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SUBSTITUIÇÃO DA CARNE MECANICAMENTE SEPARADA POR DIFERENTES CONCENTRAÇÕES DE HIDROLISADO PROTEICO EM MORTADELA

DISSERTAÇÃO DE MESTRADO

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Santa Maria, RS, Brasil
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SUBSTITUIÇÃO DA CARNE MECANICAMENTE SEPARADA POR DIFERENTES CONCENTRAÇÕES DE HIDROLISADO PROTÉICO EM MORTADELA

Carlos Pasqualin Cavalheiro

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

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Orientador (a): Profª. Ph.D Leadir Lucy Martins Fries

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Universidade Federal de Santa Maria
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SUBSTITUIÇÃO DA CARNE MECANICAMENTE SEPARADA POR DIFERENTES CONCENTRAÇÕES DE HIDROLISADO PROTÉICO EM MORTADELA

elaborada por

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

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Santa Maria, 19 de julho de 2012.

Dedico este trabalho a minha família,
por todo o carinho, dedicação
e apoio que sempre demonstraram.

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RESUMO

Dissertação de Mestrado

Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos

Universidade Federal de Santa Maria

SUBSTITUIÇÃO DA CARNE MECANICAMENTE SEPARADA POR DIFERENTES CONCENTRAÇÕES DE HIDROLISADO PROTEICO EM MORTADELA

Autor: Carlos Pasqualin Cavalheiro

Orientador: Leadir Lucy Martins Fries

Data e Local da Defesa: Santa Maria, 19 de julho de 2012.

O objetivo deste trabalho foi desenvolver um hidrolisado proteico líquido de carne mecanicamente separada (CMS) de frango, através de hidrólise enzimática e avaliar a adição de diferentes concentrações (10, 20 e 30%) em mortadela com teor de gordura reduzido. Primeiramente, foi avaliada a adição de antioxidante BHT (0,01%) e sal de cura (0,25%) na produção do hidrolisado proteico em diferentes tempos de hidrólise (5, 15, 30 e 60 minutos) com o objetivo de reduzir a oxidação lipídica e a formação da coloração amarronzada. Avaliou-se o grau de hidrólise, pH, composição centesimal, oxidação lipídica e cor instrumental. Foi possível observar que a adição de antioxidante BHT e sal de cura não afetou o grau de hidrólise ao final de 60 minutos. O pH foi similar ao encontrado na CMS original e a composição centesimal não foi afetada pelo processo de hidrólise nem pela adição de antioxidante BHT e sal de cura. Entretanto, houve uma ação sinergística entre o antioxidante BHT e o sal de cura na prevenção da oxidação lipídica dos hidrolisados durante todo o período de hidrólise. Também, foi observada uma redução na formação da coloração amarronzada pelo uso principalmente do sal de cura, evidenciada pelos maiores teores de vermelho (a^*). Posteriormente, foi avaliada a adição do hidrolisado proteico com antioxidante BHT e sais de cura, em mortadela com teor de gordura reduzido, contendo 0, 10, 20 e 30% de hidrolisado proteico de CMS. Foram avaliadas a composição centesimal, pH, oxidação lipídica, parâmetros de cor e as características microbiológicas, sensoriais e perfil de textura durante 60 dias em armazenamento a 4 ºC. A composição centesimal, pH e as características microbiológicas foram consideradas normais para o produto em questão. Os valores de oxidação lipídica dos produtos contendo hidrolisado proteico de CMS aumentaram até o 30º dia de armazenamento com posterior decréscimo até o final do período de armazenamento (60 dias). Os produtos contendo hidrolisado proteico de CMS apresentaram valores de luminosidade (L^*) e valores de a^* menores e textura mais amolecida, evidenciada tanto pelos provadores quanto pelo perfil de textura instrumental. A adição de até 10% do hidrolisado proteico se mostrou viável na fabricação de mortadela com teor de gordura reduzido, pois apresentou características de qualidade mais próximas ao tratamento controle.

Palavras-chave: hidrolisado proteico líquido, carne mecanicamente separada, hidrólise enzimática, oxidação lipídica, mortadela.

ABSTRACT

Master Dissertation

Graduate Program in Food Science and Technology

Federal University of Santa Maria

REPLACEMENT OF THE MECHANICALLY DEBONED CHICKEN MEAT FOR DIFFERENT CONCENTRATIONS OF PROTEIN HYDROLYSATE IN COOKED MEAT SAUSAGE.

AUTHOR: Carlos Pasqualin Cavalheiro

ADVISOR: Leadir Lucy Martins Fries

Date and Defense place: Santa Maria, July 19th, 2012.

The objective of this study was to develop a liquid protein hydrolysate from Mechanically Deboned Chicken Meat (MDCM) by enzymatic hydrolysis and to evaluate the addition of different concentrations (10, 20 and 30%) in a reduced-fat mortadella-type sausage. First, it was evaluated the addition of BHT antioxidant (0.01%) and curing salts (0.25%) in the production of protein hydrolysate at different hydrolysis times (5, 15, 30 and 60 minutes) in order to reduce lipid oxidation and brownish color formation. There were evaluated the degree of hydrolysis, pH, proximate composition, lipid oxidation and instrumental color. It was observed that the addition of BHT antioxidant and curing salts did not affect the degree of hydrolysis at 60 minutes. The pH was similar to the MDCM and the proximate composition was not affected by the hydrolysis process or by the addition of BHT antioxidant and curing salts. However, there was a synergistic action between the BHT antioxidant and the curing salts in lipid oxidation prevention of the hydrolysates during the whole period of hydrolysis. Also, there was observed a reduction in the formation of a brownish color mainly by the use of curing salts, evidenced by high levels of values of a^* (redness). Subsequently, there was evaluated the addition of different concentrations protein hydrolysate containing BHT antioxidant and curing salts in a reduced-fat mortadella-type sausage. Thus, four treatments were made containing 0, 10, 20 and 30% of MDCM protein hydrolysate. There were evaluated the proximate composition, pH, lipid oxidation, colorimetric parameters, texture profile and microbiological and sensory characteristics during 60 days of storage at 4 ºC. The proximate composition, pH and microbiological characteristics were considered normal for this kind of product. The values of lipid oxidation of products containing MDCM protein hydrolysate increased up to 30th day of storage with subsequent decrease until the end of the storage period (60 days). The products containing MDCM protein hydrolysate had low values of L^* (lightness) and a^* (redness), higher lipid oxidation and soft textured, evidenced both by the panelists and by instrumental texture profile. The addition of up to 10% protein hydrolysate proved to be viable in the production of reduced-fat mortadella-type sausage, showing quality characteristics closer to the control treatment.

Key-words: liquid protein hydrolysate, mechanically deboned chicken meat, enzymatic hydrolysis, lipid oxidation, mortadella.

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1 INTRODUÇÃO

O Brasil produziu 12,23 milhões de toneladas de carne de frango em 2010, sendo hoje o segundo maior produtor mundial, ficando atrás apenas da China (UBABEF, 2011). Conforme Negrão et al. (2005), pelo menos 20% das carcaças de frango de corte frescas são transformadas em carne mecanicamente separada (CMS) de ave. Desta forma, é possível estimar que aproximadamente 2,45 milhões de toneladas de CMS de ave foram produzidas no Brasil em 2010.

Com o aumento do número de abatedouros e o consequente aumento da quantidade de cortes e produtos de frango desossados manualmente, mais matérias primas com baixo valor comercial são produzidos. Entre estas, podemos citar o dorso, pescoço, ossos do peito, pontas de asa, recortes com osso, caixa torácica e carcaças lesionadas, cujo valor alimentar e comercial são menores. Apesar disso ainda há neles significativa quantidade de carne, cuja retirada manual é difícil e economicamente inviável. Daí a necessidade do uso da tecnologia de recuperação mecânica, visando o aproveitamento da carne presente nestas partes, que representa cerca de 15 a 25% da carne existente na carcaça (BERAQUET, 2000).

A CMS é uma matéria-prima amplamente utilizada pela indústria cárnea, pois é de baixo custo e possui textura pastosa, firme e uniforme (SOUSA et al., 2003). No entanto, sua utilização é limitada pelas suas propriedades tecnológicas como capacidade de ligação de água, poder emulsificante e estabilidade de emulsão, além da sua vida útil limitada devido às suas características físico-químicas e microbiológicas (KRAUTIL; TULLOCH, 1987).

Conforme a legislação brasileira é possível utilizar até 60% de CMS na formulação de produtos cárneos cozidos. Dentre estes, destaca-se a mortadela que segundo a legislação é "o produto cárneo industrializado, obtido de uma emulsão das carnes de animais de açougue, acrescido ou não de toucinho, adicionado de ingredientes, embutido em envoltório natural ou artificial, em diferentes formas e submetido ao tratamento térmico adequado" (BRASIL, 2000).

A hidrólise de proteínas é uma das formas de melhorar a qualidade da proteína inicial. O hidrolisado proteico é resultado da solubilização das proteínas que podem ser obtidas a partir da hidrólise química (ácida ou alcalina) ou por hidrólise enzimática (KRISTINSSON; RASCO, 2000; MARTONE et al., 2005). O processo de hidrólise resulta na produção de

peptídeos e aminoácidos com menor peso molecular, aumento do número de grupos ionizáveis e exposição de grupos hidrofóbicos. Diversos estudos mostram que a hidrólise de proteínas é capaz de produzir peptídeos com propriedades bioativas como antihipertensiva, antioxidante, imunomodulatória, anticancerígena e antitrombótica (KIM; MENDIS, 2006; NIETO et al., 2009; CONTRERAS et al., 2011; LI et al., 2011; ZHAO et al., 2011; LIN et al., 2012).

Esta dissertação teve como objetivo desenvolver um hidrolisado proteico líquido de Carne Mecanicamente Separada (CMS) de frango, através de hidrólise enzimática e avaliar sua utilização em diferentes concentrações na fabricação de produto cárneo cozido (mortadela).

Os objetivos específicos desse trabalho foram:

- Obter um hidrolisado proteico líquido de CMS, utilizando a enzima Alcalase, para ser utilizado na formulação de mortadela;
- Avaliar a ação de antioxidante BHT e sal de cura na produção de hidrolisado proteico líquido de CMS visando diminuir a oxidação lipídica e a formação da coloração amarronzada e nas características físico-químicas, composição centesimal e grau de hidrólise em diferentes tempos de hidrólise;
- Elaborar produto cárneo mortadela utilizando diferentes concentrações do hidrolisado proteico líquido de CMS (10, 20 e 30%) em substituição da CMS original e com teor de gordura reduzido (10%);
- Avaliar as características físico-químicas, composição centesimal, oxidação lipídica, perfil de textura, coloração instrumental, características microbiológicas e sensoriais de mortadelas produzidas com o hidrolisado proteico líquido de CMS.

2 REVISÃO DE LITERATURA

2.1 Produção de Carne de Frango

A produção de carne de frango no Brasil chegou a 12,23 milhões de toneladas em 2010, crescendo aproximadamente 11,38% em relação a 2009. Com este desempenho o Brasil se aproxima da China, hoje o segundo maior produtor mundial ficando abaixo apenas dos Estados Unidos (UBABEF, 2011).

O sul do Brasil é a região do país que corresponde à maior porcentagem de produção e abate de frangos. O Paraná é o estado com maior participação (27,77%), seguido por Santa Catarina e Rio Grande do Sul (Figura 1).

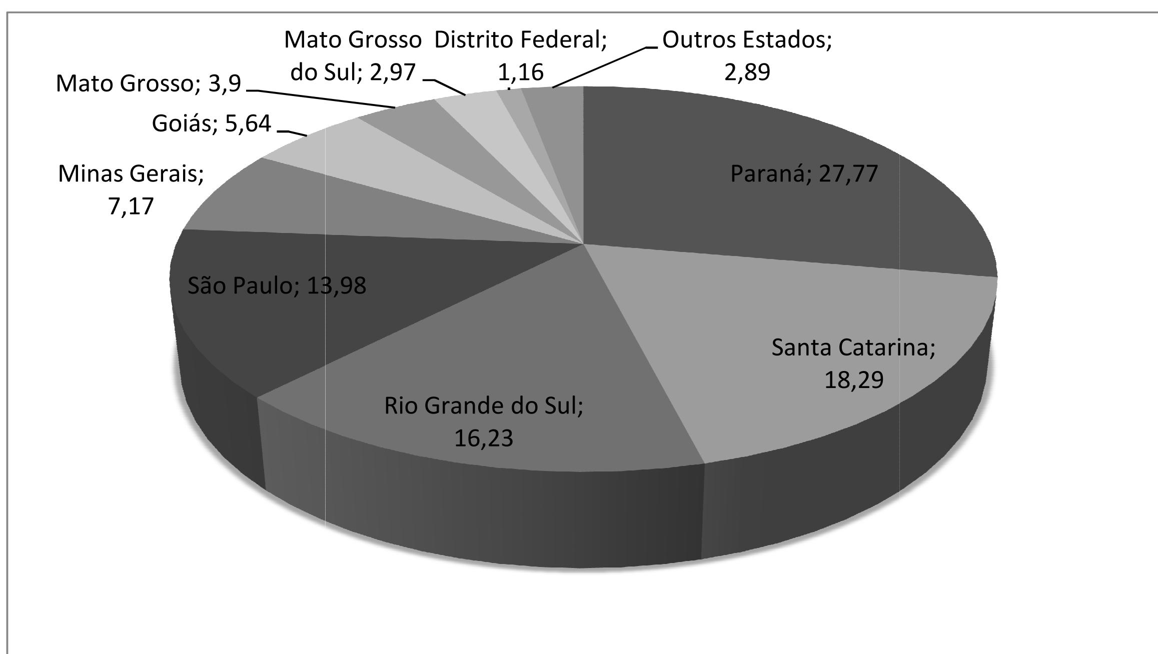

Figura 1: Participação por estados do Brasil no abate de frangos no ano de 2010, em porcentagem (UBABEF, 2010).

Muitos fatores contribuíram para o crescimento do consumo da carne de aves nos últimos anos. A diminuição do custo de produção e a modernização da tecnologia fizeram com que produtos diferenciados, que utilizam a carne mecanicamente separada de aves como matéria-prima, fossem introduzidos no mercado atual (MORI et al., 2006).

Após a retirada dos cortes nobres da carcaça do frango (peito, coxas, asas, etc.), a mesma, juntamente com o residual de carne aderido aos ossos, é submetida a um processo de trituração, onde é extraída a CMS, que é comumente utilizada na elaboração de produtos cárneos como patês e embutidos cozidos, como uma forma de reduzir custos (KIJOWSKI; NIEWIAROWICZ, 1985; STADELMAN et al., 1988).

2.2 Carne Mecanicamente Separada de Frango

A CMS de frango tornou-se uma importante matéria-prima para a elaboração de produtos cárneos no Brasil. Conforme Field (1988), o início da separação mecânica da carne se deve a empresas japonesas no período Pós-Segunda Guerra Mundial. A partir desta época, houve uma preferência dos consumidores por cortes e filés de frango ao invés das carcaças inteiras. Assim, surgiu a necessidade de se encontrar meios para o aproveitamento de dorso, pescoço e ossos resultantes da desossa manual (TRINDADE et al., 2004).

A legislação brasileira define a CMS como sendo "a carne obtida por processo mecânico de moagem de separação de ossos de animais de açougue, destinada a elaboração de produtos cárneos específicos" (BRASIL, 2000). Devido sua composição, estado físico e o elevado pH, a CMS constitui-se em um meio adequado para a proliferação bacteriana gerando como consequência uma menor vida útil.

A CMS é uma matéria prima muito utilizada pela indústria cárnea, pois é de baixo custo e possui textura pastosa, fina e uniforme (SOUSA et al., 2003). No entanto, na fabricação de produtos cárneos, alguns limites devem ser respeitados quanto aos níveis de CMS que podem ser incorporados ao produto. A legislação permite a utilização dessa matéria prima apenas em produtos cárneos industrializados cozidos, pois garante uma maior segurança para o consumidor. Pode-se utilizar no máximo 60% de CMS na fabricação de mortadela e salsicha, 30% para fiambre, almôndega e hambúrguer cozidos e 20% para linguiças cozidas (BRASIL, 2000).

2.3 Produtos Cárneos Emulsionados

No passado, a fabricação de embutidos emulsionados era considerada mais uma arte do que uma ciência. No entanto, com o crescimento da industrialização de carnes e sua relevância econômica, tornou-se necessário um maior entendimento dos princípios envolvidos na elaboração destes produtos, visto que novas tecnologias e equipamentos promoveram

novas e eficazes maneiras de expor as proteínas, para, após, emulsificá-las com a gordura (OLIVO; SHIMOKOMAKI, 2006).

Conforme Olivo e Shimokomaki (2006), os produtos cárneos emulsionados são bastante populares, sendo consumidos tanto a nível doméstico como no mercado de alimentação rápida, representando um importante segmento da industrialização de carnes. Estima-se que o consumo *per capita* no Brasil seja de aproximadamente 5 kg de produtos cárneos emulsificados, sendo 1,15 kg apenas de mortadela (GUERRA, 2010), mostrando que este tipo de alimento tem uma importante parcela na nossa dieta e considerável importância econômica.

Uma emulsão pode ser definida como sendo uma suspensão coloidal de dois líquidos imiscíveis, mas que, no entanto, mantêm-se dispersos um no outro, pela ação de um agente emulsificante, a proteína. A proteína, por possuir uma porção hidrofílica (polar) e outra hidrofóbica (apolar), atua na interface entre gordura e água, diminuindo a tensão entre as duas, unindo-as e evitando a coalescência da gordura (BAILEY; LIGHT, 1989; OLIVO; SHIMOKOMAKI, 2006).

Quando a carne, a gordura, água e sal são misturados e submetidos à alta velocidade de cominuição, uma massa homogênea é formada, com características de emulsão. A formação da emulsão consiste de duas transformações: o entumescimento das proteínas e formação da matriz viscosa e, a emulsificação das proteínas solubilizadas com os glóbulos de gordura e água (HEDRICK et al., 1994).

O principal fator de qualidade de uma emulsão cárnea é a estabilidade final, que está relacionada com a retenção de água e gordura. Uma importante característica dos produtos cárneos é sua habilidade de ligar vários componentes e proporcionar a coesividade do produto (BAILEY; LIGHT, 1989). Já para a indústria processadora, do ponto de vista econômico, é importante que a estabilidade da emulsão se mantenha durante todas as etapas de processamento do produto (OLIVO; SHIMOKOMAKI, 2006). Jones (1985) relata que numerosas energias de ligação e outras forças físicas são essenciais para manter a estabilidade e a integridade da suspensão coloidal antes, durante e após o tratamento térmico.

Diversos fatores podem influenciar na estabilidade da emulsão, como o tipo e condições dos equipamentos, temperatura e tempos de processo, tipo e tamanho das partículas de gordura, pH, momento de utilização de sal e suas quantidades, tipo e porcentagem de

proteínas, viscosidade da massa e formação da matriz geilificada (OLIVO; SHIMOKOMAKI, 2006; BARRETTO, 2007).

2.3.1 Mortadela

No Brasil, os produtos de salsicharia equivalem a um total de 44,78% em relação aos demais tipos de carnes processadas (FERREIRA et al., 2003). Conforme Olivo e Shimokomaki (2006), a família das mortadelas, devido sua excelente relação custo/benefício, representa expressiva parcela do total do volume comercializado de produtos cárneos emulsionados.

Segundo a legislação brasileira, entende-se por Mortadela como "o produto cárneo industrializado, obtido de uma emulsão das carnes de animais de açougue, acrescido ou não de toucinho, adicionado de ingredientes, embutido em envoltório natural ou artificial, em diferentes formas e submetido ao tratamento térmico adequado" (BRASIL, 2000). Ainda, no Brasil, a mortadela possui cinco classificações. O produto denominado mortadela pode ser adicionado de carne mecanicamente separada, até o limite máximo de 60%, miúdos comestíveis, pele e tendões no limite máximo de 10% e gorduras. Os produtos podem ainda ser classificados conforme seus ingredientes e processamento em Mortadela Tipo Bologna, Mortadela Italiana, Mortadela Bologna e Mortadela de Carne de Ave. No entanto, os requisitos estabelecidos para mortadelas são teores máximos de carboidratos totais de 10%, amido de 5%, umidade de 65%, gordura de 30% e proteína mínima de 12%.

2.4 Hidrólise de Proteínas

A hidrólise de proteínas é um importante bioprocesso para elevar as propriedades físicas, químicas, funcionais e nutricionais em relação à proteína original. É também um método efetivo para preparar peptídeos ativos que possuem muitas propriedades fisiológicas como ligação de minerais, efeito opióide, meio de cultura para bifidobacteria, efeito anti-cancerígeno e regulador da pressão sanguínea e sistema imunológico (SCHLIMME; MEISE, 1995).

Atualmente, o estudo da hidrólise de proteínas tem se concentrado em pescados, como uma alternativa para recuperar proteínas comestíveis dos processos de filetagem (KIM et al., 2007; CENTENARO et al., 2009; ZAVAREZE et al., 2009; JIN et al., 2011; KHALED et al., 2011; SEE et al., 2011; YANG et al., 2011; ZHAO et al., 2011). Ainda, a hidrólise de outras

matérias primas tem sido estudada, como soro de leite (PAGNO et al., 2009; SILVA et al., 2009; CONTRERAS et al., 2011), soja (SUN, 2011b; XU et al., 2012) e trigo (LEE et al., 2012). Porém, ainda são escassos estudos com outras matérias primas como a carne de frango e a CMS e ainda, a utilização de hidrolisados proteicos na fabricação de produtos cárneos.

2.4.1 Formas de hidrolisar a proteína

É possível hidrolisar proteínas através da utilização de substâncias ácidas, alcalinas ou através de enzimas. A utilização de substâncias ácidas ou alcalinas para clivar as ligações peptídicas foi o método escolhido por anos pela indústria por ser relativamente barato e simples de conduzir. No entanto, existem limitações na utilização dos seus hidrolisados como ingredientes de alimentos, além da dificuldade de controle do processo, que pode gerar produtos com composição química variável e diferentes propriedades funcionais (SKANDERBY, 1994; BLENFORD, 1994).

A hidrólise ácida pode ser realizada com uso de ácidos minerais, orgânicos ou mistura de ambos. Alguns ácidos, como o clorídrico e o sulfúrico, possuem a necessidade de neutralização antes do alimento ser consumido, gerando mais uma etapa no processo de hidrólise (OETTERER, 1994). Já a hidrólise alcalina é realizada com soluções de bases fortes, como o hidróxido de sódio (NaOH) e hidróxido de potássio (KOH) e resultam em produtos com baixa funcionalidade e pouco valor nutritivo (KRISTINSSON; RASCO, 2000).

Atualmente, a utilização de proteases específicas tem se mostrado mais vantajosa do que a hidrólise ácida ou alcalina, devido à maior especificidade, controle do grau de hidrólise, condições moderadas de ação e o menor conteúdo de sal no hidrolisado final. Além disso, as enzimas podem ser empregadas geralmente em concentrações muito baixas, sendo desnecessária sua remoção (ZAVAREZE et al., 2009). A hidrólise enzimática tem sido usada para a modificação das propriedades funcionais e nutricionais de proteínas alimentares. Estas modificações são desejáveis quando se quer aumentar a incorporação de proteínas em formulações específicas. Este tipo de hidrólise tem mostrado aumento na solubilidade, nas propriedades emulsificantes e liberação de peptídeos biologicamente ativos de certas proteínas (SPELLMAN et al., 2003).

Enzimas proteolíticas representam um importante grupo de enzimas industriais. Elas têm uma ampla faixa de aplicações biotecnológicas como a produção de alimentos, detergentes, couro, hidrolisados proteicos e fármacos (AL-SHEHRI; MOSTAFA, 2004).

As enzimas utilizadas na hidrólise de proteínas extraídas de reservas vegetais mais conhecidas são a papaína (HOYLE; MERRIT, 1994), bromelina (ASPMO et al., 2005) e algumas queratinas. Estas enzimas possuem maior atividade em faixas de pH entre 5 e 9 e são ativas em temperaturas acima de 70 ºC. As proteases de origem animal mais conhecidas são a tripsina pancreática, quimotripsina (SIMPSON et al., 1998), pepsina (VIERA et al., 1995) e as reninas que são utilizadas na composição de meios de cultivo e produção de queijo. Estas variam bastante em sua faixa de pH ótimo e sua temperatura compreende a faixa de 30 a 50 ºC. A tripsina tem pouca aplicação na indústria de alimentos, pois sua ação gera proteínas hidrolisadas com elevado amargor (RAO et al., 1998).

As proteases de origem microbiana são as mais importantes, comparadas com as de origem vegetal e animal e são mais adequadas ao uso em hidrolisados proteicos, pois oferecem uma ampla maior de atividades catalíticas e pH e temperatura mais estáveis (DINIZ; MARTIN, 1997). A mais utilizada é a Alcalase que é uma protease bacteriana alcalina, produzida pelo *Bacillus licheniformis* e é considerada por muitos pesquisadores como uma das melhores enzimas para a preparação de hidrolisados protéicos (HOYLE; MERRIT, 1994; SHAHIDI et al., 1995; KRISTINSSON; RASCO, 2000). Sua atividade declarada é de 2,4 AU/g (Unidades de Anson por grama) e suas condições ótimas de desempenho são temperaturas entre 55 e 70 ºC e pH entre 6,5 e 8,5. A Flavourzyme é uma protease fúngica complexa produzida pela fermentação submersa de uma linhagem selecionada de *Aspergillus oryzae* que não foi geneticamente modificada, atuando na hidrólise sob condições neutras ou ligeiramente ácidas (SLIZYTE et al., 2005). Seu pH ótimo está na faixa de 5,0 a 7,0 e temperatura em torno de 50 ºC. A Flavourzyme pode ser usada para remoção do amargor de hidrolisados proteicos a baixos graus de hidrólise.

2.4.2 Importância da proteína hidrolisada

Proteínas hidrolisadas possuem um grande número de propriedades funcionais, que as tornam uma fonte atrativa para uso em nutrição humana, tanto para fins médicos como para produtos de uso geral (FROKJAER, 1994).

Conforme LAHL e BRAUN (1994), os critérios mais importantes para a seleção de proteínas em hidrolisados são o valor nutricional, custo, sabor e alergenicidade. As proteínas mais comumente utilizadas em hidrolisados proteicos são proteínas de pescado, leite e soja.

Hidrolisados proteicos são produzidos com diferentes propósitos, seja para aumentar as propriedades funcionais ou para produção de pequenos peptídeos e aminoácidos que são usados em muitos produtos como agentes flavorizantes. Na forma desidratada, estes hidrolisados são utilizados pela sua alta solubilidade em água, fácil digestibilidade, alto conteúdo proteico e longa vida de prateleira (STANLEY, 1981; PEDERSEN, 1994).

Segundo CORDLE (1994), alimentos com base em proteínas hidrolisadas são úteis no controle de alergias alimentares. Este tipo de alergia é mais frequente em crianças e mais grave em adultos. É um problema de saúde com sintomas variando desde erupções cutâneas até processos anafiláticos sérios que ameaçam a sobrevivência do indivíduo. Proteínas extensivamente hidrolisadas reduzem substancialmente a reatividade imunológica e são principalmente usadas em fórmulas infantis hipoalergênicas (MAHMOUD, 1994).

Fórmulas baseadas nas proteínas hidrolisadas e aminoácidos têm sido utilizadas também em dietas enterais. Estas fórmulas são administradas às pessoas incapazes de digerir adequada quantidade de alimento na forma convencional. SCHMIDL et al. (1994) citam que as fórmulas hidrolisadas são úteis na rápida recuperação pós-cirúrgica e no tratamento de pacientes em condições que prejudique o funcionamento do sistema digestório.

2.4.3 Propriedades funcionais da proteína hidrolisada

A hidrólise enzimática aumenta a solubilidade das proteínas, porém reduz suas propriedades emulsificantes. No entanto, a capacidade de reter água não é alterada (KRISTINSSON; RASCO, 2002; SLYZYTE et al*.,* 2005). O calor utilizado para inativar a reação enzimática e a secagem podem reduzir as propriedades funcionais e nutricionais, além dos fatores sensoriais e de qualidade. A alta solubilidade em uma ampla faixa de pH é importante para muitas aplicações em alimentos e também influencia a emulsificação e propriedades de formação de espuma.

As proteínas miofibrilares intactas do pescado são quase insolúveis em amplo intervalo de pH. Os peptídeos menores produzidos pela hidrólise podem se ligar mais facilmente a água do que a proteína intacta (KRISTINSSON; RASCO, 2000). A alta solubilidade da proteína hidrolisada de pescado em amplos intervalos de pH, a qual aumenta com o grau de hidrólise já tem sido reportado por diversos autores (GBOGOURI et al., 2004; GEIRSDOTTIR et al., 2007; KLOMPONG et al., 2007; SATHIVEL; BECHTEL, 2006; SATHIVEL et al., 2005; SHAHIDI et al., 1995; THIANSILAKUL et al., 2007).

2.4.4 Propriedades bioativas da proteína hidrolisada

Propriedades bioativas como antihipertensiva, antioxidante, imunomodulatória, anticancerígena e atividade antitrombótica têm sido documentadas nas proteínas hidrolisadas de pescado, soro de leite, casca de camarão, pele de lula e batata (KIM; MENDIS, 2006; NIETO et al., 2009; CONTRERAS et al., 2011; LI et al., 2011; ZHAO et al., 2011; LIN et al., 2012).

Uma das propriedades bioativas dos hidrolisados proteicos mais documentada é a redução da pressão sanguínea através da inibição da enzima de conversão da angiotensina (ECA). Conforme Erdös et al. (2010), a ECA é uma enzima que cliva dois aminoácidos em angiotensina-I, liberando angiotensina-II e inativando o vasodilatador braquinina. A angiotensina-II aumenta a pressão sanguínea fazendo a constrição das artérias. Os inibidores da ECA podem diminuir a pressão sanguínea por inibirem a formação da angiotensina-II (PARFREY, 2008). Atualmente os medicamentos inibidores da ECA e anti-hipertensivos que são utilizados para reduzir a pressão sanguínea causam diversos efeitos colaterais (YU et al., 2006; VERMEIRSSEN et al., 2004). No entanto, na última década diversos peptídeos derivados de proteína foram descobertos e que apresentaram ação inibitória da ECA (AHHMED; MUGURUMA, 2010). Esses peptídeos foram isolados de diferentes fontes proteicas, incluindo caseína, soro de leite, soja, pescados (YU et al.; 2006; JANG; LEE, 2005), carne de frango (IROYUKIFUJITA et al., 2000), hemoglobina suína (YU et al., 2006), carne suína (SENTANDREU; TOLDRÁ, 2007; MUGURUMA et al., 2009; KATAYAMA et al., 2007) e carne bovina (JANG; LEE, 2005; JANG et al., 2008; BERNARDINI et al.; 2012).

Um estudo recente mostrou que o hidrolisado proteico de casca de camarão de diversas espécies teve um efeito positivo contra a proliferação de células cancerígenas humanas e que ele pode ser utilizado também como uma alternativa mais barata que o tratamento convencional contra o câncer (KANNAN et al., 2011). Outros estudos mostram também que a hidrólise de diferentes matérias primas é capaz de produzir peptídeos com atividade antioxidante. Yang et al. (2011) encontraram uma excelente atividade antioxidante no hidrolisado da cabeça de albacora (*Thunnus obesus*) em quatro testes modelos *in vitro*. Sun et al. (2011a) relatam a descoberta de um novo peptídeo antioxidante, proveniente de hidrolisado de hemoglobina suína, que foi capaz de reduzir a oxidação de lipídeos e se mostrou um excelente capturador de radicais livres.

Segundo Lazzi et al. (2011), o uso de hidrolisados protéicos de restos de ossos e carne de frango como meio de cultura promoveu um crescimento de diversas espécies de

Bifidobacterium de origem humana que podem ser utilizados como probióticos. Os mesmos autores frisam que é muito promissor a inclusão deste ingrediente no meio industrial para probióticos, não apenas por aumentar o crescimento de microrganismos desejáveis, mas também por manter estas células em um alto grau de viabilidade.

2.4.5 Recuperação de proteínas por hidrólise enzimática

Diversos estudos já foram realizados para a recuperação de proteínas a partir de produtos com pouco valor comercial. A hidrólise de resíduos do processamento de origem animal, principalmente pescados e aves, contém elevado teor de proteína de alto valor biológica, que pode ser recuperada e utilizada na alimentação tanto humana quanto animal (KIJOWSKI; NIEWIAROWICZ, 1985).

A hidrólise enzimática possibilita a recuperação da proteína de produtos e subprodutos da indústria, como caseína (MAHMOUD et al., 1992), carcaças de aves domésticas (SUROWKA; FIK, 1994; LINDER et al., 1995; FONKWE; SINGH, 1996), plantas (PARRADO et al., 1993; MONTEIRO; PRAKASH, 1996) e pescados diversos (LICEADA-GESUALDO; LI-CHAN, 1999; SYNOWIECKI; AL-KHATEEB, 2000; GILDBER; STENBERG, 2001). WEBSTER et al. (1982) prepararam hidrolisados proteicos a partir de refugos bovinos, como fígado, rúmen e tecidos parcialmente desengordurados, com recuperação de proteína entre 45 e 85%. SURÓWKA e FIK (1992) hidrolisaram cabeças de frango moídas com uma protease de *Bacillus subtilis* e recuperaram 39,6% da proteína. FONKWE e SINGH (1996), ao utilizar resíduos de carne mecanicamente separada de peru, obtiveram uma recuperação protéica de 46%, enquanto NILSANG et al. (2005) atingiram níveis de proteína de até 66% utilizando enzimas industrais Kojizime e Flavourzyme em concentrado solúvel de peixe.

3. MANUSCRITOS

3.1 Manuscrito 1

Development of a liquid protein hydrolysate from mechanically deboned chicken meat for use in cooked meat products

Manuscrito em fase final de revisão para ser submetido à revista *Meat Science*.

Development of a liquid protein hydrolysate from mechanically deboned chicken meat for use in cooked meat products

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29 **Abstract**

30 Protein hydrolysates were produced from mechanically deboned chicken meat
31 (MDCM) using Alcalase® 2.4L to use in cooked meat products. There was analyzed the effect
32 of BHT antioxidant and curing salt addition on the degree of hydrolysis (DH), pH, proximate
33 composition, lipid oxidation (TBARS) and instrumental color at different hydrolysis times.
34 MDCM is a good raw material to produce protein hydrolysates. The DH was affected by the
35 hydrolysis time and addition of BHT antioxidant and curing salt. After 60 minutes of
36 hydrolysis, the values of DH were between 7.14% and 14.54%. The pH of hydrolysates was
37 similar to the MDCM pH and neither the hydrolysis process nor the BHT antioxidant and
38 curing salt affected the proximate composition. However, the lipid oxidation and the brownish
39 color formation were reduced by the use of BHT antioxidant and curing salt.

40 **Key-words:** Alcalase, enzymatic hydrolysis, mechanically deboned chicken meat, BHT
41 antioxidant, curing salt.

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1. Introduction

The use of Mechanically Deboned Chicken Meat (MDCM) has increased in the food industry because of the strong tendency to replace red meat by healthier white meat in industrialized countries, and the lower price of the latter compared with other kinds of meat (Daros et al., 2005). It is reckoned that at least 20% of chicken fresh-cut carcasses are transformed into MDCM, which could be used in processed meats, such as meat emulsion, paste meat and chicken nuggets (Negrão et al., 2005). However, there may be some drawbacks in the utilization of MDCM in meat products as the rapid onset of oxidative stability resulting in off-flavors and off-odors, being the major problem in these products (Mielnik et al., 2002). Dawson and Gartner (1983) have reported that extreme mechanical stress and extraction of important amounts of fats and heme components from the bone marrow as well as the aeration during the mechanical process produce high oxidative potential of MDCM. These factors promote the auto-oxidation of polyunsaturated fatty acids, producing secondary products of fatty acid oxidation such as aldehydes, ketones, hydrocarbons, ester, furans and lactones. These secondary products are responsible for generating rancid flavors (Ladikos and Lougovois, 1990).

Enzymatic hydrolysis of proteins is an important bioprocess to improve the physical, chemical, functional and nutritional properties of original proteins. It is also an effective method to prepare active peptides, which possess many physiological properties including mineral binding, opioid activity, growth enhancer for bifid bacteria, anticancer activity and regulation of the blood pressure or the immune system (Schlimme and Meise, 1995). Recently, fish protein hydrolysates have gained increasing attention as a nutritious fish product. The fish protein hydrolysates can serve as food ingredients and also provide desirable characteristics to food products such as athletic drinks, emulsified meat products, cereal based foods, etc. Even more, they can be used for patients with gastrointestinal tract complications (Khantaphant et al., 2011). Nevertheless, few studies have been published on the production, quality and applicability of MDCM protein hydrolysates.

Due to preliminary studies carried out in our laboratory, it was observed the trend of the fat oxidation in liquid protein hydrolysates from MDCM and the tendency of brownish color formation, which is undesirable for meat products production. Furthermore, many studies reported on a literature were performed removing the fat and moisture content but not with the purpose to use the hydrolysates in its crude form. However, to elaborate cooked sausages, it becomes necessary to observe the physico-chemical characteristics, fat oxidation

91 and color changes during the hydrolysis process. Thus, the aim of this study was to develop
92 liquid protein hydrolysates from MDMC and to evaluate the addition of BHT antioxidant and
93 curing salt on the degree of hydrolysis (DH), physico-chemical characteristics, fat oxidation
94 and instrumental color for its use in meat products.

95 **2. Materials and Methods**

96 2.1 Raw material and enzyme

97 MDCM was supplied in a freeze form by a large local poultry processing plant in
98 Southern of Brazil and had pH of 6.34 ± 0.23 and TBARS values of 0.04 ± 0.02 at the
99 moment of its use.

100 The enzyme used was Alcalase 2.4L FG, an endopeptidase from *Bacillus*
101 *licheniformis*, provided by Novozymes Latin America® (Araucária, Paraná, Brazil). It has
102 been reported to be one of the highly efficient bacterial protease used to prepare functional
103 protein hydrolysates (Adler-Nissen, 1986; Benjakul and Morrisey, 1997; Diniz and Martin,
104 1997) and show higher activities than acid or neutral proteases such as Flavourzyme
105 (Klompong et al., 2007; Rebeca et al., 1991). Alcalase has great ability to solubilize protein
106 and is nonspecific, with optimum temperature that ranged from 50 to 70 ºC and optimal pH
107 range from 8 to 10 that could reduce the risk of microbial contaminations (Chabeaud et al.,
108 2009).

109 2.2 Preparation of liquid protein hydrolysates

110 After thawing overnight in a cold room (4 ºC), 250 g of MDCM was minced in cutter
111 (G. Paniz, Caxias do Sul, Brazil) for 3 minutes and then mixed with 500 ml of distilled water
112 in a ratio 1:2 (w/v). Thus, 1 ml (0.4% in relation to meat amount) of the enzyme was added
113 and the mixture was kept at 60 ºC for 60 minutes in a water bath (Marconi, Piracicaba,
114 Brazil). The mixture was continuously stirred at 200 rpm with a mechanical stirrer (Marconi,
115 Piracicaba, Brazil). The pH was not adjusted during the hydrolysis process. To reduce the
116 lipid oxidation and the brownish color formation on the protein hydrolysates, four treatments
117 with Butylated Hydroxytoluene (BHT) antioxidant and curing salt were done as follows:
118 Treatment 1 (T1) was made without addition of curing salt and antioxidant; T2 was made
119 with addition of 0.01% of BHT antioxidant; T3 was made with addition of 0.25% of curing
120 salt; and T3 was made with addition of 0.01% of BHT antioxidant and 0.25% of curing salt.
121 The antioxidant and curing salt quantities were calculated based on MDCM amount used
122 (Terra, 1998).

123 Samples were collected at 5, 15, 30, and 60 minutes of hydrolysis to analyze the
124 degree of hydrolysis, pH, lipid oxidation and instrumental color measurements. The other
125 analyzes (proximate composition) were made only after 60 minutes of hydrolysis. The
126 hydrolysis reaction was terminated by placing the mixtures in a water bath at 95 ºC for 15
127 minutes.

128 2.3 Degree of hydrolysis

129 The DH of the protein hydrolysates was calculated according to percent of
130 trichloroacetic acid (TCA) ratio method (Hoyle and Merritt, 1994). At the end of each
131 hydrolysis time of 5, 15, 30 and 60 minutes, 20 ml of protein hydrolysates was taken and
132 mixed with 20 ml of 20% (w/v) TCA to produce 10% TCA soluble material. The mixtures
133 were left to stand for 30 minutes to allow precipitation and then centrifuged at 4500g for 30
134 minutes. The fat supernatant was discarded and soluble protein was analyzed for nitrogen by
135 the macro-Kjedahl method (AOAC, 2000). The DH (%) was calculated as the formula below:

$$\mathrm{DH}\ (\%) = \frac{10\%\ \mathrm{TCA} - \mathrm{soluble\ nitrogen\ in\ sample}}{\mathrm{total\ nitrogen\ in\ sample}} \times 100$$

136 2.4 pH determination

137 The pH determination was performed at hydrolysis time of 5, 15, 30 and 60 minutes
138 using a meter electrode (DM 22, Digimed, São Paulo, Brazil) for 5 minutes while the pH
139 readings were performed. The electrode was standardized against standard pH solution of 4
140 and 8.

141 2.5 Lipid oxidation analysis

142 The lipid oxidation during the hydrolysis process was estimated by Thiobarbituric
143 Acid Reacting Substance (TBARS) values, which was expressed as milligram malonaldehyde
144 (MA) equivalent/kg. In the treatments T1 and T2 the TBARS value was measured according
145 to the methodology of Raharjo et al. (1992), while in the treatments T3 and T4 was used the
146 method with the addition of sulphonilamide as proposed by Zipser and Watts (1962) due to
147 the addition of curing salt in these treatments.

148 2.6 Instrumental color determination

149 The determination of color parameters was performed in triplicate, using Chroma
150 Meter CR-300 (Minolta Camera Co. LTD, Osaka, Japan), which uses the color system *CIE* L^*
151 a^* b^*, determining the values of lightness (L^*), red (a^*) and yellow color intensities (b^*). The
152 aperture was 8 mm, illuminant D65 and 10º standard observer were used (CIE, 1978). The

153 instrument was calibrated using a white standard plate (L^* = 95.26, a^* = 0.89, b^* = 1.18). The
154 color parameters were also converted into Chroma $C^*=[(a^*)^2+(b^*)^2]^{1/2}$, which expresses the
155 purity or saturation of the color, and hue angle h^o=arctangent$[b^*/a^*]$, which indicates the color
156 nuances (Gonçalves et al, 2007; Wrosltad et al., 2005). The color parameters from raw-
157 material (MDCM) at the moment of its use were: L^* = 49.05 (± 1.32); a^* = 32.44 (± 2.34); b^*
158 = 15.86 (± 1.62); C^* = 36.13 (± 2.67); h^o = 25.97 (± 1.57).

159 2.6 Proximate composition analysis

160 After the hydrolysis process was done, the proximate composition was carried out
161 using the approved standard methods (AOAC, 2000). The moisture content was determined
162 according to oven method (930.15). Total nitrogen content was determined by using Kjedahl
163 method (984.13) and the crude protein was estimated by multiplying total nitrogen content by
164 the factor of 6.25. Ash content was determined by charring the predried sample in crucible at
165 600ºC until a white ash was formed (942.05). The total fat content of samples was extracted
166 by the Bligh and Dyer (1959) method.

167 2.8 Statistical analysis

168 Analyzes were performed in triplicate and each assay of all experiments was repeated
169 twice. All the results were expressed as means ± standard deviation and performed by range
170 analysis used SPSS version 17.0 statistical software for Windows. One-way analysis of
171 variance (ANOVA) and Tukey’s test were used to differentiate mean values and significant
172 differences.

173 **3. Results and Discussion**

174 3.1 MDCM hydrolysate production

175 In the initial stage of hydrolysis, the MDCM with water was difficult to stir due its
176 viscosity. Nevertheless, the viscosity dropped rapidly once the enzyme started to hydrolyse
177 the substrate. As a result, the stirring became easier and the hydrolysates became watery and
178 could be treated as a free flowing liquid as also observed by Abdul-Hamid et al. (2002).

179 3.2 Degree of Hydrolysis

180 The hydrolysis of MDCM with Alcalase proceeded at a high rate during the initial 30
181 minutes, which indicates that maximum cleavage of peptides occurred within 30 minutes of
182 hydrolysis. This result was similar to the reported for enzymatic hydrolysis of different
183 protein substrates such as fish (Benjakul and Morrissey, 1997; Guerard et al., 2002; Candido

184 and Sgarbieri, 2003; Dong et al., 2008; Li et al., 2011), whey (Mutilangi et al., 1995), wheat
185 gluten (Kong et al., 2007) and can be explained by one or more of the following phenomena:
186 (a) decrease in the availability of peptide bonds susceptible to hydrolysis; (b) possibility of
187 enzyme inhibition by products of the reaction; (c) partial denaturation of enzyme (Gonzáles-
188 Tello et al., 1994).

189 The DH values found at 60 minutes of hydrolysis were between 7.14 and 14.54%
190 (Table 1) and were considered satisfactory in relation to hydrolysis time and enzyme
191 concentration used compared to others studies: Abdul-Hamid et al. (2002) reported DH of
192 14.9% after 5 hours of hydrolysis from Black Tilapia (*Oreochromis mossambicus*) using
193 Alcalase and Jin et al. (2011) obtained DH of 14.7% after 24 hours of hydrolysis from
194 Bombay Duck (*Harpodon Nereus*) using papain and was able to increase this value for 19.7%
195 using Flavourzyme for more 4 hours.

196 Also, it was observed that the addition of BHT antioxidant and curing salt have a
197 negative effect in the DH. Up to 30 minutes of hydrolysis, where the values of DH from T2,
198 T3 and T4 were lower than T1 (Table 1). Nevertheless, at 60 minutes of hydrolysis the DH of
199 T1 and T4 did not differ statistically from each other but it was observed a significant
200 difference of both when compared to T2 and T3 (Table 1), where T3 showed the lower DH.
201 These results may indicate that with the addition of antioxidant or curing salt are necessary a
202 longer hydrolysis time to reach upper values of DH.

203 3.3 pH measurements

204 The evolution of protein hydrolysates pH during the hydrolysis time is shown in Table
205 2. It was possible to observe that the pH of treatments did not differ until 15 minutes of
206 hydrolysis. At 30 minutes of hydrolysis the pH was decreased significantly in T1 and T2 ($P <$
207 0.05). T3 and T4 showed a constant pH during all the hydrolysis time. In general, other
208 studies (Foh et al., 2011; Jin et al., 2011; See et al., 2011) adjust the pH values to achieve the
209 ideal conditions for the use of Alcalase enzyme. In this study, the pH was not adjusted and the
210 values of pH from hydrolysates were very similar to the original raw-material (MDCM) and
211 thus, these protein hydrolysates can be used in cooked meat products.

212 3.4 Lipid oxidation

213 TBARS values of some samples tended to increase throughout hydrolysis time
214 especially from 30 to 60 minutes of hydrolysis (Table 3). TBARS values have been used to
215 measure the concentration of relatively polar secondary reaction products, especially

216 aldehydes (Nawar, 1996). Higher temperature is known to accelerate lipid oxidation, so the
217 lipids and free fatty acids in MDCM probably underwent oxidation during hydrolysis at 60
218 ºC. The highest TBARS values were observed at 60 minutes of hydrolysis in T1, T2 and T3
219 (Table 3). The T4 TBARS values at 60 minutes differed from the others ($P < 0.05$) and in this
220 treatment the values did not differ throughout the hydrolysis time, indicating a synergistic
221 effect of the BHT and the curing salt on the lipid oxidation protection.

222 As the fat oxidation increase during the hydrolysis process is necessary to use methods
223 that delay this process. According to Khantaphant et al. (2011), the use of pretreatments,
224 especially phospholipid membranes separation, could decrease pro-oxidants and
225 phospholipids in the hydrolysates from the muscle of brownstripe red snapper (*Lutjanus*
226 *vitta*). Yarnpakdee et al. (2012) reported that the use of Trolox and EDTA was able to
227 decrease the lipid oxidation during hydrolysis from Nile tilapia (*Oreochromis niloticus*).
228 Thus, the results of our study indicate that the addition of BHT antioxidant and curing salts is
229 an effective way to prevent lipid oxidation using ingredients commonly used in meat industry.

230 Wu et al. (1991) reported that when the TBARS value is higher than 1 mg $MDA^{.}kg^{-1}$,
231 generally off-odors are formed and it is considered as the beginning of organoleptic
232 perception of lipid oxidation. With the objective to use protein hydrolysates in cooked meat
233 products, it is necessary to prevent the lipid oxidation on this raw material, since its
234 occurrence would be perceived in the final product. The TBARS values of all treatments
235 (Table 3) were lower than 1 mg $MDA^{.}kg^{-1}$ and are able to use in meat products, ensuring the
236 security and extending the shelf life.

237 The use of BHT antioxidant and curing salt was efficient to avoid the lipid oxidation
238 on hydrolysates, but the synthetic antioxidants currently used have been found to exhibit some
239 negative health effects in animals and primates (Clayson et al., 1986; Saito et al., 2003). The
240 meat industry is increasingly searching for natural solutions to minimize oxidative rancidity
241 like the use of alternative natural antioxidants and the extract from various plant species has
242 been recognized to have antioxidant activity, such as extracts from grains, oilseed, spices,
243 honey, fruits and vegetables (Chen et al., 1996; Naveena et al., 2007). The use of natural
244 antioxidants may be an alternative to reduce the use of synthetic antioxidants also in the
245 production of protein hydrolysates. However, the effectiveness of different species which
246 have this activity must be tested in this type of product.

247

248 3.5 Determination of color

249 Color has a major influence on the presentation value of a product and influences the
250 overall acceptability of food products. According to Table 4, the lightness (L^*) values
251 decreased during the hydrolysis process in all the treatments ($P < 0.05$). It was possible to
252 observe that the addition of antioxidant BHT and/or curing salt became the hydrolysates
253 lighter than T1.

254 Therefore, the longer hydrolysis time with higher temperature (60 ºC) accelerated the
255 pigments oxidation. For this reason, the T1 and T2 have a brown color, showed by the low
256 values for lightness (L^*), as in a^* after 60 minutes of hydrolysis (Table 4). Bueno-Solano et al.
257 (2009) found a brownish color in dry powder hydrolysates from shrimp and according to
258 Lario et al. (2004), this is due to the components that were generated in the Maillard reaction
259 during the drying period. Dong et al. (2008) related that as a consequence of longer hydrolysis
260 time with higher temperature, accelerate the pigments oxidation and Maillard reaction in the
261 muscle. In our study, the brownish color formation in T1 and T2 is probably resulted from the
262 oxidation of myoglobin pigment (Benjakul and Morrissey, 1997; Honikel, 2008) and,
263 according to the a^* values found in T3 and T4, it can be avoided with the curing salt addition,
264 forming nitrosylhemochrome, which gives to the hydrolysates the bright red color
265 characteristic of cured cooked meats (Moller and Skibsted, 2002).

266 The chroma (C^*) and the hue angle (h°) are both based on the a^* and b^* values. The
267 treatments T3 and T4 had a higher chroma and lower hue angle, indicating a more intense
268 reddish color than the treatments T1 and T2, and all the treatments were between 0º and 90º,
269 i.g., between the red purple and yellow.

270 3.6 Proximate Composition

271 The values of proximate composition of MDCM and the protein hydrolysates are
272 shown on Table 5. MDCM was excellent for use as a protein source for protein hydrolysates
273 production because it contained protein content greater than 30% in dry weight (Baerbel et al.,
274 1994). It is possible to observe small differences between MDCM and protein hydrolysates on
275 dry matter (protein, fat and ash). The biggest difference is in moisture content due to large
276 water amount that is added for the hydrolysates production. The proximate composition of
277 MDCM found in this work is similar to reported for Rossi et al. (2009).

278 There was no significant difference in the crude protein content in MDCM amongst
279 the hydrolysates. The hydrolysates protein content were between 35.26% (T4) and 45.53%

280 (T2) and were similar to obtained for Soares et al. (2000), which found 40.5% of protein in
281 hydrolysates from MDCM.

282 In relation to fat, the high content can be considered a negative aspect in terms of lipid
283 oxidation. A decrease of lipid content in protein hydrolysates might significantly increase the
284 stability of material towards lipid oxidation, which may also enhance the products stability
285 (Ovissipour et al., 2009; Kristinsson and Rasco, 2000). All the studies in literature reduce the
286 fat content during the centrifugal separation (Shahidi et al., 1995), which is not made in this
287 work because for use in meat products it could be affected the product flavor.

288 All protein hydrolysates showed a low ash content (between 2.42% and 2.92%) and it
289 was not significant different amongst the treatments and to the crude MCDM (Table 5). The
290 high ash content in the hydrolysates may be attributed to the addition of NaOH required for
291 pH adjustment, which was not done in this study. High ash content has been recognized as a
292 drawback of protein hydrolysates, making the applications limited in food matrix (Shahidi et
293 al., 1995).

294 **4. Conclusions**

295 MDMC can be used as a material of protein hydrolysates due to its high content of
296 protein and these hydrolysates is a promising raw material for cooked meat products
297 manufacture.

298 The MDCM protein hydrolysates had shown a satisfactory degree of hydrolysis,
299 observed by the hydrolysis time and enzyme concentration. The hydrolysates pH was similar
300 to the original raw material (MDCM), thus is possible its use in cooked meat products. In the
301 proximate composition, a little differences where found (moisture) and it is ought to be
302 considered when cooked meat products formulation. The lipid oxidation of protein hydrolysis
303 was avoided by using antioxidants and curing salt and the redness (a^*) was improved by the
304 addition of curing salt.

305 It is recommended to use the addition of antioxidants and curing salt (T4) when
306 working with MDCM protein hydrolysates to improve the overall qualities, furthermore if is
307 to be used in cooked meat products.

308 It is necessary another studies in relation to functional characteristics of MDCM
309 protein hydrolysates such as solubility, water holding capacity, oil holding capacity, foaming
310 and emulsifying properties. New studies are in development in our laboratory and meat pilot

311 plant about the applicability of MDCM protein hydrolysates in cooked meat products and
312 their influences on the final product.

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461 Table 1 – Degree of hydrolysis of MDCM protein hydrolysates with addition of BHT
462 antioxidant and curing salt at different times, in %.

Treatments	Time of Hydrolysis			
	5 minutes	15 minutes	30 minutes	60 minutes
T1	8.10 ± 0.32^{cA}	11.68 ± 0.58^{bA}	13.98 ± 0.47^{aA}	14.54 ± 0.13^{aA}
T2	4.89 ± 0.96^{cB}	6.31 ± 0.85^{bcB}	8.06 ± 0.17^{bC}	12.35 ± 0.37^{aB}
T3	4.92 ± 0.39^{aB}	5.01 ± 1.30^{aB}	5.45 ± 1.0^{aD}	7.14 ± 1.24^{aC}
T4	6.11 ± 0.11^{cB}	5.94 ± 0.69^{cB}	12.16 ± 0.13^{bB}	14.38 ± 0.09^{aA}

463 Values are given as mean ± SD (n=3).

464 [a] Different letters within the same row indicate significant difference ($P < 0.05$).

465 [A] Different capital letters within the same column indicate significant difference ($P < 0.05$).

466 T1 – without addition of BHT antioxidant and curing salt; T2 – with addition of 0.01% of
467 BHT antioxidant; T3 – with addition of 0.25% of curing salt; T4 – with addition of 0.01% of
468 BHT antioxidant and 0.25% of curing salt.

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485 Table 2 – Effect of addition of BHT antioxidant and curing salt on pH of MDCM protein
486 hydrolysates at different hydrolysis times.

Treatments	Time of Hydrolysis			
	5 minutes	15 minutes	30 minutes	60 minutes
T1	6.06 ± 0.09^{aA}	6.04 ± 0.08^{aA}	5.88 ± 0.04^{bBC}	5.83 ± 0.07^{bBC}
T2	5.99 ± 0.14^{aA}	6.02 ± 0.19^{aA}	5.82 ± 0.04^{bcC}	5.74 ± 0.07^{cC}
T3	6.02 ± 0.14^{aA}	5.99 ± 0.09^{aA}	5.95 ± 0.08^{aB}	5.91 ± 0.04^{aAB}
T4	6.15 ± 0.16^{aA}	6.07 ± 0.09^{aA}	6.09 ± 0.10^{aA}	6.00 ± 0.08^{aA}

487 Values are given as mean ± SD (n=3).

488 [a] Different letters within the same row indicate significant difference ($P < 0.05$).

489 [A] Different capital letters within the same column indicate significant difference ($P < 0.05$).

490 T1 – without addition of BHT antioxidant and curing salt; T2 – with addition of 0.01% of
491 BHT antioxidant; T3 – with addition of 0.25% of curing salt; T4 – with addition of 0.01% of
492 BHT antioxidant and 0.25% of curing salt.

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510 Table 3 – TBARS values of MDCM protein hydrolysates with addition of BHT antioxidant
511 and curing salt at different hydrolysis times.

Treatments	Time of Hydrolysis			
	5 minutes	15 minutes	30 minutes	60 minutes
T1	0.12 ± 0.03^{bA}	0.11 ± 0.06^{bA}	0.16 ± 0.09^{bAB}	0.36 ± 0.06^{aA}
T2	0.21 ± 0.09^{bA}	0.25 ± 0.07^{bA}	0.34 ± 0.05^{bA}	0.57 ± 0.06^{aA}
T3	0.05 ± 0.02^{cA}	0.04 ± 0.03^{cA}	0.29 ± 0.05^{bA}	0.54 ± 0.07^{aA}
T4	0.09 ± 0.02^{aA}	0.05 ± 0.02^{aA}	0.05 ± 0.09^{aB}	0.05 ± 0.00^{aB}

512 Values are given as mean ± SD (n=3).

513 Expressed as mg malonaldehyde/kg.

514 [a] Different letters within the same row indicate significant difference ($P < 0.05$).

515 [A] Different capital letters within the same column indicate significant difference ($P < 0.05$).

516 T1 – without addition of BHT antioxidant and curing salt; T2 – with addition of 0.01% of
517 BHT antioxidant; T3 – with addition of 0.25% of curing salt; T4 – with addition of 0.01% of
518 BHT antioxidant and 0.25% of curing salt.

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Table 4 – Effects of addition of BHT antioxidant and curing salt on lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*) and hue angle (h^o) of MDCM protein hydrolysates at different hydrolysis times.

		Time of Hydrolysis			
		5 Minutes	15 Minutes	30 Minutes	60 Minutes
L^*	T1	54.45 ± 1.01^{aA}	52.24 ± 0.79^{abB}	51.26 ± 2.26^{bAB}	47.16 ± 1.40^{cB}
	T2	55.99 ± 1.37^{aA}	54.68 ± 2.68^{abAB}	48.76 ± 4.86^{bB}	52.92 ± 3.24^{abA}
	T3	56.61 ± 0.50^{aA}	57.51 ± 2.66^{aA}	54.98 ± 1.79^{aA}	51.37 ± 1.15^{bA}
	T4	56.72 ± 1.82^{aA}	54.86 ± 1.17^{abAB}	54.33 ± 1.01^{bA}	50.78 ± 0.70^{cA}
a^*	T1	19.41 ± 0.30^{aA}	17.71 ± 0.47^{bA}	13.28 ± 0.56^{cAB}	10.70 ± 0.14^{dB}
	T2	16.13 ± 0.44^{aB}	13.49 ± 0.66^{bB}	9.60 ± 1.01^{cC}	8.41 ± 0.80^{cB}
	T3	12.47 ± 0.38^{bC}	11.72 ± 1.08^{bC}	12.54 ± 0.69^{bB}	15.91 ± 2.81^{aA}
	T4	12.53 ± 0.48^{cC}	12.42 ± 0.15^{cB}	14.04 ± 0.28^{bA}	16.08 ± 0.22^{aA}
b^*	T1	15.47 ± 0.59^{aA}	14.45 ± 0.84^{aB}	14.33 ± 1.40^{aB}	13.74 ± 0.99^{aC}
	T2	15.71 ± 0.44^{abA}	15.32 ± 0.67^{bB}	11.19 ± 2.14^{cC}	17.82 ± 0.51^{aA}
	T3	16.54 ± 0.52^{aA}	17.52 ± 0.60^{aA}	17.00 ± 0.64^{aA}	17.06 ± 0.54^{aAB}
	T4	15.77 ± 1.38^{aA}	15.81 ± 0.93^{aB}	17.20 ± 0.55^{aA}	16.51 ± 0.55^{aB}
C^*	T1	24.83 ± 0.45^{aA}	22.86 ± 0.77^{bA}	19.55 ± 1.11^{cC}	17.41 ± 0.75^{dC}
	T2	22.78 ± 0.28^{aB}	20.40 ± 0.10^{bB}	18.96 ± 0.56^{dC}	19.71 ± 0.36^{cB}
	T3	20.71 ± 0.58^{bC}	21.10 ± 0.42^{bB}	21.14 ± 0.23^{bB}	23.38 ± 2.17^{aA}
	T4	20.15 ± 1.39^{aC}	20.11 ± 0.69^{aB}	22.21 ± 0.27^{aA}	23.04 ± 0.35^{aA}
h^o	T1	38.4 ± 1.1^{cC}	39.1 ± 1.4^{cC}	47.1 ± 3.0^{bB}	52.0 ± 2.1^{aB}
	T2	44.7 ± 1.3^{cB}	48.7 ± 2.6^{cB}	53.6 ± 2.3^{bB}	64.7 ± 2.5^{aA}
	T3	53.0 ± 0.8^{abA}	56.3 ± 3.1^{aB}	53.6 ± 2.4^{aA}	47.3 ± 4.8^{bBC}
	T4	51.5 ± 1.8^{aA}	51.4 ± 2.5^{aA}	50.8 ± 1.4^{aB}	45.7 ± 1.2^{bC}

Values are given as mean ± SD (n=3).

[a] Different letters within the same row indicate significant difference ($P < 0.05$).

[A] Different capital letters within the same column indicate significant difference ($P < 0.05$).

T1 – without addition of BHT antioxidant and curing salt; T2 – with addition of 0.01% of BHT antioxidant; T3 – with addition of 0.25% of curing salt; T4 – with addition of 0.01% BHT antioxidant and 0.25% of curing salt.

545 Table 5 – Changes on proximate composition of raw-material and MDCM protein
546 hydrolysates with addition of BHT antioxidant and curing salt.

	Treatments				
	MDCM	T1	T2	T3	T4
Dry Matter	30.01	11.23	14.56	13.06	12.39
Moisture	69.99±0.61[c]	90.77±0.23[a]	87.44±1.08[b]	86.94±1.43[b]	87.62±1.42 [b]
Protein[d]	39.63±0.38[ab]	38.55±0.33[ab]	45.53±3.89[a]	39.33±1.87[ab]	35.26±4.22[b]
Fat[d]	57.70±0.40[ab]	58.78±0.16[ab]	52.05±3.83[b]	57.90±1.82[ab]	61.97±4.46[a]
Ash[d]	2.68±0.04[a]	2.67±0.15[a]	2.42±0.04[a]	2.92±0.24[a]	2.77±0.24[a]

547 Values are given as mean ± SD (n=3).

548 [d] Values in dry matter.

549 [a] Different letters within the same row indicate significant difference ($P < 0.05$).

550 T1 – without addition of BHT antioxidant and curing salt; T2 – with addition of 0.01% of
551 BHT antioxidant; T3 – with addition of 0.25% of curing salt; T4 – with addition of 0.01% of
552 BHT antioxidant and 0.25% of curing salt.

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3.2 Manuscrito 2

Effect of protein hydrolysate from mechanically deboned chicken meat on quality properties of reduced-fat mortadella-type sausages

Manuscrito em fase final de revisão para ser submetido à revista *Meat Science*.

1 **Effect of protein hydrolysate from mechanically deboned chicken meat on quality**
2 **properties of reduced-fat mortadella-type sausages**

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29 **Abstract**

30 This study had as objective to test the use of different levels of liquid protein
31 hydrolysate from Mechanically Deboned Chicken Meat (MDCM) in a reduced-fat mortadella-
32 type sausage. Four treatments were made containing 0, 10, 20 and 30% of protein hydrolysate
33 in proportional substitution to raw MDCM. The proximate composition, pH, lipid oxidation
34 and colorimetric, microbiological, sensorial and texture characteristics were evaluated during
35 60 days of storage at 4º C. The proximate composition, pH and microbiological characteristics
36 were considered normal for this kind of product. The values of lipid oxidation of products
37 containing MDCM protein hydrolysate increased up to 30th day of storage with subsequent
38 decrease until the end of the storage period (60 days). The products containing MDCM
39 protein hydrolysate had low values of lightness (L^*) and redness (a^*), higher lipid oxidation
40 and soft texture, evidenced both by the panelists and by instrumental texture profile. The
41 addition of up to 10% protein hydrolysate proved to be viable in the production of reduced-fat
42 mortadella-type sausage, showing quality characteristics closer to the control treatment.

43 **Key-words:** protein hydrolysate, mortadella-type sausage, texture properties, mechanically
44 deboned chicken meat, fat reduction.

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59 **1. Introduction**

60 Mortadellas are non-fermented, emulsion type cooked sausages composed of water,
61 muscle proteins, fat particles, salt and small amounts of non-meat ingredients, where the meat
62 proteins serve as natural emulsifier. In this group of processed meat products, fat and protein
63 concentration and their chemical interactions, especially those occurring during the
64 emulsification process, exert a marked impact on final product quality (Nieto et al., 2009).

65 The Mechanically Deboned Chicken Meat (MDCM) is a principal ingredient used in a
66 meat products elaboration. In Brazil, according to the legislation, it is possible to use up to
67 60% of MDCM in cooked meat sausages (Brasil, 2000). However, there may be some
68 drawbacks in the usage of MDCM. A rapid onset of oxidative stability resulting in off-flavors
69 and off-odors is a major problem for products manufactured with MDCM (Mielnik et al.,
70 2002).

71 Several studies have shown the antioxidant potential of protein hydrolysates from
72 many raw materials such as potato, whey (Peña-Ramos & Xiong, 2003; Wang & Xiong,
73 2005; Contreras et al., 2011), fish (Dekkers et al., 2011; Khaled et al., 2011; Khantaphant et
74 al., 2011; Li et al., 2011; Yang et al., 2011), shrimp waste (Dey & Dora, 2011) and
75 hemoglobin porcine (Sun et al., 2011; Sun et al., 2012). Protein hydrolysates may possess
76 physicochemical characteristics and bioactivities not found in the original proteins, such as
77 antioxidant activity and greater water-holding capacity (Cumby et al., 2008). The generated
78 peptides could inhibit the harmful changes induced by lipid oxidation, due to the presence of
79 certain amino acid residues, such as tyrosine, methionine, histidine, tryptophan and proline.
80 Even more, studies have been demonstrated that shrimp shell hydrolysate can inhibit human
81 cancer cell proliferation (Kannan et al., 2011) and poultry bone and meat trimming decrease
82 serum cholesterol (Hosomi et al., 2011).

83 The meat industry is constantly undergoing transformations, driven mainly by
84 consumer. Formulation of healthier meat products based on processing strategies is one of the
85 most important current approaches to the development of potential meat-based functional
86 foods (Lopez-Lopez et al., 2009a; Lopez-Lopez et al., 2009b). Particularly, health concerns
87 about fat consumption and changes in consumer's preferences have led to extensive research
88 on low-fat meat products (Carrapiso, 2007; Kumar & Sharma, 2004; Pinero et al., 2008; Yang
89 et al., 2007).

90 Thus, the aim of this work was to use the MDCM protein hydrolysate as a substitute
91 for the raw MDCM in the different concentrations of reduced-fat mortadella-type sausages
92 elaboration.

93 **2. Materials and Methods**

94 2.1 Production of MDCM protein hydrolysate

95 The MDCM was supplied in a freeze form by a large poultry processing plant. The
96 enzyme utilized was Alcalase 2.4L FG, an endopeptidase from *Bacillus licheniformis*,
97 provided by Novozymes Latin America® (Araucária, Paraná, Brazil). After thawing overnight
98 in a cold room (4 ºC), 250 g of MDCM was minced in cutter (G. Paniz, Caxias do Sul, Brazil)
99 for 3 minutes and then, mixed with distilled water in a proportion of 1:2 (w/v). Thus, 1 ml
100 (0.4% in relation to meat amount) of the enzyme, 0.25% of curing salt and 0.01% of BHT
101 antioxidant were added and the mixture was held at 60 ºC for 60 minutes in a water bath
102 (Marconi, Piracicaba, Brazil). The mixture was continuously stirred at 200 rpm with a
103 mechanical stirrer (Marconi, Piracicaba, Brazil). The pH was not adjusted during the
104 hydrolysis process to maintain the original MDCM pH. The degree of hydrolysis obtained
105 was 14.38% and the hydrolysis reaction was terminated by placing the mixtures at boiling
106 water for 15 minutes.

107 2.2 Reduced-fat mortadella-type sausage elaboration

108 The pork meat and fat were obtained from a local slaughterhouse and frozen
109 separately at -18 ºC. Before use, it was maintained at 4 ºC for approximately 18 hours.
110 Batches of reduced-fat mortadella-type sausages were formulated with different
111 concentrations of MDCM protein hydrolysate as follow: 0%, (TC), 10% (T1), 20% (T2) and
112 30% (T3). Each batch was prepared using a typical Brazilian formula as shown in Table 1.
113 Subsequently, the following ingredients were added: salt (1.9%), ice/water (29.0%) (the water
114 amount was adjusted as the quantity of MDCM protein hydrolysate added), isolated soy
115 protein (2.0%), spices (1.0%), curing salt (0.35%), polyphosphate (0.25%), sodium
116 erythorbate (0.25%), cassava starch (5.0%) and black pepper (0.1%). The total amount of
117 each treatment was 6 kg and the batters were stuffed using 80 mm diameter water
118 impermeable plastic casings. Sausages were cooked at 80 ºC for 90 min, until to reach 72 ºC
119 in the inner part. After they were cooled in current water, storage at 4 ºC and analyzed every
120 15 days up to the end of storage period.

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122 2.3 Weight losses

123 The sausages weight was estimated before and after the cooking process to determine
124 the weight yield. It was also recorded after 60 days of storage under refrigeration at 4 ºC and
125 expressed as a percentage of the initial weight, according to Lee et al. (2008). The
126 measurements were made by triplicate for each batch.

127 2.4 Proximate composition

128 The proximate composition was carried out using the approved standards methods
129 (AOAC, 2000). The moisture content was determined according to oven method. The total
130 crude protein content was determined using *Kjedahl* method. A nitrogen conversion factor of
131 6.25 was used for calculation of crude protein content of samples. Ash content was
132 determined by charring the predried sample in crucible at 600ºC until a white ash was formed.
133 The total fat content of samples was extracted by the Bligh and Dyer (1959) method.
134 Carbohydrates were calculated from the difference. The calorific value (kcal) was calculated
135 using the values corresponding to fat (9 kcal/g), protein (4.02 kcal/g) and carbohydrates (3.87
136 kcal/g).

137 2.5 pH measurement

138 The pH was determined each 15 days of storage using 10 g of each sample and
139 homogenizing with 100 ml of distilled water (1:10) (w/v) using a meter electrode (DM 22,
140 Digimed, São Paulo, Brazil) for 5 minutes while the pH readings were performed. The
141 electrode was standardized against standard pH solution of 4 and 8.

142 2.6 Lipid oxidation

143 The lipid oxidation was evaluated using the thiobarbituric acid reactive substances
144 (TBARS) method (Raharjo, Sofos & Schmidt, 1992), with the addition of sulphonilamide,
145 proposed by Zipser and Watts (1962) and was determined each 15 days of storage.

146 2.7 Instrumental color evaluation

147 The determination of color parameters was performed in quintuplicate each 15 days of
148 storage, using Chroma Meter CR-300 (Minolta Camera Co. LTD, Osaka, Japan), which uses
149 the color system *CIE* L^* a^* b^*, determining the values of lightness (L^*), redness (a^*) and
150 yellowness (b^*). The aperture was 8 mm, illuminant D65 and 10º Standard observer were used
151 (CIE, 1978). The instrument was calibrated using a white standard plate (L^* = 95.26, a^* =
152 0.89, b^* = 1.18). The color parameters measured were also converted into Chroma

153 $C^*=[(a^*)^2+(b^*)^2]^{1/2}$, which expresses the purity or saturation of the color, and hue angle
154 h^o=arctangent$[b^*/a^*]$, which indicates the color nuances (Gonçalves et al., 2007; Wrosltad et
155 al., 2005).

156 2.7 Microbiological analysis

157 Two sausages per batch were used to evaluate the microbiological characteristics
158 according to the methodology described by Vanderzant and Splittstoesser (1992). Mesophilic
159 aerobic bacteria (MAB) were analyzed in Plate Count Agar (PCA), incubated at 37 ºC for 24
160 h. Lactic acid bacteria (LAB) were quantified using De Man, Rogosa and Sharpe (MRS) agar
161 at 37 ºC for 48h. The total coliforms were quantified in crystal-violet neutral-red bile agar at
162 37 ºC for 24 h. Termotolerant coliforms were quantified in EC broth at 45 ºC for 48 h.
163 *Staphylococcus aureus* was carried out using Baird Parker agar with egg yolk tellurite
164 emulsion and incubation at 37 ºC for 48 h. *Salmonella* were analyzed to detect presence or
165 absence in *Salmonella Shigella* agar incubated at 37 ºC for 24 h after an initial enrichment
166 with Buffered peptone water and a secondary enrichment with Rappaport-Vassiliadis broth.
167 *Clostridium* sulphite-reducer count in the samples was performed by the plate technique using
168 sulfite polymyxin sulfadiazine (SPS) agar. The plate samples were kept in anaerobic jar and
169 incubated at 37 ºC for 48 h. MAB and LAB were analyzed on the day of production and each
170 15 days of storage while the others microorganisms were analyzed on the day of production
171 and after 60 days of storage. All culture media used for microbiological analyses belonged to
172 Himedia (Himedia Ltd., Curitiba, Brazil).

173 2.8 Sensory analysis

174 The sensory evaluation was made after the microbiological safety has been assessed.
175 This study protocol was approved by the Ethics in Research Committee of The Federal
176 University of Santa Maria (RS, Brazil) under the number 01228612.6.0000.5346. The sensory
177 analysis was conducted by 52 untrained panelists recruited among students, faculty and staff
178 members from the University Campus whose ages ranged from 18 to 50 years old. They were
179 asked to express their opinion about the color, aroma, taste, texture and appearance of the
180 product. All data were recorded on a questionnaire designed to indicate the degree of
181 likeability for each sample using a structured scoring scale of seven points (1=disliked
182 extremely and 7=liked extremely). The panelists also expressed their opinion in relation to the
183 purchase future intention, in a structured scoring scale of five points (1=certainly not purchase
184 and 5=certainly purchase) (Meilgaard, Civille, & Carr, 1999).

185 2.9 Textural profile analysis

186 Texture profile analysis (TPA) was performed in a TA-XT.plus Texture Analyzed and
187 Texture Expert Exponent Software (Stable Microsystems Ltd., Surrey, England). The cooked
188 sausages were prepared (diameter 2.0 cm, height 1.5 cm) and a double compression cycle test
189 was performed up to 50% compression of the original portion height with an aluminum
190 cylinder probe of 45 mm diameter. For each batch, nine determinations were made. The
191 conditions of texture analysis were as follows: pre-test speed 1 mm/s, post-test speed 5 mm/s,
192 force 1 g, trigger 5 g. The following parameters were quantified: hardness, springiness,
193 cohesiveness, gumminess and chewiness (Bourne, 1978).

194 2.10 Statistical analysis

195 Each assay of all experiments was performed in duplicate. All the results were
196 expressed as means ± standard deviation and performed by range analysis used SPSS version
197 17.0 statistical software for Windows. One-way analysis of variance (ANOVA) and Tukey's
198 test were used to differentiate mean values and significant differences.

199 **3. Results and Discussion**

200 3.1 Weight losses

201 The type of casing used in mortadella-type sausage is a very important factor to avoid
202 the weight losses occurring during the manufacture and storage of cooked sausages. Losses up
203 to 5% have ever been described in cooked sausages vacuum-packaged in polyethylene bags
204 after two months of storage under refrigeration (Grigelmo et al., 1999). In the experiments
205 described here, no weight losses were observed after the cooking procedure. After 2 months
206 of storage, minimal weight losses were observed (between 0.45 and 0.85%) (data not shown).
207 Similar results were observed by Caceres et al. (2008) using Multibar casings in *Mortadella*.

208 3.2 Proximate composition

209 The proximate composition and calorific value of the cooked sausages made with
210 different levels of MDCM protein hydrolysate and fat reduction are presented in Table 2. The
211 moisture content of the sausages made with MDCM protein hydrolysate were between 65.41
212 and 66.98% and was significantly lower ($P < 0.05$) than the control samples (68.22%). In
213 relation to the protein content, the values were between 10.63 and 12.00% (Table 2).
214 Nevertheless, the protein content was higher in the treatments with protein hydrolysate
215 showing that the addition of protein hydrolysate can be an alternative to improve the protein
216 content in meat products.

217 The values of fat ranged from 10.41 and 11.71% and it was possible to observe
218 differences between the treatments, showing that the addition of protein hydrolysates
219 increased the fat content. However, all the fat contents observed in the results (Table 2) were
220 similar to the percentage of fat that was added in the formulation (10%).

221 The calorific values were between 168.13 kcal/100g (Control) and 183.13 kcal/100g
222 (T2) and the addition of higher levels of protein hydrolysate (T2 and T3) significantly
223 increased ($P < 0.05$) these values. Nevertheless, the fat-reduced sausages with the addition of
224 MDCM protein hydrolysate showed a lower calorific value compared to traditional sausages.
225 Caceres et al. (2008) found calorific values between 191 and 308 kcal/100 g of *mortadella*
226 with fat reduction up to 40% and Mohammadi and Oghabi (2012) reported calorific values
227 ranged from 117 to 203 kcal/100g in a beef cooked sausage with fat reduction up to 60%. As
228 there was no fat removal from MDCM protein hydrolysates, the addition of higher quantities
229 of protein hydrolysates reflected in cooked meat sausages with higher fat content and
230 consequently, higher calorific value. The treatments T2 and T3 had a higher calorific value (P
231 < 0.05) compared to the Control and T1, and this is due to the higher fat and protein content
232 in these treatments that higher levels of protein hydrolysate was added.

233 The carbohydrate content did not change between the treatments and the total ash
234 increased in the treatments with higher levels of MDCM protein hydrolysate ($P < 0.05$). Since
235 curing salt were added to the MDCM protein hydrolysate elaboration to avoid the brownish
236 color formation thus, probably the higher levels of MDCM protein hydrolysates in the
237 treatments resulted in higher levels of cured salt and consequently higher levels of ash (Table
238 2). The proximate composition and calorific value are similar to those found by Herrero et al.
239 (2008), Ventanas et al. (2010), Hayes et al. (2011) and Terns et al. (2011) working with
240 different types of emulsified cooked sausages.

241 3.3 pH measurement

242 The evolution of pH during the storage of reduced-fat mortadella-type sausages is
243 presented in Table 3. The results at day 0 ranged from 6.65 to 6.72 not showing significant
244 difference among the treatments. During the 60 days of storage, there were some variations in
245 pH values between treatments. Nevertheless, all results are within the normal range for such
246 products. At day 60 there was difference ($P < 0.05$) between all the treatments, even though
247 the values are close to those observed at day 0. The higher pH value observed in control (TC)
248 probably is due to higher amount of raw MDCM in the mortadella-type sausages formulation

(Table 1). Similar results were reported by Herrero et al. (2008) that observed pH values among 6.61 and 6.85 when analyzing different types of mortadella.

3.4 Lipid oxidation

During the storage period, the TBARS values differed significantly ($P < 0.05$) (Table 4). For the control treatment (TC) the TBARS values did not differed throughout the storage period. However, in the treatments with addition of MDCM protein hydrolysates were possible to observe an increase in TBARS values up to 30 days of storage with a decrease in the days 45 and 60. At the end of storage period the TC, T1 and T2 did not show significant difference ($P < 0.05$) among each other, while the T3 had the highest TBARS value. These results agree with Georgantelis et al. (2007) who observed the highest amount of MDA in pork sausage after 15 days of storage at 4 ºC and then it decreased. The same behavior was reported by Ghiretti et al. (1997). This reduction of TBARS values is due to MDA decomposition by some microorganisms (Smith and Alford, 1968; Moerck and Ball, 1974), further oxidation of MDA to other products like alcohols and acids, which can not react with thiobarbituric acid (Fernández et al., 1997).

Overall the reduced-fat mortadella-type sausages showed good oxidative stability during 60 days of storage (maximum TBARS value = 0.688). Hayes et al. (2011) observed values of up to 1.49 MDA·kg^{-1} in cooked sausages stored under aerobic conditions and in modified atmosphere packs for 21 days.

Wu et al. (1991) reported that when the TBARS value is higher than 1 mg MDA·kg^{-1}, generally off-odors are formed and it is considered as the beginning of organoleptic perception of lipid oxidation. Thus, assuming this value as a rancidity detection threshold, we can state that all treatments did not achieved this value even at 60 days of storage.

Several studies had shown that protein hydrolysates have an important antioxidant capacity (Klompong et al., 2007; Dong et al., 2008; Khantaphant et al., 2011). The antioxidant properties of protein hydrolysates typically result from their capability to stabilize or terminate radicals, donate a hydrogen atom, and/or chelate pro-oxidative metal ions. Hydrolysis of the polypeptide into various portions increases the accessibility of amino acids to the solvent, which results in increased radical-scavenging and metals chelating activity (Amarowicz, 2008). According to Raghavan & Kristinsson (2008), the capability of different protein hydrolysates to chelate metal ions depends on the enzymes used in hydrolysis and the degree of hydrolysis of the hydrolysate. To obtain protein hydrolysates with better antioxidant

281 capacity, it is necessary to apply methods that selected and concentrate peptides which
282 possess antioxidant activity. Peña-Ramos & Xiong (2003) and Wang & Xiong (2005) could
283 inhibit fat oxidation in patties using soy and whey, and potato hydrolysates, respectively.
284 Nevertheless, all this studies have been use freeze-dried during the hydrolysates preparation.

285 3.5 Color measurements

286 The color parameters of different reduced-fat mortadella-type sausages made with
287 MDCM protein hydrolysate and fat reduction are shown in Table 5. The lightness (L^*) values
288 are between 56.98 and 63.04 at day 0, being the T3 treatment darker ($P < 0.05$) than the
289 others. Nevertheless, the same behavior was not observed in the other days of storage. At the
290 day 60, the values are between 58.66 and 63.36, being the T2 treatment lighter ($P < 0.05$) than
291 the others. Fat reduction produces darker sausages as described in mortadella (Cáceres et al.,
292 2004; Cáceres et al., 2006) and other different cooked meat products (Claus & Hunt, 1991;
293 Grigelmo et al., 1999; Troutt et al., 1992).

294 The redness parameter (a^*) of the control treatment (TC) were higher than the
295 treatments with MDCM protein hydrolysate during all days of storage (Table 5). According to
296 Bueno-Solano et al. (2009), the protein hydrolysis produces peptides with brownish color and
297 the addition of large quantities can shift this to the product and this may be the main reason
298 for the treatments containing MDCM protein hydrolysate to show lower values of a^*. At 60
299 days of storage, the redness values of the TC significantly decreased ($P < 0.05$), while the
300 other treatments (T1, T2 and T3) did not significantly differed from day zero.

301 In relation to the yellowness parameter (b^*), the TC had the higher value at day 0
302 among all the treatments (Table 5) and remained constant up to the end of storage period
303 without significant difference between the days 0 and 60. In the treatments containing MDCM
304 protein hydrolysates, the yellowness parameter was significantly increasing during the storage
305 period and at 60 days of storage, the T2 showed the highest b^* values ($P < 0.05$).

306 The chroma (C^*) and the hue angle (h^*) are both based on the a^* and b^* values. The
307 control treatment (TC) had a higher chroma and lower hue angle, indicating a more intense
308 reddish color than the treatments containing MDCM protein hydrolysates. However, all the
309 treatments are losing the intense reddish color during the storage period.

310

3.6 Microbiological analysis

The microbiological characteristics of reduced-fat mortadella-type sausages made with MDCM protein hydrolysate are shown in Table 6. Few alterations in the growth of LAB and MAB were observed during storage period of mortadella-type sausages. In relation to the LAB, there was no significant difference between the treatments up to the day 45 of storage. At day 60, the TC showed lower counts of LAB ($P < 0.05$) when compared to other treatments. The storage not changed the LAB counts in the control treatment and T1. However, it was observed a count reduction at day 60 for the T2 and T3 treatments. An increased in the count of MAB was observed in TC during the storage being higher ($P < 0.05$) than the others treatments at 60 days of storage. This increase in MAB counts was not observed in treatments containing MDCM protein hydrolysates and the values were not significantly different during the storage period.

The values of total coliforms, thermotolerants coliforms, *Staphylococcus aureus*, *Clostridium* sulphite-reducer and *Salmonella* were very low or absent in all treatments (Table 6). The excellent microbiological quality of the products was due to the quality of raw materials used, in combination with good practices in preparing the products and also the cooking process was effective to reduce the bacterial count.

3.7 Sensory evaluation

The increase amount of MDCM protein hydrolysate added to the treatments changed the sensorial characteristics of reduced-fat mortadella-type sausages (Table 7). In relation to color and taste, the addition of MDCM protein hydrolysate in reduced-fat mortadella-type sausage above 20% caused a rejection by the panelists. According to Bueno-Solano et al. (2009), the protein hydrolysis produces peptides with brownish color and the addition of large quantities can shift this to the product. Even more, FitzGerald and O'Cuinn (2006) and Spellman et al. (2009) report that the major disadvantage of protein hydrolysate is the occurrence of bitter peptides. The bitter taste of protein hydrolysates limits their utilization for human consumption. It is reported that the bitter taste of protein hydrolysates is mainly caused by hydrophobic amino acid residues and oligopeptides, and therefore the specificity of the enzyme used is decisive for the amount of such peptides produced during hydrolysis (Kim and Li-Chan, 2006; Gildberg et al., 2002). At present there are many methods to limit the formation of bitterness, such as plastein reaction, controlling the degree of hydrolysis, masking by cyclodextrin, etc. (Nilsang et al., 2005). However, all these methods have various limitations for application, including need for specific equipment, low protein recovery yield,

344 loss of essential functions of peptides, and high cost (FitzGerald and O'Cuinn, 2006). Thus, in
345 this study, the brownish color formation and the bitterness taste are the main reasons for the
346 rejection of products containing higher levels of protein hydrolysates.

347 The panelists also reported that the treatments with high amount of protein hydrolysate
348 (T2 and T3) showed a softer texture than the control. The values for the attributes of odor and
349 appearance and the future purchase intention also reflected the same behavior of others,
350 especially by those that changed the color, texture and appearance.

351 3.8 Texture profile analysis

352 The results of the textural profile analysis in reduced-fat mortadella-type sausages are
353 shown in Table 8**.** In this study, the addition of MDCM protein hydrolysate in reduced-fat
354 mortadella-type sausages caused a significant decrease in all texture parameters. According to
355 Mittal and Barbut (1994), a fat decrease produces an increase in the hardness of cooked
356 sausages. However, there are other studies in which the results obtained lower hardness values
357 in reduced-fat products (Cáceres et al., 2008; Cáceres et al., 2006; Cáceres et al., 2004;
358 Grigelmo et al., 1999).

359 The hardness values (Table 8) were between 27.128 and 3.927 and there was a
360 significant difference ($P < 0.05$) between the control treatment (TC) and the others with
361 MDCM protein hydrolysate addition, but no significant difference between T2 and T3. The
362 control treatment had a higher value than the others and the reduction in hardness was
363 proportional to the amount of MDCM protein hydrolysate added.

364 Springiness represents the extent of recovery of sausage height and sometimes is
365 referred to "elasticity" (Yang et al., 2007). The springiness values also showed difference ($P <$
366 0.05) as the addition of protein hydrolysate was increased in the sausages elaboration (Table
367 8). The secondary parameters of cohesiveness, gumminess and chewiness behaved similarly
368 to those parameters showing a decrease as the MDCM protein hydrolysate levels increased.
369 Lower values recorded for hardness, gumminess and chewiness of cooked meat
370 sausages with the addition of MDCM protein hydrolysates may be associated with weaker
371 internal structure of hydrolysate than the MDCM. Yang et al. (2007) have pointed out that a
372 decrease in hardness by the addition of tofu in low fat sausages is related to weaker internal
373 structure of tofu than pork loin.

374

375

376 **4. Conclusions**

377 The MDCM liquid protein hydrolysate proved a viable alternative as raw material for
378 the reduced-fat mortadella-type sausages manufacture. The treatment containing 10% (T1) of
379 hydrolysate showed little characteristic changes compared to the control, being the T1 the
380 most indicated for the reduced-fat mortadella-type sausage production. Although, the products
381 had a good acceptability, showed great microbiological characteristics and a stable lipid
382 oxidation throughout the 60 days of storage.

383 Due to the soft texture of the reduced-fat mortadella-type sausages, an alternative to
384 addition of MDCM protein hydrolysates in meat products would be application in patties.
385 Thus, another alternative would be to use the MDCM protein hydrolysate in powder form.

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557 Table 1 – Formulation of reduced-fat mortadella-type sausages produced with different levels
558 of MDCM protein hydrolysate (in %).

Ingredients	Treatments			
	TC	T1	T2	T3
MDCM	60.0	50.0	40.0	30.0
MDCM protein hydrolysate	-	10.0	20.0	30.0
Pork meat	30.0	30.0	30.0	30.0
Pork fat	10.0	10.0	10.0	10.0

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Table 2 – Proximate composition and calorific value of fat-reduced mortadella-type sausages produced with different levels of MDCM protein hydrolysate.

	TC	T1	T2	T3
Moisture	68.22±0.10[a]	66.98±0.25[b]	65.41±0.17[c]	65.41±0.07[c]
Protein	10.63±0.33[c]	11.09±0.52[bc]	12.00±0.20[a]	11.49±0.09[ab]
Fat	10.66±0.33[b]	10.41±0.36[b]	11.71±0.09[a]	11.58±0.24[a]
Carbohydrate	7.62±0.58[a]	8.40±0.36[a]	7.63±0.26[a]	8.04±0.31[a]
Ash	2.88±0.02[d]	3.12±0.00[c]	3.26±0.02[b]	3.48±0.03[a]
Calorific Value (kcal)	168.13±1.88[b]	170.75±2.68[b]	183.13±1.08[a]	181.55±1.39[a]

Values are given as mean±SD (n=3).

[a]Different letters within the same row indicate significant difference ($P < 0.05$).

Control Treatment (TC) – without addition of protein hydrolysate; T1 – 10% addition of protein hydrolysate; T2 – 20% addition of protein hydrolysate; T3 – 30% addition of protein hydrolysate.

Table 3 – pH values of reduced-fat mortadella-type sausages produced with different levels of MDCM protein hydrolysate.

	TC	T1	T2	T3
Day 0	6.72±0.04^{aAB}	6.66±0.04^{aA}	6.65±0.03^{aA}	6.66±0.08^{aAB}
Day 15	6.54±0.09^{bcB}	6.51±0.09^{cB}	6.64±0.07^{bA}	6.74±0.05^{aA}
Day 30	6.69±0.10^{aAB}	6.60±0.05^{bcA}	6.59±0.10^{cAB}	6.67±0.05^{abAB}
Day 45	6.50±0.32^{aB}	6.24±0.02^{bC}	6.33±0.06^{abC}	6.42±0.07^{abC}
Day 60	6.79±0.03^{aA}	6.59±0.03^{cAB}	6.52±0.02^{dB}	6.62±0.03^{bB}

Values are given as mean±SD (n=9).

^{a}Different letters within the same row indicate significant difference ($P < 0.05$).

^{A}Different capital letters within the same column indicate significant difference ($P < 0.05$).

Control Treatment (TC) – without addition of protein hydrolysate; T1 – 10% addition of protein hydrolysate; T2 – 20% addition of protein hydrolysate; T3 – 30% addition of protein hydrolysate.

624 Table 4 – TBARS values of reduced-fat mortadella-type sausages produced with different
625 levels of MDCM protein hydrolysate.

Days	TC	T1	T2	T3
Day 0	0.260±0.080^{cA}	0.315±0.012^{abB}	0.313±0.022^{bcBC}	0.465±0.043^{aC}
Day 15	0.312±0.074^{cA}	0.411±0.053^{bcA}	0.464±0.046^{aA}	0.516±0.023^{abBC}
Day 30	0.294±0.038^{cA}	0.438±0.036^{bA}	0.478±0.018^{bA}	0.688±0.040^{aA}
Day 45	0.300±0.027^{bA}	0.290±0.053^{bC}	0.360±0.052^{bB}	0.560±0.028^{aB}
Day 60	0.240±0.051^{bA}	0.282±0.024^{bC}	0.263±0.020^{bC}	0.571±0.046^{aB}

626 Values are given as mean±SD (n=5).

627 Expressed as mg malonaldehyde/kg.

628 ^{a}Different letters within the same row indicate significant difference ($P < 0.05$).

629 ^{A}Different capital letters within the same column indicate significant difference ($P < 0.05$).

630 Control Treatment (TC) – without addition of protein hydrolysate; T1 – 10% addition of
631 protein hydrolysate; T2 – 20% addition of protein hydrolysate; T3 – 30% addition of protein
632 hydrolysate.

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Table 5 – Colorimetric parameters (L^*, a^*, b^*, C^* and h^o) of reduced-fat mortadella-type sausages produced with different levels of MDCM protein hydrolysate.

		Days of Storage				
		0	15	30	45	60
L^*	TC	63.04±2.59^{aA}	59.20±0.70^{bB}	58.98±1.39^{bA}	60.68±0.79^{abA}	58.66±1.26^{bB}
	T1	62.11±0.17^{abA}	62.73±1.15^{aA}	61.03±1.28^{abA}	60.56±1.25^{abA}	60.09±0.66^{bB}
	T2	61.16±0.97^{bA}	60.16±1.48^{bB}	59.86±0.55^{bA}	60.24±0.51^{bA}	63.36±1.20^{aA}
	T3	56.98±1.09^{bcB}	59.40±0.57^{abB}	56.15±1.29^{cB}	60.56±1.14^{aA}	58.66±2.10^{abB}
a^*	TC	23.18±0.27^{aA}	21.33±0.20^{cdA}	21.96±0.39^{bcA}	21.23±0.36^{dA}	22.15±0.22^{bA}
	T1	20.37±0.37^{aB}	20.55±0.41^{aA}	20.66±0.31^{aB}	20.14±0.51^{aB}	20.98±0.41^{aBC}
	T2	20.39±0.40^{abB}	19.29±0.41^{cdB}	20.10±0.62^{bcB}	18.93±0.28^{dC}	21.18±0.22^{aB}
	T3	20.43±0.50^{aB}	19.59±0.19^{aB}	19.96±0.51^{aB}	19.97±0.63^{aB}	20.35±0.61^{aC}
b^*	TC	13.50±0.21^{aA}	12.68±0.10^{bA}	13.13±0.23^{aA}	12.44±0.28^{bC}	13.50±0.08^{aBC}
	T1	11.79±0.13^{cC}	12.78±0.21^{bA}	12.81±0.22^{bA}	12.99±0.17^{abB}	13.27±0.26^{aC}
	T2	12.40±0.10^{cB}	12.40±0.23^{cA}	13.16±0.34^{bA}	12.90±0.19^{bB}	14.17±0.14^{aA}
	T3	11.59±0.23^{cC}	12.59±0.19^{bA}	13.24±0.19^{aA}	13.51±0.22^{aA}	13.60±0.09^{aB}
C^*	TC	27.49±0.84^{aA}	24.81±0.22^{cdA}	25.58±0.42^{bcA}	24.60±0.45^{dA}	25.93±0.21^{bA}
	T1	23.53±0.38^{bB}	24.20±0.46^{abAB}	24.31±0.38^{abB}	23.96±0.51^{abA}	24.82±0.48^{bBC}
	T2	23.86±0.31^{bcB}	22.93±0.64^{cC}	24.02±0.70^{bB}	22.90±0.34^{cB}	25.48±0.17^{aAB}
	T3	23.48±0.54^{abB}	23.29±0.34^{bBC}	24.06±0.61^{abB}	24.10±0.64^{abA}	24.47±0.52^{aB}
h^o	TC	30.2±0.3^{cB}	30.7±0.1^{bcC}	30.9±0.4^{abC}	30.3±0.2^{bcC}	31.4±0.2^{aC}
	T1	30.0±0.2^{dB}	31.8±0.2^{bcB}	31.7±0.1^{cB}	32.8±0.4^{aB}	32.3±0.2^{abB}
	T2	31.3±0.7^{dA}	32.7±0.4^{cA}	33.2±0.2^{bcA}	34.3±0.2^{aA}	33.7±0.4^{abA}
	T3	29.5±0.2^{cB}	32.7±0.2^{bA}	33.5±0.4^{abA}	34.1±0.4^{aA}	33.7±0.8^{abA}

Values are given as mean±SD (n=5).

^{a}Different letters within the same row indicate significant difference ($P < 0.05$).

^{A}Different capital letters within the same column for the same parameter indicate significant difference ($P < 0.05$).

L^*: 0 = black and 100 = white; a^*: -60 = green and +60 = red; b^*: -60 = blue and +60 = yellow.

Control Treatment (TC) – without addition of protein hydrolysate; T1 – 10% addition of protein hydrolysate; T2 – 20% addition of protein hydrolysate; T3 – 30% addition of protein hydrolysate.

Table 6 – Microbiological counts of reduced-fat mortadella-type sausages produced with different levels of MDCM protein hydrolysate.

	Days	TC	T1	T2	T3
LAB^{c}	0	3.22 ± 0.59^{aA}	2.93 ± 0.08^{aA}	3.22 ± 0.24^{aAB}	3.03 ± 0.05^{aA}
	15	2.25 ± 0.09^{bA}	2.95 ± 0.17^{aA}	3.39 ± 0.37^{aA}	2.97 ± 0.12^{aA}
	30	3.33 ± 0.86^{aA}	3.01 ± 0.17^{aA}	3.32 ± 0.15^{aA}	3.32 ± 0.20^{aA}
	45	2.49 ± 0.24^{aA}	3.28 ± 0.85^{aA}	3.39 ± 0.23^{aA}	3.09 ± 0.25^{aA}
	60	2.17 ± 0.08^{bA}	2.58 ± 0.15^{aA}	2.72 ± 0.05^{aB}	2.52 ± 0.17^{aB}
MAB^{c}	0	3.33 ± 0.67^{aB}	3.50 ± 0.38^{aA}	3.46 ± 0.48^{aA}	3.75 ± 0.11^{aA}
	15	2.82 ± 0.05^{bB}	3.36 ± 0.33^{aA}	3.67 ± 0.13^{aA}	3.52 ± 0.14^{aA}
	30	3.52 ± 0.64^{aB}	3.43 ± 0.02^{aA}	3.58 ± 0.05^{aA}	3.55 ± 0.07^{aA}
	45	2.88 ± 0.15^{aB}	3.57 ± 0.59^{aA}	3.19 ± 0.97^{aA}	3.53 ± 0.07^{aA}
	60	4.71 ± 0.18^{aA}	3.31 ± 0.15^{bA}	3.52 ± 0.10^{bA}	3.62 ± 0.26^{bA}
Total coliforms^{c}	0	≤1.0	≤1.0	≤1.0	≤1.0
	60	≤1.0	≤1.0	≤1.0	≤1.0
Termotolerant	0	≤1.0	≤1.0	≤1.0	≤1.0
coliforms^{d}	60	≤1.0	≤1.0	≤1.0	≤1.0
Staphylococcus	0	≤1.0	≤1.0	≤1.0	≤1.0
aureus^{c}	60	≤1.0	≤1.0	≤1.0	≤1.0
Clostridium	0	≤1.0	≤1.0	≤1.0	≤1.0
sulphite-reducers^{c}	60	≤1.0	≤1.0	≤1.0	≤1.0
Salmonella	0	Absence	Absence	Absence	Absence
	60	Absence	Absence	Absence	Absence

Values are given as mean±SD (n=4).

^{a}Different letters within the same row indicate significant difference ($P < 0.05$).

^{A}Different capital letters within the same column for each microorganism indicate significant difference ($P < 0.05$).

^{c}Expressed as CFU/g (Colony Forming Units per g).

^{d}Expressed as MPN/g (Most Probable Number per g).

Control Treatment (TC) – without addition of protein hydrolysate; T1 – 10% addition of protein hydrolysate; T2 – 20% addition of protein hydrolysate; T3 – 30% addition of protein hydrolysate.

Table 7 – Sensory characteristics and future purchase intention of reduced-fat mortadella-type sausages produced with different levels of MDCM protein hydrolysate.

	TC	T1	T2	T3
Color	5.19 ± 0.84^{a}	4.83 ± 0.90^{ab}	4.31 ± 1.04^{c}	4.46 ± 1.02^{bc}
Taste	5.48 ± 0.70^{a}	5.50 ± 0.90^{a}	3.98 ± 1.42^{b}	4.37 ± 1.28^{b}
Odor	5.04 ± 0.84^{ab}	5.29 ± 0.75^{a}	4.58 ± 0.87^{bc}	4.48 ± 0.92^{c}
Texture	5.60 ± 0.80^{a}	4.00 ± 1.31^{b}	2.92 ± 1.13^{c}	3.04 ± 1.10^{c}
Appearance	5.21 ± 0.89^{a}	4.40 ± 1.01^{b}	3.71 ± 1.12^{c}	3.92 ± 1.18^{bc}
Future purchase intention	4.16 ± 0.67^{a}	3.53 ± 0.86^{b}	2.37 ± 1.00^{c}	2.57 ± 1.02^{c}

Values are given as mean±SD (n=52).

[a]Different letters within the same row indicate significant difference ($P < 0.05$).

Control Treatment (TC) – without addition of protein hydrolysate; T1 – 10% addition of protein hydrolysate; T2 – 20% addition of protein hydrolysate; T3 – 30% addition of protein hydrolysate.

693 Table 8 – Textural properties of reduced-fat mortadella-type sausages made with different
694 levels of MDCM protein hydrolysate.

	TC	T1	T2	T3
Hardness	27.128±3.919[a]	8.332±0.901[b]	4.681±0.818[c]	3.927±0.677[c]
Springiness	0.935±0.033[a]	0.652±0.071[b]	0.490±0.109[c]	0.471±0.059[c]
Cohesiveness	0.756±0.038[a]	0.360±0.044[b]	0.293±0.017[c]	0.300±0.017[c]
Gumminess	20.565±3.566[a]	3.013±0.593[b]	1.367±0.223[b]	1.181±0.232[b]
Chewiness	19.238±3.621[a]	1.986±0.539[b]	0.685±0.265[b]	0.563±0.154[b]

695 Values are given as mean±SD (n=9).

696 [a]Different letters within the same row indicate significant difference ($P < 0.05$).

697 Control Treatment (TC) – without addition of protein hydrolysate; T1 – 10% addition of
698 protein hydrolysate; T2 – 20% addition of protein hydrolysate; T3 – 30% addition of protein
699 hydrolysate.

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4 DISCUSSÃO GERAL

A hidrólise de proteínas tem se mostrado um método eficaz para melhorar as propriedades físicas, químicas e biológicas em relação à proteína. Estudos revelam que hidrolisados proteicos de diferentes substratos apresentam diversas características desejáveis e benéficas para a saúde dos humanos. Entre essas características, estão a capacidade de reduzir a pressão sanguínea por inibição da ECA (AHHMED; MUGURUMA, 2010), a capacidade de reduzir a proliferação de células cancerígenas humanas (KANNAN et al., 2010) e capacidade antioxidante (YANG et al., 2011).

Estudos tem se concentrado na utilização de proteínas de pescado (JIN et al., 2011; KHALED et al., 2011; SEE et al., 2011; YANG et al., 2011; ZHAO et al., 2011), soro de leite (CONTRERAS et al., 2011), soja (XU et al., 2012) e trigo (LEE et al., 2012). Entretanto, a CMS de frango é uma matéria prima pouco utilizada para a fabricação de hidrolisados proteicos. É uma matéria prima bastante instável e rapidamente pode sofrer oxidação lipídica, formando compostos desagradáveis do ponto de vista sensorial.

A adição de antioxidante BHT e sais de cura, não afetou o grau de hidrólise dos hidrolisados. Os resultados mostraram que a maior clivagem dos peptídeos ocorreu durante os primeiros trinta minutos do processo de hidrólise, alcançando os maiores valores aos 60 minutos de hidrólise (entre 7.14 e 14.54%). O pH e a composição centesimal não foram afetados pela adição do antioxidante BHT e dos sais de cura e mantiveram-se semelhantes aos da CMS original.

Em relação à oxidação lipídica nos hidrolisados proteicos de CMS, os resultados mostraram que quanto maior o período de hidrólise, maior a ocorrência de oxidação. A temperatura utilizada para a hidrólise é um agente facilitador para a oxidação. Estudos mostram que a aplicação de pré-tratamentos como a separação das membranas fosfolipídicas diminui a formação de substâncias pró-oxidantes (KHANTAPHANT et al., 2011). Ainda, YARNPAKDEE et al. (2012) conseguiram reduzir a oxidação lipídica de hidrolisados proteicos de pescado utilizando Trolox e EDTA. A utilização de antioxidante BHT e sais de cura, mostrou um efeito sinergístico que também foi capaz de reduzir a oxidação lipídica durante todo o período de hidrólise. Estes aditivos levam vantagem em relação aos outros, por já serem comumente utilizados pela indústria de carnes. No entanto, verificou-se que nos

tratamentos em que foram utilizados apenas um desses aditivos, essa redução na oxidação lipídica não foi tão pronunciada.

Durante o processo de hidrólise enzimática, outro fator desagradável é a formação da coloração amarronzada devido à ocorrência da reação de Maillard e oxidação de pigmentos (BENJAKUL; MORRISSEY, 1997; DONG et al., 2008; HONIKEL, 2008). O uso de antioxidante BHT e sais de cura evitou a formação da coloração amarronzada, evidenciada pelos maiores valores do teor de vermelho (a^*): 10,70 para o tratamento controle e 16,08 para o tratamento contendo associação de antioxidante BHT e sais de cura. Os sais de cura são os responsáveis pela formação de nitrosohemocromo que confere aos produtos a cor vermelha característica de carnes cozidas curadas (MOLLER & SKIBSTED, 2002).

Os hidrolisados proteicos de CMS se mostraram uma matéria prima possível de ser utilizada para a fabricação de produtos cárneos cozidos. Houve poucas alterações em relação à composição centesimal da mortadela. A adição de hidrolisados proteicos promoveu um aumento significativo nos teores de proteína, umidade, gordura e no valor calórico do produto. O pH não foi afetado e se mostrou estável durante todo o período de armazenamento, apresentando valores entre 6,24 e 6,79, considerado o normal para o produto em questão.

Os produtos apresentaram boa estabilidade oxidativa durante os 60 dias de armazenamento. As mortadelas apresentaram valores inferiores a 1 mg de malonaldeído por kg de produto, considerado como limite na percepção de odores e sabores estranhos pelo consumidor (WU et al., 1991).

A adição de hidrolisado proteico de CMS produziu mortadelas mais escuras (L^* menores) que o tratamento controle. Os teores de vermelho (a^*) também foram inferiores. No entanto, todos os tratamentos apresentaram cor vermelha característica, evidenciado pelos valores de C^* e h^o.

A adição de hidrolisados proteicos afetou as características sensoriais do produto. Os provadores atribuíram notas inferiores aos tratamentos contendo maiores quantidades de hidrolisado proteico para os atributos cor, sabor e textura. A formação de peptídeos com coloração amarronzada durante o processo de hidrólise pode promover alterações de cor no produto (BUENO-SOLANO et al., 2009). FITZGERALD e O'CUINN (2006) e SPELLMAN et al. (2009) relatam que a hidrólise enzimática pode produzir peptídeos com sabor amargo que pode ter sido transferido para o produto. Em relação à textura, tanto os provadores quanto

à textura instrumental mostraram que os produtos contendo hidrolisado proteico apresentaram textura mais amolecida do que o controle.

5 CONCLUSÃO

O estudo permitiu as seguintes conclusões:

- A carne mecanicamente separada (CMS) de frango é uma matéria prima viável para a produção de hidrolisados proteicos;

- O antioxidante BHT e os sais de cura apresentaram um efeito sinergístico e reduziram a oxidação lipídica e a formação da coloração amarronzada nos hidrolisados proteicos de CMS;

- O tempo de hidrólise de 60 minutos à temperatura de 60 ºC é o mais indicado para a produção de hidrolisados proteicos de CMS. O grau de hidrólise foi satisfatório, levando em consideração o curto tempo de hidrólise, a temperatura e a concentração da enzima utilizada;

- O hidrolisado proteico de CMS pode ser utilizado para a fabricação de produtos cárneos cozidos, porque apresentaram características de qualidade similares ao controle;

- A adição de 10% de hidrolisado proteico de CMS na forma líquida é a mais indicada para a produção de produtos cárneos cozidos. Os produtos tiveram boa aceitabilidade sensorial e as características físico-químicas, microbiológicas e textura foram semelhantes às do tratamento controle;

- Os produtos contendo hidrolisado proteico de CMS apresentaram boa estabilidade lipídica e microbiológica durante o período de armazenamento de 60 dias.

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7 APÊNDICES

Apêndice A – Modelo de ficha utilizada na avaliação sensorial de produto cárneo cozido contendo diferentes concentrações de hidrolisado proteico de CMS.

Nome: ____ Data: _________________________
Idade: ____ anos Sexo: ( ) M ( ) F

Você está recebendo uma amostra de mortadela. Por favor, avalie a amostra e indique o quanto você gostou ou desgostou do produto em relação aos atributos.

Código da Amostra: ___

	Atributos				
	Cor	**Sabor**	**Odor**	**Textura**	**Aparência**
Gostei extremamente					
Gostei muito					
Gostei					
Nem gostei/nem desgostei					
Desgostei					
Desgostei muito					
Desgostei extremamente					

Em relação à compra do produto, você:

( ) Certamente compraria;

( ) Provavelmente compraria;

( ) Tenho dúvidas se compraria;

( ) Provavelmente não compraria;

( ) Certamente não compraria.

Comentários: