

**UNIVERSIDADE FEDERAL DE SANTA MARIA  
CENTRO DE CIÊNCIAS RURAIS  
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA  
DOS ALIMENTOS**

**INFLUÊNCIA DA AMÔNIA E OXIGÊNIO DA ÁGUA *IN VIVO* E DA ERVA-MATE *POST MORTEM* SOBRE A ESTABILIDADE LIPÍDICA DE FILÉS DE DOURADO**

**DISSERTAÇÃO DE MESTRADO**

**Ana Paula de Lima Veeck**

**Santa Maria, RS, Brasil  
2008**

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**INFLUÊNCIA DA AMÔNIA E OXIGÊNIO DA ÁGUA *IN VIVO* E  
DA ERVA-MATE *EX VIVO* SOBRE A ESTABILIDADE  
LIPÍDICA DE FILÉS DE DOURADO**

**por**

**Ana Paula de Lima Veeck**

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Área de Concentração em Ciência e Tecnologia de Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de  
**Mestre em Ciência e Tecnologia dos Alimentos**

**Orientador: Prof<sup>a</sup>. Dr<sup>a</sup>. Tatiana Emanuelli**

**Santa Maria, RS, Brasil**

**2008**

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CENTRO DE CIÊNCIAS RURAIS  
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA  
DOS ALIMENTOS**

A Comissão Examinadora, abaixo assinada, aprova a Dissertação de  
Mestrado

**INFLUÊNCIA DA AMÔNIA E OXIGÊNIO DA ÁGUA *IN VIVO* E DA  
ERVA-MATE *EX VIVO* SOBRE A ESTABILIDADE LIPÍDICA DE FILÉS  
DE DOURADO**

Elaborada por

**Ana Paula de Lima Veeck**

como requisito parcial para a obtenção do grau de  
**Mestre em Ciência e Tecnologia dos Alimentos**

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Santa Maria, 09 de maio de 2008.

**OFEREÇO e DEDICO**  
*À minha Mãe Irene,  
Ao meu Pai Neri,  
As Duas Pessoas que Sempre Lutaram na Vida para que Eu Atingisse meus  
Objetivos  
e Pudesse Alcançar a Vitória... Amo Vocês Eternamente.*

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## RESUMO

Dissertação de Mestrado  
Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos  
Universidade Federal de Santa Maria

### **INFLUÊNCIA DA AMÔNIA E OXIGÊNIO DA ÁGUA *IN VIVO* E DA ERVA-MATE *EX VIVO* SOBRE A ESTABILIDADE LIPÍDICA DE FILÉS DE DOURADO**

AUTORA: ANA PAULA DE LIMA VEECK

ORIENTADORA: TATIANA EMANUELLI

Local e Data de Defesa: Santa Maria, 09 de maio de 2008.

Este trabalho teve como objetivo avaliar os efeitos da exposição *in vivo* a concentrações estressantes de amônia e oxigênio na água e do tratamento com extrato de erva-mate (*Ilex paraguariensis*) *post mortem* sobre a estabilidade lipídica de filés de dourado (*Salminus brasiliensis*). Foi avaliada a influência de diferentes níveis de amônia e oxigênio sobre a composição centesimal, composição de ácidos graxos e a peroxidação lipídica de filés de dourado, assim como a estabilidade desses filés durante o armazenamento congelado (12 meses a  $-7\pm 1^{\circ}\text{C}$ ). Avaliou-se também o efeito do extrato de erva-mate sobre alterações lipídicas e de cor de filés crus de dourado durante o armazenamento congelado e sobre as características sensoriais e a oxidação lipídica de filés cozidos de dourado durante o armazenamento refrigerado. A exposição do dourado durante 15 dias a alta concentração de amônia (0,1 mg/L) afetou a composição dos filés e a exposição a alto oxigênio ( $>6,0$  mg/L) aumentou os valores de substâncias reativas ao ácido tiobarbitúrico (TBARS) medidos logo após o abate. No entanto, a exposição *in vivo* a níveis estressantes de amônia e oxigênio por 12 horas, não afetou a formação de TBARS durante o armazenamento congelado. A exposição durante 12 horas à alta amônia aumentou a susceptibilidade dos filés a oxidação lipídica durante o armazenamento congelado (maiores valores de dienos conjugados e peróxidos). Por outro lado, a exposição à baixa concentração de oxigênio (4,5 mg/L) não aumentou a taxa de oxidação lipídica dos filés. O extrato aquoso de erva-mate 10% (p/v) apresentou uma capacidade antioxidante equivalente a 25,8 mg trolox/ml extrato no ensaio de remoção do radical DPPH. O tratamento dos filés com este extrato (filés mergulhados por 1 min no extrato) reduziu as alterações de cor durante o armazenamento congelado (menor alteração de luminosidade e ângulo de matiz) e os filés tratados com o extrato de erva-mate apresentaram menor aumento nos valores de ácidos graxos livres, dienos conjugados e TBARS durante o congelamento (12 meses a  $-7\pm 1^{\circ}\text{C}$ ). O tratamento dos filés de dourado com extrato bruto de erva-mate (10 e 20%) não alterou o sabor, mas provocou importantes alterações de cor (redução na luminosidade e aumento na tendência ao amarelo) dos filés cozidos de dourado. Como o extrato bruto alterou a cor, decidiu-se purificar o extrato de erva-mate para eliminar a clorofila e outros pigmentos através de partição líquido-líquido, obtendo-se uma fase superior límpida com atividade antioxidante no ensaio de remoção do radical DPPH (1,2 vs. 0,7 mg equivalentes de trolox/ml extrato, fase superior vs. fase inferior). O extrato purificado de erva-mate



reduziu o valor de peróxidos, sem efeito significativo no conteúdo de TBARS de filés cozidos de dourado durante a armazenagem refrigerada (6 dias a  $7\pm 1^{\circ}\text{C}$ ). Não foi observado efeito da amônia ou do oxigênio sobre a composição de ácidos graxos, da mesma maneira que não houve influência do armazenamento congelado ou do tratamento com extrato de erva-mate. Os resultados indicam que a exposição a altos teores de amônia aumentou a susceptibilidade dos filés a oxidação lipídica durante o armazenamento congelado e a erva-mate apresentou atividade antioxidante nos filés congelados e certa proteção contra a oxidação de filés de dourado cozidos e armazenados sob refrigeração. Isto demonstra o possível uso da erva-mate para estender a vida útil de filés de pescados.

Palavras-chave: qualidade da água; *Salminus brasiliensis*; *Ilex paraguariensis*; atividade antioxidante; oxidação lipídica.

## ABSTRACT

Master Dissertation  
Graduate Program on Food Science and Technology  
Federal University of Santa Maria

### **INFLUENCE OF AMMONIA AND OXYGEN OF WATER *IN VIVO* AND THE ERVA-MATE *EX VIVO* ON LIPID STABILITY LIPÍDICA OF FILLETES FROM DOURADO**

AUTHOR: ANA PAULA DE LIMA VEECK

ADVISER: TATIANA EMANUELLI

Place and date of defense: Santa Maria, May 09, 2008.

This work was aimed at evaluating the effect of the exposure to stressing ammonia and oxygen levels *in vivo* as well as the effect of the treatment with erva-mate extract (*Ilex paraguariensis*) *post mortem* on the lipid stability of fillets of dourado (*Salminus brasiliensis*). The influence of different levels of ammonia and oxygen on the proximate composition, fatty acid composition and lipid peroxidation of dourado fillets, as well as on the stability of these fillets during the frozen storage (12 months at  $-7\pm 1^{\circ}\text{C}$ ) was evaluated. The effect of erva-mate extract on lipid and colour changes of raw fillets of dourado during the frozen storage and on the sensory characteristics and lipid oxidation of cooked dourado fillets during chilled storage was also evaluated. The exposure of dourado during 15 days at high ammonia concentration (0.1 mg/L) affected the composition of fillets, while the exposure to high oxygen ( $> 6.0$  mg/L) increased the value of thiobarbituric acid reactive substances (TBARS) measured immediately after slaughtering. However, the *in vivo* exposure to stressing ammonia and oxygen levels for 12 hours, did not change TBARS formation during the frozen storage. A 12-hours exposure to high ammonia increased the susceptibility of fillets to lipid oxidation during the frozen storage (higher conjugated dienes and peroxides values). On the other hand, the exposure to low oxygen concentration (4.5 mg/L) did not increase the lipid oxidation of fillets. The aqueous 10% (w/v) erva-mate extract had an antioxidant capacity equivalent to 25.8 mg trolox/mL extract in the DPPH radical scavenging assay. The treatment of dourado fillets with this extract (fillets dipped in the extract for 1 min) reduced the colour changes during frozen storage (lower changes in luminosity and hue values) and the fillets treated with erva-mate extract had lower increase of free fatty acids, conjugated dienes and TBARS values during the frozen storage (12 meses at  $-7\pm 1^{\circ}\text{C}$ ). The treatment of dourado fillets with aqueous crude 10 or 20% erva-mate extract did not modify the taste, but caused important changes in the colour (decrease of luminosity and increase in the yellowness) of cooked dourado fillets. Since crude erva-mate extract changed the colour, it was decided to purify the extract in order to eliminate chlorophyll and other pigments. Liquid-liquid partition yielded a clear upper phase with antioxidant activity in the DPPH radical scavenging assay (1.2 vs. 0.7 mg trolox equivalents/ml extract, upper phase vs. lower phase). The purified erva-mate extract reduced peroxides, but had no significant effect on the content of TBARS of cooked dourado fillets during chilled storage (6 dias at  $7\pm 1^{\circ}\text{C}$ ). No effect of ammonia or oxygen was observed on the fatty acid composition.

Similarly, there was no effect of the frozen storage or of the treatment with erva-mate extract. The results indicate that the exposure to high ammonia levels increased the susceptibility of fillets to lipid oxidation during the frozen storage. In addition, erva-mate had antioxidant activity in frozen fillets and some protective effect against lipid oxidation of cooked dourado fillets under chilling storage. This demonstrates the possible use of erva-mate to extend the shelf-life of fish fillets.

Keywords: water quality; *Salminus brasiliensis*; *Ilex paraguariensis*; antioxidant activity; lipid oxidation.

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## LISTA DE ABREVIATURAS

**ABNT** – Associação Brasileira de Normas Técnicas

**AE** – Extrato aquoso de erva-mate

**AGL** – Ácido graxo livre

**ANOVA** – Análise de variância

**ATP** – Adenosina trifosfato

**BHA** – Butil hidroxianisol

**BHT** – Butil hidroxitolueno

**CD** – Dienos conjugados

**DHA** – Ácido docosaheptaenóico

**DO** – Oxigênio dissolvido

**EE** – Extrato etanólico bruto de *Ilex paraguariensis*

**EM** – Erva-mate

**EPA** – Ácido eicosapentaenóico

**ERO** – Espécies reativas de oxigênio

**FAME** – Metil éster de ácido graxo

**MDA** – Malondialdeído

**PUFAS** – Ácidos graxos poliinsaturados

**PV** – Valor de peróxidos

**TBARS** – Substâncias reativas ao ácido tiobarbitúrico

**UV** – Ultravioleta

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## 1. INTRODUÇÃO

Os peixes e outras espécies marinhas têm grande importância econômica em muitos países além de seu uso na alimentação trazer benefícios em algumas doenças. De acordo com a Associação Americana do Coração ao menos duas porções de peixe por semana são recomendadas para conferir efeitos protetores ao coração (KRAUSS et al., 2000). Estes efeitos benéficos são atribuídos em particular à composição de ácidos graxos presentes nos peixes (MATAIX e GIL, 2002).

Para o cultivo de peixes os parâmetros de qualidade da água são extremamente importantes, sendo o oxigênio dissolvido e a amônia os principais fatores limitantes (BOYD e WATTEN, 1989; BOYD, 1990). O conhecimento dos efeitos provocados pelas diversas variáveis de qualidade da água é essencial para adotar medidas de manejo, avaliar os possíveis impactos ambientais e manter a qualidade dos organismos cultivados (BOYD e TUCKER, 1998). O dourado, *Salminus brasiliensis*, apresenta ampla distribuição geográfica, sendo encontrado nos rios das bacias do Pantanal, e dos rios Paraná, Uruguai e São Francisco (PAIVA, 1986; GODOY, 1987). Ele apresenta os requisitos necessários para que possa ser considerada uma espécie com potencial para a piscicultura, possui elevado valor de mercado, boa aceitação e demanda de consumo (BALDISSEROTTO e GOMES, 2005) além de ser bastante apreciado pela excelente qualidade de sua carne (KOCH et al., 2000; ZANIBONI FILHO, 2003).

Durante o armazenamento e o processamento dos pescados ocorrem alterações microbiológicas, bioquímicas e sensoriais que estão associadas com a perda de qualidade (EHIRA e UCHIYAMA, 1986). A oxidação lipídica é um dos principais fatores de deterioração dos alimentos. Ela promove o desenvolvimento de sabores e odores desagradáveis assim como alterações na cor, nas propriedades reológicas, textura e valor nutritivo (ADDIS e PARK, 1989; BUCKLEY et al., 1995), que podem levar a rejeição dos produtos pelos consumidores. Os pescados são mais susceptíveis a essas reações, pois possuem grande quantidade de ácidos graxos poliinsaturados em sua composição (KHAYAT e SCHWALL, 1983).

Vários métodos são utilizados para prevenir ou retardar a oxidação lipídica de alimentos, tal como a utilização de antioxidantes. Antioxidantes naturais podem ser

utilizados como uma alternativa na indústria de alimentos. Compostos como os flavonóides, ácidos fenólicos e o tocoferol, naturalmente presentes nos vegetais, são alguns dos responsáveis pela atividade antioxidante (MELLO e GUERRA, 2002). A *Ilex paraguariensis*, conhecida popularmente como erva-mate, é uma planta muito comercializada no sul da América, apresenta em sua composição altas concentrações de ácidos clorogênicos e de flavonóides, que passam para a bebida durante o processo de infusão da erva (BASTOS e TORRES, 2003). Porém, poucos estudos avaliam sua atividade antioxidante em sistemas alimentares (CAMPOS et al., 2007). Assim, o extrato de erva-mate poderia ser utilizado na indústria de alimentos em substituição aos antioxidantes sintéticos, ou em associações com estes, com intuito de diminuir a quantidade de produtos sintéticos nos alimentos.

São escassos os estudos sobre a influência de fatores fisiológicos ante-mortem na qualidade da carne de pescados, e não foram encontrados estudos dessa natureza para o dourado. Também não foram encontrados estudos avaliando a estabilidade de filés de dourado e buscando estender a sua vida de prateleira. Assim, o objetivo do presente estudo foi avaliar os efeitos da exposição a concentrações estressantes de amônia e oxigênio na água *in vivo* e do tratamento com extrato de erva-mate (*Ilex paraguariensis*) *post mortem* sobre a estabilidade lipídica de filés de dourado (*Salminus brasiliensis*).

## 2. REVISÃO BIBLIOGRÁFICA

### 2.1 Qualidade da água

Os parâmetros de qualidade da água, tais como a temperatura, salinidade, pH, dureza, resíduos nitrogenados, disponibilidade de oxigênio e turbidez, são extremamente importantes em aquicultura, podendo afetar a sobrevivência, crescimento e reprodução dos organismos (BALDISSEROTTO, 2002). Dentre esses parâmetros de qualidade de água, o oxigênio dissolvido e a amônia representam os principais fatores limitantes em sistemas de cultivo (BOYD e WATTEN, 1989; BOYD, 1990). As respostas bioquímicas às diferentes concentrações destes na água variam bastante entre as espécies. Estas respostas incluem alterações nas células sanguíneas, formação de compostos que levam ao estresse oxidativo, efeitos sobre a performance de crescimento e eficiência de conversão alimentar (HALLIWELL e GUTTERIDGE, 1999; RITOLA et al., 2000; WOOD, 2001; FOSS et al., 2003).

Um dos fatores que pode levar ao acúmulo de substâncias que alteram a qualidade da água, tais como sólidos em suspensão, amônia, dióxido de carbono, além da diminuição dos níveis de oxigênio dissolvido, é quando os peixes são cultivados em altas densidades, especialmente quando a renovação da água é restrita (STREIT, 2006).

A amônia pode ser originada de várias fontes que incluem efluentes de água de esgotos, descargas industriais, resíduos agrícolas e como um produto natural do catabolismo protéico de peixes teleósteos (FOSTER et al., 1969), existindo em duas formas, a ionizada ( $\text{NH}_4^+$ ) menos tóxica (TOMASSO, 1994) e a forma não-ionizada ( $\text{NH}_3$ ). A forma não-ionizada pode facilmente se difundir através do epitélio das brânquias tornando-se mais tóxica (PERSON-LE RUYET et al., 1997). O aumento da concentração de amônia na água provoca uma redução no gradiente de difusão entre o sangue e o meio externo ocasionando um aumento na quantidade de amônia no sangue e nos tecidos, que produz sérios problemas fisiológicos (BOYD e TUCKER, 1998). Dentre esses problemas destacam-se alterações na transformação da energia alimentar em ATP, provocando a desaminação dos aminoácidos, o que, por sua vez, impossibilita a formação de proteínas essenciais para manter o

crescimento dos animais (PARKER e DAVIS, 1981). Foram observadas alterações fisiopatológicas nos rins, fígado, baço (WOOD, 2001). Tem sido relatadas reduções da taxa de crescimento de peixes expostos à amônia em algumas espécies, como o pregado, *Scophthalmus maximus*, atualmente *Psetta maxima* (RASMUSSEN e KORSGAARD, 1996; PERSON-LE RUYET et al., 1997), dourada, *Sparus aurata* (WAJSBROT et al., 1993), linguado-legítimo, *Solea solea* (ALDERSON, 1979), bagre americano, *Ictalurus punctatus* (TOMASSO, 1994) e perca prateada, *Bidyanus bidyanus* (ROWLAND et al., 1995; FRANCÊS et al., 2000). Além disso, a amônia pode agir diretamente no sistema nervoso central, causando hiperventilação (MCKENZIE et al., 1993), hiperexcitabilidade, coma, convulsões e finalmente a morte (IP et al., 2001).

As principais causas da redução de oxigênio na água são: presença de matéria orgânica, decomposição aeróbia, respiração de animais e plantas, principalmente à noite ou em dias nublados, quando não há atividade fotossintetizante pelo fitoplâncton, e aumento da temperatura, que causa redução na solubilidade do oxigênio e aumento do consumo pelos peixes, devido ao aumento na taxa metabólica (RANTIN e MARINS, 1984; BALDISSEROTTO, 2002). Em casos de sobrevivência em hipóxia severa ( $O_2$  reduzido) ou anóxia freqüentemente (privação total de  $O_2$ ), os peixes transformam a via anaeróbica como preferencial no metabolismo energético. Nesses casos ocorre a formação das espécies reativas de oxigênio (ERO) (HALLIWELL et al., 1999). Entre as ERO estão o radical superóxido ( $O_2^- \cdot$ ), peróxido de hidrogênio ( $H_2O_2$ ) e radical hidroxil ( $\cdot OH$ ). Quando a quantidade de ERO formadas for excessiva em relação à capacidade antioxidante do organismo, estará criado o estresse oxidativo (SIES, 1986). O ideal é criar os peixes em ambientes com alta concentração de oxigênio dissolvido, mas uma redução da pressão parcial de oxigênio ocorre com freqüência em ambientes com pouca circulação de água, como lagos e tanques de cultivo com pouca renovação da mesma (RANTIN e MARINS, 1984).

A amônia é altamente tóxica sozinha, mas interage com o oxigênio tornando-se mais tóxica principalmente em condições em que o oxigênio é reduzido (LLOYD, 1961; ALABASTER et al., 1979; THURSTON et al., 1981; WAJSBROT et al., 1991). Níveis de oxigênio acima da saturação normal podem reduzir a toxicidade da amônia (COLT et al., 1991), e estudos com o peixe-lobo-malhado (*Anarhichas minor*) e bacalhau do Atlântico (*Gadus morhua*) demonstram que essas espécies de peixes

são mais tolerantes a amônia em casos de hiperóxia, quando comparado com concentrações normais de saturação de oxigênio na água (FOSS et al., 2003; REMEN, 2006).

## **2.2 Dourado (*Salminus brasiliensis*)**

O dourado, *Salminus brasiliensis* (Cuvier, 1816), pertencente à família Characidae, gênero *Salminus*, ordem Characiformes e a classe Actinopterygii. Ele habita os rios das bacias do Pantanal, e dos rios Paraná, Uruguai e São Francisco (PAIVA, 1986; GODOY, 1987), sendo considerado o maior peixe de escamas da bacia do Prata e uma das espécies mais nobres dos peixes nativos de água doce. É um peixe carnívoro de coloração típica amarelo-dourado que vive em ambientes lóticos e apresenta hábitos diurnos migrando longas distâncias durante o seu ciclo de vida reprodutivo. Possui grande porte, pois na vida adulta chega a medir em torno de 100.0 cm (BRITSKI et al., 1999) e o peso máximo publicado é de 31,4 kg (MACHACEK, 2007). É considerado um peixe com grande potencial para a aqüicultura como peixe de mesa, na pesca esportiva ou mesmo como ornamental (KUBITZA, 1995) e é bastante apreciado pela excelente qualidade de sua carne (KOCK et al., 2000; ZANIBONI FILHO, 2003).

Por ser sensível a variações da qualidade da água e pela sua posição como consumidor final na cadeia alimentar, o dourado é susceptível a biocumulação de substâncias nocivas oriundas das atividades antrópicas, sendo, por isso, considerado um bom bioindicador da presença de xenobióticos nos corpos de água (BALDISSEROTTO e GOMES, 2005). O dourado necessita que as populações das outras espécies de peixes estejam em equilíbrio para a manutenção de sua população, pois é uma espécie ictiófaga, situada no topo da cadeia trófica. Por essas características, associadas ao fato de ser um migrador de grandes distâncias e ocupar diferentes ambientes nas distintas fases da vida, o dourado pode ser considerado uma “espécie bandeira”, ou seja, uma espécie indicada para estudos de avaliação da qualidade dos ecossistemas aquáticos de água doce (BALDISSEROTTO e GOMES, 2005).

Em relação a composição química dos peixes, alguns autores relatam que ela pode variar consideravelmente devido a fatores como: espécie, idade do animal, sexo, estação do ano e fatores ambientais (GERI et al., 1995; SHIRAI et al., 2002), além disso a composição centesimal (umidade, proteína, lipídios, carboidratos e cinzas) de peixes pode informar sobre hábitos locomotores e adaptações energéticas (CHILDRESS e NYGAARD, 1973; CHILDRESS et al., 1990). Não foram encontrados estudos que avaliam a composição química do dourado.

### **2.3 Armazenamento e processamento de peixes**

Durante o processamento e armazenamento dos peixes ocorrem alterações microbiológicas, bioquímicas e sensoriais que estão associadas com a perda de qualidade (EHIRA e UCHIYAMA, 1986). A taxa e o grau das reações são muito dependentes da espécie do peixe, do tratamento ou do armazenamento utilizado, das condições em que o peixe é manipulado e do conteúdo de ácidos graxos presentes (PETILLO et al., 1998; UNDELAND et al., 1998; OETTERER, 2002). Os ácidos graxos insaturados são as estruturas mais suscetíveis ao processo oxidativo, havendo uma dependência direta entre o grau de insaturação e a susceptibilidade à oxidação (COSGROVE et al., 1987).

Os peixes, na maioria das vezes, são refrigerados ou congelados antes do consumo, sendo que após são consumidos em maior quantidade cozidos em relação ao cru. Durante o armazenamento refrigerado destacam-se alterações nas frações protéica e lipídica, formação de aminas (voláteis e biogênicas) (BENNOUR et al, 1991; NUNES et al, 1992; OLAFSDÓTTIR et al., 1997) e a proliferação de microrganismos (AUBOURG, 1999). Sendo assim, a avaliação da estabilidade de peixes refrigerados está mais focalizada na determinação de alterações causadas principalmente por microrganismos, formação de aminas voláteis, degradação de nucleotídeos e proteínas (AUBOURG, 1999). Já no armazenamento congelado, reações de hidrólise lipídica e oxidação ocorrem em maior frequência além de ocorrer desnaturação protéica e alterações de textura (MACKIE, 1993; VERMA et al., 1995). Sendo assim, a rejeição de pescados congelados susceptíveis a oxidação, se dá com o aparecimento de sabores de ranço, mudanças na textura, na

cor, das propriedades reológicas e no valor nutritivo (BUCKLEY et al., 1995; ADDIS e PARK, 1989).

O aquecimento é aplicado nos alimentos de diferentes maneiras para melhorar a qualidade higiênica pela inativação de microrganismos patogênicos e melhorar o flavor e o sabor dos produtos (BOGNAR, 1998; POKORNY, 1999). Durante o cozimento acontecem alterações químicas e físicas que podem melhorar ou prejudicar o valor nutricional, por ex. digestibilidade é aumentada devido à desnaturação protéica nos alimentos, mas a quantidade de compostos termolábeis, vitaminas lipossolúveis ou conteúdo de ácidos graxos poliinsaturados são frequentemente reduzidos (BOGNAR, 1998; FINOT, 1997). Além disso, o processamento térmico poder promover a oxidação lipídica devido à ruptura das células e à liberação de agentes pró-oxidantes, desse modo induzindo a formação de aroma de requeimado (“warm-over flavor”) em produtos cárneos cozidos durante o armazenamento refrigerado e subsequente reaquecimento (SATO e HEGARTY, 1971).

## **2.4 Estabilidade lipídica**

Os lipídios desempenham um papel importante em relação a qualidade de certos produtos alimentares, particularmente em relação às propriedades sensoriais que os tornam desejáveis, tais como flavor, cor, textura. Por outro lado, conferem valor nutritivo aos alimentos, constituindo uma fonte de energia metabolizável, de ácidos graxos essenciais (ácidos linoléico, linolênico e araquidônico) e de vitaminas lipossolúveis (A, D, E e K) (St. ANGELO, 1996).

A oxidação lipídica é um fenômeno espontâneo e inevitável, com uma implicação direta no valor comercial dos óleos e gorduras, bem como de todos os produtos formulados a partir deles (alimentos, cosméticos, medicamentos) (SILVA, et al., 1999). As reações de oxidação lipídica dependem de mecanismos reacionais diversos e extremamente complexos, os quais estão relacionados com o tipo de estrutura lipídica e o meio onde esta se encontra. O número e a natureza das insaturações presentes, o tipo de interface entre os lipídios e o oxigênio, a exposição à luz e ao calor, bem como a presença de pró-oxidantes (íons metálicos

de transição) ou de antioxidantes, são fatores determinantes para a estabilidade oxidativa dos lipídios (FRANKEL et al., 1994; BERSET et al., 1996.).

Os peixes são mais susceptíveis a essas reações que carne de frango ou de suíno principalmente durante o armazenamento congelado (KHAYAT e SCHWALL, 1983), pois possuem alto conteúdo de ácidos graxos insaturados (SÁNCHEZ-ALONSO et al, 2007), como ácido oléico (18:1), ácido linoléico (18:2), ácido linolênico (18:3) e os ácidos graxos poliinsaturados n-3 (PUFAs) como o ácido eicosapentaenóico, EPA (20:5 $\omega$ -3) e o ácido docosahexaenóico, DHA (22: 6 $\omega$ -3) (MEDINA et al, 2007). Esses ácidos graxos ao longo do tempo sofrem redução em seu conteúdo, decorrentes de reações como a lipólise e a peroxidação (ACKMAN et al., 1986). A lipólise envolve a hidrólise da ligação éster por certas enzimas (lípsases) ou pela combinação do calor e da umidade, que tem como resultado a liberação de ácidos graxos livres (FENNEMA, 2000). Em pescados, a metodologia mais comumente utilizada na determinação de ácidos graxos livres consiste na determinação colorimétrica da formação de complexos acetato cúprico/piridina (LOWRY e TINSLEY, 1976).

Já a peroxidação lipídica é um processo complexo que ocorre em múltiplos estágios (HALLIWELL e GUTTERIDGE, 1989), sendo os três estágios principais denominados como a iniciação, propagação, e a terminação. O estágio da iniciação (auto-oxidação) começa com um período de indução, que é geralmente muito lento e sucedido por um período de uma reação mais rápida; o estágio da propagação (GUNSTONE, 1996). Na auto-oxidação um átomo de hidrogênio é removido do grupo metileno (- CH = CH -) do ácido graxo insaturado, formando um radical livre (- CH = C $\cdot$ -). Este processo ocorre a partir de uma variedade de diferentes iniciadores presentes no alimento, tais como íons metálicos de transição, luz UV e enzimas (ARAÚJO, 2004).

O estágio da propagação sucede o estágio da iniciação quando os radicais livres formados reagem com o oxigênio molecular para formar o radical peroxil (- CH - COO $\cdot$ -), estes radicais são altamente reativos e capazes de remover átomos de hidrogênio de outros ácidos graxos insaturados formando os hidroperóxidos (ROOH) e novos radicais livres. Os radicais livres formados reagem com o oxigênio e a seqüência se repete. Os hidroperóxidos são denominados os produtos primários da oxidação lipídica sendo que a metodologia mais utilizada para a determinação desses compostos em pescados baseia-se na oxidação do ferro (Fe $^{2+}$ ) a íon férrico



(Fe<sup>3+</sup>) por ação dos peróxidos, o qual é dosado por colorimetria sob a forma de tiocianato férrico (CHAPMAN e MACKAY, 1949). Durante esse estágio ocorre uma troca na posição das ligações duplas dos ácidos graxos poliinsaturados, devido a estabilização por ressonância, levando a formação de hidroperóxidos isoméricos denominados de dienos conjugados (FENNEMA, 2000). O teor de dienos conjugados pode ser determinado através de absorção de luz ultravioleta a 233 nm (RECKNAGEL e GLENDE, 1984). A duração do estágio de propagação é dependente de muitos fatores incluindo a relação dos lipídios às proteínas, a composição de ácidos graxos, e a presença e as concentrações de um ou mais antioxidantes (HALLIWELL e CHIRICO, 1993).

O processo de terminação da oxidação acaba por formar na maioria das vezes álcoois, cetonas ou aldeídos de cadeia curta provenientes da clivagem dos peróxidos formados na etapa de propagação. Vários desses compostos são voláteis e, portanto, contribuem com o odor característico associado à oxidação de lipídios. Um exemplo é o hexanal, produto da oxidação do ácido linoléico. O hexanal na quantidade de  $2 \times 10^{-6}\%$ , ou mais, já é suficiente para conferir odor característico da rancidez (KRINSKY, 1989). Esses compostos podem ser avaliados nos alimentos através da dosagem de substâncias reativas ao ácido tiobarbitúrico (TBARS), que é um índice da concentração de malondialdeído (MDA).

## 2.5 Antioxidantes

Vários métodos são utilizados para prevenir ou retardar a oxidação lipídica de alimentos, como o armazenamento em baixas temperaturas, uso de embalagens adequadas e a utilização de antioxidantes. Os antioxidantes são compostos capazes de complexar íons metálicos, de estabilizar, ou de inativar radicais livres (PERCIVAL, 1998). Smith (1987) demonstrou que os antioxidantes sintéticos butil hidroxianisol e galato de propila adicionados em carne de peru anteriormente ao congelamento, inibem consideravelmente a oxidação lipídica. Lin (2000) demonstrou que antioxidantes como o ácido ascórbico,  $\alpha$ -tocoferol e trolox C são eficazes em manter a qualidade de filés de mackerel refrigerados.

Antioxidantes naturais podem ser utilizados como uma alternativa na indústria de alimentos, já que os antioxidantes sintéticos têm sido alvos de questionamentos quanto a sua inocuidade-toxicidade (ANESINI et al., 2006). Estudos recentes revelam que esses compostos podem implicar em vários riscos para a saúde, como o desenvolvimento de câncer (HOU, 2003; PRIOR, 2004). Por estas razões o seu uso é regulamentado na legislação da maioria dos países. Na União Européia, por exemplo, a quantidade de antioxidantes sintéticos é limitada a 0,01% (0,1 g/kg) para cada antioxidante se usado individualmente e a 0,02% da quantidade total se os antioxidantes forem usados em misturas (DIARIO OFICIAL de LAS COMUNIDADES EUROPEAS, Lei nº61 de 1995). No Brasil a legislação determina que antioxidantes como BHA e BHT devam ser adicionados em alimentos nas quantidades máximas de 0,02/100g do produto (RESOLUÇÃO nº 4, de 24 de novembro de 1988).

Os antioxidantes naturais são muitas vezes mais baratos, e menos prejudiciais que os antioxidantes sintéticos, e com efeito equivalente na inibição da oxidação dos tecidos (CAO et al., 1996). Compostos como os flavonóides, ácidos fenólicos e o tocoferol, naturalmente presentes nos vegetais, são alguns dos responsáveis pela atividade antioxidante, e dependendo do substrato lipídico em que atuam e das características inerentes de cada um, exercem atividade antioxidante diferenciada (MELLO e GUERRA, 2002). Está bem estabelecido que flavonóides e ácidos fenólicos apresentam atividade antioxidante similar aos antioxidantes sintéticos como o butil hidroxitolueno (BHT) e o butil hidroxianisol (BHA) (FUKUMOZO et al., 2000).

A *Ilex paraguariensis* Saint Hilaire (Aquifoliaceae), conhecida popularmente como erva-mate, é uma planta muito comercializada no sul da América, que cresce naturalmente ou é cultivada no Brasil, Argentina, Uruguai e Paraguai. Entretanto, cerca de 80% da área de ocorrência pertence ao Brasil, distribuindo-se entre os Estados do Mato Grosso do Sul, São Paulo, Paraná, Santa Catarina e Rio Grande do Sul (ESMELINDRO et al, 2002). A região Sul é a maior produtora, onde 596 municípios desenvolvem a atividade ervateira, envolvendo um total de, aproximadamente, 710.000 pessoas, para uma produção anual aproximada de 650.000 toneladas de folhas (MACCARI et al., 2000).

A erva-mate é consumida como bebida através da adição simples de água quente ao material seco da planta ou preparada por adições repetidas de água aquecida no mesmo material (chimarrão). Ela apresenta em sua composição altas

concentrações de ácido clorogênico e de flavonóides, que passam para a bebida durante o processo de infusão da erva (BASTOS e TORRES, 2003) e são os responsáveis por suas propriedades antioxidantes (FILIP et al., 2000). Numerosos compostos fitoquímicos tem sido identificados na *Ilex paraguariensis*, como derivados cafeoil, principalmente 3,5-dicafeoilquínico, 4,5-dicafeoilquínico, 3,4-dicafeoilquínico, ácido clorogênico, ácido caféico e flavonóides (FILIP et al., 2001). Segundo Medina et al. (2007) o ácido caféico apresenta alta eficiência antioxidante na prevenção da oxidação de peixes e pode inibir significativamente a formação de sabores estranhos, peróxidos e TBARS. A ordem de eficiência antioxidante dos compostos estudados foi ácido caféico > ácido ferúlico = ácido clorogênico >> ácido *o*-coumárico (MEDINA et al., 2007). A atividade antioxidante da *Ilex paraguariensis* tem sido estudada em sistemas biológicos *in vivo* e *in vitro* (GUGLIUCCI e STAHL, 1995; CAMPOS et al., 1996; GUGLIUCCI, 1996; FILIP et al., 2000; SCHINELLA et al., 2000; BRACESCO et al., 2003), porém poucos estudos avaliam a atividade antioxidante em sistemas alimentares. Campos et al. (2007) recentemente demonstrou que o extrato etanólico de erva-mate reduziu a peroxidação em salames.

Em relação à cor, a erva-mate apresenta coloração típica verde devido a presença de clorofila em suas folhas, que permanece após o seu processamento (ANDRADE, 2004). Os argentinos consomem geralmente um produto com cor verde-oliva a amarelo-dourado, enquanto os brasileiros preferem um tom verde-brilhante (MORAWICKI et al, 1999; GALEANO et al, 2006). A clorofila é constituída por uma mistura de dois compostos denominados de clorofila (*a*) e clorofila (*b*), que ocorrem aproximadamente na proporção de 3:1 (STEET e TONG, 1996). Embora ambas sejam verdes, os seus espectros de absorção são ligeiramente diferentes, de maneira que para o olho humano a clorofila *a* apresenta uma tonalidade verde-azulada, e a clorofila *b*, verde-amarelada (SCHWARTZ e LORENZO, 1990; LEHNINGER et al, 1995; STEET e TONG, 1996). A clorofila é sensível ao pH, enzimas, temperatura, luz e oxigênio, os quais têm maior ou menor influência na sua degradação de acordo com a atividade de água do meio (LAJOLO et al, 1971; SCHWARTZ e LORENZO, 1990; BOHN e WALCZYK, 2004). Em virtude disso, a constituição do alimento e as condições de processamento, bem como o ambiente de armazenagem, influenciam grandemente no curso da degradação da clorofila em alimentos processados (HEATON e MARANGONI, 1996; MORAWICKI et al, 1999),

o que pode prejudicar a aparência de alimentos ricos em clorofilas (ELBE e SCHWARTZ, 2000).

### **3. ARTIGOS CIENTÍFICOS**

#### **3.1 Artigo 1**

**QUALITY PARAMETERS AND STABILITY OF FILLETS FROM  
DOURADO (*Salminus brasiliensis*) EXPOSED TO DIFFERENT  
AMMONIA AND OXYGEN LEVELS *IN VIVO***

**Artigo em fase final de revisão pelos autores para ser submetido ao Journal of  
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(configurado conforme as normas da revista)

**Quality parameters and stability of fillets from dourado (*Salminus brasiliensis*) exposed to different ammonia and oxygen levels *in vivo***

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## ABSTRACT

The effects of exposure to stressing ammonia and oxygen levels *in vivo* were evaluated on the composition and thiobarbituric acid reactive substances (TBARS) of fillets from dourado (*Salminus brasiliensis*), as well as on the lipid oxidation of these fillets during frozen storage. A 15-days exposure to the high ammonia level reduced ash and increased protein, while a 15-days exposure to low oxygen and high ammonia reduced the fat of fillets. No change was observed on the fatty acid composition, but a 15-days exposure to the high oxygen level increased TBARS. During frozen storage conjugated dienes increased in fillets from fish that had been exposed to the high levels of oxygen and ammonia (12-h), while peroxides increased only in those exposed to the high ammonia level. These results indicate that exposure to high ammonia levels *in vivo* affects composition and increases the susceptibility of fillets to lipid oxidation during frozen storage.

**KEYWORDS:** lipid oxidation, fatty acid composition, proximate composition, TBARS, conjugated dienes, peroxide value.

## INTRODUCTION

Dourado (*Salminus brasiliensis*) is an inhabitant of the Prata basin, central and southern Brazil (1, 2). It is a large carnivorous species that has aquaculture potential to be used as a food, sport and ornamental fish (2, 3). Although Dourado is much appreciated by the excellent quality of its flesh, studies concerning the quality and stability of its flesh are lacking.

Ammonia and oxygen levels of the water are physicochemical parameters of great importance for fish survival and growth. The biological response to different concentrations of these compounds in the water varies widely among the species. These responses include changes in blood cells, generation of species that lead to oxidative stress, and impairment of growth performance and food conversion efficiency (4, 5, 6, 7). According to Geri et al. (8) and Shirai et al. (9) the proximate composition of fish may vary due the factors such as: species, age of the animal, sex, season, and environmental factors.

Ammonia can be found in two forms, the ionized form ( $\text{NH}_4^+$ ), which is less toxic and the unionized form ( $\text{NH}_3$ ) (10). The unionized form can easily diffuse across the gill epithelium (11). The increase of ammonia concentration in the water provokes a reduction in the diffusion gradient between the blood and the environment, causing an increase of ammonia in the blood and the tissues, which produces serious physiological disturbances (12) because it impairs ATP synthesis and depletes aminoacids. This, in turn, disables synthesis of essential protein for animal growth (13). Moreover, ammonia can cause renal and hepatic dysfunctions (6). Ammonia is remarkably toxic to central nervous system. Current data suggests that elevated  $\text{NH}_4^+$  displaces  $\text{K}^+$  and depolarizes neurons, causing excessive activation of



glutamate receptors, which leads to excessive calcium influx and subsequent cell death in the central nervous system (14).

The availability of oxygen in water can limit growth and performance of fish (15, 16) besides affecting cellular reactive oxygen species (ROS) levels and respectively induce oxidative stress (17, 18, 19). In cases of survival to severe hypoxia or to frequent anoxia fish will preferentially use the anaerobic pathway in the energy metabolism, which may lead to the formation of ROS (4). When the amount of reactive oxygen species formed exceeds the endogenous antioxidant capacity of the organism, there is oxidative damage to lipids, DNA and proteins, leading to oxidative stress (20).

Oxidation is an important cause of the loss of quality of frozen foods, because it can lead to the development of unpleasant tastes and off-flavours as well as changes in colour, texture and nutritive value. Also, lipid oxidation results in the generation of toxic compounds such as 4-hydroxynonenal (21). Fish are more susceptible to oxidative degradation during frozen storage due to the abundance of polyunsaturated fatty acids present in its flesh (22).

Although there are numerous studies evaluating the effect of ammonia and oxygen levels on biological response of various fish species, studies concerning the influence of these parameters on the quality of the fish fillets are lacking. The objective of this work was to study the influence of exposure to stressing levels of ammonia and oxygen through the water on the proximate composition, fatty acid composition and lipid peroxidation of fillets from dourado, as well as to evaluate the stability of these fillets during frozen storage.

## MATERIALS AND METHODS

### Treatments

One hundred and twenty juveniles of dourado (average weight: 166.9g and length: 25.43 cm) were obtained from the Department of Aquaculture of the Universidade Federal de Santa Catarina (UFSC) and carried to the Laboratory of Fish Physiology at the Universidade Federal de Santa Maria (Brazil). After the transport, fish were conditioned in 12 continuously aerated (2 air pumps of 20 W each) 250-L tanks (10 fish per tank) and acclimated to the laboratory conditions for seven days.

After acclimation water was conditioned for fish exposure to one of the following four treatments: 1) minimum  $\text{NH}_3$  (approximately 0.01 mg/L) + dissolved oxygen (DO) > 6.0 mg/L (approximately 6.25 mg/L) (control=ideal water conditions); 2) minimum  $\text{NH}_3$  + 4.5-5 mg/L DO; 3) 0.1 mg/L  $\text{NH}_3$  + DO > 6.0 mg/L; 4) 0.1 mg/L  $\text{NH}_3$  + 4.5-5 mg/L DO. Fish were submitted to treatments for 12 hours or 15 days. Three independent replicates were conducted for each treatment. Water dissolved oxygen (DO) and ammonia levels were monitored throughout all experimental period. Total ammonia levels were monitored three times per day, and levels were kept within the desired range for each treatment using the method described by Greenberg et al. (23). The unionized ammonia was calculated as described by Piper et al. (24). Water  $\text{NH}_3$  levels were increased by ammonium chloride addition. DO levels and temperature ( $25 \pm 1^\circ\text{C}$ ) were monitored three times per day using an YSI Oxymeter (Y5512 model). Water pH was monitored with DMPH-2 pHmeter (Digimed, São Paulo, Brazil) and was in average  $7.6 \pm 0.2$  during all the experimental period. DO levels were kept within the desired range for each treatment by aeration with either air or nitrogen. In all the treatments fish were fed once a day (at 15:00) with the

same commercial fish diet used in UFSC: Supra carnivorous 42% crude protein, 2-4 mm (Alisul Alimentos, São Leopoldo, Brazil), at 5.0% body mass. Uneaten food as well as other residues and feces were siphoned daily after feeding and consequently approximately 50% of the water in the tanks was replaced by water with previously adjusted DO and ammonia levels.

After the exposure period (12 h or 15 days) fish were slaughtered by hypothermia and filleted using common household practices. Fillets were immediately used to determine proximate composition, fatty acid composition and TBARS levels. A set of fillets from dourado that were exposed for 12 h were immediately packed in polystyrene trays covered with low density polyethylene film and stored at  $-7\pm 1^{\circ}\text{C}$ . These samples were analyzed after 3, 6 and 12 months of frozen storage to assess lipid oxidation.

### **Analysis of fresh fillets**

Moisture was determined by the weight loss after 4 hours at  $60^{\circ}\text{C}$  in an assisted air circulation oven, followed by 8 hours at  $105^{\circ}\text{C}$ . Ash content was determined at  $550^{\circ}\text{C}$  according to AOAC (25). Crude protein ( $\text{N} \times 6.25$ ) was determined by the microKjeldahl procedure (25). Fat content was determined using chloroform and methanol as described by Bligh and Dyer (26).

For evaluation of fatty acid composition fat was extracted according to Bligh and Dyer (26) followed by saponification in methanolic KOH solution and esterification in methanolic  $\text{H}_2\text{SO}_4$  solution (27). The fatty acid methyl esters (FAME) were analyzed using an Agilent Technologies gas chromatograph (HP 6890) fitted with a capillary column DB-23 (50% cyanopropyl-methylpolysiloxane, 60 m x 0.25 mm x 0.25  $\mu\text{m}$ ) and flame ionization detection. The injection and detector

temperature were set at 250°C and the carrier gas was nitrogen (0.6 mL/min). After injection (1 µL, split ratio 50:1) the column temperature was hold at 120°C for 5 min, then increased to 240°C at 4°C/min, and hold at this temperature for 10 min. Standard fatty acid methyl esters were run under the same conditions and the subsequent retention times were used to identify fatty acids. The fatty acids were expressed as percentages of the total fatty acid content in the standard.

Samples (1g) were homogenized with 1.5% KCl (1:5, w/v), centrifuged at 3,000 *g* for 10 min and the supernatant was used for determination of TBARS as described by Buege and Aust (28). Briefly, samples were incubated at 100°C for 15 minutes in a medium containing trichloroacetic acid and thiobarbituric acid. After incubation, butyl alcohol was used to extract the reaction product that was determined at 535 nm.

### **Analysis of frozen fillets**

TBARS was determined as described for fresh fillets. Conjugated dienes (CD) peroxides value (PV), and free fatty acids (FFA) were determined in the fat that was extracted by the Bligh and Dyer method (26). PV value was determined using a ferric thiocyanate method according to Chapman and Mackay (29). No preliminary dilution with benzene/methanol solution was necessary. A standard curve using ferric iron solution was used to calculate the content of peroxides in the sample's fat. CD value was determined using cyclohexane as solvent and recording optical density (1 cm light path) at 233 nm against a cyclohexane blank (30). FFA content was determined according to Lowry and Tinsley (31). Toluene was used as solvent (2.5 ml), 0.5 ml of 5% cupric acetate-pyridine reagent was added to the tube and shaken for 2 min. The biphasic system was centrifuged for 10 min, and the upper layer was read at 725 nm.

A standard curve using oleic acid solution was used to calculate the content of free fatty acid in the sample's fat.

### Statistical analysis

Data were analyzed using the Statistica 6.0 software package (32). Results were submitted to three-way factorial analysis of variance (ANOVA) (2 ammonia levels x 2 oxygen levels x 2 exposure times for fresh fillets or 2 ammonia levels x 2 oxygen levels x 3 storage times for frozen fillets) and the differences between the averages were post hoc evaluated using Tukey's test. Differences were considered to be significant when  $p < 0.05$ .

## RESULTS AND DISCUSSION

Proximate composition of fillets from dourado submitted to different levels of  $\text{NH}_3$  and  $\text{O}_2$  *in vivo* are shown in **Table 1**. Fish are often classified on the basis of their fat content (33). Lean fish have less than 5% fat by weight, medium fat fish have 5-10% fat, while fatty fish have more than 10% fat by weight. The dourado samples evaluated were classified as lean fish (**Table 1**), similar to other freshwater fish as tilapia (*Oreochromis mossambicus*) (2.75%) and snakehead (*Channa striatus*) (3.25%) (34). Fat values found were within the range of fat content previously reported for dourado (*Salminus maxillosus*):  $4.6 \pm 2.8\%$  (35) and  $0.88 \pm 0.01\%$  (36). A significant effect of exposure time was observed on the moisture content of fillets and post hoc analysis revealed that moisture increased during the experimental period (12 hours vs. 15 days), without significant effect of ammonia or oxygen treatment (**Table 1**).

A significant ammonia x time interaction and oxygen x time interaction was observed on the ash content. Post hoc analysis revealed that ash content of fillets

diminished during the experimental period (12 hours vs. 15 days), but only when fish were exposed to high ammonia level (0.1 mg/L) (Table 1,  $p < 0.05$ ). Besides, ash content reduced when the oxygen concentration was reduced ( $> 6.0$  mg/L vs. 4.5 mg/L), but only in fish that were exposed to high ammonia level (0.1 mg/L) for 12 h (Table 1).

ANOVA revealed a significant ammonia x time interaction on protein content and ammonia x oxygen x time interaction on fat content. Post hoc analysis revealed that protein content of fillets increased during the experimental period (12 hours vs. 15 days), but only when fish were exposed to high ammonia levels (0.1 mg/L) (Table 1;  $p < 0.05$ ). Post hoc analysis also revealed that fat content of fillets decreased during the experimental period (12 hours vs. 15 days), but only when fish were exposed to low oxygen level (4.5 mg/L) and high ammonia level (0.1 mg/L) (Table 1,  $p < 0.05$ ). These findings reveal that a short-term exposure (12 h) to environmental stressing conditions (low oxygen level and high ammonia level) did not change the composition of flesh. However, a longer exposure period (15 days) to these stressing conditions caused significant changes in flesh composition. We found no previous reports on the impact of stressing environmental conditions to the quality of fish fillets. However, we could propose that stored fat could have been mobilized to provide energy for mediating a biological response to the stressing condition. This reduction of fat content would be responsible for the relative increase of the protein content. In fact, exposure to ammonia has been reported to reduce the growth rate of various other fish species, as turbot, *Scophthalmus maximus* (11, 37), gilthead seabream, *Sparus aurata* (38), Dover sole, *Solea solea* (39), American catfish, *Ictalurus punctatus* (10) and silver perch, *Bidyanus bidyanus* (40, 41), which is in agreement with our findings.

Fifteen fatty acids were identified in dourado fillets with the following distribution: 1.0% myristic (14:0), 21.2% palmitic (16:0), 2.8% palmitoleic (16:1  $\omega$ -7 *cis*), 0.5% palmitolaidic (16:1  $\omega$ -7 *trans*), 10.6% stearic (18:0), 34.0% oleic (18:1  $\omega$ -9), 18.8% linoleic (18:2  $\omega$ -6), 1.45%  $\alpha$ -linolenic (18:3,  $\omega$ -3), 0.2% arachidic (20:0), 0.9% gondoic (20:1  $\omega$ -9), 2.3% arachidonic (20:4  $\omega$ -6), 0.3% erucic (22:1  $\omega$ -9), 0.2% lignoceric (24:0), 0.5% docosapentaenoic (DPA, 22:5  $\omega$ -3) and 4.4% docosahexaenoic acid (DHA, 22:6  $\omega$ -3). Myristoleic acid (14:1  $\omega$ -5), docosanoic acid (22:0) and eicosapentaenoic acid (20:5  $\omega$ -3) were not detected in the dourado fillets evaluated. No significant effect of ammonia, oxygen or exposure time was observed on the fatty acid composition (data not shown). The distribution of the fatty acid groups was 6.4% of  $\omega$ -3, 21.2% of  $\omega$ -6, 33.5% of saturated, 38.7% monounsaturated and 27.8% polyunsaturated acids. Results of saturated and polyunsaturated fatty acids are similar to those of Özogul et al. (42) for freshwater fish species from Turkey and those of Rahnan et al. (34) that studied 20 freshwater species from Malaysia. However, fatty acid composition was somewhat different from that previously reported for *Salminus maxillosus*: 2.5% myristic, 39.4% palmitic, 5.2% palmitoleic, 9.2% stearic, 9.8% oleic, 5.0% linoleic,  $\alpha$ -linolenic not detected, arachidic not detected, 7.1% docosahexaenoic acid and 4.8% eicosapentaenoic acid (36). This discrepancy may be related to differences in the fish diet, since fish of the present study were cultivated with commercial feed, while fish from the study of Andrade et al. (36) were purchased from fish markets and were probably wild samples (caught from river).

TBARS value is an index of lipid peroxidation, because it evaluates one of the major products of lipid peroxidation (malonaldehyde, MDA). There was a significant oxygen x time interaction on TBARS value of dourado fillets evaluated immediately

after exposure to stressing levels of ammonia and oxygen (**Table 2**). Post hoc analysis revealed that TBARS increased during the experimental period (12 hours vs. 15 days), but only when fish were exposed to the higher oxygen level (>6.0 mg/L) (**Table 2**,  $p < 0.05$ ). The antioxidant enzymes, which trigger the antioxidant defenses and which are intrinsically linked, are expected to increase under hypoxia or anoxia in order to detoxify ROS (43, 44). This functions as an anticipatory strategy in which the fish enhances antioxidant capacity during the hypoxic/anoxic state in order to prepare for oxidative stress that will occur when oxygen levels rise again (43, 45). Possibly it justifies the fact that TBARS did not increase significantly in the fish exposed to low oxygen level.

Lipid oxidative changes during frozen storage of dourado fillets submitted to different levels of ammonia and oxygen *in vivo* were determined by measuring conjugated dienes (CD) and peroxide value (PV) that are the primary oxidation products, as well as thiobarbituric acid reactive substances (TBARS) that are secondary oxidation products. Free fatty acids (FFA) were evaluated as an index of triglycerides hydrolysis. There was a significant ammonia x oxygen x time interaction on both CD and PV of dourado fillets (**Tables 3 and 4**). CD values increased after 6 months of frozen storage and then decreased at 12 months, but this change was significant only for fillets from fish that had been exposed to the high levels of both oxygen and ammonia ( $p < 0.05$ ; Table 3). The decrease of CD levels after 6 months of storage is possibly due to its decomposition into smaller molecules (46; 47). PV increased during frozen storage (12 vs. 3 months), but this increase was higher in fillets from fish that had been exposed to the high ammonia level ( $p < 0.05$ ; Table 4). This way, after 12 months of storage, fillets from fish exposed to the high ammonia level had higher PV when compared to those from fish exposed to the low ammonia



level ( $p < 0.05$ ; Table 4). These results indicate that exposure to a stressing ammonia level (0.1 mg/L) before slaughtering may result in fish fillets with increased susceptibility to lipid oxidation, possibly reducing its shelf-life. Conversely, exposure to low oxygen levels, that is also supposed to be stressful, did not increase lipid oxidation rate of fillets possibly due the “preparation for oxidative stress” through increase of the antioxidant potential of fish under anoxia or hypoxia (43). Ammonia is highly toxic alone, but interacts with oxygen to become more toxic at low oxygen levels (48; 49; 50; 51). However, in the present study, decreasing water dissolved oxygen level did not increase the lipid oxidation of fillets from fish exposed to the high ammonia level during the frozen storage.

TBARS values increased during frozen storage ( $1.51 \pm 0.05$ ,  $1.51 \pm 0.08$ , and  $2.09 \pm 0.19$  mg MDA/kg meat for 3, 6 and 12 months, respectively,  $p < 0.05$ ). However, no significant effect of ammonia or oxygen was observed on TBARS levels (data not shown). According to Al-Kahtani et al. (52) meat products can be considered in a good conservation state, concerning to oxidative changes, when they had less than 3 mg MDA/kg. Dourado fillets were below this threshold after 12 months of frozen storage, indicating that lipid oxidation chain reaction did not progress substantially to the formation of secondary products yet. No significant effect of ammonia, oxygen or storage time were observed on free fatty acid content (data not shown), indicating that triglyceride hydrolysis was not affected by treatment and did not change during frozen storage.

In conclusion, our findings revealed that a 15-days exposure of dourado to high ammonia levels affects composition of fillets, while a 12-h exposure to high ammonia levels immediately before slaughtering increases the susceptibility of fillets to lipid oxidation during frozen storage and may reduce the shelf-life of fillets. In

contrast, 12-h exposure to low oxygen levels did not increase lipid oxidation rate of fillets possibly because dourado enhanced its antioxidant capacity when exposed to low oxygen levels.

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**Table 1:** Proximate composition (%) of fillets from *S. brasiliensis* submitted to different levels of NH<sub>3</sub> and DO *in vivo*

Treatments		Exposure	Moisture	Protein	Ash	Fat
NH <sub>3</sub>	DO					
Minimum (0.01mg/L)	>6.0 mg/L	12 hours	75.01 <sup>b</sup> (0.40)	13.69 <sup>c</sup> (0.33)	1.74 <sup>a,b</sup> (0.02)	3.29 <sup>a,b</sup> (0.09)
		15 days	77.96 <sup>a</sup> (0.66)	13.81 <sup>c</sup> (0.34)	1.60 <sup>b,c,d</sup> (0.02)	2.54 <sup>b,c</sup> (0.16)
	4.5-5.0 mg/L	12 hours	74.27 <sup>b</sup> (0.05)	14.11 <sup>c</sup> (0.44)	1.60 <sup>b,c,d</sup> (0.05)	3.37 <sup>a,b</sup> (0.10)
		15 days	78.03 <sup>a</sup> (0.53)	14.22 <sup>b,c</sup> (0.55)	1.58 <sup>c,d,e</sup> (0.00)	2.80 <sup>a,b</sup> (0.08)
	>6.0mg /L	12 hours	75.20 <sup>b</sup> (0.28)	13.60 <sup>c</sup> (0.09)	1.82 <sup>a</sup> (0.03)	3.15 <sup>a,b</sup> (0.00)
		15 days	77.63 <sup>a</sup> (0.06)	16.08 <sup>a,b</sup> (0.13)	1.45 <sup>e</sup> (0.02)	3.44 <sup>a</sup> (0.23)
0.1 mg/L	4.5-5.0 mg/L	12 hours	75.20 <sup>b</sup> (0.26)	14.13 <sup>c</sup> (0.30)	1.66 <sup>b,c</sup> (0.03)	2.97 <sup>a,b</sup> (0.09)
		15 days	77.99 <sup>a</sup> (0.40)	16.78 <sup>a</sup> (0.94)	1.49 <sup>d,e</sup> (0.02)	1.87 <sup>c</sup> (0.25)

Values are mean (SE) of three independent assays. Means within the same column that have no common letters differ significantly ( $p < 0.05$ ). DO: Water dissolved oxygen.

**Table 2:** TBARS value (mg MDA/kg meat) of fillets from *S. brasiliensis* submitted to different levels of NH<sub>3</sub> and DO *in vivo*

Treatments		Duration of exposure	
DO	NH <sub>3</sub>	12 hours	15 days
> 6.0 mg/L	Minimum (0.01 mg/L)	0.32 <sup>b,c</sup> ± 0.00	0.59 <sup>a</sup> ± 0.06
	0.1mg/L	0.17 <sup>c</sup> ± 0.02	0.53 <sup>a,b</sup> ± 0.09
4.5-5.0 mg/L	Minimum (0.01 mg/L)	0.36 <sup>a,b,c</sup> ± 0.02	0.46 <sup>a,b</sup> ± 0.10
	0.1mg/L	0.12 <sup>c</sup> ± 0.02	0.30 <sup>b,c</sup> ± 0.02

Values are means ± standard error, n=3. Means that have no common letters differ significantly ( $p < 0.05$ ). DO: Water dissolved oxygen.

**Table 3:** Changes in conjugated dienes value (optical density/mg lipid/mL cyclohexane) of fillets from *S. brasiliensis* submitted to different levels of NH<sub>3</sub> and DO *in vivo* (12 h exposure), during frozen storage (-7°C)

Treatments		Storage (months)		
DO	NH <sub>3</sub>	3	6	12
> 6.0 mg/L	Minimum (0.01mg/L)	1.93 <sup>b,c,d</sup> ± 0.06	3.34 <sup>b</sup> ± 0.67	1.37 <sup>c,d</sup> ± 0.20
	0.1mg/L	2.93 <sup>b,c</sup> ± 0.56	5.70 <sup>a</sup> ± 0.27	1.28 <sup>d</sup> ± 0.09
4.5-5.0 mg/L	Minimum (0.01mg/L)	1.29 <sup>d</sup> ± 0.08	1.64 <sup>c,d</sup> ± 0.08	1.84 <sup>b,c,d</sup> ± 0.67
	0.1mg/L	2.34 <sup>b,c,d</sup> ± 0.28	1.29 <sup>d</sup> ± 0.03	1.31 <sup>d</sup> ± 0.13

Values are means ± standard error, n=3. Means that have no common letters differ significantly ( $p < 0.05$ ). DO: Water dissolved oxygen.

**Table 4:** Changes in peroxide value (mEq/kg lipid) of fillets from *S. brasiliensis* submitted to different levels of NH<sub>3</sub> and DO *in vivo* (12 h exposure), during frozen storage (-7°C).

Treatments		Storage (months)		
DO	NH <sub>3</sub>	3	6	12
> 6.0 mg/L	Minimum (0.01mg/L)	3.52 <sup>d</sup> ± 0.05	5.71 <sup>c,d</sup> ± 1.75	12.92 <sup>b,c</sup> ± 1.15
	0.1mg/L	6.50 <sup>c,d</sup> ± 1.60	8.76 <sup>c,d</sup> ± 0.38	47.26 <sup>a</sup> ± 1.98
4.5-5.0 mg/L	Minimum (0.01mg/L)	2.80 <sup>d</sup> ± 0.15	1.70 <sup>d</sup> ± 0.21	20.37 <sup>b</sup> ± 1.88
	0.1mg/L	7.21 <sup>c,d</sup> ± 1.43	1.37 <sup>d</sup> ± 0.09	43.69 <sup>a</sup> ± 2.99

Values are means ± standard error, n=3. Means that have no common letters differ significantly ( $p < 0.05$ ). DO: Water dissolved oxygen.

### 3.2 Artigo 2

**EFFECT OF ERVA-MATE (*Ilex paraguariensis*) EXTRACT ON LIPID  
AND COLOUR CHANGES OF DOURADO (*Salminus brasiliensis*)  
FILLETS DURING FROZEN STORAGE**

**Artigo em fase final de revisão pelos autores para ser submetido à revista**

**Food Chemistry**

(configurado conforme as normas da revista)

**Effect of erva-mate (*Ilex paraguariensis*) extract on lipid and colour changes of dourado (*Salminus brasiliensis*) fillets during frozen storage**

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## ABSTRACT

This study evaluated the influence of erva-mate (*Ilex paraguariensis*) on lipid and colour changes of dourado (*Salminus brasiliensis*) fillets during frozen storage. Fish fillets were dipped during 1 min in distilled water (control) or in 10% (w/v) aqueous crude erva-mate extract and were immediately analyzed or stored at -7°C for up to 12 months until analysis. No significant effect of erva-mate treatment or storage time was observed on the fatty acid composition. Free fatty acid content and conjugated dienes value increased in control fillets after 6 months of storage and then decreased, while TBARS value increased during all the experimental period. Erva-mate was effective to reduce free fatty acid content, as well as conjugated dienes and TBARS value. Dourado fillets tended to yellow during all the experimental period ( $H^* = 72.55$ ) and erva-mate treatment reduced the increase of luminosity ( $L^*$ ) and yellowness ( $H^*$ ) triggered by frozen storage. Results indicate that 10% aqueous erva-mate extract has antioxidant activity in dourado fillets.

**KEYWORDS:** conjugated dienes, TBARS, CIELab, fatty acid composition, DPPH, vegetable extract

## 1. Introduction

Dourado (*Salminus brasiliensis*) is an inhabitant of the Prata basin, central and southern Brazil (Morais Filho & Schubart, 1955; Koch, Milani, & Grosser, 2000). It is a large carnivorous species that has aquaculture potential to be used as a food, sport and ornamental fish (Koch et al., 2000; Zaniboni Filho, 2003). Although dourado is much appreciated by the excellent quality of its flesh, studies concerning the processing and stability of its flesh are lacking.

Oxidation is an important cause of the loss of quality of frozen foods, because it can lead to the development of unpleasant tastes and off-flavours as well as changes in colour, texture and nutritive value (Addis & Park, 1989). Also, lipid oxidation results in the generation of toxic compounds such as 4-hydroxynonenal (Addis et al., 1989). Fish are more susceptible to oxidative degradation during frozen storage due to the abundance of polyunsaturated fatty acids present in its flesh (Khayat & Schwall, 1983).

Some methods may be used to prevent or delay lipid oxidation during frozen storage such as the use antioxidant substances. The interest for finding naturally occurring antioxidants for use in food products has been growing, since many synthetic antioxidants have been shown to be toxic or mutagenic (Anesini, Ferraro & Filip, 2006). Some natural compounds such as anthocyanidins/anthocyanins, flavonoids and phenolic acids have antioxidant activities similar to that of synthetic antioxidants such as BHA and BHT (Cao, Sofic & Prior, 1996; Fukumozo & Mazza, 2000).

*Ilex paraguariensis* Saint Hilaire (Aquafoliaceae) is popularly known as erva-mate (Streit et al., 2007). Its natural occurrence area is restricted to Brazil, Argentina, Uruguay, and Paraguay, where it is widely consumed as a tea-like beverage



prepared by the infusion of dried and minced leaves and twigs (Clifford & Ramirez-Martinez, 1990; Bravo, Goya & Lecumberri, 2007). It contains various caffeoyl-derivatives and other polyphenols that are responsible for its antioxidant properties (Filip, Lotito, Ferraro & Fraga, 2000). The antioxidant activity of *Ilex paraguariensis* has been evaluated in biological systems both *in vivo* and *in vitro* (Gugliucci and Stahl, 1995; Campos, Escobar & Lissi, 1996; Gugliucci, 1996; Schinella, Troiani, Dávila, Buschiazzo & Tournier, 2000; Filip et al., 2000; Bracesco, Dell, Behtash, Menini, Gugliucci & Nunes, 2003). However, few studies evaluated its antioxidant activity in food systems. Campos, Hierro, Ordóñez, Bertol, Terra and la Hoz de (2007) recently demonstrated that an ethanolic erva-mate extract reduced lipid peroxidation of salami.

Considering the potential antioxidant activity of erva-mate extracts and the susceptibility of frozen fish to oxidative deterioration, the present study was aimed at evaluating the effect of erva-mate extract on lipid and colour changes of dourado (*Salminus brasiliensis*) fillets during frozen storage.

## **2. Materials and methods**

### **2.1. Treatments**

Erva-mate (dried and minced leaves and twigs) was obtained from local market. Samples were weighed (10% w/v), diluted in distilled water at room temperature and then filtered to yield the aqueous crude extract.

Samples of dourado (*Salminus brasiliensis*) were obtained from a local fish farm (Santa Maria, Brazil) slaughtered by hypothermia and immediately transported to the laboratory in ice containing boxes. Fresh fish were washed with tap water several times to remove adhering blood and slime and were filleted using common

household practices. The fillets (approximately 30g each) were dipped during 1 min in distilled water (control) or in 10% aqueous crude erva-mate extract. Some fish samples were separated and immediately used for determination of colour, fatty acid composition, fat content and TBARS value. Another set of samples were stored in polystyrene trays covered with low density polyethylene film at  $-7\pm 1^{\circ}\text{C}$  and were analyzed after 3, 6 and 12 months.

## 2.2. Analyses

Antioxidant activity of erva-mate extract was evaluated as the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity. DPPH radical scavenging assay was determined according to Brand-Williams, Cuvelier and Berset (1995) modified method. DPPH solution (0.24 mg/mL) was previously diluted until  $1.10\pm 0.02$  absorbance at 517 nm was obtained. Then, sample extract diluted (1:250) was mixed with the diluted methanolic DPPH solution. The radical scavenging power of the extracts was determined by measuring the decrease of DPPH absorbance at 517 nm after 24 hours in the dark against a blank. Trolox was used as standard for the calibration curve and results were expressed as  $\mu\text{g}$  trolox equivalents/mL extract.

For evaluation of fatty acid composition, fat was extracted according to Bligh and Dyer (1959) followed by saponification in methanolic KOH solution and esterification in methanolic  $\text{H}_2\text{SO}_4$  solution (Hartmann & Lago, 1973). The fatty acid methyl esters (FAME) were analyzed using an Agilent Technologies gas chromatograph (HP 6890) fitted with a capillary column DB-23 (50% cyanopropyl-methylpolysiloxane, 60 m x 0.25 mm x 0.25  $\mu\text{m}$ ) and flame ionization detection. The injection and detector temperature were set at  $250^{\circ}\text{C}$  and the carrier gas was nitrogen (0.6 mL/min). After injection (1  $\mu\text{L}$ , split ratio 50:1) the column temperature

was hold at 120°C for 5 min, then increased to 240°C at 4°C/min, and hold at this temperature for 10 min. Standard fatty acid methyl esters were run under the same conditions and the subsequent retention times were used to identify fatty acids. The fatty acids were expressed as percentages of the total fatty acid content in the standard.

Samples (1g) of fillets were homogenized with 1.5% KCl (1:5, w/v), centrifuged at 3,000 g for 10 min and the supernatant was used for determination of thiobarbituric acid reactive substances (TBARS) as described by Buege and Aust (1978). Conjugated dienes (CD) and free fatty acids (FFA) were determined in the fat extracted by the Bligh and Dyer method (1959). CD value was determined using cyclohexane as solvent and recording optical density (1 cm light path) at 233 nm against a cyclohexane blank (Recknagel & Glende, 1984). FFA content was determined according to Lowry and Tinsley (1976). Toluene was used as solvent (2.5 ml), 0.5 ml of 5% cupric acetate-pyridine reagent was added to the tube and shaken for 2 min. The biphasic system was centrifuged for 10 min, and the upper layer was read at 725 nm. A standard curve using oleic acid solution was used to calculate the content of free fatty acid in the sample's fat.

Sample colour was assessed using a CR-300 Chromameter (Minolta, Osaka, Japan) according to the Intl. Commission on Illumination (CIE 1976  $L^* a^* b^*$ ), using a standard illumination D65, with 10° supplementary standard observer and a standard calibration plate (number 15233011). Three measurements were recorded at each fillet, with the illumination system rotated 90° between each measurement. The  $L^*$  indicates lightness and  $a^*$  and  $b^*$  are chromaticity coordinates, where +  $a^*$  is the red direction, -  $a^*$  is the green direction, +  $b^*$  is the yellow direction, -  $b^*$  is the blue direction. From the  $a^*$  and  $b^*$  values, the chroma ( $C^*$ ) and hue ( $H^*$ ) values were

calculated. The chroma is an expression of the saturation or intensity and clarity of the colour. It is expressed by the equation;  $C^* = (a^{*2} + b^{*2})^{1/2}$  (Hunt, 1977) and it is 0 at the center and increases according to the distance from the center. Hue is the observable colour and is calculated by equation,  $H^* = \tan^{-1} (b^*/ a^*)$  (Hunt, 1977). The angle  $H^*$  is defined as starting at the  $+ a^*$  axis and is expressed in degrees;  $0^\circ$  would be  $+ a^*$  (red),  $90^\circ$  would be  $+ b^*$  (yellow),  $180^\circ$  would be  $- a^*$  (green), and  $270^\circ$  would be  $- b^*$  (blue).

## 2.9. Statistical analysis

Data were analyzed using the Statistica 6.0 software package. The results were submitted to two-way factorial analysis of variance (ANOVA) (2 treatments x 4 storage times or 2 treatments x 3 storage times) and the differences between the averages of the treatments were verified using Tukey's test. Differences were considered to be significant when  $p < 0.05$ .

## 3. Results and discussion

The DPPH radical scavenging capacity of 10% (w/v) erva-mate extract amounted to 25.8 mg trolox equivalent/mL extract. Considering that the extract was obtained as a 10% (w/v) dilution of erva-mate samples, this antioxidant equivalent represents the amount of antioxidant extracted from 0.1 g of erva-mate.

Dourado fillets used in the present study had average fat content of 2.84%, similar to other freshwater fish as red tilapia (2.41%), tilapia (2.75%) and snakehead (3.25%) (Rahnan, Huah, Hassan & Daud, 1995). Fat values found were within the range of fat content previously reported for dourado (*Salminus maxillosus*):  $4.6 \pm 2.8\%$

(Hiane, Leal Filho, Ramos Filho & Ramos, 2002) and  $0.88 \pm 0.01\%$  (Andrade, Rubira, Matsushita & Souza, 1995).

Fifteen fatty acids were identified in dourado fillets with the following distribution: 0.9% myristic (14:0), 21.4% palmitic (16:0), 2.9% palmitoleic (16:1  $\omega$ -7 *cis*), 0.5% palmitolaidic (16:1  $\omega$ -7 *trans*), 10.9% stearic (18:0), 34.6% oleic (18:1  $\omega$ -9), 18.4% linoleic (18:2  $\omega$ -6), 1.3%  $\alpha$ -linolenic (18:3,  $\omega$ -3), 0.2% arachidic (20:0), 0.9% gondoic (20:1  $\omega$ -9), 2.2% arachidonic (20:4  $\omega$ -6), 0.3% erucic (22:1  $\omega$ -9), 0.2% lignoceric (24:0), 0.4% docosapentaenoic (DPA, 22:5  $\omega$ -3) and 4.2% docosahexaenoic acid (DHA, 22:6  $\omega$ -3). Myristoleic acid (14:1  $\omega$ -5), docosanoic acid (22:0) and eicosapentaenoic acid (20:5  $\omega$ -3) were not detected in the dourado fillets evaluated. No significant effect of storage time or erva-mate treatment was observed on the fatty acid composition (data not shown). The distribution of the fatty acid groups was 6.1% of  $\omega$ -3, 20.7% of  $\omega$ -6, 33.8% of saturated, 39.4% monounsaturated and 26.8% polyunsaturated fatty acids. Results of saturated and polyunsaturated fatty acids are similar to those of Özogul, Özogul and Alagoz (2007) for freshwater fish species from Turkey and those of Rahnan et al. (1995) that studied 20 freshwater species from Malaysia. However, fatty acid composition was somewhat different from that previously reported for *Salminus maxillosus*: 2.5% myristic, 39.4% palmitic, 5.2% palmitoleic, 9.2% stearic, 9.8% oleic, 5.0% linoleic,  $\alpha$ -linolenic not detected, arachidic not detected, 7.1% docosahexaenoic acid and 4.8% eicosapentaenoic acid (Andrade et al., 1995). This discrepancy may be related to differences in the fish diet, since fish of the present study were cultivated with commercial feed, while fish from the study of Andrade et al. (1995) were purchased from fish markets and were probably wild samples (caught from river).

Lipid oxidation in dourado fillets was determined by measuring conjugated dienes (CD) that are primary oxidation products, as well as tiobarbituric acid reactive substances (TBARS) that are secondary oxidation products. Free fatty acids (FFA) were evaluated as products of triglyceride hydrolysis, which may be triggered either by enzymes (lipases) or by the combination of heating and moisture (Fennema, 2000).

There was a significant treatment x storage time interaction on FFA content (Fig. 1). No changes were observed in FFA content of fillets treated with 10% AE during storage, but FFA content of control fillets increased after 6 months of storage and then decreased ( $p < 0.05$ ; Fig. 1). Besides, at 6 months of storage the FFA content of control fillets were higher than that of AE treated fillets ( $p < 0.05$ ; Fig. 1). These results indicate higher hydrolytic activity in control samples and could be related to the water formed during lipid oxidation that could trigger lipolysis of triglycerides. Alternatively, control fillets could have absorbed more water during the treatment than fillets treated with erva-mate extract. FFA acids are more prone to undergo oxidation than fatty acids in triglycerides (Fennema, 2000). Accordingly, control samples would be expected to be more susceptible to lipid oxidation.

A significant treatment x storage time interaction was observed on CD values. No changes were observed in CD values of fillets treated with 10% AE during storage, but CD values of control fillets increased after 6 months of storage and then decreased ( $p < 0.05$ ; Fig. 2). Besides, at 6 months of storage CD values of control fillets were higher than those of AE treated fillets ( $p < 0.05$ ; Fig. 2). This finding is in agreement with the higher FFA content of control samples at this time, and indicates that these FFA are undergoing lipid oxidation yielding primary oxidation products.

A significant treatment x storage time interaction was also observed on TBARS values (Fig. 3). TBARS of control fillets increased until the 12<sup>th</sup> month of storage ( $p < 0.05$ ). However, TBARS of AE treated fillets increased until 6 months of storage and then decreased ( $p < 0.05$ ). At 12 months of storage TBARS value of AE treated fillets was significantly lower than that of control fillets ( $p < 0.05$ ). Aldehydes resulting from lipid peroxidation such as malondialdehyde and 4-OH-nonenal are responsible for the characteristic rancidity odor and rejection of oxidized foods (Hamre, Oyvind & Sandnes, 2003). According to Al-Kahtani, Abu-Tarboush, Bajaber, Atia, Abou-Arab and El-Mojaddidi (1996) meat products can be considered in a good conservation state, concerning to oxidative changes, when they had less than 3 mg MDA/kg. The samples treated with erva-mate extract had very low MDA levels after 12 months of frozen storage (0.64 mg MDA/kg), while the control fillets had MDA levels close to the rejection point (2.36 mg MDA/kg).

Colour parameters of dourado fillets are presented in Table 1. Two-way ANOVA revealed a significant treatment x storage time interaction on the luminosity ( $L^*$  values) of samples.  $L^*$  values significantly increased in control fillets during frozen storage (from 6 months onwards,  $p < 0.05$ ). However,  $L^*$  values of fillets treated with 10% aqueous extract of erva-mate (AE) did not change during storage. Consequently,  $L^*$  values of control and 10% AE fillets were not different at the start of frozen storage, but after 12 months of storage fillets treated with 10% AE had lower  $L^*$  values than control ( $p < 0.05$ ). According to Morkore (2006) the increased  $L^*$  value can be related to protein conformational changes that could change the reflection of the incident light. It is possible that protein oxidation had accompanied the significant increase of TBARS of control after 12 months of storage and could be responsible for  $L^*$  changes. This proposal is consistent with the stability of  $L^*$  values in fillets treated

with erva-mate (Table 1), since these samples had remarkably lower lipid peroxidation (CD and TBARS values) as compared to control fillets (Fig. 2 and 3). These results suggest that erva-mate extract could delay protein oxidation in dourado fillets during frozen storage.

There was a significant effect of storage time on  $a^*$  values, but no significant effect of treatment (Table 1). The decrease in redness ( $a^*$  values) observed both in control and 10% AE fillets after 3 months of storage ( $p < 0.05$ ) was possibly due to lipid and/or protein oxidation that may accelerate hemoglobin autoxidation (Wetterskog & Undeland, 2004).

There was a significant effect of storage time and AE treatment on the yellowness ( $b^*$  value) and saturation of colour (chroma value) of fillets (Table 1). Saturation indicates how much one given colour differs from the gray. Yellowness and saturation ( $C^*$  value) increased significantly during frozen storage ( $p < 0.05$ ). Yellow colour is often associated with lipid oxidation and has been reported to increase substantially during freezing in other fish species (Hamre et al., 2003; Ruff, Fitzgerald, Cross, Hamre & Kerry, 2003). Hence, the development of lipid oxidation, as indicated by the increase of TBARS value during frozen storage of our samples is probably responsible for the associated increase of yellowness and saturation. Yellowness and saturation ( $C^*$  value) were also significantly higher in AE treated fillets when compared to control ( $p < 0.05$ ), which can be related to the presence of carotenoid pigments in the erva-mate extract (Alikaridis, 1987).

Hue value indicated that fillets of dourado tended to yellowness during all the experiment (Table 1), with values similar to those reported by Hamre et al. (2003) for herring. There was a significant treatment x storage time interaction on the hue values. Hue values of samples treated with AE were higher (more tendency to



yellow) than control fillets until 3 months of storage ( $p < 0.05$ ), probably due to presence of carotenoid pigments in the erva-mate extract. During storage  $H^*$  values of both control and AE treated fillets increased, revealing a higher tendency to yellow. This probably occurred due to lipid oxidation as discussed for changes in  $b^*$  value. Accordingly, the increase of  $H^*$  value observed in the present study was higher in the control fillets as compared to AE treated fillets, which also exhibited greater increase in lipid oxidation (CD and TBARS values) as compared to AE treated fillets during frozen storage ( $p < 0.05$ ).

Results obtained demonstrate the possible usefulness of erva-mate extract to extend the shelf-life of fish fillets. Campos et al. (2007) found that salami manufactured with erva-mate extract had significantly lower TBARS values than did products manufactured without the extract during 60 days of vacuum storage. However, no other studies on the antioxidant effect of erva-mate in foods were found. Numerous active phytochemicals have been identified in *Ilex paraguariensis*, as caffeoyl derivatives, mainly 3,5-dicaffeoylquinic, 4,5-dicaffeoylquinic, 3,4-dicaffeoylquinic, chlorogenic acids, caffeic acid and flavonoids (Filip, Lopez, Giberti, Coussio & Ferraro, 2001). These compounds are probably responsible for the antioxidant activity of *Ilex paraguariensis* extract in the present study. Medina, Gallardo, Gonzalez, Lois and Hedges (2007) recently investigated the antioxidant effectiveness of various phenolic compounds (commercially available) in minced fish muscle. They found that caffeic acid showed highest antioxidant efficiency and could significantly inhibit the formation of off-flavors, peroxides, and TBARS. The overall order of antioxidant efficiency for the studied compounds was caffeic acid > ferulic acid = chlorogenic acid >> o-coumaric acid (Medina et al., 2007).

#### **4. Conclusion**

Fatty acid composition of fillets was not changed during storage and was not affected by treatment with erva-mate extract. The lower FFA, CD and TBARS values of fillets treated with erva-mate demonstrates a reduction in the lipid oxidation reactions, indicating that the extract had antioxidant activity in dourado fillets. Besides, fillets treated with erva-mate less color changes (lower changes in L\* and H\* values) during frozen storage, which is probably associated to the antioxidant activity of the extract. Further studies are required to demonstrate the viability of using *Ilex paraguariensis* extracts to extend the shelf-life of frozen fish.

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Table 1: Colour parameters of dourado fillets treated with aqueous extract of *Ilex paraguariensis* (AE) during frozen storage (-7°C)

Storage (months)	Treatments	L*	a*	b*	C*	H*
0	0% AE	56.97 <sup>b, c</sup> (0.69)	6.53 <sup>a</sup> (0.04)	7.51 <sup>d</sup> (0.35)	9.70 <sup>c</sup> (0.11)	47.48 <sup>d</sup> (0.60)
	10% AE	56.42 <sup>b, c</sup> (0.82)	5.53 <sup>a</sup> (0.17)	10.89 <sup>b, c</sup> (0.34)	12.22 <sup>a, b</sup> (0.23)	61.61 <sup>c</sup> (0.22)
3	0% AE	57.44 <sup>b, c</sup> (0.58)	4.70 <sup>a, b</sup> (0.22)	9.47 <sup>c, d</sup> (0.33)	10.33 <sup>c</sup> (0.36)	63.31 <sup>c</sup> (0.28)
	10% AE	52.54 <sup>c</sup> (1.52)	3.54 <sup>a, b</sup> (0.29)	12.61 <sup>a, b</sup> (0.37)	13.10 <sup>a, b</sup> (0.43)	74.33 <sup>b</sup> (0.79)
6	0% AE	61.22 <sup>a, b</sup> (1.65)	3.65 <sup>a, b</sup> (1.71)	12.49 <sup>a, b</sup> (0.99)	11.74 <sup>b, c</sup> (0.99)	83.50 <sup>a</sup> (2.22)
	10% AE	60.61 <sup>a, b</sup> (0.93)	1.51 <sup>b</sup> (0.45)	14.02 <sup>a</sup> (0.22)	14.10 <sup>a</sup> (0.17)	83.80 <sup>a</sup> (1.93)
12	0% AE	64.13 <sup>a</sup> (2.31)	1.38 <sup>b</sup> (0.21)	13.62 <sup>a</sup> (0.44)	13.28 <sup>a, b</sup> (0.21)	85.99 <sup>a</sup> (0.95)
	10% AE	55.67 <sup>b, c</sup> (0.83)	2.47 <sup>b</sup> (0.45)	14.03 <sup>a</sup> (0.07)	14.15 <sup>a</sup> (0.05)	80.39 <sup>a, b</sup> (1.37)

0% AE: distilled water (control). C\*: Chroma. H\*: Hue angle. Values are means (standard error) of three independent assays. Means within the same column that have no common letters are significantly different ( $p < 0.05$ ).



Fig. 1: Changes in free fatty acid content of dourado fillets treated with aqueous extract of *Ilex paraguariensis* during frozen storage (-7°C). 0% AE: control, distilled water (▲) and 10% AE (■). AE: aqueous extract of *Ilex paraguariensis*. Values are means ± standard error (n=3). \*Significantly different from all other groups (p<0.05). &Significantly different from treatment 0% AE at 3 and 6 months of storage (p<0.05).

Fig. 2: Changes in conjugated dienes value (CD) from dourado fillets treated with aqueous extract of *Ilex paraguariensis* during frozen storage (-7°C). 0% AE: control, distilled water (▲) and 10% AE (■). AE: aqueous extract of *Ilex paraguariensis*. Values are means ± standard error (n=3). OD= optical density. \*Significantly different from all other groups (p<0.05).

Fig. 3: Changes in TBARS value from dourado fillets treated with aqueous extract of *Ilex paraguariensis* during frozen storage (-7°C). 0% AE: control, distilled water (▲) and 10% AE (■). AE: aqueous extract of *Ilex paraguariensis*. Values are means ± standard error (n=3). \*\*Significantly different from the same treatment at zero months of storage (p<0.05). #Significantly different from the same treatment at 0, 3 and 6 months of storage (p<0.05). &Significantly different from 0% AE at 12 months of storage (p<0.05).

Fig. 1

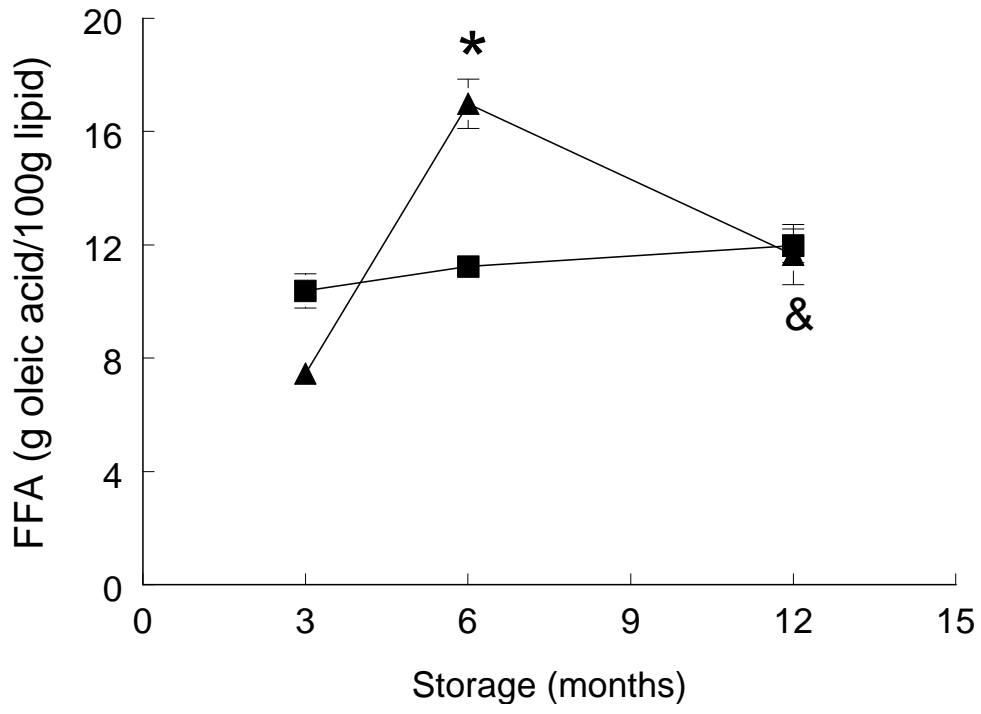


Fig. 2

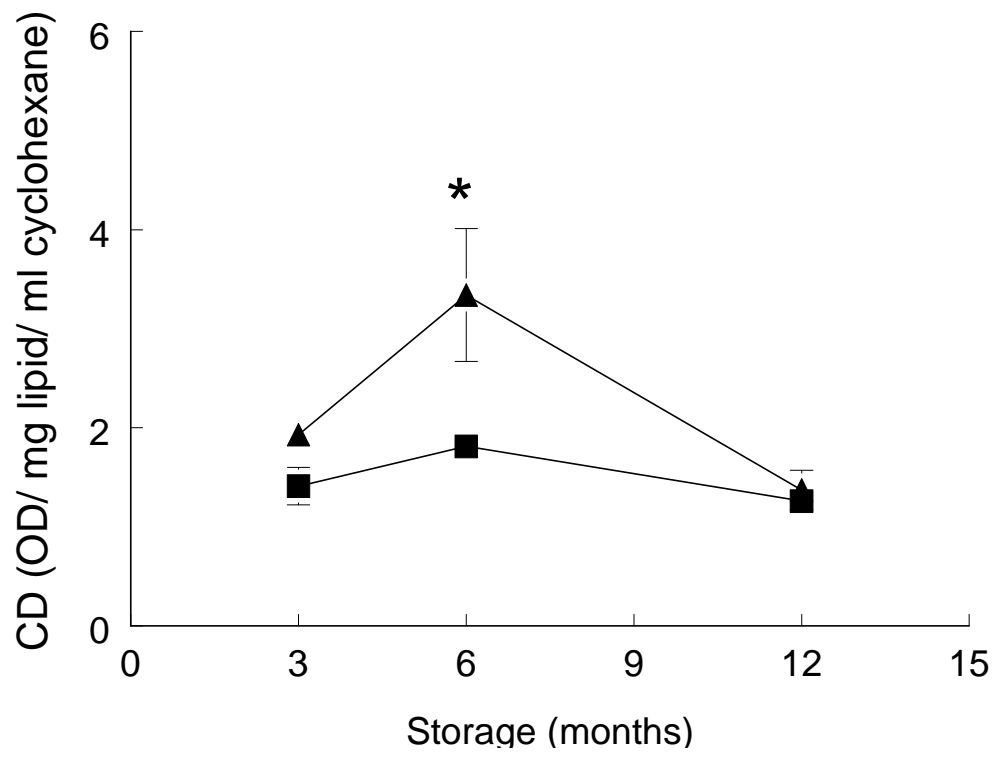
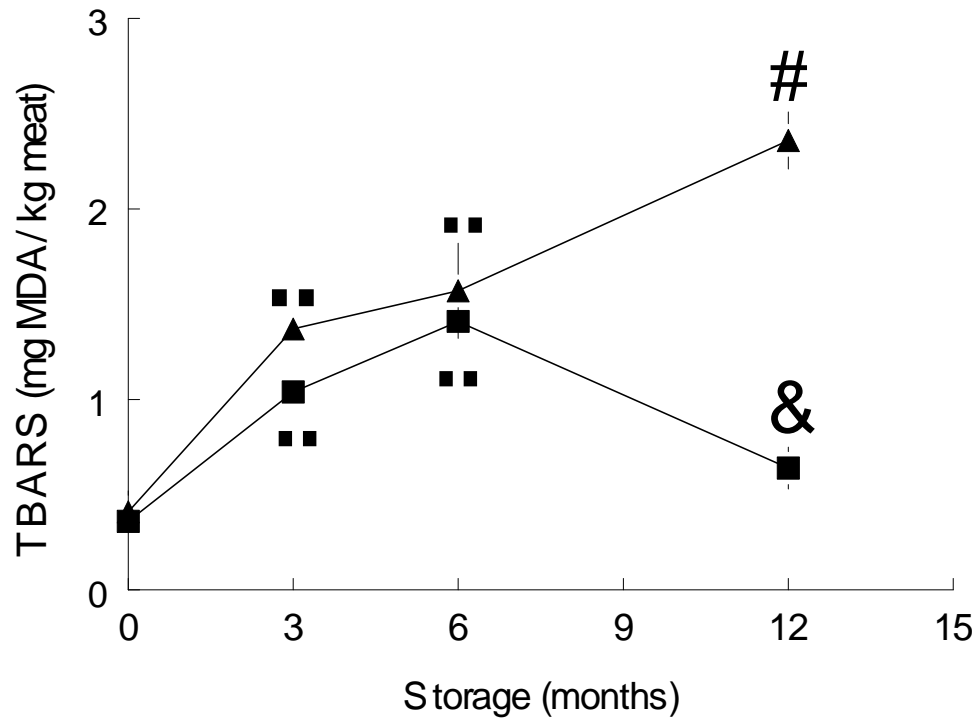


Fig. 3



### 3.2 Artigo 3

## **SENSORY CHARACTERISTICS AND LIPID OXIDATION OF COOKED DOURADO (*Salminus brasiliensis*) FILLETS TREATED WITH ERVA- MATE EXTRACT (*Ilex paraguariensis*)**

**Artigo em fase final de revisão pelos autores para ser submetido à revista LWT**

**- Food Science and Technology**

(configurado conforme as normas da revista)

**Sensory characteristics and lipid oxidation of cooked dourado  
(*Salminus brasiliensis*) fillets treated with erva-mate extract (*Ilex  
paraguariensis*)**

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## ABSTRACT

The effect of erva-mate extract on the sensory characteristics and lipid oxidation of cooked dourado fillets was evaluated during chilled storage. Dipping raw dourado fillets in crude erva-mate extract (10 or 20% aqueous solution, for 1 min) did not modify the taste of baked dourado fillets. However, a significant colour change was observed in these fillets, which was characterized by increased yellowness (reduced  $L^*$  and increased  $b^*$ ,  $C^*$  and  $H^*$  values). Besides, cooked and baked fillets previously treated with aqueous crude 10 or 20% erva-mate extract were classified as slightly worse than the control fillets in the sensory analysis of colour. Partition of crude erva-mate extract between aqueous and oily phases yielded a chlorophyll-free erva-mate extract that retained its antioxidant activity *in vitro* (DPPH assay). Dipping raw dourado fillets in the chlorophyll-free erva-mate extract (10 or 20%) had some protective effect against lipid oxidation in baked and grilled dourado fillets during chilled storage. This protection was evidenced by lower peroxide values, but no significant effect was observed on thiobarbituric acid reactive substances value.

**KEYWORDS:** Sensory analysis, chilled storage, CIELab, thiobarbituric acid reactive substances, peroxide value, vegetable extract

## 1. Introduction

Dourado (*Salminus brasiliensis*) is a freshwater fish of Characidae family that occurs in important South American rivers like the Paraná on the Uruguay (Paiva, 1983; Godoy, 1987). It is a large size carnivorous species that is sufficiently appreciated by the excellent quality of its meat (Koch, Milani, & Grosser, 2000; Zaniboni Filho, 2003).

Lipid oxidation is one of the major factors in deterioration during the storage and processing of foods. It can lead to the development of unpleasant taste and off-flavours as well as changes in colour, rheological properties, texture and nutritive value (Addis & Park, 1989; Buckley, Morrissey & Gray, 1995), which in turn lead to rejection of products by the consumer. Fish are more susceptible to these reactions due to the abundance of polyunsaturated fatty acids present in its flesh (Khayat & Schwall, 1983).

Natural antioxidants can be used as an alternative in the food industry, since many synthetic antioxidant compounds have shown to be toxic or mutagenic (Anesini, Ferraro & Filip, 2006). *Ilex paraguariensis* Saint Hilaire (Aquafoliaceae) is popularly known as erva-mate (Streit et al., 2007). Its natural occurrence area is restricted to Brazil, Argentina, Uruguay, and Paraguay, where it is widely consumed as a tea-like beverage prepared by the infusion of dried and minced leaves and twigs (Clifford & Ramirez-Martinez, 1990; Bravo, Goya & Lecumberri, 2007). The appearance is one of the main attributes of quality of foods; therefore it is the first consumer impression on a given food. Chlorophyll is responsible for the characteristic green colour of erva-mate leaves and its infusions (Andrade, 2004). Enzymes, acidic pH, heating, light and oxygen (Lajolo, Tannenbaum & Labuza, 1971; Schwartz & Lorenzo, 1990; Bohn & Walczyk, 2004) may promote the degradation of



chlorophyll that can damage the appearance of chlorophyll-rich foods (Elbe & Schwartz, 2000).

In addition, erva-mate contains various caffeoyl-derivatives and other polyphenols that are responsible for its antioxidant properties (Filip, Lotito, Ferraro & Fraga, 2000). The antioxidant activity of *Ilex paraguariensis* has been evaluated in biological systems both *in vivo* and *in vitro* (Gugliucci & Stahl, 1995; Campos, Escobar & Lissi, 1996; Gugliucci, 1996; Schinella, Troiani, Dávila, Buschiazzo & Tournier, 2000; Filip et al., 2000; Bracesco, Dell, Behtash, Menini, Gugliucci & Nunes, 2003). However, few studies evaluated its antioxidant activity in food systems. Campos, Hierro, Ordóñez, Bertol, Terra and la Hoz de (2007) recently demonstrated the antioxidant capacity of erva-mate extract to reduce lipid oxidation (TBARS values) of salami.

This study was aimed at evaluating the effect of erva-mate extract on the sensory characteristics and lipid oxidation of cooked dourado fillets during chilled storage.

## **2. Materials and methods**

### **2.1. Erva-mate extracts**

Samples of erva-mate (dried and minced leaves and twigs) were obtained from local market. The samples were weighed, diluted in distilled water at room temperature (10 and 20% w/v) and then filtered to yield the aqueous crude extracts that were used to treat fish fillets.

The separation of chlorophyll from extracts of erva-mate was achieved by their partition between aqueous and oily phases. Ethanolic (96°GL) erva-mate extracts (10 or 20% w/v) were mixed with water and chloroform in the ratio of 1:1:0.5 and shaken.

After standing, two phases were obtained, the lower one containing chloroform and chlorophyll that was characterized by the green colour, and the upper one, which was clear and contained ethanol and water. The upper phase that was free of chlorophyll was used in further assays. The chlorophyll-free extracts obtained were submitted to evaluation of their antioxidant activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, had their colour measured and were used to treat fish fillets.

## 2.2. Fish fillets

Samples of dourado (*Salminus brasiliensis*) were obtained from a local fish farm (Santa Maria, Brazil), slaughtered by hypothermia and immediately transported to the laboratory in ice containing boxes. Fresh fish were washed with tap water several times to remove adhering blood and slime and were filleted using common household practices. The fillets were cut into cubes (4 cm length x 4 cm width, weighing 20g each).

Flesh cubes were dipped during 1 min in distilled water (control) or in aqueous crude 10 or 20% erva-mate extract. Since salt could be used to hide any possible residual taste of erva-mate extract, two independent treatments were evaluated: without or with NaCl. NaCl was added at a final concentration of 1% in the water and in the erva-mate extracts used to dip flesh cubes. All samples were baked in conventional oven (250°C/20 min). These samples were submitted to sensory analysis and had their colour measured, in order to determine if the treatment with crude erva-mate extract could affect taste or colour of fillets.

In another set of experiments flesh cubes were dipped during 1 min in distilled water (control) or in aqueous chlorophyll-free 10 or 20% erva-mate extract. Samples were either oven baked or grilled before analysis. Baked fillets were prepared in a

conventional electric oven at 250°C for 20 minutes. Grilled fillets were prepared in a Black & Decker griller model G48, with thermostat set at 350°C. After set temperature was attained fillets were grilled for 10 minutes (5 min in each side). To prevent sticking, the grill was slightly greased with soybean oil, before cooking. Samples were stored in sealed plastic pots at 7±1°C and were analyzed at 1, 4 and 6 days after to evaluate lipid oxidation.

### **2.3. Sensory analysis**

The multiple comparison sensory test was used to determine whether there was a difference between the samples of flesh cubes dipped in crude erva-mate extracts (10 and 20%) and control (dipped in water) (Meilgaard, Civille & Carr, 1987; Griffiths, 1991; ABNT, 1995). Two different sessions were conducted to evaluate the effect of erva-mate extracts in fillets without or with NaCl. The degree of colour and taste difference from control was measured on a seven-points scale, where 1= extremely better than control; 2= moderately better than control; 3= slightly better than control; 4= not different from control; 5= slightly worse than control; 6= moderately worse than control; 7= extremely worse than control. The presentation of samples included a hidden control (dipped in water), so that sensory scores were obtained for the test samples and for the control sample. Samples were codified with three random numbers and evaluated by 9 trained judges.

### **2.4. Colour measurement**

Sample colour was assessed using a CR-300 Chromameter (Minolta, Osaka, Japan) according to the Intl. Commission on Illumination (CIE 1976  $L^* a^* b^*$ ), using a standard illumination D65, with 10° supplementary standard observer and a standard

calibration plate (number 15233011). Three flesh cubes of the same treatment were pooled in order to obtain a sample with an adequate area to fit the Chromameter reader. Three independent pooled samples were made for each treatment, and three measurements were recorded at each sample, with the illumination system rotated  $90^\circ$  between each measurement. For colour measurement of erva-mate extracts, samples (40 mL) were poured into Petri dishes (10 cm diameter x 1.5 cm height) and three measurements were recorded. Petri dishes were rotated to mix sample between each record. All measurements were made directly on the samples with no glasses between the sample and the equipment. The  $L^*$  indicates lightness and  $a^*$  and  $b^*$  are chromaticity coordinates, where  $+a^*$  is the red direction,  $-a^*$  is the green direction,  $+b^*$  is the yellow direction,  $-b^*$  is the blue direction. From the  $a^*$  and  $b^*$  values, the chroma ( $C^*$ ) and hue ( $H^*$ ) values were calculated. The chroma is an expression of the saturation or intensity and clarity of the colour. It is expressed by the equation;  $C^* = (a^{*2} + b^{*2})^{1/2}$  (Hunt, 1977) and it is 0 at the center and increases according to the distance from the center. Hue is the observable colour and is calculated by equation,  $H^* = \tan^{-1} (b^*/ a^*)$  (Hunt, 1977). The angle  $H^*$  is defined as starting at the  $+a^*$  axis and is expressed in degrees;  $0^\circ$  would be  $+a^*$  (red),  $90^\circ$  would be  $+b^*$  (yellow),  $180^\circ$  would be  $-a^*$  (green), and  $270^\circ$  would be  $-b^*$  (blue).

## 2.5. DPPH assay

Antioxidant activity of 10 and 20% erva-mate extracts was measured using the DPPH radical scavenging assay according to Brand-Williams, Cuvelier, and Berset (1995) modified method. DPPH solution (0.24 mg/ml) was previously diluted until  $1.10 \pm 0.02$  absorbance at 517 nm was obtained. Then, sample extract (0.1 ml) was mixed with the diluted methanolic DPPH solution (1.9 ml). The radical scavenging

power of the extracts was determined by measuring the decrease of DPPH absorbance at 517 nm after 24 hours in the dark against a blank. Trolox was used as standard for the calibration curve and results were expressed as  $\mu\text{g}$  trolox equivalents/mL extract.

## **2.6 Evaluation of lipid oxidation**

Samples of flesh cubes (1g) were homogenized with 1.5% KCl (1:5, w/v), centrifuged at 3,000 *g* for 10 min and the supernatant was used for determination of thiobarbituric acid reactive substances (TBARS) as described by Buege and Aust (1978). Briefly, samples were incubated at 100°C for 15 minutes in a medium containing trichloroacetic acid and thiobarbituric acid. After incubation, butyl alcohol was used to extract the reaction product that was determined at 535 nm.

Peroxide value was determined in the fat extracted from flesh cubes by the Bligh and Dyer method (1959), using the ferric thiocyanate method according to Chapman and Mackay (1949). No preliminary dilution with benzene/methanol solution was necessary. A standard curve using ferric iron solution was used to calculate the content of peroxides in the sample's fat.

## **2.9. Statistical analysis**

Three independent assays were conducted in all experimental steps including preparation of erva-mate extracts and treatment of fillets. Data were analyzed using the Statistica 6.0 software package. Results of sensory analysis were submitted to one-way analysis of variance (ANOVA) and means were post hoc compared using Duncan's test. The other results were submitted to two-way factorial ANOVA and

post hoc compared using Tukey's test. Differences were considered to be significant when  $p < 0.05$ .

### 3. Results and discussion

For sensory analysis of dourado fillets treated with erva-mate samples were prepared either with or without NaCl in order to verify if salt could mask a possible undesirable taste of erva-mate extracts. No significant difference in the taste was detected among samples regardless of the presence of NaCl (Table 1). A similar result was found by Campos et al. (2007) that evaluated the flavour of salami treated with ethanolic extract of erva-mate. However, samples treated with aqueous crude 10 or 20% erva-mate extract, had significantly higher scores in the sensory evaluation of colour as compared to control ( $p < 0.05$ ), being classified as slightly worse than control (Table 1). Possibly this occurred due the presence of pigments in the crude extract used to dip fillets.

The crude erva-mate extract (10 and 20%) caused a significant reduction in the luminosity ( $L^*$ ) of the samples as compared to the control (Table 2). ANOVA revealed a significant erva-mate extract x NaCl interaction on the values of  $a^*$ ,  $b^*$ , chroma and hue (Table 2). Erva-mate significantly reduced the redness of samples ( $a^*$ ), but for samples with 1% NaCl this effect was significant only for 20% AE treated samples, while for samples with no NaCl this effect was significant only for 10% AE treated samples. This reduction of  $a^*$  values is probably due to the presence of chlorophyll in the crude extract that increased the greenness of samples. Treatment with erva-mate extract significantly increased the intensity of colour (chroma) and the yellowness ( $b^*$  and  $H^*$  values) of samples, probably due to the presence of carotenoids in the extracts. However, this effect was more intense in the samples

with 1% NaCl, so that 20% AE + 1% NaCl treated fillets had significantly higher  $b^*$ ,  $C^*$  and  $H^*$  values than 20% AE + no NaCl ( $p < 0.05$ ). All samples had a tendency to yellow ( $H^*$  value near  $90^\circ$ ), but this tendency was higher in samples treated with erva-mate. The yellow colour of samples is probably related to the presence of carotenoid pigments in the extract. Also, some chlorophyll degradation through pheophytinization during baking (Teng & Chen, 1999) of erva-mate treated fillets could have contributed to increase the yellowness of samples. Alternatively, non-enzymatic browning reactions during baking of samples may have contributed to the yellowness (Fennema, 2000). These reactions could be more intense in erva-mate treated samples due to the presence of some soluble proteins and sugars in the extract, since erva-mate leaves and twigs contains 8.30-13.45% protein, 1.30-6.14% glucose and 3.60-6.90% sucrose on a dry basis (Burgstaller, 1994).

Considering that colour is one of the main attributes of quality, being responsible for the first consumer impression on a given food and that treatment with crude erva-mate extracts changed the colour of fillets we decided to purify the extracts in order to eliminate chlorophyll and other lipid soluble pigments like carotenoids. Purification yielded two phases: the lower one containing chloroform and chlorophyll that was characterized by the green colour, and the upper one, which was clear and contained ethanol and water. The upper phase had chroma and hue angle values similar to those of distilled water (control) (Table 3), demonstrating the effectiveness of the purification process. The antioxidant activity of the ethanolic crude extract and of the lower and upper phases obtained during purification are shown in Table 4. As expected 20% erva-mate extracts had higher antioxidant activity than 10% extracts. The upper phases of both 10% and 20% erva-mate extracts had greater antioxidant activity than the lower phases, demonstrating that

the purification step does not impair antioxidant activity. This probably occurred because phenolic compounds, which are responsible for the antioxidant activity (Filip et al., 2000), have greater affinity for the watery phase. Considering these results dourado fillets were treated with the chlorophyll free extracts (upper phase), cooked and stored under chilling in order to evaluate the effect of erva-mate on lipid oxidation.

Lipid oxidation during chilled storage was assessed as PV and TBARS value in baked and grilled dourado fillets (Fig. 1 and 2). During lipid peroxidation, free radicals abstract hydrogen from a double bond of fatty acids to produce fatty acid radicals, which react further with oxygen to produce fatty acid peroxy radicals. These radicals can react with a new fatty acid, generating a new fatty acid radical (new cycle of autoxidation) and a peroxide. The primary product of lipid oxidation is the fatty acid hydroperoxide, measured here as peroxide value (PV). The fatty acid hydroperoxide is unstable and decomposes readily into shorter chain hydrocarbons such as aldehydes measured through thiobarbituric acid reactive substances (TBARS), consisting mainly of malondialdehyde (Hamre, Oyvind & Sandnes, 2003). Accordingly, in the present study peroxide values decreased, while TBARS increased in the first four days of chilled storage in the baked and grilled samples (Fig. 1 and 2), indicating the occurrence of lipid peroxidation (Ackman & Takeuchi, 1986). These oxidative changes were more intense in baked samples as compared to the grilled ones.

ANOVA revealed a significant time x treatment interaction on PV. PV of all baked samples decreased at the 4<sup>th</sup> day of chilled storage, but values of control samples increased at the 6<sup>th</sup> day, so that baked fillets treated with 10% AE had lower PV than control at the 6<sup>th</sup> day ( $p < 0.05$ ; Fig. 1). PV values of grilled samples had small



changes during chilling, but there was some increase in PV of control samples at the 6<sup>th</sup> day of storage, so that grilled samples treated with 20% AE had lower PV than control at the 6<sup>th</sup> day ( $p < 0.05$ ; Fig. 1).

ANOVA revealed that TBARS values were significantly affected by the storage time both in baked and grilled samples (Fig. 2). TBARS increased in the 4<sup>th</sup> day of storage and then decreased, and this effect was more evident in baked samples ( $p < 0.05$ ). The highest TBARS values that were presented by samples at the 4<sup>th</sup> day of storage were below the limit of acceptability for meat products described by Al-Kahtani, Abu-Tarboush, Bajaber, Atia, Abou-Arab and El-Mojaddidi (1996) that is 3 mg MDA/kg. No significant effect of erva-mate treatment was observed on TBARS values (Fig. 2). This finding contrasts with results from Campos et al. (2007) who observed that salami manufactured with erva-mate extract had significantly lower TBARS values than did products manufactured without the extract during 60 days of vacuum storage. We found no other studies on the antioxidant effect of erva-mate in foods.

#### **4. Conclusions**

Although the treatment with crude erva-mate extract did not change the taste, important changes were observed in the colour of dourado fillets, which resulted in a poor sensory score as compared to control fillets. This result points to the need of removing chlorophyll from erva-mate extract before using in fish fillets. The chlorophyll-free erva-mate extract had some protective effect against lipid oxidation in baked and grilled dourado flesh, which was evidenced by lower PV, but was not effective to prevent TBARS formation.

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Table 1: Sensory analysis of baked dourado fillets treated with aqueous crude extract of *Ilex paraguariensis* (AE) using multiple comparison test with a control sample

Treatments	1% NaCl		No NaCl	
	Colour	Taste	Colour	Taste
0% AE	3.77 <sup>b</sup> ± 0.27 (2.00-5.00)	4.11 <sup>a</sup> ± 0.30 (3.00-6.00)	3.77 <sup>b</sup> ± 0.14 (3.00-4.00)	3.88 <sup>a</sup> ± 0.11 (3.00-4.00)
10% AE	4.88 <sup>a</sup> ± 0.35 (3.00-6.00)	3.66 <sup>a</sup> ± 0.28 (2.00-5.00)	5.33 <sup>a</sup> ± 0.50 (2.00-7.00)	4.33 <sup>a</sup> ± 0.47 (3.00-6.00)
20% AE	5.00 <sup>a</sup> ± 0.37 (3.00-7.00)	4.55 <sup>a</sup> ± 0.50 (2.00-6.00)	5.33 <sup>a</sup> ± 0.62 (2.00-7.00)	4.11 <sup>a</sup> ± 0.51 (2.00-6.00)

0% AE: distilled water (blind control). Values are means±standard error (minimum-maximum) of 9 trained judges. Means within the same column that have no common letters are significantly different ( $p < 0.05$ ). Results are encoded on a seven-points scale (1= much better than control sample, 7= much worse than control sample).

Table 2: Colour parameters of baked dourado fillets treated with aqueous crude extract of *Ilex paraguariensis* (AE) with or without NaCl

Treatments		L*	a*	b*	C*	H*
1%	0% AE	72.53 <sup>a</sup> (0.67)	4.33 <sup>a,b</sup> (0.00)	15.79 <sup>d</sup> (0.05)	16.37 <sup>c</sup> (0.04)	74.73 <sup>d</sup> (0.06)
	10%AE	61.37 <sup>b</sup> (0.28)	4.67 <sup>a</sup> (0.06)	16.86 <sup>c,d</sup> (0.12)	17.51 <sup>b,c</sup> (0.12)	74.60 <sup>d</sup> (0.10)
	20% AE	62.87 <sup>b</sup> (0.22)	3.87 <sup>c</sup> (0.09)	21.51 <sup>a</sup> (0.08)	21.85 <sup>a</sup> (0.07)	79.86 <sup>a</sup> (0.24)
No NaCl	0% AE	71.96 <sup>a</sup> (1,14)	4.23 <sup>b</sup> (0.07)	16.38 <sup>d</sup> (0.32)	16.89 <sup>c</sup> (0.32)	75.56 <sup>c</sup> (0.06)
	10%AE	62.41 <sup>b</sup> (0.59)	3.87 <sup>c</sup> (0.10)	18.09 <sup>b,c</sup> (0.36)	18.50 <sup>b</sup> (0.37)	77.96 <sup>b</sup> (0.17)
	20% AE	63.35 <sup>b</sup> (0.42)	4.50 <sup>a,b</sup> (0.05)	18.22 <sup>b</sup> (0.40)	18.77 <sup>b</sup> (0.40)	76.20 <sup>c</sup> (0.20)

0% AE: distilled water (control). C\*: chroma. H\*: hue angle. Values are means (standard error) of three independent assays. Means within the same column that have no common letters are significantly different ( $p < 0.05$ ).



Table 3: Colour parameters of extract of *Ilex paraguariensis*

	Samples		
	Control	10% EM Upper phase	20% EM Upper phase
C*	4.57	3.44	1.68
H*	277.67	291.90	267.60

C\*: chroma. H\*: hue angle. Control: distilled water. EM: erva-mate. Upper phase: distilled water + ethanol. Values are means of three independent assays.

Table 4: Antioxidant activity of extracts of *Ilex paraguariensis*

	10% EM			20% EM		
	EE	Lower phase	Upper phase	EE	Lower phase	Upper phase
DPPH ( $\mu\text{g trolox.ml}^{-1}$ )	2463.72	563.74	975.74	5053.14	845.96	1424.82

EM: erva-mate. EE: ethanolic crude extract of *Ilex Paraguariensis*. Lower phase: chloroform + chlorophyll. Upper phase: distilled water + ethanol. DPPH: 1,1-diphenyl-2-picrylhydrazyl. Values are means of three independent assays.

## Figure captions

Fig. 1: Changes in peroxide value (PV) of cooked dourado fillets treated with aqueous chlorophyll free extract of *Ilex paraguariensis* (AE) during chilled storage. 0% AE: control, distilled water (▲), 10% AE (●) and 20% AE (■). Values are means  $\pm$  standard error (n=3). \*Significantly different from 1<sup>st</sup> day of storage. &Significantly different from 0% AE at the same time (p<0.05).

Fig. 2: Changes in TBARS of cooked dourado fillets treated with aqueous chlorophyll free extract of *Ilex paraguariensis* (AE) during chilled storage. 0% AE: control, distilled water (▲), 10% AE (●) and 20% AE (■). Values are means  $\pm$  standard error (n=3). \*Significantly different from the respective treatments at 1 and 6 days of storage (p<0.05).

Fig. 1

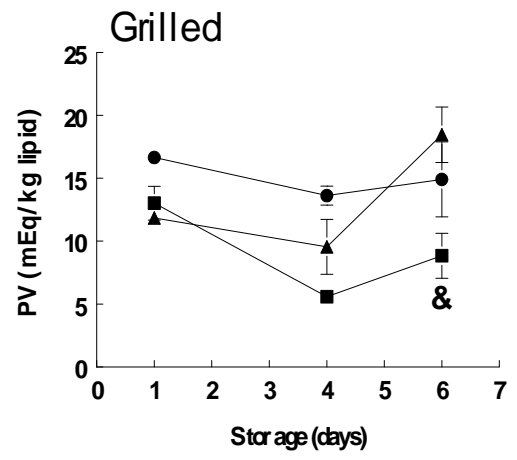
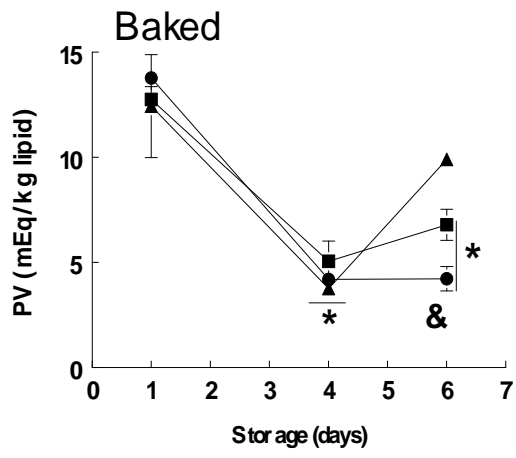
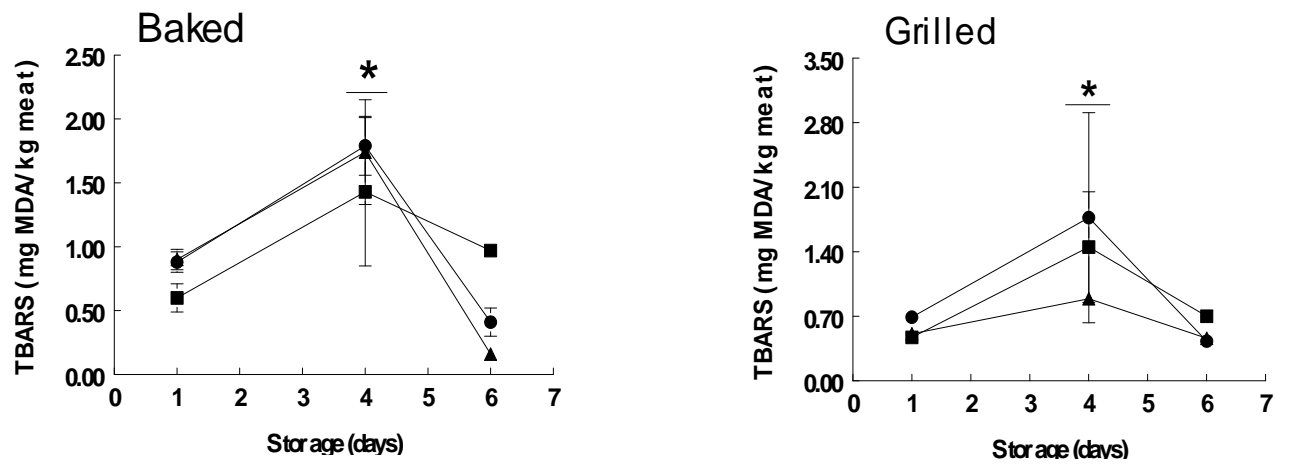


Fig. 2



## 4. DISCUSSÃO

Os peixes são consumidos em muitas partes do mundo devido ao seu alto conteúdo protéico, baixa gordura saturada e também por conterem ácidos graxos ômega-3 (ERKAN et al., 2007). A composição centesimal (umidade, proteína, lipídios, carboidratos e cinzas) de peixes pode informar sobre hábitos locomotores e adaptações energéticas (CHILDRESS e NYGAARD, 1973; CHILDRESS et al.1990). A composição de filés de peixes de água doce geralmente apresenta as seguintes variações em relação ao teor de umidade, proteínas e lipídios: 70,5-80,9%, 12,7-17,6% e 0,8-15,3%, respectivamente (ROMVÁRI et al., 2002). Menores oscilações são encontradas em relação ao conteúdo mineral (0,96-1,25%) e de carboidratos, com teores inferiores a 1% (ROMVÁRI et al., 2002). Contreras-Gusmán (1994) relata que a fração de cinzas em peixes de água doce apresenta variações em quantidades que vão de 0,90 a 3,39%. Os teores de umidade, proteína, gordura e cinzas de filés de dourado foram 76,41%, 14,55%, 2,92% e 1,61%, respectivamente. Segundo Bennion (1980), os peixes são classificados com base em seu conteúdo de gordura: peixes magros (menos que 5% de gordura), peixes com teor médio de gordura (5-10%) e peixes gordos (mais que 10% de gordura). Os exemplares de dourado avaliados foram classificados como peixes magros (2,92g/% de gordura).

Os diferentes teores de amônia e oxigênio utilizados no presente estudo (Artigo 1) não alteraram os valores de umidade, apenas houve influência do tempo de exposição (12 horas vs. 15 dias). Com relação aos minerais, a carne de pescado é considerada uma fonte valiosa de cálcio e fósforo particularmente, apresentando também quantidades razoáveis de sódio, potássio, manganês, cobre, cobalto, zinco, ferro e iodo (SIMÕES et al., 2007). As cinzas diminuíram e o conteúdo de proteína aumentou durante o período experimental (12 horas vs. 15 dias), sendo que essas alterações foram significativas quando os peixes foram expostos a altos níveis de amônia (0,1 mg/L). A gordura dos filés diminuiu com o aumento do tempo de exposição, sendo que essa alteração foi significativa nos peixes que foram expostos a baixas concentrações de oxigênio (4,5 mg/L) e altos níveis de amônia (0,1 mg/L). Alguns autores relatam que a exposição à amônia reduz a taxa de crescimento de várias espécies de peixes como o pregado, *Scophthalmus maximus*, atualmente *Psetta maxima* (RASMUSSEN e KORSGAARD, 1996; PERSON-LE RUYET et al.,

1997), dourada, *Sparus aurata* (WAJSBROT et al., 1993), linguado-legítimo, *Solea solea* (ALDERSON, 1979), bagre americano, *Ictalurus punctatus* (TOMASSO, 1994) e a perca prateada, *Bidyanus bidyanus* (ROWLAND et al., 1995; FRANCÊS et al., 2000), o que está de acordo com os nossos resultados.

Dezesseis ácidos graxos foram identificados no dourado, sendo que os ácidos palmítico (16:0), oléico (18:1  $\omega$ -9), e linoléico (18:2  $\omega$ -6) foram os predominantes. Esses ácidos graxos também foram encontrados em maior quantidade em bagres Tailandeses e japoneses (SHIRAI et al, 2002). Não foi observado efeito da amônia, oxigênio ou do tempo de exposição sobre a composição de ácidos graxos (Artigo 1), da mesma maneira que não houve influência do armazenamento congelado ou do tratamento com extrato de erva-mate (Artigo 2). SANT'ANA e MANCINI-FILHO (2000) demonstraram que a adição de antioxidantes *in vivo* como o tocoferol, BHT e extrato de alecrim na dieta de pacu (*Piaractus mesopotamicus*) protegeram contra a oxidação lipídica *post mortem*. O tocoferol foi o composto mais efetivo e a composição de ácidos graxos saturados, monoinsaturados e poliinsaturados foi semelhante entre os diferentes antioxidantes estudados.

A cor é um dos principais atributos de qualidade, constituindo-se na primeira impressão que o consumidor tem de um determinado produto. Por esse motivo foram realizadas análises de cor no presente estudo, para avaliar o efeito da erva-mate sobre os filés de dourado. Os valores de L\* dos filés tratados com extrato aquoso 10% e do controle foram semelhantes (Artigo 2). No entanto, a luminosidade dos filés do grupo controle aumentou durante o congelamento, sendo que no décimo segundo mês os filés controle apresentaram maior luminosidade do que aqueles tratados com extrato de erva-mate. Segundo Morkore (2006) o aumento do valor de L\* pode ser relacionado com alterações conformacionais em proteínas, que podem alterar a incidência e a reflexão da luz. Possivelmente a oxidação protéica tenha acompanhado o aumento significativo de TBARS do controle após 12 meses de armazenamento congelado. Esta proposta é consistente com a estabilidade dos valores de L\* dos filés tratados com extrato de erva-mate, os quais apresentaram menor oxidação lipídica (valor de CD e TBARS) quando comparado com os filés controle.

O tratamento dos filés com extrato de erva-mate não alterou os valores de a\*, houve apenas influência do tempo de armazenamento provocando uma diminuição desses valores durante o congelamento, possivelmente devido à oxidação da

mioglobina e hemoglobina (Artigo 2). Por outro lado, nos filés assados de dourado a erva-mate reduziu significativamente os valores de ( $a^*$ ), provavelmente devido à presença de clorofila no extrato bruto que por ação do calor sofre degradação e leva a formação de compostos que apresentam cor verde oliva (TENG e CHEN, 1999), o que pode ter aumentado a tendência para o verde das amostras ( $-a^*$ ) (Artigo 3). Os valores de ângulo de matiz ( $H^*$ ) indicaram que os filés de dourado tenderam ao amarelo durante todo o experimento. Houve aumento nos valores de  $H^*$  durante o congelamento, e este aumento foi maior nos filés controle, os quais apresentaram também maior aumento na oxidação lipídica (CD e TBARS), quando comparados com os filés tratados com erva-mate ( $p < 0.05$ ) (Artigo 2). Esses resultados concordam com dados prévios da literatura que indicam que a cor amarela está associada com a oxidação lipídica e aumenta durante o armazenamento congelado (HAMRE et al., 2003; RUFF et al., 2003).

Nos filés assados de dourado a erva-mate causou uma redução significativa na luminosidade dos filés em relação ao controle. A ANOVA revelou uma interação do tratamento com extrato de erva-mate x NaCl sobre os valores de  $b^*$ , croma e  $H^*$ . A erva-mate aumentou os valores de croma,  $b^*$  e  $H^*$  das amostras, e esses efeitos foram mais acentuados nas amostras com 1% de NaCl. Todas as amostras que foram processadas termicamente tenderam ao amarelo (valor de  $H^*$  próximo de  $90^\circ$ ), sendo esta tendência mais acentuada nas amostras tratadas com erva-mate. A cor amarela é provavelmente relacionada com reações de escurecimento não enzimático durante o aquecimento das amostras (FENNEMA, 2000). Essas reações podem ser mais intensas nas amostras tratadas com erva-mate devido à presença de proteínas solúveis e açúcares no extrato, já que a erva-mate contém cerca de 8,30-13,45% de proteína, 1,30-6,14% de glicose e 3,60-6,90% de sacarose (BURGSTALLER, 1994). Além disso, a presença de carotenóides e a degradação da clorofila através de reações de feofitinação durante o aquecimento (TENG e CHEN, 1999) também podem ter contribuído para o aumento da cor amarela dos filés tratados com erva-mate.

Na análise sensorial a erva-mate não alterou o sabor dos filés de dourado, independente da adição de NaCl (Artigo 3). Em relação a cor as amostras tratadas com extrato aquoso de erva-mate 10 e 20%, foram classificadas como levemente pior que o controle, possivelmente devido a presença de pigmentos no extrato bruto de erva-mate usado para mergulhar os filés.



Como o extrato bruto alterou a cor dos filés assados, decidiu-se purificar o extrato de erva-mate para eliminar a clorofila e outros pigmentos lipossolúveis como os carotenóides. A partir da purificação obtiveram-se duas fases, uma fase inferior contendo clorofórmio e clorofila que foi caracterizada pela cor verde, e uma fase superior límpida contendo etanol e água. A fase superior apresentou valores de cromas e ângulo de matiz semelhante ao controle (água destilada), demonstrando a eficiência do processo de purificação.

Na peroxidação lipídica, os radicais livres seqüestram o hidrogênio da dupla ligação dos ácidos graxos para produzir radicais livres, que reagem com o oxigênio molecular formando os radicais peroxil. Estes radicais são altamente reativos e capazes de remover átomos de hidrogênio de outros ácidos graxos insaturados formando os hidroperóxidos. Os novos radicais reagem com o oxigênio e a seqüência se repete. Durante essa seqüência ocorre uma troca na posição das ligações duplas dos ácidos graxos poliinsaturados, devido à estabilização por ressonância, levando a formação de hidroperóxidos isoméricos denominados de dienos conjugados (FENNEMA, 2000). O processo da oxidação acaba quando os compostos formados anteriormente se decompõem liberando hidrocarbonetos de cadeia curta como álcoois, cetonas ou aldeídos. Vários desses compostos são voláteis e, portanto, contribuem com o odor característico associado à oxidação de lipídios (HAMRE et al., 2003).

A oxidação lipídica nos filés de dourado foi determinada através dos produtos primários da oxidação determinados a partir dos valores de peróxidos (PV) e dienos conjugados (CD), assim como pelas substâncias reativas ao ácido tiobarbitúrico (TBARS), denominados produtos finais da oxidação. Os ácidos graxos livres foram determinados como indicadores da hidrólise de triglicerídios. Segundo Santos-Yap (1995) a formação de ácidos graxos livres pode acelerar o processo de oxidação, além de provocar alterações na textura por promover a desnaturação de proteínas miofibrilares.

Os filés dos peixes expostos ao nível alto de oxigênio (Artigo 1) apresentaram um aumento nos valores de TBARS durante o período experimental (12 horas vs. 15 dias). No entanto, a exposição *in vivo* a níveis estressantes de amônia e oxigênio por 12 horas, não afetou a formação de TBARS durante o armazenamento congelado. Durante condições de hipóxia ou anóxia os peixes aumentam as defesas antioxidantes através da síntese de enzimas antioxidantes. Essa estratégia

antecipatória funciona como uma preparação para o estresse oxidativo gerado quando os níveis de oxigênio são reestabelecidos (HERMES-LIMA e ZENTENO-SAVIN, 2002; LUSHCHAK et al., 2001). Isso possivelmente justifica o fato dos níveis de TBARS não terem aumentado significativamente nos peixes expostos a baixos teores de oxigênio após 15 dias de exposição. Da mesma maneira que a exposição dos peixes a baixos níveis de oxigênio não aumentaram a taxa de oxidação lipídica durante o armazenamento congelado.

Os níveis de dienos conjugados (CD) dos filés de dourado aumentaram até o sexto mês de armazenamento congelado e após diminuíram, sendo que este aumento foi significativamente maior nos filés dos peixes que foram expostos a níveis elevados tanto de amônia como de oxigênio. A diminuição nos valores de CD após seis meses de armazenamento é possivelmente devido à decomposição em moléculas menores (CHO et al., 1989; AUBOURG et al., 1998) para dar continuidade no processo de oxidação lipídica. Os níveis de peróxidos aumentaram ao longo do armazenamento congelado, sendo que após 12 meses de armazenamento os filés dos peixes que foram expostos a alta concentração de amônia apresentaram maior valor de peróxidos quando comparado com os daqueles que foram expostos a baixa concentração (0,01mg/L). A amônia é altamente tóxica sozinha, mas interage com o oxigênio tornando-se mais tóxica principalmente em condições em que o oxigênio é reduzido (LLOYD, 1961; ALABASTER et al., 1979; THURSTON et al., 1981; WAJSBROT et al., 1991). No entanto, em nosso estudo a diminuição do oxigênio da água não aumentou a oxidação lipídica dos filés de peixes expostos a altos níveis de amônia, durante o armazenamento congelado. A exposição a diferentes níveis de amônia e oxigênio *in vivo* não alterou a composição de ácidos graxos livres, assim como não houve efeito do tempo de congelamento.

Para tentar minimizar as reações de oxidação lipídica durante o armazenamento e processamento dos filés de dourado avaliou-se o efeito do extrato de erva-mate (*Ilex paraguariensis*). As propriedades antioxidantes desta planta têm sido demonstradas por vários autores (GUGLIUCCI, 1996; SCHINELLA et al., 2000; VANDERJAGT et al., 2002; BRACESCO et al., 2003). Em nosso trabalho, um dos métodos utilizados para avaliar a capacidade antioxidante da erva-mate foi o ensaio espectrofotométrico com o radical 2,2 difenil-1-picril-hidrazil (DPPH), no qual se evidenciou que o extrato aquoso obtido a partir de uma diluição a 10% (p/v) da amostra de erva-mate, apresenta uma capacidade de remoção do radical DPPH

equivalente a 25,8 mg trolox/ml extrato (Artigo 2). A atividade antioxidante dos extratos que foram submetidos à purificação através de partição líquido-líquido, para eliminação de pigmentos, também foi avaliada (Artigo 3). Como já era esperado a atividade antioxidante do extrato etanólico bruto 20% foi maior que a do extrato 10% e a fase superior apresentou maior atividade antioxidante em relação a fase inferior (1,2 vs. 0,70 mg equivalentes de trolox/ml extrato), demonstrando que o processo de purificação não prejudicou a capacidade antioxidante da erva-mate, provavelmente devido aos compostos fenólicos apresentarem maior afinidade pela fase aquosa.

O tratamento com extrato de erva-mate preveniu o aumento dos valores de ácidos graxos livres e dienos conjugados durante o armazenamento congelado (Artigo 2), evidenciando uma menor tendência a oxidação nas amostras tratadas com extrato de erva-mate. O valor de TBARS dos filés de dourado aumentou de forma similar nas amostras controle e tratadas com extrato de erva-mate até o sexto mês de congelamento. No entanto, após esse período os valores de TBARS continuaram aumentando apenas nas amostras controle, sendo que após 12 meses de congelamento estas apresentavam valores de TBARS superiores aos das amostras tratadas com extrato de erva-mate (2,36 mg MDA/Kg vs. 0,64 mg MDA/kg,  $p < 0.05$ ). Esses resultados demonstram que possivelmente a erva-mate pode estender a vida de útil de filés de dourado congelados.

No artigo 3 os filés de dourado foram tratados com o extrato livre de clorofila (fase superior), processados termicamente e armazenados sob refrigeração com a finalidade de avaliar o efeito da erva-mate sobre a oxidação lipídica. O valor de peróxidos diminuiu, enquanto o valor de TBARS aumentou no quarto dia de armazenamento refrigerado nos filés assados e grelhados, indicando a ocorrência de oxidação lipídica (ACKMAN & TAKEUCHI, 1986). Em relação ao processamento térmico, as amostras assadas e grelhadas apresentaram comportamento semelhante em relação à oxidação lipídica. Os filés tratados com extrato de erva-mate 10% apresentaram o menor valor de peróxidos nas amostras assadas no sexto dia de armazenamento, enquanto que nas amostras grelhadas a proteção contra a oxidação lipídica foi maior nas amostras tratadas com extrato de erva-mate 20%. Não houve efeito da erva-mate sobre os valores de TBARS, apenas houve influência do tempo de armazenamento, sendo que no quarto dia as amostras apresentaram os maiores valores, porém ficaram dentro do limite de aceitabilidade para produtos cárneos descrito por Al-Kahtani et al. (1996) de 3 mg MDA/kg.

## 5. CONCLUSÕES

- A exposição do dourado durante 15 dias a uma concentração elevada de amônia *in vivo* afetou a composição dos filés, enquanto que a exposição durante 12 horas a essa mesma concentração aumentou a susceptibilidade dos filés a oxidação lipídica durante o armazenamento congelado, o que pode reduzir a sua vida útil. Por outro lado, a exposição *in vivo* a uma concentração baixa de oxigênio não aumentou a taxa de oxidação lipídica dos filés, possivelmente porque o dourado aumentou sua capacidade antioxidante quando exposto a baixa concentração de oxigênio na água.
- Os filés tratados com o extrato de erva-mate apresentaram menor aumento nos valores de ácidos graxos livres, dienos conjugados e substâncias reativas ao ácido tiobarbitúrico durante o congelamento, demonstrando a atividade antioxidante da erva-mate nos filés de dourado, e o seu potencial para estender a vida útil desse produto. Além disso, o extrato de erva-mate reduziu as alterações de cor durante o armazenamento congelado (menor alteração de luminosidade e ângulo de matiz), o que está provavelmente associado a sua atividade antioxidante.
- O tratamento dos filés de dourado com extrato de erva-mate não provocou alterações perceptíveis de sabor, mas provocou importantes alterações de cor (redução na luminosidade e aumento na tendência ao amarelo), o que reduziu a aceitabilidade dos filés.
- Através de partição líquido-líquido, foi possível eliminar os pigmentos do extrato de erva-mate, mantendo a sua atividade antioxidante. Este extrato purificado de erva-mate conferiu certa proteção contra a oxidação lipídica em filés de dourado cozidos armazenados sob refrigeração. A proteção foi evidenciada pelo menor conteúdo de peróxidos, sem efeito significativo no conteúdo de TBARS.

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