UNIVERSIDADE FEDERAL FLUMINENSE
PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA
ÁREA DE CONCENTRAÇÃO EM HIGIENE VETERINÁRIA E PROCESSAMENTO TECNOLÓGICO DE PRODUTOS DE ORIGEM ANIMAL

MARIANA BACELLAR RIBAS RODRIGUEZ

EFEITO DA EMBALAGEM EM ATMOSFERA MODIFICADA SOBRE A VALIDADE COMERCIAL DE FILÉ DE PEITO DE FRANGO COZIDO E DESFIADO ESTOCADO EM REFRIGERAÇÃO

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Dissertação apresentada ao Programa de Pós Graduação em Medicina Veterinária da Universidade Federal Fluminense como requisito parcial para obtenção do Grau de Mestre. Área de Concentração: Higiene Veterinária e Processamento Tecnológico de Produtos de Origem Animal**.**

Orientador: PROF. DR. SÉRGIO BORGES MANO

Co-orientador: PROF. DR. CARLOS ADAM CONTE JÚNIOR

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Niterói
2013

Com muito amor e gratidão,

dedico à minha avó Jandyra Bacellar, o maior exemplo de força e dedicação incondicional.

AGRADECIMENTOS

A Deus, por estar sempre ao meu lado, iluminando minha caminhada.

À minha mãe, Eliane Bacellar Rodriguez, pelo apoio incondicional, por acreditar no meu potencial.

À minha irmã e grande amiga Juliane por estar ao meu lado em todos os momentos, pelo carinho e força para que esse sonho se realizasse.

Ao meu namorado Fernando Montenegro, pelo carinho, amizade, paciência e por estar sempre ao meu lado. Obrigada por fazer com que cada dia se torne especial.

Ao Prof. Dr. Sérgio Borges Mano, meu orientador, pela amizade, pelos ensinamentos, por depositar confiança em mim desde a graduação, e me auxiliar nos momentos que precisei.

Ao Prof. Dr. Carlos Adam Conte Junior, meu co-orientador, pela amizade e competência, por todos os elogios e críticas construtivas, e principalmente pela valiosa orientação.

Ao Prof. Dr. Robson Maia Franco, por ter apoiado esse projeto de pesquisa, por compartilhar seu conhecimento.

À Médica Veterinária Carla da Silva Carneiro, por me apoiar em diversos momentos desta pesquisa, pela amizade e contribuição com seus valiosos ensinamentos.

Aos demais professores do curso por dividir seus conhecimentos.

Aos estagiários, mestrandos, doutorandos e pós doutorandos, companheiros de laboratório, pela agradável convivência.

À coordenação do Programa de Pós Graduação em Higiene Veterinária e Processamento Tecnológico de Produtos de Origem Animal e aos secretários

Dráusio, André e Mariana, pela constante boa vontade em todos os auxílios necessários a conclusão do curso.

Aos funcionários da empresa Rica Alimentos, em especial ao médico veterinário Ociron Vasques pela disponibilidade em fornecer as amostras sem as quais este trabalho não seria realizado.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES pelo apoio financeiro

À todos, que direta ou indiretamente, colaboraram com a realização deste trabalho.

BIOGRAFIA

Mariana Bacellar Ribas Rodriguez, brasileira, filha de Julio André Ribas Rodriguez e Eliane Bacellar Rodriguez, nasceu em 07 de Julho de 1984, na cidade do Rio de Janeiro. No ano de 2004 ingressou na Universidade Federal Fluminense, onde cursou Medicina Veterinária até o ano de 2009. Durante a graduação, em 2008, foi monitora da disciplina de Anatomia Patológica Veterinária II. No ano seguinte, iniciou estágio no Laboratório de Controle Físico-Químico de Produtos de Origem Animal da Faculdade e estágio no Controle de Qualidade do abatedouro de aves Rica Alimentos (Reginaves Ind. e Com. de Aves). Trabalhou durante o ano de 2010 na mesma empresa como Analista do Controle de Qualidade. No mesmo ano, ingressou no Curso de Especialização em Ciência dos Alimentos, na Faculdade de Farmácia da Universidade Federal Fluminense. No ano de 2011, iniciou o Curso de Mestrado em Higiene e Processamento Tecnológico de Produtos de Origem Animal, na Faculdade de Veterinária da Universidade Federal Fluminense como bolsista da CAPES. Enviou trabalhos de pesquisa para III Mostra UFF em Higiene e Tecnologia de Alimentos. Em 2012 enviou trabalho para o XXIII Congresso Pan-Americano de Ciências Veterinárias. Atualmente, a autora foi aprovada no processo seletivo do Curso de Doutorado em Higiene e Processamento Tecnológico de Produtos de Origem Animal, na Faculdade de Veterinária da Universidade Federal Fluminense, o qual iniciará em Março de 2013.

RESUMO

Devido aos novos estilos de vida o consumidor busca cada vez mais produtos prontos para o consumo, como o filé de peito de frango cozido, desfiado e resfriado, entretanto, sua validade comercial é relativamente baixa. Assim, objetivou-se neste estudo, avaliar a extensão da validade comercial desse produto embalado em atmosfera modificada (EAM) com diferentes concentrações de dióxido de carbono (CO_2), através da contagem de bactérias heterotróficas aeróbicas mesófilas (CBHAM), psicrotróficas (CBHAP), *Enterobacteriaceae*, bactérias ácidas láticas (BAL) e pH. Correlacionar o crescimento bacteriano com a concentração CO_2 e a formação das aminas biogênicas (AB) e, verificar a aplicabilidade da análise de quantificação de AB como indicador do índice de qualidade das amostras. Foram obtidos 6,3 kg da amostra, dividida em sete tratamentos e assim submetidas a diferentes embalagens: aerobiose, vácuo e 10, 30, 50, 70 e 90% de CO_2 complementadas com N_2. Todas as amostras foram estocadas a 4±2°C por 28 dias. Os parâmetros de crescimento dos grupos estudados foram determinados mediante a equação de regressão de Baranyi. Observou-se tendência crescente nos valores de pH nas amostra em aerobiose, nos outros tratamentos foi observado comportamento inverso, ao longo do período de estocagem. A concentração de CO_2 apresentou decréscimo nas primeiras 24 horas, após este período os valores permaneceram estáveis. Observou-se um aumento progressivo da contagem de BHAM, BHAP, enterobactérias e BAL no decorrer do tempo de estocagem, o qual foi mais acelerado na embalagem em aerobiose do que nos demais tratamentos. Nos tratamentos com concentrações do gás superiores a 10% observou-se menor crescimento de enterobactérias e, no caso das BAL, o crescimento foi discreto em todas as amostras tratadas, independente da concentração de CO_2 utilizada. Baseada na contagem de BHAM, o aumento na validade comercial das amostras embaladas com 90% de CO_2 (28 dias) foi 3 vezes maior do que as embaladas em aerobiose (9 dias). Os níveis de putrescina e cadaverina aumentaram progressivamente no decorrer da estocagem, com o aumento da concentração do CO_2, a produção dessas aminas ocorreu de forma mais lenta. Conclui-se que o aumento na concentração do gás carbônico relaciona-se com a diminuição do desenvolvimento de bactérias produtoras de putrescina e cadaverina. Ademais, a quantificação das aminas demonstrou ser um parâmetro adequado para a avaliação do índice de qualidade do filé de peito de frango cozido e desfiado.

Palavras-chave: carne de frango, qualidade, método de conservação, atmosfera modificada, aminas biogênicas, cromatografia líquida.

ABSTRACT

The new consumer lifestyles is searching for read-to-eat products, like shredded cooked chicken breast fillet. However this products shelf life are relatively low. The aim of this study was to evaluate the extent of the shelf life of this commercial product packaged in modified atmosphere (MAP) with different concentrations of carbon dioxide (CO_2), through the aerobic heterotrophic mesophyll bacteria (AHMB) count, psychotropic (AHPB), *Enterobacteriaceae*, lactic acid bacteria (LAB) and pH. Correlate bacterial growth with CO_2 concentration and formation of biogenic amines (BA) and, verify if BA quantification can be used to indicate the quality index of samples. 6,3 kg of the sample was divided into seven groups with different packaging conditions: aerobiosis, vacuum and 10, 30, 50, 70 and 90% CO_2 completed with N_2. All samples were stored at 4±2°C for 28 days. The bacteriological growth parameters studied were adjusted through the Baranyi regression equation. The pH of the aerobic packages increased during storage, but the other treatments presented an opposite trend, where the pH values decreased during storage. CO_2 concentration decreased over the first 24 hours, but after this period, the values remained constant. A gradual increase of AHMB, AHPB, *Enterobacteriaceae* and LAB was observed during storage which was faster in the package under aerobiosis than in the other treatments. Treatments with CO_2 concentration above 10% showed smaller *Enterobacteriaceae* growth, while LAB growth was discrete in all the treated samples, independent of CO_2 concentration. The shelf life of the samples packed with 90% CO_2 (28 days), determined based on AHMB count, was three times higher than that of the samples packed under aerobiosis (9 days).The levels of putrescine and cadaverine gradually increased during storage, increasing CO_2 concentration caused a slower production of those amines. It was concluded that increasing carbon dioxide concentration causes a reduction of the growth of putrescine and cadaverine producing bacteria. Amine quantification proved to be an adequate parameter to evaluate the quality index of shredded cooked chicken breast fillet.

keywords: chicken meat, quality, preservation method, modified atmosphere, bioactive amines, liquid chromatography.

SUMÁRIO

1 **INTRODUÇÃO**

A produção e o consumo da carne de frango têm aumentado significativamente em todo o mundo, o que estimula sua utilização como matéria-prima para o processamento de novos produtos. Alimentos saudáveis e de qualidade, contendo apenas ingredientes naturais tem crescido constantemente devido aos novos estilos de vida dos consumidores, cada vez mais conscientes em relação à saúde. No entanto, a falta de tempo para preparar as refeições é um fator limitante neste processo. Assim, produtos prontos para o consumo são muito procurados.

O filé de peito de frango cozido desfiado é um produto de grande praticidade, uma vez que pode ser consumido direto ou utilizado na elaboração de novos pratos e saladas. Destaca-se por ser um alimento rico em proteínas de alto valor biológico e baixa caloria. Entretanto, como todo produto de origem animal é um produto perecível, com características fisiológicas e bioquímicas favoráveis ao crescimento bacteriano, e alterações físico-químicas e sensoriais.

Diversas são as alterações de natureza química que ocorrem na matriz alimentar durante o período de estocagem, como a formação das aminas biogênicas. Presentes em diversos tipos de alimentos são descritas como bases orgânicas de baixo peso molecular com potencial risco à saúde. A concentração das aminas formadas nos alimentos depende do tipo de microbiota presente, da ação de enzimas descarboxilases produzidas por microrganismos sobre aminoácidos específicos e de condições favoráveis para atividade dessas enzimas. Diversos autores tem indicado a formação desses compostos como critérios para avaliação de qualidade nos alimentos.

O controle da temperatura de estocagem é um dos mais importantes parâmetros usados para prolongar a validade comercial de carnes. O armazenamento sob refrigeração retarda os efeitos da deterioração, mas não promove um incremento suficiente na validade comercial do produto para a distribuição e exposição nos locais de venda. Sendo assim, novos métodos de conservação vêm sendo estudados com o objetivo de reduzir o crescimento de microrganismos instalados antes e durante o processamento.

A adoção de tecnologia da embalagem com atmosfera modificada revolucionou e modernizou os frigoríficos e as indústrias de processamento de

produtos de origem animal. Seu benefício possibilita o transporte de carne fresca por longas distâncias, com maior relevância principalmente para as exportações. Além disso, atende a crescente demanda dos consumidores por alimentos frescos, de boa qualidade, com maior prazo comercial, porém sem conservantes e aditivos.

Diante deste fato, este trabalho foi desenvolvido com o objetivo de determinar a validade comercial de filés de peito de frango cozidos e desfiados armazenados sob refrigeração e embalados em atmosfera modificada com diferentes concentrações de CO_2, verificando a influencia da concentração deste gás no comportamento de bactérias mesófilas, psicrotróficas, enterobactérias e bactérias láticas. Objetivou-se ainda avaliar a formação de aminas biogênicas nas amostras, correlacionando a formação destes compostos químicos com a contagem bacteriana e, verificando a aplicabilidade da análise de quantificação de aminas biogênicas como indicadora do índice de qualidade.

2 **REVISÃO DE LITERATURA**

A seguir serão abordadas informações sobre a evolução da avicultura, aspectos de qualidade do filé de peito de frango cozido e desfiado, utilização da tecnologia de embalagem em atmosfera modificada, os principais microrganismos envolvidos na deterioração dos alimentos, assim como sua relação com a formação de aminas biogênicas e microbiologia preditiva.

2.1 POTENCIAL DA AVICULTURA NACIONAL

Nas últimas três décadas, a avicultura brasileira tem apresentado altos índices de crescimento. Em 2009, o Brasil alcançou uma posição entre os três maiores produtores mundiais de carne de frango, com a marca de 10,9 milhões de toneladas (UBABEF, 2011). Em 2011, a produção da carne de frango chegou a 13,058 milhões de toneladas, com incremento de 6,8% em relação ao ano anterior. Com este resultado o Brasil se aproxima da China, hoje segundo maior produtor mundial, cuja produção em 2011 teria somado 13,2 milhões de toneladas, abaixo apenas dos Estados Unidos (UBABEF, 2012).

O desempenho da produção está relacionado com diversos fatores como qualidade, sanidade e preço. Além da implantação de programas sanitários, o Brasil buscou modernizar e empregar instrumentos como o manejo adequado do aviário, alimentação balanceada, melhoramento genético com aumento nos índices de conversão alimentar, e produção integrada, tornando-se mais competitivo no mercado interno e internacional (LIMA et al., 2012; SOUSA; OSAKI, 2008).

A maior parte das exportações ainda é de produtos *in natura*, que no último ano foi de 92% do volume exportado, sendo 8% referentes às exportações de produtos processados (UBABEF, 2012).

Atualmente, o mercado de produtos industrializados é promissor, pois ao analisar o número de destinos das exportações brasileiras no período entre 2000 e 2010, houve um incremento de 254%, enquanto para frango inteiro e em partes e miúdos de frango foi de 125% e 70%, respectivamente. Além disto, ao comparar o preço médio pago pela tonelada em 2010, alimentos processados e conservas de carne registraram valores 80,8% e 53,4% superiores ao pago pela tonelada de

frango inteiro e partes e miúdos de frango, respectivamente (CAMAROTTI, 2010; UBABEF, 2011).

A preferência do consumidor tem sido por produtos frescos, partes congeladas e alimentos industrializados de conveniência. Devido a este tipo de demanda, as empresas têm oferecido produtos prontos para cozer, produtos semipreparados, reduzindo o tempo de dedicação caseira no preparo dos alimentos. Produtos prontos para consumo, cozidos ou assados, possuem mercado crescente apesar da também crescente oferta de alimentos em redes de cozinhas rápidas (LIMA,1995; WANG et al., 2004).

2.2 FILÉ DE PEITO DE FRANGO COZIDO DESFIADO

A produção do filé de peito de frango cozido desfiado vem aumentando nos últimos anos. Comercializado em supermercados sob refrigeração ou congelados com o objetivo principal de atingir um novo perfil do mercado consumidor que busca conveniência e praticidade ao adquirir produtos prontos para o consumo (CONTE-JUNIOR et al., 2010; KATZ, 1999).

Entretanto existe um grande risco de contaminação durante o seu processamento. Antes do abate, os tecidos comestíveis de um animal podem ser considerados estéreis, uma vez que se encontram protegidos pela pele e o trato intestinal é uma barreira contra a flora bacteriana presente em seu interior (GILL, 1991).

Durante o abate, na sangria e nas etapas que a procede, a carne é exposta a diferentes espécies de bactérias. Assim, o filé de peito de frango apresenta uma microbiota heterogênea consistindo de bactérias mesófilas e psicrotróficas característica do produto (BOURGEOIS et al., 1994).

Para se obter o filé de peito de frango cozido e desfiado é necessário realizar outras etapas de processamento: cozimento, desfiamento e embalagem. No cozimento ao se utilizar um tratamento térmico ocorre uma seleção da microbiota, ao eliminar ou diminuir o número de microrganismos do produto (SILVA JUNIOR, 1996).

Devlieghere e colaboradores (2000) descrevem que produtos cárneos cozidos são frequentemente pós-contaminados por causa das etapas de desfiamento e embalagem do produto após o processo de pasteurização. Assim, torna-se imprescindível ressaltar a importância do sistema de inspeção realizado em conjunto

com outras ferramentas da qualidade, como Boas Práticas de Fabricação (BPF), o Procedimento Padrão de Higiene Operacional (PPHO) e a Análise de Perigos e Pontos Críticos de Controle (APPCC) para garantia dos processo e qualidade do produto final (OLIVEIRA et al., 2012).

A carne fresca refrigerada possui um alto valor de atividade de água. Este ambiente é muito adequado para o crescimento de grande quantidade de microrganismos deteriorantes que se desenvolvem na superfície da carne. Outro fator que contribui para o desenvolvimento de bactérias é a cominuição do produto, quando uma maior área se torna exposta em contato com o ar atmosférico. Caso não se utilize no produto uma embalagem protetora ou se são protegidos por películas permeáveis ao oxigênio, favorecerá o desenvolvimento de bactérias e consequentemente sua deterioração (CORTEZ-VEGA et al., 2012; HUDA et al., 2012).

2.3 EMBALAGEM COM ATMOSFERA MODIFICADA

A atmosfera modificada consiste no acondicionamento de um determinado alimento em uma embalagem com composição gasosa diferente do ar, hermeticamente fechada. No momento da embalagem o ar atmosférico é substituído por uma combinação específica de gases. Em geral os gases mais utilizados são o gás carbônico e nitrogênio (CALDERON, BARKAI-GOLAN, 1990; ZARDETTO, 2005).

Ao implementar essa tecnologia se objetiva aumentar o tempo de validade comercial ao retardar reações químicas, enzimáticas e microbiológicas e, incrementar a coloração do alimento (JEREMIAH, 2001; TANIWAKI et al., 2009).

O período de estocagem dos alimentos é consideravelmente prolongado pela modificação da atmosfera que circunda o produto, a qual reduz a taxa de respiração dos alimentos, controla as reações químicas e enzimáticas e diminui a atividade dos microrganismos presentes (JAYAS; JAYAMKONDAN, 2002; TEODORO et al., 2007).

A eficácia do uso de atmosfera modificada depende de vários fatores como, tipo de alimento, qualidade inicial da matéria-prima, mistura de gases utilizada, temperatura de armazenamento, condições de higiene durante o processamento e

das propriedades de barreira do material utilizado na embalagem (SIVERTSVIK et al., 2002).

Os fatores intrínsecos ao produto como, atividade de água, pH, teores de lipídeos, características sensoriais e principalmente, a carga microbiana e a presença de microrganismos deteriorante e patogênicos, irão determinar a velocidade de deterioração microbiológica, bioquímica e física (SARANTÓPOULOS et al., 1998).

É imprescindível que a qualidade do produto a ser embalado seja boa, e o processamento seja realizado com a aplicação das boas práticas de fabricação, pois a embalagem com atmosfera modificada, não tem a função de melhorar a qualidade do produto, apenas retardar a deterioração. Além disso, as embalagens com atmosfera modificada não reduzem ou eliminam a necessidade de refrigeração. O controle rígido de temperatura durante todo o ciclo de preparo, distribuição e comercialização do produto é um fator decisivo para o sucesso da aplicação deste método (JEREMIAH; GIBSON, 2001).

2.3.1 Variação na composição dos gases

Nos produtos de origem animal a variação da atmosfera modificada se dá de forma lenta, ocasionada principalmente pelo crescimento de microrganismos, mas também pode ocorrer pela influência de outros fatores, como a solubilidade do CO_2 na parte aquosa e gordurosa da matriz, mudanças bioquímicas, e a lenta difusão dos gases através da embalagem, por tanto a atmosfera gasosa muda continuamente durante todo o período de armazenamento (PARRY, 1993).

Seideman e colaboradores (1980) indicaram que a concentração de CO_2 diminui durante o armazenamento de carne suína (a 2ºC) embalada em atmosferas inicialmente compostas por 20/5% e 40/10% de CO_2/O_2. No entanto, outros autores asseguram que, nas mesmas condições, os níveis de CO_2 aumentam no interior das embalagens no decorrer da estocagem (SPAHL et al. 1981). Fang e Lin (1994) constataram uma diminuição de O_2 e um aumento de CO_2 durante o armazenamento a 4ºC de carne suína cozida embalada em aerobiose, observando também uma diminuição de CO_2 nas amostras embaladas em atmosfera modificada. Resultados similares foram observados em carne fresca de suíno (MCMULLEN; STILES, 1991; SORHEIM et al., 1995).

Segundo Mano et al. (2002) em estudo realizado com carne suína embalada em atmosfera modificada indica que, ainda que seja normal observar modificações na composição da atmosfera que rodeia o alimento, principalmente, naqueles com maior atividade metabólica (como hortaliças e frutas, devido à atividade respiratória das mesmas), não foram observados variações no estudo realizado. O fato é atribuído a sensibilidade do analisador de gases e a relação volume de gás/massa de alimento. Esta relação apesar de diferente das práticas comerciais teve o objetivo de evitar que a modificação da atmosfera durante o armazenamento mascarasse o efeito da própria atmosfera no comportamento dos microrganismos psicrotróficos, ou seja, objetivou criar condições próximas à das atmosferas controladas.

Estudo realizado com carne de frango embalada em atmosfera modificada nas concentrações de 75/25 O_2/CO_2 e 75/25 N_2/CO_2, armazenados a temperatura de +3,2 a +0,7ºC, durante 14 dias, apresentaram mudanças na concentração de O_2, do início do período de estocagem ao final, de 74,8% para 55,9% e no outro tratamento, o oxigênio residual foi de 0,4 % para 0,7% (GALLAS et al., 2010).

Conte-Junior (2011) em estudo realizado com diferentes concentrações de gás carbônico e oxigênio na validade comercial de carne bovina moída observou que em todas as atmosferas modificadas ocorreu redução do percentual de oxigênio ao longo do tempo, enquanto que o dióxido de carbono elevou-se, fato este atribuído à atividade microbiana e atividade bioquímica da carne.

2.3.2 Parâmetros analíticos bacteriológicos

O gás carbônico tem efeito inibitório sobre metabolismo aeróbio e anaeróbio e sua ação sobre os microrganismos pode ser atribuída à alteração das funções da membrana celular, como a captura e absorção de nutrientes. A permeabilidade celular bacteriana também é influenciada pela presença do gás. Além da inibição direta das enzimas ou diminuição da velocidade das reações enzimáticas, altera as propriedades físico-químicas das proteínas (CHURCH, 1993; SARANTÓPOULOS; SOLLER, 1994; SARANTÓPOULOS et al., 1998).

A efetividade do CO_2 depende, também, da fase de crescimento do organismo presente. O dióxido de carbono aumenta a duração da fase de adaptação e reduz a taxa de crescimento durante a fase logarítmica. O efeito sobre a primeira é maior e, portanto, à medida que a bactéria passa da fase lag para a fase log, o efeito

inibitório do crescimento é reduzido (CHURCH, 1995). Sua atividade inibitória aumenta quando a temperatura de armazenamento diminui, devido à maior solubilidade do gás em água sob baixas temperaturas. (JAY, 2005; PARRY, 1993; SIVERSTIVIK et al., 2002).

O efeito antimicrobiano do dióxido de carbono ocorre quando o gás está na concentração em torno de 10% e aumenta com maiores concentrações (MANO et al., 2000). Floros e Matsos (2005) ao utilizarem 20% de CO_2 na embalagem foi possível controlar o crescimento de muitos aeróbios, incluindo *Pseudomonas* spp*., Acenatobacter* spp.e *Moraxella* spp., embora altas concentrações possam estimular o crescimento de *Clostridium botulinum.*

Em geral, as bactérias Gram negativas são mais sensíveis à inibição pelo gás carbônico do que as Gram positivas, sendo as pseudomonas classificadas como as mais sensíveis, e os clostrídeos como os mais resistentes. Durante o armazenamento prolongado, o gás carbônico provoca uma mudança considerável na microbiota da carne, variando de uma microbiota predominantemente formada por microrganismos Gram negativos, nos produtos frescos, para uma principalmente, ou exclusivamente formada por Gram positivos (JAY, 2005).

As bactérias Gram positivas ácido-láticas, *Brochothrix thermosphacta e Lactobacillus* spp. se tornam os organismos dominantes (CHURCH, 1994), sendo que *B. thermosphacta* podem tolerar níveis de gás carbônico acima de 75% e as bactérias ácido láticas podem crescer sob condições de 100% de gás carbônico (SMITH et al, 1990).

As bactérias ácido láticas apresentam a capacidade de se desenvolver em diversas composições de gases, inclusive cooperando com o aumento do prazo comercial da carne embalada nessas condições (MANO et al., 2002). Também estão relacionadas com a redução da ameaça de patógenos, garantindo a segurança do alimento (JAYAS; JAYAMKONDAN, 2002).

Em concentrações superiores a 5% de gás carbônico, a atmosfera provoca inibição do crescimento de bolores e bactérias psicrotróficas Gram negativas como *Pseudomonas* spp*., Acinetobacter* spp*., Moraxella* spp*., Enterobacteriaceae* e *Shewanella* spp., importantes deteriorantes de produtos de origem animal e de alimentos refrigerados (CHURCH, 1994; FRANCO; LANDGRAF, 2004).

Taniwaki et al. (2009) verificaram que altas concentrações de gás carbônico inibiram o crescimento de fungos e a formação de micotoxinas. Altas concentrações

de gás carbônico tem efeito inibitório sobre microrganismos patogênicos como *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli* e *Bacillus cereus* (SARANTÓPOULOS; SOLLER, 1994).

Listeria monocytogenes, Yersinia enterocolítica e *Aeromonas hydrophila* são mais competitivas e consequentemente uma concentração de 40% de gás carbônico é necessária para sua inibição efetiva (CHURCH; PARSONS, 1995).

Concentrações de 80% de CO_2 e 20% de N_2 é a melhor escolha para as lingüiças de frango. Esta atmosfera controla o crescimento de aeróbios mesófilos e também é capaz de inibir o crescimento de *Y. enterocolítica* (CONTE-JUNIOR et al., 2010).

A concentração de gás carbônico nas embalagens não afeta apenas os microrganismos, mas também pode causar alterações na cor e no sabor dos produtos, favorecer a exsudação de carnes frescas e pescados, devido à alteração na capacidade de retenção de água. Além disso, atmosferas com altas concentrações deste gás podem acarretar o colapso da embalagem, pois o gás carbônico permeia o material de embalagem mais rapidamente que o oxigênio e nitrogênio e dissolve-se na água e na gordura do alimento (PARRY, 1993; SARANTÓPOULOS et al., 1998).

Patsias et al. (2006) constataram que embora as carnes de frango pré-cozidas armazenadas em aerobiose apresentaram um prazo de validade comercial de 14 a 15 dias, as amostras foram melhor conservadas sob as misturas de gases tiveram a validade comercial estendida para mais seis dias quando comparada com o controle, conservando as características de odor e sabor desejáveis.

Os microrganismos deteriorantes mais importantes em carnes frescas estocadas sob refrigeração pertencem ao gênero *Pseudomonas*, inibido pelo vácuo e por concentrações de CO_2 em torno de 10 a 20%. Nessas condições, outros microrganismos se tornam dominantes como, por exemplo, *B. thermosphacta* e *Lactobacillus* em aerobiose e anaerobiose, respectivamente (CHURCH, 1995).

Segundo Franco e Landgraf (2004), a quantidade e tipo de microrganismos que se desenvolvem na carne dependerão das condições de abate, estresse do animal, evisceração correta, entre outros. Jeremiah e Gibson (2001) também observaram que a validade comercial depende da microbiota inicial do produto, ou seja, quanto maior a contagem de microrganismos, menor a validade do alimento devido ao aumento da atividade microbiana.

2.3.3 Parâmetros analíticos físico-químicos

2.3.3.1 pH

O pH de um determinado meio interfere de maneira significativa no crescimento e desenvolvimento de microrganismos, uma vez que altos valores de pH criam um ambiente propício ao crescimento microbiano (JEREMIAH, 2001).

Diversos autores descrevem que altas concentrações de CO_2 são capazes de manter os valores do pH inicial do músculo por mais tempo, possivelmente pela transformação deste CO_2 em ácido carbônico (H_2CO_3) ao solubilizar-se na parte aquosa do alimento (STILES, 1990; HOOD; MEAD, 1993; MANO et al., 1995). Outra explicação para esta manutenção do pH, seria o crescimento predominante de *Lactobacillus*, os quais acidificam o meio através da produção de ácido lático (BANKS et al., 1980; OGRYDZIAK; BROWN, 1982).

2.3.3.2 Aminas biogênicas

As aminas biogênicas são produzidas em alimentos e bebidas como consequência principal da descarboxilação de aminoácidos por enzimas bacterianas (HALÁSZ et al., 1994; SHALABY, 1996). As principais aminas biogênicas encontradas frequentemente em carne fresca e processada são putrescina, cadaverina, histaminas e tiraminas (DEMEYER et al., 2000). Enquanto as poliaminas naturais espermidina e espermina apresentam pequenas alterações durante o armazenamento e processamento (HAGEN et al., 2005;HERNÁNDEZ-LOVER et al., 1997).

A determinação desses compostos químicos em carne fresca e processada torna-se de grande interesse não apenas pelo potencial risco a saúde humana (EDWARDS; SANDINE, 1981; ÕNAL, 2007; SAAID et al., 2009), mas também porque diversos autores sugerem que o perfil das aminas biogênicas poderia ser um indicador químico de contaminação no controle de qualidade de carne fresca e processada (BAUER, 2006; BOVER-CID ET AL., 2006; GOUVEIA, 2009; JIMÉNEZ-COLMENERO, 2004; ROKKA et al., 2004; RIGUEIRA, 2010; RUIZ-CAPILLAS; TAO

et al., 2011). Por essa razão esse tema será abordado de forma mais aprofundada no capítulo 3.1.

2.4 MICROBIOLOGIA PREDITIVA

A análise microbiológica é de extrema importância na avaliação da qualidade dos alimentos uma vez que fornece informações quanto as condições de processamento, armazenamento, distribuição, validade comercial e risco a saúde dos consumidores (FRANCO; LANDGRAF, 2004).

O comportamento das populações microbianas é determinado pelas características dos alimentos como atividade de água, potencial redox e pH, além das condições de armazenamento, como temperatura, umidade relativa e atmosfera (NAKASHIMA et al., 2000).

A microbiologia preditiva está baseada na hipótese de que o efeito dessas propriedades pode ser previsto por modelos matemáticos derivados de estudos quantitativos dos microrganismos, o que torna a microbiologia preditiva uma área promissora da microbiologia de alimentos, recebendo significante atenção científica nos últimos anos (MARKS, 2008).

O objetivo da microbiologia preditiva é descrever matematicamente o crescimento ou a diminuição dos microrganismos sob condições ambientais específicas, considerando os fatores intrínsecos e extrínsecos dos alimentos. Ou seja, predizer a resposta de crescimento de um determinado microrganismo frente a variações de fatores como temperatura, condições de armazenamento, umidade e pH (ROBERTS, 1992).

O primeiro relato da utilização da microbiologia preditiva foi descrito por Esty e Meyer, em 1992, ao descreverem através de um modelo linear a morte térmica dos esporos de *Clostridium botulinum* tipo A, demonstraram a relação entre a taxa de mortalidade da bactéria com o tempo é constante (BARANYI; ROBERTS, 1994).

A partir da década de 80 em decorrência de surtos de toxinfecções alimentares ocorreu uma maior consciência da necessidade da produção de alimentos seguros, aumentando o interesse pela microbiologia preditiva (MCMEEKIN et al., 2002).

De modo geral, os modelos preditivos podem predizer parâmetros cinéticos de crescimento ao longo do tempo através de equações matemáticas, dentre eles:

duração da fase “lag” (fase de adaptação ao meio em que o microrganismo se encontra); velocidade específica máxima de crescimento (velocidade de crescimento dentro da fase exponencial) e densidade populacional máxima de crescimento final (maior contagem microbiana na fase estacionária) (BATY; DELIGNETTE-MULLER; 2004; MASSAGUER, 2006; TORTORA et al, 2012). Os parâmetros cinéticos podem ser visualizados na Figura.

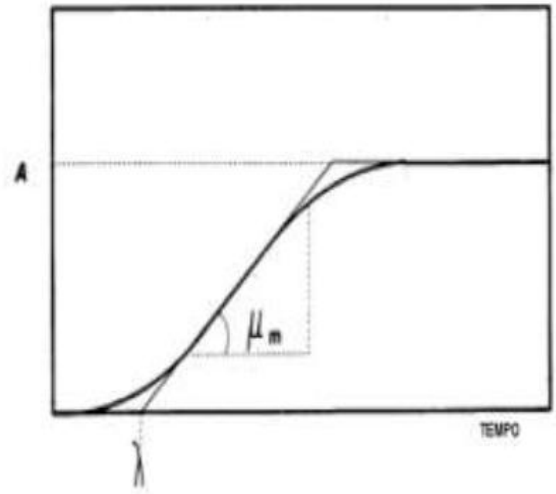

Figura. Curva de crescimento microbiano com indicação dos parâmetros cinéticos. Fonte Zwietering (1990).

μm (velocidade de crescimento exponencial)

λ (duração da fase lag)

A (log da densidade máxima da população)

É importante ressaltar que as análises microbiológicas não podem ser substituídas, sendo a microbiologia preditiva uma ferramenta adicional na busca pela garantia da segurança alimentar (MCMEEKIN et al., 2002).

As vantagens dos modelos preditivos são inúmeras e incluem avaliar a validade comercial, eficiência da higiene durante o processamento e distribuição de alimentos, determinar o efeito dos lapsos das condições de armazenamento e predizer a segurança microbiológica do alimento (MCDONALD; SUN, 1999).

A aplicação efetiva da microbiologia preditiva requer a seleção apropriada de modelos que reflitam o efeito dos parâmetros de crescimento dos microrganismos nos alimentos (WHITING; BUCHANAN, 1993).

Com a necessidade de garantir a inocuidade e a qualidade dos alimentos, há um crescente interesse na ampliação da utilização dos conceitos de modelagem

preditiva tanto para microrganismos deteriorantes quanto para microrganismos patogênicos (ROBERTS, 1992).

Modelos preditivos atualmente estão sendo utilizados para prever o comportamento dos microrganismos nos alimentos, através de modelos matemáticos computadorizados sofisticados, os quais podem avaliar múltiplos parâmetros de crescimento (ANASTÁCIO et al., 2009; DANNENHAUER, 2010).

O verdadeiro poder nas abordagens feitas pela microbiologia preditiva, ao contrário do processo tradicional de avaliação, está nos modelos desenvolvidos. Uma vez validados, podem ser utilizados para predizer com rapidez e segurança a resposta dos microrganismos em várias condições. Desta forma, a microbiologia preditiva pode ser considerada uma ferramenta preciosa para os microbiologistas de alimentos na tomada de decisões diárias (MCMEEKIN et al., 2008).

3 **DESENVOLVIMENTO**

O desenvolvimento do trabalho foi baseado na elaboração de três artigos envolvendo uma revisão sobre as aminas biogênicas, o desenvolvimento de bactérias deteriorantes em embalagem em atmosfera modificada e a formação de aminas biogênicas na matriz estudada.

Ressalta-se que a formatação dos artigos enviados está de acordo com as normas exigidas pelas respectivas revistas de envio.

3.1 BIOACTIVE AMINES: ASPECTS OF QUALITY AND SAFETY IN FOOD. Enviado para Ciência Rural em 18 de janeiro de 2013.

O comprovante de envio se encontra no apêndice 6.1.

Bioactive amines: aspects of quality and safety in food

Aminas bioativas: aspectos de qualidade e segurança em alimentos

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- REVIEW-

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RESUMO

Presentes em diversos tipos de alimentos as aminas bioativas são descritas como bases orgânicas de baixo peso molecular. Com potencial risco à saúde, apresentam características vasoativas, psicoativas e toxicológicas. A concentração de aminas formadas nos alimentos depende do tipo de microbiota presente, da ação de enzimas descarboxilases produzidas por microrganismos sobre aminoácidos específicos e de condições favoráveis para atividade dessas enzimas. Em análises de rotina para o controle na produção e comercialização de alimentos a presença desses metabólitos químicos, tem sido sugerida

como indicadora de qualidade. A prevenção da formação destas ocorre principalmente através da adoção de boas praticas de fabricação de alimentos, porém, outros métodos também podem ser utilizados pela indústria, como: o controle da temperatura na cadeia produtiva, o uso de embalagens em atmosfera modificada e a irradiação dos alimentos. Esta revisão objetiva abordar a formação de aminas bioativas em alimentos, enfatizando a formação e classificação destes metabólitos, os aspectos relacionados à saúde, os limites aceitáveis e os métodos de controle utilizados na indústria para garantir a segurança e qualidade dos alimentos. O sucesso desta abordagem está ligado à importância das aminas bioativas como indicadores de qualidade, assim como da discussão sobre parâmetros aceitáveis em alimentos.

Palavras-chave: *aminas bioativas, toxicidade, qualidade dos alimentos.*

ABSTRACT

Present in several types of food, bioactive amines are described as organic bases of low molecular weight. They have vasoactive, psychoactive and toxicological characteristics and constitute a potential health risk. The concentration of amines formed in foods depends on the type of microorganisms present, the action of decarboxylase enzymes produced by microorganisms on specific amino acids and favorable conditions for enzymatic activity. The presence of these chemical metabolites has been suggested as a quality indicator in routine analyzes for food production and marketing monitoring. Bioactive amine formation can be prevented mainly through the adoption of good manufacturing practices, but the industry can also use other methods such as temperature control in the production chain, modified atmosphere packaging and food irradiation. This review aims to address the formation of bioactive amines in foods, emphasizing the formation and classification of these metabolites, aspects related to health, acceptable limits and control methods used in the industry to ensure

food safety and quality. The success of this approach is linked to the importance of bioactive amines as quality indicators, as well as the discussion of acceptable parameters in food.

Key-words: *bioactive amines, toxicity, food quality.*

INTRODUCTION

Bioactive amines are low molecular weight organic bases produced by the metabolism of plants, animals and microorganisms (HALÁSZ et al., 1994; SALAZAR et al., 2000; BRINK et al., 2002). These amines can be detected in fresh and processed foods and can be formed by transamination of aldehydes and ketones, hydrolysis of nitrogen compounds, thermal decomposition or by decarboxylation of amino acids (HALÁSZ et al., 1994; SAAID et al., 2009). Amino acid decarboxylation is the main route of biogenic amine formation and consists on the removal of the α-carboxyl group from the amino acid structure forming the correspondent amine (SHALABY, 1996). This reaction may occur through two biochemical routes: by the action of endogenous decarboxylase enzymes, i.e. enzymes naturally present in food or by exogenous decarboxylase enzymes produced by microorganisms (SILLA-SANTOS, 1996; FLICK & GRANATA, 2005). These microorganism may constitute the characteristic microbiota of the product or may be introduced before, during or after food processing (ROKKA et al., 2004). The concentration and formation of different types of amines is directly related to the nature of the food and type of microorganism present (BRINKER et al., 2002; INNOCENTE et al., 2007).

Biogenic amines are present in low concentrations or are not detected in fresh food; however, in food of animal origin such as: fish, meat, eggs, cheese and fermented foods they can be present in high concentration able to induce a chemical poisoning (FLICK & GRANATA, 2005). In the case of fish, histamine poisoning should be enhanced, historically

known as scombroid poisoning for its association with the intake of fish in the Scombridae family which includes tuna and sardines (BRINKER et al., 2002).

The accumulation of biogenic amines in food depends on the availability of free amino acids and the presence of microorganisms with decarboxylase activity on amino acids (ÕNAL, 2007). In addition to the availability of precursor amino acids, amine formation depends on food intrinsic and extrinsic parameters such as: temperature and pH, oxygen tension, availability of carbon sources, presence of vitamins, co-enzymes, concentration of free amino acids and fermentable carbohydrates (BOVER-CID et al., 2008; BUNKOVÁ et al., 2010; GLÓRIA, 2005; GREIF et al., 2006). Thus, the main factors that influence the biosynthesis of these compounds are storage conditions, good manufacturing practices (KOMPRDA et al., 2001; KORDIOVSKA et al., 2006), the amount of microorganisms with decarboxylase activity (SILLA-SANTOS, 1996; JAY, 2005), the quality of the raw material and the availability of free amino acids (MAIJALA & NURMI., 1995; LANDETE et al., 2008; CHEN et al., 2010).

In this context, the goal of this study is to present a review on the bioactive amines present in food, including formation and classification of these metabolites, health related aspects, acceptable limits and control methods used in the food industry.

Nomenclature, classification and biosynthesis of bioactive amines

According to their biosynthetic pathway, bioactive amines are classified into (BARDÓCZ, 1995; SHALABY, 1996; LIMA & GLÓRIA, 1999; KALAČ et., 2009): enic: they are formed by bacterial enzymatic decarboxylation of amino acids (histamine, serotonin, tyramine, phenylethylamine, tryptamine, putrescine, cadaverine and agmatine); 2) natural: spermine and spermidine are formed "*in situ*" in the cells as they are required. It is noteworthy that since histamine is stored in mast cells and basophils, it could be classified as biogenic and natural.

With respect to nomenclature, most amines are named according to their precursor amino acids. The most important biogenic amines found in food are: histamine, tyramine, tryptamine, phenylethylamine and cadaverine, the synthesis of which occurs by decarboxylation of the precursor amino acids histidine, tyrosine, tryptophan, phenylalanine and lysine, respectively. In serotonin synthesis, tryptophan is transformed by tryptophan hydroxylase into 5-hydroxytryptophan, which is enzymatically decarboxylated into 5-hydroxytryptamine or serotonin. Tyrosine is the precursor of phenolic amines such as octopamine and dopamine (GLÓRIA & VIEIRA, 2007). Cadaverine and putrescine are associated to products under decomposition or putrefaction like spermine and spermidine are associated to seminal fluids where these amines were found for the first time (HALÁSZ et al., 1994; GLÓRIA, 2005; GLÓRIA & VIEIRA, 2007; KALAČ et al., 2009).

Biogenic amines can be classified according to the number of amine groups present in the molecule, the chemical structure, the action in the body, and the biosynthetic pathway. According to the number of amine groups they are classified into (BARDÓCZ, 1995; SILLA-SANTOS, 1996; VIDAL-CAROU et al., 2009):

1) monoamines (tyramine, phenylethylamine); diamines (histamine, serotonin, tryptamine, putrescine, cadaverine);

2) polyamines (spermine, spermidine and agmatine).

Regarding their chemical structure, they are classified into three major groups: (SILLA-SANTOS, 1996; SAAID et al., 2009):

1) aliphatic (putrescine, cadaverine, spermine and spermidine);

2) aromatic (tyramine and phenylethylamine); 3) heterocyclic (histamine and tryptamine).

Regarding the action in the body, they are classified into (EEROLA & MAIJALA 2004; CÖISSON et al., 2004; KALAČ et al., 2009):

1) vasoactive (tyramine, tryptamine, phenylethylamine, isoamylamine, histamine and serotonin); 2) psychoactive (norepinephrine, serotonin and dopamine).

Regardless their classification, there is a natural mechanism for bioactive amine catabolism. They are oxidized by monoamine oxidases (MAO) and diamine oxidases (DAO). On the other hand, polyamines are oxidized by DAO and polyamine oxidases (PAO) after acetylation reaction (BARDÓCZ, 1995). Thus, these amines do not represent a health hazard unless they are consumed in large quantities or if the natural catabolism mechanism of one or more amines is inhibited (HALÁSZ et al., 1994). In this case, poisoning occurs and thus it is important to determine amine profile and content in food since these substances can trigger toxicological processes (ÖNAL, 2007; SAAID et al., 2009).

Physiological and toxicological aspects

Many amines such as histamine, serotonin, dopamine and tyramine play important roles in maintaining the physiology of living organisms (SHALABY, 1996; KALAČ & KRAUSOVÁ 2005). Besides, although the bioactive amines spermidine and spermine do not have direct toxicological effects they can potentiate tyramine and histamine toxicity by competing with detoxifying enzymes (MORET et al., 2005).

The bioactive amines present in food represent a health hazard when consumed in large amount if the natural mechanism for their catabolism is impaired by disease or pharmacological agents or if the individual is genetically deficient (DONHAUSER et al., 1993; OHTA et al. 1993; KALAČ et al., 2009). Under normal conditions, amines ingested through foods are quickly metabolized by conjugation or by oxidation reactions by amine oxidase enzymes such as monoamine oxidase (MAO), diamine oxidase (DAO), polyamine oxidases (PAO) and N-methyl transferase (RICE & KOEHLER, 1976; BJELDANES et al., 1978; BARDÓCZ, 1995; HERNÁNDEZ-JOVER et al., 1997; BENEDETTI, 2001;

ORTOLANI & PASTORELLO, 2006). Individuals with respiratory and coronary heart diseases, hypertension problems or vitamin B12 deficiency are at risk because they are sensitive to smaller amounts of amines (SHALABY, 1996; SILLA-SANTOS, 1996; DADÁKOVÁ et al., 2009). People with gastrointestinal problems (gastritis, irritable bowel syndrome, Crohn's disease, gastric and colon ulcers) are also at risk since the activity of the oxidases in their bowels is generally lower than in healthy individuals. People using drugs MAO, DAO and PAO activity inhibitors may also be affected (ORTOLANI & PASTORELLO, 2006) because they prevent amine catabolism. These MAO and DAO inhibitors are used to treat stress, depression, Alzheimer and Parkinson, pulmonary tuberculosis, malaria, panic disorder and social phobia. Histamine is the amine most often related to food poisoning (LEHANE & OLLEY, 2000; CHEN et al., 2010; ÖNAL, 2007).

Histamine poisoning

Histamine poisoning causes an allergic reaction characterized by difficult breathing, vomiting, rash, itching, fever and hypertension Histamine alone at low levels does not cause poisoning but the presence of other biogenic amines such as putrescine and cadaverine, in concentration five times greater than histamine, enhance its toxicity (STRATTON at al., 1991; HERNÁNDEZ-JOVER et al., 1997; EMBORG & DALGAARD, 2008). The amines putrescine and cadaverine can potentiate histamine toxic effect, inhibiting DAO enzymes, increasing its transport through the gastrointestinal wall (TAYLOR, 1990; SALAZAR, 2002; RAPAPORT, 2007). The presence of these potentiating substances can explain why, in some cases, spoiled fish and aged cheeses are more toxic than the same amount of histamine ingested alone (GLÓRIA, 2005).

Usually bioactive amines are absent or in minimum concentration, below 10µg/g, in fresh foods. However, in fish, fish products, cheese, meat, egg and fermented food they can be

present in significant amounts, above 50µg/g, capable of inducing a chemical intoxication (SHALABY, 1996; FLICK & GRANATA, 2005; BRINKER et al., 2002). The symptoms can appear within minutes or up to an hour after ingestion and they include strange taste, headache, dizziness, nausea, facial swelling and flushing, abdominal pain, rapid, weak pulse besides diarrhea. It is worthy to note that once histamine is formed, it is not destroyed by cooking (CAMPBELL- PLATT & MAY, 2009). The concentration of histamine capable of producing poisoning varies in accordance with the susceptibility of each individual. In susceptible individuals, values between 5 and 10mg/100g will cause symptoms (LIMA & GLÓRIA, 1999). Histamine exerts its effects by binding to cell membrane receptors of skin and respiratory, cardiovascular, gastrointestinal and immunologic systems (SHALABY, 1996). Clinical signs are more severe in people taking drugs that inhibit histamine detoxifying enzymes in the intestine, in immunosuppressed individuals and individuals that use drugs and/or alcohol and the symptoms of histamine poisoning usually appear shortly after the ingestion of food, lasting approximately 24 hours (FOOD AND DRUG ADMINISTRATION - FDA, 1996).

Physiological and toxicological effects of other amines

High levels of tyramine can lead to poisoning known as cheese reaction caused by the concomitant consumption of food containing this amine (BRINK et al., 1990). Its intake is associated to headache, decreased movement of the gastrointestinal tract and action on the hunger center promoting hunger satiety sensation (FOOD AND AGRICULTURE ORGANIZATION - FAO, 1994).

The amines tryptamine, tyramine and phenylethylamine can cause headache, migraine, increased blood pressure due to constriction of the vascular system and increased heart rate (GLÓRIA, 2005). Theses amines are potent vasoconstrictors of both arteries and veins, with

tyramine and tryptamine more potent in veins that in arteries, i.e. they are selective vasoconstrictors (ELLIOT et al., 2003). Putrescine and cadaverine cause hypotension and bradycardia, besides potentiating the toxicity of other amines (SHALABY, 1996). These amines together with tyramine, can potentiate the action of histamine, because they inhibit DAO and N-HMT (N-methyl transferase) increasing its intestinal absorption (TAYLOR, 1990; LEHANE & OLLEY, 2000).

Putrescine, cadaverine, tyramine, tryptamine, phenylethylamine, spermine and spermidine are present in fish, aged cheese, red wine and meat products (SHALALBY, 2000). Tkachenko at al. (2000) highlight the importance of putrescine as cell protector against oxidative stress and modulator in gene expression.

Cadaverine is a volatile amine, associated to spoiled food. According to Goldberg et al. (1994), cadaverine is also associated to halitosis in patients with periodontal disease, and is found in tongue and saliva samples.

Polyamines as spermidine and spermine are essential components of living cells, universally occurring in animals and plants and present in most bacteria. These amines are important in regulating nucleic acid function and protein synthesis, as well as in membrane stabilization (GLÓRIA, 2005). These two amines are part of muscles in normal physiological conditions, where they act as hormone or growth factor, essential for cells (SILVA & GLÓRIA, 2002; VASCONCELOS-NETO, 2003; GLÓRIA, 2005). According to Bardócz (1995), all cells require such amines for growth, regeneration and metabolism. In addition, spermine, spermidine and putrescine, can accelerate the development of tumors, because they are found in tissues with high growing rate, and thus, their ingestion is forbidden to patients under treatment for cancer (LIMA &GLÓRIA, 1999).

Serotonin, formed from the amino acid tryptophan is synthesized in the central nervous system and enteric cells. It acts as neurotransmitter in the central nervous system and

it is an endogenous vasoconstrictor involved in the regulation of several physiological functions such as sleep, thirst, hunger, mood and sexual activity, acting on the regulatory system of emotions (COUTTS et al., 1986; LENZ, 2000).

Agmatine derives from the amino acid arginine and acts as antidepressant (BUDNI et al., 2007). This amine is present in the brain and is widely distributed in tissues and particularly in the stomach and blood, but also in the spinal cord, suggesting that it could be an endogenous modulator of pain regulation, i.e. a neurotransmitter/ neuromodulator of the central nervous system (RAASCH et al., 1995; FENG et al., 1997; BUDNI et al., 2007).

Toxic levels

The toxic levels of bioactive amines in humans are still uncertain and the toxic dose depends on the efficiency of individual metabolism. The toxic dose of histamine is 10mg/100g of food; however susceptible individuals, asthma and ulcer patients are more susceptible to the toxic effects of this amine. The toxic dose of tyramine is 10 mg/100g in normal individuals and 6 mg/100g in those under treatment with monoamine oxidase inhibitors. The toxic dose of phenylethylamine is 3 mg/100g (HALÁSZ et al., 1994).

Acute and sub-acute toxicity levels were investigated in rats, the approximate values of lethal dose (LD50) for tyramine and cadaverine are higher than 2000 mg/kg of body weight, and thus their acute toxicity is low. The approximate LD50 for putrescine is 2000 mg/kg, while spermine and spermidine present higher acute toxicity with lethal dose of 600 mg/kg of body weight. Doses that do not cause adverse effects are 2000 ppm (180 mg/kg/day) for tyramine, cadaverine and putrescine; 1000 ppm (83 mg/kg/day) for spermidine and 200 ppm (19mg/kg/day) for spermine (TIL et al. 1997; GLÓRIA, 2005).

In Brazil, the Regulation of Industrial and Sanitary Inspection of Animal Products - RIISPOA (BRAZIL, 2008), does not mention the amine maximum level allowed in products

of animal origin. However, MERCOSUR Resolution (1994) and the Technical Regulation on the Identity and Quality of Fresh Fish (whole and gutted) (BRAZIL, 1997) establish a maximum level of 100 ppm (10mg/100g) of histamine in the muscles of species of the Scombridae, Scomberesocidae, Clupeidae, Coryphaenidae and Pomatomidae families.

Control of histamine levels was not previously included in the technical norms. The maximum acceptable histamine levels in fish were also established in other countries. In the European Union the regulatory limit is 100 mg/kg (INTERNATIONAL COMMISSION REGULATION, 2005) and in USA, the Food and Drug Administration - FDA (1996), established a maximum limit of 5 mg histamine/100 g product (50 ppm) at the port and 10 mg histamine/100 g product (100 ppm) in pickled fish for species susceptible to form histamine. There are no established standards for cadaverine, putrescine or other biogenic amines with the exception of histamine.

Bioactive amines as quality index and control methods used in the food industry

Amine formation in foods rich in protein and amino acids may be inherent to the product or due to the action of added microorganisms (starter cultures) and/or contaminants (inadequate sanitary conditions). Thus, amine quantification may be used as quality parameter or index, because it can reflect poor quality of the raw materials and/or sanitary conditions during the manufacture of certain products (HALÁSZ et al., 1994; KALAČ et al., 2002; GLÓRIA, 2005; NYCHAS et al., 2008; TAO et al., 2011; CUNHA et al., 2012; ANDRADE et al., 2012).

The analyses used to determine food quality include sensory and microbiological methods. The first are fast, well accepted but depend on trained panelists and do not detect specific food contamination. On the other hand, microbiological analyses are usually slow, requiring 2-5 days to obtain results. For this reason, alternative methods involving chemical

changes caused by microbial activity are being studied (SOUZA et al., 2005; OZOGUL &OZOGUL, 2007; NTZIMANI et al., 2008; OLIVEIRA et al., 2012).

The presence of chemical metabolites, the biogenic amines, produced by the microbiological spoilage of food is suggested as quality indicator of food in addition to routine analyses (DAINTY, 1996; ROKKA et al., 2004; OLIVEIRA et al., 2012). High content of histamine and other amines can be used as quality indicators and also as indicators of potential food poisoning (BRINK et al., 1990; HALASZ et al., 1994; EEROLA & MAIJALA, 2004). This is due to the fact that microbial deterioration can be accompanied by an increase of decarboxylase production (HALÁSZ et al., 1994; COUTATE et al.,2004). It should be highlighted that these substances are reported as heat stable compounds (TAPINGKAE et al., 2010) and, cooking or prolonged exposure to heat do not eliminate the toxin (SHALABY, 1996; DUFLOS, 2009; GONZAGA et al., 2009). Thus, an advantage in using biogenic amines as quality index is that they are thermo resistant and thus, they remain in the food even after heat treatment (LIMA, GLÓRIA, 1999).

Chemical quality indices, through the relation between the bioactive amines histamine, putrescine, cadaverine, spermine and spermidine, were characterized and suggested by some authors for fish and poultry meat (MIETZ & KARMAS, 1977; YAMANAKA , 1990; SILVA & GLÓRIA, 2002; KIM et al., 2009; LÁZARO DE LA TORRE et al., 2012).

Prevention of the formation of bioactive amines in food has been mainly performed through production and storage temperature control, quality of the raw material, good manufacturing practices, use of starter cultures for fermentation, use of enzymes to oxidize amines, use of microbial modeling to assess favorable conditions to delay amine formation, use of adequate packaging techniques and food irradiation (NEUMEYER et al., 1997; DAPKEVICIUS et al., 2000; KIM et al., 2003; EMBORG & DALGARD 2008; MOHAN et al. 2009; NIETO-ARRIBAS et al. 2009).

CONCLUSIONS

Bioactive amines are chemical components of a variety of foods which call attention for different reasons since, as well as they cause positive biological effects, being essential for maintaining the physiological activities, they can, in certain concentrations have adverse effects, mainly toxicological. Amine identification and quantification is an important indicator of the sanitary condition of both, the raw material and the final product. Its control is important to reduce damages to the production chain and improves commercial relations with other countries reducing export barriers. Thus, further studies are necessary to establish safety limits for bioactive amines in animal products, vegetables and beverages.

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3.2 EFFECT OF CARBON DIOXIDE ON THE SHELF LIFE OF READY-TO-EAT SHREDDED COOKED CHICKEN BREAST FILLET STORED UNDER REFRIGERATION. Enviado para Poultry Science em 18 de janeiro de 2013.

O comprovante de envio se encontra no apêndice 6.2.

EFFECT OF CO_2 ON SHREDDED COOKED CHICKEN

Effect of carbon dioxide on the shelf life of ready-to-eat shredded cooked chicken breast fillet stored under refrigeration

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Scientific section of the paper: Processing, Products and Food Safety.

ABSTRACT

The objective of the present study was to determine the shelf life of ready-to-eat shredded cooked chicken breast fillet stored in atmosphere modified with different concentrations of carbon dioxide (CO_2), and establish a relationship between the concentration of this gas and bacterial growth. The samples were divided into seven groups with different packaging conditions: aerobiosis, vacuum and 10, 30, 50, 70 and 90% CO_2 completed with N_2. All samples were stored at 4±2°C for 28 days. During this period the following tests were performed: pH, Aerobic Heterotrophic Mesophyll Bacteria (AHMB) count, Aerobic Heterotrophic Psychotropic Bacteria (AHPB) count, *Enterobacteriaeae* count, Lactic Acid Bacteria (LAB) count and the gas composition of packaging atmospheres was verified. The

pH of the aerobic packages increased during storage, but the other treatments presented an opposite trend, where the pH values decreased during storage. CO_2 concentration decreased over the first 24 hours, but after this period, the values remained constant. A gradual increase of AHMB, AHPB, *Enterobacteriaceae* and LAB was observed during storage which was faster in the package under aerobiosis than in the other treatments. Treatments with CO_2 concentration above 10% showed smaller *Enterobacteriaceae* growth, while LAB growth was discrete in all the treated samples, independent of CO_2 concentration. The shelf life of the samples packed with 90% CO_2 (28 days), determined based on AHMB count was three times higher than that of the samples packed under aerobiosis (9 days). The increase in CO_2 concentration present in the package caused a reduction of the growth rate of the studied bacteria ($r = 0.9973$), and treatment with 90% CO_2 seems promising to increase the product's shelf life.

Keywords: chicken meat, preservation method, modified atmosphere packaging, carbon dioxide, shelf life.

INTRODUCTION

The search for high quality, fresh, ready-to-eat products containing only natural ingredients, has steadily grown due to the new lifestyles of the consumers (Katz, 1999; Conte Junior et al., 2010). Shredded cooked chicken breast fillet is a very practical product because it can be eaten as it is or used to cook new dishes. However there is risk of contamination during processing, because meat comminution, increases the exposed surface area facilitating contamination and consequent deterioration (Cortez-Vega et al., 2012; Huda et al., 2012). It should be noted that chicken meat is highly perishable, susceptible to changes, and may result inadequate for use and a risk to consumer health, due to physical and chemical alterations and bacterial growth (Tsola et al., 2008). The storage temperature, type of packing and the species

and number of psychotropic bacteria are the main factors affecting poultry meat deterioration (Tuncer and Sireli, 2008).

New preservation methods are being studied to reduce the growth of the microorganisms that appear before or during process (Conte Junior et al., 2010; Fraqueza and Barreto, 2011; Novaes et al., 2012; Ahn et al., 2013). The effect of atmospheric oxygen and growth of aerobic microorganisms are important factors that influence the shelf-life of perishable foods kept in aerobiosis (Parry, 1993; Sarantópoulos et al., 1998; Mano et al., 2002). Thus, fresh food preservation, particularly meat in modified atmosphere packed (MAP) greatly improved during the last twenty years (Mano et al., 2000; Lopes et al., 2004; Mantilla et al., 2009). MAP consists in substituting the atmosphere surrounding the product by another atmosphere that can be a gas or a mixture of gases, specially prepared for each type of food, allowing a better control of chemical, physical and microbiological reactions, avoiding or minimizing the main deteriorations that occur during the storage period. (Parry, 1993; Ordóñez, 1996; Monteiro et al., 2012).

Modified atmosphere packaging is very important for food because it may extend product shelf life and particularly, the use of carbon dioxide should be enhanced because it has some bacteriostatic or bactericidal effects for some microorganisms (Mano et al., 2000; Jeremiah and Gibson, 2001; Malavota et al., 2006). Although MAP use has increased during the last years, optimizing gas mixture composition and concentration for each product is still a challenge to ensure food quality and safety (Narasimha and Sachindra, 2002; Novaes et al., 2012). Thus, the purpose of the present study was to determine the shelf life of shredded cooked chicken breast fillet stored under refrigeration and packed in modified atmosphere with different CO_2 concentrations, and to evaluate the influence of the concentration of this gas on the behavior of mesophyll and psychrotrophic bacteria, enterobacteria and lactic bacteria.

MATERIALS AND METHODS

Samples

6.3 Kg of chicken breast fillet (*Pectoralis major*) were cooked at 100ºC and 2Kgf/cm² pressure for 20 minutes. Then, the fillets were automatically shredded using a stainless steel crusher machine (Cozix®, Equipamentos e Serviços Industriais Ltda) and cooled at 4±2ºC. This stage was performed in a slaughterhouse located in the state of Rio de Janeiro and, next the shredded cooked fillets were packed with ice (1±1ºC) and sent to the laboratory, to pack them in modified atmosphere (treatments), and in aerobiosis (control) and to conduct the bacteriological tests, pH measure and determine gas composition inside the packages.

Sample Treatment

One hundred five samples of approximately 60g of sheered cooked chicken fillet were packed in multilayer high barrier plastic bags (´Cryovac` BB4L) filled with approximately 1L of the following atmospheres: T1 (aerobiosis packaging - control), T2 (vacuum packaging), T3, T4, T5, T6 and T7 (packaging in modified atmosphere with 10, 30, 50, 70, and 90% CO_2 and ther volume completed with N_2). The samples packed in aerobiosis were placed in expanded polystyrene trays, wrapped with polyvinyl chloride film. All samples were stored at 4±2ºC for 28 days. The intervals for pH and bacteriological tests and evaluation of the gas composition inside the packages were established based on the evolution of the results observed for each parameter (Monteiro et al., 2012).

Bacteriological Analysis

The method established by the American Public Health Association (APHA 2001) was followed for Aerobic Heterotrophic Mesophyll Bacteria (AHMB) and Aerobic Heterotrophic Psychrotrophic Bacteria (AHPB), using plate count agar (PCA) for culture, incubating the inverted plates at 35±1ºC and reading after 48±2 hours, and at 7±1ºC and reading after 10

days, respectively. Lactic acid bacteria (LAB) count was performed using the plating in depth method, using De Man-Rogosa-Sharpe agar, and incubating at 30±1ºC for 120 hours (APHA, 2001). Double layer pour plate method in Crystal Violet Neutral Red Bile Glicose (VRBG) agar, incubating at 35±1ºC for 18/24 hours (APHA, 2001) was used for *Enterobacteriaceae* count.

pH Measurement

pH was measured in triplicate by the potentiometric method, using a digital pH meter (Digimed®, DM-32 Model, São Paulo, Brasil) according to technique described by the Association of Analytical Communities (AOAC, 2005), after the microbiological tests.

Determination of gas concentration inside the packages

Gas concentration inside the packages with modified atmosphere was determined by gas analysis equipment (PBI-Dansensor, Check Pointer O_2/CO_2, Ringsted, Dinamarca) and expressed as O_2% and CO_2%. The remaining gas was Nitrogen (N_2) (Esmer et al., 2011).

Statistical Analysis

The bacterial growth curves were adjusted through the DMFit 2.0 (IFR, Norwich, United Kingdom) statistical program based in predictive microbiology and idealized by Baranyi and Roberts (1994). pH results were statistically treated through second order polynomial linear regression. Pearson correlations were used to examine the relationship between CO_2 concentration and AHMB doubling time in T3, T4, T5, T6 and T7 groups. When a significant *F* was found, additional post hoc tests with Bonferroni adjustment were performed. Statistical significance was set at the 0.001 level of confidence. All analyses were performed using a commercially available statistical package (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA).

RESULTS AND DISCUSSION

Variations in gas composition during the 28-day storage are shown in figure 1. Carbon dioxide reduction during the first 24-hour storage was observed in all the assessed treatments. This behavior can be explained because CO_2 dissolves in the aqueous and fatty phases of the product resulting in package volume contraction (Jakobsen and Bertelsen, 2002; Rotabakk, 2008). However, this reduction is not further observed after the first storage hours. CO_2 concentration remains stable after the first storage day, and a significant variation of atmosphere composition is not observed in any of the treatments during the remaining storage period. This fact can be explained because of the decrease of CO_2 dissolution in the food matrix and the increase of bacterial growth with resultant CO_2 production. (Esmer et al. 2011). Similar results were described by Friedrich et al. (2008) and Degirmencioglu et al. (2012). The residual oxygen percent available inside the packages submitted to modified atmosphere decreased during storage reaching values below 1% at the end of the storage period of all the treatments with CO_2. This is related to the fact that most microorganism present in meat such as *Brochothrix thermosphacta* and LAB, that produce carbon dioxide as metabolite (Nychas, 1994), use the available residual oxygen. In addition, meat biochemical activity and plastic impermeability help to maintain O_2 concentration reduced inside the packages (Mano et al., 2002).

The pH initial value of shredded cooked chicken breast fillets was 6.3 (Figure 2). This pH is in accordance with values reported by Balamatsia et al. (2007) in fresh chicken muscle. The average pH value of the samples in aerobiosis presented an increasing trend during the storage period, indicating a decrease of sample quality. Insofar the storage period and the bacterial count increase, an intense metabolic activity occurs on the food leading to the production of alkaline products that increase the pH of the food matrix (Fang and Lin, 1994). In the present study, pH accompanied the growth of AHMB.

The average pH values obtained for the other groups T2-T7 decreased during storage, this decrease being more evident between the 12th and the 16th days. Several authors observed decrease of this parameter as a consequence of CO_2 solubility in the food matrix (McMullen and Stiles, 1991; Leygonie at al., 2011). Other authors relate pH decrease with an increase of the number of lactic acid bacteria (Gill, 1996; Gómez and Lorenzo, 2012). Bacteria belonging to the LAB group were the dominant AHMB population. As the LAB count increases in the T2-T7 groups, pH decreased, presenting values between 5.8 and 6.0.

AHMB of shredded cooked chicken breast fillet was 5.1 log cfu g^{-1} (day 0). The high initial count is related to the processing stages and the handling conditions of the product, once during meat comminution, the contact surface increases and in addition, packing is manually done, increasing the risk of contamination. AHMB reached the value of 7 log cfu g^{-1}, which is considered as the upper acceptable limit for fresh poultry meat as defined by the Commission on Microbiological Specifications for Foods (ICMSF, 1988).

Figure 3 and Table 1 show the bacterial growth under the different studied treatments.

AHMB presented 7.0 log cfu g^{-1} in T1 at the 9th storage day. Bacterial growth curve in T3 was similar, achieving the same value at the 10th storage day. Similar values were found for the vacuum packaging (T2) and the 30/70 CO_2/N_2 packaging (T4), which presented shelf life of 13 or 14 days.

The action of carbon dioxide reducing the bacterial growth rate was evident in T5, T6 and T7 samples, which presented shelf life of 21, 23 and 28 days respectively. Table 1 shows that duplication time, in hours, was inversely proportional to CO_2 concentration, T7 presented log phase of 3.6 hours while the bacterial population of samples with 10% CO_2 (T3) duplicated in 1.2 hours. This seems to be the growing stage where carbon dioxide has a bacteriostatic function. We can observe that the count at the end of T7 treatment (90%

CO_2) was 10^7 log cfu g^{-1} while at the end of T3 treatment with smaller gas concentration (10% CO_2), achieved values of 10^9 log cfu g^{-1}, thus demonstrating the effect of CO_2.

Among the treatments used in the present study, T7 was the most effective in reducing AHMB growth. The influence of the modified atmosphere packaging on AHMB growth is attributed to oxygen restriction inside the package and CO_2 bacteriostatic and bactericide activity since it causes alteration of the cell membrane functions, direct enzyme inhibition and reduction of the speed of enzymatic reactions (Daniels et al., 1985; Church, 1993; Sarantópoulos et al., 1998).

Figure 1B shows that the initial count of aerobic heterotrophic psychrotrophic bacteria (AHPM) was 5.0 log cfu g^{-1}. The bacterial growth curves obtained during the experiment were similar for all the treatments studied. However, growth was slower on treatments with CO_2 enriched atmospheres than on the control group. It should be noted the existence of strict aerobic psychrotrophic genera, that better develop in O_2 rich atmosphere (Gram and Huss, 1996). The comparison of treatments T1 and T7 in figure 1B, shows that it was more difficult for this bacterial group to develop in products packaged in CO_2 enriched atmospheres.

The initial *Enterobacteriacea* count was of the order of 10^3 log cfu g^{-1} (figure 1C). The presence of this bacterial group is traditionally associated to the hygienic and sanitary quality of foods (Del-Río et al., 2007). *Enterobacteriacea* growth presented variable behavior depending on the composition of the packaging atmosphere. This group of bacteria presented growth on treatments T1, T2 and T3 during the storage period. However, treatments T4, T5, T6 and T7, with higher concentration of CO_2, did not present development of this bacterial group while treatments T6 and T7 presented decreasing values at the end of the storage period.

LAB showed growth during storage (figure 1D), however they were not the AHMB dominant group on treatments with low CO_2 concentration. LAB performance in treatment T7 was similar to AHMB (figures 1A and 1D), indicating that LAB prevailed in this treatment. In an anaerobic environment, lactic acid-producing bacteria prevail because they are more tolerant to CO_2 than pseudomonads or *Enterobacteriaceae* (Stiles, 1991; Dainty and Mackey, 1992). According to Veimeiren et al. (2004), LAB prevail in the deterioration process of meat products stored under refrigeration and anaerobic conditions as modified atmosphere packaging.

Shelf life increase at high CO_2 concentrations is observed because the deterioration caused by LAB occurs later than the deterioration caused by aerobic bacteria such as *Pseudomonas* spp. In general, one of the alterations caused by LAB is described as "acidification" of the product, different from putrefaction caused by AHMB (Stiles, 1991; Smolander, 2004). pH decrease was also observed in the present study.

The bacteriological tests and pH results show that the use of modified atmosphere packaging extended the shelf life of the product. The bacterial groups studied presented smaller growth rate on the treatments enriched with CO_2 when compared to the remaining treatments, and the best preservation results were obtained in the packages with high CO_2 concentration. Thus, we suggest the use of 90% CO_2 atmosphere for the preservation of ready-to-eat shredded cooked chicken breast fillets because it presented the greatest self life when compared to the other studied treatments.

ACKNOWLEDGMENTS

The authors are thankful for the financial support of the State of Rio de Janeiro Carlos Chagas Filho Research Foundation (FAPERJ), process numbers E-26/111.610/2010 and E-

26/103.003/2012. M.B.R. Rodriguez was supported by the Coordination of Enhancement of People of a Superior Level (CAPES).

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Figure 1

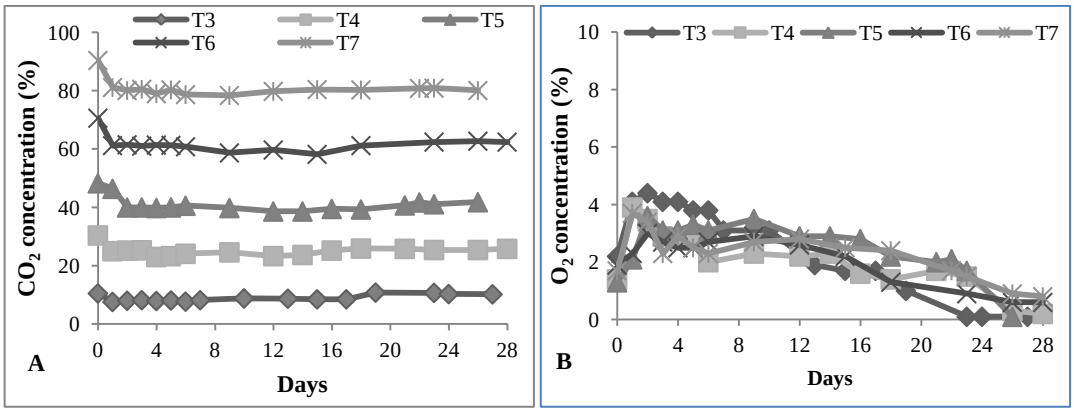

Figure 1 - CO_2 (A) and O_2 (B) composition variation of shredded cooked chicken breast fillet packages in T1 (packaging in aerobiosis - control), T2 (vacuum packaging), T3, T4, T5, T6 and T7 (MAP with 10/90, 30/70, 50/50, 70/30, 90/10 of CO_2/N_2, respectively) kept under refrigeration (4±2ºC).

Table 1 – Parameters of growth of aerobic heterotrophic mesophyll bacteria (lag phase, doubling time, count in the stationary phase and shelf life) in shredded cooked chicken breast fillet packed under different treatments at 4±2ºC during 28 days.

Samples	Lag phase (days)	Doubling time (hours)	Stationary phase (log cfu g^{-1})	Shelf life (days)
T1 (aerobiosis)	1.8	1.1	9.1	9
T2 (vacuum packaging)	1.9	1.8	8.8	13
T3 (10%CO_2/90%N_2)	1.8	1.2	7.4	10
T4 (30%CO_2/70%N_2)	2.5	1.8	7.7	14
T5 (50%CO_2/50%N_2)	0.5	2.5	7.6	21
T6 (70%CO_2/30%N_2)	2.1	2.9	7.2	23
T7 (90%CO_2/10%N_2)	2.6	3.6	7.0	28

Figure 2

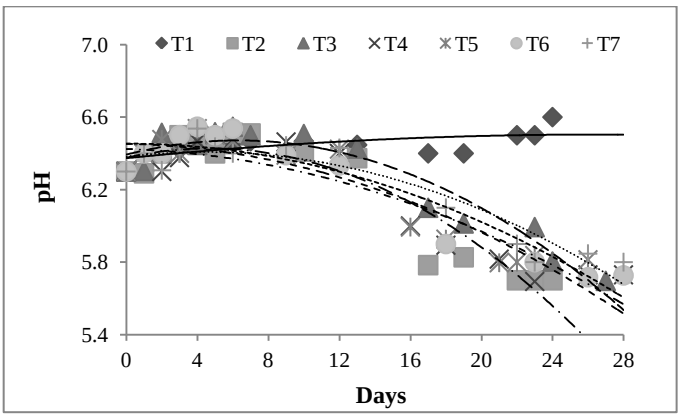

Figure 2 – pH values of shredded cooked chicken breast fillet in T1 (packaging in aerobiosis - control), T2 (vacuum packaging), T3, T4, T5, T6 and T7 (MAP with 10/90, 30/70, 50/50, 70/30, 90/10 of CO_2/N_2, respectively) kept under refrigeration (4±2ºC).

Figure 3

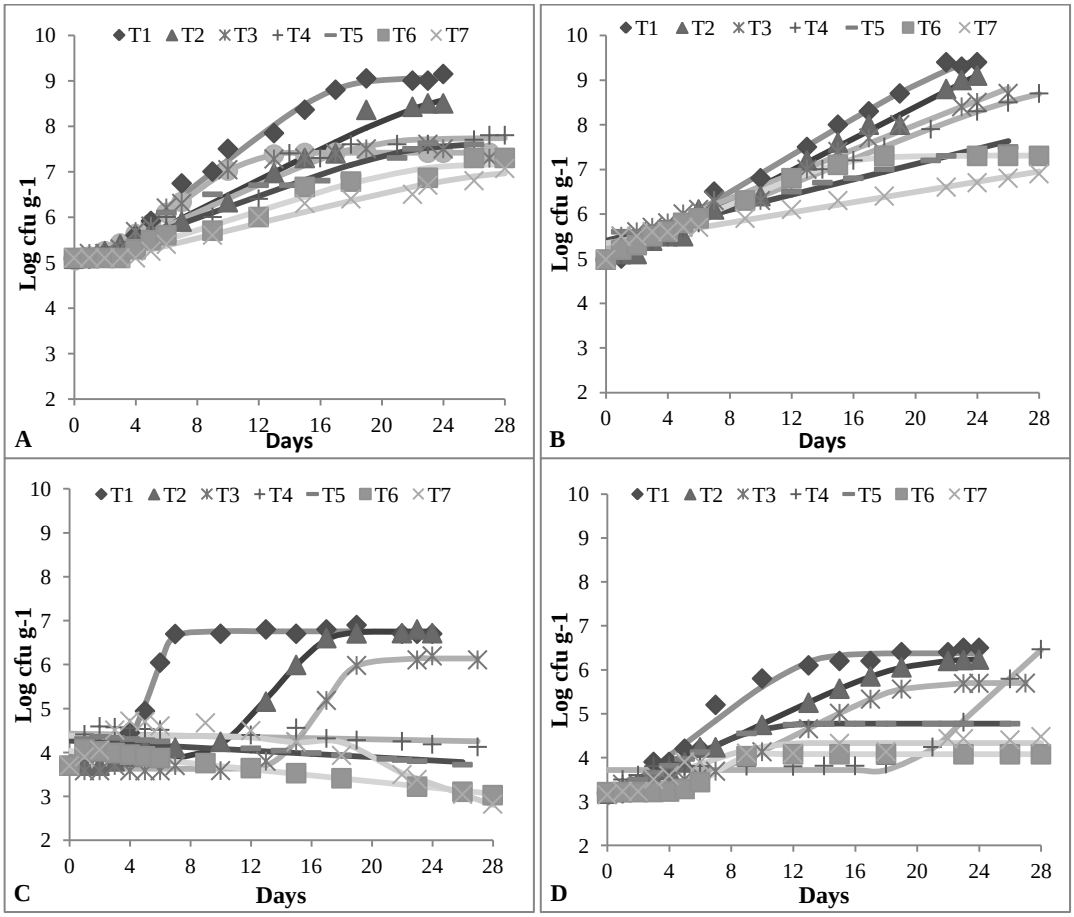

Figure 3- Average values of AHMB (A), AHPB (B), *Enterobacteriaceae* (C), and LAB (D) count in shredded cooked chicken breast fillet in T1 (packaging in aerobiosis - control), T2 (vacuum packaging), T3, T4, T5, T6 and T7 (MAP with 10/90, 30/70, 50/50, 70/30, 90/10 of CO_2/N_2, respectively) kept under refrigeration (4±2ºC).

3.3 BIOGENIC AMINES AS QUALITY INDEX IN SHREDDED COOKED CHICKEN BREAST FILLETS STORED UNDER MODIFIED ATMOSPHERE. Enviado para Food Control em 19 de janeiro de 2013.

O comprovante de envio se encontra no apêndice 6.3

Biogenic amines as quality index in shredded cooked chicken breast fillets stored under modified atmosphere

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Abstract

The aim of the present study was to assess the effect of carbon dioxide on the formation of biogenic amines (BAs) in shredded cooked chicken breast fillets packed in modified atmosphere, relating biogenic amine formation with total viable count and the possible role of BAs as indicators of shredded cooked chicken breast fillet spoilage. 6.3 kg of sample were used in the experiment separated in groups according to the treatment used: T1 (packed in atmospheric air), T2 (vacuum packaging), T3, T4, T5, T6 and T7 (packaging in modified atmosphere with 10, 30, 50, 70 and 90% CO_2, respectively, and their total volume completed with nitrogen). Total viable count was determined and biogenic amines (tyramine, spermidine, putrescine and cadaverine) were identified and quantified. The results showed a gradual reduction in the growth rate of the bacteria under study as the concentration of CO_2 increased. The levels of putrescine and cadaverine gradually increased during storage reaching values of 58.5 and 27.3 mg kg^{-1} in the packaging with atmospheric air and 41.99 and 26.32 mg kg^{-1} in the vacuum packaging on the 23rd day. In the remaining treatments, increasing CO_2 concentration caused a slower production of those amines. We concluded that changes in the concentration of the studied amines in shredded cooked chicken breast fillets were related to the growth of the studied bacteria. In addition, increasing the concentration of

carbon dioxide causes a reduction of the growth of putrescine and cadaverine producing bacteria. Amine quantification proved to be an adequate parameter to evaluate the quality index of shredded cooked chicken breast fillet.

Keywords: preservation method, modified atmosphere, bioactive amines, liquid chromatography, meat products, quality.

1.Introduction

Nowadays, people are increasingly aware of the importance of diet for health, and hence, any issue relating to food safety has a considerable impact on consumer behavior and state policies. At the same time, consumers increasingly prefer high-quality products that are safe and minimally processed, with less additives and ingredients, with a long shelf-life and ready-to-eat. The meat industry is, therefore, looking for emerging processing and storing technologies to reach this goal (Conte-Junior, Souza, Baptista, Mársico, & Mano, 2010; Jang, & Lee, 2011; Kilinc, Cakli, & Tolasa, 2008; Lee, Park, Kim, Oh, Lee, Kim, & Byun, 2005).

In the last decade, chicken-based meat products have become increasingly popular worldwide due to their nutritional quality and low cost and are available as either or precooked chicken, which after subsequent packaging are usually stored under refrigeration (Barbut, 2002). But chicken meat is highly perishable depending on conditions such as the initial load of microorganisms of the poultry carcasses, supply and production chains. It usually deteriorates within 1 week after slaughtering, in spite of having been stored under refrigeration, depending on the initial microbiology (Phillips, 1996; Russell, Fletcher, & Cox, 1996). The microorganisms present in the packages significantly influence sensory properties of the packaged meat, such as its color, smell and shelf life (Balamatsia, Paleologos, Kontominas, & Savvaidis, 2006).

Protective atmospheres are preservation systems that are becoming increasingly significant (Esmer, Irkin, Degirmencioglu, & Degirmencioglu, 2011). Most of the gases used for food packaging exhibit various degrees of bacteriostatic or bactericidal effects (Jeremiah, & Gibson, 2001, Mano, Ordoñez, & Garcia de Fernando, 2000).

Chicken meat quality evaluation includes sensory analysis, bacterial count and physico-chemical assessment. The first method is fast, well accepted but depends on panelist training, and perception of spoilage is to some extent subjective. On the other hand, traditional bacterial count and the techniques that identify the growth of specific microorganism are slow, taking from two to five days to obtain the results. For this reason, a series of chemical

indicators has been suggested to assess meat quality, including the determination and quantification of biogenic amines (Balamatsia et al., 2006; Botta, 1995; Dainty, 1996).

In some cases biogenic amines can be produced by certain decarboxylase-producer microorganisms acting on free amino acids present in food forming low molecular weight organic bases. Thus, the products with greater possibility of containing these compounds are those with high protein content and presence of microorganisms with decarboxylase activity on amino acids (Halász et al., 1994; Min, Lee, Jang, Lee, & Kim, 2004).

Usually, amines are not health hazardous, unless ingested in large amounts or if the natural mechanism for the catabolism of one or more amines is inhibited (Halász et al., 1994), in which case poisoning occurs. Therefore, it is important to determine amine profile and content in food since these substances can trigger toxic processes In addition to precursor (amino acids) availability, amine formation depends on food intrinsic and extrinsic parameters such as: temperature, pH, aerobiosis, anaerobiosis, and availability of a carbon source (Bover-cid, Miguélez-Arrizado, Becker, Holzapfel, & Vidal-Carou, 2008; Bunková, Bunka, Klcovská, Mrkvicka, Dolezalová, & Krácmar, 2010; Cunha, Conte-Junior, Lázaro de La Torre, Santos, Másrico, & Mano, 2012; Greif, Greifová, & Karovocová, 2006).

The present study aims at assessing biogenic amine formation in shredded cooked chicken breast fillets, packaged in modified atmosphere, using vacuum and carbon dioxide (CO_2), correlating the formation of these chemical compounds with bacteria count and verifying if biogenic amine quantification can be used to indicate the quality index of samples.

2. Materials and methods

2.1 Samples

The shredded cooked chicken breast fillets were obtained from a local poultry processing plant. 6.3 Kg of chicken breast fillets (*Pectoralis major*) were cooked at 100ºC and 2Kgf/cm² pressure for 20 minutes Then, the fillets were automatically shredded using a stainless steel crusher machine (Cozix®, Equipamentos e Serviços Industriais Ltda) and cooled at 4±2ºC.. Samples were transported to the laboratory in insulated polystyrene boxes on ice.

One hundred five samples of approximately 60g of shredded cooked chicken fillet were packaged in multilayer high barrier plastic bags (´Cryovac` BB4L) filled using a PBI-Dansensor model mix 9000 gas mixer (Ringsted, Denmark) connected to a BOSS model N48 vacuum sealer (Boss GmbH, Germany) with approximately 1L of the following atmospheres: T1 (aerobiosis packaging - control), T2 (vacuum packaging), T3, T4, T5, T6 and T7

(packaging in modified atmosphere- MAP with 10, 30, 50, 70, and 90% CO_2 respectively and their volume completed with N_2). The samples packed in aerobiosis were placed in expanded polystyrene trays, wrapped with polyvinyl chloride film.

Then, the samples were kept under refrigeration at 4±2ºC for 28 days until microbiological and chemical analyses. The analytical procedures were performed in duplicate and without previously determining the time interval, i.e. the analysis frequency was established based on the evolution of the results of each procedure (Monteiro, Mársico, Teixeira, Mano, Conte Junior, & Vital, 2012).

2.2 Total viable count (TVC)

A sample (25g) was taken aseptically from the package, transferred aseptically to a stomacher bag (Seward Medical, London, UK), containing 225 ml of sterile 0.1% peptone water, and homogenized using a stomacher (Lab Blender 400; Seard Medical) for 60s at room temperature. For microbial enumeration, 0.1 ml samples of serial decimal dilutions of poultry homogenates were spread on the surface of the Plate Count Agar media (PCA, Merck code 1.05463, GmbH, Germany), and incubated at 35±1ºC for 48±2 h (APHA, 2001).

2.3 Determination of bioactive amines

Amine extraction was performed according to High Performance Liquid Chromatography (HPLC) technique modified by Cunha et al. (2012), 5 g of sample were weighted and added of 5% 1:1 (v:p) perchloric acid solution ($HClO_4$), and then homogenized inn Vortex for two minutes. The solution was kept under refrigeration (4±2ºC) for one hour, periodically stirring every 10 minutes. Next, it was centrifuged at 3.000 rpm for ten minutes at 4±1ºC (Rodríguez, López, & Chaves, 2001). The supernatant was subjected to filtration (Whatman nº 1), followed by addition of 2M sodium hydroxide until reaching pH>6. Then the solution was kept in ice bath for 20 minutes and filtered again in the same previous conditions and the 2M sodium hydroxide was added until reaching pH>12.

The second step consisted in solution derivatization by addition of 40µL of benzoyl chloride and homogenization in vortex for 15 seconds. This solution was left to stand at room temperature for 20 minutes (Mei, 1994). Then, 1 mL diethyl ether was added twice; the supernatant was removed with the aid of an automatic pipette and evaporated under a stream of nitrogen. Then, the residue was resuspended with 500 µL of mobile phase.

The resuspension was transferred to vials and placed in the compartment of the Shimadzu® LC/10 AS model high performance liquid chromatograph, coupled to a UV

SPD/10 AV detector, programmed at 198 nm, with C-R6A Chromatopack integrator . A Teknokroma, TR-016057 N26243 TracerExtrasil ODS2 (15 x 0,46 cm, id. 5µm) column and a Supelco, Ascentis C18 (2 x 0,40 cm, id. 5µm) pre-column were used.

Then 20µL of resuspension were automatically injected and the run time for each sample was 15 minutes. The chromatographic conditions used were 1 mL min^{-1} flow and isocratic 42:58 (v:v) acetonitrile:water mobile phase, prepared with ultra-pure water obtained by UV Milli-Q Simplicity System (Millipore).

The external standards for biogenic amine quantification were prepared dissolving serial concentrations of cadaverine, putrescine, spermidine and tyramine (Sigma Aldrich) in 0.1N hydrochloric acid (HCl) stock solution followed by derivatization and analysis similar to the samples.

3 Statistical analysis

Bacterial growth curves were adjusted through the DMFit 2.0 (IFR, Norwich, United Kingdom) statistical program. Pearson correlations were used to examine the relationship between microbiological count and biogenic amines in each treatment. Statistical significance was set at the 0.05 level of confidence for T1 and T2, and 0.1 for the other treatments. This analysis was performed using a commercially available statistical package (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA).

4 Results

Figure 1 and 2 shows the results of the quantitative analyses of the amines tyramine, putrescine, cadaverine and spermidine in shredded cooked chicken breast fillets. The levels of tyramine and spermidine remained low during the storage period for all treatments. Putrescine and cadaverine concentrations increased in all the treatments, with the highest values in the samples with low CO_2 concentration (T1, T2, T3, T4 and T5), with putrescine as the main BA formed.

Spermidine was the amine with initial (day 0) highest concentration in shredded cooked chicken fillet stored in aerobiosis, vacuum and MAP at 4±2ºC (12.05 mg kg^{-1}). The level of this amine decreased steadily with storage, but the values were similar for all the packaging conditions.

The concentration of tyramine at the beginning of the experiment was 3.2 mg kg^{-1} (day 0). The levels of tyramine remained low (<6.0 mg kg^{-1}) for all the treatments throughout the entire storage period at 4±2ºC.

The levels of putrescine in aerobiosis, vacuum and MAP stored shredded cooked chicken fillet samples progressively increased form initial basal values of 0.2 mg kg^{-1} to values 58.52 mg kg^{-1} in the aerobiosis packaging and 41.99 mg kg^{-1} in the vacuum packaging on the 23rd storage day. In the treatments enriched with CO_2 the production of these amines was slower as the concentration of CO_2 increased. The concentrations of putrescine were 32.26 (T3), 23.20 (T4), 19.69 (T5), 10.16 (T6) and 2.15 mg kg^{-1} (T7) on the 23rd storage day. Similar results were obtained for cadaverine. Cadaverine was not initially detected in the samples, however during the storage period its concentration increased reaching values of 27.3 mg kg^{-1} in aerobiosis and 26.32 mg kg^{-1} in vacuum packaging on the 23rd storage day. In the same period, cadaverine concentration values found for CO_2 treatments were 8.3 (T3), 3.23 (T4), 2.7 (T5), 0.7 (T6) and 0.5 mg kg^{-1} (T7). These data show the effect of carbon dioxide on cadaverine production.

Significant linear correlation was observed between TVC and each biogenic amine quantified in the aerobiosis packaging (T1) assessed during storage (P=0.05). In the other treatments, vacuum packaging (P=0.05) and packaging in modified atmosphere (P=0.1), direct correlation with bacterial growth was observed only for putrescine and cadaverine.

5 Discussion

The present results on the microflora of shredded cooked chicken fillet stored in air, vacuum and MAP are similar to those reported for poultry meat (Cortez-Vega, Pizato, & Prentice, 2012; Esmer et al., 2011). In this study, putrescine, cadaverine and to a lesser degree tyramine, were the BAs that progressively increased in different types of packaging.

At the beginning, the samples contained higher concentration of spermidine; Moreira, Giombelli, Labanca, Nelson and Glória (2008) also found high levels of this biogenic amine in broiler breast immediately after slaughter. This can be explained because this amine occurs naturally in the organism (Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997). The concentration of spermidine decreases over the storage period because this BA in taken from the food matrix as nitrogen source by the microorganisms present there. Gallas, Standarová, Steinhauserová, Steinhauser and Vorlová (2010) suggests that the development of microflora with decarboxylase activity begins only after some days of storage, when other biogenic amines began to be produced by microbial action.

The levels of tyramine in different samples stored aerobically, vacuum and MAP were low. Balamatsia, Paleologos, Kontominas and Savvaidis (2006) also found low tyramine levels (tenths of milligram) in chicken meat at the beginning of the experiment and its

concentration increased during the storage period to levels of 4 mg. kg^{-1} (aerobiosis) and 8.9 mg kg^{-1} (30% CO_2 and 70% N_2) at the end of a 17-day storage period, once this amine is produced mainly by coliform and lactic acid bacteria (Min et al., 2004).

In relation to putrescine and cadaverine increasing concentrations, similar results were reported by Rokka, Eerola, Smolander, Alakomi and Ahvenainen (2004). When these authors studied biogenic amine formation in broiler packaged in modified atmosphere (80% CO_2 and 20% N_2) stored for 12 days, they observed that the increase of putrescine concentration was very slow when the samples were stored at low temperatures (3.4ºC), detecting 2.2 mg kg^{-1} putrescine at the end of the experiment. Those results are in agreement with those obtained in the present study, where putrescine concentration in shredded cooked chicken breast fillets packaged in 70% CO_2 and 30% N_2 atmosphere, stored at 4±2ºC, reached a value of 2.37 mg kg^{-1} on the 12^{th} storage day. However, different concentrations of CO_2 were not used in that study. In the present study we can conclude that only CO_2 concentrations above 50% are really effective to drastically reduce putrescine production during the storage period.

Rokka et al. (2004) also reported that samples stored in 80% CO_2 atmosphere presented cadaverine concentration below 10 mg kg^{-1}, similar to the values obtained for T4, T5, T6 and T7 in the present study. In this study, we can state that packaging in CO_2 concentration above 30% is efficient to reduce the bacterial production of cadaverine. The results obtained in the present study show that cadaverine-producer bacteria are more sensitive to CO_2 than putrescine producer bacteria.

Although Vince & Antonelli (2002) reported that among the assessed biogenic amines, cadaverine was the amine produced in the greatest quantity in chicken in aerobiosis condition, in the present study, cadaverine was the second amine to present the greatest increase, remaining behind putrescine in T1 treatment. The differences in the results of our study and those reported by other authors may be due to differences in the microbial flora and bacterial counts in chicken samples, although a comparison is not possible since no information is available on microflora types and counts in samples (Bunková et al., 2010; Curiel, Ruiz-Capillas, de las Rivas, Carrascosa, Juménez-Colmenero, & Muñoz, 2011; Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994).

The difference observed on the correlation values between bacterial growth and amine production (putrescine, cadaverine, tyramine and spermidine) in the treatment with atmospheric air (T1), that correlated with the four amines, and the other treatments (vacuum and CO_2 enriched atmosphere packaging) which correlate only with cadaverine and putrescine, is related to the fact that when vacuum packaging and modified atmosphere

packaging are used oxygen is restricted and CO_2 is present (MAP) causing an alteration of the cell membrane of some types of bacteria, besides reduction of the speed of enzyme reactions and direct enzyme inhibition (Church, 1993; Mano, Garcia de Fernando, Lopez-Galves, Selgas, Garcia, Cambero, & Ordóñez, 1995; Sarantópoulos, Alves, Contreras, Galvão, & Gomes., 1998). Thus, possible some types of bacteria responsible for the role of putrescine and cadaverine were selected when modified atmosphere and vacuum packaging was used, however, when CO_2 concentration was increased, the bacteria that produce these amines were also restricted.

6. Conclusions

Putrescine and cadaverine biogenic amines proved to be influenced by storage time and CO_2 concentration, and putrescine-producer bacteria are more CO_2 resistant. The correlation between biogenic amine formation and bacterial growth showed that putrescine and cadaverine concentrations can be used as quality indicators of this matrix, since their formation is related to TVC. Further studies can be conducted to apply these indicators to other ready-to-eat products.

Acknowledgements

The authors are thankful for the financial support of the State of Rio de Janeiro Carlos Chagas Filho Research Foundation (FAPERJ), process numbers E-26/111.610/2010 and E-26/103.003/2012. M.B.R. Rodriguez was supported by the Coordination of Enhancement of People of a Superior Level (CAPES).

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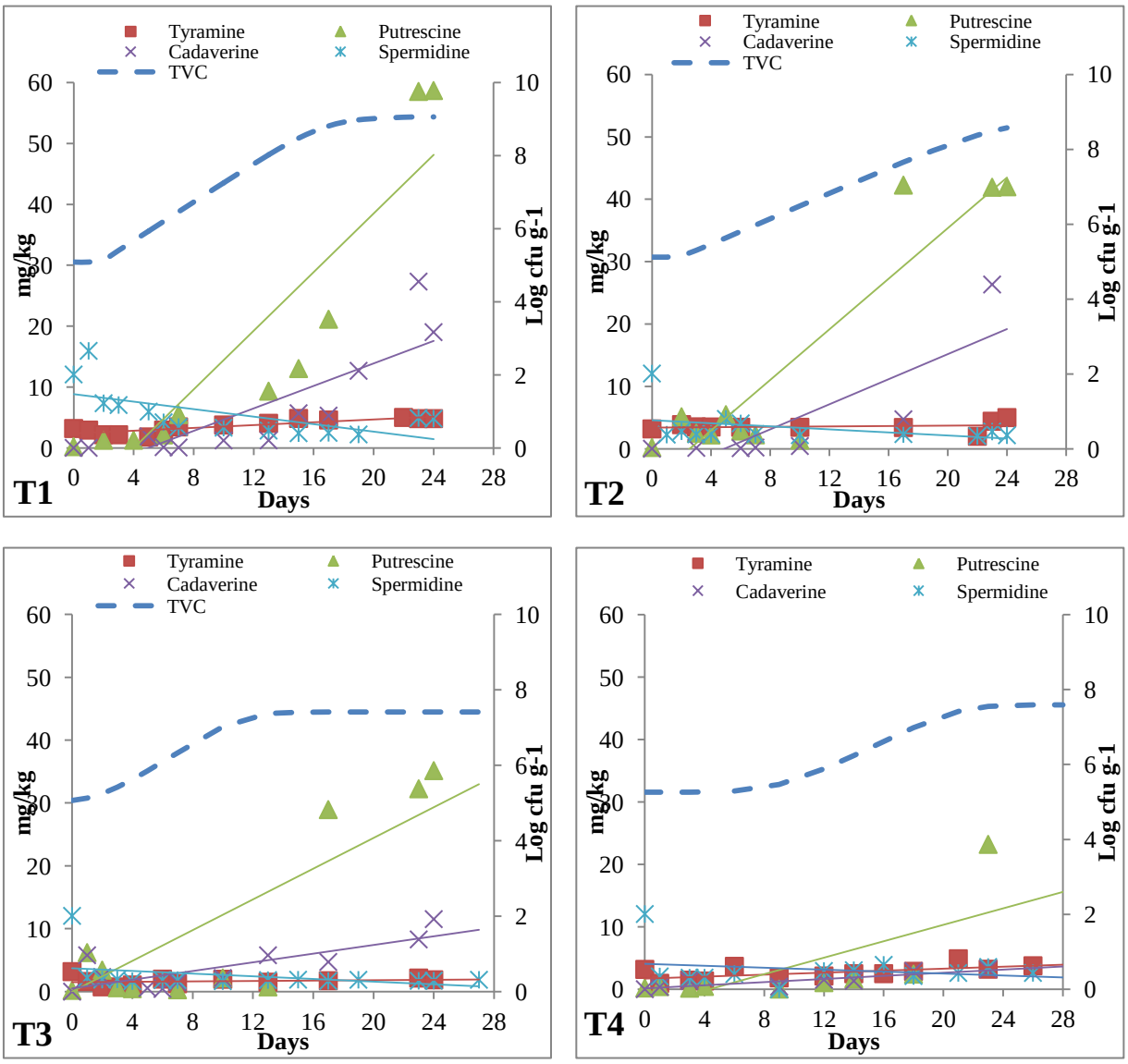

Figure 1. Average values of biogenic amines and TVC in shredded cooked chicken breast fillet samples packages in T1 (aerobiosis), T2 (vacuum), T3 and T4 (MAP with 10/90, 30/70 of CO_2/N_2, respectively) kept under refrigeration (4±2ºC).

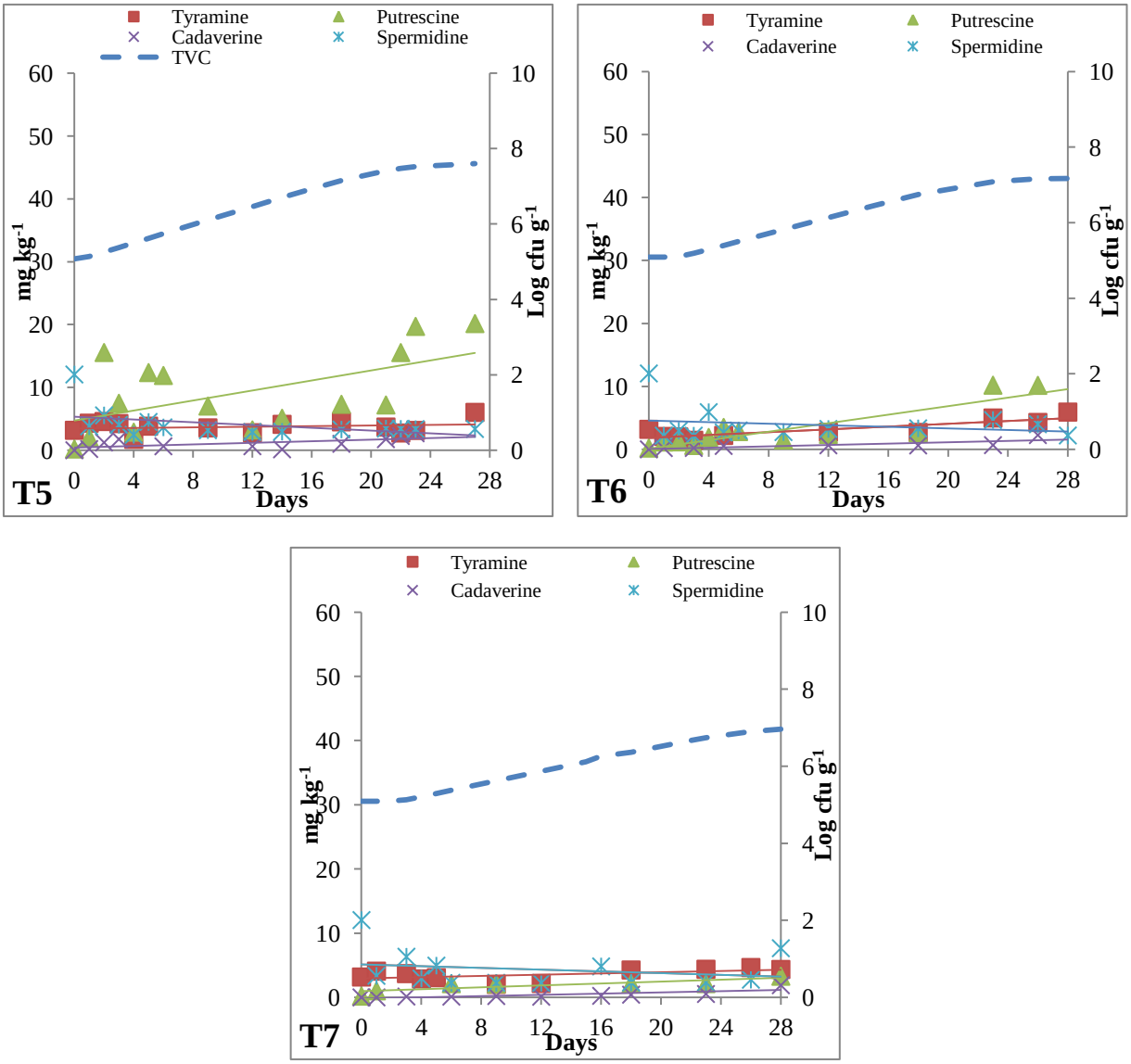

Figure 2. Average values of biogenic amines and TVC in shredded cooked chicken breast fillet samples packages in T5, T6 and T7 (MAP with 50/50, 70/30, 90/10 of CO_2/N_2, respectively) kept under refrigeration (4±2ºC).

4 **CONSIDERAÇÕES FINAIS**

De acordo com as análises bacteriológicas e com os resultados de pH, as amostras de filé de peito de frango cozido e desfiado embalados em ar atmosférico e armazenados sob refrigeração apresentaram validade comercial de nove dias, e a maior extensão de validade comercial, 28 dias, foi observada nas amostras com 90% de CO_2. Conclui-se que o uso de embalagens em atmosfera modificada prolongou a validade comercial do produto. Os grupos bacterianos estudados apresentaram taxa de crescimento inferior nos tratamentos enriquecidos com CO_2 quando comparados aos demais, de modo que, os melhores resultados em termos de conservação foram, proporcionalmente, evidenciados nas embalagens com maiores concentrações do gás.

As aminas biogênicas putrescina e cadaverina demonstraram ser influenciadas pelo tempo de armazenamento e pela concentração de CO_2, sendo as bactérias produtoras de putrescina mais resistentes ao gás. A correlação da formação de aminas biogênicas e o crescimento bacteriano encontrado sugere que a análise das aminas putrescina e cadaverina apresenta potencial para ser utilizada como parâmetro de qualidade dessa matriz, uma vez que sua formação está correlacionada com contagem de bactérias heterotróficas aeróbias mesófilas.

Recomenda-se, portanto, o uso da atmosfera contendo altas concentrações de CO_2 como forma de conservação do filé de peito de frango cozido e desfiado, por apresentar melhores parâmetros de validade comercial em detrimento das outras composições estudadas. Assim como, os resultados quantitativos de aminas biogênicas no produto podem ser explorados para a aplicação como indicadores de qualidade em outros produtos prontos para o consumo.

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6 **APÊNDICES**

6.1 CONFIRMAÇÃO DE ENVIO ONLINE DO ARTIGO PARA REVISTA CIÊNCIA RURAL

Preview

From: vagner.neujahr@gmail.com

To: carlavet.s@gmail.com

CC: marianabacellar@gmail.com, carlavet.s@gmail.com, marciabfeijo@globo.com, carlosconte@hotmail.com, mtasbm@vm.uff.br

Subject: Ciência Rural - Manuscript ID CR-2013-0074

Body: @@date to be populated upon sending@@

Dear Prof. CARNEIRO:

Your manuscript entitled "Bioactive amines: aspects of quality and safety in food" has been successfully submitted online and is presently being given full consideration for publication in the Ciência Rural.

Your manuscript ID is CR-2013-0074.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts at http://mc04.manuscriptcentral.com/cr-scielo and edit your user information as appropriate.

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Thank you for submitting your manuscript to the Ciência Rural.

Sincerely,
Ciência Rural Editorial Office

Date Sent: 18-Jan-2013

6.2 CONFIRMAÇÃO DE ENVIO ONLINE DO ARTIGO PARA REVISTA POULTRY SCIENCE

Preview

From: shaunam@assochq.org

To: marianabacellar@gmail.com

CC: marianabacellar@gmail.com, mtaconte@vm.uff.br, carlavet.s@gmail.com, robsonmf@vm.uff.br, mtasbm@vm.uff.br

Subject: Manuscript Submitted to Poultry Science - Submission Form Needed

Body: @@date to be populated upon sending@@

Dear Miss Rodriguez:

Your manuscript titled "Effect of carbon dioxide on the shelf life of ready-to-eat shredded cooked chicken breast fillet stored under refrigeration" by Rodriguez, Mariana; Conte-Junior, Carlos; Carneiro, Carla; Franco, Robson; Mano, Sérgio has been successfully submitted online and is presently being given consideration for publication in the Poultry Science.
Because publication in the Poultry Science requires the time and expertise of at least 2 reviewers, all manuscript authors and co-authors are requested to accept the responsibility to review submissions from other authors. Your contribution to the journal is extremely important in order to maintain high scientific standards, so your fulfillment of this important responsibility is anticipated if you are asked by editors.

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Sincerely,
Prof. Tom Porter
Editor-in-Chief

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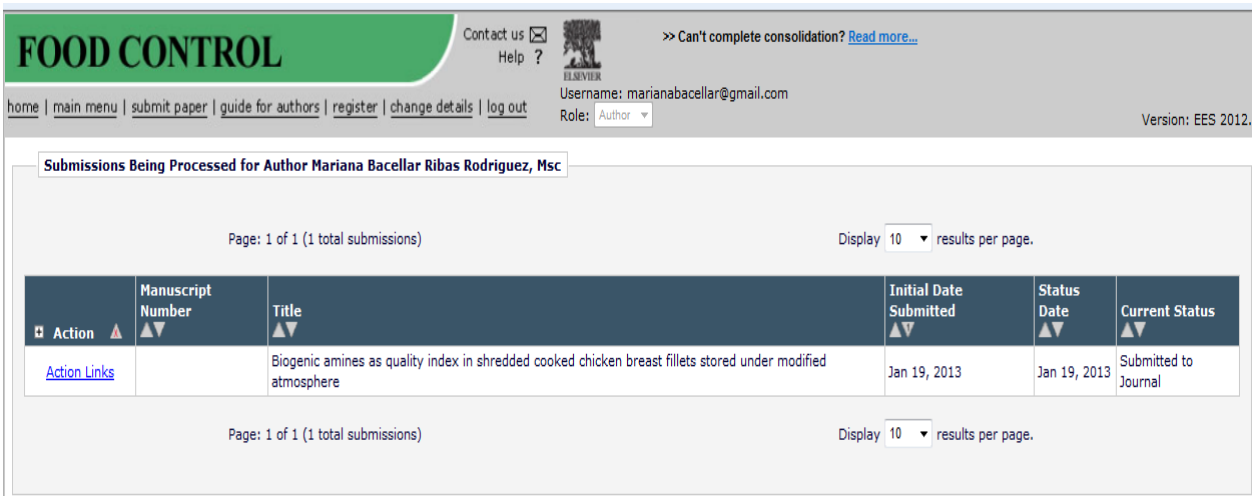
6.3 CONFIRMAÇÃO DE ENVIO ONLINE DO ARTIGO PARA REVISTA FOOD CONTROL



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Submissions Being Processed for Author Mariana Bacellar Ribas Rodriguez, Msc

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