp. and *P. citrinum.* The occurrence of aflatoxigenic fungi and aflatoxins in rice was low, but two samples of red rice from markets showed a level much higher than the maximum tolerable limit established by ANVISA (5µg/kg); one sample had a level about 5 times higher and the other had a level 14 times higher. The cooking showed an average reduction of 77.42% of aflatoxin B, the aflatoxin intake range was from 0.016 to 4.862 ng/kg bw/day and MOE range was from 346,939 to 10,625,000, which indicates low risk of exposure to aflatoxin by rice consumption.

Key-words: rice, mycobiota, aflatoxins, reduction, cooking, risk exposure evaluation.

ÍNDICE

Lista de Abreviaturas e siglas

AF	Aflatoxinas
AFB_1	Aflatoxina B_1
AFB_2	Aflatoxina B_2
AFG_1	Aflatoxina G_1
AFG_2	Aflatoxina G_2
AFPA	*Aspergillus flavus-parasiticus* Agar
AFT	Aflatoxinas totais
AI	Average of Infection
ANOVA	Análise de variância
ANVISA	Agência Nacional de Vigilância Sanitária
a_w	Atividade de água
BMDL	Benchmark Dose Modelling
BMDL10	Benchmark Dose Modelling 10%
CCD	Cromatografia de Camada Delgada
CFU	Colony forming unit
CLAE	Cromatografia Líquida De Alta Eficiência
CONAB	Companhia Brasileira do Abastecimento
Cooperja	Cooperativa Agropecuária de Jacinto Machado
CTV	Citreoviridina
CY20S	Czapek yeast extract agar with 20% sucrose
CYA	Czapek Yeast Agar
DG18	Dichloran 18% Glycerol Agar
DL_{50}	Dose Letal 50
DON	Desoxinivalenol
DRBC	Ágar Dicloran Rosa Bengala Cloranfenicol
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immuno Sorbent Assay
FAO	Food and Agriculture Organization of the United Nations
FO	Frequency of Occurrence
FSO	Food Safety Objective
FUM	Fumonisina
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
IARC	International Agency for Research on Cancer
IBGE	Instituto Brasileiro de Geografia e Estatística
IDMT	Ingestão Diária Máxima Tolerável
INMET	Instituto Nacional de Meteorologia
KBr	Brometo de Potássio
Lb	Lower Bound
LOD	Limit Of Detection

LOQ	Limit Of Quantification
MA	Maranhão
MAPA	Ministério da Agricultura Pecuária e Abastecimento
MEA	Malt Extract Agar
MOE	Margem De Exposição
ND	Não Detectado
NIV	Nivalenol
ODS	Octadecylsilyl
OTA	Ocratoxina A
PBS	Phosphate Buffered Saline
PDA	Potatoe Dextrose Agar
POF	Pesquisa de Orçamentos Familiares
RDC	Resolução da Diretoria Colegiada
RI	Range Of Infection
RLPC	Reversed-phase Liquid Chromatography
RS	Rio Grande do Sul
SC	Santa Catarina
SP	São Paulo
TFC	Turkish Food Codex
TLC	Thin Layer Chromatography
Ub	Upper Bound
UV	Ultravioleta
YESA	Yeast Extract Sucrose Agar
ZEA	Zearalenone
ZON	Zearalenona

1. INTRODUÇÃO

O arroz (*Oryza sativa*) originou-se na Ásia e atualmente possui uma importante função social e econômica, sendo uma das principais fontes de carboidrato na dieta de milhões de pessoas em todo o mundo.

O Brasil é o nono maior produtor de arroz mundial, destacando-se como o maior produtor fora da Ásia. O arroz está presente na dieta diária da população brasileira, sendo de máxima importância garantir a qualidade e sanidade do arroz, porque qualquer contaminante presente pode afetar a saúde do consumidor.

Um fator preocupante é que o arroz é um ótimo substrato para a produção de micotoxinas e quando os fungos encontram condições favoráveis, estes as produzem em grandes quantidades. Alguns estudos têm utilizado métodos inadequados de isolamento fúngico, como a diluição em placas, plaqueamento direto sem desinfecção superficial, resultando somente em dados sobre a contaminação superficial dos grãos de arroz.

Conhecer a micobiota em todas as etapas e frações do arroz é importante para compreender quais os pontos críticos do processo em que pode ocorrer a infecção por fungos toxigênicos e a produção de micotoxinas. As micotoxinas são metabólitos secundários produzidos por fungos filamentosos e dentre as que são encontradas no arroz, destacam-se as aflatoxinas que são extremamente tóxicas, o análogo B_1 é considerado o mais tóxico, sendo carcinogênico para humanos e o fígado é o principal órgão afetado após a ingestão.

O arroz polido tem se mostrado isento de crescimento fúngico devido a alta temperatura durante o polimento do arroz que pode destruir esporos fúngicos e/ou selecionar espécies resistentes, porém alguns estudos sobre a presença de micotoxinas têm revelado níveis elevados de aflatoxinas no arroz em várias etapas da produção: arroz com casca, farelo, arroz polido e arroz parboilizado.

Na presente dissertação, o primeiro capítulo trata da micobiota encontrada nas várias etapas da produção de arroz, do campo ao comércio. O segundo capítulo refere-se aos fungos aflatoxigênicos e à determinação de aflatoxinas nas mesmas amostras. O terceiro capítulo relata o teste de redução de aflatoxinas durante o cozimento comum do arroz polido, a estimativa de ingestão diária de aflatoxina B considerando a redução durante o cozimento e a margem de exposição (MOE).

2. OBJETIVOS

O objetivo da presente dissertação foi acompanhar as diversas etapas do processamento do arroz, do campo até o comércio, de modo a avaliar a micobiota e a presença de aflatoxinas. De maneira mais específica, os objetivos foram:

- Analisar a micobiota e presença de fungos produtores de aflatoxinas em amostras do campo até a comercialização, incluindo amostras de solo nos estados do Maranhão e Rio Grande do Sul, e amostras comerciais do estado de São Paulo;
- Determinar a atividade de água das amostras;
- Otimizar a metodologia de determinação de aflatoxinas;
- Determinar a presença de aflatoxinas no arroz e suas frações;
- Verificar a redução de aflatoxinas no arroz durante o processo de cozimento;
- Estimar a ingestão diária de aflatoxina B proveniente do arroz, considerando a redução da mesma durante o cozimento;
- Determinar a margem de exposição (MOE) da população brasileira pelo consumo do arroz.

3. REVISÃO BIBLIOGRÁFICA

3.1 Arroz

3.1.1 Aspectos gerais do arroz

O arroz (*Oryza sativa*) originou-se na Ásia e difundiu-se para os outros continentes, desempenhando um importante papel econômico e social, por ser uma das principais fontes de carboidrato e alimento básico predominante na dieta mundial.

É uma planta herbácea pertencente à família das gramíneas, que necessita de calor e umidade para crescer. Pode medir de 30 a 180 cm de altura, é lisa, tem pontos de floração e hastes arredondadas onde são produzidos os grãos de arroz.

O grão de arroz (Figura 1) é composto pela casca, constituída pela pálea e a lema, correspondendo a 20% do peso do grão e pela cariopse, constituída pelo pericarpo, tegumento, aleuroma, endosperma de amido e o embrião, que é composto pelo escutelo, epiblasmo, plúmula e radícula (Walter et al., 2008).

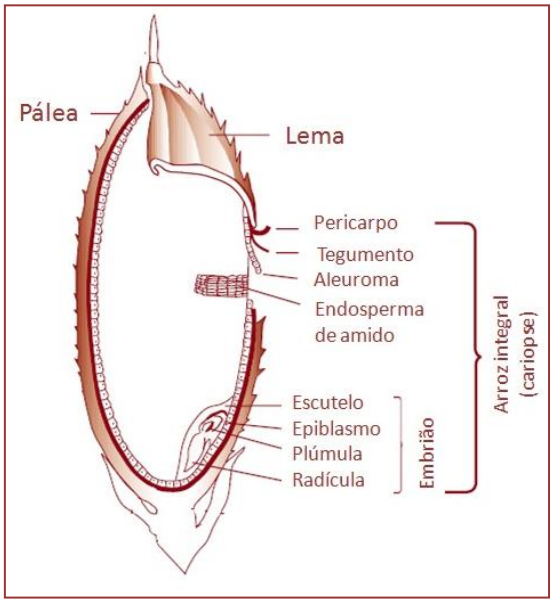

Figura 1. Estrutura do grão de arroz (Mclean et al., 2002).

O arroz é classificado pelo Ministério da Agricultura Pecuária e Abastecimento (MAPA) de acordo com a Instrução Normativa, Nº 6 de 16/02/2009, como: arroz em casca natural, beneficiado (integral, polido,

parboilizado); dividido em classes longo fino, longo, médio, curto e misturado. Após a colheita o arroz é seco para atingir o grau de umidade de 12%, armazenado em silos e encaminhado para o beneficiamento (Brasil, 2009).

O consumo médio mundial é de 60kg/pessoa/ano, sendo que em países asiáticos, onde são produzidos 90% da produção de arroz, as médias são mais elevadas, situadas entre 100 e 150kg/pessoa/ano. Na América Latina, são consumidos, em média, 30kg/pessoa/ano, o Brasil destaca-se como grande consumidor, com média de 45kg/pessoa/ano (FAO, 2016) a 58kg/pessoa/ano (IBGE, 2011).

3.1.2 Produção de arroz no Brasil e no mundo

O Brasil é o nono maior produtor mundial, destacando-se como o maior produtor fora do continente asiático. A produção mundial de arroz tem apresentado certa estagnação nos últimos anos devido a efeitos climáticos do El Niño e La Niña (Figura 2). A produção brasileira de arroz em casca na safra de 2015/2016 foi de 7,5 milhões de toneladas, decaindo 11,6% em relação à safra do ano anterior (FAO, 2016). A estimativa de produção brasileira de arroz em casca para a safra 2016/2017 é de 11.506,6 mil toneladas (Companhia Brasileira do Abastecimento, CONAB, 2016).

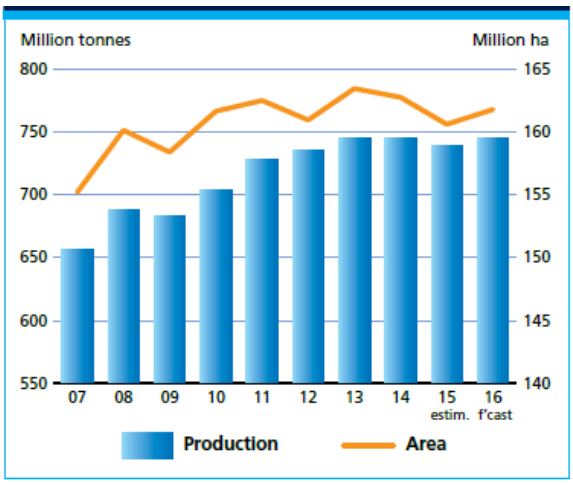

Figura 2. Produção de arroz em casca e área (FAO, 2016).

Existem dois tipos de ecossistemas de plantio de arroz no Brasil: (i) o ecossistema várzeas (irrigado), no qual é irrigado por inundação controlada e, (ii) o de terras altas (sequeiro) em que o arroz pode ser produzido sem irrigação

(água totalmente dependente da precipitação pluvial) ou com irrigação suplementar por aspersão o que diminui o estresse hídrico da planta, propiciando maior qualidade do grão.

Apesar do arroz ser uma cultura comum em quase todo o país, a maior parte da produção ocorre em 5 Estados: Rio Grande do Sul concentra a maior parte da produção (66,5%), seguido de Santa Catarina (8,6%) e Tocantins (4,7%), nestes três estados predominam o arroz irrigado; no Maranhão (5,3%) e Mato Grosso (4,4%) predominam o arroz de sequeiro. No Nordeste, especialmente no Estado do Ceará o arroz se concentra em perímetros de irrigação. Uma pequena quantidade também é produzida nos Estados por onde passa o Rio São Francisco, como Bahia, Sergipe, Alagoas e Pernambuco, essas áreas recebem a irrigação do rio (CONAB, 2016).

O grande volume produzido nos Estados do Rio Grande do Sul e Santa Catarina é considerado estabilizador para o mercado brasileiro de arroz e garante o seu suprimento à população brasileira. Quase todo o arroz produzido nestes dois Estados apresenta grãos da classe longo-fino e com alta qualidade de cocção, características exigidas pelo mercado brasileiro, principalmente nas regiões Sul e Sudeste (Sociedade Sul-Brasileira de Arroz Irrigado, 2014).

Boa parte do arroz de terras altas é cultivado no cerrado, na região central do Brasil. Nesta região ocorre a rotação de cultura do arroz com pasto ou soja (dois anos de cultivo de arroz, dois anos de pasto ou soja) para evitar o “colapso de rendimento” que ocorre com o cultivo contínuo de arroz. Após dois anos de cultivo de arroz, o rendimento declina cerca de 10% e no terceiro ano pode declinar até 70%, devido à autotoxicidade, à degradação e as pragas do solo, portanto a rotação de culturas é essencial para que o manejo agrícola seja rentável (Mclean et al., 2002).

3.1.3 Definição dos produtos

O arroz é conhecido pelos tipos e sub-produtos, conforme mostra a Figura 3. A seguir serão dadas as definições dos mais comuns (Mclean et al., 2002; Bassinello et al., 2008):

Arroz em casca: que pode ser o arroz verde recém-colhido da lavoura ou o arroz de armazenamento que já passou pelo processo de secagem.

Arroz integral: arroz que no processo de beneficiamento foi retirada a casca do grão inteiro.

Arroz polido: arroz obtido através do descasque e polimento do grão integral, é a principal forma de consumo na maioria das regiões brasileiras.

Arroz parboilizado: o arroz é submetido, ainda em casca, a um processo hidrotérmico que provoca a gelatinização total ou parcial do amido, passando posteriormente pelo descasque e polimento. É possível encontrar arroz parboilizado integral e polido.

Arroz vermelho: pertence à mesma espécie do arroz cultivado, porém é considerado uma planta daninha, o acúmulo de tanino ou antocianina deixa o pericarpo com coloração avermelhada, este tipo de arroz pode ser cultivado e consumido.

Arroz negro: de linhagem diferente do arroz comum, possui casca escura com acúmulo de antocianina no pericarpo, na película da semente e na fibra exterior do grão.

Quirera (arroz quebrado): arroz resultante do processamento que se quebra em fragmentos pequenos. Pode ser usado para ração de animais, fabricação de cerveja, pasta de arroz, vinagre, biscoitos, macarrão, farinha, amido e serve como substrato para a fermentação alcoólica para obtenção de etanol.

Farinha: produto da moagem dos grãos de arroz previamente selecionados por intermédio de um moinho.

Floco: resultado da extrusão dos grãos de arroz.

Farelo: é o subproduto do polimento do arroz, contém em média 20% de lipídeos, 14% de proteínas e bons teores de vitamina e fibras, utilizado em ração de animais, extração de óleo comestível, produção de farinhas e concentrado proteico.

Casca: resultado do descasque, não tem aplicação alimentar.

Figura 3. Tipos e sub produtos do arroz: 1. Arroz em casca; 2. Arroz integral; 3. Arroz polido; 4. Arroz parboilizado; 5. Arroz vermelho; 6. Arroz negro; 7. Quirera; 8. Farinha; 9. Floco; 10. Farelo; 11. Casca.

3.1.4 Processamento do arroz

No Brasil, em geral a colheita do arroz é feita mecanicamente, através de colhedeiras próprias para este fim que já separa no campo o grão de arroz da planta. Em seguida o arroz com casca é transportado por caminhões para os locais de processamento. O arroz é pesado diretamente do caminhão e encaminhado para o tombador e moegas onde é realizada a descarga e a coleta de uma amostra para a sua classificação. Desta amostra, são determinados o percentual de impurezas, umidade, rendimento e defeitos. Do tombador, o arroz é transportado através de caracóis helicoidais e elevadores até um silo pulmão, onde é feita a limpeza primária com uma peneira para a retirada de impurezas maiores. Após a limpeza primária, o arroz é armazenado em silos secadores para a secagem. O processo de secagem é feito até que o arroz atinja uma umidade de 13%-14% para depois ser estocado em silos de armazenamento (Figura 4).

Figura 4. (A) Silos secadores (B) Silos de armazenamento (Cooperja, acesso em 23/12/2016, www.cooperja.com.br/produtos/producaodearroz.pdf).

Processamento do arroz parboilizado: para a produção do arroz parboilizado, o arroz que estava armazenado passa por uma limpeza secundária, onde o arroz é peneirado para retirar as impurezas, em seguida é encaminhado aos tanques de encharcamento. Neste tanque, o arroz é encharcado com água quente a uma temperatura em torno de 68°C-72°C por aproximadamente 6 a 7 horas. Após esse período, o arroz passa por uma estufa de 400-500ºC por um período de 2 a 3 min. Após a estufa, o arroz segue para o secador, até a umidade baixar em torno de 12,5%-13%, a fim de evitar a proliferação de fungos e insetos. Deste ponto é feito o descasque seguindo para o separador de marinheiros (grãos de arroz não descascados). Após essa separação, o arroz passa pelo brunidor, onde é feito o polimento, extraindo-se o farelo e deixando a superfície lisa. O arroz é levado ao resfriador devido a elevação da temperatura no brunidor. O arroz passa por uma seleção eletrônica para retirar os grãos considerados defeituosos. Finalmente, os grãos selecionados são empacotados e levados ao local de transporte.

Processamento do arroz polido: o arroz que estava armazenado no silo, passa por uma limpeza secundária, onde o arroz é peneirado para retirar as impurezas, em seguida é encaminhado para o descascador, separador de marinheiros, brunidor e polidor, onde ocorre o acabamento do grão com jato de água e a retirada total do farelo. Após esta etapa, o arroz segue para o resfriador, classificador cilíndrico rotativo, que retira os grãos rajados, as matérias primas estranhas e as impurezas restantes, além de retirar a quirera (arroz quebrado).

Deste ponto o arroz passa para o classificador plano rotativo que tem a função de separar a quirera que restou da etapa anterior. Em seguida é feita a seleção eletrônica, passando para o brilhador, que adiciona o talco, composto por silicato de magnésio, e a glicose no grão, a fim de deixar o grão com um aspecto brilhoso. Finalmente os grãos são empacotados e levados ao local de transporte.

A Figura 5 apresenta o fluxograma do processamento do arroz, da chegada da matéria prima até o carregamento dos caminhões para transporte e distribuição dos produtos.

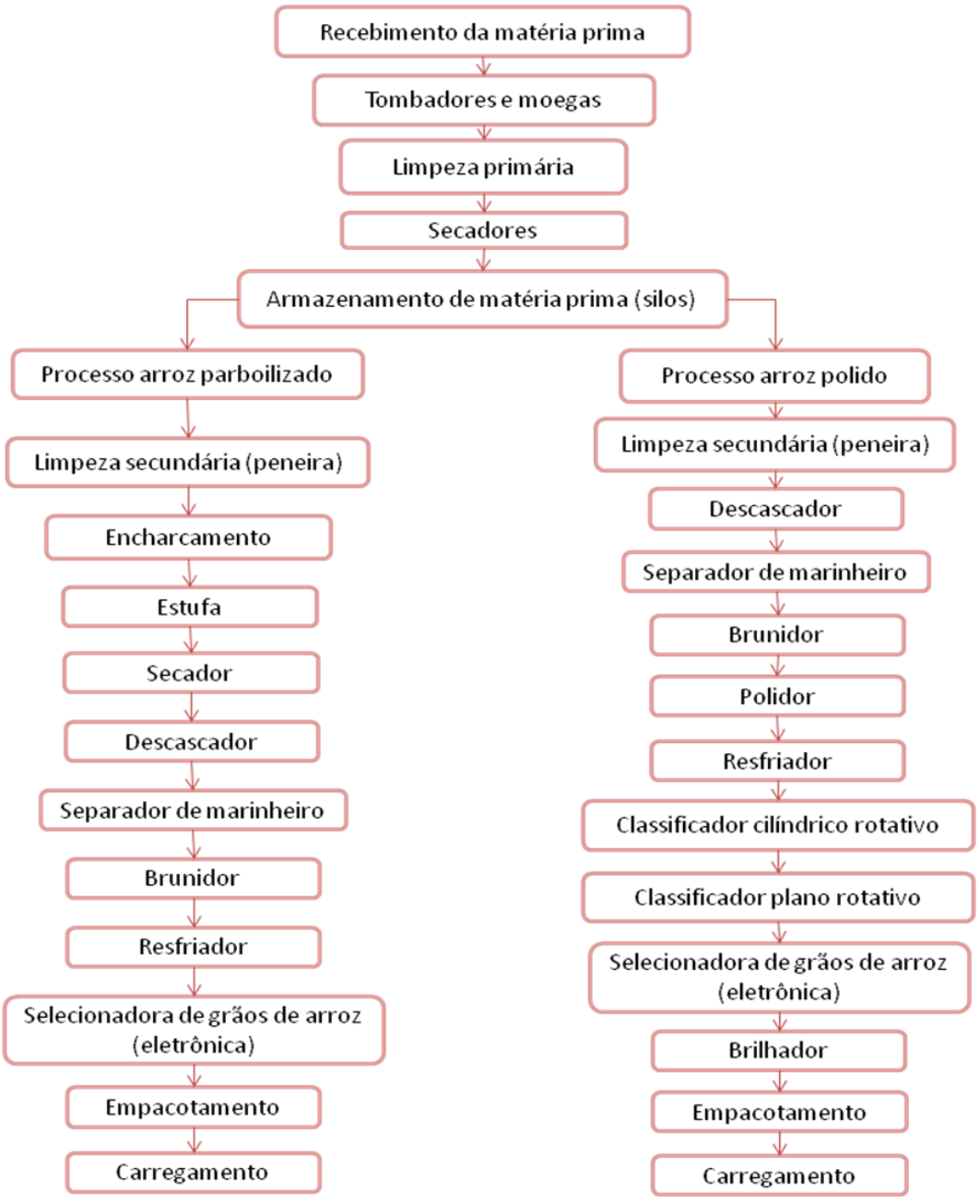

Figura 5. Fluxograma do processamento do arroz (Cooperja, acesso em 23/12/2016, www.cooperja.com.br/produtos/producaodearroz.pdf)

3.2 Aflatoxinas

Micotoxinas são metabólitos secundários produzidos por fungos filamentosos. Apresentam estruturas químicas distintas de baixo peso molecular e podem causar doenças ou morte quando ingeridas por animais ou humanos. Os fatores relacionados à sua produção pelo fungo envolvem o genótipo e fisiologia, caracterizando-o como produtor ou não, e fatores extrínsecos como umidade, temperatura e pH. Dentre as micotoxinas encontradas no arroz, as aflatoxinas são as mais comuns. Quimicamente, as aflatoxinas são moléculas de dihidrofuranos unidas a anéis cumarínicos e, assim como outros compostos heterocíclicos, fluorescem sob luz ultravioleta e são distinguidos por suas propriedades fluorescentes (Hussein & Brasel, 2001). As quatro principais aflatoxinas são chamadas de B_1, B_2, G_1 e G_2 baseado na coloração da fluorescência azul (*Blue*) ou verde (*Green*) emitida sob luz ultravioleta a 365nm.

As aflatoxinas são pouco solúveis em água, insolúveis em solventes apolares e solúveis em solventes orgânicos, como clorofórmio e metanol, e especialmente em dimetil sulfóxido (IARC, 2002). São metabólitos extremamente tóxicos, sendo o análogo B_1 considerado o mais tóxico, classificado pela International Agency for Research on Cancer (IARC, 1993) como pertencente à classe 1, i.e. composto carcinogênico ao homem, sendo o fígado o principal órgão atingido após sua ingestão. A resolução RDC 07/2011 publicada pela ANVISA (ANVISA, 2011) determina que o limite máximo tolerável de aflatoxinas em cereais não ultrapasse 5µg/kg.

A exposição às aflatoxinas pode causar uma intoxicação aguda ou crônica. Estima-se que a DL_{50} para intoxicação aguda em humanos seja de 5mg/kg, (Moss, 2002), entretanto estes casos são raros. Em 2004 houve um surto de aflatoxicose aguda no Quênia com 317 casos e 125 mortes, envolvendo milho contaminado, sendo que as amostras de milho apresentaram uma média de 354,53µg/kg (Azziz-Baumgartner et al, 2005). Por outro lado, a ocorrência de carcinoma hepatocelular devido à exposição crônica da aflatoxina tem sido bem documentada, com maior frequência em áreas onde a infecção pelo vírus da hepatite B é endêmica ou em associação com fatores de risco (Qian et al, 1994; Wang et al., 2001; Chen et al., 2001; Henry et al., 2002).

3.2.1 Fungos produtores de aflatoxinas

As aflatoxinas são produzidas principalmente por *Aspergillus flavus, A. parasiticus* e *A. nomius* (Pitt & Hocking, 2009). Outras espécies aflatoxigênicas menos comuns são: *A. pseudotamarii*, *A. bombycis, A. toxicarius, A. parvisclerotigenus, A. arachidicola*, *A. minisclerotigenes, A. ochraceoroseus, A. rambelii, Emericella astellata, E. venezuelensis, A. pseudonomius, A. pseudocaelatus, A. togoensis , A. mottae, A. sergii, A. transmontanensis,* e *A. novoparasiticus* (Ito et al., 2001, Peterson et al., 2001, Murakami, 1971, Pildain et al., 2008, Frisvad et al., 1999, Frisvad et al., 2004, Frisvad et al., 2005, Varga et al., 2011, Soares et al., 2012, Gonçalves et al., 2012).

O gênero *Aspergillus* pertence à classe dos hifomicetos, que se reproduz assexuadamente através de estruturas denominadas conídios, possui colônias que diferem na coloração e estruturas de frutificação. Apresenta uma formação de conidióforos com estipes grandes e largas e vesícula geralmente esférica na extremidade (Pitt & Hocking, 2009). Na porção fértil da vesícula ocorre a formação das fiálides, estruturas responsáveis pela produção de conídios (esporos mitóticos). O gênero pode ser classificado como monosseriado quando apresenta apenas fiálides e bisseriado quando apresenta métula e fiálide (Pitt & Hocking, 2009). O tamanho, arranjo e a coloração do conídio são características importantes para identificação das espécies.

Aspergillus flavus possui uma colônia caracteristicamente verde amarelada, cerca de 50% das cepas de *A. flavus* produzem esclerócios pretos. Apresenta vesículas globosas a subglobosas, as fiálides nascem diretamente na vesícula ou na métula. Os conídios são globosos a subglobosos, verde pálidos, finamente enrugados. Apresenta temperatura mínima de crescimento por volta de 12ºC, máxima próxima de 48ºC e ótimo em torno de 33ºC. O pH ótimo para crescimento é de 7,5 e a atividade de água mínima para crescimento é próxima a 0,80 (Pitt & Hocking, 2009). As micotoxinas mais importantes produzidas por *A.flavus* são as aflatoxinas B_1 e B_2, alguns isolados também produzem ácido ciclopiazônico (Klich & Pitt, 1988).

Aspergillus parasiticus é mais restrito geograficamente quando comparado ao *A. flavus*. Apresenta temperatura de crescimento entre 12°C e 42°C com valor

ótimo a 32°C. A atividade de água mínima para crescimento é de 0,82 a 25°C, 0,81 a 30°C e 0,80 a 37°C. O crescimento pode ocorrer numa faixa de pH de 2,4 a 10 (Pitt & Hocking, 2009). A maioria das cepas são produtoras de aflatoxinas B_1, B_2, G_1 e G_2 (Frisvad et al., 2007).

Aspergillus nomius é estritamente relacionado à *A. flavus*, na ausência de esclerócios, as duas espécies são morfologicamente indistinguíveis. A maior diferença é que *A. nomius* produz aflatoxinas B e G e seu esclerócio tem forma de bala, não esférica como de *A. flavus*. *A. nomius* cresce a atividade de água mínima de 0,83 a 25°C e 30°C e 0,81 a 37°C (Pitt & Hocking, 2009).

Existe uma diferença na porcentagem de isolados capazes de produzir aflatoxinas dentro de uma espécie, e também a quantidade e tipo de aflatoxinas produzidas por cada espécie. Cerca da metade dos isolados de *A. flavus* são aflatoxigênicos, enquanto que 100% de *A. parasiticus* possuem tal capacidade (Klich & Pitt, 1988). A temperatura e a atividade de água são fatores que além de influenciar o crescimento de cepas aflatoxigênicas, tem papel importante na expressão dos genes responsáveis pela biossíntese de aflatoxinas, estimulando a produção ou não. Enquanto a condição para crescimento de *A. flavus* é de 10°C-12°C a 43°C-48°C e atividade de água entre 0,78-0,84, a condição para a produção de aflatoxinas é de 16°C-31°C e a_w 0,95-0,99 (Pitt & Hocking, 2009).

3.3 Ocorrência de fungos, aflatoxinas e micotoxinas no arroz do Brasil e do mundo

O arroz pode ser infectado por fungos antes da colheita como *Fusarium* spp. e após a colheita durante a fase de secagem, ou armazenamento por *Aspergillus* spp. e *Penicillium* spp (Pitt & Hocking, 2009), que podem ser produtores de micotoxinas.

Dentre as micotoxinas que já foram encontradas no arroz, destacam-se as aflatoxinas (AFs), Ocratoxina A (OTA), Desoxinivalenol (DON), Zearalenona (ZON) e Fumonisina (FUM) que podem ocorrer conjuntamente.

Almeida et al. (2012) analisaram 230 amostras de arroz e frações processadas como: quebrados, farelo de arroz e casca de arroz, avaliando a ocorrência das seguintes micotoxinas: AFs, ZON, OTA, DON e citreoviridina

(CTV). Neste trabalho, as amostras de arroz estavam contaminadas com AFs totais (58,7%), OTA (40%), ZON (45,2%), DON (8,3%) e CTV (22,5%). Das166 amostras de arroz analisadas, 55% apresentaram um nível maior que 0,11 µg/kg de aflatoxinas totais. Para OTA e ZON, das 165 amostras de arroz analisadas, 28% e 29% estavam contaminadas com níveis de 0,20 a 0,24 µg/kg e de 3,6 a 290 µg/kg, respectivamente. Uma amostra (0,6%) estava contaminada com 4872 µg/kg de ZON. A maioria das amostras de arroz não continha DON, embora em uma amostra foi encontrado até 244 µg/kg desta toxina. Das 65 amostras de arroz analisadas, 94% não apresentaram níveis detectáveis de CTV (<0,9 µg/kg), mas em 6% das amostras o nível de contaminação foi de 0,9 a 31,1 µg/kg. Os maiores níveis de contaminação foram encontrados nos sub produtos de arroz ou produtos derivados como casca e farelo, havendo co-ocorrência de: AFs e ZON em 17%, AFs e OTA em 24,2%, AFs e CTV em 6,2%, OTA e CTV em 4,6%, e ZON e CTV em 3,1%.Neste trabalho, Almeida et al. (2012) não analisaram a micobiota e assim os fungos produtores destas toxinas não foram conhecidos.

Estudos sobre a exposição das aflatoxinas pelo consumo de alimentos têm sidos realizados em algumas partes do mundo. Kuiper-Goodman (1998) recomenda que a ingestão diária máxima tolerável (IDMT) não deve ultrapassar 1,0 ng/kg de peso corpóreo/dia para adultos e crianças sem hepatite B e 0,4 ng/kg de peso corpóreo/dia para adultos com hepatite B.

Na Coréia do Sul, Park et al. (2004) investigaram a exposição diária à aflatoxina B_1 através do consumo de alimentos e concluiu que o arroz foi o alimento que mais contribuiu para a ingestão desta toxina. Neste estudo, das 88 amostras comerciais de arroz polido, 5 (6%) amostras apresentaram uma média de 4,8 µg/kg de AFB_1. A estimativa de ingestão diária de aflatoxinas pelos coreanos foi de 0,89 a 5,37 ng/kg de peso corpóreo/dia, o que excede a estimativa ingestão máxima diária tolerável (IDMT), causando uma preocupação na saúde dessa população (Park et al., 2004).

Mais tarde, Park et al. (2005) estudaram a presença de várias micotoxinas como: fumonisinas, ocratoxina A, desoxinivalenol, nivalenol (NIV) e zearalenona; além da micobiota do arroz cultivados em sistema de rotação de cultura com cevada. Estes autores verificaram que 45% das amostras apresentaram infecção fúngica, sendo 17% com *A. flavus;* entretanto apenas um dos 15 isolados foi

produtor de AFB. A quantidade de amostras contaminadas com FUM B_1, OTA, DON, NIV e ZON foi baixa, sendo o percentual de contaminação de 2,27%, 9,09%, 3,41%, 5,68% e 3,41%, com média de 54,4µg/kg, 3,9µg/kg, 139µg/kg, 352µg/kg e 38,5µg/kg, respectivamente.

Um estudo realizado na Uganda por Taligoola et al. (2011) sobre a micobiota e presença de aflatoxinas no arroz polido importado do Paquistão durante 270 dias de armazenamento, oi observada uma alta incidência de fungos xerofílicos como *Eurotium amstelodami*, *E. chevalieri*, *E. rubrum* e *Aspergillus candidus. A. flavus* foi encontrado em apenas duas amostras nos oito períodos de armazenamento. Oito das dez amostras apresentaram presença de aflatoxinas, sendo que uma estava acima do limite permitido na Uganda, que é de 20µg/kg, numa faixa de 20-50µg/kg, duas estavam no intervalo de 10-20µg/kg e cinco no intervalo de ND-10µg/kg, considerando o limite permitido no Brasil (5µg/kg), a maior parte destas amostras não seriam aceitas no Brasil.

Na Coreia do Sul, devido à busca por uma alimentação mais saudável, o consumo do arroz polido tem decrescido com o aumento do consumo do arroz integral. Ok et al. (2014) analisaram 80 amostras de arroz recém colhido de diferentes regiões do país e os gêneros mais frequentes foram: *Fusarium* spp., *Penicillium* spp., *Phoma* spp., *Alternaria* spp., *Myrothecium* spp. e *Cladosporium* spp., isolados a partir de plaqueamento direto com desinfecção superficial. Foram isolados também *A. flavus* e *A. clavatus,* não produtores de toxinas. O arroz polido não apresentou aflatoxinas, enquanto que 7,5% das amostras de arroz integral apresentaram aflatoxinas no intervalo de 0,7-2,7 µg/kg.

Países como o Iraque, importam arroz de vários países como Estados Unidos, Uruguai, Argentina, Brasil e Índia por não poderem suprir a demanda interna. Ali et al. (2016) analisaram a micobiota de 50 amostras de arroz consumida no Iraque, através de plaqueamento direto sem desinfecção superficial. Os gêneros mais comuns foram *Bipolaris spicifera*, *Curvularia lunata*, *Fusarium* spp., *Exserohillum rostratum*, *Nigrospora oryzae*, *Thanatephorus cucumeris* e *Alternaria* spp. Neste trabalho não foi realizada nenhuma pesquisa das micotoxinas que poderiam estar presentes.

Um estudo interlaboratorial realizado na Índia com 581 amostras de arroz parboilizado, detectou AFB_1 em 38% com níveis ≥ 5 µg/kg e 17% com níveis

acima de 30 µg/kg, sendo que o limite máximo para AFB_1 permitido na Índia é de 30 µg/kg (Toteja et al., 2006).

Reddy et al. (2009a) analisaram 1200 amostras de arroz da Índia, 675 amostras de arroz em casca e 525 amostras de arroz polido, quanto à infecção por *Aspergillus* spp. e presença de AFB_1. Todas as amostras de arroz em casca estavam infectadas com *A. flavus*, 67,7% e apresentaram AFB_1 de 0,1 a 308,0 µg/kg, dentre estas amostras 2% apresentaram contaminação maior que o limite permitido (30 µg/kg). As amostras de arroz polido apresentaram AFB_1 no intervalo de 0,5 a 3,5 µg/kg.

Em Cleveland-EUA, foram analisadas amostras de arroz e suas frações durante as etapas de processamento. Estas amostras apresentaram níveis muito altos de aflatoxina, numa média de 114µg/kg de arroz com casca, 39µg/kg na casca, 158µg/kg no arroz integral, 357µg/kg no farelo e 56µg/kg no arroz polido (Trucksess et al., 2011).

Nas Filipinas, amostras de arroz integral e polido tiveram uma maior incidência de *Aspergillus* section *Flavi* do que o arroz com casca, porém o arroz com casca apresentou maior taxa de fungos produtores de aflatoxinas. O maior nível de aflatoxinas foi encontrado no farelo (9,97 µg/kg), seguido da casca de arroz (5,02 µg/kg) e arroz integral (4,32 µg/kg) (Sales & Yoshizawa, 2005).

Makun et al. (2011) na Nigéria, analisaram 21 amostras de arroz, sendo 10 do campo, 6 do armazenamento e 5 comerciais. Todas as amostras apresentaram níveis de aflatoxinas acima dos limites aceitáveis de 77 países que regulamentam aflatoxinas, incluindo a União Europeia (10µg/kg), a média de aflatoxinas totais encontrada foi de 82,5 µg/kg (no intervalo de 27,7 e 371,9 µg/kg). Estas amostras também não seriam aceitas no Brasil.

O limite máximo de aflatoxinas no arroz estabelecido pelo Turkish Food Codex (TFC) é de 4µg/kg, um estudo realizado por Aydin et al. (2011), revelou que 56% das 100 amostras de arroz produzidas na Turquia estavam contaminadas com aflatoxinas num intervalo de 0,05 a 21,4µg/kg, sendo que 32% estavam acima do limite máximo estabelecido pelo TFC.

A ocorrência de aflatoxinas em arroz consumido por militares do exército brasileiro foi analisada por Silva et al. (2008) em Curitiba, utilizando duas técnicas de detecção: (i) cromatografia de camada delgada (CCD) com limite de

quantificação de 3µg/kg e (ii) cromatografia líquida de alta eficiência (CLAE) com limite de quantificação de 0,5µg/kg. Pela técnica de CCD, nenhuma das 30 amostras apresentou aflatoxinas e por CLAE, seis das 26 amostras apresentaram aflatoxinas no intervalo de 0,54 a 2,04µg/kg. Já um estudo realizado na região Sul do Brasil em 2009, com 32 lotes de arroz parboilizado, revelou que três amostras estavam contaminadas por aflatoxinas no intervalo de 11,53 a 74 µg/kg (Dors et al., 2011).

Em 2010, Carvalho et al. (2010) avaliaram a incidência de fungos toxigênicos e aflatoxinas no arroz comercializado em Belo Horizonte e algumas cidades do sul de Minas Gerais, sendo 48 amostras de arroz polido, 3 de arroz parboilizado, 7 de arroz integral e 2 de arroz orgânico. O método utilizado foi o plaqueamento direto com desinfecção superficial. O maior índice de infecção fúngica no arroz foi de 100%, 42%, 32% e 13% nas amostras de arroz orgânico, polido, integral e parboilizado, respectivamente. Foram isolados 184 fungos, dentre eles *A. candidus*, *A. carbonarius*, *A. flavus*, *A. niger*, *A. tamarii*, *Alternaria alternata*, *Eurotium* spp., *Fusarium* spp., *P. citrinum*, *P. glabrum*, *Rhizopus* spp., *Trichoderma* spp. Dos 48 isolados dos *A.* seção *Flavi*, 17 foram identificados como *A. parasiticus*, sendo 14 produtores de AFB e AFG, e 31 como *A. flavus*, sendo apenas 8 produtores de AFB. Apenas uma das 36 amostras apresentou aflatoxinas com nível de 1,2µg/kg.

Beber-Rodrigues & Scussel (2013) analisaram a micobiota do arroz, pelo método de diluição seriada e a presença de micotoxinas (aflatoxinas, ocratoxina A e zearalenona) em 4 cultivares diferentes de arroz em casca, sendo 10 amostras de cada cultivar, recém colhidos da cidade de Massaranduba (SC). Os autores investigaram a relação do tempo entre a colheita e a recepção na indústria, concluindo que não houve influência significativa do tempo de espera dos caminhões no aumento da umidade do arroz, atividade de água e infecção fúngica. Os gêneros que tiveram maior incidência foram *Penicillium* spp. e *Aspergillus* spp., neste trabalho não foi detectada a presença das micotoxinas estudadas (aflatoxinas, ocratoxina A e zearalenona).

Uma das preocupações da presença de aflatoxinas no arroz dá-se pela presença das mesmas nos subprodutos como farelo, farinha e seus derivados. Jayaraman & Kalyanasundaram (2009) investigando a presença de aflatoxinas

nas frações de óleo de farelo de arroz, encontraram AFB_1 em 75% das amostras de óleo cru, com uma média de 618 µg/kg (236-956 µg/kg); em 30% do óleo refinado havia uma média de 20 µg/kg (traço-28 µg/kg) e 66,7% das amostras de “de-oiled” (óleo com menos gordura e alta quantidade de proteínas) apresentaram uma média de 33 µg/kg (7-114ppb). Quanto ao potencial de produção de aflatoxinas por *A. flavus,* 20 dos 30 isolados e 9 dos 15 isolados das amostras de “de-oiled” e óleo refinado, respectivamente, foram produtores desta toxina. Neste trabalho, os autores sugerem que o período de tempo entre o descascamento, processamento, comercialização, transporte e armazenamento pode favorecer o desenvolvimento de fungos toxigênicos e o início da produção de toxinas. Neste estudo foi verificado que a alta incidência de aflatoxinas no óleo cru foi removida durante o processo de refinamento, mas 30% das demais amostras de óleo ainda apresentaram nível acima de 30 µg/kg (nível máximo tolerado na Índia).

Na Tabela 1 podem ser visualizados os dados disponíveis na literatura sobre a ocorrência de aflatoxinas totais em arroz e produtos de arroz, bem como o método de detecção usado.

Tabela 1. Ocorrência de aflatoxinas totais em arroz e produtos de arroz.

Produto de arroz	N° amostras	Incidência de AFT (%)	Mínimo-Máximo (µg/kg)	Método	País	Referência
Arroz polido	166	40	ND – 176,31	CLAE	Brasil	Almeida et al., 2011
Arroz em casca	27	97	ND – 31,72	CLAE	Brasil	Almeida et al., 2011
Farelo	19	89	ND – 207,04	CLAE	Brasil	Almeida et al., 2011
Quirera	18	94	ND – 19,42	CLAE	Brasil	Almeida et al., 2011
Arroz polido	88	6	2,1 - 7,7	CLAE	Coreia	Park et al., 2004
Arroz polido	10	80	ND - 50	CLAE	Uganda	Taligoola et al., 2011
Arroz polido	80	0	ND	CLAE	Coreia do Sul	Ok et al., 2014
Arroz integral	80	7,5	ND - 2,7	CLAE	Coreia do Sul	Ok et al., 2014
Arroz parboilizado	1511	38	<5 - >30	HPTLC	Índia	Toteja et al, 2006
Arroz integral	9	100	0,025 – 8,7	CLAE	Filipinas	Sales & Yoshizawa, 2005
Arroz polido	68	94	ND - 8,7	CLAE	Filipinas	Sales & Yoshizawa, 2005
Arroz em casca	16	100	27,7 - 371,9	CLAE	Nigéria	Makun et al., 2011
Arroz polido	5	100	27,7 - 371,10	CLAE	Nigéria	Makun et al., 2011
Arroz polido	100	56	<0,05 - >21,4	ELISA	Turquia	Aydin et al., 2011
Arroz parboilizado	32	9	ND - 74	CCD	Brasil	Dors et al., 2011
Arroz polido	26	23	ND - 2,04	CCD/CLAE	Brasil	Silva et al., 2008
Arroz polido	36	2	ND - 1,2	CLAE	Brasil	Carvalho et al., 2010
Óleo de farelo de arroz cru	20	75	236 - 956	CCD	Índia	Jayaraman & Kalyanasundaram, 2009
Óleo de farelo de arroz refinado	20	30	traço - 28	CCD	Índia	Jayaraman & Kalyanasundaram, 2009
Óleo de farelo de arroz ("de-oiled")	30	66,7	7 - 114	CCD	Índia	Jayaraman & Kalyanasundaram, 2009
Arroz em casca	675	70,7	0,1 - 308	ELISA	Índia	Reddy et al., 2009ª
Arroz polido	525	64,1	0,5 – 3,5	ELISA	Índia	Reddy et al., 2009ª
Arroz em casca	40	0	ND	CCD	Brasil	Beber-Rodrigues & Scussel, 2013

3.4 Métodos de redução de aflatoxinas no arroz

No sistema de arroz irrigado, este é cultivado sob a água nos primeiros estágios do desenvolvimento, assim os níveis de *A. flavus* dos solos são baixos, com a baixa contaminação dos grãos maduros e consequentemente no produto final. Entretanto, se os grãos forem colhidos úmidos e se houver demora na secagem pode haver um aumento na infecção por *A. flavus* (Pitt et al., 2013). A Figura 6 mostra a aplicação do conceito de objetivo da segurança do alimento (FSO - Food Safety Objective) para grãos como trigo, cevada e arroz com casca. Por outro lado, no sistema sequeiro, o arroz é cultivado em solos com umidade moderada, em períodos de chuvas regulares, o que pode favorecer o desenvolvimento de *A. flavus*.

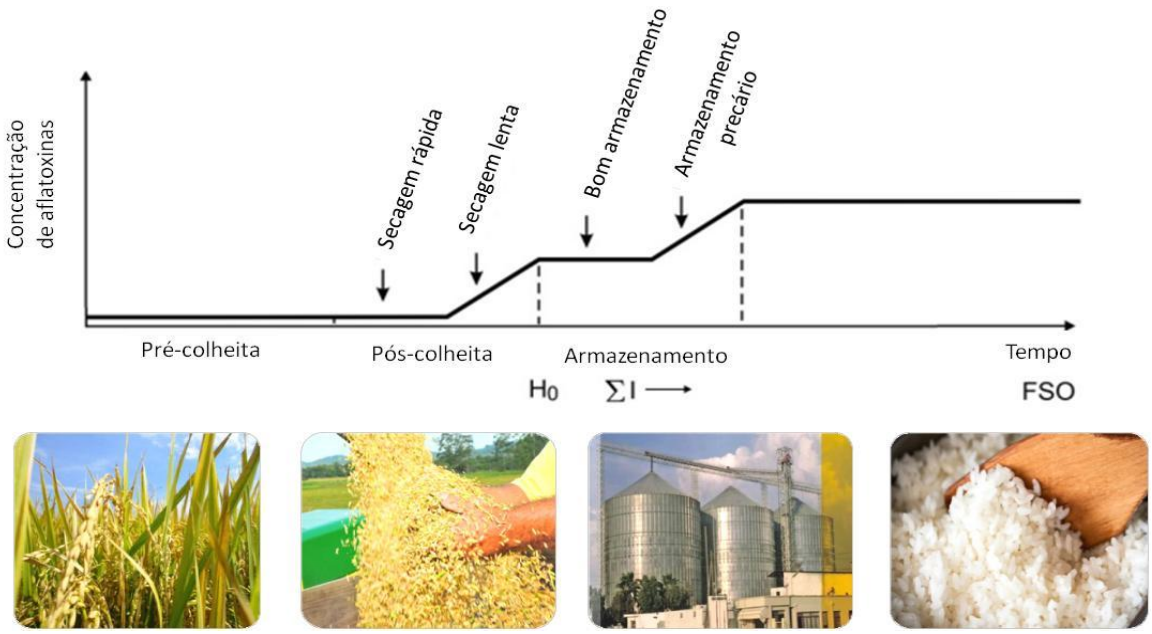

Figura 6. Formação de aflatoxinas durante a cadeia produtiva de pequenos grãos (Pitt et al., 2013)

O FSO estabelece de forma flexível, metas que devem ser atingidas na cadeia produtiva para obter uma frequência ou concentração máxima permitida de um perigo em um alimento no momento do consumo. No caso do arroz, este limite seria a concentração máxima permitida pela RDC 07/11 de 5 µg/kg e os pontos críticos para o aumento da concentração de aflatoxinas se encontram na secagem e no armazenamento.

Alguns estudos mostraram que é possível reduzir os níveis de aflatoxinas no arroz durante o beneficiamento e também durante o cozimento no momento do consumo.

Nas Filipinas foi observada uma redução de 78% de aflatoxinas no processo de polimento do arroz integral para o arroz polido; e uma redução de 38% do polimento comum para o arroz com maior polimento (Sales & Yoshizawa, 2005).

Um estudo realizado por Park & Kim (2006) concluiu que houve uma redução de 31-36% de AFB_1 no cozimento comum (panela elétrica) e uma redução de 78-83% no cozimento com pressão. Hussain & Luttfullah (2009) observaram uma redução de 84% de AFB_1 no cozimento comum, 87,5% com excesso de água e 72,5% no cozimento por microondas. Sani et al. (2012) observou uma redução de 24,8% de AFB_1 no cozimento com panela elétrica e uma redução de 17,5% no cozimento local, em fogão à gás .

Além do polimento e cozimento, há estudos de biocontrole sob o crescimento de *A. flavus* e produção de aflatoxina B_1 (AFB_1) nos grãos de arroz. Na Índia, Reddy et al. (2009b) realizaram um estudo com extratos de plantas e outros agentes de biocontrole, verificando uma inibição do crescimento de *A. flavus* e a produção de AFB_1 nos grãos de arroz. Neste estudo, *Syzigium aromaticum* (cravo-da-Índia) na concentração de 5g/kg foi capaz de inibir completamente o crescimento e a produção de AFB_1. *Curcuma longa* (cúrcuma), *Allium sativum* (alho) e *Ocimum sanctum* (tulsi) na mesma concentração inibiram cerca de 65-78% do crescimento de *A. flavus* e cerca de 72,2-85,7% da produção de AFB_1. Dentre os microrganismos estudados, *Rhodococcus erythropolis* na concentração de 25ml/kg inibiu completamente o crescimento e a produção de AFB_1. *Pseudomonas fluorescens*, *Trichoderma virens* e *Bacillus subtilis* na concentração de 200ml/kg apresentaram 93%, 80% e 68% de redução do crescimento de *A. flavus* e 83,7%, 72,2% e 58% de redução de AFB_1, respectivamente (Reddy et al., 2009b).

3.5 Avaliação da exposição

Para realizar a avaliação da exposição (ingestão diária) são considerados dados de concentração da substância no alimento, o consumo do alimento e o peso corpóreo, como representado na equação:

$$Exposição = \frac{concentração\ da\ substância\ x\ consumo\ do\ alimento}{peso\ corpóreo}$$

A concentração da substância no alimento pode ser determinada através de levantamento bibliográfico de estudos anteriores. Os dados de consumo e peso corpóreo podem ser obtidos através de balanços alimentares, dados de Pesquisa de Orçamentos Familiares (POF) e questionários alimentares individuais (FAO). No modelo determinístico, os dados de concentração da substância, consumo e peso corpóreo são valores pontuais como média, mediana, percentil ou valor máximo. As grandes vantagens desse modelo são a rapidez e facilidade dos cálculos, porém a interpretação dos dados gerados pode não ser tão representativa, pois os dados utilizados são pontuais. Este modelo é indicado quando o objetivo é identificar se o consumo de alimento oferece risco à saúde (Jardim & Caldas, 2009).

3.6 Margem de exposição (MOE)

O cálculo da margem de exposição (MOE) é uma das metodologias utilizadas para a caracterização do risco em substâncias carcinogênicas e genotóxicas e pode ser calculada segundo a fórmula:

$$MOE = \frac{Referência\ toxicológica}{Exposição}$$

A BMDL (Benchmark dose modelling) é a referência toxicológica utilizada nesse cálculo, preferencialmente a BMDL10 (Jardim & Caldas, 2009). Valores de MOE iguais a 10.000 ou maiores, quando baseados em BMDL10 proveniente de um estudo de animais, indicariam uma baixa preocupação de risco do ponto de vista de saúde pública.

Andrade et al. (2013) desenvolveram uma avaliação da exposição de risco às aflatoxinas em produtos consumidos no Brasil, o arroz apresentou exposição

de 6,4-7,5 ng/kg peso corpóreo/dia e um MOE (28.286) que representa baixa preocupação no ponto de vista da saúde pública.

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CAPÍTULO I

Mycobiota of rice: from field to market

Abstract

Rice is one of the most consumed cereals in Brazil and in the world. Due to the big impact of rice consumption on the population, studies about its quality have great importance. The present study determined the mycobiota of soil, field, processing and market rice samples from two production systems, the dryland (state of Maranhão-MA) and wetland (state of Rio Grande do Sul-RS, the biggest Brazilian producer), and market samples from São Paulo (SP). A total of 210 samples were analyzed (187 rice and 23 soils) There was a difference between the mycobiota of the two production systems with higher occurrence of *Aspergillus* section *Flavi* and species of *Penicillium* in wetland field and processing samples. The prevalence of *Aspergillus candidus*, *Eurotium chevalieri* and black moulds were observed in market samples of the three states.

1. Introduction

Rice (*Oryza sativa*) is an important carbohydrate source and is consumed all over the world. The guarantee of its quality is important because any problem may affect consumer health, mainly in countries such as Brazil where rice is present in the daily diet. Brazil is ranked as the ninth rice producer in the world and the largest producer outside Asia (FAO, 2016). There are two production systems in Brazil, the wetland system, prevalent in the South region, where the annual average temperature is 18-20°C and the average annual rainfall is 1400-1600mm^3 (INMET, 2016), and the dryland system, prevalent in the Northeast region, where the annual average temperature is 28-30°C and the average annual rainfall is 1000-1200mm³ (INMET, 2016).

In the wetland system, the rice is irrigated by controlled flooding and in the dryland system, there are two processes: one that occurs without irrigation and depends totally on rainfall and the other on supplementary irrigation that decreases the drought stress, providing higher grain quality. Rice production is common in most of Brazil, but the largest plantations are in five states: Rio Grande do Sul (66.5%), Santa Catarina (8.6%) and Tocantins (4.7%), where the wetland system predominates, and Maranhão (5.3%) and Mato Grosso (4.4%), where the dryland system predominates. In Rio Grande do Sul (RS), the rice is harvested in the autumn, when the average temperature is 20-24°C and the average rainfall is

200-400mm³. In Maranhão (MA), the rice is harvested in the summer, when the average temperature is 30-32°C and the average rainfall is 200-400mm³.

Reports on mycotoxin producing fungi in rice destined for human consumption, such as *Penicillium citrinum*, *P. islandicum*, *Aspergillus flavus*, *A. ochraceus*, *A. parasiticus*, *Fusarium* spp. have been found in several parts of the world, such as Uganda (Taligoola et al., 2004, 2011), South Korea (Park et al., 2005) Turkey (Aydin et al., 2010), Brazil (Carvalho et al., 2010), Argentina (Pinciroli et al., 2013) and India Reddy et al., 2009).

At storage, *Eurotium* spp. and *Aspergillus candidus* have been reported as having the highest incidence and increase according to the storage period. *A. fumigatus*, an important pathogen has also been found in storage samples in Uganda (Taligoola et al., 2011). Knowing about rice mycobiota is important at all stages in order to understand the fungal succession throughout the rice chain. The aim of this research was to evaluate the mycobiota of rice from field to market and rice soil from two different production systems: wetland and dryland.

2. Materials and methods

2.1 Samples

A total of 187 rice samples each of approximately 1kg was collected from dryland in Maranhão (MA), which has latitude of 05º31'35"S and longitude of 47º 29' 30" W, and wetland in Rio Grande do Sul (RS) with latitude of 30º51'04"S and longitude of 51º48'44"W, at the stages of field, processing (paddy rice, husk, husked rice, bran, broken rice, brown, polished, parboiled and red rice) and market (polished, brown, parboiled, flake, flour, red and brown with red rice). Only two samples from the drying yard were collected in Maranhão because most of the rice is dried in mechanical dryers in processing plants. In São Paulo state (SP), samples were collected from markets (polished, brown, parboiled, flake, flour, red, brown with red rice and black rice), since this state does not grow rice, but is a high rice consumer. Twenty-three soil samples from the states of Maranhão and Rio Grande do Sul were collected. Table 1 shows the number of samples from each stage and the states from where the samples were collected.

2.2 Water activity

The water activity was determined using an Aqualab Series 3TE instrument (Decagon, Pullman, Wa, USA) at 25°C±1, in triplicate.

2.3 Fungal isolation

Each sample of approximately 100 g was surface-disinfected with sodium hypochlorite solution (0.4%) for 1 min. Fifty grains were distributed in 5 Petri dishes (10 grains per dish) containing Dichloran 18% Glycerol Agar (DG18) and incubated at 25°C for 5 to 7 days, according to Pitt & Hocking (2009). Bran, flour and soil samples were analyzed by serial dilution plating technique, 25g of each sample was diluted in 225 ml of peptone water 0.1%; an aliquot of 0.1mL was inoculated onto DG18 plates and incubated at 25°C for 5 to 7 days (Pitt & Hocking, 2009). After incubation, the plates were examined and all the fungal species were first isolated in Petri dishes containing Czapek Yeast Autolysate (CYA) agar to be later identified by specific protocols for each genus.

2.4 Morphological examination

The isolated fungi were grown on the CYA and malt extract agar (MEA). The genus *Penicillium* was identified according to Pitt (2000) and Samson et al. (2010), and the identification of genera *Aspergillus* and *Eurotium* was performed according to Klich (2002), Pitt and Hocking (2009) and Samson et al. (2010). The other fungi were identified according to descriptions of Pitt and Hocking (2009) and Samson et al. (2010), supplemented with other sources when necessary.

Isolated *Aspergillus* sp. were inoculated at 3 points on the CYA and MEA plates and incubated for 7 days at 25 °C. Its teleomorphic state *Eurotium* sp. was cultivated in Czapek yeast extract agar with 20% sucrose (CY20S) for 14 days at 25ºC. The *Penicillium* species were grown by following the conditions above, and were also inoculated on CYA at 5ºC and 37ºC. After the cultivation period, the diameters of the colonies were measured and the macro and microscopic features observed in each culture medium were used for species identification.

The frequence of occurrence (FO) was calculated by the number of samples that contained a fungal species divided by the total of samples evaluated, the average of infection (AI) was calculated by the sum of infection level divided by the

total number of samples and the range of Infection (RI) is the range of infected grains in a sample.

3. Results

Tables 2, 3 and 4 show the number of samples of each rice production stage, average of water activity, frequency of occurrence, average infection rate and the range of infection from wetland system, dryland system and markets of São Paulo states (SP), respectively. There was a bigger variety of fungi from wetland field samples than from dryland; the average of water activity was higher in wetland samples. The genus *Fusarium* spp. was the most prevalent in both systems, followed by *Nigrospora oryzae*, *Alternaria* spp., *Phoma* spp. and *Bipolaris* spp. and other black moulds. *Aspergillus* section *Flavi* was more prevalent in wetland field samples (50%) than those in dryland (8.33%). In wetland samples the infection by *A.* section *Flavi* reached 100% and in dryland 2%. Dryland samples had other genera such as *Curvularia* spp., *Acremonium strictum* and hyphomycetes while wetland samples had yeasts, *Cladosporium* spp., *Penicillium aethiopicum*, *P. chrysogenum*, filamentous yeasts, *P. citrinum*, *P. expansum*, *Penicillium* spp., *Pestalotiopsis* spp. and *Trichoderma* spp.

At drying, dryland samples showed *Alternaria* spp., *E. chevalieri*, *Fusarium* spp., hyphomycetes, filamentous yeast, *Nigrospora oryzae*, *Pestalotiopsis* spp., *Phoma* spp and other black moulds.

In processing, wetland samples had a bigger variety of fungi than dryland, but both had a prevalence of *E. chevalieri*. There was a higher occurrence of *Aspergillus* section *Flavi* (36.84%) in wetland processing samples than in dryland (14.29%).

In market samples, there were occurrences of *A. candidus, A. penicillioides, A. restrictus,* black moulds*, E. chevalieri, E. rubrum, Fusarium* spp*.* and *P. citrinum* in the three states. *A. fumigatus* and *Cladosporium* spp. occurred only in wetland and dryland samples. *A.* section *Flavi*, *A.* section *Nigri*, hyphomycetes and *Syncephalastrum racemosum* occurred only in dryland and SP samples. The samples from wetland and SP had the most similar mycobiota. Flake and flour samples from dryland did not show any fungal contamination. A wetland flake sample had *Cladosporium* spp. (2%) and *Chrysonita crassa* (20%), a flour sample

showed *Cladosporium* spp., *E. chevalieri*, yeasts, *P. citrinum* and *P. cosmopolitanum*. Figure 1 shows the main fungi isolated in field, processing and market, on CYA medium and CY20S for *E. chevalieri*.

Table 5 shows the isolated species from soil samples with the number of samples in which they were present. The dryland samples had a higher number of *Aspergillus* section *Flavi* and *Aspergillus terreus* than wetland and showed *Aspergillus* section *Nigri.* Wetland samples had a higher number of *Fusarium* spp. and *Penicillium* spp.

4. Discussion

In the wetland system, the soil is prepared by flooding the area while in the dryland system the soil must not be too dry or too moist, which results in a higher water activity in field rice from the wetland than in the dryland. In our study, as we expected, the field rice samples' water activity from wetland (Rio Grande do Sul) was higher than that from dryland (Maranhão). The field samples, from both systems, showed typical mycobiota, with field fungi, such as *Fusarium* spp., black moulds and other genera.

Different methodologies and culture media have been used on the study of rice mycobiota. Beber-Rodrigues & Scussel (2013) analyzed 40 samples of paddy rice freshly harvested from the state of Santa Catarina (Brazil) using a series of dilution onto the surface of PDA media with antibiotic and reported the presence of *Acremonium* spp., *Botrytis* spp., *Fusarium* spp., *Mucor* spp., *Phialophora* spp., *Rhizomucor* spp., *Rhizopus* spp., *Saprolegnia* spp., *Trichoderma* spp. and the more common genera were *Aspergillus* spp. and *Penicillium* spp. In rice freshly harvested from South Korea the more frequent genera were *Fusarium* spp., *Penicillium* spp., *Phoma* spp. and *Alternaria* spp. (Ok et al., 2014) as well as in field samples from our study, except for dryland samples that did not show *Penicillium* spp. Ok et al. (2014) used the direct plating with surface-desinfection as well as our study, but they used DRBC instead of DG18.

Rice can be infected pre-harvest by fungi such as *Fusarium* spp. and post-harvest by fungi such as *Aspergillus* spp. and *Penicillium* spp. (Pitt & Hocking, 2009). In our study, *Fusarium* spp. occurred at all the stages of the two systems

and in SP comercial samples. *Penicillium* spp. occurred at almost all the stages of wetland but not in the field and drying stages of dryland. Field samples from both states had only *Aspergillus* section *Flavi*, while dryland drying samples did not show any species of *Aspergillus* spp. Processing and market samples from the three states showed a higher variety of *Aspergillus* spp and *Penicillium* spp. Jayaraman & Kalyanasundaram (1990) analyzed rice bran and reported the presence of *Aspergillus candidus, A. flavus, A. fumigatus, E. amstelodami, E. chevalieri, P. citrinum, Penicillium* spp., that were also found in bran rice samples from our study and other species such as *A. parasiticus, A. nidulans, A. niger, A. ornatus, A. terreus* and *P. funiculosum*, but the methodology used was the dilution onto 50% CDA media (Czapek Dox Agar 50% sucrose).

Fredlund et al. (2009) analyzed rice from the Swedish retail market using the dilution technique onto DG18 and isolated *A. candidus*, *Penicillium* spp. and *Eurotium* spp., fungi which were found in the market samples of our study from the three states. *A. flavus* and *A. niger* were isolated in the market samples from dryland and SP, *A. fumigatus* was found in the wetland and dryland market samples and other species *P. polonicum* and *P. chrysogenum* that were not found in the market samples of our study. Park et al. (2005) described the mycobiota of South Korean polished rice destined for humans, where the production system is the wetland, using the same methodology that we used in our study, and the four main fungal species were *P. citrinum*, *A. candidus*, *Alternaria* spp. and *A. versicolor*. In our study, *P. citrinum* and *A. candidus* were present in market samples of the three states. *Alternaria* spp. was present in SP and wetland market samples and *A. versicolor* only in SP samples. The polished rice imported from Pakistan by Uganda showed a mycobiota similar to our study using the same fungal isolation methodology but without surface-disinfection, with prevalence of *Eurotium* spp. and *Aspergillus candidus* (Taligoola et al., 2011). Pinciroli et al. (2013) reported the presence of the genera *Alternaria* spp., *Epicoccum* spp., *Bipolaris* spp., *Curvularia* spp., *Cladosporium* spp., and *Penicillium* spp. in paddy, brown and polished rice from Argentina with direct plating with surface-desinfection onto PDA with antibiotic. They found *Nigrospora* spp. and *Fusarium* spp. in paddy and brown rice, and *Aspergillus* spp. only in brown rice. Carvalho et al. (2010) analyzed market samples by direct plating with surface-desinfection

onto DRBC from five cities from Minas Gerais (Brazil), polished, parboiled, brown and organic rice samples and they found *A. flavus* in all the sample types, *A. candidus*, *A. parasiticus* and *Fusarium* spp. in polished and brown rice, and *A. fumigatus*, *Alternaria* spp., *Eurotium* spp. *P. citrinum* and *Penicillium* spp. in polished rice.

There are no previous reports investigating the mycobiota of rice soil. In the present study, dryland soil samples showed some relevant fungi that the rice samples from field did not show such as *P. citrinum*, *Aspergillus terreus* and *A.* section *Nigri*. The majority of species from wetland soil samples appeared in rice samples from fields.

The rice mycobiota described in our study resemble those described in literature, even using different methodologies and culture media; however, some uncommon fungi were found in our market samples such as: *Aspergillus ustus* (SP), *Chrysonilia crassa* (wetland), *Colletrotrichum* spp. (wetland) and *Monascus ruber* (SP and dryland).

5. Conclusion

The Brazilian rice mycobiota showed a more common diversity compared to previous reports. There was a difference between the two production systems observed in field samples, mainly in the percentage of *Aspergillus* section *Flavi.* Wetland had a bigger occurrence than dryland, and regarding the presence of *Penicillium* spp., dryland did not show this genus in the field. The higher presence of *Aspergillus* section *Flavi* in wetland samples and low *Penicillium* spp. in dryland samples were observed in the processing samples too. The fungi distribution and occurrence in market samples were more homogeneous in the three states. The presence of aflatoxin producing fungi indicates the importance of a future study on these species of fungi and mycotoxins.

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CAPÍTULO I

Mycobiota of rice: from field to market

(Figures and Tables)

Table 1. Number of rice samples and soil, collected from 3 Brazilian states:Dryland, Wetland and São Paulo (SP).

Stages	Number of samples		
	Wetland	**Dryland**	**SP**
Field	12	12	-
Drying	-	2	-
Processing	38	14	-
Market	25	30	54
Soil	12	11	-

Table 2: Number of samples, average of water activity, frequency of occurrence, average and range of infection in rice samples from wetland.

Stages (number of samples)	Field (12)			Processing (38)			Market (54)		
Mean a_w (range)	0.932(0.894-0.973)			0.614 (0.465-0.865)			0.693(0.503-0.719)		
Fungi	FO(%)	AI(%)	RI(%)	FO (%)	AI (%)	RI (%)	FO(%)	AI(%)	RI(%)
A. aureolatus	0	0	0	2.63	0.05	0 - 2	0	0	0
A. candidus	0	0	0	28.95	1.61	0 - 10	16	2.16	0-26
A. clavatus	0	0	0	5.26	0.11	0 - 2	0	0	0
A. fumigatus	0	0	0	0	0	0	4	0.08	0-2
A. penicillioides	0	0	0	0	0	0	4	0.08	0-2
A. restrictus	0	0	0	2.63	0.05	0 - 2	4	0.08	0-2
A. section *Flavi*	50	51.8	0-100	36.84	6.16	0 - 58	0	0	0
A. terreus	0	0	0	7.89	0.16	0 - 2	0	0	0
A. wentii	0	0	0	5.26	0.58	0 - 20	8	0.24	0-4
Absidia spp.	0	0	0	0	0	0	4	0.08	0-2
Alternaria spp.	33.33	3.33	0-20	10.53	0.58	0 - 10	4	0.48	0-12
Aspergillus spp.	0	0	0	2.63	0.05	0 - 2	0	0	0
Bipolaris spp.	8.33	0.17	0-2	5.26	0.16	0 - 4	8	0.16	0-2
Black Moulds	66.67	11.17	0-32	34.21	3.92	0 - 40	36	5.6	0-30
Chaetonium spp.	0	0	0	2.63	0.16	0-6	0	0	0
Chrysonilia crassa	0	0	0	0	0	0	4	0.8	0-20
Cladosporium spp.	16.67	0.67	0-6	23.68	0.79	0 - 8	8	0.16	0-2
Colletrotrichum spp.	0	0	0	0	0	0	4	0.08	0-2
Curvularia spp.	0	0	0	13.16	0.74	0 - 14	12	0.24	0-2
E. amstelodami	0	0	0	2.63	0.11	0 - 4	4	0.08	0-2
E. chevalieri	0	0	0	39.47	13.68	0 - 84	24	1.2	0-8
E. herbariorum	0	0	0	5.26	0.11	0 - 2	0	0	0
E. repens	0	0	0	7.89	0.21	0 - 4	4	0.08	0-2
E. rubrum	0	0	0	10.53	0.42	0 - 8	12	0.56	0-8
Emericella nidulans	0	0	0	2.63	0.05	0 - 2	0	0	0
Eupenicillium cinnamopurpureum	0	0	0	2.63	0.11	0 - 4	0	0	0
Fusarium spp.	91.67	27.83	0-90	26.32	4.53	0 - 100	16	1.28	0-16
Yeast	33.33	0.67	0-2	15.79	2.95	0 -88	0	0	0
Filamentous yeast	8.33	0.17	0-2	5.26	0.16	0 - 4	0	0	0
Mucor spp.	0	0	0	2.63	0.16	0-6	4	0.08	0-2
Nigrospora oryzae	58.33	4.5	0-18	23.68	2.76	0 - 38	8	0.24	0-4
P. aethiopicum	16.67	0.5	0-4	7.89	2.37	0 - 84	0	0	0
P. chrysogenum	16.67	8.33	0-94	2.63	0.11	0 - 4	0	0	0
P. citreonigrum	0	0	0	0	0	0	4	0.08	0-2
P. citrinum	8.33	0.5	0-6	10.53	0.47	0 - 8	4	0.08	0-2
P. expansum	8.33	0.17	0-2	2.63	0.74	0-28	0	0	0
P. islandicum	0	0	0	2.63	0.05	0 - 2	0	0	0
P. paxilli	0	0	0	7.89	0.16	0 - 2	0	0	0
Penicillium spp.	8.33	0.17	0-2	7.89	0.16	0 - 2	4	0.08	0-2
Pestalotiopsis spp.	8.33	0.17	0-2	0	0	0	0	0	0
Phoma spp.	58.33	8.17	0-38	31.58	4.18	0 - 42	0	0	0
Rhizopus spp.	0	0	0	5.26	0.16	0 - 4	0	0	0
Syncephalastrum racemosum	0	0	0	15.79	1.16	0 - 20	0	0	0
Trichoderma spp.	8.33	0.17	0-2	0	0	0	0	0	0

FO = Frequency of occurrence % (number of samples that contained a fungal species/total of samples evaluated; AI = Average of infection % (sum of infection level/total number of samples); RI = Range of Infection % (range of infected grains in a sample).

Table 3: Number of samples, average of water activity, frequency of occurrence, average and range of infection in rice samples from dryland.

Stages (Number of samples)	Field (12)			Drying (2)			Processing (14)			Market (30)		
Mean a_w (range)	0.825 (0.770 - 0.953)			0.708 (0.596 - 0.821)			0.665 (0.607-0.799)			0.603 (0.509-0.694)		
Fungi	FO (%)	AI (%)	RI (%)	FO (%)	AI (%)	RI (%)	FO (%)	AI (%)	RI (%)	FO (%)	AI (%)	RI (%)
A. candidus	0	0	0	0	0	0	0	0	0	20	0.73	0-6
A. fumigatus	0	0	0	0	0	0	0	0	0	6.67	0.13	0-2
A. penicillioides	0	0	0	0	0	0	0	0	0	10	3.53	0-86
A. restrictus	0	0	0	0	0	0	7.14	0.57	0-8	6.67	0.27	0-6
A. section *Flavi*	8.33	0.17	0-2	0	0	0	14.29	3.57	0-28	13.33	0.2	0-2
A. section *Nigri*	0	0	0	0	0	0	0	0	0	13.33	0.27	0-2
A. tamarii	0	0	0	0	0	0	0	0	0	3.33	0.07	0-2
Acremonium strictum	8.33	0.17	0-2	0	0	0	0	0	0	0	0	0
Alternaria spp.	75	4.67	0-14	50	6	0-12	7.14	0.86	0-12	0	0	0
Aspergillus spp.	0	0	0	0	0	0	7.14	0.29	0-4	0	0	0
Bipolaris spp.	8.33	0.17	0-2	0	0	0	0	0	0	0	0	0
Black Moulds	100	17.67	0 - 50	50	8	0-16	21.43	1.43	0-12	10	0.20	0-2
Cladosporium spp.	0	0	0	0	0	0	0	0	0	6.67	0.13	0-2
Curvularia spp.	75	7	0-28	0	0	0	21.43	0.43	0-2	0	0	0
E. chevalieri	0	0	0	50	42	0-84	28.57	5.29	0-38	30	1.80	0-20
E. rubrum	0	0	0	0	0	0	0	0	0	3.33	0.07	0-2
Fusarium spp.	100	47.33	12-84	50	27	0-54	21.43	5.29	0-54	3.33	0.07	0-2
Hyphomycetes	66.67	8.5	0-44	50	4	0-8	7.14	2.14	0-18	3.33	0.07	0-2
Yeast	0	0	0	0	0	0	7.14	0.14	0-2	6.67	0.13	0-2
Filamentous Yeast	0	0	0	50	2	0-4	0	0	0	6.67	0.13	0-2
Monascus ruber	0	0	0	0	0	0	0	0	0	3.33	0.07	0-2
Nigrospora oryzae	75	10.83	0-50	50	1	0-2	14.29	0.86	0-10	0	0	0
P. citrinum	0	0	0	0	0	0	0	0	0	3.33	0.07	0-2
P. digitatum	0	0	0	0	0	0	7.14	0.14	0-2	0	0	0
P. restrictum	0	0	0	0	0	0	0	0	0	3.33	0.07	0-2
Pestalotiopsis spp.	0	0	0	50	2	0-4	7.14	0.29	0-4	0	0	0
Phoma spp.	25	1.17	0-6	50	4	0-8	0	0	0	0	0	0
Rhizopus spp.	0	0	0	0	0	0	21.43	1.57	0-10	3.33	0.07	0-2
Syncephalastrum racemosum	0	0	0	0	0	0	7.14	0.29	0-4	3.33	0.07	0-2
Wallemia sebi	0	0	0	0	0	0	0	0	0	3.33	0.33	0-10

FO = Frequency of occurrence % (number of samples that contained a fungal species/total of samples evaluated; AI = Average of infection % (sum of infection level/total number of samples); RI = Range of Infection % (range of infected grains in a sample).

Table 4: Number of samples, average of water activity, frequency of occurrence, average and range of infection in rice samples from São Paulo (SP).

Fungi (number of samples)	Market (54)		
Mean a_w (range)	0.599 (0.474-0.696)		
Fungi	FO(%)	AI(%)	RI(%)
A. candidus	14.81	1.48	0-18
A. clavatus	3.70	0.07	0-2
A. penicillioides	1.85	0.04	0-2
A. restrictus	5.56	0.19	0-6
A. section *Flavi*	20.37	1.19	0-18
A. section *Nigri*	3.70	0.15	0-6
A. sydowii	1.85	0.04	0-2
A. terreus	1.85	0.04	0-2
A. ustus	1.85	0.04	0-2
A. versicolor	1.85	0.04	0-2
A. westerdijkiae	1.85	0.04	0-2
Alternaria spp.	7.41	0.19	0-4
Aspergillus spp.	3.70	0.11	0-4
Bipolaris spp.	1.85	0.19	0-10
Black Moulds	12.96	0.96	0-14
Curvularia spp.	12.96	0.59	0-14
E. amstelodami	7.41	0.41	0-10
E. chevalieri	31.48	5.26	0-100
E. herbariorum	5.56	0.22	0-8
E. repens	1.85	0.33	0-18
E. rubrum	12.96	0.70	0-14
Fusarium spp.	3.70	0.07	0-2
Hyphomycetes	16.67	1.48	0-30
Monascus ruber	1.85	0.37	0-20
Nigrospora oryzae	1.85	0.04	0-2
P. citrinum	1.85	0.04	0-2
P. paxilii	1.85	0.11	0-6
Penicillium spp.	1.85	0.04	0-2
Phoma spp.	3.70	0.07	0-2
Syncephalastrum racemosum	3.70	0.07	0-2

FO = Frequency of occurrence % (number of samples that contained a fungal species/total of samples evaluated; AI = Average of infection % (sum of infection level/total number of samples); RI = Range of Infection % (range of infected grains in a sample).

Table 5: Isolated species from soil samples and the number of samples in which they were present.

State (number of samples) Fungal species	Wetland(12)	Dryland (11)
Aspergillus section *Flavi*	3/12[a]	9/11
Aspergillus section *Nigri*	-	8/11
Aspergillus tamarii	-	1/11
Aspergillus terreus	1/12	6/11
Absidia spp.	1/12	-
Botrytis cinerea	1/12	-
Cladosporium spp.	5/12	-
Eupenicillium cinnamopurpureum	1/12	-
Eupenicillium ludwigii	1/12	-
Black Moulds	-	1/11
Fusarium spp.	4/12	1/11
Hyphomycetes	1/12	2/11
Yeast	3/12	-
Filamentous yeast	1/12	1/11
Monascus ruber	1/12	-
Mucor spp.	1/12	-
Penicillium janthinellum	1/12	-
Penicillium oxalicum	1/12	-
Paecilomyces lilacinus	3/12	-
Penicillium citrinum	-	4/11
Penicillium cluniae	-	1/11
Penicillium zoratum	-	1/11
Penicillium spp.	11/12	2/11
Pestalotiopsis spp.	1/12	-
Talaromyces spp.	1/12	5/11
Trichoderma spp.	1/12	1/11

[a] = number of samples with fungi/total number of samples evaluated

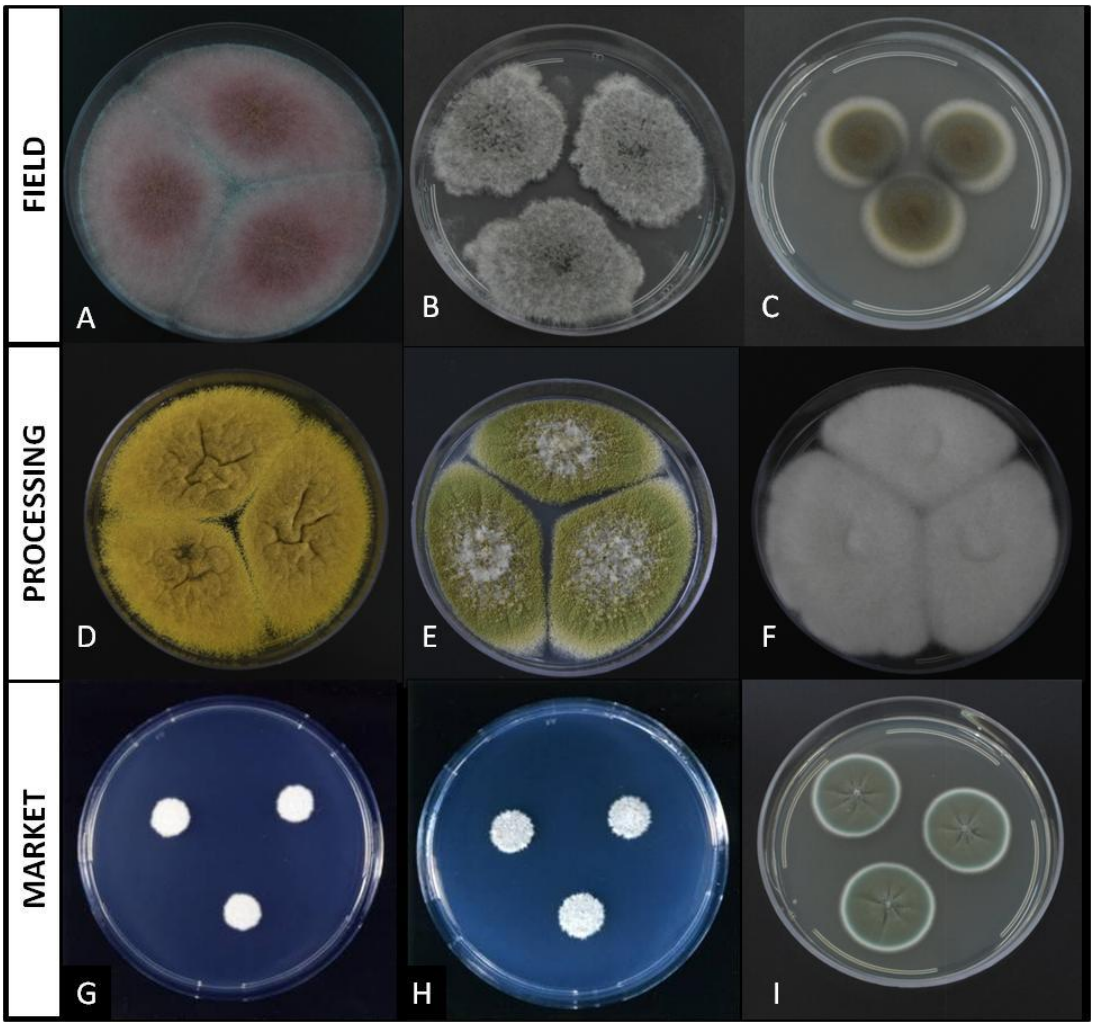

Figure 1. Main isolated fungi from field, processing and market: A. *Fusarium* spp.; B. *Nigrospora oryzae*; C. *Alternaria* spp.; D. *Eurotium chevalieri*; E. *Aspergillus* section *Flavi* F. *Fusarium* sp.; G. *Aspergillus candidus*; H. *Aspergillus restrictus*; I. *Penicillium citrinum*; A, B, C, E, F, and I: CYA/25°C/7 days; D: CY20S/25°C/14 days; G and H: CYA/25°C/14 days.

CAPÍTULO II

Occurrence of *Aspergillus* section *Flavi* and aflatoxins in Brazilian rice: from field to market

Abstract

The guarantee of the quality of rice is of utmost importance because any contaminant may affect consumer health, especially in countries such as Brazil where rice is part of the daily diet. A total of 187 rice samples, from field, processing and market from two different production systems, dryland (state of Maranhão-MA) and wetland (state of Rio Grande do Sul-RS), and market samples from the state of São Paulo (SP), were analyzed for fungi belonging to *A.* section *Flavi* and the presence of aflatoxins. Twenty-three soil samples from MA and RS were also analyzed. A total of 383 *Aspergillus* section *Flavi* were isolated; 39 aflatoxin B producers (34 from MA soil, 1 from MA bran, 3 from SP red rice and 1 from SP brown rice) and 5 aflatoxin B and G producers (1 from RS soil, 3 from MA soil and 1 from SP black rice). Only 13.37% of rice samples were contaminated with aflatoxins while 2 samples were above the maximum tolerable limit (5µg/kg), established by the National Health Surveillance Agency (ANVISA).

1. Introduction

Brazil is the ninth biggest rice producer in the world and the largest outside Asia (FAO, 2016). There are two production systems in Brazil, the wetland system, prevalent in the South region, and the dryland system, prevalent in the Northeast region. In the wetland system, the rice is irrigated by controlled flooding and in the dryland system, there are two processes: one that occurs without irrigation and depends totally on rainfall and the other on supplementary irrigation that decreases the drought stress, providing higher grain quality.

Aflatoxins are toxic metabolites. The analogue B_1 is classified by the International Agency of Research on Cancer (IARC, 1993) as class 1, a carcinogenic compound for humans in which the liver is the main affected organ after ingestion. The resolution RDC 07/2011 of the National Health Surveillance Agency (ANVISA, 2011) determines the maximum tolerable limit of 5µg/kg of aflatoxins in cereals including rice.

Rice is an excellent substrate for fungal growth and mycotoxin production (Kjer et al., 2010). There are some reports about *Aspergillus* section *Flavi* and aflatoxins in rice in several countries such as Brazil (Silva et al., 2008; Dors et al., 2009; Carvalho et al., 2010; Almeida et al., 2012; Beber-Rodrigues & Scussel,

2013), South Korea (Park et al., 2005; Ok et al., 2014), India (Toteja et al, 2006; Jayaraman & Kalyanasundaram, 2009; Reddy et al., 2009), Uganda (Taligoola et al., 2011), Philippines (Sales & Yoshizawa, 2005), Nigeria (Makun et al., 2011) and Turkey (Aydin et al., 2011), but some studies have used inadequate fungal isolation methods, such as plate dilution or direct plating without surface disinfection, resulting only in data on the surface contamination of rice grains (Fredlund et al., 2009; Aydin et al., 2011) and methods for aflatoxin determination with a high limit of detection such as thin layer chromatography (TLC), with a limit of detection (LOD) of total aflatoxins ≤ 2.0 µg/kg (Dors et al., 2009; Jayaraman & Kalyanasundaram, 2009; Beber-Rodrigues & Scussel, 2013) and enzyme-linked immunosorbent assay (ELISA) with LOD of 2.0µg/kg of AFB_1 (Reddy et al., 2009). A review on the occurrence of aflatoxigenic fungi and aflatoxins in rice from different parts of the world has been revised elsewhere (Katsurayama & Taniwaki, 2017).

Thus, the aims of this study were to analyze the presence of aflatoxin producing fungi, to optimize the methodology of aflatoxin determination and investigate the presence of aflatoxins in field, drying, storage, processing and market rice samples from two different production systems: dryland, in the state of Maranhão (MA), Northeast region, and wetland in the state of Rio Grande do Sul (RS), South region. Market samples from São Paulo (SP) were also analyzed. Soil samples from MA and RS were also analyzed for the presence of aflatoxin producing fungi.

2. Materials and methods

2.1.Samples

A total of 187 rice samples each of approximately 1kg was collected in dryland Maranhão (MA) where the latitude is 05º31'35"S and longitude is 47º 29' 30" W, and wetland in Rio Grande do Sul (RS) with latitude of 30º51'04"S and longitude of 51º48'44"W, at the stages of field, processing (paddy rice, husk, husked rice, bran, broken, brown, polished, parboiled and red rice) and market (polished, brown, parboiled, flake, flour, red and brown with red rice). Only two samples from drying yard were collected in Maranhão because most of the rice is

dried in mechanical dryers, in processing plants. In São Paulo state (SP), samples were collected from markets (polished, brown, parboiled, flake, flour, red, brown with red and black rice), since this state does not grow rice but is a high rice consumer. Twenty-three soil samples from the states of Maranhão and Rio Grande do Sul were collected. Table 1 indicates the amount from each step and the states from where the samples were collected.

2.2 Water activity

The water activity was determined using an Aqualab Series 3TE instrument (Decagon, Pullman, Wa, USA) at 25°C±1, in triplicate.

2.3 Fungal isolation

Each sample of approximately 100 g was surface-disinfected with sodium hypochlorite solution (0.4%) for 1 min. Fifty grains were distributed in 5 dishes (10 grains per dish) containing Dichloran (18%) Glycerol Agar (DG18) and incubated at 25°C for 5 to 7 days, according to Pitt & Hocking (2009). Bran, flour and soil samples were analyzed by serial dilution plating technique, 25g of each sample was diluted in 225 ml of peptone water 0.1%. An aliquot of 0.1mL was inoculated onto DG18 plates and incubated at 25°C for 5 to 7 days (Pitt & Hocking, 2009).

2.4 Fungi identification and toxigenic capacity analysis

Isolates that had the appearance of belonging to *Aspergillus* section *Flavi* were isolated in Czapek Yeast Extract Agar (CYA) and incubated at 25ºC for 7 days. Isolates were then examined on standard identification media for *Aspergillus* species CYA, at 25ºC and 37°C, Malt Extract Agar (MEA) and *Aspergillus flavus-parasiticus* Agar (AFPA), at 25°C (Pitt & Hocking, 2009). The incubation time for all media and conditions was 7 days. Although Pitt & Hocking (2009) recommended incubation of AFPA at 30ºC for 48h, in the present study, AFPA was incubated in the same way as the other media, as AFPA was used for identification, not isolation.

2.5 Potential for aflatoxin production by Aspergillus section Flavi isolates

Fungi identified as potential producers of aflatoxins were inoculated onto yeast extract sucrose agar (YESA) for 7 days at 25ºC and then the agar plug technique (Filtenborg et al., 1983) was used to evaluate the capability of isolates to produce aflatoxins. Fungal extracts taken as plugs with a cork borer were placed on thin layer chromatography (TLC) plates, developed in a toluene: ethyl acetate: formic acid 90%: chloroform (7:5:2:5, v/v/v/v) mobile phase, and visualized under UV light at 365 nm. A mixture of standard preparations of aflatoxins B_1, B_2, G_1 and G_2 (Sigma Aldrich, St. Louis, MO, USA) was used for comparison.

2.6 Aflatoxin determination in raw rice

Aflatoxin analysis was carried out on rice, based on the method of Stroka et al. (2000) as follows.

2.6.1 Clean-up

Approximately 200g of rice was finely ground using a laboratory mill (IKA A11 basic, Brazil), and passed through a sieve of 18 mesh (1.0mm). An aliquot of 25g was taken and extracted with 100 mL of methanol solution: water (8: 2, v/v) added with 2.5 g of NaCl and homogenized in a shaker for 30 minutes. The solution was first filtered using a quantitative filter (Nalgon, Germany) and a glass microfiber filter (VICAM, USA). The filtrate (10 mL) was diluted in 60 mL of phosphate buffered saline (PBS) and was applied to an immunoaffinity column for aflatoxins (R-Biopharm Rhône Ltd, UK) at a flow rate of 2-3 ml/min. The column was then washed with distilled water (30 mL) and aflatoxins eluted with methanol (1250 µL) and diluted with milli Q water (1750 µL). This method was used for polished rice, paddy rice, rice flakes, black rice, brown rice and parboiled rice samples. For red rice, rice flour and bran rice samples, the PBS was replaced by PBS tween (0.01%) and the elution was carried out with 2mL of methanol-HPLC and 1mL of milli Q water. For red+brown rice mix and rice husk, 150mL of methanol:water (8:2) for extraction, PBS tween instead of PBS (0.01%) was used, and eluted with 2mL of methanol, dried in nitrogen flow and re-suspended with 3mL of methanol:water (2:3, v.v).

2.6.2 Chromatographic conditions

An Agilent 1260 Infinity model system (Agilent, USA) of High Performance Liquid Chromatography (HPLC) was used, with a fluorescence detector set at 362 nm excitation and 455 nm emission for aflatoxins G_1 and G_2 and 425 nm emission for aflatoxins B_1 and B_2. An ODS (1.8µm, 40x15mm) guard column and a Zorbax Eclipse Plus C18 column (5 um, 4.6 x 150 mm) were employed. The mobile phase was water:acetonitrile:methanol (6:2:3, v/v/v), containing KBr (119 mg) and nitric acid (4M, 350 µL/L) at a flow rate of 1mL/min with injection volume of 20 µL. A post-column derivatization of aflatoxins B_1 and G_1 was performed with bromine using a KobraCell (R-Biopharm Rhône Ltd, UK).

Aflatoxin B_1, B_2, G_1 and G_2 standard (Sigma, USA) curves were prepared for quantification. The concentration of aflatoxins in the sample was determined by interpolation of the resulting peak area of each standard curve.

2.6.3 Methodology validation

The methodology validation of the method described above was carried out considering the recovery, detection and quantification limits following the Eurachem Guides (1998).

In a rice sample free of aflatoxins, a spike of 0.5µg/kg of total aflatoxins was added. Eight parallel extractions were performed to calculate the recovery and the standard deviation. The limit of detection (LOD) was determined according to Eurachem's formula (1998):

$$s_0' = \frac{s_0}{\sqrt{n}}$$

Where: s_0 estimated standard deviation of the number of replicates

s_0' standard deviation to calculate the LOD and limit of quantification (LOQ)

n number of repetitions

To calculate the LOD, s_0' value was multiplied by 3 and for LOQ by 10.

Besides that, in a rice sample free of aflatoxins, a spike of 5µg/kg and 20µg/kg was added. Three parallel extractions of each level were performed to calculate the recovery.

3. Results

3.1 Aspergillus section Flavi infection and water activity of rice samples

Water activity of rice samples at different stages (field, drying, processing and markets) in dryland system, wetland system and São Paulo (SP) is shown in Table 2. Wetland field samples showed higher water activity than dryland field samples, processing samples had similar water activity in both systems, wetland market samples showed higher water activity than dryland and SP market samples. *Aspergillus* section *Flavi* infection of these samples is shown in Table 3. Data on bran, flour and flake are shown in Table 4. In wetland field samples, 7 of 12 (58.33%) samples showed *A.* section *Flavi* and only 1 of 12 (8.33%) samples from dryland field had *A.* section *Flavi* occurrence. Samples from drying in dryland were not infected with *A.* section *Flavi*. In wetland processing samples, 17 of 38 samples (44.7%) had *A.* section *Flavi.* In dryland processing samples 4 of 14 samples (28.57%) had *A.* section *Flavi* occurrence. The occurrence of *A.* section *Flavi* in market samples was 1 of 30 (3.33%) dryland samples, 11 of 54 (20.37%) SP samples and none of the wetland samples showed *A.* section *Flavi* infection.

A total of 383 *A.* section *Flavi* were isolated; 44 were aflatoxin producers, 39 aflatoxin B (34 from dryland soil, 1 from dryland bran, 3 from SP red rice and 1 from SP brown rice) and 5 aflatoxin B and G (1 from wetland soil, 3 from dryland soil and 1 from SP black rice). Table 5 indicates the distribution of the isolates of *Aspergillus* section *Flavi* at all the steps of the rice production.

There was high occurrence of *A.* section *Flavi* in field samples (182 isolates) in wetland, but in dryland there was only 1 isolate. All the isolates from both states did not produce aflatoxins. In processing, wetland and dryland samples showed 70 and 44 isolates, respectively. All the isolates except for one isolate from dryland bran sample were negative for aflatoxin production. In market samples, dryland had only 3 isolates in the same polished rice sample and SP showed 27 isolates in samples of polished, parboiled, red, black and brown rice. Only five isolates were positive for aflatoxin production, all from SP samples, 4 isolates were aflatoxin B producers (3 from red rice and 1 from brown rice) and 1 isolate was an aflatoxins B and G producer from black rice.

3.2 Fungal infection in soil samples

Table 4 shows the infection of soil by *A.* section *Flavi* in dryland and wetland.

The dryland rice soil had high occurrence of *A.* section *Flavi* and the majority were aflatoxin producers (37 isolates aflatoxin B producers, 3 isolates aflatoxins B and G producers and 10 no producers), while wetland soil samples had only 3 isolates (2 negative and 1 aflatoxin B and G producer).

3.3 Aflatoxin analysis

The presence of aflatoxins from field to processing and from market is shown in Tables 6 and 7, respectively. The detection and quantification limit for aflatoxin B_1, B_2, G_1 and G_2 were 0.016, 0.012, 0.011 and 0.004µg/kg and 0.054, 0.039, 0.038 and 0.012µg/kg, respectively. The recovery of aflatoxins percentage of samples contaminated with 0.5µg/kg was 81.6%. The mean values of contamination were presented as lower bound (Lb), where the values below the limit of detection (LOD) were replaced by zero and upper bound (Ub) where the values were replaced by LOD divided by 2. The occurrence of aflatoxin in samples was low as only 25 from 187 were positive (13.37%). Aflatoxins were absent in field samples from Maranhão.

The average of contamination of wetland field samples was 0.35µg/kg, of dryland drying 0.23µg/kg, of all wetland processing was 0.04µg/kg, of all dryland processing 0.05µg/kg, of all wetland market was 0.01µg/kg, of all dryland market 0.10µg/kg and of all SP market was 1.89µg/kg. The wetland field, dryland drying, wetland processing, wetland and dryland market samples showed only aflatoxin B, while dryland processing and SP market samples showed B and G contamination.

The highest concentration of total aflatoxin was found in a SP market sample of red rice with 70.91µg/kg, followed by another market sample of red rice in SP, with 23.37µg/kg. Both values are higher than the maximum tolerable level of aflatoxins in cereals established by ANVISA (5µg/kg). The other 23 samples had levels between 0.04µg/kg and 4.93µg/kg, below the tolerable limit determined by ANVISA. The following types of rice did not show aflatoxin occurrence: paddy rice, husk, husked rice, polished from wetland and dryland processing, brown, red and parboiled rice from wetland processing, brown and flour rice from wetland and

dryland market, red and flake rice form wetland market, polished rice from wetland and SP market and parboiled rice from dryland and SP market.

4. Discussion

The rice cultivated using the irrigation system in the first stages of development shows low levels of *Aspergillus flavus* in soil, with low contamination of mature grains and there is low contamination in the final product, but if the grains are collected wet and then dried, the infection by *A. flavus* becomes more probable (Pitt et al., 2013). This is the case of Rio Grande do Sul region, where the production system is the wetland. In the present study, the wetland soil samples had low *A.* section *Flavi* contamination and the field samples had contamination with low levels of aflatoxins. In the dryland production system, the seeds are cultivated when the rain becomes more regular, so the soil is not flooded as in the wetland system and fungi can develop. Dryland soil samples showed high contamination by *A.* section *Flavi* but the field had low contamination, with levels of aflatoxins below the limit of detection. These data may indicate that wetland field samples were collected wet and the dried and dryland field samples were collected at the correct moment. There are no previous reports on rice soil. In South Korea, where the wetland system predominates, in Ok et al. (2013) only one non-toxigenic *Aspergillus flavus* was found in 80 fresh harvest samples. But in India, where the dryland system predominates, Reddy et al. (2009) reported the occurrence of *A. flavus* in all the 675 paddy rice samples analyzed.

Drying and storage are stages that require attention because if the drying is slow or the storage is poor, fungi can develop and produce mycotoxins (Pitt et al., 2013). In this study, dryland drying samples did not show *A.* section *Flavi* and one sample had a low level of aflatoxins (0.45µg/kg); 7 (53.8%) wetland paddy rice samples showed *A.* section *Flavi* but none was an aflatoxin producer and only one (7.69%) sample had a low level of aflatoxins. These data indicate that drying and storage were carried out in a safe way.

At the processing stage there is evidence that the milling process can decrease the contamination by aflatoxins. Sales & Yoshizawa (2005) observed a reduction of 78% of aflatoxins from brown rice to polished rice and a reduction of

38% of aflatoxins from milled rice to well-milled rice. The milling process removes the bran from rice and in our study bran samples from both states had aflatoxins but in low quantities. Trucksess et al. (2011) carried out a study on aflatoxins during shelling and milling and noticed that the bran was the fraction with the highest contamination (357µg/kg), followed by brown rice (158µg/kg), paddy rice (114µg/kg), polished rice (56µg/kg) and husk (39µg/kg). The presence of *A.* section *Flavi* at milling was low in our study while the types of samples with higher contamination were paddy rice, husk and bran rice. However Sales & Yoshizawa (2005) in the Philippines reported *A.* section *Flavi* in brown and polished rice during the milling process.

Polished, brown and parboiled rice are the market types of rice most analyzed by previous researchers and had low or absence of contamination by *A.* section *Flavi* (Ok et al., 2014; Taligoola et al., 2011; Carvalho et al., 2010). In our study, black and red rice showed higher contamination by *A.* section *Flavi*. There are some reports about aflatoxin contamination in polished rice that show high levels such as 176.31µg/kg (Almeida et al., 2012), 50µg/kg (Taligoola et al., 2011) and reports that show low levels such as 2.04µg/kg (Silva et al., 2008) and 1.2µg/kg (Carvalho et al., 2010). Brown rice has shown low levels such as 2.7µg/kg (Ok et al., 2014) and parboiled rice high levels such as 30µg/kg (Toteja et al., 2006) and 74µg/kg (Dors et al., 2009). In the present study, the highest levels were from red rice, 23.37µg/kg and 70.91µg/kg. Polished, parboiled, brown, brown with red, flake, flour, and black rice had levels lower than the LOD to 4.93µg/kg.

5. Conclusion

The present research revealed the low occurrence of aflatoxin-producing fungi in Brazilian rice. The most analyzed types of rice from market are polished, brown and parboiled rice because they are the most consumed by the world's population. In this study, flake, flour, red and black rice were analyzed and the type that presented higher contamination by aflatoxins was the red rice, with levels above the maximum tolerable limit established by ANVISA. However, this type of rice is not commonly consumed in Brazil. These data are of concern for those who

include this type of rice in their diet. Polished rice, brown rice and parboiled rice are the main types of rice consumed in Brazil and all the samples of these types of rice had limits below the maximum tolerable limit.

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CAPÍTULO II

Occurrence of *Aspergillus* section *Flavi* and aflatoxins in Brazilian rice: from field to market

(Tables)

Table 1. Number of rice and soil samples, collected from 3 Brazilian states: Maranhão (dryland), Rio Grande do Sul (wetland) and São Paulo (SP).

Stages	Type of rice	Number of samples		
		Wetland	Dryland	SP
Field	Paddy rice	12	12	-
Drying	Paddy rice	-	2	-
Processing	Paddy Rice	13	2	-
	Husk	4	1	-
	Husked rice	2	2	-
	Bran	4	2	-
	Broken rice	-	-	-
	Brown Rice	4	-	-
	Polished Rice	4	3	-
	Parboiled Rice	5	-	-
	Red rice	2	-	-
Market	Polished	6	19	10
	Brown	12	2	19
	Parboiled	1	6	4
	Flake	1	2	-
	Flour	1	1	-
	Red rice	1	-	10
	Brown with red	3	-	3
	Black rice	-	-	8
Soil	-	12	11	-

Table 2. Water activity of rice samples at different stages (field, drying, processing and markets) in Maranhão (dryland), Rio Grande do Sul (wetland) and São Paulo (SP).

Stage	State	Mean a_w (range)
Field	Wetland	0.932 (0.894-0.973)
	Dryland	0.825 (0.770 – 0.953)
Drying	Dryland	0.708 (0.596 – 0.821)
Processing	Wetland	0.614 (0.465-0.865)
	Dryland	0.665(0.607-0.799)
Market	Wetland	0.693 (0.503-0.719)
	SP	0.599 (0,474-0.696)
	Dryland	0.603 (0.509-0.694)

Table 3. Percent of infection of rice samples by *Aspergillus* section *Flavi,* at different processing stages (fields, processing and markets) in three Brazilian regions: Maranhão (Dryland), Rio Grande do Sul (Wetland) and São Paulo (SP).

Processing Stage	Dryland		Wetland		SP	
	Number of samples/ Number of positive samples	Range of infection (%)	Number of samples/ Number of positive samples	Range of infection (%)	Number of samples/ Number of positive samples	Range of infection (%)
Field	12/1	0 – 2	12/7	24 - 100	-	-
Drying	2/0	0	-	-	-	-
Processing						
Paddy Rice	2/1	0 – 22	13/7	0 - 38	-	-
Husk	1/1	0 - 28	4/3	0 - 58	-	-
Husked rice	2/0	0	2/1	0 - 2	-	-
Broken rice	4/0	0	-	-	-	-
Brown rice	-	-	4/1	0 - 12	-	-
Polished rice	3/0	0	4/1	0 - 2	-	-
Parboiled rice	-	-	5/1	0 - 2	-	-
Red rice	-	-	2/1	0 - 2	-	-
Market						
Polished	19/1	0 - 6	6/0	0	10/0	0
Brown	2/0	0	12/0	0	19/2	0 - 2
Parboiled	6/0	0	1/0	0	4/2	0 - 4
Red rice	-	-	1/0	0	10/3	0 - 18
Brown with red	-	-	3/0	0	3/0	0
Black rice	-	-	-	-	8/4	0 - 10

Table 4. Infection of rice by *Aspergillus* section *Flavi* in soil, bran, flake and flour samples in Dryland and Wetland

Stage	Type of rice	Dryland		Wetland	
		Number of samples/ Number of positive samples	Range of infection (CFU/g)	Number of samples/ Number of positive samples	Range of infection (CFU/g)
Soil	-	11/9	<100 - 2x10³	12/3	<100 - 2x10³
Processing	Bran	2/2	<100 - 2x10³	4/2	<100 - 3x10³
Market	Flake	2/0	0	1/0	0
	Flour	1/0	0	1/0	0

Table 5. Distribution of *Aspergillus* section *Flavi* in the rice and soil samples

Stage	System (number of isolates)	Negatives	AFB +	AFB AFBG +
Soil	Wetland (3)	2	-	1
	Dryland (47)	10	34	3
Field	Wetland (182)	182	-	-
	Dryland (1)	1	-	-
Processing	Wetland (70)	70	-	-
	Dryland (45)	44	1	-
Market	Dryland (3)	3	-	-
	SP (32)	27	4	1

Table 6. Aflatoxins incidence (µg/kg) in rice from field to processing samples in wetland and dryland

State	Wetland					Dryland				
Stage (number of samples)	Field (12)					Field (12)				
	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins
mean (Lb)	0.337	0.011	<LOD	<LOD	0.349	<LOD	<LOD	<LOD	<LOD	<LOD
mean (Ub)	0.343	0.016	0.006	0.002	0.374	0.008	0.006	0.006	0.002	0.02
Median	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Range	<LOD - 2.826	<LOD - 0.085	<LOD	<LOD	<LOD - 2.95	<LOD	<LOD	<LOD	<LOD	<LOD
No of positive samples	3 (25%)	2 (16.7%)	0	0	3 (25%)	0	0	0	0	0
Stage (number of samples)						Drying (2)				
						Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins
mean (Lb)						0.224	<LOD	<LOD	<LOD	0.225
mean (Ub)						0.228	0.006	0.006	0.002	0.235
Median			-			0.224	<LOD	<LOD	<LOD	0.225
Range						<LOD - 0.448	<LOD	<LOD	<LOD	<LOD - 0.45
No of positive samples						1 (50%)	0	0	0	1 (50%)
Stage (number of samples)	Processing - Paddy Rice (13)					Processing - Paddy Rice (2)				
	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins
mean (Lb)	0.042	<LOD	<LOD	<LOD	0.046	<LOD	<LOD	<LOD	<LOD	<LOD
mean (Ub)	0.050	0.006	0.006	0.002	0.065	0.008	0.006	0.006	0.002	0.02
Median	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Range	<LOD - 0.551	<LOD	<LOD	<LOD	<LOD - 0.60	<LOD	<LOD	<LOD	<LOD	<LOD
No of positive samples	1 (7.69%)	0	0	0	1 (7.69%)	0	0	0	0	0
Stage (number of samples)	Processing - Bran (4)					Processing - Bran (2)				
	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins
mean (Lb)	0.164	0.012	<LOD	<LOD	0.175	0.167	<LOD	0.007	<LOD	0.175
mean (Ub)	0.17	0.122	0.006	0.002	0.19	0.167	0.006	0.007	0.002	0.175
Median	<LOD	<LOD	<LOD	<LOD	<LOD	0.167	<LOD	0.007	<LOD	0.175
Range	<LOD - 0.656	<LOD - 0.047	<LOD	<LOD	<LOD - 0.70	0.101 - 0.232	<LOD	<LOD - 0.014	<LOD	0.10 - 0.25
No of positive samples	1 (25%)	1 (25%)	0	0	1 (25%)	2 (100%)	0	1 (50%)	0	2 (100%)
Stage (number of samples)						Processing - Broken rice (4)				
						Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins
mean (Lb)						0.084	<LOD	<LOD	<LOD	0.084
mean (Ub)						0.088	0.006	0.006	0.002	0.094
Median			-			0.121	<LOD	<LOD	<LOD	0.12
Range						<LOD – 0.23	<LOD	<LOD	<LOD	<LOD – 0.23
No of positive samples						2 (50%)	0	0	0	2 (50%)

Table 7. Aflatoxins incidence (µg/kg) in rice from market samples in wetland, dryland and São Paulo (SP).

State	Wetland					Dryland					SP				
Type of rice (number of samples)	Polished (6)					Polished (19)					Polished (10)				
	AFB_1	AFB_2	AFG_1	AFG_2	AFT	AFB_1	AFB_2	AFG_1	AFG_2	AFT	AFB_1	AFB_2	AFG_1	AFG_2	AFT
mean (Lb)	<LOD	<LOD	<LOD	<LOD	<LOD	0.136	0.007	<LOD	<LOD	0.143	<LOD	<LOD	<LOD	<LOD	<LOD
mean (Ub)	0.008	0.006	0.006	0.002	0.02	0.142	0.012	0.006	0.002	0.157	0.008	0.006	0.006	0.002	0.02
median	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Range	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD - 1.463	<LOD - 0.128	<LOD	<LOD	<LOD - 1.59	<LOD	<LOD	<LOD	<LOD	<LOD
No of positive samples	0	0	0	0	0	5 (26.32%)	1 (5.26%)	0	0	5 (26.32%)	0	0	0	0	0
Type of rice (number of samples)	Brown (2)					Brown (2)					Brown (19)				
	AFB_1	AFB_2	AFG_1	AFG_2	AFT	AFB_1	AFB_2	AFG_1	AFG_2	AFT	AFB_1	AFB_2	AFG_1	AFG_2	AFT
mean (Lb)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.004	<LOD	<LOD	<LOD	0.004
mean (Ub)	0.008	0.006	0.006	0.002	0.02	0.008	0.006	0.006	0.002	0.02	0.011	0.006	0.006	0.002	0.023
median	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Range	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD - 0.069	<LOD	<LOD	<LOD	<LOD - 0.069
No of positive samples	0	0	0	0	0	0	0	0	0	0	1 (5.26%)	0	0	0	1 (5.26%)
Type of rice (number of samples)	Parboiled (1)					Parboiled (6)					Parboiled (4)				
	AFB_1	AFB_2	AFG_1	AFG_2	AFT	AFB_1	AFB_2	AFG_1	AFG_2	AFT	AFB_1	AFB_2	AFG_1	AFG_2	AFT
mean (Lb)	0.102	<LOD	<LOD	<LOD	0.102	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
mean (Ub)	0.102	0.012	0.11	0.004	0.102	0.008	0.006	0.006	0.002	0.02	0.008	0.006	0.006	0.002	0.02
median	0.051	<LOD	<LOD	<LOD	0.051	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Range	0.102	<LOD	<LOD	<LOD	0.102	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
No of positive samples	1 (100%)	0	0	0	1 (100%)	0	0	0	0	0	0	0	0	0	0
Type of rice (number of samples)	Flake (1)					Flake (2)									
	AFB_1	AFB_2	AFG_1	AFG_2	AFT	AFB_1	AFB_2	AFG_1	AFG_2	AFT					
mean (Lb)	<LOD	<LOD	<LOD	<LOD	<LOD	0.108	<LOD	<LOD	<LOD	0.108					
mean (Ub)	0.008	0.006	0.006	0.002	0.02	0.058	0.006	0.006	0.002	0.118					
median	<LOD	<LOD	<LOD	<LOD	<LOD	0.108	<LOD	<LOD	<LOD	0.108			-		
Range	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD - 0.215	<LOD	<LOD	<LOD	<LOD - 0.215					
No of positive samples	0	0	0	0	0	1 (50%)	0	0	0	1 (50%)					
Type of rice (number of samples)	Red Rice (1)										Red Rice (10)				
	AFB_1	AFB_2	AFG_1	AFG_2	AFT						AFB_1	AFB_2	AFG_1	AFG_2	AFT
mean (Lb)	<LOD	<LOD	<LOD	<LOD	<LOD						8.440	1.078	<LOD	<LOD	9.428
mean (Ub)	0.008	0.006	0.006	0.002	0.02						8.446	1.093	0.006	0.002	9.444
median	<LOD	<LOD	<LOD	<LOD	<LOD			-			<LOD	<LOD	<LOD	<LOD	<LOD
Range	<LOD	<LOD	<LOD	<LOD	<LOD						<LOD - 63.32	<LOD - 8.591	<LOD	<LOD	<LOD - 70.91
No of positive samples	0	0	0	0	0						2 (20%)	2 (20%)	0	0	2 (20%)
Type of rice (number of samples)	Brown w/ red (3)										Brown w/ red (3)				
	AFB_1	AFB_2	AFG_1	AFG_2	AFT						AFB_1	AFB_2	AFG_1	AFG_2	AFT
mean (Lb)	0.098	<LOD	<LOD	<LOD	0.098						2.111	0.358	<LOD	<LOD	2.467
mean (Ub)	0.100	0.006	0.006	0.002	0.101						2.114	0.360	0.006	0.002	2.473
median	<LOD	<LOD	<LOD	<LOD	<LOD			-			2.243	0.23	<LOD	<LOD	2.47
Range	<LOD - 0.283	<LOD	<LOD	<LOD	<LOD - 0.283						<LOD - 4.09	<LOD - 0.845	<LOD	<LOD	<LOD - 4.93
No of positive samples	1 (33.3%)	0	0	0	1 (33.3%)						2 (66.67%)	2 (66.67%)	0	0	2 (66.67%)
Type of rice (number of samples)											Black Rice (8)				
											AFB_1	AFB_2	AFG_1	AFG_2	AFT
mean (Lb)											0.011	<LOD	0.047	<LOD	0.059
mean (Ub)											0.018	0.006	0.050	0.002	0.069
median			-					-			<LOD	<LOD	0.067	<LOD	0.067
Range											<LOD - 0.09	<LOD	<LOD - 0.133	<LOD	<LOD - 0.133
No of positive samples											1 (12.5%)	0	4 (50%)	0	4 (50%)

CAPÍTULO III

Reduction of aflatoxin B in rice after cooking and the aflatoxin intake

Abstract

Rice is one of the most consumed grains in the world. It can be contaminated with mycotoxins such as aflatoxins. The present study analyzed the effect of cooking to reduce the level of aflatoxin B_1, the aflatoxin intake and the margin of exposure to the Brazilian population. A sample of polished raw rice was inoculated with *Aspergillus flavus* producing aflatoxin B_1 and B_2 and placed under favorable conditions to produce the toxin. Two levels of aflatoxin B_1 + B_2, 2µg/kg and 20µg/kg, were tested and the average of reduction after a common Brazilian home cooking process was 77.17% and 77.67%, respectively. The aflatoxin intake by rice consumption ranged from 0.016 to 4.862 ng/kg bw/day and the margin of exposure (MOE) ranged from 346,939 to 10,625,000 indicating a low concern from the public health point of view.

1. Introduction

Rice has an important economic and social function, being one of the most consumed foods. The world average consumption is 60kg/person/year. In Latin America an average of 30kg/person/year is consumed with Brazil being the major consumer with an average of 45kg/person/year (FAO, 2016) to 58kg/person/year (IBGE, 2011).

Rice is a great substrate for mycotoxin production and fungi can produce them in large quantities (Kjer et al., 2010). Among the mycotoxins found in rice, aflatoxins (AF), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEA) and fumonisins (FUM) have been reported (Almeida et al., 2012). The aflatoxins are produced mainly by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Pitt & Hocking, 2009), which are extremely toxic metabolites. The analogue B_1 is considered the most toxic, classified by the International Agency of Research on Cancer (IARC, 1993) as a class 1 carcinogenic compound for humans with the liver being the main affected organ after ingestion.

There are few studies on aflatoxin reduction during a home cooking process in rice. An experiment carried out by Park et al. (2005) and Park & Kim (2006) in polished rice reported a reduction of 31 to 36% and 78 to 88% of aflatoxin B_1 using a common and under pressure cooking process, respectively. Another study on

reduction obtained 87.5%, 82.5% and 77.6% of aflatoxin B_1 in rice using different cooking processes: with excess of water, common and microwave, respectively (Hussain & Luttfullah, 2009). Park et al. (2004) analyzed the daily exposure of Koreans to aflatoxin B_1 through rice consumption of 0.89 to 5.37 ng/kg bw/day, but in their study the reduction of aflatoxin during the cooking was not considered. Since rice is consumed cooked, the present study aimed to verify the effect of a Brazilian ordinary cooking process on reduction of aflatoxin B_1 in rice, the aflatoxin intake and the margin of exposure to the Brazilian population.

2. Materials and methods

2.1 Spore suspension of Aspergillus flavus

A strain of *Aspergillus flavus* # 683 producing aflatoxin B_1 and B_2 was isolated from a rice sample from a São Paulo market.

A. flavus 683 was grown on CYA (Czapek Yeast Extract Agar) and transferred by a disposable loop to a bottle containing 100mL of peptone water 0.1% with glass beads. Then, the bottle was shaken, the content was filtrated and 1mL of the suspension diluted in 9 mL of peptone water 0.1% successively. An aliquot of 0.1 mL of spore suspension was plated on DG18 for colony forming units CFU/mL count (Figure 1).

2.2 Inoculation of A. flavus # 683 in rice and cooking test

Approximately 200g of raw polished rice (a_w=0.557) was placed on a glass plate and put into a closed container (desiccators) with water maintained at 25°C until the rice reached water activity of approximately 0.90. Then, a spore suspension of *Aspergillus flavus* # 683 producing aflatoxin B_1 and B_2 (1µL of spore suspension/1g of rice) was inoculated and incubated at the same conditions for 7 days. The flow diagram can be visualized in Figure 1. The process was repeated six times in order to have enough rice with aflatoxin.

After production of aflatoxin B_1 and B_2 by the *A. flavus* # 683 in polished rice, 100g of this rice was placed in a pan and 200 mL of water with 1% of salt was added. The pan was placed in an electric cooker until all the water had evaporated. The temperature was monitored by a thermo resistant thermometer

(TESTO 735-1, thermocouple Pt100, Brazil) and the temperatures registered every 30 seconds. The test was carried out with three samples of 100g contaminated with 20µg/kg and three samples of 100g contaminated with 2µg/kg (Figure 1).

2.3 Water activity

The water activity was determined using an Aqualab Series 3TE instrument (Decagon, Pullman, Wa, USA) at 25°C±1, in triplicate.

2.4 Aflatoxin determination in raw rice

Aflatoxin analysis was carried out on rice, based on the method of Stroka et al. (2000) as follows:

2.4.1 Clean-up

Approximately 200g of rice was finely ground using a laboratory mill (IKA A11 basic, Brazil), and passed through a sieve of 18 mesh (1.0mm). An aliquot of 25g was taken and extracted with 100mL of methanol solution: water (8:2, v/v) added with 2.5g of NaCl and homogenized in a shaker for 30 minutes. The solution was first filtered using a quantitative filter (Nalgon, Germany) and a glass microfiber filter (VICAM, USA). The filtrate (10mL) was diluted in 60mL of phosphate buffered saline (PBS) and was applied to an immunoaffinity column for aflatoxins (R-Biopharm Rhône Ltd, UK) at a flow rate of 2-3 mL/min. The column was then washed with distilled water (30mL) and aflatoxins eluted with methanol (1250µL) and diluted with milli Q water (1750µL).

2.4.2 Chromatographic conditions

An Agilent 1260 Infinity model system (Agilent, USA) of High Performance Liquid Chromatography (HPLC) was used, with a fluorescence detector set at 362nm excitation and 455nm emission for aflatoxins B_1 and B_2. An ODS (1.8µm, 40x15mm) guard column and a Zorbax Eclipse Plus C18 column (5 um, 4.6 x 150 mm) were employed. The mobile phase was water:acetonitrile:methanol (6:2:3, v/v/v), containing KBr (119 mg) and nitric acid (4M, 350µL/L) at a flow rate of 1mL/min with injection volume of 20µL. A post-column derivatization of aflatoxins

B_1 and B_2 was performed with bromine using a KobraCell (R-Biopharm Rhône Ltd, UK).

Aflatoxin B_1 and B_2 standard (Sigma, USA) curves were prepared for quantification. The concentration of aflatoxins in the sample was determined by interpolation of the resulting peak area of each standard curve.

2.4.3 Methodology validation

The methodology validation of the method described above was carried out considering the recovery, detection and quantification limits following the Eurachem Guides (1998).

In a rice sample free of aflatoxins, a spike of 0.5µg/kg of total aflatoxins was added. Eight parallel extractions were performed to calculate the recovery and the standard deviation. The limit of detection (LOD) was determined according to Eurachem's formula (1998):

$$s_0' = \frac{s_0}{\sqrt{n}}$$

Where: s_0 estimated standard deviation of the number of replicates

s_0' standard deviation to calculate the LOD and limit of quantification (LOQ)

n number of repetitions

To calculate the LOD, s_0' value was multiplied by 3 and for LOQ by 10.

2.5 Aflatoxin determination in cooked rice

All cooked rice was placed on a glass plate and dried in an oven at 50°C with ventilation until it reached the initial water activity of the raw rice (about 5 hours). Then, the entire sample was milled and analyzed for aflatoxins according to the methodology described in item 2.4.

2.6 Statistical analysis

The percentage of reduction was examined with Assistat 7.7 Software through variance analysis (ANOVA) and t test, comparing the percentage of reduction of the two different contamination levels.

2.7 Aflatoxin intake estimates

The daily intake of aflatoxin through rice depends on the concentration in the food and the amount consumed.

2.7.1 Consumption data

Rice consumption data were obtained from IBGE (Brazilian institute of Geography and Statistics) from the household budget survey (POF) in 2008-2009. The personal food consumption data were collected from all residents, 10 years old or over, from 13,569 homes selected, corresponding to 34,003 residents in total (IBGE, 2011).

2.7.2 Aflatoxin occurrence

Data of rice contamination with aflatoxin were obtained from Brazilian studies on aflatoxin occurrence in polished rice, Almeida et al. (2012), Carvalho et al. (2010) and our study shown in Chapter 2.

2.7.3 Assessment of aflatoxin exposure

A deterministic approach was used to evaluate aflatoxin exposure through rice consumption. The mean values of aflatoxin concentration in rice, reduction of aflatoxin in rice cooking, rice consumption and body weight were used.

The aflatoxin intake was estimated by the ratio between aflatoxin in rice (ng/g) less the percentage of aflatoxin reduction in cooking, then multiplied by consumption data (g/day) and divided by body weight (kg)

$$Aflatoxin\ intake\ \left[\frac{ng}{kg\ bw\ day}\right] = \frac{\left(concentration\ of\ aflatoxin\ in\ rice\left[\frac{ng}{g}\right] - \%\ of\ aflatoxin\ reduction\right) x\ rice\ consumption\left[\frac{g}{day}\right]}{body\ weight[kg]}$$

2.8 Margin of exposure (MOE)

The margin of exposure approach was calculated through the ratio between a defined point on the dose-response curve for the adverse effect and the aflatoxin intake (EFSA, 2005). The point on the dose-response curve used in this study was the lower bound value of a benchmark dose level (BMDL) from cancer incidence

of 10% in rodents (BMDL10). A BMDL10 of 0.17 mg kg^{-1} bw day^{-1} was estimated by EFSA (2007) based on a study involving aflatoxin B_1 administration at a range of dietary doses to male Fischer rats.

3. Results

3.1 Detection and quantification limit

The detection and quantification limit for aflatoxin B_1, and B_2 were 0.016µg/kg and 0.012µg/kg and 0.054µg/kg and 0.039µg/kg, respectively. The recovery percentage of samples contaminated with 0.5µg/kg was 88.47%.

3.2 Cooking test

Almost 80% of aflatoxin B_1 and B_2 was destroyed after rice cooking (Table 1). Samples with contamination of 20µg/kg showed an average reduction of 77.92% and a similar reduction of 77.38% was obtained in a sample with 2µg/kg. The statistic (t test) showed no significant difference between the results. The temperature x time profile during the cooking test is shown in Figure 2. As can be observed, the boiling point started at 8 minutes and all the water evaporated at 16 minutes.

3.3 Aflatoxin intake

Table 2 shows the mean values of aflatoxin concentration in rice with the references, the rice consumption, body weight, aflatoxin intake and MOE. The POF 2008-2009 described a rice consumption of 160g/day (IBGE, 2011). The body weight considered was 60kg. Almeida et al. (2012), our previous study shown in Chapter 2 and Carvalho et al. (2010) reported an average of aflatoxin contamination in rice of 9.09, 0.07 and 0.03, respectively. The aflatoxin intake range was 0.016-4.862 ng/kg bw/day and MOE range was 346,939-10,625,000, which indicates low concern from the public health point of view, since a MOE value of 10,000 or higher would indicate low exposure of aflatoxins to consumers (EFSA, 2007).

4. Discussion

There are several reports on occurrence of aflatoxins in raw rice (Park et al., 2004; Sales & Yoshizawa, 2005; Silva et al, 2008; Reddy et al., 2009; Carvalho et al., 2010; Makun et al., 2011; Ok et al., 2014); however, studies on the effects of cooking rice as it is consumed are few. Park et al. (2005), in South Korea, used the ordinary cooking method (160°C for 20 minutes, in an electric cooker) to evaluate the reduction of aflatoxin B_1 in polished rice, naturally contaminated. They obtained a much lower reduction (31-38%) than we had in our experiment. Later, Park & Kim (2006) obtained a similar reduction to ours (78-88%), using an electric pressure cooker fixed at 15lb/in2 (0.10MPa).

In Pakistan, Hussain & Lattfullah (2009) analyzed the effect of different rice cooking methods to reduce AFB_1. The ordinary cooking (50g of rice, 100mL of water in a gas stove for 9 minutes after boiling start) showed 84% of reduction, the cooking with excess of water (50g of rice, 200mL of water in a gas stove for 9 minutes after boiling start, after cooking the excess of water was drained off) showed 87.5% of reduction and microwave oven cooking (50g of rice, 100ml of water for 9 minutes at medium potency – 0.45kW) showed reduction of 72.5%. Hussain & Lattfullah (2009) spiked the rice samples with aflatoxin B_1 standard, which does not represent the natural contamination by fungi because it is not homogeneous but superficial. In our study, the samples were contaminated with *A. flavus* producing aflatoxin B_1 and B_2 isolated from a rice sample and incubated under favorable conditions to produce the toxin.

The ordinary cooking in Brazil, Korea and Pakistan is similar, but there are countries where the local method is very different, such as Iran. In this country a 250g rice amount is washed four times with 500mL of water and then the rice is soaked for 2 hours in this water. Separately, water is heated and once the water starts boiling, the rice is placed in the water and cooked for about 10min, during this time the rice is stirred a couple of times from bottom up. After cooking, the rice is drained in a colander with a quick rinse with cold water (10–15°C) to stop additional cooking. The cooked rice is poured into a pot and then 1 spoon of oil (about 10g) is added onto the rice. After that, a towel is placed over the lid of the pot to cover it. Finally, cooking is done at a medium low flame for 1h. Sani et al. (2012) analyzed the reduction of aflatoxins in rice by the Iran local cooking method

and the electric rice cooker method (250g of rice was washed with 500mL of drinking water and soaked in 500mL of water for 1h, then the water was drained and the rice was placed on the rice cooker with 250mL of water for 30 minutes). The local cooking method showed reduction of 17.5% and the rice cooker method showed reduction of 24.8%; there was no statistical difference between the results. The lower reduction of AFB_1 when this study is compared to the studies in other countries can be explained by the very different cooking method, where rice is washed and soaked for 1 or 2 hours in water.

Park et al. (2004) estimated the daily exposure of Koreans to aflatoxin B_1 through rice consumption on 0.89-5.37 ng/kg bw/day, but considered the aflatoxin reduction in the cooking process equal to zero. If this study considered 80% of aflatoxin reduction in the cooking process, the values would be 0.18 and 1.07 ng/kg bw/day, the higher level of aflatoxin intake (1.07 ng/kg bw/day) would be lower than the higher level of aflatoxin intake in our study (4.862 ng/kg bw/day).

5. Conclusion

The present research showed a similar reduction of aflatoxin B (77%) when compared to previous reports with similar cooking methods. Cooking can reduce the aflatoxin content in rice. The daily exposure of Brazilians to aflatoxin B_1 through rice consumption shows low concern from the public health point of view

6. References

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CAPÍTULO III

Reduction of aflatoxin B in rice after cooking and the aflatoxin intake

(Figures and Tables)

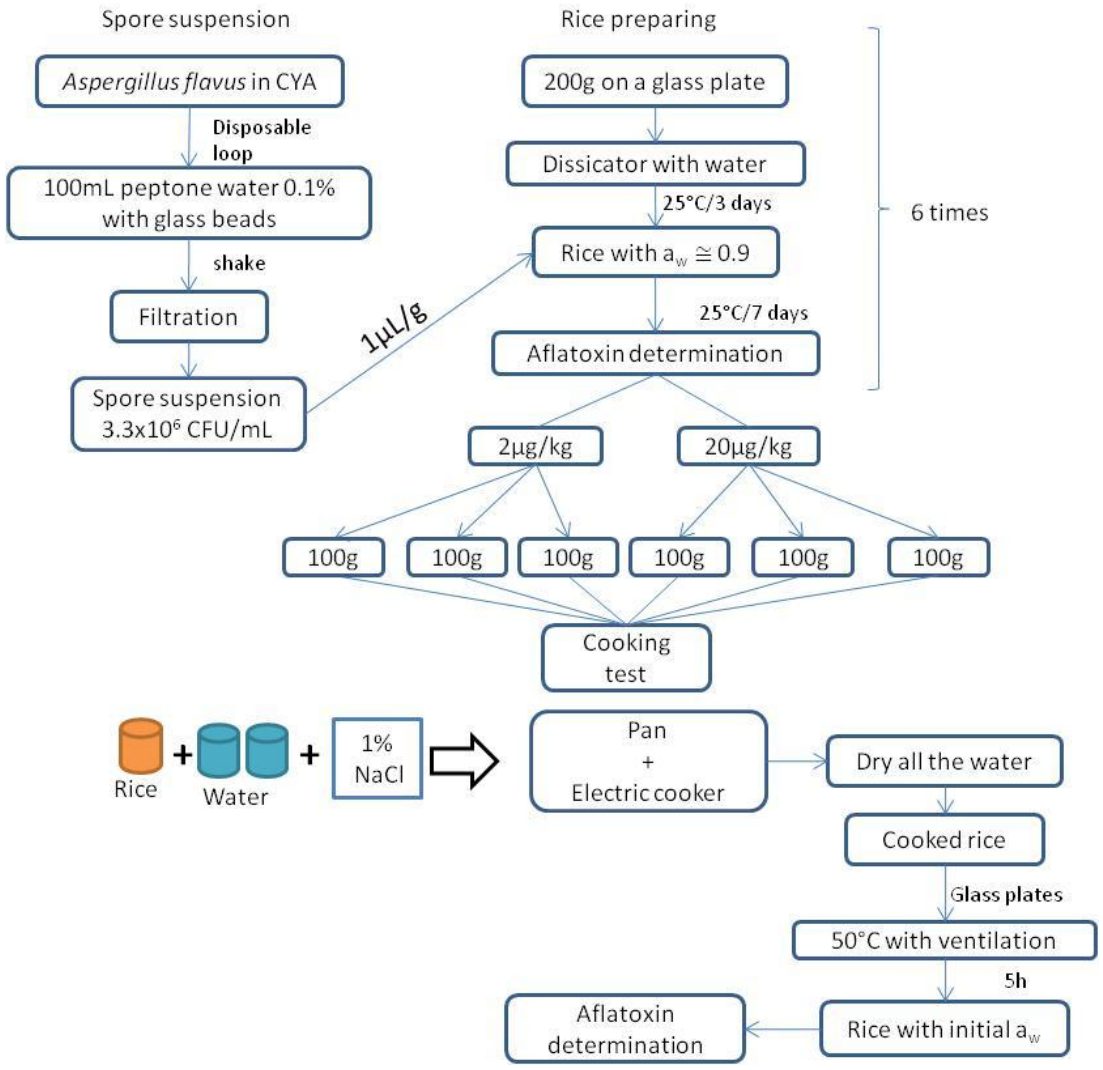

Figure 1. Flow diagram of methodology.

Table 1. Percentage of reduction of aflatoxin B_1 and B_2 levels

Samples	Initial level (µg/kg)			Level after cooking (µg/kg)			Reduction (%)			Average (%)		
	B_1	B_2	B_{TOTAL}	B_1	B_2	B_{TOTAL}	B_1	B_2	B_{TOTAL}	B_1	B_2	B_{TOTAL}
100g	19.81	0.42	20.23	4.22	ND	4.22	78.70	100	79.14			
100g	19.81	0.42	20.23	4.40	ND	4.40	77.79	100	78.25	77.45	100	77.92[a]
100g	19.81	0.42	20.23	4.78	ND	4.78	75.87	100	76.37			
100g	2.02	ND	2.02	0.46	ND	0.46	77.22	-	77.22			
100g	2.02	ND	2.02	0.45	ND	0.45	77.72	-	77.72	77.38	-	77.38[a]
100g	2.02	ND	2.02	0.46	ND	0.46	77.22	-	77.22			

Averages followed by the same letter do not differ statistically from each other.

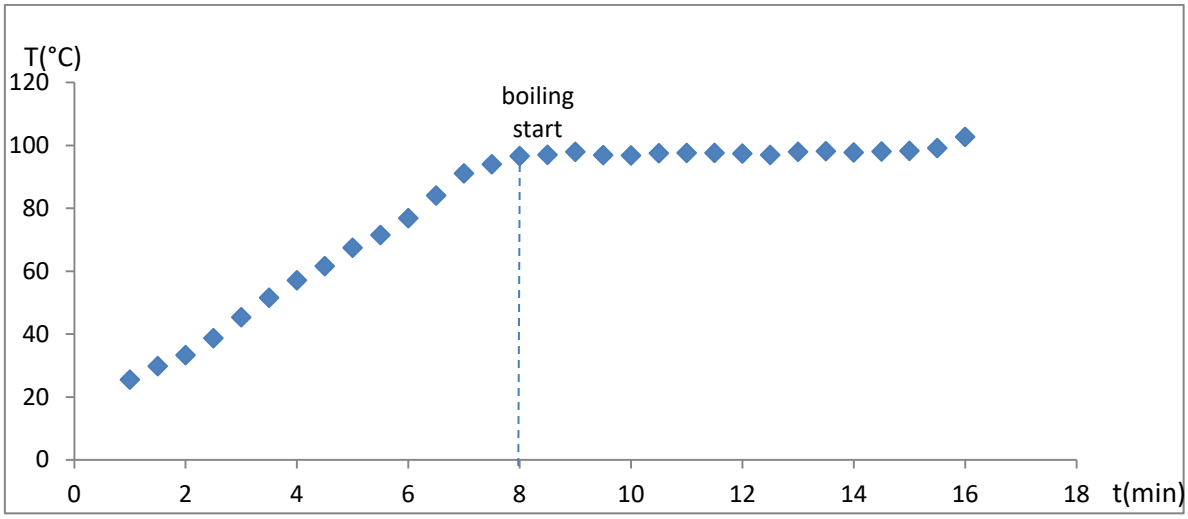

Figure 1. Time and temperature record of cooking test

Table 2. Aflatoxin intake and MOE.

Reference	Aflatoxin Average (ng/g)	After cooking (-80%)	Brazilian Rice Consumption (g)	Body Weight (kg)	Aflatoxin Intake (ng/kg body weight/day)	MOE
Almeida et al., 2012	9.09	1.82			4.862	346,939
Our study (Chapter 2)	0.07	0.014	160.3	60	0.037	4,594,595
Carvalho et al., 2010	0.03	0.006			0.016	10,625,000

4. CONCLUSÕES

Nesta dissertação as seguintes conclusões puderam ser obtidas:

- Houve uma diferença entre a micobiota característica dos dois sistemas de plantio no arroz de irrigação no Rio Grande do Sul e no sistema de arroz sequeiro no Maranhão, principalmente quanto à porcentagem de infecção por *Aspergillus* section *Flavi,* que foi maior nas amostras de campo do Rio Grande do Sul do que de Maranhão, embora em Maranhão as amostras de solo apresentaram uma alta presença de *A.* section *Flavi*.

- A ocorrência de fungos produtores de aflatoxinas foi baixa tanto nas amostras do Rio Grande do Sul como nas amostras do Maranhão.

- Nos tipos de arroz mais consumidos pela população brasileira (polido, integral e parboilizado) a maioria das amostras estava contaminada com níveis menores que o limite de detecção do método para aflatoxinas (<0,04 µg/kg).

- As amostras de arroz que apresentaram nível de aflatoxinas maior que o limite máximo tolerável estabelecido pela ANVISA (< 5 µg/kg) foram as amostras comerciais de arroz vermelho de São Paulo.

- O cozimento do arroz polido apresentou redução de 78% de aflatoxinas B1 e B2, indicando que o cozimento é um método eficaz para diminuir a ingestão de aflatoxinas.

- A estimativa de ingestão diária apresentou uma faixa de 0,016 a 4,862 ng/kg peso corpóreo/dia e a margem de exposição (MOE) na faixa de 346.939 a 10.625.000 o que indica um baixo risco de exposição da população brasileira pelo consumo de arroz.

5. ANEXO: Trabalhos advindos desta dissertação

Trabalhos apresentados em congressos

Trabalho intitulado “Mycobiota of rice and fungi producing aflatoxins” com a autoria de: Katsurayama, A. M., Nicolusi, J. A. L., Martins, L. M., iamanaka, B. T., Taniwaki, M. H. foi apresentado na forma de pôster durante o 28º Congresso Brasileiro de Microbiologia realizado no Centro de Convenções de Florianópolis, na cidade de Florianópolis, SC, no período de 18 a 22 de outubro de 2015.

Trabalho intitulado “Fungi and aflatoxins in Brazilian rice: occurrence and significance in human health” no Workshop 2016 da ICFM (International Comission on Food Mycology) em Freising – Alemanha, no período de 13 a 15 de junho de 2016.

Trabalhos a serem publicados

Katsurayama, A.K. & Taniwaki, M.H. 2017. Fungos e aflatoxinas no arroz: ocorrência e significado na saúde do consumidor. Brazilian Journal of Food Technology. (aceito – artigo de revisão).

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Katsurayama, A.K., Martins, L.M., Iamanaka, B.T. & Taniwaki, M.H. Reduction of aflatoxin B in rice after cooking and the aflatoxin intake. Em preparação para ser enviado ao Food Research International.