UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

MODELAGEM DE FATORES QUE AFETAM A ESTABILIDADE OXIDATIVA E A SUSCEPTIBILIDADE AO CRESCIMENTO FÚNGICO EM *PET FOOD*

Autora: Mayara Uana da Silva
Orientador: Prof. Dr. Ricardo Souza Vasconcelos
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Tese apresentada, como parte das exigências para obtenção do título de DOUTOURA EM ZOOTECNIA, no programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá – Área de concentração Produção Animal ou Nutrição de Animais de Companhia.

MARINGÁ
Estado do Paraná
Fevereiro- 2022

Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá - PR, Brasil)

S586m

Silva, Mayara Uana da

Modelagem de fatores que afetam a estabilidade oxidativa e a susceptibilidade ao crescimento fúngico em *pet food* / Mayara Uana da Silva. -- Maringá, PR, 2022.

117 f.color., figs., tabs.

Orientador: Prof. Dr. Ricardo Souza Vasconcellos.

Coorientadora: Profa. Dra. Joyce Sato.

Tese (Doutorado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Departamento de Zootecnia, Programa de Pós-Graduação em Zootecnia, 2022.

1. Alimentos para animais - Oxidação. 2. Alimentos para animais - Crescimento fúngico. 3. Pet food . I. Vasconcellos, Ricardo Souza, orient. II. Sato, Joyce, coorient. III. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. IV. Título.

CDD 23.ed. 636

Marinalva Aparecida Spolon Almeida - 9/1094

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TITULAÇÃO: Doutora em Zootecnia - Área de Concentração Produção Animal

APROVADA em 24 de fevereiro de 2022.

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“(...) E quando isso acontecer, quando permitirmos que a liberdade ressoe, quando a deixarmos ressoar de cada vila e cada lugar, de cada estado e cada cidade, seremos capazes de fazer chegar mais rápido o dia em que todos os filhos de Deus, negros e brancos, judeus e gentios, protestantes e católicos, poderão dar-se as mãos e cantar as palavras da antiga canção espiritual negra:

Finalmente livres! Finalmente livres!

Graças a Deus Todo Poderoso, somos livres, finalmente."

(“Eu tenho um sonho”, Martin Luther King)

À minha família, em especial a minha avó Tereza,

Ao meu filho de quatro patas,

Ao meu namorado,

Aos meus amigos

DEDICO ESTE TRABALHO.

AGRADECIMENTOS

A Deus, pela minha salvação, redenção e todas as bençõe concedidas em minha vida;

Aos meus pais, Gilson Aparecido da Silva e Vera Neide Feitosa da Silva, pelo cuidado, amor e orações. Por todas as horas de trabalho dedicadas a me oferecer uma educação privilegiada, pela companhia e ajuda no laboratório. Por todo o incentivo, exemplo e força;

À minha irmã, Maelly Thais da Silva, pelo incentivo, ajuda no laboratório e principalmente por sempre estar do meu lado;

Ao meu namorado Diego Olher, por toda sua paciência, ajuda e amor. Por ser um dos meus maiores incentivadores e acreditar muito em meu potencial;

Ao meu cachorro Aslam, por todo amor incondicional, por me alegrar todas as vezes que cheguei cansada ou triste;

À minha família em geral, por todo amor e admiração;

Ao meu orientador Dr. Ricardo Souza Vasconcellos, pela confiança, ensinamentos e amizade. Pela interminável paciência, pelo tempo, dedicação e ajuda em meu experimento, pelo exemplo de pessoa e profissional;

À minha amiga e coorientadora Joyce Sato, pela imensa ajuda nas análises, ensinamentos, disponibilidade e amizade dentro e fora do campus;

Ao professor Dr. Vanderly Janeiro, com toda ajuda na parte estatística, ensinamentos, disponibilidade e amizade;

Aos meus colegas de vida acadêmica, pela companhia, apoio, ajuda durante toda a pós-graduação;

A toda equipe do Centro de Ensino e Estudos Nutricionais em Felinos (CEENUFEL), pela ajuda, companheirismo, ensinamentos e risadas;

Aos meus amigos de vida e profissão, por todo carinho e parceria;

À Universidade Estadual de Maringá, pela formação em Zootecnia e oportunidade de me tornar mestre;

A todos os professores do Departamento de Zootecnia, pelas valiosas lições que me foram passadas ao longo da Graduação e Pós-Graduação;

Aos técnicos do Laboratório de Análises de Alimentos e Nutrição Animal *(LANA)*, Osvaldo e Ulisses, por toda a ajuda nas análises, pelas orientações e paciência;

À Solange, do Programa de Pós-Graduação em Zootecnia, pela ajuda com as "papeladas" de todo o processo do mestrado;

Aos funcionários do gatil, em especial ao seu Vilson, por sempre deixar nosso setor organizado;

Aos nossos colaboradores e colaboradoras (gatos), por todo carinho, afeto e inclusive os arranhões;

A empresa Biorigin-Zilor, pelo financiamento da pesquisa;

A CAPES, pela concessão da bolsa.

BIOGRAFIA

MAYARA UANA DA SILVA, filha de Gilson Aparecido da Silva e Vera Neide Feitosa da Silva, nasceu em Rondon, Paraná, no dia 28 de dezembro de 1991.

Em março de 2010, iniciou no Curso de Graduação em Zootecnia, na Universidade Estadual de Maringá.

Em 2011, iniciou sua jornada na pesquisa como estagiária no grupo de cunicultura, sob orientação do Professor Doutor Claudio Scapinello, atuando como bolsista de Iniciação Cientifica do CNPq, na área de nutrição de monogástricos.

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Em março de 2016, iniciou no Programa de Pós-Graduação em Zootecnia, nível de mestrado, na área de Produção Animal, na Universidade Estadual de Maringá, realizando estudos em Nutrição Animais de Companhia. Em fevereiro de 2018, submeteu-se à banca para defesa da sua Dissertação, como parte das exigências para obtenção do título de mestre em Zootecnia.

Em março de 2018, dando continuidade a seus estudos, ingressou no Programa de Pós-Graduação em Zootecnia, nível de doutorado, na área de Produção Animal, na Universidade Estadual de Maringá, realizando estudos em Nutrição Animais de Companhia, sob a orientação do Prof. Dr. Ricardo Souza Vasconcellos.

Em fevereiro de 2022, submeteu-se à banca para defesa da sua Tese.

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1. INTRODUÇÃO GERAL

A garantia da qualidade é componente fundamental a ser trabalhado na indústria de alimentos para animais de companhia, visando assegurar os padrões nutricionais, as características sensoriais e físicas dos produtos e ainda a sua vida de prateleira. Por definição, *shelf-life* é o tempo que determinado produto, mantido em condições adequadas de armazenamento, se mantém próprio para o consumo, apresentando alterações que são consideradas aceitáveis pelo fabricante, consumidor e a legislação alimentar vigente (Vitali et al., 2004). Em alimentos secos extrusados para cães e gatos, controlar os fatores que levam a oxidação lipídica e deterioração microbiana são fundamentais para garantir a vida de prateleira, que é geralmente de 12 a 18 meses.

Presente em todos os alimentos a água desempenha papel importante em sua conservação (Belitiz et al., 2004). Ela define os processos de fabricação do extrusado, confere palatabilidade e melhora a textura do alimento (Bone & Shannon, 1977). A porcentagem de água de um produto em relação à porcentagem de matéria-prima é definida como umidade. A água dentro de um alimento se apresenta de duas formas, ligada ou livre (Fellows, 2006, Krabee et al., 2009). Mesmo análise de determinação de umidade sendo rotineira na indústria *Pet food* a mesma não deve ser usada isoladamente no controle de qualidade (Krabbe et al., 2009). Isso porque a umidade não necessariamente é um indicador de susceptibilidade a degradação microbiana. Como exemplo desta afirmação, pode-se ver em alimentos semiúmidos, que apresentam entre 25-30% de água e são estáveis do ponto de vista de crescimento microbiano, enquanto alimentos secos entre 10-12% de umidade apresentam alta susceptibilidade ao crescimento microbiano. Estas diferenças de comportamento se devem às diferenças na atividade de água (aw), que realmente é indicador da quantidade de água disponível ao crescimento de microrganismos.

Um teor máximo e seguro adotado por indústrias para alimentos secos e extrusados é de 14% para umidade e 0,65 de atividade de água no produto acabado (FEDIAF, 2014). Á partir desta aw, fungos e leveduras em geral irão se desenvolver na deterioração de um alimento e, por isto a aw é um fator importante a ser controlada pela indústria *Pet food*, sendo considerado um Ponto Crítico de Controle (PCC).

Ácidos orgânicos se apresentam como alternativa de antifúngicos, para a indústria alimentícia, os ácidos possuem dupla finalidade: acidulante e conservante. Tais substâncias produzem acidez, a qual por sua vez age como flavorizante e retarda a degradação enzimática. Atuam como agentes quelantes que se ligam a metais formando os quelatos metálicos, os

quais previnem ou reduzem a oxidação oriunda da catálise dos íons metálicos (Bellaver & Scheuermann, 2004). Entretanto, testes para verificação dos efeitos antifúngicos sobre o *shelf-life* de alimentos extrusados preservados com ácidos orgânicos se torna interessante uma vez que a lista de substâncias químicas com ação antifúngica é extensa, mas, ainda muito restrita ao ser comparada com o número de drogas antibacterianas disponíveis (Nobre, 2002), e gera dúvidas quanto a melhor dosagem e tipos de antifúngicos a serem adicionados. Além disso entender as relações existentes entre as variáveis (umidade e atividade de água) são fundamentais para se conhecer a vida útil do produto. Essa relação pode ser definida através de isotermas de sorção. Essas são expressas graficamente, relacionando teor de umidade e atividade de água quando essa é adsorvida e dessorvida a uma temperatura constante. No entanto, a literatura científica ainda é carente de trabalhos que mostrem a validação dessa análise, correlacionando os valores encontrados a partir dos gráficos com valores reais.

Outra preocupação constante para assegurar a qualidade em *Pet food* é a prevenção da oxidação. Tem-se demonstrado que processos oxidativos reduzem o valor nutricional, prejudicam a palatabilidade e comprometem a saúde dos animais. Do ponto de vista oxidativo, a vida de prateleira pode sofrer influência de alguns fatores como as concentrações de metais de transição, teor de antioxidante, de gordura e ácidos graxos poli-insaturados, da temperatura, características da embalagem, teor de aw, entre outros.

Os metais de transição possuem a habilidade de catalisar a formação de radicais livres, como na autoxidação. Suas moléculas contêm hidrogênio alélico que podem formar radicais livres, que por sua vez reagem com o oxigênio formando hidroperóxidos. Pequenas quantidades de metais de transição podem iniciar a decomposição dos hidroperóxidos, assim como acelerar a velocidade da autoxidação, e consequentemente diminuir a vida útil do produto (Santos, 2012).

Íons de metais de transição como o ferro e o cobre, podem participar do processo de peroxidação lipídica catalisando a formação de radicais lipídicos como: alcoxila, peroxila e hidroxila a partir dos hidroperóxidos (Lima & Abdalla, 2001).

Lipídeos são muito instáveis quando conservados em condições desfavoráveis a sua preservação. São afetados pela presença de ar (oxigênio), luz, umidade e calor (o tratamento térmico aumenta a velocidade de oxidação). A adição de tal nutriente pode afetar a estabilidade oxidativa do produto acabado. A fração lipídica apresenta elevada susceptibilidade aos processos deteriorativos, como autoxidação. A oxidação lipídica leva a formação de radicais livres (Turek et al. 2003; Ramalho & Jorge, 2006; Lima, 2015).

Diante do exposto acima, nesta Tese de doutorado realizaram-se 3 estudos relacionando importantes aspectos que podem comprometer a vida de prateleira de alimentos secos extrusados para cães e gatos, visando ampliar o conhecimento acerca destes fatores e favorecer a produção de alimentos mais seguros..

2. REVISÃO BIBLIOGRÁFICA

2.1 Fatores que diminuem o *shelf-life* de alimentos para animais de companhia

Popularmente conhecido como "prazo de validade" *shelf-life* é o tempo de vida útil de qualquer alimento. O tempo que mantido em condições adequadas de exposição ao ar e luminosidade, o produto se mantém próprio para o consumo, apresentando alterações que são consideradas aceitáveis pelo fabricante, consumidor e a legislação alimentar vigente (Vitali et al., 2004; Calligaris et al., 2015).

Com exigência maior dos tutores em relação à qualidade e segurança alimentar dos alimentos voltados a seus animais de estimação, e expectativas de que a qualidade será mantida da compra até o consumo total do produto, análises de *shelf-life* (tempo de prateleira) se tornam essenciais para os fabricantes de alimentos.

Tal especificação pode ser encontrada nos rótulos dos alimentos, cada produto possui um tempo específico de vida útil. Alimentos secos e extrusados para animais de companhia normalmente devem manter características adequadas de consumo pelo período de 12 meses, podendo se estender a 18 meses (Chanadang et al., 2016).

Diversos fatores podem afetar o *shelf-life* de alimentos, sendo classificados como fatores intrínsecos e extrínsecos. Os fatores intrínsecos são as propriedades do produto, e isso inclui: atividade da água (a_w), valor de pH e acidez total, potencial redox, oxigênio disponível, nutrientes, composição química do alimento, bioquímica natural da formulação do produto (enzimas, reagentes químicos) e uso de conservantes na formulação do produto (por exemplo, sal, antioxidantes). Estes fatores intrínsecos são influenciados pelas características e qualidade da matéria-prima (Food Ingredientes Brasil, 2011).

Os fatores extrínsecos são as características do processamento e ambientais às quais o alimento é submetido durante sua fabricação, transporte e armazenamento, sendo esses: perfil e tempo de temperatura durante o processamento, UR (umidade relativa) do processamento, armazenagem e distribuição, exposição à luz (UV e IV) na armazenagem e distribuição, controle de temperatura do processamento e da temperatura ambiente na armazenagem,

composição da atmosfera dentro da embalagem e por fim o manuseio do consumidor (Food Ingredientes Brasil, 2011).

Tais fatores, intrínsecos e extrínsecos, podem criar uma série de processos que afetam o *shelf-life*, causando sua diminuição. Dentre alguns fatores que podem influenciar na qualidade de alimentos voltados a animais de companhia, e consequentemente, diminuir seu *shelf-life* estão: umidade, atividade de água (a_w), autoxidação lipídica e proteica e crescimento de microrganismos.

De acordo com Brandão et al., (2011), a contaminação de rações por micro-organismos é um dos principais responsáveis por danos na saúde de animais de companhia, esta contaminação pode ocorrer durante o processamento, estocagem e manuseio de matérias-primas. As alterações microbiológicas que os alimentos podem sofrer dependem de diversos fatores, sendo os que mais se destacam: a carga microbiana inicial no começo do armazenamento, as propriedades físico-químicas dos alimentos, como teor de umidade, pH e presença de conservantes; o método de processamento utilizado na produção dos alimentos; e o ambiente externo do alimento, como as composições de gás circundantes e a temperatura de armazenamento (Food Ingredientes Brasil, 2011).

Outro fator importante ligado diretamente ao tempo de *shelf-life* do produto é a absorção, principal causa da alteração física em alimentos. Mesmo sendo fatores fundamentais para a palatabilidade, a umidade e atividade de água podem proporcionar o crescimento de microrganismos (Krabbe, 2009). No caso de alimentos secos, a absorção de água pode ainda fazer com que o alimento perca sua crocância (Food Ingredientes Brasil, 2011), quanto maior a absorção, mais rapidamente o alimento pode deteriorar, interferindo diretamente em seu tempo de vida útil (Krabbe, 2009).

As alterações químicas podem ocorrer dentro dos alimentos ou a partir de reações dos componentes dos alimentos com fatores externos, como oxigênio, luz, metais de transição, desenvolvendo a rancidez). Reações oxidativas lipídicas e proteicas estão diretamente ligadas, pois são iniciadas por radicais livres, em que grande parte dos produtos da oxidação lipídica são radicais livres para a oxidação proteica (Wang et a., 2019). A oxidação faz com que o alimento perca em qualidade nutricional com a degradação de ácidos graxos essenciais e vitaminas lipossolúveis, e atrativa com a formação de *off flavors* e *off odors*, diminuindo a vida de prateleira do alimento (Silva et al., 1999; Tian et al., 2013). Além da produção de compostos nocivos, como citotóxicos e genotóxicos, podendo causar dados biológicos ao organismo dos animais (Barriuso et al., 2013).

2.2 Água nos alimentos

Presente em todos os alimentos a água desempenha papel importante em sua conservação (Belitiz et al., 2004). Ela define os processos de fabricação do extrusado, confere palatabilidade e melhora a textura do alimento (Bone & Shannon, 1977). A porcentagem de água de um produto em relação à porcentagem de matéria-prima é definida como umidade. A água dentro de um alimento se apresenta de duas formas: água ligada e livre (Fellows, 2006, Krabee et al., 2009). A água ligada está combinada com as moléculas do produto, assim é dificilmente removida e utilizada para reações, porém a água livre se encontra disponível para reações físicas e químicas, assim como para o crescimento microbiológico, favorecendo a deterioração do produto (Uboldi Eiroa, 1981).

A água livre também é conhecida como atividade água do produto, análise de tal fator é imprescindível na indústria *pet food*, pois através dela é possível controlar a reprodução microbiana, reações enzimáticas, oxidativas e hidrolíticas do alimento, fazendo com que sua qualidade seja maior e consequentemente a vida de prateleira prolongada (Brito, 2010).

Fisicamente, a atividade de água (a_w) é conhecida como a medida do estado da água nos alimentos e pode ser descrito de forma termodinâmica, pelo coeficiente de fugacidade,

$$a_w = \frac{f}{f_0}$$

Em que f é a fugacidade da água no alimento e f_0 é a fugacidade da água pura a uma determinada temperatura (Guilbert & Morin, 1986).

A determinação do teor de água de um alimento é fundamental para estimar a vida de prateleira de um produto, pois se apresenta como fator importante para controle de taxa de deterioração. A disponibilidade de água para reações enzimáticas, químicas e atividade microbiológica é que determina a vida de prateleira de um alimento, e isso é medido através da atividade de água do alimento (Fellows, 2006). Barbosa-Canovas et al., (2007) mostram como a função da água na estabilidade dos alimentos é importante pelo potencial que esta tem para contribuir nos processos de deterioração, como ilustrado na Figura 1.

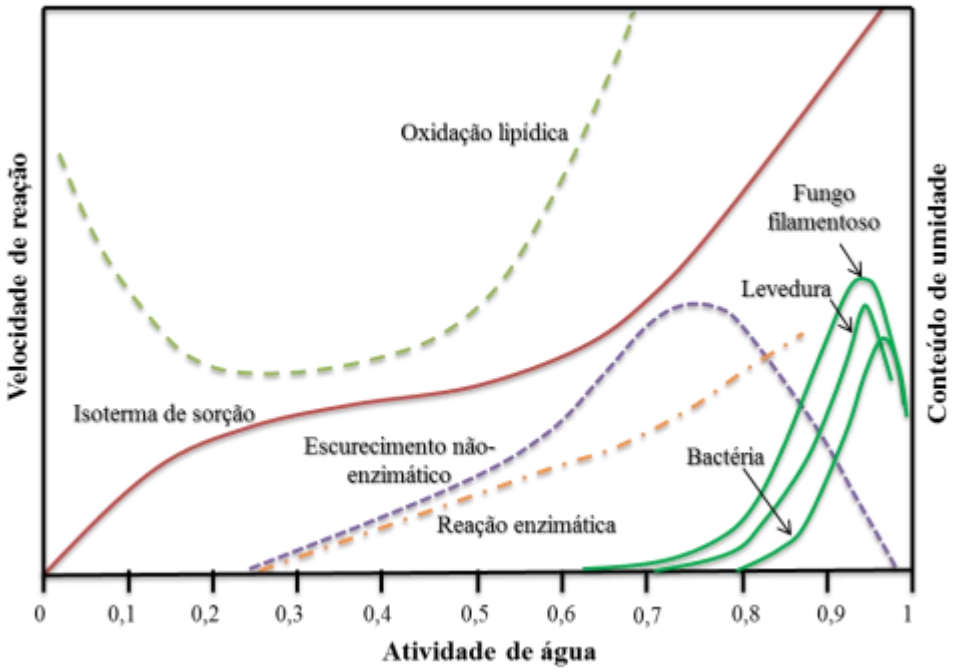

Figura 1. Esquema de estabilidade dos alimentos em função da atividade de água (Barbosa-Cánovas et al., 2007).

Mesmo a análise de determinação de umidade sendo a mais comum na indústria *pet food* a modo de evitar contaminações microbiológicas não é recomendado que seja usada isoladamente como parâmetro (Krabbe et al., 2009), podendo ser explicado pelo fato de alimentos úmidos (30% de umidade) serem poucos susceptíveis a contaminações por conta da adição de umectantes, enquanto alimentos secos (10% de umidade) apresentam uma susceptibilidade maior. Neste sentido, a atividade de água é uma medida mais adequada, pois indica a quantidade de água disponível ao crescimento de microrganismos (Tabela 1).

Para cães e gatos se adota uma umidade padrão de no máximo 14% e atividade de água de 0,65 (FEDIAF, 2014). No entanto esses valores podem variar de acordo com composição química, processamento e armazenagem (Park, et al., 2008). Por isso se torna interessante estudar a relação entre essas duas variáveis (umidade e atividade de água).

Tabela 1. Atividade de água mínima para desenvolvimento de alguns micro-organismos em alimentos.

Micro-organismos	Atividade de água mínima
Bactérias	0,91
Leveduras	0,88
Bolores	0,80
Bactérias halófilos	0,75
Bolores xerófilos	0,61
Leveduras osmotolerantes	0,60
Staphylococcus aureus	0,85

Fonte: Alves (2003).

2.3 Isotermas de sorção

Isotermas consistem em curvas que descrevem a relação entre o conteúdo de umidade dos alimentos e atividade de água em condições de temperatura constante (Kaymak-Ertekin & Gedik, 2005; Kurozawa, et al., 2005; Samapundo et al., 2007). Segundo o autor Ordóñez et al., (2005), as isotermas são diferentes dependendo do grupo dos alimentos e permitem estimar a estabilidade de um produto diante de diversos agentes alterantes. Para Park et al., (2008), a relação entre a umidade e atividade de água encontrada a partir de curvas de isotermas depende da composição química do alimento (proteínas, gordura, amido, açúcar, entre outros). E, partir dessas curvas se consegue determinar o teor final de água necessário para que alimento se mostre estável e obtenha a vida de prateleira desejada.

Essas curvas podem ser obtidas em duas formas: Isotermas de adsorção: obtidas em material completamente seco, assim o incremento do conteúdo de umidade de equilíbrio a várias atividades de água, em temperatura constante, faz com que este material ganhe massa pela entrada de água em sua estrutura. Isotermas de dessorção: obtidas no material quando este está completamente úmido e, dependendo da atividade de água do sistema, a umidade de equilíbrio do material diminui na medida em que este cede água para o sistema. Essas curvas de isotermas de adsorção e dessorção não coincidem, e a diferença entre elas é chama de histerese (Al-muhtaseb et al., 2002; Kurozawa, et al., 2005). Uma curva típica de isoterma de sorção é mostrada na figura 2 (Park & Nogueira, 1992).

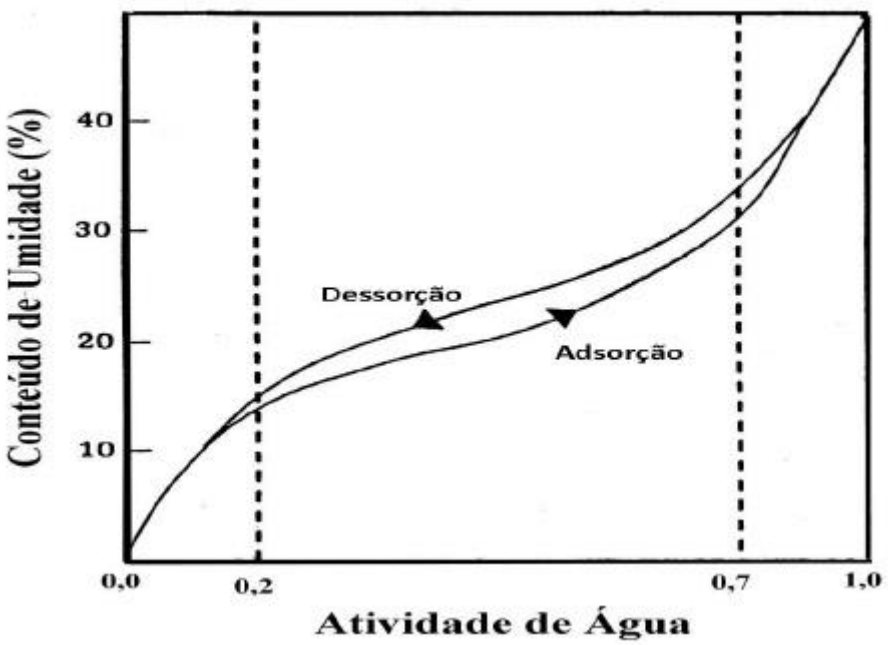

Figura 2. Curva típica de isoterma de sorção.
Fonte: Park & Nogueira (1992).

As isotermas de sorção (adsorção e dessorção) fornecem dados específicos com aplicação direta na predição de tempo de secagem do alimento, na vida de prateleira, no crescimento microbiano, na determinação do tipo de embalagem, predição do efeito do abuso da temperatura, nas especificações de controle de qualidade, definições de pontos críticos, caracterização do produto inclusive quando é constituído por componentes de atividade de água diferentes (Gal, 1987; Pena, et al., 2000).

De acordo com Brunauer et al., (1938), existem cinco tipos de isotermas de sorção de acordo com o formato de curva obtido (Figura 3). Materiais que apresentam amido, normalmente apresentam curvas do tipo II, em que o formato é sigmoide. Ainda de acordo com o autor, esse formato de curva permite entender o tipo de força existente da ligação da água com o material higroscópico, permitindo avaliar a estrutura superficial do material.

Alimentos que apresentam elevadas concentrações de açúcar, como a maior parte das frutas, possuem isotermas de sorção com formato de J, com classificação do tipo III (Gregg & Sing, 1967). Conforme Brunauer et al., (1938), as isotermas do tipo I, IV e V não são de interesse para a área de alimentos.

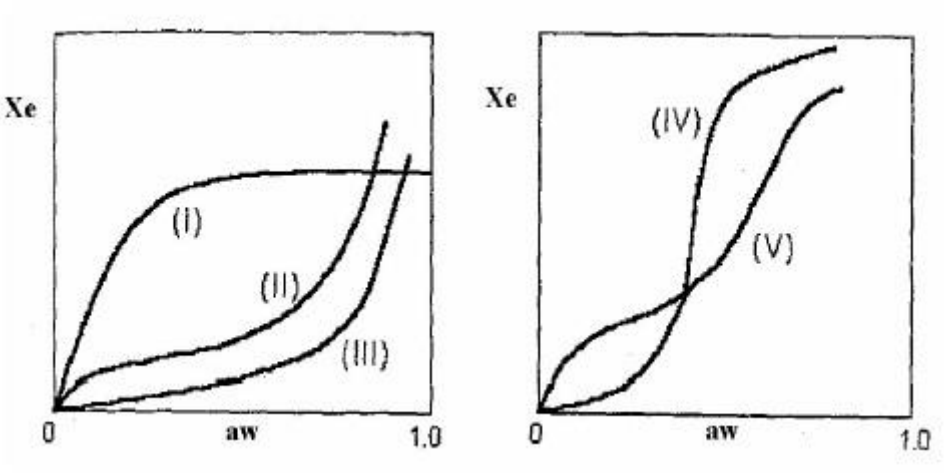

Figura 3. Formatos de curvas de isotermas de sorção (Brunauer et al., 1938).

As isotermas de sorção da maioria dos alimentos são não lineares, geralmente de forma sigmoidal, com variações de acordo com a estrutura física, composição química, temperatura e capacidade de retenção de água do alimento (Al-muhtaseb et al., 2002; Ordóñez et al., 2005). Na literatura, existem diversos modelos matemáticos capazes de predizer isotermas dos mais distintos produtos (Kurozawa, et al., 2005). Esses modelos são usados para determinar a umidade de um alimento em relação a atividade de água (Al-muhtaseb et al., 2002). Mais de 270 modelos são descritos na literatura para modelagem dos dados de equilíbrio, diferindo em caráter empírico ou teórico e no número de parâmetros envolvidos, entre esses os mais usados para curvas de sorção de alimentos são GAB, Halsey, Oswin e Peleg (Kurozawa, et al., 2005).

Lomauro et al., (2005) verificaram que a equação de GAB pode representar com grande precisão mais de 50% de isotermas de frutas, vegetais e carnes, quando comparado com equações de dois parâmetros. Alguns dos modelos mais usados para modelagem de dados de isotermas de alimentos estão apresentados na figura 4.

Nome do modelo	Modelo
GAB	$X = \frac{X_m C_{GAB} K_{GAB} a_w}{(1 - K_{GAB} a_w)(1 - K_{GAB} a_w + C_{GAB} K_{GAB} a_w)}$
BET	$\frac{X}{X_m} = \left(\frac{1 - (n+1) a_w^{\,n} + n.a_w^{\,n+1}}{1 - (1 - C) a_w - C.a_w^{\,n+1}} \right)$
BET linearizado	$\frac{a_w}{(1 - a_w) X} = \frac{1}{X_m C_{BET}} + \frac{a_w (C_{BET} - 1)}{X_m C_{BET}}$
HALSEY	$a_w = \exp(A / X^b)$
OSWIN	$X = A \left(\frac{a_W}{1 - a_W} \right)^B$
PELEG	$X = k_1 \, a_W^{\,n_1} + k_2 \, a_W^{\,n_2}$

Figura 4. Modelos para ajuste de isotermas de alimentos.
Fonte: Prado et al., (1999).

Em estudo de isotermas de *snacks* extrusados Wani & Kumar., (2016), observou que o modelo de GAB seguido de Oswin, BET e Smith melhor se ajustaram aos dados. Apesar de pouco usadas na rotina da indústria *pet food*, o conhecimento das isotermas pode contribuir com o aumento no *shelf-life* de produtos e com economia no processo de secagem nos processos industriais.

2.4 Oxidação lipídica

Os lipídeos estão presentes em quase todas as matérias-primas alimentícias, sendo as principais classes os triglicerídeos e os fosfolipídios. A fração lipídica dos alimentos está ligada diretamente a qualidade do produto, pois tem relação com as propriedades organolépticas, como aroma, cor, sabor, textura, suculência, estabilidade das proteínas e vida de prateleira. Além de ser fonte de ácidos graxos essenciais e vitaminas lipossolúveis (Ferrari, 1998; Silva et al., 1999; Silva et al., 2017).

A oxidação lipídica em alimentos é um conjunto complexo, involuntário e inevitável de reações (Barriuso et al., 2013). A sua ocorrência pode levar a formação *off flavors, off odors*, mudança de textura e coloração, fazendo com que o alimento diminua seu valor

nutritivo (Tian et al., 2013). Além da depleção de qualidade pela degradação de vitaminas e ácidos graxos essenciais, a oxidação lipídica pode causar a formação de compostos tóxicos, como por exemplo, os produtos avançados de lipoxidação (ALEs) sendo estes compostos citotóxicos e genotóxicos (Barriuso et al., 2013; Calligares et al., 2015).

A reação espontânea do oxigênio atmosférico com o lipídeo, conhecido como autoxidação é o processo mais comum de deterioração lipídica. Dinâmico, puramente químico e complexo, a autoxidação se divide em três etapas: iniciação, propagação e terminação, como mostrado na figura 5 (Gray et al., 1978; Silva et al.; 1999; Barden & Decker, 2013).

Na iniciação, ocorre a perda de um radical de Hidrogênio, assim como desaparecimento dos produtos de oxidação (oxigênio e ácidos graxos insaturados) e a formação o radical alquil do ácido graxo (Silva et al., 1999; Medina-Meza et al., 2014; Leão et al., 2017). Este reagirá com o oxigênio para formar radicais de peróxidos na etapa de propagação, fase da evolução oxidativa. Estes radicais formados reagem com ácidos graxos insaturados e formam hidroperóxidos. A segunda etapa se caracteriza pelo aparecimento dos produtos primários da oxidação, sendo estes os peróxidos e os hidroperóxidos (Silva et al., 1999; Leão et al., 2017). Por serem produtos instáveis se tornam susceptíveis a decomposição dando origem a produtos secundários, conhecida como etapa de terminação (Angelo,1996; Silva et al., 1999; Tian et al., 2013; Leão et al.,2017).

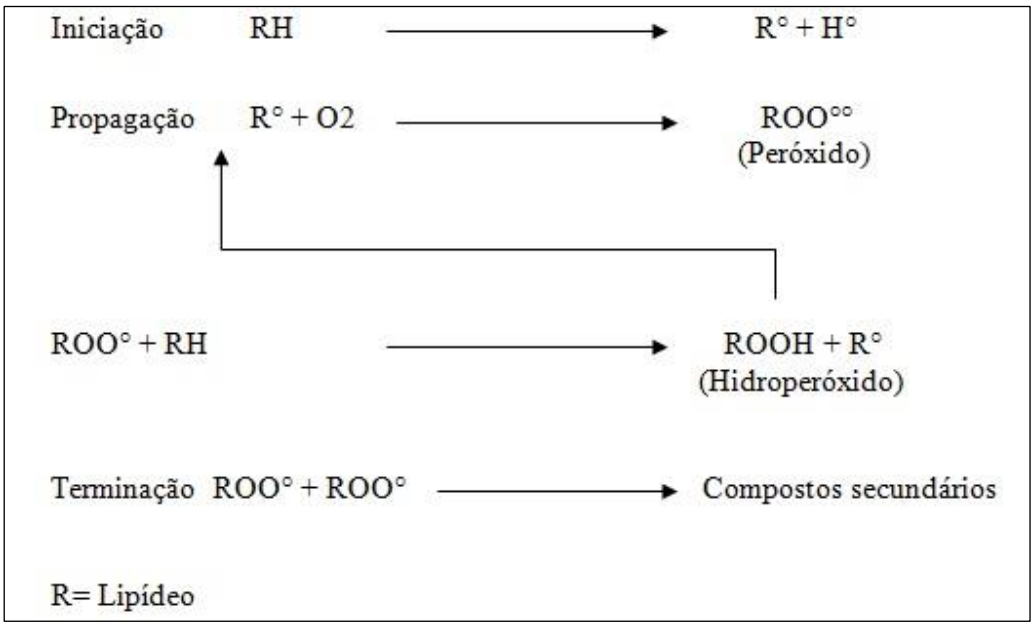

Figura 5. Reações do processo de autoxidação lipídica (adaptado de Gray, 1978).

Os processos envolvendo oxidação lipídica já foram revisados por diversos autores (Barden & Decker, 2013). Além da autoxidação existem outros mecanismos que levam a deterioração lipídica, como por exemplo, a fotoxidação, causado pela luz. Alternativamente a presença de metais de transição, como o ferro e o cobre que podem atuar como catalisadores, e a oxidação enzimática como a presença da enzima lipoxigenase atuando no processo pelo qual a deterioração oxidativa é iniciada (Silva et al., 1999; Barden & Decker, 2013).

2.5 Oxidação proteica

As proteínas se apresentam como um dos principais alvos do dano oxidativo, por seu alto teor nos tecidos e alimentos, assim como sua habilidade de neutralizar algumas espécies reativas (Davies, 2005). Diferente da oxidação lipídica e suas respectivas reações, a oxidação proteica só veio ser investigada nas últimas décadas (Estévez, 2015). Contudo, ambas reações (lipídica e proteica) estão diretamente ligadas, pois são iniciadas por radicais livres, e grande parte dos produtos da oxidação lipídica são radicais livres para a oxidação proteica (Wang et a., 2019). Mesmo com os estudos crescentes, há pouco sobre oxidação proteica em alimentos. Diversas podem ser as razões para esse fato, como: a complexidade química das reações, a falta de metodologias mais específicas e precisas e a falsa percepção que a oxidação lipídica e a contaminação microbiológica seriam as principais explicações para a deterioração de alimentos

A oxidação proteica tem início quando as espécies reativas de oxigênio e nitrogênio (ERO's e ERN's), buscando a estabilidade retiram um hidrogênio da cadeia peptídica principal ou de aminoácidos de cadeias laterais, formando um radical proteico (P•). Esse radical em presença de oxigênio formará rapidamente um radical peroxil (POO•), que por sua vez, buscando a estabilidade, passará a "atacar" outro alvo proteico, formando um peróxido de alquil ou hidroperóxidos proteicos (POOH). Existem três formas de ocorrência da oxidação proteica, sendo essas a modificação de um aminoácido específico, a clivagem do peptídeo mediado por radicais livres e por fim a formação da proteína de ligação transversal originada pela peroxidação lipídica. Além disso, a presença de aminoácidos como metionina, cisteína, arginina e histidina possivelmente tornam as proteínas mais sensíveis à oxidação (Ribeiro et al., 2018).

Reações de oxidação proteica induzem modificações nos resíduos de aminoácidos, alterando estrutura e função (Bachi et al., 2013). Os principais produtos formados, de forma irreversível, a partir da oxidação direta de proteínas, são as carbonilas (aldeídos e cetonas).

Estudos mostram que sua formação ocorre através da presença de espécies reativas e metais de transição, sendo que de todas as carbonilas formadas, cerca de 70% são os semialdeídos α-aminoadípico (AAS) e o γglutâmico (GGS). Metais de transição (Mnn^{+}) como cobre (Cu^{+}) e ferro ($Fe2^{+}$) também levam a formação de radicais alcoxil (PO•) e derivados hidroxil (POH). Além disso, um radical peroxil (LOO•), proveniente da oxidação lipídica, também pode atingir proteínas, formando um radical proteico (P•).

2.6 Metais de Transição

Os metais de transição, particularmente aqueles que possuem dois ou mais estados de valência, são um dos maiores agentes pró-oxidantes encontrados em alimentos. Eles reduzem a estabilidade oxidativa dos alimentos e tecidos biológicos por sua habilidade de decompor hidroperóxidos em radicais livres. (Mcclements & Decker, 2007). A reação catalisada por cobre e ferro é provavelmente a mais importante do ponto de vista prático (Yanishlievamaslarova, 2001).

Pequenas quantidades de metais já são suficientes para iniciar a decomposição de hidroperóxidos, assim como aumentar a velocidade da autoxidação. As reações de oxidação catalisadas por metais são determinadas por um conjunto de fatores, como o metal, o complexo formado, o quelante ou agente complexante, potencial redox do metal, hidroperóxidos e seus complexos, solventes, e a disponibilidade de oxigênio (Shahidi, 2005). Portanto, a etapa de iniciação da reação de oxidação catalisada por metais pode ocorrer por diferentes mecanismos.

Metais no estado de valência alta: envolve a transferência eletrônica direta do ácido graxo para o metal. Radicais de ácidos graxos são formados pela remoção de um elétron de uma ligação dupla (Equação 1) ou a partir de qualquer hidrogênio alílico do ácido graxo (Equação 2) (Shahidi, 2005).

Metais no estado de valência baixa: Produz um complexo ativado com molécula de O2, e como os metais redutores estão livres eles reagem rapidamente com o oxigênio.

$$RCH = CHR + M^{(n+1)+} \rightarrow R\dot{C}H - CHR + M^{n+} \quad (1)$$

$$RH + M^{(n+1)+} \rightarrow R \cdot + M^{n+} + H^{+} \quad (2)$$

Além disso, as reações de iniciação da oxidação lipídica por metais redutores (Co^{2+}, Fe^{2+}, V^{2+}, Cr^{2+}, Cu^{2+}, Mn^{2+}) ocorre por dois motivos diferentes, dependendo da disponibilidade do oxigênio e de hidroperóxidos no sistema (Shahidi, 2005). A primeira é a autoxidação de metais redutores, produzindo radicais de oxigênio que, então, irão reagir com lipídeos, como nas equações abaixo, usando o exemplo do ferro:

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{-\cdot}$$

$$O_2^{-\cdot} \leftrightarrow HOO^{-\cdot}$$

$$HOO^{-\cdot} \xrightarrow{H^+} L\cdot + H_2O_2$$

$$2O_2^{-\cdot} ou\ O_2^{-\cdot}/HOO\cdot \leftrightarrow H_2O_2 + O_2$$

$$H_2O_2 + Fe^{2+} \rightarrow HO\cdot + OH^- + Fe^{3+}$$

$$HO\cdot + LH \rightarrow H_2O + L\cdot$$

A segunda forma seria a redução ou oxidação de qualquer fonte de hidroperóxidos para RO• ou ROO•, respectivamente, que, então, reage com lipídeos, demonstrado nas equações abaixo.

$$Fe^{2+} + ROOH \xrightarrow{\text{Rápida}} Fe^{3+} + RO\cdot + OH^- \xrightarrow{LH} ROH + L\cdot$$

$$Fe^{3+} + ROOH \xrightarrow{\text{lenta}} Fe^{2+} + ROO\cdot + H^+ \xrightarrow{LH} ROOH + L\cdot$$

De fato, em presença de metais, os hidroperóxidos (ROOH) podem decompor-se para formar radicais alcoxila intermediários (RO• e ROO•), contribuindo para a propagação das reações em cadeia que, consequentemente, diminuem a estabilidade do alimento.

2.7 Aditivos

Segundo a Instrução Normativa No 13 de 30 de novembro de novembro de 2004 do Ministério da Agricultura Pecuária e Abastecimento (MAPA), em vigência, aditivos destinados a alimentação animal são substâncias ou microrganismos adicionados intencionalmente às dietas dos animais, em pequenas proporções, tendo ou não valor

nutritivo, para melhorar os índices produtivos e a saúde dos animais (MAPA, 2004). Tais aditivos não podem contaminar o meio ambiente, deixar resíduos nos produtos a serem consumidos e são usados sob determinadas normas (Butolo, 2010).

Os aditivos químicos inibidores (preservadores) são as substâncias que apresentam a principal função de controlar reações químicas e/ou biológicas indesejáveis. Entre os principais produtos o mais utilizado é o composto nitrogenado conhecido como ureia (CH_4N_2O). No entanto, o carbonato de cálcio ($CaCO_3$), hidróxido de sódio (NaOH), o benzoato de sódio (C_6H_5COONa), o pirossulfito de sódio (NaS_2O_5), o ácido fórmico (CH_2O_2), o formol (HCO_2) e misturas compostas por formol e ácido fórmico, também são exemplos de aditivos químicos (Neumann et al., 2010). Além do ácido acético e propiônico em menor escala.

Já quando falamos dos aditivos microbiológicos, tais como: as bactérias homofermentativas, heterofermentativas ou a combinação de ambas, o principal foco é a fermentação dentro do silo e inibição do crescimento de microrganismos aeróbios (leveduras) e anaeróbios indesejáveis (enterobactérias e clostrídeos) (Zopollato et al., 2009). Também, pensando na inibição da atividade de proteases e deaminases no período de fermentação e durante a fase de abertura do silo (Kung Jr. et al., 2003).

Os sequestradores de umidade atuam na redução do teor de água na forragem, concentra os carboidratos solúveis, diminui a ocorrência de fermentações clostrídicas, favorece o abaixamento do pH, reduz a quebra de proteína em amônia e diminui a produção de gases e efluentes (Andrade et al., 2010).

2.8 Ácidos orgânicos

Pode-se definir como conservante toda a substância que impede ou retarda a alteração dos alimentos provocada por microrganismos ou enzimas. O controle do crescimento de microrganismos em alimentos por conservantes químicos à base de ácidos orgânicos está relacionada com o pH do meio. A forma não dissociada da molécula é que confere a característica antimicrobiológica dos conservantes. Os valores de pKa (pH nos quais 50% da molécula se encontra na forma dissociada) da maioria dos conservantes encontram-se na faixa de pH entre 3,0 e 5,0, portanto a concentração da forma não dissociada aumenta com o aumento da acidez, garantindo maior eficiência no controle dos microrganismos (Almeida, 211). A escolha de um agente de conservação deve ser baseada no conhecimento do seu espectro antimicrobiano, as propriedades químicas e físicas tanto do alimento quanto do

conservante, as condições de manuseio, processo e estocagem e, a segurança de alta qualidade inicial do alimento a ser conservado.

Como aditivos para a indústria alimentícia, os ácidos possuem uma dupla finalidade: acidulante e conservante. O ácido fosfórico é usado na indústria de refrigerantes do tipo cola para reduzir o pH. O ácido acético é usado na fabricação de maioneses e molhos para saladas para dar aos mesmos sabor levemente picante. Outros ácidos orgânicos tais como o cítrico, tartárico, málico, láctico e o fumárico são utilizados em grande variedade de alimentos, em funções similares. Os ácidos propiônico e sórbico são usados pela sua ação antimicrobiana, é particularmente usado pelas suas propriedades fungicidas. O ácido propiônico, em solução de 10%, é aplicado na superfície de queijos para evitar a formação de mofos. O efeito como fungicida é maior em pH por volta de 4,0 que em pH 5,0. Os sais de cálcio e de sódio do ácido propiônico também apresentam propriedades antimicrobianas.

As drogas antifúngicas exercem ações fungistáticas e fungicidas, direta ou indiretamente. Os antifúngicos têm características especiais quanto ao mecanismo de ação, via de administração, ação em micoses superficiais e ou sistêmicas, podendo ser classificados com base no sítio-alvo e estrutura química, sendo que estes atuam em sua maioria na membrana celular, excetuando-se a fluocitosina e a griseofulvina, que atuam na síntese do ácido nucleico (Lacaz et al, 1991). Krabbe et al. (1996), avaliando a eficiência de antifúngicos observaram que o sulfato de cobre adicionado ao milho triturado durante o armazenamento foi ineficiente. Por outro lado, estes mesmos autores observaram que o ácido propiônico utilizado em dosagem correta foi eficiente no controle de fungos, reduzindo perdas de valor nutricional em milho. Os danos causados pelos fungos estão relacionados às perdas nutricionais em matérias-primas e alimentos completos e, dependendo da espécie e condições favoráveis estes produzem toxinas. Krabbe & Maciel, (1995), observaram que o ácido propiônico foi eficiente no controle de fungos, reduzindo perdas do valor nutricional em milho. Entretanto, o uso de antifúngico à base de ácido propiônico é pouco utilizado em alimentos para cães e não há informações publicadas disponíveis, apesar de sua inocuidade para a saúde animal e humana. Gomez et al. (2008), avaliaram o desenvolvimento fúngico em milho contaminado, durante sete semanas. Os testes foram em nível de laboratório, com uso de três produtos que tinham como base o ácido propiônico, todos foram eficientes na redução dos fungos ao longo do tempo.

2.8.1 Ácido propiônico e lático

O ácido propiônico apresenta a fórmula molecular C3H6O2, com sua utilização em conservação de alimentos se mostra um dos ácidos orgânicos mais eficientes no controle de fungos do gênero *Aspergillus* ssp. Pelo fato de os ácidos fracos apresentarem melhor ação antimicrobiana na forma não dissociada, que facilita a entrada na célula e a dissociação no citoplasma causando o desequilíbrio celular dos microrganismos (Weiss, Loeffler & Terjung, 2015). Esse ácido atua atravessando a membrana plasmática de forma não dissociada e causando acidificação no citoplasma das células dos microrganismos, levando à depleção de energia e morte celular. O ácido tem um pKa de 4,88, significando que metade do ácido está na forma não dissociada neste pH (Dijksterhuis et al., 2018). A atividade antifúngica eficaz (MIC, concentração inibitória mínima) do ácido propiônico contra vários fungos está tipicamente na faixa de 7,8 a 39 mM (0,1 a 0,5%, p / v) quando cultivado em caldo Sabouraud a um pH de 5. Assim, como o ácido propiônico pode ser usado em diversas concentrações como conservante em alimentos, os ácidos orgânicos em geral são bons antifúngicos e podem ser utilizados em diversos alimentos da dieta humana, já na dieta animal, principalmente em grãos e cerais que são utilizados da produção de ração.

Por sua vez o ácido lático (C3H6O3) tem pka de 3,88, apresenta-se na forma líquida, com sabor suave, sem odor. Pode ser aplicado em alimentos em conservas ou bebidas com função palatabilizante (Belitz et al., 2009; Baltes, 2007). Esse ácido cadeia fraca (CH3-CHOH-COOH) com mecanismo de ação semelhante ao ácido propiônico, no entanto, é menos eficiente, sua ação está mais voltada ao controle de bactérias (Muynck et al., 2004). O controle de fungos, por sua vez, requer a maior quantidade de ácido lático. Para melhor atividade antifúngica, é necessário que o ácido esteja predominantemente na sua forma não ionizada. Porém, de acordo com Stanojevic-Nikolic et al. (2020), o ácido láctico pode ser considerado bom agente de controle do crescimento microbiano para fungos da família *Aspergillus spp*.

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4. OBJETIVOS GERAIS

Neste trabalho, objetivou-se estudar e modelar matematicamente alguns componentes que influenciam a vida de prateleira em *Pet food* extrusado, considerando a estabilidade oxidativa e susceptibilidade microbiana. Para isto, a tese foi dividida em três capítulos, apresentados na forma de artigo científico, com os seguintes objetivos em cada um deles:

- Determinar as relações entre os níveis de ferro e teor de gordura com a estabilidade oxidativa dos alimentos.

- Estudar as relações entre umidade e atividade de água em alimentos secos extrusados para cães e gatos, por meio das curvas de isotermas de sorção.

- Determinar a relação entre a atividade de água em alimentos secos extrusados e a susceptibilidade ao desenvolvimento de bolores e verificar os efeitos de misturas de ácidos orgânicos na prevenção da deterioração microbiana

Artigos redigidos conforme as normas da revista LWT - *Food Science and Technology*

Modelling the oxidative stability of dry pet foods with different levels of iron and fat

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ABSTRACT

Extruded pet foods have varying concentrations of oxidizing and oxidizable compounds, such as lipids, proteins and transition metals. Regardless of these existing differences, the antioxidants doses used are often similar. It´s important to quantify the relationship between oxidizable substrates and pro-oxidant substances to improve its shelf-life. The aim of this study was to evaluate the influence of fat content and iron concentration in Pet foods, and their interaction, on the oxidative stability. A basal dry extruded food was formulated following the recommendations of FEDIAF (2019), without additional inclusion of fat and iron sources. Twenty-four treatments were developed from basal diet, including different levels of poultry fat or iron sulphate, by the statistical approach of a central compound planning. The treatments included: Negative Control (NC) without inclusion of transition metals, antioxidant and fat in the finished product, and iron and/or fat content of 62.3, 153.7, 245 and 307.3 mg/kg for iron, and 3.0, 7.5, 12 and 15.7% for poultry fat. Samples were packaged and maintained at room temperature for 12 months to determine the oxidative stability, measured by Peroxide value (PV) and acidity index. The iron concentration affected the oxidative

stability, presenting a good fit of the mathematical model, and showing the importance of controlling the transition metals in Pet food. Those relationship were also observed for fat content, but to a lesser extent. Although iron and fat contribute for oxidation in Pet foods, these factors explained less than 30% of all variation in the PV, suggesting that other factors like antioxidant concentration, processing and oxygen presence are also important to determine the Pet food shelf-life.

Keywords: Oxidation, *shelf-life*, oxidative reactions.

1. Introduction

Lipid oxidation is one of the main causes of nutritional value depletion during the processing, handling, and storage of foods with a high fat or oil content (Mussinan et al., 1998). Its occurrence can lead to off flavors, off odors, change in texture and color, as well as the formation of various products such as peroxides, hydroperoxides, aldehydes and ketones, directly affecting the food shelf life (Zhou et al., 2013; Amaral et al. al., 2018). Furthermore, it has recently been shown that protein oxidation processes can cause deleterious effects on protein functionality and structure. Protein damage impairs the meat and animal products quality, such as offal, which are ingredients used in large amount in Pet foods (Gallego et al., 2018). The adequate control of Pet food oxidation is important to produce safer and healthier products.

Extruded pet foods have varying concentrations of oxidants and oxidizable compounds, such as, respectively, transition metals and lipids, proteins, and vitamins. To prevent the oxidation and, to extend the shelf-life, antioxidant compounds, like BHT, BHA, TBHQ and tocopherols are widely used in food for human and for animal consumption (Hu et al., 2020). However, the prevention of oxidation is effective controlled by using the principle of hurdle technology. This principle consists to controlling the main factors that favor the food oxidation, like temperature, pH, water activity, transition metals, antioxidant concentration, packaging conditions, and others (Singh &Shalini, 2016). Transition metals, particularly those that have two or more valence states, are one of the major pro-oxidants found in foods. They reduce the oxidative stability of foods and biological tissues due to their ability to break down hydroperoxides into free radicals. (Mcclements & Decker, 2007). Foods intended for pets may have a maximum legal content of 142 mg of iron per 100 g of DM (FEDIAF, 2019). The reaction catalyzed by copper and iron is probably the most important from a

practical point of view (Yanishlievamaslarova, 2001). As with metals, the high lipid content is susceptible to deteriorative processes, such as autoxidation. Lipid oxidation leads to the formation of free radicals (Turek et al. 2003).

Knowing the relationships between the concentrations of oxidizable substrates and pro-oxidant substances in a food and relating them to the doses of antioxidants is essential to extend its shelf life. According to the justification above, this experiment aimed to evaluate the effect of adding increasing levels of transition metals and fat on the shelf-life of pet food.

2. Material and methods

2.1. Experimental design

Based on more treatment designs complexes, which use all combinations of factors under study, several attempts were made to reduce the number of experimental points, through techniques such as fractional repetition. In addition to this, with the objective of reducing the number of points experiments, the technique was created using the composite projects. The design applied was the completely randomized with repeated measures over time. With a central compound planning, it was possible to define the interactions between the levels of agents (Iron and fat), totaling 24 treatments. The following addition of iron were performed in a negative Control diet (NC): 62.3, 153.7, 245 and 307.3 mg/kg. The fat was included at the following levels: 3.0, 7.5, 12.0 and 15.7%. The agents were applied to the already extruded product, using the spray method.

2.2. Extrusion processing and treatments

A standard dry extruded food for adult cats was formulated following the recommendations of FEDIAF (2019), with ingredients conventionally used in Pet food

(maize, broken rice, poultry by-product meal, corn gluten feed, cellulose, poultry fat, mineral and vitamin premix). The poultry by-product meal and poultry fat were produced with no antioxidant addition. BHA (BHA P.A., ACS 99.9%, Labsynth Produtos para Laboratório, Diadema, Brazil) was diluted in the Poultry fat and added in the finished product by coating. For the extrusion of the NC, the ingredients were weighed, mixed, and ground in a hammer mill equipped with a 0.8mm sieves. The NC diet was extruded in a Pet food company (Danês Alimentos SA, Apucarana, Brazil) in a single screw extruder (E200-AR, Ferraz Máquinas e Engenharia Ltda., Ribeirão Preto, Brazil). After processing, the diet was coated with poultry fat (1%) containing BHA as antioxidant, for all treatments. The additional poultry fat was added without antioxidant, to reach the fat content of the experimental treatments. A water solution of iron sulphate (Iron sulphate P.A., ACS, Labsynth Produtos para Laboratório, Diadema, Brazil) was prepared and applied by coating before poultry fat addition, to reach the iron levels of the experimental treatments. All the treatments were added by 1% of palatable liquid (D'tech 12L, Diana Pet Food, Descalvado, Brazil) after iron and fat inclusion.

The treatments were analyzed for Crude Protein (method: 954.01), Ethereal Extract by Acid Hydrolysis (method: 954.02), Crude Fiber (method: 978.10), Ash content (method: 942.05), Moisture (method 930.15) and Water Activity (method 978.18), and all non-nitrogen extractives were determined by the difference of the other determined components of the ration (moisture, CP, EEHA, MM and FB) to 100%.

2.3 Shelf-life test

The shelf-life determination was carried out at the Laboratory of Nutrition and Metabolism of Domestic Cats, at State University of Maringá (UEM). Each treatment was stored in a 100 grams polypropylene packaging, properly sealed to avoid direct contact with atmospheric air. The samples were kept at room temperature in their

respective packages in a 3.5 mx 3.5 mx 2.8 m (width x depth x height) room, with natural and artificial lighting. Temperature and relative moisture were daily recorded, with an average of 24.1 ºC ± 3.4 for temperature and 66% ± 12 for moisture. Every 45 days, a package of each treatment was withdrawn, frozen at -20ºC until the laboratory analysis, totaling nine collection periods: 0, 45, 90, 135, 180, 225, 270, 315 and 360 days.

2.4 Analyzes

The following analyzes were performed: Peroxide value (PV), Acidity index, Iron concentration and Protein oxidation by Fourier Transformed Infrared Spectroscopy (FTIR), as described below.

2.4.1 Peroxide Value (PV)

To evaluate the oxidation parameter, analysis is usually carried out to determine the peroxide value (IP). For Peroxide value analysis in samples, the official method of the Brazilian Animal Feed Compendium, 2009, was used in an adapted way.

First, 20g of the sample was weighed in a 250 ml Erlenmeyer flask, enough to contain at least 2g of oil. Next to the sample, 50 ml of methanol, 25 ml of chloroform and 18.2 ml of water were added, the amount of water was calculated so that the sum of the water in the sample (from moisture) resulted in 20 ml of water, then the Erlenmeyer flasks were hermetically capped and subjected to agitation for a period of 30 minutes. After the stirring time, another 25 ml of chloroform and 25 ml of 1.5% sodium sulfate solution were added, capped again, and stirred for another 2 minutes. Subsequently, the solution, with the sample, was transferred to a separating funnel, where the layers were naturally separated. The lower layer (chloroform + lipid) was allowed to pour into a smaller funnel containing paper and a little anhydrous sodium sulfate, to remove the

traces of water that were invariably carried away, collecting the filtrate in a 125 ml Erlenmeyer flask. With the aid of a volumetric pipette, exactly 20 ml of the filtrate was pipetted into another 125 ml Erlenmeyer flask and 20 ml of concentrated acetic acid and 0.5 ml of a fresh and saturated solution of potassium iodide were added. The solution was gently shaken and left in the dark for exactly 1 minute. Finally, 30 mL of distilled water and 1 mL of 1% starch solution were added, if any change, even if small, in color was noticed when adding the starch, changing from yellow to purple, the solution was titrated with a solution of 0.1N Sodium Thiosulphate, until the purple color disappeared. With the confirmation of peroxide, 5 ml of the filtrate was pipetted onto previously tared fat plates and placed in an oven at 105°C for one hour. After this the fat plates were cooled in a desiccator and weighed. A blank test was performed using the reagents, but without the presence of the sample.

With the volume of Sodium Thiosulfate spent in the titration of each sample, the peroxide index was calculated using the following formula:

$$Perodixe\ Value \frac{mEq}{1000g} FAT\ = \frac{(A-B)x\ M\ x\ F\ x\ 1000}{Px4}$$

On what:

A: Volume of 0.1M Sodium Thiosulfate used in the titration of the sample, in ml;

B: Volume of 0.1 M Sodium Thiosulfate used in the titration of the blank test, in ml;

M: Molarity of Sodium Thiosulfate Solution.

F: Sodium thiosulfate solution correction factor.

P: Weight of fat extracted in aliquot x 4 (weight of plate with fat – weight of empty plate), in grams.

1000: Conversion to milliequivalents.

2.4.2 Acidity Index

For acidity index analyses, the methodology of Instituto Adolfo Lutz (IAL, 2005) was used. About 2.5 g of sample was weighed in a 125 ml Erlenmeyer flask and 100 ml of ethyl ether-ethyl alcohol solution (2: 1, v / v) was added, then the flask was subjected to preparation for 10 minutes. Then, the solution was filtered on qualitative filter paper and 5 drops of the indicator solution containing phenolphthalein were added. Finally, it was titrated with a 0.01 mol/L NaOH solution, until reaching a pinkish color. A blank test was performed under the same conditions, without the presence of the sample.

Acidity index calculations were made following the following formula:

$$\frac{IA = ((A - B)\ x\ M\ x56.1)}{m}$$

On what:

A: volume of NaOH solution (ml) used in sample titration.

B: volume of the NaOH solution (ml) spent on blank titration.

M: Molarity of the NaOH solution.

M: Sample mass (g).

2.4.3 Determination of Iron concentration

The determination of iron (Fe) concentration was carried out by preparing the mineral solution, according to the methodology described by Silva and Queiroz (2002). With the mineral matter (ash) previously obtained, the mineral solution was prepared, which consists of dissolving the ash in a hydrochloric acid solution (1:1), to solubilize the mineral present, and then readings of the solutions were taken in colorimetric spectrophotometer, recording the concentration to prepare the standard curve with the

readings obtained. All readings were performed in duplicate and repeated when the differences between them were greater than 5%.

2.5 Statistical analysis

For data statistical analysis, multiple regression models were considered. According to the data from the analyzes carried out in the present study, predictive equations for the oxidative stability of pet food were elaborated. The adjustment of the models was performed using the R program version 3.3.3 (2017). The goodness of fit of the models was measured using R2 and Akaike's information criterion. For diagnosis and verification of normality and heteroscedasticity, residual graphs were performed, including a simulated envelope graph implemented in the HNP package (version 1.2-2).

3. Results

The treatments composition for iron and ethereal extract levels approached the predicted, as shown in table 1.

Table 1

Chemical composition (g/kg) of the 24 treatments (as fed basis).

Item	Parameter (g/Kg)								
	Moisture	Ash	Crude protein	Added Fat	Analysed Fat	Added Iron	Analysed Iron	Crude fiber	NFE
1	82	62	344	30	84	62	88	33	395
2	83	62	338	30	91	245	269	33	393
3	78	56	308	120	143	62	86	33	382
4	81	55	321	120	155	245	268	33	354
5	87	59	336	30	87	62	90	33	398
6	88	62	341	30	85	245	269	33	391
7	77	57	322	120	139	62	87	33	372
8	84	56	287	120	144	245	270	33	396
9	75	61	329	75	117	154	179	33	385
10	83	58	318	75	117	154	180	33	391
11	79	59	329	75	122	154	180	33	378
12	82	59	329	75	121	154	179	33	376
13	88	60	326	75	125	154	181	33	369
14	87	59	329	75	125	0	28	33	367
15	83	58	331	75	120	307	335	33	375
16	82	63	344	0	74	154	179	33	403
17	81	55	320	151	166	154	180	33	345
18	83	58	336	75	119	154	181	33	371
19	91	58	331	75	126	154	180	33	360
20	88	60	340	0	72	0	31	33	407
21	85	61	346	0	65	0	31	33	409

22	85	62	356	0	68	0	31	33	396
23	86	62	355	0	70	0	31	33	395
24	88	65	345	0	75	0	31	33	394

NFE: Nitrogen-free extract.

The iron concentration affected the oxidative stability of all samples (Figure 1), evidencing the catalytic influence of transition metals on lipid oxidation. Note that there was a linear and corresponding increase in the peroxide index when the iron concentration was increased, fixing the ether extract and antioxidant content, as shown in Table 2, through the results it was possible to generate equations, where the effect of iron addition in the samples for 180 and 365 days was worked. The generated equations obtained excellent adjustments of R^2 (Figure 2).

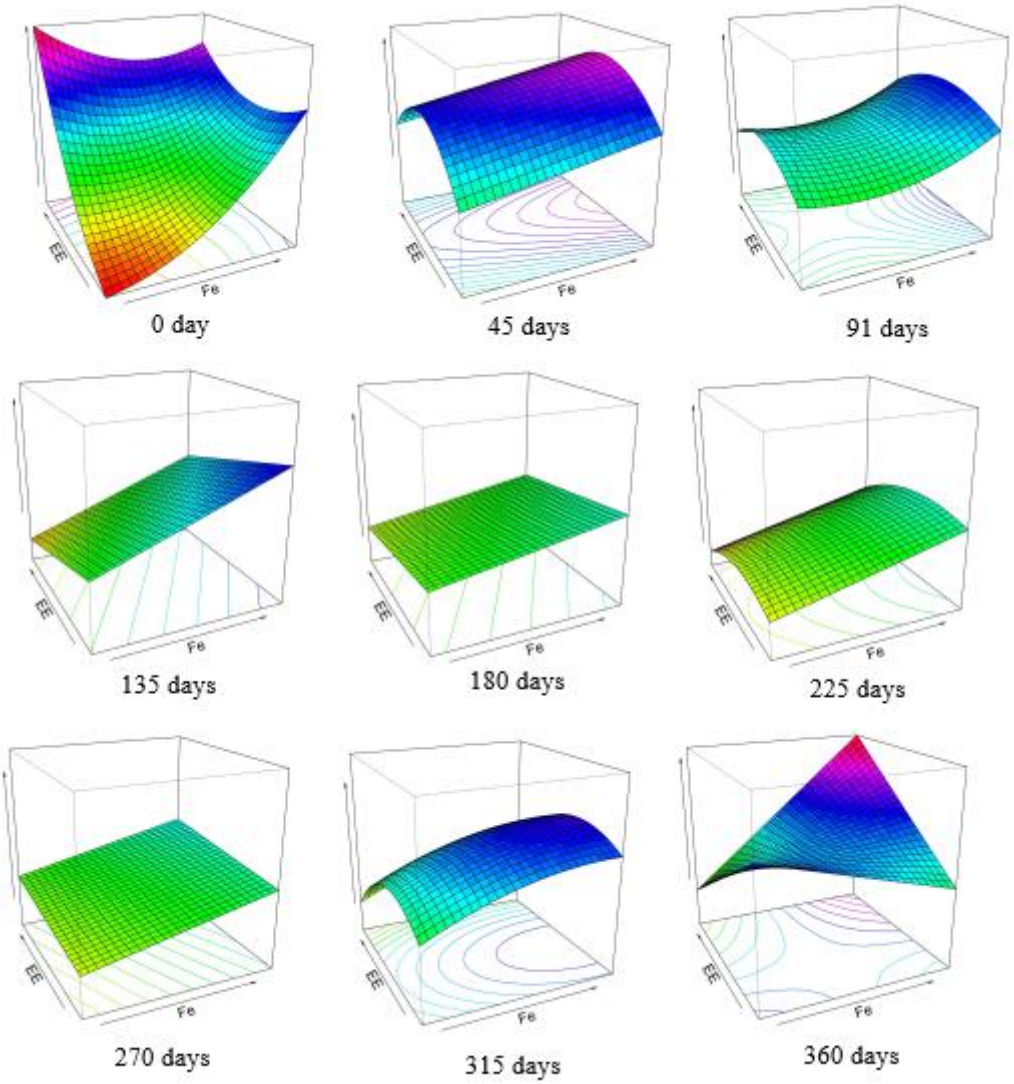

Figure 1. Response surface of the Peroxide value (PV, mEq/kg) in the interval of 360 days, where EE: ether extract and Fe: Iron content.

Table 2

Predictive peroxide values (PV, mEq/kg of fat) depending on the iron concentration (mg/kg of diet, as fed basis), considering the fat content of 120 g/kg and the synthetic antioxidant (BHA) concentration of 50 mg/kg of diet.

Days	Iron concentration (mg/kg)						
	50	100	150	200	250	300	Increment*
	PV (mEq/ Kg of fat)						
0	2.50	2.64	3.29	4.43	6.08	8.22	1.14
45	9.11	9.55	10.00	10.44	10.89	11.33	0.44
90	10.87	10.75	11.14	12.02	13.41	15.29	0.88
135	8.31	9.22	10.14	11.05	11.97	12.88	0.91
180	10.45	10.84	11.23	11.62	12.01	12.40	0.39
225	13.62	14.01	14.40	14.79	15.18	15.57	0.39
270	6.46	6.76	7.05	7.35	7.64	7.94	0.30
315	10.63	11.45	11.76	11.58	10.89	9.71	-0.18
360	10.86	11.56	12.26	12.96	13.66	14.36	0.70

*Increment on the PV by each 50 mg/kg of increased iron concentration by evaluation period.

In comparative terms, the peroxide value was higher in treatments with higher iron inclusions (Table 3). On the other hand, the acidity level remained regular as seen in table 4.

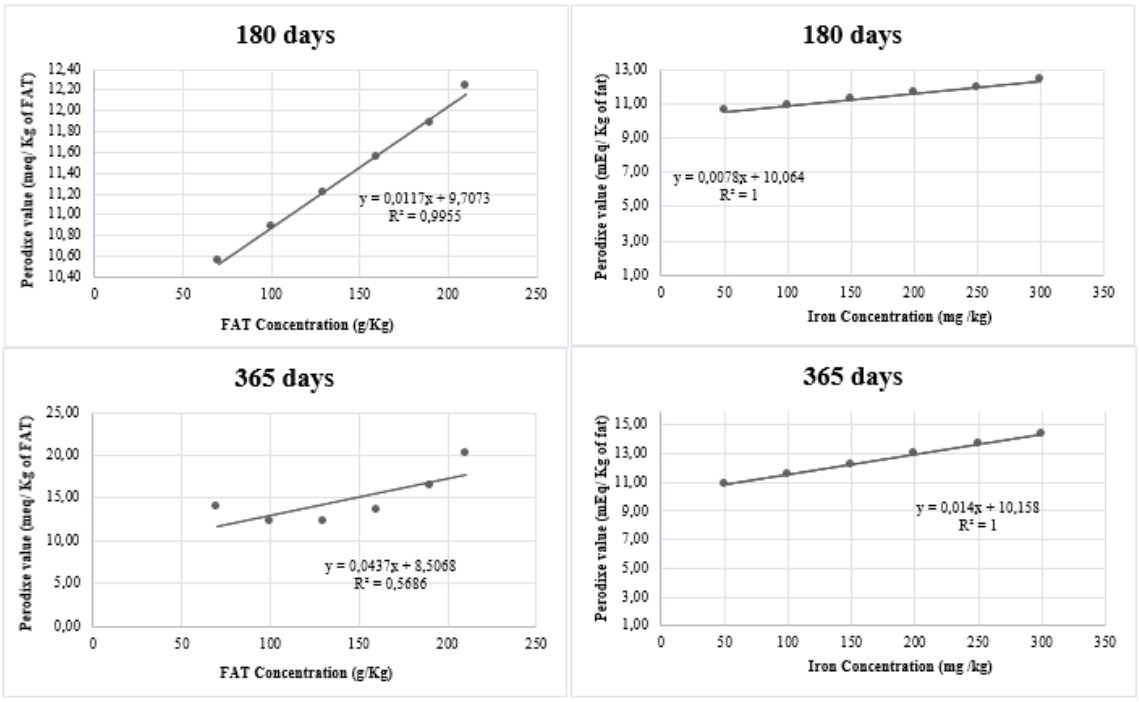

Figure 2. Predictive equations to estimate the Peroxide value (PV, mEq/kg of fat) of the diets depending on the Iron (mg/kg of diet, as fed basis) or fat (% of diet, as fed basis) concentration.

Table 3

Oxidative evolution according to peroxide value.

Item	PV (meq FAT/ Kg)								
	0 day	45 days	91 days	135 days	180 days	225 days	270 days	315 days	360 days
1	2.7	9.2	9.2	8.1	11.1	11.6	7.9	7.4	7.4
2	2.9	10.8	12.8	11.2	11.9	11.9	12.3	13.0	10.8
3	4.7	9.4	11.0	7.5	10.9	10.5	8.0	8.4	10.1
4	4.6	9,.6	10.7	10.1	12.2	12.9	12.7	12.5	15.4
5	0.0	13.0	16.8	17.3	18.5	16.8	15.2	14.2	13.7
6	0.0	14.2	19.1	19.1	26.2	21.8	22.8	19.2	20.6
7	4.8	12.0	11.6	11.4	14.8	13.3	13.7	13.4	13.6
8	5.5	12.2	14.3	16.3	16.0	17.9	17.4	18.9	16.2
9	2..4	12..6	13..8	14..0	12..5	13..6	15.6	15.8	14.5
10	2..5	10.7	11.2	12.9	14.8	14.7	15.7	16.6	16.3
11	2.4	13.6	13.2	13.7	14.0	15.6	16.0	16.9	17.1
12	3.0	14.3	14.8	16.2	17.0	16.0	16.3	16.6	16.3
13	2.2	12. 2	12.0	13.5	15.0	14.7	14.1	14.6	14.3
14	2.6	13.7	13.8	9.0	11.8	11.1	11.5	11.2	11.0
15	4.9	15.4	15.7	15.9	15.7	18.2	17.5	18.1	17.9
16	5.3	10.6	10.1	13.2	13.4	13.6	13.3	15.6	15.1
17	6.1	10.8	11.0	11.1	12.3	11.5	16.1	10.6	14.4
18	2.2	4.6	8.1	8.9	8.4	9.7	9.6	9.4	24.6
19	3.2	16.1	16.1	16.0	17.8	18.7	18.8	18.3	18.5
20	0.0	0.0	4.3	5.0	8.0	9.6	9.9	9.1	9.7
21	0.0	0.0	1.7	7.4	5.4	7.6	7.0	7.4	9.0

22	0.0	0.0	5.5	6.7	9.1	8.1	8.5	7.7	8.3
23	0.0	0.0	4.4	5.3	6.5	6.1	8.7	8.2	9.5
24	0.0	0.0	2.2	5.7	7.1	9.5	8.4	8.8	8.9

PV: Perodixe value

Table 4

Acidy index of treatments.

Item	Acidity (g NaOH/ g FAT)						
	0 day	45 days	90 days	135 days	180 days	270 days	360 days
1	0.7	1.1	1.1	1.1	1.1	1.4	1.3
2	0.9	0.9	1.2	1.1	1.2	1.4	1,5
3	1.2	1.1	1.1	1.3	1.4	1.4	1.5
4	1.1	1.1	1.1	1.5	1.5	1.5	1.5
5	0.7	1.0	1.0	0.9	1.1	1.3	1.3
6	0.8	0.9	1.1	1.0	1.1	1.8	1.8
7	1.0	1.0	1.4	1.3	1.5	1.5	1.5
8	0.9	1.2	1.4	1.4	1.8	1.7	1.7
9	0.7	1.2	1.4	1.2	1.3	1.3	1.4
10	0.4	1.0	1.3	1.2	1.5	1.5	1.5
11	0.3	1.2	1.3	1.1	1.5	1.5	1.5
12	0.4	1.2	1.2	1.1	1.3	1.4	1.5
13	1. 0	1.0	1.8	1.1	1.3	1.5	1.5
14	0.9	0.8	1.5	1.2	1.7	1.7	1.7
15	0.9	1.1	1.7	1.3	1.2	1.5	1.5
16	0.9	1.0	1.1	1.1	1.1	1.1	1.1
17	1.2	1.1	1.8	1.6	1.5	1.7	1.7
18	1.3	1.0	1.4	1.3	1.3	1.3	1.3
19	1.1	1.0	1.5	1.5	1.5	1.5	1.7
20	0.9	1.1	1.1	1.0	1.3	1.1	1.1
21	0.8	1.0	1.0	0.8	1.0	1.0	1.3

22	0.8	0.8	1.0	0.9	1..0	1.2	1.1
23	0.8	0.9	0.9	0.8	0.9	1.0	1.1
24	0.9	0.9	0.9	0.8	1.0	1.1	1.1

1. Discussion

With the results observed by iron and fat inclusion, it was possible to observe how much the pro-oxidizing and oxidizable agents interfere in the pet food stability.

Fats and oils are essential components of dry pet foods. They contribute to the taste, nutritional value, texture, and palatability of foods. However, these components are highly sensitive to the oxidation phenomenon. The first problem arising from oxidation is that this process represents a source of unpleasant odors generated by rancidity (Morelli et al., 2021).

The prevention of lipid oxidation is necessary for animal performance, health, as well as to maintain the quality of animal products. Pet foods are products that contain fat, and lipids can range from 5 to 40% (Hu et al., 2020). The high lipid content is susceptible to deteriorative processes, such as autoxidation. Lipid oxidation leads to the formation of free radicals (Turek et al. 2003).

The fat presence can influence lipid oxidation, serving as a substrate for the reactions, however, it was observed that in this study the presence of iron exerted a greater influence.

Transition metals are oxidation catalysts because they have the ability to catalyze the formation of free radicals as in autoxidation (Miller et al., 1990; Shahidi, 2005; Azri et al., 2021). They have the ability to catalyze the formation of free radicals, as in autoxidation. Its molecules contain allelic hydrogen that can form free radicals, which in turn react with oxygen to form hydroperoxides. Small amounts of transition metals can initiate the decomposition of hydroperoxides as well as accelerate the autoxidation rate (Shahidi, 2005).

The linear and corresponding increase in the peroxide index in relation to the iron concentration can be explained by the fact that in a homogeneous catalysis system, where the catalytic agent is in the same phase as the reactants, the metal ions concentration is the same as that of active sites, present in the reaction system. Thus, the increase in active sites will lead to an increase in catalytic activity, therefore, increasing the metals concentration and the catalytic activity (Shahidi, 2005).

According to the literature, several factors may be responsible for the metal catalytic effect, one of which is linked to their valence. In the present study, ferrous sulfate, a typical example of a bivalent iron compound, was used as an iron source. Trivalent metals lead to less reduction in oxidative stability compared to bivalents. This fact can be explained by the greater attraction exerted by trivalent cations, leading to the formation of inner sphere complexes with lower catalytic activity. In this sense, divalent cations can lead to external sphere complexes, with rapid electron transfer and, consequently, a reduction in oxidative stability (Silva et al., 2011).

This may explain the negative correlation between mineral content and oxidative stability. Sarin et al., (2010) evaluated the effect of transition metals in palm biodiesel, considered to have good stability. The authors observed that the biodiesel stability was impaired with the presence of metals.

Iron has a strong pro-oxidant effect in various food systems. It is capable of generating reactive oxygen species (ROS), including hydroxyl (•OH) and superoxide anion radicals ($O_2^{\bullet-}$), which can react directly with unsaturated fatty acids to produce hydroperoxides and promote oxidative damage at different levels (Saiga et al., 2003). According to Daoud et al., (2020), iron acts as a catalyst for lipid oxidation in oil samples. Where the interactions of iron with oxygen can constitute the first step for

oxidative reactions. Thus, an absolute reduction of oxygen could limit oxidation, even with the iron presence.

Studies carried out by Jain & Sharma (2014) also indicated this catalytic effect of metals, demonstrating that its influence is detrimental to the stability of Jatropha biodiesel.

The peroxide value (PV) in general among all treatments, increased throughout the experimental period. Gross et al. (1994) also found a linear increase in the peroxide index when working with test dog food at room temperature at 22.2°C.

The data found in the present study showed linearity, demonstrating that the peroxide value is a reliable and accurate analysis for assessing the degree of lipid oxidation of dry and extruded foods for dogs and cats. Despite the existing criticism of the peroxide value determination, as this is a subjective method, since the titration is performed visually, it proved to be a good indicator of lipid oxidation under normal conditions (Jung-Min et al., 2016). The acid index (AI) measures the number of acidic functional groups present in the sample, according to the results presented in the present study, this variable remained regular over time, not being influenced by the variables tested (fat and iron).

Despite the promising results, indicating levels of iron and fat that affect the food stability for companion animals, more studies need to be carried out to bring more accurate results, as an example in the use of antioxidants. Some limitations were observed in this study, such as the form of iron application, non-preserving fat and viscera flour with antioxidant, which left the PV high in the first days of shelf-life.

5. Conclusions

Iron concentration in dry Pet food contributes significantly with the oxidative stability during the shelf-life and should be controlled. Although the fat also present effect on the oxidation, this influence was less important than the iron.

Acknowledgments

The authors would like to thank the CEENUFEL research group, the professor of the statistics department Vanderly Janeiro and CAPES for funding the research.

Conflict of interest statement

The authors declare no conflict of interest.

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Sorption isotherm curves in dry extruded pet foods

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A B S T R A C T

The Guggenheim-Anderson-De Boer (GAB) model was used to characterize the adsorption and desorption and to compare these curves with drying curves of six dry extruded foods for dogs (n=3) and cats (n=3). The equilibrium moisture content was determined at a 30ºC, using the gravimetric method by using six saturated saline solutions (lithium chloride, potassium acetate, sodium nitrite, magnesium chloride, sodium chloride and potassium chloride), respectively from 0.11 to 0.83 water activities. Twenty grams of each pet food sample was placed over each solution in airtight flasks containers (without direct contact) and weighted each 48 hours until constant weight. The isotherm curves between water activity (w_a) and moisture content were plotted by GAB model. An additional curve for each food was plotted between w_a and moisture from direct data during the samples drying after extrusion in an air oven furnace at 60°C, in the way to compare this result with desorption curve modelled by isotherm method. This procedure was evaluated, since it represents a simple method compared to the isotherm. All pet foods exhibited a type II isotherm. The average R^2 was 0.99 for the adsorption curves, 0.87 for desorption and 0.99 for drying curves, showing a good fit of the models. The adsorption and desorption isotherm curves

presented different predicted equilibrium moisture, with higher values predicted by desorption method. The lag between these two curves, called hysteresis, can occur due to several factors, such as capillary condensation, changes in the physical structure of the material and phase change. Similarly, the results predicted by drying curve and desorption isotherm were also different between them. In conclusion, the adsorption activities are suitable for completely dry materials, as the increase in the equilibrium moisture content at various water activities, at constant temperature, causes this material to gain mass by the entry of water into its structure. In this study it was not possible to recommend the use of sorption isotherm predicted by drying curve, as its present different results from standard adsorption and desorption isotherms.

Keywords: Equilibrium moisture, Food safety, Monolayer moisture, Shelf-life, Water activity

1. Introduction

Pet foods are usually nutritionally complete and should present long shelf-life, from 12 to 18 months. Dry Pet foods are susceptible to microbial spoilage (Baser & Yalçin, 2017), and it has long been recognized the relationship between its water content and safety (Fennema, 1996). Thus, controlling the water available for microbial growth is one of the main concerns of dry food manufacturers. Although the moisture measurement is one of the most used methods (Ozbekova & Kulmyrzaev, 2019), this measurement alone is not a reliable indicator to predict the microbial growth and hydrolytic reactions in food (Fennema, 1996). Foods with the same moisture content can differ between their susceptibility to the microbial spoilage, turning it much more reliable to predict the stability of a given food by measuring water activity (Ozbekova & Kulmyrzaev, 2019). As the water activity (w_a) can change in function of the temperature, it is important to apply techniques such as the sorption isotherm that correlate moisture content and water activity at constant temperature, to predict the limits of moisture in a food that water activity remains in the safe limits (Baser & Yalçin, 2017).

Isotherms describe the integrated hygroscopic properties of the various food constituents and biopolymers with water (Kaymak-Ertekin & Gedik, 2004). These curves can be obtained in two ways: Adsorption isotherms: obtained in completely dry material, so the increase in the equilibrium moisture content to various water activities, at constant temperature, makes this material gain mass due to the ingress of water into its structure. Desorption isotherms: obtained in the material when it is completely wet and, depending on the water activity of the system, the equilibrium moisture of the material decreases as it gives water to the system (Al-muhtaseb et al., 2002).

The isotherm curves are important tools that provide specific data to control the food processing conditions, to predict the susceptibility to the microbial growth, to prevent the undesirable organoleptic changes, and to determine the most adequate food packaging system (Alhamdan & Hassan, 1999). Isotherm curves are calculated from different methods and GAB and Peleg models are commonly used for dry foods. These models allow to estimate the equilibrium moisture at safe water activities, important to extend the shelf-life. The GAB model also allows determining monolayer moisture content. This parameter indicates the amount of water strongly adsorbed on specific sites of foods (Wani & Kumar, 2016). Monolayer moisture contributes to the physical and chemical stability of foods, as it is not available for enzymatic activity, nonenzymatic browning, lipid oxidation, or other reactions that compromise shelf-life (Goula et al., 2008; Wani and Kumar, 2016; Arslan-Tontul, 2020).

Although isotherms are used for many materials and foods, it is important to evaluate the adequacy of mathematical models to describe the relationship between moisture and water activity in Pet foods. For this reason, this study aimed to characterize the adsorption and desorption isotherms of commercial dry dog and cat foods, by using GAB model.

2. Material and methods

2.1. Pet food samples

Six complete extruded dry commercial Pet foods were used. The Pet foods were manufactured by three companies located in Paraná, Brazil, with two samples from each establishment. The feed production flowchart followed according to the establishments' routine, seeking non-interference in it. The samples were collected at the extruder output (high moisture samples) and after the drying process (low moisture samples).

After collection, all samples were cooled at room temperature, conditioned in a polipropylene packaging, sealed, and frozen until the analysis.

All commercial foods had a basic composition based on ground whole corn, wheat bran, poultry byproduct meal and appropriate premix.

2.2. Moisture sorption isotherms

Moisture sorption isotherms (adsorption and desorption) were calculated from the data obtained by using the gravimetric method (Yogendrarajah et al., 2015; Wani et al., 2016). For this, saturated saline solutions with different water activities (0.11–0.83) were prepared in deionized water and placed in airtight plastic containers (Figure 1). Six salts were used: sodium chloride (25010090, ACS reagent, ≥95.0%; Synth, Diadema, SP, Brazil), lithium chloride (28273960, ACS reagent, ≥95.0%; Synth, Diadema, SP, Brazil), potassium chloride (28273999, ACS reagent, ≥95.0%; Synth, Diadema, SP, Brazil), magnesium chloride (28273190, ACS reagent, ≥95.0%; Synth, Diadema, SP, Brazil), potassium acetate (29153999, ACS reagent, ≥95.0%; Synth, Diadema, SP, Brazil), and sodium nitrite (28341010, ACS reagent, ≥95.0%; Synth, Diadema, SP, Brazil). About 500 mL of each saturated solution was prepared, and a 50 mL aliquot was used to test each food sample. Bottom appearance was used as a criterion to establish the saturation point of saline solutions. The a_w of saturated saline solutions was determined by the dew point method using a water activity analyzer (LabSwift-aw, Novasina, Lachen, Switzerland), according to AOAC method 978.18.

The isothermal curves were determined at a temperature of 30ºC. The low moisture samples, collected at the dryer output were used for the adsorption isotherms. Additionally, these samples were dried in an oven at 70°C for 24 hours, in order to standardize the initial moisture. On the other hand, the samples collected at the exit of the extruder barrel were used for the desorption isotherms without prior drying.

For the isotherm (adsorption and desorption), intact kibbles (20 g) were weighed and placed in small chambers at the top of the plastic containers without direct contact with the saline solution (Figure 1). Samples were maintained in a forced-air oven (320-SE, FANEM, São Paulo, Brazil) at 30ºC until weight stabilization for 3 consecutive weighing (Greenspan, 1977). A digital thermo hygrometer (model 7666, Incoterm, Porto Alegre, Brazil) was used to monitor the oven temperature. Samples were weighed every two days on a digital analytical scale (HR-200, A&D Company, California, USA). Weight stabilization was defined as weight changes of less than 0.01 g in three successive measurements. Moisture sorption isotherms were built by plotting the moisture content of samples after weight stabilization versus the a_w of saturated salt solutions (0.113, 0.216, 0.324, 0.634, 0.750, and 0.834)

Sorption isotherms were fitted to the exponential function described by GAB models (Eq. 1) that were used for estimating the equilibrium moisture content of pet foods at 30°C according to the following equation:

$$X_{eq} = \frac{ck a_w X}{(1 - k a_w)(1 - k a_w + ck a_w)} \quad (1)$$

where X_{eq} is the equilibrium moisture content (kg/kg DM); a_w is the water activity; c, k are model constants; and X is the monolayer moisture content (kg/kg).

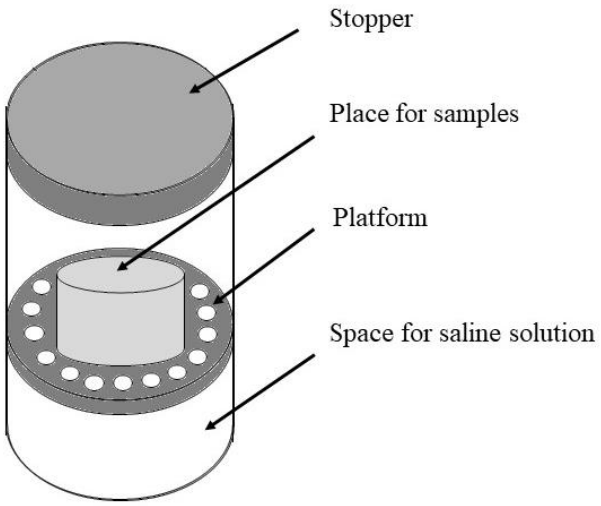

Figure. 1. Schematic representation of the airtight plastic container used to determine the moisture adsorption isotherms.

2.3. Drying curves

The drying curve was drawn from the samples collected at the exit of the extruder barrel, where they were submitted to a 60°C oven, in order to obtain moisture levels at 3%, 7%, 10-12%, 15% and greater than 15%. Every two hours, a sample was taken for moisture analysis following the methodology described by the AOAC (1995) and water activity by the dew point method, using the methodology approved by the AOAC (method 978.18) in specific equipment (Labswift -aw, Novasina, United States). The samples were kept in an oven at 60°C until reaching the desired moisture levels. All the water activity measurements were performed at 30°C. The isotherm curve was predicted from those moisture and water activity determinations by using the GAB model, following the same steps of calculations described for the adsorption and desorption curves. The purpose of use this alternative method was to compare the equilibrium moisture estimated by the drying curve with the standard method described for adsorption and desorption curves, as the drying curve is simpler (it did not require

saline solutions) and faster (24 hours) than gravimetric method of isotherm (approximately 30-40 days).

2.4. Statistical analysis

The quality of model fitting for each sample was assessed by coefficient of determination (R^2), Akaike information criterion (AIC), and standard deviation (SD). Model fitting was performed using the minpack.lm package in RStudio software version 3.4.3 (Boston, USA). Exponential equations were obtained for different water activities. To compare the drying curves with the desorption isotherm, normality tests (Shapiro-Wilk's and Lilliefors) of the area under the curve were used and subsequently the Wilcoxon paired t-test was applied to compare the difference between the areas of the curves from the two methodologies.

3. Results

Moisture sorption isotherms presented a good fit by exponential Guggenheim–Anderson–de Boer (GAB) model, evidenced by AIC, adjusted R^2, and SD indicators (Table 1 and 2).

Table 1

Adjusted parameters for estimating equilibrium moisture content (Xeq) in pet food samples subjected to drying curve and adsorption isotherms by the Guggenheim – Anderson – de Boer (GAB) model.

Item	Fit quality[1]			Model parameter[2]		
	AIC	R^2	SD	X	c	k
Adsorption isotherm						
Pet food 1	17.82	0.99	0.42	4.236	22.101	0.958
Pet food 2	6.10	0.99	0.26	3.973	39.695	0.991
Pet food 3	2.77	0.99	0.22	3.442	14.763	1.016
Pet food 4	10.64	0.99	0.31	3.979	32.582	0.963
Pet food 5	8.36	0.99	0.28	4.031	72.437	0.966
Pet food 6	-11.12	0.99	0.13	3.647	29.699	0.987
Drying curve						
Pet food 1	-10.14	0.99	0.16	4.866	10.170	0.944
Pet food 2	33.50	0.99	0.54	4.754	11.486	0.947
Pet food 3	29.65	0.99	0.48	3.258	85.000	1.083
Pet food 4	48.24	0.97	0.94	5.108	10.852	0.955
Pet food 5	43.37	0.98	0.81	4.970	15.543	0.988
Pet food 6	24.80	0.99	0.45	4.618	13.129	0.929

[1] AIC, Akaike information criterion; R^2, coefficient of determination; SD, standard deviation. [2] GAB model: $X_{\mathrm{eq}} = (\mathrm{ck}a_{\mathrm{w}}X)/(1-\mathrm{k}a_{\mathrm{w}})(1-\mathrm{k}a_{\mathrm{w}}+\mathrm{ck}a_{\mathrm{w}})$, where a_{w} is the water activity; a, c, and k are model constants; and X is the monolayer moisture content (kg/kg).

Table 2

Adjusted parameters for estimating equilibrium moisture content (Xeq) in pet food samples subjected to drying curve and desorption isotherms by the Guggenheim – Anderson – de Boer (GAB) model.

Item	Fit quality[1]			Model parameter[2]		
	AIC	R^2	SD	X	c	k
Desorption isotherm						
Pet food 1	46.55	0.89	1.39	6.644	16.012	0.784
Pet food 2	40.25	0.93	1.07	5.908	140.573	0.831
Pet food 3	43.37	0.88	1.22	6.001	22.187	0.766
Pet food 4	32.26	0.89	0.97	12.631	14.293	0.267
Pet food 5	49.89	0.80	1.60	7.776	15.331	0.672
Pet food 6	46.99	0.82	1.42	6.329	13.418	0.721
Drying curve						
Pet food 1	-10.14	0.99	0.16	4.866	10.170	0.944
Pet food 2	33.50	0.99	0.54	4.754	11.486	0.947
Pet food 3	29.13	0.99	0.48	3.220	146.542	1.085
Pet food 4	48.24	0.97	0.94	5.108	10.852	0.955
Pet food 5	43.37	0.98	0.81	4.970	15.543	0.988
Pet food 6	24.80	0.99	0.45	4.618	13.129	0.929

[1] AIC, Akaike information criterion; R^2, coefficient of determination; SD, standard deviation. [2] GAB model: $X_{\mathrm{eq}} = (\mathrm{ck}a_{\mathrm{w}}X)/(1-\mathrm{k}a_{\mathrm{w}})(1-\mathrm{k}a_{\mathrm{w}}+\mathrm{ck}a_{\mathrm{w}})$, where a_{w} is the water activity; a, c, and k are model constants; and X is the monolayer moisture content (kg/kg).

According to the GAB model, the parameter X of the model represent the monolayer moisture content. This parameter ranged from 3.44 to 4.24 gH2O / 100g DM for the adsorption curves, and from 5.91 to 12.63 gH2O / 100g DM for the desorption curves. For drying curves, this parameter ranged from 3.22 to 5.10 g H2O/100g DM. The mean equilibrium moisture (Xeq) estimation of the foods differed according to the method, ranging from 3.50 ± 0.42 to 14.22% ± 0.47 for adsorption curves, from 5.26 ± 0.52 to 14.95% ± 1.10 for desorption curves and 4.08 ± 0.30 to 17.37% ± 1.11 for drying curves. The adsorption curves presented lower Xeq than desorption curves over

the entire range of water activity (Figure 2; Table 3), characterizing the hysteresis. The drying curve presented intermediate values (Figure 2).

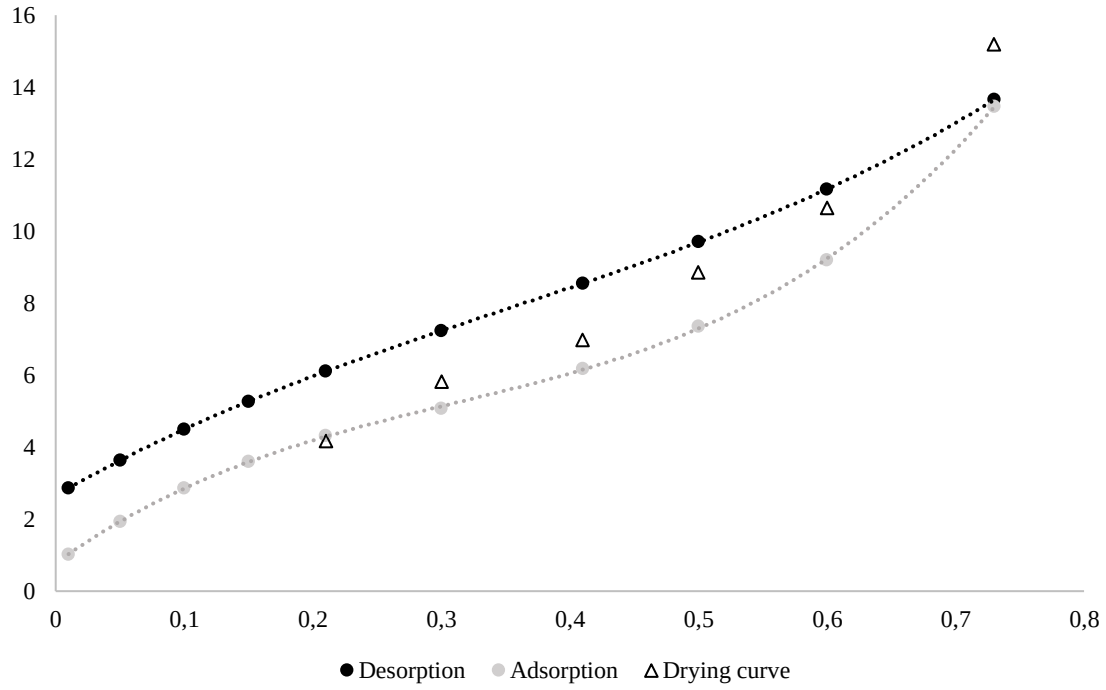

Figure. 2. Moisture sorption isotherms and drying curve of extruded dry pet food (*n* = 6) at 30 ºC temperature estimated by Guggenheim–Anderson–de Boer (GAB) models. GAB model at 30 °C. Adsorption curve (gray line); Desorption curve (black line) and Drying curve (triangle). Dashed lines are trend lines.

As they present similar curves, a comparison between the drying curve and the desorption isotherm was carried out, with the aim of using the drying curve to replace the desorption method. However, when comparing the two methods, the areas below the curve between them were different. Thus, it would not be possible to recommend the drying curve in replacement of desorption isotherm, as faster and simpler method (Table 4).

Table 3

Average of the moisture values (%) of the GAB model in certain water activities for extruded dog and cat food.

Item	Water activity					
	0.21	0.30	0.41	0.50	0.60	0.73
	Adsorption curve					
Pet food 1	4.50	5.35	6.52	7.75	9.64	13.83
Pet food 2	4.58	5.34	6.45	7.68	9.63	14.22
Pet food 3	3.50	4.29	5.39	6.57	8.45	13.02
Pet food 4	4.45	5.20	6.28	7.42	9.21	13.21
Pet food 5	4.80	5.49	6.54	7.68	9.49	13.58
Pet food 6	4.08	4.80	5.84	6.96	8.74	12.88
	Desorption curve					
Pet food 1	6.04	7.22	8.65	9.96	11.72	14.84
Pet food 2	6.92	7.70	8.84	10.01	11.70	14.95
Pet food 3	5.79	6.77	7.96	9.07	10.55	13.14
Pet food 4	6.14	7.61	9.03	10.01	10.99	12.16
Pet food 5	6.48	7.74	9.16	10.37	11.88	14.29
Pet food 6	5.26	6.36	7.63	8.74	10.16	12.51
	Drying curve					
Pet food 1	4.34	5.43	6.87	8.30	10.43	14.98
Pet food 2	4.39	5.44	6.83	8.23	10.32	14.81
Pet food 3	4.08	4.71	5.75	7.00	9.20	15.47
Pet food 4	4.67	5.82	7.34	8.88	11.19	16.22
Pet food 5	5.03	6.13	7.63	9.21	11.68	17.37
Pet food 6	4.37	5.35	6.64	7.93	9.85	13.86

Table 4

Area below the adjusted Peleg and Gab model curves.

Model	Curves	Item						Value-p^t	Value-p^w
		Pet food 1	Pet food 2	Pet food 3	Pet food 4	Pet food 5	Pet food 6		
Peleg	Drying curve	5.9599	6.0718	5.6812	6.4291	6.8635	5.6703		
	Adsorption	5.387	5.4800	4.7367	5.2301	5.4476	4.9875	0.0052	0.0313
	Desorption	6.8516	7.1236	6.243	6.4213	6.959	5.9025	0.0456	0.0625
GAB	Drying curve	5.9645	5.9312	5.6784	6.4219	6.8127	5.6814		
	Adsorption	5.4572	5.5231	4.7363	5.2626	5.4897	4.9825	0.0080	0.0313
	Desorption	6.8421	7.1341	6.2465	6.4225	6.9751	5.9036	0.0447	0.0313

Value-p^t: probability of significance of the paired t test

Value-p^w: probability of significance of the Wilcoxon signed rank test.

Despite the normality of the areas below the curve, by the Shapiro-Wilk's and Lilliefors tests, two comparison tests were applied to compare the difference between the areas of the drying curve method and desorption isotherm. From the results presented in Table 4, it can be concluded that the two methods produce curves that lead to different areas.

4. Discussion

The model proposed by Guggenheim-Anderson-De Boer (GAB) satisfactorily adjusted to the experimental data, accurately describing the equilibrium sorption isotherms in the analyzed condition, since resulted in values close to 1 for R2, low AIC values and standard error (SD) values close to 0. The application of the GAB model is advantageous, as it allows determining the moisture content of the monolayer. This parameter indicates the amount of water strongly adsorbed in specific food locations (Wani and Kumar, 2016). The value of this water content is directly linked to the microbial and oxidative stability of the product (Yao et al., 2020). The water values in the monolayer ranged from 3.220 to 12.631 g H_2O/g DM according to the type of isotherm, with the values of the desorption curves being the highest. Such results are similar to those found by Mutlu et al. (2020) that worked with roasted, green and ground coffee beans. For the adsorption curves, values between 3.06–3.28 g H_2O/ g DM were found, close to the values found in this work for adsorption curves. Yao et al. (2020) studied the desorption and adsorption curves in wheat grains, and they found results between 5.262- 9.873 H_2O/ g DM, where the highest values are those of the desorption isotherms. The desorption curves were generated using feed from the extruder exit, that is, with a high-water content. This characteristic results in higher values for the water parameter in the monolayer. According to Yao et al. (2020) greater

mobility in the monolayer translates into lower product stability, and according to Moreira et al. (2010) a lower vapor pressure is needed to reach a certain moisture content by a desorption process than by adsorption. Hysteresis is related to the nature and state of the components in food. The adsorption activities are suitable for completely dry materials, as the increase in the equilibrium moisture content at various water activities, at constant temperature, causes this to gain mass by the entry of water into its structure (Al-muhtaseb et al., 2002).

Using the GAB model, from its constants it is possible to carry out a more detailed study of the heat and mass transfer that occurs during the sorption phenomenon. Furthermore, it is considered an adequate model to describe experimental data in water activity ranges up to 0.90 (Oliveira et al., 2017). There are several studies in the literature on food sorption isotherms, which describe that the GAB model presented a good fit for the data found. such as 1–2 mm thick fruit slices oven-dried at 70 °C for 24 h, vegetables, extruded snacks, roasted beans, dry-cured ham and green and ground coffee (Tsami et al., 1990; Wang and Brennan, 1991; Kiranoudis et al., 1993; Maroulis et al., 1988; McLaughlin and Magee, 1998; Kaymark-Ertekin and Gedik, 2005; Wani and Kumar, 2016; Mutlu et al., 2020; Betiol et al., 2020).

It is possible to observe that the values of the C and K parameters tend to be greater than 2 and less than 1, respectively, at a temperature of 30ºC, classifying, therefore, the isotherms as type II, as stated by Blahovec (2004). The isothermal form is unique for each type of product, materials that have starch, usually have type II curves, with a sigmoid shape. According to Brunauer et al., (1938), this curve format allows us to understand the type of existing force of the water bond with the hygroscopic material, allowing us to assess the material's surface structure. Type II isotherms are characterized by a sigmoidal curve (Al-muhtaseb et al., 2002), commonly observed for

dry products such as extruded biscuits (Lazou and Krokida, 2011; Wani and Kumar, 2016) and cake flours (Al-Muhtaseb et al., 2010).

The graphs generated and presented in Figure 2, show that the adsorption isotherm curves are below the desorption curve for all foods and in all evaluated water activity range. This phenomenon is also observed in the equilibrium moisture calculations in table 3. The equilibrium moisture obtained through desorption is greater than that obtained when the food is subjected to conditions that provide a gain in moisture, that is, to adsorption conditions. This phenomenon, common to all hygroscopic materials, is known as hysteresis (Al-muhtaseb et al., 2002; Moreira et al., 2010).

It is due to the structural characteristics of the reagents, which change the energy accessibility in the polar sites, providing a movement or change in the moisture of the system (Al-Muhtaseb et al., 2002). Caurie (2007) reported that a decrease in hysteresis or its absence is related to a greater stability of stored products, because reversibility principles in thermodynamics help to understand the food-water interactions for dehydration and storage.

However, there are some limitations to this statement, mainly due to the presence of errors in the calculations of thermodynamic functions, due to some phenomena that can occur with food depending on its chemical composition. Supersaturated solutes are below the crystallization point at a given water activity and thus can contain more water in the same water activity. Foods with a high sugar content exhibit this phenomenon. Another description is about capillarity, which is related to pores that can absorb more water below the water activity, in which adsorption occurs, due to the fact that porous materials attract a greater amount of water (Rizvi, 2005).

Similar results were found by Moreira et al. (2010) who worked with type II adsorption and desorption isotherms of chestnut and wheat flour at different temperatures (20, 35, 50 and 65 ◦C).

Usually dry and extruded food for dogs and cats has a low moisture content, with drying processes decreasing the moisture content at maximum of 13 g / 100 g (Leiva et al., 2019). And low water activity value (about 0.5 in the final product) to control microbial spoilage (Lambertini et al., 2016). According to Bueno et al. (2001), a moisture content lower than 11.5% is already sufficient to inhibit the fungi growth in dry and extruded dog and cat food. All six foods presented moisture within what was considered safe when water activity levels as 0.50 and 0.60 were applied to the model (Table 3). This fact shows how moisture sorption isotherms are relevant tools for studying the moisture and water activity relationships in foods, providing specific data with direct application in predicting food drying time, shelf life, microbial growth, in quality control specifications and definitions of critical points (Alhamdan & Hassan, 1999).

When comparing the two methods (drying curve and desorption isotherms) it was possible to observe through comparison tests that even with areas below the curves showing normality, it would not be possible to validate the desorption isotherm according to the drying curve, because the two methods produce curves that lead to different areas. Even the two methods presented similar equilibrium moisture results within the proposed water activity range, where for the desorption curves the values were between 6.11 ± 0.57 and 14.15% ± 1.26 and 4.48 ± 0.33 and 15.45% ± 1.22. However, it is worth remembering that through the drying curves it is possible to relate the drying time with the moisture content. Where it is noticed that the moisture content presents a constant variation between the points drawn on the curve, which represents

the period in which the water diffusion inside the solid is equal to the amount of water that vaporizes on the surface. This is a different behavior of water sorption isotherms, which are graphs that relate the amount of water in a food (moisture) with its water activity, as a function of constant temperature (Wani & Kumar, 2016; Tejada-Ortigoza et al., 2017; Chen, 2019; Renshaw et al., 2019; Arslan-Tontul, 2020).

5. Conclusions

The isotherm curves predicted by GAB model presents a good fit for dry kibble diets for dogs and cats. The desorption and adsorption curves present the hysteresis phenomena, predicting different equilibrium moisture, with higher values for desorption curves. It seems that adsorption isotherm is suitable for dry Pet food, as it present better fit than desorption. However, it's necessary to develop future studies for this validation. In this study it was not possible to recommend the use of sorption isotherm predicted by the drying curve, as its present different results from standard adsorption and desorption isotherms.

Acknowledgments

The authors would like to thank the CEENUFEL research group, the professor of the statistics department Vanderly Janeiro and CAPES for funding the research.

Conflict of interest statement

The authors declare no conflict of interest.

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Organic acids derived from bacterial fermentation as mold-inhibitors in pet food

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A B S T R A C T

Dry extruded Pet foods are not favorable for microbial overgrowth due to their low water activity conditions. Despite this, the relation between water activity, moisture content and mold-inhibitors concentration to prevent the spoilage is a little studied. Thus, the aim of this study was to determine a dose-response effect of two organic acid blends derived from bacterial fermentation on the mold inhibition of extruded Pet food in different moisture contents. Two blends of organic acids, mold-inhibitor A and B, with 37% organic and 60% organic acids, respectively, were evaluated. Two studies were designed, respectively, for the evaluation of mold-inhibitor A and B. Each experiment presented a 4x5x4+1 factorial design, with 4 moisture levels (5%, 10%, 15% and 20%), 5 evaluation periods (days 0, 15, 30, 45 and 60), and 4 doses of each mold-inhibitor A or B (three inclusion levels plus no addition) with a positive control (0.15% of calcium propionate). For mold-inhibitor A the inclusion was 0.44, 0.66 and 1%, for mold-inhibitor B the inclusion was 0.22, 0.44 and 0.66%, totaling 32 treatments, with repeated measures over time (days 0, 15, 30, 45 and 60 days).

Chemical composition analyses, determination of adsorption isotherms, microorganism growth count analysis and food preference test were performed in all treatments. The effect of water activity proves to be an important parameter in fungal growth conditions. Thus, it is possible to observe that the moisture of 10 and 5% does not present favorable conditions for microbial growth, and throughout the experimental period, the fungus inoculated concentration (106 CFU / g) was reduced regardless of the treatment, even in the negative control. For treatments submitted to 15 and 20% moisture, there was a gradual increase in mold counts throughout the experimental period. In these conditions of high moisture, it was possible to verify that the mold inhibitors A and B had lower molds count in relation to the CN and CP at 15% of moisture and, at 20% of moisture, the mold inhibitor A presented greater effectiveness in fungal control in relation to the others. The moisture content and aw data were well explained by the exponential models of GAB and Peleg with a good general fit, as evidenced by the AIC, adjusted R2 and DPm values for adsorption isotherms, and the actual moistures were close to those determined by this analysis, proving its effectiveness as a tool in quality control. As noted, animals tended to consume the negative control (no antifungal). Regarding the first choice for smell and taste, the animals did not show linearity, showing that these results did not show great relevance regarding the intake relationship. Thus, it was possible to conclude that organic acids are effective in controlling fungi, but water is an extremely important factor for the shelf-life of pet food.

Keywords: *Shelf -life*, microorganisms, quality control.

1. Introduction

Pet food safety is the most important point for manufacturers since the food contamination can compromise the animal health with negative effect on the company's reputation. Dry extruded Pet food is widely produced and, contributes with around 80% of global Pet food production (Euromonitor, 2019). Although extrusion sterilizes food, finished products may still become contaminated or spoiled. The United States Food and Drug Agency (FDA), in the last two years, notified 14 pet food recalls due to microbial contamination, with dry products representing around 30% of them. Thus, additives are used in these foods, to ensure food safety, ensuring stability and resistance to deterioration. (Craig, 2021).

Water is one of the main factors that determine the risk of contamination, shelf life, physical properties and food safety in the finished product (Charoen et al., 2015). Thus, the microbial growth is closely linked to the amount of free water in foods (Jin et al., 2019). Alternative to limit microbial growth in a food product is the use of additives based on organic acids. The action of these additives is mainly by decreasing the pH (Coban, 2019). Organic acids have the third largest production market in the world and are widely used as antimicrobial agents in the food industry (Ali et al., 2011).

The use of organic acids as antimicrobial agents in the food industry will depend on its properties, such as: chemical formula, physical form, pKa value, molecular weight, minimum inhibitory concentration, buffer properties, among others (Thompson et al., 1997). The pKa value is one of the factors that most interferes with its efficiency, being a very important criterion. Many organic acids range between values of 3.0 and 5.0, such as propionic acid with a value of 4.87 and lactic acid with a value of 3.86. These values are satisfactory for microbial control, it is advantageous to use the acidulants between this range (Shadini et al., 2014; Coban, 2019).

As additives for the food industry, organic acids have a dual purpose: acidulant and preservative. Propionic acid is normally applied to the surface of food to prevent mold from forming. The effect as a fungicide is greater at pH around 4.0 than at pH 5.0. In turn, the fungi control by lactic acid requires a greater amount. For best antifungal activity, it is necessary that the acid is predominantly in its non-ionized form. However, according to Stanojevic-Nikolic et al. (2020), lactic acid can be considered a good microbial growth control agent for *Aspergillus spp*. (Stanojevic-Nikolic et al, 2020).

The aim of this study was to determine a dose-response effect of two organic acid blends derived from the bacterial fermentation on the mold inhibition of extruded Pet food in different moisture contents.

2. Material and methods

2.1 Experimental design

Two studies were designed, respectively, for evaluation of mold inhibitor A and B. Each experiment presented a 4x5x4+1 factorial design, with 4 moisture levels (5%, 10%, 15% and 20%), 5 evaluation periods (days 0, 15, 30, 45 and 60), and 4 doses of each mold inhibitor A or B (three inclusion levels plus none addition) with a positive control (0.15% of calcium propionate). For mold inhibitor A the inclusion was 0.44, 0.66 and 1%, for mold inhibitor B the inclusion was 0.22, 0.44 and 0.66%. Thus, the study presented a completely randomized design with repeated measures over time, totaling 16 treatments (additive versus moisture level) for each mold-inhibitor (A or B) plus positive control. Each treatment presented three repetitions.

2.2 Experimental diets and processing

A reference diet (Negative Control), without any antimicrobial additive inclusion was formulated following the recommendations of FEDIAF (2019), with

ingredients conventionally used in dry Pet food (maize, broken rice, poultry by-product meal, maize gluten feed, poultry fat, cellulose and vitamin premix). The mold-inhibitors were added in this diet to compose the treatments.

Mold inhibitors evaluated were provided by a Brazilian company (Zilor-Biorigin, Lençóis Paulista, Brazil). Two blends were used, denominated mold-inhibitor A (37% organic acids of which 25% of propionic acid) and mold-inhibitor B (60% of organic acids of which 35% of lactic acid). A positive control diet formulated with pure calcium propionate was used, as this salt of organic acid is largely used in extruded Pet foods. Mold inhibitors were added in the negative control diet before the processing.

All treatments were prepared and processed (Extrusion plant from State University of São Paulo, Jaboticabal, Brazil) in a 250 kg/h single screw extruder (MEX-250, Manzoni Industrial Ltda, Campinas, Brazil). The negative Control (NC) diet was prepared in amount to guarantee the same composition for all treatments. Thus, the mold-inhibitors were added and mixed by sub-sampling the NC, before the extrusion.

To compose the treatments with different moisture contents, each treatment, during processing had a sample collected at the extruder barrel output, with approximately 20% moisture, where it was dried in an oven at 60 °C in a forced ventilation oven until it reaches the 15% of moisture level. The treatments with moisture levels of 10% and 5% were possible by sampling the diet after drying, with adjustment of the conveyor speed of the dryer. All treatments were submitted to laboratory analysis, shelf-life tests and adsorption isotherm. For the food preference test, samples with 5% moisture received externally the inclusion of fat and palatability.

2.3 Analysis

2.3.1 Chemical analysis

Water activity (WA) of pet food samples was determinate using specific equipment (Labswift-aw, Novasina, Lachen, Switzerland). The chemical composition was performed following the methodologies; moisture (MO) and dry matter (DM, Method 930.15), ash (MM, Method 942.05), crude protein (CP, Method 954.01), acid-hydrolyzed ether extract (AHEE, Method 954.02), crude fiber (CF, Method 962.09), described by the Association of Official Analytical Chemists (AOAC, 1995). Nitrogen free extract (NFE) were calculated by the equation NFE = (100 − CP − EE − CF − MM). (Table 1).

Table 1

Chemical composition of basal diet.

Item	Chemical composition[1] (g/kg)						
	Moisture	DM	Ash	CP	AHEE	CF	NFE
Basal diet	76	924	65	304	103	21	507

[1] DM, dry matter; CP, crude protein; AHEE, acid-hydrolyzed ether extract; CF, crude fiber; NFE, nitrogen-free extract.

2.3.2 Moisture sorption isotherms

Determination of moisture adsorption isotherms was performed according to a gravimetric method (Yogendrarajah et al., 2015; Wani & Kumar, 2016). Saturated saline solutions with different water activities (0.11–0.89) were prepared in deionized water and placed in airtight plastic containers (Figure 1).

Six salts were used: sodium chloride (25010090, ACS reagent, ≥95.0%; Synth, Diadema, SP, Brazil), lithium chloride (28273960, ACS reagent, ≥95.0%; Synth, Diadema, SP, Brazil), potassium chloride (28273999, ACS reagent, ≥95.0%; Synth, Diadema, SP, Brazil), magnesium chloride (28273190, ACS reagent, ≥95.0%; Synth, Diadema, SP, Brazil), potassium acetate (29153999, ACS reagent, ≥95.0%; Synth, Diadema, SP, Brazil), and sodium nitrite (28341010, ACS reagent, ≥95.0%; Synth,

Diadema, SP, Brazil). About 500 mL of each saturated solution was prepared, and a 50 mL aliquot was used to test each food sample. Bottom appearance was used as a criterion to establish the saturation point of saline solutions. The a_w of saturated saline solutions was determined by the dew point method using a water activity analyzer (LabSwift-aw, Novasina, Lachen, Switzerland), according to AOAC method 978.18. The saturation solutions had the following water activities 0.171, 0.283, 0.336, 0.685, 0.764 and 0.870.

Isothermal curves were determined at a temperature of 30°C. One sample of each treatment (Negative control, positive control, 0.33, 0.66 and 1% inclusion of mold-inhibitor A and 0.22, 0.44 and 0.66% inclusion of mold-inhibitor B) were subjected to a pre-drying in an oven at 70 °C for a period of 24 hours, to standardize the initial moisture of the samples. The samples collected at the exit of the extruder were subjected to desorption isotherms without prior drying. Intact kibbles (20 g) were weighed and placed in small chambers at the top of the plastic containers without direct contact with the saline solution. Samples were then dried in a forced-air oven (320-SE, FANEM, São Paulo, Brazil) at 30ºC until weight stabilization (Greenspan, 1977). A digital thermo hygrometer (model 7666, Incoterm, Porto Alegre, Brazil) was used to monitor the oven temperature. Samples were weighed every two days on a digital analytical scale (HR-200, A&D Company, California, USA) until constant weight was achieved. Weight stabilization was defined as weight changes of less than 0.01 g in three successive measurements. Moisture sorption isotherms were constructed by plotting the moisture content of samples after weight stabilization versus the a_w of saturated salt solutions.

Moisture sorption isotherms was adjusted according to the exponential function described by Peleg (1993). Peleg (1) and Guggenheim-Anderson-de Boer - GAB

models (2) for each temperature (30 and 40°C) were used to estimate of the moisture equilibrium (Xeq):

$$\mathrm{Xeq} = \mathrm{a}.wa^{\mathrm{b}} + \mathrm{c}.wa^{\mathrm{d}} \quad (1)$$

$$\mathrm{Xeq} = \frac{\mathrm{X.c.k}.wa}{(1-\mathrm{K}.wa)(1-\mathrm{K}.wa+\mathrm{C.k}.wa)} \quad (2)$$

where, *Xeq* is the equilibrium moisture content, kg/kg; *wa* is the water activity; *a*, *b*, *c*, *d*, *K* are constants of model; *X* is the water in the monolayer, kg/kg.

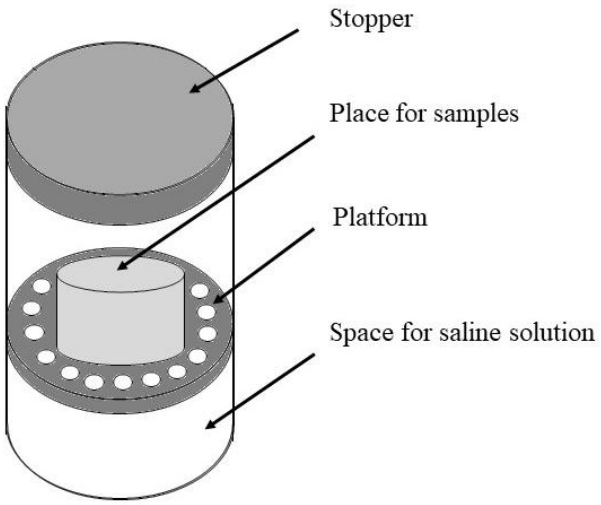

Figure. 1. Schematic representation of the airtight plastic container used to determine the moisture adsorption isotherms.

2.3.3 Analysis of Moisture and Water Activity

The initial moisture of samples was determined in an oven at 105ºC as described by AOAC (1995) and the water activity by the dew point method, using a methodology approved by the AOAC (method 978.18) in specific equipment (Labswift-aw, Novasina, USA) (Table 2).

Table 2

Actual moisture and water activity values of pet food samples.

Item	Moisture (%)			
	5	10	15	20
Pet food 1	6.44	9.02	14.06	19.81
Pet food 2	5.72	9.48	15.50	14.37
Pet food 3	6.20	10.08	17.26	17.46
Pet food 4	5.29	9.76	14.20	17.20
Pet food 5	6.23	8.10	14.33	15.20
Pet food 6	4.40	9.52	14.39	18.02
Pet food 7	4.88	9.57	12.92	20.38
Pet food 8	6.17	10.30	13.50	20.43
Item	Water activity			
	5	10	15	20
Pet food 1	0.439	0.567	0.688	0.749
Pet food 2	0.436	0.566	0.695	0.731
Pet food 3	0.412	0.575	0.708	0.759
Pet food 4	0.420	0.574	0.671	0.760
Pet food 5	0.431	0.64	0.699	0.737
Pet food 6	0.422	0.570	0.713	0.767
Pet food 7	0.433	0.571	0.654	0.774
Pet food 8	0.413	0.579	0.635	0.760

Pet food 1: Nagative Control- NC (no antifungal); Pet food 2: Positive Control- PC (0.15% calcium propionate); Pet food 3: 1% mold-inhibitor A; Pet food 4: 0.66% mold-inhibitor A; Pet food 5: 0.33% mold-inhibitor A; Pet food 6: 0.66% mold-inhibitor B; Pet food 7: 0.44% mold-inhibitor B; Pet food 8: 0,22% mold-inhibitor B.

2.3.4Microbiology of feed samples (shelf life)

For microbiology, all 32 foods were inoculated with strains of the fungus *Aspergillus brasiliensis* -16404 Kwik Stik (392P) at a concentration of 10^6. TSA Irr. Triple Pack 90mm was used to cultivate the fungus growth (25ºC per 72 hours).

Packages with 25g of feed were opened and diluted in 225 ml of 0.1% peptone water, homogenized, followed by serial dilutions 10^{-1} to 10^{-5} in test tubes containing 9 ml of 0.1 then 1-ml peptone water of the dilutions were inoculated in Sabouraud Dextrose 4% Agar - Irradiated - 90mm plates, waiting for 72 hours at a controlled

temperature of 25º C to count the colonies. Two replicates of each treatment were used to count colonies over the period of 0, 15, 30, 45 and 60 days, totaling 5 counting periods.

2.3.5 Food preference test

The food preference test was used to assess the organoleptic perception of cats when exposed to foods with organic acid-based additives (mold-inhibitor A and B) and their different levels. For this, all treatments with 5% moisture were compared with the negative control. The food preference test was used, the two-bowl method (Food A x B). All challenges were made regarding the negative control treatment. For the tests, the energy requirements for maintenance of cats were determined according to the NRC (2006). For this demand, an additional 30% was adopted, in order to have leftovers to calculate the intake ratio. Food A and B were exposed to the animals simultaneously, four times a day (8:00 am; 10:00 am; 2:00 pm; 4:00 pm). During these periods, the animals at each of these meals had access to the feeders for 20 minutes. The positions of feeders A and B were alternated with each feeding, aiming to minimize laterality problems. Food demands and their respective leftovers were weighted on a bench scale (Prix 3 fit, Toledo do Brasil, São Bernardo do Campo, Brazil). First choice and intake ratio (RI-A) were measured. The first choice was measured in two moments (8:00 and 14:00). For this, the first diet tasted (food A or B) in each trial was recorded. The intake ratio of foods A and B was calculated from the consumption of each food in relation to the total daily intake. For this, food intake ration A (RI-A) = (food A intake / food A intake + food B intake). During exposure to the feed, the animals were kept in individual cages measuring 0.30 m2 (60 x 50 cm). Between each meal and day of the experiment, the cats were kept in a collective, environmentally enriched cattery, measuring 49 m2 with ad libitum access to fresh water and natural and artificial light.

Twenty-one neutered adult cats were used in this study. Where 10 cats (5 females and 5 males), were approximately 4.4 kg ± 1.6, with 7 years of age. And the remaining 11 cats (4 females and 7 males), were 4.1 kg ± 0.4, with 2 years of age. The animals belonged to the Domestic Cat Nutrition and Metabolism Laboratory at the State University of Maringá, Brazil. All cats were adapted to the two-bowl test to quantify food preference.

2.4 Statistical analysis

The values observed will be assumed to be independent, since after counting fungi, the sample does not return to the experiment for further counting. Also, it will be worked with logarithm in base 10 of the observed count. For data analysis, a model will be considered as a completely randomized design assembled in a split-plot scheme in which the main factor is the treatment, in 32 levels, and the secondary factor is the time in 5 levels. So, the used model is:

$$yijk = \mu + \tau i + \varphi(i)k + \gamma j + (\tau\,\gamma)ij + \varepsilon ijk$$

on what:

yijk: is the observation that received the level i of the treatment factor at time j in repetition k;

μ: is the general mean associated with all observations;

τi: is the effect of the i-th level of treatment;

φ(i)k: is the error of the parcel that received the level i of the factor in the repetition k, calling it error(a);

γj: is the effect of level j time;

(τ γ) ij: is the effect of the interaction between treatment and time;

εijk: is the error of the k subplot that received the i-th level of the treatment factor and in the j-th time, calling error(b).

Considering the methodology of analysis of variances (ANOVA), it is necessary to impose some assumptions about the model. Thus, this model will have as a basic assumption that the terms of the error(a) are independent with normal distribution of mean and constant variance and the error terms(b) are independent with normal distribution of mean and constant variance.

The fit quality of the models to experimental data was assessed by the adjusted coefficient of determination (R^2), Akaike information criterion (AIC), and maximum standard deviation (DPm). Model fitting was performed using the minpack.lm package in RStudio software version 3.4.3 (Boston, USA). Exponential equations were obtained for different water activities. The data obtained during the food preference tests, were submitted to the Anderson-Darling and Shapiro-Wilks tests, at the 5% significance level. This were used to verify the normality assumption of the intake ratio data for all trials. For the intake ratio, data from trials one and two were underwent to paired T-test. For this the test of proportion in choice in each one of the test days were used to verify if different or similar in 50%, considering ($p < 0.05$).

3. Results

The effect of water activity (Table 3) is shown to be an important parameter in fungal growth conditions. Thus, it is possible to observe that the moisture of 10 and 5% does not present favorable conditions for microbial growth, and throughout the experimental period, the inoculated fungus concentration (10^6 CFU/g) was reduced regardless of the treatment, even in the control negative. However, on day 0, the immediate effect of the antifungal agents was positive in reducing the fungal count

compared to the negative control (Figures 2 and 3). Mold-inhibitors A and B had the lowest counts, even when compared to the positive control, on the initial day of the study, showing an immediate fungicidal effect on mold control in recent contaminations. The highest counts were obtained in the negative control, as expected.

Table 3

Application of the mathematical models generated considering the moisture content (%) and antifungal level (%), in the prediction of mold growth (Log-CFU/g), on days 0 and 30 of the study.

			Mold-inhibitor A (%)						
Day	Moisture (%)	Positive Control	0.00	0.10	0.20	0.40	0.60	0.80	1.00
0	5	3.24	4.30	3.92	3.60	3.14	2.92	2.94	3.19
	10	3.60	4.32	3.93	3.60	3.12	2.87	2.86	3.10
	15	3.42	4.49	4.08	3.73	3.21	2.93	2.88	3.08
	20	3.54	4.80	4.37	4.00	3.42	3.09	2.99	3.13
	11		4.34	3.95	3.61	3.12	2.87	2.86	3.09
	12		4.37	3.97	3.63	3.14	2.88	2.86	3.08
30	5	2.42	1.63	1.70	1.78	1.93	2.08	2.23	2.38
	10	2.98	2.19	2.15	2.11	2.02	1.94	1.86	1.77
	15	5.84	3.33	3.17	3.01	2.69	2.38	2.06	1.74
	20	6.16	5.04	4.76	4.49	3.94	3.39	2.84	2.29
	11		2.37	2.31	2.24	2.11	1.98	1.85	1.72
	12		2.57	2.49	2.40	2.22	2.05	1.87	1.69
			Mold-inhibitor B (%)						
0	5	3.24	4,63	3,80	3.18	2.56	2.76	3.78	5.63
	10	3.60	4.18	3.66	3.26	2.86	2.98	3.61	4.77
	15	3.42	4.31	4.00	3.73	3.36	3.21	3.27	3.54
	20	3.54	5.02	4.81	4.59	4.06	3.45	2.74	1.94
	11		4.16	3.69	3.33	2.95	3.02	3.56	4.55
	12		4.17	3.74	3.40	3.04	3.07	3.50	4.32
30	5	2.42	2.12	2.20	2.25	2.28	2.20	2.02	1.73
	10	2.98	3.44	3.15	2.94	2.78	2.98	3.52	4.41
	15	5.84	5.14	4.47	4.00	3.67	4.13	5.40	7.47
	20	6.16	7.22	6.18	5.45	4.93	5.67	7.66	10.91
	11		3.75	3.38	3.12	2.93	3.18	3.86	4.99
	12		4.08	3.63	3.32	3.09	3.39	4.23	5.59

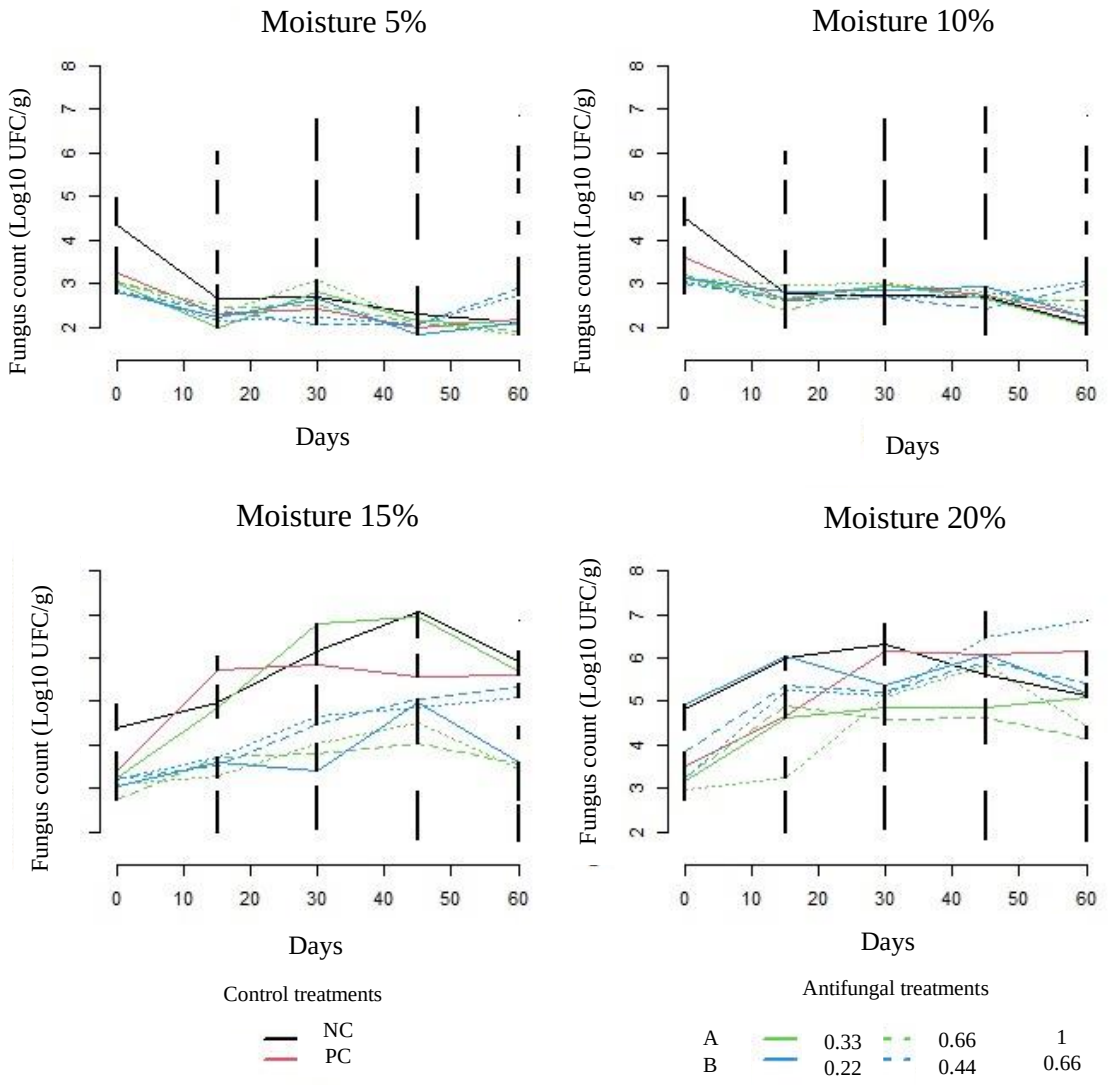

Figure. 2. Fungus counts according to the moisture of pet food samples, where: Negative Control - NC (no antifungal); Positive Control- PC (0.15% calcium propionate); 0.33% mold-inhibitor A; 0.66% mold-inhibitor A; 5: 1% mold-inhibitor A; 0.22% mold-inhibitor B, 0.44% mold-inhibitor B and 0.66% mold-inhibitor B.

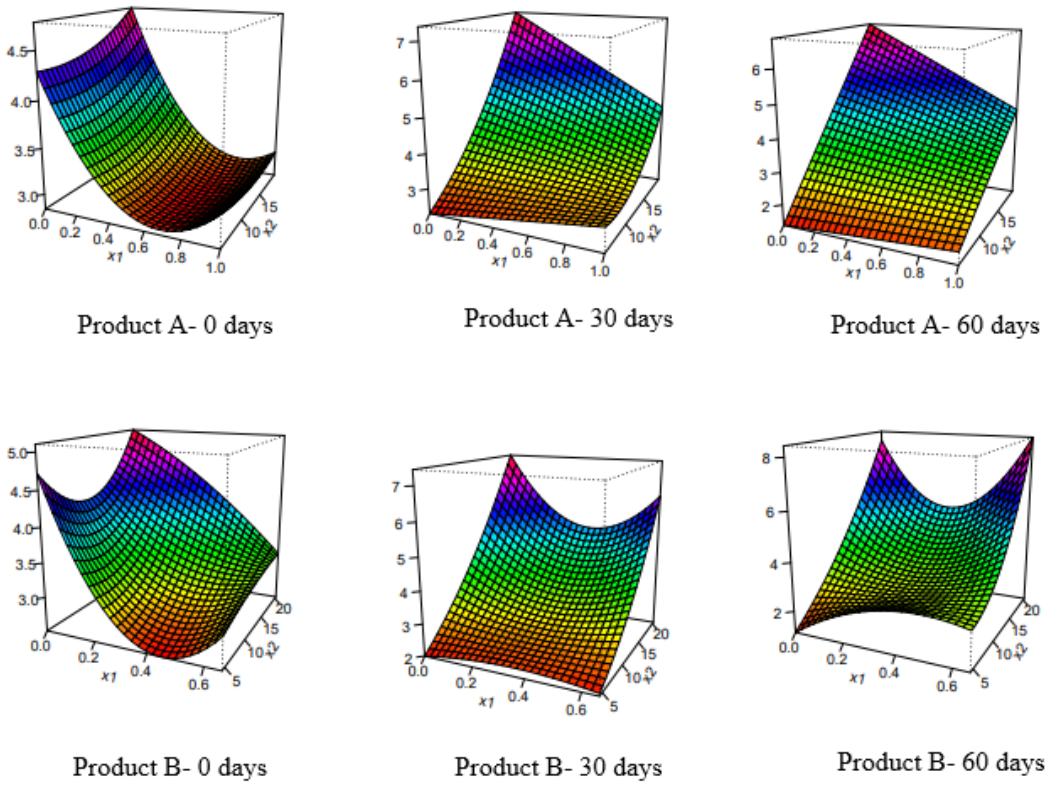

Figure. 3. Contour and surface graph for models fitted to the results of mold-inhibitors A and B on days 0, 30 and 60 respectively.

For treatments submitted to 15 and 20% moisture, there was a gradual increase in mold counts throughout the experimental period. In these conditions of high moisture, it was possible to verify that the mold-inhibitors A and B had lower counts of molds in relation to the CN and CP at 15% of moisture and, at 20% of moisture, the mold-inhibitor A presented greater effectiveness in fungal control in in relation to the others (Figure 2).

Since this study was designed to provide mathematical modeling to predict fungal growth as a function of the antifungal level, day of evaluation and sample moisture content, in Table 4 it is possible to apply the equations developed and verify that, in in general, even under high moisture conditions, mold-inhibitor A proved to be effective from a concentration of 0.6% in the food, to inhibit microbial growth, even after 30 days, different from positive control and mold-inhibitor B, which did not were

effective in high moisture condition after 30 days. On day 0, mold-inhibitor B was also effective. With Figure 3, it is possible to observe the relationships in each mold-inhibitor A or B, between dose, mold count and moisture content, as an illustrative application of the models shown in Table 4.

Table 4

Adjusted parameters for estimating equilibrium moisture content (Xeq) in pet food samples subjected to adsorption isotherms by the Guggenheim – Anderson – de Boer (GAB) and model.

Item	Fit quality[1]			Model parameter[2]		
	AIC	R^2	DPm	X	c	k
Pet food 1	26.86	0.94	12.31	5.244	-5.00E+08	9.00E-01
Pet food 2	27.66	0.92	14.45	4.987	-5.00E+08	9.00E-01
Pet food 3	27.7	0.94	14.02	4.982	-6.00E+08	9.00E-01
Pet food 4	26.58	0.94	13.06	5.042	-4.00E+08	9.00E-01
Pet food 5	29.55	0.91	21.12	4.616	-2.00E+08	9.00E-01
Pet food 6	22.01	0.97	9.77	5.040	-3.00E+08	9.00E-01
Pet food 7	26.37	0.94	13.64	4.874	-3.00E+08	9.00E-01
Pet food 8	23.74	0.96	9.50	4.951	-1.00E+08	9.00E-01

[1] AIC, Akaike information criterion; R^2, coefficient of determination; DPm, maximum standard deviation. [2] GAB model: $X_{\mathrm{eq}} = (\mathrm{ck}a_{\mathrm{w}}X)/(1-\mathrm{k}a_{\mathrm{w}})(1-\mathrm{k}a_{\mathrm{w}}+\mathrm{ck}a_{\mathrm{w}})$, where a_{w} is the water activity; a, c, and k are model constants; and X is the monolayer moisture content (kg/kg).

Moisture content and a_{w} data were well-explained by exponential GAB and Peleg models, with an overall good fit, as evidenced by AIC, adjusted R^2, and DPm values for moisture sorption isotherms (Table 4 and 5). A prediction equation was modeled for foods at 30 °C.

Table 5

Adjusted parameters for estimating equilibrium moisture content (Xeq) in pet food samples subjected to adsorption isotherms by the Peleg model.

Item	Fit quality[1]			Model parameter[2]			
	AIC	R^2	DPm	A	B	C	D
Pet food 1	12.95	0.99	2.15	13.05	0.612	13.06	3.193
Pet food 2	19.86	0.98	5.19	12.554	0.626	13.217	3.272
Pet food 3	15.13	0.99	2.74	9.682	0.475	18.978	2.978
Pet food 4	6.88	0.99	1.44	14.150	0.691	13.921	3.885
Pet food 5	20.93	0.98	8.19	14.855	0.836	13.445	4.066
Pet food 6	19.91	0.98	5.89	12.967	0.544	18.503	5.240
Pet food 7	-3.71	0.99	0.68	15.134	0.763	13.204	4.467
Pet food 8	11.75	0.99	1.98	13.486	0.629	16.246	4.583

[1] AIC, Akaike information criterion; R^2, coefficient of determination; DPm, maximum standard deviation.[2] Peleg model: $X_{\mathrm{eq}} = \mathrm{a}a_{\mathrm{w}}{}^{\mathrm{b}} + \mathrm{c}a_{\mathrm{w}}{}^{\mathrm{d}}$, where a_{w} is the water activity and a, b, c, and d are model constants.

The equilibrium moisture content of foods with water activity of 0.5 to 0.6 at a temperature of 30°C was 9.03 and 10.80% moisture, respectively, according to the GAB and 9.50 and 11.59%, respectively, according to Peleg (Table 6).

Table 6

Average of the moisture values (%) of the GAB and Peleg model in certain water activities for extruded dog and cat food.

Water activity	GAB model							
	1	2	3	4	5	6	7	8
0.10	5.76	5.48	5.47	5.54	5.07	5.54	5.36	5.44
0.20	6.40	6.08	6.08	6.15	5.63	6.15	5.94	6.04
0.30	7.18	6.83	6.82	6.91	6.32	6.90	6.68	6.78
0.40	8.19	7.79	7.78	7.88	7.21	7.88	7.62	7.74
0.50	9.53	9.07	9.06	9.17	8.39	9.16	8.86	9.00
0.60	11.40	10.84	10.83	10.96	10.03	10.96	10.60	10.76
0.70	14.17	13.48	13.46	13.63	12.48	13.62	13.17	13.38
0.80	18.72	17.81	17.79	18.01	16.49	18.00	17.41	17.68
0.90	27.60	26.25	26.22	26.54	24.29	26.53	25.65	26.06
	Peleg model							
	1	2	3	4	5	6	7	8
0.10	3.20	2.98	3.26	2.88	2.17	3.71	2.61	3.17
0.20	4.95	4.65	4.66	4.68	3.89	5.41	4.44	4.91
0.30	6.53	6.17	5.99	6.29	5.53	6.77	6.10	6.39
0.40	8.15	7.73	7.50	7.91	7.23	8.03	7.74	7.82
0.50	9.97	9.50	9.37	9.71	9.12	9.38	9.52	9.40
0.60	12.10	11.60	11.74	11.85	11.38	11.09	11.60	11.34
0.70	14.67	14.16	14.73	14.54	14.18	13.53	14.21	13.93
0.80	17.79	17.29	18.47	17.98	17.75	17.23	17.64	17.56
0.90	21.56	21.12	23.08	22.40	22.36	22.90	22.21	22.65

The monolayer moisture contents for the 8 foods evaluated here ranged from 4.616 to 5.244 g H2O / g DM. With water activity values within the safe level, it is possible to observe actual product moisture results close to that estimated by the isotherm curves. Proving the effectiveness of this analysis as a tool in quality control, in estimating a safe level of moisture for the product. It is noted that the adsorption isotherm proved to be a very efficient tool in determining safe levels of moisture and water activity in extruded dry cat foods. In this way, in a water activity of 0.60, it was possible to verify by the microbiological data in this work that the fungal growth is not favored and, in this way, the antifungals can be used in a preventive way only,

confirming the isotherm findings that show this moisture condition and water activity to be safe

In the palatability test, as observed, the animals tended to consume the negative control (without antifungal), in relation to the products with antifungal, but in general these differences were not significant (Figure. 4).

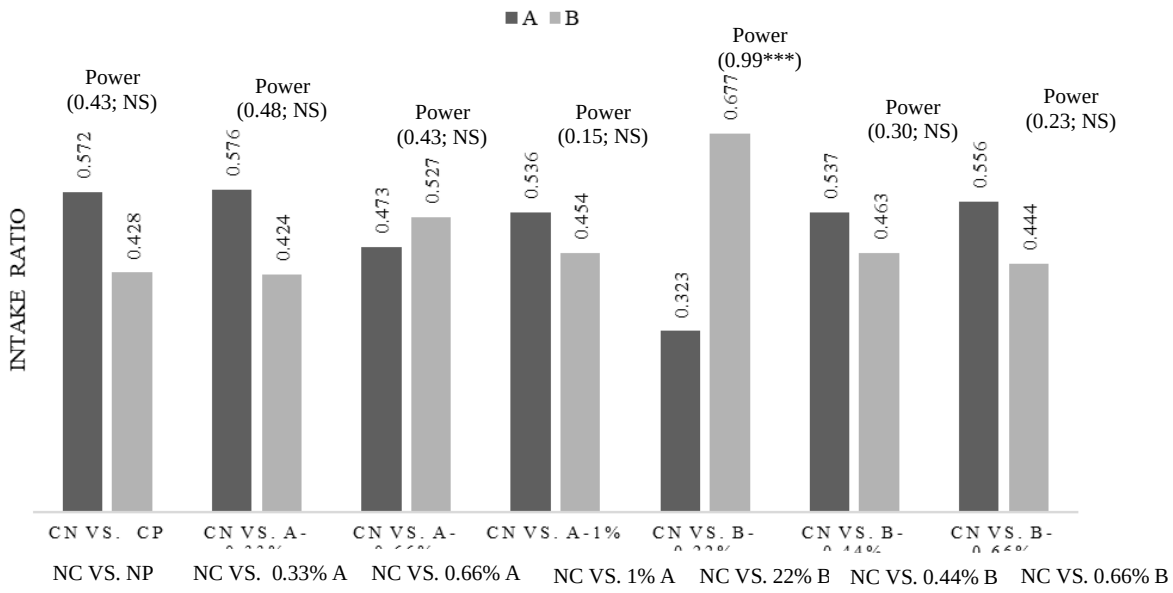

Figure. 4. Food preference test of dry and extruded commercial cat foods with organic acids at different levels, where:

Negative Control - NC (no antifungal); Positive Control- PC (0.15% calcium propionate); 0.33% mold-inhibitor A; 0.66% mold-inhibitor A; 5: 1% mold-inhibitor A; 0.22% mold-inhibitor B, 0.44% mold-inhibitor B and 0.66% mold-inhibitor B.

3. Discussion

In this study, we inoculated *Aspergillus Brasilienses* in dry extruded cat food for the evaluation of the interaction between moisture content and mold-inhibitor efficacy. The prevalence of fungal contamination in pet food is mainly given by the genus *Aspergillus spp.*, representing about 62% of all mold contamination in dog foods (Bueno et al., 2001).

Propionic acid is one of the most efficient and widely used organic acid for controlling mold contamination in foods by *Aspergillus ssp*. This is explained because weak acids, as propionic acid, are efficient antimicrobials in the non-dissociated form, which facilitates the entry into the cell and the dissociation in the cytoplasm causing the cell imbalance of microorganisms (Weiss, Loeffler & Terjung, 2015). This compound presents a pKa = 4.88, which means that half of the acid is in the undissociated form at this pH (Dijksterhuis et al., 2019). The effective antifungal activity (MIC, minimal inhibitory concentration) of propionic acid against various fungi is typically in the range of 7.8 to 39 mM (0.1 to 0.5%, w/v). These include various *Aspergillus* and *Penicillium* species (Haque et al., 2009).

Unlike propionic acid, lactic acid works with a lower efficiency. Lactic acid is also considered a weak chain acid (CH3-CHOH-COOH) with a mechanism of action similar to propionic acid, with an action more related to the bacteria control (Muynck et al., 2004). Mold control, in turn, requires the highest lactic acid. For a better antifungal activity, it is necessary that the acid is predominantly in its non-ionized form. According to Stanojevic-Nikolic et al. (2020), lactic acid is a good microbial growth controlling agent for fungi of the *Aspergillus* spp family. The results obtained showed that organic acid can be efficient in protecting foods against microbiological contamination.

However, in this study both acids presented effect on the mold contamination in Cat food, with this effectiveness more evidenced by mold-inhibitor based on propionic acid. The water activity plays an essential role on the physical, chemical and mainly microbiological conditions of pet food, being considered an important parameter for production, packaging and shelf life of dry foods (Baser & Yalçin, 2017). Dry and extruded foods generally have a water activity range from 0.45 to 0.60 (Timmons, 2006; Novasina, 2011; Decagon, 2003).

The relation between water activity and moisture was evidenced in this study. Lower moistures (5.66 ± 0.74 and 9.48% ± 0.68) presented water activities between 0.43 ± 0.10 and 0.57 ± 0.01, respectively. Those conditions did not favor the microbial growth, since al the treatments presented a decreasing in the mold count over time (Figure 02). Despite this, at the start of the study (day 0), all the mold inhibitors presented effectiveness to decrease the mold count compared to the Negative Control. Although there are no favorable conditions for microbial growth, in these conditions of low moisture (<10%) and water activity (<0.6), antifungals are widely used, as they have a preventive purpose in case of challenges with recent contamination or even under challenging conditions of heterogeneous drying, high relative moisture after open packaging, among others. On the other hand, moistures with 15 and 20% had favorable conditions for microbial growth, where it was possible to observe a differentiated effect of mold-inhibitor A in relation to the others. In moistures above 10%, mold-inhibitor A showed effectiveness at a concentration of approximately 0.5% (equivalent to 0.18% of the active ingredient) to maintain fungal contamination at levels below $3x10^6$, as generally accepted in Pet food in Brazil. (ABINPET, 2021). Water activity is one of the main tools in forecasting the survival of microorganisms in food due to their direct influence on product quality and stability (Tapia et al., 2020). According to Beuchat

(2002), it is not possible for microorganisms to grow in water activities lower than 0.61 and the growth inhibition of the *Aspergillus* genus fungi is in the range of 0.65 to 0.61, which corroborates the results found in this study, since the molds growth in food occurred in moisture content above 13%, where they had water activities higher than 0.65.

In this study, the GAB and Peleg models provided a good fit to the data, according to the values of the quality of models fit parameters. However, according to R^2, Peleg had a slightly better fit for these foods than GAB.

However, the GAB model has an advantage, as with it is possible to determine the moisture content of the monolayer and GAB equation can be used to predict the water sorption isotherms for biological material at wide range: 0.05 < aw> 0.8–0.9 (Timmermann, 2003). The monolayer water is the amount of water strongly adsorbed in specific food locations (Wani and Kumar, 2016). Monolayer moisture contributes to the physical and chemical stability of foods, as it is not available for enzymatic activity, non-enzymatic browning, lipid oxidation or other reactions that compromise shelf life (Shrestha et al., 2007; Goula et al., 2008; Wani and Kumar, 2016; Arslan-Tontul, 2020). The monolayer moisture contents for the 8 foods evaluated here ranged from 4.616 to 5.244 g H2O / g MS. Such results are similar to those found by Sahu & Patel (2020) when they worked with extruded products based on a mixture of corn and millet with incorporation of defatted soybean. For the adsorption curves at a temperature of 30ºC, mean values of 5.33 H2O / g DM were found.

Through the parameter values of the GAB model, it was possible to classify the isotherms as type II, as stated by Blahovec (2004). Its C and K parameters tend to be greater than 2 and less than 1, respectively. The isothermal form is unique for each type of product and materials that contain starch generally have type II curves, with a

sigmoid shape. According to Brunauer, Emmett, & Teller (1938), this curve shape allows us to understand the type of force existing in the water connection with the hygroscopic material, allowing us to assess the surface structure of the material. Type II isotherms are characterized by a sigmoidal curve (Al-muhtaseb, Mcminn, & Magee, 2002), similar types of findings have also been reported by researchers Norajit, Gu & Ryu (2011), Wani & Kumar (2016) and Sahu & Patel (2020) for extruded foods and cake flours (Al-Muhtaseb et al., 2010).

Due to the drying process, dry and extruded foods for dogs and cats have a low moisture content, reaching an average content of 13 g of water for every 100 g of food (Leiva et al., 2019). These reduced levels are also found in the water activity, around 0.5 in the final product, in order to control microbial deterioration (Lambertini et al., 2016). According to Bueno et al. (2001), a moisture content lower than 11.5% is sufficient to inhibit the fungi growth in dry and extruded feed for dogs and cats. All foods with mean water moisture values of 9.48% ± 0.679, are considered safe to prevent microbial growth and had mean water activity values of 0.57 ± 0.01. When compared with the values obtained through the sorption isotherm, we note similarity in the result. With values of 0.5 and 0.6 of water activity, foods would have 9.03 and 10.80% moisture respectively, according to GAB and 9.50 and 11.59% respectively, according to Peleg, values very close to reality. This shows us that isotherms are great tools that can be used in quality control, as they provide specific data with direct application in predicting food drying time, shelf life, microbial growth, in determining the type of packaging, prediction of the effect of temperature abuse, quality control specifications, definitions of critical points, product characterization even when it is constituted by different water activity components (Alhamdan & Hassan, 1999).

As observed, the animals consumed more of the negative control, with no addition of organic acid, except in negative control vs. 0.22% of mold-inhibitor B (60% organic acids and 35% lactic acid), where the difference was significant. In general, lactic acid is believed to improve the palatability of the feed (Van Winsen et al., 2001, Prohaszka et al., 1990). In relation to mold-inhibitor A, with the presence of propionic acid, such results are contrary to those obtained by De Brito et al., (2010), who work with diets for dogs containing two different levels of moisture (low and high), in addition to the inclusion or not of propionic acid. The authors found that diets containing propionic acid (0.65 or 1.3 g / kg) and high moisture content (102 g / kg) positively influenced food intake and, when comparing diets with high and low moisture without organic acid studied, did not notice statistical difference, proving the positive effect of propionic acid on palatability.

5. Conclusions

Moisture content in cat food above 10% are critical for mold growth. Modeling adsorption isotherms is an important tool for the safe control of moisture and water activity with high predictive capacity and, additionally, allowing to reduce economic losses and environmental impacts with excessive drying.

Among the mold-inhibitors, the blend based on mold-inhibitor A containing propionate showed effectiveness in controlling fungal growth in Pet food, even under high moisture conditions. The conditions of this study allow the extrapolation of antifungal doses in extruded food as a function of its moisture, given that the applied modeling showed a good fit and predictive capacity.

Acknowledgments

The authors thank Biorigin- Zilor Energy and Foods (Lençóis Paulista, Brazil) for preparing the experimental diets and funding the research, CEENUFEL research group, the professor of the statistics department Vanderly Janeiro and CAPES for funding the research.

Conflict of interest statement

The authors declare no conflict of interest.

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