UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

DANIELA MIOTTO BERNARDI

"ADDITION OF NATURAL PRODUCTS WITH ANTIOXIDANT ACTION AND FLAXSEED OIL IN SWINE DIETS: EFFECTS ON MEAT AND MEAT PRODUCT"

"ADIÇÃO DE PRODUTOS NATURAIS COM AÇÃO ANTIOXIDANTE E DE ÓLEO DE LINHAÇA EM DIETAS DE SUÍNOS: EFEITOS SOBRE A CARNE E PRODUTO CÁRNEO"

CAMPINAS
2016

DANIELA MIOTTO BERNARDI

"ADDITION OF NATURAL PRODUCTS WITH ANTIOXIDANT ACTION AND FLAXSEED OIL IN SWINE DIETS: EFFECTS ON MEAT AND MEAT PRODUCT"

"ADIÇÃO DE PRODUTOS NATURAIS COM AÇÃO ANTIOXIDANTE E DE ÓLEO DE LINHAÇA EM DIETAS DE SUÍNOS: EFEITOS SOBRE A CARNE E PRODUTO CÁRNEO"

Thesis presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food and Nutrition, in the area of Experimental Nutrition and Nutrition Applied to Food Technology.

Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Doutora em Alimentos e Nutrição, na área de Nutrição Experimental e Aplicada à Tecnologia de Alimentos.

Orientador: Dr. Valdemiro Carlos Sgarbieri

Coorientadora: Dra. Teresinha Marisa Bertol

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL. TESE DEFENDIDA PELA ALUNA DANIELA MIOTTO BERNARDI E ORIENTADA PELO PROFESSOR DR. VALDEMIRO CARLOS SGARBIERI

CAMPINAS

2016

Agência(s) de fomento e nº(s) de processo(s): CNPq, 453854/2013-3

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Claudia Aparecida Romano - CRB 8/5816

B456a

Bernardi, Daniela Miotto, 1986-
Addition of natural products with antioxidant action and flaxseed oil in swine diets : effects on meat and meat product / Daniela Miotto Bernardi. – Campinas, SP : [s.n.], 2016.

Orientador: Valdemiro Carlos Sgarbieri.
Coorientador: Teresinha Marisa Bertol.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Omega-3. 2. Antioxidantes naturais. 3. Oxidação lipídica. 4. Hidrolisados. 5. Suíno. I. Sgarbieri, Valdemiro Carlos. II. Bertol, Teresinha Marisa. III. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. IV. Título.

Informações para Biblioteca Digital

Título em outro idioma: Adição de produtos naturais com ação antioxidante e de óleo de linhaça em dietas de suínos : efeitos sobre a carne e produto cárneo
Palavras-chave em inglês:
Omega-3
Natural antioxidants
Lipid oxidation
Hydrolysates
Swine
Área de concentração: Nutrição Experimental e Aplicada à Tecnologia de Alimentos
Titulação: Doutora em Alimentos e Nutrição
Banca examinadora:
Valdemiro Carlos Sgarbieri [Orientador]
Juliana Burger Rodrigues
Leandro Daniel de Paris
Maria Teresa Bertolo Pacheco
Marise Aparecida Rodrigues Pollonio
Data de defesa: 07-12-2016
Programa de Pós-Graduação: Alimentos e Nutrição

BANCA EXAMINADORA

_____

Dr. Valdemiro Carlos Sgarbieri
Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas
(Orientador)

_____

Dra. Juliana Büger Rodrigues
Pesquisadora – Campinas, SP
(Membro Titular)

_____

Dr. Leandro Daniel De Paris
Pesquisador – Toledo, PR
(Membro Titular)

_____

Dra. Maria Teresa Bertolo Pacheco
Instituto de Tecnologia de Alimentos
(Membro Titular)

_____

Dra. Marise Aparecida Rodrigues Pollonio
Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas
(Membro Titular)

_____

Dr. Daniel Barrera Arellano
Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas
(Membro Suplente)

_____

Dr. Rogério Manoel Lemes de Campos
Universidade Federal de Santa Catarina
(Membro Suplente)

_____

Dr. Sérgio Bertelli Pflanzer Júnior
Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas
(Membro Suplente)

Ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno

Dedico esta tese aos meus pais Gilso Bernardi e Anelita Silvana Miotto Bernardi, e ao meu noivo André Luiz Martins Pereira, que sempre me apoiaram e incentivaram à concretizar mais esta etapa.

Dedico também à todos os criadores de suínos do Brasil, que assim como meus pais, meus tios e meus primos, mesmo em tempos de crise não desistem, pois acreditam na atividade, têm amor pelo que fazem e empenham-se ao máximo para o crescimento da suinocultura no país.

AGRADECIMENTOS

À Deus por ter permitido a conclusão desta etapa de minha jornada, que além de um doutorado, é um sonho que se torna realidade, uma realização pessoal e a oportunidade de crescimento profissional.

Aos meus orientadores

Ao meu querido orientador professor Dr. Valdemiro Carlos Sgarbieri, por novamente honrar-me com a oportunidade ser sua aluna durante o doutorado. Por ter acreditado na viabilidade deste trabalho. Por compartilhar tanta sabedoria, experiência, amizade e pela grande dedicação que teve ao me orientar, receba minha gratidão!

À minha querida coorientadora a pesquisadora Dra. Teresinha Marisa Bertol, por ter me recebido muito bem na Embrapa Suínos e Aves, pela grande contribuição científica neste trabalho, por toda atenção, dedicação, incentivo, amizade e pelo valoroso exemplo de pesquisadora, aqui declaro meu reconhecimento!

Às empresas parceiras

À Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA, unidade Suínos e Aves de Concórdia –SC, por ter fornecido estrutura para a realização do experimento animal. Agradeço imensamente a atenção, solicitude e respeito de todos os pesquisadores, técnicos e assistentes, que contribuíram tanto para a realização da pesquisa e também para garantir um excelente ambiente de trabalho. Agradeço em especial à equipe da granja e fábrica de ração (Luiz Carlos Ajala, Cleiton Marcos Schuck, Valdir Hegler, Hedo Haupt, Vilson Becker, Levino Bassi, Iles Pilonetto, Miguel Klassmann) e à todos dos laboratórios (João Alberto Suzin Marini, Vicky Lilge Kawaski, Anildo Cunha Júnior, Carlos Roberto Bernardi, Diego Zurek) que contribuíram para a realização do trabalho. Minha gratidão aos pesquisadores Dr. Arlei Coldebella pela grande contribuição com todas as análises estatísticas e ao Dr. Elsio

Antônio Pereira de Figueiredo que encaminhou meu primeiro e-mail a Dra. Teresinha Marisa Bertol, o que resultou nesta parceria.

À Falbom Agroindustrial Ltda. por ter contribuído com a realização da pesquisa, desde as primeiras etapas de produção do hidrolisado de carcaça de tilápia, até o fornecimento de todo hidrolisado para o experimento animal, bem como, por ter indicado meu nome para receber uma bolsa de pesquisador RHAE em projeto aprovado pela empresa junto ao Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq. Agradeço em especial a Dra. Fabiane Dieterich por proporcionar esta parceria. Agradeço também à todos os colaboradores e pesquisadores da empresa (Leandro Daniel de Paris, Júlio César Adams Haab e Pedro Oliveira Moreira) que contribuíram na produção e secagem do hidrolisado. Manifesto meu reconhecimento pelo respeito e atenção que todos da empresa tiveram comigo e com a pesquisa.

À NUTRON, marca da Cargill do Brasil, pela parceria nesta pesquisa e por ter fornecido ingredientes necessários para a formulação das rações experimentais. Meu especial agradecimento aos Srs. Alvaro Dubois e Julio Cesar Carrera Carvalho por terem proporcionado esta parceria.

Aos órgãos de fomento, universidades, departamentos, laboratórios e professores

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, pela bolsa de estudos durante o doutorado.

Ao Grupo de Estudos de Manejo na Aquicultura – GEMAQ, da Universidade Estadual do Oeste do Paraná - UNIOESTE, pela parceria na realização do projeto de produção e análise do hidrolisado de carcaça e cabeça de tilápia. Agradeço em especial ao professor Dr. Wilson Rogério Boscolo pela receptividade e viabilização da parceria junto à Falbom e principalmente pala contribuição científica nesta tese. Agradeço a todos os professores e alunos do grupo que contribuíram para a realização deste trabalho.

Ao Departamento de Alimentos e Nutrição – DEPAN, da Universidade Estadual de Campinas – UNICAMP, por toda estrutura disponibilizada para a realização do trabalho. A todos os professores do departamento, aos colaboradores e todos os alunos que ajudaram, meu reconhecimento e gratidão. Agradeço em especial:

- Ao Dr. Mario Maróstica Júnior por ter disponibilizado equipamento para leitura em diferentes análises e por ter contribuído na apresentação de trabalhos no "12th European Nutrition Conference (FENS)".

- Aos professores Dra. Helena Maria Bolini e Dr. Jorge Herman Behrens pelas orientações em relação a análise sensorial.

- Ao Dr. Nilo Sérgio Sabbião Rodrigues por toda ajuda, especialmente no primeiro semestre do doutorado.

- Às secretárias do DEPAN Sra. Maria Aparecida Vasconcellos Osteti e Srta. Suelen de Almeida, por toda gentileza e solicitude.

Ao laboratório de fontes proteicas do Departamento de Alimentos e Nutrição – DEPAN, UNICAMP e em especial à professora Dra. Flavia Neto, por ter disponibilizado reagentes e estrutura para a realização das análises de proteína, grau de hidrólise e atividade antioxidante do hidrolisado. Gentilmente agradeço a professora Flavia Neto, Eliana Maria Pettirossi Motta, Elizabeth Freitas Silva, Fernanda Guimarães Drummond e Silva, Natália Caldeira de Carvalho, Fabiana de Oliveira Martins, Maria Elisa Caetano Silva Abreu e Tássia Batista Pessato, todas vocês me trataram com respeito e carinho, me ajudaram nas análises e me fizeram sentir parte do grupo.

À professora Dra. Marise Aparecida Rodrigues Pollonio pela orientação, apoio e incentivo na escrita do artigo de revisão desta tese, durante a disciplina TP354 (Atributos de qualidade funcional em produto cárneo). Obrigada por juntamente com sua aluna Camila de Souza Paglarini, me ajudarem com relação à análises de TBARS.

Ao laboratório de óleos e gorduras do departamento de Tecnologia de Alimentos – DTA, UNICAMP. Em especial agradeço ao professor Dr. Daniel Barrera-Arelano pela valorosa orientação em relação às análises realizadas no equipamento Rancimat, bem como ao seu aluno Dr. Gustavo G. Pereira pela ajuda durante a análise.

Ao laboratório de química de Alimentos do Departamento de Ciência de Alimentos – DCA, UNICAMP e à professora Dra. Helena Godoy e à Dra. Adriana Dillenburg Meinhart pelas análises de Tocoferol.

Ao Centro Universitário Assis Gurgacz – FAG por ter cedido estrutura de laboratório para a realização das análises sensoriais do produto cárneo desenvolvido na presente tese. Em especial agradeço à coordenadora do curso de Nutrição Sra. Nanci Rouse Teruel Berto.

À professora Dra. Juliana Burger pela orientação e ajuda na realização das análises estatísticas da análise sensorial.

Aos os meus familiares e amigos

Aos meus queridos pais Gilso Bernardi e Anelita Silvana Miotto Bernardi e à meus irmãos Gabriela Bernardi e Gilso Bernardi Júnior manifesto minha eterna gratidão por todo amor e carinho que me dedicam desde tenra idade, pelas orações e demonstrações de fé, pelo incentivo e paciência ao longo do doutorado e de toda vida. Sou grata também pela cooperação nos dias de análise sensorial do doutorado.

Ao meu amado noivo, André Luiz Martins Pereira, obrigada por sempre me tratar com tanto amor e carinho, por toda compreensão e incentivo e pela cooperação nos dias de análise sensorial.

Ao meu tio Alcides Miotto da Biriba's Genética de Suínos, por toda ajuda e incentivo no início do doutorado.

À minha tia Lurdes Maria Borghelot e à minha mãe pela companhia nas viagens de carro para Concórdia-SC, Chapecó-SC, Toledo-PR e Eneas Marques-PR.

À querida amiga Dra. Fernanda Guimarães Drummond e Silva por todo apoio, orientação e parceria na realização das análises de atividade antioxidante, pela amizade e pelo aconselhamento durante o doutorado. Receba minha gratidão.

Às amigas e parceiras de laboratório na Embrapa, Bárbara Cristina Silveira-Almeida e Daniele Camillo, pelo auxílio inigualável nas análises de TBARS e de qualidade de carne, bem como pelos laços de amizade que construímos nesse convívio.

Às queridas amigas (Fernanda Guimarães Drummond e Silva, Glaucia Carielo Lima, Natália Machado Alencar, Taís Mazzola, Ana Paula Barth, Guirminda Maria Butka e Maria de Lurdes Bernardi Buganca) que me receberam sempre com tanto carinho em suas casas durante essa jornada.

À todos os alunos que contribuíram para a realização da análise sensorial do produto cárneo da tese.

Estendo meu reconhecimento e gratidão à todos os professores, aos colegas de laboratório, aos funcionários das universidades e empresas por onde estive, pois juntos desenvolvemos além da pesquisa, criamos uma rede de conhecimento, compartilhamos sonhos, atamos laços de amizade e formamos uma comunidade de pessoas que buscam a qualidade de vida para as gerações futuras. Juntos escrevemos e comprovamos que ciência, dedicação e parceria garantem um futuro melhor para todos! Recebam meu reconhecimento e votos que continuemos atuando pelo futuro da humanidade.

“O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo.
Mesmo não atingindo o alvo, quem busca e vence obstáculos,
no mínimo fará coisas admiráveis.”
José de Alencar

RESUMO GERAL

A adição de produtos naturais com ação antioxidante na ração animal, assim como o uso de diferentes fontes lipídicas, especialmente de ω-3, são estratégias nutricionais que vêm sendo estudadas nos últimos anos para incorporar estes compostos na carne. A presença de ω-3 em carnes, tem como principal finalidade promover o apelo fisiológico-funcional destes ácidos graxos, ao passo que a presença de antioxidantes naturais pode resultar em efeitos positivos na vida de prateleira do produto, além de promover uma conotação mais saudável. Assim, o objetivo do presente trabalho foi avaliar o efeito da adição de óleo de linhaça, como fonte de ω-3 e de hidrolisado de carcaça e cabeça de tilápia do Nilo, bagaço de uva, extrato de semente de uva e vitamina E, como fontes de antioxidantes naturais, na dieta de suínos. Para tanto, foi produzido um hidrolisado de carcaça e cabeça de tilápia do Nilo e avaliado seu potencial antioxidante por meio de testes químicos (ORAC, FRAP e ABTS). Após a confirmação da atividade antioxidante do hidrolisado, este foi utilizado na experimentação animal. O experimento com animais foi conduzido com 96 suínos, distribuídos em 6 tratamentos: C- ração controle; L- ração com 3% de óleo de linhaça (OL); LBU- ração com 3% de OL + 10% bagaço de uva; LEU- ração com 3% de OL + 0,0022% de extrato de semente de uva; LH- ração com 3% de OL+ 5% hidrolisado de carcaça e cabeça de tilápia do Nilo e LVitE- ração com 3% de OL + 200 ppm de suplemento de vitamina E. Avaliaram-se parâmetros de desempenho de crescimento, qualidade de carcaça e qualidade de carne, antioxidantes totais no soro sanguíneo, perfil lipídico do toucinho e lombo, tocoferóis no lombo, estabilidade oxidativa da gordura (Rancimat®, 90^{o}C). Da carne dos animais, foram elaborados mini-hamburgueres os quais foram avaliados em relação à substâncias reativas ao ácido tiobarbitúrico (TBARS), cor e aceitabilidade. A inclusão de óleo de linhaça e antioxidantes na dieta não produziu efeito sobre o desempenho, qualidade da carcaça e antioxidantes totais no soro. Verificou-se que os tratamentos com linhaça promoveram incorporação de ω-3, aumento de ácidos graxos poli-insaturados (PUFA) totais e redução de ácidos graxos saturados (SFA) totais na carne e na gordura. Em relação à qualidade da carne, os resultados de escore de cor do tratamento LEU sugeriram uma possível atividade antioxidante do extrato de semente de uva. Os resultados de TBARS e estabilidade oxidativa da gordura (Rancimat®) revelaram que a Vitamina E foi o antioxidante mais eficiente, porém os tratamentos LH e LEU apresentaram efeito antioxidante nos produtos. A análise sensorial apresentou resultados que corroboram com os supracitados. Verificou-se ainda, aumento significativo de tocoferol total especialmente no tratamento LVitE

e também nos tratamentos LH e LEU. Portanto, a vitamina E parece apresentar alto potencial antioxidante, mesmo quando a carne apresenta maiores concentrações de PUFAs e mais estudos são necessários para entender o efeito antioxidante *in vivo* dos peptídeos produzidos com a hidrólise de carcaças de pescados e também dos produtos ricos em compostos fenólicos como os subprodutos derivados do processamento da uva.

ABSTRACT

The addition of natural antioxidants in animal feed and the use of different lipid sources, particularly ω-3 fatty acids, are strategies widely studied for years, to incorporate these compounds in the meat. The presence of ω-3 fatty acids in meat aims at physiological and functional appeals, whereas the presence of natural antioxidants can lead to positive effects in shelf life of the products and promote a healthier connotation. Thus, the objective of this study was to evaluate the effect of flaxseed oil as a source of ω-3 fatty acids, and Nile tilapia carcass and head hydrolysate, grape pomace, grape seed extract, and vitamin E as sources of natural antioxidants in swine diets. Nile tilapia carcass and head hydrolysate was produced and its antioxidant potential was evaluated by chemical assays (ORAC, FRAP and ABTS). After confirming its antioxidant activity, the hydrolysate was used in animal experimentation. The experiments with animals were performed with 96 pigs, divided into 6 treatments: C- control diet; F- feed containing 3% flaxseed oil (FO); FGP-, feed containing 3% FO + 10% grape pomace; FGE- feed containing 3% FO + 0.0022% grape seed extract; FH-, feed containing 3% FO + 5% Nile tilapia carcass and head hydrolysate; and FVitE- feed containing 3% FO + 200 ppm vitamin E supplement. Growth performance, carcass, and meat quality parameters, serum total antioxidant status, lipid profile of backfat and loin, tocopherol content in the loin, oxidative stability of lipids (measured by the Rancimat equipment), as well as thiobarbituric acid reactive substances (TBARS), color, and acceptability of mini-hamburgers. Dietary inclusion of flaxseed oil and antioxidants had no influence on growth performance, carcass quality and serum total antioxidant status. The treatments containing flaxseed oil promoted a significant incorporation of ω-3 fatty acids, an increase in poly-unsaturated fatty acids (PUFA), and a reduction of saturated fatty acids (SFA) in backfat and loin. Regarding the meat quality, the color scores of the treatment FGE suggested a possible antioxidant activity of the grape seed extract. Results of TBARS and oxidative stability of lipids (Rancimat) revealed that vitamin E was the most effective antioxidant, although the treatments FH and FGE also showed evidence of antioxidant activity. The sensory evaluation corroborated these findings. A significant increase was also observed in the total tocopherols, especially in the treatment FVitE, followed by FH, and FGE. Therefore, vitamin E has proven to have high antioxidant potential, even in meat containing higher concentrations of PUFAs and further studies are needed to better evaluate the antioxidant effect *in vivo* of the peptides produced by hydrolysis of fish carcasses

and products rich in phenolic compounds, such as those derived from grape processing by products.

SUMÁRIO

1. INTRODUÇÃO GERAL

O aumento do consumo de ômega-3 (ω-3) está associado a inúmeros benefícios à saúde (ALBERT et al., 2005; SEO; BLANER; DECKELBAUM, 2005; APPLETON et al., 2006; SUBLETTE et al., 2006; PAJK et al., 2006; DUNSTAN et al., 2007, 2008; VEDIN et al., 2008; BLOEDON et al., 2008; POUDEL-TANDUKAR et al., 2009; DAWCZYNSKI et al., 2010; DANGOUR et al., 2012) e as recomendações para a ingestão destes ácidos graxos (AG) sugerem que representem cerca de 0,5% a 1,2% da ingestão energética total do indivíduo (TRUMBO et al., 2005; EFSA, 2010), entretanto, eles estão naturalmente presentes em baixas concentrações nos alimentos, especialmente, em carnes e produtos cárneos (TACO, 2011) que são importantes fontes de gordura da dieta humana. Por este motivo, muitos estudos têm se concentrado em incorporar ω-3 em diferentes alimentos e especialmente em matérias primas cárneas, como a carne suína (ROSENVOLD; ANDERSEN, 2003; BERNARDI et al., 2016).

Apesar dos benefícios nutricionais da incorporação de ω-3 em alimentos, muitos obstáculos tecnológicos devem ser considerados (BERNARDI et al., 2016), dentre os quais a oxidação lipídica é destaque, pois os ω-3 são menos estáveis, mais reativos e portanto, mais susceptíveis aos radicais livres (DECKER; AKOH; WILKES, 2012).

As reações de oxidação são processos naturais e inevitáveis em sistemas biológicos e são as principais causas não microbianas de deterioração de carnes e produtos cárneos, pois induzem a modificações lipídicas e proteicas, que afetam significativamente as propriedades nutricionais e sensoriais da carne, resultando em deterioração do aroma, cor, sabor e textura (JAMILAH et al., 2009; DECKER; AKOH; WILKES, 2012).

Os antioxidantes são substâncias naturais ou sintéticas, que atuam na prevenção e/ou redução de reações de oxidação (SAMARANAYAKA; LI-CHAN, 2011; BERNARDI et al., 2016). Antioxidantes sintéticos são efetivos, entretanto, os questionamentos sobre a segurança do uso destas substâncias alimentos, têm motivado pesquisas cujo objetivo é identificar compostos naturais com ação antioxidante (FALOWO; FAYEMI; MUCHENJE, 2014; SHAH et al., 2014; BERNARDI et al., 2016). É importante ressaltar também, que muitos estudos têm se concentrado na identificação de compostos com potencial antioxidante em subprodutos da indústria de alimentos (SÁYAGO-AYERDI et al., 2009; CHALAMAIAH et al., 2012; DAL BOSCO et al., 2012; HOSSAIN; KO; YANG, 2012) e quando o potencial antioxidante é confirmado, o uso destas matérias primas é recomendável, pois estimula um destino mais sustentável para subprodutos alimentícios, bem como aumenta a viabilidade econômica do estudo.

Vitamina E, vitamina C, compostos fenólicos, taninos, carotenoides, certos peptídios e alguns metais cofatores enzimáticos, são exemplos de compostos bioativos com propriedade

antioxidante e podem estar presentes nos alimentos e subprodutos da indústria alimentícia (SAMARANAYAKA; LI-CHAN, 2011; SHAH et al., 2014; BERNARDI et al., 2016).

Neste contexto, o uso de antioxidantes naturais em produtos com maiores teores de ω-3 pode ser uma estratégia eficaz, tanto para retardar as reações de oxidação, favorecidas pela presença destes AG, como para aumentar o potencial fisiológico-funcional do alimento (BERNARDI et al., 2016).

Desta forma, no presente estudo foram testados na alimentação de suínos uma fonte de ω-3 associado à diferentes produtos naturais fontes de antioxidantes, a fim de avaliar ao mesmo tempo, a incorporação do ω-3 na carne e o potencial dos antioxidantes adicionados na ração sobre as reações de oxidação e vida de prateleira da carne e produto cárneo. Foi utilizado como fonte de ω-3 o óleo de linhaça, pois ele possui altas concentrações de α-linolênico, precursor dos demais AG da família ω-3 (VAZ et al., 2006; TACO, 2011). Como fontes de antioxidantes foram utilizadas a vitamina E e outros produtos naturais com características distintas: hidrolisado de carcaça e cabeça de tilápia do Nilo e subprodutos do processamento da uva (bagaço desidratado e o extrato de semente).

A vitamina E, foi escolhida por ser uma referência de antioxidante na alimentação animal, uma vez que estudos recentes evidenciam que em condições normais, quando esta vitamina é ingerida, ela é incorporada aos tecidos, o que promove aumento da estabilidade oxidativa da gordura (SALES; KOUKOLOVÁ, 2011).

Hidrolisados de proteínas de pescados vêm sendo estudados devido ao seu potencial antioxidante, sendo que esta bioatividade decorre da presença de certos peptídeos produzidos durante a hidrolise (SAMARANAYAKA; LI-CHAN, 2011; CHALAMAIAH et al., 2012). Muitos ensaios químicos comprovaram o potencial antioxidante de hidrolisados de proteínas musculares de tilápia do Nilo (RAGHAVAN; KRISTINSSON; LEEUWENBURGH, 2008; DAUD; BABJI; YUSOP, 2013; CHUESIANG; SANGUANDEEKUL, 2015), porém o potencial antioxidante *in vivo* ainda não foi estudado em suínos. O hidrolisado desenvolvido no presente estudo utilizou como matéria prima, carcaça e cabeça de Tilápia do Nilo, portanto, subprodutos do processamento deste peixe. Foi feito a caracterização *in vitro* do potencial antioxidante do hidrolisado produzido, antes de sua utilização *in vivo* no experimento com suínos.

Subprodutos do processamento da uva são fontes de compostos fenólicos (LAFKA; SINANOGLOU; LAZOS, 2007). O potencial antioxidante sobre a carne decorrente da incorporação de subprodutos da uva na dieta animal, foi comprovado em experimentos com frangos e cordeiros (BRENES et al., 2008; SÁYAGO-AYERDI et al., 2009; JERÓNIMO et al.,

2012; CHAMORRO et al., 2015), porém o efeito antioxidante da adição destes produtos na dieta de suínos ainda não têm resultados conclusivos (CARPENTER et al., 2007; YAN; KIM, 2011; BERTOL et al., 2016). O bagaço de uva utilizado na pesquisa foi obtido a partir da desidratação do bagaço da uva integral gerado na fabricação de vinho e o extrato de semente de uva foi produzido e fornecido por empresa privada da área de alimentação animal.

Desta forma, o presente estudo foi dividido em quatro etapas, que estão apresentadas nesta tese na forma de capítulos, sendo que cada capítulo resultou em um artigo:

- **Capítulo 01**: Apresenta uma revisão de literatura que trata de aspectos relevantes da incorporação de ω-3 em carnes e produtos cárneos. São abordados temas sobre a importância nutricional da incorporação de ω-3 em alimentos, as estratégias que vêm sendo empregadas para promover o incremento destes AG em carnes e produtos cárneos, bem como, o impacto que a presença de ω-3 têm sobre a estabilidade oxidativa do produto. O trabalho aborda também, produtos naturais com potencial antioxidante que estão sendo testados como uma alternativa para minimizar a oxidação em carnes e produtos cárneos. O artigo de revisão bibliográfica apresentado no capítulo foi aceito e publicado pela revista "*Journal of the Science of Food and Agriculture*" (volume 96, número 8 de junho de 2016, páginas 2620-2634).

- **Capítulo 02:** Apresenta o artigo original com os dados originais referentes ao processo de produção do hidrolisado de carcaça e cabeça de tilápia do Nilo. No artigo estão apresentados também os resultados de caracterização proteica do produto, bem como seu potencial antioxidante *in vitro*. São discutidos aspectos sobre principal mecanismo de ação antioxidante do hidrolisado de carcaça e cabeça de tilápia do Nilo. O artigo foi aceito para publicação na revista "*Food Science and Technology (Campinas)*" no dia 13 de agosto de 2016. DOI: http://dx.doi.org/10.1590/1678-457X.15216 .

- **Capítulo 03:** Apresenta o artigo original com os detalhes do protocolo utilizado no experimento com suínos, onde foi adicionado na dieta animal óleo de linhaça e produtos naturais com potencial antioxidante. No artigo, estão descritas informações de como foram formuladas as dietas experimentais e os resultados da atividade antioxidante dos produtos testes. Também são discutidos os resultados desempenho de crescimento, qualidade de carcaça, qualidade de carne e antioxidantes totais no soro sanguíneo dos

animais. O artigo foi submetido para a revista "*Journal of the Science of Food and Agriculture*".

- **Capítulo 04:** Apresenta um artigo original com os resultados da suplementação de óleo de linhaça e produtos naturais com potencial antioxidante sobre o perfil lipídico da carne *in natura* e toucinho, teor de tocoferol na carne *in natura* e estabilidade oxidativa da gordura. Também são apresentados os resultados dos testes realizados ao longo do armazenamento congelado por seis meses, de mini-hambúrgueres produzidos a partir da carne e gordura dos animais experimentais, foram avaliados a estabilidade oxidativa (TBARS), a cor e a aceitabilidade dos produtos. O artigo será submetido para avaliação na revista *Food Research International*.

Após os quatro capítulos, segue uma discussão geral, cujo objetivo é integrar e relacionar o que foi abordado ao longo da tese. No final, os autores apresentam as principais conclusões do trabalho, bem como sugestões e indicações para trabalhos futuros.

2. OBJETIVOS

2.1. OBJETIVO GERAL

Determinar a eficiência da suplementação de óleo de linhaça e de produtos naturais com ação antioxidante na dieta de suínos, sobre a incorporação de ω-3 na carne e seus efeitos na estabilidade oxidativa, bem como nas propriedades funcionais tecnológicas e sensoriais.

2.1. OBJETIVOS ESPECÍFICOS

- Produção em planta piloto industrial de um hidrolisado de carcaça e cabeça de tilápia do Nilo e avaliação de sua atividade antioxidante *"in vitro"*;
- Avaliação da atividade antioxidante *"in vitro"* de subprodutos industriais: bagaço de uva e extrato de semente de uva;
- Avaliação de dietas experimentais contendo óleo de linhaça e produtos naturais com ação antioxidante (hidrolisado de carcaça e cabeça de tilápia do Nilo, bagaço de uva, extrato de semente de uva e vitamina E) sobre o desempenho, qualidade da carcaça de suínos e qualidade de carne;
- Determinar o efeito do óleo de linhaça na dieta de suínos sobre a incorporação de ácidos graxos ω-3 e sobre as características funcionais tecnológicas na carne;
- Avaliação da eficiência dos produtos naturais com ação antioxidante na dieta de suínos sobre a estabilidade oxidativa da gordura e de produto cárneo congelado por 6 meses, bem como sobre o status antioxidante no soro sanguíneo dos animais;
- Avaliar, por meio de análises sensoriais, se a suplementação dietética com os produtos naturais com ação antioxidante provoca alterações na aceitabilidade de um produto cárneo (mini-hambúrguer) armazenado congelado por seis meses.

3. CAPÍTULO 01

"ω-3 IN MEAT PRODUCTS: BENEFITS AND EFFECTS ON LIPID OXIDATIVE STABILITY"

Daniela Miotto Bernardi, Teresinha Marisa Bertol, Sérgio Bertelli Pflanzer Jr., Valdemiro Carlos Sgarbieri, Marise Aparecida Rodrigues Pollonio

Artigo de revisão de literatura publicado na revista "*Journal of the Science of Food and Agriculture*", volume 96, número 8 de junho de 2016, páginas 2620-2634.

ABSTRACT

Although ω-3 intake has been associated with numerous health benefits, its addition to certain food matrices, and in particular meat products, may involve various technological barriers influencing the final quality of the products. Lipid oxidation must be highlighted due to the modification of both the sensory characteristics and the shelf-life of meat products. In order to reduce the impact of chemical changes and promote oxidative stability, the use of natural antioxidants has gained ground due to the health and safety advantages linked to its effectiveness at reducing lipid oxidation. Many natural compounds have also been successfully tested in animal feed, in order to protect the raw meat materials and reduce the risk of lipid oxidation in processed products. This review aims to address the challenges and advantages of the incorporation of ω-3 fatty acids in raw meat materials and processed meat products, and to describe the use of different compounds to enhance lipid oxidative stability.
Keywords: ω-3 fatty acids, lipid oxidation, natural antioxidants, healthy meat products, lipid reformulation

3.1. INTRODUCTION

Most fatty acids may be synthesized in the body to undergo elongation and desaturation reactions. However, the precursors of the ω-6 and ω-3 families are defined as essential as they cannot be synthesized endogenously in humans and in some animals, due to a deficiency of certain desaturases able to insert double bonds between positions △12 and △15 (1).

Essential fatty acids may be modified by mammals and the enzymes involved in the elongation, desaturation and decarboxylation processes for both α-linolenic (ALA) and linoleic acid (LA) fatty acids are the same. Although these enzymes have a greater affinity for ω-3 fatty acids, a high amount of ω-6 may inhibit the conversion of ALA to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are the most biologically active ω-3 fatty acids (2).

The main benefits associated with EPA and DHA fatty acids are their anti-inflammatory properties (3,4), cardiovascular protective effects (5,6,7), reduction of the risk of depression and suicide (8,9), delaying of the onset of aging-associated neurological degeneration (10), and reduction of the risks for certain cancers (11). They also promote fetal development (12) and improve infant cognitive functions (13).

The ratio of ω-6 to ω-3 fatty acids in dietary intake has become of great significance in human nutrition because of those multiple health benefit and a ratio equal to or less than 5:1

has been recommended (14,15,16,17,18). However, a higher dietary intake of ω-6 has been observed over recent decades, resulting in a ω-6/ω-3 ratio higher than 20:1 (19,20), due to the low amounts of ω-3 found in food. This has led to a surge of interest in its enrichment in food products. As these compounds are highly susceptible to lipid oxidation, and particularly so in meat products, many parameters must be considered before ω-3 fatty acids may be added to foods in order to produce stable products that are acceptable to consumers.

This review addresses strategies for increasing ω-3 fatty acid content in meat and its derivatives, as well as the impact on the oxidative stability of meat products. Moreover, considering that oxidative preservation is an important aspect of consumer acceptability, the use of natural antioxidants as protective agents in meat and meat products will also be addressed.

3.2. STRATEGIES FOR LIPID REFORMULATION OF MEAT AND MEAT PRODUCTS

Meat and meat products are important sources of fat in the human diet; however the natural concentration of polyunsaturated fatty acids (PUFA) in red meat, especially the ω-3 family, is relatively low. Enser et al. (21), compared the lipid composition of beef, mutton, and pork, and found high amounts of saturated fat in all meat samples. In terms of PUFA, although low concentrations were observed for all samples, pork had a higher PUFA content than the other meats. The ω-6/ω-3 ratio in pork is however greater than those observed for the other red meats, thus the higher PUFA content in this meat is due to the presence of ω-6 fatty acid. Low ω-3 content in beef, mutton, pork, and even chicken meat was reported, so it is very important to implement strategies to increase the levels of this fatty acid in these meats, given its importance in the human diet.

By means of a metabolic pathway, meat's lipid profile may be modified by increasing the amount of certain fatty acids in the animals' diets, as well as changing the manufacturing processes (22). With regard to the lipid profile of meat products, reformulation strategies may be implemented mainly through the direct addition of fats and oils to the product of interest (23).

In both cases, there are many factors contributing to the success of the operation, as well as factors that may produce a negative impact. The main strategies used for lipid reformulation of meat and meat products are discussed below, as are the main factors influencing the process.

3.2.1. Increasing ω-3 in raw meat materials

Many factors affect the quantity and quality of lipids present in muscle tissues and raw meat materials. Breed, genotype, age and sex may influence lipid content, with animal feed the main factor affecting meat's lipid profile (22).

Tests have been ongoing for a number of years on the use of different lipid sources in animal diets aimed at modifying meat's lipid profile. One of the earliest studies in this area was by Ellis & Isbell (24), who increased ω-6 content in pigs' subcutaneous adipose tissue by including soybean in the animals' diets. In recent years, research has focused on increasing ω-3 fatty acid, conjugated linoleic acid (CLA), and certain monounsaturated fatty acids, especially among non-ruminants, due to their increased incorporation of fatty acids from their diet.

Matthews et al. (25) supplemented pigs' diets with ground flaxseed at concentrations of 0, 50 and 100 g kg^{-1}, resulting in an ALA concentration of 33.0, 205.4 and 327.8 g kg^{-1} of ether extract respectively, an ALA concentration in the muscle of 11, 33 and 39 g kg^{-1}, and a ω-6/ω-3 ratio of 7.2, 3.5, and 3.9, respectively. Turner et al. (26) also added flaxseed (0, 50 and 100 g kg^{-1}) to pigs' diets for 11 weeks, resulting in a ALA concentration in the loin of 4.1, 23.7, 61.5 g kg^{-1}, and a ω-6/ω-3 ratio of 7.19, 1.78, and 0.95, respectively.

Similar results were obtained by Kouba et al. (27) who added linseed to swine diets and compared the results to a control diet (based on soybean meal, barley, and wheat). The ALA content and ω-6/ω-3 ratio were 291 and 61 g kg^{-1}, and 1.1 and 7.0 for the modified and control diet, respectively. The meat had 30 g kg^{-1} of ALA after 60 days of supplementation, and a ω-6/ω-3 ratio of 3.0 when compared to the control group, with 6.5 g kg^{-1} of ALA and a ω-6/ω-3 ratio of 7.3. It is worth noting that the ω-6/ω-3 ratio for the control group was much lower than that recorded for the control groups in other studies, mainly due to the addition of a blend of soybean oil and tallow (20:80) as a lipid source to the diet.

Realini et al. (28) evaluated the effect of different sources of fat (tallow, high oleic acid sunflower oil, regular sunflower oil, linseed oil and a blend containing fats and oils including fish oil) in swine diets, at concentrations ranging from 97 to 110 g kg^{-1} depending on the lipid source. Animals fed higher quantities of oleic acid sunflower oil, sunflower oil, and flaxseed oil had higher contents of monounsaturated and polyunsaturated fatty acids, and the group fed flaxseed oil had a greater increase in ALA, followed by the group that ingested the blend (linseed oil + fish oil). It is worth noting that the group that ingested the blend (linseed oil + fish oil) had the highest concentrations of EPA and DHA.

Bertol et al. (29) supplemented swine diets in the finishing phase with soybean oil, canola oil, and a blend of canola oil and linseed oil at a concentration of 30 g kg^{-1} for each source, and had a significant increase in ω-3 fatty acids, particularly for the group supplemented with the blend containing canola oil and flaxseed oil. The lipid profile of the *Longissimus dorsi* revealed that the concentration of ALA were 11.6, 12.5 and 21.1 g kg^{-1} and the ω-6/ω-3 ratios were 14.61, 11.30, and 6.46, respectively. Partially replacing corn with rice bran (620 g kg^{-1} corn and 380 g kg^{-1} rice bran) in swine diets resulted in increased levels of ALA and LA in meat, as reported by Campos et al. (30). Other results also showed that dietary modification factors did not influence feed conversion, weight gain, and meat quality.

Missotten et al. (31) demonstrated that swine diets rich in ω-3 fatty acids may influence the incorporation of fatty acids in muscle tissues, indirectly affecting fatty acid composition by modulating lipogenic enzyme expression.

The addition of linseed oil and fish oil to chicken diets also increased the ω-3 fatty acid content and decreased the ω-6/ω-3 ratio in the meat (32,33). Anjum et al. (34) added extruded flaxseed to chicken diets and evaluated the meat's growth performance, lipid profile and oxidative stability. The authors concluded that the addition of extruded flaxseed to the amounts of 0, 50, 100, 150 g kg^{-1} resulted in a significant increase of ω-3 fatty acids in the meat, with ALA concentrations in leg meat of 39.8, 61.0, 84.1 and 95.2 g kg^{-1}, respectively.

Another test focused on the addition of linseed associated with sunflower oil to lamb diets (35). The authors found that the higher the proportion of linseed oil in the diet, the greater the ALA content in body fat, while higher CLA concentrations were observed in diets containing higher sunflower oil content, thus the (linseed and sunflower) oil blend promoted an increase of both ω-3 and CLA in the meat.

A review reported by Bas & Sauvant (36) discussed the main effects of different lipid sources in the composition of fatty acids among cattle, stating that animals fed with flaxseed had higher proportions of ALA when compared with those fed with other fat sources.

The addition of fish oil also resulted in an increase of ω-3 fatty acid in beef (37). These authors added a fish oil blend (anchovy, sardine and salmon) to heifer diets and recorded a significant increase in both EPA and DHA concentrations without changes to meat color. He et al. (38) evaluated the effects of six diets for confined cattle as follows: 0 oil seed, 100 g kg^{-1} flaxseed, and 100 g kg^{-1} sunflower seed, with or without the addition of 300 g kg^{-1} triticale. The authors reported an increase of ALA in diets containing flaxseed, which was higher for the diet with flaxseed + triticale. A significant increase in oleic acid was observed in diets containing sunflower seed (38).

Wachira et al. (39) compared the effect of dietary fat sources on the levels of ω-3 in muscle and adipose tissue in lambs, and found that the ALA content was higher in animals fed linseed oil, when compared with animals fed the control diet or a diet containing fish oil, while both the EPA and DHA concentrations were higher in animals fed fish oil. It is notable that animals fed linseed oil showed higher EPA and DHA concentrations than the control group, proving that ALA conversion into EPA and DHA does occur in ruminants, although to a lesser degree.

Table 1 displays the main studies designed to modify the lipid profile of animal tissues by changing the lipid profile of the animals' diets.

Table 1

Despite the fact that adding fish oil is more effective at increasing EPA and DHA in the carcass, terrestrial sources of ω-3 such as linseed are highly effective at increasing the ALA. The advantage of including terrestrial sources of ω-3 are due to the strong correlation between the addition of fish oil to animal diets and the development of off-flavor in meat, therefore many studies have used terrestrial sources of ω-3 because this reduces off-flavor, promoting better product acceptability (40). Juárez et al. (41), however, reported that increasing ω-3 levels in pork through dietary co-extruded flaxseed supplementation negatively affected the meat's texture and flavor.

Several studies have focused on determining the length of time required for supplementing lipid sources in animal diets to reach a maximum increment of ω-3 fatty acid in meat. Kouba et al. (27) added 60 g kg^{-1} crushed flaxseed to swine diets for 20, 60 and 100 days before slaughter, and found a lower ω-6/ω-3 ratio and a greater incorporation of ALA after a feeding period of 60 days. The best conversion of ALA to DHA was observed after 20 days of supplementation.

The length of the period of supplementation of lipid sources to diets in studies designed to manipulate the lipid profile of meat varies greatly. Some authors reported a period of supplementation ranging from 30 to 60 days (42, 29, 43, 28, 44), or from 60 to 100 days (45, 46, 47, 25, 48, 26) before slaughter for pigs. There is also significant variation in the period of supplementation for cattle and lambs; however it usually ranges from 40 to 100 days before slaughter (37, 35, 49, 50).

Production systems also influence the lipid composition of animal carcasses, although to a lesser extent than feed composition. Nilzen et al. (51) demonstrated that pigs reared in

extensive systems showed a higher content of unsaturated fat than meat from animals reared in intensive systems. Similarly, Tejerina et al. (52) found that meat from pigs reared in *Montanera* (outdoor) systems had higher PUFA as well as better color and tocopherol contents than meat from animals reared in intensive systems. Feeding is largely responsible for these results, as pigs reared outdoors usually have access to pasture, and under the *Montanera* system they also have access to *bellota*, a natural fruit of the oak tree, which serves as a great differential and confers special characteristics to the final product (52).

Wiecek et al. (53) evaluated the effect of feed restriction on growing and finishing pigs and found that animals under longer restriction periods had lower carcass fat content, with less saturated and more mono and polyunsaturated fat. Rauw et al. (54) also reported that high intake and low feed frequency resulted in an increase in the content of saturated fat on the carcass, as fatter animals generally have higher levels of saturated fat.

The percentage and type of oil added, the composition of the basal diet, the period of supplementation, the production system and the effect of feed restriction all influence carcass lipid composition, but these factors also vary greatly from one study to another, as do the results obtained. There is thus a real need for more studies and further discussion in the area, as many questions remain unanswered, such as the existence of interaction between the oil added to the diet or oil contained in the ingredients and the period of supplementation.

3.2.2. Increases in ω-3 fatty acid content in processed meat products

Meat products with a high ω-3 fatty acid content may be produced by using meat with higher levels of this fatty acid provenient from animals fed with special diets, as described previously, or by modifying the formulation of meat products through the addition of external fat sources such as vegetable or fish oils (23).

Oils used for increasing ω-3 in meat products have physicochemical characteristics that are different to animal fats, and therefore the processing conditions must be adjusted so that the reformulated product features the desired quality attributes. In some products, this is particularly challenging because the functional quality attributes of texture, stability of lipid oxidation, and therefore sensory properties may be affected. These oils may be incorporated by adding liquid, pre-emulsified or encapsulated oil to fresh, cooked or fermented products (23).

Many studies on the incorporation of ω-3 use pre-emulsified oils, which seems to be due to the challenges involved in adding these oils directly to different meat batters. In 1989, Park et al. (55) produced low-fat frankfurter sausages enriched with monounsaturated and ω-3

fatty acids, using fish oil and high oleic sunflower oil, and different fat sources have been used in formulations based on this study ever since.

Paneras et al. (56) produced frankfurter sausages containing pre-emulsified olive, corn, sunflower, and soybean oil. The results showed that the sausages made with soybean oil showed the lowest ω-6/ω-3 ratio, equal to 7.7. Cofrades et al. (57) also developed a formulation of frankfurters enriched with ω-3 and a natural antioxidant of olive oil known as hydroxytyrosol. Camara & Pollonio (58) used pre-emulsified linseed oil with sodium caseinate to partially replace pork fat in commercial formulations of bologna sausage, with treatments containing up to 39 g kg^{-1} of linseed oil producing satisfactory sensory scores and good technological properties.

Berasategi et al. (59) also succeeded in developing a new formulation of reduced-energy and reduced-fat bologna sausages, enriched with ω-3 and *Melissa officinalis* extract. The healthier product had 850 kcal kg^{-1}, 36 g kg^{-1} fat, 6 g kg^{-1} ALA and 4.4 g kg^{-1} DHA, an ω-6/ω-3 ratio of 0.4 and did not induce significant negative effects on sensory quality.

Salami with a higher ω-3 fatty acid content has been produced with pre-emulsified fish oil. Muguerza et al. (60) used fish oil extract, while Valencia et al. (61) used deodorized fish oil. The addition of pre-emulsified linseed oil has also proven effective in producing salami with higher levels of ω-3 (62).

More recently, Garcia-Iñiguez de Ciriano et al. (63) suggested a more significant alteration to salami formulations, containing high levels of ω-3 fatty acids and low sodium and higher calcium content. To increase ω-3 fatty acids, these authors partially replaced pork fat with pre-emulsified linseed oil, obtaining a healthier product with good sensory acceptability. In terms of the oxidative stability, no significant difference was observed when compared with the control group, since 0.2 g kg^{-1} BHA was added to the pre-emulsion of linseed oil.

Delgado-Pando et al. (64) developed a pork liver pâté enriched with ω-3 fatty acids by adding three different oil sources to the lipid emulsion; including olive oil (443.9 g kg^{-1}), linseed oil (378.7 g kg^{-1}), and fish oil (177.4 g kg^{-1}). The amount of oil emulsion varied between 0, 123, 246 g kg^{-1}. Formulations with a higher emulsion content had a higher increase of the ω-3 family, as well as lower lipid stability to oxidation.

Pietrasik et al. (65) evaluated the addition of rapeseed oil emulsion by means of the quality parameters of matured beef, and found that the emulsion not only increased the ω-3 content of the product, but also promoted lower shear force, higher juiciness and tenderness scores, as well as less perceived connective tissue. It is worth noting that the ω-3 content remained high even after cooking.

Meadus et al. (66) added 31 g kg^{-1} DHA oil to mixed brine and injected it into pork loins at 100 mL kg^{-1} to increase the ω-3 content of the meat. After grilling (at 205°C), the loins contained approximately 1.46 mg g^{-1} DHA.

Adding encapsulated oil was tested by Pelser et al. (67), who produced Dutch type salami by replacing 150 to 200 g kg^{-1} pork fat with linseed oil and encapsulated fish oil or canola and flaxseed pre-emulsified oil. Apart from a significant increase in ω-3 content, the authors found that the sensory characteristics of salami containing encapsulated oil were similar to the control.

Mairesse et al. (68) produced a cured ham with a higher ω-3 fatty acid content and low susceptibility to oxidation, by feeding pigs with a diet containing flaxseed associated with two antioxidants. The ham manufactured with meat from these animals had a significantly higher ω-3 content when compared with the ham produced with meat of animals fed the control diet. Furthermore, the lipid oxidation was lower in hams made with meat from animals supplemented with the flaxseed associated with the antioxidant agents.

Campos et al. (69) also obtained a product with higher ω-3 and ω-6 contents by using dietary modified meat. The authors partially replaced corn with rice bran, producing salami with the modified meat. The products showed higher ω-3 and ω-6 contents, and no differences in either sensory profile or texture were observed when compared with those produced with meat without modifications. Table 2 shows the main studies carried out to evaluate the lipid profile reformulation of processed meat products by adding lipid sources to the product matrix.

Table 2

The greatest challenge involved in incorporating ω-3 in animal tissues consists of determining the adequate level to which the precursor source of the fatty acid should be added, given that the amount varies significantly across different studies and that the levels of metabolic incorporation to the tissues depend on instrinic and extrinsic factors. These include the source added to the diet, the duration of the supplementation period, the animal species, the rearing system, animal health, and digestibility of the product. When the aim is to directly incorporate ω-3 into the meat product, however, the greatest challenge is maintaining the technological and sensory properties while the product is being processed and during its stay on the shelf. This involves an appropriate selection of parameters, including the concentration supported by the meat matrix before its stability is lost to lipid oxidation, emulsion stability,

textural properties and those related to color, aroma, flavor and microbiological safety, particularly for products including a stage featuring heat treatment.

3.2.3. Effects of processing conditions on ω-3 levels in meat products

Few studies have focused on evaluating the real increase of ω-3 fatty acids upon cooking and the effect of the different stages in the manufacture of meat products. Guillevic et al. (70) added 42 g kg^{-1} of extruded flaxseed to swine diets, obtaining an increase in ω-3 fatty acid content in both raw meat (chops) and processed products (sausages, pâtés, smoked bacon and cooked chops). When comparing the percentage of PUFA and ω-6/ω-3 ratio between fresh and cooked chops, it was also observed that heat treatment resulted in no significant changes to these parameters.

Martínez et al. (71) produced burgers with a better lipid profile by adding pre-emulsified olive oil, corn oil, and deodorized fish oil to the formulations. The authors found no significant difference to the ω-3 fatty acid content between the raw and cooked products.

Pietrasik et al. (65) injected canola oil emulsion into mature beef and studied the ω-3 fatty acid content upon cooking by means of two different methods: dry heat and wet heat. The results demonstrated that the heat treatments resulted in no significant changes to the ω-3 content of the meat. Meadus et al. (66) also registered high concentrations of DHA in the meat, even upon cooking.

Lee et al. (72), however, developed meat products with a high ω-3 content by adding 250 g kg^{-1} pre-emulsified algae oil into ground turkey, pork sausages, and restructured ham, assessing the effect of the processing and cooking stages on the stability of fatty acids. Antioxidants were also used (citrate, erythorbate and rosemary extract). The results revealed that heat treatment reduced between 31 and 15% of the ω-3, while storage did not affect the ω-3 fatty acid content in raw and cooked products, probably due to the addition of antioxidants to the formulations.

3.3. IMPACT OF THE ADDITION OF ω-3 FATTY ACIDS ON THE OXIDATIVE STABILITY OF MEAT PRODUCTS

The reformulation of meat products targeting an increase of ω-3 fatty acids is a technological challenge for the food industry, since it implies in a number of adverse effects impairing the acceptability and shelf-life of the processed products (40).

Rheological parameters such as consistency, texture, and viscosity are strongly affected by the addition of PUFA, because the fat may become liquid or softened at room temperature (73).

The oxidative stability of ω-3 enriched foods is also impacted, since the product is more susceptible to lipid oxidation due to the double bonds in the ω-3 fatty acid molecule, rendering them more accessible to free radicals and oxygen reactive species, resulting in damage to the product's sensory properties (74).

It is worth noting that protein oxidation in foods is directly affected by lipid oxidation, since the products of lipid oxidation react with proteins leading to their subsequent oxidation, primarily causing changes in aroma and color (75). The mechanisms of lipid oxidation and its effects on meat products are outlined below.

3.3.1. Lipid oxidation in meat products

Autoxidation of lipids and the generation of free radicals are natural phenomena in biological and food systems, and may be catalyzed by environmental factors and/or the presence of metals, enzymes and pigments. However, as mentioned briefly in the previous section, the presence of double bonds may promote lipid oxidation due to their high reactivity and lower chemical stability and strength (74), which strongly affects products containing a high ω-3 fatty acid content.

Lipid oxidation in meat products is commonly measured by the thiobarbituric acid reactive substances (TBARS) method, with the principle based on the fact that the products of primary oxidation mainly consist of hydroperoxides, which are quickly broken down into several substances reactive to the thiobarbituric acid, particularly carbonyls, with malondialdehyde the most important element (76). Tarladgis and co-workers (77) described a method using a distillation technique for the quantitative determination of malonaldehyde in meat, and this has become the most commonly used index of lipid oxidation (76).

In a study by Delgado-Pando et al. (64), an increase of ω-3 fatty acids in pork liver pâté strongly interfered with the product's oxidative stability, as higher TBARS values were observed for products with a higher ω-3 content, with these being more pronounced during storage and thus reducing the product's shelf-life.

The addition of flaxseed oil, flaxseed, camelina oil and camelina seed to lamb diets produced increased TBARS in meat, however the increased amount of lipid oxidation was not

high enough to cause sensory interferences, as no changes in the color, pH, and texture of the meat were observed (50).

Many authors have argued that there is interdependence between lipid oxidation and color oxidation in meat. Pigment oxidation can catalyze lipid oxidation and free radicals produced during lipid oxidation can oxidize iron atoms or denature myoglobin molecules, adversely affecting the color of meat products (78).

In a study developed by Anjum et al. (34) involving increasing the percentage of extruded linseed in chicken diets, it was found that the higher the ω-3 content in the meat, the higher the TBARS values. The amount of free radicals also increased, while color intensity of both breast and thigh was reduced. Nuggets produced with chest and leg meat from animals fed diets containing 100 and 150 g kg^{-1} extruded linseed (higher ω-3 increments in meat) also had lower acceptability, which was mainly attributed to aroma and flavor. Furthermore, increased storage time reduced acceptability primarily due to changes in aroma and flavor. Increasing the percentage of PUFA, and especially the ω-3 family, thus resulted in lower oxidative stability of both the fresh meat and the processed product, and a lower acceptability for nuggets (34). It has also been shown that lipid oxidation exerts a strong impact on the characteristics of flavor, aroma, color, shelf-life, functionality, and the nutritional quality of meat products (74).

Lipid oxidation also affects a food's nutritional value, resulting in vitamin loss and changes to essential fatty acids, as well as promoting the production of toxic compounds (62).

Strategies for avoiding or minimizing oxidation taking place in food include oxygen removal, inactivation of enzymes, and protection against light and metal ions (74). Many of these are thus applied during processing and entail the use of packaging to protect the product from the light, vacuum packaging or modified atmosphere, heat treatment, or the use of certain additives with antioxidant properties. An antioxidant agent may be defined as any substance that, when present at a low concentration in terms of the oxidizable substrate, effectively delays or inhibits this oxidation process. In foods, antioxidant agents reduce lipid oxidation and hence protein oxidation (79).

Nitrates and phosphates are two types of additive with antioxidant activity commonly used in meat products. Nitrates' antioxidant action is due to the formation of nitrosylmyoglobin, which forms n*itrosylhemochrome* during heating, blocking the catalytic activity of heme iron and thus preventing its release and initiation of oxidation. Nitrites also appear as a non-heme iron chelator and eventually as a copper and cobalt chelator (80). Phosphates' antioxidant properties derive from their ability to chelate ions of pro-oxidant metals (81) and to increase

pH and ionic strength (82), which induces conformation changes in myofibrillar proteins, partially inhibiting reactions between proteins and peroxidized lipids.

In addition to these components, the meat industry also uses additives known as synthetic antioxidants, such as butylatedhydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (83). The use of synthetic compounds has however been subject to concerns regarding safety, and thus the search for strategies that inhibit lipid oxidation and make meat products healthier and well accepted globally has increased considerably in recent years, especially in the form of research into natural antioxidants (84).

3.4. NATURAL ANTIOXIDANTS

The food industry, and in particular the meat industry, has conducted various studies to identify natural antioxidants which act either alone or synergistically with other additives that may be applied to products in order to reduce oxidation without harming the product's sensory characteristics.

Considering the importance of the consumption of PUFA and especially the ω-3 family, as well as these fatty acids' implications for lipid oxidation, this review will therefore discuss several natural products to have produced positive effects on inhibiting lipid oxidation in meat products. It should however be noted that although most of the studies presented in this review do not provide evidence of an increase of ω-3 fatty acids in meat or meat products, the results may be useful for studies aimed at manufacturing meat products with a high ω-3 fatty acid content and high oxidative stability.

3.4.1. Vitamin E

Vitamin E's antioxidant activity is due to its action as a potent peroxyl radical scavenger, meaning that when lipid hydroperoxides are oxidized to peroxyl radicals, these react 1000 times more quickly with vitamin E than PUFA, thus promoting considerable protection for these fatty acids (85).

Sales & Koukolová (85) performed a meta-analysis by using Mitscherlich's mathematical model to investigate the relationship between vitamin E intake vs. the concentration of vitamin E in muscles and the concentration of vitamin E in muscles vs. fat oxidation and stability of meat color. The meta-analysis was conducted by means of studies on cattle and pigs, with the vitamin E supplementation period in studies on cattle ranging from 38

to 285 days and the period in studies on pigs ranging from 21 to 109 days. They found that vitamin E accumulation in muscles produces a significant reduction in lipid oxidation, with the average concentration of vitamin E in the meat effective at reducing the production of TBARS 3.6 μg g^{-1} for cattle and 3.2 μg g^{-1} for pigs, thereby confirming the protective effect of this compound on meat, even after the animal's slaughter (85).

The same authors also found that an average supplementation of 810 IU/d for cattle and 393,8 IU/d for pigs provided an average accumulation of vitamin E in the meat of 3.5 μg g^{-1} and 3.6 μg g^{-1} for cattle and pigs, respectively. The results also revealed that a maximum concentration of vitamin E may be reached by means of supplementation in both pork and beef, as the concentration of the vitamin in muscle tissue will plateau even after an increase in supplementation (85).

Cardenia et al. (45) increased oleic acid content in pork by increasing the amount of oleic acid sunflower oil in the animals' diet, followed by supplementation with vitamin E in order to improve the lipid stability of the meat, which would have been harmed due to the increased amount of unsaturated fat. The results showed that adding vitamin E to the diet reduced lipid oxidation in meat.

Rivas-Cañedo et al. (86) added ω-3 fatty acids to lamb diets, assessing the effectivness of both vitamin E and polyphenol-rich wine extract added to the diet upon reducing lipid oxidation of beef refrigerated in an O_2 rich atmosphere for a period of 0 to 6 days. The authors found that vitamin E was more effective than the wine extract at reducing lipid oxidation.

3.4.2. Antioxidant compounds derived from fruit processing

Many fruits have been successfully tested as antioxidants in meat products, with their antioxidant power generally attributed to their polyphenolic compounds (87).

Grape has been extensively studied to determine its role in lipid preservation in meat products. Research mainly involving seed extracts has demonstrated that it can inhibit lipid oxidation in raw beef (88), chicken (89,90) and pork (92), as well as in cooked products such as ground beef (91, 93), precooked beef sausages (94), turkey breast (95), cooked pork (92,96) and precooked chicken breast (97).

Pateiro et al.(98) evaluated the shelf-life of refrigerated pork liver pâté with grape extract, green tea, and chestnut added to it. The results obtained for the TBARS index showed that grape and green tea extract were more effective than BHT, but that the chestnut showed no antioxidant effect. The same group also evaluated the antioxidant effect of grape, green tea,

chestnut and seaweed extracts in pork patties while stored for 20 days in modified atmosphere packs, observing that the most effective antioxidant was grape, followed by BHT and then green tea. The seaweed and chestnut extracts were not effective at increasing the product's oxidative stability (99). Despite the fact that chestnut did not show antioxidant activity in these studies, a previous study by Lorenzo et al. (100) concluded that chestnut extract had antioxidant activity in dry-cured sausages.

The grape seed extract was as effective as the synthetic antioxidant propyl gallate and more efficient than the rosemary oleoresin, BHA and BHT, respectively, at reducing the formation of TBARS in cooked, frozen and reheated beef (101). Under a model system, Kulkarni et al. (94) investigated pre-cooked, frozen, and reheated sausages, confirming the antioxidant effect of grape seed extract equivalent to propyl gallate and greater than ascorbic acid. Sasse et al. (102) compared the efficacy of grape seed extract, oregano extract, and rosemary extract at preventing lipid oxidation in cooked and frozen pork. The results confirmed grape seed extract's positive effect on lipid preservation.

Grape seed extract at concentrations above 10 g kg^{-1} altered the color of cooked beef, evidenced by CIELab measurements which showed that meat remained darker (L*), redder (a*) and less yellow (b*) when compared with the sample treated with BHA/BHT (91). No adverse effects were observed regarding color, aroma, or reheated flavor at concentrations equal to or below 2 g kg^{-1} (96). However, more studies are needed to assess the real impact of the concentration of this extract on the sensory characteristics of meat.

Along with the studies on grape seed extract, Selani et al. (103) also performed research in to waste produced by wineries containing seeds and skins. The authors compared the antioxidant effect of BHT with seed and skin extracts from Isabel and Niagara grapes. The study was performed on raw and cooked poultry meat stored in a freezer. The results showed that both types of grape extract were as effective as BHT in reducing the formation of TBARS, however negative changes to product color were observed, although these were less noticeable for the extracts from the Isabel grape. Özvural & Vural (104) also evaluated the effect of grape seed flour obtained from wine by-products as an antioxidant agent in frankfurter sausages, proving the effectiveness of the product.

In another study, Chamorro et al. (105) evaluated the effect of including phenolic compounds from grape pomace (0, 50 and 100 g kg^{-1}) and α-tocopheryl acetate (0.2 g kg^{-1}) in broiler chicken diets. The oxidative stability of the meat and the polyunsaturated fatty acid content increased with the dietary addition of α-tocopheryl acetate and grape pomace. Meat α-

tocopherol content was higher for the group with tocopherol in the diet and there was also a slight increase in the groups supplemented with grape pomace.

Studies have also been carried out on the use of plum (extract, purée and concentrated juice) as an antioxidant agent in meat products. Positive results were reported for cooked sausages (106), ham (107), roast beef (108), lean beef cuts (109) and turkey breast (110). Evidence on the stability of the antioxidant capacity during storage was also recorded (106), however the processed plum produced negative effects on color (110,107), and higher cooking loss and shear strength (107).

Cranberry has been widely studied due to its high levels of anthocyanins, which are phenolic compounds with a strong ability to inhibit lipid oxidation (111). Its ability to reduce lipid oxidation in meat products was confirmed by (112) by means of mechanically separated turkey meat and cooked ground pork. Raghavan & Richards (113) compared the lipid oxidation inhibition capacity of cranberry juice and cranberry cake extracts. The evaluation was performed on vacuum-packed mechanically separated turkey meat. The authors found that the juice powder was significantly effective at inhibiting lipid oxidation.

Larrain et al. (114) also evaluated cranberry's antioxidant effect in processed pork products, by feeding animals powdered juice concentrate. The results were inconclusive, however, with only a protective effect on the bacon color observed.

Blackcurrant is rich in anthocyanins and has proven antioxidant activity (115). Jia et al. (116) evaluated blackcurrant extract's activity in meat products, reporting a protective effect on lipid oxidation in refrigerated uncooked pork meatballs. At concentrations of 5 g kg^{-1}, the extract showed antioxidant activity similar to BHA (0.2 g kg^{-1}), and at concentrations of 10 and 20 g kg^{-1}, the antioxidant activity was higher than BHA (0.2 g kg^{-1}).

Pomegranate (in the form of seeds and peels) was also investigated for antioxidant activity in meat products. Studies demonstrated a reduction in lipid oxidation in fresh and cooked chicken (117,118, 119, 120) and raw and cooked goat meat (121). Naveena et al. (117) reported that the addition of both the peel powder and powdered juice had little effect on the sensory characteristics of the product. Kanatt et al. (120) demonstrated that the seed extract had no antioxidant effect, unlike the peel extract that was significantly effective at protecting against lipid oxidation.

Citrus extracts (lemon and orange) are also effective at maintaining lipid stability in beef (122), bologna sausage (123) and beef burger meat (124).

Hydroethanolic extract of persimmon (*Diospyros kaki L.)* positively inhibited TBARS in heat-treated ground chicken meat containing NaCl. Apart from its positive effects as an antioxidant agent, the fruit extract did not alter color parameters and acceptability (125).

Devatkal et al. (126) also evaluated the antioxidant properties of banana (*Musa paradisiaca*) and sapodilla (*Manilkara zapota*) peel extracts in chicken patties, with a 20 g kg^{-1} concentration recording a similar antioxidant activity to a 1 g kg^{-1} BHT concentration.

Lychee seed water extract was effective at slowing lipid oxidation of meat paste and improving the sensory properties of the product during the later stages of the storage period (127).

3.4.3. Condiments, leaves, herbs, and spices

The antioxidant properties of rosemary seasoning have been widely tested in meat products. Many studies have confirmed its efficacy in beef and raw pork (128), cooked beef (91,96,101,129), cooked pork (96,130,131), mechanically deboned turkey meat (132), frozen sausages (133), irradiated beef burgers (134,135), and liver pâté (136).

When added to lamb diets, rosemary extract was effective at promoting oxidative preservation of meat (137, 138). Rosemary extracts acted as an effective antioxidant when introduced to lamb diets, and also stabilized the sensory quality, TBARS value and color of cooked and chill-stored patties, without the addition of synthetic preservatives (139). Similarly, the addition of rosemary and α-tocopherol acetate to chicken diets revealed that both improved the oxidative stability of meat (140).

Despite many positive results, several commercially manufactured rosemary-based products such as oleoresin were not as effective at reducing lipid oxidation, resulting in various divergences in literature (132). More recently, Teruel et al. (141) confirmed that the type of extracting solvent influences rosemary's antioxidant activity. The group studied three kinds of rosemary extract (powder-acetone, liquid-methanol and liquid-acetone) and recorded the highest antioxidant activity for the powder-acetone extract followed by the liquid methanol and liquid acetone extracts.

Garlic's antioxidant activity has also been reported in literature, and has been attributed to the presence of sulfur compounds. Yin & Cheng (142) registered antioxidant and antimicrobial activities for certain garlic-derived organosulfur compounds in ground beef. In chicken sausages, the addition of garlic resulted in a reduction of TBARS (143). On the other hand, the addition of fresh garlic (10 g kg^{-1}) to irradiated and vacuum packaged steaks had a

pro-oxidant effect (144). The use of methanol and water extract of garlic in fresh pork resulted in a decrease in TBARS, an improvement in color and an antimicrobial effect (145). The addition of garlic powder to chicken diets significantly reduced TBARS in meat (146).

Mariutti et al. (147) evaluated the antioxidant effect of garlic and sage added to salted chicken meat that was heat treated and stored in a freezer. The results revealed that the addition of garlic did not result in antioxidant activity, unlike sage, which reduced lipid and cholesterol oxidation.

Adding 0.5 g kg^{-1} sage to pork burgers increased the stability of lipid oxidation (148). Estévez et al. (149) added 10 g kg^{-1} essential sage oil to pork liver pâté and found a protective effect on PUFA. Buffalo salami containing sage oil (0.25 g kg^{-1} and 0.50 g kg^{-1}) also induced significant reductions in TBARS and an improvement in the sensory characteristics (150). Fasseas et al. (151) demonstrated that sage's antioxidant action was more effective on cooked meat than raw meat.

Marjoram, rosemary, and sage extracts were also tested for inhibition of lipid oxidation and off-flavor in irradiated samples of ground pork loin. The results showed that both extracts were significantly effective, but marjoram extract showed a better antioxidant effect (152).

Green tea catechins have significant antioxidant power when compared to other natural antioxidants (153), with their action appearing to be dose dependent. Concentrations between 0.2 and 0.4 g kg^{-1} provided the best results for cooked and raw red meat and poultry (154,155), while concentrations greater than 1 g kg^{-1} reduced red color in raw pork (156). The addition of green tea to chicken diets (0.3 g kg^{-1}) also reduced lipid oxidation in meat (157). Mitsumoto et al. (154) reported that catechins' antioxidant power is due to its capacity to bind to the Fe-component of myoglobin.

Tea dregs are a waste product of tea processing. Zhao et al. (158) hydrolyzed and tested the antioxidant power of tea dreg hydrolyzate in chicken meat products, with the results showing strong antioxidant activity. The authors suggest that the enzymatic hydrolysis of this waste produces peptides/amino acids which stabilized or terminated the radicals through the donation of hydrogen.

Although oregano was also effective at reducing lipid oxidation in raw and cooked pork and beef (96,128,159), its antioxidant activity was lower than that observed for grape seed extract (102,96), and rosemary (135). Supplementation with 0.1 g kg^{-1} oregano oil and vitamin E in chicken diets reduced lipid oxidation in meat when compared to the control, and the effectiveness of both antioxidants was similar (160). In contrast, supplementation of swine diets with oregano oil did not promote an antioxidant effect on meat (161).

Cooked pork patties with olive leaf extract were produced with *Longissimus dorsi* muscle from pigs reared under a diet supplemented with linseed oil, with results demonstrating that the dietary linseed oil increased ALA in meat and the olive leaf extract reduced lipid and protein oxidation (162). In frankfurters enriched with ω-3 fatty acids, the effect of hydroxytyrosol was more effective than BHA/BHT at reducing TBARS production and increasing antioxidant activity in the product (57).

Basil and horseradish (Galangal) also demonstrated antioxidant activity *in vitro*, thus they were tested (in the form of powder and ethanol extract) in ground and cooked pork. The results showed that basil had a higher antioxidant effect than horseradish, which was potentiated by using the product in powder form (163).

Cinnamon (*Cinnamomum sp.)* was also effective at inhibiting reheated flavor in cooked pork, beef and lamb meat (164). Chan et al. (165) tested cinnamon bark deodorized aqueous extract in refrigerated chicken meatballs, with results showing that cinnamon improved the color and oxidative stability of meat without adversely affecting the sensory characteristics.

Cinnamon extract, grape seed, oregano, pomegranate peel, and clove showed antioxidant effects and antimicrobial activity in raw pork (166).

Curry also demonstrated antioxidant activity, with a concentration of 20 g kg^{-1} added to raw poultry seasoned with salt (20 g kg^{-1}) resulting in a significant decrease in TBARS upon 0, 2, 4, 6 and 8 days of refrigerated storage (167). Biswas et al. (168) also proved this ability to protect against lipid oxidation in refrigerated ground pork.

García-Iñiguez de Ciriano et al. (169) studied the addition of lemon balm (*Melissa officinalis L.*) as an antioxidant agent to salami containing a high ω-3 fatty acid content, and found that the seasoning was effective at inhibiting lipid oxidation. In a later study, the authors produced a lyophilized extract of lemon balm to evaluate the oxidative stability of oil/water emulsions rich in ω-3, concluding that the extract was as effective as BHA at inhibiting TBARS (170). The authors made bologna sausage meat with high ω-3 fatty acids, stabilizing lipid oxidation by adding lemon balm extract to the formulation (171).

The addition of commercial lemon balm extract to roast pork burger also conferred an antioxidant effect, although to a lesser degree than that observed for the commercial rosemary extract (131). The addition of lemon balm and a combination of hawthorn (*Crataegus oxyacantha L.*) and yarrow (*Achillea millefolium L.*) to chicken diets also conferred a protective effect, reducing lipid oxidation in meat (172).

Dried yerba mate (*Ilex paraguariensis*) extracts protected against lipid oxidation in poultry (173), with a concentration of 0.5 g kg^{-1} the best from a sensory point of view, associated

with the prevention of lipid oxidation (174). Beal et al. (175) also confirmed yerba mate extract's protective effect against lipid oxidation in Italian-style fermented sausages, finding that the extract inhibited TBARS without affecting the product's sensory characteristics.

Campos et al.(69) also observed a reduction in TBARS in salami containing yerba mate extract as an antioxidant agent, as well as a lower concentration of volatile compounds from oxidation, with a predominance of volatile compounds during fermentation.

Hydroethanolic extracts of yerba mate leaves were investigated by Milani et al. (125) in heat-treated ground chicken meat with salt. The authors found that despite preventing the formation of TBARS, from a sensory point of view the product was not well accepted by the judges due to changes in flavor and color. The addition of an aqueous extract in chicken diets in the finishing phase conferred a protective effect on meat lipids (176).

Peppers are also used as antioxidant agents in meat products. One study compared the antioxidant power of black pepper (*Piper guineense*) essential oil with rosemary oleoresin in raw and cooked ground pork, and found that black pepper oil was effective at reducing TBARS (177) . The addition of different species of the genus *Capsicum* also conferred an antioxidant effect in raw and cooked pork (178).

3.4.4. Other products with antioxidant activity

Many recent studies have focused on the use of flower extracts to reduce lipid oxidation in meat and meat products. Gallo et al. (179) studied *Echinacea angustifolia* supercritical extracts aimed at preventing lipid and protein oxidation in cooked and frozen chicken meatballs. The results revealed strong and specific antioxidant action.

Extract of edible lotus (*Nelumbo nucifera*) was tested on cooked and uncooked ground beef and pork, with the authors finding that both the leaf extract and the extract of rhizomes prevented lipid oxidation (180).

Dog rose (*Rosa canina* L.) is rich in polyphenols and ascorbic acid, and exhibited a protective effect on lipid oxidation when tested in pork sausages (181).

Certain protein isolates and their hydrolysates may also be used to prevent lipid oxidation in meat products. Soy protein isolate and whey proteins, for example, not only reduce cooking losses, but may also reduce lipid oxidation in meat products. Peña-Ramos & Xiong (182) evaluated both soy protein and whey isolates and their hydrolysates in preventing lipid oxidation in refrigerated pork meatballs. Lipid oxidation was measured according to the formation of TBARS and conjugated dienes. The authors concluded that both isolates

contributed to the reduction of lipid oxidation, with the hydrolysis only favoring the protective effect of the whey protein isolate.

Barbosa-Pereira et al. (183) evaluated the antioxidant activity of a natural extract obtained from residual waste from a brewery and compared it with a commercial rosemary extract and two synthetic antioxidants (BHT and propyl gallate). The antioxidants were added directly to beef samples and in active packaging films. They were effective in both forms, with the residual waste extract showing a similar antioxidant effect to propyl gallate and a more effective result than BHT and rosemary extract.

Honey is another food product to have been tested as an antioxidant agent in cooked meats. Positive results have been obtained regarding oxidative stability in chicken (184) and turkey (185). Sampaio et al. (186) evaluated the synergistic effect of oregano, sage, and honey in the prevention of lipid oxidation in cooked chicken breast and thigh refrigerated for 96 and 48 hours. The results confirmed the protective effect of all of the natural antioxidants tested, with good acceptability by consumers.

Natural antioxidants' synergistic effect was also verified by Krishnan et al. (187), in evaluating the antioxidant power of Dianthus (*Syzygium aromaticum*), cinammon, oregano, black mustard and their combinations in raw chicken meat. The results showed that all spice extracts feature a potential as a natural antioxidant, but the blend with Dianthus, cinnamon and oregano had a higher antioxidant effect.

As discussed above, although many studies have been conducted (Table 3) and many products have been shown to have an antioxidant effect on meat or meat products, more research is required in this field. It must be highlighted that before natural antioxidants (in the form of extracts, oil, flour or even the food in its whole format) may be added to meat products, several parameters must be carefully evaluated, such as: the form of extraction, the extractor agent and the concentration of the bioactive compound. The importance of the form of extraction and the extractor agent has been confirmed by recent studies on rosemary extract (141). The addition of natural antioxidants may also produce negative sensory effects on meat products (91,156,125), therefore this factor must also be considered.

Table 03

When adding natural antioxidants to animal diets (Table 04) with the aim of increasing meat's oxidative stability, it is not just the afore mentioned that must be considered, but also

the effects of digestion, absorption, dynamic incorporation in tissues and the mechanism of antioxidant action, as all of these factors may vary among species. Meat's lipid composition also varies greatly between species, heavily influencing its oxidative stability and meaning that these factors may directly influence the effect of the natural antioxidants.

Table 04

An understanding of the mechanism of activity of vitamin E as an antioxidant agent may aid and orient knowledge on the antioxidant activity of other products. Frank (188) suggested that one of the mechanisms of antioxidant activity in certain compounds (catechins, epicatechins and quercitins) is the increase of concentrations of vitamin E in the tissues, even without extra supplementation, therefore performing as a vitamin E economizer (188,105).

Considering the nutritional importance of incorporating ω-3 in meat and meat products, discussion on the effects of such incorporation on the physico-chemical and sensorial characteristics of the products is therefore vital in this context, with the oxidative stability of the products playing a key role, as well as strategies to increase it, such as the use of natural antioxidants. Several studies have therefore been launched simultaneously on the increase of ω-3 and the use of natural antioxidants in meat products (43,169,170,171,63,86,49), with very promising results, undoubtedly representing a strong trend for the meat and meat product industry in the years to come.

3.5. FINAL CONSIDERATIONS

Increasing ω-3 fatty acid consumption through foods is of utmost importance, thus strategies promoting the increase of this fatty acid in foods are gaining ground.

Meat and meat products are important sources of dietary fat, and hence the increase of ω-3 fatty acids in these products is particularly significant. Altering lipid profile may however lead to technological issues such as shelf-life reduction and lower lipid oxidation stability.

The use of antioxidant agents to prevent and inhibit these oxidative processes has been highly recommended. Many studies have been carried out to identify natural compounds with antioxidant effects including fruit-derived products, spices, flowers, and hydrolysates, for use in meat products. Due to promising results obtained in these studies and concerns about food safety, natural antioxidants have been recommended for use in industrial processes.

Despite the fact that many of these natural compounds have been proven to possess antioxidant effects, many questions must be asked before the meat industry will be able to incorporate both ω-3 and natural antioxidants into products. These issues include the limits to which these components may be added in order to produce beneficial effects; the best means of incorporation, whether by animal diet or directly into the processed meat products or even in both, and finally, the industrial viability and supply of natural antioxidants required to ensure the consistency of the implementation of such a reformulation of meat products.

The strategies described in the present review for enhancing the healthfulness of meat, such as increasing ω-3 fatty acid content and using natural antioxidants, should be conducted simultaneously, but studies with a focus on human health and wellness are still lacking on the subject.

3.6. REFERENCES

1. Moreira NX, Curi R and Mancini-Filho J. Fatty acids: review. *Nutrire* 24:105-123 (2002).
2. Vaz JS, Deboni F, Azevedo MJ, Gross JL and Zelmanovitz T. Ácidos graxos como marcadores biológicos da ingestão de gorduras. *Rev Nutr* 19(4):489-500 (2006).
3. Vedin I, Cederholm T, Freund Levi Y, Basun H, Garlind A, Faxén Irving G, Jönhagen ME, Vessby B, Wahlund LO and Palmblad J. Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the Omega study. *Am J Clin Nutr* 87(6):1616-1622 (2008)
4. Furuhjelm C, Warstedt K, Larsson J, Fredriksson M, Böttcher MF, Fälth-Magnusson K and Duchén K. Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy. *Acta Paediatr* 98(9):1461-1467 (2009).
5. Kris-Etherton PM, Harris WS and Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 106:2747-2757 (2002).
6. Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, Di Mascio R, Franzosi Mg, Geraci E, Levantesi G, Maggioni Ap, Mantini L, Marfisi Rm, Mastrogiuseppe G, Mininni N, Nicolosi Gl, Santini M, Schweiger C, Tavazzi L, Tognoni G, Tucci C, and Valagussa F. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of The Gruppo Italiano Per Lo Studio Della Sopravvivenza Nell'infarto Miocardico (Gissi)-Prevenzione. *Circulation* 105(16):1897-903 (2002).

7. Dawczynski C, Martin L, Wagner A and Jahreis G. n-3 LC-pufa-enriched dairy products are able to reduce cardiovascular risk factors: A double-blind, cross-over study. *Clin Nutr* 29(5):592-9 (2010).
8. Appleton KM, Hayward RC, Gunnell D, Peters TJ, Rogers PJ, Kessler D and Ness AR. Effects of n-3 long-chain polyunsaturated fatty acids on depressed mood: systematic review of published trials. *Am J Clin Nutr* 84(6):1308-1316 (2006).
9. Sublette ME, Hibbeln JR, Galfalvy H, Oquendo MA and Mann JJ.Omega-3 polyunsaturated essential fatty acid status as a predictor of future suicide risk. *Am J Psychiatry* 163(6):1100-2 (2006).
10. Dangour AD, Andreeva VA, Sydenham E and Uauy R. Omega 3 Fatty Acids and Cognitive Health in Older People.*Br J Nutr* 107(Suppl 2):S152-8 (2012)
11. Seo T, Blaner WS and Deckelbaum RJ. Omega-3 Fatty Acids: Molecular Approaches To Optimal Biological Outcomes. *Curr Opin Lipidol* 16(1):11-8 (2005).
12. Dunstan JA, Mitoulas LR, Dixon G, Doherty DA, Hartmann PE, Simmer K and Prescott SL. The effects of fish oil supplementation in pregnancy on breast milk fatty acid composition over the course of lactation: A randomized controlled trial. *Pediatr Res* 62(6):689-694 (2007).
13. Dunstan JA, Simmer K, Dixon G, and Prescott SL. Cognitive Assessment of Children at Age 2(1/2) Years After Maternal Fish Oil Supplementation in Pregnancy: A Randomised Controlled Trial. *Arch Dis Child Fetal Neon* 93(1):F45-50 (2008)
14. Simopoulos AP, Leaf A and Salem N. Essentiality and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Ann Nutr Metabol* 43(3):127-30 (1999).
15. Schaefer EJ. Lipoproteins, nutrition, and heart disease. *Am J Clin Nutr* 75(2):191-212 (2002).
16. Chardigny JM, Bretillon L and Sébédio JL. New insights in health effects of trans alpha-linolenic acid isomers in humans. *Eur J Lipid Sci Technol* 103(7):478-82 (2001).
17. Kris-Etherton PM, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G and Etherton TD. Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr* 71(1 Suppl):179-188 (2000).
18. World Health Organization. Joint Consultation: fats and oils in human nutrition. *Nutr Rev* 53(7):202-205 (1995)
19. Simopoulos AP. Omega-6/Omega-3 essential fatty acid ratio and chronic diseases. *Food Rev Inter* 20(1):77-90 (2004).

20. Simopoulos AP. Omega-3 fatty acids in wild plants, nuts and seeds. *Asia Pacific J Clin Nutr* 11(S6):S163–S173 (2002)
21. Enser M, Hallett K, Hewett B, Fursey GAJ and Wood JD. Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Sci* 44:443-458 (1996).
22. Rosenvold K and Andersen HJ. Factors of significance for pork quality-a review. *Meat Sci* 64:219–237 (2003)
23. Jiménez-Colmenero F. Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats.*Trends Food Sci Techn* 18:567-578 (2007)
24. Ellis NR and Isbell HS. Soft pork studies. The effect of food fat upon body fat, as shown by the separation of the individual fatty acids of the body fat. *J Biol Chem* 69:239-248 (1926).
25. Matthews KR, Homer DB, Thies F and Calder PC. Effect of whole linseed (*Linum usitatissimum*) in the diet of finishing pigs on growth performance and on the quality and fatty acid composition of various tissues. *Br J Nutr* 83(6):637-43 (2000).
26. Turner TD, Mapiye C, Aalhus JL, Beaulieu AD, Patience JF, Zijlstra RT, and Dugan MER. Flaxseed fed pork: n−3 fatty acid enrichment and contribution to dietary recommendations. *Meat Sci* 96:541-547 (2014)
27. Kouba M, Enser M, Whittington FM, Nute GR and Wood JD. Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. *J Anim Sci* 81(8):1967-79 (2003).
28. Realini CE, Duran-Montgé P, Lizardo R, Gispert M, Oliver MA and Esteve-Garcia E. Effect of source of dietary fat on pig performance, carcass characteristics and carcass fat content, distribution and fatty acid composition. *Meat Sci* 85(4):606-612 (2010)
29. Bertol TM, de Campos RM, Ludke JV, Terra NN, de Figueiredo EA, Coldebella A, dos Santos Filho JI, Kawski VL and Lehr NM. Effects of genotype and dietary oil supplementation on performance, carcass traits, pork quality and fatty acid composition of backfat and intramuscular fat. *Meat Sci* 93(3):507-16 (2013)
30. Campos RML, Hierro E, Ordóñez JÁ, Bertol TM and Hoz L. A note on partial replacement of maize with rice bran in the pig diet on meat and backfat fatty acids. *J Anim Feed Sci*15:427-433 (2006)
31. Missotten J, De Smet S, Raes K and Doran O. Effect of supplementation of the maternal diet with fish oil or linseed oil on fatty-acid composition and expression of δ5- and δ6-desaturase in tissues of female piglets. *Animal* 3(8):1196-204 (2009)

32. López-Ferrer S, Baucells MD, Barroeta AC and Grashorn MA. N-3 enrichment of chicken meat. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil. *Poult Sci* 80:741-752 (2001).
33. Crespo N and Esteve-Garcia E. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. *Poult Sci* 80:71-78 (2001).
34. Anjum FM, Haider MF, Khan MI, and Sohaib M. Impact of extruded flaxseed meal supplemented diet on growth performance, oxidative stability and quality of broiler meat and meat products. *Lipids health dis* DOI:10.1186/1476-511X-12-13 (2013)
35. Jerónimo E, Alves SP, Prates JA, Santos-Silva J and Bessa RJ. Effect of dietary replacement of sunflower oil with linseed oil on intramuscular fatty acids of lamb meat. *Meat Sci* 83(3):499-505 (2009).
36. Bas PandSauvant D. Variations de la composition des dépôts lipidiques chez les bovins. *INRA Prod Anim* 14(5):311-322 (2001)
37. Dunne PG, Rogalski J, Childs S, Monahan FJ, Kenny DA and Moloney AP. Long chain n-3 polyunsaturated fatty acid concentration and color and lipid stability of muscle from heifers offered a ruminally protected fish oil supplement. *J Agric Food Chem* 11;59(9):5015-25 (2011).
38. He ML, McAllister TA, Hernandez-Calva LM, Aalhus JL, Dugan MER and McKinnon JJ. Effect of dietary inclusion of triticale dried distillers' grain and oilseeds on quality and fatty acid profile of meat from feed lot steers. *Meat Sci* 97:76-82 (2014)
39. Wachira AM, Sinclair LA, Wilkinson RG, Enser M, Wood JD and Fisher AV. Effects of dietary fat source and breed on the carcass composition, n-3 poly-unsaturated fatty acid and conjugated linoleic acid content of sheep meat and adipose tissue. *Br J Nutr* 88:697-709 (2002)
40. Kouba M and Mourot J. A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. *Biochimie* 93(1):13-17 (2011).
41. Juárez M, Dugan MER, Aldai N, Aalhus JL, Patience JF, Zijlstra RT, and Beaulieu AD. Increasing omega-3 levels through dietary co-extruded flaxseed supplementation negatively affects pork palatability. *Food Chem* 126:1716-1723 (2011)
42. Alonso V, Najes LM, Provincial L, Guillén E, Gil M, Roncalés P, Beltrán JA. Influence of dietary fat on pork eating quality. *Meat Sci* 92(4):366-373 (2012).
43. Daza A, Rey AI, Ruiz J and Lopez-Bote CJ. Effects of feeding in free-range conditions or in confinement with different dietary MUFA/PUFA ratios and α-tocopheryl acetate,

on antioxidants accumulation and oxidative stability in Iberian pigs. *Meat Sci* 69(1):151-63 (2005).

44. Teye GA, Sheard PR, Whittington FM, Nute GR, Stewart A and Wood JD. Influence of dietary oils and protein level on pork quality. Effects on muscle fatty acid composition, carcass, meat and eating quality. *Meat Sci* 73(1):157-65 (2006).
45. Cardenia V, Rodriguez-Estrada MT, Cumella F, Sardi L, Della Casa G and Lercker G. Oxidative stability of pork meat lipids as related to high-oleic sunflower oil and vitamin E diet supplementation and storage conditions. *Meat Sci* 88(2):271-9 (2011).
46. Enser M, Richardson RI, Wood JD, Gill BP and Sheard PR. Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Sci* 55(2):201-12 (2000).
47. Mas G, Llavall M, Coll D, Roca R, Diaz I, Gispert M, Oliver MA and Realini CE. Carcass and meat quality characteristics and fatty acid composition of tissues from pietrain-crossed barrows and gilts fed an elevated monounsaturated fat diet. *Meat Sci* 85(4):707-14 (2010).
48. Mitchaothai J, Yuangklang C, Wittayakun S, Vasupen K, Wongsutthavas S, Srenanul P, Hovenier R, Everts H and Beynen AC. Effect of dietary fat type on meat quality and fatty acid composition of various tissues in growing-finishing swine. *Meat Sci* 76(1):95-101 (2007)
49. Luciano G, Pauselli M, Servili M, Mourvaki E, Serra A, Monahan FJ, Lanza M, Priolo A, Zinnai A and Mele M. Dietary olive cake reduces the oxidation of lipids, including cholesterol, in lamb meat enriched in polyunsaturated fatty acids. *Meat Sci* 93(3):703-14 (2013).
50. Moloney AP, Kennedy C, Noci F, Monahan FJ and Kerry JP. Lipid and colour stability of M. *longissimus* muscle from lambs fed camelina or linseed as oil or seeds. *Meat Sci* 92(1):1-7 (2012).
51. Nilzen V, Babol J, Dutta PC, Lundeheim N, Enfalt AC and Lundstrom K. Free range rearing of pigs with access to pasture grazing- effect on fatty composition and lipid oxidation products. *Meat Sci* 58:267-275 (2001).
52. Tejerina D, García-Torres S, de Vaca MC, Vázquez FM and Cava R. Study of variability in antioxidant composition and fatty acids profile of *Longissimus dorsi* and *Serratus ventralis* muscles from Iberian pigs reared in two different *Montanera* seasons. *Meat Sci* 90(2):414-9 (2012)

53. Więcek J, Rekiel A, Batorska M and Skomiał J. Effect of restricted feeding and realimentation periods on pork quality and fatty acid profile of M. *longissimus thoracis* . *Meat Sci* 87(3):244-9 (2011).
54. Rauw WM, Diaz I, Francés F, Corella D, Soler J, Tibau J and Gomez-Raya L The relationship between feed intake behaviour with intramuscular fat, cholesterol and fatty acid composition in pork. *J Anim Breed Genet* 129(4):289-97 (2012)
55. Park J, Rhee KS, Keeton JT and Rhee KC. Properties of low-fat frankfurters containing monounsaturated and omega-3 polyunsaturated oils. *J Food Sci* 54(3):500-504 (1989).
56. Paneras ED and Bloukas JG. Vegetable oils replace pork backfat for low-fat frankfurters. *JFood Sci* 59(4):725-728 (1994)
57. Cofrades S, Sandoval LS, Delgado-Pando G, López-López I, Ruiz-Capillas C and Jiménez-Colmenero F. Antioxidant activity of hydroxytyrosol in frankfurters enriched with n-3 polyunsaturated fatty acids. *Food Chem* 129:429-436 (2011)
58. Câmara AKFI and Pollonio MAR. Reducing animal fat in bologna sausage using pre-emulsified linseed oil: technological and sensory properties. *J Food Quality* 38:201-212 (2015)
59. Berasategi I, Navarro-Blasco I, Calvo MI, Cavero RY, Astiasarán I and Ansorena D. Healthy reduced-fat Bologna sausages enriched in ALA and DHA and stabilized with *Melissa officinalis* extract. *Meat Sci* 96:1185-1190 (2014)
60. Muguerza E, Ansorena D and Astiasaran I. Functional dry fermented sausages manufactured with high levels of n-3 fatty acids: nutritional benefits and evaluation of oxidation. *JSci Food Agricul* 84(9):1061-1068 (2004).
61. Valencia I, Ansorena D and Astiasaran I. Nutritional and sensory properties of dry fermented sausages enriched with n-3 PUFAs. *Meat Sci* 72(4):727-733 (2006).
62. Ansorena D and Astiasaran I. The use of linseed oil improves nutritional quality of the lipid fraction of dry-fermented sausages. *Food Chem* 87(1):69-74 (2004).
63. García-Íñiguez de Ciriano M, Berasategi I, Navarro-Blasco I, Astiasarán I and Ansorena D. Reduction of sodium and increment of calcium and ω-3 polyunsaturated fatty acids in dry fermented sausages: effects on the mineral content, lipid profile and sensory quality. *J Sci Food Agric* 15;93(4):876-81 (2013).
64. Delgado-Pando G, Cofrades S, Ruiz-Capillas C, Triki M and Jiménez-Colmenero F. Enriched n−3 PUFA/konjac gel low-fat pork liver pâté: Lipid oxidation, microbiological properties and biogenic amine formation during chilling storage. *Meat Sci* 92(4):762-7 (2012).

65. Pietrasik Z, Wang H and Janz JA. Effect of canola oil emulsion injection on processing characteristics and consumer acceptability of three muscles from mature beef. *Meat Sci* 93(2):322-8 (2013).
66. Meadus W, Turner TD, Dugan MER, Aalhus JL, Duff P, Rolland D, Uttaro B and Gibson LL. Fortification of pork loins with docosahexaenoic acid (DHA) and its effect on flavour. *J Anim Sci Biotechnol* 4:1-8
67. Pelser WM, Linssen JPH, Legger A and Houben JH. Lipid oxidation in n-3 fatty acid enriched Dutch style fermented sausages. *Meat Sci* 75(1):1-11 (2007).
68. Mairesse G, Benet M, Méteau K, Juin H, Durand D and Mourot J. Effect of plant antioxidant in n-3 polyunsaturated fatty acid–enriched diet on fatty acid composition and sensorial attributes of dry-cured ham. *Intern J Food Sci Techn* 46:2656-62 (2011)
69. Campos RML, Hierro E, Ordóñez JÁ, Bertol TM, Terra NN and Hoz L. Fatty acid and volatile compounds from salami manufactured with yerba mate (*Ilex paraguariensis*) extract and pork back fat and meat from pigs fed on diets with partial replacement of maize with rice bran. *Food Chem* 103:1159–1167 (2007)
70. Guillevic M, Kouba M and Mourot J. Effect of a linseed diet on lipid composition, lipid peroxidation and consumer evaluation of French fresh cooked pork meats. *Meat Sci* 81(4):612-8 (2009)
71. Martínez B, Miranda JM, Vázquez BI, Fente CA, Franco CM, Rodríguez JL and Cepeda A. Development of a Hamburger Patty with Healthier Lipid Formulation and Study of its Nutritional, Sensory, and Stability Properties. *Food Bioprocess Technol* 5:200-8 (2012)
72. Lee S, Hernandez P, Djordjevic D, Faraji H, Hollender R and Faustman C. Effect of antioxidants and cooking on stability of n-3 fatty acids in fortified meat products. *J Food Sci* 71(3):C233-C238 (2006b).
73. Warnants N, Van Oeckel MJ and Boucqué CV. Effect of incorporation of dietary polyunsaturated fatt acids in pork backfat on the quality of salami. *Meat Sci* 49(4):435-45 (1998).
74. Decker EA, Akoh CC and Wilkes RS. Incorporation of (N-3) Fatty Acids in Foods: Challenges and Opportunities. *J Nutr* 142(3):610s-613s (2012).
75. Viljanen K, Kivikari R and Heinonen M. Protein-lipd interations during liposome oxidation with added antocyanin and other phenolic compounds. *J Agric Food Chem* 52:1104-11 (2004)

76. Osawa CC, Felício PE and Gonçalves LAG. Teste de TBA aplicado a carnes e derivados: métodos tradicionais, modificados e alternativos. *Quim Nova* 28(4): 655-663 (2005)
77. Tarladgis B, Watts BM, and Yonathan M. Distillation method for determination of malonaldehyde in rancidity food. *J Am Oil Chem Soc* 37:44-48 (1960).
78. O'Grady MN, Monahan FJ, Burke RM and Allen P. The effect of oxygen level and exogenous *α-tocopherol* on the oxidative stability of minced beef in modified atmosphere packs. *Meat Sci* 55:39-45 (2000)
79. Moreira EAM and Shami NJIE. Licopeno como agente antioxidante. *Rev Nutri* 17(2):227-236 (2004)
80. Morrissey PA and Tichivangana JZ. The antioxidant activities of nitrite and nitrosyl-myoglobin in cooked meats. *Meat Sci* 14:175-90 (1985)
81. Tims MJ and Watts BM. Protection of cooked meats with phosphates. *Food Technol* 12:240-243 (1958)
82. Miller MF, David GW, Seidemann SC, Ramsey CB and Rolan TL. Effects of various phosphates on the palatability, appearance, and storage traits of flaked and formed restructured beef steaks. *J Food Sci* 51:1435-1438 (1986)
83. Decker EA and Xu Z. Minimizing rancidity in muscle foods. *Food Tecnol* 52(10):54-59 (1999).
84. Melo EA, Mancini-Filho J, Guerra NB and Maciel GR. Atividade antioxidante de extratos de coentro (*Coriandrum sativum L.*). *Ciênc Tecn Alimen* 23:195-199 (2003)
85. Sales J and Koukolová V. Dietary vitamin E and lipid and color stability of beef and pork: Modeling of relationships. *J Anim Sci* 89(9):2836-2848 (2011).
86. Rivas-Cañedo A, Apeleo E, Muiño I, Pérez C, Lauzurica S, Pérez-Santaescolástica C, Díaz MT, Cañeque V and Fuente J. Effect of dietary supplementation with either red wine extract or vitamin E on the volatile profile of lamb meat fed with omega-3 sources. *Meat Sci* 93:178–186(2013)
87. Zuo Y, Wang C and Zhan J. Separation, characterization, and quantitation of benzoic and phenolic antioxidants in American cranberry fruit by GC– MS. *J Agric Food Chem* 50:3789-3794 (2002)
88. Bañón S, Diaz P, Rodriguez M, Garrido MD and Price A. Ascorbate, green tea and grape seed extracts increase the shelf-life of low sulphite beef patties. *Meat Sci*. 77:626-633 (2007)

89. Lau DW and King AJ. Pre- and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. *J Agric Food Chem* 51:1602-1607 (2003).
90. Brannan RG. Effect of grape seed extract on descriptive sensory analysis of ground chicken during refrigerated storage. *Meat Sci* 81(4):589-95 (2009).
91. Ahn J, Grun IU and Mustapha A. Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. *Food Microbiol* 24:7-14 (2007)
92. Carpenter R, O'Grady MN, O'Callaghan YC, O'Brien NM and Kerry JP. Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Sci* 76:604–610 (2007).
93. Ahn J, Grun IU and Fernando LN. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *J Food Sci* 67:1364-1369 (2002)
94. Kulkarni S, DeSantos FA, Kattamuri S, Rossi SJ and Brewer, MS. Effect of grape seed extract on oxidative, color and sensory stability of pre-cooked, frozen, re-heated beef sausage model system. *Meat Sci* 88:139-144 (2011).
95. Mielnik MB, Olsen E, Vogt G, Adeline D and Skrede G. Grape seed extract as antioxidant in cooked, cold stored turkey meat. *LWT–Food Sci Techn* 39:191–198 (2006)
96. Rojas MC and Brewer MS. Effect of natural antioxidants on oxidative stability of cooked, refrigerated beef and pork. *J Food Sci* 72:S282–S288 (2007).
97. Brannan RG. Effect of grape seed extract on physicochemical properties of ground, salted, chicken thigh meat during refrigerated storage at different relative humidity levels. *J Food Sci* 73:C36–C40 (2008).
98. Pateiro M, Lorenzo JM, Amado IR and Franco D. Effect of addition of green tea, chestnut and grape extract on the shelf-life of pig liver patê. *Food Chem* 147:386-394 (2014)
99. Lorenzo JM, Sineiro J, Amado IR and Franco D. Influence of natural extracts on the shelf-life of modified atmosphere-packaged pork patties. *Meat Sci* 96:526-534 (2014)
100. Lorenzo JM, González-Rodríguez RM, Sánchez M, Amado IR and Franco D. Effects of natural (grape seed and chestnut extract) and synthetic antioxidants (*buthylatedhydroxytoluene*, BHT) on the physical, chemical, microbiological and sensory characteristics of dry cured sausage "*chorizo*". *Food Res Int* 54:611-620 (2013)

101. Colindres P and Brewer MS. Oxidative stability of cooked, frozen, reheated beef patties: effect of antioxidants. *J Sci Food Agric* 30;91(5):963-8 (2011).

102. Sasse A, Colindres P and Brewer MS. Effect of natural and synthetic antioxidants on the oxidative stability of cooked, frozen pork patties. *J Food Sc* 74(1):S30-5 (2009).

103. Selani MM, Contreras-Castillo CJ, Shirahigue LD, Gallo CR, Plata-Oviedo M and Montes-Villanueva ND. Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage. *Meat Sci* 88(3):397-403 (2011).

104. Özvural EB and Vural H. Grape seed flour is a viable ingredient to improve the nutritional profile and reduce lipid oxidation of frankfurters. *Meat Sci* 88(1):179-83 (2011).

105. Chamorro S, Viveros A, Rebolé A, Rica BD, Arija I and Brenes A. Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace. *Food Res Intern* http://dx.doi.org/10.1016/j.foodres.2014.11.054 (2014)

106. Nuñez de Gonzalez MT, Boleman RM, Miller RK, Keeton JT and Rhee KS. Antioxidant Properties of Dried Plum Ingredients in Raw and Precooked Pork Sausage. *J Food Sci* 73(5):H63-71 (2008).

107. Nuñez de Gonzalez MT, Hafley BS, Boleman RM, Miller RM, Rhee KS and Keeton JT. Qualitative effects of fresh and dried plum ingredients on vacuum-packaged, sliced hams. *Meat Sci* 83(1):74-81 (2009).

108. Nuñez de Gonzalez MT, Hafley BS, Boleman RM, Miller RK, Rhee KS and Keeton JT. Antioxidant properties of plum concentrates and powder in precooked roast beef to reduce lipid oxidation. *Meat Sci* 80(4):997-1004 (2008).

109. Yildiz-Turp G and Sedaroglu M. Effects of using plum puree on some properties of low fat beef patties. *Meat Sci* 86:896-900 (2010).

110. Lee EJ and Ahn DU. Quality characteristics of irradiated turkey breast rolls formulated with plum extract. *Meat Sci* 71:300-30 (2005)

111. Vinson JA, Su XH, Zubik L and Bose P. Phenol antioxidant quantity and quality in foods: Fruits. *J Agric Food Chem* 49(11):5315–5321 (2001).

112. Lee CH, Reed JD and Richards MP. Ability of various polyphenolic classes from cranberry to inhibit lipid oxidation in mechanically separated turkey and cooked ground pork. *J Muscle Foods* 17:248-266 (2006a).

113. Raghavan S and Richards MP. Comparison of solvent and microwave extracts of cranberry press cake on the inhibition of lipid oxidation in mechanically separated turkey. *Food Chem* 102:818-826 (2007).

114. Larrain RE, Krueger CG, Richards MP and Reed JD. Color changes and lipid oxidation in pork products made from pigs fed with cranberry juice powder. *J Muscle Foods* 19:17-33 (2008).

115. Wu QK, KoponenJM, Mykkänen HM and Törrönen AR. Berry phenolic extracts modulate the expression of p21 WAF1 and Bax but not Bcl- 2 in HT-29 colon cancer cells. *J Agric Food Chem* 55:1156-1163 (2007)

116. Jia N, Kong B, Liu Q, Diao X and Xia X. Antioxidant activity of blackcurrant (*Ribes nigrum L.*) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. *Meat Sci* 91(4):533-9 (2012).

117. Naveena BM, Sen AR, Kingsly RP, Singh DB and Kondaiah N. Antioxidant activity of pomegranate rind powder extract in cooked chicken patties. *Intern J Food Sci Techn* 43:1807-1812 (2008).

118. Naveena BM, Sen AR, Vaithiyanathan S, Babji Y and Kondaiah N. Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. *Meat Sci* 80:304-308 (2008).

119. Vaithiyanathan S, Naveena BM, Muthukumar M, Girish PS and Kondaiah N. Effect of dipping in promegranate (*Punica granatum*) fruit juice phenolic solution on the shelf-life of chicken meat under refrigerated storage (4°C). *Meat Sci* 88:409-414 (2011).

120. Kanatt SR, Chander R and Sharma A. Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf-life of chicken products. *Intern J Food Sci Techn* 45:216-222 (2010)

121. Devatkal SK, Narsaiah K and Borah A. Antioxidant effect of extracts of kinnow rind, pomegranate rind and seed powder in cooked goat meat patties. *Meat Sci* 85:155-159 (2010).

122. Fernández-López J, Zhi N, Aleson-Carbonell L, Pérez-Alvarez JA and Kuri K. Antioxidant and antibacterial activities of natural extracts application in beef meatballs. *Meat Sci* 69:371-380 (2005)

123. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J and Pérez-Alvarez JA. Effect of adding citrus fibre washing water and rosemary essential oil on the quality characteristics of a bologna sausage. *LWT- Food Sci Techn* 43:958-963 (2010)

124. Aleson-Carbonell L, Fernández-López J, Pérez-Alvarez JA and Kuri K. Characteristics of beef burger as influenced by various types of lemon albedo. *Innov Food Sci Emerg Technol* 6:247-255 (2005)

125. Milani LIG, Terra NN, Fries LLM, Rezer APS, Ferreira SF, Cichoski AJ and Valente CRF. Oxidação lipídica, características sensoriais e cor da carne de frango adicionada de extratos de caqui (*Diospyros kaki, L.*) e submetida a tratamento térmico. *Braz J Food Technol* 13(4)-242-250 (2010)

126. Devatkal SK, Kamboj R and Paul D. Comparative antioxidant effect of BHT and water extracts of banana and sapodilla peels in raw poultry meat. *J Food Sci Technol* 51:387-391 (2014)

127. Qi S, Huang H, Huang J, Wang Q and Wei Q. Lychee (*Litchi chinensis Sonn.*) seed water extract as potential antioxidant and anti-obese natural additive in meat products. *Food Control* 50:195-201 (2015)

128. Rojas MC and Brewer MS. Effect of natural antioxidants on oxidative stability of frozen, vacuum-packaged beef and pork. *J Food Quality* 31:173-188 (2008)

129. Bozkurt H and Belibaglı KB. Use of rosemary and *Hibiscus sabdariffa* in production of kavurma, a cooked meat product. *J Sci Food Agric* 89:1168–1173 (2009)

130. Nissen LR, Byrne DV, Bertelsen G and Skibsted LH. The antioxidative activity of plant extracts in cooked pork patties as evaluated by descriptive sensory profiling and chemical analysis. *Meat Sci* 68:485-495 (2004).

131. Lara MS, Gutierrez JI, Timón M and Andrés AI. Evaluation of two natural extracts (*Rosmarinus officinalis L.* and *Melissa officinalis L.*) as antioxidants in cooked pork patties packed in MAP. *Meat Sci* 88(3):481-488 (2011).

132. Mielnik MB, Aaby K and Skrede G. Commercial antioxidants control lipid oxidation in mechanically deboned turkey meat. *Meat Sci* 65:1147-1155 (2003)

133. Sebranek JG, Sewalt VJH, Robbins KL and Houser TA. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. *Meat Sci* 69:289-296 (2005).

134. Trindade RA, Mancini-Filho J and Villavicencio ALCH. Effects of natural antioxidants on the lipid profile of electron beamirradiated beef burgers. *Europ J Lipid Sci Techn* 111(11):1161-1168 (2009)

135. Trindade RA, Mancini-Filho J and Villavicencio ALCH. Natural antioxidants protecting irradiated beef burgers from lipid oxidation. *LWT-Food Sci Techn* 43:98104 (2010)

136. Doolaege EHA, Vossen E, Raes K, Meulenaer BD, Verhé R, Paelinck H and Smet SD. Effect of rosemary extract dose on lipid oxidation, colour stability and antioxidant concentrations, in reduced nitrite liver pâtés. *Meat Sci* 90:925-931 (2012)

137. Nieto G, Estrada M, Jordán MJ, Garrido MD and Bañón S. Effects in ewe diet of rosemary by-product on lipid oxidation and the eating quality of cooked lamb under retail display conditions. *Food Chem* 124:1423-1429 (2011)

138. Bañón S, Méndez L and Almela E. Effects of dietary rosemary extract on lamb spoilage under retail display conditions. *Meat Sci* 90(3):579-83 (2012).

139. Serrano R, Ortuño J and Bañón S. Improving the Sensory and Oxidative Stability of cooked and Chill-Stored Lamb Using Dietary Rosemary Diterpenes. *J Food Sci* 79:1805-1810 (2014)

140. Lee SM, Park WH and Kim K. Effects of Dietary Supplementation with Rosemary and *α–tocopherol acetate* on Performance and Meat Quality of Chicken Meat during Refrigerated Storage. *Korean J Food Sci Ani Resour* 30(3):472-478 (2010)

141. Teruel MR, Garrido MD, Espinosa MC and Linares MB. Effect of different format-solvent rosemary extracts (*Rosmarinus officinalis)* on frozen chicken nuggets quality. *Food Chem* 172:40-46 (2015)

142. Yin MC and Cheng WS. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. *Meat Sci* 63(1):23-28 (2003).

143. Sallam KI, Ishloroshi M and Samejima K. Antioxidant and antimicrobial effects of garlic in chicken sausage. *LWT-Food Sci Technol* 37:849–855 (2004).

144. Wong PYY and Kitts DD. The effects of herbal pre-seasoning on microbial and oxidative changes in irradiated beef steaks. *Food Chem* 76:197-205 (2002).

145. Park SY and Chin KB. Evaluation of pre-heating and extraction solvents in antioxidant and antimicrobial activities of garlic, and their application in fresh pork patties. *Intern J Food Sci Techn* 45:365-373 (2010)

146. Choi IH, Park WY and Kim YJ. Effects of dietary garlic powder and *α-tocopherol* supplementation on performance, serum cholesterol levels, and meat quality of chicken. *Poult Sci* 89(8):1724-31 (2010).

147. Mariutti LR, Nogueira GC and Bragagnolo N. Lipid and Cholesterol Oxidation in Chicken Meat are Inhibited by Sage but not by garlic. *J Food Sci* 76(6):C909-15(2011).

148. McCarthy TL, Kerry JP, Kerry JF, Lynch PB and Buckley DJ. Evaluation of the antioxidant potential of natural food/plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. *Meat Sci* 58(1):45-52 (2001).

149. Estévez M, Ventanas S and Cava R. Effect of natural and synthetic antioxidants on protein oxidation and colour and texture changes in refrigerated stored porcine liver pâté.*Meat Sci* 74(2):396-403 (2006)

150. Salem FMA and Ibrahim HM. Dry fermented buffalo sausage with sage oil extract: Safety and quality. *Grasas aceites* 61(1):76-85 (2010)

151. Fasseas MK, Mountzouris KC, Tarantilis PA, Polissiou M and Zervas G. Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chem* 106:1188–1194 (2007)

152. Mohamed HM, Mansour HA and Farag MD. The use of natural herbal extracts for improving the lipid stability and sensory characteristics of irradiated ground beef. *Meat Sci* 87(1):33-9 (2011).

153. Sullivan CM, Lynch AM, Lynch PB, Buckley DJ and Kerry JP. Assessment of the antioxidant potential of food ingredients in fresh, previously frozen and cooked chicken patties. *Intern J Poultry Sci* 3(5):337-344 (2004)

154. Mitsumoto M, O'Grady MN, Kerry JP and Buckley DJ. Addition of tea catechins and vitamin C on sensory evaluation, colour and lipid stability during chilled storage in cooked or raw beef and chicken patties. *Meat Sci* 69:773−779 (2005).

155. Tang SZ, Kerry JP, Sheehan D, Buckley DJ and Morrisey PA. Antioxidative effect of added tea catechins on susceptibility of cooked red meat, poultry and fish patties to lipid oxidation. *Food Res Inter* 34:651-657 (2001)

156. Jo C, Son JH, Son CB and Byun MW. Functional properties of raw and cooked pork patties with added irradiated, freeze-dried green tea leaf extract powder during storage at 4°C. *Meat Sci* 64:13-17 (2003).

157. Tang SZ, Kerry JP, Sheehan D, Buckley DJ and Morrisey PA. Dietary tea catechins and iron-reduced lipid oxidation in chicken meat, liver and heart. *Meat Sci* 56:285-90 (2000).

158. Zhao L, Wang S and Huang Y. Antioxidant function of tea dregs protein hydrolysates in liposome–meat system and its possible action mechanism International. *J Food Sci Technol* 49:2299-2306 (2014)

159. Scramlin SM, Newman MC, Cox RB, Sepe HA, Alderton AL, O'Leary J and Mikel WB. Effects of oregano oil brine enhancement on quality attributes of beef

Longissimus dorsi and *Semimembranosus* muscles from various age animals. *J Food Sci* 75(2):S89-94 (2010).

160. Avila-Ramos F, Pro-Martínez A, Sosa-Montes E, Cuca-García JM, Becerril-Pérez CM, Figueroa-Velasco JL and Narciso-Gaytán C. Effects of dietary oregano essential oil and vitamin E on the lipid oxidation stability of cooked chicken breast meat. *Poult Sci* 91(2):505-11 (2012).

161. Simitzis PE, Symeon GK, Charismiadou MA, Bizelis JA and Deligeorgis SG. The effects of dietary oregano oil supplementation on pig meat characteristics. *Meat Sci* 84(4):670-6 (2010).

162. Botsoglou E, Govaris A, Ambrosiadis I, Fletourisc D and Papageorgioud G. Effect of olive leaf (*Olea europea L.*) extracts on protein and lipid oxidation in cooked pork meat patties enriched with n-3 fatty acids. *J Sci Food Agric* 94:227-234 (2014)

163. Juntachote T, Berghofer E, Siebenhandl S and Bauer F. The antioxidative properties of Holy basil and Galangal in cooked ground pork. *Meat Sci* 72(3):446-56 (2006).

164. Jayathilakan K, Sharma GK, Radhakrishna K and Bawa AS. Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-over-flavour in different species of meat. *Food Chemi* 105:908–916 (2007)

165. Chan KW, Khong NMH, Iqbal S, Ch'ng SE, Younas U and Babji AS. Cinnamon bark deodorised aqueous extract as potential natural antioxidant in meat emulsion system: a comparative study with synthetic and natural food antioxidants. *J Food Sci Technol.* DOI:10.1007/s13197-012-0818-5 (2012)

166. Shan B, Cai Y, Brooksb JD and Corke H. Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. *J Sci Food Agric* 89(11):1879-1885 (2009).

167. Devatkal SK, Thorat PR, Manjunatha M and Anurag RK. Comparative antioxidant effect of aqueous extracts of curry leaves, fenugreek leaves and butylated hydroxytoluene in raw chicken patties. *J Food Sci Technol* 49(6):781-785 (2012)

168. Biswas AK, Chatli MK and Sahoo J. Antioxidant potential of curry (*Murraya koenigii L.*) and mint (*Mentha spicata*) leaf extracts and their effect on colour and oxidative stability of raw ground pork meat during refrigeration storage. *Food Chemis* 133:467-472 (2012)

169. García-Iñiguez de Ciriano M, Larequi E, Rehecho S, Calvo MI, Cavero RY, Navarro-Blasco I, Astiasarán I and Ansorena D. Selenium, iodine, ω-3 PUFA and

natural antioxidant from *Melissa officinalis L.*: A combination of components from healthier dry fermented sausages formulation. *Meat Sci* 85(2):274-9 (2010a).

170. García-Iñiguez de Ciriano M, Rehecho S, Calvo MI, Cavero RY, Navarro I, Astiasarán I and Ansorena D. Effect of lyophilized water extracts of *Melissa officinalis* on the stability of algae and linseed oil-in-water emulsion to be used as a functional ingredient in meat products. *Meat Sci* 85(2):373-7 (2010b).

171. Berasategi I, Legarra S, de Ciriano MG, Rehecho S, Calvo MI, Cavero RY, Navarro-Blasco I, Ansorena D and Astiasarán I. "High in omega-3 fatty acids" bologna-type sausages stabilized with an aqueous-ethanol extract of *Melissa officinalis*. *Meat Sci* 88(4):705-11(2011).

172. Marcinčáková D, Čertík M, Marcinčák S, Popelka P, Šimková J, Klempová T, Petrovič V, Tučková M and Bača M. Effect of dietary supplementation of *Melissa officinalis* and combination of *Achillea millefolium* and *Crataegus oxyacantha* on broiler growth performance, fatty acid composition and lipid oxidation of chicken meat. *Italian J Anim Sci* 10(4):165-170 (2011)

173. Racanicci AMC, Danielsen B and Skibsted LH. Mate (*Ilex paraguariensis*) as a source of water extractable antioxidant for use in chicken meat. *Eur Food Res Technol* 227:255-260 (2008)

174. Racanicci AMC, Allesen-Holm BH and Skibsted LH. Sensory evaluation of precooked chicken meat with mate (*Ilex paraguariensis*) added as antioxidant. *Eur Food Res Technol* 229:277-280 (2009)

175. Beal P, Faion AM, Cichoski AJ, Cansian RL, Valduga AT, de Oliveira D and Valduga E. Oxidative stability of fermented Italian-type sausages using mate leaves *(Ilex paraguariensis St. Hil)* extract as natural antioxidant. *Int J Food Sci Nutr* 62(7):703-10 (2011).

176. Racanicci AMC, Menten JFM, Alencar SM, Buissa RS and Skibsted LH. Mate (*Ilex paraguariensis*) as dietary additive for broilers: performance and oxidative stability of meat. *Eur Food Res Technol* 232:655-661 (2011)

177. Ugwuona F. Effect of piper extracts on pork stability. *J Food Proces Preserv*34 :235–246 (2010).

178. Olorunsanya OA, Olorunsanya EO, Aliu OT and Kayode RMO. Effects of different species of Pepper (*Capsicum*) on oxidative stability of raw and cooked pork patties. *Pakistan J Nutr* 8(10):1588-91 (2009).

179. Gallo M, Ferracane R and Naviglio D. Antioxidant addition to prevent lipid and protein oxidation in chicken meat mixed with supercritical extracts of *Echinacea angustifolia*. *J Supercrit Fluids* 72:198–204(2012)

180. Huang B, He J, Ban X, Zeng H, Yao X and Wang Y. Antioxidant activity of bovine and porcine meat treated with extracts from edible lotus (*Nelumbo nucifera*) rhizome knot and leaf. *Meat Sci* 87(1):46-53 (2011).

181. Vossen E, Utrera M, De Smet S, Morcuende D and Estévez M. Dog rose (*Rosa canina L.*) as a functional ingredient in porcine frankfurters without added sodium ascorbate and sodium nitrite. *Meat Sci* 92(4):451-7 (2012).

182. Peña-Ramos EA and Xiong .YL Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Sci* 64(3):259-63 (2003).

183. Barbosa-Pereira L, Aurrekoetxea GP, Angulo I, Paseiro-Losada P and Cruz JM. Development of new active packaging films coated with natural phenolic compounds to improve the oxidative stability of beef. *Meat Sci* 97:249-254 (2014)

184. Avila-Ramos F, Pro-Martínez A, Sosa-Montes E, Cuca-García JM, Becerril-Pérez C, Figueroa-Velasco JL, Ruiz-Feria CA, Hernández-Cázares AS and Narciso-Gaytán C. Dietary supplemented and meat-added antioxidants effect on the lipid oxidative stability of refrigerated and frozen cooked chicken meat. *Poult Sci* 92(1):243-9 (2013).

185. McKibben J and Engeseth NJ. Honey as a Protective Agent against Lipid Oxidation in Ground Turkey. *J Agric Food Chem* 30;50(3):592-5 (2002).

186. Sampaio GR, Saldanha T, Soares RA and Torres EA. Effect of natural antioxidant combinations on lipid oxidation in cooked chicken meat during refrigerated storage. *Food Chem* 135(3):1383-90 (2012).

187. Krishnan KR, Babuskin S, Babu PAS, Sasikala M, Sabina K, Archana G, Sivarajan M and Sukumar M. Antimicrobial and antioxidant effects of spice extracts on the shelf-life extension of raw chicken meat. *Intern J Food Microb* 171:32-40 (2014)

188. Frank J. Beyond vitamin E supplementation: An alternative strategy to improve vitamin E status. *J Plant Physiol* 162:834-843 (2005).

3.6. TABLES

Table 1: Main studies conducted on reformulation of the lipid profile of meat by changing the lipid profile of animal diets

Lipid source	Inclusion in animal diets	Species	References
Linseed	50 and 100 g kg^{-1}	Swine	Matthews et al.(25)
Linseed	6 g kg^{-1}	Swine	Kouba et al.(27)
(A) beef tallow, (B) sunflower oil, (C) High-oleic sunflower oil, (D) linseed oil (E) fat blend - Tallow, Sunflower oil, Linseed oil (F) oil blend - Linseed oil and Fish oil	(A) 110 g kg^{-1}, (B) 100 g kg^{-1}, (C) 96 g kg^{-1}, (D) 97 g kg^{-1}, (E) 100 g kg^{-1}, (F) 97 g kg^{-1}	Swine	Realini et al.(28)
Soybean oil, canola oil, flaxseed oil and canola oil blend	30 g kg^{-1}	Swine	Bertol et al.(29)
Partial replacement of corn by rice bran (corn/rice bran)	62/38	Swine	Campos et al.(30), Campos et al.(69)
Linseed oil (A) linseed seed (B), camelina oil (C) and camelina seed (D)	(A) 60 g kg^{-1}, (B) 179 g kg^{-1}, (C) 60 g kg^{-1}, (D) 193 g kg^{-1}	Lamb	Moloney et al.(50)
Animal fat (A), soybean oil(B), palm oil (C)	(A) 10 g kg^{-1} and 30 g kg^{-1}, (B) 10 g kg^{-1}, (C) 10 g kg^{-1}	Swine	Alonso et al.(42)
Palm kernel, palm, soybean	28 g kg^{-1}	Swine	Teye et al.(44)
High α-linolenic acid	45 g kg^{-1}	Swine	Enser et al.(46)
Flaxseed	50 and 100 g kg^{-1}	Swine	Turner et al.(26)

Table 1 (Continued)

Fish oil	20 and 40 g kg^{-1}	Chicken	López-Ferrer et al.(32)
Tallow, olive oil, sunflower oil and linseed oil	60 and 100 g kg^{-1}	Chicken	Crespo et al.(33)
Extruded flaxseed	50, 100 and 150 g kg^{-1}	Chicken	Anjum et al.(34)
Extruded flaxseed	50 and 100 g kg^{-1}	Swine	Juárez et al.(41)
Linseed (A), olive cake (B) and linseed + olive cake (C)	(A) 200 g kg^{-1}, (B) 350 g kg^{-1}, (C) 100 g kg^{-1} linseed + 170 g kg^{-1} olive cake	Lamb	Luciano et al.(49)
Linseed oil (A),sunflower oil (B), blend linseed oil 333 g Kg^{-1} + sunflower oil 666 g Kg^{-1} (C)	6 g kg^{-1}	Lamb	Jerónimo et al.(35)
Linseed oil (A), and fish oil (B)	60 g kg^{-1}	Lamb	Wachira et al. (39)
Flaxseed and sunflower	100 g kg^{-1}	Cattle	He et al.(38)
Fish oil	46 g kg^{-1}, 92 g kg^{-1} and 183 g kg^{-1}	Cattle	Dunne et al.(37)

Table 2: Main studies conducted to evaluate the effect of lipid sources on ω-3 fatty acids levels in processed meat products

Lipid source	Matrix	References
Pre-emulsified olive (A), corn (B), sunflower (C), and soybean oil (D)	Frankfurter sausage	Paneras et al.(56)
Fish oil and high oleic sunflower oil	Frankfurter sausage	Park et al.(55)
Oil in water emulsion with healthier oil combination (olive 443.9 g kg^{-1}, linseed 378.7 g kg^{-1}, fish oil 177.4 g kg^{-1})	Frankfurter sausage	Cofrades et al.(57)
Fish oil extract	Salami	Muguerza et al.(60)
Deodorized fish oil	Salami	Valencia et al.(61)
Pre-emulsified linseed oil	Salami	Ansorena & Astiasaran (62), Garcia-Iñiguez de Ciriano et al.(63)
Pre-emulsified linseed oil	Bologna sausages	Câmara & Pollonio(58)
Pre-emulsified linseed oil and algae oil	Bologna sausages	Berasategi et al.(59)
Olive, flaxseed, and fish oil blends	Pork liver pate	Delgado-Pando et al.(64)
DHA oil to mixed to brine	Pork loin	Meadus et al.(66)
Canola oil emulsion	Matured beef	Pietrasik et al.(65)
Pre-emulsified Algae oil	Turkey burger, and fresh pork sausage	Lee et al.(112)
Pre-emulsified Algae oil	Ground turkey meat, restructured ham, and fresh pork sausage	Lee et al.(72)

Table 2 (Continued)

Pre-emulsified and deodorized corn, olive, and fish oil	Burger	Martínez et al.(71)
Linseed oil and encapsulated fish oil or canola and flaxseed pre-emulsified oil	Salami	Pelser et al.(67)
Modified pork (inclusion of linseed in pigs diet)	Sausage, pate, smoked bacon, cooked chops	Guillevic et al.(70)
Modified pork (inclusion of flaxseed in pigs diet)	Cured ham	Mairesse et al.(68)
Modified pork (inclusion of rice bran in pigs diet)	Salami	Campos et al.(69)
Modified chicken meat (inclusion of extruded flaxseed in chickens diet)	Nuggets	Anjum et al.(34)

Table 3: Main studies conducted to evaluate the effect of fruits, spices, herbs, and seasonings as natural antioxidants added in meat products

Antioxidant source	Matrix	References
Grape seed	Pork products	Carpenter et al.(92), Shan et al.(166), Rojas & Brewer(96), Sasse et al.(102), Brannan (97), Pateiro et al. (98), Lorenzo et al. (100), Lorenzo et al. (99)
	Beef products	Kulkarni et al. (94), Bañón et al. (88), Rojas & Brewer (96), Ahn et al. (91), Colindres & Brewer (101)
	Poultry meat products	Law & King (89), Brannan (90), Mielnik et al. (132)
Waste produced by winery	Pork and poultry meat products	Selani et al.(103), Özvural & Vural (104)
Plum	Beef and pork products	Nuñez de Gonzales et al. (106), Nuñez de Gonzales et al. (107), Nuñez de Gonzales (108), Yildiz-Turp & Serdaroglu (109)
Cranberry	Pork and poultry meat products	Lee et al. (112), Raghavan & Richards (113)
Blackcurrant	Pork products	Jia et al. (116)
Pomegranate	Pork and poultry meat products	Navenna et al. (117), Navenna et al. (118), Vaithiyanathan et al. (119), Kanatt et al. (120), Shan et al. (166)
Chestnut	Pork product	Pateiro et al. (98), Lorenzo et al. (99), Lorenzo et al. (100)
Citrus	Beef and pork products	Fernández-López et al.(122), Viuda-Martos et al. (123), Aleson-Carbonell et al. (124)
Persimmon, Banana, sapodilla and lychee	Poultry meat products	Milani et al.(125), Devatkal et al.(126), Qi et al. (127)

Table 3 (Continued)

Seaweed	Pork product	Lorenzo et al. (99)
Rosemary extract	Beef products	Rojas & Brewer (128), Ahn et al. (91), Colindres & Brewer (101), Mohamed et al. (152), Trindade et al. (134), Trindade et al. (135), Barbosa-Pereira et al.(183)
	Pork products	Rojas & Brewer (128), Nissen et al. (130), Lara et al. (131), Sebranek et al. (133), Doolaege et al. (136), Sasse et al. (102), Lara et al. (131), Ugwuona (177), McCarthy et al. (148)
	Poultry meat products	Mielnik et al. (132), Lee et al. (140)
Garlic	Beef, pork and poultry meat products	Yin & Cheng (142), Sallam et al. (143), Park & Chin (145), Wong & Kitts (144), Mariutti et al. (147)
Sage	Beef, pork and poultry meat products	Mariutti et al. (147), Mohamed et al. (152), McCarthy et al. (148), Estévez et al.(149), Salem & Ibrahim (150), Fasseas et al. (151), Sampaio et al. (186)
Marjoram	Pork product	Mohamed et al. (152)
Green tea	Beef, pork and poultry meat products	Mitsumoto et al. (154), Tang et al. (155), Pateiro et al. (98), Lorenzo et al.(100), Lorenzo et al. (99), Jo et al. (156), McCarthy et al. (148)
Tea dregs	Poultry meat products	Zhao et al. (158)
Oregano	Beef, pork and poultry meat products	Rojas & Brewer (96), Sasse et al. (102), Rojas & Brewer (128), Sacramlin et al. (159), Shan et al. (166), Trindade et al. (135), Sampaio et al. (186), Krishnan et al. (187), Avila-Ramos et al. (160)

Table 3 (Continued)

Basil and Horseradish	Pork product	Juntachote et al. (163)
Cinnamon and clove	Beef, pork and poultry meat products	Jayathilakn et al. (164), Shan et al. (166), Chan et al. (165), Krishnan et al. (187)
Curry	Pork and poultry meat products	Devatkal et al. (167), Biswas et al. (168)
Pepper	Pork products	Ugwuona (177), Olorunsanya et al. (178)
Lemon balm	Pork products	Lara et al. (131)
Lemon balm	Products with high ω-3 fatty acids	García-Iñíguez de Ciriano et al. (169), García-Iñíguez de Ciriano et al. (170) , Berasategi et al. (171)
Yerba mate	Pork and poultry meat products	Racanicci et al. (173), Racanicci et al. (174), Milani et al. (125), Beal et al. (175), Campos et al. (69)
Flowers	Beef, pork and poultry meat products	Gallo et al. (179), Huang et al. (180), Vossen et al. (181)
Protein isolates and hydrolysates	Pork products	Penã-Ramos & Xiong (180), McCarthy et al. (148)
Waste from brewery	Beef products	Barbosa-Pereira et al. (183)
Honey	Poultry meat products	Avila-Ramos et al. (184), McKibben & Engeseth (185), Sampaio et al.(186)

Table 4: Main studies on natural antioxidants supplied in the diet to reduce lipid oxidation in meat of swine, lamb and chickens

Antioxidant source	Inclusion	Supplying period	Species	References
Grape pomace and vitamin E	0, 50 and 100 g kg^{-1} grape pomace and 0.2 g kg^{-1} α- tocopheryl acetate	21 days	Broiler chicks	Chamorro et al. (105)
Rosemary	0.6 mg kg^{-1} Rosemary extract	21 days	Lamb	Bañón et al. (138)
Rosemary	100 and 200 g kg^{-1}dietary rosemary leaf	240 days	Pregnant ewes	Nieto et al. (137)
Rosemary	0, 0.2, 0.4 and 0.6 g kg^{-1}	80 days	Lamb	Serrano et al. (139)
Rosemary and Vitamin E	5 g kg^{-1} rosemary, 10 g kg^{-1} rosemary 0.2 g kg^{-1} α-tocopherol and 5 g kg^{-1} rosemary + 0.2 g kg^{-1} α-tocopherol	35 days	Chicken	Lee et al. (140)
Garlic powder	10, 30, 50 g kg^{-1} garlic powder, and 30 g kg^{-1} garlic powder + 200 IU kg^{-1} α-tocopherol	35 days	Chicken	Choi et al. (146)
Green tea	0.05, 0.1, 0.2, 0.3 g kg^{-1}	42 days	Chicken	Tang et al. (157)
Oregano and vitamin E	0.1 g kg^{-1} Oregano essential oil, and 0.01 or 0.1 g kg^{-1} α-tocopherol	42 days	Chicken	Ávila-Ramos et al. (160)
Oregano	0.25, 0.50 and 1.0 ml kg^{-1} Oregano essential oil	35 days	Swine	Simitzis et al. (161)

Table 4 (Continued)

Lemon balm and hawthorn + yarrow	20 g kg^{-1}	41 days	Chicken	Marcinčáková et al. (172)
Yerba Mate	Yerba mate infusions (1, 5, 10 g kg^{-1}) added to drinking water	14 days	Chicken	Racanicci et al. (176)
Olive Cake	170 or 350 g kg^{-1} olive cake	40 days	Lamb diet with high ω-3	Luciano et al. (49)
Vitamin E	0.04 and 0.20 g kg^{-1} α-tocopherol	56 days	Iberian pigs	Daza et al. (43)
Vitamin E& and red wine extract	0.3 g kg^{-1} α-tocopherol 0.9 g kg^{-1} red wine extract	Days	Lamb	Rivas-Cañedo et al. (86)

3.8. ABBREVIATIONS

ALA: α-linolenic acid

BHA: butylatedhydroxyanisole

BHT: butylated hydroxytoluene

CLA: conjugated linoleic acid

DHA: docosahexaenoic acid

EPA: eicosapentaenoic acid

LA: linoleic acid

PUFA: polyunsaturated fatty acids

TBARS: thiobarbituric acid reactive substances

4. CAPÍTULO 02

"PRODUCTION OF HYDROLYSATE FROM PROCESSED NILE TILAPIA (*Oreochromis niloticus*) RESIDUES AND ASSESSMENT OF ITS ANTIOXIDANT ACTIVITY"

Daniela Miotto Bernardi, Leandro Daniel de Paris, Fabiana Dieterich, Fernanda Guimarães Dummond e Silva, Wilson Rogério Boscolo, Cezar Sary, Altevir Signor, Teresinha Marisa Bertol, Valdemiro Carlos Sgarbieri

Artigo original aceito para publicação na revista "*Food Science and Technology (Campinas)*" no dia 13 de agosto de 2016. DOI: http://dx.doi.org/10.1590/1678-457X.15216 .

ABSTRACT

The objective of this work was to produce protein hydrolysates from by-products of the Nile tilapia fileting process, and to assess the effects of different hydrolysis times on the antioxidant activity of the hydrolysed animal-based protein, in free form and incorporated into a food matrix. Gutted tilapia heads and carcasses were hydrolysed by Alcalase® for different hydrolysis times producing six hydrolysates. The protein content, degree of hydrolysis, reverse-phase high-performance liquid chromatography, and antioxidant activity by the ORAC, FRAP and TEAC methods were analysed. Three mini-hamburger formulations were produced and the lipidic oxidation of mini-hamburger was determined by TBARS. The protein contained in the residue was completely recovered in the process. The hydrolysates varied in their degree of hydrolysis, but presented similar levels of antioxidant activity. In the mini-hamburgers the hydrolysate was capable of delaying oxidation after 7 days of storage. Hydrolysis of tilapia processing by-products produced peptides may be used in the formulation of functional foods. Practical application: The practical application of this work aims at production of peptides with antioxidant activity using protein recovery from a raw material considered a residue or by-product of Nile Tilapia processing
Keywords: fish, protein, hydrolysis, antioxidants

4.1. INTRODUCTION

Antioxidants play an important role in the reduction of the oxidative process, both in food systems and in the human body. In foodstuffs, its use contributes towards delaying lipidic and protein oxidation, helping to maintain the colour, aroma, taste, texture and nutritional value of food products (Bernardi et al., 2016). In the human body the presence of antioxidants, whether endogenous or exogenous, reduces oxidative damage caused by reactive oxygen and nitrogen species, thus reducing the risk of developing certain diseases (Samaranayaka & Li-Chan, 2011).

Due to questions regarding the safety of using synthetic substances in food, there has been a growing interest in identifying natural compounds that possess antioxidant properties and can be used as natural additives in food or as physiological-functional ingredients in food products (Bernardi et al., 2016). Of the compounds studied, the bioactive peptides stand out, since they can be produced from several protein sources through different methods (enzymatic hydrolysis, digestion in the gastrointestinal tract and microbial fermentation), as well as being

considered safe, nutritionally healthy, low cost and highly antioxidant (Samaranayaka & Li-Chan, 2011).

The production of bioactive peptides from the hydrolysis of fish product proteins is feasible and intensely studied primarily due to the wide availability of this raw material (Samaranayaka & Li-Chan, 2011). In this context, the Nile tilapia (*Oreochromis niloticus*) is a freshwater fish whose intensive breeding has increased substantially in recent years (Roslan et al., 2014), with the filet being its product of greatest commercial value. The head, carcass, bones, skin, fins and viscera represent roughly 60 to 70% of the weight of the tilapia, considered waste or by-products of its processing, which if not properly discarded or used, can have a significant environmental impact, bearing in mind the considerable volume (Roslan et al., 2014; Silva et al., 2014). These residues are sources of high nutritional value protein, therefore making use of them is of nutritional, economic and environmental interest (Roslan et al., 2014; Silva et al., 2014).

As mentioned above, enzymatic hydrolysis of proteins produces peptides with antioxidant potential. Furthermore, it's a form of transforming raw material and, therefore, can be used as an alternative for recycling tilapia processing residues. Hence, the objective of this work was to produce a hydrolysate at a pilot industrial plant from the by-product of the fileting process of Nile tilapia, to evaluate the effect of the hydrolysis time on the protein recovery of the material and on the *in vitro* antioxidant activity, as well as to assess the capacity of the hydrolysate to maintain the oxidative stability of a meat product.

4.2. MATERIALS AND METHODS

4.2.1. Production of the hydrolysate

The hydrolysate was produced from by-products resulting from the fileting process of Nile tilapia. The head and gutted carcass were used, supplied by the company Falbom Agroindustrial Ltda. (Toledo-PR-Brazil). The hydrolysis was performed in accordance with the description given by Dieterich et al. (2014).

Tilapia heads and carcasses were ground in a meat grinder (5.0 mm) and placed in an industrial reactor equipped with an electric heater. The composition was 80% ground material (head and gutted carcass) and 20% water. The material was homogenized and heated up to 50ºC, at which time the Alcalase® (2.75 AU-A/g) from *Bacillus licheniformis* (Novozymes Latino Americana Ltda., Paraná, Brazil) was added at a proportion of 0.2% (w/w). The total

duration of the hydrolysis was 240 minutes and the homogenization process remained constant until the end of the reaction. The temperature and pH were monitored and controlled throughout the hydrolysis process, the temperature varied from 50ºC to 64ºC and the pH varied between 5.8 and 6.1. Every 40 minutes of reaction time 2 litre samples of the hydrolysate were collected, which were subjected to thermal inactivation of the enzyme (heated to 90ºC for 10 minutes) and filtered (through a 1mm mesh screen) to remove remaining bones. By the end, samples with six different hydrolysis times had been obtained: H40) 40 min; H80) 80 min; H120) 120 min; H160) 160 min; H200) 200 min and H240) 240 min.

Half of each portion of hydrolysate was frozen in an aluminium tray and lyophilized (Liobrás Freeze-Dryer, model LP810) and named whole lyophilized hydrolysates (WLH). The other half was centrifuged at 13700 x g for 30 minutes in RC5C centrifuge (Sorvall Instruments Dupont, Wilmington, USA) with the temperature controlled at 20ºC to 26ºC. The insoluble material was discarded and the soluble material was frozen and lyophilized in the same conditions as cited above, these products were named centrifuged lyophilized hydrolysates (CLH). All the hydrolysates were frozen and stored (-20ºC) in hermetically sealed pots until they were analysed and all the assays were carried out in triplicate. The complete flow chart of the processing and analysis of the products is shown in Figure 1.

Figure 1

4.2.2. Determination of protein and degree of hydrolysis (DH)

The protein content of the samples was determined according to Kjeldahl methodology described by the "Association of Official Analytical Chemists" (AOAC, 1995), considering a factor of 6.25 for the conversion of % N to protein (N x 6.25).

The degree of hydrolysis (DH) is defined as the percentage of cleaved peptide bond: DH (%) = (h/htot) x 100. Where, h is the number of hydrolysed bonds and htot is the total number of peptide bonds per equivalent of the protein studied.

The DH was determined by the o-phthaldialdehyde (OPA) method (Nielsen et al., 2001). This method is based on the reaction of primary amino groups with OPA, using serine as the standard solution. Briefly, the OPA reagent was prepared with 3.81 g sodium tetraborate decahydrate and 100 mg sodium dodecyl sulfate dissolved in 80 mL deionized water added of 88 mg dithiothreitol. Eighty mg OPA was dissolved in 2 mL ethanol, mixed with the above solution and the volume completed with 100 mL with distilled water. Twenty mg serine was

dissolved in 200 mL deionized water (0.9516 mmole/L). Three mL OPA reagent was added into 400 µL of serine standard solution or protein hydrolysate, mixed for 5 seconds and incubated for 2 minutes at room temperature. The absorbance was spectrophotometrically measured at 340 nm.

4.2.3. High-performance liquid chromatography (HPLC)

For the characterization of protein in the centrifuged lyophilized hydrolysates (CLH) chromatographic analysis (HPLC) was carried out using a high-performance liquid chromatography system with an automatic injector and a diode-array absorbance detector (Agilent, 1200 Series, Washington, USA). Separation was carried out on a Luna C18 column (250 mm x 4.6 mm, Phenomenex, Torrance, CA) at a flow rate of 1 mL/min. The mobile phase was composed of solvent A (0.04% *trifluoroacetic acid* in water) and solvent B (0.03% *trifluoroacetic acid* in acetonitrile). The gradient was from 0 to 80% of solvent B over 40 min. The absorbance was measured at 214 and 280 nm. The hydrolysates (8 mg/mL) were filtered through a 45-µm membrane and 50 µL were injected. The Star Chromatography Workstation software (Agilent) was used for recording and processing the data.

4.2.4. Determination of antioxidant activity

The antioxidant capacity of the hydrolysates (CLH) was measured in an aqueous extract. To obtain the extract, the powdered samples were suspended in deionized water 1% (w/v). The extracts were homogenized for 30 minutes, centrifuged in RC5C (Sorvall Instruments Dupont, Wilmington, USA) at 35735 x g (30 min, 10°C), then filtered in a Whatman #1 filter and stored in a dark environment at -20° C. Three repetitions of the aqueous extract were produced for each hydrolysate.

The Oxygen Radical Absorbance Capacity (ORAC) method was employed according to the description given by Dávalos et al. (2004). A microplate was used to mix 20 µL of the sample extract (or blank, or standard solutions), 120 µL of sodium fluorescein (dissolved in potassium phosphate buffer pH 7.4, in the final concentration of 0.378 µg/mL) and 60 µL AAPH [(2.2'-azobis (2-methylpropionamidine) dihydrochloride) were dissolved in water in the final concentration of 108 mg/mL]. The potassium phosphate buffer was used as blank. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solutions (25 - 800 µM) were used as standard. Fluorescence was measured every minute for 80 minutes using the microplate

reader (Synergy™ HT Multi-Mode Microplate Reader, Biotek®, Vermont, USA) with a 485-nm excitation filter, 520 nm emission and reaction temperature of 37° C. The calculation was based on the area under the curve (AUC) formed by the decline in fluorescence over time. The results were expressed in µM of Trolox equivalent (TE) per gram (µM TE/ g) of sample.

Measuring antioxidant activity through Ferric Reducing Antioxidant Power (FRAP) was carried out in accordance with the methodology described by Benzie & Strain (1996). In a dark environment, 30 µL of the sample extract (or blank, or standard solutions) were mixed into 90 µL of water and 900 µL of the FRAP reagent with the following composition: 450 µL of acetate buffer 0.3 M and pH 3.6; 225 µL of TPTZ 10 mmol in 40 mmol of CLH and 225 µL of $FeCl_3$ 20 mmol. The mixture was incubated at 37° C for 30 minutes. The reading was made at 593 nm (Synergy™ HT Multi-Mode Microplate Reader, Biotek®, Vermont, USA). FRAP was calculated from the Trolox standard curve (100 - 1600 µM) and expressed µM TE/ g of sample.

The determination of Trolox equivalent antioxidant capacity (TEAC) measured by the 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation was conducted in accordance with the method described by Re et al. (1999). The ABTS radical ($ABTS^{\bullet+}$) solution was produced by reacting 5 mL of the ABTS stock solution (7mM) with 88 µL of the potassium persulfate solution (140 mM). The mixture was allowed to react in the dark for 16 h at room temperature. Prior to assay, 1 mL $ABTS^{\bullet+}$solution was diluted with ethanol to obtain an absorbance of 0.70 at 734 nm. To initiate the reaction, 15 µL of sample extract (or blank, or standard solutions) was mixed with 1.5 mL of $ABTS^{\bullet+}$solution, and after 6 minutes absorbance was read at 734 nm (Synergy™ HT Multi-Mode Microplate Reader, Biotek®, Vermont, USA). Trolox standard curve (25 - 1000 µM) was prepared and the results were expressed in µM TE/ g of sample.

4.2.5. Antioxidant effect in meat product

Mini hamburgers of pork meat and pork fat were made, using loin and backfat with the addition of 3% of flaxseed oil in the diet. The average percentage of polyunsaturated fatty acids (PUFA) in the ether extract of the backfat was 23.16 ± 0.97% and of the loin 15.79 ± 0.84% and the average percentage of ω-3 in the ether extract of these two cuts was 5.33 ± 0.34% and 2.81±0.08%, respectively. Considering the results obtained for the antioxidant activity of the product, the H200 sample of the centrifuged lyophilized hydrolysates (CLH) was selected to be added to the meat product.

Loin, backfat and NaCl were weighed in the proportions of 78.4%, 19.6% and 2%, respectively. Three formulations of mini hamburgers were produced: MHC: control (no added antioxidant); MHH: with addition of 1% (w/w) of centrifuged and dry hydrolysate (H200); MHBHT: with addition of 0.02% (w/w) of butylhydroxytoluene (BHT).

The meat and backfat were placed, together with the NaCl and other ingredients, into a domestic food processor (Philips Walita, model Ri1364, Brazil) for 30 seconds. In sequence the samples were weighed in portions of 12.5g and moulded. At the end they were placed in individual plastic bags and maintained in the presence of oxygen under refrigeration (4º C). Determinations of thiobarbituric acid-reactive substances (TBARS) were done at T0 (production day) and T4, T7 and T10 days of refrigerated storage, respectively. Three repetitions of the mini hamburgers were produced for each time of storage and formulation.
The TBARS analysis was conducted in accordance with the Vyncke (1970) methodology. Briefly, a 2.5 g sample was homogenized (T25 digital Ultra-Turrax®, IKA, German) with 10 mL of 7.5% trichloroacetic acid solution and 0.25 mL of 0.2% BHT solution. Subsequently the homogenate was filtered using Whatman #1 filter paper. The filtrate was mixed in equal amounts to 0.02 M thiobarbituric acid solution, and the mixture was heated in a water bath (80ºC) for 40 min. The sample was then cooled and the color was spectrophotometrically measured at 538 nm. The results were expressed in mg of malondialdehyde (MDA) per Kg of sample (mg MDA/Kg).

4.2.6. Statistical analysis

The results were expressed as mean (±) standard deviation. The effect of the hydrolysis time on protein values, DH and antioxidant activity was assessed (ORAC, FRAP, TEAC), as well as the effect of adding antioxidants on the production of TBARS in the mini hamburgers. The data were tested by analysis of variance (ANOVA), followed by the Tukey test. The level of $p<0.05$ was considered significant.

4.3. RESULTS AND DISCUSSION

4.3.1. Characterization of the Hydrolysates

The percentage of crude protein in the ground feedstock (head and carcass) was 42.35% in a dry base (11.82% in wet base) and these results agreed with those reported in the literature (Roslan et al., 2014; Silva et al., 2014).

Table 1

The hydrolysis ensured total recovery of the protein from the raw material; after the enzymatic reaction the mean protein content of the WLH samples was 45.33% (Table 1). It was also found that the hydrolysis time did not affect significantly the protein recovery from the samples ($p>0.05$), corroborating with the literature which shows that enzymatic hydrolysis does not promote any change in protein nitrogen content, but does increase protein digestibility (Li et al, 2010). The proteolysis of fish by-products promotes the breaking of peptide bonds, thus contributing toward the solubilisation of the protein fraction and resulting in two fractions: one soluble and the other insoluble. The insoluble fraction consists of bones and other components not hydrolysed by proteases, whereas the soluble fraction is composed mainly of peptides (Silva et al., 2014).

In the present study it was found that hydrolysis associated with centrifugation and freeze drying (CLH) promoted a high concentration of protein content, which reached a final mean percentage of 78.97%. Therefore, based on these results one can assert that the processing used was efficient in protein recovery and concentration, ensuring total usage of the proteins considered as industrial waste. Other authors (Roslan et al., 2014; Silva et al., 2014) have obtained similar results for hydrolysate of same type of raw material.

As was expected, the hydrolysis time promoted differences in the degree of hydrolysis (DH) of the samples ($p=0.001$). The DH results were demonstrated by the difference in the intensity of the hydrolysate peaks observed in the chromatographic profiles detected at 214 and 280 nm (Figure 2). The chromatograms showed that the peptides related to the majority of the peaks eluted between 7 and 15 minutes, a region of average hydrophobicity. However, the increase in hydrolysis time promoted an increase in the intensity of the peaks, primarily those eluted at 8, 9, 11, 13 and 16 minutes in the profiles with detection at 214 nm and at 8, 9, 11 and 15 minutes with detection at 280 nm. This hydrolysate profile might be explained by the broad specificity of Alcalase®, an endopeptidase that splits bonds within the polypeptide chain, mainly producing small and medium-sized peptides (Liu et al., 2010).

Figure 2

The DH is an indicator of the efficiency of the hydrolysis (Foh et al., 2010) and normally after quick initial reaction phase the rate of hydrolysis tends to diminish, subsequently entering the stationary phase (Hoyle & Merritt, 1994), which can be observed in this study, considering that at 40 minutes of hydrolysis (H40) the DH was 19.5%, at 160 minutes (H160) it was 24.85% and at 240 minutes of reaction the DH was 25.72%. In relation to other studies involving tilapia protein hydrolysates with Alcalase®, the results of DH were highly variable (from 7.5% to 85.3%). This variation suggests differences in the methods, as well as in the hydrolysis conditions used (Daud et al., 2013; Daud et al., 2015; Fan et al., 2012; Foh et al., 2010; Roslan et al., 2014; Silva et al., 2014). In this context, the DH results obtained by Roslan et al. (2014) support those of the present study, since the reaction conditions, DH analysis method, as well as the enzyme and feedstock were similar.

4.3.2. Antioxidant activity of the hydrolysates

Fish products are sources of high quality and highly digestible proteins and hydrolysis of these proteins produces biological active peptides, with antioxidant potential being one of the main factors to generate its bioactivity (Samaranayaka & Li-Chan, 2011).

The amino acid composition of the feedstock and the hydrolysis conditions are determinant factors in the peptide profile of the hydrolysates, which directly influences the antioxidant capacity (Girgih et al., 2015; Wiriyaphan et al., 2015).

To determine the antioxidant potential of the hydrolysates, the FRAP and ABTS tests were used to assess the electron transfer capacity and the ORAC test was used to assess the H+ transfer capacity (Huang et al., 2005). The results of the antioxidant activity of the hydrolysates are presented in Table 2.

Table 2

In all the methods used it was verified that the hydrolysis time and consequently the DH had no interference in the antioxidant activity of the hydrolysates ($p>0.05$). However some authors (Daud et al., 2013; Daud et al., 2015; Raghavan et al., 2008) have shown that the DH can influence the antioxidant potential of peptides formed in the hydrolysis of tilapia muscle proteins.

The ORAC results obtained were similar to those achieved for mussel hydrolysate (Park et al., 2014), as well as for hydrolysates of flaxseed protein (Silva et al., 2013) and oat (Tsopmo

et al., 2010) which used Alcalase® as the enzyme. Girgih et al (2013) and Girgih et al. (2015) found that salmon and cod hydrolysates presented higher amounts of ORAC than those found in the present study.

The evaluated hydrolysates of tilapia processing by-products showed high FRAP activity and ABTS radical cation. Other authors (Choonpicharn et al., 2014; Yarnpakdee et. al., 2014) have shown elevated FRAP activity in hydrolysates produced with tilapia muscle and skin. The ABTS results presented here support those obtained by Raghavan et al. (2008) and are lower than those of Yarnpakdee et al. (2014), both based on tilapia muscle hydrolysates.

As a rule low molecular weight peptides display a greater capacity to eliminate hydroxyl radicals (Lee et al., 2010) and are able to expose more lateral chains, able to donate electrons with greater facility and therefore become more accessible to free radicals (Wiriyaphan et al., 2015). On the other hand, it is important to underline that despite smaller peptides being more efficient, some authors (Girgih et al., 2015) have found that non fractioned hydrolysates, like that produced in our study, show a greater FRAP, indicating a synergistic effect between peptides of different molecular weights.

The size of the peptide and the sequence of amino acids in the structure are all essential factors for their antioxidant activity (Samaranayaka et al., 2010; Wiriyaphan et al., 2015). Literature shows that peptides containing hydrophobic residues, including His, Met and Cys, are determinant in the hydroxyl radical neutralization capacity (Hernández-Ledesma et al., 2005), while the presence of aromatic residues (Tyr, Trp and Phe) give the peptide higher capacity to donate electrons (Girgih et al., 2015; Wiriyaphan et al., 2015).

In the hydrolysates of the present study the antioxidant activity probably derives from both the presence of aromatic amino acid residues, exhibited by the presence of peaks in the profiles with detection at 280 nm, and the presence of peptides of average hydrophobicity eluted in the profiles with detection at 214 nm. (Figure 2).

High quantities of hydrophobic amino acids (Ala, Val, Leu, Cys, Trp, Pro and Ile) in the peptides also offers structural properties that can improve interactions with lipidic foods, and help increase the admission of peptides in target organs by means of hydrophobic interactions with membranes (Girgih et al., 2015).

Comparing the three methods to assess antioxidant activity, it was found that the lowest results in uM TE per g of sample were those determined by the FRAP and ABTS methods, and the highest results by the ORAC method. Therefore, the main mechanism of antioxidant action of the tilapia hydrolysates is possibly by means of hydrogen atom transfer.

Furthermore, the greatest ORAC values suggest that the tilapia hydrolysate peptides may be good *in vivo* antioxidants, since this is the method that presented the best correlation with physiological conditions (Magalhães et al., 2008).

4.3.3. Application of the hydrolysate in meat product and assessment of TBARS production

Lipidic oxidation causes nutritional losses and reduces the shelf life of the food products, furthermore the consumption of oxidized products can cause cellular injury (Girgih et al., 2015). Free radicals are normally formed in the aqueous phase of foods, primarily by Fenton reactions, and then migrate to the lipidic phase where they stimulate oxidation (Brewer 2011). Amino acids, peptides and proteins possess a surfactant capacity and are capable of acting in the oil-water interface, where they present antioxidant activity and could be an excellent antioxidant option for application in food systems.

Lipidic oxidation is a spontaneous and inevitable process in meat and meat products. The mini hamburger produced with pork meat and fat with a high PUFA and especially ω-3 content, is a good product model to assess this process. The presence of PUFA and ω-3, as well as the grinding and addition of NaCl contributed toward the generation of a pro-oxidant state. The choice of H200 for this test was made randomly, considering that all the hydrolysates produced presented statistically similar antioxidant activity. Figure 3 shows the results of the TBARS analyses of the mini hamburgers (MHC, MHBHT, MHH) stored for 10 days.

Figure 3

With 4 days' and 7 days' storage only BHT (0.02%) presented antioxidant activity capable of delaying lipidic oxidation ($p<0.001$), with the malondialdehyde (MDA) concentration being statistically equal in the MHC and MHH samples and statistically lower in the MHBHT sample. With 10 days' storage the hydrolysate and BHT presented a significant antioxidant effect and all the samples differed ($p<0.001$), with the lowest MDA value being found in the MHBHT sample, followed by the MHH sample and the greatest MDA value being in the MHC sample.

The results suggest that the hydrolysate of tilapia processing by-products has an antioxidant effect, however, as expected, it is less expressive than that found in the synthetic

antioxidant (BHT). It was also found that the hydrolysate had a delayed antioxidant effect (after 7 days of storage at 4ºC).

Similar results regarding delayed antioxidant effect of protein hydrolysates on food products models, were also observed by other authors (Peña-Ramos & Xiong, 2003; Sakanakaet al., 2004). Sakanaka et al. (2004) prepared cookies with a high concentration of linoleic acid and observed that from the third day of storage there was a considerable increase in the amount of peroxide in the samples. The same authors found that hydrolysed egg protein significantly reduced the production of peroxides only after the seventh day of storage, under the same conditions. Likewise, Peña-Ramos & Xiong (2003) also found that pork patties added of hydrolysed soybean protein showed significantly lower production of conjugated dienes compared to the control after 7 days of storage.

Other authors (Dekkers et al., 2011; Raghavan & Kristinsson, 2008) also reported antioxidant effect of the protein hydrolysate of tilapia on food systems. Raghavan & Kristinsson (2008) used a model system of washed tilapia muscle, and found that the tilapia protein hydrolysate was capable of acting as antioxidant agent only after the third day of storage, moreover, suggested that the intensity and speed of antioxidation in the system was dependent on the type of enzyme and the degree of hydrolysis. However in a later study (Dekkers et al. 2011) demonstrated that tilapia protein hydrolysate, showed antioxidant effect in mahi mahi (*Coryphaena hippurus*) filets, but found that the fractionation of peptides by their molecular weight had no effect on increasing the hydrolysate's capacity to delay oxidation.

Despite the promising results at 10 days of storage, further studies are still required in different food matrixes to provide greater understanding of the antioxidant activity of peptides produced during fish protein hydrolysis. It should be highlighted that there are many *in vitro* studies in this area, yet *in vivo* works and studies in food systems remain scarce. Furthermore, consumer evaluations are also scarce, but of great importance, especially in the case of adding these hydrolysates to food products, as it is known that the increased DH can produce bitter-tasting peptides, which can reduce product acceptability (Halldorsdottir et al., 2014; Yarnpakdee et al., 2014).

4.4. CONCLUSIONS

By means of hydrolysis with Alcalase®, it was possible to recover proteins that were considered residues or by-products of the Nile tilapia processing. In addition to protein

recovery, hydrolysis also promoted the formation of highly antioxidant peptides *in vitro* and the enzymatic reaction time did not interfered in the results.

The hydrolysate produced revealed potential for use as an antioxidant in human diet, bearing in mind that its antioxidant action was engaged primarily through hydrogen atom transfer reactions, as well as presenting promising results regarding its application as an antioxidant for food products.

Therefore, the results obtained have strengthened the economic, environmental, and nutritional importance of the work and reinforced the need for new investigations in order to achieve a better understanding of the actions and usefulness of these compounds *in vivo* in animal diets and in food products.

4.5. REFERENCES

AOAC. (1995). *Official methods of analysis of AOAC International* (16th ed.). Virgínia.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76.

Bernardi, D. M., Bertol, T. M., Pflanzer, S. B., Sgarbieri, V. C., & Pollonio, M. A. R. (2016). ω-3 in meat products: Benefits and effects on lipid oxidative stability. *Journal of the Science of Food and Agriculture*, *96*(8), 2620 – 2634.

Choonpicharn, S., Jaturasitha, S., Rakariyatham, N., Suree, N., & Niamsup, H. (2014). Antioxidant and antihypertensive activity of gelatin hydrolysate from Nile tilapia skin. *Journal of Food Science and Technology*, *52*, 3134–3139.

Daud, N. A., Babji, A. S., & Yusop, S. M. (2013). Antioxidant activities of red tilapia (Oreochromis niloticus) protein hydrolysates as influenced by thermolysin and alcalase. *AIP Conference Proceedings*, *1571*, 687–691.

Daud, N. A., Babji, A. S., & Yusop, S. M. (2015). Effects of Enzymatic Hydrolysis on the Antioxidative and Antihypertensive Activities from Red Tilapia Fish Protein. *Journal of Nutrition & Food Sciences*, *5*(387). doi:10.4172/2155-9600.1000387

Dávalos, A., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending Applicability of the Oxygen Radical Absorbance Capacity (ORAC-Fluorescein) Assay. *Journal of Agricultural and Food Chemistry*, *52*(1), 48–54.

Dekkers, E., Raghavan, S., Kristinsson, H. G., & Marshall, M. R. (2011). Oxidative stability of mahi mahi red muscle dipped in tilapia protein hydrolysates. *Food Chemistry*, *124*(2), 640–645.

Dieterich, F., Boscolo, W. R., Pacheco, M. T. B., Silva, V. S. N., Gonçalves, G. S., & Vidotti, R. M. (2014). Development and Characterization of Protein Hydrolysates Originated from Animal Agro Industrial Byproducts. *Journal of Dairy, Veterinary & Animal Research*, *1*(2), 1–7.

Fan, J., He, J., Zhuang, Y., & Sun, L. (2012). Purification and identification of antioxidant peptides from enzymatic hydrolysates of tilapia (oreochromis niloticus) frame protein. *Molecules*, *17*(11), 12836–12850.

Foh, M. B. K., Amadou, I., Foh, B. M., Kamara, M. T., & Xia, W. (2010). Functionality and antioxidant properties of tilapia (Oreochromis niloticus) as influenced by the degree of hydrolysis. *International Journal of Molecular Sciences*, *11*(4), 1851–1869.

Girgih, A. T., He, R., Hasan, F. M., Udenigwe, C. C., Gill, T. A., & Aluko, R. E. (2015). Evaluation of the in vitro antioxidant properties of a cod (Gadus morhua) protein hydrolysate and peptide fractions. *Food Chemistry*, *173*, 652–659.

Halldorsdottir, S. M., Sveinsdottir, H., Gudmundsdottir, A., Thorkelsson, G., & Kristinsson, H. G. (2014). High quality fish protein hydrolysates prepared from by-product material with Fucus vesiculosus. *Journal of Functional Foods*, *9*, 10–17.

Hernández-Ledesma, B., Dávalos, A., Bartalomé, B., & Amigo, L. (2005). Preparation of antioxidant enzymatic hydrolysates from alpha-lactalbumin and beta-lactoglobulin. Identification of active peptides by HPLC-MS/MS. *Journal of Agricultural and Food Chemistry*, *53*(3), 588–593.

Hoyle, N. T., & Merritt, J. H. (1994). Quality of fish protein hydrolysates from herring (Clupea harengus). *Journal of Food Science*, *59*(1), 76–79.

Huang, D., Boxin, O. U., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*(6), 1841–1856.

Lee, S.-J., Kim, E.-K., Hwang, J.-W., Oh, H.-J., Cheong, S.-H., Moon, S.-H., … Park, P.-J. (2010). Purification and characterisation of an antioxidative peptide from enzymatic hydrolysates of duck processing by-products. *Food Chemistry*, *123*(2), 216–220.

Li, Z.-Y., Youravong, W., & H-Kittikun, A. (2010). Protein hydrolysis by protease isolated from tuna spleen by membrane filtration: A comparative study with commercial proteases. *LWT - Food Science and Technology*, *43*(1), 166–172.

Liu, Q., Kong, B., Xiong, Y. L., & Xia, X. (2010). Antioxidant activity and functional properties of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis. *Food Chemistry*, *118*(2), 403–410.

Magalhães, L. M., Segundo, M. A., Reis, S., & Lima, J. L. F. C. (2008). Methodological aspects

about in vitro evaluation of antioxidant properties. *Analytica Chimica Acta*, *613*(1), 1–19.

Nielsen, P. m., Petersen, D., & Dambmann, C. (2001). Improved Method for Determining Food Protein Degree of Hydrolysis. *Journal of Food Science*, *66*(5), 642–646.

Park, S. Y., Ahn, C., & Je, J. (2014). Antioxidant and Anti-Inflammatory Activities of Protein Hydrolysates from Mytilus Edulis and Ultrafiltration Membrane Fractions. *Journal of Food Biochemistry*, *38*(5), 460–468.

Peña-Ramos, E. A., & Xiong, Y. L. (2003). Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Science*, *64*(3), 259–263.

Raghavan, S., & Kristinsson, H. G. (2008). Antioxidative efficacy of alkali-treated tilapia protein hydrolysates: A comparative study of five enzymes. *Journal of Agricultural and Food Chemistry*, *56*(4), 1434–1441.

Raghavan, S., Kristinsson, H. G., & Leeuwenburgh, C. (2008). Radical scavenging and reducing ability of tilapia (Oreochromis niloticus) protein hydrolysates. *Journal of Agricultural and Food Chemistry*, *56*(21), 10359–10367.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231–1237.

Roslan, J., Faezah, K., Abdullah, N., & Mazlina, S. (2014). Characterization of Fish Protein Hydrolysate from Tilapia (Oreochromis niloticus) by-Product. *Agriculture and Agricultural Science Procedia*, *2*, 312–319.

Sakanaka, S., Tachibana, Y., Ishihara, N., & Juneja, L. R. (2004). Antioxidant activity of egg-yolk protein hydrolysates in a linoleic acid oxidation system. *Food Chemistry*, *86*(1), 99–103.

Samaranayaka, A. G. P., Kitts, D. D., & Li-Chan, E. C. Y. (2010). Antioxidative and angiotensin-I-converting enzyme inhibitory potential of a pacific hake (merlucclus productos) fish protein hydrolysate subjected to simulated gastrointestinal digestion and caco-2 cell permeation. *Journal of Agricultural and Food Chemistry*, *58*(3), 1535–1542.

Samaranayaka, A. G P, & Li-Chan, E. C. Y. (2011). Food-derived peptidic antioxidants: A review of their production, assessment, and potential applications. *Journal of Functional Foods*, *3*(4), 229–254.

Silva, F. G. D., O'Callagahan, Y., O'Brien, N. M., & Netto, F. M. (2013). Antioxidant Capacity of Flaxseed Products: The Effect of In vitro Digestion. *Plant Foods for Human Nutrition*, *68*(1), 24–30.

Silva, J. F. X., Ribeiro, K., Silva, J. F., Cahú, T. B., & Bezerra, R. S. (2014). Utilization of

tilapia processing waste for the production of fish protein hydrolysate. *Animal Feed Science and Technology*, *196*, 96–106.

Tsopmo, A., Cooper, A., & Jodayree, S. (2010). Enzymatic hydrolysis of oat flour protein isolates to enhance antioxidative properties. *Advance Journal of Food Science and Technology*, *2*(4), 206–212.

Vyncke, W. (1970). Direct Determination of the Thiobarbituric Acid Value in Trichloracetic Acid Extracts of Fish as a Measure of Oxidative Rancidity. *Fette Seifen Anstrichmittel*, *12*(12), 1084–1087.

Wiriyaphan, C., Xiao, H., Decker, E. A., & Yongsawatdigul, J. (2015). Chemical and cellular antioxidative properties of threadfin bream (Nemipterus spp.) surimi byproduct hydrolysates fractionated by ultrafiltration. *Food Chemistry*, *167*, 7–15.

Yarnpakdee, S., Benjakul, S., Kristinsson, H. G., & Kishimura, H. (2014). Antioxidant and sensory properties of protein hydrolysate derived from Nile tilapia (Oreochromis niloticus) by one- and two-step hydrolysis. *Journal of Food Science and Technology*, *52*(2004), 3336–3349.

4.6. TABLES AND FIGURES

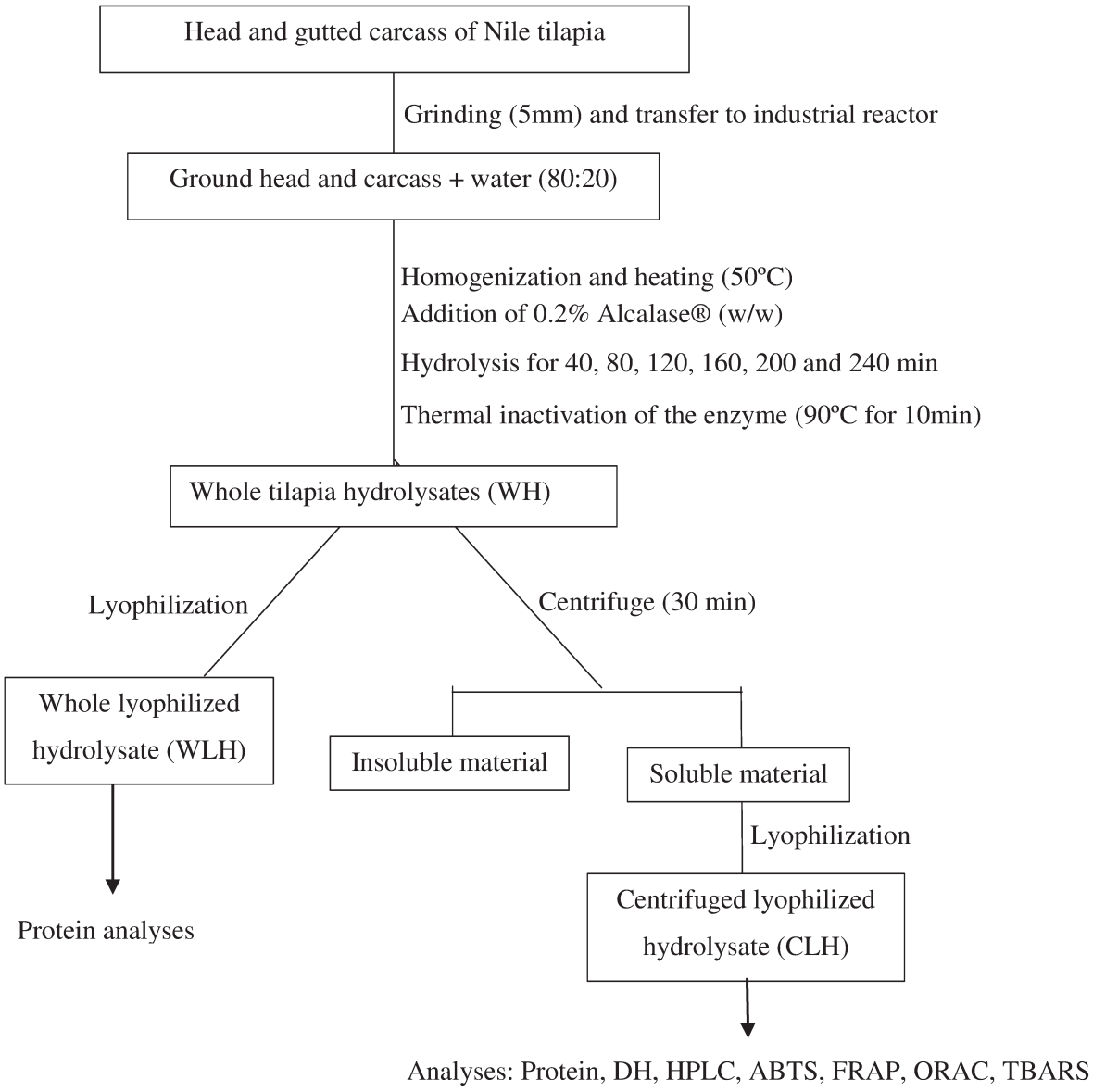


Figure 1. Flow chart of the processing of Nile tilapia carcasses hydrolysates

Table 1. Percentage of protein and degree of hydrolysis of the Nile tilapia head and carcass hydrolysate produced with different times of hydrolysis

	WLH	CLH	
Sample	Protein (%)	Protein (%)	Degree hydrolysis (%)
H40	46.82±3.78	76.20±3.24	$19.5±1.07^{CD}$
H80	45.95±7.04	79.07±5.07	$21.52±0.56^{BCD}$
H120	44.45±1.28	78.68±2.15	$23.53±1.99^{ABCD}$
H160	44.96±8.7	80.96±5.00	$24.85±2.25^{ABC}$
H200	44.2±1.65	78.88±1.61	$26.13±0.75^{AB}$
H240	45.57±2.05	80.01±2.03	$25.72±1.28^{AB}$
p	0.995	0.859	*0.001*

WLH: Whole lyophilized hydrolysate; CLH: centrifuged lyophilized hydrolysate; H40: hydrolysate with 40 minutes of hydrolysis; H80: hydrolysate with 80 minutes of hydrolysis; H120: hydrolysate with 120 minutes of hydrolysis; H160: hydrolysate with 160 minutes of hydrolysis; H200: hydrolysate with 200 minutes of hydrolysis; H240: hydrolysate with 240 minutes of hydrolysis.

p: p value by ANOVA. The mean averages with values $p<0.05$ were tested by Tukey and follow, represented by uppercase letters.

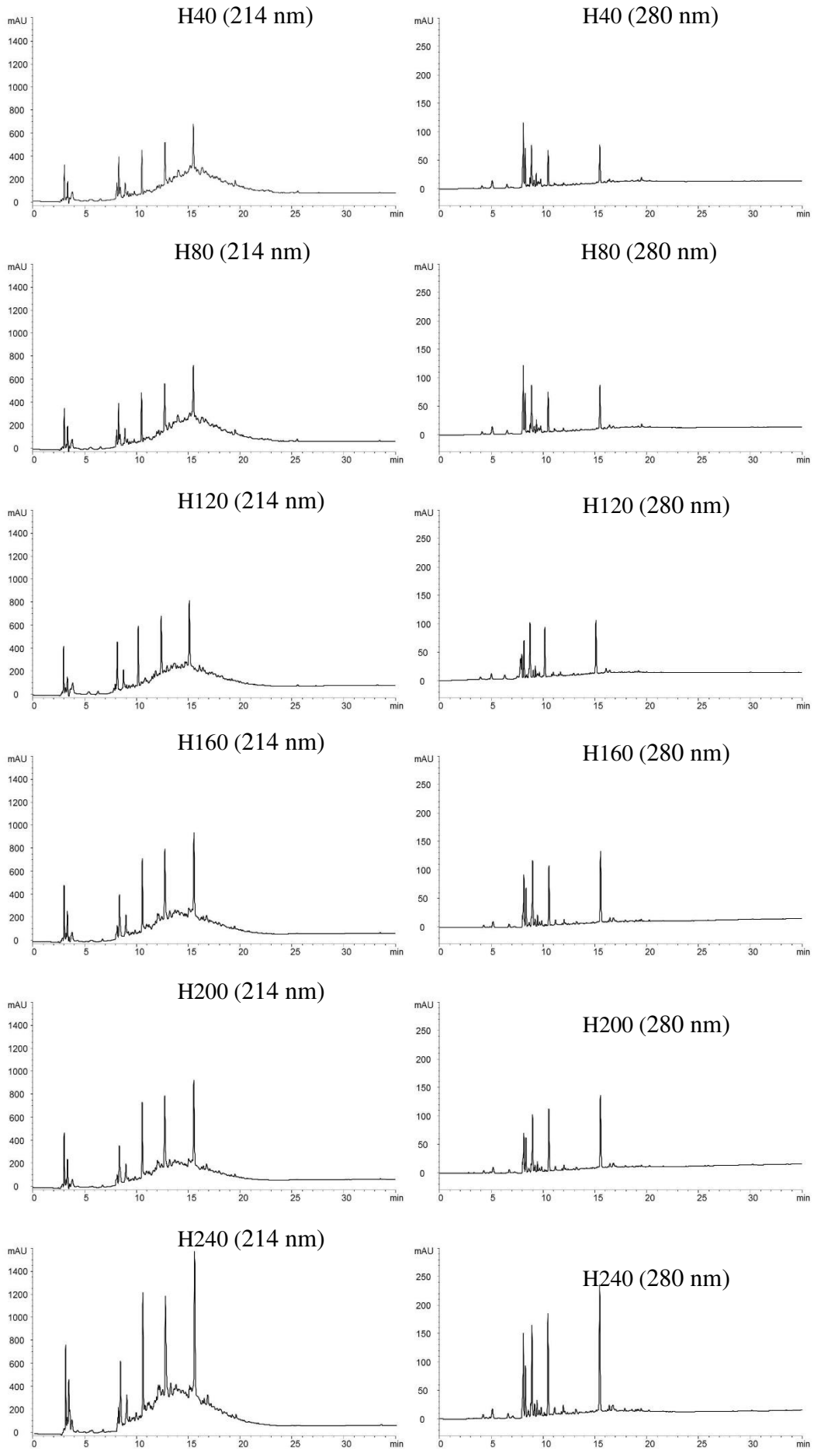


Figure 2. High-performance liquid chromatography of Nile tilapia hydrolysates produced by Alcalase® with different times of hydrolysis.

Table 2. Antioxidant activity measured by ORAC, ABTS and FRAP of (centrifuged and dried) hydrolysate of Nile tilapia head and carcass produced by Alcalase® with different times of hydrolysis.

Sample	ORAC (µM TE/ g*)	ABTS (µM TE/ g*)	FRAP(µM TE/ g*)
H40	331.25±9.87	61.48±7.22	74.83±7.63
H80	305.26±35.10	69.77±10.53	77.27±10.39
H120	327.48±43.96	64.73±16.75	67.89±12.1
H160	349.36±59.92	69.27±8.32	68.96±4.2
H200	337.95±40.66	70.10±11.37	64.09±16.49
h240	303.43±10.38	72.26±18.31	87.91±8.41
P	*0.564*	*0.234*	*0.086*

H40: hydrolysate with 40 minutes of hydrolysis; H80: hydrolysate with 80 minutes of hydrolysis; H120: hydrolysate with 120 minutes of hydrolysis; H160: hydrolysate with 160 minutes of hydrolysis; H200: hydrolysate with 200 minutes of hydrolysis; H240: hydrolysate with 240 minutes of hydrolysis

p: p value by ANOVA. * The results are expressed in µM of Trolox equivalent (TE) per gram (µM TE/ g) of sample .

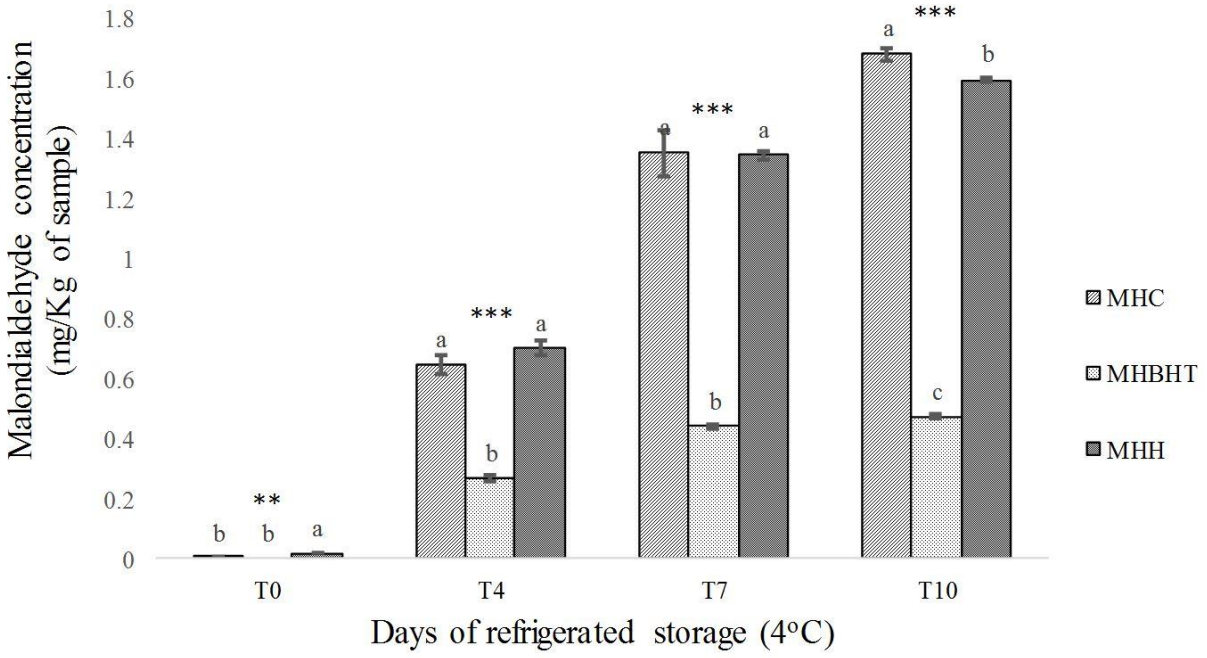


Figure 3. Presence of thiobarbituric acid-reactive substances (TBARS), expressed in mg of malondialdehyde (mg/Kg of sample). The samples analysed were: a mini-hamburgers produced without antioxidant (MHC), same product added of 1% tilapia hydrolysate (MHH) and same mini-hamburger added of 0.02% of butylhydroxytoluene (MHBHT). (**: $p<0.01$, ***: $p<0,001$).

4.7. ABBREVIATIONS

ABTS: 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
BHT: butylhydroxytoluene
CLH: centrifuged lyophilized hydrolysate;
DH: degree of hydrolysis
FRAP: Ferric Reducing Antioxidant Power
H120: hydrolysate with 120 minutes of hydrolysis;
H160: hydrolysate with 160 minutes of hydrolysis;
H200: hydrolysate with 200 minutes of hydrolysis;
H240: hydrolysate with 240 minutes of hydrolysis.
H40: hydrolysate with 40 minutes of hydrolysis;
H80: hydrolysate with 80 minutes of hydrolysis;
HPLC: High-performance liquid chromatography
MDA: malondialdehyde
MHBHT: mini hamburgers with addition of 0.02% (w/w) BHT
MHC: mini hamburgers control (no added antioxidant);
MHH: mini hamburgers with addition of 1% (w/w) of H200;
OPA: o-phthaldialdehyde
ORAC: Oxygen Radical Absorbance Capacity
PUFA: polyunsaturated fatty acids
TBARS: thiobarbituric acid-reactive substances
TE: Trolox equivalent
TEAC: Trolox equivalent antioxidant capacity
WLH: Whole lyophilized hydrolysate;

4.8. ACKNOWLEDGEMENTS

The authors would like to thank the National Counsel of Technological and Scientific Development (CNPq - process 453854/2013-3) and the company Falbom Agroingustrial Ltda. for all their support in the development of this study.

5. CAPÍTULO 03

"ADDITION OF NATURAL ANTIOXIDANTS IN SWINE DIETS CONTAINING HIGH CONTENT OF α-LINOLENIC ACID : EFFECTS ON PERFORMANCE, CARCASS TRAITS AND QUALITY OF MEAT"

Daniela Miotto Bernardi, Teresinha Marisa Bertol, Arlei Coldebella, Bárbara Cristina Silveira-Almeida, Leandro Daniel de Paris and Valdemiro Carlos Sgarbieri

Artigo original submetido para avaliação na revista "*Journal of the Science of Food and Agriculture*".

ABSTRACT

The inclusion of natural antioxidants in animal diets, as well as the use of different lipid sources have been tested in order to improve the nutritional quality of meat. The aim of this work was to assess the effect of adding natural antioxidants to swine diets containing flaxseed oil (FO) on growth performance, total antioxidant status (TAS) in the blood serum, carcass traits and meat quality. Ninety-six animals were alloted in six treatments: C- control diet; F- diet with 3% FO; FGP- diet with 3% FO + 10% grape pomace; FGE- diet with 3% FO + 0.0022% grape seed extract; FH- diet with 3% FO + 5% tilapia carcass hydrolysate; and FVitE- diet with 3% FO + 200 ppm vitamin E supplement. The treatments had no effect on growth performance and on serum TAS. Low magnitude effects were observed in the carcass traits. Regarding the meat quality, the colour score of the FGE treatment suggested a possible antioxidant effect of the grape seed extract. However, further studies are required to take into account the shelf life of the meat, as well as different percentages of added natural antioxidants and different administration times.
Keywords: polyunsaturated fatty acids, tilapia hydrolysate, grape pomace, grape seed extract, oxidative stability.

5.1. INTRODUCTION

In recent years the principal recommendations for human diet in relation to lipid ingestion have underlined the importance of reducing excessive consumption of fats and increasing ingestion of polyunsaturated fatty acids (PUFA), especially omega-3 (ω-3) (1). At the same time the pork meat industry, by means of genetic improvement and nutrition, has also developed considerably, ensuring lower fat content and better fatty acid (FA) profile in the meat, such as the increase of ω-3 and also an increase in monounsaturated fatty acid (MUFA) (2).

Previous studies have shown that the FA content of pig meat and fat reflect the FA profile of the diet (3–5). However, increased PUFA content in the meat can promote sensory alterations in the product, which among other factors may be associated to lower oxidative stability, resulting in greater lipidic oxidation and, consequently, greater protein oxidation (6,7).

The addition of natural antioxidants to the animal's diet might offer a solution for increasing the oxidative stability of meat (7), especially in those with high PUFA levels. Vitamin E, vitamin C, carotenoids, phenolic compounds, thiols and certain peptides are some

examples of antioxidants found in food and often in processed food derivatives (8). Natural antioxidants can be extracted and purified prior to being added to the diet, or the source of these compounds (food or by-product) can be directly incorporated into the diet (7).

Processed grape derivatives contain significant quantities of phenolic compounds, which are important free radical sequestrants, that can act in the delay of oxidation reactions(9). Grape pomace and grape seed extract have been tested in poultry diet and displayed a residual antioxidant effect on the meat (10,11), however in swine diets the effect of these products remain partially unclear (12,13).

It has recently been demonstrated that peptides formed from fish protein hydrolysis and their by-products possess antioxidant potential (8). An *in vitro* study (14) showed that following the action of gastrointestinal enzymes, these peptides maintain or increase their antioxidant activity. Furthermore, they contain significant quantities of hydrophobic amino acids that could facilitate their entry into the target organs by means of hydrophobic interactions with membranes (15). However, although displaying antioxidant effect in *in vitro* tests, very few studies have evaluated this product in animal diet (16,17).

Vitamin E is the most tested natural antioxidant in animal diet (18). Its effect on the oxidative stability of meats over the shelf life has been widely reported in the literature (19). On the other hand, some authors suggest that the efficiency of this vitamin might be compromised when the diet contains high PUFA concentrations (18).

Therefore, considering the scarcity of *in vivo* studies with pigs, as well as the lack of consensus regarding the effect of natural antioxidants, especially when associated to heightened levels of PUFA in the meat, there is a clear necessity to evaluate the effect of dietary inclusion of the aforementioned natural antioxidants on meat quality. Moreover, considering the inclusion level of some sources of these antioxidants in the diet, their contribution with essential nutrients, the presence of anti-nutritional factors and the possible negative interaction with components of the diet, it is also important to evaluate the effect of these products on the growth performance and carcass characteristics of the pigs.

In this context, the aim of this study was to evaluate the effect of including recognised sources of natural antioxidants (grape pomace, grape seed extract, Nile tilapia carcass hydrolysate and Vitamin E) associated to flaxseed oil supplementation in pigs' diets on the growth performance, total antioxidants in the serum, carcass traits and the quality of *in natura* meat.

5.2. MATERIALS AND METHODS

5.2.1. Animals, experimental diets evaluation of performance, "in vivo" body composition and serum total antioxidant analysis (TAS)

The experimental procedures were approved by the Ethics Committee on Animal Use in Research (CEUA / CNPSA) (file reference 002/2014), in accordance with the ethical principles for animal experimentation. Prior to the experimental period, the animals were reared at a confined pig production unit at Embrapa Suínos e Aves, Concórdia, Santa Catarina, Brazil.

Ninety-six pigs of the Embrapa MS115 x F1 genotype, with average initial age of 127.39 ± 4.29 days and average initial weight of 80.01 ± 2.43 kg were distributed into treatments according to initial weight (block) in a randomized complete block design with six treatments: C- control diet with no added oil; F- diet with 3% flaxseed oil (FO); FGP- diet with 3% FO + 10% grape pomace; FGE- diet with 3% FO + 0.0022% grape seed extract (Cargill, reference code F400851912501); FH- diet with 3% FO + 5% tilapia protein hydrolysate; and FVitE- diet with 3% FO + 200 ppm vitamin E (α-tocopheryl acetate - Cargill, reference code 1920101011); and two sexes: gilts and barrows.

The experimental diets were formulated to be isocaloric and isoproteic and meet the nutritional requirements for pigs with a live weight of 70 to 120 kg established by Rostagno et al. (20). A detailed description of the diets and their physicochemical composition is presented in Table 1. Chemical composition of the diets and ingredients used in the diets formulationwas analysed in accordance with the AOAC (21) and the FA composition was analysed following lipid extraction (22), saponification and esterification (23). The FA methyl-esters were separated in a Varian CP-3800 gas chromatograph (WalnutCreek, CA, USA) equipped with a Varian CP-8410 autosampler, CP-1177 split/splitless injector and flame ionization detector (H2 flow of 30 mL/min, air flow of 300 mL/min). The management of the instrument was conducted by Varian Star Workstation 5.0 software. The separation was performed using a fused silica capillary column Supelco SP-2380, with 100 m length, 0.25 mm internal diameter and 0.2 mM of film thickness. The heating furnace was operated by programming the temperature from 140 to 190° C at a rate of 4° C/min, held at 190° C for 15 min, and further increased from 190 to 220° C at a rate of 1° C/min, kept the temperature at 220° C for 17.5 min. The temperatures of the injector and detector were 240 and 280° C, respectively. The liner used was "split / splitless with single taper" with 78.5 mm length and 4 mm internal diameter (SGE Analytical Science).

The split ratio was 1: 100 and the injected volume was 0.2-0.5 uL. Ultrapure Nitrogen was used as carrier gas with a flow of 1.6 mL/min.

Table 1

Crude flaxseed oil used in the diets was purchased in local commerce, coming from production by cold pressing. The grape pomace, composed of skin and seeds, was obtained from red wine processing, dehydrated in a fixed-bed dryer (4 h, at 45 to 60º C) and contained 9.12% moisture, 12.58% crude protein, 11.25% ether extract, 8.82% ash and 34.48% crude fibre.

The tilapia hydrolysate was obtained from hydrolysis (with Alcalase®, 2.75 AU-A/g, from *Bacillus licheniformis,* Novozymes Latino Americana Ltda., Paraná, Brazil) from by-products (carcass and head) of Nile tilapia (Falbom Agroindustrial Lta, Paraná, Brazil). The hydrolysis time was 2 hours followed by drying in a Spray dryer (R.M. Máquinas Frigoríficas, model CTS-01) using maltodextrin as drying aid agents. Details of the experimental protocol used for production of this product are described by De Paris et al. (24). The tilapia hydrolysate had 10.0% moisture, 33.9% crude protein, 1.1% ether extract and 13.2% ash.

The vitamin E supplement (Cargill, reference code 1920101011) used contained 500 mg of the vitamin/g of product and was added to the diet of the FVitE treatment at the required proportion to obtain a concentration of 200 ppm of vitamin E. In accordance with the manufacturer's specifications, every 1 g of grape seed extract (Cargill, reference code F400851912501) corresponds to an antioxidant power equivalent to 9 g of vitamin E, or 18 g of the vitamin E supplement used in the experiment. Hence, the concentration of grape seed extract used in the FGE treatment was that recommended (0.0022%) to attain the antioxidant potential equivalent to 200 ppm of vitamin E.

The antioxidant activity of the grape pomace, grape seed extract and Nile tilapia carcass hydrolysate was determined in aqueous extract. The oxygen radical absorption capacity (ORAC), the determination of Ferric Reducing Antioxidant Power (FRAP) and total antioxidant activity of the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation, were executed in accordance with the methods proposed by Dávalos et al. (25), Benzie and Strain (26) and Re et al. (27), respectively. The results were expressed in μmol Trolox equivalent (TE) per gram of sample.

The experiment lasted 42 days and throughout this period the animals were kept in individual pens, equipped with nipple drinkers and semi-automatic feeders, therefore, with

water and feed *ad libitum*. The animals were individually weighed at the start of the experiment (IW), at day 21 and at the end of the experiment (FW).

Backfat thickness and loin depth were measured *in vivo* at the start (BFaloka1 and LDaloka1) and on the final day (BFaloka2 e LDaloka2) of the experiment, with the use of an ultrasound equipment (ALOKA SSD-500 V), coupled to a linear array transducer (UST 5011-3.5 MHz) and operated through the software BioSoft Toolbox® for Swine by Biotronics Inc (West Lafayette, USA). The evaluations were made with the animal kept standing on a flat surface. The P2 point was established, where two images were taken and the backfat thickness and loin depth were calculated by the aforementioned software.

Blood samples (10 mL) were taken from the animals the day before slaughter. After the material was centrifuged, the serum was separated and stored at -70ºC until the time of the analyses. Total antioxidant (TAS) determination involved the use of a total antioxidant analysis kit (reference NX2332), control (reference NX2331) and standard (reference NX2615), both produced by Randox Laboratories Ltd., Co. (Antrim, UK).

After 42 days the pigs were subjected to 15 hours pre-slaughter fasting with *ad libitum* supply of water. Loading and transport to the slaughterhouse were carried out at the start of the morning and the distance travelled was 15 km. At the abattoir, the animals rested for 4 hours and the slaughter was executed by bleeding in the horizontal position following electrical stunning, in accordance with the industry standards. Following evisceration, the carcasses were stored in a cold chamber, with an average temperature of 2 to 4° C for 24 hours.

5.2.2. Evaluations of the carcass characteristics

Immediately after the slaughter and evisceration the carcasses were weighed, obtaining the hot carcass weight (HCW). After 24 hours of cold storage, the measurements of carcass traits were taken in accordance with ABCS (28). Backfat thickness (BF) was measured using a digital calliper at the following points: first rib (BF1), last rib (BF2), first sacral vertebra (BF3) and P2 (at the last rib, in the region of the insertion of the last thoracic vertebra with the first lumbar vertebra, six centimetres from the midline) (BF4a) (28).

The loin depth (LD) and a second measurement of backfat thickness at P2 (BF4b), mimicking the electronic grading probe reading position, were obtained from the drawing of the loin eye area and fat area in the last rib. The loin eye area (LEA) and subcutaneous fat area (FArea) were measured, using a planimeter (28).

Based on the LEA and FArea measurements, the fat-meat ratio (FMR) was calculated (FMR = FArea/LEA). The percentage of lean meat (PLM) was calculated using BF4b and LD, according to the equation established by Guidoni et al. (29):

$$PLM = 58.408 – (0.5886 \times BF\ P2) + (0.1739 \times LD) – (0.0189 \times HCW)$$

5.2.3. Meat quality analysis

Measurements of pH were performed 45 minutes (pH45') and 24 hours (pH24h) after slaughter, in the loin (*Longissimus thoracics)* at the last rib and in the ham (*semimembranosus)* using a portable digital pH meter.

Twenty-four hours after slaughter the colour and marbling scores were assessed, in accordance with the standard (30). Based on the CIELAB system (L*, a*, b*, Spectrophotometer, MinoltaLTD., Japan), colour assessments of the loin and ham were also made after the samples were exposed to air for 20 minutes. Colour saturation was calculated according to Little (31).

$$\text{Colour saturation index} = \sqrt{a^{*2}+b^{*2}}$$

Loin and ham samples were collected for analysis of drip loss and cooking loss (CL), as per methodology proposed by Honikel (32).

To determine moisture, ash and ether extract content, loin samples were lyophilized (Liobrás Freeze-Dryer, model LP810) and ground in a cooling grinder (FossTecator 1095 — Knifetec Sample) and stored at -20° C. The analyses were conducted in accordance with AOAC (21).

5.2.4. Statistical Analysis

The data were subjected to analysis of variance using the GLM procedure of SAS (33). The mathematical model used included the effects of block (initial weight) within sex, treatment and treatment *vs*. sex interaction. Protected t test was used for comparison of multiple means whenever the F test detected significant effect ($p \leq 0.05$).

5.3. RESULTS AND DISCUSSION

No interaction was detected between treatment and sex ($p>0.05$) in the variables evaluated in this study.

5.3.1. Antioxidant activity

The results of the antioxidant activity of the grape pomace, grape seed extract and Nile tilapia carcass hydrolysate are shown in Table 2. The grape seed extract showed the greater antioxidant capacity among the products evaluated. Both the grape seed extract and the grape pomace showed greater antioxidant activity measured by FRAP, whereas the principal antioxidant mechanism of the hydrolysate was elimination of the peroxyl radical, measured by ORAC. The ORAC method presented the best correlation with physiological conditions (25) .

Table 2

5.3.2. Growth performance and blood serum TAS

The treatments did not influence the FW, average daily weight gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FC) ($p>0.05$; Table 3). The absence of any effect on growth performance is probably due to the fact that the diets were isoenergetic and isoproteic.

Regarding the inclusion of flaxseed oil in the pigs diets, there were also no effects on growth performance as reported in other studies (4) in which different sources of oils were evaluated using isoenergetic and isoproteic diets.

Like in the present study, Realini et al. (3) observed no interference of the sources of oil and fats on the ADFI, ADG, and FW of the animals, however these authors found that the inclusion of a blend of fats (bovine tallow, sunflower oil, and flaxseed oil) in the diet (10% inclusion rate) improved the FC compared to the treatment with no added fat.

Table 3

Just like in the other treatments, no alterations were observed in the growth performance of the animals fed the diets with grape pomace, grape seed extract, supranutritional levels of

vitamin E and fish hydrolysate ($p>0.05$). The results obtained are in agreement with other authors who added grape products in swine (13) and chicken diets (10,11), supranutritional levels of vitamin E in the swine diet (34–36) and fish hydrolysate in swine diets (16).

On the other hand, gender did influence weight, feed consumption and feed conversion ratio, barrows displayed a greater IW ($p<0.001$), FW ($p<0.001$) and ADFI ($p<0.001$). Gilts showed a better FC ($p<0.001$). These results were expected and are in line with those reported in the literature (4,37,38).

The addition of antioxidants to the diet caused no difference on blood serum TAS levels between treatments ($p=0.2025$). O`Grady et al. (39) also found no increase in TAS levels in the plasma of beef cattle and pigs fed with natural antioxidants. Likewise, Gladine et al. (18) found no increase in the plasma TAS of rats fed with natural antioxidants, but found that these compounds were capable of effectively reduce the lipidic oxidation of the plasma.

5.3.3. Carcass traits

The treatments had no effect on the carcass traits parameters ($p>0.05$), except for the backfat thickness measured by the calliper at P2 (BF4a), which was greater in the animals under the control treatment ($p<0.05$) (Table 4).

However, it should be underlined that this result is an effect of minor magnitude and does not reflect the reality of the carcass as a whole, since the other BF measurements taken in the carcass (BF1, BF2, BF3, BF4b) and also in the live animal (BFaloka1, BFaloka2) showed no differences between treatments ($p>0,05$).

Table 4

The absence of any effect of the antioxidants added to the diets on the carcass traits is in line with the findings presented in the literature regarding the inclusion of grape pomace (13) and vitamin E (34–36). On the other hand, Liaset et al. (17) found that the presence of fish hydrolysate in rat diet reduced the weight of the animals and body fat percentage.

The antioxidant sources assessed in the present study with levels of 5% (tilapia hydrolysate – FH) or 10% (grape pomace – FGP) of inclusion in the diet are ingredients that are still the subject of very few studies in animal diet and their precise values of metabolizable energy (ME) and available amino acids remain unknown. Therefore, the values used in the formulation of the diets may not correspond precisely to the product used, and this may

influence the deposition of protein and fat in the carcass. The same applies to the flaxseed oil regarding to its ME value and should be considered as a possible reason for the lower BF4a measurements found in treatments F, FGP, FGE, FH and FVitE.

The sex of the animals had a strong influence on the parameters of carcass yield and quality. The HCW and carcass yield (CY) were greater for the barrows ($p<0.001$). The BF at all measurement points of the carcass (BF1, BF2, BF3, BF4a, BF4b) and also in the *in vivo* evaluations (BFaloka1 and BFaloka2), as well as the FArea and FMR were also greater in barrows ($p<0.001$). On the other hand, the gilts displayed a higher PLM ($p<0.001$) and a greater LEA ($p=0.05$). These results agree with the literature (4,37,38) and confirm that the female carcasses have a higher proportion of muscle, whereas the barrows carcasses have a higher proportion of fat. This greater body fat deposition in the barrows can be explained by the fact that castration significantly contributes to increased body fat deposition (40).

5.3.4. Meat quality

The marbling score and ether extract, dry matter and ash content of the loin were not affected by treatments ($p>0.05$; Table 5). Teye et al. (5) also found no effect of the lipidic source of the diet on marbling, however some studies have demonstrated that the kind of fat added to the diet can interfere in the marbling score of the loin (4,38) and on fat (3,38) and protein (3) content of the carcass.

As in present study, others authors (13,34,35) showed that the presence of natural antioxidants in swine diet (supranutritional doses of vitamin E and 3% grape pomace, respectively) had no effect on the marbling score of the loin, lipid percentage and moistness of the meat.

Table 5

There was no difference between the treatments in the pH45 minutes of the loin and ham ($p>0.05$) and pH24h of the loin ($p>0.05$), however the pH24h of the ham differed ($p<0.05$), with the highest values observed in the FGE and FGP treatments and lowest values in the FVitE treatment.

The loin colour scores also differed between treatments. The highest scores were observed in the FGE and control treatments, while the FVitE treatment had the lowest score

(p=0.05). In the ham, the colour score followed the same trend as in the loin what can be justified by the value of p obtained (p=0.057).

On the other hand, regarding the colour measured by the Cielab system (L*, a*, b* and colour saturation) there was no difference between the treatments (p>0.05). The same occurred for drip loss and for cooking loss (p>0.05).

Considered in isolation, the statistical difference observed in pH24h is not corroborated in the literature. The main effects of the animal diet on this parameter are related to the ingestion of carbohydrates, as well as fasting periods (2). Furthermore, other studies with natural antioxidants (13,34–36,41) and other lipidic sources (4,5) in swine diets have not proven any effect of these ingredients on the pH of meat.

However, when analysing the pH and colour results together, a similar behaviour was found between the treatments, that is, in the treatment with grape seed extract, higher pH24h (ham) and colour score (loin and ham) values were observed, whereas in FVitE treatment lower pH and colour scores were found. Therefore, despite the pH results not being in accord with those reported in the literature regarding the effect of treatments, as mentioned above, they may have influenced the colour.

The effect of pH on the colour of meat is well known, as it affects the eletrostatic repulsion of proteins and consequently the water holding capacity (WHC) of the myofibril proteins. The greater the gap between the final pH of the meat and the isoelectric point of the myofibril proteins the greater the electrostatic repulsion in these proteins, as well as the greater the solubility in the middle and, consequently, the greater the WHC of these proteins will be. A higher WHC of the myofibril proteins results in less liquid loss from the cell and therefore greater retention of sarcoplasmic proteins, such as myoglobin, promoting a better colour score. Hence, the pH may have influenced the colour in the FGE and FVitE treatments.

As well as the pH, another factor that can interfere in the colour of the meat is the redox state of the myoglobin (42) and this fact might explain part of the colour score results obtained, for the presence of PUFA and antioxidants in the diet can interfere directly in the oxidation of this protein.

The presence of PUFA in the diet increases the PUFA levels in the meat (3–5), which reduces its oxidative stability (6). Therefore, the high colour scores in the control treatment could be due to a greater SFA content and lesser PUFA content in the meat, which may have resulted in lower lipidic oxidation and, consequently, lower myoglobin oxidation. Juárez et al. (2011) also found that the addition of flaxseed reduced the colour parameters of meat.

Some authors (9,18,43) suggest that when phenolic compounds ((+)-catechins, (-) epicatechins and quercitins) are present in the animal diet, they are absorbed and incorporated and may act on the tissues in such a way as to promote a "saving" and "recycling" effect of tissue vitamin E, resulting in oxidative protection. Therefore, the higher colour score observed in the treatment with grape seed extract may be due to this protective effect of the phenolic compounds on lipidic oxidation and consequently on the redox state of the myoglobin. Some authors who added products rich in these antioxidants to animal diet have described positive effects on the a* and b* values and colour saturation index (13,44). Other authors did not find any effect of grape seed extract on meat colour (12).

It has been reported in the literature that supranutritional doses of vitamin E in the animal diet foster protection against oxidation reactions in meats (19). However, based on color results obtained for the animals under FVitE treatment it was not possible to prove this effect, therefore the redox state of the myoglobin would not seem to explain the colour results obtained in fresh meat cold stored for 24 hours in this study. Guo et al. (35) also found no protective effect of supranutritional doses of vitamin E on the colour of the meat *in natura* with 24 hours of cold storage. Therefore, this colour stability effect reported on the meat of animals fed with higher concentrations of vitamin E is likely more evident over the course of the meat storage (34,36).

Wang et al. (36) also reported that the presence of natural antioxidants in the diet, especially vitamin E, can protect the membrane phospholipids from oxidation, thus ensuring greater integrity of this membrane and consequently less liquid losses. However, the drip loss results of the treatments with antioxidants failed to confirm this, and corroborate the results of some authors (34,35). Other authors (38,45), just like in the present study, also failed to find any effect of the lipidic source of the diet on drip loss of the meat.

The meat quality evaluations made in this study were performed with the meat *in natura* stored for 24 hours post slaughter, and this imposes few challenges for the tested antioxidants, as well as very little time for most of the PUFAs present in meat to promote a pro-oxidant effect. Therefore, from a point of view of oxidative reactions, more investigations are necessary. Some indications of antioxidant activity have been observed, such as in the treatments with grape seed extract, yet assessments that consider the shelf life of the meat and meat products are recommended to evaluate oxidative stability and confirm this, as well as so the other antioxidants tested can be sufficiently challenged to delay oxidation reactions.

It is important to highlight that although the tilapia hydrolysate (FH) did not display a protective effect, it cannot be disregarded as a potential antioxidant for animal diet, considering

its antioxidant capacity *in vitro* (14,46), as well as considering the economic, environmental, and nutritional importance of the product. New approaches are necessary, with more detailed investigations about how the peptides resulting from hydrolysis of fish proteins behave *in vivo*, during digestion, absorption and distribution in target tissues, as well as the magnitude of their bioactivity after passing through all these stages, and how they might affect the oxidative balance of the tissues so as to cause residual post mortem effect.

With respect to the influence of the sex on the meat quality parameters evaluated, it was found that the percentage of ether extract ($p<0.005$) and marbling score ($p<0.001$) of the loin were greater in the barrows, corroborating the carcass traits results and thus confirming that barrows possess a higher body fat content. These results are in agreement with those reported by Dungan et al. (45). Other studies however (4,37) have not verified any differences in marbling between barrows and gilts.

No significant differences were observed in the pH45 minutes and pH24h values between barrows and gilts ($p>0.05$), and this result is in line with that reported in the literature (37,38,45). There was also no difference between the sexes for meat colour score and L* and b* values, however, higher a* values were found in the ham of barrows ($p<0.05$) and the same trend was found in the loin ($p=0.07$). The colour saturation index also differed, with the highest values being found in the loin ($p<0.05$) and ham ($p<0.01$) of the barrows. Eggert et al. (37) did not find any statistical difference between the sexes for colour scores, whereas Dugan et al. (45) found higher L*, a*, b* values and colour saturation indices for barrows. Bertol et al. (4) reported higher b* values for barrows.

The sex significantly influenced drip loss of the loin, with greater losses being observed in the barrows ($p<0.05$). This result is not in agreement with the literature, which shows that there is no statistical difference in the drip loss between barrows and gilts (37,45). On the other hand, drip loss in the ham and cooking loss in the loin did not statistically differ between barrows and gilts ($p>0.05$).

5.4. CONCLUSION

The adding natural antioxidants in swine diets containing flaxseed oil did not cause any detrimental effect on growth performance and only caused low magnitude effects on the carcass traits.

As regards the meat quality, the pH and colour results followed a very similar pattern, suggesting a possible antioxidant effect of the grape seed extract. However, despite the

promising results new studies are required to prove this antioxidant effect of the grape by-products, as well as to better assess the other tested products, such as tilapia hydrolysate. New approaches using different supplementation times and concentrations of bioactive products should be considered in future experimental designs.

Additional studies are also recommended to improve the estimative of the metabolizable energy and the coefficients of digestibility of amino acids of these new ingredients for use in dietary formulation.

5.5. REFERENCES

1. EFSA. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. Efsa J [Internet]. 2010;8(3):1461.
2. Rosenvold K, Andersen HJ. Factors of significance, for pork quality - a review [Review]. Meat Sci. 2003;64(3):219–37.
3. Realini CE, Duran-Montgé P, Lizardo R, Gispert M, Oliver M a., Esteve-Garcia E. Effect of source of dietary fat on pig performance, carcass characteristics and carcass fat content, distribution and fatty acid composition. Meat Sci, 2010;85(4):606–12.
4. Bertol TM, de Campos RML, Ludke J V, Terra NN, de Figueiredo EAP, Coldebella A, et al. Effects of genotype and dietary oil supplementation on performance, carcass traits, pork quality and fatty acid composition of backfat and intramuscular fat. Meat Sci, 2013;93(3):507–16.
5. Teye GA., Sheard PR, Whittington FM, Nute GR, Stewart A, Wood JD. Influence of dietary oils and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. Meat Sci. 2006;73(1):157–65.
6. Decker EA, Akoh CC, Wilkes RS. Incorporation of (n-3) Fatty Acids in Foods: Challenges and Opportunities. J Nutr. 2012;
7. Bernardi DM, Bertol TM, Pflanzer SB, Sgarbieri VC, Pollonio MAR. ω-3 in meat products: Benefits and effects on lipid oxidative stability. J Sci Food Agric. 2016;
8. Samaranayaka AGP, Li-Chan ECY. Food-derived peptidic antioxidants: A review of their production, assessment, and potential applications. J Funct Foods. 2011;3(4):229–54
9. Lafka T-I, Sinanoglou V, Lazos ES. On the extraction and antioxidant activity of phenolic compounds from winery wastes. Food Chem. 2007;104(3):1206–14.

10. Brenes A, Viveros A, Goñi I, Centeno C, Sáyago-Ayerdy SG, Arija I, et al. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. Poult Sci. 2008;87(2):307–16.
11. Sáyago-Ayerdi SG, Brenes A, Viveros A, Goñi I. Antioxidative effect of dietary grape pomace concentrate on lipid oxidation of chilled and long-term frozen stored chicken patties. Meat Sci . 2009;83(3):528–33.
12. O'Grady MN, Carpenter R, Lynch PB, O'Brien NM, Kerry JP. Addition of grape seed extract and bearberry to porcine diets: Influence on quality attributes of raw and cooked pork. Meat Sci. 2008;78(4):438–46.
13. Yan L, Kim IH. Effect of Dietary Grape Pomace Fermented by Saccharomyces boulardii on the Growth Performance, Nutrient Digestibility and Meat Quality in Finishing Pigs. Asian-Australasian J Anim Sci. 2011;24(12):1763–70
14. Wiriyaphan C, Xiao H, Decker EA, Yongsawatdigul J. Chemical and cellular antioxidative properties of threadfin bream (Nemipterus spp.) surimi byproduct hydrolysates fractionated by ultrafiltration. Food Chem. 2015;167:7–15.
15. Girgih AT, He R, Hasan FM, Udenigwe CC, Gill TA, Aluko RE. Evaluation of the in vitro antioxidant properties of a cod (Gadus morhua) protein hydrolysate and peptide fractions. Food Chem. 2015;173:652–9.
16. Nørgaard J V., Blaabjerg K, Poulsen HD. Salmon protein hydrolysate as a protein source in feed for young pigs. Anim Feed Sci Technol. 2012;177(1-2):124–9.
17. Liaset B, Madsen L, Hao Q, Criales G, Mellgren G, Marschall HU, et al. Fish protein hydrolysate elevates plasma bile acids and reduces visceral adipose tissue mass in rats. Biochim Biophys Acta - Mol Cell Biol Lipids. 2009;1791(4):254–62.
18. Gladine C, Morand C, Rock E, Gruffat D, Bauchart D, Durand D. The antioxidative effect of plant extracts rich in polyphenols differs between liver and muscle tissues in rats fed n-3 PUFA rich diets. Anim Feed Sci Technol 2007;139(3-4):257–72.
19. Sales J, Koukolová V. Dietary vitamin E and lipid and color stability of beef and pork: Modeling of relationships. J Anim Sci. 2011;89(9):2836–48.
20. Rostagno HS, Allbino LFT, Donzele JL, Gomes PC, Oliveira RF de, Lopes DC, et al. Tabelas brasileiras para aves e suínos. 2005;189.
21. AOAC. Official methods of analysis of AOAC International. 16th ed. Virgínia; 1995.
22. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 1957;226(1):497–509
23. Hartman L, Lago RCA. Rapid preparation of fatty acid methyl esters from lipids. Lab

Pr. 1973;22:475–6,494.

24. De Paris LD, Adams Haab JC, Sary C, Bernardi DM, Boscolo WR, Signor A. Production and spray drying of protein hydrolyzate obtained from tilapia processing by-products. Acta Sci Technol. 2016;38(1):89.
25. Dávalos A, Gómez-Cordovés C, Bartolomé B. Extending Applicability of the Oxygen Radical Absorbance Capacity (ORAC-Fluorescein) Assay. J Agric Food Chem. 2004;52(1):48–54.
26. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70–6.
27. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9-10):1231–7.
28. ABCS AB de criadores de suínos. Método brasileiro de classificação de carcaças. RS: Estrela; 1973. 17p p.
29. Guidoni AL, Dallacosta OA, Bertol TM. Preditores e Predição do Peso, Porcentagem e Quantidade de Carne de Carcaça Suínas e Suas Partes. Relatório Técnico da Empresa Brasileira de Pesquisa Agropecuária. EMBRAPA; 2007.
30. NPPC. Pork quality standards. Council. NPP, editor. Des Moines, IA; 1999.
31. Little AC. Off on a tangent. J Food Sci [Internet]. 1975;40(2):410–1. Available from: http://dx.doi.org/10.1111/j.1365-2621.1975.tb02213.x
32. Honikel KO. Reference methods for the assessment of physical characteristics of meat. Meat science. 1998. p. 447–57.
33. SAS Institute Inc. System for Microsoft Windows, Release 9.4, Cary, NC, USA, 2002-2012
34. Boler DD, Gabriel SR, Yang H, Balsbaugh R, Mahan DC, Brewer MS, et al. Effect of different dietary levels of natural-source vitamin E in grow-finish pigs on pork quality and shelf life. Meat Sci. 2009;83:723–30.
35. Guo Q, Richert BT, Burgess JR, Webel DM, Orr DE, Blair M, et al. Effects of dietary vitamin E and fat supplementation on pork quality. J Anim Sci. 2006;84(11):3089–99.
36. Wang H, Wang LS, Shi BM, Shan AS. Effects of dietary corn dried distillers grains with solubles and vitamin E on growth performance, meat quality, fatty acid profiles, and pork shelf life of finishing pigs. Livest Sci. 2012;149(1-2):155–66.
37. Eggert JM, Grant AL, Schinckel AP. F actors Affecting Fat Distribution in Pork Carcasses. 2007;23(16301):42–53.

38. Juárez M, Dugan MER, Aldai N, Aalhus JL, Patience JF, Zijlstra RT, et al. Increasing omega-3 levels through dietary co-extruded flaxseed supplementation negatively affects pork palatability. Food Chem. 2011;126(4):1716–23.
39. O`Grady MN, Maher M, Troy DJ, Moloney a. P, Kerry JP. An assessment of dietary supplementation with tea catechins and rosemary extract on the quality of fresh beef. Meat Sci. 2006;73:132–43.
40. Kouba M, Sellier P. A review of the factors influencing the development of intermuscular adipose tissue in the growing pig. Meat Sci. 2011;88(2):213–20
41. Larraín RE, Krueger CG, Richards MP, Reed JD. Color Changes and Lipid Oxidation in Pork Products Made From Pigs Fed With Cranberry Juice Powder. 2006;19(2008):17–33.
42. Mancini R a., Hunt MC. Current research in meat color. Meat Sci. 2005;71(1):100–21.
43. Frank J. Beyond vitamin E supplementation: An alternative strategy to improve vitamin E status. J Plant Physiol. 2005;162(7):834–43.
44. Bertol TM, Al. E. Effects of the inclusion of grape pomace in the diet of pigs on pork quality and oxidative stability of omega-3 enriched fat. Ciência Rural. 2016;accepted a.
45. Dugan MER, Aalhus JL, Robertson WM, Rolland DC, Larsen IL. Practical dietary levels of canola oil and tallow have differing effects on gilt and barrow performance and carcass composition. Can J Anim Sci. 2004;84(4):661–71.
46. Girgih AT, He R, Hasan FM, Udenigwe CC, Gill TA, Aluko RE. Evaluation of the in vitro antioxidant properties of a cod (Gadus morhua) protein hydrolysate and peptide fractions. Food Chem. 2015;173:652–9.

5.6. TABLES

Table 1. Percentage composition and nutrients content, energy and fatty acids of the experimental diets fed from days 0 to 21 and 22 to 42 of the experimental period.

Ingredients %	Days 0 to 21						Days 22 to 42					
	C[d]	F[d]	FGP[d]	FGE[d]	FH[d]	FVitE[d]	C[d]	F[d]	FGP[d]	FGE[d]	FH[d]	FVitE[d]
Corn	76.837	62.046	64.124	62.049	62.778	62.104	81.894	67.518	69.608	67.521	68.903	67.576
Soybean meal	20.217	18.096	19.564	18.098	15.350	18.131	15.569	13.564	15.041	13.566	11.204	13.600
Wheat bran	0	13.400	0.542	13.992	11.325	13.865	0	13.473	0	13.466	9.271	13.339
Flaxseed oil	0	3,000	3,000	3,000	3,000	3,000	0	3,000	3,000	3,000	3,000	3,000
Grape pomace	0	0	10,000	0	0	0	0	0	10,000	0	0	0
Tilapia hydrolysate	0	0	0	0	5,000	0	0	0	0	0	5,000	0
Vitamin E	0	0	0	0	0	0.040	0	0	0	0	0	0.040
Grape seed extract	0	0	0	0.0022	0	0	0	0	0	0.0022	0	0
Limestone	0.861	1.102	0.690	1.102	1.326	1.100	0.780	1.012	0.656	1.011	1.607	1.009
Dicalcium Phosphate	0.811	0.439	0.764	0.439	0	0.442	0.689	0.330	0.599	0.330	0	0.334
Salt	0.298	0.296	0.298	0.296	0.248	0.296	0.179	0.177	0.179	0.177	0.130	0.177
Vitamin mix[a]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Mineral mix[b]	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
L-Lysine	0.232	0.266	0.241	0.266	0.231	0.265	0.207	0.238	0.215	0.238	0.197	0.237
L-Threonine	0.047	0.050	0.050	0.057	0.050	0.057	0.052	0.0592	0.065	0.059	0.058	0.059
DL-Methionine	0.016	0.020	0.037	0.020	0.005	0.020	0	0	0.007	0	0	0
Choline Chloride	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030

Table 1 (Continued)

Ingredients, %	Days 0 to 21						Days 22 to 42					
	C[d]	F[d]	FGP[d]	FGE[d]	FH[d]	FVitE[d]	C[d]	F[d]	FGP[d]	FGE[d]	FH[d]	FVitE[d]
Mycotoxin binder	0.450	0.450	0.450	0.450	0.450	0.450	0.450	0.450	0.450	0.450	0.450	0.450
Colistin sulfate	0.050	0.0570	0.061	0.050	0.057	0.050	0	0	0	0	0	0
Calculated nutrient content												
ME (kcal/kg)	3288	3300	3300	3300	3300	3300	3305	3322	3321	3322	3322	3322
Crude Protein (%)	15.68	16.5	15.36	16.02	15.88	16.21	14.95	14.44	14.57	14.16	14.63	14.21
Ether Extract (%)	3.44	6.38	6.94	6.58	6.21	6.36	3.47	6.14	6.98	6.37	6.11	6.45
Ca (mg/kg)	5642	5647	5156	5335	6190	5112	5280	5374	4605	5732	6771	5125
Total P (mg/kg)	4976	5093	4759	5170	6520	5209	4375	4860	4197	4522	5866	4415
Digestible lysine (%)	0.85	0.85	0.85	0.85	0.85	0.85	0.72	0.72	0.72	0.72	0.72	0.72
Σ SFA[c] (mg/100g)	629	991	1028	997	999	978	634	1015	1096	1056	1045	1020
Σ MUFA[c] (mg/100g)	1060	1754	1825	1747	1710	1797	1062	1705	1919	1808	1740	1781
Σ PUFA[c] (mg/100g)	1602	3356	3782	3547	3227	3305	1623	3151.5	3659	3216	3049	3365
C18:2(mg/100g)	1529	2017	2385	2078	1967	1945	1343	1959	2261	1983	1791	2093
C18:3 (mg/100g)	74	1339	1397	1469	1260	1361	277	1193	1398	1225	1249	1267

[a] Vitamin mix: supplied by Cargill, reference code F30420. Minimum content per kg : Pholic acid (250mg), pantothenic acid (9333,5mg), niacin (16g), selenium (300mg), vitamin A (3200000 UI), Vitamin B1 (500mg), Vitamin B12 (10500mcg), Vitamin B2 (2800 mg) Vitamin B6 (600 mg), Vitamin D3 (650000 UI), Vitamin E (minimum 8500UI), Vitamin K3 (1000mg), Ethoxyquin (208.13mg).

[b] Mineral mix: supplied by Cargill, reference code F30702. Minimum content per kg: Cu (15.97g), Fe (99g), I (600mg), Mn (28.87g), Zn (160g).

[c]Σ SFA: sum of saturated fatty acids; Σ MUFA: sum of monounsaturated fatty acids; Σ PUFA: sum of polyunsaturated fatty acids

[d] Treatments: C) control diet without added oil; F) 3% flaxseed oil (FO); FGP) 3% FO + 10% grape pomace; FGE) 3% FO + 0.0022% grape seed extract; FH) 3% FO + 5% tilapia protein hydrolysate; and FVitE) 3% FO + 200ppm vitamin E.

Table 2. Antioxidant activity of the grape seed extract, grape pomace and Nile tilapia hydrolysate.

	ABTS (umol TE/g*)	FRAP (umol TE/g*)	ORAC (umol TE/g*)
Grape seed extract	6710.25 ± 10.05	13562.52 ± 664.18	1907.65 ± 415.02
Grape pomace	21.56 ± 5.3	66.1 ± 1.69	29.44 ±7.45
Tilapia hydrolysate	12.68 ± 2.65	19.39 ± 1.76	42.97 ± 4

*µmol Trolox equivalent (TE) per gram of sample

Table 3. Effects of adding natural antioxidants to swine diets containing flaxseed oil and gender effect on growth performance and serum total antioxidant status (TAS)

									P value		
Variables	C	F	FGP	FGE	FH	FVitE	Gilts	Barrows	Treatment	Sex	Treatment vs. sex
Ages (days)	127.75 ± 1.03	126.88 ± 1.16	127.06 ± 1.09	128.00 ± 1.36	127.44 ± 1.05	127.19 ± 0.84	127.71 ± 0.69	127.06 ± 0.55	0.926	0.336	0.901
IW (kg)	80.01 ± 0.69	80.01 ± 0.64	80.03 ± 0.63	79.98 ± 0.63	80.03 ± 0.53	79.98 ± 0.62	78.93 ± 0.31	81.08 ± 0.32	0.999	0.001	0.999
FW (kg)	122.52 ± 1.19	121.82 ± 1.70	121.44 ± 1.63	119.76 ± 1.48	121.65 ± 1.31	122.31 ± 1.10	119.80 ± 0.84	123.37 ± 0.69	0.715	0.001	0.917
ADG(kg)	1.012 ± 0.024	0.995 ± 0.036	0.986 ± 0.031	0.947 ± 0.028	0.991 ± 0.026	1.008 ± 0.025	0.973 ± 0.018	1.007 ± 0.014	0.715	0.181	0.912
ADFI(kg)	3.384 ± 0.064	3.189 ± 0.097	3.276 ± 0.090	3.111 ± 0.073	3.168 ± 0.072	3.248 ± 0.064	3.072 ± 0.045	3.387 ± 0.033	0.108	0.001	0.649
FC	3.35 ± 0.04	3.23 ± 0.07	3.34 ± 0.06	3.31 ± 0.09	3.21 ± 0.07	3.23 ± 0.05	3.18 ± 0.04	3.38 ± 0.031	0.444	0.001	0.375
TAS (mmol/L)	1.389 ± 0.031	1.409 ± 0.097	1.525 ± 0.102	1.393 ± 0.036	1.407 ± 0.041	1.590 ± 0.057	1.428 ± 0.035	1.476 ± 0.044	0.202	0.728	0.476

IW: initial weight; FW: final weight; ADG: daily weight gain; ADFI: daily feed consumption; FC: feed conversion; TAS: Serum total antioxidant status.
Treatments: C: control diet without added oil; F: 3% flaxseed oil (FO); FGP: 3% FO + 10% grape pomace; FGE: 3% FO + 0.0022% grape seed extract; FH: 3% FO + 5% tilapia protein hydrolysate; FVitE: 3% FO + 0.04% vitamin E.

Table 4. Effects of adding natural antioxidants to swine diets containing flaxseed oil and gender effect on carcass traits.

									P value		
Variables	C	F	FGP	FGE	FH	FVitE	Gilts	Barrows	Treatment	Sex	Treatment vs. sex
HCW (kg)	89.59 ± 0.88	88.35 ± 1.20	88.18 ± 1.24	87.42 ± 1.06	88.36 ± 0.95	88.93 ± 0.76	86.65 ± 0.58	90.23 ± 0.47	0.737	0.001	0.872
CY(%)	72.76 ± 0.34	72.54 ± 0.31	72.61 ± 0.26	73.01 ± 0.22	72.64 ± 0.26	72.72 ± 0.24	72.26 ± 0.16	73.15 ± 0.13	0.823	0.001	0.454
BF_1 (mm)	34.07 ± 1.00	33.31 ± 1.60	31.96 ± 1.72	33.61 ± 1.45	34.56 ± 1.48	36.25 ± 1.58	31.86 ± 0.83	36.01 ± 0.79	0.385	0.001	0.181
BF_2 (mm)	22.85 ± 0.94	21.23 ± 1.00	21.38 ± 1.51	20.83 ± 1.19	21.59 ± 1.19	21.10 ± 1.07	18.92 ± 0.53	24.05 ± 0.58	0.715	0.001	0.114
BF_3 (mm)	19.46 ± 0.72	18.53 ± 0.80	18.74 ± 1.15	17.86 ± 1.07	19.10 ± 0.70	19.62 ± 0.81	17.12 ± 0.49	20.63 ± 0.39	0.536	0.001	0.207
BF_{4a} (mm)	23.11± 0.96^{A}	18.63 ± 0.91^{B}	19.64 ± 1.19^{B}	19.99 ± 1.48^{B}	20.50 ± 1.21^{B}	19.53 ± 1.17^{B}	17.52 ± 0.56	22.90 ± 0.58	0.031	0.001	0.383
BF_{4b} (mm)	24.89 ± 1.44	22.16 ± 1.01	21.74 ± 1.42	23.47 ± 1.53	22.37 ± 1.19	23.24 ± 1.28	20.38 ± 0.69	25.53 ± 0.63	0.352	0.001	0.398
LDc (mm)	59.96 ± 1.58	62.26 ± 1.39	63.42 ± 1.98	62.31 ± 1.25	61.94 ± 1.93	65.88 ± 2.33	63.38 ± 1.05	61.86 ± 1.00	0.325	0.367	0.264
LDaloka1 (mm)	62.11 ± 1.53	62.26 ± 1.53	59.64 ± 1.20	62.84 ± 0.94	59.31 ± 1.36	61.74 ± 1.47	60.95 ± 0.81	61.68 ± 0.77	0.326	0.507	0.129
LDaloka2 (mm)	74.76 ± 1.54	75.07 ± 1.19	73.74 ± 1.64	74.80 ± 1.07	74.36 ± 1.01	77.10 ± 1.21	74.69 ± 0.81	75.26 ± 0.68	0.527	0.578	0.090
BFaloka1 (mm)	14.11 ± 0.55	13.48 ± 0.42	13.51 ± 0.42	13.30 ± 0.47	13.81 ± 0.71	13.81 ± 0.57	12.60 ± 0.24	14.75 ± 0.28	0.827	0.001	0.504
BFaloka2 (mm)	18.44 ± 0.76	17.81 ± 0.78	18.10 ± 0.78	17.93 ± 0.87	18.89 ± 1.06	18.96 ± 0.85	16.55 ± 0.41	20.17 ± 0.41	0.810	0.001	0.802
FArea (cm^2)	24.28 ± 0.88	23.50 ± 1.16	23.14 ± 1.25	22.53 ± 1.22	23.58 ± 1.20	24.31 ± 0.96	21.29 ± 0.53	25.79 ± 0.58	0.667	0.001	0.230
LEA (cm^2)	40.26 ± 1.23	42.94 ± 1.16	40.66 ± 1.34	40.94 ± 0.55	40.58 ± 1.30	43.37 ± 1.49	42.48 ± 0.75	40.42 ± 0.63	0.318	0.053	0.581
FMR	0.613 ± 0.031	0.553 ± 0.03	0.575 ± 0.033	0.556 ± 0.037	0.588 ± 0.035	0.571 ± 0.030	0.507 ± 0.015	0.644 ± 0.017	0.667	0.001	0.759
PLM (%)	52.49 ± 1.04	54.52 ± 0.72	54.97 ± 0.92	53.78 ± 0.98	54.34 ± 0.84	54.51 ± 1.06	55.80 ± 0.50	52.43 ± 0.45	0.299	0.001	0.855

HCW: Hot carcass weight; CY: carcass yield; BF_1: backfat thickness at first rib; BF_2: backfat thickness at last rib; BF_3: backfat thickness at first sacral vertebra; BF_{4a}: backfat thickness at P2 measured on the carcass; BF_{4a}: backfat thickness at P2 measured in the drawing; LDc: loin depth on the carcass; LDaloka1: initial loin depth measured by Aloka equipment; LDaloka2: final loin depth measured by Aloka equipment; BFaloka1: initial backfat thickness measured by Aloka equipment; $BFaloka_2$: final backfat thickness measured by Aloka equipment; FArea: subcutaneous fatty area; LEA: loin eye area; FMR: fat meat ratio; PLM: percentage of lean meat. Treatments: C: control diet without added oil; F: 3% flaxseed oil (FO); FGP: 3% FO + 10% grape pomace; FGE: 3% FO + 0.0022% grape seed extract; FH: 3% FO + 5% tilapia protein hydrolysate; FVitE: 3% FO + 0.04% vitamin E.

Table 5. Effects of adding natural antioxidants to swine diets containing flaxseed oil and gender effect on meat quality parameters.

									P value		
Variables	C	F	FGP	FGE	FH	FVitE	Gilts	Barrows	Treatment	Sex	Treatment vs. sex
					Loin						
DM (%)	25.01 ± 0.33	24.99 ± 0.20	25.02 ± 0.31	24.90 ± 0.30	24.91 ± 0.26	25.25 ± 0.21	24.84 ± 0.15	25.18 ± 0.16	0.960	0.095	0.198
EE (%)	1.92 ± 0.12	1.72 ± 0.12	1.80 ± 0.15	1.85 ± 0.15	2.14 ± 0.13	2.03 ± 0.17	1.73 ± 0.07	2.09 ± 0.08	0.265	0.003	0.983
Ash (%)	1.25 ± 0.03	1.26 ± 0.02	1.22 ± 0.02	1.25 ± 0.02	1.21 ± 0.02	1.21 ± 0.02	1.24 ± 0.01	1.23 ± 0.01	0.246	0.738	0.444
Marbling[1]	2.20 ± 0.18	2.00 ± 0.16	1.81 ± 0.19	1.81 ± 0.10	1.81 ± 0.19	2.13 ± 0.26	1.75 ± 0.09	2.17 ± 0.10	0.333	0.001	0.409
pH45`	6.42 ± 0.068	6.42 ± 0.04	6.40 ± 0.05	6.40 ± 0.05	6.36 ± 0.05	6.43 ± 0.07	6.43 ± 0.03	6.38 ± 0.03	0.847	0.28	0.385
pH24h	5.4 ± 0.03	5.41 ± 0.02	5.44 ± 0.03	5.44 ± 0.02	5.38 ± 0.02	5.38 ± 0.03	5.41 ± 0.01	5.408 ± 0.02	0.382	0.983	0.998
DL (%)	4.71 ± 0.49	4.06 ± 0.27	3.68 ± 0.40	3.44 ± 0.38	4.09 ± 0.4	4.46 ± 0.55	3.71 ± 0.23	4.41 ± 0.25	0.182	0.038	0.845
CL (%)	35.71 ± 0.22	35.98 ± 0.24	35.50 ± 0.27	36.06 ± 0.27	35.37 ± 0.30	35.50 ± 0.36	35.71 ± 0.18	35.67 ± 0.15	0.303	0.655	0.746
Colour score[1]	3.80 ± 0.15^{A}	3.44 ± 0.16^{AB}	3.63 ± 0.13^{AB}	3.81 ± 0.14^{A}	3.44 ± 0.18^{AB}	3.27 ± 0.12^{B}	3.66 ± 0.08	3.47 ± 0.09	0.055	0.094	0.635
L*	46.48 ± 0.70	46.74 ± 0.47	46.34 ± 0.63	46.4 6 ± 0.61	47.75 ± 0.65	46.96 ± 0.55	46.73 ± 0.37	46.85 ± 0.33	0.599	0.831	0.937
a*	4.16 ± 0.19	3.87 ± 0.3	3.48 ± 0.20	3.94 ± 0.24	3.96 ± 0.21	4.11 ± 0.27	3.74 ± 0.13	4.09 ± 0.14	0.341	0.071	0.588
b*	1.70 ± 0.38	1.65 ± 0.31	1.45 ± 0.31	1.64 ± 0.41	2.21 ± 0.43	2.01 ± 0.25	1.63 ± 0.2	1.93 ± 0.21	0.353	0.166	0.960
Colour sat	4.66 ± 0.26	4.36 ± 0.32	3.93 ± 0.24	4.47 ± 0.32	4.84 ± 0.20	4.70 ± 0.24	4.25 ± 0.15	4.73 ± 0.16	0.149	0.026	0.747
					Ham						
pH45min	6.49 ± 0.04	6.54 ± 0.06	6.45 ± 0.03	6.51 ± 0.04	6.51 ± 0.05	6.46 ± 0.04	6.51 ± 0.03	6.48 ± 0.02	0.623	0.417	0.786
pH24h	5.46 ± 0.04^{CB}	5.46 ± 0.02^{CB}	5.52 ± 0.03^{AB}	5.54 ± 0.02^{A}	5.45 ± 0.02^{CB}	5.43 ± 0.02^{C}	5.47 ± 0.02	5.48 ± 0.02	0.045	0.49	0.929
DL (%)	3.38 ± 0.38	2.93 ± 0.26	2.92 ± 0.3	2.72 ± 0.31	3.24 ± 0.32	3.22 ± 0.27	2.93 ± 0.2	3.19 ± 0.15	0.615	0.338	0.834
Colour score[1]	3.80 ± 0.18	3.56 ± 0.13	3.81 ± 0.16	3.94 ± 0.14	3.50 ± 0.18	3.27 ± 0.15	3.68 ± 0.092	3.62 ± 0.099	0.057	0.623	0.918

Table 5 (Continued)

Variables	C	F	FGP	FGE	FH	FVitE	Gilts	Barrows	P value		
									Treatment	Sex	Treatment vs. sex
L*	45.90 ± 0.69	46.65 ± 0.58	45.51 ± 0.84	45.22 ± 0.76	46.61 ± 0.60	46.88 ± 0.69	46.45 ± 0.41	45.79 ± 0.40	0.432	0.201	0.186
a*	6.77 ± 0.30	6.53 ± 0.38	6.69 ± 0.37	6.46 ± 0.36	5.96 ± 0.37	5.93 ± 0.38	5.98 ± 0.19	6.80 ± 0.21	0.452	0.006	0.838
b*	3.02 ± 0.34	3.2 ± 0.33	2.57 ± 0.31	2.4 ± 0.36	2.88 ± 0.41	3.04 ± 0.37	2.81 ± 0.21	2.88 ± 0.20	0.397	0.773	0.184
Colour sat	7.51 ± 0.33	7.35 ± 0.43	7.29 ± 0.33	7.02 ± 0.35	6.82 ± 0.36	6.81 ± 0.37	6.74 ± 0.20	7.53 ± 0.20	0.634	0.006	0.992

DM: dry matter; EE: ether extract; DL: drip loss; CL: cooking loss; Colour sat: colour saturation index

C: control diet without added oil; F: 3% flaxseed oil (FO); FGP: 3% FO + 10% grape pomace; FGE: 3% FO + 0.0022% grape seed extract; FH: 3% FO + 5% tilapia protein hydrolysate; FVitE: 3% FO + 0.04% vitamin E.

[1] NPPC scoring system. Color: 1= pale pinkish gray to white, ..., 6= dark purplish red. Marbling: 1= devoid to practicaly devoid, …, 10= abundant (NPPC, 1999)

5.7. ABBREVIATIONS

a*: colour measured by the CIELAB system. Positive a* is red and negative a* is green.

ABTS: 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)

ADFI: average daily feed intake

ADG: average daily weight gain

b*: colour measured by the CIELAB system. Positive b* is yellow and negative b* is blue.

BF: Backfat thickness

BF1: Backfat thickness at first rib

BF2: Backfat thickness at last rib,

BF3: Backfat thickness at first sacral vertebra

BF4: Backfat thickness at point P2

BFaloka: Backfat thickness measured *in vivo*

C: control diet;

CL: cooking loss

DL: drip loss;

DM: dry matter;

EE: ether extract;

F: diet with 3% flaxseed oil

FA: fatty acid

FAarea: fat area

FC: feed conversion ratio

FGE: diet with 3% FO + 0.0022% grape seed extract;

FGP: diet with 3% flaxseed oil + 10% grape pomace;

FH: diet with 3% LO + 5% tilapia carcass hydrolysate;

FLVitE: diet with 3% FO + 200 ppm vitamin E supplement

FMR: fat-meat ratio

FO: flaxseed oil

FRAP: Ferric Reducing Antioxidant Power

FW: Final weight

HCW: hot carcass weight

IW: Initial weight

L*: colour measured by the CIELAB system. The maximum for L* is 100, which represents a perfect reflecting diffuser. The minimum for L* is zero, which represents black.
LD: loin depth
LDaloka: loin depth measured *in vivo*
LEA: loin eye area
ME: metabolizable energy
ORAC: Oxygen Radical Absorbance Capacity
P2: at the last rib, in the region of the insertion of the last thoracic vertebra with the first lumbar vertebra, six centimetres from the midline
pH24h: 24 hours after slaughter
pH45': 45 minutes after slaughter
PLM: percentage of lean meat
PUFA: polyunsaturated fatty acids
SFA: saturated fatty acids
TAS: total antioxidant status;
TE: Trolox equivalent
WHC: water holding capacity

5.8. ACKNOWLEDGEMENTS

The authors would like to thank Embrapa, National Counsel of Technological and Scientific Development (CNPq), Falbom Agroindustrial Ltd. Company and the company Cargill Foods Brazil for all their support in the development of this study.

6. CAPÍTULO 04

"ADDITION OF NATURAL ANTIOXIDANTS IN SWINE DIETS CONTAINING HIGH CONTENT OF α-LINOLENIC ACID: EFFECTS ON FAT OXIDATIVE STABILITY AND MEAT PRODUCT ACCEPTABILITY"

Daniela Miotto Bernardi, Teresinha Marisa Bertol, Anildo Cunha Junior, Arlei Coldebella, Daniel Barrera-Arellano, Helena Godoy, Adriana Dillenburg Meinhart, Juliana Burgüer Rodrigues, Valdemiro Carlos Sgarbieri

O artigo original que será submetido para a revista *Food Research International*.

ABSTRACT

The aim of this study was to evaluate the effect of the addition of natural antioxidants and flaxseed oil in the diet of pigs on the oxidative stability and acceptability of fat and meat product. Ninety-six animals were used, distributed in six treatments: C- control diet; F- 3% flaxseed oil (FO); FGP- 3% FO + 10% grape pomace; FGE- 3% FO + 0.0022% grape seed extract; FH- 3% FO + 5% tilapia protein hydrolysate; and FVitE- 3% FO + 200 ppm vitamin E. Assessments were made of the lipidic profile (loin and backfat), tocopherols in the loin, oxidative stability of the fat and TBARS (thiobarbituric acid reactive substances), color and sensory analysis of mini-hamburgers made with loin and backfat. Flaxseed oil addition in the diet was effective to increase the ω-3 and total PUFA content and to decrease SFA and MUFA in meat and fat. The oxidative stability of backfat and the TBARS and sensory analysis of mini-hamburgers revealed that vitamin E was the most efficient antioxidant, although FH and FGE treatments have presented a mild effect on the oxidative stability of backfat and sensorial characteristics, respectively. There was an increase in the total tocopherol content, especially in the FVitE treatment, but also in FH and FGE treatments. Despite some promising results, further studies are recommended to understand the *in vivo* antioxidant effect of peptides produced from the hydrolysis of fish and products rich in phenolic compounds such as grape-derived products.

Keywords: ω-3 fatty acids, lipid oxidation, natural antioxidants, TBARS, Vitamin E

6.1. INTRODUCTION

The consumption of omega-3 (ω-3) is associated to several health benefits and the incorporation of these fatty acids (FA) in meat and meat products has gained interest in recent years (Bernardi, Bertol, Pflanzer, Sgarbieri, & Pollonio, 2016). Pork is widely consumed around the world and the addition of known ω-3 sources in the diet of pigs has presented promising results, especially with the addition of flaxseed, an important source of alpha-linolenic acid (ALA) (Bertol et al., 2013; Juárez et al., 2011; Turner et al., 2014).

Increasing the ω-3 content, as well as other polyunsaturated fatty acids (PUFA), may lead to several technological challenges, which can damage the final quality of the meat. Reducing the oxidative stability is one of the negative effects and can result in deterioration of the aroma, color, flavor, texture and nutritional value (Decker, Akoh, & Wilkes, 2012).

In some cases antioxidants can represent an effective strategy to reduce oxidation in meats with higher ω-3 content. Synthetic antioxidants can be used, however, concerns over the safety of these substances have encouraged the development of investigations with the objective of identifying natural compounds with antioxidant properties (Bernardi, Bertol, Pflanzer, et al., 2016).

Grape pomace is a processing residue that represents approximately 20% of the total weight of the fruit and has a high concentration of phenolic compounds, primarily responsible for its antioxidant activity (Lafka, Sinanoglou, & Lazos, 2007). The antioxidant effect of the pomace and grape seed extracts has been successfully tested in the diets for chickens (Chamorro et al., 2015; Sáyago-Ayerdi, Brenes, Viveros, & Goñi, 2009) but in swine diets its antioxidant effect on the meat is of no consensus (O'Grady, Carpenter, Lynch, O'Brien, & Kerry, 2008; Yan & Kim, 2011) .

Roughly 60% of the total weight of Nile tilapia products are considered residues and the literature has recently shown that hydrolysates of these residues have antioxidant potential (Chalamaiah, Dinesh Kumar, Hemalatha, & Jyothirmayi, 2012). Hydrolysates from Nile tilapia have demonstrated antioxidant activity *in vitro* (Fan, He, Zhuang, & Sun, 2012), however *in vivo* studies with these compounds remain scarce.

Vitamin E is a natural antioxidant which is commonly supplied in pig diets (Sales & Koukolová, 2011; Wang, Wang, Shi, & Shan, 2012). Sales & Koukolová (2011) showed that there is a strong correlation between vitamin E intake, its incorporation into the tissues and increased oxidative stability of the fat. However, some authors suggest that the effectiveness of vitamin E as an antioxidant may be compromised when high amounts of PUFA are present in the animal's diet (Gladine et al., 2007).

Therefore, the aim of this study was to evaluate the effect of addition flaxseed oil and one of the following antioxidants: grape pomace, grape seed extract, Nile tilapia hydrolysate and vitamin E in swine diets, and evaluate the effects on the oxidative stability and sensory properties of backfat and mini-hamburguers.

6.2. MATERIALS AND METHODS

6.2.1. Animals and experimental diets

This study was approved by the Ethics Committee on Animal Use in Research (CEUA / CNPSA) (file reference 002/2014), in accordance with the ethical principles established by the Brazilian College of Animal Experimentation.

The study was conducted in a randomized complete block design with six treatments: C- control diet without added oil; L- diet with 3% flaxseed oil (FO); FGP- diet with 3% FO + 10% grape pomace; FGE- diet with 3% FO + 0.0022% grape seed extract; FH- diet with 3% FO + 5% tilapia protein hydrolysate; and FVitE- diet with 3% FO + 200 ppm vitamin E (α-tocopheryl acetate); and two sexes: gilts and barrows. In the FGE treatment the amount of grape seed extract added was that recommended by the manufacturer to achieve the same antioxidant potential as 200 ppm of vitamin E. The experimental diets, based in corn and soybean meal-, were formulated to be isocaloric and to meet the nutritional requirements of pigs in the finishing phase (Rostagno et al., 2005). The description of the experimental diets and evaluation of antioxidant ingredients are presented by Bernardi, Bertol, Coldebella, et al. (2016).

Ninety-six pigs (48 gilts and 48 barrows) of the genotype Embrapa MS115xF1 (127.39 ± 4.29 days of age and 80.01 ± 2.43 kg) were distributed in blocks according to their initial weight, in a total of 8 replications of gilts and 8 of barrows per treatment. The animals were kept in individual pens, equipped with nipple drinkers and semi-automatic feeders. The experiment lasted 42 days.

At the end of the experimental period, the pigs were subjected to approximately 15 hours of fasting before being transported to the abattoir and a 4-hour pre-slaughter resting. The slaughter was executed by bleeding in the horizontal position following insensibilization by electronarcosis, in accordance with the industry standards. After the toilet and evisceration the carcasses were stored in a cold chamber, with an average temperature of 2 to 4°C for 24 hours. After this period, loin and backfat samples were collected and stored at -20°C.

6.2.2. Analysis

Prior to perform ether extract and lipidic profiles the loin samples were lyophilized (Liobrás Freeze-Dryer, model LP810), ground in a cooling grinder (FossTecator 1095 — Knifetec Sample) and stored at -20 °C. The backfat was kept frozen at -20 °C until the time of the analysis.

Ether extract analysis of loin samples was conducted in accordance with AOAC (1995). Fatty acids (FA) composition was determined following extraction of lipids (Folch, Lees, & Stanley, 1957), saponification and esterification (Hartman & Lago, 1973). The FA methyl-

esters were separated in a Varian CP-3800 gas chromatograph (WalnutCreek, CA, USA) equipped with a Varian CP-8410 autosampler, CP-1177 split/splitless injector and flame ionization detector (H_2 flow of 30 mL/min, air flow of 300 mL/min). The management of the instrument was conducted by Varian Star Workstation 5.0 software. The separation was performed using a fused silica capillary column Supelco SP-2380, with 100 m length, 0.25 mm internal diameter and 0.2 mM of film thickness. The heating furnace was operated by programming the temperature from 140 to 190° C at a rate of 4° C/min, held at 190° C for 15 min, and further increased from 190 to 220° C at a rate of 1° C/min, kept the temperature at 220° C for 17.5 min. The temperatures of the injector and detector were 240 and 280° C, respectively. The liner used was "split / splitless with single taper" with 78.5 mm length and 4 mm internal diameter (SGE Analytical Science). The split ratio was 1: 100 and the injected volume was 0.2-0.5 uL. Ultrapure Nitrogen was used as carrier gas with a flow of 1.6 mL/min.

For analysis of tocopherols in the loin samples, lipid extraction was performed as in Folch et al., (1957). Tocopherol analysis was conducted in accordance with Pinheiro-Sant'Ana et al., (2011).

To determine the fat stability, the backfat samples were ground in a chilling grinder (Foss Tecator 1095 — Knifetec Sample), put in an oven (50 ºC) for three hours and manually pressed. The oxidative stability was determined in accordance with AOCS (1992), 90ºC, air=10L/h (893 Professional Biodiesel Rancimat®). The results were expressed in terms of induction time in hours (h).

6.2.3. Preparation and analysis of the mini-hamburgers

The mini-hamburgers were prepared one day after the sample collection, with the samples being processed while still frozen. The loin, backfat, and salt were weighed in accordance with the proportions 78.37%, 19.66% and 1.96%, respectively. The loin and backfat were diced and homogenized in a domestic food processor (Philips Walita, model Ri1364) together with the salt for 30 seconds. Portions of 12.5g were obtained and molded as mini-hamburgers with a diameter of approximately 3 cm. The samples were stored frozen (-20ºC) in bags with the presence of oxygen. The measurements of color and thiobarbituric acid-reactive substances (TBARS) in the mini-hamburgers were performed at: T0 (day after freezing); T2 (two months after freezing); T4 (four months after freezing) and T6 (six months after freezing).

The color assessment was executed by the CIELAB system (a*, b*, Spectrophotometer, Minolta LTD., Japan) after the samples had been removed from freezing and exposed to air for

20 minutes. The TBARS analysis was conducted in accordance with the Vyncke (1970) methodology and the results are expressed in mg of malonaldialdehyde (MDA) per Kg of sample.

All the assessors who voluntarily participated on the mini-hamburger sensorial analysis signed the Free and Informed Consent Form, as approved by the Research Ethics Committee of the Assis Gurgacz University (report number 1.158.963), in compliance with Directive DFCM 20/97, of 21/05/1997. The analysis were conducted on the mini-hamburger samples at three storage times: T2 (two months after freezing), T4 (four months after freezing) and T6 (six months after freezing). At each time (T2, T4, T6) 106 untrained assessors participated in the analysis, all pork eaters and aged over 18 years. The frozen mini-hamburgers were placed on a Philips Walita domestic electric grill (model RI4417/21) at full power (250ºC) and without any added oil, for 5 minutes and 20 seconds, with each side of the mini-hamburger in contact with the grill for 2 minutes and 40 seconds.

The samples were presented monadically in complete and balanced blocks, on disposable white plates, coded with a 3-digit number and in standard quantities of 12.5 g, with no sample repetition. The overall acceptance, appearance, aroma, flavor and texture were evaluated on a non-structured 9 cm hedonic scale, with the limit markers of "dislike extremely" on the left and "like extremely" on the right. Detection of unusual flavor and unusual aroma in the samples was also evaluated, using a non-structured 9 cm scale, with the limit markers corresponding to "none" and "extremely strong".

6.2.4. Statistical analysis

Statistical analysis of the fatty acids profile data was performed by means of analysis of variance, considering the treatment as a variation source. Multiple means comparison was done using the protected t test , whenever the F test detected significant effect ($p \leq 0.05$) (SAS, 2012).

For TBARS and hamburger color, the statistical analysis was executed by analysis of repeated measures, considering the effect of block within sex, treatment, storage time, and the interactions between the latter three factors and 16 types of variance and covariance matrix structures, using PROC MIXED (SAS, 2012). Means comparison for the effect of the interaction between treatment and storage time was done using the F test to compare the treatment effect within time and vice versa, followed by the t test whenever the F test detected significant effects ($P \leq 0.05$).

Analysis of variance (ANOVA) was used for analyzing the acceptance scale using

consumers and samples as a fixed source of variation. Additionally, principal component analysis (PCA) was done using a correlation matrix with the means of the samples. Hierarchical cluster analysis (HCA) was also performed to find groups of samples with similar sensory characteristics in each methodology, having as input samples coordinates in the first and second dimension of the sensory maps produced by each technique, considering Euclidean distances (dissimilary) and Warld's techniques (agglomeration method) and automatic truncation (Moussaoui & Varela, 2010). The statistical analyses of sensory tests were done by the software XLSTAT (2012).

6.3. RESULTS AND DISCUSSION

6.3.1. Fatty acids profile

The lower SFA content was found in the backfat of the animals of the treatments with flaxseed oil when compared to the C treatment ($p<0.005$) (Table 1). This same behavior was found in the loin, however without any significant difference (Table 2). In the backfat it was also found that the C16:0 and C18:0 content ($p<0.005$) was higher in treatment C compared to the others.

Table 1

Table 2

The treatments with flaxseed oil presented lower MUFA levels both in the loin ($p<0.05$) and backfat ($p<0.005$). The backfat of treatment C also presented higher levels of C16:1 ($p<0.05$), C17:1 ($p<0.05$) and C18:1 ($p<0.005$).

Several authors (Duran-Montgé, Realini, Barroeta, Lizardo, & Esteve-Garcia, 2008; Juárez et al., 2011; Turner et al., 2014) have obtained similar results, indicating that the addition of sources of PUFA to the diet may result in reduced MUFA and SFA levels in the meat. This effect may be associated to the reduction in the expression of lipogenic genes (Duran-Montgé, Theil, Lauridsen, & Esteve-Garcia, 2009) and therefore reduction of *de novo* synthesis of fatty acids. Furthermore, the incorporation of the dietary FAs into tissues may also be facilitated (Bertol et al., 2013).

The addition of flaxseed oil in the diet promoted a significant increase in total PUFAs in the loin ($p<0.05$) and backfat ($p<0.0001$). The PUFA/SFA ratio in the loin of the animals fed with flaxseed oil was greater than that found in the C treatment ($p=0.05$), being near the 0.4 ratio which is considered adequate by the British Department of Health (1994). This same ratio in the backfat was 0.7 ($p<0.001$) in the treatments with flaxseed oil.

The ω-3 were the PUFAs which increased most with the addition of flaxseed oil in the diet. The total ω-3 concentration in the loin of the flaxseed treatments was 3.41±0.21 times greater than those found in the loin of the C treatment ($p<0.0001$). In the backfat, the total ω-3 content in the flaxseed oil treatments were 4.59 ± 0.19 times greater than those found in the C treatment ($p<0.0001$). The incorporation of ALA was primarily responsible for the increased ω-3, both in the loin and in the backfat, and the high content of this FA in these tissues is due to its high concentration in flaxseed, which, when added to animal diet, promotes increased tissue ALA (Bertol et al., 2013; Juárez et al., 2011; Turner et al., 2014).

The inclusion of flaxseed in the diet also resulted in increased EPA levels in both the loin ($p<0.0001$) and backfat ($p<0.0001$), however, significant amounts of DHA were not detected. Some authors (Turner et al., 2014) have reported that the addition of flaxseed to the diet promotes increased DHA in the tissues, yet others (Juárez et al., 2011) have observed only an increase in the EPA levels.

The ALA concentration in the backfat was higher than in the loin, on the other hand, in the loin the EPA concentration was higher than in the backfat. In the treatments with flaxseed oil, the average quantity of EPA in the loin and backfat was 9.91 ± 0.95 and 116.1 ± 12.47 times smaller than the ALA concentration, respectively. Kloareg, Noblet, & Van Milgen (2007) found a concentration of EPA that was 2.1 times smaller than that of ALA, with the ALA to EPA conversion rate being greater than that observed in the present study. The results of this study have shown that there is a difference in ALA incorporation and EPA conversion between the lipids from the backfat and from the intramuscular fat from the loin. The addition of flaxseed oil promoted increased linoleic acid (LA) concentration in the diet, however no LA increase ($p>0.05$) was observed in the loin or backfat in our work. Conversely, Turner et al., (2014) found that the addition of flaxseed in the diet increased LA in the meat.

As expected, the ω-6/ω-3 ratio in the backfat and in the loin was lower in the treatments with flaxseed oil ($p<0.0001$), the average ratio of these treatments was 4.91±0.21 and 3.22±0.14, in the loin and backfat, respectively. This ratio is important since the enzymes involved in the elongation and desaturation processes of the ω-6 and ω-3 FAs are the same, and

therefore excess LA can inhibit ALA to EPA and DHA conversion (Vaz, Deboni, Azevedo, Gross, & Zelmanovitz, 2006).

The tested natural antioxidants did not cause any alterations in the FAs ($p>0.05$) except in the C18:1n7c of the backfat, which showed lower quantities in the treatments with antioxidants ($p<0.01$). Haak, Raes, Van Dyck, & De Smet (2008) also found no effect of antioxidants on the FAs of the meat. On the other hand, in swine diets (Yan & Kim, 2011) and chicken diets (Chamorro et al., 2015) it has been found that the presence of grape pomace (3% for pigs, 5% and 10% for chickens) increased PUFAs, especially ALA. Mairesse et al., (2011) observed that supplementing swine diets with natural antioxidants compromised ALA incorporation in the meat.

The effect of the vitamin E in the diet on the FAs of the meat is not entirely clear. Lauridsen, Theil, & Jensen (2013) found that greater quantities of vitamin E (150 and 300 mg/Kg) in the diet resulted in more SFAs in the meat.

Recent studies have shown that the addition of fish protein hydrolysate in the diet of rats can alter the FA composition of fat and especially liver tissue, due to the product's capacity to modify the genetic expression of the desaturases Δ6 and Δ9 (Bjørndal et al., 2013). However, as mentioned previously, in this study the tilapia hydrolysate, as well as the vitamin E, grape pomace and grape seed extract don't promote changes in the FA composition of the meat.

6.3.2. Oxidative stability of fat and mini-hamburgers

Lipidic oxidation is a spontaneous and inevitable *post mortem* process. The mini-hamburger is a good product for assessing this process, as grinding the meat causes muscular and fat tissue disintegration, which increases the contact surface with the oxygen, thus favoring oxidation chain reactions. Furthermore, the addition of NaCl contributes to create a pro-oxidant state. This same strategy was used by Sáyago-Ayerdi et al. (2009) when evaluating the antioxidant effect of grape pomace in chicken diet.

Table 3 shows TBARS results during the storage period of the mini-hamburgers. There were increased concentrations of MDA in all the treatments throughout the stocking period (-20ºC) ($p<0.05$) and the presence of flaxseed oil in the diet significantly contributed to this result. At T2 and T4 the MDA concentration in the F treatment was greater than in the C treatment ($p<0.05$). Of the antioxidants tested, vitamin E was the most efficient, since MDA production in the FVitE treatment was lower than in the F treatment at all times ($p<0.05$). It was also found

that compared to the C treatment, at T0 and T2 the MDA production was lower ($p<0.05$) for FVitE, whereas at T4 and T6 they were not significant.

Table 3

The results of the fat stability analysis by Rancimat are shown in Table 4 and also indicate that the addition of flaxseed oil to the diet generated a less stable fat and none of the antioxidants (FGE, FGP, FH, FVitE) were able to completely revert this effect, that is, they were unable to afford the same stability verified in treatment C. However, the presence of vitamin E increased the oxidative stability of the fat with ω-3, in other words, when compared to the F treatment, the oxidative stability of the fat from the FVitE treatment was two times higher, therefore promoted a slight increase in product shelf life.

Table 4

Increased lipidic oxidation of pork enriched with ω-3 by the addition of flaxseed in the diet of pigs has been observed by other authors (Juárez et al., 2011). Therefore, the addition of ω-3 sources in the animal diet increases lipidic oxidation, due to the incorporation of these FAs in the meat, which increases desaturation and reactivity and weakens the strength of the connections, promoting greater instability and susceptibility to the action of oxidant agents (Decker et al., 2012).

The results of our study are in line with Santos, Hoz, Cambero, Cabeza, & Ordóñez (2008) and Haak, De Smet, Fremaut, Van Walleghem, & Raes (2008), who found that the addition of vitamin E to swine diets with flaxseed oil reduced oxidation of the meat. Supra-nutritional doses of vitamin E have been successfully tested by Wang et al. (2012). On the other hand, other authors (Musella et al., 2009) have found that the supra-nutritional presence of vitamin E in swine diets with extruded flaxseed did not reduce lipidic oxidation. Gladine et al. (2007) reported that the effectiveness of the vitamin E was compromised when high doses of PUFA were added to the diet.

At T0 MDA production in the FH treatment was equal to that in the FGE and FVitE treatments and lower than in F treatment ($p<0.05$). At T2, T4, and T6 the FH treatment did not differ from the F. On the other hand, in the analysis conducted in the Rancimat the FH treatment had a greater induction time than the F treatment. Despite the effects presented by FH treatment

were of low magnitude, the tilapia hydrolysate is an interesting product and worthy of greater investigation regarding to *in vivo* antioxidant potential. The peptides formed from fish protein hydrolysis contain significant quantities of hydrophobic amino acids that could facilitate their entry into the target organs by means of hydrophobic interactions with membranes. Furthermore, following the action of gastrointestinal enzymes they maintain or increase their antioxidant activity (Girgih et al., 2015; Wiriyaphan, Xiao, Decker, & Yongsawatdigul, 2015).

The TBARS results for the FGE and FGP treatments revealed that at T0 the MDA concentration in the FGE treatment was lower than in the F, whereas it did not differ between the FGP and F treatments. At T2 the results demonstrated a pro-oxidant effect of the FGE and FGP treatments, in which the MDA production was greater than and equal to, respectively, the F treatment ($p<0.0001$). At T4 and T6 the MDA concentration in the FGP and FGE treatments was equal to the F treatment. The fat stability showed that the FGP treatment had lower induction time than the F, whereas in the FGE treatment the stability was similar to the F. O'Grady et al. (2008) observed no reduction in the lipidic oxidation in the meat of pigs fed with grape seed extract. Other authors (Larraín, Krueger, Richards, & Reed, 2006) added cranberry juice (rich in polyphenols) to swine diets and found a pro-oxidant effect in the meat. On the other hand, Mairesse et al. (2011) found that pigs fed with polyphenols from grape had lesser meat oxidation. A reduction in the MDA concentration of the meat has also been observed in pigs with grape pomace in their diet (Yan & Kim, 2011). The *in vivo* bioavailability of polyphenols may be one of the factors capable of affecting its efficiency when supplied through the diet (Gladine et al., 2007). Furthermore, some of the phenolic compounds may be degraded by the colonic microflora in the intestine even before being absorbed (Ward, Croft, Puddey, & Hodgson, 2004).

6.3.3. Color stability of the mini-hamburgers

As expected, all the samples showed a reduction of a* during storage (Table 5). The reduced intensity of the red is due to myoglobin oxidation (AMSA, 1991). The FGP treatment samples showed lower a* values at times T0 ($p<0.001$) and T2 ($p<0.0001$).

The results obtained by other authors (Larraín et al., 2006; O'Grady et al., 2008) also showed that the a* values of the pork were diminished by storage, even with natural antioxidants in the diet. On the other hand, grape pomace in the pig diet increased the a* values of the meat (Yan & Kim, 2011).

Table 5

An increase was also found in the b* values over time. Greater b* values are related to yellower tonality, resulting from myoglobin oxidation (AMSA, 1991). At T6 greater b* values ($p<0.01$) were observed in the treatments with flaxseed oil. Juárez et al. (2011) found a lower hue value (a*/b*) in pigs fed with flaxseed.

The results of the color analyses did not allow us to confirm the effect of the tested antioxidants on meat color stability. Likewise, it remained unclear if the increase of ω-3 in meat and fat had any effect on these parameters.

6.3.4. Sensory analysis of the mini-hamburgers

The sensory analysis data are presented in Table 6. The consumers perceived few alterations in the samples as storage extended, however it was found that overall acceptance diminished ($p<0.05$) in the samples from the FVitE and FH treatments and strange flavor increased ($p<0.05$) in the samples from the FVitE and F treatments.

Table 06

Regarding the comparison between treatments, the samples differed for overall acceptance at times T2 and T4 ($p<0.05$). At T2 the samples from the FVitE treatment were those that reported the highest score, followed by treatments C, FH and FGE and the lowest scores were for the treatments F and FGP. At T4 the results followed the same trend, but the highest scoring samples were from treatment C, followed by FGE and the lowest scoring samples were for treatments F, FGP and FH.

The aroma of the samples differed at T6 ($p<0.05$), the highest scores for this attribute were for treatment FVitE and the lowest scores were for treatment FH. Difference ($p<0.05$) in the flavor of the samples was found at times T2 and T6, with the highest scoring samples at T2 being for the FVitE treatment and the lowest scores for treatments F, FH and FGP. At T6 the highest scores were for treatment C and the lowest for treatments FGP and FH.

The presence of unusual flavor differed between the treatments at times T4 and T6 ($p<0.05$). At both times the samples which presented the lowest unusual flavor ratings were those from treatment C. The least intense unusual flavor, as well as the highest scores for aroma and flavor are probably related to the lesser presence of lipidic oxidation products (Decker et

al., 2012). In treatments FH and FGP the strange flavor may be related both to oxidation products, and to the residual taste provoked by the concentration of fish hydrolysate (5%) and grape pomace (10%) in the animal diet.

In general it was found that the samples from the C and FVitE treatments were those that presented the best acceptability, whereas the samples from the F, FH and FGP treatments were the least well-accepted and the samples from the FGE treatment reported intermediate scores. This behavior of the samples is reinforced by cluster analyses, presented in the form of a dendogram (Figure 1 A) and by principal component analysis (Figure 1 B).

Figure 1

The cluster analysis revealed a very similar sample segmentation regarding the acceptability and oxidative stability results of the mini-hamburgers, where the samples were split into three large groups of similar behavior: group 01) samples from treatment F; group 02) samples from treatments C and FVitE; group 03) samples from treatments FGP, FH and FGE. Group 3 can be subdivided into two smaller groups, where treatments FH and FGE are in one group and FGP is separate.

The principal component analysis explains 70.03% of the results and shows a similar segmentation to that previously proposed, with the difference being that the FGE treatment remains in an intermediary region and close to the FH and FVitE treatments. This analysis also allows us to verify that the attributes of flavor, aroma, unusual flavor and unusual aroma, especially at times T4 and T6, were the main reasons for the separation of the F treatment and the FH and FGP treatments.

The sensory results corroborate those obtained in the TBARS analysis and also the data reported in the literature which suggest that the presence of flaxseed oil in swine diet reduces acceptability of the meat products and that the presence of vitamin E in the diet can improve product acceptance (Santos et al., 2008). In this study it was also found that the seed extract promoted improved acceptability of the tested product, however in another study, Mairesse et al. (2011) observed no significant effects of adding natural antioxidants (rich in phenolic compounds) to the diet on the sensory attributes of meat product with higher PUFA content.

6.3.5. Vitamin E concentrations in the loin

The vitamin E concentrations in the loin are shown in Table 7. As expected, due to the high alpha tocopherol concentration in the diet, the FVitE treatment resulted in higher alpha tocopherol and total tocopherol contents in the tissues ($p<0.0001$). In the FH, FGE and FGP treatments there were higher γ-tocopherol concentrations. It was also found that the F, FH, FGP and FGE treatments resulted in higher total tocopherol contents ($p<0.0001$) than the C treatment.

Table 7

Gladine et al. (2007) found that natural antioxidant supplementation promoted increased vitamin E concentrations in the liver. The increased vitamin E found in the FGE and FGP treatments might be explained by the fact that the phenolic compounds in the diet can act as "savers" and "recyclers" of vitamin E, from its oxidized form (Gladine et al., 2007) and this mechanism could explain the positive results obtained in the sensory analysis of the FGE treatment. The total tocopherol concentration in the FH treatment may have contributed positively to the results of oxidative stability of the fat achieved by this treatment.

6.4. CONCLUSIONS

The inclusion of flaxseed oil in the pig diet promoted significant modifications in the lipidic profile of the meat and backfat, with increased PUFA concentrations, especially ALA, and reduced SFA and MUFA concentrations, thus contributing to increase the oxidation of lipids.

Of the antioxidants used, vitamin E was the most efficient in reducing oxidation and improving acceptability. The tilapia hydrolysate and grape seed extract showed some evidence of antioxidant effect on the meat and fat, but further studies *in vivo* are needed to assess these effects. New approaches with different concentrations and supplementation times are recommended.

6.5. REFERENCES

AMSA. (1991). Guidelines for Meat Color Evaluation. *Proceedings of the Reciprocal Meat Conference*, *44*(Volume 44), 1–17.

AOAC. (1995). *Official methods of analysis of AOAC International* (16th ed.). Virgínia.

AOCS. (1992). Method Cd 12b-92. In *Official Methods and Recommended Practices of the American Oil Chemists' Society*.

Bernardi, D. M., Bertol, T. M., Coldebella, A., Silveira-Almeida, B. C., De Paris, L. D., & Sgarbieri, V. C. (2016). Inclusion of natural antioxidants in swine diets containing ω-3: effects on performance, carcass quality and quality of meat. *Submitted for Evaluation in the Journal of the Science of Food and Agriculture*.

Bernardi, D. M., Bertol, T. M., Pflanzer, S. B., Sgarbieri, V. C., & Pollonio, M. A. R. (2016). ω-3 in meat products: Benefits and effects on lipid oxidative stability. *Journal of the Science of Food and Agriculture*, *96*(8), 2620–2634.

Bertol, T. M., de Campos, R. M. L., Ludke, J. V, Terra, N. N., de Figueiredo, E. a P., Coldebella, A., … Lehr, N. M. (2013). Effects of genotype and dietary oil supplementation on performance, carcass traits, pork quality and fatty acid composition of backfat and intramuscular fat. *Meat Science*, *93*(3), 507–16.

Bjørndal, B., Berge, C., Ramsvik, M. S., Svardal, A., Bohov, P., Skorve, J., & Berge, R. K. (2013). A fish protein hydrolysate alters fatty acid composition in liver and adipose tissue and increases plasma carnitine levels in a mouse model of chronic inflammation. *Lipids in Health and Disease*, *12*(1), 143.

British Department of Health. (1994). *Nutritional aspects of cardiovascular disease. Report on health and social subjects*. London.

Chalamaiah, M., Dinesh Kumar, B., Hemalatha, R., & Jyothirmayi, T. (2012). Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. *Food Chemistry*, *135*(4), 3020–38.

Chamorro, S., Viveros, A., Rebolé, A., Rica, B. D., Arija, I., & Brenes, A. (2015). Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace. *Food Research International*, *73*, 197–203.

Decker, E. A., Akoh, C. C., & Wilkes, R. S. (2012). Incorporation of (n-3) Fatty Acids in Foods: Challenges and Opportunities. *The Journal of Nutrition*. http://doi.org/10.3945/jn.111.149328

Duran-Montgé, P., Realini, C. E., Barroeta, a C., Lizardo, R., & Esteve-Garcia, E. (2008). Tissue fatty acid composition of pigs fed different fat sources. *Animal : An International Journal of Animal Bioscience*, *2*(12), 1753–62.

Duran-Montgé, P., Theil, P. K., Lauridsen, C., & Esteve-Garcia, E. (2009). Dietary fat source affects metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. *Animal*, *3*(4), 535.

Fan, J., He, J., Zhuang, Y., & Sun, L. (2012). Purification and identification of antioxidant peptides from enzymatic hydrolysates of tilapia (oreochromis niloticus) frame protein. *Molecules*, *17*(11), 12836–12850.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*, *226*(1), 497–509.

Girgih, A. T., He, R., Hasan, F. M., Udenigwe, C. C., Gill, T. A., & Aluko, R. E. (2015). Evaluation of the in vitro antioxidant properties of a cod (Gadus morhua) protein hydrolysate and peptide fractions. *Food Chemistry*, *173*, 652–659.

Gladine, C., Morand, C., Rock, E., Gruffat, D., Bauchart, D., & Durand, D. (2007). The antioxidative effect of plant extracts rich in polyphenols differs between liver and muscle tissues in rats fed n-3 PUFA rich diets. *Animal Feed Science and Technology*, *139*(3–4), 257–272.

Haak, L., De Smet, S., Fremaut, D., Van Walleghem, K., & Raes, K. (2008). Fatty acid profile and oxidative stability of pork as influenced by duration and time of dietary linseed or fish oil supplementation. *Journal of Animal Science*, *86*(6), 1418–1425.

Haak, L., Raes, K., Van Dyck, S., & De Smet, S. (2008). Effect of dietary rosemary and α-tocopheryl acetate on the oxidative stability of raw and cooked pork following oxidized linseed oil administration. *Meat Science*, *78*(3), 239–47.

Hartman, L., & Lago, R. C. A. (1973). Rapid preparation of fatty acid methyl esters from lipids. *Lab. Pract.*, *22*, 475–476,494.

Juárez, M., Dugan, M. E. R., Aldai, N., Aalhus, J. L., Patience, J. F., Zijlstra, R. T., & Beaulieu, a. D. (2011). Increasing omega-3 levels through dietary co-extruded flaxseed supplementation negatively affects pork palatability. *Food Chemistry*, *126*(4), 1716–1723.

Kloareg, M., Noblet, J., & van Milgen, J. (2007). Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. *The British Journal of Nutrition*, *97*(1), 35–44.

Lafka, T.-I., Sinanoglou, V., & Lazos, E. S. (2007). On the extraction and antioxidant activity of phenolic compounds from winery wastes. *Food Chemistry*, *104*(3), 1206–1214.

Larraín, R. E., Krueger, C. G., Richards, M. P., & Reed, J. D. (2006). Color Changes and Lipid Oxidation in Pork Products Made From Pigs Fed With Cranberry Juice Powder, *19*(2008), 17–33.

Lauridsen, C., Theil, P. K., & Jensen, S. K. (2013). Composition of α-tocopherol and fatty acids in porcine tissues after dietary supplementation with vitamin E and different fat sources. *Animal Feed Science and Technology*, *179*(1–4), 93–102.

Mairesse, G. . d, Benet, M. ., Méteau, K. ., Juin, H. ., Durand, D. ., & Mourot, J. . (2011). Effect of plant antioxidant in n-3 polyunsaturated fatty acid-enriched diet on fatty acid composition and sensorial attributes of dry-cured ham. *International Journal of Food Science and Technology*, *46*(12), 2656–2662.

Moussaoui, K. A., & Varela, P. (2010). Exploring consumer product proï¬ling techniques and their linkage to a quantitative descriptive analysis. *Food Quality and Preference*, *21*(8), 1088–10 99.

Musella, M., Cannata, S., Rossi, R., Mourot, J., Baldini, P., & Corino, C. (2009). Omega-3 polyunsaturated fatty acid from extruded linseed influences the fatty acid composition and sensory characteristics of dry-cured ham from heavy pigs. *Journal of Animal Science*, *87*(11), 3578–88.

O'Grady, M. N., Carpenter, R., Lynch, P. B., O'Brien, N. M., & Kerry, J. P. (2008). Addition of grape seed extract and bearberry to porcine diets: Influence on quality attributes of raw and cooked pork. *Meat Science*, *78*(4), 438–46.

Pinheiro-Sant'Ana, H. M., Guinazi, M., Oliveira, D. D. S., Della Lucia, C. M., Reis, B. D. L., & Brandão, S. C. C. (2011). Method for simultaneous analysis of eight vitamin E isomers in various foods by high performance liquid chromatography and fluorescence detection. *Journal of Chromatography A*, *1218*(47), 8496–8502.

Rostagno, H. S., Allbino, L. F. T., Donzele, J. L., Gomes, P. C., Oliveira, R. F. de, Lopes, D. C., … Barreto, S. L. de T. (2005). Tabelas brasileiras para aves e suínos, 189.

Sales, J., & Koukolová, V. (2011). Dietary vitamin E and lipid and color stability of beef and pork: Modeling of relationships. *Journal of Animal Science*, *89*(9), 2836–48.

Santos, C., Hoz, L., Cambero, M. I., Cabeza, M. C., & Ordóñez, J. a. (2008). Enrichment of dry-cured ham with α-linolenic acid and α-tocopherol by the use of linseed oil and α-tocopheryl acetate in pig diets. *Meat Science*, *80*, 668–674.

SAS. (2012). Statistical Analysis Sytem.

Sáyago-Ayerdi, S. G., Brenes, A., Viveros, A., & Goñi, I. (2009). Antioxidative effect of dietary grape pomace concentrate on lipid oxidation of chilled and long-term frozen stored

chicken patties. *Meat Science*, *83*(3), 528–33.

Turner, T. D., Mapiye, C., Aalhus, J. L., Beaulieu, A. D., Patience, J. F., Zijlstra, R. T., & Dugan, M. E. R. (2014). Flaxseed fed pork: n-3 fatty acid enrichment and contribution to dietary recommendations. *Meat Science*, *96*(1), 541–7.

Vaz, J. dos S., Deboni, F., Azevedo, M. J. de, Gross, J. L., & Zelmanovitz, T. (2006). Ácidos graxos como marcadores biológicos da ingestão de gorduras. *Revista de Nutrição*, *19*(4), 489–500.

Vyncke, W. (1970). Direct Determination of the Thiobarbituric Acid Value in Trichloracetic Acid Extracts of Fish as a Measure of Oxidative Rancidity. *Fette Seifen Anstrichmittel*, *12*(12), 1084–1087.

Wang, H., Wang, L. S., Shi, B. M., & Shan, A. S. (2012). Effects of dietary corn dried distillers grains with solubles and vitamin E on growth performance, meat quality, fatty acid profiles, and pork shelf life of finishing pigs. *Livestock Science*, *149*(1–2), 155–166.

Ward, N. C., Croft, K. D., Puddey, I. B., & Hodgson, J. M. (2004). Supplementation with grape seed polyphenols results in increased urinary excretion of 3-hydroxyphenylpropionic Acid, an important metabolite of proanthocyanidins in humans. *Journal of Agricultural and Food Chemistry*, *52*(17), 5545–9.

Wiriyaphan, C., Xiao, H., Decker, E. A., & Yongsawatdigul, J. (2015). Chemical and cellular antioxidative properties of threadfin bream (Nemipterus spp.) surimi byproduct hydrolysates fractionated by ultrafiltration. *Food Chemistry*, *167*, 7–15.

XLSTAT. (2012). Software XLSTAT for Windows. Paris, France: Adinsoft.

Yan, L., & Kim, I. H. (2011). Effect of Dietary Grape Pomace Fermented by Saccharomyces boulardii on the Growth Performance, Nutrient Digestibility and Meat Quality in Finishing Pigs. *Asian-Australasian Journal of Animal Sciences*, *24*(12), 1763–1770.

6.6. TABLES AND FIGURES

Table 1. Effects of adding flaxseed oil and natural antioxidants to swine diets on fatty acids composition (% of ether extract) in pork backfat.

Variables	C	F	FGP	FGE	FH	FVtitE	P value
C10:0	0.065 ± 0.002	0.063 ± 0.004	0.059 ± 0.004	0.058 ± 0.003	0.060 ± 0.001	0.056 ± 0.003	0.1377
C12:0	0.070 ± 0.003	0.069 ± 0.004	0.063 ± 0.002	0.061 ± 0.002	0.064 ± 0.003	0.061 ± 0.003	0.1087
C14:0	1.228 ± 0.071	1.103 ± 0.047	1.069 ± 0.024	1.033 ± 0.037	1.082 ± 0.027	1.067 ± 0.045	0.0681
C15:0	0.056 ± 0.003	0.051 ± 0.003	0.053 ± 0.006	0.049 ± 0.005	0.056 ± 0.004	0.052 ± 0.005	0.9147
C16:0	22.12 ± 0.41^{a}	19.90 ± 0.53^{b}	19.76 ± 0.33^{b}	19.75 ± 0.38^{b}	20.34 ± 0.16^{b}	20.23 ± 0.52^{b}	0.0031
C16:1	1.672 ± 0.102^{a}	1.476 ±0.070^{ab}	1.419 ± 0.082^{b}	1.314 ± 0.093^{b}	1.453 ± 0.042^{b}	1.342 ± 0.078^{b}	0.0217
C17:0	0.384 ± 0.020	0.300 ± 0.012	0.301 ± 0.035	0.321 ± 0.040	0.342 ± 0.023	0.322 ± 0.038	0.3929
C17:1	0.295 ± 0.014^{a}	0.210 ± 0.011^{b}	0.210 ± 0.027^{b}	0.226 ± 0.027^{b}	0.240 ± 0.012^{ab}	0.221 ± 0.029^{b}	0.0381
C18:0	11.19 ± 0.18^{a}	9.775 ± 0.262^{b}	9.738 ± 0.320^{b}	10.58 ± 0.39^{ab}	10.58 ± 0.27^{ab}	10.64 ± 0.29^{ab}	0.0276
C18:1n7c	1.866 ± 0.074^{a}	1.667 ± 0.048^{b}	1.636 ± 0.078^{bc}	1.555 ± 0.055^{bc}	1.670 ± 0.051^{bc}	1.559 ± 0.043^{c}	0.0017
C18:1n9c	38.86 ± 0.40^{a}	36.29 ± 0.58^{b}	36.60 ± 0.40^{b}	37.15 ± 0.43^{b}	36.67 ± 0.51^{b}	36.39 ± 0.39^{b}	0.0037
C18:2n6c	14.73 ± 0.43	17.02 ± 0.64	16.64 ± 0.61	15.73 ± 0.43	15.60 ± 0.36	16.20 ± 0.62	0.0645
C18:3n3_ALA	1.158 ± 0.061^{b}	5.280 ± 0.338^{a}	5.579 ± 0.227^{a}	5.372 ± 0.164^{a}	5.046 ± 0.206^{a}	5.072 ± 0.302^{a}	<.0001
C20:0	0.245 ± 0.014	0.228 ± 0.017	0.227 ± 0.011	0.224 ± 0.009	0.225 ± 0.010	0.231 ± 0.012	0.8821
C20:1n9c	0.780 ± 0.034	0.684 ± 0.040	0.715 ± 0.040	0.703 ± 0.016	0.740 ± 0.032	0.702 ± 0.023	0.3842
C20:2n6c	0.654 ± 0.035	0.728 ± 0.035	0.732 ± 0.027	0.700 ± 0.026	0.696 ± 0.030	0.691 ± 0.032	0.4777
C20:4n6c	0.080 ± 0.005	0.073 ± 0.004	0.069 ± 0.002	0.065 ± 0.006	0.068 ± 0.006	0.077 ± 0.004	0.2014
C20:5n3c_EPA	0.000 ± 0.000^{c}	0.052 ± 0.005^{a}	0.045 ± 0.004^{ab}	0.047 ± 0.004^{a}	0.038 ± 0.004^{b}	0.047 ± 0.003^{a}	<.0001
C22:1n9c	0.146 ± 0.018^{b}	0.628 ± 0.039^{a}	0.679 ± 0.030^{a}	0.657 ± 0.040^{a}	0.631 ± 0.040^{a}	0.637 ± 0.022^{a}	<.0001
Σ SFA	35.36 ± 0.43^{a}	31.49 ± 0.78^{b}	31.27 ± 0.59^{b}	32.08 ± 0.65^{b}	32.75 ± 0.36^{b}	32.66 ± 0.73^{b}	0.0018
Σ MUFA	43.62 ± 0.37^{a}	40.96 ± 0.62^{b}	41.26 ± 0.53^{b}	41.60 ± 0.46^{b}	41.41 ± 0.58^{b}	40.85 ± 0.44^{b}	0.0032

Table 1 (Continued)

Variables	C	F	FGP	FGE	FH	FVtitE	P value
Σ PUFA	16.62 ± 0.50[b]	23.16 ± 0.97[a]	23.07 ± 0.81[a]	21.92 ± 0.57[a]	21.44 ± 0.51[a]	22.09 ± 0.90[a]	<.0001
Σ ω-3	1.158 ± 0.061[b]	5.332 ± 0.341[a]	5.624 ± 0.230[a]	5.420 ± 0.165[a]	5.083 ± 0.207[a]	5.118 ± 0.305[a]	<.0001
Σ ω-6	15.46 ± 0.46	17.82 ± 0.67	17.44 ± 0.62	16.50 ± 0.45	16.36 ± 0.37	16.97 ± 0.65	0.0644
MUFA/SFA	1.235 ± 0.020	1.305 ± 0.037	1.322 ± 0.029	1.300 ± 0.037	1.266 ± 0.027	1.254 ± 0.028	0.4780
PUFA/SFA	0.471 ± 0.019[b]	0.741 ± 0.046[a]	0.741 ± 0.038[a]	0.686 ± 0.029 [a]	0.655 ± 0.018[a]	0.680 ± 0.040[a]	<.0001
ω6/ω3	13.49 ± 0.62[a]	3.374 ± 0.123[b]	3.111 ± 0.084[b]	3.050 ± 0.069 [b]	3.237 ± 0.111[b]	3.345 ± 0.139[b]	<.0001

C: control diet without added oil; F) 3% flaxseed oil (FO); FGP) 3% FO + 10% grape pomace; FGE) 3% FO + 0.0022% grape seed extract; FH) 3% FO + 5% tilapia protein hydrolysate; and FVitE) 3% FO + 0.04% vitamin E.

[A,B] Means followed by different letters differ (P≤0.05) by the protected t test.

Table 2. Effects of adding flaxseed oil and natural antioxidants to swine diets fatty acids composition (% of ether extract) and fat content (%) in pork loin.

Variables	C	F	FGP	FGE	FH	FVtitE	P value
Ether Extract %	1.923 ± 0.123	1.717 ± 0.122	1.800 ± 0.154	1.854 ± 0.146	2.139 ± 0.126	2.028 ± 0.172	0.2654
C10:0	0.104 ± 0.004	0.107 ± 0.005	0.089 ± 0.005	0.100 ± 0.005	0.107 ± 0.005	0.110 ± 0.004	0.0578
C12:0	0.081 ± 0.005	0.083 ± 0.006	0.079 ± 0.006	0.077 ± 0.004	0.082 ± 0.002	0.086 ± 0.004	0.9411
C14:0	1.221 ± 0.073	1.236 ± 0.081	1.104 ± 0.045	1.170 ± 0.057	1.194 ± 0.027	1.205 ± 0.045	0.7295
C15:0	0.945 ± 0.119	0.847 ± 0.135	0.808 ± 0.151	0.813 ± 0.098	0.596 ± 0.106	0.634 ± 0.038	0.2730
C16:0	22.58 ± 0.41	22.26 ± 0.46	21.71 ± 0.20	21.81 ± 0.33	21.61 ± 0.17	21.80 ± 0.44	0.3404
C16:1	2.617 ± 0.128	2.256 ± 0.117	2.183 ± 0.139	2.390 ± 0.171	2.309 ± 0.087	2.336 ± 0.139	0.2931
C17:0	0.240 ± 0.011	0.223 ± 0.013	0.259 ± 0.022	0.215 ± 0.015	0.230 ± 0.011	0.230 ± 0.014	0.3328
C17:1	0.165 ± 0.006	0.140 ± 0.009	0.154 ± 0.009	0.141 ± 0.018	0.167 ± 0.008	0.169 ± 0.018	0.5406
C18:0	10.33 ± 0.21	10.19 ± 0.22	10.50 ± 0.31	9.889 ± 0.412	10.08 ± 0.24	9.967 ± 0.320	0.8443
C18:1n7c	3.535 ± 0.063	2.901 ± 0.154	2.977 ± 0.236	2.911 ± 0.263	3.035 ± 0.199	2.781 ± 0.280	0.2098
C18:1n9c	36.24 ± 0.65	34.01 ± 0.75	34.58 ± 0.65	36.02 ± 0.37	35.59 ± 0.69	35.26 ± 0.56	0.0921
C18:2n6c	10.80 ± 0.68	12.43 ± 0.76	12.42 ± 0.62	11.67 ± 0.71	11.96 ± 0.82	12.31 ± 0.96	0.4669
C18:3n3_ALA	0.728 ± 0.041[b]	2.534 ± 0.089[a]	2.390 ± 0.195[a]	2.129 ± 0.099[a]	2.340 ± 0.111[a]	2.461 ± 0.29[a]	<.0001
C20:0	0.169 ± 0.010	0.168 ± 0.014	0.196 ± 0.012	0.148 ± 0.012	0.159 ± 0.013	0.156 ± 0.008	0.1033
C20:1n9c	0.563 ± 0.017	0.519 ± 0.015	0.554 ± 0.021	0.522 ± 0.020	0.540 ± 0.036	0.514 ± 0.017	0.4105
C20:2n6c	0.325 ± 0.015	0.346 ± 0.026	0.359 ± 0.023	0.339 ± 0.023	0.367 ± 0.022	0.339 ± 0.023	0.7244
C20:4n6c	0.230 ± 0.030	0.194 ± 0.028	0.167 ± 0.022	0.164 ± 0.021	0.172 ± 0.022	0.156 ± 0.013	0.2459

Table 2 (Continued)

Variables	C	F	FGP	FGE	FH	FVtitE	P value
C20:5n3c_EPA	0.037 ± 0.013[b]	0.278 ± 0.034[a]	0.214 ± 0.036[a]	0.241 ± 0.040[a]	0.224 ± 0.029[a]	0.246 ± 0.036[a]	<.0001
C22:1n9c	0.086 ± 0.015[b]	0.270 ± 0.012[a]	0.248 ± 0.015[a]	0.253 ± 0.016[a]	0.245 ± 0.008[a]	0.238 ± 0.018[a]	<.0001
Σ SFA	35.67 ± 0.54	35.11 ± 0.61	34.75 ± 0.46	34.22 ± 0.66	34.05 ± 0.34	34.19 ± 0.77	0.4147
Σ MUFA	43.21 ± 0.65[a]	40.10 ± 0.92[b]	40.70 ± 0.65[b]	42.24 ± 0.44[ab]	41.89 ± 0.81[ab]	41.30 ± 0.88[ab]	0.0396
Σ PUFA	12.12 ± 0.73[b]	15.79 ± 0.84[a]	15.55 ± 0.75[a]	14.54 ± 0.84[a]	15.06 ± 0.97[a]	15.51 ± 1.29[a]	0.0318
Σ ω-3	0.765 ± 0.037[b]	2.812 ± 0.083[a]	2.604 ± 0.192[a]	2.370 ± 0.128[a]	2.563 ± 0.131[a]	2.707 ± 0.325[a]	<.0001
Σ ω-6	11.35 ± 0.71	12.97 ± 0.81	12.95 ± 0.65	12.17 ± 0.73	12.50 ± 0.85	12.80 ± 0.99	0.5225
MUFA/SFA	1.213 ± 0.028	1.145 ± 0.038	1.172 ± 0.025	1.236 ± 0.026	1.230 ± 0.022	1.210 ± 0.034	0.2964
PUFA/SFA	0.341 ± 0.024[b]	0.451 ± 0.027[a]	0.449 ± 0.025[a]	0.428 ± 0.031[a]	0.443 ± 0.031[a]	0.458 ± 0.044[a]	0.0542
ω6/ω3	14.87 ± 0.71[a]	4.620 ± 0.277[b]	5.077 ± 0.387[b]	5.136 ± 0.155[b]	4.858 ± 0.124[b]	4.861 ± 0.261[b]	<.0001

C: control diet without added oil; F) 3% flaxseed oil (FO); FGP) 3% FO + 10% grape pomace; FGE) 3% FO + 0.0022% grape seed extract; FH) 3% FO + 5% tilapia protein hydrolysate; and FVitE) 3% FO + 0.04% vitamin E. [A,B] Means followed by different letters differ (P≤0.05) by the protected t test.

Table 3. Effect of adding flaxseed oil and natural antioxidants to swine diets and storage time (T0, T2, T4, T6) on the presence of thiobarbituric acid-reactive substances (TBARS) expressed by the concentration of malondialdehyde (mg/Kg of sample) in mini-hamburgers

Storage time in months	Treatments						P value
	C	F	FGP	FGE	FH	FVtitE	
T0	0.21 ± 0.03^{Dab}	0.27 ± 0.03^{Da}	0.22 ± 0.03^{Cab}	0.18 ± 0.02^{Cb}	0.20 ± 0.03^{Db}	0.15 ± 0.02^{Db}	0.0296
T2	0.86 ± 0.08^{Cd}	1.06 ± 0.07^{Cbc}	1.22 ± 0.07^{Bab}	1.26 ± 0.10^{Ba}	0.97 ± 0.08^{Ccd}	0.65 ± 0.05^{Ce}	<.0001
T4	1.21 ± 0.10^{Bbc}	1.52 ± 0.12^{Ba}	1.35 ± 0.07^{Bab}	1.38 ± 0.07^{Bab}	1.32 ± 0.08^{Bab}	1.04 ± 0.10^{Bc}	0.0186
T6	1.61 ± 0.16^{Abc}	1.96 ± 0.17^{Aab}	1.68 ± 0.07^{Aabc}	2.03 ± 0.11^{Aa}	1.95 ± 0.16^{Aab}	1.47 ± 0.12^{Ac}	0.0307

C: control diet without added oil; F) 3% flaxseed oil (FO); FGP) 3% FO + 10% grape pomace; FGE) 3% FO + 0.0022% grape seed extract; FH) 3% FO + 5% tilapia protein hydrolysate; and FVitE) 3% FO + 0.04% vitamin E.

T0: time 0; T2: two months storage; T4: four months storage; T6: six months storage.

Means in rows followed by distinct lowercase letters differ significantly by the t test ($p \leq 0.05$);

Means in columns followed by distinct uppercase letters differ significantly by the t test ($p \leq 0.05$).

Table 4. Effects of adding flaxseed oil and natural antioxidants to swine diets on oxidative stability (Rancimat*) of fat.

Treatments	Induction time (h)	Stability compared to F group
C	7.84 ± 0.08	2.49
F	3.15 ± 0.01	1.00
FGP	2.98 ± 0.24	0.95
FGE	3.20 ± 0.02	1.01
FH	3.53 ± 0.16	1.12
FVitE	6.70 ± 0.02	2.13

*90ºC, air=10L/h

C: control diet without added oil; F) 3% flaxseed oil (FO); FGP) 3% FO + 10% grape pomace; FGE) 3% FO + 0.0022% grape seed extract; FH) 3% FO + 5% tilapia protein hydrolysate; and FVitE) 3% FO + 0.04% vitamin E.

Table 5. Effect of adding flaxseed oil and natural antioxidants to swine diets and storage time (T0, T2, T4, T6) on the color (a*, b*) of mini-hamburgers.

Storage time in months	Treatments						P value
	C	F	FGP	FGE	FH	FVitE	
				a*			
T0	5.74 ± 0.28 ^{Aa}	5.71 ± 0.45 ^{Aa}	4.70 ± 0.31 ^{Ab}	6.26 ± 0.44 ^{Aa}	5.93 ± 0.43 ^{Aa}	5.71 ± 0.61 ^{Aa}	0.0022
T2	5.06 ± 0.37 ^{Aa}	4.14 ± 0.36 ^{Bab}	2.82 ± 0.33 $^{B c}$	4.19 ± 0.35 ^{Bb}	4.20 ± 0.29 ^{Bb}	3.88 ± 0.37 ^{Bb}	<.0001
T4	2.40 ± 0.36 ^{B}	2.15 ± 0.30 ^{C}	2.27 ± 0.27 ^{B}	2.62 ± 0.38 ^{C}	2.80 ± 0.26 ^{C}	2.84 ± 0.40 ^{C}	0.7863
T6	2.40 ± 0.24 ^{B}	2.64 ± 0.17 ^{C}	2.81 ± 0.29 ^{B}	2.41 ± 0.39 ^{C}	1.82 ± 0.27 ^{D}	2.89 ± 0.41 ^{BC}	0.0788
				b*			
T0	12.23 ± 0.34 ^{C}	13.06 ± 0.26 ^{B}	12.36 ± 0.29 ^{C}	12.19 ± 0.22 ^{C}	12.20 ± 0.45 ^{C}	11.87 ± 0.28 ^{B}	0.3172
T2	12.98 ± 0.42 ^{B}	13.32 ± 0.20 ^{B}	13.52 ± 0.19 ^{B}	12.90 ± 0.28 ^{B}	13.14 ± 0.22 ^{B}	12.49 ± 0.24 ^{B}	0.1915
T4	12.70 ± 0.25 ^{BC}	13.03 ± 0.31 ^{B}	13.52 ± 0.15 ^{B}	12.84 ± 0.25 ^{B}	12.97 ± 0.18 ^{B}	12.40 ± 0.22 ^{B}	0.0769
T6	13.61±0.28 ^{Ab}	14.18 ± 0.31 ^{Aab}	14.83 ± 0.22 ^{Aa}	14.37 ± 0.23 ^{Aa}	14.31 ± 0.15 ^{Aa}	14.13 ± 0.19 ^{Aab}	0.0230

C: control diet without added oil; F) 3% flaxseed oil (FO); FGP) 3% FO + 10% grape pomace; FGE) 3% FO + 0.0022% grape seed extract; FH) 3% FO + 5% tilapia protein hydrolysate; and FVitE) 3% FO + 0.04% vitamin E.

T0: time 0; T2: two months' storage; T4: four months' storage; T6: six months' storage.

Means in rows followed by distinct lowercase superscritps differ significantly by the t test ($p \leq 0.05$);

Means in columns followed by distinct uppercase superscripts differ significantly by the t test ($p \leq 0.05$).

Table 6. Effect of adding flaxseed oil and natural antioxidants to swine diets and storage time (T2, T4, T6) on the acceptability of mini-hamburgers

Storage time in months	Treatments						P value Treatment
	C	F	FGP	FGE	FH	FVitE	
				Overall acceptance			
T2	6.90 ± 2.04ab	6.30 ± 2.14b	6.30 ± 2.35b	6.53 ± 2.08ab	6.61 ± 2.11Aab	7.07 ± 1.81Aa	0.0005
T4	6.83 ± 2.08[a]	6.10 ± 2.31b	5.84 ± 2.53b	6.42 ± 2.29ab	6.12 ± 2.39ABb	6.15 ± 2.29Bab	0.0014
T6	6.20 ± 2.25	6.20 ± 2.40	5.78 ± 2.31	6.2 ± 2.19	5.63 ± 2.26B	6.13 ± 2.21B	0.0600
P value Time	0.0901	0.7844	0.2243	0.4906	0.0087	0.0029	
				Appearance			
T2	6.92 ± 1.97	6.595 ± 2.22	6.57 ± 2.15	6.86 ± 2,00	6.86 ± 2.2	7.12 ± 1.87	0.0547
T4	7.17 ± 1.93	6.72 ±2.19	6.88 ± 2.03	6.89 ± 1.93	6.64 ± 2.23	6.62 ±2.16	0.0552
T6	6.80 ± 2.14	6.50 ± 2.04	6.51 ± 2.19	6.77 ± 1.95	6.27 ± 2.34	6.88 ± 1.93	0.0631
P value Time	0.4051	0.7715	0.3584	0.8152	0.1778	0.3621	
				Aroma			
T2	6.94 ± 2.04	6.56 ± 2.09	6.55 ± 2.05	6.86 ± 2.04	6.63 ± 2.14	7.10 ± 1.78	0.0997
T4	6.70 ± 2.14	6.42 ± 2.43	6.24 ± 2.38	6.60 ± 2.22	6.44 ± 2.22	6.53 ± 2.23	0.4077
T6	6.69 ± 2.14ab	6.59 ± 2.04ab	6.26 ± 2.31ab	6.60 ± 2.13ab	6.14 ± 2.28b	6.82 ± 1.85[a]	0.0332
P value Time	0.6815	0.8497	0.4840	0.7030	0.2994	0.2046	
				Flavor			
T2	6.84 ± 2.11ab	6.25 ± 2.28b	6.21 ± 2.37b	6.45 ± 2.35ab	6.22 ± 2.23b	7.01 ± 2.00a	0.0026
T4	6.48 ± 2.42	5.93 ± 2.59	5.99 ± 2.51	6.25 ± 2.52	6.11 ± 2.67	6.35 ± 2.33	0.2647
T6	6.49 ± 2.26[a]	6.37 ± 2.18ab	5.64 ± 2.54b	5.91 ± 2.46ab	5.63 ± 2.52b	6.34 ± 2.26ab	0.0027
P value Time	0.5298	0.4170	0.2235	0.2723	0.2587	0.0632	

Table 6 (Continued)

Storage time in months	Treatments						P value Treatment
	C	F	FGP	FGE	FH	FVitE	
				Texture			
T2	7.09 ± 1.97	6.64 ± 2.14	6.49 ± 2.27	6.61 ± 2.31	6.58 ± 2.16	7.08 ± 1.85	0.0565
T4	6.98 ± 2.12	6.47 ± 2.23	6.42 ± 2.29	6.62 ± 2.01	6.58 ± 2.32	6.46 ± 2.46	0.1100
T6	6.67 ± 2.14	6.35 ± 2.43	6.46 ± 2.43	6.44 ± 2.31	6.46 ± 2.31	6.82 ± 2.20	0.4060
P value							
Time	0.3234	0.6830	0.8987	0.7197	0.9451	0.1826	
				Unusual aroma			
T2	0.52 ± 1.22	0.58 ± 1.52	0.84 ± 0.85	0.64 ± 1.39	0.85 ± 1.72	0.49 ±1.06	0.3078
T4	0.75 ± 1.41	1.03 ± 1.96	0.76 ± 1.61	0.63 ± 1.33	0.89 ±1.99	0.86 ± 1.66	0.4174
T6	0.70 ± 1.73	0.67 ± 1.60	0.93 ± 1.90	0.54 ± 1.24	0.83 ± 1.62	0.63 ± 1.36	0.2905
P value							
Time	0.4373	0.0914	0.8054	0.8483	0.8929	0.2605	
				Unusual flavor			
T2	0.66 ± 1.50	1.15 ± 2.02B	1.22 ± 2.05	0.83 ± 1.75	0.99 ± 1.85	0.78 ± 1.65B	0.1586
T4	0.78 ± 1.62b	1.73 ± 2.5Aa	1.63 ± 2.39[a]	1.08 ± 1.97ab	1.34 ± 2.39ab	1.42 ± 2.26Aab	0.0033
T6	0.87 ± 1.74b	1.01 ± 2.05Bab	1.28 ± 2.02ab	1.35 ± 2.11ab	1.57 ± 2.32a	1.17 ± 1.97ABab	0.0562
P value							
Time	0.9037	0.0028	0.0966	0.6550	0.5837	0.0105	

C: control diet without added oil; F) 3% flaxseed oil (FO); FGP) 3% FO + 10% grape pomace; FGE) 3% FO + 0.0022% grape seed extract; FH) 3% FO + 5% tilapia protein hydrolysate; and FVitE) 3% FO + 200ppm vitamin E.

T2: two months' storage; T4: four months' storage; T6: six months' storage.

Means in rows followed by distinct lowercase superscripts differ significantly by the Tukey ($p \leq 0.05$);

Means in columns followed by distinct uppercase superscripts differ significantly by the Tukey ($p \leq 0.05$).

Table 7. Effects adding flaxseed oil and natural antioxidants to swine diets on tocopherol content (ug/100g meat) of loin

Variables	C	F	FGP	FGE	FH	FVtitE	p value
α-Tocopherol	98.21 ± 13.1^{c}	112.87 ± 8.61^{bc}	128.46 ± 17.8^{bc}	128.42 ± 15.5^{bc}	147.37 ± 15.2^{b}	347.10 ± 19.9^{a}	<.0001
γ- Tocopherol	19.74 ± 2.56^{dc}	25.52 ± 3.91^{dbc}	30.24 ± 3.80^{ab}	25.73 ± 2.45^{abc}	34.77 ± 3.21^{a}	18.07 ± 1.58^{d}	0.0042
Tocopherol total	117.95 ± 14.6^{c}	138.39 ± 11.7^{bc}	158.70 ± 20.7^{bc}	154.15 ± 17.8^{b}	182.14 ± 17.5^{b}	365.17 ± 21.2^{a}	<.0001

δ-tocopherol and β-tocopherol were not detected.

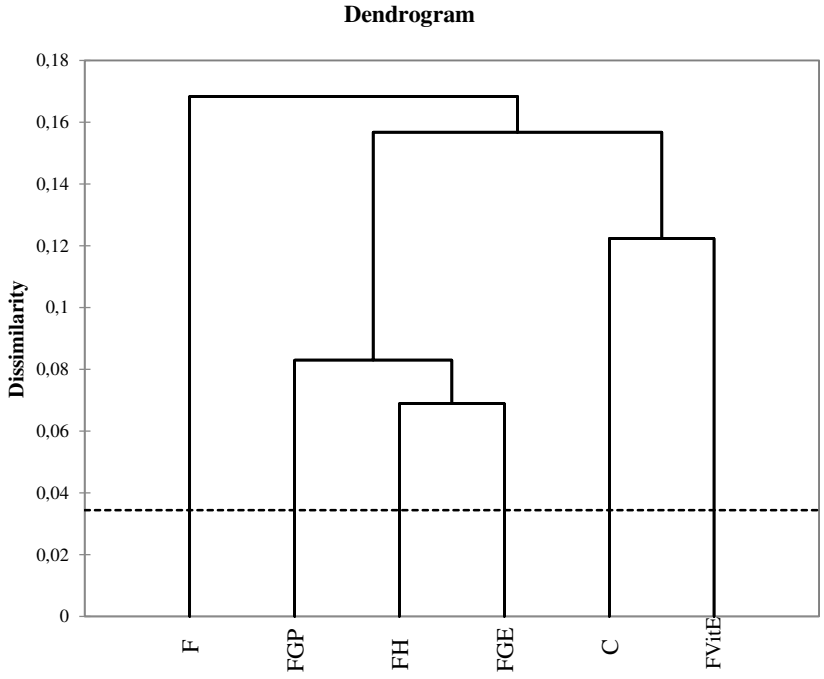
The overall acceptance, appearance, aroma, flavor and texture were evaluated on a non-structured 9 cm hedonic scale, with the limit markers of "dislike extremely" on the left and "like extremely" on the right.

Detection of unusual flavor and unusual aroma in the samples was also evaluated, using a non-structured 9 cm scale, with the limit markers corresponding to "none" and "extremely strong".

C: control diet without added oil; F) 3% flaxseed oil (FO); FGP) 3% FO + 10% grape pomace; FGE) 3% FO + 0.0022% grape seed extract; FH) 3% FO + 5% tilapia protein hydrolysate; and FVitE) 3% FO + 0.04% vitamin E.

[a,b] Means in row followed by different superscript differ (P≤0.05) by the protected t test

1 A



1 B

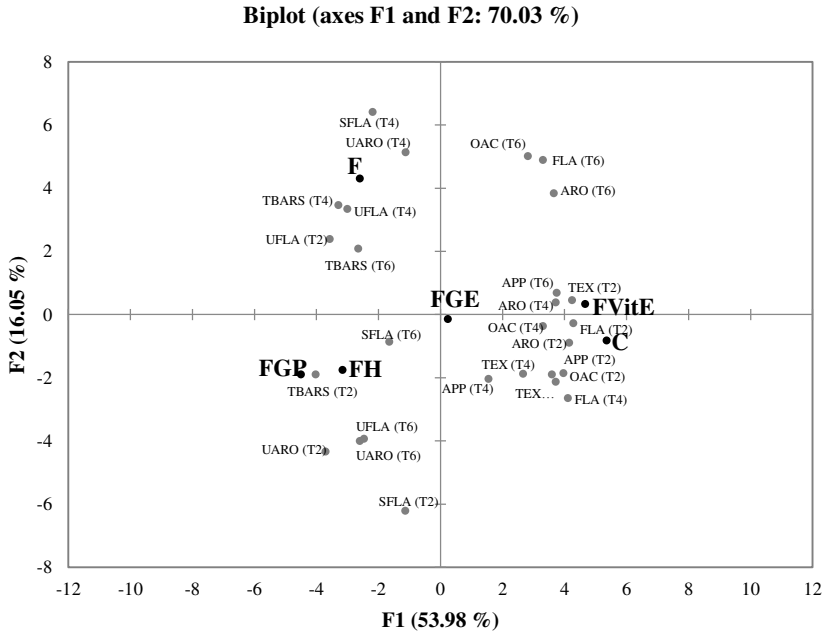


Figure 1. Cluster analysis (Figure 1A) and Principal Component Analysis (Figure 1B: appearance - APP, aroma - ARO, flavor - FLA, texture - TEX, overall acceptance - OAC, unusual flavor - UFLA and unusual aroma - UARO, and Storage time - T2, T4, T6) in the data obtained from sensory analysis of mini-hamburgers made with pork from treatments with natural antioxidants and linseed oil. C: control diet without added oil; F) 3% flaxseed oil (FO); FGP) 3% FO + 10% grape pomace; FGE) 3% FO + 0.0022% grape seed extract; FH) 3% FO + 5% tilapia protein hydrolysate; and FVitE) 3% FO + 0.04% vitamin E.

6.7. ABBREVIATIONS

a*: colour measured by the CIELAB system. Positive a* is red and negative a* is green.

ALA: alpha-linolenic acid

APP: appearance

ARO: aroma

b*: colour measured by the CIELAB system. Positive b* is yellow and negative b* is blue.

C: control diet

DHA: docosahexaenoic acid

EPA: eicosapentaenoic acid

F: diet with 3% flaxseed oil

FA: fatty acids

FGE: diet with 3% FO + 0.0022% grape seed extract;

FGP: diet with 3% flaxseed oil + 10% grape pomace;

FH: diet with 3% FO + 5% tilapia carcass hydrolysate;

FLA: flavor

FO: flaxseed oil

FVitE: diet with 3% FO + 200 ppm vitamin E supplement

HCA: Hierarchical cluster analysis

LA: linoleic acid

MDA: malonaldehyde

MUFA: monounsaturated fatty acids

OAC: overall acceptance,

PCA: principal component analysis

PUFA: polyunsaturated fatty acids

PUFA: polyunsaturated fatty acids

SFA: saturated fatty acids

TBARS: thiobarbituric acid reactive substances

TEX: texture

UARO: unusual aroma

UFLA: unusual flavor

6.8. ACKNOWLEDGEMENTS

The authors would like to thank Embrapa, National Counsel of Technological and Scientific Development (CNPq), Falbom Agroingustrial Ltda. and Cargill Foods Brazil for all their support in the development of this study.

7. DISCUSSÃO GERAL

Verificou-se no presente trabalho, que a incorporação de óleo de linhaça na ração de suínos, promoveu alterações no perfil lipídico, tanto do lombo como do toucinho. Altas concentrações de ácidos graxos (AG) ω-3 foram observadas nas amostras supracitadas, sendo o AG α-linolênico (ALA) o ω-3 presente em maior concentração, além disso, também foram detectadas concentrações significativas de AG eicosapentaenoico (EPA).

Kloareg et al. (2007) reportaram que em suínos cerca de 40% do ALA suplementado pode ser aproveitado pelo organismo, onde parte pode ser armazenada em sua forma original ou pode ser elongado, dessaturado e convertido em outros ω-3, como o EPA e o docosahesaenoico (DHA). Como verificado no presente estudo, a incorporação de ALA e sua conversão em EPA também foi observada por outros autores (MATTHEWS et al., 2000; KOUBA et al., 2003; REALINI et al., 2010; JUÁREZ et al., 2011).

A suplementação com óleo de linhaça contribuiu para reduzir a relação ω-6/ω-3 em lombo e toucinho. Esta relação é de grande importância, pois as enzimas envolvidas no processo de elongamento e dessaturação dos ácidos graxos ω-6 e ω-3 são as mesmas e a alta concentração do AG linoléico (precursor de ω-6) pode resultar em menor conversão do ALA em EPA e DHA (VAZ et al., 2006).

Observou-se também, nos animais alimentados com óleo de linhaça um maior teor total de ácidos graxos poli-insaturados (PUFAs) e uma redução no teor de ácidos graxos saturados (SFA), tanto no lombo como no toucinho, esta alteração de perfil lipídico resultou em aumento da relação PUFA/SFA. Em humanos, a substituição de parte do SFA ingerido, por PUFA, pode reduzir o risco de doenças cardiovasculares (JAKOBSEN et al., 2009).

A incorporação de ω-3 e de PUFAs em geral, apresenta muitos obstáculos tecnológicos em produtos cárneos (BERNARDI et al., 2016) e no presente estudo foi possível verificar que esta incorporação favoreceu a oxidação. Foram observados menores escores de cor no lombo dos animais que receberam óleo de linhaça na dieta, assim como verificou-se que o toucinho apresentou uma gordura menos estável.

Também foi verificado maior oxidação lipídica, detectada pela alta concentração de malonaldeído (MDA), nas amostras de mini-hambúrgueres congelados produzidos com a carne dos animais do tratamento com óleo de linhaça, além disso, verificou-se maiores valores de b* e menores valores de a* nestes mini-hamburgueres, sendo que maiores valores de b* estão relacionados à maior tonalidade amarela e menores valores de a* estão relacionados à menor intensidade de vermelho, portanto, possivelmente o resultado obtido na avaliação de cor do produto cárneo está relacionado à oxidação da mioglobina (AMSA, 1991).

Dessa forma, em produtos enriquecidos com ω-3 é importante a utilização de estratégias que busquem a redução/retardo da oxidação no produto e no presente estudo a adição na dieta de produtos naturais com potencial antioxidante foi a estratégia empregada.

A vitamina E é o antioxidante natural mais utilizado na alimentação animal (GLADINE et al., 2007) e embora seu efeito sobre a estabilidade oxidativa de carnes ao longo do armazenamento esteja fartamente relatado na literatura (SALES; KOUKOLOVÁ, 2011), alguns autores sugerem que a eficiência antioxidante desta vitamina pode ficar comprometida em animais alimentados com maiores concentrações de PUFA (GLADINE et al., 2007).

Os resultados do presente trabalho mostraram que a Vitamina E foi o antioxidante mais efetivo em relação às avaliações que consideraram a vida de prateleira da carne, como a determinação da estabilidade oxidativa (TBARS) e a aceitação sensorial dos mini-hamburgueres armazenados congelados por seis meses. Além disso, a vitamina E também mostrou grande efetividade no teste de estabilidade oxidativa da gordura, mensurada pelo tempo de indução à oxidação realizada no equipamento Rancimat. Estes resultados corroboram com os obtidos por outros autores que também adicionaram óleo de linhaça e vitamina E na dieta de suínos (HAAK et al., 2008; SANTOS et al., 2008).

Por outro lado, verificou-se nas avaliações de escore de cor da carne fresca (armazenada por 24h sob refrigeração) que a presença de vitamina E na dieta não contribuiu para melhorar este escore. Portanto, o efeito relatado na literatura sobre a estabilidade de cor conferido à carne de animais alimentados com maiores concentrações de vitamina E provavelmente fica mais evidente ao longo do armazenamento (BOLER et al., 2009; WANG et al., 2012), ou seja, avaliações realizadas na carne *in natura* (24horas após o abate), impõe poucos desafios à vitamina E.

O bagaço e as sementes de uva são subprodutos que apresentam alto teor de compostos fenólicos que possuem atividade antioxidante (LAFKA; SINANOGLOU; LAZOS, 2007), sendo que de acordo com o verificado no presente estudo, o principal mecanismo antioxidante tanto do bagaço de uva desidratado, como do extrato de semente de uva é a capacidade de atuar como agentes redutores (determinado pelo método de FRAP).

Mesmo com a atividade antioxidante comprovada nos testes químicos, a adição de bagaço de uva (10%) na dieta de suínos com maior teor de ω-3, não apresentou efeito antioxidante sobre a carne, gordura ou produto cárneo, pelo contrário mostrou um indicativo de efeito pró-oxidante. Outros autores também mostraram que a adição de altas concentrações de produtos ricos em compostos fenólicos na dieta de suínos também resultou em efeitos pró-oxidantes (LARRAÍN et al., 2006) ou em nenhum efeito antioxidante (O'GRADY et al., 2008).

Por outro lado, diferente do observado nos dados com o bagaço de uva, a incorporação de extrato de semente de uva na dieta dos suínos apresentou resultados positivos em relação ao potencial antioxidante sobre a carne. Foram observados maiores escores de cor no lombo fresco (24h armazenamento), sendo que este resultado pode estar relacionado ao estado redox da mioglobina (MANCINI; HUNT, 2005). Em relação à análise sensorial, verificou-se que os mini-hamburgueres produzidos com a carne do tratamento com extrato de semente de uva, ficaram com aceitabilidade superior ao tratamento com óleo de linhaça sem antioxidante. Porém, apesar dos efeitos positivos supracitados, o extrato de semente de uva não apresentou efetividade nas análises de TBARS realizadas nos mini-hamburgueres ao longo do armazenamento, bem como no teste de estabilidade da gordura (Rancimat).

Gladine et al. (2007) sugerem que os compostos fenólicos têm maior atividade em órgãos mais sensíveis, como o fígado, além disso, ao passar pelos processos de digestão, absorção, metabolismo e transporte, os antioxidantes podem perder parte da bioatividade e também biodisponibilidade *in vivo*. Entretanto, quando compostos fenólicos ((+)-catequinas, (-) epicatequinas e quercitinas) presentes na dieta animal, conseguem ser absorvidos e incorporados aos tecidos, podem promover um efeito "poupador" e "reciclador" de Vitamina E tecidual, resultando em proteção oxidativa (FRANK, 2005; GLADINE et al., 2007; LAFKA; SINANOGLOU; LAZOS, 2007), portanto este fato reforça a necessidade de estudos adicionais sobre a incorporação de subprodutos da uva na dieta de suínos.

No presente estudo foi produzido um hidrolisado de carcaça e cabeça da tilápia do Nilo e avaliado seu potencial antioxidante por testes químicos, bem como seu efeito na alimentação de suínos. Os resultados mostraram que a hidrólise com Alcalase® foi capaz de recuperar toda a proteína presente na carcaça e na cabeça da tilápia, resultando em um hidrolisado com potencial antioxidante.

Os resultados de atividade antioxidante do hidrolisado de carcaça e cabeça da tilápia do Nilo mostraram-se compatíveis com os reportados por outros autores que também avaliaram hidrolisados de pescados (RAGHAVAN; KRISTINSSON; LEEUWENBURGH, 2008; CHOONPICHARN et al., 2014; YARNPAKDEE et al., 2014; GIRGIH et al., 2015). Além disso, verificou-se que o principal mecanismo de atividade antioxidante do hidrolisado de carcaça e cabeça da tilápia do Nilo é a transferência de átomos de hidrogênio (determinada no método de ORAC), o que o torna um antioxidante com potencial de apresentar bons efeitos antioxidantes *in vivo*.

Apesar dos bons resultados de atividade antioxidante *in vitro,* verificou-se que a presença de hidrolisado de carcaça e cabeça da tilápia do Nilo na dieta dos suínos não contribuiu

para reduzir a produção de MDA em mini-hamburgueres congelados e também não interferiu nos escores de cor da carne fresca. Por outro lado, na análise realizada no Rancimat a gordura deste tratamento apresentou maior tempo de indução que o tratamento que tinha apenas óleo de linhaça na dieta. Verificou-se também que na análise sensorial, os mini-hamburgeres produzidos a partir da carne do tratamento com hidrolisado ficou em regiões de aceitabilidade intermediárias e distantes do tratamento contendo somente óleo de linhaça.

Os mecanismos antioxidantes *in vivo* utilizados pelos peptídeos ainda não estão totalmente claros, porém sabe-se que após a ação de enzimas gastrointestinais, estes peptídeos mantém ou aumentam sua atividade antioxidante, além disso, podem ser absorvidos de forma intacta no intestino e transportados até orgãos alvo, onde a presença de resíduos de aminoácidos hidrofóbicos podem facilitar sua entrada por meio de interações hidrofóbicas com membranas (GIRGIH et al., 2015).

Conforme esperado, os resultados do presente trabalho mostraram que a concentração de tocoferol tecidual foi maior no tratamento com adição dietética de vitamina E, seguido pelos tratamentos com hidrolisado de carcaça e cabeça da tilápia do Nilo e extrato de semente de uva. Isso reforça a hipótese do efeito "poupador" e "reciclador" da vitamina E tecidual, promovida pelos compostos fenólicos do extrato de semente de uva. Em relação ao hidrolisado, novas abordagens são necessárias, com investigações mais detalhadas sobre como os peptídeos resultantes da hidrólise de proteínas de pescados se comportam *in vivo,* durante a digestão, absorção e distribuição em tecidos-alvo, bem como, qual a magnitude de sua bioatividade após passar por todas estas etapas, e como podem ter seu potencial antioxidante afetado.

Também foram realizadas avaliações dos índices zootécnicos dos animais alimentados com óleo de linhaça e produtos naturais com potencial antioxidante e, de maneira geral, verificou-se que os tratamentos não influenciaram nos parâmetros de desempenho de crescimento e qualidade de carcaça, sendo que estes resultados corroboram com os obtidos por outros autores (GUO et al., 2006; MITCHAOTHAI et al., 2007; BOLER et al., 2009; GUILLEVIC; KOUBA; MOUROT, 2009; YAN; KIM, 2011; WANG et al., 2012; BERTOL et al., 2013). É importante ressaltar ainda que as fontes de antioxidante com maior nível de inclusão na ração (bagaço de uva e hidrolisado de carcaça e cabeça da tilápia do Nilo), bem como o óleo de linhaça, são ingredientes pouco estudados na alimentação animal e não se conhece seus valores exatos de energia metabolizável (EM) e de aminoácidos digestíveis, sendo que os valores usados na formulação das rações podem não ter sido os mais indicados.

8. CONCLUSÃO GERAL E PERSPECTIVAS FUTURAS

A inclusão de óleo de linhaça na dieta de suínos promoveu modificações significativas no perfil lipídico da carne e toucinho, onde verificou-se aumento das concentrações totais de ω-3, com significativa incorporação de ALA e sua conversão à EPA, além disso, maiores teores de PUFA e menores de SFA foram observados.

Esta modificação no perfil de ácidos graxos aumentou a suscetibilidade oxidativa na carne e gordura, demonstrado pelas análises de estabilidade oxidativa, TBARS, cor e sensorial, o que evidenciou ainda mais a importância da suplementação conjunta de ω-3 e antioxidantes.

Foi possível elaborar um produto com potencial antioxidante *in vitro* a partir de subprodutos do processamento da Tilápia e que quando este foi adicionado na dieta dos suínos apresentou indicativos de potencial antioxidante, observados pelas análises de estabilidade oxidativa da gordura, sensorial dos mini-hambúrgueres e tocoferol na carne.

Da mesma forma, verificou-se que o extrato de semente de uva também apresentou resultados positivos para a cor da carne fresca, sensorial do mini-hamburguer e tocoferol no lombo, indicando que o produto pode atuar como antioxidante *in vivo.* Por outro lado, o bagaço de uva adicionado na dieta, mostrou-se ineficiente como agente antioxidante e apresentou indicativos de efeito pró-oxidante.

A vitamina E foi o produto testado na ração que apresentou maior eficiência antioxidante sobre a carne, gordura e produto cárneo. Portanto pode ser considerada um padrão de antioxidante na dieta de suínos, especialmente quando é considerado o efeito ao longo do armazenamento e mesmo que maiores concentrações de PUFA estejam presentes na dieta.

É importante ressaltar que os resultados encontrados em testes químicos de atividade antioxidante, do hidrolisado de carcaça e cabeça de tilápia do Nilo, do extrato de semente de uva e do bagaço de uva, não foram reproduzidos com a mesma eficiência nos testes *in vivo*, o que provavelmente deve-se à interferência de fatores como a digestão, absorção e transporte dos compostos bioativos*,* portanto, no protocolo experimental estes são fatores importantes de serem considerados.

Sugerem-se novas abordagens, com os mesmos antioxidantes testados, utilizando-se diferentes tempos de suplementação, diferentes concentrações e quantidades nas dietas, bem como, a combinação destes produtos, considerando que eles apresentam mecanismos de ação antioxidante distintos, portanto, poderiam ter ações complementares.

Recomenda-se, ainda, estudos para determinar o valor exato de energia metabolizável e de aminoácidos biodisponíveis dos produtos naturais testados. Em relação ao hidrolisado de carcaça e cabeça de tilápia do Nilo, também são recomendados estudos que determinem os

aminoácidos e peptídeos de maior efeito antioxidante no produto, de forma que possam ser concentrados no produto teste, para aumentar seu potencial antioxidante.

Em relação às avaliações dos efeitos dos antioxidantes naturais sobre a carne, em estudos futuros, poderiam se consideradas avaliações de oxidação lipídica (TBARS) não apenas na carne crua, mas também no produto após a cocção, uma vez que o aquecimento é um fator que afeta significativamente a estabilidade oxidativa do produto, o que poderia resultar em maior efeito dos antioxidantes testados.

Como feito neste estudo, recomenda-se que os próximos trabalhos com produtos naturais com ação antioxidante, busquem e estudem os compostos bioativos em matérias primas sustentáveis e economicamente viáveis, como os subprodutos da indústria de alimentos, de forma à contribuir na redução do impacto ambiental causado por estes resíduos, bem como para garantir o máximo aproveitamento dos alimentos. Além disso, a utilização destas matérias primas pode aumentar a viabilidade econômica dos projetos e consequentemente viabilizar a sua aplicação comercial.

9. REFERÊNCIAS GERAIS

ABCS, A. B. DE CRIADORES DE SUÍNOS. **Método brasileiro de classificação de carcaças**. RS: Estrela, 1973.

AHN, J.; GRUN, I. U.; FERNANDO, L.N. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. **Journal of Food Science**. v.67, p.1364-1369, 2002.

AHN, J.; GRUN. I. U.; MUSTAPHA, A. Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. **Food Microbiology**. v.24, p.7-14, 2007

ALBERT, C. M.; OH, K.; WHANG, W.; MANSON, J. E.; CHAE, C. U.; STAMPFER, M. J.; WILLETT, W. C.; HU, F. B. Dietary α-Linolenic Acid Intake and Risk of Sudden Cardiac Death and Coronary Heart Disease. **Circulation** , v. 112, n. 21, p. 3232–3238, 2005..

ALESON-CARBONELL, L.; FERNÁNDEZ-LÓPEZ, J.; PÉREZ-ALVAREZ, J. A.; KURI, K. Characteristics of beef burger as influenced by various types of lemon albedo. **Innovative Food Science and Emerging Technologies**. v. 6, p. 247–255, 2005.

ALONSO, V.; NAJES, L. M.; PROVINCIAL, L.; GUILLÉN, E.; GIL, M.; RONCALÉS, P.; BELTRÁN, J. A. Influence of dietary fat on pork eating quality. **Meat Science.** v. 92, p. 366–373, 2012.

AMSA. Guidelines for Meat Color Evaluation. **Proceedings of the Reciprocal Meat Conference**, v. 44, n. Volume 44, p. 1–17, 1991.

ANJUM, F. M.; HAIDER, M. F.; KHAN, M. I.; SOHAIB, M.; ARSHAD, M. S. Impact of extruded flaxseed meal supplemented diet on growth performance , oxidative stability and quality of broiler meat and meat products. **Lipids in Health and Disease**, v. 12, n. 1, p. 1, 2013.

ANSORENA, D.; ASTIASARAN, I. The use of linseed oil improves nutritional quality of the lipid fraction of dry-fermented sausages. **Food Chemistry.** v.87, p.69-74, 2004.

AOAC. **Official methods of analysis of AOAC International**. 16. ed. Virgínia: [s.n.].

AOCS. Method Cd 12b-92. In: **Official Methods and Recommended Practices of the American Oil Chemists' Society**. [s.l: s.n.].

APPLETON, K. M.; HAYWARD, k d; GUNNELL, D.; PETERS, T. J.; ROGERS, P.;

KESSLER, d; A.R., N. Effects of n-3 long-chain polyunsaturated fatty acids on depressed mood: Systematic review of published trials. **American Journal of Clinical Nutrition**, v. 84, n. 6, p. 1308–1316, 2006.

AVILA-RAMOS, F.; PRO-MARTÍNEZ, A.; SOSA-MONTES, E.; CUCA-GARCÍA, J. M.; BECERRIL-PÉREZ, C.; FIGUEROA-VELASCO, J.L.; RUIZ-FERIA, C. A.; HERNÁNDEZ-CÁZARES, A. S.; NARCISO-GAYTÁN, C. Dietary supplemented and meat-added antioxidants effect on the lipid oxidative stability of refrigerated and frozen cooked chicken meat. **Poultry Science.** v.92, p.243-9, 2013.

AVILA-RAMOS, F.; PRO-MARTÍNEZ, A.; SOSA-MONTES, E.; CUCA-GARCÍA, J. M.; BECERRIL-PÉREZ, C. M.; FIGUEROA-VELASCO, J. L.; NARCISO-GAYTÁN, C. Effects of dietary oregano essential oil and vitamin E on the lipid oxidation stability of cooked chicken breast meat. **Poultry science**, v. 91, n. 2, p. 505–511, 2012.

BAÑÓN, S.; DIAZ, P.; RODRIGUEZ, M.; GARRIDO, M. D.; PRICE, A. Green tea and grape seed extracts increase the shelf-life of low sulphite beef patties. **Meat Science**. v.77, n.1, p.626-633, 2007.

BAÑÓN, S.; MÉNDEZ, L.; ALMELA, E. Effects of dietary rosemary extract on lamb spoilage under retail display conditions. **Meat Science**, v. 90, n. 3, p. 579–583, 2012.

BARBOSA-PEREIRA, L.; AURREKOETXEA, G. P.; ANGULO, I.; PASEIRO-LOSADA, P.; CRUZ, J. M. Development of new active packaging fi lms coated with natural phenolic compounds to improve the oxidative stability of beef. **Meat Science.** v. 97, p. 249–254, 2014.

BAS, P.; SAUVANT, D. Variations de la composition des dépôts lipidiques chez les bovins. **INRA Production** Animal, v.14, n.5, p.311-322, 2001.

BEAL, P.; FAION, A. M.; CICHOSKI, A. J.; CANSIAN, R. L.; VALDUGA, A. T.; DE OLIVEIRA, D.; VALDUGA, E. Oxidative stability of fermented Italian-type sausages using mate leaves *(Ilex paraguariensis St. Hil)* extract as natural antioxidant. **International Journal of Food Science Nutrition,** v.62, n.7, p. 703-10, 2011.

BENZIE, I. F.; STRAIN, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. **Analytical biochemistry**, v. 239, n. 1, p. 70–76, 1996.

BERASATEGI, I.; LEGARRA, S.; GARCIA-IGUEZ DE CIRIANO, M.; REHECHO, S.; CALVO, M. I.; CAVERO, R. Y.; NAVARRO-BLASCO, I.; ANSORENA, D.; ASTIASARÁN, I. “High in omega-3 fatty acids” bologna-type sausages stabilized with an aqueous-ethanol extract of Melissa officinalis. **Meat Science**, v. 88, n. 4, p. 705–711, 2011..

BERASATEGI, I.; NAVARRO-BLASCO, Í.; ISABEL, M.; YOLANDA, R.; ASTIASARÁN, I.; ANSORENA, D. Healthy reduced-fat Bologna sausages enriched in ALA and DHA and stabilized with Melissa of fi cinalis extract. **Meat Sience**, v. 96, n. 3, p. 1185–1190, 2014.

BERNARDI, D. M. et al. ω-3 in meat products: Benefits and effects on lipid oxidative stability. **Journal of the Science of Food and Agriculture**, v. 96, n. 8, p. 2620 – 2634, 2016a.

BERTOL, T. M.; DE CAMPOS, R. M. L.; LUDKE, J. V; TERRA, N. N.; DE FIGUEIREDO, E. a P.; COLDEBELLA, A.; DOS SANTOS FILHO, J. I.; KAWSKI, V. L.; LEHR, N. M. Effects of genotype and dietary oil supplementation on performance, carcass traits, pork quality and fatty acid composition of backfat and intramuscular fat. **Meat science**, v. 93, n. 3, p. 507–16, 2013.

BERTOL, T. M.; et al. Effects of the inclusion of grape pomace in the diet of pigs on pork quality and oxidative stability of omega-3 enriched fat. **Ciência rural**, v. accepted a, 2016.

BISWAS, A. K.; CHATLI, M. K.; SAHOO, J. Antioxidant potential of curry (Murraya koenigii L .) and mint (Mentha spicata) leaf extracts and their effect on colour and oxidative stability of raw ground pork meat during refrigeration storage. **Food Chemistry**, v. 133, n. 2, p. 467–472, 2012.

BJØRNDAL, B.; BERGE, C.; RAMSVIK, M. S.; SVARDAL, A.; BOHOV, P.; SKORVE, J.; BERGE, R. K. A fish protein hydrolysate alters fatty acid composition in liver and adipose tissue and increases plasma carnitine levels in a mouse model of chronic inflammation. **Lipids in health and disease**, v. 12, n. 1, p. 143, 2013.

BLOEDON, L. T.; BALIKAI, S.; CHITTAMS, J.; CUNNANE, S. C.; BERLIN, J. a; RADER, D. J.; SZAPARY, P. O. Flaxseed and cardiovascular risk factors: results from a double blind, randomized, controlled clinical trial. **Journal of the American College of Nutrition**, v. 27, n. 1, p. 65–74, 2008.

BOLER, D. D.; GABRIEL, S. R.; YANG, H.; BALSBAUGH, R.; MAHAN, D. C.; BREWER,

M. S.; MCKEITH, F. K.; KILLEFER, J. Effect of different dietary levels of natural-source vitamin E in grow-finish pigs on pork quality and shelf life. **Meat Science**, v. 83, p. 723–730, 2009.

BOTSOGLOU, E.; GOVARIS, A.; AMBROSIADIS, I.; FLETOURIS, D.; BOTSOGLOU, N. Effect of olive leaf (Olea europea L .) extracts on protein and lipid oxidation of long-term frozen n -3 fatty acids-enriched pork patties. **Journal of Science of Food and Agriculture** v. 94, p.227-234, 2014.

BOZKURT, H.; BELIBAGLI, K. B. Use of rosemary and *Hibiscus sabdariffa* in production of kavurma, a cooked meat product. **Journal of Science of Food and Agriculture,** v.89, p.1168–1173, 2009.

BRANNAN, R. G. Effect of grape seed extract on descriptive sensory analysis of ground chicken during refrigerated storage. **Meat Science**, v. 81, p. 589–595, 2009.

BRANNAN, R. G. Effect of grape seed extract on physicochemical properties of ground, salted, chicken thigh meat during refrigerated storage at different relative humidity levels. **Journal of Food Science,** v.73, p.C36–C40, 2008.

BRENES, A.; VIVEROS, A.; GOÑI, I.; CENTENO, C.; SÁYAGO-AYERDY, S. G.; ARIJA, I.; SAURA-CALIXTO, F. Effect of Grape Pomace Concentrate and Vitamin E on Digestibility of Polyphenols and Antioxidant Activity in Chickens. **Poultry science**, v. 87, n. 2, p. 307–16, 1 mar. 2008.

BRITISH DEPARTMENT OF HEALTH. **Nutritional aspects of cardiovascular disease. Report on health and social subjects**. London: [s.n.].

CÂMARA, A. K. F. I.; POLLONIO, M. A. R. Reducing animal fat in bologna sausage using pre-emulsified linseed oil: technological and sensory properties. **Journal of Food Quality,** v.38, p.201-212, 2015

CAMPOS, R. M. L. De; HIERRO, E.; ORDÓÑEZ, J. A.; BERTOL, T. M. A note on partial replacement of maize with rice bran in the pig diet on meat and backfat fatty acids. **Journal of Animal Feed Science**. p. 427–433, 2006.

CAMPOS, R. M. L.; HIERRO, E.; ORDÓÑEZ, J. A.; BERTOL, T. M.; TERRA, N. N.; HOZ,

L. De. Food Chemistry Fatty acid and volatile compounds from salami manufactured with yerba mate (Ilex paraguariensis) extract and pork back fat and meat from pigs fed on diets with partial replacement of maize with rice bran. **Food Chemistry**, v. 103, p. 1159–1167, 2007.

CARDENIA, V.; RODRIGUEZ-ESTRADA, M. T.; CUMELLA, F.; SARDI, L.; DELLA CASA, G.; LERCKER, G. Oxidative stability of pork meat lipids as related to high-oleic sunflower oil and vitamin E diet supplementation and storage conditions. **Meat Science**, v. 88, n. 2, p. 271–279, 2011.

CARPENTER, R.; O`GRADY, M. N.; O`CALLAGHAN, Y. C.; O`BRIEN, N. M.; KERRY, J. P. Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. **Meat Science**, v. 76, n. 4, p. 604–610, 2007.

CHALAMAIAH, M.; DINESH KUMAR, B.; HEMALATHA, R.; JYOTHIRMAYI, T. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. **Food chemistry**, v. 135, n. 4, p. 3020–38, 2012.

CHAMORRO, S.; VIVEROS, A.; REBOLÉ, A.; RICA, B. D.; ARIJA, I.; BRENES, A. Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace. **Food Research International**, v. 73, p. 197–203, 2015.

CHAN, K. W.; KHONG, N. M. H.; IQBAL, S.; EE, S.; YOUNAS, U.; BABJI, A. S. Cinnamon bark deodorised aqueous extract as potential natural antioxidant in meat emulsion system : a comparative study with synthetic and natural food antioxidants. **Journal of Food Science and Technology.** 2012. DOI:10.1007/s13197-012-0818-5

CHARDIGNY, J. M.; BRETILLON, L.; SÉBÉDIO, J. L. New insights in health effects of trans alpha-linolenic acid isomers in humans. **European Journal of Lipid Science and Technology**, v. 103, n. 7, p. 478–482, 2001.

CHOI, I. H.; PARK, W. Y.; KIM, Y. J. Effects of dietary garlic powder and ?-tocopherol supplementation on performance, serum cholesterol levels, and meat quality of chicken. **Poultry science**, v. 89, p. 1724–1731, 2010.

CHOONPICHARN, S.; JATURASITHA, S.; RAKARIYATHAM, N.; SUREE, N.; NIAMSUP, H. Antioxidant and antihypertensive activity of gelatin hydrolysate from Nile tilapia skin. **Journal of Food Science and Technology**, v. 52, n. May, p. 3134–3139, 2014.

CHUESIANG, P.; SANGUANDEEKUL, R. Protein hydrolysate from tilapia frame: antioxidant and angiotensin I converting enzyme inhibitor properties. **International Journal of Food Science & Technology**, v. 50, n. 6, p. 1436–1444, 2015.

COFRADES, S.; SANDOVAL, L. S.; DELGADO-PANDO, G.; LÓPEZ-LÓPEZ, I.; RUIZ-CAPILLAS, C.; JIMÉNEZ-COLMENERO, F. Antioxidant activity of hydroxytyrosol in frankfurters enriched with n -3 polyunsaturated fatty acids. **Food Chemistry**, v. 129, n. 2, p. 429–436, 2011.

COLINDRES, P.; BREWER, M. S. Oxidative stability of cooked , frozen , reheated beef patties : effect of antioxidants. **Journal of Science of Food and Agriculture**, p. 963–968, 2011.

CRESPO, N.; ESTEVE-GARCIA, E. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. **Poult Science**, v.80, p.71-78, 2001.

DAL BOSCO, A.; MOURVAKI, E.; CARDINALI, R.; SERVILI, M.; SEBASTIANI, B.; RUGGERI, S.; MATTIOLI, S.; TATICCHI, A.; ESPOSTO, S.; CASTELLINI, C. Effect of dietary supplementation with olive pomaces on the performance and meat quality of growing rabbits. **Meat Science**, v. 92, p. 783–788, 2012.

DANGOUR, A. D.; ANDREEVA, V. a; SYDENHAM, E.; UAUY, R. Omega 3 fatty acids and cognitive health in older people. **The British journal of nutrition**, v. 107 Suppl, p. S152-8, 2012.

DAUD, N. A.; BABJI, A. S.; YUSOP, S. M. Antioxidant activities of red tilapia (Oreochromis niloticus) protein hydrolysates as influenced by thermolysin and alcalase. **AIP Conference Proceedings**, v. 687, n. 2013, p. 687–691, 2013.

DAUD, N. A.; BABJI, A. S.; YUSOP, S. M. Effects of Enzymatic Hydrolysis on the Antioxidative and Antihypertensive Activities from Red Tilapia Fish Protein. **Journal of Nutrition & Food Sciences**, v. 05, n. 04, 2015.

DÁVALOS, A.; GÓMEZ-CORDOVÉS, C.; BARTOLOMÉ, B. Extending Applicability of the Oxygen Radical Absorbance Capacity (ORAC-Fluorescein) Assay. **Journal of Agricultural and Food Chemistry**, v. 52, n. 1, p. 48–54, 2004.

DAWCZYNSKI, C.; MARTIN, L.; WAGNER, A.; JAHREIS, G. n-3 LC-PUFA-enriched

dairy products are able to reduce cardiovascular risk factors: a double-blind, cross-over study. **Clinical nutrition (Edinburgh, Scotland)**, v. 29, n. 5, p. 592–599, 2010.

DAZA, a.; REY, a. I.; RUIZ, J.; LOPEZ-BOTE, C. J. Effects of feeding in free-range conditions or in confinement with different dietary MUFA/PUFA ratios and α-tocopheryl acetate, on antioxidants accumulation and oxidative stability in iberian pigs. **Meat Science**, v. 69, p. 151–163, 2005.

DE PARIS, L. D.; ADAMS HAAB, J. C.; SARY, C.; BERNARDI, D. M.; BOSCOLO, W. R.; SIGNOR, A. Production and spray drying of protein hydrolyzate obtained from tilapia processing by-products. **Acta Scientiarum. Technology**, v. 38, n. 1, p. 89, 2016.

DECKER, E. A.; AKOH, C. C.; WILKES, R. S. Incorporation of (n-3) Fatty Acids in Foods: Challenges and Opportunities. **The Journal of Nutrition**, v.142, n.3, p.610s-613s, 2012.

DECKER, E. A.; XU, Z. Minimizing rancidity in muscle foods. **Food and Tecnology,** v.52, n.10, p.54-59, 1999.

DEKKERS, E.; RAGHAVAN, S.; KRISTINSSON, H. G.; MARSHALL, M. R. Oxidative stability of mahi mahi red muscle dipped in tilapia protein hydrolysates. **Food Chemistry**, v. 124, n. 2, p. 640–645, 2011.

DELGADO-PANDO, G.; COFRADES, S.; RUIZ-CAPILLAS, C.; TRIKI, M. JIMÉNEZ-COLMENERO, F. Enriched n−3 PUFA/konjac gel low-fat pork liver pâté: Lipid oxidation, microbiological properties and biogenic amine formation during chilling storage. **Meat Science**, v.92, n.4, p.762-7, 2012.

DEVATKAL, S. K.; KAMBOJ, R.; PAUL, D. Comparative antioxidant effect of BHT and water extracts of banana and sapodilla peels in raw poultry meat. **Journal of Food Science and Technology,** v. 51, p. 387–391, 2014.

DEVATKAL, S. K.; NARSAIAH, K.; BORAH, A. Anti-oxidant effect of extracts of kinnow rind , pomegranate rind and seed powders in cooked goat meat patties. **Meat Science**, v. 85, n. 1, p. 155–159, 2010.

DEVATKAL, S. K.; THORAT, P. R. Comparative antioxidant effect of aqueous extracts of curry leaves , fenugreek leaves and butylated hydroxytoluene in raw chicken patties. **Journal**

of Food Science and Technology, v. 49, p. 781–785, 2012.

DIETERICH, F.; BOSCOLO, W. R.; PACHECO, M. T. B.; SILVA, V. S. N.; GONÇALVES, G. S.; VIDOTTI, R. M. Development and Characterization of Protein Hydrolysates Originated from Animal Agro Industrial Byproducts. **Journal of Dairy, Veterinary & Animal Research**, v. 1, n. 2, p. 1–7, 2014.

DOOLAEGE, E. H. A.; VOSSEN, E.; RAES, K.; MEULENAER, B. De; VERHÉ, R.; PAELINCK, H.; SMET, S. De. Effect of rosemary extract dose on lipid oxidation , colour stability and antioxidant concentrations , in reduced nitrite liver pâtés. **Meat Science**, v. 90, n. 4, p. 925–931, 2012.

DUGAN, M. E. R.; AALHUS, J. L.; ROBERTSON, W. M.; ROLLAND, D. C.; LARSEN, I. L. Practical dietary levels of canola oil and tallow have differing effects on gilt and barrow performance and carcass composition. **Canadian Journal of Animal Science**, v. 84, n. 4, p. 661–671, 2004.

DUNNE, P. G.; ROGALSKI, J.; CHILDS, S.; MONAHAN, F. J.; KENNY, D. A.; MOLONEY, A. P. Long Chain n-3 Polyunsaturated Fatty Acid Concentration and Color and Lipid Stability of Muscle from Heifers Offered a Ruminally Protected Fish Oil Supplement. **Journal of Agriculture and Food Chemistry,** p. 5015–5025, 2011.

DUNSTAN, J. A.; MITOULAS, L. R.; DIXON, G.; DOHERTY, D. A.; HARTMANN, P. E.; SIMMER, K.; PRESCOTT, S. L. The effects of fish oil supplementation in pregnancy on breast milk fatty acid composition over the course of lactation: A randomized controlled trial. **Pediatric Research**, v. 62, n. 6, p. 689–694, 2007.

DUNSTAN, J. A.; SIMMER, K.; DIXON, G.; PRESCOTT, S. L. Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. **Archives of disease in childhood. Fetal and neonatal edition**, v. 93, n. 1, p. F45–F50, 2008.

DURAN-MONTGÉ, P.; REALINI, C. E.; BARROETA, a C.; LIZARDO, R.; ESTEVE-GARCIA, E. Tissue fatty acid composition of pigs fed different fat sources. **Animal : an international journal of animal bioscience**, v. 2, n. 12, p. 1753–62, 2008.

DURAN-MONTGÉ, P.; THEIL, P. K.; LAURIDSEN, C.; ESTEVE-GARCIA, E. Dietary fat

source affects metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. **Animal**, v. 3, n. 4, p. 535, 2009.

EBRANEK, J.G.; SEWALT, V. J. H.; ROBBINS, K. L.; HOUSER, T. A. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. **Meat Science**, v.69, p.289-296, 2005.

EFSA, P. on D. P. N. and A. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. **Efsa J**, v. 8, n. 3, p. 1461, 2010.

EGGERT, J. M.; GRANT, A. L.; SCHINCKEL, A. P. F actors Affecting Fat Distribution in Pork Carcasses. v. 23, n. 16301, p. 42–53, 2007.

ELLIS, N. R.; ISBELL, H. S. Soft pork studies. The effect of food fat upon body fat, as shown by the separation of the individual fatty acids of the body fat. **Journal of Biological and Chemistry,** v.69, p.239-248, 1926.

ENSER, M.; HALLETT, K.; HEWETT, B.; FURSEY, G. A. J.; WOOD, J. D. Fatty acid content and composition of English beef, lamb and pork at retail. **Meat Science**, v.44, p.443-458, 1996.

ENSER, M.; RICHARDSON, R. I.; WOOD, J. D.; GILL, B. P.; SHEARD, P. R. Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages. **Meat Science**, v. 55, n. 2, p. 201–212, 2000.

ESTÉVEZ, M.; VENTANAS, S.; CAVA, R. Effect of natural and synthetic antioxidants on protein oxidation and colour and texture changes in refrigerated stored porcine liver pâté. **Meat Science,** v.74, n.2, p.396-403, 2006.

FALOWO, A. B.; FAYEMI, P. O.; MUCHENJE, V. Natural antioxidants against lipid – protein oxidative deterioration in meat and meat products : A review. **FRIN**, v. 64, p. 171–181, 2014.

FAN, J.; HE, J.; ZHUANG, Y.; SUN, L. Purification and identification of antioxidant peptides from enzymatic hydrolysates of tilapia (oreochromis niloticus) frame protein. **Molecules**, v. 17, n. 11, p. 12836–12850, 2012.

FASSEAS, M. K.; MOUNTZOURIS, K. C.; TARANTILIS, P. A.; POLISSIOU, M.; ZERVAS, G. Antioxidant activity in meat treated with oregano and sage essential oils. **Food Chemistry**,

v. 106, p. 1188–1194, 2007.

FERNÁNDEZ-LÓPEZ, J.; ZHI, N.; ALESON-CARBONELL, L.; PÉREZ-ALVAREZ, J. A.; KURI, K. Antioxidant and antibacterial activities of natural extracts application in beef meatballs. **Meat Science**, v.69, p.371-380, 2005.

FOH, M. B. K.; AMADOU, I.; FOH, B. M.; KAMARA, M. T.; XIA, W. Functionality and antioxidant properties of tilapia (Oreochromis niloticus) as influenced by the degree of hydrolysis. **International Journal of Molecular Sciences**, v. 11, n. 4, p. 1851–1869, 2010.

FOLCH, J.; LEES, M.; STANLEY, G. H. S. A simple method for the isolation and purification of total lipids from animal tissues. **J Biol Chem**, v. 226, n. 1, p. 497–509, 1957.

FRANK, J. Beyond vitamin E supplementation: An alternative strategy to improve vitamin E status. **Journal of Plant Physiology**, v. 162, n. 7, p. 834–843, 2005.

FURUHJELM, C.; WARSTEDT, K.; LARSSON, J.; FREDRIKSSON, M.; BÖTTCHER, M. F.; FÄLTH-MAGNUSSON, K.; DUCHÉN, K. Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy. **Acta Paediatrica**, v.98, n.9, p.1461-1467, 2009.

GALLO, M.; FERRACANE, R.; NAVIGLIO, D. Antioxidant addition to prevent lipid and protein oxidation in chicken meat mixed with supercritical extracts of Echinacea angustifolia. **Journal of Supercritical Fluids**, v. 72, p. 198–204, 2012.

GARCÍA-ÍÑIGUEZ DE CIRIANO, M.; BERASATEGI, I.; NAVARRO-BLASCO, I.; ASTIASARÁN, I.; ANSORENA, D. Reduction of sodium and increment of calcium and ω-3 polyunsaturated fatty acids in dry fermented sausages: effects on the mineral content, lipid profile and sensory quality. **Journal of Science of Food and Agricuture,** v.93, n.4, p.876-81, 2013.

GARCÍA-IÑIGUEZ DE CIRIANO, M.; LAREQUI, E.; REHECHO, S.; CALVO, M. I.; CAVERO, R.Y.; NAVARRO-BLASCO, I.; ASTIASARÁN, I.; ANSORENA, D. Selenium, iodine, ω-3 PUFA and natural antioxidant from *Melissa officinalis L.*: A combination of components from healthier dry fermented sausages formulation. **Meat Science**, v.85, n.2, p.274-9, 2010.

GARCÍA-IÑIGUEZ DE CIRIANO, M.; REHECHO, S.; CALVO, M. I.; CAVERO R. Y.; NAVARRO, I.; ASTIASARÁN, I.; ANSORENA, D. Effect of lyophilized water extracts of *Melissa officinalis* on the stability of algae and linseed oil-in-water emulsion to be used as a functional ingredient in meat products. **Meat Science** v.85, n.2, p.373-7, 2010.

GIRGIH, A. T.; HE, R.; HASAN, F. M.; UDENIGWE, C. C.; GILL, T. A.; ALUKO, R. E. Evaluation of the in vitro antioxidant properties of a cod (Gadus morhua) protein hydrolysate and peptide fractions. **Food Chemistry**, v. 173, p. 652–659, 2015.

GLADINE, C.; MORAND, C.; ROCK, E.; GRUFFAT, D.; BAUCHART, D.; DURAND, D. The antioxidative effect of plant extracts rich in polyphenols differs between liver and muscle tissues in rats fed n-3 PUFA rich diets. **Animal Feed Science and Technology**, v. 139, n. 3–4, p. 257–272, 2007.

GUIDONI, A. L.; DALLACOSTA, O. A.; BERTOL, T. M. **Preditores e Predição do Peso, Porcentagem e Quantidade de Carne de Carcaça Suínas e Suas Partes. Relatório Técnico da Empresa Brasileira de Pesquisa Agropecuária**EMBRAPA, , 2007. .

GUILLEVIC, M.; KOUBA, M.; MOUROT, J. Effect of a linseed diet on lipid composition, lipid peroxidation and consumer evaluation of French fresh and cooked pork meats. **Meat Science**, v. 81, p. 612–618, 2009.

GUO, Q.; RICHERT, B. T.; BURGESS, J. R.; WEBEL, D. M.; ORR, D. E.; BLAIR, M.; FITZNER, G. E.; HALL, D. D.; GRANT, a. L.; GERRARD, D. E. Effects of dietary vitamin E and fat supplementation on pork quality. **Journal of Animal Science**, v. 84, n. 11, p. 3089–3099, 2006.

HAAK, L.; DE SMET, S.; FREMAUT, D.; VAN WALLEGHEM, K.; RAES, K. Fatty acid profile and oxidative stability of pork as influenced by duration and time of dietary linseed or fish oil supplementation. **Journal of Animal Science**, v. 86, n. 6, p. 1418–1425, 2008a.

HAAK, L.; RAES, K.; VAN DYCK, S.; DE SMET, S. Effect of dietary rosemary and α-tocopheryl acetate on the oxidative stability of raw and cooked pork following oxidized linseed oil administration. **Meat science**, v. 78, n. 3, p. 239–47, 2008b.

HALLDORSDOTTIR, S. M.; SVEINSDOTTIR, H.; GUDMUNDSDOTTIR, A.; THORKELSSON, G.; KRISTINSSON, H. G. High quality fish protein hydrolysates prepared

from by-product material with Fucus vesiculosus. **Journal of Functional Foods**, v. 9, p. 10–17, 2014.

HARTMAN, L.; LAGO, R. C. A. Rapid preparation of fatty acid methyl esters from lipids. **Lab. Pract.**, v. 22, p. 475–476,494, 1973.

HE, M. L.; MCALLISTER, T. A.; HERNANDEZ-CALVA, L. M.; AALHUS, J. L.; DUGAN, M. E. R.; MCKINNON, J. J. Effect of dietary inclusion of triticale dried distillers' grain and oilseeds on quality and fatty acid profile of meat from feed lot steers. **Meat Science,** v.97, p.76-82, 2014

HERNÁNDEZ-LEDESMA, B.; DÁVALOS, A.; BARTALOMÉ, B.; AMIGO, L. Preparation of antioxidant enzymatic hydrolysates from alpha-lactalbumin and beta-lactoglobulin. Identification of active peptides by HPLC-MS/MS. **Journal of agricultural and food chemistry**, v. 53, n. 3, p. 588–593, 2005.

HONIKEL, K. O. **Reference methods for the assessment of physical characteristics of meat.Meat science**, 1998.

HOSSAIN, E.; KO, S. Y.; YANG, C. J. Dietary supplementation of green tea by-products on growth performance , meat quality , blood parameters and immunity in finishing pigs. v. 6, n. 12, p. 2458–2467, 2012.

HOYLE, N. T.; MERRITT, J. H. Quality of fish protein hydrolysates from herring (Clupea harengus). **Journal of Food Science**, v. 59, n. 1, p. 76–79, 1994.

HUANG, B.; HE, J.; BAN, X.; ZENG, H.; YAO, X.; WANG, Y. Antioxidant activity of bovine and porcine meat treated with extracts from edible lotus (Nelumbo nucifera) rhizome knot and leaf. **Meat Science**, v. 87, n. 1, p. 46–53, 2011.

HUANG, D.; BOXIN, O. U.; PRIOR, R. L. The chemistry behind antioxidant capacity assays. **Journal of Agricultural and Food Chemistry**, v. 53, n. 6, p. 1841–1856, 2005.

JAKOBSEN, M. U.; REILLY, E. J. O.; HEITMANN, B. L.; PEREIRA, M. a; BA, K.; FRASER, G. E.; GOLDBOURT, U.; KNEKT, P.; LIU, S.; PIETINEN, P.; SPIEGELMAN, D.; STEVENS, J.; VIRTAMO, J.; WILLETT, W. C.; ASCHERIO, A. Major types of dietary fat and risk of coronary heart disease : a pooled analysis of 11 cohort studies 1 – 3. **The American**

journal of clinical nutrition, v. 89, p. 1425–1433, 2009.

JAMILAH, B.; MOHAMED, A.; ABBAS, K. A.; RAHMAN, R. A.; KARIM, R. A review on the effect of animal diets and presence of selected natural antioxidants on lipid oxidation of meat. v. 7, n. April, p. 5–10, 2009.

JAYATHILAKAN, K.; SHARMA, G. K.; RADHAKRISHNA, K.; BAWA, A. S. Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-over-flavour in different species of meat. **Food Chemistry**, v. 105, p. 908–916, 2007.

JERÓNIMO, E.; ALFAIA, C. M. M.; ALVES, S. P.; DENTINHO, M. T. P.; PRATES, J. A. M.; VASTA, V.; SANTOS-SILVA, J.; BESSA, R. J. B. Effect of dietary grape seed extract and Cistus ladanifer L. in combination with vegetable oil supplementation on lamb meat quality. **Meat Science**, v. 92, n. 4, p. 841–847, 2012.

JIA, N.; KONG, B.; LIU, Q.; DIAO, X.; XIA, X. Antioxidant activity of blackcurrant (*Ribes nigrum L.*) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. **Meat Science**, v.91, n.4, p.533-9, 2012.

JIMÉNEZ-COLMENERO, F. Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. **Trends of Food Science and Technology,** v.18, p.567-578, 2007

JO, C.; SON, J. H.; SON, C. B.; BYUN, M. W. Functional properties of raw and cooked pork patties with added irradiated, freeze-dried green tea leaf extract powder during storage at 4°C. **Meat Science**, v.64, p.13-17, 2003.

JUÁREZ, M.; DUGAN, M. E. R.; ALDAI, N.; AALHUS, J. L.; PATIENCE, J. F.; ZIJLSTRA, R. T.; BEAULIEU, a. D. Increasing omega-3 levels through dietary co-extruded flaxseed supplementation negatively affects pork palatability. **Food Chemistry**, v. 126, n. 4, p. 1716–1723, 2011.

JUNTACHOTE, T.; BERGHOFER, E.; SIEBENHANDL, S.; BAUER, F. The antioxidative properties of Holy basil and Galangal in cooked ground pork. **Meat Science**, v.72, n.3, p.446-56, 2006.

KANATT, S. R.; CHANDER, R.; SHARMA, A. Original article Antioxidant and antimicrobial

activity of pomegranate peel extract improves the shelf life of chicken products. **International Journal of Food Science and Technology,** v.45, p.216–222, 2010.

KLOAREG, M.; NOBLET, J.; VAN MILGEN, J. Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. **The British journal of nutrition**, v. 97, n. 1, p. 35–44, 2007.

KOUBA, M.; ENSER, M.; WHITTINGTON, F. M.; NUTE, G. R.; WOOD, J. D. Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. **Journal of Animal Science**, v. 81, n. 8, p. 1967–1979, 2003.

KOUBA, M.; MOUROT, J. A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. **Biochimie**, v. 93, n. 1, p. 13–17, 2011.

KOUBA, M.; SELLIER, P. A review of the factors influencing the development of intermuscular adipose tissue in the growing pig. **Meat Science**, v. 88, n. 2, p. 213–220, 2011.

KRIS-ETHERTON, P. M.; HARRIS, W. S.; APPEL, L. J. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. **Circulation***,* v.106, p.2747-2757, 2002.

KRIS-ETHERTON, P. M.; TAYLOR, D. S.; YU-POTH, S.; HUTH, P.; MORIARTY, K.; FISHELL, V.; HARGROVE, R. L.; ZHAO, G.; ETHERTON, T. D. Polyunsaturated fatty acids in the food chain in the United States. **The American journal of clinical nutrition**, v. 71, n. 1 Suppl, p. 179S–88S, 2000.

KRISHNAN, K. R.; BABUSKIN, S.; BABU, P. A. S.; SASIKALA, M.; SABINA, K.; ARCHANA, G.; SIVARAJAN, M.; SUKUMAR, M. Antimicrobial and antioxidant effects of spice extracts on the shelf-life extension of raw chicken meat. **International Journal of Food Microbiology,** v.171, p.32-40, 2014.

KULKARNI, S.; DESANTOS, F. A.; KATTAMURI, S.; ROSSI, S. J.; BREWER, M. S. Effect of grape seed extract on oxidative , color and sensory stability of a pre-cooked , frozen , re-heated beef sausage model system. **Meat Science**, v. 88, n. 1, p. 139–144, 2011.

LAFKA, T.-I.; SINANOGLOU, V.; LAZOS, E. S. On the extraction and antioxidant activity of phenolic compounds from winery wastes. **Food Chemistry**, v. 104, n. 3, p. 1206–1214,

2007.

LARA, M. S.; GUTIERREZ, J. I.; TIM??N, M.; ANDR??S, A. I. Evaluation of two natural extracts (Rosmarinus officinalis L. and Melissa officinalis L.) as antioxidants in cooked pork patties packed in MAP. **Meat Science**, v. 88, n. 3, p. 481–488, 2011.

LARRAÍN, R. E.; KRUEGER, C. G.; RICHARDS, M. P.; REED, J. D. Color Changes and Lipid Oxidation in Pork Products Made From Pigs Fed With Cranberry Juice Powder. v. 19, n. 2008, p. 17–33, 2006.

LAU, D. W.; KING, A. J. Pre- and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. **Journal of Agricultural and Food Chemistry**, v. 51, n. 6, p. 1602–1607, 2003.

LAURIDSEN, C.; THEIL, P. K.; JENSEN, S. K. Composition of α-tocopherol and fatty acids in porcine tissues after dietary supplementation with vitamin E and different fat sources. **Animal Feed Science and Technology**, v. 179, n. 1–4, p. 93–102, 2013.

LEE, C. H.; REED, J. D.; RICHARDS, M. P. Ability of various polyphenolic classes from cranberry to inhibit lipid oxidation in mechanically separated turkey and cooked ground pork. **Journal of Muscle Foods,** v.17, p.248-266, 2006.

LEE, E. J.; AHN, D. U. Quality characteristics of irradiated turkey breast rolls formulated with plum extract. **Meat Science** v. 71, p. 300–305, 2005.

LEE, S. M.; PARK, W. H.; KIM, K. Effects of dietary supplementation with rosemary and alfa-tocopherol acetate on performance and meat quality of chicken meat during refrigerated storage. **Korean J Food Sci Anim Resour**, v. 30, p. 472–478, 2010.

LEE, S.; HERNANDEZ, P.; DJORDJEVIC, D.; FARAJI, H.; HOLLENDER, R. FAUSTMAN, C. Effect of antioxidants and cooking on stability of n-3 fatty acids in fortified meat products. **Journal of Food Science,** v.71, n.3, p.C233-C238, 2006.

LEE, S.-J.; KIM, E.-K.; HWANG, J.-W.; OH, H.-J.; CHEONG, S.-H.; MOON, S.-H.; JEON, B.-T.; LEE, S. M.; PARK, P.-J. Purification and characterisation of an antioxidative peptide from enzymatic hydrolysates of duck processing by-products. **Food Chemistry**, v. 123, n. 2, p. 216–220, nov. 2010.

LI, Z.-Y.; YOURAVONG, W.; H-KITTIKUN, A. Protein hydrolysis by protease isolated from tuna spleen by membrane filtration: A comparative study with commercial proteases. **LWT - Food Science and Technology**, v. 43, n. 1, p. 166–172, jan. 2010.

LIASET, B.; MADSEN, L.; HAO, Q.; CRIALES, G.; MELLGREN, G.; MARSCHALL, H. U.; HALLENBORG, P.; ESPE, M.; FRØYLAND, L.; KRISTIANSEN, K. Fish protein hydrolysate elevates plasma bile acids and reduces visceral adipose tissue mass in rats. **Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids**, v. 1791, n. 4, p. 254–262, 2009.

LITTLE, A. C. Off on a tangent. **Journal of Food Science**, v. 40, n. 2, p. 410–411, 1975.

LIU, Q.; KONG, B.; XIONG, Y. L.; XIA, X. Antioxidant activity and functional properties of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis. **Food Chemistry**, v. 118, n. 2, p. 403–410, 2010.

LÓPEZ-FERRER, S.; BAUCELLS, M. D.; BARROETA, A. C.; GRASHORN, M. A. N-3 enrichment of chicken meat. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil. **Poult Science**, v.80, p.741-752, 2001.

LORENZO, J. M.; GONZÁLEZ-RODRÍGUEZ, R. M.; SÁNCHEZ, M.; AMADO, I. R.; FRANCO, D. Effects of natural (grape seed and chestnut extract) and synthetic antioxidants (buthylatedhydroxytoluene , BHT) on the physical , chemical , microbiological and sensory characteristics of dry cured sausage “ chorizo ”. **Food Research International**, v. 54, n. 1, p. 611–620, 2013.

LORENZO, J. M.; SINEIRO, J.; AMADO, I. R.; FRANCO, D. Influence of natural extracts on the shelf life of modi fi ed atmosphere-packaged pork patties. **Meat Science,** v. 96, p. 526–534, 2014.

LUCIANO, G.; PAUSELLI, M.; SERVILI, M.; MOURVAKI, E.; SERRA, A.; MONAHAN, F. J.; LANZA, M.; PRIOLO, A.; ZINNAI, A.; MELE, M. Dietary olive cake reduces the oxidation of lipids , including cholesterol , in lamb meat enriched in polyunsaturated fatty acids. **Meat Science,** v. 93, n. 3, p. 703–714, 2013.

MAGALHÃES, L. M.; SEGUNDO, M. A.; REIS, S.; LIMA, J. L. F. C. Methodological aspects about in vitro evaluation of antioxidant properties. **Analytica Chimica Acta**, v. 613, n. 1, p. 1–

19, 2008.

MAIRESSE, G. . d; BENET, M. .; MÉTEAU, K. .; JUIN, H. .; DURAND, D. .; MOUROT, J. . Effect of plant antioxidant in n-3 polyunsaturated fatty acid-enriched diet on fatty acid composition and sensorial attributes of dry-cured ham. **International Journal of Food Science and Technology**, v. 46, n. 12, p. 2656–2662, 2011.

MANCINI, R. a.; HUNT, M. C. Current research in meat color. **Meat Science**, v. 71, n. 1, p. 100–121, 2005.

MARCHIOLI, R.; BARZI, F.; BOMBA, E.; CHIEFFO, C.; DI GREGORIO, D.; DI MASCIO, R.; FRANZOSI, M. G.; GERACI, E.; LEVANTESI, G.; MAGGIONI, A. P.; MANTINI, L.; MARFISI, R.; MASTROGIUSEPPE, G.; MININNI, N.; NICOLOSI, G. l; SANTINI, M.; SCHWEIGER, C.; TAVAZZI, L.; TOGNONI, G.; TUCCI, C.; VALAGUSSA, F. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of The Gruppo Italiano Per Lo Studio Della Sopravvivenza Nell'infarto Miocardico (Gissi)-PrevenzioneNo Title. **Circulation**, v. 105, n. 16, p. 1897–1903, 2002.

MARCINČÁKOVÁ, D.; ČERTÍK, M.; MARCINČÁK, S.; POPELKA, P.; ŠIMKOVÁ, J.; KLEMPOVÁ, T.; PETROVIČ, V.; TUČKOVÁ, M.; BAČA, M. Effect of dietary supplementation of Melissa officinalis and combination of Achillea millefolium and Crataegus oxyacantha on broiler growth performance, fatty acid composition and lipid oxidation of chicken meat. **Italian Journal of Animal Science**, v. 10, n. 4, p. 165–170, 2011.

MARIUTTI, L. R.; NOGUEIRA, G. C.; BRAGAGNOLO, N. Lipid and Cholesterol Oxidation in Chicken Meat are Inhibited by Sage but not by garlic. **Journal of Food Science,** v.76, n.6, p.C909-15, 2011

MARTÍNEZ, B.; MIRANDA, J. M.; VÁZQUEZ, B. I.; FENTE, C. A.; FRANCO, C. M.; RODRÍGUEZ, J. L.; CEPEDA, A. Development of a Hamburger Patty with Healthier Lipid Formulation and Study of its Nutritional , Sensory , and Stability Properties. **Food Bioprocess Technology,** p.200–208, 2012.

MAS, G.; LLAVALL, M.; COLL, D.; ROCA, R.; DIAZ, I.; GISPERT, M.; OLIVER, M. A.; REALINI, C. E. Carcass and meat quality characteristics and fatty acid composition of tissues

from pietrain-crossed barrows and gilts fed an elevated monounsaturated fat diet. **Meat Science**, v.85, n.4, p.707-14, 2010.

MATTHEWS, K. R.; HOMER, D. B.; THIES, F.; CALDER, P. C. Effect of whole linseed (Linum usitatissimum) in the diet of finishing pigs on growth performance and on the quality and fatty acid composition of various tissues. **The British journal of nutrition**, v. 83, n. 6, p. 637–43, 2000.

MCCARTHY, T. L.; KERRY, J. P.; KERRY, J. F.; LYNCH, P. B.; BUCKLEY, D. J. Evaluation of the antioxidant potential of natural food / plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. **Meat Science,** v. 57, p. 45–52, 2001.

MCKIBBEN, J.; ENGESETH, N. J. Honey as a Protective Agent against Lipid Oxidation in Ground Turkey. **Journal of Agriculture of Food Chemistry,** v. 50, n.3, p.592-5, 2002).

MEADUS, W. J.; TURNER, T. D.; DUGAN, M. E.; AALHUS, J. L.; DUFF, P.; ROLLAND, D.; UTTARO, B.; GIBSON, L. L. Fortification of pork loins with docosahexaenoic acid (DHA) and its effect on flavour. **Journal of Animal Science and Biotechnology**, v. 4, n. 1, p. 46, 2013.

MELO, E. A.; MANCINI-FILHO, J.; GUERRA, N. B.; MACIEL, G. R. Atividade antioxidante de extratos de coentro (*Coriandrum sativum L.*). **Ciência e Tecnologia de Alimentos,** v.23, p.195-199, 2003.

MIELNIK, M. B.; AABY, K.; SKREDE, G. Commercial antioxidants control lipid oxidation in mechanically deboned turkey meat. **Meat Science**, v.65, p.1147-1155, 2003.

MIELNIK, M. B.; SEM, S.; EGELANDSDAL, B.; SKREDE, G. By-products from herbs essential oil production as ingredient in marinade for turkey thighs. **LWT–Food Science and Technology,** v. 41, p. 93–100, 2008.

MILLER, M. F.; DAVID, G.W.; SEIDEMANN, S. C.; RAMSEY, C. B.; ROLAN, T. L. Effects of various phosphates on the palatability, appearance, and storage traits of flaked and formed restructured beef steaks. **Journal of Food Science,** v.51, p.1435-1438, 1986

MISSOTTEN, J.; DE SMET, S.; RAES, K.; DORAN, O. Effect of supplementation of the

maternal diet with fish oil or linseed oil on fatty-acid composition and expression of Δ5- and Δ6-desaturase in tissues of female piglets. **Animal**, v. 3, n. 8, p. 1196–204, 2009.

MITCHAOTHAI, J.; YUANGKLANG, C.; WITTAYAKUN, S.; VASUPEN, K.; WONGSUTTHAVAS, S.; SRENANUL, P.; HOVENIER, R.; EVERTS, H.; BEYNEN, a. C. Effect of dietary fat type on meat quality and fatty acid composition of various tissues in growing-finishing swine. **Meat Science**, v. 76, n. 1, p. 95–101, 2007.

MITSUMOTO, M.; O'GRADY, M. N.; KERRY, J. P.; BUCKLEY, D. J. Addition of tea catechins and vitamin C on sensory evaluation, colour and lipid stability during chilled storage in cooked or raw beef and chicken patties. **Meat Science**, v. 69, p. 773−779, 2005.

MOHAMED, H. M. H.; MANSOUR, H. A.; FARAG, M. D. E. H. The use of natural herbal extracts for improving the lipid stability and sensory characteristics of irradiated ground beef. **Meat Science**, v. 87, n. 1, p. 33–39, 2011.

MOLONEY, A. P.; KENNEDY, C.; NOCI, F.; MONAHAN, F. J.; KERRY, J. P. Lipid and colour stability of M . longissimus muscle from lambs fed camelina or linseed as oil or seeds. **Meat Science**, v. 92, n. 1, p. 1–7, 2012.

MOREIRA, E. A. M.; SHAMI, N. J. I. E. Licopeno como agente antioxidante. **Revista de Nutrição,** v.17, n.2, p.227-236, 2004

MOREIRA, N. X.; CURI, R.; MANCINI-FLHO, J. Fatty acids: review. **Nutrire**, v. 24, p. 105–123, 2002.

MORRISSEY, P. A.; TICHIVANGANA, J. Z. The antioxidant activities of nitrite and nitrosyl-myoglobin in cooked meats. **Meat Science**, v.14, p.175-90, 1985.

MOUSSAOUI, K. A.; VARELA, P. Exploring consumer product proï¬ling techniques and their linkage to a quantitative descriptive analysis. **Food Quality and Preference**, v. 21, n. 8, p. 1088–10 99, 2010.

MUGUERZA, E.; ANSORENA, D.; ASTIASARAN, I. Functional dry fermented sausages manufactured with high levels of n-3 fatty acids: nutritional benefits and evaluation of oxidation. **Journal of Science of Food Agriculture,** v.84, n.9, p.1061-1068, 2004.

MUSELLA, M.; CANNATA, S.; ROSSI, R.; MOUROT, J.; BALDINI, P.; CORINO, C.

Omega-3 polyunsaturated fatty acid from extruded linseed influences the fatty acid composition and sensory characteristics of dry-cured ham from heavy pigs. **Journal of animal science**, v. 87, n. 11, p. 3578–88, 2009.

NAVEENA, B. M.; SEN, A. R.; KINGSLY, R. P.; SINGH, D. B.; KONDAIAH, N. Antioxidant activity of pomegranate rind powder extract in cooked chicken patties. **International Journal of Food Science and Technology,** p. 1807–1812, 2008a.

NAVEENA, B. M.; SEN, A. R.; VAITHIYANATHAN, S.; BABJI, Y.; KONDAIAH, N. Comparative efficacy of pomegranate juice , pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. **Meat Science,** v. 80, p. 1304–1308, 2008b.

NAVEENA, S.; NAVEENA, B. M.; MUTHUKUMAR, M.; GIRISH, P. S.; KONDAIAH, N. Effect of dipping in promegranate (*Punica granatum*) fruit juice phenolic solution on the shelf-life of chicken meat under refrigerated storage (4°C). **Meat Science**, v.88, p.409-414, 2011.

NIELSEN, P. m.; PETERSEN, D.; DAMBMANN, C. Improved Method for Determining Food Protein Degree of Hydrolysis. **Journal of Food Science**, v. 66, n. 5, p. 642–646, 2001.

NIETO, G.; ESTRADA, M.; JORDÁN, M. J.; GARRIDO, M. D.; BAÑÓN, S. Effects in ewe diet of rosemary by-product on lipid oxidation and the eating quality of cooked lamb under retail display conditions. **Food Chemistry**, v. 124, p. 1423–1429, 2011.

NILZEN, V.; BABOL, J.; DUTTA, P. C.; LUNDEHEIM, N.; ENFALT, A. C.; LUNDSTROM, K. Free range rearing of pigs with access to pasture grazing- effect on fatty composition and lipid oxidation products. **Meat Science,** v.58, p.267-275, 2001.

NISSEN, L. R.; BYRNE, D. V.; BERTELSEN, G.; SKIBSTED, L. H. The antioxidative activity of plant extracts in cooked pork patties as evaluated by descriptive sensory profiling and chemical analysis. **Meat Science,** v.68, p.485-495, 2004.

NØRGAARD, J. V.; BLAABJERG, K.; POULSEN, H. D. Salmon protein hydrolysate as a protein source in feed for young pigs. **Animal Feed Science and Technology**, v. 177, n. 1–2, p. 124–129, 2012.

NPPC. **Pork quality standards**. Des Moines, IA: [s.n.].

NUÑEZ DE GONZALEZ, M. T. N. De; HAFLEY, B. S.; BOLEMAN, R. M.; MILLER, R.

K.; RHEE, K. S.; KEETON, J. T. Antioxidant properties of plum concentrates and powder in precooked roast beef to reduce lipid oxidation. **Journal of Food Science,** v. 80, p. 997–1004, 2008.

NUÑEZ DE GONZALEZ, M. T. N. De; HAFLEY, B. S.; BOLEMAN, R. M.; MILLER, R. M.; RHEE, K. S.; KEETON, J. T. Qualitative effects of fresh and dried plum ingredients on vacuum-packaged , sliced hams. **Meat Science**, v. 83, n. 1, p. 74–81, 2009.

NUÑEZ DE GONZALEZ, M. T.; BOLEMAN, R. M.; MILLER, R. K.; KEETON, J. T. RHEE, K. S. Antioxidant Properties of Dried Plum Ingredients in Raw and Precooked Pork Sausage. **Journal of Food Science,** v.73, n.5, p.H63-71, 2008.

O`GRADY, M. N.; MAHER, M.; TROY, D. J.; MOLONEY, a. P.; KERRY, J. P. An assessment of dietary supplementation with tea catechins and rosemary extract on the quality of fresh beef. **Meat Science**, v. 73, p. 132–143, 2006.

O'GRADY, M. N.; MONAHAN, F. J.; BURKE, R. M.; ALLEN, P. The effect of oxygen level and exogenous *α-tocopherol* on the oxidative stability of minced beef in modified atmosphere packs. **Meat Science,** v.55, p.39-45, 2000

O'GRADY, M. N.; CARPENTER, R.; LYNCH, P. B.; O'BRIEN, N. M.; KERRY, J. P. Addition of grape seed extract and bearberry to porcine diets: Influence on quality attributes of raw and cooked pork. **Meat science**, v. 78, n. 4, p. 438–46, 2008.

OLORUNSANYA, O. A.; OLORUNSANYA, E. O.; ALIU, O. T.; KAYODE, R. M. O. Effects of different species of Pepper (*Capsicum*) on oxidative stability of raw and cooked pork patties. **Pakistan Journal of Nutrition,** v.8, n.10, p.1588-91, 2009.

OSAWA, C. C.; FELÍCIO, P. E.; GONÇALVES, L. A. G. Teste de TBA aplicado a carnes e derivados: métodos tradicionais, modificados e alternativos. **Química Nova** v.28, n.4, p.655-663, 2005

ÖZVURAL, E. B.; VURAL, H. Grape seed fl our is a viable ingredient to improve the nutritional pro fi le and reduce lipid oxidation of frankfurters. **Meat Science,** v. 88, n. 1, p. 179–183, 2011.

PAJK, T.; PH, D.; REZAR, V.; PH, D.; LEVART, A.; PH, D.; SALOBIR, J.; PH, D. Efficiency

of apples , strawberries , and tomatoes for reduction of oxidative stress in pigs as a model for humans. v. 22, p. 376–384, 2006.

PANERAS, E. D.; BLOUKAS, J. G. Vegetable oils replace pork backfat for low-fat frankfurters. **Journal of Food Science,** v.59, n.4, p.725-728, 1994.

PARK, S. Y.; AHN, C.; JE, J. Antioxidant and Anti-Inflammatory Activities of Protein Hydrolysates from Mytilus Edulis and Ultrafiltration Membrane Fractions. **Journal of Food Biochemistry**, v. 38, n. 5, p. 460–468, 2014.

PARK, S. Y.; CHIN, K. B. Evaluation of pre-heating and extraction solvents in antioxidant and antimicrobial activities of garlic, and their application in fresh pork patties. **International Journal of Food Science,** v.45, p. 365-373, 2010

PATEIRO, M.; LORENZO, J. M.; AMADO, I. R.; FRANCO, D. Effect of addition of green tea , chestnut and grape extract on the shelf-life of pig liver pâté. **Food Chemistry**, v. 147, p. 386–394, 2014.

PELSER, W. M.; LINSSEN, J. P. H.; LEGGER, A.; HOUBEN, J. H. Lipid oxidation in n-3 fatty acid enriched Dutch style fermented sausages. **Meat Science,** v.75, n.1, p.1-11, 2007.

PEÑA-RAMOS, E. A.; XIONG, Y. L. Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. **Meat Science**, v. 64, n. 3, p. 259–263, 2003.

PIETRASIK, Z.; WANG, H.; JANZ, J. A. M. Effect of canola oil emulsion injection on processing characteristics and consumer acceptability of three muscles from mature beef. **Meat Science**, v. 93, n. 2, p. 322–328, 2013.

PINHEIRO-SANT'ANA, H. M.; GUINAZI, M.; OLIVEIRA, D. D. S.; DELLA LUCIA, C. M.; REIS, B. D. L.; BRANDÃO, S. C. C. Method for simultaneous analysis of eight vitamin E isomers in various foods by high performance liquid chromatography and fluorescence detection. **Journal of Chromatography A**, v. 1218, n. 47, p. 8496–8502, 2011.

POUDEL-TANDUKAR, K.; NANRI, A.; MATSUSHITA, Y.; SASAKI, S.; OHTA, M.; SATO, M.; MIZOUE, T. Dietary intakes of alpha-linolenic and linoleic acids are inversely associated with serum C-reactive protein levels among Japanese men. **Nutrition research (New York, N.Y.)**, v. 29, n. 6, p. 363–70, 2009.

QI, S.; HUANG, H.; HUANG, J.; WANG, Q.; WEI, Q. Lychee (Litchi chinensis Sonn .) seed water extract as potential antioxidant and anti-obese natural additive in meat products. **Food Control**, v. 50, p. 195–201, 2015.

RACANICCI, A. M. C.; DANIELSEN, B.; SKIBSTED, L. H. Mate (*Ilex paraguariensis*) as a source of water extractable antioxidant for use in chicken meat. European Food Research ***and*** Technology, v.227, p.255-260, 2008

RACANICCI, A. M. C.; HELENE, B.; SKIBSTED, L. H. Sensory evaluation of precooked chicken meat with mate (Ilex paraguariensis) added as antioxidant. European Food Research ***and*** Technology, v.229, p. 277–280, 2009.

RACANICCI, A. M. C.; MENTEN, J. F. M.; ALENCAR, S. M.; BUISSA, R. S.; SKIBSTED, L. H. Mate (Ilex paraguariensis) as dietary additive for broilers : performance and oxidative stability of meat. European Food Research ***and*** Technology, n. 232, p. 655–661, 2011.

RAGHAVAN, S.; KRISTINSSON, H. G.; LEEUWENBURGH, C. Radical scavenging and reducing ability of tilapia (Oreochromis niloticus) protein hydrolysates. **Journal of Agricultural and Food Chemistry**, v. 56, n. 21, p. 10359–10367, 2008.

RAGHAVAN, S.; RICHARDS, M. P. Comparison of solvent and microwave extracts of cranberry press cake on the inhibition of lipid oxidation in mechanically separated turkey. **Food Chemistry,** v.102, p.818-826, 2007.

RAUW, W. m.; DIAZ, I.; FRANCÉS, F.; CORELLA, D.; SOLER, J.; TIBAU, J.; GOMEZ-RAYA, L. The relationship between feed intake behaviour with intramuscular fat, cholesterol and fatty acid composition in pork. **Journal of Animal Breeding and Genetics**, v. 129, n. 4, p. 289–297, 2012.

RE, R.; PELLEGRINI, N.; PROTEGGENTE, A.; PANNALA, A.; YANG, M.; RICE-EVANS, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radical Biology and Medicine**, v. 26, n. 9–10, p. 1231–1237, 1999.

REALINI, C. E.; DURAN-MONTGÉ, P.; LIZARDO, R.; GISPERT, M.; OLIVER, M. a.; ESTEVE-GARCIA, E. Effect of source of dietary fat on pig performance, carcass characteristics and carcass fat content, distribution and fatty acid composition. **Meat Science**, v. 85, n. 4, p. 606–612, 2010.

RIVAS-CAÑEDO, A.; APELEO, E.; MUIÑO, I.; PÉREZ, C.; LAUZURICA, S.; PÉREZ-SANTA ESCOLÁSTICA, C.; DÍAZ, M. T.; CAÑEQUE, V.; FUENTE, J. Effect of dietary supplementation with either red wine extract or vitamin E on the volatile profile of lamb meat fed with omega-3 sources. **Meat Science,** v.93, p.178–186, 2013

ROJAS, M. C.; BREWER, M. S. Effect of natural antioxidants on oxidative stability of cooked, refrigerated beef and pork. **Journal of Food Science,** v.72, p.S282–S288, 2007.

ROSENVOLD, K.; ANDERSEN, H. J. Factors of significance, for pork quality - a review [Review]. **Meat Science**, v. 64, n. 3, p. 219–237, 2003.

ROSLAN, J.; FAEZAH, K.; ABDULLAH, N.; MAZLINA, S. Characterization of Fish Protein Hydrolysate from Tilapia (Oreochromis niloticus) by-Product. **Agriculture and Agricultural Science Procedia**, v. 2, p. 312–319, 2014.

ROSTAGNO, H. S.; ALLBINO, L. F. T.; DONZELE, J. L.; GOMES, P. C.; OLIVEIRA, R. F. de; LOPES, D. C.; FERREIRA, A. S.; BARRETO, S. L. de T. Tabelas brasileiras para aves e suínos. p. 189, 2005.

SALEM, F. M. A.; IBRAHIM, H. M. Dry fermented buffalo sausage with sage oil extract: Safety and quality. **Grasas y aceites,** v.61, n.1, p.76-85, 2010.

SALES, J.; KOUKOLOVÁ, V. Dietary vitamin E and lipid and color stability of beef and pork: Modeling of relationships. **Journal of Animal Science**, v. 89, n. 9, p. 2836–48, 2011.

SALLAM, K. I.; ISHLOROSHI, M.; SAMEJIMA, K. Antioxidant and antimicrobial effects of garlic in chicken sausage. **LWT-Food Science and Technology,** v.37, p.849–855, 2004.

SAMARANAYAKA, a. G. P.; KITTS, D. D.; LI-CHAN, E. C. Y. Antioxidative and angiotensin-I-converting enzyme inhibitory potential of a pacific hake (merlucclus productos) fish protein hydrolysate subjected to simulated gastrointestinal digestion and caco-2 cell permeation. **Journal of Agricultural and Food Chemistry**, v. 58, n. 3, p. 1535–1542, 2010.

SAMARANAYAKA, A. g p; LI-CHAN, E. C. Y. Food-derived peptidic antioxidants: A review of their production, assessment, and potential applications. **Journal of Functional Foods**, v. 3, n. 4, p. 229–254, 2011.

SAMPAIO, G. R.; SALDANHA, T.; SOARES, R. A. M.; TORRES, E. A. F. S. Effect of natural

antioxidant combinations on lipid oxidation in cooked chicken meat during refrigerated storage. **Food Chemistry**, v. 135, n. 3, p. 1383–1390, 2012.

SANTOS, C.; HOZ, L.; CAMBERO, M. I.; CABEZA, M. C.; ORDÓÑEZ, J. a. Enrichment of dry-cured ham with α-linolenic acid and α-tocopherol by the use of linseed oil and α-tocopheryl acetate in pig diets. **Meat Science**, v. 80, p. 668–674, 2008.

SAS. **Statistical Analysis Sytem**, 2012.

SASSE, A.; COLINDRES, P.; BREWER, M. S. Effect of Natural and Synthetic Antioxidants on the Oxidative Stability of Cooked, Frozen Pork Patties. **Journal of Food Science**, v. 74, n. 1, p. S30–S35, 2009.

SÁYAGO-AYERDI, S. G.; BRENES, A.; VIVEROS, A.; GOÑI, I. Antioxidative effect of dietary grape pomace concentrate on lipid oxidation of chilled and long-term frozen stored chicken patties. **Meat science**, v. 83, n. 3, p. 528–33, 2009.

SCHAEFER, E. J. Lipoproteins, nutrition, and heart disease. **American Journal of Clinical Nutrition**, v. 75, n. 2, p. 191–212, 2002.

SCRAMLIN, S. M.; NEWMAN, M. C.; COX, R. B.; SEPE, H. A.; ALDERTON, A. L.; O'LEARY, J.; MIKEL. W. B. Effects of oregano oil brine enhancement on quality attributes of beef *Longissimus dorsi* and *Semimembranosus* muscles from various age animals. **Journal of Food Science,** v.75, n.2, p. S89-94, 2010.

SELANI, M. M.; CONTRERAS-CASTILLO, C. J.; SHIRAHIGUE, L. D.; GALLO, C. R.; PLATA-OVIEDO, M.; MONTES-VILLANUEVA, N. D. Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage. **Meat Science**, v.88, n.3, p.397-403, 2011

SEO, T.; BLANER, W. S.; DECKELBAUM, R. J. Omega-3 fatty acids: molecular approaches to optimal biological outcomes. **Current opinion in lipidology**, v. 16, n. 1, p. 11–8, 2005.

SERRANO, R.; ORTUÑO, J.; BAÑÓN, S. Improving the sensory and oxidative stability of cooked and chill-stored lamb using dietary rosemary diterpenes. **Journal of food science**, v. 79, n. 9, 2014.

SHAH, M. A.; JOHN, S.; BOSCO, D.; MIR, S. A. Plant extracts as natural antioxidants in meat

and meat products. **MESC**, v. 98, n. 1, p. 21–33, 2014.

SHAN, B.; CAI, Y.; BROOKS, D.; CORKE, H. Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. **Journal of Science and Food Agriculture,** p. 1879–1885, 2009.

SILVA, F. G. D.; O'CALLAGAHAN, Y.; O'BRIEN, N. M.; NETTO, F. M. Antioxidant Capacity of Flaxseed Products: The Effect of In vitro Digestion. **Plant Foods for Human Nutrition**, v. 68, n. 1, p. 24–30, 2013.

SILVA, J. F. X.; RIBEIRO, K.; SILVA, J. F.; CAHÚ, T. B.; BEZERRA, R. S. Utilization of tilapia processing waste for the production of fish protein hydrolysate. **Animal Feed Science and Technology**, v. 196, p. 96–106, 2014.

SIMITZIS, P. E.; SYMEON, G. K.; CHARISMIADOU, M. A.; BIZELIS, J. A.; DELIGEORGIS, S. G. The effects of dietary oregano oil supplementation on pigmeat characteristics. **Meat Science**, v. 84, p. 670–676, 2010.

SIMOPOULOS, A. P. Omega-3 fatty acids in wild plants , nuts and seeds. **Asia Pacific Journal of Clinical Nutrition,** v. 11, p. S163–S173, 2002.

SIMOPOULOS, A. P. Omega-6/Omega-3 essential fatty acid ratio and chronic diseases. **Food Reviews Interernational,** v.20, n.1, p.77-90, 2004.

SIMOPOULOS, A. P.; LEAF, A.; SALEM, N. Essentiality of and Recommended Dietary Intakes for Omega-6 and Omega-3 Fatty Acids. **Annals of nutrition & metabolism**, v. 43, n. 3, p. 127–133, 1999.

SUBLETTE, M. E.; HIBBELN, J. R.; GALFALVY, H.; OQUENDO, M. a; MANN, J. J. Omega-3 polyunsaturated essential fatty acid status as a predictor of future suicide risk. **The American journal of psychiatry**, v. 163, n. 6, p. 1100–1102, 2006.

SULLIVAN, C. M.; LYNCH, A. M.; LYNCH, P. B.; BUCKLEY, D. J.; KERRY, J. P. Assessment of the antioxidant potential of food ingredients in fresh, previously frozen and cooked chicken patties. **International Journal of Poutry Science**, v. 3, p. 337–344, 2004.

TACO. Tabela brasileira de composição de alimentos. **NEPA - Unicamp**, p. 161, 2011.

TANG, S. Z.; KERRY, J. P.; SHEEHAN, D.; BUCKLEY, D. J.; MORRISEY, P. A. Antioxidative effect of added tea catechins on susceptibility of cooked red meat, poultry and fish patties to lipid oxidation. **Food Research International,** v.34, p.651-657, 2001

TANG, S. Z.; KERRY, J. P.; SHEEHAN, D.; BUCKLEY, D. J.; MORRISSEY, P. A. Dietary tea catechins and iron-induced lipid oxidation in chicken meat , liver and heart. **Meat Science,** v.56, p. 285-90, 2000.

TARLADGIS, B.; WATTS, B. M.; YONATHAN, M. Distillation method for determination of malonaldehyde in rancidity food. **Journal of American Oil Chemists Society** 37:44-48 (1960).

TEJERINA, D.; GARCÍA-TORRES, S.; VACA, M. C. De; VÁZQUEZ, F. M.; CAVA, R. Effect of production system on physical – chemical , antioxidant and fatty acids composition of Longissimus dorsi and Serratus ventralis muscles from Iberian pig. **Food Chemistry**, v. 133, n. 2, p. 293–299, 2012.

TERUEL, M. R.; GARRIDO, M. D.; ESPINOSA, M. C.; LINARES, M. B. Effect of different format-solvent rosemary extracts (Rosmarinus officinalis) on frozen chicken nuggets quality. **Food Chemistry**, v. 172, n. 5957, p. 40–46, 2015.

TEYE, G. a.; SHEARD, P. R.; WHITTINGTON, F. M.; NUTE, G. R.; STEWART, a.; WOOD, J. D. Influence of dietary oils and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. **Meat Science**, v. 73, n. 1, p. 157–165, 2006.

TIMS, M. J.; WATTS, B. M. Protection of cooked meats with phosphates. ***Food Technology,*** v.12, p.240-243, 1958

TRINDADE, R. A.; MANCINI-FILHO, J.; VILLAVICENCIO, A. L. C. H. LWT Natural antioxidants protecting irradiated beef burgers from lipid oxidation. **LWT - Food Science and Technology**, v. 43, n. 1, p. 98–104, 2010.

TRINDADE, R.A.; MANCINI-FILHO, J.; VILLAVICENCIO, A. L. C. H. Effects of natural antioxidants on the lipid profile of electron beamirradiated beef burgers. **European Journal of Lipid Science Technology,** v.111n, 11, p.1161-1168, 2009.

TRUMBO, P.; SCHLICKER, S.; YATES, A. a.; POOS, M. **Dietary reference intakes for**

energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. [s.l: s.n.]v. 102

TSOPMO, a.; COOPER, a.; JODAYREE, S. Enzymatic hydrolysis of oat flour protein isolates to enhance antioxidative properties. **Advance Journal of Food Science and Technology**, v. 2, n. 4, p. 206–212, 2010.

TURNER, T. D.; MAPIYE, C.; AALHUS, J. L.; BEAULIEU, A. D.; PATIENCE, J. F.; ZIJLSTRA, R. T.; DUGAN, M. E. R. Flaxseed fed pork: n-3 fatty acid enrichment and contribution to dietary recommendations. **Meat science**, v. 96, n. 1, p. 541–7, 2014.

UGWUONA, F. U. Effect of piper extracts on pork stability. **Journal of Food Process Preservation,** v. 34, n. 2010, p. 235–246, 2009.

VALENCIA, I.; ANSORENA, D.; ASTIASARAN, I. Nutritional and sensory properties of dry fermented sausages enriched with n-3 PUFAs. **Meat Science,** v.72, n.4, p.727-733, 2006.

VAZ, J. dos S.; DEBONI, F.; AZEVEDO, M. J. de; GROSS, J. L.; ZELMANOVITZ, T. Ácidos graxos como marcadores biológicos da ingestão de gorduras. **Revista de Nutrição**, v. 19, n. 4, p. 489–500, ago. 2006.

VEDIN, I.; CEDERHOLM, T.; FREUND LEVI, Y.; BASUN, H.; GARLIND, A.; FAXÉN IRVING, G.; JÖNHAGEN, M. E.; VESSBY, B.; WAHLUND, L.-O.; PALMBLAD, J. Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study. **The American journal of clinical nutrition**, v. 87, n. 6, p. 1616–22, 2008.

VILJANEN, K.; KIVIKARI, R.; HEINONEN, M. Protein-lipd interations during liposome oxidation with added antocyanin and other phenolic compounds. **Journal of Agriculture Food Chemistry,** v.52, p.1104-11, 2004

VINSON, J.A.; SU, X. H. ; ZUBIK, L.; BOSE, P. Phenol antioxidant quantity and quality in foods: Fruits. **Journal of Agriculture Food Chemistry,** v.49, n.11, p.5315–5321, 2001.

VIUDA-MARTOS, M.; RUIZ-NAVAJAS, Y.; FERNÁNDEZ-LÓPEZ, J.; PÉREZ-ALVAREZ, J. A. Effect of adding citrus fibre washing water and rosemary essential oil on the

quality characteristics of a bologna sausage. **LWT- Food Science and Technology,** v.43, p.958-963, 2010

VOSSEN, E.; UTRERA, M.; DE SMET, S.; MORCUENDE, D.; ESTÉVEZ, M. Dog rose (Rosa canina L.) as a functional ingredient in porcine frankfurters without added sodium ascorbate and sodium nitrite. **Meat Science**, v. 92, n. 4, p. 451–457, 2012.

VYNCKE, W. Direct Determination of the Thiobarbituric Acid Value in Trichloracetic Acid Extracts of Fish as a Measure of Oxidative Rancidity. **Fette Seifen Anstrichmittel**, v. 12, n. 12, p. 1084–1087, 1970.

WACHIRA, A. M.; SINCLAIR, L. A.; WILKINSON, R. G.; ENSER, M.; WOOD, J. D.; FISHER, A.V. Effects of dietary fat source and breed on the carcass composition, n-3 poly-unsaturated fatty acid and conjugated linoleic acid content of sheep meat and adipose tissue. **The British journal of nutrition,** v.88, p.697-709, 2002.

WANG, H.; WANG, L. S.; SHI, B. M.; SHAN, A. S. Effects of dietary corn dried distillers grains with solubles and vitamin E on growth performance, meat quality, fatty acid profiles, and pork shelf life of finishing pigs. **Livestock Science**, v. 149, n. 1–2, p. 155–166, 2012.

WARD, N. C.; CROFT, K. D.; PUDDEY, I. B.; HODGSON, J. M. Supplementation with grape seed polyphenols results in increased urinary excretion of 3-hydroxyphenylpropionic Acid, an important metabolite of proanthocyanidins in humans. **Journal of agricultural and food chemistry**, v. 52, n. 17, p. 5545–9, 2004.

WARNANTS, N.; VAN OECKEL, M. J.; BOUCQUÉ, C. V. Effect of incorporation of dietary polyunsaturated fatt acids in pork backfat on the quality of salami. **Meat Science**, v.49, n.4, p.435-45, 1998.

WIĘCEK, J.; REKIEL, A.; BATORSKA, M.; SKOMIAŁ, J. Effect of restricted feeding and realimentation periods on pork quality and fatty acid profile of M. longissimus thoracis. **Meat science**, 2011.

WIRIYAPHAN, C.; XIAO, H.; DECKER, E. A.; YONGSAWATDIGUL, J. Chemical and cellular antioxidative properties of threadfin bream (Nemipterus spp.) surimi byproduct hydrolysates fractionated by ultrafiltration. **Food chemistry**, v. 167, p. 7–15, 2015.

WONG, P. Y. Y.; KITTS, D. D. The effects of herbal pre-seasoning on microbial and oxidative changes in irradiated beef steaks. **Food Chemistry**, v.76, p.197-205, 2002.

WORLD HEALTH ORGANIZATION. Joint Consultation: fats and oils in human nutrition. **Nutr Rev,** v.53, n.7, p.202-205, 1995.

WU, Q. K.; KOPONEN, J. M.; MYKKÄNEN, H. M.; TÖRRÖNEN, A. R. Berry phenolic extracts modulate the expression of p21 WAF1 and Bax but not Bcl- 2 in HT-29 colon cancer cells. **Journal of Agriculture Food Chemistry,** v.55, p.1156-1163, 2007

XLSTAT. **Software XLSTAT for Windows**Paris, FranceAdinsoft, , 2012. .

YAN, L.; KIM, I. H. Effect of Dietary Grape Pomace Fermented by Saccharomyces boulardii on the Growth Performance, Nutrient Digestibility and Meat Quality in Finishing Pigs. **Asian-Australasian Journal of Animal Sciences**, v. 24, n. 12, p. 1763–1770, 2011.

YARNPAKDEE, S.; BENJAKUL, S.; KRISTINSSON, H. G.; KISHIMURA, H. Antioxidant and sensory properties of protein hydrolysate derived from Nile tilapia (Oreochromis niloticus) by one- and two-step hydrolysis. **Journal of Food Science and Technology**, v. 52, n. 2004, p. 3336–3349, 2014.

YILDIZ-TURP, G.; SEDAROGLU, M. Effects of using plum puree on some properties of low fat beef patties. **Meat Science,** v.86, p.896-900, 2010.

YIN, M.; CHENG, W. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. **Meat Science,** v. 63, p. 23–28, 2003.

ZHAO, L.; WANG, S.; HUANG, Y. Original article Antioxidant function of tea dregs protein hydrolysates in liposome – meat system and its possible action mechanism. **Journal of Food Science and Technology,** p.2299–2306, 2014.

ZUO, Y.; WANG, C.; ZHAN, J. Separation, characterization, and quantitation of benzoic and phenolic antioxidants in American cranberry fruit by GC– MS. **Journal of Agriculture Food Chemistry,** v.50, p.3789-3794, 2002.

10. APÊNDICES

10.1. FICHA DE REGISTRO DE AVALIAÇÃO SENSORIAL

Nome: _____Idade:_____anos
Você está recebendo uma amostra de HAMBÚRGUER DE CARNE SUÍNA. Por favor, prove a amostra codificada e avalie o quanto você gostou e desgostou da amostra com relação à ACEITAÇÃO GLOBAL, APARÊNCIA, AROMA e SABOR.

Amostra: _____

ACEITAÇÃO GLOBAL |_____|
Desgostei muitíssimo Gostei muitíssimo

APARÊNCIA |_____|
Desgostei muitíssimo Gostei muitíssimo

AROMA |_____|
Desgostei muitíssimo Gostei muitíssimo

SABOR |_____|
Desgostei muitíssimo Gostei muitíssimo

TEXTURA |_____|
Desgostei muitíssimo Gostei muitíssimo

Indique na escala abaixo, sua opinião com relação ao sabor SALGADO das amostras.

SABOR SALGADO |_____|_____|
Abaixo do ideal Ideal Acima do ideal

Indique na escala abaixo se você percebeu algum AROMA ESTRANHO na amostra

AROMA ESTRANHO |_____|
Nenhum Extremamente forte
Poderia descrever este AROMA ESTRANHO : _____

Indique na escala abaixo se você percebeu algum SABOR ESTRANHO na amostra

SABOR ESTRANHO |_____|
Nenhum Extremamente forte
Poderia descrever este SABOR ESTRANHO :
_____

10.2. TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado (a) a participar, como voluntário(a), da pesquisa **PRODUTOS CÁRNEOS ELABORADOS COM CARNE SUÍNA COM MAIOR TEOR DE OMEGA-3 E ANTIOXIDANTES NATURAIS: ANÁLISE SENSORIAL**, no caso de você concordar em participar, favor assinar ao final do documento.

Sua participação não é obrigatória, e, a qualquer momento, você poderá desistir de participar e retirar seu consentimento. Sua recusa não trará nenhum prejuízo em sua relação com o pesquisador(a) ou com a instituição.

Você receberá uma cópia deste termo onde consta o telefone e endereço do pesquisador (a) principal, podendo tirar dúvidas do projeto e de sua participação.

TÍTULO DA PESQUISA: Produtos cárneos elaborados com carne suína com maior teor de ômega -3 e antioxidantes naturais: análise sensorial

PESQUISADOR(A) RESPONSÁVEL: Daniela Miotto Bernardi

ENDEREÇO: Rodovia Municipal Ff 306, Km 01, Tatu-Jupy, Céu Azul-PR

TELEFONE: (45) 8404-6232

PATROCINADOR: não há

OBJETIVOS Avaliar a aceitabilidade de produtos (hamburguer) produzidos com e carne de suínos com maior teor de omega-3 e antioxidantes naturais.

JUSTIFICATIVA: A adição de ômega-3 em produtos cárneos pode ser uma alternativa para aumentar o consumo de ômega na dieta.

PROCEDIMENTOS DO ESTUDO: Os provadores serão convidados experimentar seis amostras de linguiça lingüiça toscana ou seis amostras de hambúrguer de carne suína. Após provar as amostras, os provadores darão sua opinião sobre as amostras por meio do preenchimento de uma ficha de avaliação sensorial.

RISCOS E DESCONFORTOS: Os provadores estão sujeitos ao risco de não gostarem do produto avaliado, além de se sentirem desconfortáveis no preenchimento das fichas de análise sensorial. Os provadores também podem ter reação alérgica devido ao consumo de carne suína ou de quaisquer ingredientes utilizados na elaboração dos produtos. IMPORTANTE: se você tiver qualquer alergia à produtos cárneos não continue esta pesquisa.

BENEFÍCIOS: Serão a elaboração de produtos cárneos com maior teor de Omega-3 e antioxidantes naturais, com potencial de serem lançados no mercado. Tais produtos poderão auxiliar no aumento do consumo de Omega-3, além disso poderão ser uma alternativa de fonte natural de omega-3, pois estará naturalmente presente na carne.

CUSTO/REEMBOLSO PARA O PARTICIPANTE: Não haverão custos, para os participantes da análise sensorial.

CONFIDENCIALIDADE DA PESQUISA: Os dados pessoais não serão divulgados, serão de total sigilo dos pesquisadores.

EXPOSIÇÃO PESSOAL: Não haverá exposição pessoal

Assinatura do Pesquisador Responsável: _____

Eu,**_____,** declaro que li as informações contidas nesse documento, fui devidamente informado(a) pelo pesquisador(a) – **Daniela Miotto Bernardi**– dos procedimentos que serão utilizados, riscos e desconfortos, benefícios, custo/reembolso dos participantes, confidencialidade da pesquisa, concordando ainda em participar da pesquisa.

Foi-me garantido que posso retirar o consentimento a qualquer momento, sem qualquer penalidade. Declaro ainda que recebi uma cópia desse Termo de Consentimento.

Poderei consultar o pesquisador responsável (acima identificado) ou o CEP/FAG, com endereço na Faculdade Assis Gurgacz, Av. das Torres, 500, Cep 85807-030, Fone: (45) 3321-3871, no e-mail: comitedeetica@fag.edu.br sempre que entender necessário obter informações ou esclarecimentos sobre o projeto de pesquisa e minha participação no mesmo.

Os resultados obtidos durante este estudo serão mantidos em sigilo, mas concordo que sejam divulgados em publicações científicas, desde que meus dados pessoais não sejam mencionados.

LOCAL E DATA: _____, _____, _____.

_____ **_____**

(Nome por extenso) **(Assinatura)**

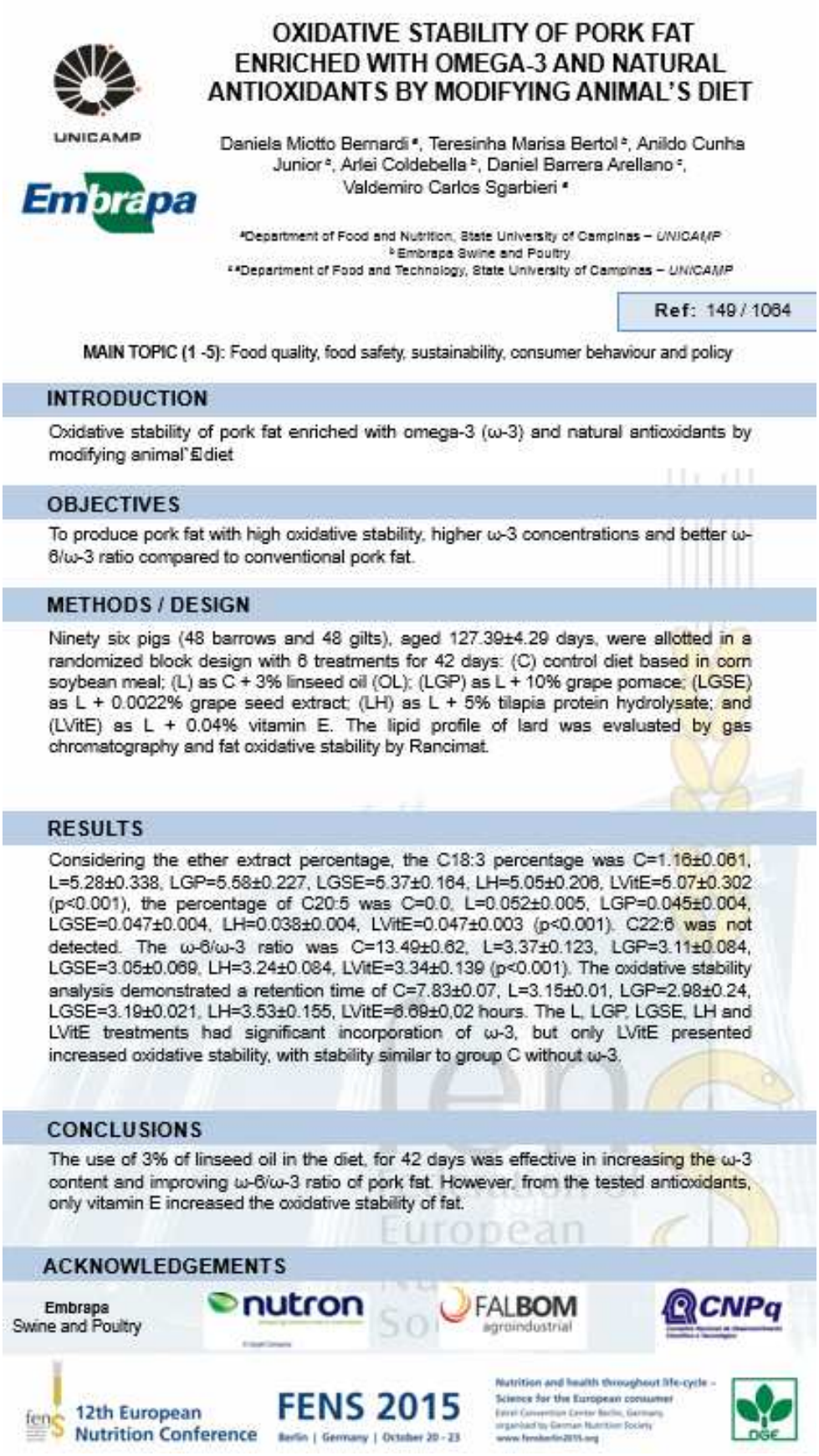
Presenciamos a solicitação de consentimento, esclarecimentos sobre a pesquisa e aceite do sujeito em participar.

Testemunhas (não ligadas à equipe de pesquisadores):

Nome: _____Assinatura:_____

Nome: _____Assinatura:_____

10.3. RESUMOS PUBLICADOS EM CONGRESSOS



OXIDATIVE STABILITY OF PORK FAT ENRICHED WITH OMEGA-3 AND NATURAL ANTIOXIDANTS BY MODIFYING ANIMAL'S DIET

Daniela Miotto Bernardi [a], Teresinha Marisa Bertol [b], Anildo Cunha Junior [a], Arlei Coldebella [b], Daniel Barrera Arellano [c], Valdemiro Carlos Sgarbieri [a]

[a]Department of Food and Nutrition, State University of Campinas – *UNICAMP*
[b]Embrapa Swine and Poultry
[c]Department of Food and Technology, State University of Campinas – *UNICAMP*

Ref: 149 / 1084

MAIN TOPIC (1 -5): Food quality, food safety, sustainability, consumer behaviour and policy

INTRODUCTION

Oxidative stability of pork fat enriched with omega-3 (ω-3) and natural antioxidants by modifying animal's diet

OBJECTIVES

To produce pork fat with high oxidative stability, higher ω-3 concentrations and better ω-6/ω-3 ratio compared to conventional pork fat.

METHODS / DESIGN

Ninety six pigs (48 barrows and 48 gilts), aged 127.39±4.29 days, were allotted in a randomized block design with 6 treatments for 42 days: (C) control diet based in corn soybean meal; (L) as C + 3% linseed oil (OL); (LGP) as L + 10% grape pomace; (LGSE) as L + 0.0022% grape seed extract; (LH) as L + 5% tilapia protein hydrolysate; and (LVitE) as L + 0.04% vitamin E. The lipid profile of lard was evaluated by gas chromatography and fat oxidative stability by Rancimat.

RESULTS

Considering the ether extract percentage, the C18:3 percentage was C=1.16±0.061, L=5.28±0.338, LGP=5.58±0.227, LGSE=5.37±0.164, LH=5.05±0.206, LVitE=5.07±0.302 ($p<0.001$), the percentage of C20:5 was C=0.0, L=0.052±0.005, LGP=0.045±0.004, LGSE=0.047±0.004, LH=0.038±0.004, LVitE=0.047±0.003 ($p<0.001$). C22:6 was not detected. The ω-6/ω-3 ratio was C=13.49±0.62, L=3.37±0.123, LGP=3.11±0.084, LGSE=3.05±0.069, LH=3.24±0.084, LVitE=3.34±0.139 ($p<0.001$). The oxidative stability analysis demonstrated a retention time of C=7.83±0.07, L=3.15±0.01, LGP=2.98±0.24, LGSE=3.19±0.021, LH=3.53±0.155, LVitE=6.69±0,02 hours. The L, LGP, LGSE, LH and LVitE treatments had significant incorporation of ω-3, but only LVitE presented increased oxidative stability, with stability similar to group C without ω-3.

CONCLUSIONS

The use of 3% of linseed oil in the diet, for 42 days was effective in increasing the ω-3 content and improving ω-6/ω-3 ratio of pork fat. However, from the tested antioxidants, only vitamin E increased the oxidative stability of fat.

ACKNOWLEDGEMENTS

Embrapa
Swine and Poultry

OXIDATIVE STABILITY OF PORK PATTIES ENRICHED WITH OMEGA-3 AND NATURAL ANTIOXIDANTS BY MODIFYING ANIMAL'S DIET

Daniela Miotto Bernardi[a], Teresinha Marisa Bertol[b], Arlei Coldebella[b], Fabiana Dieterich[c], Leandro Daniel de Paris[c], Valdemiro Carlos Sgarbieri[a]

[a]Department of Food and Nutrition, State University of Campinas – *UNICAMP*
[b]Embrapa Swine and Poultry
[c]State University of West Paraná – *UNIOESTE*

Ref: 149 / 1030

MAIN TOPIC (1 -5): Food quality, food safety, sustainability, consumer behaviour and policy

INTRODUCTION

Omega-3 (ω-3) has low oxidative stability, thus the use of antioxidants is an alternative for increasing the stability of products enriched with this fatty acid

OBJECTIVES

To evaluate the effect of the addition of natural antioxidants in the diet of pigs on the oxidative stability of ω-3-enriched patties made with pork loin and back fat, over six months of storage

METHODS / DESIGN

Ninety six pigs (48 barrows and 48 gilts), aged 127.39±4.29 days, were allotted in a randomized block design with 6 treatments for 42 days: (C) control diet based in corn soybean meal; (L) as C + 3% linseed oil (OL); (LGP) as L + 10% grape pomace; (LGSE) as L + 0.0022% grape seed extract; (LH) as L + 5% tilapia protein hydrolysate; and (LVitE) as L + 0.04% vitamin E. The patties contained on average 78.37% loin, 19.66% pork back fat, and 1.96% salt. Lipid oxidation was assessed by TBARS (thiobarbituric acid reactive substances) assay at 0, 2, 4, and 6 months of frozen storage.

RESULTS

The malonaldehyde levels (mg MDA/kg) were: C=0.21±0.03ab, L=0.27±0.03a, LGP=0.22±0.03ab, LGSE-=0.18±0.02b, LH=0.20±0.03b, LVitE=0.15±0.02b($p<0.05$) at time 0; C=0.86±0.08d, L=1.06±0.07bc, LGP=1.22±0.07ab, LGSE=1.26±0.10a, LH=0.97±0.08cd, LVitE=0.65±0.05e ($p<0.001$) after two months of storage; C=1.21±0.10bc, L=1.52±0.12a, LGP=1.35±0.07ab, LGSE=1.38±0.07ab, LH=1.32±0.08ab, LVitE=1.04±0.10c($p<0.05$) after 4 months of storage; and C=1.61±0.16bc, L=1.96±0.17ab, LGP=1.68±0.07abc, LGSE=2.03±0.11a, LH=1.95±0.16ab, LVitE=1.47±0.12c($p<0.05$) at the end of the storage period. Although lower oxidation values were observed in the treatment LVitE when compared to the treatment C after 2 months of storage, no significant differences were observed in the other storage periods.

CONCLUSIONS

Vitamin E was the most effective and LH the second most effective in maintaining oxidative stability of ω-3-enriched patties at the concentrations used in this study.

ACKNOWLEDGEMENTS

Embrapa
Swine and Poultry

ANTIOXIDANT ACTIVITY OF PROTEIN HYDROLYSATES FROM NILE TILAPIA PROCESSING RESIDUES

Daniela Miotto Bernardi*[A], Fabiana Dieterich[B], Leandro Daniel de Paris[B], Fernanda Guimarães Drummond e Silva[A], Cezar Sary[C], Wilson Rogério Boscolo[C], Altevir Signor[C], Teresinha Marisa Bertol[D], Valdemiro Carlos Sgarbieri[A]

*dani_miotto@yahoo.com.br

[A]Universidade Estadual de Campinas, Departamento de Alimentos e Nutrição, Campinas-SP, Brasil; [B]Falbom Agroindustrial Ltda., Toledo-PR, Brasil; [C]Universidade Estadual do Oeste do Paraná, Grupo de Estudos de Manejo na Aqüicultura, Toledo-PR, Brasil; [D]Embrapa Suínos e Aves, Concórdia-SC, Brasil

INTRODUCTION

In recent decades there is an increasing demand for fish processing by-products, due to the high amount produced, intrinsic nutritional value, and low cost of the raw material.

OBJECTIVE

To produce protein hydrolysates using by-products from the filleting process of Nile tilapia and to evaluate its antioxidant activity.

METHODS

Heads and carcasses of eviscerated tilapia were milled and mixed with 20% water and 0.2% Alcalase® enzyme (Bacillus licheniformis protease) in an industrial reactor. The hydrolysis reaction was carried out for 240 min at 60°C. Six samples were collected at different reaction times: A-40 min; B-80 min; C-120 min; D-160 min; E-200 min; and F-240 min. Thermal inactivation of the enzyme was performed at 90 °C for 2 min for each aliquot, followed by filtration to remove the remaining bones. Subsequently, all samples were lyophilized and analyzed for total protein[1], degree of hydrolysis[2], and antioxidant activity by the oxygen radical absorbance capacity (ORAC)[3], ferric reducing antioxidant power (FRAP)[4], and free radical capture ABTS•+ (ABTS)[5].

RESULTS

Table 01 shows the protein content and the degree of hydrolysis of the samples and Table 02 shows the antioxidant activity of the samples determined by the different assays.

Table 01: The protein content and the degree of hydrolysis of the samples

Hydrolysates	Protein content (%)	Degree of hydrolysis (%)
A	46.82±3.78	19.5±1.07
B	45.95±7.04	21.52±0.56
C	44.45±1.28	23.53±1.99
D	44.96±8.7	24.85±2.25
E	44.2±1.65	26.13±0.75
F	45.57±2.05	25.72±1.28

A: 40 minutes of hydrolysis; B-80 minutes of hydrolysis; C-120 minutes of hydrolysis; D-160 minutes of hydrolysis; E-200 minutes of hydrolysis; and F-240 minutes of hydrolysis.

Table 02: Antioxidant activity of protein hydrolysates from Nile tilapia processing residues

Hydrolysates	ORAC (uMTE/g)	FRAP (uMTE/g)	ABTS (uMTE/g)
A	159.46±3.7	21.68±2.29	36.29±5.98
B	150.57±21	18.89±0.88	31.43±8.02
C	168.43±23	17.24±0.76	33.63±8.14
D	151.28±14	19.6±1.39	35.69±5.01
E	152.08±18	22.66±3.27	36.29±2.69
F	164.26±18	22.22±1.3	30.69±1.54

A: 40 minutes of hydrolysis; B-80 minutes of hydrolysis; C-120 minutes of hydrolysis; D-160 minutes of hydrolysis; E-200 minutes of hydrolysis; and F-240 minutes of hydrolysis.

No significant changes were observed in the antioxidant activity of the hydrolysates for the periods studied. The main mechanism of antioxidant activity of the hydrolysate was the hydrogen atom transfer reactions, evidenced by the ORAC assay.

CONCLUSION

It is possible to make a product with high antioxidant activity using by-products from the processing of tilapia fish.

REFERENCES

1. Nielsen P.m., Petersen D., Dambmann C. Improved Method for Determining Food Protein Degree of Hydrolysis. J Food Sci [Internet]. 2001;66(5):642–6.
2. AOAC. Official methods of analysis of AOAC International. 16th ed. Virginia; 1998.
3. Dávalos A, Gómez-Cordovés C, Bartolomé B. Extending Applicability of the Oxygen Radical Absorbance Capacity (ORAC-Fluorescein) Assay. J Agric Food Chem. 2004;52(1):48–54.
4. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70–6.
5. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9–10):1231–7.

ACKNOWLEDGEMENTS

FALBOM agroindustrial · GEMAq · UNICAMP · Embrapa Suínos e Aves · CNPq

EFEITO DA INCLUSÃO DE ÓLEO DE LINHAÇA E ANTIOXIDANTES NATURAIS NO DESEMPENHO E QUALIDADE DA CARCAÇA E DA CARNE DE SUÍNOS EM TERMINAÇÃO

DM BERNARDI[1*]; TM BERTOL[2]; A COLDEBELLA[2], BC SILVEIRA-ALMEIDA[3], F DIETERICH[4], LD PARIS[4], VC SGARBIERI[1]
[1] Universidade Estadual de Campinas, Departamento de Alimentos e Nutrição, Campinas-SP; [2]Embrapa Suínos e Aves, Concórdia-SC; [3] Universidade Federal Rural de Pernambuco, Departamento de Zootecnia, Recife-PE; [4]Falbom Agroindustrial Ltda., Toledo-PR

INTRODUÇÃO

A incorporação de ω-3 em carnes vêm ganhando interesse nos últimos anos, porém o aumento deste ácido graxo está associado à redução da estabilidade oxidativa no alimento, assim, para minimizar este efeito uma boa estratégia é o uso de antioxidantes naturais. O objetivo do trabalho foi avaliar o efeito da inclusão de óleo de linhaça e antioxidantes naturais na alimentação de suínos, sobre o desempenho, características de carcaça e qualidade da carne.

MATERIAL E MÉTODOS

Foram utilizados 96 suínos (48 machos e 48 fêmeas), com peso médio inicial de 80,01±2,43 Kg, distribuídos em blocos casualizados, com 6 tratamentos: (C) ração controle a base de milho e farelo de soja sem incorporação de óleo; (L) ração com 3% de óleo de linhaça (OL); (LBU) ração com 3% de OL + 10% bagaço de uva; (LEU) ração com 3% de OL + 0,0022% de extrato de semente de uva; (LH) ração com 3% de OL+ 5% hidrolisado proteico de tilápia e (LVitE) ração com 3% de OL + 0,04% de vitamina E. O experimento teve a duração de 42 dias. Avaliou-se o ganho de peso diário (GPD), consumo diário de ração (CDR) e conversão alimentar (CA). Quarenta e cinco minutos após o abate foi medido o pH do lombo (pH45L) e após 24 horas realizou-se as avaliações de: espessura de toucinho (ET), profundidade de lombo (ProfL), porcentagem de carne magra (PCM), pH do lombo (pH24L), perda por gotejamento (*Drip*L) e escore de marmoreio (Marm). Os dados foram submetidos à análise de variância, através do procedimento GLM do SAS e as médias comparadas pelo teste t protegido (p≤0,05).

RESULTADOS

Tabela 1. Médias dos valores de peso final, ganho de peso diário, consumo de ração diário, conversão alimentar, espessura de toucinho, profundidade de lombo, porcentagem de carne magra, pH do lombo 45 min após o abate, pH do lombo 24 horas após o abate, perda por gotejamento e marmoreio.

Variável	Tratamentos						Sexo		CV	Probabilidade F		
	C	L	LBU	LEU	LH	LVitE	F	M	(%)	Tto	Sexo	TtoXSexo
PF (Kg)	122,5	121,8	121,4	119,8	121,7	122,3	119,8	123,4	4,24	0,715	0,0011	0,9174
GPD (Kg)	1,012	0,995	0,986	0,947	0,991	1,008	0,973	1,007	12,32	0,715	0,1805	0,9117
CRD (Kg)	3,384	3,189	3,276	3,111	3,168	3,248	3,072	3,387	8,63	0,108	<,0001	0,6485
CA	3,35	3,23	3,34	3,31	3,21	3,23	3,18	3,38	7,73	0,4435	0,0002	0,3747
ET (mm)	24,89	22,16	21,74	23,47	22,37	23,24	20,38	25,53	19,19	0,3520	<,0001	0,3977
ProfL(mm)	59,96	62,26	63,42	62,31	61,94	65,88	63,38	61,86	11,18	0,3254	0,3671	0,2642
PCM (%)	52,49	54,52	54,97	53,78	54,34	54,51	55,80	52,43	5,94	0,2985	<,0001	0,8549
pH45L	6,42	6,42	6,40	6,40	6,36	6,43	6,43	6,38	2,55	0,8468	0,2799	0,3850
pH24L	5,39	5,41	5,44	5,44	5,38	5,38	5,41	5,41	1,93	0,3824	0,9834	0,9977
***Drip*L (%)**	4,71	4,06	3,68	3,44	4,09	4,46	3,71	4,41	38,33	0,1817	0,0382	0,8449
Marm	2,20	2,00	1,81	1,81	1,81	2,13	1,74	2,17	31,22	0,3328	0,0012	0,4092

Não houve interação tratamento x sexo nem efeito dos tratamentos (P>0,05) sobre as variáveis de desempenho, características de carcaça e qualidade da carne. Portanto, a inclusão de óleo de linhaça e antioxidantes na dieta, nas respectivas concentrações, não influenciou as respostas avaliadas. Os machos apresentaram maior PF, CRD, CA e ET(p≤0,05). Por outro lado, fêmeas apresentaram maior PCM e menor *Drip*L (p≤0,05).

CONCLUSÃO

A inclusão de 3% de óleo de linhaça e diferentes antioxidantes naturais na dieta de suínos não provocou alterações significativas no desempenho, qualidade de carne e de carcaça.

AGRADECIMENTOS

Embrapa Suínos e Aves
nutron
FALBOM agroindustrial
CNPq

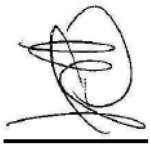
11. ANEXOS

11.1. PARECER DO COMITÊ DE ÉTICA EM PESQUISA ANIMAL

Embrapa	Parecer Substanciado	ETICA 1/2

Protocolo do projeto junto ao CEUA / CNPSA	Nº002
Título do Projeto	
INCREMENTO DE ω-3 NA CARNE SUÍNA E USO DE ANTIOXIDANTES NATURAIS NA PREVENÇÃO DA OXIDAÇÃO LIPÍDICA	
Pesquisador Responsável	
Teresinha Marisa Bertol	
Delineamento (Ok/Ver parecer)	Ok
Tamanho de amostra (Ok/Ver parecer)	Ok
Cálculo do tamanho da amostra (Ok/Ver parecer)	Ok
Normas e Diretrizes de ética (Ok/Ver parecer)	Ok
Data de início prevista (dd/mm/aaaa)	16/06/2014
Data de término prevista (dd/mm/aaaa)	18/08/2014
Recomendação (A) Aprovado/AP) Aprovado com pendências/ R) Reprovado)	A

Comentários Gerais sobre o Projeto
Recomendações/ Sugestões - Indicar o veterinário responsável pelo cuidado dos animais. - Ponderar se a coleta de sangue via veia Jugular não seria uma alternativa mais indicada que a coleta de sangue via orelha.

RELATOR	
Paulo Augusto Esteves Nome  Visto	DATA 28/ 02/ 2014

11.2. PARECER DO COMITÊ DE ÉTICA EM PESQUISA COM SERES HUMANOS

FACULDADE ASSIS GURGACZ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: PRODUTOS CÁRNEOS ELABORADOS COM CARNE SUÍNA COM MAIOR TEOR DE OMEGA-3 E ANTIOXIDANTES NATURAIS: ANÁLISE SENSORIAL

Pesquisador: Daniela Miotto Bernardi

Área Temática:

Versão: 1

CAAE: 46772615.1.0000.5219

Instituição Proponente:

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.158.963

Data da Relatoria: 30/07/2015

Apresentação do Projeto:

A pesquisa intitulada PRODUTOS CÁRNEOS ELABORADOS COM CARNE SUÍNA COM MAIOR TEOR DE OMEGA-3 E ANTIOXIDANTES NATURAIS: ANÁLISE SENSORIAL sob responsabilidade da pesquisadora Daniela Miotto Bernardie CAEE 46772615.1.0000.5219 encontra-se de acordo com as normas regulamentadoras de pesquisa envolvendo seres humanos

Objetivo da Pesquisa:

O Objetivo da pesquisa PRODUTOS CÁRNEOS ELABORADOS COM CARNE SUÍNA COM MAIOR TEOR DE OMEGA-3 E ANTIOXIDANTES NATURAIS: ANÁLISE SENSORIAL encontra-se de acordo com a proposta metodológica do estudo.

Avaliação dos Riscos e Benefícios:

A pesquisa encontra-se de acordo a resolução 466/12 quanto aos Riscos e Benefícios conforme:

I.3 - assistência ao participante da pesquisa:

II.3.1 - assistência imediata - é aquela emergencial e sem ônus de qualquer espécie ao participante da pesquisa, em situações em que este dela necessite; e

II.3.2 - assistência integral - é aquela prestada para atender complicações e danos decorrentes,

Endereço: Avenida das Torres, 500
Bairro: FAG **CEP:** 85.806-095
UF: PR **Município:** CASCAVEL
Telefone: (45)3321-3890 **Fax:** (45)3321-3900 **E-mail:** debora@fag.edu.br

FACULDADE ASSIS GURGACZ

Continuação do Parecer: 1.158.963

direta ou indiretamente, da pesquisa;

II.4 - benefícios da pesquisa - proveito direto ou indireto, imediato ou posterior, auferido pelo participante e/ou sua comunidade em decorrência de sua participação na pesquisa.

Comentários e Considerações sobre a Pesquisa:

Pesquisa relevante para a área temática proposta.

Considerações sobre os Termos de apresentação obrigatória:

Todos os itens necessários estão descritos e adequados.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

aprovado

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

O comitê acata o parecer do relator por entender que o projeto está adequado, no que se refere à ética em pesquisa com seres humanos.

CASCAVEL, 24 de Julho de 2015

Assinado por:
Débora Goulart Bourscheid Dorst
(Coordenador)

Endereço: Avenida das Torres, 500
Bairro: FAG **CEP:** 85.806-095
UF: PR **Município:** CASCAVEL
Telefone: (45)3321-3890 **Fax:** (45)3321-3900 **E-mail:** debora@fag.edu.br

11.3. AUTORIZAÇÃO DE REPRODUÇÃO DO ARTIGO: "ω-3 IN MEAT PRODUCTS: BENEFITS AND EFFECTS ON LIPID OXIDATIVE STABILITY"

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

Oct 05, 2016

This Agreement between Daniela Bernardi ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	3962491076692
License date	Oct 05, 2016
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Journal of The Science of Food and Agriculture
Licensed Content Title	ω-3 in meat products: benefits and effects on lipid oxidative stability
Licensed Content Author	Daniela Miotto Bernardi,Teresinha Marisa Bertol,Sérgio Bertelli Pflanzer,Valdemiro Carlos Sgarbieri,Marise Aparecida Rodrigues Pollonio
Licensed Content Date	Jan 19, 2016
Licensed Content Pages	15
Type of Use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Print and electronic
Portion	Full article
Will you be translating?	No
Title of your thesis / dissertation	ADIÇÃO DE ANTIOXIDANTES NATURAIS E DE ÓLEO DE LINHAÇA NA DIETA DE SUÍNOS E AVALIAÇÃO DOS EFEITOS DA SUPLEMENTAÇÃO SOBRE A CARNE E PRODUTO CÁRNEO
Expected completion date	Dec 2016
Expected size (number of pages)	180
Requestor Location	Daniela Bernardi rod. Municipal FF 306, Km 01, Tatu-Jupi Caixa postal 48 Céu Azul, Paraná 85840000 Brazil Attn: Daniela Bernardi
Publisher Tax ID	EU826007151
Billing Type	Invoice
Billing Address	Daniela Bernardi rod. Municipal FF 306, Km 01, Tatu-Jupi Caixa postal 48 Céu Azul, Brazil 85840000 Attn: Daniela Bernardi
Total	**0.00 USD**
Terms and Conditions	

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a"Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http //myaccount copyright com).

Terms and Conditions

- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.
- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.
- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.
- These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.
- In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.
- WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.
- This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.
- This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

WILEY OPEN ACCESS TERMS AND CONDITIONS

Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

The Creative Commons Attribution License

The Creative Commons Attribution License (CC-BY) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

Creative Commons Attribution Non-Commercial License

The Creative Commons Attribution Non-Commercial (CC-BY-NC)License permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.(see below)

Creative Commons Attribution-Non-Commercial-NoDerivs License

The Creative Commons Attribution Non-Commercial-NoDerivs License (CC-BY-NC-ND) permits use, distribution and reproduction in any medium, provided the original work is properly cited, is not used for commercial purposes and no modifications or adaptations are made. (see below)

Use by commercial "for-profit" organizations

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee.

Further details can be found on Wiley Online Library http://olabout.wiley.com/WileyCDA/Section/id-410895.html

Other Terms and Conditions:

v1.10 Last updated September 2015

11.4. AUTORIZAÇÃO DE REPRODUÇÃO DO ARTIGO: "*PRODUCTION OF HYDROLYSATE FROM PROCESSED NILE TILAPIA (Oreochromis niloticus) RESIDUES AND ASSESSMENT OF ITS ANTIOXIDANT ACTIVITY*"

sbCTA
www.sbcta.org.br

Sociedade Brasileira de Ciência e Tecnologia de Alimentos

Orgão de Utilidade Pública
Lei Municipal No. 4919 de 31/08/1979 - Fundada em 08/04/1967

Av. Brasil, 2880
Caixa Postal 271
13001-970 - Campinas/SP

C.G.C: 46.113.742/0001-24
Fone/Fax: (019)3241-0527
Fone: (019)3241-5793

Campinas, 07 de Outubro de 2016

DECLARAÇÃO

Declaramos para os devidos fins que autorizamos, para fins de tese do autor, a reprodução do artigo *"PRODUCTION OF HYDROLYSATE FROM PROCESSED NILE TILAPIA (Oreochromis niloticus) RESIDUES AND ASSESSMENT OF ITS ANTIOXIDANT ACTIVITY"* Ref. 152/2016, aceito para publicação na Revista Food Science and Technology.

Atenciosamente,

Diretoria de Publicações/sbCTA