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

POTENCIAL DA SEMENTE DE NÊSPERA (*Eriobotrya japonica*) NA ESTABILIDADE OXIDATIVA DE PRODUTOS DE JUNDIÁ (*Rhamdia quelen*)

TESE DE DOUTORADO

Jaqueline Piccolo

Santa Maria, RS, Brasil
2014

POTENCIAL DA SEMENTE DE NÊSPERA (*Eriobotrya japonica*) NA ESTABILIDADE OXIDATIVA DE PRODUTOS DE JUNDIÁ (*Rhamdia quelen*)

Jaqueline Piccolo

Tese apresentada ao Curso de Doutorado 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
Doutor em Ciência e Tecnologia dos Alimentos.

Orientador: Prof. Dr. Ernesto Hashime Kubota
Coorientadora: Prof[a]. Dr[a]. Tatiana Emanuelli

Santa Maria, RS, Brasil

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A Comissão Examinadora, abaixo assinada, aprova a Tese de Doutorado

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Elaborada por
Jaqueline Piccolo

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

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“O correr da vida embrulha tudo. A vida é assim: esquenta e esfria, aperta e daí afrouxa, sossega e depois desinquieta. O que ela quer da gente é coragem.”

João Guimarães Rosa.

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos
Universidade Federal de Santa Maria

POTENCIAL DA SEMENTE DE NÊSPERA (*Eriobotrya japonica*) NA ESTABILIDADE OXIDATIVA DE PRODUTOS DE JUNDIÁ (*Rhamdia quelen*)

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COORIENTADORA: TATIANA EMANUELLI
Local e Data de Defesa: Santa Maria, 28 de novembro de 2014.

Este trabalho teve como objetivo avaliar o efeito de extratos de semente de nêspera sobre a estabilidade oxidativa em filés, patê e almôndega à base de jundiá, ao longo do armazenamento. Foi avaliada a capacidade antioxidante e atividade antimicrobiana *in vitro* de extratos de semente de nêspera obtidos através de diferentes extrações, utilizando diferentes solventes e ultrassom. Seguiu-se com a avaliação da aplicabilidade dos extratos de semente de nêspera na estabilidade oxidativa de filés de jundiá congelados (extrato obtido com acetona 70% e rotaevaporado; EA); em patês refrigerados à base de pescado refrigerado (extrato hidretanólico; EE) e de almôndegas à base de pescado, pré-cozidas e armazenadas congeladas (EA). Os extratos acetônicos apresentaram o maior conteúdo de compostos fenólicos totais e também de taninos totais. O tratamento com ultrassom melhorou a extração de compostos fenólicos totais na extração com acetona 70% e a atividade FRAP nos extratos de acetona 35% e 70%. Nenhuma atividade antimicrobiana foi observada nos extratos. O extrato de acetona 70% foi apontado como o extrato com propriedades antioxidantes mais expressivas. Nos filés tratados com o EA, a formulação contendo ácido ascórbico apresentou maiores valores de dienos conjugados (DC) que o controle e que os filés tratados com EA aos 6 meses de armazenamento, além de apresentar valores maiores de substâncias reativas ao ácido tiobarbitúrico (TBARS) que a formulação tratada com o extrato 400 ppm aos 9 meses de armazenamento. Contudo, os valores de DC e TBARS foram semelhantes entre os tratamentos aos 12 meses de armazenamento. O teor de proteínas carboniladas (PC) aumentou até os 12 meses, contudo, não teve influência dos tratamentos. Houve diminuição dos valores de a* em todos os períodos avaliados e aumento de b* nos tempos 9 e 12 meses. Nas concentrações avaliadas o EA não foi capaz de retardar a oxidação lipídica e proteica em filés armazenados congelados, contudo, não alterou a composição centesimal ou aceitabilidade dos filés avaliadas no tempo 0 meses. Nos patês tratados com o EE, os teores de DC e peróxidos (PV) aumentaram ao longo do armazenamento, contudo, foram similares entre todos os tratamentos aos 35 dias. O conteúdo TBARS não foi afetado pelo EE e houve aumento linear no conteúdo de PC nos patês ao longo do armazenamento. Nas concentrações avaliadas, o EE não foi capaz de inibir ou reduzir as oxidações

lipídicas e proteicas em patês à base de pescado armazenados refrigerados. Nas almôndegas desenvolvidas com filés previamente tratados com o EA, PV e TBARS diminuíram ao longo do tempo de armazenamento devido aos altos valores nos tempos iniciais atribuídos à cocção e manipulação. As formulações contendo ácido ascórbico e 800 ppm apresentaram maiores valores de PV que a formulação controle, decréscimo da tendência ao vermelho e aumento da tendência ao amarelo ao longo do armazenamento congelado. O conteúdo de proteínas carboniladas aumentou ao longo do tempo de armazenamento, similar ao ocorrido com a dureza das almôndegas cozidas, sem efeito dos tratamentos. Nas concentrações avaliadas, o EA não foi capaz de inibir a oxidação de lípidos e proteínas ou de prevenir a alteração de cor das almôndegas à base de pescado, sendo que as formulações contendo ácido ascórbico e 800 ppm apresentaram efeitos pró-oxidantes.

Palavras-chave: Antioxidante. *Eribotrya japonica*. Extrato. Oxidação lipídica. Oxidação proteica. *Rhamdia quelen*. Textura.

ABSTRACT

Doctoral Thesis
Graduate Program on Food Science and Technology
Federal University of Santa Maria

LOQUAT (*Eriobotrya japonica*) SEED POTENTIAL ON OXIDATIVE STABILITY OF JUNDIÁ (*Rhamdia quelen*) FISH PRODUCTS

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CO-ADVISER: TATIANA EMANUELLI
Place and date of defense: Santa Maria, november 28, 2014.

This study aimed to evaluate the effect of the loquat seed extract on oxidative stability of catfish fillets, pate and meatballs, during storage. We evaluated the antioxidant and antimicrobial *in vitro* activity of loquat seed extracts obtained by different extraction using different solvents and ultrasound treatment. This was followed by evaluating the applicability of loquat seed extracts on oxidative stability of frozen catfish fillets (extracts obtained with 70% acetone and rotaevaporated; AE); on catfish based-pates refrigerated stored (hydroethanolic extract; EE) and on catfish based-meatballs, pre-cooked and frozen stored. Acetone extracts showed the highest total phenolics and total tannins content. Ultrasound treatment improved total phenolic content in 70% acetone extraction and FRAP activity in 35% and 70% acetone extracts. No antimicrobial activity was observed in extracts. The 70% acetone extract was indicated as the one with more significant antioxidant properties. In fish fillets treated with AE, ascorbic acid containing formulation had higher conjugated dienes (CD) than the control and AE-treated pates at 6 months of storage, and presented higher thiobarbituric acid reactive substances (TBARS) than the 400 ppm treated fish fillet at 9 months of storage. However, CD and TBARS had similar values among treatments at 12 months. The protein carbonyl (PC) content increased until 12 months compared to 0 months, however, had no treatment effect. There was a decrease of a* values in all periods and an increase of b* values at 9 and 12 months. The evaluated concentrations of AE were not able to slow lipid and protein oxidation in fish fillets frozen stored, however, did not alter chemical composition or acceptability of fish fillets at 0 months of storage. In catfish based-pates treated with EE, the CD and peroxide values (PV) increased during storage, however, were similar in all treatments after 35 days. TBARS content was not affected by EE. There was a linear increase in PC content in pates over storage. At evaluated concentrations, EE was not able to inhibit or reduce the lipid and protein oxidation in fish pates refrigerated stored. In catfish based-meatballs prepared with AE-treated fish fillets, PV and TBARS decreased over storage time due to high values at initial times assigned to cooking and mincing. Ascorbic acid and 800 ppm containing formulations had higher PV than control formulation, decrease of a* values and increase of b* values over frozen storage. PC content increased over storage time, paralleled to the hardness, with no treatment effect. In the tested

concentrations, AE was not able to inhibit the lipid and protein oxidation or to prevent the color change of pre-cooked fish meatballs, and ascorbic acid and 800 ppm formulations showed pro-oxidant effects.

Keywords: Antioxidant. *Eribotrya japonica*. Extract. Lipid oxidation. Protein oxidation. *Rhamdia quelen*. Texture.

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$^{\bullet}HO_2$ – Radical hidroperoxil
$^{\bullet}NO$*x* – Óxido de nitrogênio
$^{\bullet}O_{2-}$ – Radical superóxido
$^{1}O_2$ – Oxigênio singlete
AAS – Semiladeído α-aminoadípico
ANOVA – Análise de variância
BHA – Butil hidroxianisol
BHT – Butil hidroxitolueno
DC – Dienos conjugados
DHA – Ácido docosahexaenóico
DPA – ácido docosapentaenóico
DPPH – 1,1-difenil-2-picrilhidrazil
EPA – Ácido eicosapentaenóico
GGS – Semiladeído γ-glutâmico
GP – Galato de propila
$GS^{\bullet}$ - Radical til glutationa
H_2O_2 – Peróxido de hidrogênio
HNE - 4-hidroxi-2-nonenal
$HO^{\bullet}$ - Radical hidroxil
$MbFe^{2+}$ - Oximioglobina
$MbFe^{3+}$ - Metamioglobina
MCO – Methal catalyzed oxidation
MDA – Malondialdeído
MUFA – Ácidos graxos monoinsaturados
$NO^{\bullet}$ - Óxido nítrico
O_3 – Oxigênio triplete
PUFA – Ácidos graxos poli-insaturados
$RO^{\bullet}$ – Radical alcoxil
$ROO^{\bullet}$ - Radical peroxil
ROOH – Hidroperóxido
ROS – Espécies reativas de oxigênio
SFA – Ácidos graxos saturados
$SO3^{-\bullet}$ - Óxido sulfúrico
TBARS – Substâncias reativas ao ácido tiobarbitúrico
TBHQ – Terc-butil-hidroquinona
VP – Valor de peróxidos

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INTRODUÇÃO

O emprego de substâncias antioxidantes tem sido amplamente difundido na indústria de alimentos na tentativa de manter a qualidade do produto, uma vez que os antioxidantes podem, efetivamente, retardar as reações oxidativas ocorridas nos alimentos ao longo de seu armazenamento. Assim, a preocupação dos consumidores com a segurança e a toxicidade dos antioxidantes sintéticos tem levado à substituição destes pelos antioxidantes naturais, havendo um crescente aumento do interesse em explorar novas fontes naturais destes compostos.

As plantas são as fontes mais abundantes dos antioxidantes naturais, contudo, as sementes de frutas não recebem a devida atenção como fontes de antioxidantes. A nêspera (*Eriobotrya japonica*) fruta de origem asiática da região da China, vem sendo também cultivada no Brasil com o estado de São Paulo sendo o líder da produção nacional. Sua exploração comercial é destinada ao consumo *in natura*, sendo as sementes descartadas. Entretanto, estudos demonstram que a aplicação de extrato de sementes de nêspera tem poder de supressão de doenças e redução de lipídios em estudos *in vivo* e *in vitro*, o que pode ser devido ao seu maior conteúdo de polifenóis em relação à polpa e casca.

A oxidação lipídica é a maior causa da perda de qualidade nas carnes durante o armazenamento e processamento e devido à presença de ácidos graxos poliinsaturados, os pescados são mais suscetíveis à oxidação lipídica do que as demais carnes. Além disso, a oxidação lipídica pode induzir mudanças nas estruturas proteicas levando à oxidação proteica, isto é, tornando as proteínas menos solúveis e influenciando na capacidade de ligação de água e textura dos pescados. Contudo, a avaliação da oxidação proteica ainda é escassa nas pesquisas envolvendo alimentos e ainda menos frequente na área de pescados.

Assim, seria viável verificar a aplicabilidade de extratos de semente de nêspera em pescados e seus produtos considerando-se a necessidade da indústria de alimentos em encontrar novas fontes de aditivos alimentares oriundas de fontes naturais, a predisposição dos pescados ao desenvolvimento de reações oxidativas, e a viabilização da produção de espécies nativas de pescado e de espécies amplamente distribuídas de partes subutilizadas de frutas na oxidação de alimentos.

REVISÃO DE LITERATURA

1. Aditivos alimentares e antioxidantes

Aditivo alimentar é definido pela legislação brasileira (BRASIL, 1997) como "qualquer ingrediente adicionado intencionalmente aos alimentos, sem propósito de nutrir, com o objetivo de modificar as características físicas, químicas, biológicas ou sensoriais, durante a fabricação, processamento, preparação, tratamento, embalagem, acondicionamento, armazenagem, transporte ou manipulação de um alimento". Dentre as 23 funções dos aditivos alimentares, categorizadas pela legislação brasileira, uma delas é agir como antioxidante, retardando o aparecimento de alterações oxidativas no alimento, promovendo assim sua vida útil (BRASIL, 1997).

Segundo Halliwell (2007), antioxidante é definido como "toda substância que atrasa, previne ou remove danos oxidativos a uma molécula alvo". Além disso, podem ser referidos como "qualquer substância que remove diretamente ROS ou age indiretamente na regulação positiva das defesas antioxidantes ou então inibe a produção de ROS" (KHLEBNIKOV et al., 2007). Deve-se considerar também que, após remover um radical, um antioxidante deve ser capaz de formar radicais estáveis frente à ligação intramoleculares de hidrogênio e à continuação da oxidação (HALLIWELL, 1990).

A atividade antioxidante ocorre através de várias vias: como inibidores de radicais livres (oxidantes preventivos) inibindo a formação de radicais livres a partir de lipídios; como interruptores da propagação da cadeia de auto-oxidação; como sequestradores de oxigênio singlete; através de sinergismo com outros antioxidantes; como agentes redutores que convertem hidroperóxidos em compostos estáveis; como quelantes de metais e também como inibidores de enzimas pró-oxidativas (DARMANYAN et al., 1998; HEIM et al., 2002; MIN e BOFF, 2002; POKORNÝ, 2007; KANCHEVA, 2009).

Devido às suas propriedades protetoras em diferentes estágios do processo oxidativo, os antioxidantes podem ser classificados em primários e secundários. Os antioxidantes primários cessam ou retardam a oxidação doando átomos de hidrogênio ou elétrons aos radicais livres convertendo-os a moléculas menos

reativas. Já os antioxidantes secundários agem incluindo a ligação de íons metálicos, convertendo hidroperóxidos a espécies não-radicais, absorvendo radiação UV ou desativando o oxigênio singlete (MAISUTHISAKUL et al., 2005).

Compostos antioxidantes sintéticos como BHA (butilhidroxianisol), BHT (butilhidroxitolueno) e TBHQ (terc-butil-hidroquinona) são utilizados na indústria de alimentos a fim de inibir os processos oxidativos (FASSEAS et al., 2007). Contudo, estudos têm evidenciado seus efeitos tóxicos ao organismo apresentando riscos à saúde como danos ao fígado e carcinogênese (WICHI, 1988; SHERWIN, 1990), além de mutagenicidade (SHAHIDI e WANASUNDARA, 1992).

Assim, seus efeitos toxicológicos danosos ao organismo humano aumentaram a demanda por antioxidantes naturais em substituição aos sintéticos na indústria de alimentos por parte dos consumidores preocupados com a saúde e a segurança alimentar (KARRE et al., 2013).

1.1 Compostos fenólicos

Os polifenóis são largamente encontrados nas plantas e contribuem para a cor e o sabor dos vegetais (BELITZ et al., 2009) e são constituídos de um grupamento hidroxila (-OH) ligado diretamente a um hidrocarboneto aromático (KRICHER, 2011). Os compostos fenólicos são classificados em ácidos fenólicos (ácido hidroxibenzóico e hidroxicinâmico), flavonoides (antocianinas, flavonoides e flavonas), taninos diterpenos (hidrolisáveis e condensados), estilbenos, curcuminóides, cumarinas, lignanas, quinonas e outros (alcaloides fenólicos, terpenóides fenólicos, glicosídeos fenólicos e óleos voláteis) (FRESCO et al., 2006; HUANG et al., 2009). As posições e números de grupamentos hidroxila em relação ao grupamento carboxílico funcional determinam a capacidade da atividade antioxidante de cada composto (RICE-EVANS et al., 1996).

Os compostos fenólicos são os principais constituintes das plantas que podem contribuir com a capacidade antioxidante e nos processos oxidativos nos alimentos devido a sua capacidade de inibir ou retardar esses processos, sendo os ácidos fenólicos, flavonoides, estilbenos e lignanas os mais abundantes nos alimentos (D’ARCHIVIO et al., 2007).

Os ácidos hidrocinâmico e hidroxibenzóico agem como quelantes e removedores de radicais livres com impacto maior nos radicais peroxil e hidroxil,

ânios superóxido e peroxinitritos. Um dos compostos mais promissores é o ácido gálico, precuros de muitos taninos (KRIMMEL et al., 2010).

Os flavonoides são estruturas constituídas de grupos fenólicos hidroxil ligados a estruturas aromáticas. Os flavonoides são divididos em 6 classes dependendo do estado de oxidação do anel pirano central: flavonóis, flavonas, flavanonas, isoflavonas, antocianidinas e flavanóis (catequinas e proantocianidinas) (D'ARCHIVIO et al., 2007). Sua atividade antioxidante está sustentada pela ação como agentes redutores, doadores de hidrogênio, sequestradores de oxigênio singlete, removedores de radicais superóxidos e quelantes de metais. Alguns dos flavonoides mais importantes são a catequina, catequina-galato, quercetina e kaempferol (RICE-EVANS et al., 1996; PROCHÁZKOVÁ et al., 2011).

Antioxidantes naturais provindos de matérias-primas de diversas fontes vegetais têm sido testados quanto à aplicabilidade em alimentos e especificamente seu uso em pescados e seus subprodutos como uvas (PAZOS et al., 2005); sementes de uva (LUTHER et al., 2007; YERLIKAYA e GOKOGLU, 2010); sementes de romã (SÁNCHEZ-ALONSO et al., 2007); semente de pomelo (ZARINA e TAN, 2013); chá verde (LIN e LIN, 2005; YERLIKAYA e GOKOGLU, 2010; GAI et al., 2014); chá preto (LIN e LIN, 2005); alecrim (OZOGUL et al., 2010; UÇAK et al., 2011) e tomilho e orégano (BENSID et al., 2014).

2. Nêspera

A nêspera (*Eriobotrya japonica* Lindl) é uma fruta pertencente à família Rosaceae, originária da China, sendo cultivada na China e no Japão desde a antiguidade (VAUGHAN e GEISSLER, 1997). Pode ser também encontrada no norte da Índia, na região do Mediterrâneo, Inglaterra, América do Norte, Central e do Sul (MORTON, 1987). Devido a sua ampla distribuição recebe várias denonminações sendo chamada também de "Pipa" ou "Luju" na China, "japanese plum" ou "japanese medlar" nos Estados Unidos, nespola na Itália, níspero na Espanha, ameixa do Japão em Portugal, ameixa amarela no Brasil, entre outros (LIN et al., 2007; MORTON, 1987).

Apenas alguns países a exploram como fonte econômica como é o caso da China que produz 42.000 hectares gerando 200.000 toneladas do fruto, o que

representa 64% da produção mundial, seguida da Espanha, Paquistão, Turquia e Japão (CABALLERO e FERNÁNDEZ, 2003). Devido ao fato de ser uma planta de clima subtropical, a nêspera é adaptada no Brasil, sendo o Estado de São Paulo, mais especificamente a região de Mogi das Cruzes, o líder da produção nacional (GOMES, 2000; CABALLERO e FERNÁNDEZ, 2003).

O fruto da nespereira é redondo, oval ou elíptico (Fig. 1), com diâmetro de cerca de 2 a 5 cm e média de peso de 30 a 40 g, com algumas cultivares atingindo até 70 a 170 g. Sua casca é fina com coloração branca ou alaranjada, sua polpa é branca ou alaranjada, sendo o fruto consitutuído de 3 a 4 sementes relativamente grandes pesando em torno de 1,2 a 3,6 g cada (LIN et al., 2007). Devido a sua polpa aromática, suculenta e saborosa, pode ser consumida fresca ou processada na forma de sucos, geleias ou até mesmo vinho (LIN et al., 2007).

Figura 1 - Nêsperas (A) e sementes de nêspera (B). Fonte: Autor.

Os frutos de nêspera possuem importância nutricional devido à presença de compostos bioativos como flavonóides (LOUATI et al., 2003), fenólicos (DING et al., 2001), amigdalina (ZHUANG et al., 2002), ácidos triterpênicos (LIANG et al., 1990) e carotenóides (GODOY et al., 1995), taninos, vitamina B, ácido ascórbico e arsênico (MORTON, 1987). Derivados dos ácidos hidroxicinâmico e benzóico foram encontrados nos frutos de nêspera, além da cianidina glicosídeo (DING et al., 2001; KOBA et al., 2007). Os triterpenos ácido oleanólico, ursólico e maslínico foram encontrados nas folhas de nêspera e apontados como protetores de doenças e se

deve a compostos como esses o amplo uso de suas folhas como medicinais para doenças de pele, inflamatórias e tosse. Além disso, há estudos evidenciando a ação de suas folhas na redução da glicemia sanguínea (DE TOMMASI et al., 1991; ALIPPI et al., 1990), ação anti-inflamatória (YOUNG et al., 1994; DE TOMMASI et al., 1992), na prevenção de hepatopatias (NISHIOKA et al, 2002), câncer (ITO et al., 2000; KIM et al., 2009).

Contudo, suas sementes não são comestíveis e tampouco utilizadas no plano farmacêutico, sendo descartadas. Entretanto, pesquisas recentes envolvendo as sementes de *Eriobotrya japonica* apontam a presença de amigdalina, esteróis, β-sitosterol, trigicerídios, esteroléster, diglicerídios, e ácidos graxos saturados como o palmítico e poli-insaturados linoléico e linolênico (NISHIOKA et al. 2002) e oleico (MORTON, 1987), além de ácido clorogênico, cianidina glicosídeo, epicatequina (EC), epigalocatequinagalato (EGCG) e procianidina B2 nos extratos etanólicos de sementes de *Eriobotrya japonica* (KOBA et al., 2007).

Devido à presença de compostos com amplas habilidades frente a radicais livres nas sementes de nêsperas, vários estudos demonstraram o potencial dos seus extratos na redução de hepatopatias (NISHIOKA et al., 2002), nefropatias (HAMADA et al., 2004), efeitos hipoglicemiantes (TANAKA et al., 2008), redução de triglicerídios (SHIH et al., 2013), atividade inibitória na peroxidação lipídica em ratos (YOKOTA et al., 2008). Além disso, o alto conteúdo de epigalocatequina-3-galato (EGCG) e procianidina B2 encontrada nas sementes de nêspera seria o responsável pela habilidade de remover os radicais livres e reduzir a oxidação do LDL colesterol *in vitro* dos extratos etanólicos de semente de nêspera (KOBA et al., 2007).

Contudo, o fato de a nêspera ser uma espécie amplamente difundida no território brasileiro, de algumas regiões se apresentarem como exportadoras deste fruto e seus importantes efeitos farmacológicos se contrapõem aos escassos estudos desta espécie na área de alimentos. Deste modo, o estudo da semente de nêspera na oxidação de alimentos se faz importante considerando-se as necessidades da indústria de alimentos em encontrar novas fontes de aditivos alimentares oriundos de fonte naturais e a viabilização da sua produção e uso de seus subprodutos.

3. Pescados

3.1 Aquicultura

A produção mundial de pescado teve um crescimento constante nas últimas cinco décadas com um aumento anual no abastecimento do mercado de peixes de 3,2%, ultrapassando o crescimento populacional de 1,6%. O Brasil está entre os 10 maiores produtores mundiais de pescado com uma produção de 266.000 toneladas no ano de 2012. Já o consumo mundial de pescado *per capita* aumentou de uma média de 9,9 kg em 1960 para 19,2 kg em 2012, o que é devido à combinação do crescimento populacional, aumento de renda e da urbanização, e facilitado pela forte expansão da produção de pescados e canais de distribuição mais eficientes (FAO, 2014).

O pescado é uma mercadoria muito heterogênea e mudanças nas espécies consumidas se devem ao crescimento da produção na aquicultura, o que também está relacionado ao aumento da demanda por pescados e produtos à base de pescado. A aquicultura levou ao aumento da demanda e consumo de espécies como camarão, salmão, bivalvos, tilápia, *Pangasius* e bagres, com um decréscimo em seus preços e um grande aumento na sua comercialização (FAO, 2014).

No entanto, o consumo de pescado pode ser influenciado por padrões de consumo alimentar, fatores socioeconômicos, além de características pessoais, estado de saúde e dimensões atitudinais (TRONDSEN et al., 2003).

3.2 Importância nutricional dos pescados

O pescado possui elevado valor nutricional sendo uma excelente fonte de proteínas, em torno de 15 a 25%, além de apresentar todos os aminoácidos essenciais e alta digestibilidade. Também, são fontes de minerais como zinco, magnésio, cobre, manganês e vanádio e vitaminas A, D, E, K e do complexo B (OETTERER et al., 2006).

Quanto ao teor de gordura, os pescados possuem baixo teor de colesterol e são ricos em ácidos graxos poli-insaturados (PUFA), principalmente os da família n-3 como os ácidos pentaenóico (EPA, C20:5n-3) e docosahexaenóico (DHA, C22:6n-3) (HORROCKS E YEO, 1999; SUÁREZ-MAHECHA et al., 2002). Aos ácidos graxos

poli-insaturados é atribuída importância como agentes anti-inflamatórios (CONTRERAS-GUZMÁN, 1994; SUÁREZ-MAHECHA et al., 2002). Na cascata de oxidação do ácido linoleico é obtido o ácido araquidônico (C20:4n-6), o qual é precursor de citocinas e eicosanóides anti-inflamatórios e de mediadores da vasoconstrição e agregação plaquetária. O EPA age na redução de efeitos inflamatórios, sendo precursor de citocinas e outros derivados anti-inflamatórios. O DHA é outro agente anti-inflamatório precursor de resolvinas e protectinas, sendo o principal componente das membranas da retina e fosfolipídios cerebrais (DECKELBAUM e TORREJON, 2012). A importância dos ácidos graxos poli-insaturado n-3 está pautada durante a formação fetal no desenvolvimento de células nervosas (neurônios e células gliais), sendo sua carência razão de sérias consequências (CALDER, 2012) e atuam na prevenção de cânceres de mama (JUDÉ et al., 2006) e de colo de útero (ROYNETTE et al., 2004).

O organismo humano sintetiza os ácidos graxos através do sistema enzimático ácido graxo sintase, elongase e dessaturase. No entanto, os mamíferos não são capazes de sintetizar o ácido linoléico (n-6), necessário para a formação de membranas biologicamente ativas; nem o ácido α-linolênico (n-3), que é sintetizado somente pelas plantas (BELITZ e GROSCH, 1997). Assim, a denominação de ácido graxo essencial é utilizada para os ácidos graxos que não podem ser sintetizados pelo organismo humano e por isso, precisam ser fornecidos pela dieta (CARVALHO et al., 2003). Ambos os ácidos graxos supracitados devem ser supridos pela dieta, e a partir da elongação e dessaturação de suas cadeias serão formados os outros ácidos graxos das famílias n-6 e n-3 como o araquidônico, o EPA e o DHA, os quais desempenham importantes funções biológicas (STRYER, 1994; BELITZ e GROSCH, 1997).

Assim, os ácidos graxos da família n-3 têm importância na pressão sanguínea e na resistência vascular (SUDHEENDRAN et al., 2010). Na infância, os ácidos graxos n-3 exercem importantes papéis na no desenvolvimento cognitivo e aprendizagem bem como desenvolvimento visual (CALDER, 2012). EPA e DHA têm sido apontados como benéficos na redução do risco de depressão e suicídio e no atraso de degeneração neurológica no envelhecimento (APPLETON et al., 2010; SUBLETTE et al., 2006). Embora não se tenha evidenciado efeitos na diabetes tipo 2, os ácidos graxos n-3 exercem efeitos na síndrome metabólica (CARPENTIER et al.,

2006), além de agirem no decréscimo de infartos e doenças coronarianas (SUDHEENDRAN et al., 201; MAYURASAKORN, 2011).

3.3 Alteração pós-abate nos pescados

O músculo do peixe vivo é geralmente estéril, porém pode haver penetração das bactérias presentes na pele e nas vísceras nos músculos após o abate. Pouco após o abate, é iniciado o processo de *rigor mortis*, definido como uma alteração física na carne, que resulta da complexa modificação bioquímica do músculo após a morte do animal, ou seja, o enrijecimento progressivo do músculo e redução do pH (KUBOTA e EMANUELLI, 2004). Com a morte e consequente falência sanguínea, o músculo passa a utilizar a via anaeróbica para obtenção de energia, transformando o glicogênio em glicose, gerando ácido lático que se acumula no músculo, gernado queda de pH. Com o gasto dos depósitos energéticos, o processo contrátil tende a cessar formando um complexo irreversível denominado acto-miosina, atingindo o *rigor mortis*. A instalação do rigor mortis é acompanhada por mudanças físicas como perda de extensibilidade e elasticidade, e encurtamento muscular (OGAWA, 1999).

Assim, o pescado torna-se suscetível a deterioração após o abate, o que pode ser decorrente da atividade de microorganismos ou pela deterioração química (oxidação lipídica e atividade enzimática).

A redução do pH durante o *rigor mortis* favorece a ação de enzimas proteolíticas endógenas (autólise), responsáveis pelo amaciamento da carne. O processo de hidrólise das proteínas e gorduras devido à ação das enzimas proteolíticas e lipídicas nos tecidos é denominado autólise e inicia uma vez que o músculo está rígido e o pH baixo, condição necessária para a ação das catepsinas (BEIRÃO et al., 2004).

O desenvolvimento de microorganismos é um dos principais fatores que levam à deterioração do pescado, sendo que a maioria das bactérias apresenta atividade proteolítica e lipolítica, levando a formação de substâncias indesejáveis. A microbiota bacteriana de deterioração do pescado é constituída principalmente por microoganismos gram-negativos não esporulados pertencentes aos gêneros: *Pseudomonas, Acetinobacter, Moraxella, Flavobacterium*, além dos coliformes frequentemente podem estar relacionados à contaminação e à deterioração dos alimentos. Os principais produtos finais da decomposição bacteriana em pescados

são amoníaco, compostos sufurados, H_2S e mercaptanos; ácidos graxos de cadeia curta (acético, propiônico, valérico, láctico, succínico), ácidos aromáticos (benzóico, fenil propiônico e seus sais amoniacais) e bases orgânicas (metilamina, dimetilamina, trimetilamina, histamina, feniltilamina, putrescina e cadaverina) (ORDOÑEZ, 2005).

Devido a sua composição química constituída de elevado teor de água e gorduras insaturadas, o pescado se caracteriza por ser um produto altamente perecível. A oxidação dos lipídios pode ser desencadeada por via enzimática (ação da enzima lipoxigenase) ou não enzimática (ação de luz, calor, oxigênio, metais, etc.).

A rancidez hidrolítica induzida pela via enzimática é caracterizada pela hidrólise dos triglicerídios e consequente liberação de ácidos graxos livres durante o processamento e o armazenamento, devido à ação de enzimas hidrolíticas naturalmente presentes no peixe (BRAGADÓTTIR et al., 2004), produzindo características organolépticas indesejáveis ao alimento, sendo o off-flavor a principal delas (GATTA et al., 2000).

Já a oxidação lipídica via não enzimática ocorre quando se dá a remoção de átomos de elétrons, sendo causada pela reação dos lipídios insaturados com o oxigênio atmosférico. A oxidação lipídica origina radicais livres, promovendo alterações principalmente de ordem sensorial (sabor, aroma, textura e cor) (ARAÚJO, 1995).

3.3.1 Oxidação lipídica em carnes

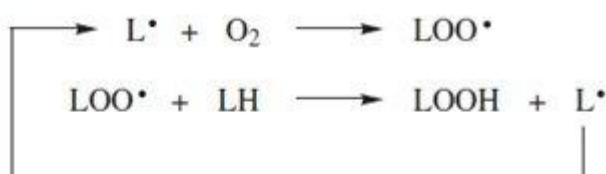
A oxidação lipídica é a principal causa de deterioração em alimentos e um desafio para a indústria de alimentos. Trata-se de um complexo processo em que ocorre a reação dos ácidos graxos instaurados com o oxigênio molecular por meio de um mecanismo de radicais livres em cadeia, formando hidroperóxidos e desenvolvendo um mecanismo em cadeia (GRAY, 1978). Os problemas associados à oxidação lipídica envolvem a alteração de sabor, perda de valor nutritivo e segurança alimentar, danos biológicos, alterações de propriedades funcionais (FRANKEL, 1984).

A oxidação dos lipídios ocorre em 3 fases (Fig. 2): iniciação, propagação e terminação. A iniciação é caracterizada pela perda de um átomo de hidrogênio a

partir de um ácido graxo insaturado, produzindo um radical livre. Na reação de propagação, o radical livre gerado na molécula de lipídio reage com o oxigênio para formar radical peroxil. Em seguida, o peroxil abstrai um átomo de hidrogênio de outra cadeia de lipídios, dando origem ao radical livre hidroperóxido. A peroxidação dos ácidos graxos insaturados é acelerada pelos radicais gerados da degradação dos hidroperóxidos por um mecanismo de reação monomolecular (BELITZ et al., 2009). Além disso, durante a formação de hidroperóxidos são também formados os dienos conjugados, produtos do rearranjo molecular das ligações duplas dos lipídios. Na terminação, o radical hidroperóxido vai perpetuar as demais reações e promover a continuação da oxidação lipídica (PEARSON et al, 1977;. ENSER, 1987). A decomposição dos hidroperóxidos promove a formação de produtos não radicais como aldeídos, cetonas, álcoois, hidrocarbonetos, furanos, lactonas, ácidos orgânicos voláteis e compostos epóxi, conhecidos como compostos secundários da oxidação (FRANKEL, 1984). Muitos destes compostos gerados a partir da decomposição dos hidroperóxidos são voláteis e contribuem para a alteração de sabor e odor do alimento. Propanal, pentanal, hexanal e 4-hidroxinonenal (HNE) estão entre os compostos voláteis gerados em carnes a partir da oxidação dos lipídios (LYNCH e FAUSTMAN, 2000).

Iniciação:

Propagação:



Terminação:

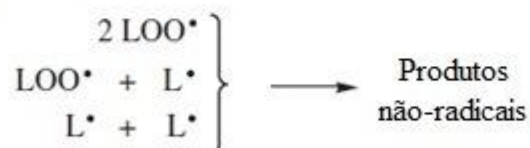


Figura 2 - Mecanismo de auto-oxidação dos lipídios. Fonte: SHAHIDI e ZHONG, 2005.

Além do oxigênio molecular, outros agentes podem promover a degradação dos lipídios como os produtos de decomposição de espécies endógenas (H_2O_2, ROOH) ou radicais ($ROO^{\bullet}$, $HO^{\bullet}$, $NO^{\bullet}$, $GS^{\bullet}$) ou espécies endógenas ($^1O_2$, O_3), radicais endógenos ($^{\bullet}NOx$, $SO3^{\bullet -}$) ou agentes físicos (radiação UV e ionizante, aquecimento) (SIMIC et al., 1988; PACKER e GLAZER, 1990). Contudo, outros fatores como foto-oxidação, íons de metais pesados, catálise enzimática e oxigênio ativo a partir de reações enzimáticas parecem estar envolvidos na iniciação da auto-oxidação dos lipídios (BELITZ et al., 2009).

Os íons metálicos, ferro e cobre, são geralmente aceitos como agentes principais na catálise de mudanças oxidativas nos tecidos. O ferro é o metal mais abundante nos sistemas biológicos e promove o início da oxidação lipídica pela geração de radicais livres capazes de abstrair um próton dos ácidos graxos insaturados (KANNER, 1994; GUTTERIDGE e HALLIWELL, 1990). No entanto, a presença de hidroperóxidos é um pré-requisito para a atividade do íon metálico, que decompõe o hidroperóxido a radical livre (BELITZ et al., 2009).

Além disso, hemoglobina e mioglobina, compostos responsáveis pela distribuição de oxigênio aos tecidos e com alta prevalência de ferro, são os compostos mais abundantes encontrados *in vivo* (BARON E ANDERSEN, 2002). A transformação da oximioglobina ($MbFe^{2+}$) em metamioglobina ($MbFe^{3+}$) é reportada como altamente relacionada com a extensão da oxidação lipídica em carnes (ANDERSEN E SKHIBSTED, 1991; GREENE, 1969; RHEE et al., 1987) sendo a metamioglobina apontada como um potencial antioxidante em pH entre 5,3 e 6,2, pH ótimo das carnes. Assim, estudos demonstram o efeito pró-oxidativo da metamioglobina em presença de pH acidificado e de hidroperóxidos (HOGG et al., 1994; BARON et al., 1997), dando suporte ao mecanismo proposto por Tapel (1995) para os hidroperóxidos dependentes de heme proteína-catalisada na oxidação lipídica.

Os produtos primários gerados a partir da oxidação dos lipídios não apresentam odor nem sabor, contudo, os produtos secundários são poderosos compostos que afetam tanto o odor quanto o sabor dos alimentos. O ácido linoléico é o precursor do hexanal que um composto volátil, e alcanos e alquenos como e etano e o pentano também são voláteis gerados na oxidação lipídica. Entretanto, o

malondialdeído (MDA) é o composto preferencialmente formado pela auto-oxidação dos ácidos graxos com três ou mais duplas ligações, não pertencente a fração volátil dos compostos gerados (BELITZ, 2009).

As interações dos hidroperóxidos e dos produtos secundários da oxidação lipídica com proteínas e aminoácidos têm um impacto considerável na estabilidade durante o processamento, cocção e armazenamento. Os compostos secundários da oxidação lipídica (aldeídos e epóxidos) reagem com grupamentos amino e sulfidrila das proteínas e as ligações induzidas por radicais e cisão das proteínas podem ser responsáveis pelas maiores perdas nutricionais dos produtos durante o armazenamento (GARDNER, 1979).

Também ocorrem reações de escurecimento não-enzimático (Maillard) durante a oxidação lipídica devido aos compostos secundários gerados durante a reação e sua interação com proteínas, provocando perdas nutricionais e mudanças organolépticas. Compostos carbonilados, como os aldeídos e dialdeídos gerados na oxidação dos lipídios, formam bases de Schiff com aminas e a polimerização dessas bases que são instáveis produz as melanoidinas, compostos de alto peso molecular que conferem coloração escura ao produto além de novos compostos voláteis que afetam as características de sabor, especialmente durante o processamento e cocção (FRANKEL, 1984). Deste modo, a interação das proteínas com produtos da oxidação lipídica afeta as propriedades de emulsificação, solubilidade, capacidade de retenção de água, textura e características reológicas do produto (HALL, 1987).

Assim, a oxidação lipídica resulta em desenvolvimento de off-flavor, odor de ranço, descoloração, perdas por purga, perda de valor nutricional, decréscimo na vida útil e na acumulação de compostos tóxicos, que podem ser trazer prejuízos aos consumidores (CHAIJAN, 2008; RICHARDS et al., 2002; MAPIYE et al., 2012).

3.3.2 Oxidação proteica em carnes

A importância da avaliação da oxidação proteica está apoiada no alto teor de proteína presente no tecido muscular e a suscetibilidade das proteínas musculares a reações de oxidação levando a efeitos prejudiciais na qualidade da carne (DECKER et al., 1993; MERCIER et al., 1995). A oxidação proteica é relatada como iniciada por espécies reativas de oxigênio (ROS), semelhante ao ocorrido na oxidação dos

lipídios, por metais de transição, bem como pela reação com metabólitos secundários do estresse oxidativo (SHACTER, 2000; STADTMAN e LEVINE, 2003).

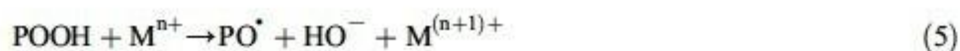
Segundo Estévez (2011), o processo de oxidação das proteínas se inicia com a abstração de um átomo de hidrogênio da proteína por meio de radicais livres, gerando um radical de proteína ($P^{\bullet}$) (Fig. 3; reação 1) que na presença de oxigênio é convertido a radical peroxil ($POO^{\bullet}$) e, consequentemente, a alquilperóxido (POOH) pela abstração de um átomo de hidrogênio de outra molécula suscetível (Reações 2 e 3). Reações com radicais hidroperóxido ($HO_2^{\bullet}$) ou com metais de transição (Mn^+) também produzem radicais alcoxil (Reações 4 e 5) e seus derivados hidroxil (POH) (Reações 6 e 7). Além disso, a interação das proteínas com hidroperóxidos e produtos secundários da oxidação lipídica, especialmente aldeídos e açúcares redutores, também promove a oxidação das proteínas (VILJANEN, 2005; BORON, 2010).

$$PH + HO^{\bullet} \rightarrow P^{\bullet} + H_2O \quad (1)$$

$$P^{\bullet} + O_2 \rightarrow POO^{\bullet} \quad (2)$$

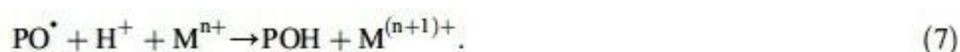
$$POO^{\bullet} + PH \rightarrow POOH + P^{\bullet} \quad (3)$$

$$POOH + HO_2^{\bullet} \rightarrow PO^{\bullet} + O_2 + H_2O \quad (4)$$



$$POOH + M^{n+} \rightarrow PO^{\bullet} + HO^{-} + M^{(n+1)+} \quad (5)$$

$$PO^{\bullet} + HO_2^{\bullet} \rightarrow POH + O_2 \quad (6)$$

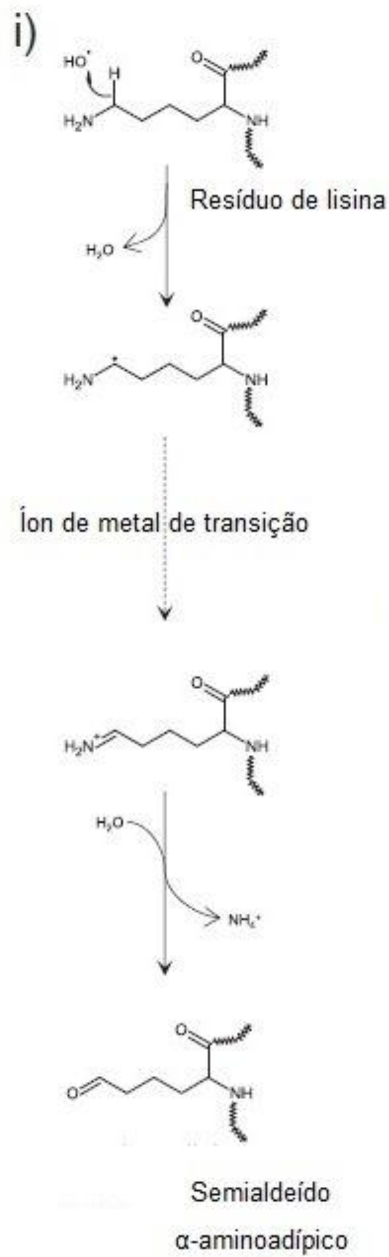


$$PO^{\bullet} + H^{+} + M^{n+} \rightarrow POH + M^{(n+1)+}. \quad (7)$$

Figura 3 - Cadeia dos processos envolvidos na oxidação proteica em sistemas biológicos. Fonte: ESTÉVEZ, 2011.

Estévez (2011) também aponta que a modificação das cadeias laterais de aminoácidos resulta na fragmentação dos peptídeos, na formação de ligações cruzadas intra e intermoleculares e na conversão dos aminoácidos em diferentes aminoácidos (STADTMAN e LEVINE, 2000). Quanto à fragmentação dos aminoácidos, a cisteína e a metionina seriam os primeiros a serem oxidados devido à alta suscetibilidade de seus centros sulfurados, juntamente com os resíduos de triptofano que seriam oxidados em presença de metais de transição (ESTÉVEZ et al., 2008a; VILJANEN et al., 2004). A ligação cruzada está ligada à formação de cistina (pontes dissulfeto) e ditirosinas a partir de dois resíduos de cisteína e de tirosina, respectivamente (LUND et al., 2011). Já a formação de compostos carbonilados se deve à oxidação dos resíduos de treonina, prolina, arginina e lisina (STADTMAN e LEVINE, 2003).

As alterações geradas pelos danos oxidativos nas proteínas incluem perda de fluorescência do triptofano (ESTÉVEZ et al., 2008; GANHÃO et al., 2010a), perda de grupamentos sulfidrílicos (FREDERIKSEN et al., 2008), formação de ligações cruzadas (XIONG et al., 2009) e ganho de derivados carbonilados (ESTÉVEZ et al., 2005; GANHÃO et al., 2010b). Segundo Berlet e Stadtman (1997), “a carbonilação é a modificação não enzimática e irreversível das proteínas envolvendo a formação de porções carbonílicas induzidas pelo estresse oxidativo e outros mecanismos”. Estévez (2011) aponta quatro vias de geração de compostos carbonilados: a) oxidação direta das cadeias laterais de lisina, treonina, arginina e prolina (REQUENA et al., 2001); b) glicação não-enzimática na presença de açúcares redutores (AKAGAWA et al., 2005); c) clivagem oxidativa do esqueleto dos peptídeos via α-amidação ou oxidação de cadeias laterais de glutamil (BERLET e STADTMAN, 1997; GARRISON, 1987); e d) ligação covalente a compostos carbonilados não-proteicos como 4-hidroxi-2-nonenal (HNE) ou malondialdeído (MDA) (FEENEY et al., 1975) (Fig. 4A e 4B).



ii)

CH_2OH

Glicose

Resíduo de lisina

$-H_2O$ $+H_2O$

Base de Schiff

Íon de metal de transição

Produto de Amadori

Íon de metal de transição

α-Dicarbonil

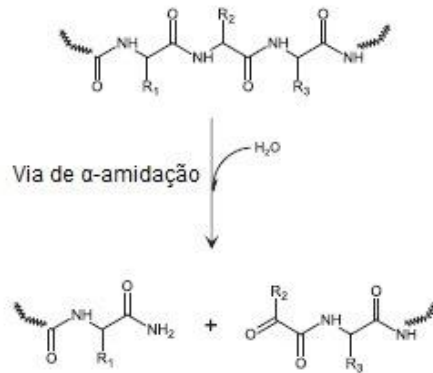
$+H_2O$ $-H_2O$

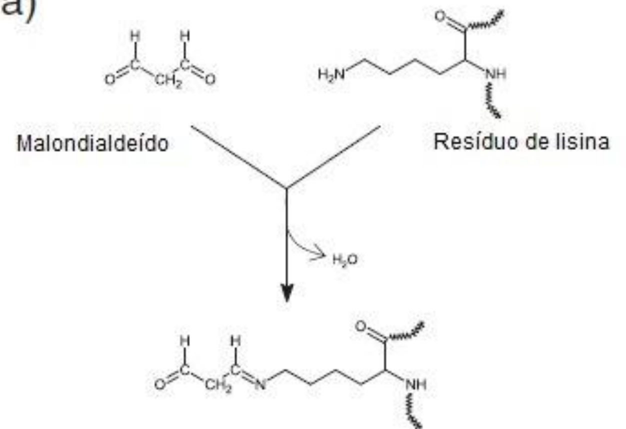
Íon de metal de transição

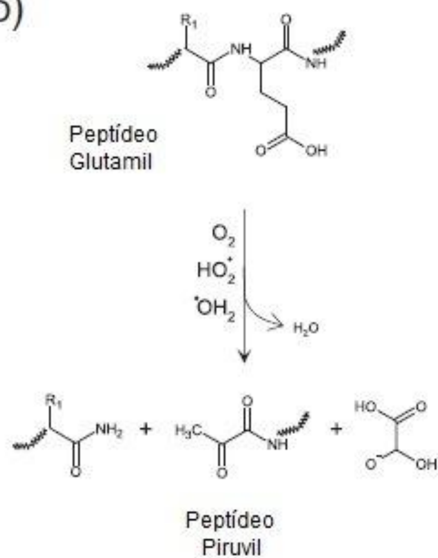
NH_3^+

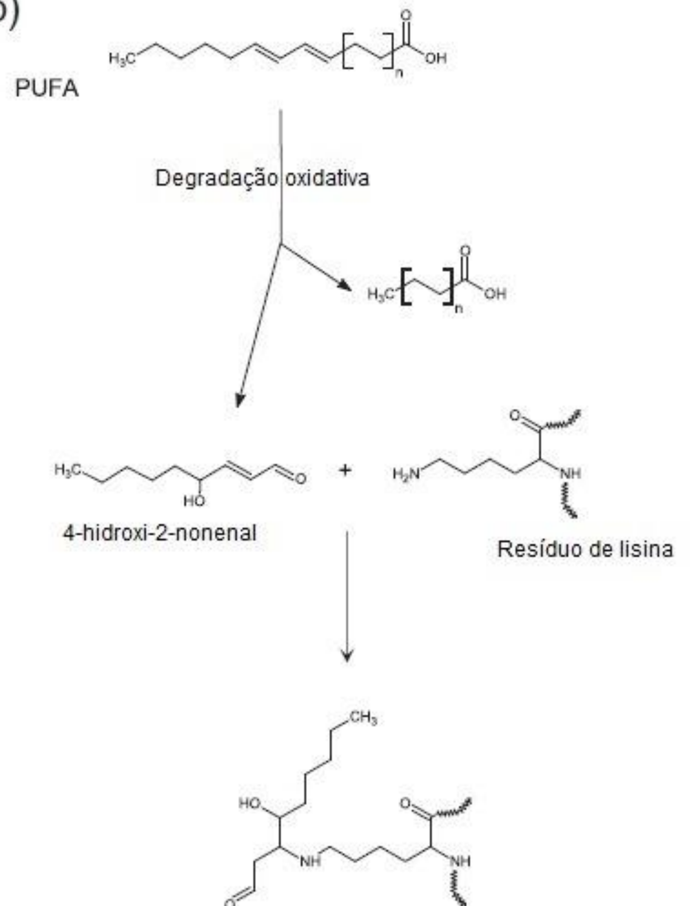
Semialdeído

α-aminoadípico









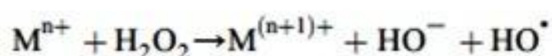
B

Figura 4 - Vias de formação de compostos carbonilados. (A) i) Oxidação catalisada por metais de cadeias laterias de aminoácidos básicos (REQUENA et al., 2001); (A)

ii) Glicação não enzimática (AKAGAWA et al., 2005); (B) iii) Clivagem de esqueleto de peptídeos por amidação (a) e por resíduos de glutamil (b) (BARLETT e STADTMAN, 1997; GARISON, 1987); (B) iv) Ligação a compostos não-proteicos como hidroxinonenal (a) e malondialdeído (b) (FEENEY et al., 1975). Fonte: ESTÉVEZ, 2011.

No entanto, a oxidação direta dos aminoácidos é o único mecanismo que tem efeito comprovado no aumento de grupos carbonil a partir de proteínas cárneas e é comprovadamente a principal rota de carbonilação e a mais potente fonte de ataque direto a proteínas (ESTÉVEZ e HEINONEN, 2010; SHACTER, 2000).

Estévez (2011) evidencia que a formação dos derivados carbonílicos está relacionada com o sistema MCO (methal catalyzed oxidation - catálise por metais), segundo o qual formas reduzidas de metais de transição poderiam reduzir o peróxido de hidrogênio (H_2O_2) para formar intermediários reativos, como os radicais hidroxil, na proximidade de cadeias laterais de aminoácidos (STADTMAN e LEVINE, 2003) (Fig. 5). Dessa forma, a treonina seria convertida em ácido α-amino-3-keto butírico, a lisina ao semialdeído α-amino adípico (AAS) e a arginina e a prolina em semiladeído γ-glutâmico (GGS), sendo o AAS e o GGS propostos como biomarcadores do dano proteico (ESTÉVEZ, 2011; DANESHVAR et al., 1997).



$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + HO^- + HO^\bullet$$

Figura 5 - Sistema MCO relacionado à formação de compostos carbonilados. Fonte: ESTÉVEZ, 2011.

Além dos metais de transição promoverem a modificação de proteínas, a mioglobina (Mb) também pode atuar na carbonilação. O mecanismo no qual a mioglobina atua seria via transformação da oximioglobina em metamioglobina. Nessa conversão há a formação de radicais superóxido, os quais tem a capacidade de dismutar o peróxido de hidrogênio, seguindo a mesma via de redução catalítica descrita para os metais de transição (figura descrita acima), o que evidencia o papel da mioglobina, principalmente na forma de metamioglobina, como potencial promotor da oxidação proteica (ESTÉVEZ, 2011; PARK et al., 2006).

Do mesmo modo, radicais peroxil ($ROO^{\bullet}$), formados na oxidação lipídica, também podem ser iniciadores da carbonilação proteica. Quando é excedida a capacidade antioxidante das proteínas em função do estresse oxidativo sofrido, tanto lipídios quanto proteínas sofrem danos causados pelas reações com os radicais livres (GARDNER, 1979). Os lipídios manifestarão mais rapidamente sua degradação que as proteínas, no entanto, os radicais livres e hidroperóxidos formados a partir dos ácidos graxos insaturados poderiam atacar as cadeias laterais dos aminoácidos mais suscetíveis, gerando compostos carbonilados (PARK et al., 2006).

3.3.3 Atuação dos antioxidantes nas reações de oxidação

Segundo Falowo et al. (2014), as reações dos antioxidantes com os agentes oxidativos poderiam ocorrer por meio de 3 etapas (Fig. 6): a) pela doação de elétrons na etapa de propagação prevenindo a formação de radicais formados a partir de lipídios e proteínas (DANGLES e DUFOUR, 2006; ALLEN e CORNFORTH, 2010) ou b) pela remoção dos radicais livres iniciadores da oxidação por meio do sequestro dos catalisadores da iniciação da cadeia (ANTOLOVICH et al., 2002) ou c) limitando os radicais iniciadores por metais de ligação, quelando estes metais, estabilizando-os e tornando-os inativos (ALLEN e CORNFORTH, 2010; DAI e MUMPER, 2010) (Fig. 6).

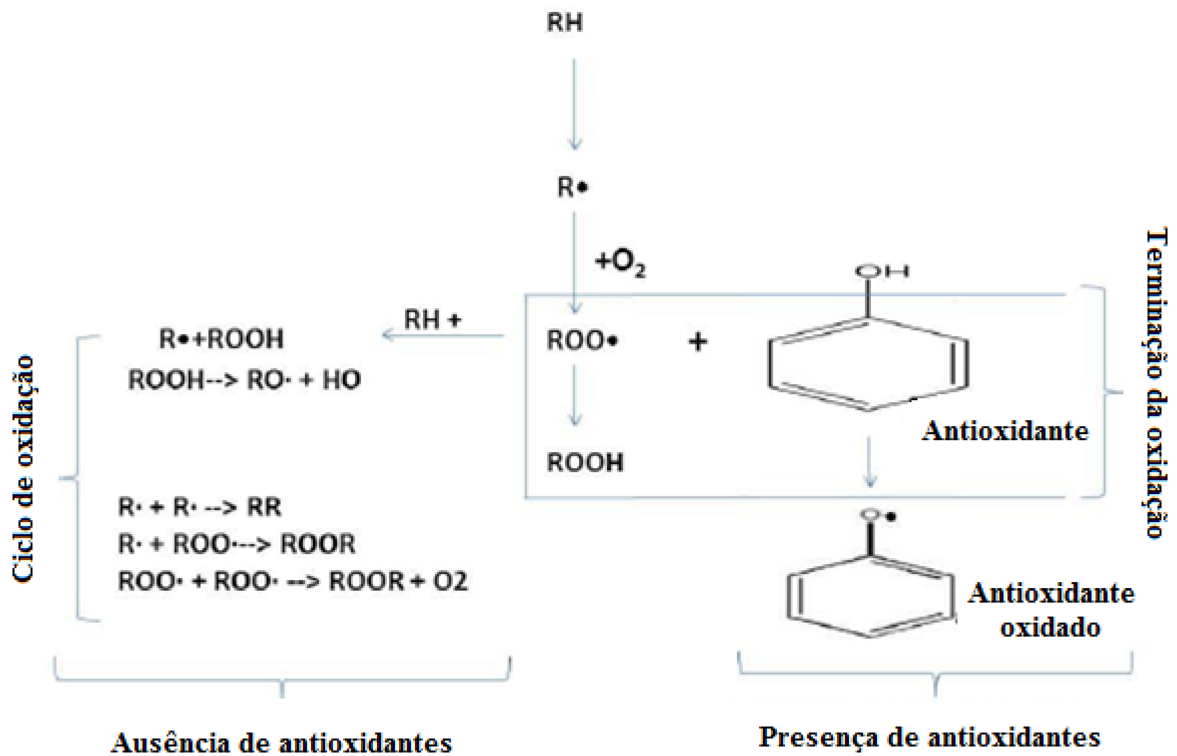


Figura 6 - Reação dos antioxidantes na fase de propagação da cadeia de oxidação lipídica. Fonte: FALOWO et al., 2014.

Dessa forma, os antioxidantes interfeririam na cadeia de oxidação lipídica de maneiras diferenciadas, de acordo com sua especificidade, contribuindo para a redução/ inibição das reações de oxidação tanto lipídica quanto proteica, uma vez que ambas podem estar associadas.

3.4 Processamento de pescados

A comercialização do pescado é feita por meio da obtenção de filés ou postas ou pelo processamento de suas carnes ou resíduos de carnes (KUBOTA E EMANUELLI, 2004). A retirada de vísceras, espinhos, pele e escamas devem ser realizadas objetivando máxima eficiência e menor custo (MOREIRA et al., 2001).

Os resíduos gerados no processamento de pescados são atualmente aproveitados na produção de óleo bruto e farinha de peixe para alimentação animal, os quais têm baixo valor comercial. Entretanto, diversos produtos para consumo

humano podem ser elaborados a partir desses resíduos, tais como, concentrados protéicos, farinhas, gelatina e *minced* (OETTERER et al., 2006).

A polpa de pescado pode ser definida como “o músculo integral, mecânica ou manualmente obtida pela separação das espinhas, ossos, pele e escamas, num processo de manufatura”, podendo se apresentar com coloração branca ou avermelhada, dependendo da espécie de peixe (MOREIRA et al., 2001). Segundo a FAO/WHO (1995), a polpa ou carne mecanicamente separada (CMS) de pescado pode ser definida como sendo um “produto obtido a partir de uma única espécie, ou mistura de espécies de peixes com características sensoriais similares, através do processo de separação mecânica da parte comestível, gerando partículas de músculo isentas de ossos, vísceras, escamas e pele”.

O processamento da polpa de peixe inicia com os pescados passando pelo desossador, que consta de um cinto de borracha o qual pressiona os peixes contra um tambor perfurado (orifícios de 3 a 5 cm) forçando a carne a passar para o interior do tambor, separando os ossos e a pele da parte comestível (OETTERER et al., 2006) (Fig. 7). Além disso, para a produção de polpa de pescado pode-se utilizar consideráveis quantidades de carne desperdiçada e de resíduos provindos do processo de filetagem (aparas e espinhaços) e é possibilitado o uso de espécies de baixo valor comercial e que não são comercializadas, viabilizando a produção de espécies de baixo valor econômico (MOREIRA et al., 2001).

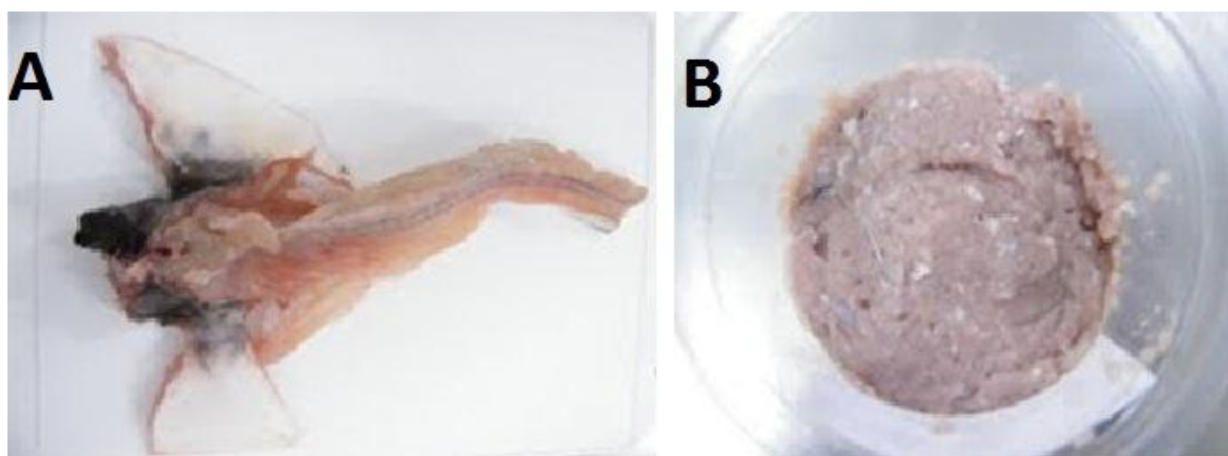


Figura 7 - Obtenção da polpa de resíduos da filetagem de jundiá (B) a partir da carcaça sem cabeça, nadadeiras e barbatanas (A). Fonte: Autor.

No entanto, durante o processamento da polpa de pescados ocorre o rompimento das células, maior superfície de exposição, incorporação de oxigênio e a conseqüente exposição à oxidação, afetando o sabor, coloração e textura (OETTERER et al., 2006), necessitando de técnicas de conservação como o congelamento rápido, que seria um dos processos mais indicados para retardar a deterioração da polpa de pescado, aumentando sua vida útil e proporcionando sua comercialização em blocos (OETTERER, 2002; KUBOTA e EMANUELLI , 2004).

3.4.1 Produtos cárneos à base de pescado

Os produtos cárneos são obtidos, preferencialmente, a partir de carne fresca que sofra um ou mais tipos de processo como salga, defumação, cozimento ou adição de condimentos e temperos. O processamento da carne atribui características de cor, sabor e aroma e tem como objetivo reduzir a perecibilidade e aumentar a viabilização destas carnes, criando alternativas para sua comercialização, além de agregar valor ao produto.

Os produtos cárneos embutidos cozidos como o patê são caracterizados por, uma vez embalados, submetidos a tratamento térmico (TERRA, 1998). Sendo assim, patê trata-se do “produto cárneo industrializado obtido a partir de carnes e/ou produtos cárneos e/ou miúdos comestíveis, das diferentes espécies de animais de açougue, transformados em pasta, adicionado de ingredientes e submetido a um processo térmico adequado” (BRASIL, 2000a).

As almôndegas são caracterizadas por ser um “produto cárneo industrializado, obtido a partir da carne moída de uma ou mais espécies de animais de açougue moldada na forma arredondada, adicionada de ingredientes e submetido ao processo tecnológico adequado, podendo ser comercializada na sua forma crua, frita, semi-frita, cozida ou esterilizada” (BRASIL, 2000b).

Os produtos cárneos são interessantes fontes de proteína e de ferro, porém, geralmente possuem grandes quantidades de gordura, saturada e colesterol, os quais têm sido relacionados com algumas doenças crônicas como doenças cardiovasculares e câncer (SIERI et al., 2008; SIRI-TARINO, et al., 2010; GONZALEZ e RIBOLI, 2010). Deste modo, o uso de pescado como fonte de proteínas na elaboração destes produtos cárneos poderia favorecer nutricionalmente

suas composições, incorporando maior quantidade de lipídicos benéficos. Atualmente, os produtos cárneos utilizando carne de pescado em sua composição estão bastante difundidos na literatura como patês (AQUERRETA et al., 2002), croquetes (GOKOGLU et al., 2012), empanados (TOKUR et al., 2006), hambúrguer (TANG et al., 2001); SANCHEZ-ALONSO et al., 2008) e apresentam características interessantes de aceitabilidade, composição química e estabilidade.

Contudo, a composição das carnes em geral torna-as um ambiente para crescimento de microrganismos deteriorantes e patogênicos, além de favorecer e acelerar as mudanças oxidativas e de deterioração do produto. Assim, são necessárias estratégias para adequada preservação das carnes e seus produtos para manutenção da sua qualidade e segurança (AYMERICH et al., 2008) como o uso de métodos físico como refrigeração e congelamento, além do uso de aditivos químicos que retardem ou minimizem essas alterações.

3.5 Técnicas de conservação de alimentos

A técnica de redução de temperatura na conservação de alimentos é utilizada para retardar ou inibir reações químicas e atividades enzimáticas e a ação de microrganismos, sendo as técnicas mais empregadas a refrigeração e o congelamento.

De acordo com o Regulanto de Inspeção Industrial e Sanitária de Produtos de Origem Animal do Ministério da Agricultura, o pescado pode ser comercializado fresco, resfriado ou congelado. Os pescados frescos e resfriados são mantidos em gelo, sendo que os resfriados são acondicionados à temperatura de -0,5 e -2,0 ºC. Já os pescados congelados são os tratados por processos adequados de congelamento em temperatura não superior a -25 °C e depois de submetido ao congelamento devem ser mantidos em câmara fria a -15 °C (-18 °C) (MAPA, 1952).

Para o armazenamento do pescado, são reportadas duas formas de armazenamento em baixas temperaturas que incluem o resfriamento a -1°C a 4 °C, inibindo o crescimento de microrganismos; e o congelamento a -18 a -30 °C, que cessam completamente o crescimento de bactérias (BERKEL et al., 2004), contudo, as alterações devido ao crescimento enzimático e não enzimático continuam a ocorrer em baixas taxas (GHALY et al., 2010).

O processo de congelamento visa à diminuição da velocidade das reações físico-químicas e enzimáticas e à paralisação do desenvolvimento microbiano, retardando o processo de deterioração do pescado (KUBOTA e EMANUELLI, 2004). Os peixes contêm cerca entre 60 e 80% de umidade e o processo de congelamento converte a maior parte da água em gelo (JOHNSTON et al., 1994). Contudo, o ponto de congelamento do peixe é a -1 e -2 °C, e mesmo a -30 °C uma proporção de água no peixe ainda se mantém descongelada. A qualidade final do músculo principalmente da taxa de congelamento (rápida e lenta). O congelamento rápido produz carnes de maior qualidade em comparação com as taxas de congelamento lento, que geram grandes cristais de gelo durante o processo, os quais danificam as paredes das células e causam desnaturação das proteínas (RAHMAN, 1999; GARTHWAITE, 1997). O processo de congelamento rápido forneceria uma vida útil em torno de 12 meses ao pescado (-35 °C), enquanto que o lento (-18 °C) não seria muito superior a 6 meses (KUBOTA e EMANUELLI, 2004).

Deste modo, as técnicas de conservação dos alimentos utilizando as baixas temperaturas são empregadas visando à redução da atividade de água e seu impacto no retardo das reações oxidativas nos alimentos.

4. Jundiá (*Rhamdia quelen*)

O jundiá (*R. quelen*) é um peixe de água doce, pertencente à ordem Siluriforme, do gênero *Rhamdia* e à família Heptapteridae, sendo uma das maiores radiações de bagres com distribuição neotropical de água doce. Sua distribuição é ampla, abrangendo a América do Sul e a Central (México até Argentina) (SILFVERGRIP, 1996). Outros nomes também são atribuídos para o jundiá no Brasil como jundiá-tinga, jandiá, jandiá-tinga, mandi, sapipoca; na Argentina é também chamado de bagre, bagre-negro, bagre-sapo, bagre-sul-americano; e em inglês seu nome comum é silver catfish (BALDISSEROTO E RADÜNZ NETO, 2004).

O jundiá é um peixe de couro, de coloração variando entre marrom-avermelhado claro a cinza, com a parte do corpo ventral mais clara, sendo seu habitat em lagos e poços fundos de rios, com preferência a águas calmas com fundo

de lama e areia, com hábitos oportunistas e noturnos (BALDISSEROTO E RADÜNZ NETO, 2004) (Fig. 8).

Figura 8 - Exemplar de jundiá. Fonte: Autor.

Dentre as espécies nativas brasileiras com potencial para a piscicultura, o jundiá destaca-se por apresentar facilidade na reprodução, rusticidade, boa eficiência alimentar, resistência ao manejo, crescimento acelerado e carne saborosa sem espinhos intramusculares (GOMES et al., 2000; CARNEIRO, 2002; FRACALOSSI, 2002; 2004).

Assim como os demais pescados, a composição química do jundiá é influenciada por fatores como idade, sexo, espécie, estação do ano, fatores ambientais, alimentação e porção do peixe analisada, contudo, seu conteúdo de proteínas varia de 11 a 20%, enquanto o de gorduras varia de 2,5 a 16% (EMANUELLI e PICCOLO, 2013). Quanto a qualidade nutricional de seus lipídios, os ácidos graxos mais abundantes encontrados em filés de jundiá são os ácidos oléico (C18:1n-9), linoléico (C18:2n-6) e palmítico (C16:0), com teores de 30, 19 e 25%, respectivamente, enquanto que o DPA (docosapentaenóico C22:5n-3) e o DHA (docohexaenóico C22:6n-3) são encontrados em quantidades menores (1,2 e 3,9%, respectivamente) (WEBER et al., 2008).

No entanto, os lipídios poli-insaturados presentes no jundiá favorecem a oxidação lipídica e deterioração do pescado e seus produtos, o que pode ser minimizado e/ ou retardado por técnicas de conservação por meio do frio e uso de aditivos alimentares com propriedades antioxidantes.

Assim, a proposta desse estudo está embasada na aplicação de aditivos alimentares de fontes naturais em pescado, os quais são fontes de ácidos graxos poli-insaturados, elementos desencadeadores das reações oxidativas, utilizando como modelo experimental o jundiá, uma espécie nativa do Brasil e considerada promissora devido a seus fatores zootécnicos e de qualidade da carne.

OBJETIVOS

Como objetivo geral deste trabalho propôs-se avaliar o efeito de extratos de semente de nêspera sobre a estabilidade oxidativa em filés, patê e almôndega à base de jundiá, ao longo do armazenamento.

1.7.1 Objetivos específicos

- ✓ Avaliar a capacidade antioxidante e a atividade antimicrobiana *in vitro* de extratos de semente de nêspera obtidos através de diferentes extrações, utilizando diferentes solventes e ultrassom.
- ✓ Estudar o efeito da imersão de filés de jundiá no extrato acetônico de semente de nêspera sobre as reações oxidativas (lipídica e proteica), os parâmetros de coloração e sensoriais durante o armazenamento congelado.
- ✓ Estudar a estabilidade lipídica e proteica de patês à base de pescado (filé e polpa de resíduo da filetagem) tratados com o extrato hidroetanólico de semente de nêspera, ao longo do armazenamento refrigerado.
- ✓ Estudar as reações de oxidação (lipídica e proteica), parâmetros de cor, de textura e sensoriais em almôndegas pré-cozidas à base de pescado (filé e polpa de resíduo da filetagem) tratadas com o extrato de acetônico semente de nêspera, ao longo do armazenamento congelado.

DESENVOLVIMENTO

MANUSCRITO 1

EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF LOQUAT (*Eriobotrya japonica*) SEED EXTRACTS

Artigo em fase final de revisão pelos autores para ser submetido à revista Alimentos e Nutrição - Araraquara

(configurado conforme as normas da revista)

ORIGINAL ARTICLE

Evaluation of antioxidant and antimicrobial properties of loquat (*Eriobotrya japonica*) seed extracts

Avaliação das propriedades antioxidantes e antimicrobianas de extratos de semente de nêspera (*Eriobotrya japonica*)

Loquat seed extracts: antioxidant and antimicrobial properties

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Abstract: Currently, much of food industry research is focused on natural antimicrobial and antioxidant compounds for replacing the synthetic ones in food preservation. Fruits are a promising source of phytochemicals with antioxidants activity that remains underutilized possibly due to the lack of studies on this area. The loquat seed extract has been identified with important pharmacological effects in animal models which could also play a role in oxidative reactions of food. **Objectives:** To evaluate the antioxidant and antimicrobial activities in loquat seed extracts using different solvents and ultrasound treatments. **Material and methods:** Loquat seeds powder was extracted with 99.8% methanol, 80% methanol, 80% ethanol, 35% acetone, 70% acetone or distilled water, either using an ultrasound-assisted procedure or not. The content of total phenolics and total tannins content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity, ferric reducing antioxidant power, and *in vitro* antimicrobial activity of extracts were evaluated. **Results:** Acetone extracts showed the highest phenolic compounds content and also total tannin content compared to others. Ultrasound treatment improved total phenolic content in 70% acetone extraction and FRAP activity in 35% and 70% acetone extracts. Acetone extracts exhibited the highest FRAP activity, while acetone and ethanol extracts had the highest DPPH antioxidant capacity. No antimicrobial activity was observed for the extracts of loquat seeds regardless of the solvent or extraction procedure. **Conclusion:** Although the extracts show any antimicrobial activity, 70% acetone extract was indicated as the extract with more expressive antioxidant properties.
Key-words: phenolic compounds, DPPH, FRAP, bacteria, tannin.

Resumo: Atualmente, grande parte das pesquisas da indústria alimentícia está voltada para os compostos antioxidantes e antimicrobianos naturalmente presentes em frutas e vegetais para substituir os sintéticos na preservação de alimentos. O uso de sementes de frutas poderia ser interessante para a indústria, mas essas continuam sendo subutilizadas devido à falta de estudos. O extrato de sementes de nêspera tem sido apontado com importantes efeitos farmacológicos em modelos animais que poderiam também desempenhar um papel importante nas reações oxidativas em alimentos. **Objetivos:** Avaliar a atividade antioxidante e antimicrobiana em extratos de semente de nêspera usando diferentes solventes e tratamento com ultrassom. **Material e métodos:** O pó de sementes de nêspera foi extraído usando metanol 99,8%, metanol 80%, etanol 80%, acetona 35%, acetona 70% ou água destilada, submetidos ao tratamento com ultrassom ou não. O conteúdo de fenólicos totais, taninos totais, atividade de remoção do radical 2,2-difenil-1-picrilhidrazila (DPPH), poder de redução do ferro e a atividade antimicrobiana *in vitro* foram avaliados. **Resultados:** Extratos acetônicos apresentaram o maior conteúdo de compostos fenólicos totais e também de taninos totais em comparação aos demais. O tratamento com ultrassom melhorou a extração de compostos fenólicos totais na extração com acetona 70% e a atividade FRAP nos extratos de acetona 35% e 70%. Os extratos de acetona exibiram a maior atividade FRAP, enquanto extratos de acetona e etanol obtiveram a maior atividade antioxidante contra o radical DPPH. Nenhuma atividade antimicrobiana foi observada nos extratos de semente de nêspera no que diz respeito ao solvente ou procedimento de extração. **Conclusão:** Embora os extratos não tenham apresentado nenhuma atividade antimicrobiana, o extrato de acetona 70% foi indicado como o extrato contendo as propriedades antioxidantes mais expressivas.
Palavras-chave: compostos fenólicos, DPPH, FRAP, bactérias, taninos.

Introduction

The increasing demand for processed foods has led to many changes in the quality and safety of food. Also, it is remarkable that consumers tend to prefer clean labeling and food additives or natural ingredients perceived as healthy (Hillmann, 2010; Joppen, 2006). Thus, food industry motivation in the use of natural antioxidants and antimicrobial compounds has been intensified for improving shelf life of food products (Basile et al., 2005; Benkeblia et al., 2004; Sokmen et al., 2004; Wong et al., 2006).

Natural antioxidants compounds that are found in plants include phenolic acids, flavonoids, carotenoids and tocopherols (Charles, 2013) and are able to delay autoxidation (Nawar, 1996). These natural antioxidants, primarily phenolic compounds, are recognized as potential free radical scavengers, reducing agents, metal chelators and singlet oxygen quenchers (Chew et al., 2009).

Ultrasound-assisted extraction is an emerging prospective technology that has been successively employed in plant extraction field and components such as aromas, pigments, and antioxidants have been extracted and analyzed efficiently from a variety of matrices (Xia et al., 2006; Chen et al., 2007; Ma et al., 2009; Rodrigues et al., 2008). The principle of ultrasound waves is altering physical and chemical properties of plant and their cavitational effect enables the release of extractable compounds intensifying the mass transport by disrupting the plant cell walls (Chemat et al., 2011).

Antimicrobial agents are widely used for food preservation and to control and prevent foodborne pathogens. Despite the fact that most antimicrobials used in the food industry are synthetic preservatives (Montville & Matthews, 2008), a range of natural antimicrobials can be found in a variety of plants including fruits, vegetables, spices and herbs (Lee et al., 2003; Windson et al., 2011; Tajkarimi et al., 2010).

Fruit seeds are considered by-products of the fruit processing and can be source of valuable beneficial compounds (Parry et al. 2005). The complete utilization of fruits is desirable and would be interesting if seeds could be used as a source of natural food additives and ingredients (Soong & Barlow 2004). However, the use of seeds for commercial purposes, especially as a source of antioxidants, remains low possibly due to few studies with this part of plant (Norshazila et al., 2010).

Loquat (*Eriobotrya japonica*) is an Asian fruit that belongs to the Rosaceae family (Morton, 1987). It has been cultivated in China and Japan since ancient times and can also be found in the Mediterranean region, South Africa, Australia, South America, California and India (Vaughan & Geissler, 1997). Loquat seeds are reported to contain interesting compounds as benzoic acid, caffeic acid, chlorogenic acid, benzaldehyde, unsaturated fatty acids, amygdalin and plant sterols (Gray & Fowden, 1972; Yokota et al., 2006; Kim et al., 2009). Loquat seed extracts has shown significant pharmacological effects in animal models as reducing oxidative stress on renal disorders (Hamada et al., 2004), suppressing the oxidation of linoleic acid and of human LDL-cholesterol when compared with extracts from the peel and pulp (Koba et al., 2007). However, the studies with the seed of loquat, which are still scarce, could help to unravel the industrial potential of this fruit byproduct and stimulate its utilization. In this context, the aim of this research was to evaluate the antioxidant and antimicrobial activities in loquat seed extracts using different solvents and ultrasound treatments.

Material and methods

Preparation of extracts

Loquat (*E. japonica*) fruits were collected from native plants in Santa Maria city at Rio Grande do Sul state, Brazil (29°41’03’’S; 53°48’25’’W) during winter and spring of

2011. After hand-harvest fruits were transferred to the laboratory, cleaned, sorted and seeds were handled separated from pulp.

The extraction procedure was performed following Koba et al. (2007) methodology with modifications. As proposed by Koba et al. (2007), the dark coat of seeds were removed prior to extraction as its contribution to overall antioxidant activity is negligible and it could bring undesirable color to the extract. The selection of solvents was based on the evidence that acetone, methanol, ethanol and their combination with water have beeno ften used for the extraction of phenolics from plants (Dai & Mumper, 2010).

E. japonica (EJ) seeds were placed at room temperature and then grounded in a micro mill (MA-630/I, Marconi, Piracicaba, SP, Brazil). For the common extraction, ten grams of seeds were homogenized with 70 mL of solvent (99.8% methanol, 80% methanol, 80% ethanol, 35% acetone, 70% acetone or distilled water). Samples were then either submitted either to an ultrasound-assisted extraction or not. For the ultrasound-assisted extraction, samples were submitted to an ultrasound bath (MaxiClean 1600, Unique, Indaiatuba, SP, Brazil) during 30 min at room temperature (40 kHz of ultrasonic frequency and 135 Watts RMS of ultrasonic potency) immediately after the addition of the solvent. Thereafter, both ultrasound-assisted or not samples were heated to 50ºC for 5 min (except for the water extract that was heated to 85ºC for 5 min). After cooling down to room temperature, the homogenate was centrifuged and filtered through a No. 5B filter paper into a volumetric flask. The volume of the filtrate was adjusted to 100 mL with the respective solvent extraction.

The extracts were then concentrated in a rotary evaporator and re-suspended to proper concentration with distilled water (except for water extract that was not evaporated). Three independent repetitions were made for each solvent extraction (totaling 36 extracts) and these were stored at -18ºC until analysis.

Total phenolic compounds

Total phenolic compounds content were determined according to Singleton & Rossi (1965) with some modification in the proportion of reagents. A 0.4 mL aliquot of the extract was mixed with 2 mL of the Folin–Ciocalteu reagent. After 3 min at room temperature, 1.6 mL of a saturated (Na_2CO_3) solution was added and the mixture was placed in dark room for 2 h. The absorbance was measured at 765 nm using a model visible spectrophotometer and results were expressed as mg of gallic acid equivalent.100 g^{-1} WS (wet seed weight) with the concentration range of 10-50 $mg.mL^{-1}$ (r^2=0.9999).

Total tannins

The total tannin was assessed by the determination of non-tannin phenolic content using the method of Makkar et al., (1993). PVPP (polyvinypolypyrrolidone) (100 g) was weighed in test tubes before being added with 1.0 mL distilled water and 1.0 mL of the extracted sample. The tubes were vortex-mixed before kept at 4°C for 15 min. Then, the tubes were vortex-mixed again before centrifugation at 1559.6 *g* for 10 min. The supernatant was collected (0.1 mL) and added by 0.4 mL distilled water, 0.25 mL Folin-Ciocalteu reagent (1 N). After 3 min, 1.25 mL of 7.5% Na_2CO_3 were added and stirred in vortex. The reaction occurred in the dark during 40 min and simple phenols were measured for absorbance at 725 nm using spectrophotometer.

Tannin content was calculated as the difference between total phenolics and non-tannin phenolic compounds. Total phenolic and tannin content were expressed as tannic acid equivalents using a calibration curve of tannic acid (Sigma, USA) with the concentration range of 5-25 $mg.mL^{-1}$ (r^2=0.9960). Total tannin was expressed as mg tannic acid equivalent.g^{-1} WS.

DPPH free radical scavenging activity

The antioxidant capacity of the EJ seed extracts was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method according to Brand-Williams et al. (1995). A 0.05 mL aliquot of previously diluted extract and 1.95 mL of DPPH (0.024%) were shaken and after 24 h the absorbance was measured at 517 nm. The control consisted of 1.95 mL of methanolic solution of DPPH and 0.050 ml of distilled water and the blank consisted of methanol. Trolox (6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid) was used as standard for the calibration curve with the concentration range of 8.3-83.3 $\mu M.mL^{-1}$ (r^2=0.9876) and the results were expressed as mmol trolox equivalents.100g^{-1} WS.

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power test was conducted according to Benzie & Strain (1996). Aliquots (0.09 ml) of each extract were mixed with 2.7 mL of FRAP reagent and the absorbance of the reaction mixture was measured at 593 nm after incubation at 37°C for 15 min. Trolox was used as standard for the calibration curve and the results were expressed as mmol trolox equivalents.100 g^{-1} of WS with the concentration range of 8.3-333.3 $\mu M.mL^{-1}$ (r^2=0.9914).

In vitro antimicrobial activity

EJ seed extracts were tested individually against *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC14579, *Salmonella enterica subsp. Enterica serovar Enteritidis* ATCC 13076, *Salmonella enterica subsp. enterica serovar Choleraesuis* ATCC 10708, *Salmonella enterica subsp. enterica serovar Typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 25922. A disc-diffusion test was performed according to the procedures described by the National Committee for Clinical Laboratory

Standards (NCCLS, 2003). Test bacteria were grown in Mueller-Hinton broth and the bacteria suspension was diluted with sterile physiological solution (NaCl 0.85%) to 10^8 CFU.mL^{-1} (turbity = MacFarland standard 0.5). The suspensions were swabbed uniformly on the surface of MHA (Mueller-Hinton Agar) in a Petri dish and, subsequently, sterilized paper discs (Whatman, 6 mm in diameter) were impregnated with 0.01 mL of each extract (1.6, 3.1, 3.4, 4.3, 5.4 and 2.3 µg of phenolic compounds/ 0.01 mL of extract respectively to EM-N, EM80-N, ET80-N, EC35-N, EC70-N and EA-N; and 2.6, 3.4, 4.1, 5.3, 8.0 and 2.5 µg of phenolic compounds/ 0.01 mL of extract respectively to EM-U, EM80-U, ET80-U, EC35-U, EC70-U and EA-U) and plated on agar previously inoculated with the test microorganism. Soaked discs with distilled water were used as negative controls and 30 mg chloramphenicol discs were used as positive controls. After 24 h of incubation at 36°C the diameter (mm) of the inhibition zones were measured.

Statistical analysis

All analyses were run in duplicate and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using the Statistica software package version 6.0 (Copyright StatSoft, Inc 1984-2001). Differences between means were first analyzed within the same extraction procedure (ultrasound-assisted or not) using the ANOVA test. To evaluate the effect of ultrasound-assistance, differences between means of the same solvent extraction and different extraction procedure were performed using ANOVA. For both, post-hoc Tukey´s test was applied (P<0.05). Pearson's coefficient was determined between phenolic compounds and antioxidant capacity tests.

Results

Total phenolic compounds in EJ seed extracts are presented in Table 1. Both 70% acetone ultrasound-assisted and non-assisted extracts (EC70-U and EC70-N) showed the highest values of total phenolics (803.8±11.5 and 545.1±24.6 mg gallic acid.100 g^{-1}, respectively). Among the ultrasound-assisted extracts, EC70-U was followed by EC35-N, which also presented great amount of phenolic compounds (533.0±35.5 mg gallic acid.100g^{-1} seed) (*P*<0.05). Both for the non-assisted and the ultrasound-assisted extracts, EM and EA extracts (EM-N, EM-U, EA-N and EA-U) were similar, being significantly lower than the other solvents (*P*<0.05). Ultrasound treatment only improved total phenolic content in 70% acetone extraction compared to the same non-assisted extraction (P<0.05).

Tannins are part of phenolic compounds, called complex polyphenols, and are shown in Table 1. Both for the non-assisted and the ultrasound-assisted extracts, the highest content of total tannins was found in acetone extracts (except for EC35-U) and other treatments obtained similar values to each other (*P*<0.05). In this context, ultrasound treatment reduced the total tannin content of 35% acetone extracts compared to the same solvent non-assisted extraction (P<0.05).

[Insert Table 1]

The antioxidant capacity of EJ seed extract was tested by its ability to donate a hydrogen atom to of the stable DPPH radical and generate a reduced molecular form (DPPH method) and by its ability to reduce iron ion (FRAP method), presented in Table 1. Among the extracts obtained with no ultrasound treatment, EC70-N, EC35-N, ET80-N and EM80-N, had significantly higher antioxidant capacity against DPPH radical than EM-N and EA-N (*P*<0.05). Almost the same behavior was found among the extracts obtained with the ultrasound-assisted treatment, but EC70-U, EC35-U and ET80-U were only higher than EA-U

extracts (*P*<0.05). Thus, ethanol and acetone extracts showed significantly higher antioxidant capacity against DPPH radical than the aqueous extracts (*P*<0.05).

In the FRAP method, among the non-assisted extracts, EM80-N showed significantly higher antioxidant capacity in the FRAP assay than EM-N, EC35-N and EA-N. Among the ultrasound-assisted extracts, ET80-U showed significantly higher value of FRAP than EM-U, EM80-U and EA-U (*P*<0.05). Furthermore, both for the non-assisted and the ultrasound-assisted extracts, 70% acetone extracts (EC70-N and EC70-U) had the highest antioxidant capacity than the other extracts (Table 1, *P*<0.05).

Thus, ultrasound treatment did not affect antioxidant activity against DPPH radical, however, FRAP activity was significantly higher in 35% and 70% acetone extracts ultrasound-assisted compared to the same solvent non-assisted extraction (Table 1, P<0.05).

The growth inhibition halo against the disc impregnated with 30 mg chloramphenicol (positive control) in the disk diffusion test is shown in Figure 1. Methanol, ethanol, acetone and aqueous extracts of loquat seed obtained either with non-assisted or ultrasound-assisted procedures showed no antimicrobial activity against any tested microorganism according to the disk diffusion test (data not shown) (Figure 1).

[Insert Figure 1]

Discussion

The solvent used in the extraction can largely influence the extraction of phenolic compounds, once higher is the polarity of the solvent higher is the amount of phenolic compounds that can be extracted (Gaméz-Meza et al., 1999). Our results showed that acetone was able to extract higher amount of phenolic compounds from the EJ seed than the other solvents (*P*<0.05). Similar results were found for extracts from goiaba residues (Nascimento

et al., 2010), avocado (Rodríguez-Carpena et a., 2011) and grape pomace (Rockenbach et al., 2008). Thus, physicochemical characteristics of acetone are closer to the phenolic compounds characteristics of the evaluated samples.

A mechanical effect exerted by ultrasound treatment promotes greater penetration of solvent into the sample matrix, increasing the contact surface area between solid and liquid phase (Rostagno et al., 2003). In this context, ultrasound treatment together with 70% acetone solvent was the most able to interact with sample matrix once ultrasound-assisted treatment only improved total phenolic content in 70% acetone extraction compared to the same non-assisted extraction ($P<0.05$).

Comparing with edible seeds, the EJ seed extract had higher values of phenolic compounds than pistachio seeds (113 µg gallic acid.g^{-1} extract) (Martorana et al., 2013). Also, methanolic and aqueous EJ seed extracts had phenolic content similar to raw pinhão seeds (296 mg.100 g^{-1} seed) (Koehnlein et al., 2012). Comparing with other vegetal residues, EJ seed extracts had phenolic content similar to grape pomace (Rockenbach et al., 2008) and much higher than those found in agroindustrial residues of pineapple, mango and passion fruit (2.4, 4.5 and 3.4 mg gallic acid equivalents.g^{-1} of dry matter) (Infante et al., 2013). Except for the EM-N, the phenolic content of EJ seed extracts are mostly higher than that of some fruits, like grapefruit and strawberries (182 and 148 mg gallic acid.100g^{-1}, respectively), both considered potential phenolic sources (Sun et al., 2002).

DPPH radical is reduced in the presence of a molecule of antioxidant that donates hydrogen, changing its violet color by yellow. Our results show that acetone and ethanol extracts presented significantly higher antioxidant capacity against DPPH radical than the aqueous extracts ($P<0.05$). This result can be explained by the low extraction of phenolic compounds in the extracts obtained with 99.8% methanol and water compared to other extracting methods.

All EJ seed extracts had higher DPPH values than purple and red pitanga seed extracts (14.6-16.7 mmol trolox.100 g^{-1} seed) (Bagetti, 2009) and cupuassu commercial seeds (130-148 μmol trolox equivalent.g^{-1} sample) (Pugliese et al., 2013), except for the methanolic and aqueous ones.

According to Pulido et al. (2000), the antioxidant efficiency determined by the FRAP method depends on the redox potential of the analyzed compounds, characterized by complexity of their molecules, such as the degree of hydroxylation and length of the conjugations. Antioxidant activity of phenolic acids increase with increasing the degree of hydroxylation, as is the case of trihidroxil gallic acid, which has a high antioxidant activity (Balasundram et al., 2006). Ultrasound treatment can degrade and oxidize antioxidants by radicals formed during cavitation (Pingret et al., 2013). Thus, the higher antioxidant activity of ultrasound-assisted acetone extracts compared to non-ultrasound treatments can be attributed to tannins hydrolysis into gallic acid caused by ultrasonication, which influenced the redox potential and consequently the FRAP activity.

A moderated correlation found between total phenolic content and FRAP ($r=0.66$, $P<0.05$) compared to a weak correlation between total phenolic content and DPPH ($r=0.37$, $P<0.05$) suggests that the phenolics extracted from EJ seeds have a greater ability to transfer electrons (FRAP activity) than to scavenge free radicals (DDPH activity).

Koba et al. (2007) reported the major compounds in ethanol extract of loquat seeds as flavonoids, such as epicatechin and epigalocatechin gallate (EGCG) and procyanidin B2. These compounds are condensed tannins which exhibit strong antioxidant properties in comparison to low molecular weight phenolic compounds (Hagerman et al., 1998). Tannins are soluble in water and polar organic solvents, with 70% acetone being considered the most efficient for extraction of condensed tannins (proanthocyanidins) (Jones et al., 1976), what explains the higher content of these compounds in the acetone extract.

Beyond its antioxidant capacity, tannins can also affect bacterial growth by depriving the substrates required for microbial growth, inhibiting the extracellular microbial enzymes or acting directly on microbial metabolism through inhibition of oxidative phosphorylation (Scalbert, 1991).

Despite the antimicrobial and antifungal effects that can be attributed to tannins due to protein binding, the ethanol, methanol, acetone and water extracts of EJ seeds, at the tested concentrations, did not show antimicrobial activity against the tested microorganisms according to the disc-diffusion test. In contrast, benzoic, caffeic and chlorogenic acids from loquat seeds were found to exhibit antibacterial effects against against *S. aureus*, *S. epidermidis*, *B. cereus* and *P. aeruginosa* (Jeong et al., 2014). It denotes that phenolic compounds found in the extracts are not able to inhibit the growth of tested microorganisms in the tested concentrations.

Conclusion

Although the extracts did not show any antimicrobial activity, ultrasound procedure improved the extraction of phenolic compounds and FRAP activity only in the acetone extraction and 70% acetone extract showed the highest total phenolics content, total tannins content and FRAP activity, and was indicated as the extract with more expressive antioxidant properties.

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Table 1 - Total phenolics, total tannins content and antioxidant activity of EJ seed extracts.

Extracts	Total phenolics (mg gallic acid.100g^{-1} WS)	Total tannins (mg tannic acid.100g^{-1} WS)	DPPH (mmol trolox.100g^{-1} WS)	FRAP (mmol trolox.100g^{-1} WS)
No treatment				
EM-N	159.2 ± 26.1^{e}	99.5 ± 30.0^{b}	12.2 ± 4.9^{b}	92.4 ± 7.7^{c}
EM80-N	314.9 ± 15.0^{cd}	93.7 ± 33.9^{b}	24.6 ± 0.9^{a}	214.5 ± 31.5^{b}
ET80-N	339.9 ± 14.2^{bc}	101.9 ± 27.3^{b}	27.0 ± 0.0^{a}	149.9 ± 2.5^{bc}
EC35-N	430.2 ± 52.6^{b}	$202.2 \pm 46.6^{ab*}$	25.4 ± 0.5^{a}	$90.2 \pm 23.1^{c*}$
EC70-N	$545.1 \pm 24.6^{a*}$	227.0 ± 12.4^{a}	25.0 ± 2.4^{a}	$343.0 \pm 45.1^{a*}$
EA-N	226.9 ± 4.2^{de}	91.9 ± 4.6^{b}	12.7 ± 1.4^{b}	69.1 ± 11.6^{c}
Ultrasound-assisted				
EM-U	258.7 ± 75.3^{d}	83.6 ± 17.5^{b}	17.8 ± 9.2^{ab}	128.2 ± 6.5^{de}
EM80-U	338.0 ± 26.9^{cd}	106.9 ± 6.7^{b}	25.7 ± 2.1^{ab}	174.0 ± 17.1^{cd}
ET80-U	413.8 ± 16.9^{c}	120.5 ± 15.0^{b}	25.5 ± 2.9^{a}	223.6 ± 24.1^{b}
EC35-U	533.0 ± 35.5^{b}	$78.2 \pm 27.2^{b*}$	26.1 ± 0.5^{a}	$170.3 \pm 26.5^{bc*}$
EC70-U	$803.8 \pm 11.5^{a*}$	207.8 ± 8.3^{a}	27.1 ± 0.2^{a}	$466.8 \pm 9.2^{a*}$
EA-U	248.5 ± 23.7^{d}	77.6 ± 25.8^{b}	10.9 ± 4.4^{b}	82.9 ± 12.5^{e}

Results are mean ± standard deviation (n=3). Means that have no common letter within the same column and the same extraction method are different ($P<0.05$). *Significantly different from the same solvent extraction with other procedure extraction (ultrasound-assisted or not). Letters N and U indicate the extract obtained using no treatment and the ones obtained using ultrasound treatment, respectively. EM = 99.8% methanol extraction; EM80 = 80% methanol extraction; ET80 = 80% ethanol extraction; EC35 = 35% acetone extraction; EC70 = 70% acetone extraction; EA = aqueous extraction.

Figure 1 – Representative Petri dish of the disc-diffusion test for the antimicrobial activity in ultrasound-assisted 80% methanol extract (3.4 ug of phenolic compounds/ 0.01 mL of extract): only the positive control showed a growth inhibition halo. Chloramphenicol (30 mg) impregnated disc (positive control, C+), extract discs with 3 replications (R) and distilled water disc (negative control, C-).

MANUSCRITO 2

LOQUAT (*Eriobotrya japonica*) SEED EXTRACT IMMERSION EFFECT ON OXIDATIVE STABILITY OF SILVER CATFISH (*RHAMDIA QUELEN*) FILLETS STORED FROZEN

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

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ORIGINAL ARTICLE

Loquat (*Eriobotrya japonica*) seed extract immersion effect on oxidative stability of silver catfish (*Rhamdia quelen*) fillets stored frozen

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Abstract: The effect of loquat seed (EJ) extract on lipid and protein oxidation, proximate composition, color and sensory properties was assessed in 100, 200 and 400 ppm EJ-treated frozen fish fillets. Ascorbic acid fish fillets had higher conjugated dienes (CD) values than control and EJ-treated fillets at 6 months, while 400 ppm EJ-treated fillets presented lower thiobarbituric acid reactive substances (TBARS) values than ascorbic acid fillets at 9 months (P<0.05). However, at the end point CD and TBARS values became similar among treatments (*P*<0.05). The protein carbonyl content of fillets hardly increased from 3 months of storage until the end point compared to 0 months, with no treatment effect (*P*<0.05). Redness decreased over storage time in all treatments at all storage time, while yellowness increased in all treatments at 9 and 12 months compared to 0 months (P<0.05). The EJ-treated fillets and ascorbic acid containing formulations had lower ΔE_{0-12} values than control

(*P*<0.05). The immersion of fish fillets on EJ seed extract was not able to slow down lipid or protein oxidations or prevent redness and yellowness modifications over storage time. However, ΔE_{0-12} pointed a minor color modification in EJ-treated and ascorbic acid fish fillets compared to control fish fillets and did not modify proximate composition and acceptability properties of fish fillets.

Keywords: TBARS, carbonyl protein, color, sensory.

Introduction

Lipid oxidation is the main cause of quality deterioration in frozen meat as it causes discoloration, off-flavors development and texture changes (Min and Ahn, 2005). Lipid oxidation begins with the removal of a hydrogen atom from unsaturated lipids. Then, the double bonds are rearranged generating conjugated dienes. Peroxyradical, which are formed when dienes are attacked by molecular oxygen, can remove a hydrogen atom from an adjacent lipid to form hydroperoxides, which continue the propagation phase of oxidation reaction until formation of malondialdehyde. Due to the close proximity to the double carbon bonds, polyunsaturated fatty acids (PUFAs) are more sensitive to interact with hydroxyl radicals and be oxidized (Halliwell and Gutteridge, 2007). Fish are important sources of PUFAs and despite the potential health benefits provided by these lipid compounds (Lee and Lip, 2003) they also increase meat susceptibility to lipid oxidation (Frankel, 1998). Silver catfish (*Rhamdia quelen*) is a freshwater species that has become important to aquaculture of Southern Brazil because of its adaptation to subtropical and temperate climate (Barcellos et al., 2006), moreover it has a flesh tasty and absence of intramuscular bones (Fracalossi et al., 2002) that could contribute to its acceptance by consumers.

Lipid oxidation products can induce protein cross linking, resulting in a protein oxidation dependent reaction (Esterbauer and Cheeseman, 1987). Thus, protein oxidation of meat products can lead to technological changes as alterations in water holding capacity,

reduced tenderness, discoloration, flavor deterioration and loss of nutritional quality (Lund et al., 2011).

Therefore, food industry has made efforts to prevent the development of these oxidative reactions in meat and avoid its undesirable effects, what explains the increase in research around antioxidants, mainly from natural resources, once synthetic ones are noticeable by their potential toxicological effects (Naveena et al., 2008a; Wanasundara and Shahidi, 1998).

Because of the metal chelating and radical scavenging properties of phenolic compounds, extracts from plant materials have been considered good sources of natural antioxidants in meat and fish products (Reihani et al., 2014; Ganhão et al., 2010; Naveena et al., 2008b).

Loquat (*Eriobotrya japonica*) is an Asian fruit cultivated in Japan and also in other subtropical and tropical countries (Femenia et al., 1998). Loquat seeds has significant effects on the suppression of diseases and lipids in animal models as inhibition of oxidative stress on renal disorders (Hamada et al., 2004), hypoglycemic properties (Kim et al., 2009), inhibition of liver fibrosis (Nishioka et al., 2002) and suppressing LDL-cholesterol oxidation *in vitro* (Koba et al., 2007). However, little is known about the technological potential of loquat seed extracts for the food industry (Piccolo et al., 2014).

The aim of this research was to investigate the effect of loquat seed extract on lipid and protein oxidation, proximate composition, color and sensory properties of fish fillets stored frozen.

Material and methods

Preparation of extracts

Loquat seed fruits (*E. japonica;* EJ) were collected from native plants in Santa Maria region of Rio Grande do Sul state, Brazil (29°41’03’’S; 53°48’25’’W) during winter and spring of 2011. After hand-harvest, fruits were transferred to the laboratory, cleaned, sorted and

seeds were handled separated from the pulp. The dark coat that covers the seeds was removed and seeds were grounded in a micro-mill (Marconi MA-630), dried in an air-assisted oven at 60°C for 2 h, cooled to room temperature and frozen in dark vessels (-18°C) until use.

Loquat seed extract was obtained following Koba et al. (2007) procedure with modifications. Seeds (20 g) were homogenized in 70% acetone (100 mL), heated at 50°C for 10 min and sonicated (Unique, USC-1600) for 1 h (40 kHz of ultrasonic frequency and 135 Watts RMS of ultrasonic potency). After, the extract was cooled at room temperature, centrifuged at 1083.1 *x g* during 3 min and the supernatant was filtered, rotaevaporated at 40°C and kept at -18°C until use (200 mg *E. japonica* seed/mL extract).

Total phenolic content was determined at 725 nm using Folin-Ciocalteu method (Swain and Hillis, 1959). Gallic acid was used as standard for the calibration curve and the amount of phenolic compounds was expressed as mg gallic acid/mL of extract. The phenolic content of extract was 0.81 ± 0.09 mg gallic acid/mL extract (405.43 ± 43.94 mg of gallic acid/100 g seed).

Preparation of fish fillets

Silver catfish (*R. quelen* 421.9 ± 0.09 g) were acquired from a fish farm at Santa Maria, Rio Grande do Sul, Brazil. Fish were transported to the laboratory on ice, slaughtered, cleaned, gutted and filleted (22.4 ± 2.9% yield). The fillets were frozen at -18°C during 5 h before the treatment. Fillets were immersed (10g fillet/mL solution) for 10 min (5 min on each side) in extracts containing 100, 200 and 400 ppm (mg of phenolic compounds/L of extract), ascorbic acid (200 ppm, positive control) or distilled water (negative control) following the procedure adopted by Veeck et al. (2013) with modifications. Fillets were packed in polyethylene trays, wrapped with ziplock plastic bags and stored at -18±1°C.

Three fish fillets were taken from each treatment (n=3) at the sampling times (0, 3, 6, 9 and 12 months). One portion of fillets was used to evaluate the proximate composition; a second portion was to evaluate color, lipid oxidation, and protein oxidation; and a third

portion was used to assess the sensory properties, which were carried out only at 0 and 12 months of storage.

Proximate composition of fish fillets

Proximate composition analysis was performed at 0 months of storage and moisture, ash and protein contents were determined using official methods (AOAC, 2000). Moisture was determined by oven drying at 105°C for 12 h; ash content was determined in a muffle furnace at 550°C and crude protein was determined by the Kjeldahl method using the conversion factor of 6.25. The method of Bligh and Dyer (1959) was used for determining the fat content of fish fillets. Carbohydrate content was measured by difference.

Lipid oxidation

Conjugated dienes (CD) were determined in the fat extracted by the Bligh and Dyer method (1959) as described by Recknagel and Glende (1984), using cyclohexane as the solvent and the optical density recorded at 233 nm. All measurements were normalized to a uniform base of 1 mg lipid/mL of cyclohexane.

The secondary products of lipid oxidation were measured as thiobarbituric acid reactive substances (TBARS) and were determined spectrophotometrically as described by Buege and Aust (1978). Results were expressed as mg MDA/kg fish fillet.

Protein oxidation

Protein oxidation was determined as the total carbonyl content in protein as described by Levine et al. (1990) with modifications. Samples (1 g) were minced and homogenized in phosphate-buffered saline (PBS) (4 mL), using an ultraturrax homogenizer for 30 s. Proteins (300 µL supernatant) were precipitated with 40% TCA (250 µL) and centrifuged at 2122.8 *x g* during 5 min. The pellet was ressuspended and incubated with dinitrophenylhydrazine (DNPH) in the dark for 15 min at room temperature. Then, pellets were precipitated with 40% TCA (250 µL) and were washed two times with 1:1 (v/v) ethanol/ethyl acetate (500 µL). The

pellet was re-dissolved in 2% sodium dodecyl sulfate (SDS) (1.5 mL) at 37°C, and the carbonyl content was determined by measuring the absorbance at 370 nm. Protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard. The results are expressed in nmol carbonyl/mg of protein.

Color measurement

Color was assessed using a Minolta Chromameter CR-300 (Minolta Ltd., Osaka, Japan) with a standard illuminant D65, 2° observation angle. Before each session the chromameter was calibrated on the CIE color space system using a white tile (number 15233011). Color measurements were made on the surface of each fish fillet and three measurements were recorded directly on the samples after turning 90° from the previous reading. According to the International Commission on Illumination (CIE 1976, L* a* b*) the chromaticity coordinates a* and b* indicate the direction of color, where +a* is the red direction and -a* is the green direction; +b* is the yellow direction and -b* is the blue direction. L* is the luminosity that ranges from 0 (absolute black) to 100 (full white). The numerical total color difference (ΔE) between fish fillets at 0 and 12 months was calculated using the following equation: $\Delta E_{0\text{-}12} = [(L_{12}-L_0)^2 + (a_{12}-a_0)^2 + (b_{12}-b_0)^2]^{1/2}$.

Sensory analysis

This study was approved by the Ethics Committee (CAAE 07241112.4.0000.5346) of the Federal University of Santa Maria and all the panelists provided their consent prior to inclusion in the study.

An expert panel (n=8 to 14 panelists) evaluated fish fillets at 0 and 12 months. The panel was selected among students from the University based on questionnaire to determine their interest in participating and possible exclusion factors, smoking and allergies. The panelists were trained to assess the color, odor, flavor and texture characteristics of fillets. The trainings were conducted comparing these characteristics in fresh fish and oxidized

ones. Panelists were also trained to the odor and taste perception using crude and diluted extract.

Immediately before analysis, fish samples (20 g) were wrapped in wax paper and heated in a microwave oven during 1 min. The samples were coded and randomly offered to panelists. Crackers and drinking water were provided for palate cleansing between samples. The color, odor, flavor, texture and overall acceptability of fillets was evaluated at 0 months of storage using a 9-points hedonic scale (1 = dislike extremely, 9 = like extremely). Panelists were also asked about the rancid odor of fillets using a structured 10-points numeric scale at end points (1 = no rancid odor; 10 = very much rancid odor) at 0 and 12 months.

Statistical analysis

The proximate composition, ΔE and sensory analysis at 0 months of storage were analyzed using an one-way analysis of variance (ANOVA), and other results were analyzed using a two-way factorial ANOVA (5 treatments × 5 time points). Data of both sensory analyses were normalized to take account of individual scale-use differences. The differences between the averages were *post hoc* evaluated using Tukey´s HSD (honest significant difference) test. Differences were considered to be significant when $P<0.05$.

Results and discussion

Proximate composition

No significant difference was found in the proximate composition of fish fillets at 0 months of storage ($P>0.05$; Table 1). Moisture content ranged from 80.4 to 83.3%, ash content from 1.1 to 1.5%, protein from 9.8 to 10.7%, fat from 1.6 to 2.8% and carbohydrate content from 3.7 to 6.6%. Thus, immersion of fish fillets on EJ extracts had no influence on the proximate composition of fillets ($P>0.05$). This corroborates with Gai et al. (2014) who found no significant effect of green tea extract on the chemical composition of tench fillets.

[Insert Table 1]

Lipid stability

ANOVA revealed a significant treatment x storage time interaction on CD values and TBARS of fish fillets (Fig. 1 and 2). CD values increased from 3 months of storage, and ascorbic acid fish fillets had higher values than control and EJ-treated fillets at 6 months ($P<0.05$; Fig. 1). CD values remained similar among treatments in the other storage times ($P>0.05$).

[Insert Figure 1]

TBARS values significantly increased from 6 months of storage for ascorbic acid and control fish fillets ($P<0.05$). At 9 months, 400 ppm EJ-treated fillets presented lower TBARS values than ascorbic acid fillets. However, at the end point TBARS values became similar among treatments ($P<0.05$; Fig. 2).

[Insert Figure 2]

Metal ions are catalysts of oxidative reactions and iron is the most abundant metal in the tissues. The ferrous form (Fe^{2+}) accelerates lipid oxidation producing hydroxyl radicals from hydrogen peroxide via the Fenton reaction (Valko et al., 2007). Despite the antioxidant efficiency of ascorbic acid, it paradoxically may also have an effect as a pro-oxidant. Ascorbic acid is able to reduce transition metals, such as iron and copper ions, and so they are capable to interact via Fenton reaction with hydrogen peroxide generating hydroxyl radicals, which can catalyze oxidation reactions (Halliwell and Gutteridge, 2007). Thus, ascorbic acid acted as a pro-oxidant causing an increase in primary (CD values) and in secondary (TBARS levels) lipid oxidation products. The EJ extract did not decrease the formation of primary or secondary products of lipid oxidation compared to the control fillets.

Flavonoids may exert their antioxidant capacity by scavenging reactive oxygen species, preventing the formation of reactive oxygen species, interacting with other antioxidants (Martínez-Flórez et al., 2004; Heim et al., 2002). Koba et al. (2007) described the presence of chlorogenic acid, cyanidin glucoside, epicatechin, EGCG and procyanidin B2 in loquat seed ethanolic extracts. Thus, primarly galotannins and condensed tannins compose ethanolic fraction of loquat seed extracts. In a recent study based on antioxidant properties of tannins, Magalhães et al. (2014) showed gallotannins as having greater ability for reducing free radicals, while condensed tannins can scavenge peroxyl radicals, representing a potential antioxidant mechanism to prevent lipid oxidation. In addition, previous studies showed that EJ seed extracts have a higher efficiency in transferring electrons (FRAP activity) than as free radicals scavenging (DPPH scavenging activity) (data not published).

Nevertheless, EJ extract naturally present compounds were not enough for slowing down primary or secondary lipid oxidation products. Previous studies also presented no effect of EJ seed extract on fish pate refrigerated stored (Piccolo et al., 2014), which can be attributed to heating and processing of meat products that can promote lipid oxidation by disrupting cell membranes during this processes (Gray and Pearson, 1987). However, TBARS values did not reach the threshold of rancidity perception for fish (3 mg MDA/kg) at the end of storage time (Al-Kahtani et al., 1996).

Protein stability

ANOVA revealed a significant main effect of storage time, but no effect of treatments on protein carbonyl content. The protein carbonyl content of fillets increased from 3 months of storage and was more accentuated at the end point (8 to 20-fold higher than at 0 months) ($P<0.05$; Fig. 3).

[Insert Figure 3]

Protein carbonyl compounds can be formed from different pathways, in meat systems mainly resulting from the direct oxidation of side chains of arginine, lysine, proline and threonine residues (Requena et al., 2001). Carbonyl groups can also be formed by the non-enzymatic glycation of proteins with reducing sugars; oxidative cleavage of the peptide backbone and by amino acids binding to secondary lipid oxidation products (non-protein carbonyl compounds) (Estévez, 2011).

Furthermore, despite the increase in conjugated dienes, the increase in TBARS did not reach critical values indicative of randicity for fish. Thus, our results suggest that the protein oxidation occurred even before than lipid oxidation in the evaluated fish fillets and it was mainly promoted by direct oxidation of amino acids.

Therefore, phenolic compounds of EJ extracts did not act in slowing down the protein oxidation. Addition of phenolic compounds cannot always inhibit protein carbonyl, once several studies revealed no effect or even pro-oxidative effects of phenolics in meat (Utrera et al., 2014; Ganhão et al., 2010). Also in fish the beneficial effects of extracts is not found as occurred in EJ seed extracts and hydroxycinnamic acids addition that did not reduce protein modifications in fish pates refrigerated stored (Piccolo et al., 2014) and minced frozen horse mackerel white muscle (Medina et al., 2009), respectively. These finfings are corroborated by Dean et al. (1991) that explains that inconsistencies between the antioxidant and pro-oxidant activity of phenolic compounds are the result of different affinities between different phenolics, metallic ions and proteins.

Color

Lightness values showed no significant changes on treatments or over storage time (57.0 ± 0.4 at 0 months vs. 58.3 ± 0.7 at 12 months) (data not shown). Minced horse mackerel treated with potato peel extract also did not show changes over chilled storage (Sabeena Farvin et al., 2012).

ANOVA revealed a significant main effect of storage time, but no effect of treatments on the redness (a*) and yellowness (b*). A decrease in redness observed over storage time

was linear in all fish fillets, whereas yellowness increased over storage time and was significantly higher at 9 and 12 months of storage (7.19±0.34; 9.51±0.71; 9.05±0.35 at 0, 9 and 12 months of storage, respectively, for yellowness) (P<0.05; Fig. 4A and 4B).

[Insert Figure 4A and 4B]

Myoglobin is the main protein responsible for meat color and the decrease in the redness (a* value) can be attributed to myoglobin oxidation (Mancini and Hunt, 2005). Heme iron (Fe^{2+}) oxidation to ferric iron (Fe^{3+}), due to interaction with lipid oxidation products (hydroperoxides) (Yin and Faustman, 1993), enables the conversion of oxymyoglobin to metmyoglobin, changing the bright red to brownish color (Fox, 1987).

In addition, the yellow color is associated to lipid oxidation, whose products play an important role in yellow color modification of fish fillets (Khantaphant et al., 2011). The increase in the yellowness (b* values) may have a close relationship with lipid and protein oxidation, since aldehydic products of lipid oxidation, such as malonaldehyde, and of protein oxidation, such as carbonyl groups, may react via Maillard reaction, inducing the formation of melanoidins, increasing b* values (Damodaran et al., 2010; Chaijan et al., 2009). The increase in TBARS influenced color parameters in *Salminus brasiliensis* fillets treated with mate extracts (Veeck et al., 2013). However, in jundiá fish fillets increased b* values appeared to be related only to protein oxidation once TBARS did not reach critical values of lipid oxidation.

ANOVA revealed a main effect of treatment on total color difference of fish fillets between the beginning and the end of the frozen storage (ΔE_{0-12}) (P<0.05; Fig. 5). A limit to appreciate visual changes in color is below of value of 2 (Francis and Clydesdale, 1975) however, although all treatments presented higher values than the maximum, fillets treated with EJ extracts and with ascorbic acid had lower ΔE_{0-12} values than control (P<0.05).

Once the ΔE is calculated from the difference between the initial and final L*, a*, and b* values, the lightness would be the coordinated color that would have the greatest

contribution to alter the ΔE values due to its magnitude in relation to redness and yellowness. However, lightness values did not differ significantly over time and do not seem to have affected the total color difference. Thus, despite not having been detected significant differences between treatments in redness and yellowness of fish fillets, significant color changes in a* and b* over storage time compared to the initial values appear to have contributed to lower $\Delta E_{0\text{-}12}$ in ascorbic acid and EJ-treated fish fillets compared to control.

[Insert Figure 5]

Sensory analysis

Color, odor, flavor, texture and overall acceptability scores of EJ-treated fillets were similar to the control fillets at the beginning of the storage (Table 2; $P>0.05$), showing that EJ extracts did not influence the sensory characteristics of fillets.

[Insert Table 2]

ANOVA revealed no effect of treatment or storage time on the rancid odor of frozen catfish fillets (data not shown). Thus, although ascorbic acid has provided the development of lipid oxidation, it was not observed on rancid odor development of fish fillets. Al-Kahtani et al. (1996) refers the threshold of rancidity perception for fish being about 3 mg MDA/kg, which was not reached in our study and can explain no modification in rancid odor perception by panelists.

In addition, frozen storage does not avoid oxidative reactions that can affect sensory properties leading to unpleasant flavor, odor and texture. Besides malondialdehyde, also formaldehyde formed during the oxidation of lipids makes cross-links with proteins leading to loss of solubility and water-holding capacity, and moreover, to changes in odor and taste (Steen and Lambelet, 1997). However, the decrease in sensory properties appeared to be

less noticeable than changes in the chemical and physical properties, which include increase of protein oxidation and b* values, and decrease in a* values.

Conclusion

The immersion of fish fillets on EJ seed extract was not able to slow down lipid or protein oxidations. EJ seed extract was not able on preventing redness decrease and yellowness increase over storage time. However, $\Delta E_{0\text{-}12}$ pointed a minor color modification in EJ-treated and ascorbic acid fish fillets compared to control fish fillets and did not change proximate composition and acceptability properties of fish fillets.

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Table 1. Proximate composition (g/100g) of fish fillets treated with loquat seed extract.

Treatments	Moisture	Ash	Protein	Fat	Carbohydrate
Control	80.4 ± 0.5	1.2 ± 0.0	10.3 ± 0.3	1.6 ± 0.5	6.6 ± 0.4
Ascorbic acid	83.3 ± 0.7	1.1 ± 0.0	9.8 ± 0.5	2.2 ± 0.3	3.7 ± 1.0
100 ppm	80.3 ± 0.9	1.4 ± 0.2	10.7 ± 0.4	4.0 ± 0.5	4.5 ± 1.0
200 ppm	82.3 ± 0.7	1.4 ± 0.2	10.5 ± 0.5	2.4 ± 0.6	3.9 ± 0.4
400 ppm	80.9 ± 0.5	1.5 ± 0.0	10.3 ± 0.1	2.8 ± 1.1	4.4 ± 1.7

Results are mean ± standard error (n=3). ANOVA revealed no significant difference by Tukey´s test ($P>0.05$).

Table 2. Sensory characteristics of fish fillets treated with loquat seed extracts at 0 months*.

Treatments	Color	Odor	Flavor	Texture	Overall acceptability
Control	7.9 ± 0.3	7.5 ± 0.3	8.2 ± 0.2	8.4 ± 0.2	8.0 ± 0.3
Ascorbic acid	7.8 ± 0.3	7.7 ± 0.3	8.2 ± 0.3	8.4 ± 0.2	8.0 ± 0.2
100 ppm	7.4 ± 0.4	7.6 ± 0.3	7.7 ± 0.3	7.9 ± 0.3	7.7 ± 0.3
200 ppm	7.9 ± 0.3	8.0 ± 0.2	8.2 ± 0.2	8.5 ± 0.2	8.1 ± 0.2
400 ppm	8.0 ± 0.3	7.4 ± 0.4	7.8 ± 0.5	8.4 ± 0.2	8.0 ± 0.3

*Evaluated using a hedonic 9 points-scale with verbal descriptors (1=dislike extremely; 9=like extremely). Results are mean ± standard error (n=14). ANOVA revealed no treatment effect by Tukey´s test.

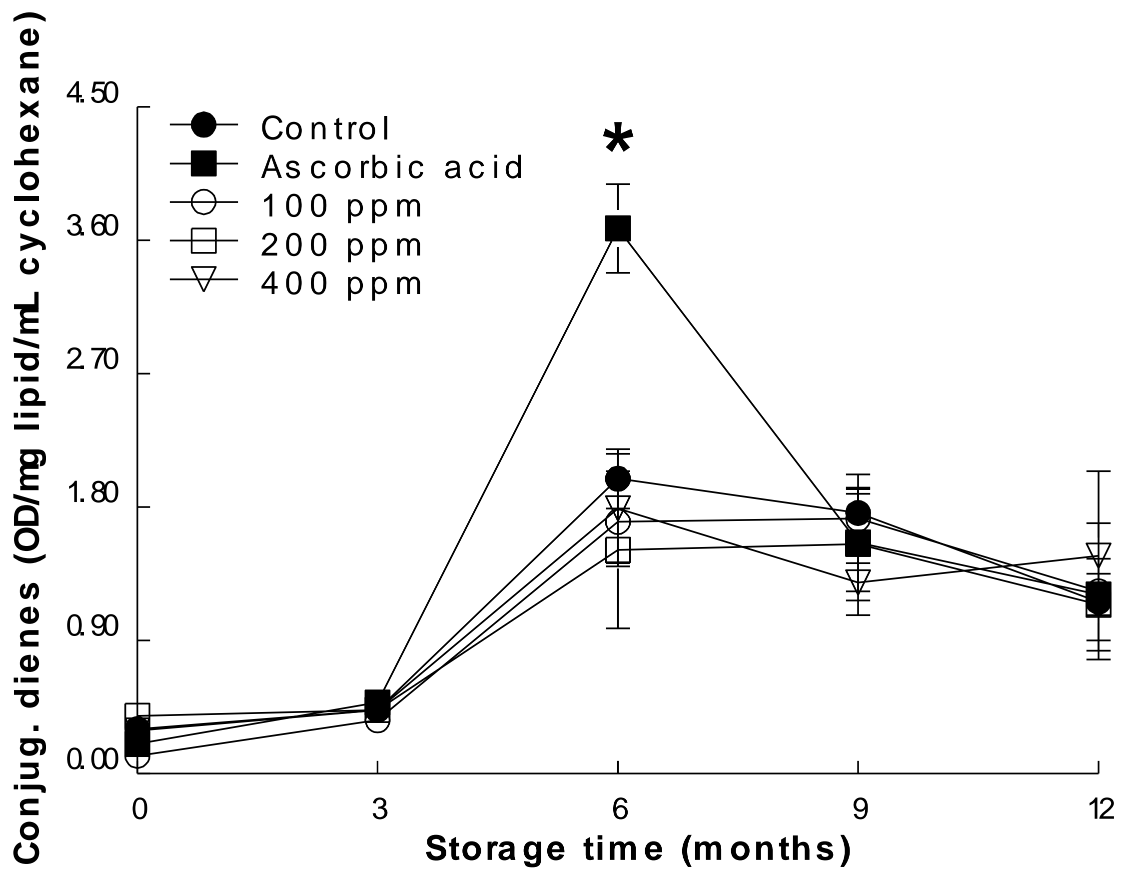

Fig 1. Effect of loquat seed extract on conjugated dienes (CD) of fish fillets during frozen storage. Results are mean ± standard error (n=3). ANOVA revealed a significant formulation x storage time interaction. *Significantly different from control at the same day ($P<0.05$).

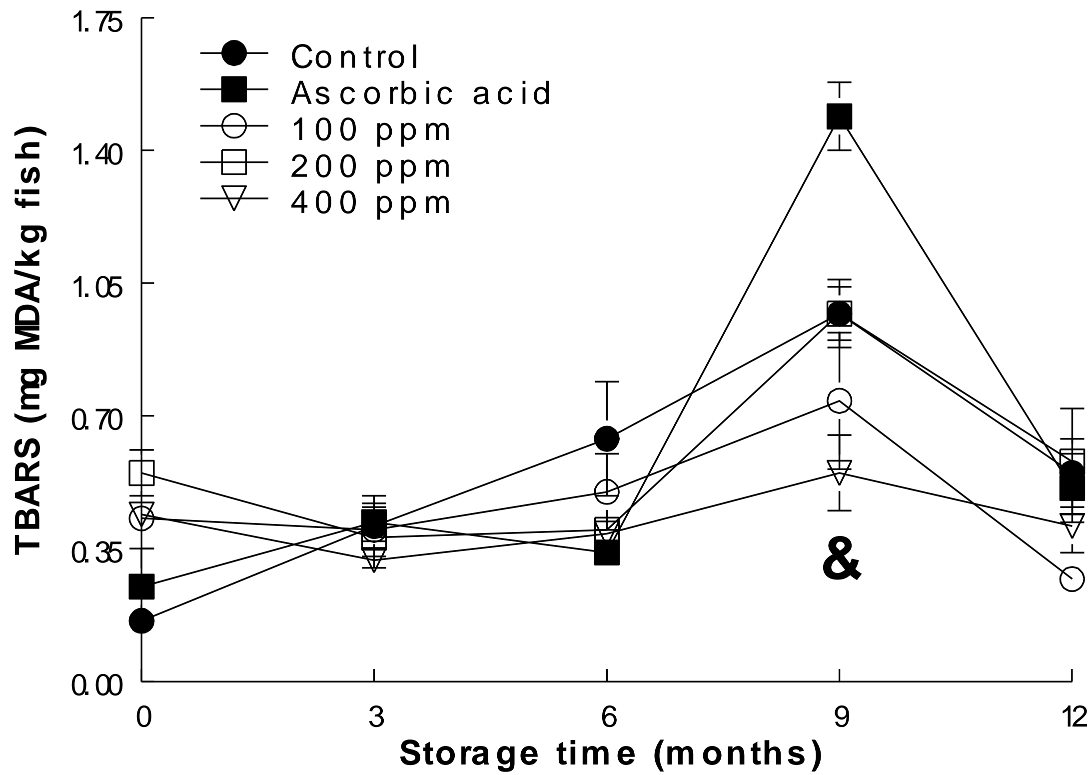

Fig 2. Effect of loquat seed extract on thiobarbituric acid reactive substances (TBARS) of fish fillets during frozen storage. Results are mean ± standard error (n=3). ANOVA revealed a significant formulation x storage time interaction. [&]Significantly different from ascorbic acid at the same day (P<0.05).

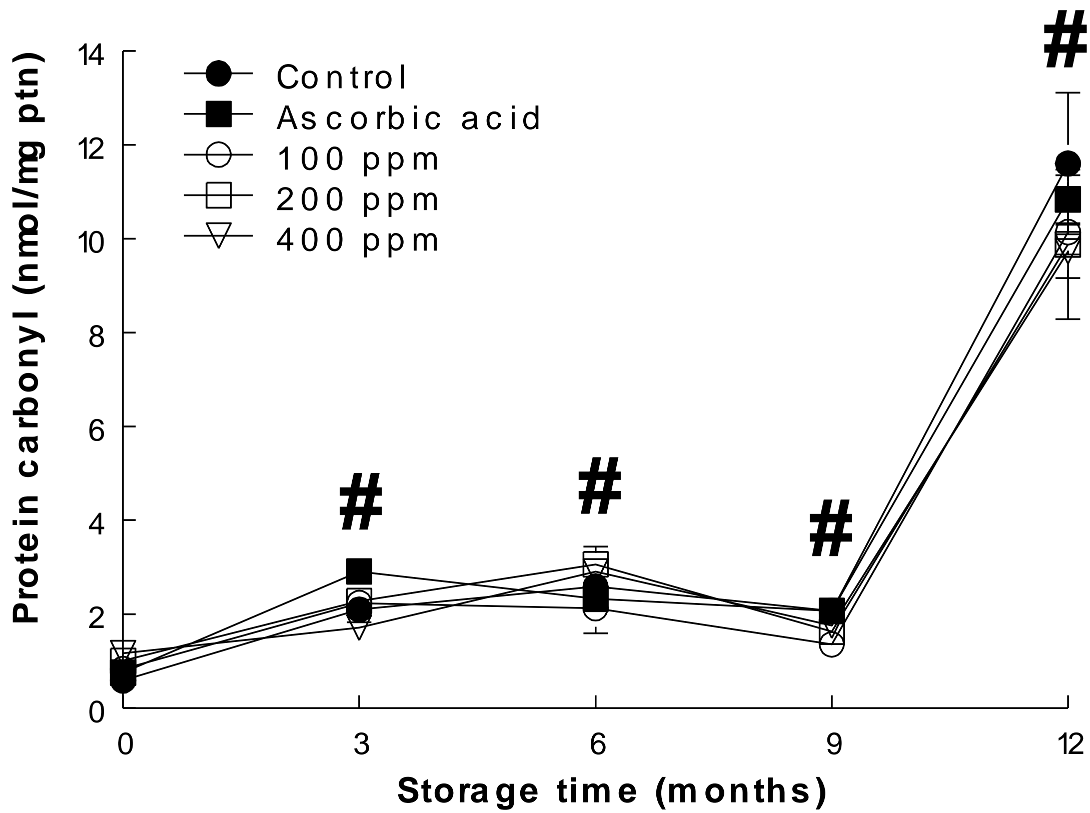

Fig 3. Effect of loquat seed extract on protein carbonyl content of fish fillets during frozen storage. Results are mean ± standard error (n=3). ANOVA revealed a significant main effect of storage time, but no effect of treatment ($P<0.05$). [#]Significantly different from 0 months.

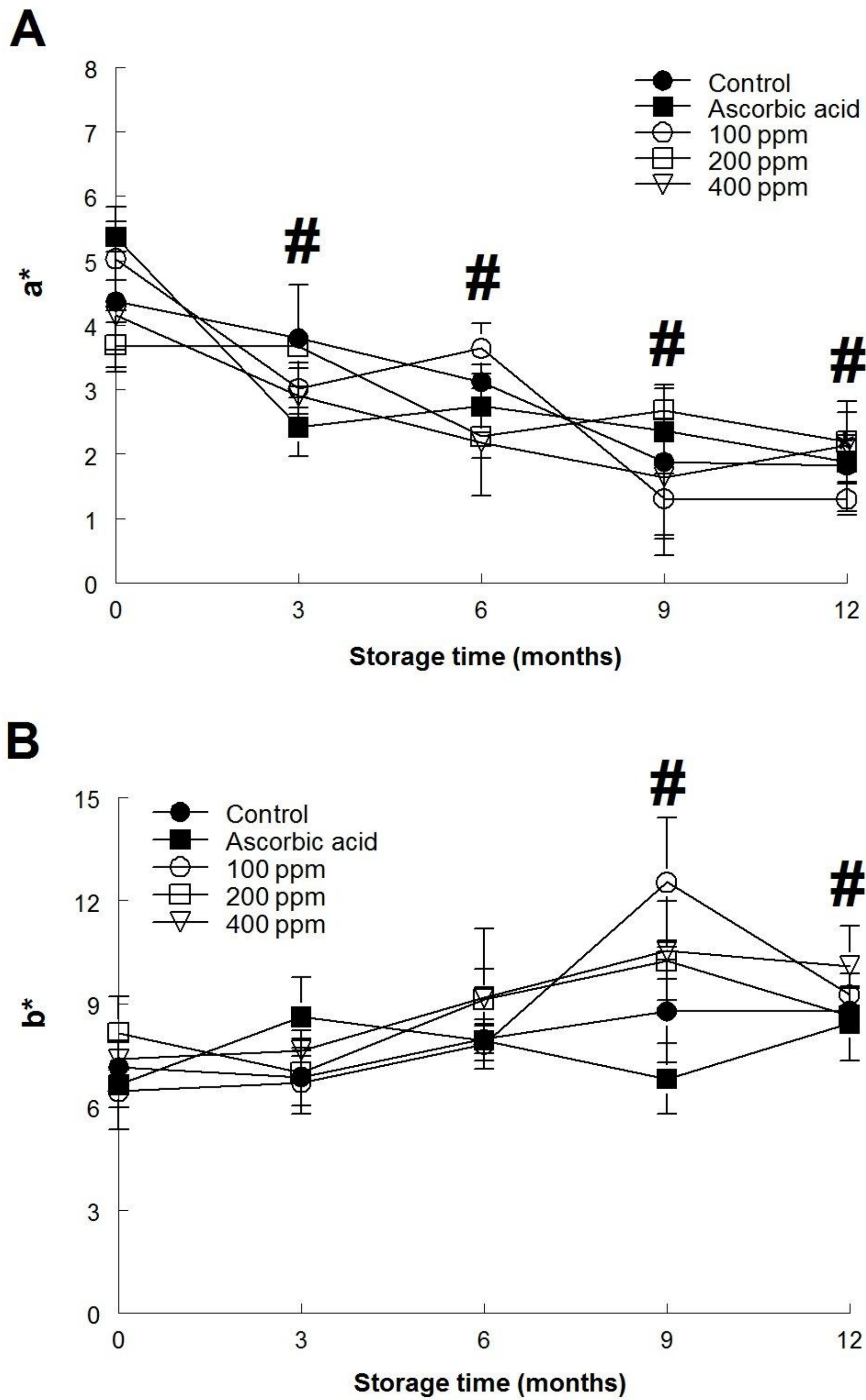

Fig 4. Effect of loquat seed extract on redness (A) and yellowness (B) of fish fillets during frozen storage. Results are mean ± standard error (n=3). ANOVA revealed a significant main effect of storage time, but no effect of treatment (P<0.05). #Significantly different from 0 months.

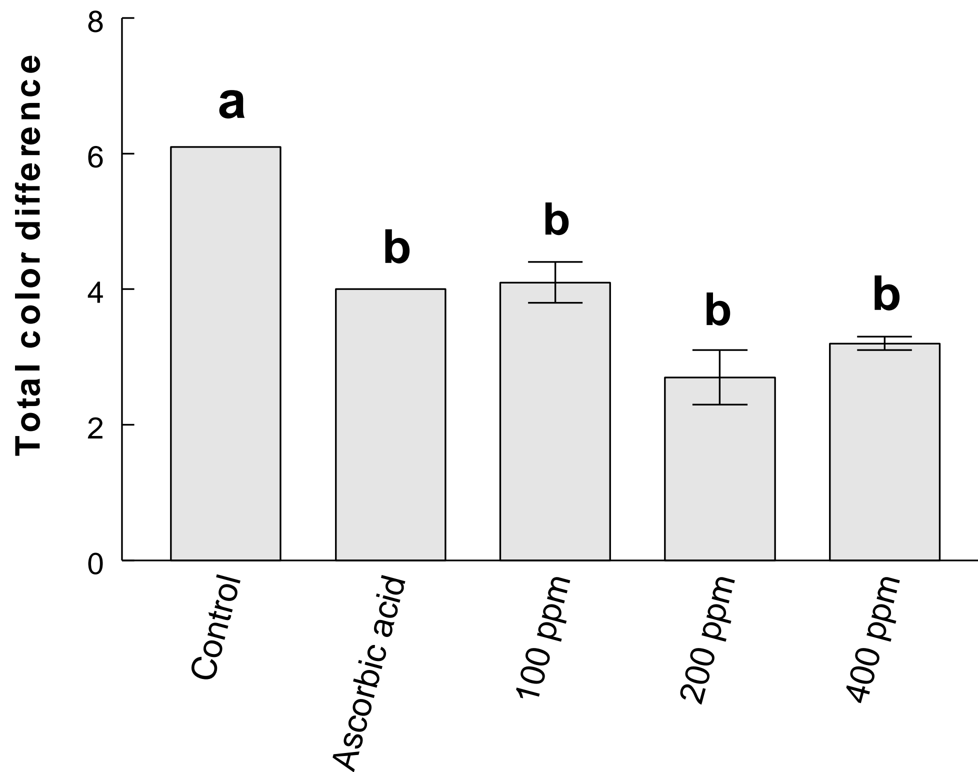

Fig 5. Effect of loquat seed extract on total color difference ($\Delta E_{0\text{-}12\ months}$) of fish fillets during frozen storage. Results are mean ± standard error (n=3). ANOVA revealed a significant main effect of treatments. Means that have no common letters are different by the Tukey´s test ($P<0.05$).

MANUSCRITO 3

OXIDATIVE STABILITY OF REFRIGERATED FISH PATES CONTAINING LOQUAT SEED EXTRACT

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Oxidative stability of refrigerated fish pates containing loquat seed extract

Estabilidade oxidativa de patês de pescado contendo extrato de semente de nêspera armazenados refrigerados

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ABSTRACT

This study investigated the effects of hydroethanolic ***E. japonica*** *seed extracts (EJSE) as inhibitors of lipid and protein oxidation on fish pates subjected to refrigerated storage. Five fish pate formulations were developed. These formulations included two control pates (water-control and ascorbic acid-control) and three pates with added EJSE (0.1, 0.2 and 0.4g of seed 100g^{-1} product, equivalent to 3.4, 6.8 or 13.6mg phenolic compounds kg^{-1} product), which were then stored under refrigeration for 35 days. Conjugated dienes (CD) and peroxide (PV) values increased along with the storage time; however, these values decreased and were similar among all samples at the end of 35 days of analysis ($P<0.05$). However, the thiobarbituric acid reactive substances levels (TBARS) did not change along the storage and were not affected by the EJSE. Additionally, there was a linear increase in the protein carbonyl content of fish pates over the storage period ($P<0.05$), but no effect of EJSE on protein oxidation. The results show that, at the concentrations evaluated, hydroethanolic* ***E. japonica*** *seed extract was unable to inhibit or reduce lipid and protein oxidation in fish pates, but the observed phenolic content emphasizes the need for further studies on the wastes of this fruit.*

Key words: ***Eriobotrya japonica, Rhamdia quelen,*** *lipid oxidation, protein oxidation, wastes.*

RESUMO

Este trabalho investigou os efeitos do extrato hidroetanólico de semente de ***E. japonica*** *(EJSE) como inibidor da oxidação lipídica e proteica em patês a base de pescado armazenados refrigerados. Foram desenvolvidas cinco formulações de patê de pescado. Estas formulações incluíram dois patês controles (controle-água e controle-ácido ascórbico) e três adicionados de EJSE (0,1; 0,2 e 0,4g de semente 100g^{-1} de produto, equivalente a 3,4; 6,8 ou 13,6mg compostos fenólicos kg^{-1} de produto) que foram armazenados refrigerados durante 35 dias. Os valores de dienos conjugados (CD) e peróxidos (PV) aumentaram ao longo do armazenamento, contudo, CD e PV diminuíram de maneira semelhante em todas as amostras aos 35 dias de análise ($P<0,05$). No entanto, o conteúdo de substâncias reativas ao ácido tiobarbitúrico (TBARS) não se modificou ao longo do armazenamento e não foi afetado pelo EJSE. Também houve aumento linear no conteúdo de proteínas carboniladas dos patês de pescado ao longo do armazenamento ($P<0,05$), sem efeito do EJSE na oxidação proteica. Os resultados mostram que, nas concentrações avaliadas, o extrato hidroetanólico de semente de* ***E. japonica*** *não foi capaz de inibir ou reduzir as oxidações lipídicas e proteicas em patês de pescado, mas seu conteúdo fenólico enfatiza para a necessidade de aprofundar as pesquisas com o resíduo desta fruta.*

Palavras-chave: ***Eriobotrya japonica, Rhamdia quelen,*** *oxidação lipídica, oxidação proteica, resíduos.*

INTRODUCTION

Lipid oxidation is the main cause of muscle food quality deterioration. It is a complex process in which unsaturated fatty acids react with molecular oxygen to generate a free radical chain mechanism, creating fatty acid hydroperoxides, which are the primary products of the oxidation and initiate the oxidative chain (GRAY, 1978). In addition, compounds resulting from lipid oxidation can modify proteins, inducing crosslinking and resulting in undesirable changes in food properties, including protein denaturation, loss of protein solubility, changes in the texture and functional properties of

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proteins and destruction of nutrient components (VERMA et al., 1995).

Fish are an important source of protein and contain high levels of n-3 polyunsaturated fatty acids (PUFA). The consumption of these fatty acids is involved in brain development of children and protection against cancer, heart disease, diabetes and mental illness (ZAMARIA, 2004). Despite the benefits of PUFAs, they are highly susceptible to oxidation due to their double bonds. The oxidation of lipids leads to discoloration, unpleasant odor and rancid taste (FRANKEL, 2005). In addition, lipid oxidation can accelerate the oxidation of proteins, which results in texture and technological changes in the product (XIONG, 2000).

Thus, the increase in the number of health-concerned consumers and the increased demand for fish products due to their health benefits have motivated the development of new seafood products with longer shelf lives using natural antioxidants. Waste products from fruits and vegetables offer an economical source of antioxidants that could replace synthetic molecules.

Loquat (***Eriobotrya japonica***) is an Asian fruit that is native to Japan and cultivated in various other tropical and subtropical countries (FEMENIA et al., 1998). Recent studies reveal that loquat seeds have the highest polyphenol content and show the most potent activity against lipid oxidation (KOBA et al., 2007). In addition, the methanol and ethanol extracts of loquat seed inhibited hepatic fibrosis in rats (NISHIOKA et al., 2002). Moreover, there has been no study on the antioxidant potential of extracts from loquat seeds against lipid oxidation in food systems, which would be an interesting use for this product. In this context, the assessment of the potential of loquat seeds to prevent the oxidation of food products has merit. The aim of this research was to investigate the effect of the addition of hydroethanolic extracts of seeds from ***E. japonica*** on lipid and protein oxidation of fish pates during refrigerated storage.

MATERIALS AND METHODS

Preparation of extracts

The loquat fruits (***E. japonica***) were collected from native plants in the Santa Maria region of Rio Grande do Sul state, Brazil. Ten grams of ground seeds (without the dark coat) were homogenized in approximately 70ml of 80% ethanol. The homogenate was then incubated at 50°C for 30min and then cooled to room temperature. The homogenate was centrifuged at 1083.1 *x g* for 3min and filtered into a volumetric flask. The extracts were re-suspended in 80% ethanol to the proper volume (100ml) and stored at -18°C until analysis. The total phenolic compound contents of three independent extracts were evaluated according to the method of SINGLETON & ROSSI (1965), with some modifications. The phenolic content of the extracts was 339.9±14.2mg gallic acid l^{-1} (equivalent to 339.9±14.2mg gallic acid $100g^{-1}$ seed).

Preparation of filleting waste pulp and fillets of fish

Fresh silver catfish (***Rhamdia quelen***) were obtained from a local market, washed in cold water with 5ppm chlorine, beheaded, gutted, and filleted. The ventral muscles and backbone (without fins) were treated as residues of the filleting process and used to prepare the filleting waste pulp as described by BOCHI et al. (2008). Fillets and filleting waste pulp were packed and kept at -18°C until use (approximately 7 days).

Manufacture of fish pate formulations

Five formulations were developed including a negative control containing distilled water, a positive control containing ascorbic acid (0.02g of ascorbic acid $100g^{-1}$ of product) and three formulations with increasing amounts of the hydroethanolic extract to yield 3.4, 6.8 or 13.6mg phenolic compounds kg^{-1} product (0.1, 0.2 and 0.4g of seed $100g^{-1}$ of product).

The control formulation had the following ingredients per kg of batter: 280g fish fillet, 260g fish pulp, 200ml cold water, 200ml soybean oil, 15g soybean isolate protein, 20g starch, 18g salt, 6g seasoning mix, 2g garlic, 1.5g sodium nitrite, 2g sodium tripolyphosphate, 2ml liquid smoke, and 2ml carmine coloring. Sodium erythorbate was not used in the formulations (except for the positive control containing ascorbic acid) so that the color and oxidation parameters were not influenced. In fish pates treated with the extract, the water was replaced with hydroethanolic extract, whereas the other ingredients were the same in controls and treated pates. All of the ingredients were minced in a cutter, transferred into artificial casings, cooked at 74-76°C, cooled with cold water (5°C), and dried and stored at 5±1°C for shelf life analysis. The lipid and protein oxidation were assessed after 1, 9, 15, 21 and 35 days of storage. Three independent replicates were prepared for each fish pate formulation for each day of evaluation and were analyzed in duplicate.

Lipid oxidation

The fat was extracted using chloroform and methanol as described by BLIGH & DYER (1959) to determine the fat content. The conjugated dienes (CD) and peroxide values (PV) were determined in the extracted fat by the BLIGH & DYER method (1959). Cyclohexane was used as the solvent, and the optical density was recorded at 233nm for the determination of CD (RECKNAGEL & GLENDE, 1984). All measurements were normalized to a uniform base of 1mg lipid/ml of cyclohexane. The PV was determined using a ferric thiocyanate method according to CHAPMAN & MACKAY (1949). A standard curve generated using ferric iron solutions was used to calculate the peroxide contents of the fat.

Thiobarbituric acid reactive substances (TBARS) were determined spectrophotometrically as described by BUEGE & AUST (1978). The samples were homogenized with 1.5% KCl, and the supernatant was incubated at 100°C for 15min in a medium containing trichloroacetic acid (TCA) and thiobarbituric acid. After incubation, butyl alcohol was used to extract the reaction product, which was measured at 535nm.

Protein oxidation

Protein oxidation was assessed based on the amount of protein carbonyl groups as described by LEVINE et al. (1994). The samples (1g) were homogenized in phosphate-buffered saline (PBS) (4ml), precipitated with TCA, and incubated with dinitrophenylhydrazine in the dark for 15min. Then, samples were precipitated with TCA, and the pellets were washed with 1:1 (v/v) ethanol/ethyl acetate. The pellet was re-dissolved in 2% sodium dodecyl sulfate (SDS), and the carbonyl content was determined by measuring the absorbance at 370nm. The results are expressed in nmoles carbonyl per mg of protein.

Statistical analysis

All analyses were run in duplicate, and the results were expressed as the mean ± standard deviation (SD). The lipid and protein oxidation were evaluated using two-way factorial ANOVA (5 formulations ×5 storage times). Post-hoc comparisons were made using Tukey's test ($P<0.05$). The statistical analysis was conducted using the Statistica software package version 6.0 (Copyright StatSoft, Inc. 1984-2001).

RESULTS AND DISCUSSION

The results of the analysis of the lipid and protein oxidation from fish pates containing loquat seed extract during refrigerated storage are shown in figure 1 and table 1. Lipids containing methylene-interrupted dienes or polyenes show a shift in the position of the double bond during the oxidation due to isomerization and the formation of CD. The formation of CD is reported to increase with the uptake of oxygen during the early stages of oxidation and to result in the formation of primary oxidation products, along with the peroxides (HALLIWELL & GUTTERIDGE, 1989).

Our results indicated an interaction between the treatment and storage time effects on the CD values of the fish pates (Figure 1A). The CD values increased during the storage period; however, this increase on the 9th and 15th days of storage was less pronounced for the EJ-0.4g% formulation, which had lower CD values compared to the W-control ($P<0.05$). On the 15th day of storage, all formulations had lower CD values than the W-control ($P<0.05$). The CD values of EJ-treated pates were similar to those found in the W-control at 1, 21 and 35 days of refrigerated storage.

The concentration of hydroperoxides formed during PUFA oxidation is used as a measure of the extent of oxidation. There was an interaction between the treatment and storage time effects on the peroxide value (PV) of fish pates (Figure 1B). The PV increased greatly over 21 days of storage only for the EJ-treated pates, which also had higher PV than the AA-control and W-control at 21 days of storage ($P<0.05$). However, the PV were similar among all samples on the 35th day of storage.

Thereby, the lower CD levels found in EJ-treated pates compared to W-control on the 15th day of analysis may be associated to the higher PV levels of EJ-treated pates on the 21st day of analysis (Figure 1), since conjugated dienes are converted to hydroperoxides in the lipid oxidation chain (GRAY, 1978). These results indicate that the progress of lipid oxidation was faster in the EJ-treated pates compared to the controls.

Secondary lipid oxidation was studied based on the TBARS value, which is an index of malonaldehyde (MDA) concentration. MDA is one of the main end products of lipid oxidation. The TBARS values showed no significant changes during storage time (0.04±0.04mg MDA kg^{-1} pate at day 1 and 0.04±0.03mg MDA kg^{-1} pate at 35 days of refrigerated storage). In addition, EJ extract did not change TBARS values of pates (data not shown). This finding contrasts with the study by KOBA et al. (2007), where the ethanol extract of loquat seed significantly lowered TBARS levels and showed an inhibitory effect on LDL oxidation *in vitro*. However, in our study, despite the moderate temperature

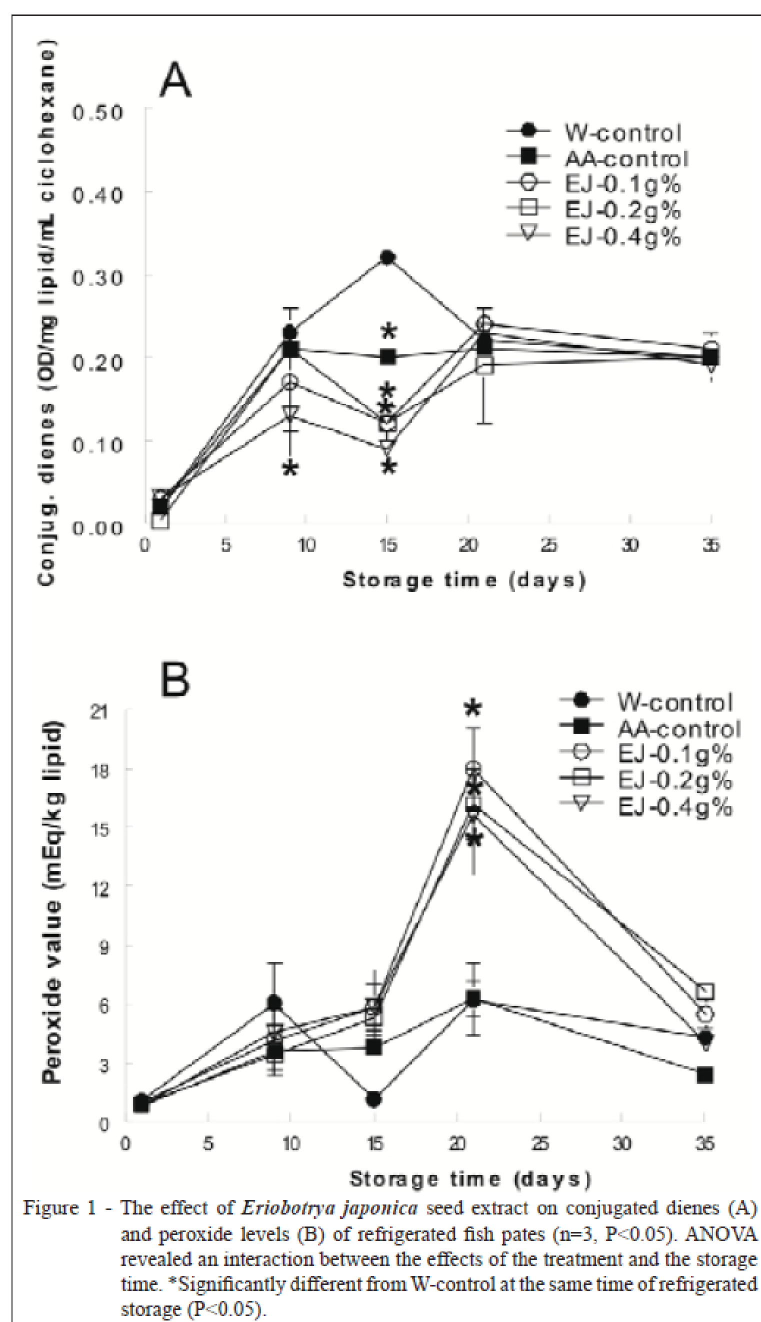

Figure 1 - The effect of ***Eriobotrya japonica*** seed extract on conjugated dienes (A) and peroxide levels (B) of refrigerated fish pates (n=3, $P<0.05$). ANOVA revealed an interaction between the effects of the treatment and the storage time. *Significantly different from W-control at the same time of refrigerated storage ($P<0.05$).

employed in the cooking of fish pates and its potential to initiate lipid oxidation, there was no increase in the TBARS value during the storage, which indicates that no appreciable amount of secondary lipid oxidation products was formed during the storage. Therefore, our TBARS results suggest that the storage time was not sufficiently long to produce secondary lipid oxidation products in the fish pates.

In contrast, there was a decrease in PV of EJ-treated pates, which indicates the degradation of these primary compounds into secondary ones. Because TBARS levels did not increase, we suggest that hydroperoxides may have been degraded into compounds that cannot be detected in the TBARS assay, such as ketones, alcohols, aldehydes (except MDA), hydrocarbons, volatile organic acids or epoxy compounds (SHAHIDI & ZHONG, 2010).

Therefore, the decrease in hydroperoxides with unchanged levels of TBARS in fish pates suggests that lipid oxidation is either in the initial

Table 1 - Effect of ***Eriobotrya japonica*** seed extract on the protein carbonyl content (nmol mg^{-1} protein) of refrigerated fish pates.

Storage time	Formulations					Average
	W-control	AA-control	EJ-0.1g%	EJ-0.2g%	EJ-0.4g%	
Day 1	3.6 ± 0.4	1.8 ± 0.8	3.7 ± 1.5	4.4 ± 0.3	4.0 ± 0.6	3.5 ± 1.1^{d}
Day 9	5.7 ± 1.0	4.8 ± 0.9	4.7 ± 0.8	4.8 ± 1.8	7.7 ± 0.9	5.6 ± 1.5^{c}
Day 15	5.6 ± 4.3	5.1 ± 1.4	8.9 ± 1.3	5.5 ± 0.3	7.9 ± 0.9	6.6 ± 2.3^{bc}
Day 21	7.8 ± 0.8	9.4 ± 2.6	8.1 ± 2.4	7.6 ± 1.0	7.2 ± 1.9	8.1 ± 1.8^{ab}
Day 35	8.8 ± 4.6	11.8 ± 2.7	8.6 ± 0.6	7.7 ± 0.6	7.5 ± 0.3	9.1 ± 2.7^{a}

Values for each formulation are mean ± standard deviation of 3 replicates, whereas the average values of all formulations are mean ± standard deviation of 15 replicates. ANOVA revealed a significant effect of storage time but no effect of treatment. Values with different superscript in a column differ significantly ($P<0.05$).

stage or yielded secondary oxidation products that cannot be detected in the TBARS tests.

The protein oxidation in the fish pates was evaluated based on the formation of protein carbonyl groups (Table 1). Oxidative reactions involving the side chains of amino acids can lead to the formation of carbonyl groups, which may result in an increased susceptibility to protein aggregation and a loss of solubility, leading to a loss of quality (STADTMAN, 1990). There was a linear increase in the protein carbonyl content of fish pates over the storage period, but no difference between the formulations (Table 1; $P<0.05$). Thus, no beneficial effect of EJ extract was observed on the protein oxidation.

Proteins can be modified by compounds resulting from the lipid oxidation, leading to amino acid changes and a decrease in protein functionality (LUND et al., 2011). Also, proteins can be modified independently of lipids and may be modified by reactive oxygen species (ROS), which include free radicals but also by non-radical species such as hydrogen peroxide and hydroperoxides. This was most likely what occurred in our study; we detected an increase in protein oxidation and no increase in secondary lipid oxidation products (TBARS) during storage. Thus, the high concentration of proteins and their proximity to hydroperoxides in this food product could have exposed proteins to oxidative reactions.

Furthermore, several studies show no effect of phenolic-containing plant extracts on the formation of protein carbonyl groups during heat treatment of meat products (ESTÉVEZ et al., 2006). This occurs because the protective effect is dependent on the composition of the raw material (meat as well as antioxidants), antioxidant concentration and production technology used (JONGBERG et al., 2013).

CONCLUSION

E. japonica seed extract, at the concentrations tested was not effective to prevent lipid or protein oxidation in fish pates. More studies are necessary to enhance the antioxidant potential of this product in fish pates and explore this waste vegetable source as a natural antioxidant.

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MANUSCRITO 4

OXIDATIVE PROPERTIES OF FISH MEATBALLS CONTAINING FILLETS TREATED WITH LOQUAT (*Eriobotrya japonica*) SEED EXTRACT

Artigo em fase final de revisão pelos autores para ser submetido à revista Ciência e Tecnologia de Alimentos/Food Science and Technology

(configurado conforme as normas da revista)

ORIGINAL ARTICLE

Oxidative properties of fish meatballs containing fillets treated with loquat (*Eriobotrya japonica*) seed extract

Propriedades oxidativas de almôndegas de pescado contendo filés tratados com extrato de semente de nêspera (*Eriobotrya japonica*)

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Abstract: Influenced by consumers, food industry is searching for natural antioxidant compounds that could replace the synthetic compounds used to delay lipid and protein oxidation in meat products. Plants are a rich source of natural antioxidants and loquat seed has been reported to have antioxidant properties in animal models but has been scarcely investigated in food science. The objective of the present study is to evaluate the effect of loquat (*Eriobotrya japonica;* EJ) seed extract on the oxidative, color, textural and sensory changes of cooked fish meatballs during frozen storage. Peroxide (PV) and thiobarbituric reactive substances (TBARS) values decreased over storage time because of higher values

at initial times attributed to cooking and mincing procedures. Ascorbic acid and 800 ppm presented higher PV than control formulation over storage time, increase of b* values and decrease of a* values over storage time ($P<0.05$). Protein carbonyl content increased over storage time, paralleled to the hardness of cooked fish meatballs. Sensory analysis was similar between all treatments at 1 day of analysis and the rancid odor had a slight increase over storage time, not so pronounced as oxidative reactions. Thus, the concentrations of EJ seed extracts applied were not efficient on retarding lipid or protein oxidation, neither to prevent color or textural changes due to oxidative reactions. Ascorbic acid and EJ 800 ppm were more prone to act as a pro-oxidant and critical procedures used for fish meatballs development may have contributed to non-antioxidative action by the EJ extracts in the formulations.

Keywords: protein carbonyl content, lipid oxidation, hardness, yellowness, antioxidant.

Introduction

Meat and meat products are indicated as one of the most important foods in the human diet and ready-to-eat products had a great increase on their demand (Mackenzie, 2011). Fish are a good alternative to meet the growing consumers´ demand for healthy products as they are good sources of protein and polyunsaturated fatty acids. However, muscle foods are susceptible to deteriorative processes and fish are more prone to lipid oxidation than the meat from other animals because of its high polyunsaturated fatty acids content (Sohn et al., 2005).

Lipid oxidation, which is related to the fat content of the product, changes texture properties and leads to discoloration and off-flavor development affecting sensory properties and food safety of meat products (Gray et al., 1996). In addition, meat processing and storage can also promote lipid oxidation as the cell disruption releases pro-oxidant solutes, which increase reactive oxygen species that can initiate oxidative reactions (Zaritsky, 2012).

Protein oxidation may also be associated to and accelerated by lipid oxidation in meat products. Protein oxidation comprises the direct oxidation of amino acid residues, formation

of carbonyl compounds, cleavage of peptide bonds and formation of intra and/or intermolecular cross-linked derivatives (Lund et al., 2011; Stadtman and Levine, 2003). Frozen storage particularly promotes the development of protein carbonyl compounds which reduces the water-holding capacity, changes the color and texture of meat proteins, and results in the loss of meat quality (Estévez et al., 2011; Utrera et al., 2012).

Aiming to reduce the impact of lipid and protein oxidation and their deleterious consequences on meat and meat products, a growing interest is perceived in food industry in development of natural powerful ingredients that can act preventing these reactions (Falowo et al., 2014). As plant materials are rich in antioxidant compounds such as polyphenols, the plant industry by-products may be great and cheap sources of these compounds (Soong and Barlow, 2004). Particularly, most fruit seeds are not commercially utilized and are discarded as waste.

Loquat (*Eriobotrya japonica* Lindl.) is a native fruit from China that has been cultivated in Mediterranean area, India, South Africa, South America, and California (Vaughan and Geissler, 1997). Loquat had been used as fresh fruit, but also processed for jams and jellies, discarding leaves and seeds. Interestingly, loquat seed extracts have been reported to present important pharmacological effects on suppression of diseases and lipids in animal models as inhibition of oxidative stress on renal disorders (Hamada et al., 2004) and fibrosis in rats (Nishioka et al., 2002), having hypoglycemic effects (Tanaka et al., 2008; Kim et al., 2009) and suppressing LDL-cholesterol oxidation *in vitro* (Koba et al., 2007) what clearly indicates an important biological activity for the natural compounds found in loquat seeds.

Despite these findings in pharmaceutical area, the studies on the use of loquat seeds in food science are still scarce. Also, a study involving hydroethanolic loquat seed extract was not effective on preventing lipid or protein oxidation in fish pates during refrigerated storage (Piccolo et al., 2014).

In this context, the objective of the present study was to evaluate the effect of loquat seed extract on oxidative (lipid and protein), chemical, color, textural and sensory changes of cooked fish meatballs during frozen storage.

Material and methods

Preparation of loquat seed extract

Loquat (*E. japonica;* EJ) fruits were collected from native plants in Santa Maria city at Rio Grande do Sul state, Brazil (29°41'03"S; 53°48'25"W) during winter and spring of 2011. After hand-harvest fruits were transferred to the laboratory, cleaned, sorted and seeds were handled separated from pulp. The dark coat that covers the seeds was removed and seeds were grounded in a micro-mill (MA-630/I, Marconi, Piracicaba, SP, Brazil), dried at 60°C in an air-assisted oven for 2 h, cooled to room temperature and frozen in dark vessels (-18°C) until use.

Loquat seed extract was obtained following Koba et al. (2007) procedure with modifications. Seeds (20 g) were homogenized with 70% acetone (100 mL), heated at 50°C for 10 min and sonicated (MaxiClean 1600, Unique, Indaiatuba, SP, Brazil) for 1 h (40 kHz of ultrasonic frequency and 135 Watts RMS of ultrasonic potency). The extract was then cooled at room temperature, centrifuged at 1083.1 *x g* during 3 min and the supernatant was filtered and rotaevaporated at 40°C. The concentrated extract was kept at -18°C until use (200 mg *E. japonica* seed/mL extract).

Total phenolic content was determined at 725 nm using Folin-Ciocalteu method (Singleton and Rossi, 1965). Gallic acid was used as standard for the calibration curve and the amount of phenolic compounds was expressed as mg gallic acid/mL of extract. The phenolic content of the extract was 0.81 ± 0.09 mg gallic acid/mL (405.4 ± 43.9 mg of gallic acid/100 g seed).

Preparation of fish meatball formulations

Silver catfish (*Rhamdia quelen*) were acquired from a fish farm at Santa Maria, Rio Grande do Sul, Brazil. Fish were transported to the laboratory on ice, the fillets without skin were obtained in the laboratory and immersed (10 g fillet/2 mL solution) for 10 min (5 min on each side) in concentrated EJ seed extracts previously diluted in distilled water until 400, 600

or 800 ppm (mg of phenolic compounds/L of extract), ascorbic acid (200 ppm, positive control) or distilled water (control). Fillets were packed in polyethylene trays, wrapped with ziplock plastic bags and stored at -18 ± 1°C for 12h.

The fish ventral muscles and backbone without fins were used to obtain a fish pulp as described by Bochi et al. (2008). Briefly, these fish filleting residues were passed once through a meat grinder fitted with a 5 mm plate and once through a 2 mm plate to obtain a fish pulp without visible fishbone, which was packed in plastic jars with lids and kept at -18 ± 1°C for 12h.

Fish fillets were thawed and minced in a meat grinder provided with a 5 mm disk plate until a homogeneous mass was obtained. The meatballs were prepared by adding ingredients in the following order: grounded fish (53%), fish pulp (22%), salt (1.2%), dehydrated parsley (0.8%), powdered garlic (0.8%), powdered onion (0.8%), oat flour (5%), bread flour (5%), non-hydrated texturized soybean protein (2%), annatto (0.2%) and powder egg (2.6%, previously hydrated in 6.5% water). Only fish fillets were treated prior to batches development and Three batches were prepared for each treatment: control (fish fillets immersed in distilled water), ascorbic acid (fish fillets immersed in ascorbic acid solution), 400, 600 and 800 ppm EJ extracts (fish fillets immersed in concentrated EJ extracts previously diluted in distilled water until respective concentration). Each batch was manually shaped into meatballs weighing 17 g each that were pre-cooked in an oven at 200°C for 25 min. After cooling at room temperature, the meatballs were packed in oxygen-permeable polyethylene bags and stored at −18°C for 150 days in the dark.

Two fish meatballs were taken from each formulation batch at 1, 30, 90 and 150 days of frozen storage (n=3 per formulation). One portion of the meatballs was used to analyze the proximate composition, and a second portion was used to evaluate the objective color, lipid oxidation, protein oxidation and texture profile. In addition to these assays, a third portion was prepared for assessing sensory properties of cooked meatballs at 1 and 150 days of storage.

Proximate composition

Proximate composition analysis was performed at 0 months of storage. Moisture content was determined by oven-drying of 5 g of cooked meatball at 105°C until a constant weight was obtained. Ash content was determined by placing samples into a muffle furnace at 550°C for 24 h and then weighing the remaining material. Crude protein (N x 6.25) was determined by the microKjeldahl procedure (AOAC, 2000). Fat content was determined using chloroform and methanol as described by Bligh and Dyer (1959). Carbohydrate content was estimated by difference.

Lipid oxidation

Conjugated dienes (CD) and peroxide value (PV) were determined in the fat, extracted by the Bligh and Dyer method (1959). In CD analysis, cyclohexane was used as the solvent and the optical density was recorded at 233 nm (Recknagel and Glende, 1984) and the results were expressed as optical density (OD)/mg lipid/mL of cyclohexane. PV was determined as described by Chapman and Mackay (1949), using a ferric thiocyanate method. A standard curve generated using ferric iron solutions was used to calculate the peroxide contents of the fat and the results were expressed as mEq peroxide/g fat.

Thiobarbituric acid-reactive substances (TBARS) were assessed using the spectrophotometric method described by Buege and Aust (1978). The results were expressed as mg of malonaldehyde (MDA)/kg sample.

Protein oxidation

Protein oxidation was determined as total protein carbonyl content as described by Levine et al. (1990) with modifications. Samples (1 g) were minced and homogenized in phosphate-buffered saline (4 mL), using an ultraturrax homogenizer for 30 s. Proteins (300 µL supernatant) were precipitated with 40% TCA (250 µL) and centrifuged for 5 min at 2122.8 *x g*. The pellet was incubated with dinitrophenylhydrazine (DNPH) in the dark for 15 min at room temperature. Then, pellets were precipitated with 40% TCA (250 µL) and were

washed two times with 1:1 (v/v) ethanol/ethyl acetate (500 µL). The pellet was re-dissolved in 2% sodium dodecyl sulfate (1.5 mL) at 37°C, and the carbonyl content was determined by measuring the absorbance at 370 nm. Total protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard. The results are expressed in nmol of carbonyl/mg of protein.

Color measurements

Color of cooked fish meatballs was assessed using CR-300 Minolta Chromameter (Minolta Ltd., Osaka, Japan) equipped with a standard illuminant D65, 2° observation angle and calibrated with a white ceramic plate (number 15233011). The cooked meatballs were homogenized in a blender and placed on a Petry dish, which was placed over a white surface. three measurements were recorded directly on the samples after turning 90° from the previous reading for evaluating lightness (L*), redness (a*) and yellowness (b*).

Texture profile analysis (TPA)

Texture profile analysis (TPA) was performed at room temperature using a TA.XT Plus Texture Analyzer (Stable Micro Systems, Surrey, UK). Cooked fish meatballs were thawed at room temperature and cut into 40 mm diameter x 10 mm thickness slices. Then, four cylindrical samples were taken from each treatment and subjected to a two-cycle compression test (n=3). The samples were compressed to 50% of their original height with a cylindrical probe of 45 mm, a cross-head speed of 1 mm/s and an interval of 5s between compressions. Texture parameters were determined following descriptions by Bourne (1978) and the SMS manual (Stable Micro Systems, Surrey, UK) and the results were expressed as hardness (N).

Sensory analysis

This study was approved by the Ethics Committee (CAAE 07241112.4.0000.5346) of the Federal University of Santa Maria and all panelists provided their consent prior to inclusion in the study.

An expert panel (n=8 to 7) composed of University students evaluated cooked fish meatballs at 1 and 150 days of storage. The panel was selected based on a questionnaire to determine their interest in participating and possible exclusion factors as smoking and allergies. The panelists were trained by color, odor, flavor and texture of fish meatballs. The trainings were conducted comparing these characteristics in fresh fish and oxidized ones. Panelists were also trained to the odor and taste perception using crude and diluted extract.

Immediately before analysis, meatball samples (17g) were wrapped in aluminum foil and heated in oven at 200°C/25 min and presented individually in a randomized order. Crackers and drinking water were provided for rinse the mouth between samples. Panelists asked to evaluate the color, odor, flavor, texture and overall acceptability of meatballs at 1 day of storage using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Panelists were also asked to evaluate the rancid odor of meatballs using a structured 10-points numeric scale at end points (1 = no rancid odor; 10 = very much rancid odor) at 1 and 150 days of storage.

Statistical analysis

Results were processed using Statistica 7.0 (Copyright® Stat Soft, Inc 1984–2001). Data obtained for proximate composition and sensory analysis at 1 day of storage were evaluated using one-way analysis of variance (ANOVA). Lipid and protein oxidation, texture profile and color measurements were evaluated using two-way factorial ANOVA (5 formulations x 4 storage times). Data of both sensory analyses were normalized to take account of individual scale-use differences. Post hoc comparisons were made by the Tukey´s test and correlation analysis was performed with the means of the data in the different storage times, at the level of 5% of probability. All measurements were made at least in duplicate and the results are means of three independent experiments.

Results and discussion

Proximate composition

Utilization of fish fillets treated with EJ seed extracts did not alter the ash, protein or carbohydrate content of meatballs formulations (Table 1; $P>0.05$). Fat content of ascorbic acid, EJ 400 and EJ 800 ppm was significantly higher than the control formulation (Table 1; $P<0.05$). Only the formulation containing EJ 600 ppm obtained similar fat content to control formulation, probably due to its lower moisture content compared to control ($P<0.05$). However, our results suggest that these changes in fat and moisture content are not arising from the extract incorporation, but were likely due to the fish, which had no weight standard and, consequently, yielded fillets with no fat standard.

[Insert Table 1]

Lipid oxidation

CD values remained quite stable over storage time ranging from 0.84 ± 0.02 at day 1 vs. 0.79 ± 0.01 OD/ mg lipid/ mL cyclohexane at day 150 ($P<0.05$) (data not shown). The low levels of CD during all storage time can be explained by the fact that they are primary compounds of lipid oxidation and, due to the high temperature used to cook fish meatballs, they may have been early degraded into hydroperoxides.

ANOVA revealed a significant formulation x storage time interaction on PV and TBARS values of cooked fish meatballs during the frozen storage (Fig. 1 and 2; $P<0.05$). PV decreased over storage time. Ascorbic acid formulation showed higher PV than control formulation at 30 and 90 days ($P<0.05$). At the end point, EJ 800 ppm showed higher PV than control formulation ($P<0.05$). Thus, ascorbic acid and EJ 800 ppm were not able to slow down the production of primary lipid oxidation compounds on fish meatballs. However, EJ 400 and EJ 600 ppm formulations had PV similar to the control formulation during all storage time.

Higher levels of TBARS were observed at day 1 and the values decreased over frozen storage (Fig. 2; *P*<0.05). All formulations containing EJ extract had lower TBARS values than the ascorbic acid formulation at day 1 (*P*<0.05). In the remaining days, there was no difference in the TBARS levels among formulations (*P*<0.05).

[Insert Fig. 1 and 2]

The high initial TBARS values in cooked fish meatballs can be explained by heat processing. Heating can induce changes in meat product components involving thermal inactivation of antioxidant enzymes (Lee et al., 1996) and also disruption of cellular compartmentalization followed by exposure of membrane lipids to a pro-oxidative environment, leading to the release of free iron from myoglobin which may catalyze lipid oxidation (Kristensen and Purslow, 2001).

Ascorbic acid has antioxidant properties preserving meat color and acting as an antioxidant, however depending on the concentration, the tocopherol content and the presence of metal ions, it can also act a pro-oxidant (Schaefer et al., 1995) by catalysing the formation of hydroxyl radicals in the presence of free iron. Thus, higher PV and TBARS values found in ascorbic acid formulation indicate that, in the present study, ascorbic acid acted as a pro-oxidant.

Natural seed extracts have been successfully employed in meat and fish products exhibiting positive effects on lipid oxidation as pomegranate seed power (Devatkal et al., 2010), peach seed extract (Yogesh and Ali, 2014); avocado seeds (Rodríguez-Carpena et al., 2011) and grape seeds (Carpenter et al., 2007; Yerlikaya and Gokoglu, 2010). Nevertheless, the capacity to delay lipid oxidation depends on the phenolic profile of the extract, thermal processing of food, food matrix and oxidative stability of its constituents (Jongberg et al., 2013).

Protein oxidation

Increased protein carbonylation indicate that the muscle proteins of meatballs are susceptible to oxidative reactions causing increase in carbonyl compounds. ANOVA revealed significant main effect of storage time, but no effect of treatment on protein carbonyl levels of cooked fish meatballs (Fig. 3; $P<0.05$). Protein carbonyl content had a marked decreased up to 30 days of storage, followed by a slight increase after 90 days and 150 days of storage ($P<0.05$).

[Insert Fig. 3]

The high protein carbonyl content at the start of storage was probably due to cooking and mincing procedures applied to cooked fish meatballs. Tissue disruption releases iron and incorporates oxygen to the system, whereas the heating degrades myoglobin and also releases iron (Ganhão et al., 2010), which plays an important role in protein oxidation (Kristensen and Purslow, 2001).

The slight decrease of protein carbonyl that occurred between days 1 and 30 of storage can be related to changing the route of production of carbonyl compounds, favored by the release of iron in the early stages, followed by oxidation-reduction reaction of iron and initiation of production of carbonyl compounds from the degradation of lysine, proline and arginine, the main route of protein carbonylation (Stadtman and Levine, 2003).

Also, lipid oxidation products can modify proteins by inducing cross-linking resulting in loss of nutritional properties and decrease in functionality of proteins (Gray et al., 1996). In this context, combination of non-protein carbonyl compound (aldehyde as MDA) to proteins through covalent bindings can lead to decrease in TBARS and increasing protein carbonylation (Estévez, 2011). However, the increase in protein carbonylation over storage time (more specifically at 90 days of storage) seems not to be related to the TBARS value, once TBARS content had decreased among 1 and 30 days of storage and remained stable from 30 days.

Thus, EJ extracts were not able to inhibit protein oxidation and the observed increase in protein carbonylation in cooked fish meatballs can be mostly attributed to the pro-oxidant effect of iron released during processing and also degradation of protein residues.

Texture profile

ANOVA revealed a main effect of storage time, but no effect of treatments, on hardness of cooked fish meatballs (Fig. 4; $P<0.05$). Hardness increased from 30 days of storage time ($P<0.05$). Hardness had a slight decrease at day 30 compared to initial values, and the significant increase was only between at 90 and 150 days compared to 30 days of storage ($P<0.05$). At the end point, the hardness of all formulations was similar among each other.

[Insert Fig. 4]

Protein oxidation seems to affect functional properties of proteins by changing its structure and integrity (Xiong, 2000) influencing water holding capacity and texture properties (Davies, 2005). Protein oxidation influence in meat tenderness is still unclear but the tenderness of meat can be related to protein oxidation inducing protein cross-linking via disulfide-bonding what causes strengthening in myofibrilar structure forming a tough muscle (Lund et al., 2007ab). This is corroborated by the moderated positive correlation found between protein carbonyl content and hardness ($r=0.441$; $P=0.01$), thus, hardness increase in cooked fish meatballs can be attributed to protein carbonylation.

Color

Color has been pointed as one of the most important indicators of the loss of quality in meat products. ANOVA revealed significant treatment x storage time interaction on lightness and yellowness of cooked fish meatballs stored frozen (Fig. 5 and 6B; $P<0.05$). Lightness increased over storage for all treatments ($P<0.05$). The formulation containing

fillets treated with 400 ppm EJ had lower L* values than control formulation at 90 days (*P*<0.05). At the end point, all treatments had similar values among each other. A similar increase in L* values has been observed for during the frozen storage of dourado fillets (Veeck et al., 2013).

ANOVA revealed a main effect of treatment and storage time on the redness of cooked fish meatballs (Fig. 6A; *P*<0.05). The a* values presented negative values during all storage and had a significant decrease at 30 and 90 days compared to day 1 (*P*<0.05). Ascorbic acid, EJ 600 ppm and EJ 800 ppm formulations had significantly lower a* values than the control formulation (*P*<0.05). Ascorbic acid formulation showed the highest initial lipid oxidation (TBARS values), which may have contributed to the lower redness over storage time.

Bhattacharya et al. (1994) found that the processing temperature and time had a negative influence on color, decreasing redness in muscle tissue of Pacific chum salmon (*Onchorynchus keta*). Thus, cooking process may have contributed to redness decrease in fish meatballs, being more expressive on ascorbic acid, EJ 600 ppm and EJ 800 ppm formulations.

[Insert Fig. 5 and 6]

ANOVA revealed a significant treatment x storage time interaction on yellowness (P<0.05). Yellowness increased up to 30 days of storage and then decreased to initial values at 90 days (Fig. 6A; *P*<0.05). Finally, slight increased b* was obtained at 150 days compared to initial values (*P*<0.05). Formulations containing ascorbic acid and EJ 800 ppm showed significantly higher b* values than control formulation during all the storage time, except for EJ 800 ppm at day 1 (*P*<0.05).

The aldehydes produced from lipid oxidation can modify some amino acid residues, such as lysine producing pyrroles that, by polymerisation reaction, are responsible for the color changes (Zamora et al., 1999). Thus, the decay of TBARS values over storage time

and a moderated negative correlation found between TBARS and lightness (r=-0.526; P=0.001) can denote that these aldehydes promoted color modifications in fish meatball formulations. However, once acetone is widely used for carotenoid extraction (Rodríguez-Bernaldo de Quirós and Costa, 2006) and EJ extract possessed a slight yellow color, it is also possible that the presence of carotenoids may have influenced the yellowness increase in EJ 800 ppm. The color perceveid by the sensory analysis is given in Table 2.

[Insert Table 2]

Sensory analysis

The incorporation of EJ seed extracts on the fish fillets used in the formulation did not change the sensory scores for color, odor, flavor, texture or the overall acceptability of fish meatballs at 1 day of storage (Table 2; $P>0.05$).

Lipid oxidation results in off-flavor, rancid odor, discoloration and other sensory detrimental consequences (Falowo et al., 2014). Thus, sensory evaluation was carried out over storage time to assess the development of rancid odor in fish meatballs. As expected, taking into consideration that lipid and protein oxidation occurred in meatballs, ANOVA revealed a main effect of storage time, but no effect of treatments, on the rancid odor scores of frozen meatballs (Table 3; $P<0.05$). Rancid odor had a significant increase over storage time, but values were similar among treatments ($P<0.05$). This increase was reasonable in proportion to protein oxidation in meatballs. Although some formulation containing EJ extract showed a pro-oxidative effect on lipid oxidation, it was not so pronounced on rancid odor development of fish meatballs.

Conclusion

High PV levels, decreasing a* values and increasing b* values on ascorbic acid and EJ 800 ppm formulations compared to other treatments over storage time indicated a

propensity to act as a pro-oxidant of these formulations. Protein carbonyl increased over storage time in all treatments as well as hardness. Also, sensory analysis was not influenced by EJ extracts and rancid odor increased over storage time, however, it was remarkably lower than oxidative reactions.

Thus, the concentrations of EJ seed extracts applied were not efficient on retarding lipid or protein oxidation, neither to prevent color and textural changes due to oxidative reactions. Critical procedures used for fish meatballs development may have contributed to non-antioxidative action by the EJ extracts in the formulations.

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Table 1. Proximate composition (g/100 g) of cooked fish meatballs containing loquat seed extract

Formulations	Moisture	Ash	Protein	Fat	Carbohydrates
Control	61.8 ± 0.1^{a}	2.6 ± 0.0	15.0 ± 0.8	9.7 ± 0.0^{a}	11.4 ± 0.0
AA	61.6 ± 0.2^{ab}	2.7 ± 0.0	14.0 ±0.5	8.1 ±0.1^{c}	14.0 ± 0.7
EJ 400 ppm	61.9 ± 0.1^{a}	2.7 ± 0.1	14.3 ± 0.4	8.7 ± 0.1^{b}	12.4 ± 0.6
EJ 600 ppm	60.8 ± 0.4^{b}	2.8 ± 0.0	14.9 ± 0.6	9.6 ± 0.1^{a}	11.9 ± 0.6
EJ 800 ppm	61.1 ± 0.1^{ab}	2.6 ± 0.1	15.4 ± 0.5	8.9 ± 0.0^{b}	12.1 ± 0.6

Results are mean ± standard error (n=3). Control: containing fish fillets treated with distilled water; AA: containing fish fillets treated with ascorbic acid (200 ppm); EJ 400 ppm: containing fillets treated with *E. japonica* extracts that had 400 ppm of phenolics/ L of extract; EJ 600 ppm: containing fillets treated with *E. japonica* extracts that had 600 ppm of phenolics/ L of extract; EJ 800 ppm: containing fillets treated with *E. japonica* extracts that had 800 ppm of phenolics/ L of extract.

$^{a\text{-}c}$Means that have no common superscript letter within the same column are significantly different (P<0.05).

Table 2. Sensory analysis of cooked fish meatballs containing loquat seed extract at day 1.

Formulations	Color*	Odor*	Flavor*	Texture*	Overall acceptability*
Control	6.9 ± 0.5	7.9 ± 0.4	8.0 ± 0.2	8.0 ± 0.6	7.8 ± 0.3
AA	7.1 ± 0.5	7.9 ±0.2	7.5 ± 0.5	7.7 ± 0.6	7.5 ± 0.5
EJ 400 ppm	7.4 ± 0.5	7.6 ± 0.3	7.9 ± 0.3	8.1 ± 0.2	8.0 ± 0.2
EJ 600 ppm	7.3 ± 0.5	7.8 ± 0.2	7.8 ± 0.2	8.0 ± 0.3	7.9 ± 0.2
EJ 800 ppm	7.0 ± 0.6	7.9 ± 0.3	7.5 ± 0.6	8.1 ± 0.2	7.6 ± 0.5

*Evaluated using a hedonic 9 points-scale with verbal descriptors (1=dislike extremely; 9=like extremely). Results are mean ± standard error (n=8). No significant diferences were observed among formulations. Control: containing fish fillets treated with distilled water; AA: containing fish fillets treated with ascorbic acid (200 ppm); EJ 400 ppm: containing fillets treated with *E. japonica* extracts that had 400 ppm of phenolics/ L of extract; EJ 600 ppm: containing fillets treated with *E. japonica* extracts that had 600 ppm of phenolics/ L of extract; EJ 800 ppm: containing fillets treated with *E. japonica* extracts that had 800 ppm of phenolics/ L of extract.

Table 3. Sensory analysis of rancid odor of cooked fish meatballs containing loquat seed extract at 1 and 150 days of frozen storage.

Formulations	Rancid odor*	
	Day 1	Day 150
Control	0.1 ± 0.1	1.7 ± 0.6
AA	0.5 ± 0.3	0.9 ± 0.4
EJ 400 ppm	0.5 ± 0.4	0.9 ± 0.3
EJ 600 ppm	0.3 ± 0.2	1.7 ± 0.8
EJ 800 ppm	0.0 ± 0.0	1.1 ± 0.6
Average	0.3 ± 0.1[a]	1.3 ± 0.2[b]

*Evaluated using a structured 10-points numeric scale at end points (0 = no rancid odor, 10 = very rancid odor). Results are mean ± standard error (n=7).

[a-b]Means with different superscript letters within the same row are significantly different (P<0.05).

Control: containing fish fillets treated with distilled water; AA: containing fish fillets treated with ascorbic acid (200 ppm); EJ 400 ppm: containing fillets treated with *E. japonica* extracts that had 400 ppm of phenolics/ L of extract; EJ 600 ppm: containing fillets treated with *E. japonica* extracts that had 600 ppm of phenolics/ L of extract; EJ 800 ppm: containing fillets treated with *E. japonica* extracts that had 800 ppm of phenolics/ L of extract.

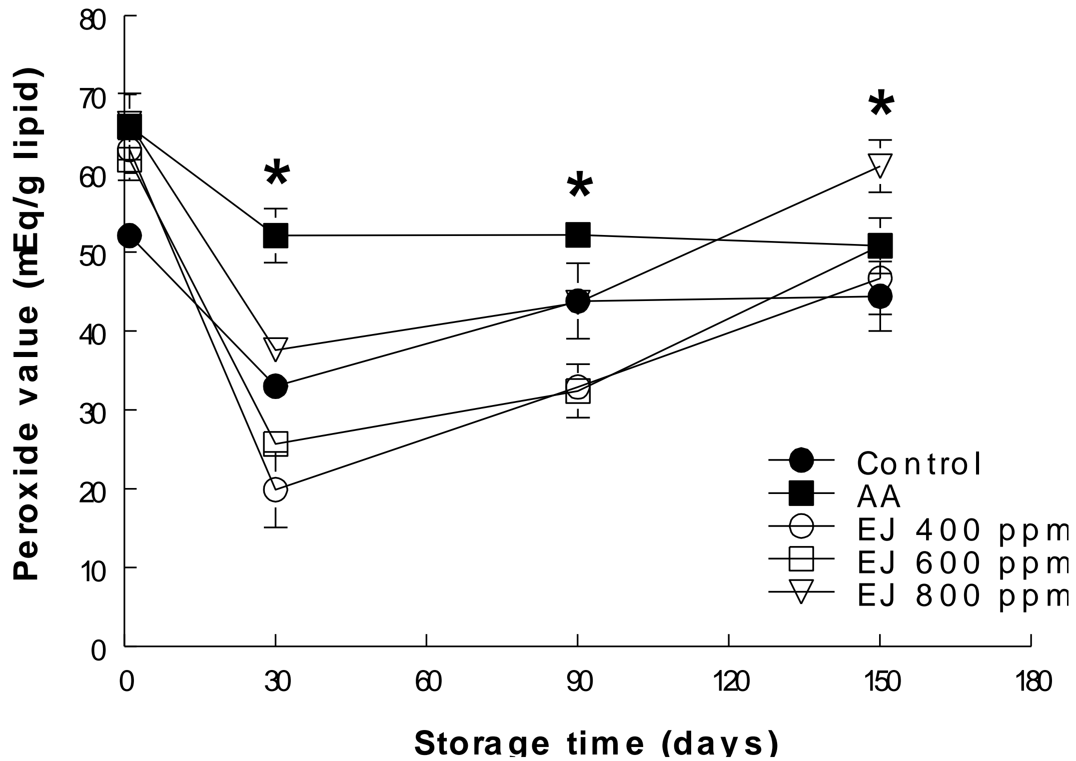

Fig 1. Effect of loquat seed extract on peroxide value (PV) of cooked fish meatballs during frozen storage. Results are mean ± standard error of three replicates. ANOVA revealed a significant formulation x storage time interaction. *Significantly different from control at the same day (P<0.05). Control: containing fish fillets treated with distilled water; AA: containing fish fillets treated with ascorbic acid (200 ppm); EJ 400 ppm: containing fillets treated with *E. japonica* extracts that had 400 ppm of phenolics/ L of extract; EJ 600 ppm: containing fillets treated with *E. japonica* extracts that had 600 ppm of phenolics/ L of extract; EJ 800 ppm: containing fillets treated with *E. japonica* extracts that had 800 ppm of phenolics/ L of extract.

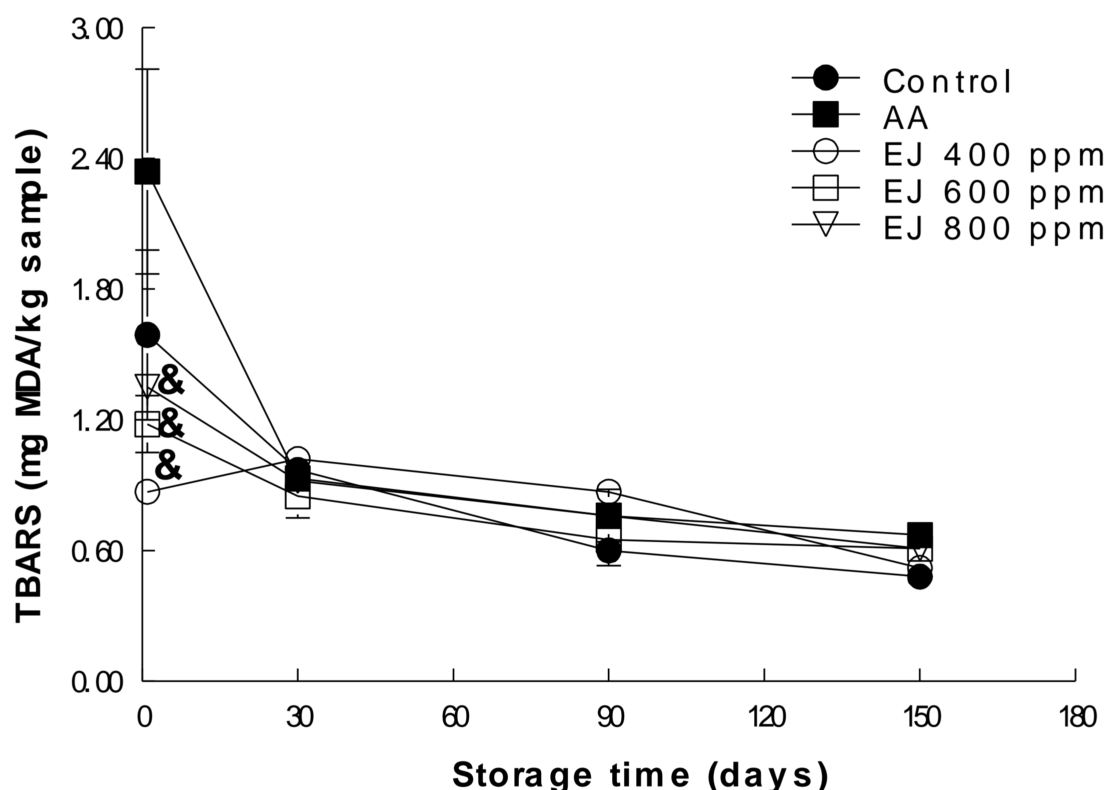

Fig 2. Effect of loquat seed extract on TBARS of cooked fish meatballs during frozen storage. Results are mean ± standard error of three replicates. ANOVA revealed a significant formulation x storage time interaction. [&]Significantly different from ascorbic acid at the same day (P<0.05). Control: containing fish fillets treated with distilled water; AA: containing fish fillets treated with ascorbic acid (200 ppm); EJ 400 ppm: containing fillets treated with *E. japonica* extracts that had 400 ppm of phenolics/ L of extract; EJ 600 ppm: containing fillets treated with *E. japonica* extracts that had 600 ppm of phenolics/ L of extract; EJ 800 ppm: containing fillets treated with *E. japonica* extracts that had 800 ppm of phenolics/ L of extract.

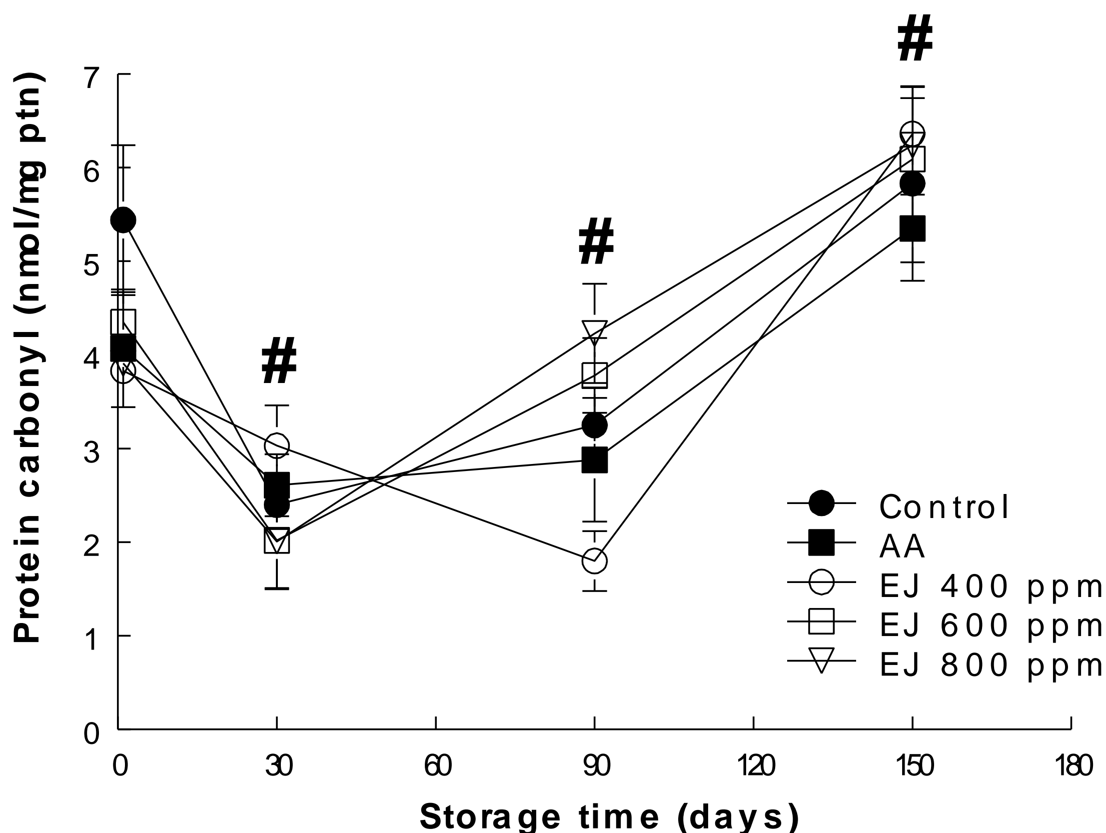

Fig 3. Effect of loquat seed extract on protein carbonyl content of cooked fish meatballs during frozen storage. Results are mean ± standard error of three replicates. ANOVA revealed a significant main effect of storage time, but no effect of treatments ($P<0.05$). Control: containing fish fillets treated with distilled water; AA: containing fish fillets treated with ascorbic acid (200 ppm); EJ 400 ppm: containing fillets treated with *E. japonica* extracts that had 400 ppm of phenolics/ L of extract; EJ 600 ppm: containing fillets treated with *E. japonica* extracts that had 600 ppm of phenolics/ L of extract; EJ 800 ppm: containing fillets treated with *E. japonica* extracts that had 800 ppm of phenolics/ L of extract. [#]Significantly different from 0 days.

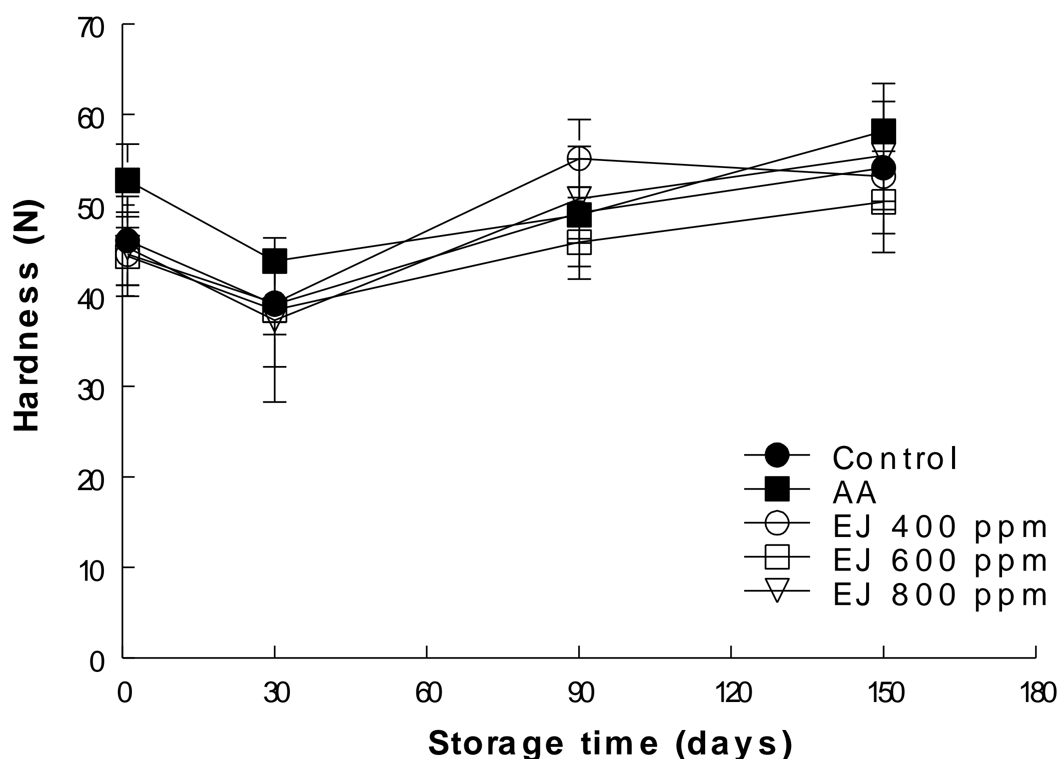

Fig 4. Effect of loquat seed extract on the hardness of cooked fish meatballs during frozen storage. Results are mean ± standard error of three replicates. ANOVA revealed a significant main effect of storage time, but no effect of treatments ($P<0.05$). Control: containing fish fillets treated with distilled water; AA: containing fish fillets treated with ascorbic acid (200 ppm); EJ 400 ppm: containing fillets treated with *E. japonica* extracts that had 400 ppm of phenolics/ L of extract; EJ 600 ppm: containing fillets treated with *E. japonica* extracts that had 600 ppm of phenolics/ L of extract; EJ 800 ppm: containing fillets treated with *E. japonica* extracts that had 800 ppm of phenolics/ L of extract.

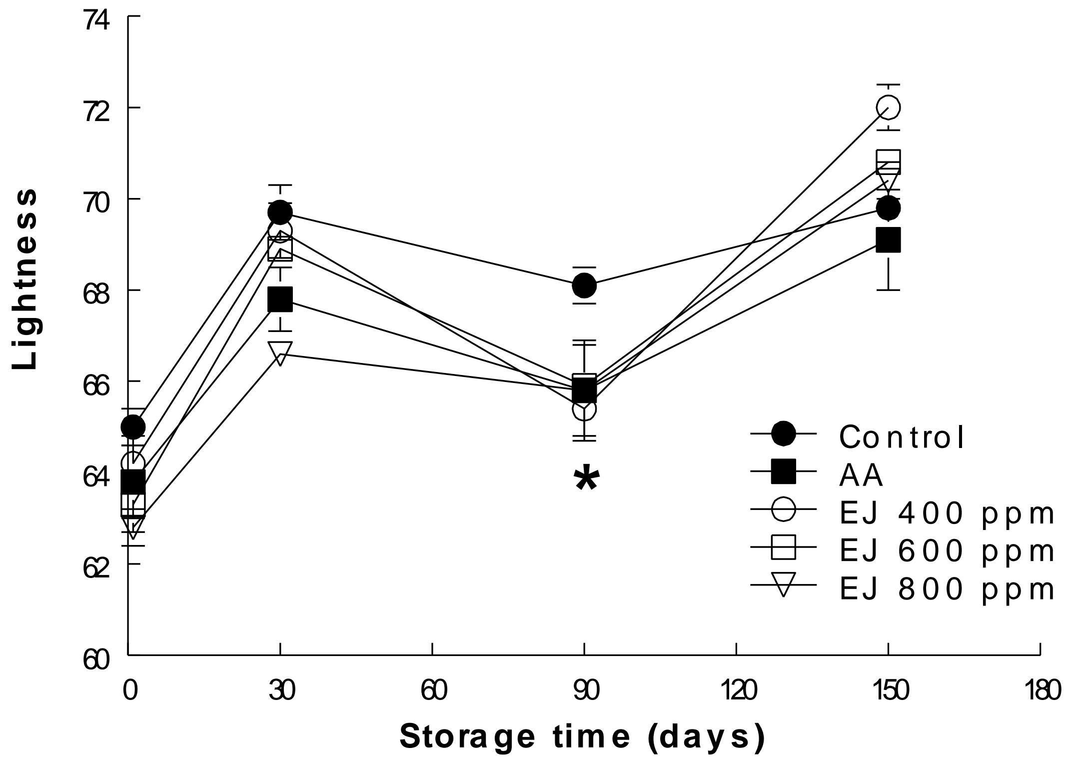

Fig 5. Effect of loquat seed extract on lightness of cooked fish meatballs during frozen storage. Results are mean ± standard error of three replicates. ANOVA revealed a significant formulation x storage time interaction. *Significantly different from control at the same day ($P<0.05$). Control: containing fish fillets treated with distilled water; AA: containing fish fillets treated with ascorbic acid (200 ppm); EJ 400 ppm: containing fillets treated with *E. japonica* extracts that had 400 ppm of phenolics/ L of extract; EJ 600 ppm: containing fillets treated with *E. japonica* extracts that had 600 ppm of phenolics/ L of extract; EJ 800 ppm: containing fillets treated with *E. japonica* extracts that had 800 ppm of phenolics/ L of extract.

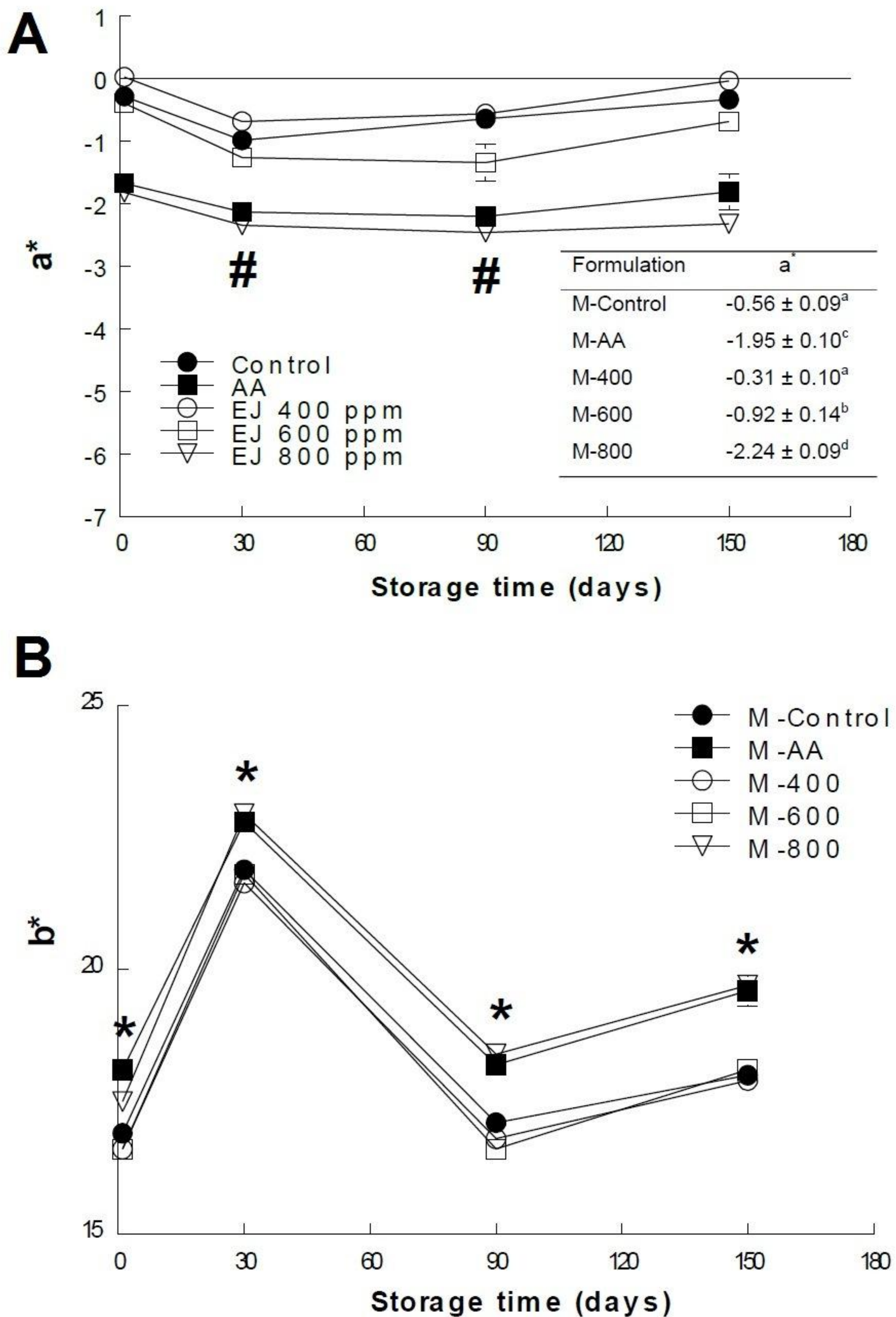

Fig 6. Effect of loquat seed extract on redness (A) and yellowness (B) of cooked fish meatballs during frozen storage. Results are mean ± standard error of three replicates.

ANOVA revealed a significant main effect of storage time and treatments on redness (panel A). ANOVA revealed a significant formulation x storage time interaction on yellowness (panel B). [*]Significantly different from control at the same day (P<0.05). [a-c]Means that have no common superscript letter within the same column are significantly different (P<0.05). Control: containing fish fillets treated with distilled water; AA: containing fish fillets treated with ascorbic acid (200 ppm); EJ 400 ppm: containing fillets treated with *E. japonica* extracts that had 400 ppm of phenolics/ L of extract; EJ 600 ppm: containing fillets treated with *E. japonica* extracts that had 600 ppm of phenolics/ L of extract; EJ 800 ppm: containing fillets treated with *E. japonica* extracts that had 800 ppm of phenolics/ L of extract. [#]Significantly different from 0 days.

DISCUSSÃO

A oxidação lipídica é a maior causa da perda de qualidade nas carnes durante o armazenamento e processamento e devido à presença de ácidos graxos poli-insaturados, os pescados são mais suscetíveis à oxidação lipídica do que as demais carnes (SOHN et al., 2005; OETTERER, 2002). Além disso, a oxidação lipídica pode induzir mudanças nas estruturas proteicas levando à oxidação proteica (POKORNY et al., 1976), isto é, tornando as proteínas menos solúveis e influenciando na capacidade de ligação de água e textura dos pescados (XIONG, 2000).

A incorporação de antioxidantes nos alimentos pode efetivamente retardar a oxidação lipídica, agindo frente à redução da atividade dos radicais livres, remoção dos radicais livres e complexação de metais pró-oxidantes, retardando assim também a oxidação das proteínas (TACHAKITTIRUNGROD et al., 2006). No entanto, devido à preocupação dos consumidores com a segurança e a conscientização quanto à toxicidade dos antioxidantes sintéticos (SUN e FUKUHARA, 1997; WICHI, 1988; SHERWIN, 1990; SHAHIDI e WANASUNDARA, 1992), tem havido um crescente aumento do interesse em explorar novas fontes de antioxidantes naturais.

As plantas são as fontes mais abundantes dos antioxidantes naturais como os compostos fenólicos (PRATT e HUDSON, 1990) e têm sido amplamente relatadas com efeitos benéficos neste sentido, contudo, as sementes de frutas não recebem a devida atenção como fontes de antioxidantes. Além disso, os compostos fenólicos podem agir como antimicrobianos prevenindo o desenvolvimento de patógenos e auxiliando na conservação dos alimentos (MONTVILLE e MATTHEWS, 2008; WINDSON et al., 2011; TAJKARIMI et al., 2010).

A nêspera (*Eriobotrya japonica*) fruta de origem asiática da região da China, vem sendo também cultivada no Brasil com o estado de São Paulo sendo o líder da produção nacional, sendo sua exploração comercial destinada ao consumo *in natura* e as sementes descartadas. Entretanto, estudos demonstram o potencial do extrato de sementes de nêspera no campo farmacológico com efeitos na redução de hepatopatias (NISHIOKA et al., 2002), nefropatias (HAMADA et al., 2004), efeitos hipoglicemiantes (TANAKA et al., 2008), redução de triglicerídios (SHIH et al., 2013), atividade inibitória na peroxidação lipídica em ratos (YOKOTA et al., 2008) e na

redução da oxidação do LDL colesterol *in vitro* (KOBA et al., 2007).

Assim, o objetivo deste estudo foi avaliar o efeito de extratos de semente de nêspera sobre a estabilidade oxidativa em filés, patê e almôndega à base de jundiá, ao longo do armazenamento, considerando-se a predisposição dos pescados ao desenvolvimento de reações oxidativas, as necessidades da indústria de alimentos em encontrar novas fontes de aditivos alimentares oriundos de fonte naturais e a viabilização da produção de espécies nativas de pescado como o jundiá e de nêspera, espécie amplamente distribuída no nosso país e subutilizada, bem como de seus produtos.

Neste contexto, como primeiro objetivo do trabalho (manuscrito 1) objetivou-se avaliar a capacidade antioxidante e a atividade antimicrobiana *in vitro* de extratos de semente de nêspera obtidos através de diferentes extrações, utilizando diferentes solventes e ultrassom. Seguiu-se com a avaliação da aplicabilidade dos extratos de semente de nêspera na estabilidade oxidativa de filés de jundiá congelados (filés tratados com extrato acetônico) (manuscrito 2); em um produto cárneo refrigerado à base de pescado (patês tratados com extrato hidroetanólico) (manuscrito 3) e de um produto cárneo congelado à base de pescado (almôndegas tratadas com extrato acetônico) (manuscrito 4).

A motivação da indústria de alimentos para o uso de antioxidantes e compostos antimicrobianos a partir de fontes naturais para uma melhor preservação dos alimentos tem se intensificado nos últimos anos (SÖKMEN et al, 2004. ; WONG et al, 2006). Os antioxidantes naturais presentes em frutas e vegetais, principalmente os compostos fenólicos, são reconhecidos por serem potenciais sequestradores de radicais livres, agentes redutores e quelantes de metais (CHEW et al., 2009). Do mesmo modo, os agentes antimicrobianos, usualmente sintéticos, utilizados industrialmente para prevenir o crescimento de microrganismos, podem também ser encontrados em uma ampla gama de fontes naturais (MONTVILLE and MATTHEWS, 2008; LEE et al., 2003; WINDSON et al., 2011). Neste contexto, a semente de nêspera possui compostos como o ácido benzóico, o ácido cafeico, o ácido clorogênico, o benzaldeído, ácidos graxos insaturados e esteróis como a amigdalina (GRAY; 1972;. YOKOTA et al., 2006; KIM et al., 2009) que podem estar relacionados com seus efeitos farmacológicos benéficos na fibrose hepática (NISHIOKA et al., 2002) e na supressão da oxidação do ácido linoleico e de oxidação de LDL-colesterol *in vitro* (KOBA et al., 2007), entre outros.

A recuperação de polifenóis das plantas é influenciada pela solubilidade dos compostos fenólicos no solvente utilizado. Assim, solventes como etanol, metanol, acetona, acetato de etila, entre outros, vem sendo usados em diferentes concentrações para a extração de compostos fenólicos a partir de produtos frescos (ALOTHMAN et al., 2009; TURKMEN et al., 2006). Além disso, o ultrassom tem sido empregado com sucesso na extração de compostos como aromas, pigmentos e antioxidantes (XIA et al., 2006; CHEN et al., 2007; MA et al., 2009).

Neste contexto, o objetivo do manuscrito 1 foi avaliar a capacidade antioxidante e a atividade antimicrobiana *in vitro* de extratos de semente de nêspera obtidos com o emprego de diferentes solventes e ultrassom. Os extratos acetônicos apresentaram o maior conteúdo de compostos fenólicos totais e também de taninos totais em comparação aos demais. O tratamento com ultrassom melhorou a extração de compostos fenólicos totais na extração com acetona 70% e a atividade FRAP nos extratos de acetona 35% e 70%. Apesar de os taninos poderem afetar o crescimento microbiano privando os microrganismos de obterem substrato para seu desenvolvimento, nenhuma atividade antimicrobiana foi observada nos extratos nas concentrações utilizadas.

Assim, o manuscrito 1 demonstrou que embora os extratos não tenham apresentado atividade antimicrobiana, o uso de ultrassom melhorou a extração de compostos fenólicos e atividade FRAP apenas na extração com acetona e que o extrato de acetona 70% apresentou o maior teor de fenólicos totais, taninos totais e atividade FRAP, sendo apontado como o extrato com propriedades antioxidantes mais expressivas.

Além disso, a composição centesimal das sementes também foi avaliada, contudo, não foi contemplada em nenhum dos artigos propostos. A semente de nêspera apresentou conteúdos de cinzas (1,37 ± 0,01 g/ 100 g de semente) e carboidratos (43,47 ± 0,15 g/ 100 g) maiores que as sementes de pitanga (0,6-0,8 g de cinzas e 36,4-38,4 g carboidratos/100 g de semente de pitanga, respectivamente) (BAGETTI, 2009). Os teores de umidade (52,69 ± 0,29 g/ 100 g), lipídios (0,35 ± 0,09 g/ 100 g) e proteínas (2,11 ± 0,16 g/ 100 g) da semente de nêspera foram menores que os encontrados nas sementes de pitanga (57-58,6 g de umidade; 0,5-0,7 g de lipídios e 3,3-3,7 g de proteínas/ 100 g de semente de pitanga) (BAGETTI, 2009). Entretanto o conteúdo de fibra alimentar encontrado na semente de nêspera (22,7 ± 1,75 g /100 g) foi semelhante ao relatado nas sementes de pitanga (23-24,7 g/ 100

g), sendo a nêspera constituída prioritariamente de fibra insolível (18,7 ± 0,77 g/ 100 de semente). Desta forma, o uso da semente de nêspera poderia ser explorado quanto ao seu teor de fibra alimentar e fornecimento de fibra insolúvel.

Fatores como o processamento e as condições de armazenamento influenciam na taxa de desenvolvimento da oxidação dos alimentos (FRANKEL, 1984). Segundo Ladikos (1990), qualquer processo que cause rompimento de membranas, como os processos de diminuição de tamanho (moagem, trituração), desossa e cozimento, resulta em exposição dos fosfolipídios ao oxigênio e acelera o desenvolvimento de rancificação.

A oxidação lipídica pode ser efetivamente retardada pelo uso de antioxidantes (FERNÁNDEZ-GINÉS et al., 2005), podendo ser estendida a oxidação proteica e protegendo a perda de qualidade dos alimentos ao longo de sua vida útil. Dessa forma, os demais manuscritos objetivaram avaliar o potencial dos extratos acetônico e hidroetanólico na estabilidade oxidativa de filés de jundiá e seus produtos (patê e almôndegas) ao longo de sua vida útil.

O manuscrito 2 avaliou o efeito do extrato acetônico de semente de nêspera (EA) na estabilidade oxidativa de filés de jundiá mergulhados em concentrações de 100, 200 e 400 ppm e armazenados congelados durante 12 meses.

Os resultados demonstraram que a formulação contendo ácido ascórbico apresentou maiores valores de dienos conjugados (DC) que os filés tratados com EA e que o controle aos 6 meses de armazenamento, além de apresentar os maiores valores de substâncias reativas ao ácido tiobarbitúrico (TBARS) que a formulação tratada com 400 ppm do EA aos 9 meses de armazenamento ($P<0,05$). Contudo, no ponto final de análise, ambos DC e TBARS apresentaram valores similares entre os tratamentos ($P>0,05$). O teor de proteínas carboniladas apresentou um grande aumento a partir dos 3 meses até os 12 meses de armazenamento, comparado ao tempo 0 meses, contudo não teve influência dos tratamentos ($P>0,05$).

Assim, o EA não foi capaz de retardar a produção de compostos primários e secundários da oxidação lipídica e o desenvolvimento de proteínas carboniladas.

Observou-se também uma alteração de cor nos filés ao longo do armazenamento, apontada pela diminuição dos valores de a* em todos os períodos avaliados e ao aumento de b* nos tempos 9 e 12 meses ($P<0,05$), causado provavelmente pela oxidação de lípidos e proteínas e a formação de melanoidinas (ESTÉVEZ, 2011). No entanto, os valores de ΔE apontaram que o extrato acetônico

de EJ foi tão eficaz na prevenção da modificação da cor em filés de jundiá quanto o ácido ascórbico. Além disso, o EA não alterou a composição centesimal ou aceitabilidade dos filés avaliadas no tempo 0 meses.

O manuscrito 3 avaliou o efeito do extrato hidroetanólico de semente de nêspera (EE) na estabilidade oxidativa de patês à base de pescado (filé e polpa de resíduo da filetagem) tratados com concentrações de 3,4; 6,8 e 13,6 mg de fenólicos/ kg de produto (0,1; 0,2 e 0,4 g de semente/ 100 g de produto), armazenados refrigerados durante 35 dias.

Os teores de DC e peróxidos (PV) aumentaram ao longo do armazenamento, contudo, diminuíram de maneira semelhante em todas as amostras aos 35 dias de análise. No entanto, o conteúdo de substâncias reativas ao ácido tiobarbitúrico (TBARS) não se modificou ao longo do armazenamento e não foi afetado pelo EE. O aumento seguido de decréscimo do conteúdo de PV e a manutenção dos níveis de TBARS sugerem que a oxidação estaria ainda nos estágios iniciais ou então os peróxidos produzidos foram degradados a outros compostos que não podem ser detectados pelo método de TBARS (SHAHIDI e ZHONG, 2010). Também houve aumento linear no conteúdo de proteínas carboniladas dos patês de pescado ao longo do armazenamento, sem efeito do EE na oxidação proteica. Segundo Estévez et al. (2006), muitos estudos não conseguem evidenciar o potencial dos compostos fenólicos de seus extratos na formação de proteínas carboniladas em produtos termicamente tratados, como é o caso do patê, e isso ocorreria devido a vários fatores dependentes como o material usado, a concentração do antioxidante e a tecnologia empregada (JONGBERG et al., 2013).

Assim, o manuscrito 3 mostrou que, nas concentrações avaliadas, o extrato hidroetanólico de semente de *E. japonica* não foi capaz de inibir ou reduzir as oxidações lipídicas e proteicas em patês à base de pescado.

O manuscrito 4 avaliou o efeito do extrato acetônico (EA) de semente de nêspera na estabilidade oxidativa de almôndegas à base de pescado tratadas com concentrações do extrato de 400, 600 e 800 ppm, pré-cozidas e armazenadas congeladas durante 150 dias.

Os resultados mostraram que os valores de PV e TBARS diminuíram ao longo do tempo de armazenamento devido aos altos valores nos tempos iniciais atribuídos à cocção e manipulação. As formulações contendo ácido ascórbico e 800 ppm apresentaram maiores valores de PV que a formulação controle, decréscimo da

tendência ao vermelho e aumento da tendência ao amarelo ao longo do armazenamento congelado ($P<0,05$). O conteúdo de proteínas carboniladas aumentou ao longo do tempo de armazenamento, similar ao ocorrido com a dureza das almôndegas cozidas.

O EA incorporado aos filés utilizado para o desenvolvimento de almôndegas não influenciou a aceitabilidade das almôndegas no dia 1 de armazenamento e o odor de ranço apresentou um leve aumento ao longo do armazenamento, contudo, nada proporcional às reações oxidativas ocorridas no produto.

Assim, as formulações tratadas com o EA não foram capazes de inibir a oxidação de lípidos e proteínas ou de prevenir a alteração de cor das almôndegas à base de pescado, sendo que as formulações contendo ácido ascórbico e 800 ppm apresentaram efeitos pró-oxidantes.

Tanto nos patês quanto nas almôndegas, acredita-se que os extratos (EA e EE) não tenham sido efetivos no retardo da oxidação lipídica e proteica ou evitado a as alterações de cor ao longo do armazenamento devido às altas temperaturas empregadas no desenvolvimento dos produtos, uma vez que o processo de cocção pode ter causado rompimento das membranas celulares e liberação de ferro heme, acelerando as reações oxidativas (GANHÃO et al., 2010b; KRISTENSEN e PURSLOW, 2001), além de aceleração da oxidação da mioglobina a metamioglobina (FAUSTMAN et al., 2010), afetando a cor do produto nas almôndegas quando empregada a maior concentração do extrato.

Nos filés, apesar de não ter sido empregada alta temperatura, acredita-se que o extrato (EA) não tenha apresentado efeito positivo no retardo da oxidação lipídica ou proteica e na descoloração devido ao fato de que o efeito protetor dos extratos é dependente da composição da matéria-prima (do pescado, bem como do antioxidante) e da concentração de antioxidante utilizada (JONGBERG et al., 2013).

Deste modo, o conteúdo de compostos fenólicos encontrados nos extratos de semente de nêspera e a atividade de redução do ferro (FRAP) evidenciada pelos estudos *in vitro* poderiam apresentar efeitos benéficos na vida útil de carnes, entretanto, mais estudos são necessários na otimização da extração de compostos das sementes de nêspera e na aplicação desses em produtos de pescado.

CONCLUSÃO

Os resultados indicam que o extrato de semente de nêspera (*Eriobotrya japonica*) não foi suficiente para inibir ou reduzir a oxidação lipídica ou proteica e a alteração de cor em filés, patê e almôndegas elaboradas a partir de jundiá (*Rhamdia quelen*) durante o armazenamento.

O conteúdo de compostos fenólicos encontrados nos extratos de semente de nêspera sugere um possível uso na vida útil de alimentos, contudo, mais estudos são necessários na otimização dos compostos extraídos e na sua aplicabilidade em produtos de pescado. Igualmente, a semente de nêspera poderia ser aplicável na indústria de produtos cárneos enfatizando-se seu uso relacionado ao seu conteúdo de fibra alimentar e insolúvel.

Sugere-se que o extrato de semente de nêspera não tenha sido efetivo nos produtos propostos devido às concentrações utilizadas e às condições críticas a que os mesmos foram submetidos (cocção, congelamento lento, processamento). Assim, em trabalhos futuros poderia-se avaliar a efetividade dos extratos de semente de nêspera aliados a compostos sintéticos, verificando-se seu efeito sinérgico.

Além disso, as evidências apresentadas por esse trabalho apontam que a oxidação proteica pode ser iniciada antes mesmo que a oxidação lipídica em carnes. Deste modo, a metodologia utilizada neste trabalho para a determinação da oxidação das proteínas otimizada especificamente para determinação em jundiá pode ser um importante marcador do início das reações oxidativas em pescados e relevante para futuros trabalhos nesta área.

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APÊNDICE I

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado a participar de um estudo intitulado “POTENCIAL DA SEMENTE DE NÊSPERA (*Eriobotrya japonica*) NA ESTABILIDADE OXIDATIVA DE PESCADO E SEUS PRODUTOS”, que tem como objetivo investigar o efeito inibitório do extrato de semente de nêspera em relação à oxidação lipídica e proteica em almôndegas à base de jundiá congeladas.

Os pescados são excelentes fontes proteicas e de ácidos graxos poliinsaturados, contudo, a degradação destes durante o armazenamento e processamento influencia na estabilidade e qualidade dos mesmos, levando a rancificação. O uso de antioxidantes naturais é um método eficaz para controle da rancidez e de limitação dos efeitos tóxicos dos antioxidantes sintéticos. Estudos relatam um maior teor de compostos fenólicos à semente da nêspera do que na polpa e casca, possuindo características que poderiam trazer consequências benéficas na estabilidade oxidativa de pescados e seus produtos.

Procedimentos a serem realizados

Serão oferecidas a você amostras de almôndegas à base de jundiá. Será solicitado que você as prove, marcando nas fichas a sua resposta com relação às características sensoriais (cor, textura, etc.) do produto oferecido.

Riscos possíveis e benefícios esperados

Fica claro que você não é obrigado a participar do projeto. No caso de recusa você não terá nenhum tipo de prejuízo. A qualquer momento da pesquisa você é livre para retirar-se da mesma.

No caso de aceite, fica claro que os produtos oferecidos são seguros e de boa qualidade, não havendo prejuízos ou riscos a sua saúde. Não haverá benefício financeiro pela sua participação e nenhum custo para você.

Você não terá benefícios diretos, entretanto, ajudará a identificar aditivos naturais e, portanto, mais saudáveis, que poderiam ser usados na conservação de alimentos.

Confidencialidade

Os dados obtidos com esta pesquisa serão publicados em revistas científicas reconhecidas. Os seus dados serão analisados em conjunto com os de outros participantes, assim, não aparecerão informações que possam lhe identificar, sendo mantido o sigilo de sua identidade.

Utilização dos dados obtidos

O material coletado e os seus dados serão utilizados somente para esta pesquisa e ficarão guardados com o pesquisador por cinco anos, após o qual serão destruídos.

Os pesquisadores responsáveis pelo estudo são a Prof. Dr. Ernesto Hashime Kubota e Jaqueline Piccolo, aluna do Programa de Pós Graduação em Ciência e Tecnologia de Alimentos da UFSM. Em qualquer etapa do estudo você terá acesso aos pesquisadores responsáveis pelo estudo para esclarecimento de eventuais dúvidas.

Este estudo obteve aprovação junto ao Comitê de Ética em Pesquisa da Universidade Federal de Santa Maria, com CAAE n° 07241112.4.0000.5346.

Telefones para contato com os pesquisadores
*Prof. Dr. Ernesto Hashime Kubota – Departamento de Tecnologia e Ciência dos Alimentos – CCR
Email: ernehk2008@yahoo.com.br
(55) 3220 8254
*Jaqueline Piccolo - Pós Graduação em Ciência e Tecnologia de Alimentos – UFSM
Email: jaquepiccolo@hotmail.com
(55) 96440702

Acredito ter sido suficientemente informado a respeito das informações que li ou que foram lidas para mim, descrevendo o estudo "POTENCIAL DA SEMENTE DE NÊSPERA (*Eriobotrya japonica*) NA ESTABILIDADE OXIDATIVA DE PESCADO E SEUS PRODUTOS". Ficaram claros para mim quais são os objetivos do estudo, os procedimentos a serem realizados, seus desconfortos e riscos, as garantias de confidencialidade e de esclarecimentos permanentes. Ficou claro também que minha participação é isenta de despesas. Concordo voluntariamente em participar deste estudo e poderei retirar o meu consentimento a qualquer momento, antes ou durante o mesmo, sem penalidades ou prejuízo.

____
Assinatura do participante

Declaro que obtive de forma apropriada e voluntária o Consentimento Livre e Esclarecido deste sujeito de pesquisa ou representante legal para a participação neste estudo.

____
Assinatura do responsável pelo estudo

Santa Maria, ________ de _________________________de 2013.

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Pesquisador: Ernesto Hashime Kubota

Área Temática:

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ANEXO II

Decision Letter (CR-2014-0066)

From: rudiweiblen@gmail.com

To: jaquelinepiccolo@gmail.com

CC: jaquelinepiccolo@gmail.com, anapauladaniel@gmail.com, laurenfferreira@gmail.com, brunaklein06@yahoo.com.br, amandarruviaro@gmail.com, tatiemanuelli@gmail.com, ernehk2008@yahoo.com.br

Subject: Ciência Rural - Decision on Manuscript ID CR-2014-0066

Body: 27-Feb-2014

Dear Miss Piccolo:

It is a pleasure to accept your manuscript entitled "Oxidative stability of refrigerated fish pates containing loquat seed extract" in its current form for publication in the Ciência Rural.
Your paper has been approved provisionally and may still need some corrections or additional information after it has been verified by our staff. So we will contact you in the near future with the suggestions and before the paper is published in the Ciência Rural.
The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

Thank you for your fine contribution. On behalf of the Editors of the Ciência Rural, we look forward to your continued contributions to the Journal.

Sincerely,
Dr. Rudi Weiblen
Editor-in-Chief, Ciência Rural
rudiweiblen@gmail.com

ANEXO III

Jaqueline Piccolo <jaquelinepiccolo@gmail.com>

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