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

BRUNA LEAL RODRIGUES

PARÂMETROS DE QUALIDADE E EFICIÊNCIA DA RADIAÇÃO UV-C EM TRUTAS ARCO-ÍRIS (*Onchorynchus mykiss*)

NITERÓI
2013

BRUNA LEAL RODRIGUES

PARÂMETROS DE QUALIDADE E EFICIÊNCIA DA RADIAÇÃO UV-C EM TRUTAS ARCO-ÍRIS (*Onchorynchus mykiss*)

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. CARLOS ADAM CONTE JÚNIOR

CO-ORIENTADOR: Prof. Dr. THIAGO DA SILVEIRA ALVARES

Niterói, RJ

2013

R696 Rodrigues,Bruna Leal
Parâmetros de qualidade e eficiência da radiação UV-C em Trutas Arco-Iris / Bruna Leal Rodrigues; orientador Carlos Adam Conte Júnior – 2013.
80f.

Dissertação (Mestrado em Higiene Veterinária e Processamento Tecnológico de Produtos de Origem Animal) – Universidade Federal Fluminense, 2013.
Orientador: Carlos Adam Conte Júnior

1. Conservação de pescado. 2. Truta. 3. Prazo de validade de produtos. 4. Embalagem em atmosfera modificada. 5. Radiação ultravioleta. I. Título.

CDD 664.94

BRUNA LEAL RODRIGUES

PARÂMETROS DE QUALIDADE E EFICIÊNCIA DA RADIAÇÃO UV- C EM TRUTAS ARCO-ÍRIS (*Onchorynchus mykiss*) **REFRIGERADAS**

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.

Aprovada em 08 de novembro de 2013.

BANCA EXAMINADORA

Prof. Dr. Carlos Adam Conte Junior – Orientador - UFF

Dra. Maria Lúcia Guerra Monteiro – PNPD/CAPES

Profa. Dra. Cárla da Silva Carneiro - UFRJ

Niterói
2013

Com imensa gratidão, amor e carinho, dedico aos meus pais Marcia T.L. Rodrigues e Nelson C.B. Rodrigues, exemplos de vida a serem seguidos.

AGRADECIMENTOS

À Deus por sempre iluminar minha caminhada e da minha família .

Aos meus pais, Marcia Terezinha Leal Rodrigues e Nelson Castello Branco Rodrigues, pelo imenso carinho, dedicação e amor que me ofereceram durante toda a vida, por me ensinarem direta e indiretamente o real sentido das palavras coragem, disciplina, determinação, dedicação e perseverança e por sempre me fazerem sonhar e acreditar que a realização de um sonho é possível e só depende de você.

Ao meu irmão e grande amigo Leonardo Leal Rodrigues, meu companheiro fiel e bem humorado que enche minha vida de alegria todos os dias.

Ao meu cavalo Moleque Hode Luã, meu eterno amor e incentivador, responsável direto pelas maiores, mais importantes e mais valiosas conquistas da minha vida.

Aos meus padrinhos, Miriam Leal e Luis Otávio Segond, pelos conselhos e por participarem ativamente da minha vida tanto pessoal como profissional.

Aos meus avós, Marlene Leal, Milton Leal, Jacyntha Rodrigues e Nelson Rodrigues, pelo carinho, conselhos e ensinamentos que levarei por toda a vida.

Ao meu orientador e grande amigo Carlos Conte, por todo o tempo dedicado, pelas ajudas, pelos brilhantes ensinamentos e conselhos tanto profissionais quanto pessoais, por acreditar e depositar em mim uma enorme confiança que vem desde a graduação.

Ao meu co-orientador Thiago Álvares, pela amizade, pela valiosa atenção, por todo o tempo dedicado, pelos ensinamentos e conselhos.

A todos os integrantes do laboratório de controle físico-químico de produtos de origem animal da UFF, pelo companheirismo, pelos ensinamentos, pelos bons momentos de trabalho divididos.

Aos amigos e bolsistas de iniciação científica, em especial a Laís Doro, Jasmim Arcanjo e Pedro Lopes, por todo o auxílio prestado, amizade e companheirismo nos momentos mais difíceis.

A todos os meus amigos, em especial ao Guilherme Sicca Lopes Sampaio, Marion Costa, Hugo Azevedo, Karoline Palmeira, Carolina Barcellos, Celso Fasura, Letícia Aquino, Cesar Lazaro, Bruna Santos, Marcelo Torres, Leonardo Gaze, Celina Zampiere, Raphael Leonardo, Beatriz Frasão, Ana Letícia Marques e Luiz Felipe Hermida, pelos ótimos momentos dentro e fora da faculdade, pelo companheirismo e por me transformarem em uma pessoa mais feliz todos os dias da minha vida.

A todos os alunos de pós-graduação, mestrandos, doutorandos, estagiários pela ótima convivência.

A todos os professores da pós-graduação pelo tempo e ensinamentos concedidos.

Ao Sr. Wilson e Sra. Gisele, proprietários da empresa Trutas da Serrinha, por concederem as amostras.

Ao Programa de Pós Graduação em Higiene Veterinária e Processamento de Produtos de Origem Animal e ao Drausio, André e Mariana, pela colaboração e simpatia.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pelo apoio financeiro.

A todos que contribuíram de alguma forma para a realização e conclusão deste trabalho.

“Que os vossos esforços desafiem as impossibilidades, lembrai-vos de que as grandes coisas do homem foram conquistadas do que parecia impossível.”

Charles Chaplin

BIOGRAFIA

Bruna Leal Rodrigues, brasileira, natural do Rio de Janeiro. Nascida em 10 de maio de 1988, filha de Marcia Terezinha Leal Rodrigues e Nelson Castello Branco Rodrigues. No ano de 2006 ingressou no curso de Medicina Veterinária da Universidade Federal Fluminense e concluiu a graduação na mesma universidade no ano de 2011. Ao concluir a graduação, recebeu prêmio do Conselho Regional de Medicina Veterinária do Rio de Janeiro- CRMV- RJ por apresentar maior coeficiente de rendimento (C.R.) da turma. No ano de 2009 foi aprovada e classificada no concurso de monitoria de Ezoognósia. No mesmo ano tornou-se bolsista CNPq onde trabalhou no projeto de pesquisa intitulado "Estimação de curvas de crescimento, consumo e produção de ovos de matrizes de linhagem tipo caipira" durante a vigência de 2009/2010. No ano de 2010 seu estudo foi classificado entre os dez melhores projetos da Área de Ciências Agrárias, apresentado no XX Seminário de Iniciação Científica e Prêmio UFF Vasconcellos Torres de Ciência e Tecnologia da Universidade Federal Fluminense. No ano de 2010 trabalhou como bolsista CNPq no projeto de pesquisa intitulado "Avaliação de modelos para estimação de curvas de crescimento, consumo e produção de ovos de matrizes de linhagem tipo caipira" durante a vigência 2010/2011. No ano de 2011 fez estágio na Vigilância Sanitária do Rio de Janeiro, no Ministério da Agricultura Pecuária e Abastecimento, no abatedouro de aves Rica Alimentos (Reginaves Ind. e Com. de Aves) com Sistema de Inspeção Estadual (SIE) e no grupo Paludo restaurantes. No mesmo ano trabalhou por 3 meses no grupo Paludo restaurantes como analista de qualidade. No final do ano de 2011 foi aprovada em primeiro lugar na seleção para Mestrado no Programa de Pós-graduação em Medicina Veterinária, Área de Concentração Higiene Veterinária e Processamento Tecnológico de Produtos de Origem Animal da Universidade Federal Fluminense, com início em março de 2012, e foi bolsista CNPq. No ano de 2012 publicou o artigo intitulado "Qualidade físico-química do pescado utilizado na elaboração de sushis e sashimis de atum e salmão comercializados no município do Rio de Janeiro, Brasil" na revista Semina: Ciências Agrárias. No ano de 2013 publicou o artigo intitulado "Concentration of Biogenic Amines in Rainbow Trout (*Oncorhynchus mykiss*) Preserved in Ice and its Relationship with Physicochemical Parameters of Quality" na revista Journal of Aquaculture Research and Development.

RESUMO

O pescado é uma fonte nutricional de alta qualidade e, por possuir, principalmente, proteínas de alto valor biológico e alta quantidade de ácidos graxos insaturados, o consumo do mesmo tem sido fomentado na dieta humana. No entanto, devido a composição química e pH próximo da neutralidade, a deterioração desta matriz ocorre rapidamente resultando na perda da qualidade e na diminuição da validade comercial do produto. Como forma de controlar a rápida deterioração e prolongar a validade desta matriz, métodos de conservação como a embalagem em atmosfera modificada e a radiação ultravioleta de ondas curtas (UV-C) tem sido estudados. Desta forma, os objetivos gerais do trabalho foram divididos em dois estudos: (1) Avaliar a utilização de aminas biogênicas e outros parâmetros físico-químicos como indicadores de qualidade em trutas arco-íris inteiras (Artigo I). Os resultados demonstraram que a concentração de aminas biogênicas e os valores de pH aumentaram significativamente durante o período de estocagem. Não foram observadas diferenças significativas nos valores de bases voláteis totais ao longo do armazenamento. A amônia foi detectada após o 11º dia de estocagem. Com base nos resultados, as aminas biogênicas putrescina e cadaverina podem ser utilizadas como índice de qualidade para trutas arco-íris inteiras. No entanto, as bases voláteis totais não são consideradas adequadas para avaliação do nível de frescor desta matriz. (2) Avaliar a influência do tipo de embalagem e composição de gases na eficiência da radiação ultravioleta (UV-C) em filés da referida espécie (Artigo II). Os resultados demonstraram que a dose de 0,1001±0,01 J/cm^2 promoveu redução de 1,8 log (UFC.g^{-1}) de *Proteus mirabilis*. Diferentes proporções dos gases CO_2 e N_2 não influenciaram os efeitos da radiação UV-C nas diferentes doses aplicadas. A passagem da luz UV reduziu significativamente em todas as embalagens estudadas (embalagem laminada (EL); embalagem com barreira aos gases (CBG); embalagem sem barreira aos gases (SBG)), quando comparado ao grupo controle (EL> CBG> SBG). Com base nos resultados, a radiação UV-C pode ser utilizada para reduzir a carga bacteriana superficial em filés de trutas arco-íris embalados. A utilização de diferentes proporções de gases não interferiu na ação dos raios UV-C sob os filés. As embalagens com e sem barreira aos gases foram consideradas adequadas para a submissão à radiação UV-C.

Palavras-chave: peixe dulcícola, índice de qualidade, validade comercial, métodos de conservação, luz UV-C, embalagem

ABSTRACT

Fish is a source of high nutritional quality and due to have mainly proteins of high biological value and high amount of unsaturated fatty acids, their consumption has been fostered in the human diet. However, due to their chemical composition and pH near neutrality, the deterioration of this matrix is quickly lead to loss of quality and decreased the duration of the product. In order to control the rapid deterioration and prolong the validity of the matrix, preservation methods such as modified atmosphere packaging and UV-C radiation has been studied. This study was divided into two parts: (1) Evaluate the use of biogenic amines and other physicochemical parameters as indicators of quality in whole rainbow trout (Article I). The results showed that the concentration of biogenic amines and the pH values increased significantly during the storage period. There were no significant differences in the values of total volatile bases during storage. Ammonia was detected after the 11th day of storage. Based on the results, biogenic amines putrescine and cadaverine can be used as a quality index for whole rainbow trout. However, the total volatile bases are not considered appropriate for assessing the level of freshness of this matrix. (2) Evaluate the influence of the type of packaging and gas composition on the efficiency of ultraviolet (UV - C) in this species of fish (Article II). The results demonstrated that a dose of 0.1001±0.01 J/cm^2 promoted reduction of 1.8 log (CFU g -1) of *Proteus mirabilis*. Different proportions of the gases CO_2 and N_2 did not affect the effects of UV-C radiation in different doses. The passage of UV light showed a significant reduction in all studied packaging (laminate packaging (LP); packaging with gas barrier (GB); package without gas barrier (WGB)) compared to control group (LP > GB > WGB). Based on the results, the UV- C radiation can be used to reduce the surface bacterial load on packed rainbow trout fillets. The use of different proportions of gases do not interfere with the effect of UV- C on the fillets. Packages with and without gas barrier were considered suitable for submission to UV- C.

Keywords: freshwater fish, quality index, commercial validity, conservation methods, UV- C light, packages.

SUMÁRIO

1 **INTRODUÇÃO**

A carne do pescado é um complexo molecular de elevado valor nutritivo, que possui alta digestibilidade, rápida absorção e baixo valor calórico, além de apresentar alto teor de aminoácidos e ácidos graxos essenciais, especialmente da série ômega 3 e vitaminas que são benéficos a saúde humana. No entanto, sua composição química, bem como o seu pH próximo a neutralidade, acelera a sua deterioração, pois favorece o desenvolvimento da microbiota natural presente nesta matriz. A deterioração no pescado ocorre como consequência da atividade enzimática e microbiológica, resultando na produção de diversos metabólitos, que levam a perda da qualidade do produto e servem como indicadores de qualidade da matéria-prima.

As bases voláteis totais (monometilamina, dimetilamina, trimetilamina e amônia) são compostos nitrogenados originados a partir da degradação de componentes do pescado (aminoácidos e nucleotídeos) durante o processo de deterioração. A determinação das bases voláteis totais (BVT) constitui-se em um dos métodos mais amplamente utilizados para avaliação da qualidade do pescado (HUSS, 1995). Em pescado dulcícola a determinação de amônia é considerada como um bom parâmetro para avaliação do índice de qualidade, uma vez que é o principal composto pertencente ao grupo de substâncias avaliadas na análise de BVT nestas espécies de peixes. Outro parâmetro também amplamente utilizado para avaliar o processo de degradação do pescado é a determinação do potencial hidrogeniônico (pH). Ao longo do processo de deteriora, ocorre a formação de compostos alcalinos, como a amônia e as aminas biogênicas, que se acumulam na musculatura aumentando os valores do pH muscular.

O desenvolvimento bacteriano é um dos principais fatores que levam à deterioração do pescado, sendo que a grande maioria desses microrganismos apresenta atividade proteolítica ou lipolítica contribuindo, portanto, para a degradação dos tecidos e para uma série de transformações bioquímicas indesejáveis, inclusive a produção de aminas biogênicas, que levam à total decomposição do pescado.

As aminas biogênicas são formadas no pescado como consequência da descarboxilação de aminoácidos e sua presença está relacionada com a existência de bactérias deteriorantes na matriz alimentar. Há uma variação na predominância

de aminas biogênicas de acordo com a espécie do pescado, sendo que de uma forma geral a concentração após a captura é vestigial podendo aumentar ao longo da estocagem devido a condições inadequadas de captura, armazenamento e processamento da matriz.

O desenvolvimento de métodos analíticos para identificação e quantificação das aminas biogênicas é de grande importância para determinar o frescor do pescado, além disso pode ser significativamente mais rápida em comparação a métodos microbiológicos tradicionais. As técnicas cromatográficas oferecem uma vantagem uma vez que garantem quantificações precisas e permitem a análise simultânea de diversas aminas biogênicas em pescado e produtos da pesca.

Como forma de controlar o rápido processo de deteriora e de aumentar a validade comercial do pescado e seus derivados, diversos métodos de conservação tem sido amplamente estudados. A embalagem em atmosfera modificada é um método de conservação que tem como princípio a substituição do ar atmosférico do interior da embalagem por uma mistura de gases. A ação dos gases reduz a taxa de crescimento microbiano, auxilia no controle da atividade enzimática e oxidação das gorduras, aumentando, com isso, a validade comercial do produto, além de manter sua qualidade microbiológica, nutricional e sensorial.

A radiação ultravioleta de ondas curtas (UV-C) é um processo não térmico utilizado para desinfecção de superfícies ou de água. Entretanto, atualmente cresce o número de evidências científicas que utilizam esta tecnologia para descontaminação superficial de alimentos, onde diversos tipos de microrganismos, inclusive vírus e fungos, podem ser inativados. Consiste em um método de conservação de fácil implementação, de baixo custo, que não gera resíduos químicos ou subprodutos indesejáveis, sendo ambientalmente segura.

Neste contexto, o objetivo do presente estudo foi estabelecer um índice de qualidade de aminas biogênicas, assim como verificar os parâmetros físico-químicos mais adequados para determinar o grau de frescor de truta arco-íris resfriada, além de avaliar a influência do tipo de embalagem e composição de gases na eficiência da radiação ultravioleta (UV-C) nesta espécie de pescado.

2 **FUNDAMENTAÇÃO TEÓRICA**

2.1 INTRODUÇÃO E DISTRIBUIÇÃO DA TRUTA ARCO-ÍRIS (*Onchorynchus mykiss*) NO BRASIL E NO MUNDO

A truta arco-íris (*Oncorhynchus mykiss)* pertencente à ordem Salmoniformes e à família Salmonidae, é uma das espécies mais difundidas mundialmente, mais cultivadas pela aquicultura comercial e mais comercializadas em todo o mundo (COLOSO, 2003; HERSHBERGER, 1992). Devido a sua grande variação fenotípica, diversas nomenclaturas já foram aplicadas às variedades da truta arco-íris (LAZZAROTTO; CARAMASCHI, 2009). Esta foi descrita originalmente como *Salmo mykiss,* porém atualmente aplica-se o nome *Oncorhynchus*, que corresponde a todos os salmões e trutas encontrados no Oceano Pacífico (SMITH; STEARLEY, 1989).

A espécie é originária da vertente Pacífica da América do Norte e Nordeste da Ásia, compreendendo os rios e as áreas costeiras (LAZZAROTTO; CARAMASCHIO, 2009). Encontra-se largamente difundida em todos os rios de água fria do mundo, com exceção do continente Antártico, uma vez que apresenta excelentes características para prática da aquicultura e da pesca esportiva (HERSHBERGER, 1992).

A truta arco-íris é, possivelmente, uma das espécies mais antigas empregadas em cultivo e apresenta hoje uma distribuição cosmopolita (WELCOME, 1988; GALL; CRANDELL, 1992). A difusão artificial da espécie iniciou-se em 1874 pela transferência de ovos embrionados do norte da Califórnia, para Nova York (EUA). Em 1879, foi introduzida na França e posteriormente difundida por todos os países da Europa, sendo que seu cultivo industrial ocorreu inicialmente na Dinamarca, na década de 1890 (GALL; CRANDELL, 1992). Os primeiros países a introduzirem esta espécie na América do Sul foram a Argentina e o Chile na primeira década do século XX, sendo posteriormente inseridas na Colômbia, Peru, Equador, Venezuela e Bolívia (WELCOME, 1988). No Brasil, as primeiras introduções ocorreram nos anos de 1913, 1942 e na década de 1950, sendo seu ingresso justificado pela ausência de espécies nativas nas regiões acidentadas e montanhosas do Brasil (LAZZAROTTO; CARAMASCHI, 2009).

Esta espécie já foi registrada em Minas Gerais, São Paulo, Rio de Janeiro, Espírito Santo, Rio Grande do Sul, Santa Catarina e Paraná (TAKINO; MAIER; STEMPNIEWSKI, 1984; TABATA, 1997; BIZERRIL; LIMA, 2001; MAGALHÃES et al., 2002; SOSINSKI, 2004; LAZZAROTTO et al., 2005; LAZZAROTTO et al., 2007). No Sudeste, esta espécie foi introduzida pelo médico veterinário Dr. Ascânio de Faria que recebeu 5.000 ovos embrionados de truta arco-íris provenientes da Dinamarca e disseminou nas regiões do Planalto da Bocaina e Parque Nacional do Itatiaia (FARIA, 1953a; SHUBBART, 1953).

O último dado encontrado na literatura relata que a truticultura no Brasil está localizada principalmente no Sul e no Sudeste e o cultivo nestas regiões totaliza 75% da produção nacional (AMARAL, 2007).

2.2 CARACTERÍSTICAS ZOOTÉCNICAS DA ESPÉCIE

Espécie dulcícola, a truta arco-íris é encontrada em regiões onde a temperatura da água é fria, mesmo no verão (GALL; CRANDELL, 1992). Na região tropical, tem a ocorrência limitada apenas às zonas de altitude (WELCOME, 1988). Os limites máximo e mínimo de temperatura da água para garantir a sobrevivência desta espécie são 0 e 25º C, todavia, a faixa de temperatura recomendada para condições de cultivo intensivo situa-se entre 10 e 20º C. A faixa térmica entre 15°C e 17°C garante as melhores taxas de crescimento e temperaturas próximas a 10°C, os melhores índices reprodutivos. O conhecimento das variações da temperatura do local proposto para o cultivo irá definir se o mesmo permite a exploração do ciclo completo de produção da truta ou apenas a engorda desses animais (TABATA, 1997).

Como requisito esta espécie requer ambientes com água em quantidade e qualidade, com correnteza moderada a forte e água bem oxigenada para a procriação, apesar de também ser capaz de viver em lagos de água fria (KAILOLA et al., 1993). Os tanques-rede são considerados mais apropriados para nossas regiões, uma vez que são mais adequados para locais de topografia mais acidentada (TABATA, 1997).

Por possuir elevado valor comercial e características zootécnicas e reprodutivas favoráveis, como boa capacidade de sobrevivência e reprodução em

ambientes naturais e artificiais como represas, tanques, tanques-rede e etc. associado ao seu alto grau de domesticação (facilidade de coleta dos gametas, amadurecimento de ambos os sexos em ambientes artificiais, aceitação de alimento artificial desde o primeiro arraçoamento e manejo em relação ao controle referente a sexualidade), considera-se esta espécie uma das mais estudadas e cultivadas em todo o mundo (TABATA, 1997).

2.3 DETERIORAÇÃO DO PESCADO

A carne do pescado apresenta diversas qualidades bastante apreciadas e valorizadas pelo mercado consumidor atualmente como o baixo valor calórico, a elevada digestibilidade e o elevado valor nutritivo, dado pelo alto teor de aminoácidos, ácidos graxos essenciais, especialmente da série ômega 3, e vitaminas (GERMANO; GERMANO; OLIVEIRA, 1998). Contudo, a composição química (alta atividade de água, alto teor de lipídeos insaturados facilmente oxidáveis, pH próximo da neutralidade, nutrientes de fácil utilização por microrganismos, rápida ação das enzimas autolíticas e alta atividade metabólica da microbiota) bem como o rápido desenvolvimento do *rigor mortis* caracterizam uma matriz alimentar de elevada perecibilidade (SANCHEZ-CASCADO, 2005).

O processo de deterioração inicia-se primeiramente por mudanças autolíticas que ocorrem devido à ação de enzimas endógenas dos músculos ou provenientes das vísceras. A manipulação do pescado, desde a sua captura ou despesca até sua distribuição, contribui para que as enzimas contidas nas vísceras extravasem e atinjam os músculos, colaborando para a deterioração e produção de metabólitos que servem como substrato para a multiplicação microbiana (GRAM; HUSS,1996; PEREIRA; TENUTA-FILHO, 2005).

À medida que o tempo avança, as alterações degradativas do pescado vão progredindo (OETTERER, 1998) e a velocidade das alterações dependem diretamente de fatores endógenos (espécie do pescado; composição química) e exógenos (manipulação; estocagem) (RODRIGUEZ et al., 2004).

A carne do pescado passa por três fases a partir do momento que o peixe é capturado até que se estabeleça a deterioração: o *pré rigor*, o *rigor* e o *pós rigor mortis*. Na etapa de pré-rigor, o glicogênio é utilizado como fonte de energia, dando origem ao ácido lático provocando uma diminuição do pH muscular (peixes de carne

branca: 6,0 a 6,2; peixes de carne vermelha: 5,6 a 5,8). Em adição, ocorre a formação do complexo acto-miosina devido a fusão das moléculas de actina e miosina, levando ao estabelecimento do *rigor mortis* caracterizado pelo enrijecimento muscular (BARROS, 2003). O *rigor mortis* do pescado pode durar de 2 a 18 horas, e o pico ocorre na 6º hora pós captura (OETTERER, 2006). Segundo Connel (1988), até o término da rigidez cadavérica não se inicia o processo de deterioração microbiana uma vez que o pH encontra-se baixo devido a produção de ácido lático.

A duração do *rigor mortis* é variável. Diversos fatores como manejo, captura, higiene e temperatura ambiente podem influenciar na durabilidade do mesmo (KUBITZA, 2000) . Segundo o mesmo autor, o fim do *rigor mortis* em peixes abatidos logo após a captura e em peixes submetidos a um descanso pré-abate variam de 20 a 65 horas e de 72 a 96 horas. Ademais, peixes submetidos a estresse pré-abate apresentam rápida fase de *rigor mortis*, uma vez que ocorre um elevado consumo das reservas de glicogênio, tornando a carne pouco ácida, o que acelera a atuação das enzimas autolíticas, a ação dos microrganismos e, consequentemente, a deterioração da carne.

Segundo Oetterer (2006) na etapa final do *rigor mortis*, ocorre proteólise, desnaturação e posteriormente degradação, levando a formação de peptídeos e aminoácidos livres, o desenvolvimento de microrganismos e a produção de metabólitos que propiciam a perda de qualidade do produto e podem servir como índice de qualidade da matéria-prima (LISTON, 1980; ARASHISAR et al., 2004).

2.3.1 **Metabólitos produzidos durante o processo de deterioração**

Após a morte, ocorre a diminuição do pH muscular do pescado devido a produção de ácido lático proveniente da glicólise *post mortem*. A diminuição do pH está diretamente ligada a quantidade de glicogênio presente na musculatura do pescado que geralmente, por está presente em baixas quantidades, faz com que esta matriz apresente pH mais elevado quando comparada a carne de mamíferos (HUSS, 1998). Segundo Barros (2003), é na fase de *rigor mortis* que, devido ao pH ácido da carne, o pescado se encontra na condição de maior frescor, uma vez que a acidez torna o meio desfavorável à ação microbiana, além de controlar a ação das

enzimas presentes na matriz. Ao chegar na fase de *rigor post mortem* ocorre a formação de peptídeos e aminoácidos livres provenientes da hidrólise protéica, que dão origem a substâncias nitrogenadas, fazendo com que o pH aumente gradativamente (BARROS, 2003).

Segundo Simeonidou et al. (1998) o pH *post mortem* pode variar de 6,0 a 7,0 dependendo de vários fatores como espécie, estação do ano, entre outros. Chytiri et al. (2004a) observaram que o pH variou de 6,34 e 6,52 em trutas inteiras estocadas por 18 dias em gelo. Rodriguez et al. (1999) também observaram altos valores de pH ao estudarem as propriedades sensoriais e os indicadores bioquímicos em trutas arco-íris, observando variação entre 6,5 e 7,0.

Dentre os metabólitos nitrogenados produzidos estão as bases voláteis totais (monometilamina, dimetilamina, trimetilamina e amônia) originadas da degradação de aminoácidos e nucleotídeos durante o processo de deterioração (HUSS, 1995; GIANNINI, 2003).

A amônia é a base volátil mais representativa no início do processo de degradação. Esta é formada pelos produtos da desaminação dos derivados da adenosina trifosfato (ATP) e posteriormente, pela degradação de compostos nitrogenados, como por exemplo, os aminoácidos, que em conjunto com a trimetilamina, passam a serem os compostos mais significativos (OGAWA; MAIA, 1999). Na fase de *pós-rigor* a desaminação bacteriana de aminoácidos também é uma via de produção de amônia, causando aumento de seus níveis após a primeira semana de armazenamento (CONTRERAS-GUZMÁN, 1994).

O óxido de trimetilamina (OTMA) é um composto nitrogenado não protéico típico de peixes marinhos e invertebrados (HUSS, 1995). Este pode ser reduzido a trimetilamina (TMA) por três vias distintas: degradação não enzimática, ação enzimática bacteriana ou por decomposição enzimática, gerando em quantidade correspondente os compostos dimetilamina e formaldeído (CINTRA et al., 1999; TIMM; JORGENSEN, 2002).

Diferentemente de peixes de água salgada, os peixes dulcícolas geralmente apresentam valores mínimos de OTMA, precursor da TMA, que é o principal composto, juntamente com a amônia, formadores das BVT. Desta forma, a concentração de BVT em peixes dulcícolas permanece baixa durante a estocagem quando comparado a peixes marinhos (ZAITSEV et al., 1969; SCHERER et al., 2006). O Regulamento Técnico de Identidade e Qualidade (RTIQ) de Peixe Fresco

(BRASIL, 1997) e o RIISPOA (BRASIL, 2008) preconizam que para o pescado ser próprio para consumo deve apresentar um limite inferior a 30mg N-BVT/ 100g de carne, excluindo os elasmobrânquios. Porém, os peixes de água doce dificilmente alcançam este valor limite, mesmo apresentando avançados sinais de deterioração. De forma a aproximar o valor limite para realidade da espécie truta arco-íris, Gimenez et al. (2002) propuseram um limite máximo para estes compostos de 25 mg N-BVT/100 g.

As aminas biogênicas também são substâncias nitrogenadas formadas durante o processo de deterioração do pescado. Compostos básicos formados pela descarboxilação enzimática dos aminoácidos livres e da transaminação dos aldeídos e cetonas (SANTOS, 1996; SHALABY, 1996; KIM; MAH; HWANG, 2009), apresentam baixo peso molecular e ocorrem naturalmente em microrganismos, plantas e animais (SANTOS, 1996; SHALABY, 1996; FERNANDES, 2001; SAAID et al., 2009). São classificadas como alifáticas, grupo onde estão incluídas as aminas putrescina, cadaverina, espermina e espermidina; aromáticas, onde incluem-se a tiramina e a feniletilamina; e as heterocíclicas, onde encontram-se a histamina e a triptamina (SANTOS, 1996; ÖNAL, 2007; SAAID et al., 2009). Cada amina biogênica é derivada de um aminoácido específico, sendo a histamina, putrescina, cadaverina, e tiramina derivadas dos aminoácidos precursores histidina, ornitina, lisina e tirosina, respectivamente (Sánchez-Cascado, 2005). Na figura 1 encontra-se a esquematização da via biossintética de cada amina e seus aminoácidos precursores.

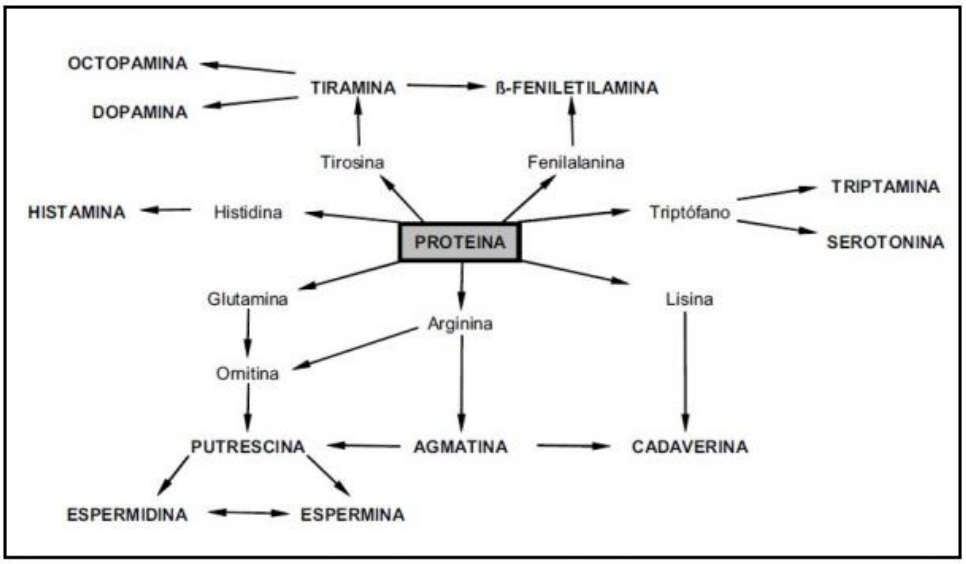


Figura 1: Síntese de aminas biogênicas derivadas dos aminoácidos precursores. Fonte: Sánchez-Cascado (2005).

Durante o período *post mortem* ocorre aumento do teor de aminoácidos livres devido à ação de enzimas proteolíticas que fazem parte do processo autolítico e que estão presentes no trato intestinal (FLICK; GRANATA, 2005; SAAID et al., 2009). Em condições normais, as aminas biogênicas estão ausentes ou encontram-se em concentrações mínimas (< 10 ppm) em alimentos frescos, sendo rapidamente metabolizadas no organismo por conjugação ou por reações de oxidação (SMITH, 1981). Contudo, em alimentos como pescado e seus derivados (SHALABY, 1996; FLICK; GRANATA, 2005; BRINKER; KEER; RAYNER, 2002), podem estar presentes em concentrações significativas (> 50 ppm), e são capazes de induzir uma intoxicação química (HALÁSZ et al., 1994).

Existe uma grande diversidade de microrganismos capazes de produzir aminas biogênicas, tais como bactérias da família *Enterobacteriacae* spp., *Clostridium* spp., *Lactobacillus* spp., *Morganella* spp., *Proteus morganii*, *Proteus* spp., *Hafnia alvei* e *Klebsiella* spp. (SHALABY, 1996; FLICK; GRANATA, 2005).

Halász et al. (1994) observaram que as bactérias da família Enterobacteriaceae estão geralmente implicadas na formação de cadaverina, assim como as do gênero *Pseudomonas* spp. são consideradas responsáveis pela formação de putrescina em trutas arco-íris.

Segundo diversos autores a quantidade de aminas biogênicas formadas depende do número de microrganismos descarboxilase positivos presentes no

produto, a disponibilidade de aminoácidos livres, a atividade das enzimas descarboxilase e a temperatura (SANTOS, 1996; SHALABY, 1996; BRINKER; KEER; RAYNER, 2002).

Como forma de averiguar as condições higiênico-sanitárias utilizadas durante a produção, processamento e armazenamento dos produtos e a qualidade das matérias – primas empregadas, utiliza-se a determinação de aminas biogênicas como ferramenta para avaliar o índice de qualidade de determinada matriz (TAYLOR, 1986; DONHAUSER; WAGNER; GEIGER, 1993; VECIANA-NOGUÉS; MARINÉ-FONT; VIDAL-CAROU, 1997). Podem da mesma forma ser empregadas como indicador de deterioração, uma vez que a ação microbiana pode ser acompanhada pelo aumento da produção de descarboxilases (HALÁSZ et al., 1994). É relevante ressaltar que as aminas são termorresistentes e permanecem no alimento mesmo após o tratamento térmico, o que torna o seu uso como indicador de qualidade, uma vantagem (LIMA; GLÓRIA, 1999).

Rodriguez et al. (1999) sugerem que a determinação das aminas cadaverina e putrescina servem como satisfatórios indicadores de qualidade para trutas arco-íris, ressaltando que a putrescina pode ser utilizada como indicador prematuro do processo de autólise muscular.

Rezaei et al. (2007) estudando a presença de aminas biogênicas em trutas arco-íris estocadas por 18 dias, observaram aumento das aminas durante o período de estocagem e sugeriram que o monitoramento dos níveis de putrescina podem servir como um índice para avaliar o frescor desta espécie de pescado.

2.4 MÉTODOS DE CONSERVAÇÃO EM PESCADO

A embalagem tem como função proteger e conservar os alimentos, uma vez que reduzem as alterações significativas de sua composição ao longo do período de estocagem e os mantém com qualidade até a chegada ao consumidor final. Diversos tipos de materiais e técnicas de embalagens podem ser utilizados para melhorar a conservação da carne do pescado durante o armazenamento. Dentre os tipos de embalagens, as embalagens laminadas (protegem a carne da ação da luz e diminuem reações de rancificação em produtos com elevado teor de gordura), as embalagens com barreira aos gases (utilizadas na tecnologia de embalagem em atmosfera modificada) e as embalagens sem barreira aos gases (utilizadas em

produtos embalados em aerobiose e que permitem trocas gasosas com o ar atmosférico) são as mais utilizadas pelas indústrias. Dentre as técnicas de embalagens, as mais empregadas são a embalagem em atmosfera modificada e a embalagem à vácuo.

A embalagem em atmosfera modificada (EAM) constitui no acondicionamento do produto em embalagens hermeticamente fechadas, com composição gasosa diferente do ar atmosférico (MANO, 1997). Segundo Genigeorgis (1985), são duas as categorias de modificação da atmosfera mais utilizadas na conservação dos alimentos: a primeira através da alteração das técnicas de embalagem e a segunda através da adição de uma mistura de gases no interior de uma embalagem.

A embalagem à vácuo é a forma mais simples do uso da atmosfera modificada. É definida como o acondicionamento do produto em embalagens impermeáveis aos gases nas quais o ar atmosférico é removido (PARRY, 1995; SOCCOL; OETTERER, 2003). A ausência do ar atmosférico promove o controle do desenvolvimento de microrganismos, da ação enzimática e da oxidação, principais mecanismos responsáveis pela deterioração dos alimentos (PRENTICE; SAINZ, 2005).

O segundo sistema de modificação da atmosfera é através da remoção do ar atmosférico e a adição de um gás ou de uma mistura de gases com variáveis combinações em uma embalagem que possua em sua composição barreira aos gases. Esta tecnologia tem se mostrado eficiente para extensão da validade comercial, por manter o frescor e a qualidade do pescado durante o período de estocagem e por facilitar a comercialização dos produtos por longas distâncias, assim como mantê-los no ponto de venda por um longo período de tempo. (GIMENEZ et al., 2002).

Os gases mais utilizados são o oxigênio (O_2), o dióxido de carbono (CO_2) e o nitrogênio (N_2). O O_2 é o gás que geralmente estimula o crescimento de bactérias aeróbias e inibe o crescimento de bactérias anaeróbias. A presença de O_2 em EAM é mais importante no armazenamento de carnes vermelhas uma vez que mantém o pigmento da carne (mioglobina) em sua forma oxigenada (oximioglobina), conferindo a cor vermelho cereja às carnes vermelhas conservadas desta forma. Porém, este gás é responsável por diversas reações indesejáveis nos alimentos, como oxidação e rancificação de gorduras, além de facilitar o processo de deterioração devido ao estímulo de crescimento de bactérias aeróbias. O O_2, portanto, é geralmente evitado

em EAM no acondicionamento de vários produtos (CHURCH, 1994; FLOROS; MATSOS, 2005).

O CO_2 é o principal gás responsável pelo efeito bacteriostático proveniente da EAM. Este gás apresenta solubilidade tanto em meio aquoso como lipídico e ao dissolver-se forma ácido carbônico, composto responsável pela redução do pH. O processo de acidificação é o principal responsável pela ação antimicrobiana promovida pelo CO_2, juntamente com as alterações de permeabilidade celular bacteriana e à inibição enzimática, resultando na redução da taxa de crescimento microbiano e alteração da microbiota, levando a desaceleração do processo de deterioração (SARANTÓPOULOS; SOLER, 1994; CHURCH; PARSONS, 1995; FELLOWS, 2006).

O N_2 é um gás quimicamente inerte e insípido, sendo utilizado como um gás de enchimento para limitar o colapso da embalagem causado pela absorção do CO_2 pelo produto, uma vez que é pouco solúvel em água e gordura. Além disso, retarda a rancificação oxidativa e a taxa de crescimento de microrganismos aeróbios (CHURCH, 1995; BLAKISTONE, 1999).

A proporção de gases utilizada no acondicionamento de pescado varia de acordo com a porcentagem de lipídeo presente na matriz. Pescado com elevado teor de lipídeos, por exemplo, não devem ser embalados com gás oxigênio, uma vez que o mesmo pode atuar como agente oxidativo induzindo a formação de aldeídos, cetonas e álcoois (SOCCOL, 2002).

2.5 RADIAÇÃO ULTRAVIOLETA DE ONDAS CURTAS (UV-C)

A radiação ultravioleta de ondas curtas (UV-C) é um processo não térmico que tem sido empregado amplamente na indústria de alimentos e hospitais para sanitização do ar e superfícies (ANDERSON et al., 2006; SOMMERS; SITES; MUSGROVE, 2010). Porém, atualmente há um grande interesse na utilização desta tecnologia para conservação de alimentos e aumento de sua validade comercial (SASTRY; DATTA; WOROBO, 2000; BINTSIS; LITOPOULOU-TZANETAKI; ROBINSON, 2000), uma vez que apresenta diversas vantagens como simplicidade de implementação, tecnologia efetiva e de baixo custo e não geração de resíduos químicos e radioativos (CHUN et al., 2009).

A radiação UV ocupa extensa faixa de comprimento de onda na região não ionizante do espectro eletromagnético (100-400nm), localizada entre os raios X e a luz visível, cuja classificação pode ser observada na Tabela 1. Apesar de ocupar ampla faixa de comprimento de onda, o intervalo considerado de maior efeito germicida é o de 200-280 nm, sendo o comprimento de onda de 253,7 nm o mais indicado para inativação de vírus, bactérias, protozoários, leveduras e algas (BINTSIS; LITOPOULOU-TZANETAKI; ROBINSON, 2000).

Tabela 1: Características da luz UV

Tipo	Comprimento de onda	Faixa (nm)	Características
UV-A	Longo	320-400	Alterações na pele humana (bronzeamento)
UV-B	Médio	280-320	Queimadura de pele (câncer)
UV-C	Curto	200-280	Faixa germicida (microrganismos)
UV-V		100-200	Região de UV de vácuo

Fonte: Guerrero-Beltrán; Barbosa-Cánovas (2004)

Seu modo de ação tem sido atribuído às ligações cross-linking entre os ácidos nucléicos timina e citosina da mesma fita de DNA microbiano, gerados após absorção da radiação a que foram expostos. Esta ligação resulta no bloqueio da transcrição e replicação do DNA microbiano, levando a redução na taxa de crescimento, indução da morte celular e decréscimo da carga bacteriana na superfície do alimento (MCDONALD et al., 2000; GUERRERO-BELTRAN; BARBOSA-CANOVAS, 2004).

No entanto, fotoreativações podem ocorrer quando as células injuriadas pela radiação UV-C são expostas a comprimentos de onda maior que 330 nm (LILTVED; LANDFALD, 2000). Enzimas denominadas fotoliases são ativadas e passam a atuar na divisão dos dímeros de timina e citosina formados pela ação da radiação UV-C, reparando os danos a nível de DNA microbiano (STEVENS et al., 1998).

É importante considerar, portanto, a dose de radiação a ser aplicada sob o produto para que haja garantia de um alimento seguro. Estudos demonstram que para que ocorra a inativação de microrganismos, a exposição de radiação UV deve ser no mínimo de 0,04J/cm^2 em todas as regiões do produto (SASTRY; DATTA; WOROBO, 2000). Além disso, fatores como a configuração geométrica do reator, a

energia transmitida, o comprimento de onda, os arranjos físicos da fonte de UV, a permeabilidade e topografia do produto, assim como os microrganismos presentes na superfície do alimento influenciam na eficácia desta tecnologia (SASTRY; DATTA; WOROBO, 2000; WOODLING; MORARU, 2005; CHUN et al., 2009; STOOPS et al., 2013).

3 **DESENVOLVIMENTO**

3.1 ARTIGO I: CONCENTRATION OF BIOGENIC AMINES IN RAINBOW TROUT (*Oncorhynchus mykiss*) PRESERVED IN ICE AND ITS RELATIONSHIP WITH PHYSICOCHEMICAL PARAMETERS OF QUALITY. Published in *Journal Aquaculture Research and Development***.**

CONCENTRATION OF BIOGENIC AMINES IN RAINBOW TROUT (*Oncorhynchus mykiss*) PRESERVED IN ICE AND ITS RELATIONSHIP WITH PHYSICOCHEMICAL PARAMETERS OF QUALITY

Bruna Leal Rodrigues[1], Thiago Silveira Alvares[2], Marion Pereira da Costa[1], Guilherme Sicca Lopes Sampaio[1], César Aquiles Lázaro de la Torre[1], Eliane Teixeira Márisco[1], Carlos Adam Conte Júnior[1]

[1] Laboratory of Physicochemical control; Department of Food Technology; Fluminense Federal University; Niterói, Brazil.

[2] Chemistry Institute; Federal University of Rio de Janeiro; Rio de Janeiro, Brazil.

Running Title: Biogenic amines in rainbow trout as parameters of quality.

Address for Correspondence:

Professor Carlos Adam Conte-Junior, Ph.D.

Laboratory of Physicochemical control - Department of Food Technology

Fluminense Federal University

Rua Vital Brazil Filho, 64, Santa Rosa Niterói, Rio de Janeiro, Brazil.

CEP: 24230-340

Phone # 55-21-2629-9545

Faz # 55-21-2629-9541

E-mail: mtaconte@vm.uff.br

ABSTRACT

Biogenic amines are formed as a result of amino acid decarboxylation and is linked to food deterioration. Analysis of these metabolites may be of great importance to determine food quality. The aim of this study was to quantify the biogenic amines (putrescine and cadaverine), and evaluate the physicochemical parameters (pH, ammonia and total volatile bases) of rainbow trout meat (*Oncorhynchus mykiss*). Fifteen samples were packed in ice and transported in a styrofoam container to the laboratory. Analyses were performed daily until the 15th day of storage. Biogenic amines concentrations and pH increased significantly throughout the storage period. No significant differences were observed in total volatile bases values over the time. Ammonia was detected after the 11th day of storage. Based on these results, cadaverine and putrescine may be used as a quality index of rainbow trout; however, total volatile bases may not be adequate parameter for this matrix.

Keywords: physicochemical parameters, biogenic amines, quality index, rainbow trout

INTRODUCTION

Fish and fishery products have played an important role in the human diet due to their high nutritional quality [1]; however, the chemical composition, high water activity, easily oxidized fat content, and pH close to neutral, accelerate its deterioration by promoting the development of the natural microbiota in this food matrix [2, 3]. The deterioration occurs as a result of enzymatic and microbial activity, resulting in the production of different metabolites, which can lead to loss of product quality and can serve as quality indicators of the raw material [4, 5].

The total volatile bases (monomethylamine, dimethylamine, trimethylamine, ammonia) are nitrogenous compounds originated from the degradation of some compounds in fish (amino acids and nucleotides) during the deterioration process [6, 7]. The determination of total volatile bases (TVB) is one of the most widely used for assessing fish quality [6].

In freshwater fish, ammonia is considered a good indicator for evaluating the quality index, since it is the main compound of the group of substances evaluated in the analysis of TVB in these species [8]. Another indicator also widely used to evaluate the degradation process of fish is the hydrogen potential (pH). During the decaying process, there is the formation of alkaline compounds such as ammonia and amines, which accumulate in the muscle, increasing the muscle pH values [9].

Biogenic amines are formed as a result of amino acid decarboxylation, which is linked to the existence of spoilage bacteria in the food matrix [6, 10, 11]. Studies have reported that the biogenic amines, especially putrescine and cadaverine, can be considered good parameters for assessing the quality and the deterioration rate of various food matrices, including fish [3, 11-15]. The development of analytical methods, faster than microbiological ones, for the identification and quantification of biogenic amines is very important to determine fish

freshness [3, 6]. Currently, chromatographic techniques offer a great advantage, since they ensure accurate measurements and allow for the simultaneous analysis of several biogenic amines in fish and fishery products [16, 17]. Among the chromatographic techniques, high-performance liquid chromatography (HPLC) is being widely used because of its sensitivity and reliability [18].

Due to the fact that there is limited scientific evidence demonstrating the effectiveness of biogenic amines as quality indicators of trout, and considering that rainbow trout (*Oncorhynchus mykiss*) is one of the main commercial aquaculture species produced and marketed worldwide [19], this study was conducted with the purpose of evaluating the use of biogenic amines and physicochemical parameters for quality assessment.

MATERIALS AND METHODS

a) Sampling

Fifteen fresh rainbow trout (*Oncorhynchus mykiss*) specimens were obtained from the *Trutas da Serrinha* Company located in Itatiaia, a region of the Serra da Mantiqueira, in the state of Rio de Janeiro, Brazil. The samples were packed in ice (0±1°C) and transported in a styrofoam container to the laboratory. The filet was obtained in sterile conditions, and all instruments used for filets dissection were previously sterilized. The samples were analyzed daily until the 15th day of storage (see figure 1). All analyses were performed in duplicate.

b) Physicochemical analyses

pH, TVB and ammonia parameters were determined in order to evaluate the state of fish freshness during storage at 0±1°C. For the analysis of pH and TVB, potentiometric and microdiffusion methods were used, respectively, as described by Conte-Júnior *et al.* and by Conway and Byrne [20, 21]. The qualitative determination of ammonia was performed by

using mercuric iodide, potassium iodide, sodium hydroxide solutions and water (Nessler reagent).

c) Biogenic amines quantification

The biogenic amines, putrescine and cadaverine, were assayed by High- Performance Liquid Chromatography (HPLC). Briefly, 5 mL of perchloric acid (5%) were added to 5 g of sample and kept 1 hour under refrigeration condition (4±2°C) with periodic stirring. Subsequently the solution was centrifuged and filtered through Whatman filter paper N^{o} 1, followed by the addition of 2N sodium hydroxide to reach pH > 6. In the next step, the homogenized solution was kept in an ice bath for 20 minutes and filtered a second time, with the subsequent addition of 2N sodium hydroxide to reach pH > 12 [22]. Under these circumstances the solution was derivatized with the addition of 40 μL of benzoyl chloride, homogenized for 15s and left to stand at room temperature for 20 min. Thereafter, 1 mL of diethyl ether was added and the supernatant was removed. The resulting sample was evaporated in a stream of nitrogen to be finally resuspended with 500 μL of mobile phase (acetonitrile: H_2O; 42:58; v:v) [23]. 20 μL of sample were injected into HPLC device coupled with UV detector; the flow rate was set at 1 mL min^{-1}. For the separation of the amine, a Teknokroma column, TR-016057 N26243 Tracer Extrasil ODS2 (15 x 0.46 cm, id. 5μm) and a Supelco precolumn, Ascentis C18 (2 x 0.40 cm, id. 5μm) were used.

d) Statistical analysis

The one-way ANOVA was performed to identify differences between biogenic amines (putrescine and cadaverine) and physicochemical parameters (TVB and pH) over the 15-day period of storage. When a significant *F* was found, additional post hoc tests with Bonferroni adjustment were performed. For the interpretation of result all the data obtained (from day 1

to day 15) were divided into three periods: Time 1 (T1) – analysis of the first five days of storage; Time 2 (T2) – analysis of the subsequent five days; Time 3 (T3) – analysis of the last five days of storage. Based on previous studies [11, 14, 24], no significant changes should occur in the biogenic amines concentrations and pH values at Time 1.

Statistical significance was set at the 0.05 level of confidence. All analyses were performed using a commercially available statistical package (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, Calif., USA).

RESULTS AND DISCUSSION

Recent studies do not report the use of quality physicochemical analyses that are considered simple and fast, and they have also not associated these analyses with biogenic amine concentrations to evaluate the quality of rainbow trout (*Oncorhynchus mykiss*). Therefore, the present study was designed to investigate the potential use of biogenic amines and physicochemical parameters – such as TVB, ammonia and pH – as a quality index for this species of fish. Overall, the major finding of the study was that the concentrations of the biogenic amine, putrescine and cadaverine, increased significantly – together with a significant increase in pH – over the 15-day storage period and the presence of ammonia was observed only after the 11^{th} day of storage. No significant difference was observed in TVB values during the 15 days of storage.

Changes in TVB and pH values over the period of the storage are shown in Table 1. There was no significant difference in TVB values throughout the 15 days of storage. A significant increase in pH was observed in T3 as compared to T1. The presence of ammonia was observed only after the 11^{th} day of storage.

The changes in cadaverine and putrescine concentrations over the days of storage are presented in Figures 2 and 3, respectively. There was a significant increase in putrescine (T1: 100.17 ± 13.28 mg/kg; T2: 136.53 ± 13.28 mg/kg; T3: 165.62 ± 7.27 mg/kg) and cadaverine (T1: 56.49 ± 0 mg/kg; T2: 59.22 ± 4.97 mg/kg; T3: 419.75 ± 295.44 mg/kg) over the storage period.

According to EC Decision 95/149, there is no maximum limit for TVB; however, the limit was fixed to 25 mg N/100g in rainbow trout as proposed by Gimenez *et al.* (2002) [25, 26]. Although the present values at the end of storage period were below the limit suggested by Gimenez *et al.*, the fish was in an evident state of sensorial deterioration, which had gone undetected by TVB analysis [26].

The compounds that form TVB are present in varying concentrations in muscle according to the kind of fish [7]. Unlike marine fish, freshwater fish generally have negligible values of trimethylamine oxide. Low levels of trimethylamine and the presence of ammonia are the main compounds that form part of TVB. Due to the low trimethylamine formation, the TVB concentration in freshwater fish species remains low during storage [8, 27]. In addition, Morishita *et al.* reported that factors such as age, location and method of cultivation may influence the non-protein nitrogen compound content in the fish muscle, which may influence the TVB levels [28].

In the present study the TVB determination was not considered a good parameter for evaluating the quality index, since the values observed in this analysis did not even change at the end of storage period (Table 1) when the fish reached a state of deterioration (Figures 2 and 3). Other studies have confirmed the present results, demonstrating that TVB is not a good parameter for evaluating the quality index of freshwater fish [24, 27]. Therefore, TVB may be considered an uncertain and unreliable decay index for freshwater fish.

Regarding ammonia, there was a correlation with the state of sensorial deterioration, since it was detected after the 11th day of storage in the samples. Ammonia is the main compound belonging to this set of volatile bases in freshwater fish species. This analysis is therefore considered satisfactory for evaluating the process of protein degradation; hence it may be used to determine the quality index on these fish species [8].

The pH values increased continuously over the storage period, reaching higher values in T3 (6.77±0.13). This increase was due to the production of basic compounds formed during the autolytic changes [6]. According to Rodriguez *et al.* the accumulation of alkaline metabolites, such as amines, promotes an increase in muscle pH, indicating a deterioration process [9].

When analyzing the results of biogenic amines, the putrescine and cadaverine concentrations increased significantly throughout the storage period. The behavior of these amines in trout flesh was also observed by other researchers [11, 15]. Studying the biochemical changes in rainbow trout stored for 12 days, Rodriguez *et al.* observed an increase of putrescine while cadaverine was detected only after 9 days of storage [11]. The authors suggest that the presence of cadaverine may serve as an indicator of muscle change, which is caused by increased activity of microorganisms. Furthermore, putrescine may be an indicator of premature muscle autolytic degradation, since it is formed during the first days of storage.

According Dawood *et al.* the rapid formation of putrescine in fish is due to high enzymatic activity (due to the microflora contaminant) that promotes both the decarboxylation of glutamic acid and arginine and the synthesis of ornithine, which results in putrescine formation [12]. The authors suggest that putrescine and cadaverine may be reliable indicators of fish spoilage. Likewise, Rezaei *et al.* studying the presence of biogenic amines in rainbow trout stored for 18 days, observed an increase of amines during the storage period and

suggested that monitoring the putrescine levels may serve as an index to evaluate the freshness of rainbow trout [15].

According to Gram and Dalgaard and Halász *et al.* the increase and the formation of these amines is related to the bacterial load present in meat [3, 29]. Halász *et al.* observed that the bacteria of the family Enterobacteriaceae are usually implicated in the formation of cadaverine [29]. On the other hand, bacteria of the genus Pseudomonas spp. are responsible for the formation of putrescine.

Other previous studies [4, 15, 30-32] have demonstrated that the formation of biogenic amines depends on several factors that may alter the concentration of these amines in the food matrix. These factors include: aquaculture conditions, food, fish species, body composition, storage and processing conditions, autolytic interactions, availability of free amino acids, and the presence of decarboxylase-active microorganisms.

Based on the results of the present study, it appears that the biogenic amines, putrescine and cadaverine, may be considered suitable indicators of the degradation process of rainbow trout meat. Furthermore, the presence of ammonia and changes in pH may be regarded as quality parameters to evaluate this species. Finally, the total volatile bases (TVB) were not useful in assessing the deterioration level of the fish studied.

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-26/111.933/2011, E-26/110.460/2012 and E-26/103.003/2012. BL Rodrigues was supported by the National Council for Scientific and Technological Development (CNPq). Trutas da Serrinha is gratefully acknowledged for providing samples. The authors would like to thank Ricky Toledano for the English revision of the manuscript.

REFERENCES

1. Fallah AA, Saei-Dehkordi SS, Nematollahi A (2011) Comparative assessment of proximate composition, physicochemical parameters, fatty acid profile and mineral content in farmed and wild rainbow trout (*Oncorhynchus mykiss*). Int J Food Sci Tech 46: 767–773.

2. Gram L, Huss HH (1996) Microbiological spoilage of fish and fish products. Int J Food Microbiol 33: 121-37.

3. Gram L, Dalgaard P (2002) Fish spoilage bacteria-problems and solutions. Curr Opin Biotechnol 13: 262–266.

4. Liston J (1980) Microbiology in fishery science. In: *Advances in fish science and technology. Fishing News Books*. Edited by JJ Connell. England: Surrey.

5. Arashisar S, Hisar O, Kaya M, Yanik, T (2004) Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorhynchus mykiss*) fillets. Int J Food Microbiol 97: 209–214.

6. Huss HH (1995) Quality and quality changes in freshwater fish. Fisheries Technical Paper N^{o}. 348. Rome: Food and Agriculture Organization (FAO) of United Nations [http://www.fao.org/docrep/V7180E/V7180E00.HTM]

7. Giannini DH (2003) Determinación de nitrógeno básico volátil (NBV) em pescado: Consideraciones Generales. Alimentaria 40: 49–54.

8. Zaitsev V, Kizevetter I, Lagunov L, Makarova T, Minder L, Podsevalov V (1969) Caracteristics of fish as a raw material for industry. In: *Fish curing and processing*. Moscou.

9. Rodríguez O, Losada V, Aubourg SP, Barros-Velázquez J (2004) Enhanced shelf-life of chilled European hake (*Merluccius merluccius*) stored in slurry ice as determined by sensory analysis and assessment of microbiological activity. Food Res Int 37: 749-757.

10. Mietz JL, Karmas E (1977) Chemical quality index of canned tuna as determined by high-pressure liquid chromatography. J Food Sci 42: 155–158.

11. Rodriguez CJ, Besteiro I, Pascual C (1999) Biochemical changes in freshwater rainbow trout (*Oncorhynchus mykiss*) during chilled storage. J Sci Food Agric 79: 1473–1480.

12. Dawood AA, Karkalas J, Roy RN, Williams CS (1988) The occurrence of non-volatile amines in chilled-stored rainbow trout (*Salmo irideus*). Food Chem 27: 33–45.

13. Krizek M, Pavlicek T, Vacha F (2002) Formation of selected biogenic amines in carp meat. J Sci Food Agric 82: 1088–1093.

14. Katikou P, Georgantelis D, Paleologos EK, Ambrosiadis I, Kontominas MG (2006) Relation of biogenic amines' formation with microbiological and sensory attributes in lactobacillus-inoculated vacuum-packed rainbow trout (*Oncorhynchus mykiss*) fillets. J Agric Food Chem 54: 4277-4283.

15. Rezaei M, Montazeri N, Langrudi HE, Mokhayer B, Parviz M, Nazarinia A (2007) The biogenic amines and bacterial changes of farmed rainbow trout (*Oncorhynchus mykiss*) stored in ice. Food Chem 103: 150–154.

16. Hwang DF, Chang SH, Shiua CY, Chai TJ (1997) High-performance liquid chromatogramphic determination of biogenic amines in fish implicated in food poisoning. J Chromatogr B 693: 23–30.

17. Cinquina AL, Calì A, Longo F, Santis L, Severoni A, Abballe F (2004) Determination of biogenic amines in fish tissues by ion-exchange chromatography with conductivity detection. J Chromatogr A 1032: 73–77.

18. Ozogul F, Taylor KDA, Quantick P, Ozogul Y (2002) Biogenic amines formation in Atlantic herring (*Clupea harengus*) stored under modified atmosphere packaging using a rapid HPLC method. Int J Food Sci Tech 37: 515–522.

19. Coloso R M, King K, Fletcher JW, Weis P, Werner A, Ferraris R P (2003) Dietary P regulates phosphate transporter expression, phosphatase activity, and effluent P partitioning in trout culture. J Comp Physiol B 173: 519–530.

20. Conte Junior CA, Peixoto BTM, Lopes MM, Franco RM, Freitas MQ, Fernández M, Mano SB (2010) Effect of modified atmosphere packaging on the growth/survival of *Yersinia enterocolitica* and natural flora on fresh poultry sausage. In: *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. *Volume 2.* Edited by Méndez-Vilas A. Badajoz: Formatex.

21. Conway EJ, Byrne A (1933) An absorption apparatus for the micro-determination of certain volatile substances: The micro-determination of ammonia. Biochem J 27: 419–429.

22. Rodríguez SC, López B, Chaves AR (2001) Effect of different treatments on the evolution of polyamines during refrigerated storage of eggplants. J Agric Food Chem 49: 4700-4705.

23. Mei YH (1994) A sensitive and fast method for the determination of polyamines in biological samples. Benzoyl chloride pre-column derivatization high-performance liquid chromatography. J Liq Chrom 17: 2413-2418.

24. Chytiri S, Chouliara I, Savvaidis IN, Kontominas MG (2004) Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. Food Microbiology 21: 157–165.

25. European Union (1995) Commission decision 95/149/EC, 8, March 1995. Off J Eur Comm. L97, 84–87.

26. Gimenez B, Roncales P, Beltran JA (2002) Modified atmosphere packaging of filleted rainbow trout. J Sci Food Agric 84: 1154–1159.

27. Scherer R, Augusti PR, Bochi VC, Steffens C, Fries LLM, Daniel AP, Kubota EH, Neto JR, Emanuelli T (2006) Chemical and microbiological quality of grass carp (*Ctenopharyngodon idella*) slaughtered by different methods. Food Chem 99: 136–142.

28. Morishita T, Uno K, Araki T, Takahashi T (1989) Comparison of the amounts of extractive nitrogenous constituents in the meats of cultured red sea bream of different localities and culture methods and those of wild fish. Nippon Suisan Gakk 55: 1565–1573.

29. Halász A, Baráth Á, Simon-Sarkadi L, Holzapfel W (1994) Biogenic amines and their production by microorganisms in food. Trends Food Sci Technol 5: 42-49.

30. Shahidi F (1994) The chemistry processing technology and quality of seafoods-an overview. In: *Seafoods chemistry, processing technology and quality of seafoods*. Edited by Shahidi F, Botta JR. Great Britain.

31. Bodmer S, Imark C, Kneubuhl M (1999) Biogenic amines in foods: histamine and food processing. Inflamm Res 48: 296–300.

32. Krizek M, Vacha F, Vorlova L, Lukasova J, Cupakova S (2004) Biogenic amines in vacuum-packed and non-vacuum packed flesh of Carp (*Cyprinus carpio*) stored at different temperatures. Food Chem 88: 185–191.

Legends to figures:

Figure 1. Experimental design of the study.

Figure 2. Cadaverine concentrations (mg/kg) over the period of storage. T1 = analysis of the first five days of storage; T2 = analysis of the subsequent five days; T3 = analysis of the last five days of storage. # significantly different from T1 and T2.

Figure 3. Putrescine concentrations (mg/kg) over the period of storage. T1 = analysis of the first five days of storage; T2 = analysis of the subsequent five days; T3 = analysis of the last five days of storage. # significantly different from T1 and T2. * significantly different from T1.

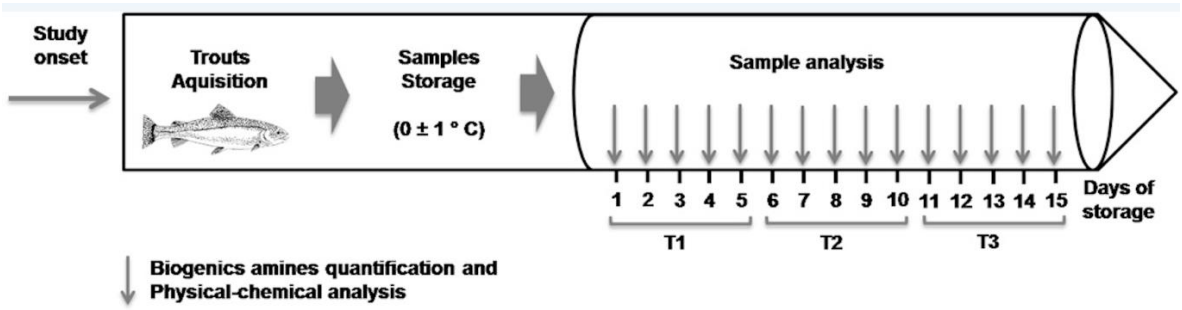


Figure 1: Experimental design of the study

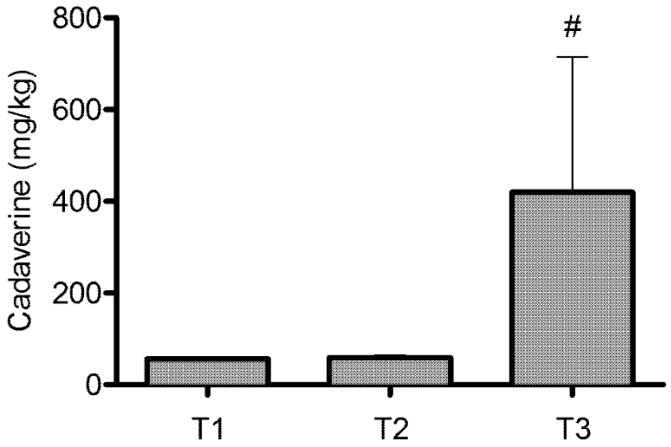


Figure 2: Cadaverine concentrations (mg/kg) over the period of storage. T1 = analysis of the first five days of storage; T2 = analysis of the subsequent five days; T3 = analysis of the last five days of storage. # significantly different from T1 and T2.

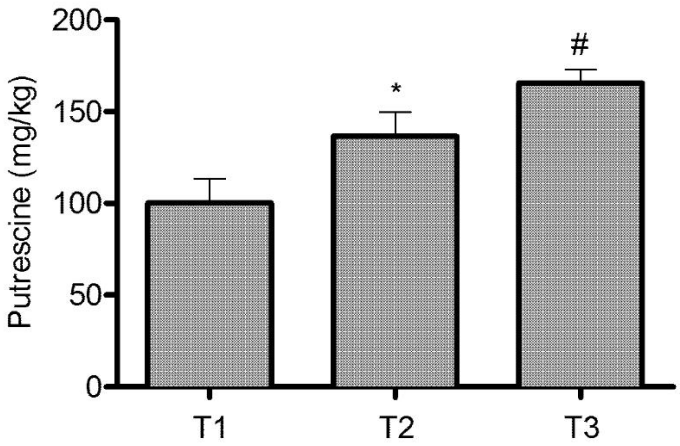


Figure 3: Putrescine concentrations (mg/kg) over the period of storage. T1 = analysis of the first five days of storage; T2 = analysis of the subsequent five days; T3 = analysis of the last five days of storage. # significantly different from T1 and T2. * significantly different from T1.

Table 1. Mean & standard deviation values for total volatile basis (TVB) and hydrogen potential (pH) over the period of storage.

	T1	T2	T3
TVB (mg N/100g)	9.30±0.58	9.07±1.19	9.07±0.0
pH	6.47±0.19	6.73±0.09	6.77±0.13*

T1 = analysis of the first five days of storage; T2 = analysis of the subsequent five days; T3 = analysis of the last five days of storage. * Significantly different from T1 (only pH; $P < 0.05$).

3.2 ARTIGO II: INFLUENCE OF PACKAGED AND GAS COMPOSITION IN UV-C RADIATION EFFECTIVENESS IN RAINBOW TROUT (*Oncorhynchus mykiss*). Sent to *Journal of Stored Products Research*.

Influence of packaging and gas composition on UV-C radiation effectiveness in rainbow trout (*Oncorhynchus mykiss*)

Bruna Leal Rodrigues[1], Thiago da Silveira Alvares[2], Marion Pereira da Costa[1], Guilherme Sicca Lopes Sampaio[1], César Aquiles Lázaro de la Torre[1], Eliane Teixeira Marsico[1] and Carlos Adam Conte Júnior[1]*

[1]Department of Food Technology, Faculty of Veterinary, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil.

[2]Nucleus of Basic Nutrition and Dietetics, Universidade Federal do Rio de Janeiro, Macaé, Rio de Janeiro, Brazil.

* Corresponding Author:

Professor Carlos Adam Conte Júnior

Faculty of Veterinary Medicine - Department of Food Technology

Universidade Federal Fluminense

Rua Vital Brazil Filho 64, CEP: 24230-340, Niterói, Rio de Janeiro, Brazil.

Phone: 55(21) 2629- 9545.

Email: mtaconte@vm.uff.br

ABSTRACT

The present study evaluated the effectiveness of short-wave ultraviolet (UV-C) radiation on rainbow trout fillets inoculated with *Proteus mirabilis*. In addition, the influence of the type of package (laminate packaging – LP; packaging with gas barrier – GB; package without gas barrier – WGB) and gas composition on the efficiency of UV-C radiation for the conservation of this species of fish was also investigated. Two pounds of rainbow trout were inoculated, packaged under aerobic conditions, under different ratios of CO_2 and N_2 gases and subjected to UV-C radiation. Microbiological analyses were performed. The results show that a 0.1001±0.01 J/cm^2 dose of UV-C promoted reduction of 1.8 log (CFU.g^{-1}) of *Proteus mirabilis.* Different ratios of CO_2 and N_2 gas did not alter the effects of UV-C radiation at different UV-C doses. The passage of UV-C radiation was significantly reduced in all packaging (LP > GB > WGB) when compared to a control. The results suggest that UV-C radiation improves the surface quality of packaged rainbow trout fillets. The use of gases (modified atmosphere technology) did not interfere with the action of UV-C rays on the fillets. Packaging with and without the gas barrier are suitable for submission to UV-C radiation.

Keywords: fresh water fish, UV-C radiation, packaged, modified atmosphere, *Proteus mirabilis*.

1.Introduction

Rainbow trout is one of the major fish species cultivated and marketed by commercial aquaculture, mainly due to the advantageous husbandry characteristics, its nutritional properties and its wide acceptance as meat by the market (Conor, 2000; Coloso et al. 2003, Sidhu, 2003). However, the quality of fresh trout is still a major concern for the food industry and consumers due to high perishability. Mainly due to chemical composition and microorganism activity, spoilage of fish occurs rapidly resulting in a loss of quality and shorter commercial shelf life (Gram L and Huss H, 1996; Gram L and Dalgaard, 2002; Rodrigues et al., 2012; Rodrigues et al., 2013).

Several methods of preservation have been studied to control the rapid deterioration of fish meat. Ultraviolet radiation is a non-thermal technology used in food processing to inactivate pathogenic and spoilage microorganisms present on food surfaces (Chun et al., 2010; Haughton et al., 2011). UV-C radiation (wavelength 253.7 nm) is used to obtain a germicidal effect and has been approved by the FDA for use on food product surfaces (Bintsis et al., 2000; US Food and Drug Administration, 2007). This technology has some technical advantages: it is an effective and relatively inexpensive process that is easy to implement, and that does not generate chemical and radioactive residues (Chun et al., 2009).

Besides UV-C radiation, conservation methods that act over a long time period, such as modified atmosphere packaging technology, have been studied for their ability to extend the commercial shelf life of products by preserving freshness and quality (Giménez et al., 2002; Monteiro et al., 2013). The combination of UV-C radiation and modified atmosphere may be a strategy to effectively reduce the microbial load and therefore enhance the shelf life of products. However, there are no studies that evaluate the influence of gas ratios on the efficiency of UV-C radiation on the product surface. Therefore, it may be that the presence of these gases may hinder the effectiveness of UV-C radiation, but such a relationship would

need closer examination. In addition, the choice of packaging also may be relevant when these two techniques are combined, thus certain packaging may impair the penetration of UV-C radiation and thereby reduce the effectiveness of this preservation method.

The application of UV-C radiation may have a special importance in pre-packaged products. Since packaging is the last step in food production without further safety treatments, UV-C radiation could be important to eliminate possible contamination by instruments or personnel and to decrease the risk of possible microorganism activity (Chun et al., 2009). However, specific studies for each microorganism and food matrix are required to evaluate the efficiency of UV-C radiation since their sensitivity may vary (Sastry et al., 2000).

Although many studies have evaluated the application of UV-C in pathogenic microorganisms (Chun et al., 2010; Haughton et al., 2011; Keklik et al., 2010; Sommers et al., 2010; Unluturk et al., 2008), there is a lack of information about the effectiveness of this method in *Proteus* sp. and in fish meat. This microorganism may either be part of the natural microbiote of fish, or its presence may be caused by contamination during food processing (Ruiz-Capillas and Jiménez- Colmenero, 2010). Moreover, these microorganisms have the capacity to produce decarboxylase enzymes and are strong producers of biogenic amines in fish, therefore constituting a danger to public health (Houicher et al., 2013).

Due to the fact that there is limited scientific evidence about the efficiency of UV-C on products that are already packaged, this study aims to evaluate the UV-C radiation effectiveness in rainbow trout fillets inoculated with *Proteus mirabilis*, packaged under aerobic conditions and different proportions of gases (CO_2 and N_2). Furthermore, analyses of packaging materials that allows the best response when applying UV-C radiation was also addressed.

We hypothesized that UV-C radiation will inactivate microorganisms present on food surfaces. In addition, the presence of gases and packaging will impair the effectiveness of UV-C radiation in fillet already packaged.

2. Material and Methods

2.1 UV-C equipment

A stainless steel barrel-shaped chamber was constructed to perform the experiments. Twelve UV-C lamps, six of 30W and six of 55W (OSRAM™ HNS, OFR, Munich, Germany), were distributed in interspersed positions inside the chamber. Nylon netting was used to position the samples in the geometrical center of the chamber. The intensities applied were 0.713 mW/cm^2 (30W lamps), 1.065 mW/cm^2 (55W lamps) and 1.668 mW/cm^2 (30 and 55W lamps), determined by UV radiometer (MRUR-203™, Instrutherm, São Paulo, Brazil). In order to determine the higher irradiance inside the chamber, different locations throughout the nylon net were tested by UV radiometer.

2.2 Packaging material

In order to evaluate the packaging that allows superior penetration of UV-C radiation, three types of low-density polyethylene commercial packaging were tested while empty with three doses (0.0428±0.00, 0.0639±0.00 and 0.1001±0.01 J/cm^2): (1) Laminate packaging– LP (20 cm x 30cm); (2) Packaging with gas barrier– GB (20cm x 35cm); (3) Packaging without gas barrier– WGB (19cm x 30cm). A radiometer was located inside the empty package and the irradiation was measured for 60 s. The measurements were performed in triplicate. Furthermore, these intensities were measured without the presence of packaging, constituting control values.

2.3 Preparation of inoculums

One serotype of *Proteus mirabilis* (INCQS 00265) was used in this study. This serotype was obtained from Oswaldo Cruz Foundation (Rio de Janeiro, Brazil). The inoculations were performed according to Sant'ana et al. (2012). In brief, the culture was grown in Falcon tubes containing 30 mL of BHI at 37°C for 24h, and the process was repeated twice. This initial step was used to obtain bacterial cultures in the stationary phase of growth at a concentration of 8 log CFU/mL. After the final incubation, cultures were centrifuged at 1000 *g* for 15 min at 4°C. Cell pellets were washed three times with sterile phosphate buffered saline (PBS, pH 6.0). The bacterial concentration was determined by measuring the optical density at 600nm (OD600) by UV spectrophotometer (Smartspec Plus, BioRad, Hercules, CA, USA).

2.4 Fish meat samples and inoculation

Two kilograms of eviscerated rainbow trout were obtained from the Trutas da Serrinha Company located in Itatiaia, Rio de Janeiro, Brazil. The samples were packed in ice (0±1°C) and transported in a Styrofoam box to the laboratory. In order to obtain fillet samples of 8 x 8 cm, the trout was filleted under sterile conditions.

Sixteen samples were aseptically packaged in packaging with gas barrier and conditioned at 4°C until inoculation, which did not exceed two hours. Each package was opened, and one milliliter of inoculum solution with OD600 (corresponding to approximately 7.0×10^8 cells) was inoculated onto the surface of each trout filet. After that, samples were packaged using different proportions of gases and submitted to UV radiation.

2.5 Different gas ratio and UV radiation

Sixteen treatments were performed using either separate or combined conservation methods (Figure 1). Nine treatments were performed by combining three doses of UV radiation (0.0428±0.00, 0.0639±0.00 and 0.1001±0.01 J/cm^2) with three different proportions of gases (30%, 50% and 70% of carbon dioxide; the remaining volume was completed by nitrogen gas and vacuum was performed prior to the addition of modified atmosphere gases (TECMAQ, Vacuum sealer, AP 450). Three treatments involved UV radiation alone (at three treatment levels with doses of 0.0428±0.00, 0.0639±0.00 and 0.1001±0.01 J/cm^2) and three involved gas ratios alone (at 30%, 50% and 70% of carbon dioxide). One study observed an extended shelf life of freshwater fish by using combinations of 40-60% CO_2 (Boziaris, et al. 2014) in order to inhibit deteriorating compounds and bacterial growth. However, others studies indicate that lower (30%) and higher (70%) concentrations of this gas can have the positive effect of the inhibiting bacterial growth (Bouletis et al., 2013; Provincial et al. 2013; Yew et al. 2013). Based on the results of the above mentioned studies, we chose to use low (30%), medium (50%) and high (70%) concentration of CO_2 gas for the present study. The control sample (positive control) was inoculated and packaged without UV radiation or modified atmosphere.

UV exposure was administered for 60 s in all treatments. Each sample was placed in a central area (10 x 40 cm^2) of the unit and irradiated simultaneously on both surfaces. After packaging at different gas proportions and after administering UV radiation doses, bacteriological tests were performed.

The UV-C doses 0.0428±0.00, 0.0639±0.00 and 0.1001±0.01 J/cm^2 were used because the UV equipment used in this study did not support the inclusion of higher power lamps. The exposure time of 60 s was chosen based on previous results by Ozer and Demirci (2006), who

found that *E. coli* and *Listeria monocytogenes* were reduced by 1 log when applying UV-C radiation.

2.6 Bacteriological analysis

A swab method was performed on the surface of fillets with the aid of a sterilized mold by autoclaving (2 x 5 cm). The swabs were placed in tubes containing saline and 6-fold serial dilutions. An amount of 1 mL was removed from each dilution and cultured by the pour plate technique in triplicate on Salmonella-Shigella agar. The plates were incubated at 37°C for 48 h. One additional fillet was tested for the initial presence of *Proteus* species (negative control). Culture forming units were counted and expressed as log $CFU.g^{-1}$ (Isohanni and Lyhs, 2009).

2.7 Statistical analysis

Two-way analysis of variance (ANOVA) with repeated measures on two factors (4 x 3; packaging material x UV-C doses) was used to identify differences in absorption of UV-C doses radiation for different types of packaging. A two-way ANOVA with repeated measures on two factors (4 x 4; UV-C doses x proportion of gases) was utilized to identify differences in the reduction of bacterial load of *Proteus mirabilis* when submitted to doses of UV-C radiation with or without the use of different proportions of gases. When a significant *F* was found, additional post-hoc tests with Bonferroni adjustment were performed. Statistical significance was set at the 0.05 level of confidence. All analyses were performed using the commercially available statistical package XLSTAT version 2013.2.03 (Addinsoft, Paris, France).

3. Results

The results of the evaluation of different packaging and different UV-C intensities are shown in Table 1.

All packages allowed the penetration of at least some UV-C radiation with exception of the laminate packaging that did not allow the passage of UV-C radiation regardless of intensity. Packages with and without gas barrier differed only significantly when the highest UV-C dose (0.1001±0.01 J/cm^2) was applied. Overall, the packaging without gas barrier allowed higher passage of UV-C radiation.

The results of log reduction of *Proteus mirabilis* on rainbow trout fillets with UV-C radiation at different doses and gas ratios are shown in Table 2. Results were statistically different only when the highest dose (0.1001±0.01 J/cm^2) of UV-C was applied under aerobic conditions, resulting in a reduction of 1.8 log (CFU/g) of *Proteus mirabilis*.

4. Discussion

All packaging, when subjected to different UV-C doses (0.0428±0.00, 0.0639±0.00 and 0.1001±0.01 J/cm^2), resulted in statistical differences compared to no packaging, which demonstrates that packaging materials may hinder the passage of UV –C radiation (Table 1).

The use of laminated packages was previously suggested as a strategy to reduce the production of free radicals derived from oxidation of unsaturated fatty acids and by exposure to sun radiation, among other factors (Zuta et al., 2007). However, our results suggest that laminated packaging blocks the passage of ultraviolet rays by 100%, even at the highest UV-C dose (0.1001±0.01 J/cm^2). This phenomenon may be due to reflectance from the lamination of the packaging, where the incident radiation is equal to reflected radiation, hindering or even preventing the passage of UV-C rays (Ringus and Moraru, 2013; Warriner et al., 2000;

Woodling and Moraru, 2005). Therefore, this packaging is not recommended for products to be subjected to the process of UV-C radiation.

Our results indicate that packaging without gas barrier allows a higher passage of UV-C compared to packaging with gas barrier, when exposed to a radiation dose of 0.1001±0.01 J/cm^2. However, it can only be used for products packed under aerobic conditions since it does not have barriers to prevent gas exchange with the environment and therefore cannot be used for vacuum packing foods or modified atmosphere packaging. Thus, gas barrier packaging is recommended for conditioned products undergoing ultraviolet radiation and modified atmosphere packaging, allowing the passage of UV-C rays and preventing gas exchange with the environment (Robertson, 2013).

Radiation of 0.1001±0.01 J/cm^2 affected bacterial growth on inoculated trout fillets, resulting in a reduction of 1.8 log (CFU.g^{-1}) of *Proteus mirabilis.* Different ratios of CO_2 and N_2 gas did not alter the effect of UV-C radiation at different doses, indicating that the use of different gas proportions does not interfere with the exposure of the product to UV-C rays.

The mode of action of UV-C has been attributed to cross-linking between the nucleic acid thymine and cytosine of DNA in microorganisms caused by absorption of UV-C radiation. This bond results in the blocking of DNA transcription and replication, inhibiting cell growth, leading to cell death and decreasing the bacterial load on the food surface (Guerrero-Beltran and Barbosa-Canovas, 2004; McDonald et al., 2000).

There are few studies that examine UV-C radiation effectiveness against *Proteus mirabilis* and on fish meat. However, our results are in agreement with previous studies that showed a reduction of other bacterial groups and food matrices. UV-C exposure of up to 0.192 J/cm^2 for 32 seconds reduced *E.coli*, *S. enteritidis* and *Enterobacteriaceae* on chicken skin by 0.77, 1.01 and 0.30 log CFU/g, respectively, and on skinless chicken fillet by 0.98, 1.34 and 1.29 log CFU/g for the same microorganisms, respectively (Haughton et al., 2011).

Chun et al. (2010) reported an initial population reduction of *S. typhimurium* by 1.19 log CFU/g in chicken breast submitted to 0.05 J/cm^2. Unluturk et al. (2010) observed a reduction of 1.403 log CFU/g in the pathogenic *E. coli* strain 0157:H7 in liquid egg white when exposed to UV-C radiation of 1.57 J/cm^2 for 20 min.

As noted, the reduction of bacterial load varies depending on the microorganism and on the food matrix. Characteristics of the food matrix and the UV-C source, such as food surface topography and composition, microorganism locality and the power and wavelength of the UV-C lamp, can interfere with the efficiency of UV-C radiation to reduce superficial bacterial load (Chun et al., 2009; Sastry et al., 2000; Stoops et al., 2013; Woodling and Moraru, 2005). Furthermore, food matrices with a greater load of gram-negative bacteria respond better to UV-C radiation since these microorganisms have only one layer or several thin layers of the outer membrane leading to a lower resistance to UV-C radiation than gram-positive bacteria (Blatchley et al., 2001; Unluturk et al., 2010).

Based on the results of the present study, UV-C radiation improves the surface quality of packaged rainbow trout fillets. Furthermore, the use of different gas proportions in modified atmosphere technology did not interfere with the action of UV-C rays on the fillets. Finally, packaging with and without gas barrier are suitable for submission to UV-C radiation.

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-26/103.003/2012 and E-26/11.673/2013. BL Rodrigues was supported by the National Council for Scientific and Technological Development (CNPq). Trutas da Serrinha is gratefully acknowledged for providing samples. The authors would like to thank Dagmar Frisch and Ricky Toledano for the English revision of the manuscript.

5. References

Bintsis, T., Litopoulou-Tzanetaki, E., Robinson, R.K., 2000. Existing and potencial applications of ultraviolet light in food industry – a critical review. Journal of the Science of Food and Agriculture 80, 637-645.

Blatchley, E.R., Dumoutier, N., Halaby, T.N., Levi, Y., Laine, J.M., 2001. Bacterial responses to ultraviolet irradiation. Water Science and Technology 43, 179-186.

Bouletis, A.D., Arvanitoyannis, I.S., Hadjichristodoulou, C., Neofitou, C., Sakkomitrou, M., Kolokythopoulou, F., 2013. The effect of modified atmosphere packaging on the microbiological, physical, chemical and sensory characteristics of broadtail squid (*Illex coindetii*). International Journal of Food Science & Technology, 2013 (IN PRESS).

Boziaris, I.S., 2014. Seafood Processing: Technology, Quality and Safety, first ed. John Wiley & Sons, UK.

Chun, H., Kim, J., Chung, K., Won, M., Song, K.B., 2009. Inactivation kinetics of *Listeria monocytogenes*, *Salmonella enterica* serovar *Typhimurium*, and *Campylobacter jejuni* in ready-to-eat sliced ham using UV-C irradiation. Meat Science 83, 599-603.

Chun, H.H., Kim, J.Y., Song, K.B., 2010. Inactivation of foodborne pathogens in ready-to-eat salad using UV-C irradiation. Food Science and Biotechnology 19, 547-551.

Chun, H.H., Kim J.Y, Lee, B.D., Yu, D.J., Song, K.B., 2010. Effect of UV-C irradiation on the inactivation of inoculated pathogens and quality of chicken breasts during storage. Food Control 21, 276-280.

Coloso, R.M., King, K., Fletcher, J.W., Weis, P., Werner, A., Ferraris, R.P., 2003. Dietary P regulates phosphate transporter expression, phosphatase activity, and effluent P partitioning in trout culture. Journal of Comparative Physiology B 173, 519-530.

Conor, W.E., 2000. Importance of n-3 fatty acids in health and disease. American Journal of Clinical Nutrition 71,171S-175S.

Giménez, B., Roncales, P., Beltran, J.A., 2002. Modified atmosphere packaging of filleted rainbow trout. Journal of the Science of Food and Agriculture 84, 1154-1159.

Gram, L., Huss, H., 1996. Microbiological spoilage of fish and fish products. International Journal of Food Microbiology 33, 121-137.

Gram, L., Dalgaard, P., 2002. Fish spoilage bacteria-problems and solutions. Current Opinion in Biotechnology 13, 262-266.

Guerrero-Beltran, J.A., Barbosa-Canovas, G.V., 2004. Review: advantages and limitations on processing foods by UV light. Food Science and Technology International 10, 137–147.

Haughton, P.N., Lyng, J.G., Cronin, D.A., Morgan, D.J., Fanning, S., Whyte, P., 2011. Efficacy of UV Light Treatment for the Microbiological Decontamination of Chicken, Associated Packaging, and Contact Surfaces. Journal of Food Protection 74, 565-572.

Houicher, A., Kuley, E., Bendeddouche, B., Ozogul, F., 2013. Histamine and tyramine production by bacteria isolated form spoiled sardine (*Sardina pilchardus*). African Journal of Biotechnology 12, 3288-3295.

Isohanni, P.M.I., Lyhs, U., 2009. Use of ultraviolet irradiation to reduce *Campylobacter jejuni* on broiler meat. Poultry Science 88, 661-668.

Keklik, N.M., Demirci, A., Puri, V.M., 2010. Decontamination of unpackaged and vacuum-packaged boneless chicken breast with pulsed ultraviolet light. Poultry Science 89, 570-581.

McDonald, K.F., Curry, R. D., Clevenger, T. E., Unklesbay, K., Eisenstark, A., Golden, J., Morgan, R.D., 2000. A comparison of pulsed and continuous ultraviolet light sources for the decontamination of surfaces. IEEE Transactions on Plasma Science 28, 1581–1587.

Monteiro, M.L.G., Mársico, E.T., Mano, S.B., Teixeira, C.E., Canto, A.C.V.C.S., De Carvalho Vital, H., Conte-Júnior, C.A., 2013. Influence of good manufacturing practices on the shelf life of refrigerated fillets of tilapia (*Oreochromis niloticus*) packed in modified atmosphere and gamma-irradiated. Food Science and Nutrition 1, 298-306.

Ozer, N.P., Demirci, A., 2006. Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* inoculated on raw salmon fillets by pulsed UV-light treatment. International Journal of Food Science and Technology 41, 354-360.

Provincial, L., Guillén, E., Gil, M., Alonso, V., Roncalés, P., Beltrán, J.A., 2013. Survival of *Listeria monocytogenes* and *Salmonella Enteritidis* in sea bream (*Sparus aurata*) fillets packaged under enriched CO_2 modified atmospheres. International Journal of Food Microbiology 162, 213-219.

Ringus, D.L., Moraru, C.I., 2013. Pulsed Light inactivation of *Listeria innocua* on food packaging materials of different surface roughness and reflectivity. Journal of Food Engineering 114, 331-337.

Robertson, G.L., 2013. Food packaging: Principles and practice, third ed. CRC Press.

Rodrigues, B.L., Santos, L.R., Márs ico, E.T., Camarinha, C.C., Mano, S.B., Conte Junior, C. A., 2012. Qualidade físico-química do pescado utilizado na elaboração de sushis e sashimis de atum e salmão comercializados no município do Rio de Janeiro, Brasil. Semina Ciências Agrárias (Impresso) 33, 1849-1856.

Rodrigues, B.L., Álvares, T.S., Costa, M.P., Sampaio, G.S.L., Lázaro De La Torre, C.A., Márs ico, E.T., Conte-Junior, C.A., 2013. Concentration of biogenic amines in rainbow trout (*Oncorhynchus mykiss*) preserved in ice and its relationship with physicochemical parameters of quality. Journal of Aquaculture Research & Development 4, 174-177.

Ruiz-Capillas, C., Jiménez-Colmenero, F., 2010. Biogenic amines in seafood products. In: L.M.L. Nollet, & F. Toldora (Eds.), Handbook of Seafood and Seafood Products Analysis. New York: Taylor & Francis Group, CRC Press, pp. 833-850.

Sant'ana, A.S., Barbosa, M.S., Destro, M.T., Landgraf, M., Franco, B.D.G.M., 2012. Growth potential of *Salmonella* spp. and *Listeria monocytogenes* in nine types of ready-to-eat

vegetables stored at variable temperature conditions during shelf-life. International Journal of Food Microbiology 157, 52-58.

Sastry, S.K., Datta, A.K., Worobo, R.W., 2000. Ultraviolet light. Journal of Food Science Supplement 65, 90-92.

Sidhu, K.S., 2003. Health benefits and potential risks related to consumption of fish or fish oil. Regulatory Toxicology and Pharmacology 38, 336–344.

Sommers, C.H., Sites, J.E., Musgrove, M., 2010. Ultraviolet light (254 nm) inactivation of pathogens on foods and stainless steel surfaces. Journal of Food Safety 30, 470-479.

Stoops, J., Jansen, M., Claes, J., Campenhout, L.V., 2013. Decontamination of powdery and granular foods using Continuous Wave UV radiation in a dynamic process. Journal of Food Engineering 119, 254-259.

Unluturk, S., Atilgan, M.R., Baysal, A.H., Tari C., 2008. Use of UV-C radiation as a non-thermal process for liquid egg products (LEP). Journal of Food Engineering 85, 561-568.

Unluturk, S., Atilgan, M.R., Baysal, A.H., Unluturk, M.S., 2010. Modeling inactivation kinetics of liquid egg white exposed to UV-C irradiation. International Journal of Food Microbiology 142, 341-347.

US Food and Drug Administration, 2007. Irradiation in the production, processing and handling of food. Available: http://www.foodsafety.gov/lrd/fcf179.html. Acessed 05 November 2013.

Warriner, K., Rysstad, G., Murden, A., Rumsby, P., Thomas, D., Waites, W.M., 2000. Inactivation of *Bacillus subtilis* spores on packaging surfaces by UV excimer laser irradiation. Journal of Applied Microbiology 88, 678-685.

Woodling, S.E., Moraru, C.I., 2005. Influence of surface topography on the effectiveness of pulsed light treatment for the inactivation of *Listeria innocua* on stainless-steel surfaces. Journal of Food Science 70, 345-351.

Yew, C.C., Bakar, F.A., Rahman, R.A., Bakar, J., Zaman, M.Z., Velu, S., Shariat, M., 2013. Effects of Modified Atmosphere Packaging with Various Carbon Dioxide Composition on Biogenic Amines Formation in Indian Mackerel (*Rastrelliger kanagurta*) stored at $5 \pm 1°C$. Packaging Technology and Science, 2013 (IN PRESS).

Zuta, P., Simpson, B., Zhao, X., Leclerc, L., 2007. The effect of a-tocopherol on the oxidation of mackerel oil. Food Chemistry 100, 800–807.

Figure legend:

Figure 1. UV-C radiation doses (0.0428, 0.0639 and 0.1001 J/cm^2) and different gas proportions (30%, 50% and 70% of carbon dioxide) used to perform sixteen treatments.

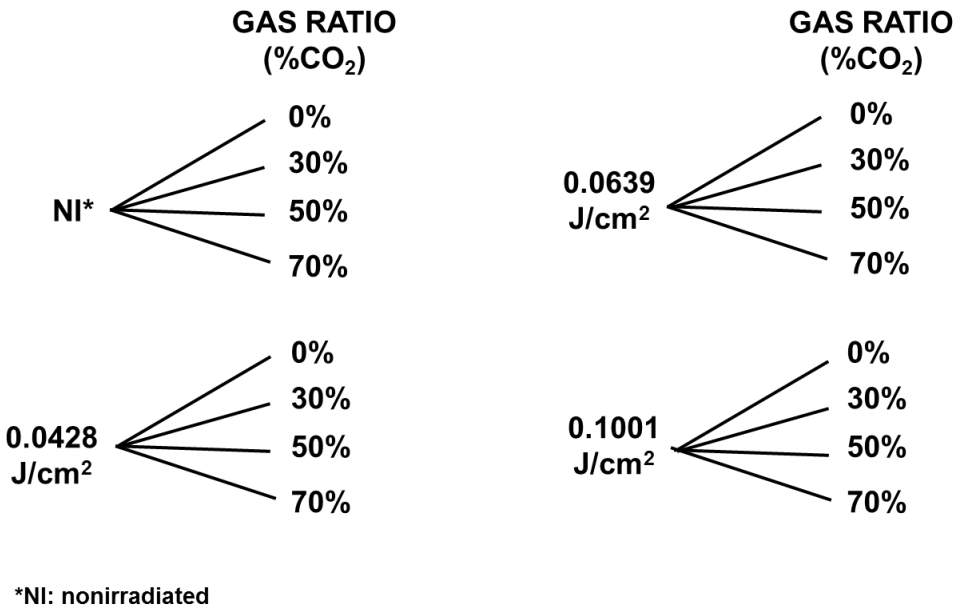


Figure 1. UV-C radiation doses (0.0428, 0.0639 and 0.1001 J/cm^2) and different gas proportions (30%, 50% and 70% of carbon dioxide) used to perform sixteen treatments.

Table 1. Residual UV-C doses (J/cm2) for different packaging (GB, WGB and LP).

UV-C Doses (J/cm^2)	Packages			
	UP	GB	WGB	LP
0.0428	0.0428 ± 0.00^{aA}	0.0316 ± 0.00^{bA}	0.0306 ± 0.01^{bA}	0.000 ± 0.00^{cD}
0.0639	0.0639 ± 0.00^{aB}	0.0435 ± 0.01^{bB}	0.0454 ± 0.00^{bB}	0.000 ± 0.00^{cD}
0.1001	0.1001 ± 0.01^{aC}	0.0692 ± 0.01^{bC}	0.0781 ± 0.00^{cC}	0.000 ± 0.00^{cD}

Values are means ±SD. UP: unpackaged; GB: gas barrier packaging; WGB: without gas barrier packaging; LP: laminate packaging. Different lowercase letters indicate significant differences (P <0.05) for values within a row. Means with different capital letters show significant differences (P <0.05) between values within a column.

Table 2. Results of log reduction of *Proteus mirabilis* on rainbow trout fillets with different UV-C light doses and gas ratios.

Gas Ratio ($\%CO_2$)	UV-C doses (J/cm^2)			
	0	0.0428	0.0639	0.1001
0	0.16 ± 0.40^{aA}	0.65 ± 0.47^{aA}	0.90 ± 0.70^{aA}	1.80 ± 0.11^{bA}
30	0.86 ± 0.76^{aA}	1.10 ± 0.72^{aA}	1.60 ± 0.70^{aA}	1.37 ± 0.58^{aA}
50	1.30 ± 1.22^{aA}	1.09 ± 0.53^{aA}	1.13 ± 0.47^{aA}	1.31 ± 0.67^{aA}
70	1.28 ± 0.25^{aA}	1.18 ± 0.38^{aA}	1.28 ± 0.42^{aA}	1.58 ± 0.72^{aA}

Values are means ±SD. Different lowercase letters indicate significant differences (P <0.05) for values within a row. Different capital letters indicate significant differences (P <0.05) between values within a column.

HIGHLIGHTS

- We evaluated the effectiveness of UV-C on trout fillets inoculated with *Proteus sp.*
- We assessed the influence of package and gas composition on the efficiency of UV-C
- UV-C radiation improves the surface quality of packaged rainbow trout fillet
- The use of gases did not interfere with the action of UV rays on the fillets
- Packaging with and without gas barrier are suitable for submission to UV radiation

4 CONSIDERAÇÕES FINAIS

Baseado nos resultados do presente estudo conclui-se que as aminas biogênicas putrescina e cadaverina podem servir como adequados indicadores de qualidade e frescor de trutas arco-íris inteiras. Além disso, a putrescina pode ser utilizada como indicador precoce de degradação muscular uma vez que é formada nos primeiros dias de estocagem, enquanto a cadaverina um indicador tardio de deterioração nesta espécie. A determinação de amônia e avaliação do pH também foram considerados adequados para indicar o estado de frescor do pescado.

A tecnologia de radiação UV-C foi considerada eficiente na redução da carga bacteriana de *Proteus mirabilis* em filés de truta arco-íris embalados e, portanto, sua utilização é eficaz na descontaminação superficial desta matriz alimentar. A utilização de diferentes proporções de gases usualmente utilizadas na tecnologia de embalagem em atmosfera modificada não influenciou na ação e eficiência da luz UV-C sob o produto. As embalagens sem e com barreira aos gases apresentaram boa resposta a ação da radiação UV-C e foram consideradas apropriadas para utilização desta tecnologia.

5 **REFERÊNCIAS BIBLIOGRÁFICAS**

AMARAL, G. F. *Análise do segmento de trutas: abordagens de cadeia produtiva e turismo rural*. Rio de Janeiro, 2007. 118f. Dissertação (Mestrado em Desenvolvimento, Agricultura e Sociedade) - Departamento de Desenvolvimento, Agricultura e Sociedade, Universidade Federal Rural do Rio de Janeiro , Rio de Janeiro, 2007.

ANDERSON, B. M.;BÅNRUD, H.; BØE, E.; BJORDAL, O.; DRANGSHOLT, F. Comparison of UV C light and chemical for disinfection of surface in hospital isolation units. *Infection Control and Hospital Epidemiology*, v. 27, 2006.

ARASHISAR, S., HISAR, O., KAYA, M.; YANIK, T. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorhynchus mykiss*) fillets. *International Journal of Food Microbiology*, v.97, 2004.

BARROS, G. C. Perda de qualidade do pescado, deterioração e putrefação. *Revista do Conselho Federal de Medicina Veterinária*, n. 30, 2003.

BINTSIS, T.; LITOPOULOU-TZANETAKI, E.; ROBINSON, R.K. Existing and potencial applications of ultraviolet light in food industry – a critical review. *Journal of the Science of Food and Agriculture*, v. 80, p.637-645, 2000.

BIZERRIL, C.R.F.S.; LIMA N.R. 2001. Espécies de peixes introduzidas nos ecossistemas aquáticos continentais do Estado do Rio de Janeiro, Brasil. *Comunicações do Museu de Ciências e tecnologia da PUC-RS*, v.14, 2001.

BLAKISTONE, B. A. Principles and applications of modified atmosphere packaging of Foods. New York: Chapman and Hall, 1999.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Portaria nº 185 de 13 de maio de 1997. Aprova o Regulamento Técnico de Identidade e Qualidade de Peixe Fresco (Inteiro e Eviscerado). *Diário Oficial [da] República Federativa do Brasil*. Brasília, DF, 1997.

_____. Ministério da Agricultura, Pecuária e Abastecimento. Decreto nº 30.691, de 29 de março de 1952. Aprova o novo Regulamento de Inspeção Industrial e Sanitária de Produtos de Origem Animal (RIISPOA). *Diário Oficial [da] União*. Brasília, DF, 1952.

BRINKER,C.; KERR, M.; RAYNER, C. Investigation of biogenic amines in fish and fish products. *Victorian Government Department of Human Services*. 1ª ed., 2002,p. 17.

BRODY, A. L. El mercado. In: PARRY, R. T. *Envasado de los alimentos en atmósfera modifi cada*. Zaragoza:Acribia, 1995. p. 32-55.

CHYTIRI, S.; CHOULIARA, I.; SAVVAIDIS, I. N.; KONTOMINAS, M. G. Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. *Food Microbiology,* v.21, 2004a.

CHUN, H.; KIM, J.; CHUNG, K.; WON, M.; SONG, K.B. Inactivation kinetics of *Listeria monocytogenes*, *Salmonella enterica* serovar *Typhimurium*, and *Campylobacter jejuni* in ready-to-eat sliced ham using UV-C irradiation. *Meat Science*, v. 83, p. 599-603, 2009.

CHURCH, N. Developments in modified-atmosphere packaging and related technologies. *Trends in Food Science and Technology*, v. 5, 1994.

CHURCH, I. J.; PARSONS, A. L. Modified atmosphere packaging tecnology: a review. *Journal of the Science of Food and Agriculture*, v. 67, 1995.

CINTRA, I. H. A.; OGAWA, N. B. P.; SOUZA, M. R.; DINIZ, F. M.; OGAWA, M. Decomposition of trimethylamine oxide related to the use of sulfites in shrimp. Ciência e Tecnologia de Alimentos, Campinas: SBCTA, V. 19, n. 3, 1999.

COLOSO, R. M.; KING, K.; FLETCHER, J.W.; WEIS, P.; WERNER, A.; FERRARIS, R. P. Dietary P regulates phosphate transporter expression, phosphatase activity, and effluent P partitioning in trout culture. *Journal of Comparative Physiology B*, v.173, n.6, 2003.

CONNEL, J. J. *Control de la calidad del pescado*. Zaragoza: Acribia, 1988.

CONTRERAS-GUZMÁN, E. S. Bioquímica de pescados e derivados. Jaboticabal: FUNEP, 1994. 409 p.

DONHAUSER, S.; WAGNER, D.; GEIGER, E. Biogenic amines: significance, occurrence and assessment. *Brawelt International*, v. 11, 1993.

DYER, W. J. Amines in fish muscle. I. Colorimetric determination of trimethylamine as the picrate salt. *Journal of the fish research board of Canada*, Canada, v. 6, 1945.

FARIA, A. Notas sobre a biologia da truta “arco-íris” – *Salmo gairdneri irideus* (Gibbons) – importada da Dinamarca e introduzida em rios do Sertão da Bocaina, Município de Bananal, Estado de São Paulo. *Rio de Janeiro: MA/DNPA*,1953a.

FELLOWS, P.J. Tecnologia do processamento de alimentos: princípios e prática. Porto Alegre: Artmed, 2006. 602p.

FERNANDES, J. O. *Desenvolvimento de metodologias de cromatografia gasosa* – espectrometria de massa para a determinação de aminas biogénicas em vinhos do Porto e em mostos. Porto, 2001. Tese (Doutoramento) - Faculdade de Farmácia, Universidade do Porto, Porto, 2001.

FLICK, G.J.; GRANATA, L.A. Biogenic Amines in Foods. In: DABROWSKI, W.M., SIKORSKI, Z.E. (Eds.) *Toxins in Food. Chemical and Functional Properties of Food Components Series*. CRC Press, 2005. p. 121-154.

FLOROS, J. D.; MATSOS, K. I. Introduction on modified atmosphere packaging. In: HAN, J. H. Innovations in food packaging. 2005. Disponível em: http://books.google.com.br/books?id=MbVtx091tCUC&pg=PA103&dq=HAN ,+J.+H.+Innovations+in+food+packaging.+2005&hl=ptBR&ei=wM_mTJTKHsWblgfDn 7iFDA&sa=X&oi=book_result&ct=result&resnum=1&ved=0CC8Q6AEwAA#v=onepag e&q&f=false>. Acesso em: 10 set. 2012.

GALL, G.A.E.; CRANDELL, P.A. The rainbow trout. *Aquaculture*,v. 100, 1992.

GENIGEORGIS, C.A. Microbial and safety implications of the use of modified atmospheres to extend the storage life of fresh meat and fish*. International Journal of Food Microbiology*, vol.1, n 5, 1985.

GERMANO, P.M.L.; GERMANO, M.I.S.;OLIVEIRA, C.A.F. Aspectos da qualidade do pescado de relevância em saúde pública. *Revista Higiene Alimentar,* v.12, 1998.

GIANNINI, D. H. Determinación de nitrógeno básico volátil (NBV) em pescado: Consideraciones Generales*. Alimentaria*, Madrid, v. 40, n. 343, 2003.

GIMENEZ, B.; RONCALES, P. ;BELTRAN, J.A. Modified Atmosphere Packaging Of Filleted Rainbow Trout. *Journal of the Science of Food and Agriculture*, v. 84, 2002.

GRAM, L.; HUSS, H. H. Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology*, v. 33, n.1, 1996.

GUERRERO-BELTRÁN, J. A.; BARBOSA-CÁNOVAS, G. V. Advantages and Limitations on Processing Foods by UV Light. *Food Science and Technology International*, v. 10, n. 3, p. 137-147, 2004.

HALÁSZ, A.; BARÁTH, Á.; SIMON-SARKADI, L.; HOLZAPFEL, W. Biogenic amines and their production by microorganisms in food. *Trends in Food Science and Technology*, v. 5, 1994.

HALL, S.R.; MILLS, E.L. Exotic species in large lakes of the world. *Aquatic Ecosystems Health and Management*, v.3, 2000.

HERSHBERGER, W.K. Genetic variability in rainbow trout populations. *Aquaculture*, Estados Unidos, v.100, 1992.

HUSS, H. H. El pescado fresco: su calidad y cambios de su calidad. FAO – Organização das Nações Unidas para Agricultura e Alimentação – Documento técnico de pesca n 348. Roma, 1998.

HUSS, H. H. Quality and quality changes in fresh fish. FAO- Food and Agriculture Organization of the United Nations - *Fisheries technical* paper n.348. Roma, 1995.

KAILOLA, P.J.; WILLIAMS, M.J.; STEWART, P.C.; REICHELT, R.E.; MCNEE, A. ; GRIEVE, C. Australian fisheries resources. *Bureau of Resource Sciences*, Canberra, Australia, 1993.

KIM M. K.; MAH J. H.; HWANG H. J. Biogenic amine formation and bacterial contribution in fish, squid and shellfish. *Food Chemistry*, v. 116, 2009.

KUBITZA, F. *Tilápia* (*Oreochromis* sp.): tecnologia e planejamento na produção comercial. Divisão de Biblioteca e Documentação, Jundiaí, São Paulo, 2000. 285p.

LAZZAROTTO, H.; EBERIENOS, D.; FARIAS, H.J.; LIMA, S.M.Q. A influência de processos atuais e históricos na riqueza e composição de espécies de peixes em

bacias costeiras da Serra do Mar. *In:* ANAIS DO VII CONGRESSO DE ECOLOGIA DO BRASIL, 2005, Caxambu, Minas Gerais.

LAZZAROTTO, H.L.; BRITO, M.F.G.; CARAMASCHI, E.P. Threatened fishes of the world: *Pareiorhaphis garbei* (Ihering, 1911) (Ostariophysii: Loricariidae). *Environmental Biology of Fishes*, 78: 91-92, 2007.

LAZZAROTTO, H.; CARAMASCHI, E.P. Introdução da Truta no Brasil e na bacia do rio macaé, Estado do Ri o de Janeiro: Histórico, Legislação e Perspectivas. *Oecologia Brasiliensis*, v.13, 2009.

LILTVED, H.; LANDFALD, B. Effects of high intensity light on ultraviolet-irradiated and non-irradiated fish pathogenic bacteria. *Water Research*, v. 34, n 2, p. 481-486, 2000.

LIMA, A.S.; GLÓRIA, M.B.A. Aminas bioativas em alimentos. *Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos*, Campinas, v. 33, 1999.

LISTON, J. Microbiology in fishery science. In: *Advances in fish science and technology*. Surrey: Fishing News Book, England, p. 138–157, 1980.

MAGALHÃES, A. L. B.; ANDRADE, R. F.; RATTON, T. F.; BRITO, M. F. G. Ocorrência da truta arco-íris *Oncorhynchus mykiss* (Walbaum, 1792) (Pisces: *Salmonidae*) no alto rio Aiuruoca e tributários, bacia do rio Grande, Minas Gerais, Brasil. *Boletim do Museu de Biologia Mello Leitão,* Minas Gerais, v. 14, 2002.

MANO, S.B. *Comportamento de la microbiota natural y Listeria monocytogenes, Aeromonas hidrophila y Yersinia enterolítica em carne envasada em atmosferas modificadas.* Tese (Doutorado em veterinária). Departamento de nutrición y bromatologia III (higiene e tecnologia de los alimentos). Universidade Computense de Madrid (España), 1997.

MCDONALD, K.F.; CURRY, R. D.; CLEVENGER, T. E.; UNKLESBAY, K.; EISENSTARK, A.; GOLDEN, J.; MORGAN, R.D. (2000). A comparison of pulsed and continuous ultraviolet light sources for the decontamination of surfaces. *IEEE Transactions on Plasma Science*, 28, 1581–1587.

OETTERER, M. Técnicas de beneficiamento e conservação do pescado de água doce. Panorama da Aquicultura, v. 8, n. 46, p. 14-20, 1998.

OETTERER. M. Proteínas do pescado - processamento com intervenção protéica. *In*: OETTERER, M.; REGITANO D'ARCE, M.A.; SPOTO, M.H.F. *Fundamentos de Ciência e Tecnologia de Alimentos*. Barueri: Manole, 2006.

OGAWA, N.B.P.; MAIA, E.L. Manual de Pesca: ciência e tecnologia do pescado. São Paulo: Livraria Varela, 1999. 430 p.

ÖNAL, A. A review: current analytical methods for the determination of biogenic amines in foods. *Food Chemistry*, v.103, 2007.

PARRY, R.T. *Envasado de los alimentos en atmósfera modificada*. Madrid (España): A. Madrid Vicente, 1995.

PEREIRA, A. A. F.; TENUTA-FILHO, A. Avaliação de condições de consumo da sardinha (*Sardinella brasiliensis*). *Ciência e Tecnologia de Alimentos*, Campinas, v. 25, n. 4, 2005.

PRENTICE, C.; SAINZ, R. L. Cinética de deterioração apresentada por filés de carpa-capim *(Ctenopharyngodon idella)* embalados a vácuo sob diferentes condições de refrigeração. *Ciência e Tecnologia de Alimentos*, vol. 25, n 1, 2005.

REZAEI, M.; MONTAZERI, N.; LANGRUDI, H.E.; MOKHAYER, B.; PARVIZ, M.; NAZARINIA, A. The biogenic amines and bacterial changes of farmed rainbow trout (*Oncorhynchus mykiss*) stored in ice. *Food Chemistry*, v.103, 2007.

RODRIGUEZ, C.J.; BESTEIRO, I.;PASCUAL, C. Biochemical changes in freshwater rainbow trout (*Oncorhynchus mykiss*) during chilled storage. *Journal of the Science of Food and Agriculture*, v.79,1999.

RODRÍGUEZ, O.; LOSADA, V.; AUBOURG, S. P.; BARROS-VELÁZQUEZ, J. Enhanced shelf-life of chilled European hake (*Merluccius merluccius*) stored in slurry ice as determined by sensory analysis and assessment of microbiological activity. *Food Research International*, v. 37, 2004.

SAAID, M.; SAAD, B.; HASHIM, N.H.; ALI, A.S.M.; SALEH,M.I. Determination of biogenic amines in selected Malaysian food. *Food Chemistry*, v. 113, 2009.

SANTOS, M. H. S. Biogenic amines: their importance in foods. *Food Microbiology*, v.29, 1996.

SÁNCHEZ-CASCADO, S. P. Estúdio de alternativas para la evolutión de la frescura y la calidade del boquerón (*Engraulis encrasisholus*) y sus derivados. Barcelona, 2005. 287 f. Tese (Programa de Doctorado Nutrición, Tecnologia e Higiene de los Alimentos) – Faculdade de Farmácia, Universitat de Barcelona, Barcelona, 2005.

SARANTÓPOULOS, C.I.G.L.; SOLER, R.M. Embalagens com atmosfera modificada/controlada. *Catálogo Brasileiro de Produtos e Serviços*. Campinas, Ital, Jul. 1994.

SASTRY, S.K.; DATTA, A.K.;WOROBO, R.W. Ultraviolet light. *JFS Supplement,* 2000.

SCHERER, R.; AUGUSTI, P.R.; BOCHI, V.C.; STEFFENS, C.; FRIES, L.L.M.; DANIEL, A.P.; KUBOTA, E.H.; NETO, J.R. ;EMANUELLI, T. Chemical and microbiological quality of grass carp (*Ctenopharyngodon idella*) slaughtered by different methods. *Food Chemistry*, v.99, 2006.

SHALABY, A. R. Significance of biogenic amines to food safety and human health. *Food Research International*. v.29, 1996.

SHUBBART, O. Estudo de material coletado pelo Dr. Ascânio de Faria na região do Itatiaia. *Rio de Janeiro: MA/DNPA*, 1953.

SIMEONIDOU, S.; GOVARIS, A.; VARELTZIS, K. Quality assessement of seven Mediterranean fish species during storage on ice. *Food Research International,* v.30, 1998.

SMITH, G.R.; STEARLEY, R.F. The classification and scientific names of rainbow and cutthroat trouts. *Fisheries*, Estados Unidos, v.14, n.1, 1989.

SOCCOL, M.C.H. *Otimização da vida útil da tilápia cultivada (Oreochromis niloticus) minimamente processada e armazenada sobre refrigeração*. Dissertação apresentada a Escola Superior de Agricultura “Luiz de Queiroz” da Universidade de São Paulo, Piracicaba, SP, 2002.

SOCCOL, M. C. H.; OETTERER, M. Use of modified atmosphere in seafood preservation. *Brazilian Archives of Biology and Technology*, v. 46, n. 4, 2003.

SOMMERS, C. H.; SITES, J. E.; MUSGROVE, M. Ultraviolet light (254 nm) inactivation of pathogens on foods and stainless steel surfaces. *Journal of Food Safety*, v.30, 2010.

SOSINSKI, L.T.W. *Introdução da Truta Arco-Íris (Oncorhynchus mykiss) e suas conseqüências para a comunidade aquática dos rios de altitude do sul do Brasil*. Rio Grande do Sul, 2004. 84p.Tese (Doutorado. em Ecologia) - Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2004.

STEVENS, C.; KHAN, V.A.; LU, J.Y.;WILSON, C.L.; PUSEY, P.L.; KABWE, M.K; IGWEGBE, E.C.K.; CHALUTZ, E.; DROBY, S. The germicidal and hormetic effect of UV-C light on reducing brown rot disease and yeast microflora of peaches. *Crop Protection* 17(1): 75-84, 1998.

STOOPS, J.; JANSEN, M.; CLAES, J.; CAMPENHOUT, L.V. Decontamination of powdery and granular foods using Continuous Wave UV radiation in a dynamic process. *Journal of Food Engineering*, 119, 254-259, 2013.

TABATA, Y.A. 1997. Truticultura: situação mundial e no Brasil. *In:* ANAIS DO WORKSHOP INTERNACIONAL DE AQUICULTURA, 1997,São Paulo, SP.

TAKINO, M.; MAIER, M.H.; STEMPNIEWSKI, H.L. Características físicas e químicas da água em ambientes de altitude elevada. *Boletim do Instituto de Pesca,* Campos do Jordão, São Paulo*,* v.11, 1984.

TAYLOR, S.L. Histamine food poisoning: toxicology and clinical aspects. *Critical Reviews in Toxicology*, v. 17, 1986.

TIMM, M.; JORGENSEN, B. M. Simultaneous determination of ammonia, dimethylamine, trimethylamine and trimethylamine-oxide in fish extracts by capillary electrophoresis with indirect UV - detection. *Food Chemistry*, Denmark, v. 76, n. 4, 2002.

VECIANA-NOGUÉS, M. T.; MARINÉ-FONT, A.; VIDAL-CAROU, M.C. Biogenic amines as hygienic quality indicators of tuna. Relationships with microbial counts,

ATP- related compounds, volatile amines, and organoleptic changes. *Journal of Agricultural and Food Chemistry*, Washington, v. 45, 1997.

WELCOME, R.L. International introductions of inland aquatic species. *FAO – Food and Agriculture Organization, Fish.Tech. Paper*, n. 294, 1988.

WOODLING, S.E.; MORARU, C.I. Influence of surface topography on the effectiveness of pulsed light treatment for the inactivation of *Listeria innocua* on stainless-steel surfaces. *Journal of Food Science*, 70, 345-351, 2005.

ZAITSEV, V.; KIZEVETTER, I.; LAGUNOV, L.; MAKAROVA, T.; MINDER, L.; PODSEVALOV, V. Caracteristics of fish as a raw material for industry. In: _____.Fish curing and processing. Moscou, 1969. p. 170-188.

6 **APÊNDICES**

6.1PAPER1

Aquaculture
Research & Development

Rodrigues et al., J Aquac Res Development 2013, 4:3
http://dx.doi.org/10.4172/2155-9546.1000174

Research Article Open Access

Concentration of Biogenic Amines in Rainbow Trout (*Oncorhynchus mykiss*) Preserved in Ice and its Relationship with Physicochemical Parameters of Quality

Bruna Leal Rodrigues[1], Thiago Silveira Alvares[2], Marion Pereira da Costa[1], Guilherme Sicca Lopes Sampaio[1], César Aquiles Lázaro de la Torre[1], Eliane Teixeira Márcico[1] and Carlos Adam Conte Júnior[1]*

[1]*Laboratory of Physicochemical Control, Department of Food Technology, Fluminense Federal University, Niterói, Brazil*
[2]*Chemistry Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

Abstract

Biogenic amines are formed as a result of amino acid decarboxylation and are linked to food deterioration. Analysis of these metabolites may be of great importance to determine food quality. The aim of this study was to quantify the biogenic amines (putrescine and cadaverine), and evaluate the physicochemical parameters (pH, ammonia and total volatile bases) of rainbow trout meat (*Oncorhynchus mykiss*). Fifteen samples were packed in ice and transported in a Styrofoam container to the laboratory. Analyses were performed daily until the 15th day of storage. Biogenic amines concentrations and pH increased significantly throughout the storage period. No significant differences were observed in total volatile bases values over the time. Ammonia was detected after the 11th day of storage. Based on these results, cadaverine and putrescine may be used as a quality index of rainbow trout; however, total volatile bases may not be adequate parameter for this matrix.

Keywords: Physicochemical parameters; Biogenic amines; Quality index; Rainbow trout

Introduction

Fish and fishery products have played an important role in the human diet due to their high nutritional quality [1]; however, the chemical composition, high water activity, easily oxidized fat content, and pH close to neutral, accelerate its deterioration by promoting the development of the natural microbiota in this food matrix [2,3]. The deterioration occurs as a result of enzymatic and microbial activity, resulting in the production of different metabolites, which can lead to loss of product quality and can serve as quality indicators of the raw material [4,5].

The total volatile bases (monomethylamine, dimethylamine, trimethylamine, ammonia) are nitrogenous compounds originated from the degradation of some compounds in fish (amino acids and nucleotides) during the deterioration process [6,7]. The determination of total volatile bases (TVB) is one of the most widely used for assessing fish quality [6].

In freshwater fish, ammonia is considered a good indicator for evaluating the quality index, since it is the main compound of the group of substances evaluated in the analysis of TVB in these species [8]. Another indicator also widely used to evaluate the degradation process of fish is the hydrogen potential (pH). During the decaying process, there is the formation of alkaline compounds such as ammonia and amines, which accumulate in the muscle, increasing the muscle pH values [9].

Biogenic amines are formed as a result of amino acid decarboxylation, which is linked to the existence of spoilage bacteria in the food matrix [6,10,11]. Studies have reported that the biogenic amines, especially putrescine and cadaverine, can be considered good parameters for assessing the quality and the deterioration rate of various food matrices, including fish [3,11-15]. The development of analytical methods, faster than microbiological ones, for the identification and quantification of biogenic amines is very important to determine fish freshness [3,6]. Currently, chromatographic techniques offer a great advantage, since they ensure accurate measurements and allow for the simultaneous analysis of several biogenic amines in fish and fishery products [16,17]. Among the chromatographic techniques, high-performance liquid chromatography (HPLC) is being widely used because of its sensitivity and reliability [18].

Due to the fact that there is limited scientific evidence demonstrating the effectiveness of biogenic amines as quality indicators of trout, and considering that rainbow trout (*Oncorhynchus mykiss*) is one of the main commercial aquaculture species produced and marketed worldwide [19], this study was conducted with the purpose of evaluating the use of biogenic amines and physicochemical parameters for quality assessment.

Materials and Methods

Sampling

Fifteen fresh rainbow trout (*Oncorhynchus mykiss*) specimens were obtained from the *Trutas da Serrinha* Company located in Itatiaia, a region of the Serra da Mantiqueira, in the state of Rio de Janeiro, Brazil. The samples were packed in ice (0 ± 1°C) and transported in a styrofoam container to the laboratory. The filet was obtained in sterile conditions, and all instruments used for filets dissection were previously sterilized.

***Corresponding author:** Dr. Carlos Adam Conte Junior, Laboratory of Physicochemical Control, Department of Food Technology, Fluminense Federal University, Rua Vital Brazil Filho, 64, Santa Rosa Niterói, Rio de Janeiro, Brazil, Tel: 55-21-2629-9545; Fax: 55-21-2629-9541; E-mail: mtaconte@vm.uff.br

Received December 23, 2012; **Accepted** January 23, 2013; **Published** February 03, 2013

Citation: Rodrigues BL, Alvares TS, da Costa MP, Lopes Sampaio GS, de la Torre CAL, et al. (2013) Concentration of Biogenic Amines in Rainbow Trout (*Oncorhynchus mykiss*) Preserved in Ice and its Relationship with Physicochemical Parameters of Quality. J Aquac Res Development 4: 174 doi:10.4172/2155-9546.1000174

Citation: Rodrigues BL, Alvares TS, da Costa MP, Lopes Sampaio GS, de la Torre CAL, et al. (2013) Concentration of Biogenic Amines in Rainbow Trout (*Oncorhynchus mykiss*) Preserved in Ice and its Relationship with Physicochemical Parameters of Quality. J Aquac Res Development 4: 174 doi:10.4172/2155-9546.1000174

The samples were analyzed daily until the 15th day of storage (Figure 1). All analyses were performed in duplicate.

Physicochemical analyses

pH, TVB and ammonia parameters were determined in order to evaluate the state of fish freshness during storage at 0 ± 1°C. For the analysis of pH and TVB, potentiometric and microdiffusion methods were used, respectively Conte-Júnior et al. and Conway and Byrne [20,21]. The qualitative determination of ammonia was performed by using mercuric iodide, potassium iodide, sodium hydroxide solutions and water (Nessler reagent).

Biogenic amines quantification

The biogenic amines, putrescine and cadaverine, were assayed by High- Performance Liquid Chromatography (HPLC). Briefly, 5 mL of perchloric acid (5%) were added to 5 g of sample and kept 1 hour under refrigeration condition (4 ± 2°C) with periodic stirring. Subsequently the solution was centrifuged and filtered through Whatman filter paper No 1, followed by the addition of 2N sodium hydroxide to reach pH>6. In the next step, the homogenized solution was kept in an ice bath for 20 minutes and filtered a second time, with the subsequent addition of 2N sodium hydroxide to reach pH>12 [22]. Under these circumstances the solution was derivatized with the addition of 40 µL of benzoyl chloride, homogenized for 15 s and left to stand at room temperature for 20 min. Thereafter, 1 mL of diethyl ether was added and the supernatant was removed. The resulting sample was evaporated in a stream of nitrogen to be finally resuspended with 500 µL of mobile phase (acetonitrile: H_2O; 42: 58; v: v) [23]. 20 µL of sample were injected into HPLC device coupled with UV detector; the flow rate was set at 1 mL min^{-1}. For the separation of the amine, a Teknokroma column, TR-016057 N26243 Tracer Extrasil ODS2 (15×0.46 cm, id. 5 µm) and a Supelco precolumn, Ascentis C18 (2×0.40 cm, id. 5 µm) were used.

Statistical analysis

The one-way ANOVA was performed to identify differences between biogenic amines (putrescine and cadaverine) and physicochemical parameters (TVB and pH) over the 15-day period of storage. When a significant *F* was found, additional post hoc tests with Bonferroni adjustment were performed. For the interpretation of result all the data obtained (from day 1 to day 15) were divided into three periods: Time 1 (T1)–analysis of the first five days of storage; Time 2 (T2)–analysis of the subsequent five days; Time 3 (T3)–analysis of the last five days of storage. Based on previous studies [11,14,24], no significant changes should occur in the biogenic amines concentrations and pH values at Time 1.

Statistical significance was set at the 0.05 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).

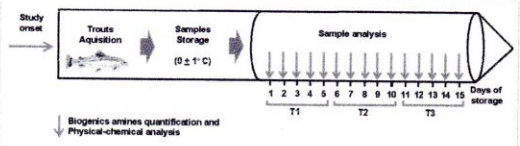


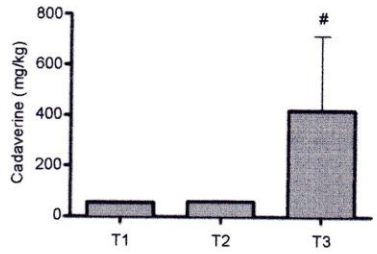
Figure 1: Experimental design of the study.

Results and Discussion

Recent studies do not report the use of quality physicochemical analyses that are considered simple and fast, and they have also not associated these analyses with biogenic amine concentrations to evaluate the quality of rainbow trout (*Oncorhynchus mykiss*). Therefore, the present study was designed to investigate the potential use of biogenic amines and physicochemical parameters–such as TVB, ammonia and pH–as a quality index for this species of fish. Overall, the major finding of the study was that the concentrations of the biogenic amine, putrescine and cadaverine, increased significantly–together with a significant increase in pH–over the 15-day storage period and the presence of ammonia was observed only after the 11th day of storage. No significant difference was observed in TVB values during the 15 days of storage.

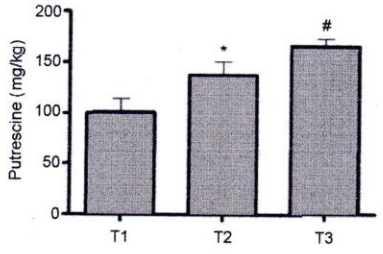
Changes in TVB and pH values over the period of the storage are shown in table 1. There was no significant difference in TVB values throughout the 15 days of storage. A significant increase in pH was observed in T3 as compared to T1. The presence of ammonia was observed only after the 11th day of storage.

The changes in cadaverine and putrescine concentrations over the days of storage are presented in figures 2 and 3, respectively. There was a



T1=analysis of the first five days of storage; T2=analysis of the subsequent five days; T3=analysis of the last five days of storage.
#Significantly different from T1 and T2.

Figure 2: Cadaverine concentrations (mg/kg) over the period of storage.



T1=analysis of the first five days of storage; T2=analysis of the subsequent five days; T3=analysis of the last five days of storage.
#Significantly different from T1 and T2.
*Significantly different from T1

Figure 3: Putrescine concentrations (mg/kg) over the period of storage.

Citation: Rodrigues BL, Alvares TS, da Costa MP, Lopes Sampaio GS, de la Torre CAL, et al. (2013) Concentration of Biogenic Amines in Rainbow Trout (*Oncorhynchus mykiss*) Preserved in Ice and its Relationship with Physicochemical Parameters of Quality. J Aquac Res Development 4: 174 doi:10.4172/2155-9546.1000174

significant increase in putrescine (T1: 100.17 ± 13.28 mg/kg; T2: 136.53 ± 13.28 mg/kg; T3: 165.62 ± 7.27 mg/kg) and cadaverine (T1: 56.49 ± 0 mg/kg; T2: 59.22 ± 4.97 mg/kg; T3: 419.75 ± 295.44 mg/kg) over the storage period.

According to EC Decision 95/149, there is no maximum limit for TVB; however, the limit was fixed to 25 mg N/100g in rainbow trout Gimenez et al. [25,26]. Although the present values at the end of storage period were below the limit suggested by Gimenez et al. the fish was in an evident state of sensorial deterioration, which had gone undetected by TVB analysis [26].

The compounds that form TVB are present in varying concentrations in muscle according to the kind of fish [7]. Unlike marine fish, freshwater fish generally have negligible values of trimethylamine oxide. Low levels of trimethylamine and the presence of ammonia are the main compounds that form part of TVB. Due to the low trimethylamine formation, the TVB concentration in freshwater fish species remains low during storage [8,27]. In addition, Morishita et al. reported that factors such as age, location and method of cultivation may influence the non-protein nitrogen compound content in the fish muscle, which may influence the TVB levels [28].

In the present study the TVB determination was not considered a good parameter for evaluating the quality index, since the values observed in this analysis did not even change at the end of storage period (Table 1) when the fish reached a state of deterioration (Figures 2 and 3). Other studies have confirmed the present results, demonstrating that TVB is not a good parameter for evaluating the quality index of freshwater fish [24,27]. Therefore, TVB may be considered an uncertain and unreliable decay index for freshwater fish.

Regarding ammonia, there was a correlation with the state of sensorial deterioration, since it was detected after the 11th day of storage in the samples. Ammonia is the main compound belonging to this set of volatile bases in freshwater fish species. This analysis is therefore considered satisfactory for evaluating the process of protein degradation; hence it may be used to determine the quality index on these fish species [8].

The pH values increased continuously over the storage period, reaching higher values in T3 (6.77 ± 0.13). This increase was due to the production of basic compounds formed during the autolytic changes [6]. According to Rodriguez et al. the accumulation of alkaline metabolites, such as amines, promotes an increase in muscle pH, indicating a deterioration process [9].

When analyzing the results of biogenic amines, the putrescine and cadaverine concentrations increased significantly throughout the storage period. The behavior of these amines in trout flesh was also observed by other researchers [11,15]. Studying the biochemical changes in rainbow trout stored for 12 days, Rodriguez et al. observed an increase of putrescine while cadaverine was detected only after 9 days of storage [11]. The authors suggest that the presence of cadaverine may serve as an indicator of muscle change, which is caused by increased activity of microorganisms. Furthermore, putrescine may be an indicator of premature muscle autolytic degradation, since it is formed during the first days of storage.

	T1	T2	T3
TVB (mg N/100g)	9.30 ± 0.58	9.07 ± 1.19	9.07 ± 0.0
pH	6.47 ± 0.19	6.73 ± 0.09	6.77 ± 0.13*

T1=analysis of the first five days of storage; T2=analysis of the subsequent five days; T3=analysis of the last five days of storage.
*Significantly different from T1 (only pH; P<0.05).

Table 1: Mean and standard deviation values for total volatile basis (TVB) and hydrogen potential (pH) over the period of storage.

According Dawood et al. the rapid formation of putrescine in fish is due to high enzymatic activity (due to the microflora contaminant) that promotes both the decarboxylation of glutamic acid and arginine and the synthesis of ornithine, which results in putrescine formation [12]. The authors suggest that putrescine and cadaverine may be reliable indicators of fish spoilage. Likewise, Rezaei et al. studying the presence of biogenic amines in rainbow trout stored for 18 days, observed an increase of amines during the storage period and suggested that monitoring the putrescine levels may serve as an index to evaluate the freshness of rainbow trout [15].

According to Gram and Dalgaard and Halász et al. the increase and the formation of these amines is related to the bacterial load present in meat [3,29]. Halász et al. observed that the bacteria of the family Enterobacteriaceae are usually implicated in the formation of cadaverine [29]. On the other hand, bacteria of the genus Pseudomonas spp. are responsible for the formation of putrescine.

Other previous studies [4,15,30-32] have demonstrated that the formation of biogenic amines depends on several factors that may alter the concentration of these amines in the food matrix. These factors include: aquaculture conditions, food, fish species, body composition, storage and processing conditions, autolytic interactions, availability of free amino acids, and the presence of decarboxylase-active microorganisms.

Based on the results of the present study, it appears that the biogenic amines, putrescine and cadaverine, may be considered suitable indicators of the degradation process of rainbow trout meat. Furthermore, the presence of ammonia and changes in pH may be regarded as quality parameters to evaluate this species. Finally, the total volatile bases (TVB) were not useful in assessing the deterioration level of the fish studied.

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-26/111.933/2011, E-26/110.460/2012 and E-26/103.003/2012. BL Rodrigues was supported by the National Council for Scientific and Technological Development (CNPq). *Trutas da Serrinha* is gratefully acknowledged for providing samples. The authors would like to thank Ricky Toledano for the English revision of the manuscript.

References

1. Fallah AA, Saei-Dehkordi SS, Nematollahi A (2011) Comparative assessment of proximate composition, physicochemical parameters, fatty acid profile and mineral content in farmed and wild rainbow trout (*Oncorhynchus mykiss*). Int J Food Sci Tech 46: 767-773.
2. Gram L, Huss HH (1996) Microbiological spoilage of fish and fish products. Int J Food Microbiol 33: 121-137.
3. Gram L, Dalgaard P (2002) Fish spoilage bacteria-problems and solutions. Curr Opin Biotechnol 13: 262-266.
4. Liston J (1980) Microbiology in fishery science. In: Edited by JJ, (eds.). Advances in fish science and technology. Fishing News Books. England: Surrey.
5. Arashisar S, Hisar O, Kaya M, Yanik T (2004) Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorhynchus mykiss*) fillets. Int J Food Microbiol 97: 209-214.
6. Huss HH (1995) Quality and quality changes in freshwater fish. Fisheries

Citation: Rodrigues BL, Alvares TS, da Costa MP, Lopes Sampaio GS, de la Torre CAL, et al. (2013) Concentration of Biogenic Amines in Rainbow Trout (*Oncorhynchus mykiss*) Preserved in Ice and its Relationship with Physicochemical Parameters of Quality. J Aquac Res Development 4: 174 doi:10.4172/2155-9546.1000174

Technical Paper No. 348. Rome: Food and Agriculture Organization (FAO) of United Nations.

7. Giannini DH (2003) Determinación de nitrógeno básico volátil (NBV) em pescado: Consideraciones Generales. Alimentaria 40: 49-54.
8. Zaitsev V, Kizevetter I, Lagunov L, Makarova T, Minder L, et al. (1969) Caracteristics of fish as a raw material for industry. In: Fish curing and processing. Moscou.
9. Rodríguez O, Losada V, Aubourg SP, Barros-Velázquez J (2004) Enhanced shelf-life of chilled European hake (*Merluccius merluccius*) stored in slurry ice as determined by sensory analysis and assessment of microbiological activity. Food Res Int 37: 749-757.
10. Mietz JL, Karmas E (1977) Chemical quality index of canned tuna as determined by high-pressure liquid chromatography. J Food Sci 42: 155-158.
11. Rodríguez CJ, Besteiro I, Pascual C (1999) Biochemical changes in freshwater rainbow trout (*Oncorhynchus mykiss*) during chilled storage. J Sci Food Agric 79: 1473-1480.
12. Dawood AA, Karkalas J, Roy RN, Williams CS (1988) The occurrence of non-volatile amines in chilled-stored rainbow trout (*Salmo irideus*). Food Chem 27: 33-45.
13. Krizek M, Pavlicek T, Vacha F (2002) Formation of selected biogenic amines in carp meat. J Sci Food Agric 82: 1088-1093.
14. Katikou P, Georgantelis D, Paleologos EK, Ambrosiadis I, Kontominas MG (2006) Relation of biogenic amines' formation with microbiological and sensory attributes in lactobacillus-inoculated vacuum-packed rainbow trout (*Oncorhynchus mykiss*) fillets. J Agric Food Chem 54: 4277-4283.
15. Rezaei M, Montazeri N, Langrudi HE, Mokhayer B, Parviz M, et al. (2007) The biogenic amines and bacterial changes of farmed rainbow trout (*Oncorhynchus mykiss*) stored in ice. Food Chem 103: 150-154.
16. Hwang DF, Chang SH, Shiua CY, Chai TJ (1997) High-performance liquid chromatogramphic determination of biogenic amines in fish implicated in food poisoning. J Chromatogr B Biomed Sci Appl 693: 23-29.
17. Cinquina AL, Cali A, Longo F, Santis L, Severoni A, et al. (2004) Determination of biogenic amines in fish tissues by ion-exchange chromatography with conductivity detection. J Chromatogr A 1032: 73-77.
18. Ozogul F, Taylor KDA, Quantick P, Ozogul Y (2002) Biogenic amines formation in Atlantic herring (*Clupea harengus*) stored under modified atmosphere packaging using a rapid HPLC method. Int J Food Sci Tech 37: 515-522.
19. Coloso R M, King K, Fletcher JW, Weis P, Werner A, et al. (2003) Dietary P regulates phosphate transporter expression, phosphatase activity, and effluent P partitioning in trout culture. J Comp Physiol B 173: 519-530.
20. Conte Junior CA, Peixoto BTM, Lopes MM, Franco RM, Freitas MQ, et al. (2010) Effect of modified atmosphere packaging on the growth/survival of *Yersinia enterocolitica* and natural flora on fresh poultry sausage. In: Méndez-Vilas A (ed.) Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology (2ndedn), spain.
21. Conway EJ, Byrne A (1933) An absorption apparatus for the micro-determination of certain volatile substances: The micro-determination of ammonia. Biochem J 27: 419-429.
22. Rodríguez SC, López B, Chaves AR (2001) Effect of different treatments on the evolution of polyamines during refrigerated storage of eggplants. J Agric Food Chem 49: 4700-4705.
23. Mei YH (1994) A sensitive and fast method for the determination of polyamines in biological samples. Benzoyl chloride pre-column derivatization high-performance liquid chromatography. J Liq Chrom 17: 2413-2418.
24. Chytiri S, Chouliara I, Savvaidis IN, Kontominas MG (2004) Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. Food Microbiology 21: 157-165.
25. European Union (1995) Commission decision 95/149/EC, 8, March 1995. Off J Eur Comm. L97, 84–87.
26. Gimenez B, Roncales P, Beltran JA (2002) Modified atmosphere packaging of filleted rainbow trout. J Sci Food Agric 84: 1154-1159.
27. Scherer R, Augusti PR, Bochi VC, Steffens C, Fries LLM, et al. (2006) Chemical and microbiological quality of grass carp (*Ctenopharyngodon idella*) slaughtered by different methods. Food Chem 99: 136-142.
28. Morishita T, Uno K, Araki T, Takahashi T (1989) Comparison of the amounts of extractive nitrogenous constituents in the meats of cultured red sea bream of different localities and culture methods and those of wild fish. Nippon Suisan Gakk 55: 1565-1573.
29. Halász A, Baráth Á, Simon-Sarkadi L, Holzapfel W (1994) Biogenic amines and their production by microorganisms in food. Trends Food Sci Technol 5: 42-49.
30. Shahidi F (1994) The chemistry processing technology and quality of seafoods-an overview. In: Shahidi F, Botta JR, (eds.). Seafoods chemistry, processing technology and quality of seafoods. Great Britain.
31. Bodmer S, Imark C, Kneubuhl M (1999) Biogenic amines in foods: histamzine and food processing. Inflamm Res 48: 296-300.
32. Krizek M, Vacha F, Vorlova L, Lukasova J, Cupakova S (2004) Biogenic amines in vacuum-packed and non-vacuum packed flesh of Carp (*Cyprinus carpio*) stored at different temperatures. Food Chem 88: 185-191.

Citation: Rodrigues BL, Alvares TS, da Costa MP, Lopes Sampaio GS, de la Torre CAL, et al. (2013) Concentration of Biogenic Amines in Rainbow Trout (*Oncorhynchus mykiss*) Preserved in Ice and its Relationship with Physicochemical Parameters of Quality. J Aquac Res Development 4: 174 doi:10.4172/2155-9546.1000174

6.2 CONFIRMAÇÃO DE SUBMISSÃO DO ARTIGO INTITULADO: INFLUENCE OF PACKAGING AND GAS COMPOSITION ON UV-C RADIATION EFFECTIVENESS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

Dear Mrs. Bruna Rodrigues,

We have received your article "Influence of packaging and gas composition on UV-C radiation effectiveness in rainbow trout (Oncorhynchus mykiss)" for consideration for publication in Journal of Stored Products Research.

Your manuscript will be given a reference number once an editor has been assigned. Once the reference number has been allocated, you can then track the status of your paper.

To track the status of your paper, please do the following:

1. Go to this URL: http://ees.elsevier.com/spr/

2. Enter these login details:
  Your username is: brunalrmlk@yahoo.com.br
If you need to retrieve password details, please go to:
http://ees.elsevier.com/spr/automail_query.asp

3. Click [Author Login]
This takes you to the Author Main Menu.

4. Click [Submissions Being Processed]

Thank you for submitting your work to this journal.

Kind regards,

Elsevier Editorial System
Journal of Stored Products Research

****
For further assistance, please visit our customer support site athttp://help.elsevier.com/app/answers/list/p/7923. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.