UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ENGENHARIA DE ALIMENTOS

Vitor Andre Silva Vidal

REDUCING SODIUM IN SALTED MEATS: EFFECTS OF PARTIAL REPLACEMENT OF SODIUM CHLORIDE BY OTHER CHLORIDE SALTS

REDUÇÃO DE SÓDIO EM CARNES SALGADAS: EFEITOS DA SUBSTITUIÇÃO PARCIAL DE CLORETO DE SÓDIO POR OUTROS SAIS CLORADOS

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Thesis presented to the School of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food Technology

Tese de doutorado à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Tecnologia de Alimentos

***Supervisor/Orientador*: PROFA. DRA. MARISE APARECIDA RODRIGUES POLLONIO**

ESTE EXEMPLAR CORRESPONDE A VERSÃO FINAL DA TESE DEFENDIDA PELO ALUNO VITOR ANDRE SILVA VIDAL E ORIENTADO PELA PROFA. DRA. MARISE APARECIDA RODRIGUES POLLONIO

Campinas

2019

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

V667r

Vidal, Vitor Andre Silva, 1991-
Redução de sódio em carnes salgadas : efeitos da substituição parcial de cloreto de sódio por outros sais clorados / Vitor Andre Silva Vidal. – Campinas, SP : [s.n.], 2019.

Orientador: Marise Aparecida Rodrigues Pollonio.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Carne salgada. 2. Sal. 3. Lipólise. 4. Proteólise. 5. Lisina. I. Pollonio, Marise Aparecida Rodrigues. II. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Reducing sodium in salted meats : effects of partial replacement of sodium chloride by other chloride salts
Palavras-chave em inglês:
Salted meat
Salt
Lipolysis
Proteolysis
Lisina
Área de concentração: Tecnologia de Alimentos
Titulação: Doutor em Tecnologia de Alimentos
Banca examinadora:
Marise Aparecida Rodrigues Pollonio [Orientador]
Dirce Yorika Kabuki
Neura Bragagnolo
Marco Antônio Trindade
Ana Lúcia da Silva Corrêa Lemos
Data de defesa: 30-09-2019
Programa de Pós-Graduação: Tecnologia de Alimentos

Identificação e informações acadêmicas do(a) aluno(a)
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- Currículo Lattes do autor: http://lattes.cnpq.br/6057198167849188

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Ata da Defesa, assinada pelos membros da Comissão Examinadora, consta no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa da Unidade.

“Todo o conhecimento humano começou com intuições, passou daí aos conceitos e terminou com ideias”.

Immanuel Kant

AGRADECIMENTOS

À Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos e ao Departamento de Tecnologia de Alimentos pelo acolhimento e estrutura fornecida para realização desta tese de doutorado.

À minha orientadora professora Doutora Marise Aparecida Rodrigues Pollonio por sempre me ensinar, orientar, apoiar, aconselhar e principalmente ter me aceitado como orientado tanto no mestrado quanto no doutorado, tenho muito orgulho de ter sido seu aluno. Sem a dedicação da senhora nada disso seria possível. Muito obrigado.

Ao técnico e amigo José Roberto dos Santos por todos os ensinamentos, ajuda, companheirismo e imprescindível competência nestes 6 anos.

A todos os professores, colegas e funcionários da Faculdade de Engenharia de Alimentos, que de alguma forma fizeram parte deste trabalho.

À Universidad de Talca e ao professor Doutor Leonardo Silva Santos pela parceria e ajuda inestimável para realização deste trabalho.

Ao Centro Tecnolóxico da Carne e ao professor Doutor Jose Manuel Lorenzo Rodriguez pelos ensinamentos e total apoio durante a fase final da pesquisa.

Ao professor Adriano Cruz pela grande ajuda nas análises sensoriais.

Ao professor Marcelo Morgano pelo grande apoio nas análises de minerais.

À professora Fabiane Nachtigall pelo grande apoio nas análises realizadas no Chile.

A minha querida avó Maria Ananias da Silva que iniciou a família e que tenho muito orgulho, admiração e saudades.

Aos meus pais Neila Maria da Silva Vidal e Hamilton Cardozo Vidal pelo incentivo em qualquer escolha que eu faça.

Aos meus irmãos Elisa, Pedro e primos/irmãos Vinicius, Thais e Henrique.

Aos meus queridos padrinhos Maristela e Manoel por estarem presentes em todos os momentos da minha vida, principalmente nos difíceis.

Aos meus sogros Vladmir e Silvia pelo apoio, principalmente durante o final da elaboração da Tese.

Aos meus tios Mariluce, Marinalva, Roberto, Marli/Nivaldo, Maciel/Poliana, Maria Helena/Éder que sempre me bajularam.

Aos meus colegas Maristela, Ana Karoline, Mirian, Wanessa, Mária, Teresa, Bibiana, Vanessa, Leandro, Iara, Rodolfo, Mayara, Rickyn, Claudia e Guilherme e aos estagiários Alef, João, Kamila e Ana.

Aos meus amigos Bernardo, Diego, Elton, André, Erik, Luiz, Lucas, Ricardo, Fábio, Kaian e Rafael por compartilharem bons momentos.

Um agradecimento especial a minha amiga Camila de Souza Paglarini pelo companheirismo e amizade desde o primeiro dia de pós-graduação e que perdurará para sempre.

Ao meu amigo Gustavo Pagotto Borin, pela ótima convivência e amizade que criamos morando quatro anos juntos.

E, finalmente à Fernanda Bristotti, obrigado por ser essa pessoa especial, fundamental e indescritível em minha vida.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001

Ao CNPq (processos: 140533/2015-0 e 406671/2016-8) pelo auxílio financeiro.

Vitor Andre Silva Vidal

RESUMO

Os produtos cárneos salgados são amplamente consumidos em todo o mundo e caracterizam-se pelo alto valor nutricional, estabilidade microbiológica e propriedades sensoriais típicas. No entanto, ao longo de seu processo de fabricação, adiciona-se uma elevada concentração de cloreto de sódio (NaCl) para garantir um processo de salga com a esperada redução da atividade de água. Dessa forma, essa categoria de produtos cárneos poderá contribuir com o aumento do consumo de sódio na dieta, particularmente, se as operações de dessalga não forem padronizadas adequadamente. Segundo a Organização Mundial de Saúde (OMS), o consumo de sódio acima do recomendado (2000 mg por dia), aumenta o risco de ocorrência de diversas doenças crônicas tais como hipertensão, doenças renais, doenças cardiovasculares e alguns tipos de câncer. Em carnes salgadas, a substitutição de NaCl é um grande desafio para a indústria de processamento devido às complexas reações que podem ser influenciadas pela utilização de outros sais clorados, particularmente, as propriedades físico-químicas, microbiológicas e sensoriais. Com base nessas considerações, este estudo teve como objetivo avaliar os efeitos da substituição parcial de 50% NaCl, com base na força iônica, por cloreto de potássio (KCl) e cloreto de cálcio ($CaCl_2$) na forma de blends em produto cárneo salgado, sobre as características de processo, propriedades físico-químicas, sensoriais, estabilidade microbiológica, reações lipolíticas e proteolíticas de carne salgada. Na primeira etapa do trabalho (capítulo 2), foram estudadas as características físico-químicas, microbiológicas e sensoriais de jerked beef elaborados com redução de 50% de NaCl através de sua substituição por diferentes sais clorados. A partir dos resultados obtidos no capítulo 2, investigaram-se os efeitos da incorporação de lisina e extrato de levedura com finalidade de melhorar os atributos sensoriais dos tratamentos de carne salgada contendo blends de $CaCl_2$ e KCl, dando origem ao capítulo 3. A fim de avaliar o comportamento da água em carne salgada durante vida de prateleira de 180 dias, no capítulo 4, foram realizadas análises físico-químicas, de microestrutura e ressonância magnética de baixo campo. No capítulo 5, as reações de lipólise foram investigadas analisando-se os ácidos graxos, compostos voláteis e oxidação lipídica em tratamentos de carne salgada contendo redução de 50% de NaCl e substituição por blends de KCl e $CaCl_2$. No capítulo 6, as reações de proteólise foram estudadas através de análise de força de cisalhamento e perfil eletroforético durante 180 dias. Finalmente, no capítulo 7, os parâmetros físicos-químicos de tratamentos de carne salgada ao longo das etapas de salga úmida, salga seca e maturação foram analisados. A substituição parcial do NaCl por KCl e $CaCl_2$ nas etapas de salga úmida e seca reduziu expressivamente o teor de sódio no produto

final e não impactou a estabilidade microbiológica. Lisina e extrato de levedura demonstraram ser uma boa opção para os efeitos sensoriais negativos ocasionados pela redução de NaCl e adição de KCl e $CaCl_2$ em produto cárneo salgado com redução de sódio. O uso de $CaCl_2$ no *blend* de sais afetou negativamente os atributos sensoriais e físico-químicos, aumentando o sabor amargo, dureza e aroma de ranço. Os resultados também evidenciaram que o $CaCl_2$ promove o aumento das reações lipolíticas e proteoliticas resultando em mudança da morfologia da carne salgada, além de prejudicar o processo de desidratação. Em geral, o *blend* contendo NaCl + KCl resultou em efeitos tecnológicos e sensoriais similares ao tratamento controle com 100% de NaCl, mostrando ser uma boa estratégia para reduzir o teor de sódio na categoria de produtos cárneos salgados. O presente estudo contribuiu efetivamente para recomendar soluções tecnológicas consistentes no âmbito industrial para redução de sódio em carnes salgadas, resultando em produto mais saudável, seguro e com aceitação sensorial.

Palavras-chave: carne salgada, cloreto de sódio, lipólise, proteólise, lisina, extrato de levedura.

ABSTRACT

Salted meat products are widely consumed all over the world, and characterized by high nutritional value, microbiological stability and typical sensory properties. However, due to its manufacturing process, a high concentration of sodium chloride (NaCl) is added to ensure a salting process with the expected reduction in water activity. Thus, this category of meat products may contribute to the increased sodium intake in the diet, particularly if the desalting operations are not standardized properly. According to the World Health Organization (WHO), high levels sodium, above the recommended 2000 mg per day, increase the risk of several chronic diseases such as hypertension, kidney and cardiovascular diseases and some type of cancers In salted meat, the replacement of NaCl is one of the most great challenge for processing industry due to the complex reactions that can be influenced when other chlorides salts are used, particularly the regarding to physicochemical, microbiological and sensory properties. Based on these considerations, this study aimed to evaluate the effects of partial substitution of 50% NaCl based on ionic strength by potassium chloride (KCl) and calcium chloride ($CaCl_2$) in the form of blends in salted meat product on process characteristics, physicochemical properties, sensory properties, microbiological stability, lipolytic and proteolytic reactions of salted meat. In the first step of the work (chapter 2), were studied the physicochemical, microbiological and sensory characteristics of jerked beef performed with 50% reduction of NaCl through its replacement by different chloride salts. From the results obtained in chapter 2, The effects of incorporating lysine and yeast extract were investigated in order to improve the sensory attributes of salted meat treatments containing $CaCl_2$ and KCl blends, giving rise to chapter 3. In order to evaluate the behavior of water in salt meat during shelf life of 180 days, in chapter 4, were performed physicochemical, microstructure and low field magnetic resonance analysis. In chapter 5, lipolysis reactions were investigated by analyzing fatty acids, volatile compounds and lipid oxidation in salt meat treatments containing 50% NaCl reduction and substitution by KCl and CaCl2 blends. In chapter 6, the proteolysis reactions were studied by shear force and electrophoretic profile analysis during 180 days. Finally, in the last chapter, the physicochemical parameters of salted meat treatments along the wet salting, dry salting and ripening steps were analyzed. Partial substitution of NaCl by KCl and $CaCl_2$ in the wet and dry salting steps significantly reduced the sodium content in the final product and did not impact microbiological stability. Lysine and yeast extract have been shown to be a good option to minimize negative sensory effects caused by NaCl reduction and addition of KCl and $CaCl_2$ in salted meat product with reduced

sodium content. The use of $CaCl_2$ in the salt blend negatively affected sensory and physicochemical attributes, increasing bitter taste, hardness and rancid aroma. The results also showed that $CaCl_2$ promotes the increase of lipolytic and proteolytic reactions resulting in a change in the morphology of salted meat, besides impairing the dehydration process. In general, the blend containing NaCl + KCl resulted in technological and sensory effects similar to 100% NaCl control treatment, proving to be a good strategy to reduce sodium content in the salted meat product category. The present study effectively contributed to recommend to industry a consistent technological solutions for sodium reduction in salted meat, resulting in a healthier and safe final product with sensorial acceptance.

Keywords: salted meat, sodium chloride, lipolysis, proteolysis, lysine, yeast extract.

SUMÁRIO

INTRODUÇÃO GERAL

A carne é consumida por humanos há centenas de milhares de anos e sua forma de apresentação e consumo mudou de acordo com o desenvolvimento de diferentes civilizações. No entanto, sempre foi um produto considerado altamente relevante desde que a ciência avançou nos mecanismos que explicam como o valor nutricional dos componentes dos alimentos influencia a manutenção da saúde humana (Dilger, 2017; Zhang, Schilling, & Owens, 2017).

A carne e produtos cárneos são excelentes fontes de macronutrientes representados pelas proteínas e lipídios e micronutrientes, incluindo principalmente, aminoácidos essenciais; Dentre os importantes nutrientes fornecidos por esta categoria, estão vitaminas do complexo B, ácidos graxos e aminoácidos essenciais, zinco, selênio, cobre e principalmente ferro (Binnie et al., 2014; De Smet & Vossen, 2016; Pereira & Vicente, 2013), responsáveis pela manutenção de uma dieta de alto valor nutricional.

A categoria de produtos cárneos salgados é muito apreciada, consumida e principalmente diversificada, variando consideravelmente suas características sensoriais, nutricionais e vida-de-prateleira (Jiménez-Colmenero, Ventanas, & Toldrá, 2010; Jones, Arnaud, Gouws, & Hoffman, 2017). Estas diferenças são provenientes da matéria-prima e processo utilizados para obtenção do produto. Muito destes produtos são característicos de uma região, cultura ou grupo de pessoas, como jerked beef no Brasil (Vidal et al., 2019), bresaola na Itália (Picone, et al., 2019) e cecina de Leon na Espanha (Molinero, Martínez, Rubio, Rovira, & Jaime, 2008).

Em geral, para a obtenção de produtos cárneos salgados tradicionais, é necessária a adição de elevados teores de cloreto de sódio (NaCl), cuja função é promover a salga e desidratação de cortes cárneos até atividade de água próxima de 0,78 ou valores menores. Como consequência, produtos cárneos salgados possuem elevado teor de cloreto de sódio em sua composição, sendo obrigatória a etapa de dessalga dos cortes salgados antes de seu cozimento e posterior consumo. Dependendo das condições dessa operação, produtos finais poderão ter concentrações muito elevadas de sódio a serem ingeridas pelos consumidores, colocando em risco diversos aspectos da saúde.

O sódio é necessário para o bom funcionamento de diversas funções no corpo humano, tais como, controlar a quantidade de água célular, regular os fluídos corpóreos e a pressão sanguínea (Domínguez et al., 2017). Porém, a ingestão excessiva de sódio aumenta o risco de

desenvolvimento de diversos males à saúde como hipertensão, doenças cardiovasculares e renais e certos tipos de câncer (Cook, Appel, & Whelton, 2016; Frieden, 2016). De qualquer forma, a importância do NaCl para o desenvolvimento das características sensoriais, tecnológicas e estabilidade microbiológica (Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017; Sharedeh, Mirade, Venien, & Daudin, 2015). Dentre os diversos efeitos indesejáveis que a diminuição de adição de NaCl causa, podem ser citados o aumento das reações de proteólise, mudanças na propriedade de textura, diminuição da estabilidade microbiológica com consequente diminuição da vida-de-prateleira e desenvolvimento de sabor e aroma não característicos (Mora et al., 2015; Toldrá, 2002; Wu et al., 2014).

Há muitas estratégias para redução de sódio em produtos cárneos. Provavelmente, a mais comum é a adição de outros sais clorados como KCl (cloreto de potássio), $MgCl_2$ (cloreto de magnésio) e $CaCl_2$ (cloreto de cálcio), sendo o KCl o sal clorado mais utilizado para esta finalidade (Desmond, 2006). KCl promove efeitos similares em comparação ao NaCl, porém, o principal limitante do uso de KCl é o sabor amargo que desenvolve (Horita, Morgano, Celeghini, & Pollonio, 2011). Além disso, em função do amplo e extensivo uso do KCl como sal substituto em muitos produtos alimentícios, estudos têm alertado para o risco de hipercalemia, sendo potencialmente perigoso para pessoas com problemas renais (Abuelo, 2018). Além do KCl, sais divalentes como $CaCl_2$ são muito utilizados, mas os efeitos sensoriais e tecnológicos são mais pronunciados, como o desenvolvimento elevado de sabor amargo e grande influência nas reações de proteólise, lipólise e oxidação lipídica se comparados com outros sais (Vidal et al., 2019). De acordo com Murphy (1981), quanto maior o peso atômico do íon, maior será o sabor amargo gerado.

Uma estratégia para minimizar os efeitos sensoriais indesejáveis da redução de NaCl e/ou adição de outros sais, é adição de realçadores de sabor, podendo aumentar consideravelmente a aceitação sensorial do produto reduzido de sódio (dos Santos, Campagnol, Morgano, & Pollonio, 2014).

Levando em conta todas as características dos produtos cárneos salgados, propriedades do NaCl e desafios de redução deste ingrediente, este estudo teve como finalidade estudar os efeitos da substituição parcial de 50% NaCl, com base na força iônica, por cloreto de potássio (KCl) e cloreto de cálcio ($CaCl_2$) na forma de blends em produto cárneo salgado, sobre as características de processo, propriedades físico-químicas, sensoriais, estabilidade microbiológica, reações lipolíticas e proteolíticas de carne salgada. Este estudo foi dividido em dez Capítulos como descrito a seguir:

Os Capítulos 1, 2, 3, 4, 5, 6 e 7 consistem em artigos publicados ou submetidos em periódicos. O Capítulo 1 é um artigo de revisão bibliográfica que aborda as características de produtos cárneos salgados (principalmente os brasileiros charque, jerked beef e carne-de-sol), processamento, efeitos da redução de NaCl e estratégias para redução de sódio em produtos cárneos salgados.

O Capítulo 2 publicado no periódico *Meat Science*, teve como objetivo avaliar os efeitos da substituição parcial do NaCl por *blends* de KCl e $CaCl_2$ nas propriedades físico-químicas, microbiológicas e sensoriais de jerked beef. A partir dos resultados obtidos no Capítulo 2, o Capítulo 3 e 4 foram elaborados. O Cápitulo 3 publicado também no periódico *Meat Science*, selecionou compostos naturais para melhorar as características sensoriais de carne salgada elaborada com diferentes sais clorados. No Capítulo 4, publicado no periódico *Food Research International*, foram investigados os efeitos dos diferentes sais na mobilidade da água durante 180 dias de armazenamento utilizando análises de ressonância magnética de baixo campo, microestrutura e físico-químicas com o propósito de fundamentar algumas das principais reações que ocorrem durante vida de prateleira desse produto.

Para avaliar as reações lipolíticas, no Capítulo 5, a oxidação lipídica, compostos voláteis e ácidos graxos foram investigados, e no Capítulo 6 foram comparadas as análises de força de cisalhamento e perfil eletroforético das proteínas miofibrilares e sarcoplasmáticas para explicar as reações proteolíticas observadas. Finalmente, no Capítulo 7, foram investigados os parâmetros físico-químicos de produtos cárneos salgados ao longo das etapas de processamento.

Além dos Capítulos em formato de artigos (1 a 7), a discussão geral foi apresentada no Capítulo 8, conclusão geral no Capítulo 9 e no Capítulo 10 as referências utilizadas na tese.

A Figura 1 apresenta os artigos contidos neste estudo.

Figura 1. Artigos contidos na tese

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CAPÍTULO 1

SALTED MEAT PRODUCTS: NUTRITIONAL CHARACTERISTICS, PROCESSING AND STRATEGIES FOR SODIUM REDUCTION

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Submitted to Food Reviews International

Abstract

The category of salted meat products is very diversified and have very particular characteristics. In addition to being widely consumed worldwide, this category have important nutritional properties and are an excellent source of protein mainly in regions where the cold chain is not fully established due to the microbiological stability that salt and dehydration provides. However, they have been criticized due to some high levels of sodium, which if consumed in excess may increase the risk of developing certain health disorders. The reduction of sodium content in salted meat products is especially difficult because in addition to many of these are considered traditional, NaCl is fundamental for the development of the physicochemical, sensorial and microbiological stability. A known strategy of sodium reduction in meat products is the use of other salts, but these substitute salts can promote significant changes in the product, mainly sensory. To minimize negative sensory effects by reducing of sodium chloride (NaCl) and adding other chloride salts, the most commom action is the use of flavor enhancers. In this context, this work has the objective of explaining in detail the characteristics, processing, different strategies for sodium reduction and the effect of NaCl reduction in the category of salted meat products.

Keywords: salted meat processing; sodium reduction; chloride salts

1. Introduction

People consume meat primarily due to its sensory characteristics, social status it provides, to enjoy special occasions, and because of nutritional benefits that meat provides (Boler & Woerner, 2017; McNeill, 2014; Murphy, Spungen, Bi, & Barraj, 2011; O'Connor, Kim, & Campbell, 2017).

Meat and meat products are one of the main sources of protein and rich in very important nutrients as vitamins and minerals (Cabrera & Saadoun, 2014). These components are essential for biological functions, antioxidative enzymatic system and good functioning of human metabolic processes (Bauchart et al., 2007; Udenigwe & Howard, 2013), and necessary to optimize growth and development (Pereira & Vicente, 2013).

Salted meat products are widely appreciated and consumed worldwide due to their unique sensory characteristics and relatively long shelf-life (Liu, Pu, Sun, Wang, & Zeng, 2014). There are numerous salted meat products around the world, and the raw material and process used to obtain the product are very diversified, varying considerably the shelf-life and nutritional characteristics (Gandemer, 2002; Jiménez-Colmenero, Ventanas, & Toldrá, 2010; Estevez, Morcuende, Ventanas, & Ventanas, 2008; Jones, Arnaud, Gouws, & Hoffman, 2017). Depending on the process, some salted meat products can be stored at room temperature for months due of their microbiological stability, making this category very important in regions where the cold chain is scarce or not used (Torres, Pearson, Gray, Ku, & Shimokomaki, 1989).

NaCl has been used for thousands of years as a food preservative. With the advent and expansion of refrigeration, improved logistics, better packaging and greater knowledge of the factors influencing microbiological stability, NaCl still remains an important ingredient to develop numerous desirable sensory and technological characteristics in meat products, mainly in salted meat products (Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017; Sharedeh, Mirade, Venien, & Daudin, 2015). In addition, sodium is important for regulating physiological functions in the human body such as body fluids and blood pressure (Delgado-Pando et al., 2018). Anyway, excessive sodium intake increases the risk of developing various health ailments as cardiovascular and renal disorders, high blood pressure and certain types of cancer (Cook, Appel, & Whelton, 2016; Frieden, 2016; Strazzullo, D'Elia, Kandala, & Cappuccio, 2009). Taking into account, the World Health Organization (WHO) recommends a daily intake of 2 grams of sodium, equivalent to 5 grams of NaCl. In order to establish the

recommendation for daily consumption, four elements were considered: development of actions to sensitize consumers about the risks of excessive sodium intake, available data on consumption and levels of sodium in foods, reformulation of processed foods and monitoring and evaluation of these measures (Zanardi, Ghidini, Conter, & Ianieri, 2010). In any case, the excessive use of NaCl during the processing of salted meat products makes this category high in sodium content and, taking into account the high consumption of this category of products worldwide, is necessary and very important the effective reduction of NaCl content added to consequently decrease the amount of sodium intake by the population.

The aim of this review is to adress the relevance and the challenges to reduce sodium in salted meats regarding their nutritional characteristics, processing and strategies to make this meat product healthier without compromising its traditional properties.

2. Physical, chemical, nutritional and microbiological characteristics of meat

Meat has been used as food for thousands of years. For example, animals such as buffalo and deer are a source of meat for more than 500,000 years. It is defined as meat the animal portion used as food. According to American Meat Science Association (AMSA), meat is skeletal muscle and its associated tissues derived from avian, amphibian, mammalian, reptilian and aquatic species (Boler & Woerner, 2017).

Meat proteins can be classified according to their solubility into three broad groups: myofibrillar, sarcoplasmic and stromal proteins. The most important for the functional and sensorial characteristics are myofibrillar and sarcoplasmic proteins. Myofibrillar proteins play a relevant role in the biochemical changes that occur after the animal is slaughtered, resulting in important functional properties. These proteins have multiple important functionalities in meat products, including: water retention capacity, flavor and fat retention (Xiong, 2005). The versatility of this group of proteins provides the development of a variety of texture in meat products (Xiong, 2005). The sarcoplasmic proteins are very important mainly for the sensorial contribution, among these proteins, it is worth mentioning the myoglobin and the hemoglobin that are responsible for the color of the meat (Przybylski et al., 2016).

Notoriously the meat is an excellent source of high quality protein. Meat proteins have a high content of essential amino acids - which are not produced by the human body, being required consumption for balanced nutrition (Pereira & Vicente, 2013).

The lipid content present in meat and meat products is a relevant source of fat-soluble vitamins and essential fatty acids being an excellent source of energy (Cabrera & Saadoun, 2014). The consumption of fat is fundamental to reduce risks of devolepment of various diseases, particularly cardiac coronary disorders (WHO). An ideal fat intake ranges from 15% to 30% of the total calories, from this total 10-15% from monounsaturated fatty acids, 6-10% from polyunsaturated fatty acids, not more than 10% saturated fatty acids and less than 1% of trans fatty acids (Jiménez-Colmenero et al., 2010). The croosbreed, sex and age of animal considerably influences the fat content (Toldrá, 2006), the fatty acids composition is influenced mainly by feed (Pastorelli et al., 2003), and meat may be an alternative for the ingestion of long-chain omega-3 polyunsaturated fatty acids (Wyness et al., 2011).

The amount of minerals intake is important for human nutrition, especially for children, elderly and pregnant, and the insufficient consumption of these micronutrients is common (Cabrera & Saadoun, 2014; Hambidge & Krebs, 2007). Meat and meat products can be a good source of minerals such as iron, zinc, selenium, among other micronutrients (Mulvihill, 2014). According to Black (2003), some minerals as copper and zinc help the enzyme system by controlling free radicals in the body. With this, the red meat can be an effective source of minerals for a health nutrition, avoiding the deficiency of certain minerals.

Iron plays a crucial role in human health, very important for the adequate development of various biological functions, being vital to normal energy metabolism, imune system and mainly in children's development (Binnie, Barlow, Johnson, & Harrison, 2014). Iron has two forms: non-heme iron and heme-iron. Non-heme iron is found in a wide variety of foods as legumes, dairy products and mainly vegetables, however, heme-iron comes from hemoglobin and myoglobin, so it is present only in animal foods (Pereira & Vicente, 2013). If compared to non-heme iron, heme iron has higher bioavailable and is easily absorbed in the intestinal lumen because it is absorbed as an intact molecule by enterocytes (Hallberg & Hulthén, 2000; Simpson & McKie, 2009; Turhan, Altunkaynak, & Yazici, 2004).

Meat is an excellent source of vitamins, especially vitamin B12 and animal products are considered the largest source of vitamin B12 (Watanabe, 2007). Low intake of vitamin B12 can cause various health problems. According to Bourre (2011), the red meat is the main source of B vitamins, especially B2, B6 and B12 in the diet at all ages. Restricted vegetarian diets are associated with low intake of vitamin B12 (Craig, 2009), vitamin B12 deficiency is one of the main causes of megaloblastic anemia, in addition to increasing the risk of certain cardiovascular disorders (Green & Miller, 2005), and also associated with depressive

symptoms and neurological impairment (Agarwal, 2011). Williams (2007) observed that 100 grams of red meat have 25% of the recommended daily amount of niacin, vitamin B6, riboflavin and pantothenic acid.

The content and proportion of nutrients in meat products are directly affected by the raw material and processing techniques used. The composition of fatty acids, minerals and vitamins of the raw material can be affected by the type of muscle, production system, geographic location, feeding, breed and age of the animals (Ammerman, Martin, Gamble, Loaiza, & Blue, 1974; Cabrera, Ramos, Saadoun, & Brito, 2010; Hintze, Lardy, Marchello, & Finley, 2002; Realini, Duckett, Brito, Dalla Rizza, & De Mattos, 2004; Wagner, Dolezal, Yates, May, & Duckett, 1993) affecting directly the nutritional characteristics of meat products.

The microbiological profile of meat and meat products is one of the main criteria for determining quality and safety for the consumer (Biswas et al., 2011). Meat is one of the most perishable foods because it has excellent characteristics for the growth of several microorganisms (Doulgeraki, Ercolini, Villani, & Nychas, 2012), the nutrient density, chemical and enzymatic activities, pH and mainly the high moisture content makes this category very susceptible to deterioration (Dave & Ghaly, 2011). The development of spoilage bacteria in the meat produces undesirable odor, taste and color, gas and slime (Iulietto, Sechi, Borgogni, & Cenci-Goga, 2015). The most common bacterial pathogens identified and associated with meat are *Bacillus cereus*, *Campylobacter*, *Clostridium perfringens*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella sp*., *Staphylococcus aureus* and *Yersinia enterocolitica* (Biswas et al., 2011). It is a fact that microbial spoilage causes a great loss of products and shorten shelf-life, even with modern knowledge, cold chain and techniques (Remenant, Jaffrès, Dousset, Pilet, & Zagorec, 2015), however, the salting technique can inhibit the growth of most microorganisms and increase shelf life in many months.

3. Sodium reduction in meat products

Reducing sodium levels in processed food has been one of the goals of the food industry (WHO, 2011). According to WHO, meat products contribute significantly to daily sodium intake, about 16–25% of total, being considered the second largest sodium contributor in the diet, followed by bakery products. In general, reducing the sodium content in meat

products is challenging due to the important technological, sensory and microbiological stability properties provided by NaCl (Inguglia et al., 2017). For example, NaCl suppress microbial growth (Yotsuyanagi et al., 2016), can inhibit the activity of proteolytic enzymes impacting physicochemical and sensory characteristics (Martín, Córdoba, Antequera, Timón, & Ventanas, 1998; Petrova, Aasen, Rustad, & Eikevik, 2015), and influences lipid oxidation and lipolysis reactions (Lorenzo, Cittadini, Bermúdez, Munekata, & Domínguez, 2015).

There are several strategies for reducing sodium in meat products. It is possible to reduce sodium content by others chloride salts (Horita, Messias, Morgano, Hayakawa, & Pollonio, 2014; Vidal et al., 2019), non-chloride salts as lactates and phosphates (Ruusunen & Puolanne, 2005), flavor enhancers as taurine, lysine, monosodium glutamate and yeast extracts (dos Santos, Campagnol, Morgano, & Pollonio, 2014) among other strategies. The most common strategy to sodium reduction is addition of chloride salts, being potassium chloride (KCl) the most used due of its similars chemical properties to NaCl, however, the addition of this substitute salt may be limited due to the development of bitter taste (McGough, Sato, Rankin, & Sindelar, 2012). Strategies as the use of NaCl blends with substitute salts and flavor enhancers are widely used to reduce the undesirable sensory and technological effects of substitute salts (Desmond, 2006).

Table 1. Average sodium content in meat products

Meat product	Sodium content (mg/100g)	Reference
Beef jerky	1785	USDA Food Composition Databases (2019)
Brazilian charqui	5875	Brazilian food composition table (TACO) (2019)
Frankfurters	976	USDA Food Composition Databases (2019)
Ham	1280	USDA Food Composition Databases (2019)

Hamburger	487	USDA Food Composition Databases (2019)
Jerked beef	5256	Vidal et al. (2019)
Meatballs	666	USDA Food Composition Databases (2019)
Mortadella	1246	USDA Food Composition Databases (2019)
Salami	1740	USDA Food Composition Databases (2019)
Sausage	739	USDA Food Composition Databases (2019)

According to the averages of sodium content in meat products presented in Table 1, salted meat products (brazilian charqui and jerked beef) have the highest sodium content. During the salting steps to obtain the salted meat product, it is necessary to use a large amount of NaCl to develop the desirable characteristics in the final product, thus making it a great challenge to reduce the sodium content without affecting the quality.

4. Salted meat products

The appearance of salted meat products is immemorial. In pre-Columbian America, the Quichuas developed knowledge about salting to perfection, transforming it into technology applied to a large number of foods (Carvalho Junior, 2002). Variations of jerky, derived from the word Quechua ch'arki, means dried, salted meat (Roberts, 2017).

The performance of salting meat was fundamental to gain access to the mineral resource and, thereby, the technique appeared in different places of the world. Humans need

salt to live, because of this fact our ancestors knew where to find suitable supplies. These sources can be saline pools, prehistoric deposits, evaporated ocean water or other forms.

The salting and/or dehydratition of meat has resulted in the appearance of a wide range of meat products in many places of the world. The category of salted meat products is very diverse, having several different characteristics from one product to another. Many of these differences in flavor, shelf life and composition occur due to the technology applied during the processing that is usually passed from generation to generation.

Many salted meat products are considered traditional, with specific characteristics and associated with a region, group of people and culture. With this, processing is often performed without control and standardization. The process for obtaining salted meat product is very wide, and it can be combined several processes as wet and dry salting, solar drying, fermentation and smoking. All these techniques are very antique, but still applied in the meat products industry.

The evolution of salted meat products elaborated with whole meats, resulted in three very distinct categories. The first includes products which, due to its low salt content, can be consumed without any culinary preparation. Among these, are cecina de Leon (Molinero, Martínez, Rubio, Rovira, & Jaime, 2008), jámon serrano (Fernández et al., 2007) and lácon (Lorenzo et al., 2015) in Spain, pastirma of Turkey (Abdallah, Mohmaed, Mohamed, & Emara, 2017), biltong (Jones et al., 2017) in South Africa, and bresaola (Picone et al., 2019) in Italia.

The second category of salted meat products includes those which do not require desalting, stable for short periods of time at room temperature but which must be cooked before consumption. The sun-dried beef, a typical artisanal brazilian salted meat with relativy high moisture (64-70%) (Ishihara, Moreira, de Souza, Salviano, & Madruga, 2013) can be an example of this category.

The third category require desalting and cooking for consumption. In this category can be included charqui (Shimokomaki et al., 1998) and jerked beef (Vidal et al., 2019) from Brazil, and tasajo (Chenoll, Heredia, Seguí, & Fito, 2007) from Cuba.

4.1 Salted meats from Brazil – brazilian charqui, jerked beef and sun-dried beef

The charqui is a brazilian salted meat produced and consumed for more than a century, the consumption is mainly in the northeast region, having its production in the majority handmade and small. According to the brazilian regulation (1962), charqui is a product of intermediary water activity (around 0.74), maximum of 15% of mineral residue and 47.5% of moisture. The use of additives (nitrite, nitrate, phosphate, etc) is prohibited and there are no determinations regarding the conditioning of the product for commercialization (Brasil, 1962). The charqui is elaborate by the performance of salting, stacking and exposure to the sun during days for drying of bovine raw meat. According to Biscola et al. (2013), during stacking, the fermentation is carried out by acid-lactic bacteria naturally present in raw meat, responsible for the development of the charqui's flavor characteristics. The product's conservation is based on dehydration and salt to prevent the growth of undesirable microorganisms, being stable for six months without packaging and does not require refrigeration. Therefore, it is an important source of protein of high biological value in rural areas, where it sometimes does not have a consistent refrigeration system (Torres, Pearson, Gray, Ku, & Shimokomaki, 1989).

Briefly, jerked beef is an adaptation of brazilian charqui (Shimokomaki et al., 1998). The jerked beef is a traditional brazilian salted meat with intermediary water activity and great importance in the brazilian meat industry. The process to obtain jerked beef is similar to charqui, but due to greater water activity (maximum of 0.78) and moisture (maximum of 55%) of jerked beef, nitrite addition and vacuum packaging is required to reach six months of shelf-life at room temperature (Brasil, 2005). Furthermore, the maximum mineral residue is 18.3% and the addition of additives such as erythorbate and phosphate during the preparation of jerked beef is allowed by brazilian regulation (Brasil, 2005). Due to the higher moisture and shorter processing time compared to charqui, jerked beef becomes more economically profitable and nowadays is the brazilian salted meat product most produced and consumed.

The sun-dried beef, although it is a salted meat product, the characteristics this product resemble more fresh meat than salted meat products as charqui and jerked beef. The sun-dried beef is very consumed in the brazilian northeast, mainly in the capitals, and the production is basically small and handmade, with lack of technological advances, and does not have established official quality standards, enabling the distribution and marketing in unsatisfactory hygienic-sanitary conditions, favoring the development of deteriorating and pathogenic microorganisms that can negatively affect consumer health (Evangelista-Barreto et

al., 2014). Due to dehydration only on the surface, high aw and not addition of preservatives as nitrite and using vacuum packaging, the sun-dried beef shelf-life varies from 2 to 4 days and may increase a few days by the use of cold chain (Ishihara, Moreira, de Souza, Salviano, & Madruga, 2013). Taking into account all characteristics mentioned above the sun-dried beef, especially the short shelf-life, its production and consumption are basically limited to the northeast of Brazil.

The volume of salted meat produced in Brazil is not officially known, there are debatable estimates of about 500.000 tons annually. Undoubtedly, a significant volume for products thought to be extinguished by refrigeration and new food options.

5. Salted meat processing

During the elaboration of salted meat product it is necessary to make one or more salting steps. The salting methods differ according to the product, purpose and directly affect the characteristics of the product. The salting method applied will define the mechanism of mass transfer and gain or loss of weight. Salting techniques can be divided into two groups: wet salting (immersion or injection) and dry salting (Shahidi & Samaranayaka, 2004).

5.1 Wet salting

The wet salting by immersion is used to standardize the salting process of the pieces of meat, immersing the pieces in brine, being able to be added curing salts and spices to enhance some characteristics of flavor and aroma (Arnau, 2004). The brine is usually saturated and has an average of 25% salt concentration (Pegg, 2004) with a minimum meat/brine ratio of 1:2 (Heinz & Hautzinger, 2007). The wet salting time varies according to the size of the meat pieces, salt concentration, product characteristics and subsequent process steps (Shahidi & Samaranayaka, 2004). In some cases, this step is performed before of dry salting to improve the quality of the final product.

In the wet salting by injection, the brine is injected through needles directly into the muscle tissue, quickly transferring brine into the meat pieces with consequent rapid reduction of microbial growth (Shahidi & Samaranayaka, 2004; Pegg, 2004). Salting time using this method is very short compared to other salting methods, but the sensorial characteristics from the cured salts are less intense (Heinz & Hautzinger, 2007). The effectiveness of salting by

injection is dependent on the equipment used, characteristics such as pressure on the muscle during the injection, amount of brine added, spacing and number of needles, impacting the muscular structure and affecting the quality of the salted meat product (Pegg, 2004).

Figure 1.Wet salting by immersion

5.2 Dry salting

Dry salting is used as a method of conservation for thousands of years. The method consist in placed meat pieces directly in contact with the salt, other ingredients and additives (Arnau, 2004). The two techniques most used for dry salting are by use of undetermined or exact salt content (Petrova, Aasen, Rustad, & Eikevik, 2015).

The undetermined dry salting is the most common technique employed. The pieces of meat are stacked on top of each other and layers of salt are added between layers of meat. It is often necessary to carry out re-stacking of the meat pieces in order to produce a uniform distribution of the salt and pressure exerted on the meat pieces.

For dry salting by use of exact salt content, a certain salt content is added on the surface of the product. The technique of addition of exact salt content requires more time compared to the other main method of dry salting because all the salt needs to be absorbed by the product. The time required varies according to the size of meat pieces (Bosse et al., 2018).

At the base of container which supports the meats pieces must be holes where the moisture can be drained (Heinz & Hautzinger, 2007). The temperature and relative humidity

is very important during the salting process, and the higher the temperature greater will be the diffusion of salt (Domínguez et al., 2017).

Figure 2. Dry salting

6. NaCl effects in salted meat products

NaCl is the most commonly ingredient used in the processing of meat products, being very important due to its technological and sensorial properties, among them ionic strength increase, microbiological stability, influence the water retention and binding capacity, modify the texture properties and provides salty taste (Desmond, 2006).

In addition to performing technological and sensory functions, NaCl is a very important nutrient for the human body due to its role as regulator of body fluids, contributes to adequate hydration supporting the transmission of nerve impulses to the brain and control of the amount of water in the cells (Domínguez et al., 2017). Anyway, as mentioned previosly, excessive sodium intake can increase the risk of numerous health problems as cardiovascular and kidney disorders, high blood pressure and certain types of cancer (Strazzullo et al., 2009).

With this in mind, it is necessary to investigate all the effects of NaCl in salted meat products, being a challenge both in technological and sensorial point of view.

6.1 Antimicrobial effect

NaCl is an excellent preservative, inhibiting the growth and survival of undesirable microorganisms, prevents rapid deterioration and increases shelf-life (Inguglia et al., 2017).

The addition of NaCl causes osmotic shock of the cells, which results in the loss of water from the cell causing microbial death or retarding its growth (Davidson & Taylor, 2007). In addition, NaCl retains water molecules with a consequent decrease of water activity, making it impossible to grow most of the microorganisms (Yotsuyanagi et al., 2016). According to Manzoni, Schimel, and Porporato (2012) and Moyano, Manzoni, and Chenu (2013), the majority of microorganisms can not multiply in water activity lower than 0.90.

The water activity of most salted meat products is low, so that few microorganisms can survive and/or develop under these conditions. For example, brazilian charqui has around 0.75 water activity and is stable for six months at room temperature without the need for packaging (Torres et al., 1994). However, the main microorganisms present in salted meat products are the halophyllic and halotolerant (Ventosa, Nieto, & Oren, 1998), decreasing the shelf-life of this category (Torres et al., 1994).

Table 2. Minimal water activity for microorganism growth

Range of water activity	Microorganisms Inhibited
1.00 – 0.95	*Bacillus, C. botulinum E, G, Clostridium perfringens, Escherichia, Klebsiella, Pseudomonas, Proteus, Shigella* and some yeasts
0.95 – 0.91	*Bacillus cereus, Clostridium botulinum A, B, Listeria monocytogenes* and *Salmonella*
0.91 – 0.87	*Micrococcus, Staphylococcus aureus (aerobic),* many yeasts *(Candida, Torulopsis, Hansenula)*
0.87 – 0.80	*Staphyloccocus aureus, Debaryomyces, most molds (mycotoxigenic penicillia)* and *most Saccharomyces (bailii) spp.*
0.80 – 0.75	*mycotoxigenic aspergilli* and *most halophilic bacteria*
0.75 – 0.65	*Saccharomyces bisporus and xerophilic molds (Aspergillus chevalieri, A. candidus, Wallemia sebi),*
0.65 – 0.61	*Aspergillus echinulatus, Monascus bisporus* and *Osmophilic yeasts (Sacharomyces rouxii)*
0.61 or less	No microbial proliferation

Adapted from Tapia, Alzamora, and Chirife (2008).

In this way, the addition of NaCl and dehydratation makes the product microbiologically stable and the loss of water during the processing of salted meat products directly influences the development of the organoleptic characteristics.

6.2 Effects on proteolytic reactions

During the processing of salted meat there are several biochemical reactions that will define the quality of the final product. Among them, are the proteolytic reactions that are dependent of variables as microbiota, processing conditions, raw material, water activity and mainly NaCl content (Mora et al., 2015). The NaCl regulates the activity of the proteolytic enzymes, being able to inhibit the enzymatic activity with the increase of the NaCl concentration during the drying process (Toldrá, 2002). Toldrá, Rico, and Flores (1992) observed that reduction of NaCl content can activate more muscular proteases, increasing the proteolytic activity and consequent release of free amino acids. The proteolytic enzymes can be grouped into two systems: a system composed of acidic lysosomal proteinases (cathepsins and cystatin), and a calcium-dependent proteolytic system or statin (D-type) (Roseiro et al., 2008). The activity of the D-type proteolytic enzyme during maturation is inhibited by the presence of calpastatin and NaCl (Toldrá, 2002).

Due to the proteolysis process, many peptides are released (Toldrá, 2016). Some of these peptides have important amino acid residues, such as AAATP, AAPLAP, KPVAAP, LAGRP, KAAAAP e TGLKP, which exert an inhibitory activity against angiotensin I-converting enzyme, reducing arterial contraction and lowering blood pressure (Escudero, Aristoy, Nishimura, Arihara, & Toldrá, 2012; Escudero et al., 2013; Escudero, Mora, & Toldrá, 2014). Mora, Escudero, Arihara, and Toldrá (2015) observed that dry-cured ham extracts have antihypertensive effect *in vivo* assays.

According to Wu et al. (2014), the reduction of NaCl causes problems as the excess of proteolysis due to the intense action of the proteases of the tissue, which can imply in texture problems. Tissue proteases may also act in excess on proteins and polypeptides forming a high content of low molecular weight nitrogen compounds such as free amino acids and peptides, which may generate undesirable flavors as bitter taste (Martín, Córdoba, Antequera, Timón, & Ventanas, 1998; Toldrá, 1998). Moreover, the reduction of NaCl added may adversely affect on sensory quality of salted meat products, especially texture properties.

6.3 Effects on lipolytic reactions and lipid oxidation

The changes that occur on the lipids affect sensorially the salted meat products (Chizzolini, Novelli, & Zanardi, 1998), being lipolysis and lipid oxidation reactions directly influenced by factors as pH, additives, water activity, metals, processing conditions, storage and salts (Demeyer, 2004).

NaCl is an excellent pro-oxidant and is usually present in high content in salted meat products. The processing conditions of the salted meat product are responsible for promoting and catalyzing lipid oxidation due to the use of NaCl, drying and dehydration. NaCl reduces the activity of antioxidant enzymes and increases the catalytic activity of iron (Fe), and oxidation reactions can produce undesirable taste and odor if not properly controlled (Domínguez et al., 2017), however, some meat products have characteristic taste and odor from the oxidation reactions. Lipid oxidation forms compounds as malonaldehyde and cholesterol oxides, which if consumed in excess may increase the risk of development of certain chronic diseases, therefore, the formation of these compounds should be monitored (Addis, 1986; Jiménez-Colmenero, Carballo, & Cofrades, 2001).

Lorenzo et al. (2015) suggest that the increase of the malonaldehyde content during the salting and post-salting steps are related to the oxidation phenomenon that can be favored by the pro-oxidant action of the salt and by metallic ions present.

Oxidation of free fatty acids is the second stage of conversion of lipids to flavor compounds, peroxides, and numerous volatile compounds or aroma percussors (Huang, Li, Huang, Li, & Sun, 2014). The salted meat product has typical flavor, aroma and texture from the proteolysis, lipolysis and oxidation reactions (Garrido, Domínguez, Lorenzo, Franco, & Carballo, 2012; Purriños, Franco, Carballo, & Lorenzo, 2012), but these reactions must be controlled in order to avoid loss of quality in the final product.

6.4 Changes on water retention capacity and texture properties

In meat and meat products the water molecule is retained between the myofilaments of the muscle fibers by the capillary phenomenon. The increase or decrease of the space between the myofilaments will define the water retention capacity (Huff-Lonergan & Lonergan, 2005).

According to Raoult-Wack (1994), one of the explanations is the entry of NaCl in the meat matrix with consequent output of water molecules, this phenomenon is called osmotic dehydration (Raoult-Wack, 1994).

As mentioned previosly, NaCl is a very important ingredient for the development of technological and sensorial characteristics in salted meat products, among these characteristics is the texture that is affected by the reduction of NaCl added in the formulation (Desmond, 2006). NaCl inhibits the activity of the proteolytic enzymes present in muscle cells, these enzymes are mainly responsible for protein degradation and affect texture properties (Roseiro et al., 2008). In addition, the texture is related to aroma and flavor, therefore, adhesive, pasty or soft texture reduce salivation, reducing the perception of flavor and aroma compounds (Garrido, Lorenzo Rodriguez, Franco, & Carballo, 2014).

7. Strategies for reduction of sodium content in salted meat products

The consumer and meat industry is alert to sodium content and the demand for products with reduced levels of sodium increases continuously year by year. Due to the various NaCl functionalities already mentioned previously, the reduction of this ingredient provides a great technological challenge to maintain product quality (Tamm, Bolumar, Bajovic, & Toepfl, 2016).

7.1 Reduction of the added NaCl content

The gradual reduction of the added NaCl content may be a good method for reducing sodium over time. According to Cobcroft, Tikellis, and Busch (2008), the reduction of 5-10% of salt content per year is a good strategy to habituate consumers to less salty flavored products. On the other hand, this strategy has some limitations. First, a relatively long time is required to effectively reduce NaCl, and it is necessary to be employed on a large scale in the industry, requiring a great joint effort and synchrony (Inguglia et al., 2017).

The reduction of sodium content should be carried out with caution, the simple reduction of NaCl may increase the reactions of proteolysis (Zhao et al., 2008) and changes in lipid reactions due to the pro-oxidant action of NaCl (Jin et al., 2012). According to Tamm et al. (2016), the reduction of less than 10% of NaCl does not significantly affect the taste.

In salted meats, this strategy is not possible to be conducted, because the high content of NaCl added have the objective to reduce water activity at safe level that results in shelf stable meat products.

7.2 Use of chloride and non-chloride salts for partial or total NaCl replacement

One of the strategies to reduce the sodium content in meat products is the use of other chloride salts as KCl, $MgCl_2$ and $CaCl_2$ for partial or total NaCl replacement, being the KCl is the most common (He & MacGregor, 2010). However, the replacement of NaCl with KCl is limited to a maximum of 30% in most products due to the development of metallic and bitter taste (Doyle & Glass, 2010; Grummer, Bobowski, Karalus, Vickers, & Schoenfuss, 2013; Toldrá & Reig, 2011). In addition, KCl increases lipid reactions and levels of volatile compounds, especially aldehydes, and consequently modifies the aroma and taste (Wu et al., 2015). According to Ripollés, Campagnol, Armenteros, Aristoy, and Toldrá (2011), the partial replacement of NaCl by other salts may affect lipid oxidation, acid lipase activity and formation of free fatty acids in dry-cured ham.

The use of $CaCl_2$ and $MgCl_2$ in porcine can be a healthy strategy with nutritional benefits (Armenteros, Aristoy, & Toldrá, 2009), therefore, the use of divalent cations promotes undesirable effects such as development of bitter, metallic and residual taste, astringency besides affecting texture in dry-cured loins (Armenteros, Aristoy, Barat, & Toldrá, 2009). According to Murphy, Cardello and Brand (1981), the greater atomic weight of the ion, the greater the bitter development. Moreover, the use of substitute salts influences salting time (Vidal et al., 2019), which may impair the quality of the final product due to changes in texture and microbiological stability (Aliño, Grau, Fuentes, & Barat, 2010; Aliño et al., 2009; Blesa et al., 2008; Lorenzo et al., 2015). Vidal et al. (2019), replacing partially the NaCl by $CaCl_2$ in jerked beef, found that the addition of $CaCl_2$ cause deleterious effects such as increased rancid aroma, hardness, fibrosity, bitter taste and aftertaste with consequent significant reduction of sensory acceptance.

Non-chloride salts such as lactates are examples of ingredients used to minimize changes in salty taste and antimicrobial effect in meat products with reduced NaCl content (Doyle & Glass, 2010). Sodium and potassium lactate, sodium diacetate, glycine and phosphates are some of the non-chlorinated salts used as NaCl substitutes (Devlieghere, Vermeiren, Bontenbal, Lamers, & Debevere, 2009; Weiss et al., 2010). Phosphates are often

used due to providing a relative salty taste and to increase the water retention capacity (Weiss et al., 2010), promoting greater yield and change on texture properties.

7.3 Use of flavor enhancers

The reduction or substitution of NaCl in meat products is not easy assignment and can significantly affect the sensory properties (Liem, Miremadi, & Keast, 2011). Several sensory interactions occur during oral processing (Delwiche, 2004), and sodium ion may have a major influence on flavor perception (Keast, Breslin, & Beauchamp, 2001). According to Gaudette and Pickering (2013), sodium can suppress the bitter taste, with consequent great importance in the flavor modification.

One of the strategies used to reduce sodium content and minimize undesirable sensory impact due to reduction or substitution of NaCl by other salts is the addition of flavor enhancers. The enhancers act by increasing taste perception due to the activation of umami flavor recipients, so these ingredients provide a unique tool to reduce sodium content in foods (McGough et al., 2012). According to Wallis and Chapman (2012), flavor enhancers allow the sodium content in the final product to be reduced by 40%. Due to the large and growing demand for sodium reduction in food products, the number of flavor enhancers available is higher each year.

Several studies have reported the ability of different flavor enhancers to reduce negative sensory impacts from substitute salts and/or NaCl reduction in meat products (Flores, Corral, Cano-García, Salvador, & Belloch, 2015; McGough et al., 2012; Pietrasik & Gaudette, 2014). The use of flavor enhancers as lysine, taurine, monosodium glutamate, yeast extract, lactates, disodium guanylate and disodium inosinate may be a good strategy to improve the sensory acceptance of meat products with sodium reduction (Campagnol, dos Santos, Wagner, Terra, & Pollonio, 2011; Desmond, 2006).

Campagnol, dos Santos, Terra, and Pollonio (2012) found that the use of lysine, disodium guanylate and disodium inosinate may decrease the undesirable sensory effects provided by KCl in fermented sausages. In frankfurters, McGough, Sato, Rankin, and Sindelar (2012) were able to reduce 20% NaCl with good sensory acceptance using natural flavor enhancer. According to Campagnol, dos Santos, Wagner, Terra, and Pollonio (2011), the use of 1 or 2% of yeast extract improves the taste of fermented sausages with replacement of 25% NaCl by KCl. Pietrasik and Gaudette (2014) obtained good sensory results reducing

the NaCl content by 40% and adding 1.25% commercial flavor enhancer (Fonterra™ "Savoury Powder") in restructured cooked hams. When using taurine, lysine, disodium inosinate, disodium guanylate and monosodium glutamate dos Santos, Campagnol, Morgano, and Pollonio (2014) found improvement in the sensorial quality of fermented cooked sausages with replacement of 50% and 75% of NaCl by KCl.

8. Final Considerations

Regular consumption of meat and meat products provides a number of benefits to human health, making it difficult to obtain the same nutrients or the same amount in other foods. The category of salted meat products even possessing a relatively high sodium content, is much consumed and important source of protein, minerals and vitamins worldwide. Taking this into account, is very important to reduce the sodium content in this category to make them healthier. Reduce NaCl content partially or completely in salted meats is very complex and challenging because its extremely important to provide functionalities and quality for final product.

Is imperative to understand all the characteristics of the product, processing and possible strategies that can be employed to minimize the negative impacts that the reduction and/or substitution of NaCl may cause in salted meat products. The success of sodium reduction will depend primarily on the characterists of product and the processing aspects.

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CAPÍTULO 2

REDUCING 50% SODIUM CHLORIDE IN HEALTHIER JERKED BEEF: AN EFFICIENT DESIGN TO ENSURE SUITABLE STABILITY, TECHNOLOGICAL AND SENSORY PROPERTIES

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Meat Science

Vol. 152, p. 49-57, 2019

DOI: 10.1016/j.meatsci.2019.02.005

Highlights

- 50% NaCl was replaced by blends of KCl and $CaCl_2$ to reduce sodium in jerked beef
- Blends with $CaCl_2$ increased bitter taste and rancid aroma in reformulated jerked beef
- Reduced sodium product stability was maintained with blends of $CaCl_2$ and KCl
- KCl was the best salt substitute to preserve sensory properties of reduced sodium jerked beef

Abstract

The objective of this study was to evaluate the effects of the partial replacement of NaCl by blends of KCl and $CaCl_2$ on the physicochemical, microbiological, and sensory properties of jerked beef. For that, in the dry and wet salting stages, 50% NaCl of the control treatment (FC1) was replaced by 50% KCl (F1), 50% $CaCl_2$ (F2), and a blend containing 25 % KCl and 25% $CaCl_2$ (F3) at equivalent concentrations based on the ionic strength. All reformulated treatments presented a significant sodium reduction when compared to the control (27.57% F1, 41.59% F2, and 36.74% F3). The $CaCl_2$ blends resulted in final products with bitter taste and rancid aroma accompanied by a higher TBARS and shear force and lower a* values ($P < 0.05$). The substitute salts did not affect the microbiological stability ($P > 0.05$). The present results demonstrate that adding 50% KCl may be a good strategy to reduce sodium in jerked beef.

Keywords: salt substitutes, salted meat, sodium reduction, jerked beef

1. Introduction

Jerked beef is a meat product very consumed in Brazil and in the world (Ishihara & Madruga, 2013), constituting a large and important source of meat protein, especially in areas with non-existent or deficient cold chain (Torres, Pearson, Gray, Ku, & Shimokomaki, 1989). According to Garcia, Mizubuti, Kanashiro, and Shimokomaki (2001), salted meats have a high protein efficiency, excellent biological value, and easy digestion.

In short, jerked beef is an adaptation of charqui (Shimokomaki *et al*., 1998), a traditional salted meat that has been produced since antiquity with a great importance in the economic development of many countries. Its preservation is based on hurdle technology, consisting of the reduction of water activity (aw), addition of nitrite, and use of vacuum packaging (Biscontini, Shimokomaki, Oliveira, & Zorn, 1996; Torres et al., 1994). Brazilian legislation for jerked beef has established that the product must be vacuum packed, with maximum limits of 55% moisture, 18.3% minerals, 50 ppm of residual sodium nitrite, and aw 0.78 in the final product (Brasil, 2005).

Recently, the World Health Organization (2012) recommends a daily intake of 2 grams of sodium equivalent to 5 grams of sodium chloride (NaCl) to reduce the risk of chronic diseases such as hypertension and cardiovascular diseases (McGough, Sato, Rankin, & Sindelar, 2012; Pires *et al*., 2017). Meat products have been characterized as one of the main sources of dietary sodium, thus their consumption may be restricted in the context of a healthy diet. Despite the technological and nutritional advantages of jerked beef, it contains very high sodium levels, requiring a desalting step for the preparation. Whereas this procedure cannot be standardized by the meat industry, consumers may be exposed to high levels of this mineral in meals that include this meat product. The use of salt substitutes to NaCl is an excellent strategy to effectively guarantee the sodium reduction in this product after the desalting operation. Although official data about the sodium levels of jerked beef in Brazil are non-existent, the average found in commercial product labels exceeds 5.000 mg Na/100 g.

The use of NaCl is especially important in meat products, particularly in salt-cured meat, due to its sensory, technological, and preservation role (Man, 2007; Tim, 2002), thus the reduction and/or partial replacement of NaCl is a great technological challenge mainly from the sensory point of view. According to Delgado-Pando et al. (2018), the quality and safety of the meat products may be impaired if the sodium chloride content is reduced without taking measures to minimize the negative impacts.

There are several strategies for reducing NaCl in meat products (Desmond, 2006). Among the possible strategies, the most performed are: reduction of the sodium content by simple reduction of NaCl added (Fellendorf, O'Sullivan, & Kerry, 2016), addition of flavor enhancers such as taurine and lysine (dos Santos, Campagnol, Morgano, & Pollonio, 2014), use of non-chlorinated salts such as phosphates and lactates (Ruusunen & Puolanne, 2005), and chlorinated salts such as potassium chloride (KCl), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$) (Desmond, 2006; Horita et al., 2016).

The use of blends containing NaCl substitutes such as KCl, $CaCl_2$, and $MgCl_2$ has been studied in different meat products, in which salt is reponsible for the sensory properties, typical texture development, and microbiological stability such as dry fermented sausages, dry-cured ham, pastirma, chorizo, etc. (Guàrdia, Guerrero, Gelabert, Gou, & Arnau, 2006; Horita et al., 2016; Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017). Guàrdia, Guerrero, Gelabert, Gou, and Arnau (2008) evaluated the reduction of 50% NaCl and the replacement by 50% KCl and KCl/K-lactate blends (40:10) in dry femented sausages, and reported no changes in the overall acceptance. Armenteros, Aristoy, Barat, and Toldrá (2012) reported good sensory results in dry-cured ham with the reduction of NaCl and addition of KCl. However, there are no studies reporting the replacement of NaCl in jerked beef or salt-cured meats with very low water activity (< 0.8), probably due to the great challenge in maintaining the sensory properties and the physicochemical quality attributes of the reformulated products.

The quality of the jerked beef is dependent on several variables including the raw material, processing conditions, and formulation components, and salt is one of the most important ingredients (Mora et al., 2015). Therefore, there is a need to understand the different effects of substitute salts on the salting process, and the evolution of lower water activity, microbiological stability, quality attributes, and other characteristics. Based on that, the objective of this study was to investigate the effects of the partial replacement of NaCl by KCl and $CaCl_2$ blends during the salting operations, and the physicochemical, microbiologicals and sensory characteristics of the reformulated jerked beef. The study also evaluated the effects of the substitute salts on the reduction of water activity throughout the salting process, and the impact of the reformulation on the overall properties of the final product.

2. Material and Methods

2.1 Treatments, raw materials, and additives

The manufacture process of jerked beef in the different treatments was carried out according to the conventional technique used in the Brazilian meat industry, which was performed in the Pilot Plant of Meat Products, Faculty of Food Engineering, at UNICAMP.

The additives sodium nitrite and sodium erythorbate were donated by the company Kerry of Brazil. The salts NaCl, KCl, and $CaCl_2$ were purchased from a food grade company (Anidrol, Brazil). The selected meat cut was outside flat (*biceps femoris*) purchased from slaughterhouses with assured hygienic quality.

Four jerked beef formulations were made, as shown in Table 1. To replace NaCl, the partial levels of KCl and $CaCl_2$ added was based on the ionic strength of NaCl. The rate of NaCl: KCl: $CaCl_2$ was 1: 1.27: 0.63, respectively, to result the same ionic strength in all treatments. Then, the blends were made in sufficient quantity for the salting steps, depending on the weight of the raw meat, using 2 kg salt per kg of meat, considering the rate described above. Similar amounts of the additives sodium nitrite and sodium erythorbate were added in the wet salting step, and the salt was the variable of the wet and dry salting steps. The experiment was performed in three replicates on different days.

Table 1. Jerked beef treatments added with NaCl substitutes.

Treatments	NaCl reduction (%)	NaCl (%)	mg/2000mg*	KCl (%)	mg/2000mg*	$CaCl_2$ (%)	mg/2000mg*
FC1	-	100	2000	-		-	
F1	50	50	881	50	1119	-	
F2	50	50	1227	-	-	50	773
F3	50	50	1026	25	651	25	323

*Blends with NaCl, KCl and $CaCl_2$ used in the wet and dry salting steps for each 1000 mg of bovine raw meat based on the ionic strength equivalent to NaCl (2.000mg salt/1000mg raw bovine meat)

2.2 Processing

The manufacture process of jerked beef is described in Figure 1. First, the excess fat and collagen were removed from the bovine raw meat (*biceps femoris*) to be submitted to the salting steps (wet and dry), which was then cut into portions approximately 40 mm thick and 300 mm long (Figure 1. A). In the wet salting step (Figure 1. B), the pieces of each treatment were submerged in a saturated solution (brine containing 150 ppm sodium nitrite and 500 ppm sodium erythorbate) for 1 hour. In the dry salting step, for each kg of meat cut (*biceps femoris*), were added 2 kg of NaCl (FC1). For F1, F2 and F3, the same amount, however composed of the blends of the substitute salts, was added in the proportion described in Table 1 to obtain the same ionic strength of FC1. The pieces were in contact with different salt blends, as also described in Table 1, for 144 hours (6 days) in a cold chamber at 13 ° C in the form of layers (Figure 1. C). During the dry salting period, the salt layers were changed after the first 24 hours and the parts changed every 24 hours in a procedure defined as re-salting, aiming to standardize the pressure exerted by the weight of the pieces. At the end of the 6 days, when the aw predicted for this product (≤ 0.78) was reached, the portions were washed with a 1% sodium hypochlorite solution to remove surface soils and reduce possible contaminations from the process steps. Finally, the drying and ripening steps (Figure 1. D) were carried out in a controlled climatic chamber (Instala Frio, Curitiba, Brazil) with 55% humidity, 25 °C and 0.5 m/s forced air ventilation for 24 hours. After the process (Figure 1. E), the pieces were vacuum packed of polyethylene (Spel, São Paulo, Brazil) and stored at 25 °C (Figure 1. F).

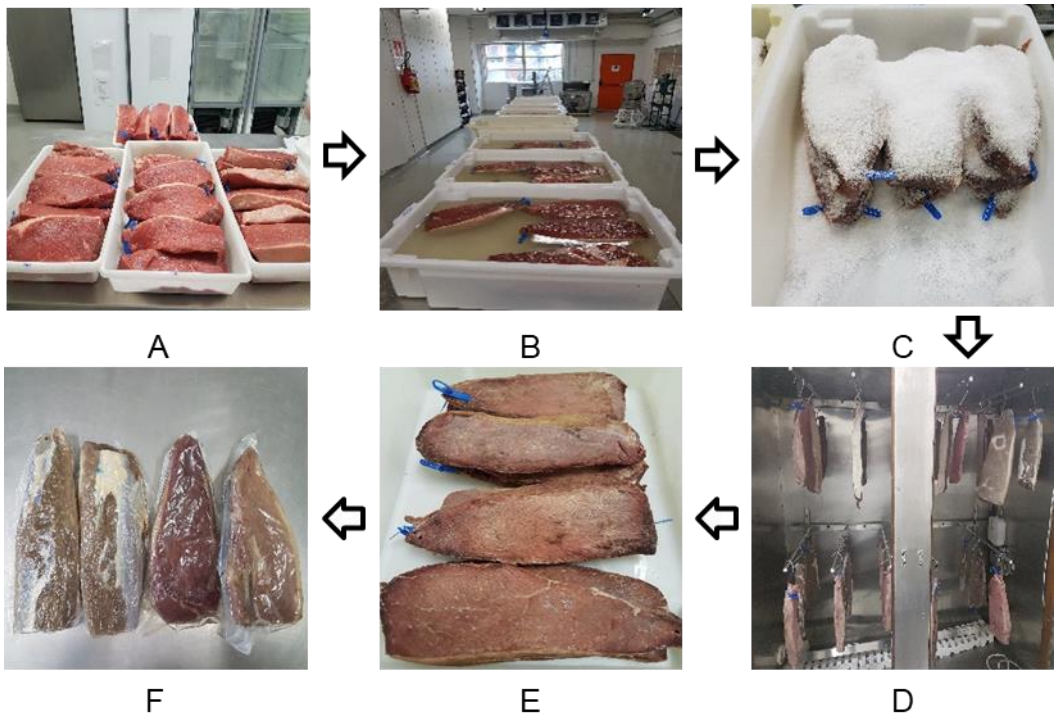

Figure 1. Processing steps for obtaining jerked beef; A: outside flat cuts; B: wet salting; C: dry salting; D: ripening; E: final product; F: vacuum packaging

All the processing steps were carried out in the Meat Laboratory of the Department of Food Technology (DTA) at University of Campinas (UNICAMP).

2.3 Physicochemical characterization

2.3.1 Proximate composition, pH, and water activity

The moisture, protein, lipids, and ash contents were determined in both raw meat (before wet salting step) and the final product according to Horwitz and Latimer (2005). The pH was determined by homogenizing 10 g sample and distilled water (1:10), followed by pH readings using an combined electrode (22 DM, Digimed, São Paulo, Brazil). The water activity was measured at 20 ° C using the Aqualab apparatus (Decagon Devices Inc., Pullman, USA). Both pH and aw were measured after wet salting, dry salting, and in the final product (salted meat after ripening). All analyses were performed in triplicate for each replicate of the experiment.

2.3.2 Instrumental color

The color was measured using the Hunter Lab colorimeter (Colourquest II, Hunter Associates Laboratory Inc., Virginia, USA) with 20 mm aperture, D65 illuminant, and standard 10 ° observer. CIELAB L*, a*, and b* parameters were determined as an indicator of luminosity, red intensity, and yellow intensity, respectively. The whiteness index (W) was calculated by the following equation: 100 - [(100-L *) 2 + a * 2 + b * 2] 1/2. The color variation was measured in four points in the central cut surface area of four slices per unit of sample. The assays were performed in triplicate for each treatment and each replicate. The color measurements were performed in raw meat, after the wet salting, dry salting, and final product. The samples were kept at room temperature (25 ° C) during analysis.

2.3.3 Desalting, cooking, and shear force

The samples of the different treatments were cut into portions of 6x6 cm and desalted using a ratio of 1: 6 (sample: water), with continuous water exchange every 2 hours for 30 hours, and then vacuum packed for later cooking. Cooking was carried out in a water bath (RSA-1708, RSA, Campinas, Brazil) at 80 ° C, and the temperature of the samples was monitored by a thermocouple from the moment the center of the sample reached 72 ° C, remaining at this temperature for 60 minutes.

The desalted and cooked samples were stored under refrigeration for 24 hours prior to the analysis of Warner-Bratzler Shear Force. Cylinders 1.27 cm in diameter parallel to the muscle fibers were sampled with the aid of a manual cutter (Stock & Board, 1995). The shear force was measured in a TA-XT 2i texture analyzer (Texture Technologies Corporation / Stable Micro Systems, Hamilton, UK), equipped with a 1 mm thick Warner-Bratzler blade. The equipment was previously calibrated with a weight of 5 kg. The ascent/descent speed was 200 mm/min and the distance to the platform was 25 mm. Each cylinder was cut only once and the result was expressed in N (Stock & Board, 1995). The analysis was performed in six replicates for each replicate of each treatment.

2.3.4 Lipid oxidation

The lipid oxidation was measured in raw meat, after the wet salting, dry salting, and final product as 2-thiobarbituric acid reactive substances (TBARS), according to the

methodology proposed by Bruna, Ordóñez, Fernández, Herranz, and De la Hoz (2001). The results were expressed as mg of malonaldehyde per kg of sample. The analysis was performed in triplicate for each replicate of each treatment.

2.3.5 Residual nitrite content

The residual nitrite was determined in final products at 24 hours after end of processing according to the official analytical methodology established in Normative Instruction 20 of July 21, 1999, of the Ministry of Agriculture, Livestock, and Supply (MAPA) (Brasil, 2005).

2.3.6 Minerals level

The determination of Ca, K, and Na and the sample preparation was performed by the dry digestion method (Horwitz & Latimer, 2005). For that, 1 g sample was weighed into porcelain capsules, burned in a heating plate, and incinerated in a muffle furnace (413, Fanem, São Paulo, Brazil) at 450 ° C until the formation of ash free of black spots. The ash was quantitatively transferred to a 50 mL volumetric flask with 5% (v v) nitric acid, and the solution was filtered on quantitative filter paper (Nalgon 3550) prior to the readings on the inductively coupled plasma emission spectrometer (Agilent 5100 VDV ICP OES, Agilent Technologies, Tokyo, Japan). The mineral levels were mensured in raw meat, after the wet salting, dry salting, final product and desalted (following the desalting step previously described in topic *2.3.*3. The analysis was performed in triplicate for each replicate of each treatment.

2.3.7 Microbiological characterization

Microbiological determinations were carried out in the raw material and in the final products at the end of processing. For that, 25 g of sample was homogenized with 225 ml of 0.1% peptone water for 2 minutes in a Stomacher (BA 7021, Seward, Easting Close, UK). Subsequent serial dilutions were performed for the following microbiological analyses: total counts, lactic acid counts, thermally tolerant coliforms, and total coliforms (Silva et al., 2017).

2.3.8 Sensory evaluation

A trained panel (7 men, 6 women, aged 20-55) with previous experience and familiarity in meat products (Santos et al., 2015, Horita et al., 2017) was used to generate the sensory profiling of the samples. Firstly, the repertory Grid method was used to generate to describe the similarities and differences in relation to the attributes appearance, aroma, flavor, and texture of the samples followed by a group discussion to select the most cited descriptors that best characterize the product and to eliminate those that were not perceived by most assessors. After the discussion and selection of the descriptors, three sessions of the intensity scale method were performed using an unstructured linear scale of 9 cm, with the term "little" at the far left and "far" at the right end of the scale for each descriptor selected. For the sensorial analyses, the samples were desalted and cooked following the steps previously described in topic *2.3.3*.

2.3.9 Statistical analysis

Three independent processes were performed using the same methodology, formulation, and technology. For each process, at least three samples were taken for each analysis. The results were expressed as the averages from all data. Data were analyzed using a general linear model considering the treatments as a fixed effect and the replicates as a random effect using 5% of significance. Significant differences were analyzed by the Tukey's test at the 5 % level of significance utilizing the commercial software Statistica v. 8 (Statsoft Inc., Tulsa, Oklahoma, USA).

For the sensory data, principal component analysis (PCA) using correlation matrix (Granato et al., 2018) and confidence ellipses were constructed using bootstrap technique (500 resamplings resulting in 500 sensory descriptions of virtual panels) (Balthazar et al., 2018). The data set consisted of a 4 x 10 matrix, in which rows represented the salted meat samples, and columns the mean values of sensory attributes. For these analyses the software XLSTAT 2018.6 (Addinsoft, Paris, France) was used.

3. Results and Discussion

3.1 Proximate composition, pH, and aw

During the processing of jerked beef, two simultaneous mass transfer phenomena take place: a gradual water release from the space inside to outside the myofibrils, and the salt absorption into the muscle tissue, resulting in a lower moisture and higher salt levels in the final product (Sabadini et al., 2001). As shown in Table 2, the treatment F2 containing 50% NaCl + 50% $CaCl_2$ (always based on the ionic strength relative to the % substitution) presented a higher moisture value ($P < 0.05$) after the drying and ripening steps comparing to treatments F1 (50% NaCl + 50% KCl) and F3 (50% NaCl + 25% KCl + 25% $CaCl_2$).

Table 2. Centesimal composition of jerked beef treatments

Treatments	Moisture (%)		Ash (%)		Protein (%)		Lipids (%)	
	Raw meat	FP	Raw meat	FP	Raw meat	FP	Raw meat	FP
FC1	75.23^{a}	50.64^{ab}	1.06^{a}	17.44^{c}	21.47^{a}	27.81^{a}	2.35^{a}	3.94^{a}
F1	75.15^{a}	50.45^{b}	1.04^{a}	18.61^{b}	21.49^{a}	27.90^{a}	2.47^{a}	3.63^{a}
F2	75.42^{a}	51.25^{a}	1.05^{a}	16.43^{d}	21.22^{a}	28.87^{a}	2.43^{a}	3.31^{a}
F3	75.37^{a}	50.14^{b}	1.08^{a}	19.27^{a}	21.36^{a}	28.49^{a}	2.34^{a}	2.56^{b}
Standard error	0.15	0.13	0.01	0.25	0.17	0.26	0.12	0.13

Values are means. $^{a, b, c, d}$ Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test. FP: final product. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$.

According to Lewicki and Michaluk (2004), calcium ions increase the mass transfer causing an increase in the dehydration rate. Several factors including the type and concentration of salt, and pH can alter the solubility of myofibrillar proteins and swelling of muscle fibers, which can lead to changes in the water retention and water binding capacity of meat products (Offer et al., 1989; Offer & Trinick, 1983; Oliveira, Pedro, Nunes, Costa, & Vaz-Pires, 2012). In jerked beef, $CaCl_2$ probably caused a high dehydration, forming a very firm and dry barrier on the surface of the flesh cut, thereby impairing the water release from the inner regions of the outside flat. Several authors have observed an increase in water loss of various products with the addition of calcium ions (Lewicki, Vu Le, & Pomarańska-Łazuka, 2002; Mastrantonio, Pereira, & Hubinger, 2005; Pereira, Carmello-Guerreiro, Bolini, Cunha,

& Hubinger, 2007). According to the results, the divalent $CaCl_2$ salt has a different effect on the dehydration process of jerked beef when compared to NaCl and KCl. Although a significant difference was observed, the moisture contents were very close from a practical point of view, despite the water activity values confirmed the surface dehydrating effect of $CaCl_2$. The treatment F2 presented higher aw value when compared to all other treatments ($P < 0.05$).

The ash content was also influenced by the nature and concentration of the salts used to reach the ionic strength equivalent to NaCl in the dry and wet salting stages. A lower ash level was observed for the treatment F2 (50% NaCl and 50% $CaCl_2$), which is in agreement with its higher moisture content. Despite the three independent replicates, the homogeneous distribution of minerals from the salting stages in the various treatments is a great challenge. Although the samples were taken from different parts of the salted cuts combining superficial and inner portions, it is believed that the differences may be due to the mass transfer phenomenon that is not always homogeneous and constant for meat cuts. However, all treatments presented values close to that described in the identity and quality standards for jerked beef industrialized in Brazil (18.3%). No differences were observed for the protein content between the control (FC1: 100% NaCl), and the modified treatments (F1, F2 and F3). Even without significant difference ($P < 0.05$), the highest protein content of the jerked beef made with 50% replacement of NaCl by $CaCl_2$ may be due to the retention of the pigment myoglobin in the meat cut, which was not removed during the osmotic dehydration in comparison to the other treatments. It was observed that the liquid drained during the salting step was transparent, which was not observed for the other treatments (Fig. 2).

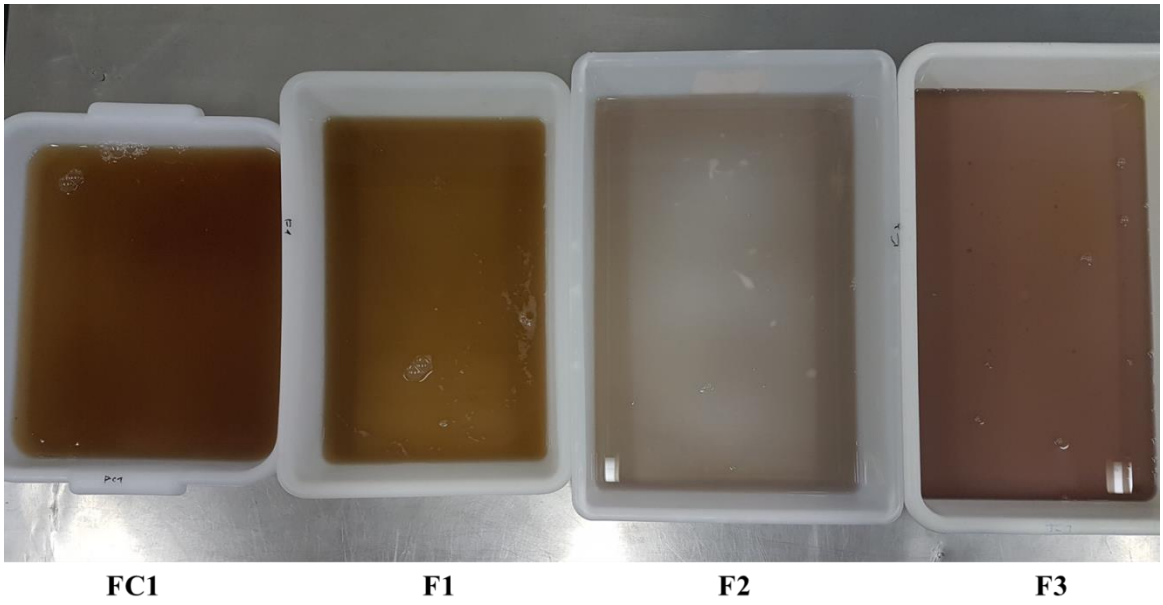

Figure 2. Liquid released by treatments of jerked beef. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

The treatment F3 (50% NaCl, 25% KCl and 25% $CaCl_2$) exhibited the lowest lipids level, differing from the other treatments, which were not different from each other. Due to the addition of several salts and their different diffusion properties, changes may occur in the centesimal composition of the treatments, which explains these results.

As observed in Table 3, there was pH reduction with the evolution of the jerked beef processing, for all treatments. The final products of the treatments containing $CaCl_2$ (F2: 50% NaCl + 50% KCl and F3: 50% NaCl + 25% KCl and 25% $CaCl_2$) exhibited the lowest pH values in relation to the treatments FC1 (100% NaCl) and F1 (50% NaCl + 50% KCl). A similar effect of $CaCl_2$ on pH reduction was reported by several authors in studies on salt reduction in meat products (Gimeno, Astiasarán, & Bello, 1999; Gimeno, Astiasarán, & Bello, 2001; Lawrence , Dikeman, Hunt, Kastner, & Johnson, 2003). It was also observed that, in general, there was no difference in pH of the meat sections at the end of wet salting, thus the reduction of water activity during the dry salting combined with the increase in ion concentration in the final product was determinant for the pH of jerked beef.

In salted-cured meats, salt increases the osmotic pressure and decreases aw during the process, which makes the product more stable and safe for consumption (Toldrá, 2006). In the manufacturing process of jerked beef, there was a significant reduction of water activity of the treatments, as shown in Table 3. The treatment F2 (50% NaCl + 50% $CaCl_2$) presented the highest aw value ($P < 0.05$) at the end of the dry salting step and in the final product. Despite

$CaCl_2$ is used in many products as a dehydrating agent (Lewicki & Michaluk, 2004), it seems to impair the decrease of aw in jerked beef. This result may be due to its dehydrating effect at a relatively high concentration, as observed in the treatment F2 (50% NaCl + 50% $CaCl_2$), leading to a probable rapid and intense superficial dehydration and thus hampering the decrease in aw. In the processed product, the treatments containing 50% NaCl + 50% KCl (F1) and 50% NaCl + 25% KCl + 25% $CaCl_2$ (F3) exhibited the lowest aw in relation to the treatments made with 100% NaCl (FC1) and 50% NaCl + 50% $CaCl_2$ (F2). Thus, the results showed that the replacement of 50% NaCl by KCl or KCl + $CaCl_2$ may be a viable alternative for the industry from a technological point of view, taking into account the safety and the necessity of reducing the sodium content in salted meat products.

Table 3. pH and aw values during processing of jerked beef treatments

Treatments	pH				Aw			
	Raw meat	AWS	ADS	FP	Raw meat	AWS	ADS	FP
FC1	5.64^{a}	5.64^{aA}	5.53^{bB}	5.28^{bC}	0.988^{a}	0.978^{aA}	0.773^{bB}	0.763^{bC}
F1	5.67^{a}	5.61^{abA}	5.59^{abA}	5.32^{aB}	0.987^{a}	0.975^{aA}	0.766^{cB}	0.754^{cC}
F2	5.63^{a}	5.39^{bA}	5.42^{cA}	5.12^{cB}	0.986^{a}	0.978^{aA}	0.794^{aB}	0.775^{aC}
F3	5.65^{a}	5.76^{aA}	5.67^{aA}	5.00^{dB}	0.988^{a}	0.975^{aA}	0.772^{bB}	0.753^{cC}
Standard error	0.02	0.38	0.12	0.13	0.001	0.001	0.002	0.002

Values are means. $^{a, b, c, d}$ Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test. ^{A,B,C,D} Values in the same line with the same capital letters do not differ significantly ($P < 0.05$) according to Tukey's test. AWS: after wet salting; ADS: after dry salting; FP: final product. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

3.2 Instrumental color

The color of meat products is a very important sensory characteristic for the consumer acceptance, and is mainly dependent on the chemical state of the pigment myoglobin and protein of the heme group. Myoglobin is the only pigment in sufficient quantity to confer red color to meat (Mancini & Hunt, 2005). There are basically two pro-oxidant mechanisms caused by the presence of salt: the increase in the oxidation potential of myoglobin by

reducing the buffering capacity of meat and the surface tension (Seideman, Cross, Smith, & Durland, 1984). The pro-oxidant mechanism may be due to the ability of salt to affect the integrity of the cell membrane, facilitating the oxidation and release of Fe ions from iron-containing molecules, such as heme proteins (Kanner, Harel, & Jaffe, 1991; Rhee, 1999).

As can be seen in Table 4, the addition of different salts affected the color characteristics of jerked beef, which was most evident for the parameter a* (red-green dimension). The intensity of the red color decreased ($P < 0.05$) in the presence of KCl (F1: 50% NaCl + 50% KCl) and substantially decreased ($P < 0.05$) in the presence of $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$) in relation to the control FC1 (100% NaCl). Lorenzo et al. (2015) found similar results when replacing NaCl with KCl, $CaCl_2$, and $MgCl_2$ in dry-cured lacón, with a significant difference ($P < 0.05$) in red color (a*). The whiteness index (W) increased significantly ($P < 0.05$) in all treatments during the processing of jerked beef. Fellendorf, Kerry, Hamill, and O'Sullivan (2018) found that replacing NaCl with substitute salts (KCl, $CaCl_2$, and $MgCl_2$) significantly affected ($P < 0.05$) the color parameters of corned beef. Cheng, Wang, and Ockerman (2007) replaced NaCl with KCl in salted pork and found a significant difference in the coordinates a* and b*.

Table 4. Color Parameters (L*, a*, b*, W) during processing of jerked beef treatments

Treatments	Raw meat	AWS	ADS	FP
		L*		
FC1	37.14^{a}	36.52^{aA}	38.17^{bA}	41.69^{bA}
F1	37.63^{a}	32.61^{bB}	43.46^{aA}	43.04^{bA}
F2	36.89^{a}	36.38^{aB}	42.41^{aA}	44.34^{bA}
F3	37.34^{a}	36.21^{aC}	45.94^{aB}	49.61^{aA}
Standard error	0.51	0.42	0.89	1.28
		a*		
FC1	16.90^{a}	9.08^{cB}	10.84^{aA}	11.78^{aA}
F1	17.52^{a}	9.91^{bA}	10.25^{aA}	9.74^{bA}
F2	17.33^{a}	11.41^{aA}	7.45^{bB}	7.05^{cB}
F3	17.17^{a}	9.82^{bA}	8.02^{bA}	6.71^{cB}
Standard error	0.67	0.17	0.31	0.41
		b*		

FC1	18.15^{a}	13.63^{aA}	14.02^{abA}	14.74^{aA}
F1	18.72^{a}	12.07^{cB}	15.74^{aA}	14.42^{aAB}
F2	18.31^{a}	12.94^{abA}	12.87^{bA}	14.17^{aA}
F3	18.44^{a}	12.71^{bcB}	15.38^{aA}	14.06^{aAB}
Standard error	0.29	0.81	0.57	0.52
		W		
FC1	32.40^{a}	34.43^{aA}	35.53^{bA}	38.39^{bA}
F1	32.70^{a}	30.82^{bB}	40.22^{aA}	40.21^{bA}
F2	31.89^{a}	34.07^{aB}	40.46^{aA}	42.00^{bA}
F3	32.41^{a}	34.21^{aC}	43.03^{aB}	47.10^{aA}
Standard error	0.66	0.39	0.76	1.13

Values are means. $^{a, b, c, d}$ Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test. ^{A,B,C,D} Values in the same line with the same capital letters do not differ significantly ($P < 0.05$) according to Tukey's test. AWS: after wet salting; ADS: after dry salting; FP: final product. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

3.3 Minerals levels

As expected, the partial replacement of NaCl by KCl and/or $CaCl_2$ in the wet and dry salting steps reduced ($P < 0.05$) the sodium level, meanwhile incresead potassium and calcium levels respectively, influencing the sensorial atributtes. A reduction of sodium of 27.57% was observed in the treatment F1 (50% NaCl + 50% KCl), 41.58% in the treatment F2 (50% NaCl + 50% $CaCl_2$) and 36.73% in the treatment F3 (50% NaCl + 25% KCl + 25% $CaCl_2$) when compared to the control treatment FC1 (100% NaCl). After desalting step, the sodium content in the control treatment (FC1) remains relatively high, but the NaCl replacement by KCl and/or $CaCl_2$ reduced approximately 45% to 50% of sodium content in all treatments. Thus, concerning the sodium reduction, the present strategy on the use of salt substitutes in jerked beef was very successful. These results have a direct impact on the procedures at the household level. Although the manufacturers recommend the number of desalting hours for these products, consumers do not always follow these indications closely. Thus, when the sodium level is reduced, a consumption with a lower sodium content is

guaranteed even if a proper desalting is not practiced. In Brazil, this is a very consumed product in North and Northeast regions, besides being much appreciated in gastronomy in other places. The sodium reduction strategy in salted meats can contribute significantly to improving the quality of the diet of many consumers.

Table 5. Ca, K and Na values in mg/100g during processing of jerked beef treatments

Treatments	Raw meat	AWS	ADS	FP	Desalted
			Ca		
FC1	4.67^{a}	7.37^{cB}	9.07^{cB}	16.35^{cA}	8.66^{cB}
F1	4.32^{a}	6.11^{cC}	10.29^{cB}	12.78^{cA}	10.29^{cB}
F2	4.59^{a}	235.49^{aD}	2102.46^{aA}	1712.77^{aB}	441.81^{aC}
F3	4.45^{a}	106.61^{bC}	718.54^{bB}	841.37^{bA}	146.08^{bC}
Standard error	0.47	16.06	144.79	119.62	29.84
			K		
FC1	305.19^{a}	269.89^{cB}	345.41^{cA}	325.19^{cA}	73.10^{dC}
F1	301.56^{a}	1112.202^{aC}	4032.91^{aA}	3584.67^{aB}	509.67^{aD}
F2	300.79^{a}	292.05^{cC}	361.70^{cB}	461.53^{cA}	124.11^{cD}
F3	303.61^{a}	643.302^{bC}	3048.19^{bA}	2889.51^{bB}	418.00^{bD}
Standard error	5.47	59.36	276.58	245.79	31.53
			Na		
FC1	63.76^{a}	556.15^{aC}	5421.19^{aA}	5256.30^{aA}	1355.97^{aB}
F1	61.04^{a}	360.06^{cD}	2943.67^{bB}	3807.05^{bA}	723.53^{cC}
F2	64.48^{a}	496.70^{abC}	3025.03^{bA}	3070.65^{cA}	792.22^{bB}
F3	66.21^{a}	457.12^{bC}	3008.74^{bB}	3325.47^{cA}	678.53^{dC}
Standard error	2.04	16.48	179.04	149.59	46.27

Values are means. $^{a, b, c, d}$ Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test. ^{A,B,C,D} Values in the same line with the same capital letters do not differ significantly ($P < 0.05$) according to Tukey's test. AWS: after wet salting; ADS: after dry salting; FP: final product. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

3.4 Shear force

Salts directly affect the texture characteristics of meat products due to changes in the water binding and water retention capacity of meat products (Offer et al., 1989; Offer & Trinick, 1983; Oliveira, Pedro, Nunes, Costa, & Vaz-Pires, 2012). In general, the composition of meat products directly affects the shear force (Yun-Sang et al., 2016).

The results of shear force (N) were: FC1: 35.79^{b}; F1: 40.70^{b}; F2: 51.39^{a}; F3: 56.98^{a}, having 1.57 of standard error. The use of $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$) significantly increased ($P < 0.05$) the force required to shear the sample when compared to the treatments FC1 (100% NaCl) and F1 (50% NaCl +50% KCl). The treatments containing NaCl (FC1: 100% NaCl) and NaCl + KCl (F1: 50% NaCl + 50% KCl) did not differ among themselves for this texture parameter. These effects may be due to the characteristic dehydrating action of the salt and the blends used. The severe superficial drying by the presence of $CaCl_2$ may be responsible for hardening of the samples, which in turn may be related to the denaturation of proteins of the outermost portion, decreasing the water retention capacity during the cooking. Duranton, Simonin, Chéret, Guillou, and de Lamballerie (2012) found that increasing the concentration of NaCl in cured meat decreased the shear force. Yun-Sang et al. (2016) found that the type of salt directly affects the shear force in cured pork loin. It should be emphasized that although there are no data in the literature to compare the present results, it is possible to correlate the results with the sensory properties, as discussed soon after.

3.5 Lipid oxidation

Lipid oxidation is one of the main causes of deterioration of meat products, promoting loss of essential fatty acids, rancidity, undesirable texture and odor (Alfaia et al., 2010; Broncano, Petrón, Parra, & Timón, 2009; Domínguez, Gómez, Fonseca, & Lorenzo, 2014). Salted meats are particularly susceptible to lipid oxidation since NaCl is considered a potent pro-oxidant and the reduction of water activity can concentrate the intermediate compounds of the lipid oxidation, determined by the quantification of 2-thiobarbituric acid reactive substances (TBARS). In the present study, the malonaldehyde values expressing the tendency to lipid oxidation are shown in Table 6. The highest malonaldehyde values were reported after the wet salting step, except for the treatment F2 (50% NaCl + 25% KCl + 25% $CaCl_2$). The dry salting step promoted a significant reduction ($P < 0.05$) of the malonaldehyde values in

all treatments, which does not represent a reduction of lipid oxidation, once in jerked beef, oxidation increases as the products are exposed to air for 24 hours of ripening, after washing with hypochlorite. The residual nitrite content at the end of the process was zero, indicating a provable antioxidant effect only during the salting steps, taking into account that the Brazilian legislation has established lower residual nitrite levels in jerked beef when compared to emulsified and restructured products (150 ppm). The transformation of the malonaldehyde generated mainly in the intermediate step into end products such as ketones and alcohols, during the oxidative process has been reported in the literature (Torres et al., 1989). Salted meats such as charqui and jerked beef are characterized by flavor and aroma resulting from desirable oxidative reactions, which can also lead to product deterioration under uncontrolled conditions.

Table 6. Malonaldehyde values (mg/kg) during processing of jerked beef treatments

Treatments	Raw meat	AWS	ADS	FP
FC1	0.04^{a}	1.43^{aA}	0.61^{bC}	0.98^{cB}
F1	0.04^{a}	1.42^{aA}	1.02^{aB}	0.90^{cB}
F2	0.05^{a}	1.47^{aA}	0.97^{aC}	1.66^{aA}
F3	0.04^{a}	1.43^{aB}	1.03^{aC}	1.12^{bB}
Standard error	0.002	0.26	0.03	0.05

Values are means. $^{a, b, c, d}$ Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test ^{A,B,C,D} Values in the same line with the same capital letters do not differ significantly ($P < 0.05$) according to Tukey's test. AWS: after wet salting; ADS: after dry salting; FP: final product test. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

In this study, the treatments with addition of $CaCl_2$ (F2: 50% NaCl + 50% KCl and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$) exhibited the highest TBARS values, thus explaining the greater lipid oxidation power of $CaCl_2$ when compared to NaCl and KCl. All results were higher than 0.5 mg/kg malonaldehyde, and values above this range are considered to be perceptible to the consumer (Kang, Park, Choi, Lee, & Auh, 2009).

3.6 Microbiological characterization

All treatments presented thermotolerant and total coliform counts < 3 most probable number/g. Low lactic acid bacteria (log colony forming unit/g) counts were observed in the

processed product (FC1: 2.62; F1: 2.92; F2: 3.18; F3: < 2), probably due to the severe dehydration during processing of jerked beef ((Lara et al., 2003), which prevents the microbial growth. In addition, water activity is one of the factors that directly influence the microbial growth (Davey, 1989).

The results of microbiological stability of this study confirm the safety of jerked beef for all treatments, indicating that blends of substitute salts can be used in this product category to reduce the NaCl level by up to 50%.

3.7 Sensory evaluation

10 sensory descriptors were enumerated by the trained panel as follows: red color, apparent fibrosity, rancid aroma, characteristic jerked beef aroma, salty taste, bitter taste, aftertaste, hardness, fibrosity, and juiciness. Overall, addition of $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$) highlighted the undesirable bitter and aftertaste, fibrosity, rancid aroma, and hardness (Table 7), without no effect on the salty taste.

PCA bidimensional map explained 97.17 % of variability using two dimensions, with the first and the second dimensions explained 88.34 % and 8.83 %, respectively (Figure 3). Regards the first dimension, sample F2 and F3 was associated with apparent fibrosity, rancid aroma, bitter taste, hardness, fibrosity, and aftertaste while FC1 and F1 were associated with red color, salty taste, juiciness and characteristic jerked beef aroma. Observing the confidence ellipses, it is noted absence of superposition among FC1 e F1 and F2 and F3, suggesting the contribution of $CaCl_2$ addition in jerked beef formulation on the sensory profiling.

These sensory vocabulary are in agreement with the texture and TBARS analyses of such treatments. According to Armenteros et al. (2001) and Lawless, Rapacki, Horne, and Hayes (2003), divalent cations such as $CaCl_2$ are characterized by promoting metallic taste, astringent, bitter and irritating sensation, directly impacting the acceptance of the product. Several authors have reported the negative effect of $CaCl_2$ on the sensory characteristics of various meat products, such as Dos Santos et al. (2015) in dry fermented sausages, Horita, Morgano, Celeghini, and Pollonio (2011) in sausages, and Armenteros et al. (2012) in cured and dried raw ham. However, the addition of NaCl and KCl (FC1: 100% NaCl and F1: 50% NaCl + 50% KCl) provided aroma and color characteristic of jerked beef, juiciness, and salty taste. Probably, KCl and NaCl exhibited similar behavior in the desalting step, with a lower content of residual ions perceptible to the consumer, unlike $CaCl_2$, which becomes more

impregnated in the muscle tissue during salting in the outer portion, being more difficult to be removed by desalting. This hypothesis, however, should be confirmed in future studies.

Table 7. Sensory evaluation of jerked beef treatments

Treatments	Red color	Apparent fibrosity	Rancid aroma	Characteristic jerked beef aroma	Salty taste	Bitter taste	After taste	Hardness	Fibrosity	Juiciness
FC1	3.13^{a}	3.62^{b}	1.83^{b}	6.00^{a}	5.81^{a}	1.10^{c}	2.33^{b}	2.95^{c}	3.21^{b}	2.65^{a}
F1	2.67^{a}	3.36^{b}	1.84^{b}	4.97^{ab}	5.62^{a}	1.13^{c}	2.14^{b}	3.69^{bc}	3.44^{b}	2.42^{a}
F2	2.41^{a}	5.18^{a}	2.78^{a}	3.54^{c}	5.54^{a}	7.37^{a}	6.28^{a}	5.30^{a}	5.17^{a}	1.26^{b}
F3	1.31^{b}	4.55^{ab}	2.49^{a}	4.41^{bc}	5.40^{a}	6.35^{b}	5.70^{a}	4.67^{ab}	4.77^{a}	1.19^{b}
Standard error	0.15	0.18	0.14	0.21	0.18	0.27	0.24	0.18	0.19	0.14

Values are means. $^{a, b, c, d}$ Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

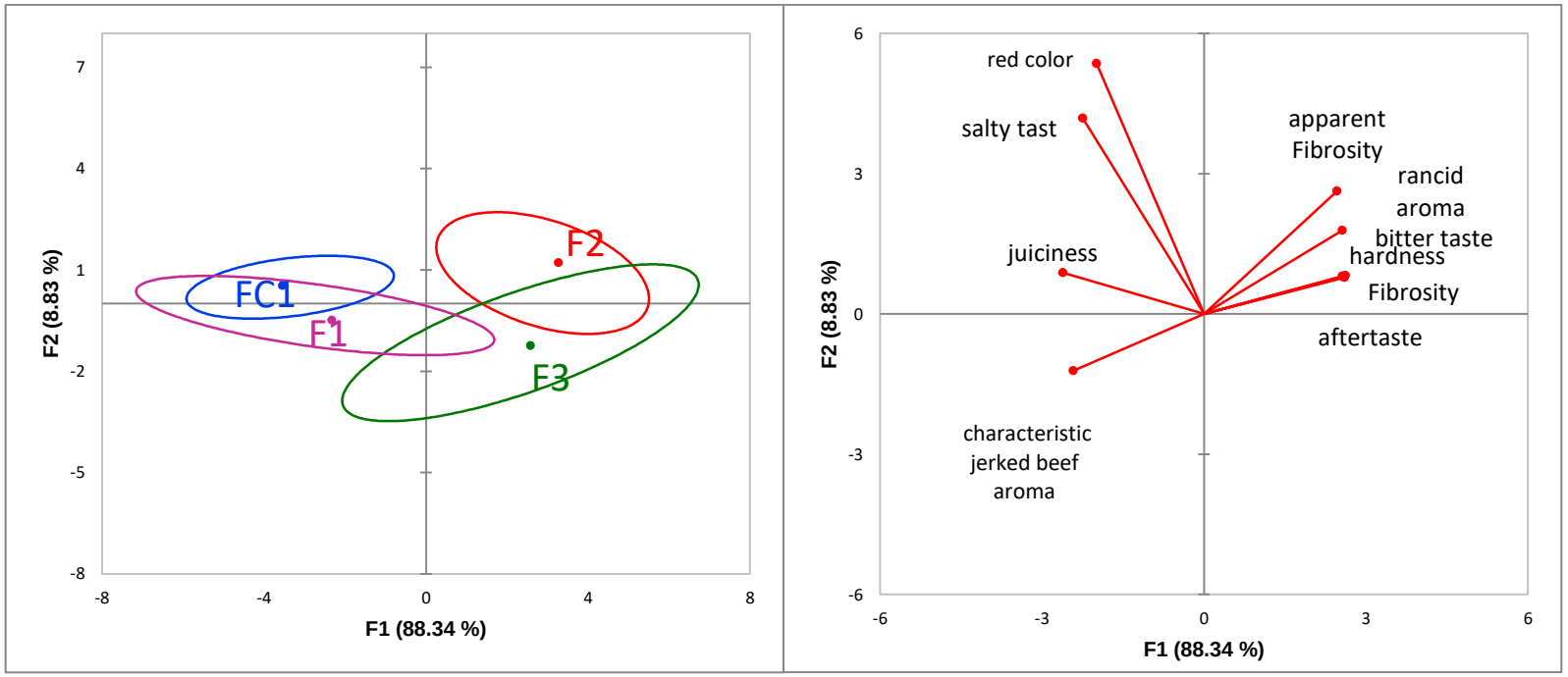

Figure 3. Representation of the samples and the attributes in the first and second dimensions of the principal component analysis (PCA) on the correlation matrix of average attribute scores of sensory attributes of jerked beef samples. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

4. Conclusion

The addition of 50% and 25% $CaCl_2$ based on the NaCl ionic strength provided undesirable characteristics to the product, particularly with respect to the sensory properties, with the occurence of bitter taste, fibrosity, rancid aroma, and aftertaste. Blends containing $CaCl_2$ also impaired the reduction of water activity when compared to NaCl, besides increasing bleaching and reducing the coordinate a* with increased shear force. On the other hand, the substitution of 50% of NaCl with KCl provided technological and sensory characteristics very similar to jerked beef traditionally made with 100% sodium chloride. Therefore, it is recommended to use up to 50% KCl rather than sodium chloride to improve the nutritional status of this relevant product in Brazil and other countries that still face the ineffective cold chain in relation to food safety.

Acknowledgment

The authors thank National Council for Scientific and Technological Development (process numbers: 140533/2015-0 and 406671/2016-8) for the financial support to this work and scholarships.

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CAPÍTULO 3

ADDING LYSINE AND YEAST EXTRACT IMPROVES SENSORY PROPERTIES OF LOW SODIUM SALTED MEAT

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Meat Science

In Press

DOI: 10.1016/j.meatsci.2019.107911

Highlights

- Use of lysine, yeast extract, KCl and $CaCl_2$ to reduce sodium content in salted meat
- The addition of lysine and yeast extract increased the sensory acceptance
- Lysine and yeast extract minimized the rancid aroma, salty taste and aftertaste

Abstract

The partial reduction of Sodium Chloride (NaCl) and the use of lysine, yeast extract and substitute salts Potassium Chloride (KCl) and Calcium Chloride ($CaCl_2$) in the characteristics of salted meat were investigated. Proximate composition, physicochemical properties (pH, water activity, lipid oxidation), instrumental analysis (color, shear force), microbiological analysis (total counts, lactic acid bacteria counts, thermally tolerant coliforms, and total coliforms) and sensory evaluation (120 consumers) were performed. The partial replacement of NaCl by KCl and $CaCl_2$ significantly reduced the sodium content of salted meat treatments, while lysine and yeast extract minimized the negative sensory effects due to the addition of KCl and $CaCl_2$. The addition of lysine and yeast extract increased the sensory acceptance and decreased rancid aroma, salty taste, and aftertaste of salted meat made with a blend of NaCl + KCl + $CaCl_2$, with no differences in the physiochemical quality parameters. Moreover, the treatments made with the blend NaCl + KCl exhibited characteristics similar to traditional salted meat formulations.

Keywords: potassium chloride; calcium chloride; salted meat; sodium reduction; lysine; yeast extract; sensory techniques.

1 Introduction

Salted meat encompasses a wide variety of products, many of them regionally produced and appreciated in several countries, due to their unique sensory characteristics and long shelf life (Liu et al., 2014). Several processing steps, including salting, drying, and ripening are involved in the manufacture of salted meat products (Mora et al., 2015), using salt and additives to promote the dehydration and microbiological stability (Leistner, 1987; Shimokomaki et al., 1998). From a nutritional and food safety perspective, the stability of salted meat products makes this category highly relevant in regions where the cold chain is scarce or non-existent (Ishihara & Madruga, 2013).

However, salted meats are characterized by high sodium level. Excessive consumption of this mineral can increase the risk of several health problems such as high blood pressure and cardiovascular diseases (Aburto et al., 2013; Delgado-Pando et al., 2018; Vollmer et al., 2001). Thus, the World Health Organization (WHO) has recommended a maximum daily intake of 2 g of Sodium (Na), which is equivalent to 5 g of NaCl. In salted meats, sodium levels remain high due to the salting procedures of the manufacturing process, making it imperative desalting before consuming. However, consumers do not always desalt the product properly, ingesting a high sodium content. Thus, the reduction of NaCl levels used in the salting steps becomes a promising strategy for an effective sodium reduction in salted meat products.

One of the main strategies to reduce the sodium content in meat products is the replacement of NaCl by other chloride salts or ingredients (Aaslyng, Vestergaard, & Koch, 2014; Aliño et al., 2010; Fellendorf, Kerry, Hamill, & O'Sullivan, 2018; Inguglia et al., 2017). Several authors have used KCl and $CaCl_2$ as salt substitutes in meat matrixes (Aliño, Grau, Toldrá, & Barat, 2010; Dos Santos, Campagnol, Fagundes, Wagner, & Pollonio, 2017; Fellendorf, O'Sullivan, & Kerry, 2017; Horita et al., 2014; Lorenzo et al., 2015; Tobin, O'Sullivan, Hamill, & Kerry, 2013). Both NaCl and KCl have similar chemical properties and are therefore the most commonly used salts to partially replace NaCl, with the advantage of reducing the sodium levels with a simultaneous increase in potassium intake (Aljuraiban et al., 2012; Fellendorf et al., 2018). However, the addition of KCl to meat products is mainly limited by the development of bitter and metallic taste (Askar, Samahy, & Tawfik, 1994; Sinopoli & Lawless, 2012; Toldrá & Reig, 2011; Zanardi, Ghidini, Conter, & Ianieri, 2010). According to Terrel (1983) and Pasin et al. (1989), the use of KCl and NaCl in a ratio of 50:50 can reduce the salty taste, increase the metallic taste and astringent taste, with a

negative impact from the sensory point of view, thus requiring new strategies to avoid such effects in the sensory quality of meat products.

Moreover, hyperkalemia has been a long-standing concern regarding the reformulation of food products to lower sodium levels (Abuelo, 2018). The use of a blend containing salts as $CaCl_2$, lactates, and different phosphates has been studied in different meat matrixes as technological strategies to enable sodium reduction without functional impairment, with special care in sensory optimization (Collins, 1997; Dos Santos et al., 2015; Guàrdia, Guerrero, Gelabert, Gou, & Arnau, 2008; Horita, Morgano, Celeghini, & Pollonio, 2011). In this context, a strategy widely used to minimize the undesirable sensory impacts of substitute salts and NaCl reduction is the addition of flavor enhancers, commercial flavor enhancers such as monosodium glutamate and lactates (Desmond, 2006). Different authors have reported that flavor enhancers such as taurine, lysine, disodium guanylate, and disodium inosinate can minimize the sensory effects caused by KCl (Berglund & Alizadeh, 1999; Kurtz & Fuller, 1997; Salemme & Barndt, 2008; Zolotov et al., 1998).

Lysine is an essential amino acid that cannot be made by the body, thus it must come from food. It has a great nutritional value (Kelly, O'Connell, O'Sullivan, & Guilbault, 2000), and is required to the synthesis of the proteins in human metabolism value (Wolfe, 2017).

Yeast extract is a natural ingredient intensively used to improve the flavor, containing a rich source of compounds or precursors (non-volatile compounds), such as peptides, nucleotides, vitamins of group B and amino acids (Festring & Hofmann, 2010; Liu et al., 2015). According to Alim et al. (2018), most of these volatile and non-volatile substances and aroma-active compounds of yeast extract are generated during heating, improving the flavor.

Lysine and yeast extract have been successfully incorporated into different meat products (Bolumar et al., 2006; Campagnol, dos Santos, Wagner, Terra, & Pollonio, 2011; Campagnol, Santos, Morgano, Terra, & Pollonio, 2011; Delgado-Pando et al., 2019; Flores et al., 2015), however, there are no studies on their application in salted meat.

Taking into account the high sodium content of salted meats and the challenge of reducing the NaCl content without significant effects on the processed product, this study evaluated the partial reduction of NaCl by the addition of substitute salts, KCl and $CaCl_2$, and lysine and yeast extract aiming to optimize the sensory, microbiological, and physicochemical attributes of reformulated salted meats.

2 Material and Methods

2.1 Treatments, raw materials, and additives

The additives sodium erythorbate and sodium nitrite were donated by the company Kerry of Brazil. The salts NaCl, KCl, and $CaCl_2$ were purchased from a food grade company (Anidrol, Brazil), the inosinate + guanylate was donated by Ajinomoto Brasil, the lysine, taurine and arginine was purchased from Now Foods (USA), and the natural ingredients Bionis YE MXE NS (yeast extract) and Bionis SFE 201 (yeast extract) were donated by Biorigin do Brasil and PuracArome NA4 by Purac-Corbion Brazil. Outside flat (*biceps femoris*) was the meat cut selected for the study, which was purchased from slaughterhouses with assured hygienic quality.

The flavor enhancers were selected using nine treatments (Table 1) as follows: two control treatments (TC1: 100% NaCl and TC2: 25% NaCl + 75% KCl) and seven treatments with 75% NaCl replacement by KCl and different flavor enhancers. The replacement of NaCl by KCl was based on the ionic strength of the control treatment (100% NaCl) aimed at obtaining the same ionic strength in all treatments.

The selection of flavor enhancers were performed utilizing overall acceptance and difference from control analysis, and the results are shown in Table 2. Seven salted meat treatments were made utilizing two flavor enhancers previously selected (lysine and Bionis YE MXE NS), NaCl, KCl, and $CaCl_2$ (Table 3). The concentration of KCl and $CaCl_2$ substitute salts was based on the calculation of ionic strength to make up the ionic strength of 50% and 25% of NaCl, obtaining the same ionic strength in all treatments. The same amount of additives was added in the wet salting step, and the salt was the variable.

Table 1. Salted meat treatments added with NaCl, KCl and differents flavor enhancers

T	NaCl	KCl	Lysine	Taurine	Arginine	Inosinate + Guanylate	Bionis YE MXE NS	Bionis SFE 201	Purac Arome NA4
TC1	100		-	-	-	-	-	-	-
TC2	25	75	-	-	-	-	-	-	-
T1	25	75	3	-	-	-	-	-	-
T2	25	75	-	0.225	-	-	-	-	-
T3	25	75	-	-	3	-	-	-	-
T4	25	75	-	-	-	0.18	-	-	-
T5	25	75	-	-	-	-	5	-	-
T6	25	75	-	-	-	-	-	5	-
T7	25	75	-	-	-	-	-	-	5

*: For each 1000mg of raw bovine meat was added 2000mg of salt. The proportion of salt added was based on the ionic strength. The different salts was added in the wet and dry salting steps. To add homogeneously the flavor enhancers, was utilized tumbler after the wet salting step

Table 2. Overall acceptance and difference from control analysis in salted meat treatments

Treatments	Overall acceptance	Difference from control
TC1	6.37[a]	-
TC2	6.24[a]	2.75
T1	6.08[ab]	3.17
T2	5.84[ab]	3.50
T3	5.97[ab]	4.83
T4	5.21[b]	3.33
T5	6.20[ab]	4.67
T6	5.74[ab]	4
T7	5.91[ab]	4

Means in the same column followed by different letters present statistically significant difference by the Tukey test ($P < 0.05$). TC1: 100% NaCl; TC2: 25% NaCl + 75% KCl; T1: 25% NaCl + 75% KCl + 3% lysine; T2: 25% NaCl + 75% KCl + 0.23% taurine; T3: 25% NaCl + 75% KCl + 3% arginine; T4: 25% NaCl + 75% KCl + 0.18% inosinate + guanylate; T5: 25% NaCl + 75% KCl + 5% Bionis YE MXE NS; T6: 25% NaCl + 75% KCl + Bionis SFE 201; T7: 25% NaCl + 75% KCl + 5% Purac Arome NA4.

2.2 Processing

The salted meat treatments were elaborated according to Vidal et al. (2019) in the Pilot Plant of Meat Products, Faculty of Food Engineering, at UNICAMP. Two salting steps, wet salting and dry salting, were carried out. For wet salting, the cuts were immersed in brine (salt concentration according to Table 1 and Table 3 + 150 ppm sodium nitrite and 500 ppm sodium erythorbate) with ionic strength corresponding to 100% NaCl (control treatment) for 1 hour at 13 ºC. The meat pieces were placed in a tumbler (MGH-20, Dorit, Klllwangen, Switzerland) for 6 minutes after the wet salting step, and the yeast extract and lysine were added.

For the dry salting step, the pieces were put in contact with the salt blends for 144 hours (6 days) in a chamber at 13 ° C in the form of intercalated layers of meat and salt. The ripening step was carried out in a climatic chamber (Instala Frio, Curitiba, Brazil) at 55%

humidity, 25 °C, and 0.5 m/s forced air ventilation for 24 hours (1 day). The final products were vacuum-packed in polyethylene packages (Spel, São Paulo, Brazil) and stored at 25 °C.

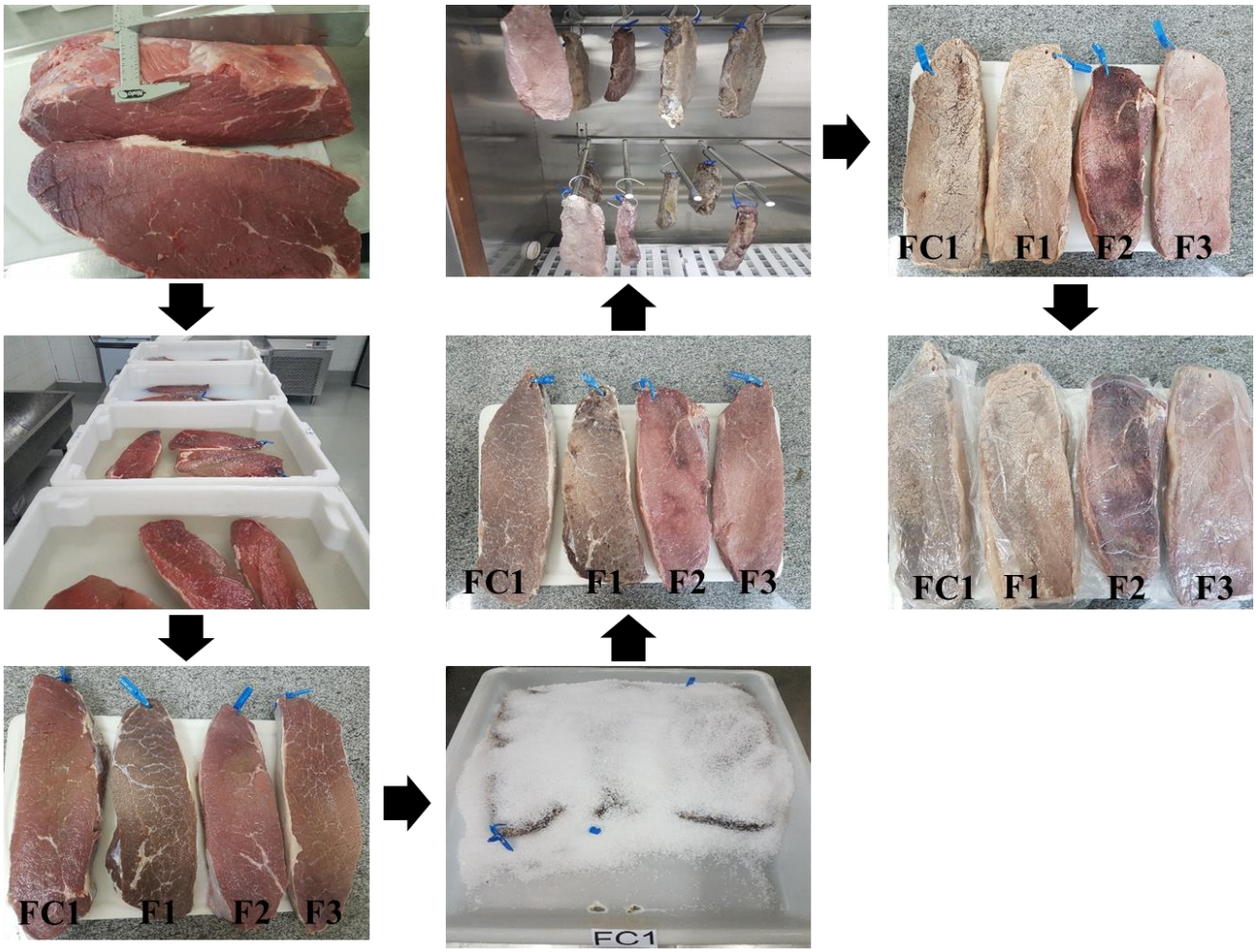

Figure 1. Processing steps for obtaining salted meat. In sequence: outside flat cuts; wet salting; treatments after wet salting; dry salting; treatments after dry salting ; ripening; treatments after ripening; final product with vacuum packaging

Table 3. Salted meat treatments added with NaCl, KCl, $CaCl_2$, lysine and yeast extract (Bionis YE MXE NS)

Treatments	NaCl reduction (%)	NaCl mg/2000mg*	KCl mg/2000mg*	$CaCl_2$ mg/2000mg*	Lysine (%)	Bionis YE MXE NS (%)
FC1	-	2000	-	-	-	-
F1	50	881	1119	-	-	-
F2	50	1026	651	323	-	-
F3	50	881	1119	-	3	-
F4	50	1026	651	323	3	-
F5	50	881	1119	-	-	5
F6	50	1026	651	323	-	5

*: For each 1000mg of raw bovine meat was added 2000mg of salt (1 meat: 2 salt). The proportion of salt added for KCl and $CaCl_2$ was based on the ionic strength of control. The different salts was added in the wet and dry salting steps. To add homogeneously the yeast extract and lysine, was utilized tumbler after the wet salting step

2.3 Overall Acceptance and difference from control – Selection of flavor enhancers

Overall acceptance and overall difference tests were performed to evaluate the flavor enhancers of this study. The compounds and the respective treatments are shown in Table 1. The salted meat with the addition of different flavor enhancers was evaluated by 120 untrained assessors (67 female and 53 male, aged from 18 to 58) at University of Campinas, in the Sensory Analysis Laboratory, Department of Food Technology, in the Faculty of Food Engineering. In this step, it was assumed a high sodium reduction through the replacement of 75% NaCl by KCl with the objective of testing the different flavor enhancers under equal conditions.

A hedonic scale of nine points (1: dislike extremely and 9: like extremely) in balanced complete blocks was used to perform the overall acceptance test (MacFie et al., 1989). The overall difference test was performed by presenting the control sample (TC1) and the other samples at the same time, and the assessors were asked to point out the differences from the control using a nine-point scale (1: no difference and 9: extremely different). The sensory tests with untreined assessors were performed on different days.

2.4 Proximate composition, pH, and water activity

The proximate composition was determined according to Horwitz and Latimer (2005). The pH was determined using a combined electrode (22 DM, Digimed, São Paulo, Brazil). For that, 10 g of salted meat sample was homogenized in distilled water (1:10) followed by pH readings. The water activity (aw) was measured using an Aqualab apparatus (Decagon Devices Inc., Pullman, USA).

2.5 Instrumental color

The parameters CIELAB L*, a*, and b* were determined as an indicator of luminosity, red intensity, and yellow intensity, utilizing the Hunter Lab colorimeter (Colourquest II, Hunter Associates Laboratory Inc., Virginia, USA) with D65 illuminant, standard 10° observer and 20 mm aperture, at room temperature. The equation: 100 - [(100-L *) 2 + a * 2 + b * 2] 1/2 was used to calculate the whiteness index (W).

2.6 Desalting, cooking, shear force and minerals level

The desalting and cooking procedures were performed according to Vidal et al (2019). The desalted and cooked samples were stored under refrigeration for 24 hours prior to Warner-Bratzler shear force analysis (Stock & Board, 1995). The shear force was measured in a TA-XT 2i texture analyzer (Texture Technologies Corporation / Stable Micro Systems, Hamilton, UK). The distance to the platform was 25 mm and the ascent/descent speed was 200 mm/min. This analysis was performed in six replicates for each replicate process.

The minerals (Calcium (Ca), Potassium (K), and Sodium (Na) were mensured in the final and desalted product by the dry digestion method (Horwitz & Latimer, 2005).

2.7 Lipid oxidation

The lipid oxidation was measured in the final products as 2-thiobarbituric acid reactive substances (TBARS), according to Bruna, Ordóñez, Fernández, Herranz, and De la Hoz (2001).

2.8 Microbiological characterization

Microbiological counts were performed in the raw meat and final products according to Silva et al., (2017), through the enumeration of lactic acid bacteria, total counts, total coliforms, and thermotolerant coliforms.

2.9 Sensory evaluation of reduced sodium salted meats added of lysine and yeast extract

Two flavor enhancers that obtained the most satisfactory results (lysine and yeast extract (Bionis YE MXE NS)) from step *2.3* were selected using overall acceptance and difference from control sensory analysis.

Two flavor enhancers with the most satisfactory results (lysine and yeast extract, Bionis YE MXE NS) in the Section *2.3* were selected using the overall acceptance and overall difference tests.

The desalted samples were evaluated by 120 consumers (53 male, 67 female, aged 18-58) comprising students and staff of the University of Campinas, and the samples were presented in a monadic and balanced order (MacFie et al., 1989). The sensory attributes were

appearance, aroma, flavor, texture, and overall impression (1: dislike extremely, 5: neither like, neither dislike, 9: like extremely) (Horita et al., 2016). In addition, the consumers evaluated the following sensory descriptors through an intensity scale method using an unstructured 9 cm linear scale: red color, dry surface, rancid aroma, characteristic salted meat aroma, salty taste, bitter taste, aftertaste, hardness, fibrosity, and juiciness. The descriptors were previously generated by consumers using a Grid method in a study with jerked beef (Vidal et al., 2019). The purchase intention was evaluated using a binomial scale (1: yes, 0: no) (Cruz et al., 2011; Taksima et al., 2015).

2.10 Statistical analysis

The process was performed in three replicates on different days using similar formulation, methodology, and technology. For each process, at least three samples were taken for analysis. The general linear model (GLM) considering the treatments as a fixed effect and the replicates as a random effect, using 5% of significance level was used to analyze the physicochemical and instrumental data, and the significant differences were analyzed by the Tukey's test at a 5% significance level. Both the GLM and Tukey's test were performed using the comercial software Statistica v. 8 (Statsoft Inc., Tulsa, Oklahoma, USA).

Data of the sensory tests were evaluated using two-way Anova, considering the samples as a fixed effect and consumers as a random effect (Dos Santos et al., 2015). Multivariate analysis of variance (MANOVA) was performed to determine whether the seven salted meat treatments were different when considering all ten sensory descriptors simultaneously. Descriptive discriminant analysis (DDA) (Beckley, Herzog, & Foley, 2017) was performed to identify the sensory attributes underlying the group differences among the formulations. Logistic regression (LR) was used to evaluate the binomial scale of purchase intention test, identifying the sensory attributes influencing the purchase intention (Cruz et al., 2011; Taksima et al. 2015). Finally, a machine learning technique, random forest (RF), was used to evaluate the sensory descriptors with the great contribution for the overall acceptance (Granitto, Gasperi, Biasioli, Trainotti, & Furlanello, 2007). All analyses were performed using XLSTAT 2018.4 (Adinsoft, Paris, France).

3 Results and Discussion

3.1 Selecting flavor enhancers compounds

The results of the overall acceptance and overall difference tests of salted meat formulations with the addition of different flavor enhancers: lysine, taurine, arginine, inosinate + guanylate, and natural flavor enhancers (Bionis YE MXE NS, Bionis SFE 201 and PuracArome NA4) are shown in Table 2.

No significant differences ($P < 0.05$) were observed in the sensory evaluation among the treatments; however, it was possible to verify that the treatment containing lysine (T1) obtained satisfactory results when compared to the treatments made with the addition of other amino acids (taurine, arginine, and inosinate + guanylate). With respect to the natural flavor enhancers, the treatment containing Bionis NS YE MXE yeast extract (T5) highlighted among the other treatments (T6 and T7).

From these results, both the lysine (T1) and Bionis NS YE MXE yeast extract (T5) were selected for the continuity of the study.

3.2 Proximate composition, pH, and aw

The results of proximate composition, pH, and aw of the salted meat treatments are shown in Table 4. A significant difference ($P < 0.05$) in moisture and ash levels was observed among the treatments. The addition of blends containing $CaCl_2$ (F2, F4, F6) decreased ($P < 0.05$) the moisture content and significantly increased ($P < 0.05$) the ash content in relation to other treatments. These results are probably due to the dehydrating action of calcium ions, which leads to an increased mass transfer with a consequent increase in the dehydration rate (Lewicki & Michaluk, 2004; Vidal et al., 2019). Some authors have reported that several factors can affect the water retention and binding capacities, such as salt concentration and type of salt, directly impacting the moisture content (Offer et al., 1989; Offer & Trinick, 1983; Oliveira, Pedro, Nunes, Costa, & Vaz-Pires, 2012). Thus, the addition of $CaCl_2$ can cause different effects on the dehydration of the salted meat when compared to NaCl and KCl as observed in a previous study (Vidal et al., 2019). In addition, the use of lysine (F4) and yeast extract (F6) significantly decreased ($P < 0.05$) the moisture content when compared to the

treatment made with the same salt blend, with no addition of lysine and yeast extract (F2), probably due to these ingredients contribute to eliminating water from the meat matrix.

Table 4. Proximate composition, pH and aw of salted meat treatments

Treatments	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)	pH	Aw
FC1	50.67^{a}	17.93^{c}	28.75^{a}	3.48^{a}	5.36^{d}	0.759^{a}
F1	50.61^{a}	18.50^{a}	27.70^{a}	3.16^{a}	5.42^{c}	0.753^{b}
F2	49.61^{c}	19.36^{a}	28.23^{a}	2.76^{b}	5.15^{f}	0.753^{b}
F3	50.43^{ab}	18.37^{b}	27.69^{a}	3.14^{ab}	5.70^{a}	0.753^{b}
F4	48.92^{d}	19.46^{a}	27.69^{a}	3.51^{a}	5.45^{bc}	0.755^{ab}
F5	49.85^{bc}	18.44^{b}	27.85^{a}	3.13^{ab}	5.48^{b}	0.751^{b}
F6	48.39^{d}	19.44^{a}	27.50^{a}	3.37^{a}	5.24^{e}	0.752^{b}
Standard error	0.12	0.65	0.12	0.04	0.04	0.001

Means in the same column followed by different letters present statistically significant difference by the Tukey test ($P < 0.05$). FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 25% KCl + 25% $CaCl_2$; F3: 50% NaCl + 50% KCl + 3% lysine; F4: 50% NaCl + 25% KCl + 25% $CaCl_2$ + 3% lysine; F5: 50% NaCl + 50% KCl + 5% Bionis YE MXE NS; F6: 50% NaCl + 25% KCl + 25% $CaCl_2$ + 5% Bionis YE MXE NS

No significant differences ($P < 0.05$) were observed in the protein contents of the salted meat treatments after the addition of different salts and lysine and yeast extract. The treatment F3 (50% NaCl + 25% KCl + 25% $CaCl_2$) exhibited the lowest lipid content, with no differences ($P < 0.05$) when compared to the other treatments. Although a careful standardized sampling was employed, small changes in fat content may occur due to intermuscular fat, which can explain the present results.

The treatments F2 (50% NaCl + 25% KCl + 25% $CaCl_2$) and F6 (50% NaCl + 25% KCl + 25% $CaCl_2$ + 5% Bionis YE MXE NS) exhibited the lowest pH values ($P < 0.05$) in relation to other treatments. The effect of the pH reduction caused by $CaCl_2$ in meat products has also been reported by other authors (Gimeno, Astiasarán, & Bello, 2001; Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2003). In the present study, the addition of lysine together with NaCl and KCl (F3) increased substantially the pH values ($P < 0.05$) when compared to the other treatments.

The addition of a high salt concentration during the manufacture of salted meat causes an increase in osmotic pressure and a decrease in aw, making the product safe even when stored at room temperature (Toldrá, 2006). After 6 days of the salting procedures, all treatments exhibited aw values around 0.75.

3.3 Instrumental color

Salts are pro-oxidants due to the increased potential oxidation of myoglobin, decreasing the buffer capacity and the surface tension of meat (Seideman, Cross, Smith, & Durland, 1984), besides affecting the cells releasing iron ions from the molecules (Kanner, Harel, & Jaffe, 1991). The results of instrumental color are presented in Table 5. The color parameters L* (luminosity), a* (red-green dimension), b* (yellow-blue dimension), and W (whiteness index) were significantly affected ($P < 0.05$) by the addition of different salts and lysine and yeast extract, which was more evident for the parameter a*, once the blend containing NaCl, KCl, $CaCl_2$(F2), with lysine (F4) and yeast extract (F6) exhibited lower ($P < 0.05$) red coordinate values in relation to the other treatments.

Table 5. Shear force values (N), malonaldehyde values (mg/kg) and instrumental color (L*, a*, b*, W) of salted meat treatments

Treatments	Shear Force (N)	Malonaldehyde (mg / kg)	Instrumental color			
			L*	a*	b*	W
FC1	40.89^{b}	1.02^{a}	50.71^{b}	7.51^{b}	11.48^{a}	48.83^{b}
F1	37.27^{b}	0.99^{a}	49.97^{cd}	7.91^{a}	11.64^{a}	48.03^{c}
F2	59.33^{a}	1.09^{a}	50.59^{bc}	6.42^{d}	10.61^{b}	49.06^{b}
F3	38.25^{b}	1.01^{a}	48.99^{e}	7.73^{ab}	11.48^{a}	47.14^{d}
F4	61.88^{a}	1.14^{a}	47.38^{f}	6.46^{cd}	10.43^{b}	45.97^{e}
F5	35.60^{b}	0.98^{a}	49.58^{de}	8.03^{a}	11.26^{a}	47.71^{c}
F6	62.37^{a}	1.01^{a}	51.95^{a}	6.80^{c}	11.43^{a}	50.14^{a}
Standard error	0.12	0.03	0.18	0.09	0.07	0.17

Means in the same column followed by different letters present statistically significant difference by the Tukey test ($P < 0.05$). FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 25% KCl + 25% $CaCl_2$; F3: 50% NaCl + 50% KCl + 3% lysine; F4: 50% NaCl

+ 25% KCl + 25% $CaCl_2$ + 3% lysine; F5: 50% NaCl + 50% KCl + 5% Bionis YE MXE NS; F6: 50% NaCl + 25% KCl + 25% $CaCl_2$ + 5% Bionis YE MXE NS

3.4 Shear force

The shear force values are shown in Table 5. A significant increase ($P < 0.05$) in shear force values (N) was observed in the treatments made with the addition of blends containing NaCl, KCl, $CaCl_2$ (F2) and lysine and yeast extract (F4, F6) when compared to the treatments containing NaCl (FC1) or NaCl + KCl (F1, F3, F5). In contrast, no significant differences ($P < 0.05$) were found between the treatments containing $CaCl_2$ (F2, F4, F6) and between the treatments containing only NaCl (FC1) or NaCl + KCl (F1, F3, F5).

The effect of $CaCl_2$ on the increase in shear force values may be probably due to the dehydration process, increasing hardness of the samples and reducing the water retention capacity. Several authors have reported that salts directly affect the texture characteristics of meat products (Aliño et al., 2009; Aliño et al., 2010; Offer et al., 1989; Offer & Trinick, 1983; Oliveira et al., 2012). According to Delgado-Pando et al. (2019), salt is the main ingredient that affects the texture. The results of the shear force may be related to the sensory properties of the products, which is discussed in Section 3.7.

3.5 Lipid oxidation

The control and minimization of lipid oxidation in meat products is of great interest due to the negative impact on the product's characteristics, which can lead to unpleasant rancidity, odor and texture, and loss of essential fatty acids (Alfaia et al., 2010; Domínguez, Gómez, Fonseca, & Lorenzo, 2014). In the present study, the lipid oxidation was determined by the quantification of 2-thiobarbituric acid reactive substances (TBARS), as shown in Table 5. There was no significant difference ($P < 0.05$) in malonaldehyde (mg/kg) values of the salted meat treatments made with the addition of different salts and lysine and yeast extract.

However, all treatments presented malonaldehyde levels higher than 0.5 mg/kg, which is perceptible to the consumers (Kang, Park, Choi, Lee, & Auh, 2009). Generally, salted meat products have characteristic taste and odor from the oxidation reactions, which can cause rapid deterioration under uncontrolled conditions.

3.6 Minerals levels

As can be seen in Table 6, the reduction of NaCl content in the salting steps and the addition of KCl and $CaCl_2$ significantly decreased ($P < 0.05$) the sodium content of the desalted product, as expected.

Table 6. Ca, K and Na values in mg/100g of salted meat treatments

Treatments	FP				Desalted			
	Ca	K	Na	Fe	Ca	K	Na	Fe
FC1	15.95^{d}	385.94^{e}	4849.44^{a}	3.71^{de}	7.71^{d}	120.98^{d}	1558.55^{a}	1.42^{b}
F1	14.99^{d}	3875.88^{a}	3534.81^{d}	3.85^{cd}	7.74^{d}	649.12^{a}	1047.40^{b}	1.42^{b}
F2	858.01^{b}	2930.86^{c}	3349.04^{e}	4.55^{a}	516.73^{a}	553.04^{b}	937.12^{b}	2.03^{a}
F3	15.74^{d}	3371.06^{b}	4001.26^{b}	3.48^{e}	8.60^{d}	623.71^{a}	1042.59^{b}	1.43^{b}
F4	567.32^{c}	2407.08^{d}	2944.37^{f}	4.22^{b}	451.33^{c}	468.21^{c}	1107.45^{b}	2.00^{a}
F5	12.74^{d}	3705.13^{a}	3771.54^{c}	3.48^{e}	8.02^{d}	650.87^{a}	1056.06^{b}	1.55^{b}
F6	1087.02^{a}	2743.20^{c}	3557.44^{d}	4.04^{bc}	640.97^{a}	479.31^{c}	1042.47^{b}	2.07^{a}
Standard error	54.74	139.19	71.55	0.05	33.85	22.08	28.21	0.04

Means in the same column followed by different letters present statistically significant difference by the Tukey test ($P < 0.05$). FP: final product. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 25% KCl + 25% $CaCl_2$; F3: 50% NaCl + 50% KCl + 3% lysine; F4: 50% NaCl + 25% KCl + 25% $CaCl_2$ + 3% lysine; F5: 50% NaCl + 50% KCl + 5% Bionis YE MXE NS; F6: 50% NaCl + 25% KCl + 25% $CaCl_2$ + 5% Bionis YE MXE NS

Salted meats require a long period of desalting in water before cooking (Shimokomaki et al., 1998). However, consumers not always follow the recommendations for this step, thus increasing the risk of high intake of Sodium from the traditional products. The present results show that is possible to guarantee a lower dietary sodium intake from the reformulated samples, even if the desalting procedures are not adequate.

3.7 Microbiological characterization

The results of total counts (log CFU/g), lactic acid bacteria (log CFU/g), thermotolerant coliforms (log MPN/g) and total coliforms (log MPN/g) are presented in Table 7. Except for raw meat, all treatments presented total and thermotolerant coliform counts < 1 log MPN/g. In addition, low total counts (from 2.64 to 3.04 log CFU/g) and lactic acid

bacteria counts (from <1 to 2.26 log CFU/g) were observed for all treatments, probably due to the great decrease of aw caused by the severe manufacture process of salted meat, which inhibited the microbial growth (Jeanson et al., 2015).

Table 7. Total counts (log CFU/g), lactic acid bacteria (log CFU/g), thermotolerant coliform (log MPN/g) and total coliform (log MPN/g) in salted meat treatments

Treatments	Total counts	Lactic acid bacteria	Thermotolerant coliform	Total coliform
Raw meat	5.41	4.20	< 1	< 1
FC1	2.64	< 1	< 1	< 1
F1	3.04	< 1	< 1	< 1
F2	2.83	< 1	< 1	< 1
F3	2.91	2.26	< 1	< 1
F4	2.46	< 1	< 1	< 1
F5	2.93	2.15	< 1	< 1
F6	2.45	< 1	< 1	< 1

FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 25% KCl + 25% $CaCl_2$; F3: 50% NaCl + 50% KCl + 3% lysine; F4: 50% NaCl + 25% KCl + 25% $CaCl_2$ + 3% lysine; F5: 50% NaCl + 50% KCl + 5% Bionis YE MXE NS; F6: 50% NaCl + 25% KCl + 25% $CaCl_2$ + 5% Bionis YE MXE NS

All analyses were negative for the presence of pathogens (data not shown), thus the reduced-sodium treatments were considered safe to perform the sensory evaluation. The results confirm the microbiological safety of the salted meat treatments made with NaCl reduction and the addition of KCl, $CaCl_2$, and lysine and yeast extract.

3.8 Sensory evaluation

The results of the sensory evaluation of the attributes color, aroma, flavor, texture and overall acceptance are presented in Table 8, and the sensory descriptors determined by the intensity scaling are shown in Table 9. Overall, the addition of the blends containing $CaCl_2$ (F2, F4, F6) to the salted meat treatments resulted in the lowest sensory scores ($P < 0.05$) for aroma, flavor, texture, and global acceptance when compared to the other treatments. On the other hand, the use of lysine (F4) and yeast extract (F6) together with the blend NaCl + KCl + $CaCl_2$ increased the sensory acceptance of the treatments when compared to the treatment F2,

with no addition of lysine and yeast extract. The salted meat treatments containing NaCl + KCl (F1), and lysine and yeast extract (F3, F5) exhibited acceptance scores similar to the control treatment FC1 (100% NaCl).

Table 8. Sensory attributes of salted meat treatments

Treatments	Color	Aroma	Flavor	Texture	Overall acceptance
FC1	6.43^{a}	6.23^{ab}	6.71^{ab}	6.05^{ab}	6.38^{ab}
F1	5.85^{b}	6.23^{ab}	6.56^{ab}	6.22^{a}	6.23^{b}
F2	6.01^{ab}	5.70^{c}	3.50^{d}	5.28^{c}	4.03^{d}
F3	6.12^{ab}	6.40^{a}	6.43^{b}	6.38^{a}	6.34^{ab}
F4	5.73^{b}	5.93^{bc}	4.63^{c}	5.23^{c}	4.73^{c}
F5	6.43^{a}	6.61^{a}	6.98^{a}	6.49^{a}	6.77^{a}
F6	5.23^{c}	5.72^{c}	4.38^{c}	5.56^{bc}	4.61^{c}
Standard error	0.17	0.15	0.21	0.19	0.01

Means in the same column followed by different letters present statistically significant difference by the Tukey test ($P < 0.05$). FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 25% KCl + 25% $CaCl_2$; F3: 50% NaCl + 50% KCl + 3% lysine; F4: 50% NaCl + 25% KCl + 25% $CaCl_2$ + 3% lysine; F5: 50% NaCl + 50% KCl + 5% Bionis YE MXE NS; F6: 50% NaCl + 25% KCl + 25% $CaCl_2$ + 5% Bionis YE MXE NS

Regarding the sensory descriptors evaluated using the intensity scaling technique, the treatment F2 (50% NaCl + 25% KCl + 25% $CaCl_2$) exhibited significantly higher values ($P < 0.05$) for rancid aroma, salty taste, bitter taste, and aftertaste. In addition, the use of $CaCl_2$ (F2, F4, F6) significantly decreased ($P < 0.05$) the characteristic salted meat aroma and succulence in relation to the other treatments. The addition of lysine (F4) and yeast extract (F6) together with the blend containing NaCl + KCl + $CaCl_2$ significantly decreased ($P < 0.05$) rancid aroma, salty taste, and aftertaste in relation to the treatment made with the same blend with no addition of lysine and yeast extract (F2). Indeed, the salted meat treatments containing NaCl + KCl (F1, F3, F5) showed similar sensory characteristics to the control treatment (FC1: 100% NaCl) except for the salty taste, which significantly ($P < 0.05$) decreased with the addition of KCl, lysine and yeast extract.

MANOVA (F: 6.762; Wilks lambda 0.625; $P< 0.0001$) suggested significant differences among the salted meat formulations when all sensory descriptors were compared simultaneously. Thus, DDA was performed to determine which attributes were mainly

responsible for the differences between samples. Six dimensions were needed to explain the total variance (100%), which accounted for 92.69% of the total variance explained (Table 10) when considering the canonical structures r values (Can 1, 2, and 3), thus identifying the constructs that largely accounted for the sample differences. The first dimension (Can 1), which accounted for 75.85% of the variance explained, showed the descriptors aftertaste, salty taste, and bitter taste (canonical correlation 0.616, 0.464, and 0.351) as the sensory descriptors contributing to the group differences among the seven treatments. In this sense, we concluded that the addition of salt substitutes and lysine and yeast extract had an impact on the flavor attribute, once it was the main construct that accounted for the group differences. Our findings confirm the challenge of sodium reduction in meat products.

Logistic regression (LR) was used to identify the sensory attributes that influenced the purchase intent of the salted meats (Table 11). Characteristic salted meat aroma, aftertaste, bitter taste, and fibrosity were the most critical attributes influencing the purchase intent ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, and P: 0.001, respectively) with odd ratio values of 0.995, 0.994, 0.866, and 1.303, respectively, indicating a purchase probability of 0.995, 0.994, 0.866, and 1.302 times higher ($P< 0.0001$ and P: 0.001). Interestingly, the salty flavor was not critical for the consumers' purchase intent (odds ratio of 0.726 and p=0.176). Based on the full logit model with ten sensory attributes, the purchase intent of the salted meats can be predicted with an accuracy of 77.14 %, indicating a good fit of the sensory data. In addition, LR was useful to check the possible different perceptions of the consumers regarding the sensory attributes and purchase intention.

Random Forest (RF) was used to build a predictive model among the sensory descriptors (independent variables) and overall acceptance (dependent variables), identifying the relative importance of the regressor variables, in this case, the sensory attribute. RF variable importance is based on how much prediction error increases when the data for that variable are permuted (noised up) while all the others are left unchanged. In other words, in RF, a variable is important if dropping it seriously affects prediction accuracy or node impurity, which can be measured by the mean decrease in accuracy value (Bi & Chung, 2011). According to the RF, the variables with the large relative importance on the overall acceptance were characteristic salted meat aroma, bitter taste, rancid aroma, salty taste and hardness, with mean increased error values of 2.584, 2.294, 1.794, 1.407, and 1.279, respectively, suggesting that drastic changes in these sensory attributes can cause changes in

the overall liking. There is no report on using machine learning techniques in the sensory and consumer science of meat products, thus our study can widely contribute to the meat industry.

Table 9. Sensory descriptors of salted meat treatments

Treatments	Red color	Dry surface	Rancid aroma	Characteristic salted meat aroma	Salty taste	Bitter taste	After taste	Hardness	Fibrosity	Juiciness
FC1	1.95^{c}	3.34^{ab}	2.28^{bc}	5.07^{a}	6.35^{ab}	2.29^{c}	2.84^{c}	4.43^{a}	4.65^{ab}	4.65^{a}
F1	2.27^{bc}	2.70^{c}	2.37^{bc}	5.06^{a}	5.26^{cd}	1.65^{c}	2.67^{c}	4.11^{a}	4.47^{abc}	4.54^{a}
F2	2.72^{b}	3.27^{abc}	3.28^{a}	4.05^{b}	6.58^{a}	5.95^{a}	5.77^{a}	4.61^{a}	4.78^{a}	3.51^{b}
F3	2.55^{b}	2.86^{bc}	2.26^{bc}	5.24^{a}	5.02^{cd}	1.53^{c}	2.92^{c}	4.32^{a}	4.06^{bc}	4.79^{a}
F4	1.99^{c}	3.43^{ab}	2.26^{bc}	3.99^{b}	5.49^{cd}	4.75^{b}	4.53^{b}	4.76^{a}	4.73^{a}	3.57^{b}
F5	2.51^{b}	3.01^{abc}	2.01^{c}	5.06^{a}	4.74^{d}	1.47^{c}	2.69^{c}	4.04^{b}	3.92^{c}	4.84^{a}
F6	3.43^{a}	3.45^{a}	2.64^{b}	3.84^{b}	5.70^{bc}	5.49^{ab}	4.58^{b}	4.27^{a}	4.76^{a}	3.73^{b}
Standard error	0.18	0.20	0.23	0.20	0.28	0.44	0.25	0.27	0.22	0.20

Means in the same column followed by different letters present statistically significant difference by the Tukey test ($P < 0.05$). FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 25% KCl + 25% $CaCl_2$; F3: 50% NaCl + 50% KCl + 3% lysine; F4: 50% NaCl + 25% KCl + 25% $CaCl_2$ + 3% lysine; F5: 50% NaCl + 50% KCl + 5% Bionis YE MXE NS; F6: 50% NaCl + 25% KCl + 25% $CaCl_2$ + 5% Bionis YE MXE NS

Table 10. Descriptive discriminant analysis (DDA) reporting the canonical structure r values for describing group differences among the six salt meats

Sensory Descriptor	Can 1	Can 2	Can 3
Red color	0.181	-0.870	0.103
Dry surface	0.002	0.059	-0.283
Rancid aroma	-0.119	-0.008	0.644
Characteristic salted meat aroma	-0.406	0.027	0.343
Salty taste	0.351[a]	0.353	0.516
Bitter taste	0.464[a]	0.086	-0.182
Aftertaste	0.516[a]	0.009	0.122
Hardness	-0.049	0.407	-0.279
Fibrosity	0.076	-0.086	0.091
Juiciness	-0.221	0.074	0.022
Cumulative variance explained (%)	75.85	87.31	92.69

* Based on the pooled within-group variances. Can 1, 2, and 3 refer to the first, second, and third canonical discriminant functions, respectively. [a] Indicates attributes that accounted for the group differences in the first canonical discriminant function

Finally, the sensory results are in agreement with other authors, who found several negative sensory effects of $CaCl_2$ in different meat products (Armenteros, Aristoy, Barat, & Toldrá, 2012; Dos Santos et al., 2015; Horita, Morgano, Celeghini, & Pollonio, 2011). The use of the blend NaCl + KCl, lysine, and yeast extract demonstrated to be a good alternative to reduce sodium content in salted meat treatments, without negatively impacting the sensory characteristics. However, the addition of salt substitutes and lysine and yeast extract to meat products is a multifactorial problem, thus a correct optimization in accordance with the consumer´s opinion is required.

Table 11. Logistic regression analysis for predicting for purchase intent of salted meat treatments

Independent Variables	Purchase Intent		
	Estimate	Prob > X^2	Odds-Ratio
Intercept	-0.231	0.547	1.079

Red color	0.076	0.105	1.048
Dry surface	0.047	0.277	0.978
Rancid aroma	-0.022	0.563	1.216
Characteristic salted meat aroma	0.196	< 0.0001	0.965
Salty taste	-0.036	0.176	0.726
Bitter taste	-0.320	< 0.0001	0.866
Aftertaste	-0.144	0.001	0.994
Hardness	-0.006	0.858	0.862
Fibrosity	-0.148	0.001	1.303
Juiciness	0.265	< 0.0001	1.079

* Based on the logistic regression analysis using a full model with all the sensory descriptors. The analysis of maximum likelihood estimates was used to obtain parameter estimates. Significance of parameter estimates was based on the Wald X2 value at $P < 0.05$

4 Conclusion

The use of lysine and yeast extract minimized the negative sensory effects provided by the addition of $CaCl_2$ without changing the physicochemical quality parameters and safety of salted meat treatments.

The salted meat treatments made with the blend NaCl + KCl exhibited sensory characteristics similar to the control treatment (100% NaCl) when NaCl was reduced in 50%, based on the ionic strengh. Although the blend NaCl + KCl exhibited a positive sensory evaluation, strategies to reduce potassium levels should also be studied, aimed at reducing the risk of hyperkalemia.

The present study contributes to make healthier salted meat, with good sensory acceptance and an expressive reduction of sodium levels, using natural ingredients, thus leading to the development of products with a clean label appeal.

Acknowledgment

The authors thank National Council for Scientific and Technological Development (process numbers: 140533/2015-0 and 406671/2016-8) for the financial support to this work

and scholarships. This work was carried out with the support of the CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil - Financing Code 001

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CAPÍTULO 4

UNDERSTANDING THE EFFECT OF DIFFERENT CHLORIDE SALTS ON THE WATER BEHAVIOR IN THE SALTED MEAT MATRIX ALONG 180 DAYS OF SHELF LIFE

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Food Research International

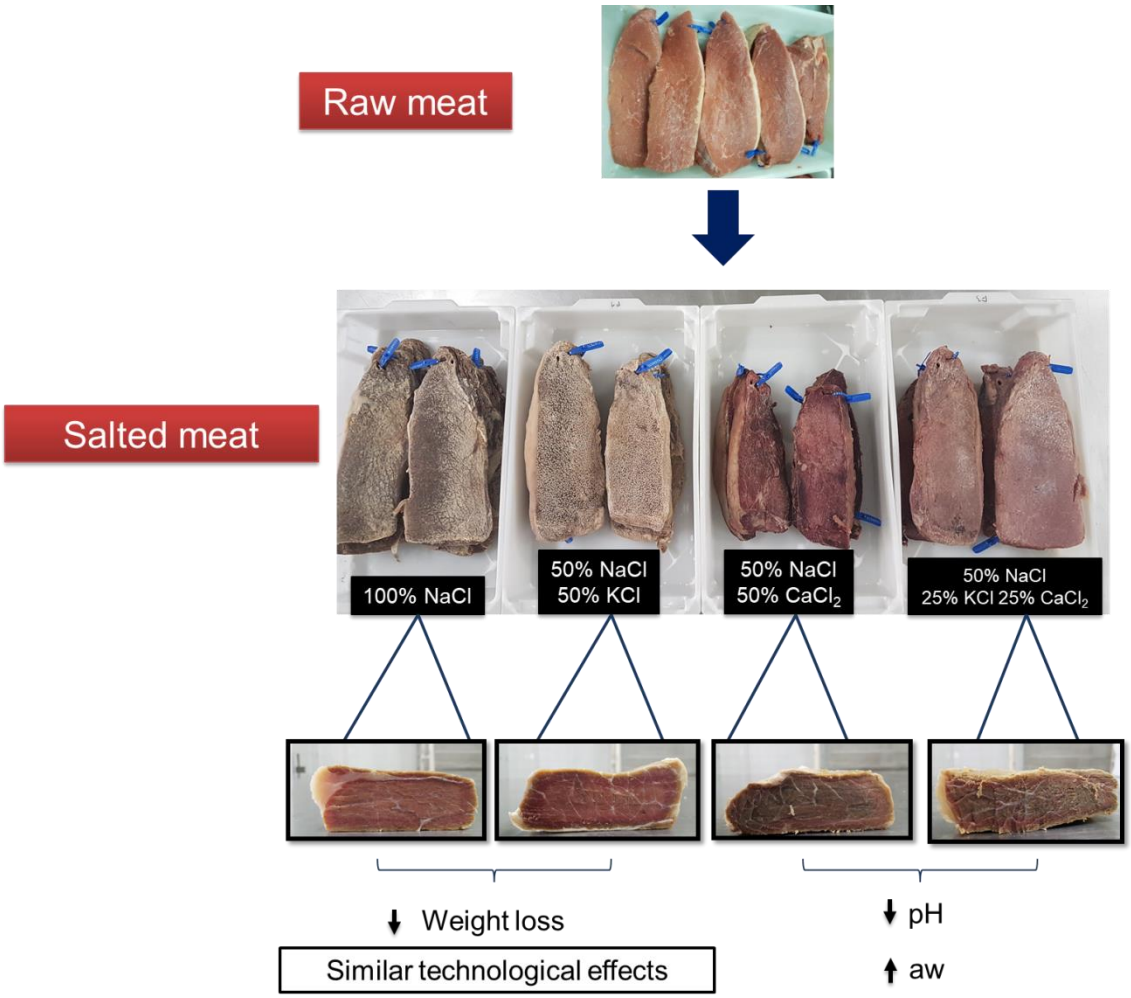
In Press

DOI: 10.1016/j.foodres.2019.108634

Highlights

- Different chloride salts influenced the physical characteristics of salted meat along 180 days of storage
- The LF-NMR was an effective method to analyze the water behavior in salted meat
- Using $CaCl_2$ resulted in a greater weight loss and the highest water activity.
- Addition of $CaCl_2$ led to lower values of entrapped, immobilized and mobile water

Graphical abstract



Abstract

The objective of this study was to evaluate the effects of different chloride salts (NaCl, KCl, and $CaCl_2$) on water behavior in salted meat during 180 days of shelf life by Low Field Nuclear Magnetic Resonance and physicochemical analysis. Four salted meat treatments were made using the following salts in the wet and dry salting steps: FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$. The analyses performed were: moisture, pH, aw, weight loss and Low Field Nuclear Magnetic Resonance. The use of $CaCl_2$ as a salt substitute to NaCl during the elaboration of salted meat caused a decrease of pH and higher values of aw and weight loss when compared with the treatments containing only NaCl or NaCl + KCl. The morphology of the salted meat changed with the addition of $CaCl_2$, possibly making the matrix structure more open and facilitating dehydration, whereas the NaCl replacement by KCl did not cause significant modifications in salted meat characteristics during 180 days of storage. In general, the results demonstrated that the addition of KCl may be a good alternative to reduce the sodium content in salted meat product, and the Low Field Nuclear Magnetic Resonance method has proved a good tool for obtaining additional information on the changes that salts can cause in the structure of salted meat products.

Keywords: chloride salts; sodium reduction; salt dehydration; bovine meat; LF-NMR.

1. Introduction

Salted meat products are widely appreciated due to its unique and characteristic flavor and longer shelf life (Ishihara & Madruga, 2013). The preservation of these products is based on the hurdle technology, and the salting and drying steps are the most important barriers along with vacuum packaging, and the use of additives in some products (Leistner, 1987; Shimokomaki et al., 1998). In general, salted meat products have high sodium content due to the salting step required during the manufacture, which requires desalting before consuming. However, consumers are not able to properly desalt the product, thus ingesting a high sodium levels.

The high sodium intake, particularly from processed foods has been strongly associated with the increasing of the risk of some chronic diseases (Aaslyng et al., 2014; Aburto et al., 2013) including hypertension and cardiovascular damages. The WHO recommends ingesting only 2 g of sodium per day, which is a great challenge to both the consumer and the meat industry. Meat products have been cited as the most significant source of dietary sodium due to the presence of sodium chloride (NaCl), which is commonly used in all categories of processed meats. This salt has technological, sensory, and safety properties, thus the NaCl replacement by simple reformulation strategies is considered a difficult task. According to Desmond (2006) and Terrel (1983), NaCl promotes microbiological stability, affects the water retention and binding capacities, increases the ionic strength, modifies the texture properties, and provides a salty taste.

The substitution of NaCl by other salts is one of the main strategies for reducing sodium content. Several authors have used potassium chloride (KCl) and calcium chloride ($CaCl_2$) as salt substitutes to NaCl in several meat products (Dos Santos et al., 2017; Horita et al., 2014; Lorenzo et al., 2015). Although KCl is the most used to partially replace NaCl due to its similar characteristics (Aljuraiban et al., 2012), it can lead to undesirable sensory characteristics such as metallic and bitter taste in meat products (Askar et al., 1993; Zanardi et al., 2010). The use of blends containing $CaCl_2$ has been successfully used as a strategy to reduce sodium in different meat products without functional impairment (Collins, 1997; Dos Santos et al., 2015; Guàrdia et al., 2008; Horita et al., 2011), however, the use of $CaCl_2$ promotes undesirable effects such as development of bitter, metallic and residual taste, affects the texture and can impairs the release of water in salted meat (Vidal, et al., 2019).

The mobility and distribution of water in meat and meat products has a major influence on the microbiological stability and product's quality, such as appearance, tenderness, firmness, and juiciness (Dhall et al., 2012; Shao et al., 2016). Therefore, it is very important to understand the water behavior during the processing and storage of salted meat, aiming at future salt replacement with satisfactory sensory and technological performance. In meat and meat products, the water molecule is held in the space between myofilaments of the muscle fibers by the capillary phenomenon, and the increase or decrease in this space can define the water retention capacity (Huff-Lonergan & Lonergan, 2005). The pH evolution can be induced by solubilization and precipitation of proteins during salting processes, and the pH values can be related to the water loss and water holding capacities (WHC) in salted and dry meat products (Hamoen et al., 2013).

Low Field Nuclear Magnetic Resonance (LF-NMR) is a rapidly and non-destructive way to investigate the chemical-physical state of water in meat and meat products (Bertram et al., 2002; Carneiro et al., 2016; Marcone et al., 2013). This technique allows determining the relaxation of hydrogen protons and therefore investigating the water mobility and distribution in salted meat products during the storage (Gudjónsdóttir et al., 2011; Sánchez-Alonso et al., 2012), however, there are no studies correlating the LF-NMR parameters and water content in salted meat products elaborated with bovine raw meat and salt substitutes.

This study aimed to investigate the water behavior on salted meat treatments with the partial replacement of NaCl by KCl and/or $CaCl_2$ based on the same ionic strength, during a shelf life of 180 days, through the LF-NMR method as a possible strategy to predict the parameters of quality assurance of these reformulated products.

2. Material and Methods

2.1 Treatments, raw materials and additives

The additives sodium nitrite and sodium erythorbate were donated by the company Kerry of Brazil. The salts NaCl, KCl, and $CaCl_2$ were purchased from a food grade company (Anidrol, Brazil). The selected meat cut was bovine raw meat (*biceps femoris*) purchased (9 samples of 5 kg for each treatment) from slaughterhouses of Campinas (São Paulo, Brazil). The bovine raw meat was carefully selected by safety and quality parameters. The pH of raw

material ranged from 5.6 to 5.9. The microbiological analyses meet the standards established by the Brazilian regulatory issues (Brasil, 2001).

The salted meat treatments can be seen in Table 1 Four treatments were performed as follows: the control (FC1: 100% NaCl) and 3 treatments with salt substitutes (F1: 50% NaCl + 50% KCl, F2: 50% NaCl + 50% $CaCl_2$, F3: 50% NaCl + 25% KCl + 25% $CaCl_2$). The concentration of KCl and $CaCl_2$ substitute salts was based on the calculation of ionic strength to make up the same ionic strength in all treatments. In the wet salting step, the proportion of satured brine with the respective salts and additives was 4:1 (brine:raw meat), similar amounts of the additives sodium nitrite and sodium erythorbate were added in all treatments. For dry salting step, 2 kg of the respective salts were used per kg of meat. The experiment was performed in three replicates on different days utilizing the same methodology and technology.

Table 1. Salts used to performed the salted meat treatments

Treatments	NaCl (%)	NaCl (mg)*	KCl (%)	KCl (mg)*	$CaCl_2$ (%)	$CaCl_2$ (mg)*
FC1	100	1000	-	-	-	-
F1	50	441	50	560	-	-
F2	50	614	-	-	50	387
F3	50	513	25	326	25	162

The amount of salt added was based on the ionic strength, all treatments obtained the same ionic strength. * Salt proportion added according to ionic strength, for each 1000 mg of bovine raw meat was utilized 2000 mg of salt

2.2 Processing

The salted meat treatments were elaborated according to Vidal et al (2019). For wet salting, the raw bovine meat were immersed for 1 hour in a saturated solution (brine containing salts according to Table 1, 500 ppm sodium erythorbate, and 150 ppm sodium nitrite) utilizing filtered water.

In the dry salting step, the pieces were put in contact with different salt blends in a cold chamber at 13 °C. At the end of the 6 days of dry salting , the ripening steps were carried out in a controlled climatic chamber (Instala Frio, Curitiba, Brazil) with 55% humidity, 25 °C

and 0.5 m/s forced air ventilation for 24 hours. The final product was vacuum packed with polyethylene (Spel, São Paulo, Brazil) and stored at 25 °C.

The replicates were carried out in the Pilot Plant of Meat Products, Faculty of Food Engineering, at UNICAMP.

2.3 Physicochemical characterization

2.3.1 Moisture, pH and aw

The moisture, pH and aw were determined according to Horwitz and Latimer (2005). To determine the moisture content, the samples were oven dried at 105 ° C until reach constant weight. The pH was determined by homogenizing of sample and distilled water (1:10), followed by pH readings using an combined electrode (22 DM, Digimed, São Paulo, Brazil). The aw was measured at 20 ° C using the Aqualab apparatus (Decagon Devices Inc., Pullman, USA). To avoid interferences in the results and make it more homogeneous, the samples for moisture and aw analyzes were collected from the center of the pieces. The moisture, pH and aw were measured at 0, 45, 90, 135 and 180 days of storage. All analyses were performed in triplicate for each replicate of the experiment.

2.3.2 Weight loss

The weight loss was expressed as a percentage (%) referring to previous weight of sample and measured at the same time using the formula: final weight x 100 / previous weight. The analysis was performed in triplicate for each replicate of the experiment.

2.3.3 Scanning electron microscopy

A high vacuum scanning electron microscope TM 3000 Tabletop Microscope, with a magnitude of 15x to 30000x and 15 kV acceleration voltage (Hitachi High Technologies, Japan) was used to evaluate the microstructure of salted meat samples utilizing magnification at 250x. The samples were cut into standard size, placed in stubs, and analyzed in the modular equipment.

2.3.4 Low Field Nuclear Magnetic Resonance

The relaxation times were evaluated using LF-NMR for portions of approximately 1 g of salted meat after salting steps (wet and dry) and at 0, 45, 90, 135 and 180 days of storage placed in a 8-mm diameter cylindrical glass tubes. About 15 min was to stabilized the temperature at 39.8 °C. The measurements of the transverse relaxation time were performed on a Bruker minispec mq20 NMR analyser (Bruker Company, Massachusetts, USA) to a proton resonance frequency of 20 MHz. The spin–spin relaxation time, T_2, was measured using the Carr-Purcell-Meiboom-Gill (CPMG) sequence, with 90 and 180 proton pulses of 8.5 and 16.6 μs, respectively, and echo time of 160 μs. The data, were obtained in triplicate for two different batches samples, in which 15000 echoes were acquired with 16 scan repetitions and the repetition time between subsequent scans was 15 s. The fitting of the CPMG decay curves was performed using multi-exponential. After inverse Laplace transform of the CPMG decay curve, the time constant for each process were detemined from the center peak position, and the area under each peak. The corresponding proportion of water molecules exhibiting a specific relaxation time was determined by cumulative integration using Log-Normal distribution.

2.4 Statistical analysis

Three independent processes were performed using the same methodology, formulation, and technology, in different days. For each process, at least three samples were taken for each analysis. The results were expressed as the averages from all data. Data were analyzed using a General Linear Model (treatments as a fixed effect and replicates as a random effect) using 5% of significance. Significant differences were analyzed by the Tukey's test at the 5 % level of significance utilizing the commercial software Statistica v. 8 (Statsoft Inc., Oklahoma, USA).

3. Results and Discussion

3.1 Moisture

With respect to the moisture results (Table 2) of the treatment F2 (50% NaCl + 50% $CaCl_2$), a higher moisture value *($P < 0.05$*) was observed at time 0 when compared to days 45,

90, 135, and 180, while the other treatments present similar moisture levels throughout the storage time.

Table 2. Moisture (%) values in salted meat treatments during storage

Treatments	0 day	45 days	90 days	135 days	180 days
FC1	50.31^{abA}	49.85^{aA}	49.25^{aA}	49.75^{aA}	49.42^{aA}
F1	49.98^{abA}	49.20^{aA}	49.13^{abA}	49.21^{abA}	49.19^{abA}
F2	51.08^{aA}	48.61^{aB}	48.54^{bB}	48.45^{cB}	48.67^{bB}
F3	49.31^{bA}	48.45^{aA}	48.75^{abA}	48.59^{bcA}	48.88^{abA}
Standard error	0.09	0.20	0.10	0.12	0.09

Values are means. $^{a, b, c, d}$ Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test. ^{A,B,C,D} Values in the same line with the same capital letters do not differ significantly ($P < 0.05$). FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

The pH, the type of salt used in the processing, and the salt content affect the solubility of myofibrillar proteins and swelling of muscle fibers, which may lead to changes in the water retention and binding capacity (Offer et al., 1989; Oliveira et al., 2012). During the salting steps, the addition of high levels of $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$) may have caused a higher superficial dehydration, forming a very firm and dry surface barrier in the meat product, thereby impairing the water release from the inner regions of the outside flat, resulting in higher moisture values. Many authors have reported an increase in the dehydration rate in several foods with the addition of $CaCl_2$ (Lewicki and Michaluk, 2004; Lewicki et al., 2002; Mastrantonio et al., 2005; Pereira et al., 2007). The present results found different effects of the divalent salt $CaCl_2$ on the dehydration of salted meat when compared to NaCl and KCl. As reported by Vidal et al. (2019), this phenomenon was responsible for reducing the sensory acceptance of the products as a direct consequence.

3.2 pH and aw

The results of pH and aw are presented in Table 3. The pH values reduced ($P < 0.05$) during the storage time for all treatments. The treatments containing $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$) obtained lower pH values in relation to the treatments containing NaCl and KCl (FC1: 100% NaCl and F1 :50% NaCl + 50% KCl). The decrease in pH values with the addition of $CaCl_2$ is related to two atoms of Cl^- (anions) in

its composition, leading to lower pH values when compared to monovalent salts NaCl and KCl Several authors have reported the effects of pH reduction due to the addition of divalent salt such as $CaCl_2$ in different meat products (Fieira, Marchi, Marafão, & Alfaro, 2018; Gimeno et al., 1999; Gimeno et al., 2001; Horita, Messias, Morgano, Hayakawa, & Pollonio, 2014; Lawrence et al., 2003; Lorenzo et al., 2015; Vidal et al., 2019). During the storage of the vacuum packaged salted meat, lactic fermentation may have occurred with a consequent gradual decrease in pH values (Dos Santos et al., 2015).

Table 3. pH and aw values in salted meat treatments during storage

Treatments	0 day	45 days	90 days	135 days	180 days
			pH		
FC1	5.55^{bA}	5.52^{aA}	5.46^{aB}	5.24^{bC}	5.22^{aC}
F1	5.68^{aA}	5.53^{aB}	5.45^{aC}	5.30^{aD}	5.25^{aE}
F2	5.13^{cA}	5.05^{bB}	4.95^{bC}	4.94^{cC}	4.70^{bD}
F3	5.05^{dA}	4.97^{cBC}	4.94^{bC}	4.95^{cC}	4.54^{cD}
Standard error	0.02	0.05	0.05	0.03	0.06
			Aw		
FC1	0.775^{bA}	0.754^{bB}	0.744^{bC}	0.748^{bBC}	0.745^{bC}
F1	0.751^{cA}	0.744^{cB}	0.732^{cC}	0.736^{cC}	0.733^{cC}
F2	0.788^{aC}	0.795^{aB}	0.800^{aA}	0.802^{aA}	0.790^{aC}
F3	0.759^{cA}	0.745^{cB}	0.741^{bB}	0.744^{bB}	0.731^{cC}
Standard error	0.003	0.003	0.004	0.004	0.004

Values are means. $^{a, b, c, d}$ Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test. ^{A,B,C,D} Values in the same line with the same capital letters do not differ significantly ($P < 0.05$). FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

Higher aw values ($P < 0.05$) were observed for the treatment F2 (50% NaCl + 50% $CaCl_2$) over the entire storage time, followed by FC1 (100% NaCl), when compared to the treatments F1 (50% NaCl + 50% KCl) and F3 (50% NaCl + 25% KCl + 25% $CaCl_2$). In all treatments, except for F2 (50% NaCl + 50% $CaCl_2$), there was a gradual decline in aw values during the storage. The KCl (F1 and F3) decreased significantly ($P < 0.05$) the aw values of the treatments while the high $CaCl_2$ content of the treatment F2 did not cause a decrease in aw values. As $CaCl_2$ is a dehydrating agent (Lewicki and Michaluk, 2004), probably rapid and

intense surface dehydration may have occurred, thus hampering the decrease in aw and entrapment within the treatment.

3.3 Weight loss

The results of weight loss can be seen in Table 4. After the wet salting, a weight loss was observed for the treatments containing $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$), while the treatments containing only NaCl and KCl exhibited a weight gain. During the dry salting, the treatments containing $CaCl_2$ (F2 and F3) showed significantly greater weight loss ($P < 0.05$) when compared to the treatments FC1 (100% NaCl) and F1 (50% NaCl + 50% KCl). During ripening, the treatments FC1 and F1 had the highest weight losses ($P < 0.05$). The treatment F2 with the highest $CaCl_2$ content had the greatest weight loss ($P < 0.05$) when compared to the other treatments.

The significant weight loss of the treatments F2 (50% NaCl + 50% $CaCl_2$) and F3 (50% NaCl + 25% KCl + 25% $CaCl_2$) during the dry salting may have caused intense surface dehydration, leading to water entrapment by the $CaCl_2$. Thus, during 24 hours of ripening, the remaining water outlet was impaired by the formation of this physical barrier. According to Vidal et al. (2019), effects on the increase of hardness, changes of color accompanied this phenomenon.

According to results in Table 3, the treatment F2 (F2: 50% NaCl + 50% $CaCl_2$) obtained the highest values of aw. As mentioned previously, probably occurred intense dehydration on the surface of meat pieces caused by $CaCl_2$. Thus, even with higher aw and moisture values, the high content of $CaCl_2$ significantly decreased ($P < 0.05$) the weight of salted meat.

Table 4. Weight loss (%) values in salted meat treatments during process

Treatments	After wet salting	1º day	2º day	3º day	4º day	5º day	6° day	After ripening	Total weight loss
FC1	-1.02^{bE}	6.55^{bB}	3.25^{bC}	2.92^{aC}	1.15^{bD}	0.87^{bD}	0.85^{bcD}	8.41^{bA}	22.98^{c}
F1	-0.61^{bE}	6.29^{bB}	2.81^{bC}	2.62^{aC}	1.10^{bD}	0.52^{bD}	0.44^{cD}	9.30^{aA}	22.47^{c}
F2	0.68^{aD}	10.68^{aA}	5.27^{aB}	3.20^{aC}	2.93^{aC}	2.55^{aC}	2.51^{aC}	5.88^{cB}	33.70^{a}
F3	0.20^{aD}	6.39^{bA}	5.25^{aA}	3.55^{aB}	2.44^{aBC}	2.32^{aBC}	1.11^{bCD}	6.38^{cA}	27.64^{b}
Standard error	0.79	0.36	1.43	0.92	0.16	1.01	0.88	0.26	0.71

Values are means. $^{a, b, c, d}$ Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test. ^{A,B,C,D} Values in the same line with the same capital letters do not differ significantly ($P < 0.05$). FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

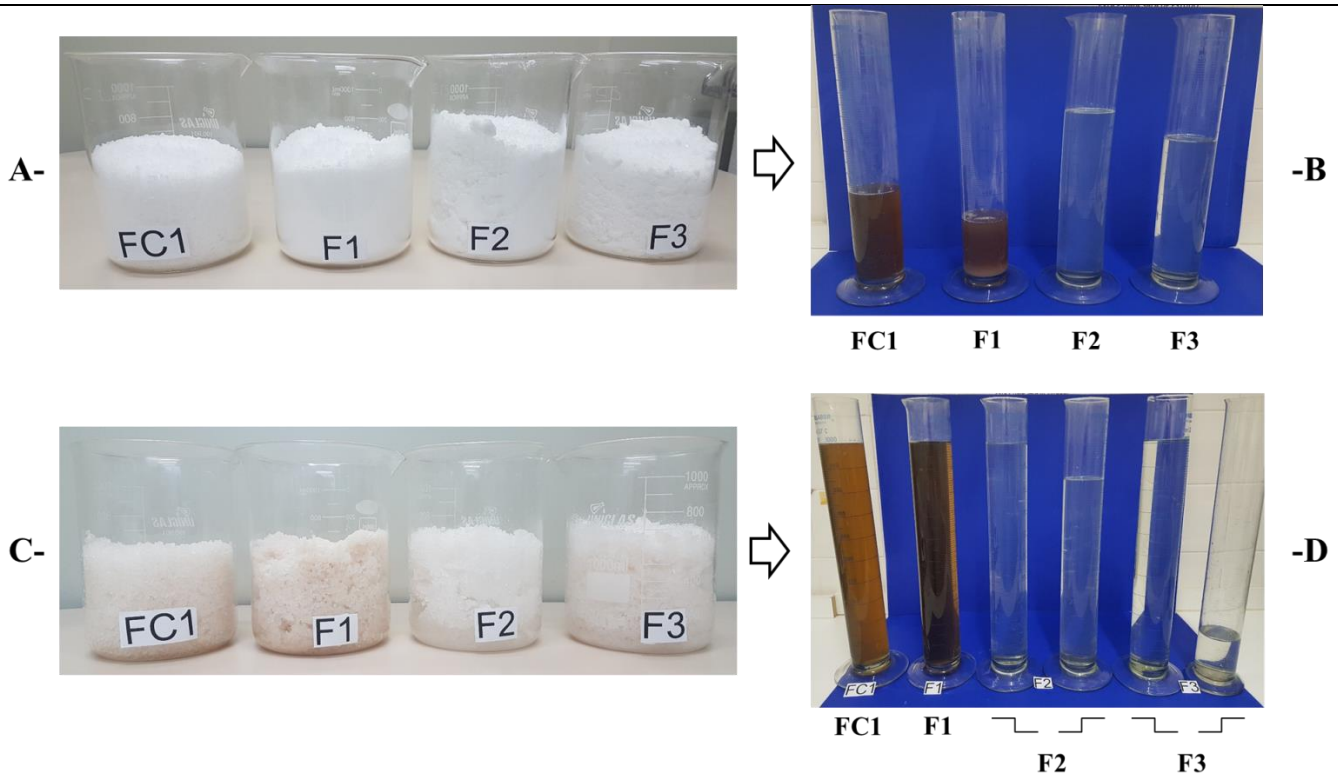


Figure 1. Salts and liquid released of salted meat treatments. A: salts used in salted meat processing; B: liquid released after first 24 hours (1 day) of dry salting – FC1: 0.41 L, F1: 0.27 L. F2: 0.78 L, F3: 0.66 L; C: salts after 144 hours (6 days) of dry salting; D: liquid released after 144 hours (6 days) of dry salting – FC1: 1 L, F1: 1 L, F2: 1.85 L, F3: 1.17 L. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

Regarding the liquid released from the samples (Figure 1. B and D), the treatments containing $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$) presented a higher amount of liquid released, which was colorless. Figure 1 confirmed the dehydrating action of $CaCl_2$ in meat products. In addition, the $CaCl_2$ is a sequestrant metal (Ropp, 2013), thereby, the seizure of iron present in myoglobin may have occurred, not allowing it to be released by the liquid.

3.4 Scanning electron microscopy

Microstructure images of different salt meat treatments can be seen in Figure 3. The treatments FC1 (100% NaCl) and F1 (50% NaCl + 50% KCl) presented a denser, compact and homogeneous topography when compared to the treatments containing $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$) which demonstrates the similar effects of NaCl and KCl on the microstructure during 180 days of storage.

The use of $CaCl_2$ (F2 and F3) resulted in salted meat with heterogeneous microstructure and apparent salt crystals, mainly in the treatment with 50% of the ionic strength provided by $CaCl_2$ (F2). The $CaCl_2$ crystal migration may be due to the intensive dehydrating effect of $CaCl_2$ on salted meat, which is responsible by the rapid superficial drying, keeping the water activity higher in the treatment F2 when compared to the other treatments, which is consistent with the results of this study.

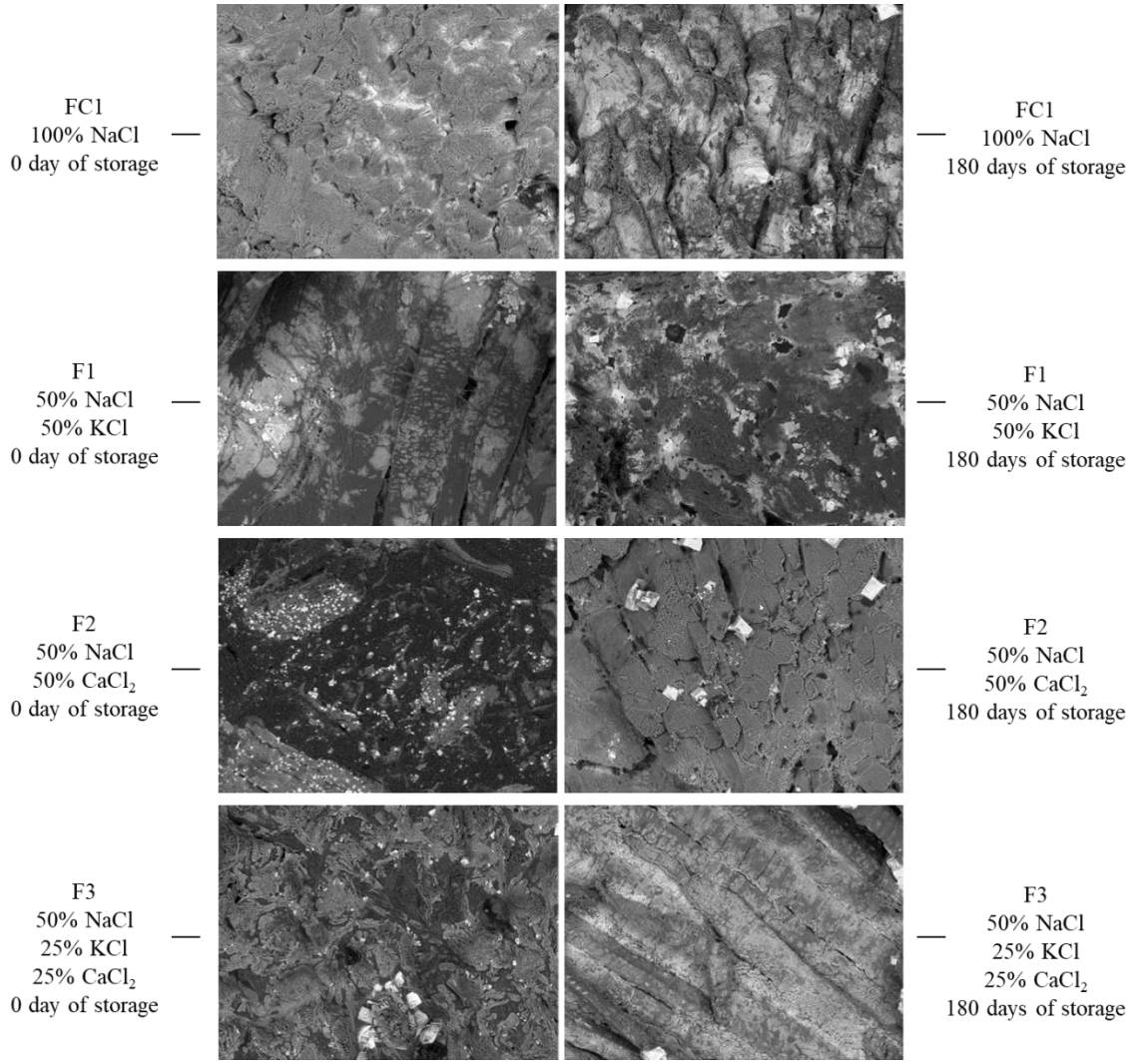


Figure 2. Scanning electron microscopy images of salted meat treatments with 250x magnification.

3.5 Low Field Nuclear Magnetic Resonance

The CPMG sequence is commonly used to study the population of mobile protons in meat, as reported in literature (Laghi et al., 2017; Møller et al., 2011; Scussat et al., 2017; Yang et al., 2016; Zheng et al., 2015). The LF-NMR T_2 relaxation curves for the salted meat samples exhibited a multiple-exponential distribution with clearly two distinct populations mainly related to water with a small contribution of fat molecules in meat, which can be ignored (Gudjónsdóttir, 2011) (Figure 2. a). These results are due to the greater amount of protons (of the order of 80%) present in the meat samples comes from water (Mungure et al., 2017).

The first population with relaxation time between 10 and 80 ms (T_{2a}) is due to the entrapped or immobilized water associated with the highly organized protein or fiber bundle structures. The second population with relaxation time between 80 and 600 ms (T_{2b}) referred to the mobile water located between fiber bundles interacting weakly with the charged groups.

After dry salting, the distribution of relaxation times for immobilized water (T_{2a}) and mobile population (T_{2b}) was dramatic shorter in time, due to the reduction of mobility or increase in the interactions between it and organized protein or fiber bundle structures for all samples, indicating muscle swelling due to the salt added (Figure 2. b). Several studies have shown that processes such as salting can affect the morphology of the sample (Aursand, Erikson, & Veliyulin, 2010; Erikson, Standal, Aursand, Veliyulin, & Aursand, 2012; Greiff, Aursand, Erikson, Josefsen, & Rustad, 2015), explaining the change in relaxation time.

From 0 to 180 days of storage, similar T_{2a} and T_{2b} values were observed between the treatments FC1 (100% NaCl) and F1 (50% NaCl + 50% KCl), and between F2 (50% NaCl + 50% $CaCl_2$) and F3 (50% NaCl + 25% KCl + 25% $CaCl_2$) as shown in Figure 2 (b and c). The treatment F2 showed lower T_{2a} and T_{2b} followed by the treatment F3, when compared to the treatments FC1 and F1, probably due to the dehydrating effect of $CaCl_2$. The dehydrating effect of $CaCl_2$ (Ca^{2+}) is due to the action of the divalent cation on the muscular structure, and therefore the proton exchange, providing an open access in the salted meat matrix, thus facilitating the outflow of water (Aliño et al., 2009).

With respect to the quantities of the two distinct populations, the faster relaxing water population T_{2a} was dominant in FC1 (100% NaCl) and F1 (50% NaCl + 50% KCl) at all processing steps. After the dry salting step, a slight decrease in T_{2a} populations was observed, corresponding to an increase in the more mobile T_{2b} population, which was recovered after storage. A similar effect was observed for the sample F1 despite being less accentuated. For the treatment F2 (50% NaCl + 50% $CaCl_2$), a significant decrease in T_{2a} populations was observed, after the dry salting step, corresponding to an increase in the more mobile T_{2b} population to reverse the majority proportion. The same effect with less prominence was observed for F3 (50% NaCl + 25% KCl + 25% $CaCl_2$), which demonstrates the stronger salt dehydration caused by $CaCl_2$.

These results show that the LF-NMR method is effective to provide additional information on changes in the structure of salted meat treatments due to the addition of different salts.

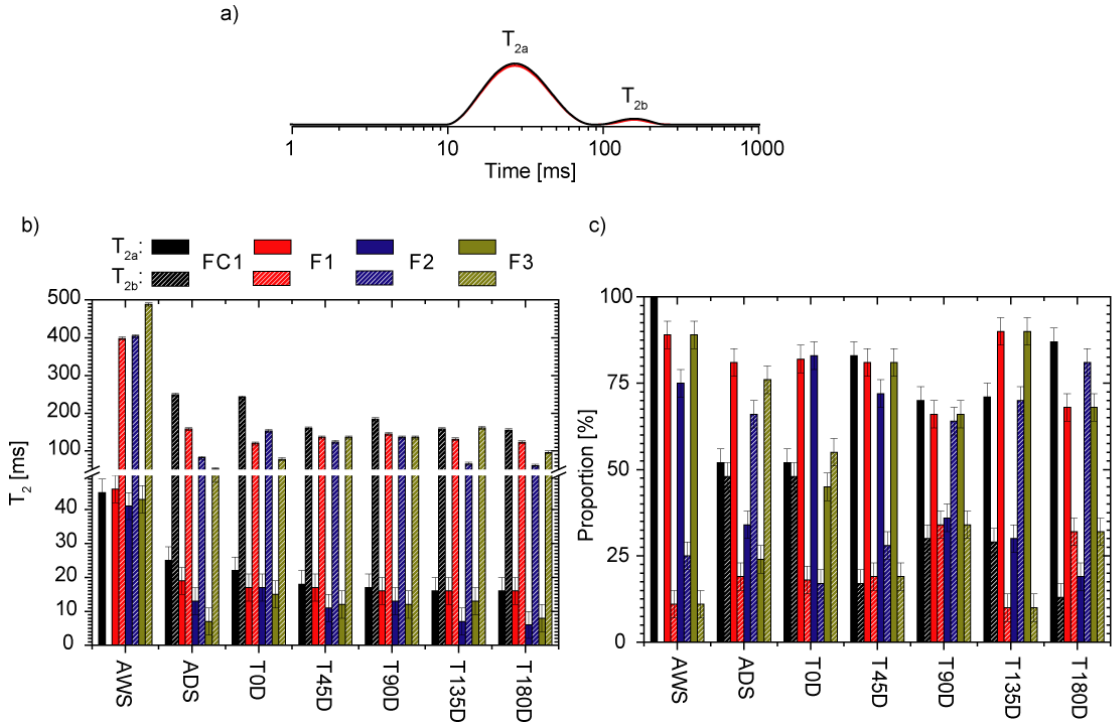


Figure 3. LF-NMR of salted meat treatments. a) The LF-NMR curve for the salted meat samples exhibited a Log-Normal distribution deconvolution in two water populations; b) T_2 center relaxation time; c) relative intensity. AWS: after wet salting; ADS: after dry salting; T0D: 0 day of storage; T45D: 45 days of storage; T90D: 90 days of storage; T135D: 135 days of storage; T180D: 180 days of storage. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

4. Conclusion

The distinct effect of the use of $CaCl_2$ in the salted meat treatments during the shelf life is evident when compared to NaCl and KCl. In general, the addition of blends containing $CaCl_2$ changed the structure of salted meat, providing an open access in the meat matrix, leading to higher weight loss, aw, and dehydration during 180 days of storage. Although similar effects were observed between NaCl and KCl, the use of KCl should be carried out with caution due to the risk of hyperkalemia in patients with renal problems.

The present results demonstrated the need for further studies, aimed to optimize the use of $CaCl_2$ in salted meat products, without significantly affecting the product's characteristics during the storage.

Conflict of interest

All authors declare that they have no conflict of interest.

Acknowledgment

The authors would like to thank National Council for Scientific and Technological Development (CNPq) (process numbers: 140533/2015-0 and 406671/2016-8) for the financial support.

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CAPÍTULO 5

SUBSTITUTION EFFECTS OF NACL BY KCL AND $CACL_2$ ON LIPOLYSIS OF SALTED MEAT

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Foods

Vol. 8, p. 595-615, 2019

DOI: 10.3390/foods8120595

Abstract

The objective of this study was to investigate the effect of sodium chloride (NaCl) reduction and partial substitution by potassium chloride (KCl) and calcium chloride ($CaCl_2$) on lipid composition of salted meat aiming at reducing sodium content. To evaluate the effect of different salts on lipid oxidation thiobarbituric acid-reactive substances (TBARs) assay was performed along 180 days. Furthermore, ESI-MS/MS and GC analysis were conducted to detect and identify oxidized lipids, volatile compounds and free fatty acids profile at end of processing (time 0). Lipid profiles from different salted meat demonstrated that NaCl and $CaCl_2$ salt have inducted more lipid oxidation when compared to the combination of NaCl and KCl salts, highlighting the implication of $CaCl_2$ on increased lipolysis reactions. However, the obtained results from both the analysis suggest that a combination of NaCl and KCl salts can be a good alternative for reducing the sodium content without compromising the quality of the salted meat.

Keywords: Salted meat; salt substitutes; lipolysis; lipid oxidation; fatty acids; ESI-MS.

1. Introduction

Salted meats are consumed and appreciated worldwide because of their unique sensory characteristics and shelf stable properties. Their consumption is an excellent alternative to improve the nutritional status of people who live in an area with deficiencies in the cold chain. However, despite all these benefits, these meat products have high sodium content and depend of good gastronomy practices to provide an adequate desalting step not always observed by consumers.

Salting is a traditional method of preservation of several meat products and undoubtedly appears as an important technology for the development of meat industry (Liu, Pu, Sun, Wang. & Zeng, 2014). Many countries have traditional salted meat such as biltong in South Africa, jerked beef in Brazil (Vidal et al., 2019), bresaola in Italy (Picone et al., 2019), and cecina in Spain (Molinero et al., 2008). Particularly, in Brazil, these products are consumed in a large scale being an important economical item for national meat industry also focused in exportation due to high acceptation sensory. In the context of public health, a significant part of population living in poorest regions finds their nutritional requirements of essential aminoacids, minerals, mainly Fe and vitamins from B complex by salted meat consumption (Cabrera & Saadoun, 2014). The Brazilian meat industry has great interest in develop healthier salted meat products by sodium reduction giving consistent claims to support the consumer of this meat product in a healthy consumption in a current scenario.

Salting process is responsible for many changes in meat characteristics along drying and consequent reducing water activity. The most important are related to color, taste, proteolysis and lipolysis. NaCl is the most important ingredient used for the development of several functional and sensorial characteristics in meat products, through influence the water retention and binding capacity, modify the texture properties and provides salty taste (Desmond, 2006), resulting in improved microbiological stability.

A disadvantage of excess of sodium intake is increasing the risk of development of many health disorders such as cardiovascular diseases and hypertension (McGough, Sato, Rankin, & Sindelar. 2012; Pires, Munekata, Baldin, Rocha, Carvalho. dos Santos et al. 2017; Ripollés, Campagnol, Armenteros, Aristoy. & Toldrá. 2011). However, to elaborate salted meat, higher amounts of NaCl are used during salting steps, which is unhealthy for consumption. Hence, an additional desalting step is required for the elaboration of salted meat. Taking into account the high sodium content, the partial NaCl replacement by others

salts in the salting steps becomes a promising strategy for effective reduction of sodium in different meat products, but this reformulation can result in some technological challenges.

Meat lipids are mainly composed of triglycerides and phospholipids, which contain saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Triglycerides are storage lipids and are composed of three fatty acids esterified to glycerol and richer in SFAs, whereas phospholipids are often functional lipids prevalent in cell membranes and as such contain more PUFAs than triglycerides (Mapiye et al., 2012). The content of unsaturated fatty acids will depend on the meat raw material and the amount used in the processing (Valsta, Tapanainen, & Männistö. 2005), that could play a significant role on sensory properties of salted meats. Oxidation of unsaturated fatty acids can occur during processing and storage of meat and to a certain extent, it is a desirable phenomenon as it produces active compounds that influences flavor (e.g. aroma and taste) (Chizzolini, Novelli, & Zanardi, 1998; Ordoñez, Hierro, Bruna, & de la Hoz, 1999). In the same way, along salted meat processing is also characterized by lipolysis that results from the breaking down of triglycerides and phospholipids through hydrolysis or by an enzymatic process, generates glycerol and FFA (Demeyer, 2004). This phenomenon is intensely observed during the salting steps and storage, being affected by different salts (Ripollés et al., 2011). FFA starts to accumulate as the process progresses and with an increased period of storage, later they tend to decrease due to the higher susceptibility to oxidation.

The reactions of lipolysis and lipid oxidation are interpreted as different phenomena and these reactions can be directly influenced by water activity (aw), pH, nitrite, metals, salts, and storage (Demeyer. 2004). The salt added to elaborate meat products contribute and accelerate lipid oxidation, which is one of the main responsible for the quality losses along shelf life (Mariutti & Bragagnolo 2017). The catalytic role of NaCl may be due to the displacement of the iron ions from macromolecules altering the reactivity and distribution, thus increasing its catalytic activity. Furthermore, NaCl may form chelate complexes with ferric iron (Decker & Zm, 1998), which is suggested to be responsible for the catalysis of lipid oxidation in biological tissues. In the salting process, the temperature also can catalyze the lipid oxidation reaction (Kristensen & Purslow. 2001). An intense process of oxidation, however, could reach the level of rancidity, and the foodstuff would no longer be acceptable for human consumption. In addition, before such a condition is reached, lipid oxidation reactions could generate toxic compounds including cholesterol oxides and malonaldehyde,

which are associated with increased risk of developing of certain types of cancer, heart disease and premature aging (Jiménez-Colmenero. Carballo. & Cofrades. 2001).

The role of NaCl in salted meats is important to maintain the typical identity of these products, and of because this, the reducing sodium content by substitution by other salts is a great technological challenger and it has been intensively studied. However, the literature is very rich in studies on lipolysis of fermented meat products combined with dehydration but very poor or nonexistent regarding salted meat reactions.

Thereby, the objective of the present study was to investigate the effect of NaCl reduction and substitution by KCl and $CaCl_2$ blends on lipolysis, aiming at sodium reduction in salted meat.

2. Material and methods

2.1 Treatments, material and additives

The NaCl, KCl and $CaCl_2$ salts used in salting step processses were food grade (Anidrol. Brazil). The bovine raw meat was the *biceps femoris* obtained from slaughterhouse with assured sanitary quality (Friboi, São Paulo, Brazil). The additives sodium erythorbate and sodium nitrite were donated by the company Kerry of Brazil. All the solvents chloroform, methanol and xylene were HPLC grade purchased from Merck (Chile), and ultra-pure water was used in all experiments. Paraffin-embedded meat sample was dewaxed with hexane solvent followed by extraction of the lipids from the meat sample.

Four treatments of salted meat were performed as described in Table 1. The NaCl replacement by KCl and $CaCl_2$ was based on ionic strength of control treatment (100% NaCl), obtaining the same ionic strength in all treatments.

2.2 Salted meat processing

The salted meat processing was performed according to Vidal et al. (2019). In the wet salting, the raw bovine meat were immersed in a respective satured solution with salts and additives (respective salt, 0.015% sodium nitrite and 0.05% sodium erythorbate) for 1 hour as described in Table 1. After wet salting, the meat pieces were put in contact with respective

salts for dry salting step (Table 1) for 144 hours (6 days) in a cold chamber at 13 °C. After that, ripening was carried out in a controlled climatic chamber (Instala Frio, Curitiba, Brazil) with 55% humidity, 25 °C and 0.5 m/s forced air ventilation for 24 hours (1 day). The final product was vacuum packed with polyethylene (Spel, São Paulo, Brazil) and stored at 25 °C in 180 days.

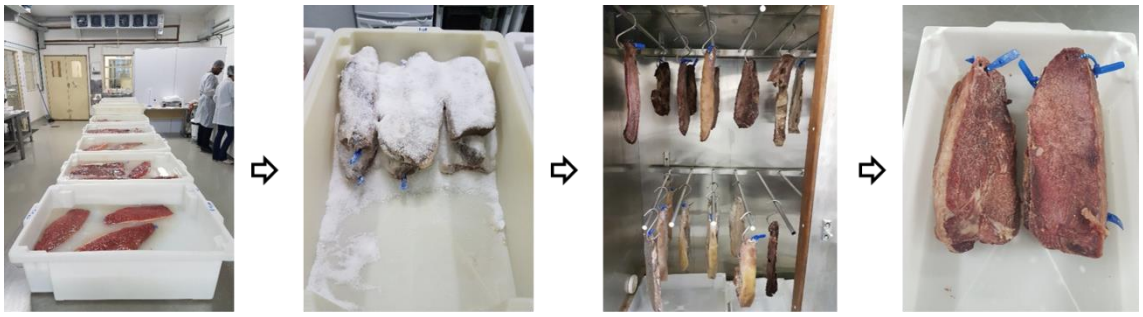


Figure 1. Processing steps for obtaining salted meat; A: wet salting; B: dry salting; C: ripening; D: final product

The processes were executed in the Pilot Plant of the Meat Area, Faculty of Food Engineering, University of Campinas. The process was carried out in triplicate with same technology and methodology in three diferent days.

Table 1. Salts and additives used to performed the salted meat treatments

Treatments	NaCl (%)	NaCl (mg)*	KCl (%)	KCl (mg)*	$CaCl_2$ (%)	$CaCl_2$ (mg)*
FC1	100	1000	-		-	
F1	50	441	50	560	-	
F2	50	614	-	-	50	387
F3	50	513	25	326	25	162

The amount of salt added was based on the ionic strength, all treatments obtained the same ionic strength. * Salt proportion added according to ionic strength, for each 1000mg of bovine raw meat was utilized 2000mg of salt

2.3 Lipid oxidation

The lipid oxidation of salted meat was measured by the amount of thiobarbituric acid-reactive substances (TBARs) as described by Bruna, Ordóñez, Fernández. Herranz, & de la Hoz (2001), using trichloroacetic acid instead of perchloric acid as the solvent. The results

were expressed in g of malonaldehyde MDA/kg for each sample. The lipid oxidation was measured at 0, 45, 90, 135 and 180 days of storage in triplicate for each treatment in each process replicate.

2.4 Instrumental color

Color measurements were determined with 20mm aperture, D65 illuminant and 10° standard observer using Hunter Lab colorimeter (Colourquest II, Hunter Associates Laboratory Inc., Reston, Virginia, USA). *L**, *a** and *b** color parameters were determined as an indicator of luminosity, red intensity, and yellow intensity, respectively. The whiteness index was calculated by the following equation:

$$100 - [(100 - L^*)\,2 + 2a^* + 2b^*]^{1/2} \qquad (1)$$

The color parameters were measured at 0, 45, 90, 135 and 180 days of storage in triplicate for each treatment in each process replicate.

2.5 Lipolysis analysis

2.5.1 Formalin-Fixed Paraffin-Embedded (FFPE) sample preparation

The samples were fixed according to Bancroft (2002). The salted meat samples were cut and placed in a flask with buffered formaldehyde solution (4%, phosphate buffer 0.075 M, pH 7.3) 1:20 ratio of salted meat formaldehyde solution, remaining in the solution for 48 hours for tissue fixation. After that, the tissues were placed in a cassette with 11% formic acid solution overnight, then, the samples were put in 70% ethanol solution. The following steps were: 1 hour in a solution of 95% ethanol and 5% methanol, 4 times of 90 min with absolute ethanol, 2 times of 1 hour with xylene, and finally 2 times of wax (Paraplast Plus, McCormick) at 58 °C for 1 hour.

2.5.2 Lipids extraction

Primarily, salted meat samples were dewaxed as reported by Wojakowska et al. (2015). In brief, FFPE salted meat samples were gently immersed in 100% xylene for 10 minutes, which was kept at 60 °C for efficient paraffin solvation. After that, the samples were rehydrated and were subsequently used for lipids extraction.

Folch protocol (Folch et al., 1957) was used for extracting total lipids from salted. Salted meat samples was sonicated with 10ml of chloroform/methanol mixture (2:1) for 30 min. After homogenization, equal volumes of chloroform and water were added to the extract so that there was phase separation. The lower phase was collected into a test tube and the upper phase was taken again for washing with 2 ml of the solvent mixture; after separation of the extract lower phase was combined with the first extract. This process was repeated for 3 times and all the organic extracts were pooled together and the solvent was removed by evaporating under vacuum in a rotary evaporator and dried lipids were stored at -20 °C in dark flask with nitrogen until use.

2.5.3 Electrospray Ionization-Tandem Mass Spectrometer (ESI-MS) analysis

Lipid analysis was performed on an electrospray ionization-tandem mass spectrometer (ESI-MS/MS) with a linear ion-trap mass analyzer (Amazon, Bruker) equipped with a Hamilton syringe pump and an electrospray source. Samples were prepared by dissolving 1 ml of the lipid sample in 100 ml of methanol. The sample was injected at an infusion rate of 5 µl/min, the ion spray voltage was set at -4.5 kV and the source temperature were at 220 °C for both positive and negative ionization modes. MS/MS experiments were conducted manually for identification of lipid species with nitrogen as collision gas and collision energy of ~50 eV. Data analysis software package (Bruker Daltonics) was used to collect full scan spectra over the range of *m/z* 100-1200 and the obtained raw data was preprocessed for smoothening baseline subtraction peak picking, and deconvolution if needed.

2.5.4 Volatile compound profile

The extraction of the volatile compounds was performed using solid-phase microextraction (SPME), followed the conditions described by Domínguez et al. (2019). For headspace SPME (HS-SPME) extraction, 1 g of each sample was weighed in a 20 ml vial,

after being ground using a commercial grinder. The conditioning, extraction and injection of the samples were carry out with an autosampler PAL-RTC 120. The extractions were carried out at 37 °C for 30 min, after equilibration of the samples for 15 min at the temperature used for extraction, ensuring a homogeneous temperature for sample and headspace. Once sampling was finished, the fiber was transferred to the injection port of the gas chromatograph–mass spectrometer (GC–MS) system. A gas chromatograph 7890B (Agilent Technologies, Santa Clara, CA, USA) equipped with a mass selective detector 5977B MSD (Agilent Technologies) and a DB-624 capillary column (30 m, 0.25 mm i.d., 1.4 μm film thickness; J&W Scientific, Folsom, CA, USA) was used for volatile analysis. Compounds were identified by comparing their mass spectra with those contained in the NIST14 (National Institute of Standards and Technology, Gaithersburg) library, and/or by comparing their mass spectra and retention time with authentic standards (Supelco, Bellefonte, PA, USA), and/or by calculation of retention index relative to a series of standard alkanes (C5-C14) (for calculating Linear Retention Index, Supelco 44585-U, Bellefonte, PA, USA). The results are expressed as area units (AU) of the Quantifier Ion x 10^4/g of sample.

2.5.5 Free fatty acids profile

Total lipids were extracted from 5 g of salted meat sample, the methodology was performed according to Folch, Lees, and Stanley (1957). Free fatty acids (FFA) were separated using NH2-aminopropyl mini-columns as described by Regueiro, Gibert, and Díaz (1994). 50 mg of the extracted lipids were transesterified with a solution of boron trifluoride (14%) in methanol, and the FAMEs were stored at -80 °C until chromatographic analysis. Separation and quantification of FAMEs was carried out using a gas chromatograph GC-Agilent 6890N (Agilent Technologies, Madrid, Spain) equipped with a flame ionization detector and an automatic sample injector HP 7683, and using a Supelco SPTM-2560 fused silica capillary column (100 m, 0.25 mm i.d., 0.2 μm film thickness, Supelco Inc., Bellafonte, PA, USA). Chromatographic conditions were as follows: initial oven temperature of 120 °C (held for 5 min), first ramp at 2 °C/min to 170 °C (held for 15 min), second ramp at 5 °C/min to 200 °C (held for 5 min) and third ramp at 2 °C/min to final temperature of 235 °C (held for 10 min). The injector and detector were maintained at 260 and 280 °C, respectively. Helium was used as carrier gas at a constant flow-rate of 1.1 ml/min, with the column head pressure set at 35.56 psi. One μL of solution was injected in split mode (1:50). The fatty acids were quantified using nonadecanoic acid methyl ester, at 0.3 mg/ml, as internal standard that was

added to samples prior to fat extraction and methylation. Identification of fatty acids was performed by comparison of the retention times with those of known FAME standard and the results expressed as g/100 g of total fatty acids.

2.6 Statistical analysis

In each process, at least three samples were collected for each analysis. The results expressed in this work are averages obtained from all data. The commercial software Statistica v.8 (Statsoft Inc., Tulsa, Oklahoma. USA) was used to perform general linear models analysis and Tukey's test ($P < 0.05$) considering the treatments as a fixed effect and the replicates as a random effect using 5% of significance.

3. Results

3.1 Lipid oxidation (TBARs)

Lipid oxidation is the major reason for deterioration of meat and meat products promoting rancidity, loss of essential fatty acids, undesirable odor and texture, besides production of toxic compounds (Alfaia, Alves, Lopes, Fernandes, Costa, Fontes et al., 2010; Broncano, Petrón, Parra, & Timón, 2009; Domínguez, Gómez, Fonseca, & Lorenzo, 2014). Salted meats products are particularly susceptible to the rapid development of lipid oxidation due to high NaCl concentration which is considered as a potent pro-oxidant and has a low or intermediate water activity (Ma. Ledward, Zamri. Frazier, & Zhou, 2007).

Table 2. Malonaldehyde (mg/kg) values in salted meat treatments during storage

Treatments	0 day	45 days	90 days	135 days	180 days
FC1	0.95^{cC}	1.61^{cA}	0.80^{bC}	0.75^{aC}	1.32^{aB}
F1	0.88^{cB}	1.78^{cA}	0.35^{cC}	0.50^{bC}	0.71^{cB}
F2	1.10^{bB}	3.58^{aA}	1.09^{aB}	0.68^{abC}	1.26^{aB}
F3	1.74^{aB}	2.40^{bA}	1.20^{aC}	0.38^{cD}	0.95^{bC}
Standard error	0.06	0.15	0.06	0.03	0.05

Values are means. $^{a.\ b.\ c.\ d}$ Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test. $^{A.B.C.D}$ Values in the same line with the same capital letters do not differ significantly ($P < 0.05$) according to Tukey's test. FC1:

100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

3.2 Lipolysis

As discussed earlier, the phenomena of lipid oxidation or lipid peroxidation are mostly observed in polyunsaturated fatty acids such as omega-3 fatty acids (α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid) and omega-6 fatty acids (linoleic acid, arachidonic acid and docosapentaenoic acid). These PUFAs produces a wide variety of oxidation products, primary products of lipid peroxidation are lipid hydroperoxides that on further oxidation forms different aldehydes such as malonaldehyde (MDA), propanal, hexanal, 4-hydroxynonenal (4-HNE) and other F2-isoprostanes such as 8-iso-prostaglandin F2α (8-iso-PGF2α).

Thus, trying to explain the effect of different salts on salted meat during lipolysis and lipid oxidation, it was employed ESI-MS without any derivatization step to analyze lipid composition and detecting and identifying the oxidized lipids. Primarily, lipid profiles of different salted meat treatments were obtained by ESI-MS in positive and negative modes at different days of storage. The full scan spectra in negative ion mode (Figure 2) demonstrates that the total fatty acid composition of FC1, F1, F2 and F3 samples at initial days of storage (T0) has majorly PUFAs especially, linolenic acid (18:2n-6), arachidonic acid (AA) (20:4n-6), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3).

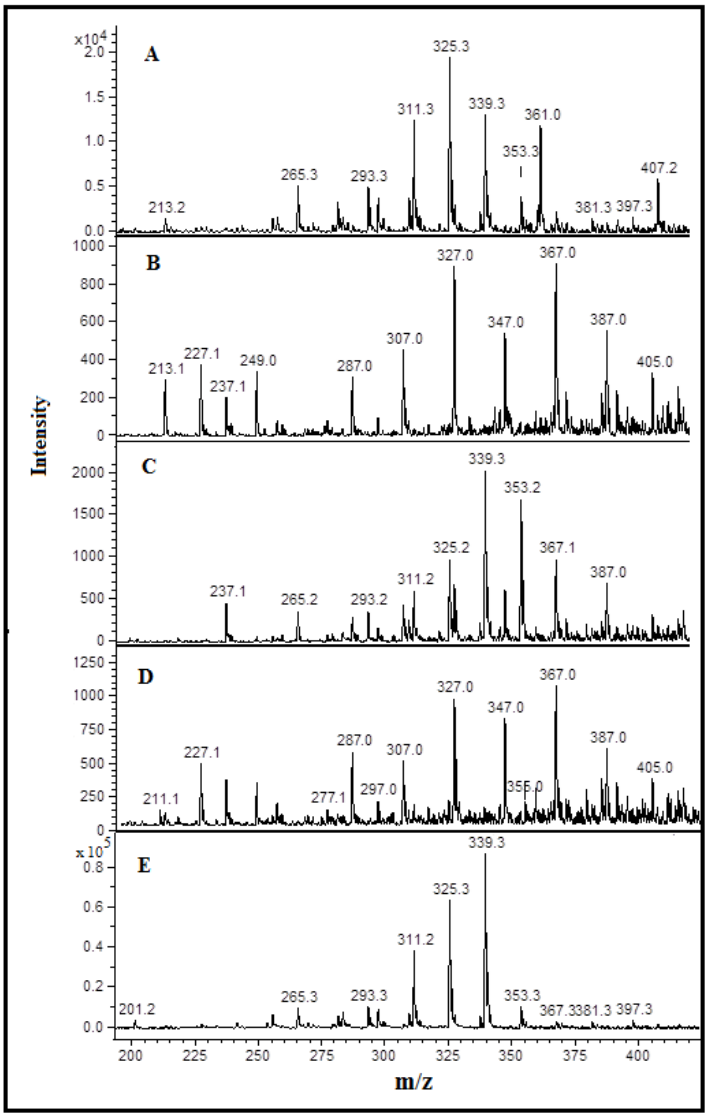


Figure 2. Lipid profiles of salted meats at initial days of storage i.e at T0, (a) control; (b) FC1: 100% NaCl; (c) F1: 50% NaCl + 50% KCl; (D) F2: 50% NaCl + 50% $CaCl_2$; (E) F3: 50% NaCl + 25% KCl + 25% $CaCl_2$ obtained in ESI-MS in negative ion mode.

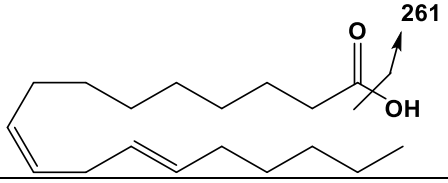
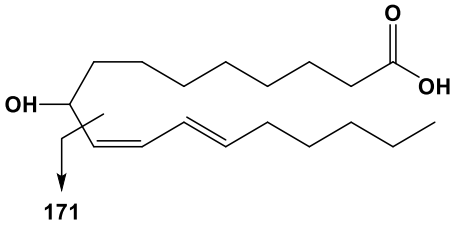
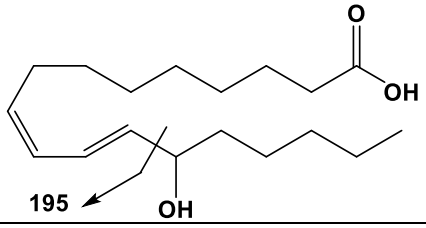
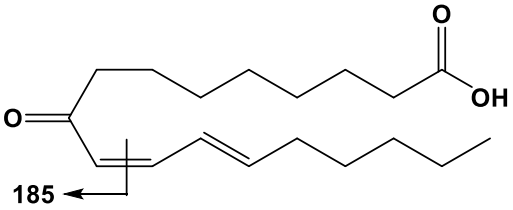
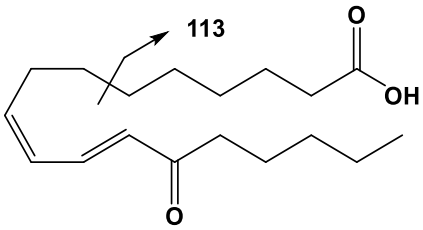
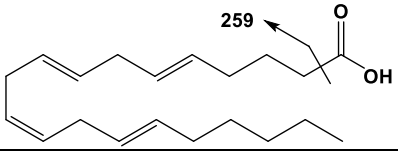
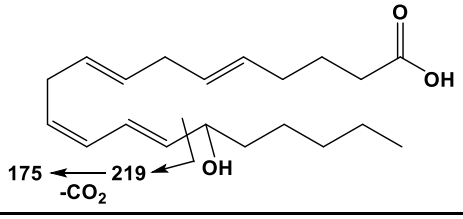
3.2.1 Detection and identification of oxidized phospholipids

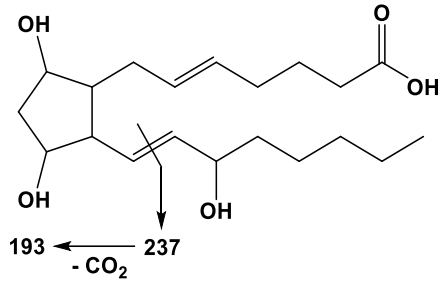
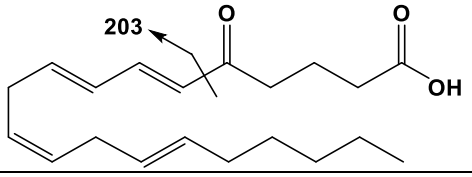
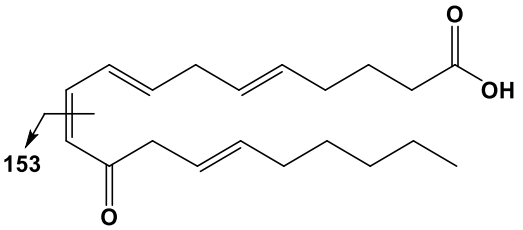
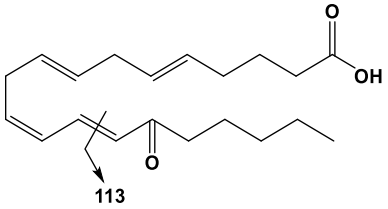
Detection and identification of oxidized phospholipids and fatty acids were performed by selecting particular oxidized phospholipid of interest and then carrying out fragmentation to obtain structural information by lipidomics protocol.

The oxidation of AA was identified by selecting the precursor ion peak m/z 353, which was readily detected with good intensity in the full scan spectra. The characteristic

fragment ions for the identification of these oxidized fatty acids and their possible structures are summarized in Table 3.

Table 3. Structures of the oxidized polyunsaturated fatty acids with their precursor and their characteristic fragment ion masses.

Carbon Annotation	Compound	Precursor (*m/z*)	Product (*m/z*)	Chemical Structure
18:2	LA	279	261	
18:2	9-HODE	295	171	
18:2	13-HODE	295	195	
18:3	9-OxoODE	293	185	
18:3	13-OxoODE	293	113	
20:4	AA	303	259	
20:4	15-HETE	319	175	

20:4	PGF2α	353	193	
20:4	5-OxoETE	317	203	
20:4	12-OxoETE	317	153	
20:4	15-OxoETE	317	113	

Lists the compounds that were detected in the ESI-MS spectra of lipids. the first column represents the carbon annotation of fatty acids. Second column shows the different fatty acids that are oxidized. Third column provides their masses and the fourth column shows their fragmented ions and the fifth column represent the structure of the oxidized fatty acids and the formation of the product ions by fragmentation. LA, linolenic acid; 9-HODE, 9-hydroxy linolenic acid; 13-HODE, 13-hydroxylinolenic acid; 9-OxoODE, 9-oxo-10E,12Z-octadecadienoic acid; 13-OxoODE, 13-Oxo-9,11-octadecadienoic acid; AA, arachidonic acid; 15-HETE, 15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid; $PGF_{2\alpha}$ prostaglandin F2 alpha; 5-OxoETE, 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid; 12-OxoETE, 12-Oxo-5Z,8Z,10E,14Z-eicosatetraenoic acid; 15-OxoETE, 15-Oxo-5Z,8Z,11Z,13E-eicosatetraenoic acid.

Selective fragmentation of the precursor ion m/z 353 (Figure 3) exhibited fragment ions m/z 237 and 309, which are the signature peak of PGF2α, and a peak with m/z value 193 corresponding to decarboxylation of the peak m/z 237. Presence of the product ion peak at m/z 193 in the MS/MS spectra of *m/z* 353 confirms oxidation of arachidonic acid (20:4).

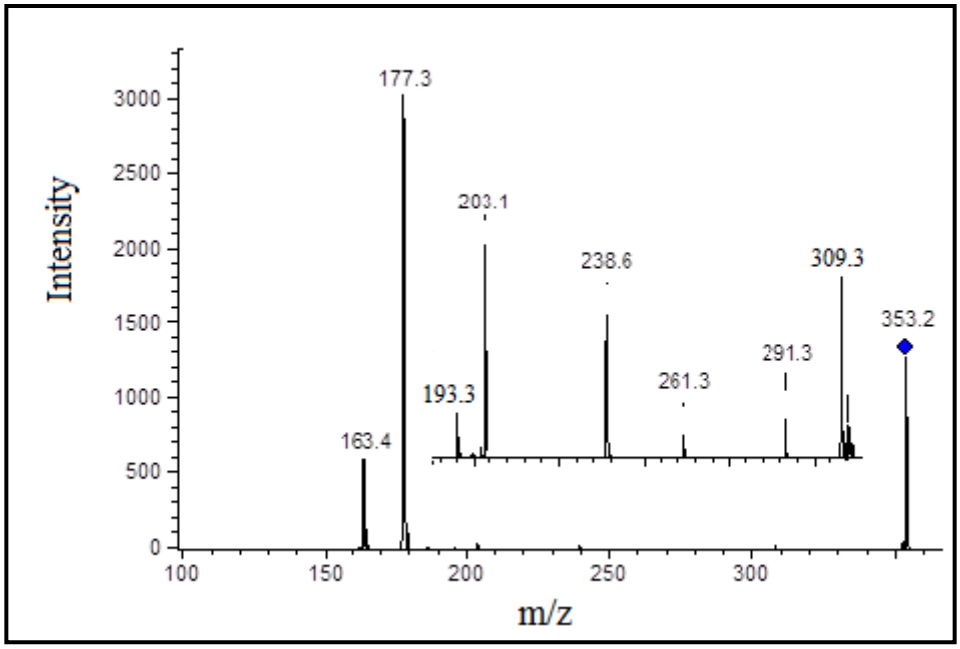


Figure 3. Structural identification of oxidized arachidonic acid by MS/MS fragmentation pattern.

3.2.2 Volatile compound profile

The volatile profile of salted meat is shown in the Table 4 (expressed as UA x 10^4/g of dry matter). A total of 57 volatile organic compounds (VOC) of 8 different chemical groups were separated and identified in the meat samples. These groups were hydrocarbons (17 compounds), alcohols (11), aldehydes (8), ketones (8), acids (6), sulphur compounds (4), esters (2) and 1 furan. The major volatile compounds identified in the present study are in line with those reported in the literature for other dry-cured products (Domínguez et al., 2019; Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004; Ventanas, Estevez, Andrés, & Ruiz, 2008).

Figure 4 shows the total VOC from each chemical family (both, as AU x 10^4/g and as percentage of total volatile compounds) in the 4 batches of salted meat analyzed. Statistical analysis using VentVed showed significant differences in the VOC contents between batches. The F2 and F3 batches exhibited higher values of total VOC (1603 and 1777 AU x 10^4/g, respectively; data not shown) than F1 (1401 AU x 10^4/g; data not shown) that present intermediate values and FC1 (967 AU x 10^4/g; data not shown) which had the lowest value.

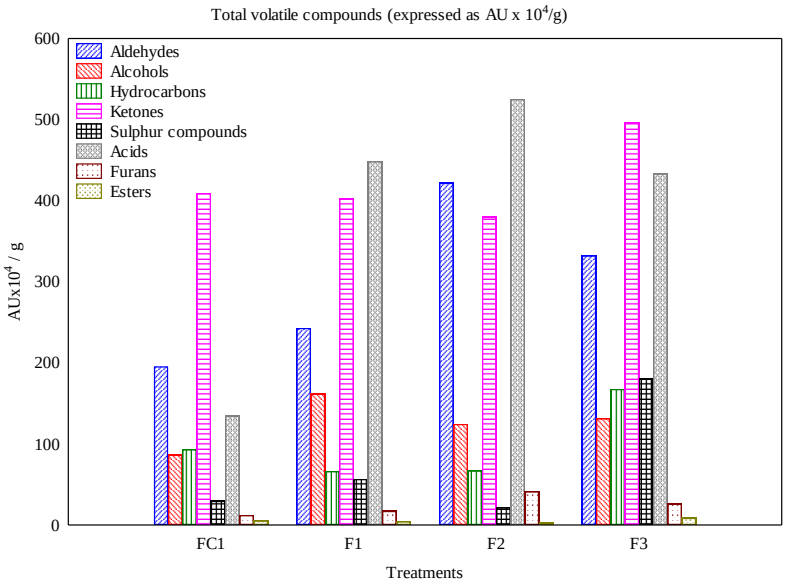
Table 4. Volatile compounds (expressed as AU of quantifier ion x 10^4/g) of salted meat (0 day of storage)

Volatile compound	LRI	*m/z*	Treatments			
			FC1	F1	F2	F3
Glycidol	506	44	8.39 ± 0.52^{b}	5.04 ± 1.02^{b}	14.08 ± 1.54^{a}	14.89 ± 1.70^{a}
Methanethiol	509	48	0.41 ± 0.06^{b}	0.93 ± 0.06^{b}	8.39 ± 1.42^{a}	6.80 ± 1.23^{a}
Pentane	522	42	8.28 ± 0.26^{a}	2.39 ± 1.05^{b}	4.06 ± 2.53^{ab}	7.93 ± 1.78^{a}
Acetone	533	58	137.24 ± 38.23	112.18 ± 14.35	113.24 ± 30.45	164.53 ± 37.41
Dimethyl sulfide	535	62	3.74 ± 0.10^{a}	3.50 ± 0.11^{a}	1.34 ± 0.13^{b}	3.13 ± 0.21^{a}
Carbon disulfide	538	76	24.85 ± 0.31^{b}	39.13 ± 3.73^{a}	14.80 ± 3.96^{c}	44.22 ± 2.22^{a}
Propanal, 2-methyl-	562	72	1.30 ± 0.07^{c}	1.36 ± 0.02^{c}	4.82 ± 0.78^{b}	5.85 ± 0.30^{a}
1-Propanol	577	59	1.30 ± 0.24^{bc}	1.74 ± 0.04^{b}	3.16 ± 0.25^{a}	0.92 ± 0.31^{c}
2,3-Butanedione	593	86	108.44 ± 3.27^{a}	70.70 ± 2.12^{c}	54.22 ± 1.25^{d}	81.52 ± 1.24^{b}
2-Butanone	599	72	10.34 ± 1.10^{b}	9.64 ± 3.16^{b}	32.66 ± 6.58^{a}	35.05 ± 8.01^{a}
Hexane, 2,2-dimethyl-	665	57	34.93 ± 0.77	34.11 ± 1.14	27.10 ± 9.37	31.50 ± 1.68
Formic acid, 2-propenyl ester	682	57	1.90 ± 0.59^{b}	1.97 ± 0.21^{b}	1.87 ± 0.49^{b}	4.61 ± 1.00^{a}
Heptane	685	71	0.72 ± 0.10^{b}	1.06 ± 0.01^{b}	1.85 ± 0.06^{a}	1.90 ± 0.19^{a}
Acetic acid	704	60	10.31 ± 0.37^{c}	31.86 ± 9.63^{b}	39.78 ± 6.43^{ab}	46.79 ± 35.52^{a}
1-Butanol	718	56	1.86 ± 0.27^{b}	2.57 ± 0.51^{ab}	4.41 ± 0.97^{a}	3.11 ± 0.75^{ab}
Pentanal	739	58	15.71 ± 0.63^{c}	11.24 ± 0.70^{c}	29.86 ± 3.25^{b}	44.44 ± 4.05^{a}
Disulfide, dimethyl	793	94	1.09 ± 0.32^{b}	13.22 ± 6.32^{b}	4.98 ± 2.27^{b}	95.68 ± 6.9^{a}
Acetoin	799	88	145.28 ± 4.40^{b}	204.05 ± 22.72^{a}	171.95 ± 1.26^{ab}	203.77 ± 12.49^{a}
Propanoic acid, 2-hydroxy-2-methyl-, ethyl ester	830	59	3.46 ± 0.16^{b}	2.47 ± 0.12^{c}	1.00 ± 0.23^{d}	4.13 ± 0.13^{a}
Octane	835	85	3.42 ± 0.39^{b}	3.98 ± 0.33^{b}	7.35 ± 1.76^{a}	6.17 ± 0.42^{a}
Propanoic acid	837	74	1.02 ± 0.35^{b}	2.68 ± 0.76^{b}	5.03 ± 1.66^{a}	2.82 ± 0.94^{b}
1-Pentanol	860	55	40.35 ± 0.92^{a}	11.08 ± 0.75^{c}	9.57 ± 0.28^{c}	14.48 ± 0.42^{b}
Prenol	869	71	2.54 ± 0.54^{b}	4.53 ± 0.30^{a}	0.89 ± 0.03^{c}	1.30 ± 0.65^{bc}
Hexanal	878	56	135.79 ± 5.78^{d}	183.42 ± 3.42^{c}	299.82 ± 25.45^{a}	238.40 ± 2.87^{b}
Propanoic acid, 2-methyl-	901	73	0.64 ± 0.2^{b}	1.99 ± 0.44^{a}	2.77 ± 0.20^{a}	1.83 ± 0.48^{a}
2,3-Butanediol	924	45	1.85 ± 0.24^{c}	10.39 ± 2.17^{a}	3.42 ± 0.47^{bc}	6.02 ± 0.71^{b}
2,3-Butanediol, [R-(R*,R*)]-	932	45	14.61 ± 7.04^{c}	107.30 ± 15.60^{a}	69.60 ± 2.26^{b}	65.07 ± 2.17^{b}
Butanoic acid	933	60	101.64 ± 5.86^{c}	377.09 ± 7.29^{b}	447.78 ±	352.37 ±

					58.78[a]	10.48[b]
Pentane, 2,2-dimethyl-	950	57	0.40 ± 0.18	0.36 ± 0.03	0.64 ± 0.18	0.54 ± 0.03
2-Heptanone	983	58	3.35 ± 0.15[b]	3.45 ± 0.27[b]	4.16 ± 0.18[a]	3.88 ± 0.16[ab]
Heptanal	990	70	8.69 ± 0.71[c]	12.75 ± 1.84[b]	14.78 ± 1.46[b]	18.42 ± 0.14[a]
.alpha.-Phellandrene	994	93	2.69 ± 0.05[b]	2.78 ± 0.12[b]	3.62 ± 0.13[a]	3.82 ± 0.02[a]
Pentanoic acid	1021	60	2.91 ± 0.59	4.32 ± 0.77	4.31 ± 0.50	4.14 ± 1.03
Dimethyl trisulfide	1052	126	0.06 ± 0.04[c]	0.84 ± 0.29[b]	0.49 ± 0.20[b]	10.19 ± 3.59[a]
Furan, 2-pentyl-	1055	81	12.19 ± 0.30[d]	17.36 ± 0.80[c]	41.45 ± 5.14[a]	26.19 ± 1.01[b]
Benzaldehyde	1063	106	20.80 ± 1.14[b]	14.98 ± 1.32[b]	49.22 ± 2.72[a]	53.63 ± 6.10[a]
1-Octen-3-ol	1069	57	11.33 ± 0.66[c]	13.87 ± 0.63[b]	22.84 ± 1.68[a]	14.42 ± 0.58[b]
5-Hepten-2-one, 6-methyl-	1074	108	2.48 ± 0.11[a]	1.84 ± 0.18[b]	2.21 ± 0.10[a]	1.79 ± 0.08[b]
Octanal	1085	84	4.97 ± 0.32[c]	6.27 ± 1.45[bc]	8.84 ± 0.85[ab]	8.92 ± 0.12[a]
Undecane, 3,6-dimethyl-	1087	57	14.29 ± 2.23[b]	5.39 ± 0.27[c]	5.82 ± 0.64[c]	49.82 ± 1.31[a]
Dodecane, 2,6,10-trimethyl-	1098	57	11.79 ± 1.24[b]	4.12 ± 0.39[c]	3.80 ± 0.28[c]	39.75 ± 3.40[a]
Hexanoic acid	1102	60	14.99 ± 0.58[b]	31.96 ± 4.88[a]	25.57 ± 3.12[a]	25.40 ± 1.98[a]
2-Ethyl-1-hexanol	1113	57	3.00 ± 0.16[c]	3.71 ± 0.11[b]	5.23 ± 0.06[a]	3.55 ± 0.20[b]
Butane, 2,2-dimethyl-	1133	57	0.53 ± 0.01[d]	0.66 ± 0.02[c]	0.87 ± 0.12[b]	1.21 ± 0.03[a]
1-Octanol	1147	55	0.95 ± 0.25	0.72 ± 0.01	0.99 ± 0.16	0.98 ± 0.18
1-Hexen-3-one	1152	70	1.53 ± 0.17[b]	0.73 ± 0.03[c]	0.53 ± 0.11[c]	4.85 ± 0.47[a]
Nonanal	1169	56	7.84 ± 0.38	12.20 ± 4.35	12.71 ± 1.49	11.90 ± 0.34
Undecane, 4,4-dimethyl-	1174	85	1.91 ± 0.17[b]	0.75 ± 0.07[c]	0.55 ± 0.03[c]	4.37 ± 0.33[a]
Undecane, 3-methyl-	1189	57	2.08 ± 0.01[b]	1.53 ± 0.09[c]	1.17 ± 0.08[d]	4.50 ± 0.12[a]
Dodecane	1210	71	3.76 ± 0.15[b]	3.05 ± 0.11[b]	3.54 ± 0.17[b]	5.67 ± 0.50[a]
Decane, 5-ethyl-5-methyl-	1226	71	2.46 ± 0.20[b]	0.73 ± 0.08[c]	0.41 ± 0.06[c]	4.87 ± 0.10[a]
2,2,5-Trimethylhexan-4-one	1236	57	0.55 ± 0.08[b]	0.14 ± 0.04[c]	0.12 ± 0.03[c]	0.85 ± 0.12[a]
Undecane, 5-ethyl-	1241	57	1.73 ± 0.24[a]	0.39 ± 0.04[b]	0.23 ± 0.10[b]	2.36 ± 0.70[a]
Tridecane	1282	57	1.16 ± 0.06[c]	3.26 ± 0.03[b]	4.42 ± 0.61[a]	1.46 ± 0.17[c]
2,2,6,6-Tetramethylheptane	1330	57	1.43 ± 0.15[a]	0.56 ± 0.04[c]	0.53 ± 0.05[c]	0.99 ± 0.10[b]
Hexane, 3,3-dimethyl-	1348	57	1.68 ± 0.24[a]	1.28 ± 0.13[ab]	1.44 ± 0.23[ab]	0.88 ± 0.10[b]
Pentadecanal	1548	82	0.56 ± 0.06[c]	0.73 ± 0.06[c]	1.97 ± 0.09[a]	1.11 ± 0.04[b]

Values are means ± standard deviation. [a, b, c, d] Means in the same column followed by different lowercase letters present statistically significant difference by the Tukey test ($P < 0.05$). LRI: linear retention index calculated for DB-624 capillary column (J&W scientific: 30 m x 0.25 mm id, 1.4 µm film thickness) installed on a gas chromatograph equipped with a mass selective detector. *m/z*: Quantifier ion. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

a)



b)

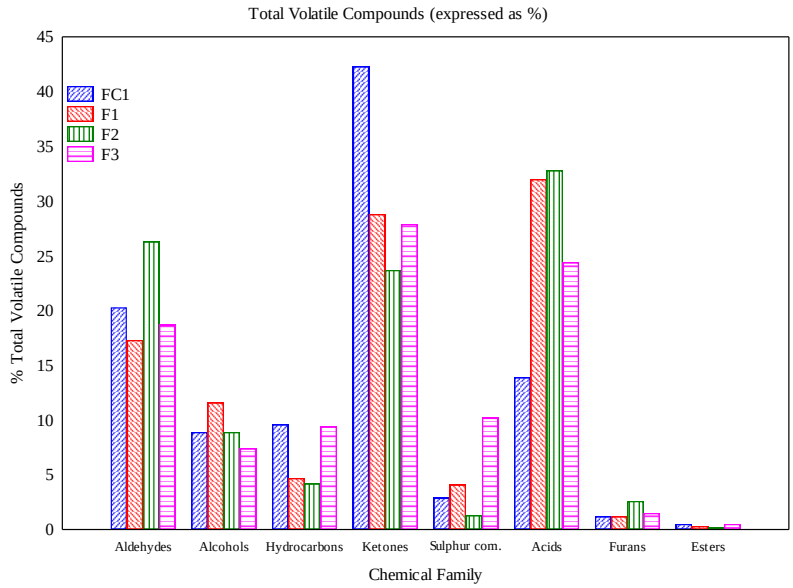


Figure 4. Graphic representation of the volatile compounds, expressed as AUx10^4/g and (a) % of total volatile compounds (b) from each chemical family detected on salted meat. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

3.2.3 Free fatty acids profile

Lipolysis generates FFA, and after the lipid oxidation process, produces several volatile compounds such as ketones, alcohols and aldehydes (Ordóñez, Hierro, Bruna, & Hoz, 1999). According to Lorenzo and Carballo (2015), each individual free fatty acid should be

the balance result between its release from phospholipids, triglycerides and its oxidative degradation. The profile of FFA can be verified in Table 5.

The main FFAs in all salted meat treatments were oleic acid (C18:1n-9), palmitic acid (C16:0) and stearic acid (C18:0). The FFA profile of these salted meat treatments is similar as described by other authors in others dry-cured meat products (Lorenzo & Carballo, 2015; Martín, Córdoba, Ventanas, & Antequera, 1999; Muriel, Andres, Petron, Antequera, & Ruiz, 2007).

Table 5. Free fatty acids (%) of salted meat (0 day of storage)

Free fatty acids	Treatments			
	FC1	F1	F2	F3
6:0	0.20 ± 0.13^{a}	0.15 ± 0.02^{a}	0.24 ± 0.05^{a}	0.12 ± 0.07^{a}
8:0	0.14 ± 0.27^{ab}	0.13 ± 0.01^{b}	0.18 ± 0.31^{a}	0.16 ± 0.12^{ab}
10:0	0.10 ± 0.02^{a}	0.11 ± 0.02^{a}	0.14 ± 0.03^{a}	0.14 ± 0.01^{a}
11:0	0.11 ± 0.01^{a}	0.13 ± 0.02^{a}	0.18 ± 0.01^{a}	0.19 ± 0.01^{a}
12:0	0.12 ± 0. 02^{a}	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	0.12 ± 0.05^{a}
13:0	0.08 ± 0.02^{a}	0.08 ± 0.01^{a}	0.09 ± 0.02^{a}	0.09 ± 0.01^{a}
14:0	1.12 ± 0.15^{a}	0.97 ± 0.07^{a}	1.22 ± 0.46^{a}	1.25 ± 0.64^{a}
14:1n-5	0.21 ± 0.01^{a}	0.16 ± 0.02^{a}	0.15 ± 0.05^{a}	0.19 ± 0.06^{a}
15:0	0.23 ± 0.02^{a}	0.24 ± 0.03^{a}	0.24 ± 0.02^{a}	0.20 ± 0.06^{a}
15:1n-5	2.43 ± 0.64^{a}	2.65 ± 0.53^{a}	3.78 ± 0.16^{a}	3.83 ± 0.27^{a}
16:0	23.43 ± 3.16^{a}	22.25 ± 1.95^{b}	22.24 ± 0.01^{b}	24.14 ± 4.45^{a}
16:1n-7	1.39 ± 0.22^{a}	1.16 ± 0.08^{a}	1.12 ± 0.02^{a}	1.40 ± 0.39^{a}
17:0	0.64 ± 0.08^{ab}	0.74 ± 0.05^{ab}	0.79 ± 0.08^{a}	0.58 ± 0.16^{b}
18:0	22.57 ± 2.81^{ab}	23.76 ± 2.02^{a}	24.43 ± 0.46^{a}	20.80 ± 2.89^{b}
9t-18:1	0.33 ± 0.05^{b}	0.67 ± 0.20^{a}	0.58 ± 0.08^{ab}	0.28 ± 0.07^{b}
11t-18:1	1.24 ± 0.14^{a}	0.98 ± 0.28^{a}	1.07 ± 0.02^{a}	1.36 ± 0.32^{a}
18:1n-9	24.19 ± 2.69^{a}	26.76 ± 2.31^{a}	26.09 ± 1.45^{a}	24.85 ± 6.99^{a}
18:1n-7	1.42 ± 0.21^{b}	1.68 ± 0.13^{a}	1.66 ± 0.02^{a}	1.37 ± 0.26^{b}
9t,11t-18:2	0.22 ± 0.04^{a}	0.16 ± 0.02^{a}	0.18 ± 0.01^{a}	0.23 ± 0.01^{a}
18:2n-6	12.65 ± 1.30^{a}	11.22 ± 0.66^{a}	9.55 ± 0.32^{b}	11.53 ± 1.60^{a}
18:3n-6	0.19 ± 0.04^{a}	0.11 ± 0.02^{b}	0.12 ± 0.03^{b}	0.18 ± 0.02^{ab}
18:3n-3	1.23 ± 0.13^{a}	0.61 ± 0.02^{b}	0.60 ± 0.02^{b}	1.15 ± 0.24^{a}
9c,11t-18:2 (CLA)	0.39 ± 0.05^{a}	0.31 ± 0.02^{ab}	0.20 ± 0.04^{b}	0.41 ± 0.13^{a}

20:0	0.19 ± 0.03^{a}	0.21 ± 0.02^{a}	0.26 ± 0.02^{a}	0.22 ± 0.01^{a}
20:1n-9	0.12 ± 0.01^{a}	0.17 ± 0.03^{a}	0.18 ± 0.01^{a}	0.18 ± 0.06^{a}
20:2n-6	0.12 ± 0.01^{a}	0.11 ± 0.02^{a}	0.13 ± 0.01^{a}	0.14 ± 0.02^{a}
20:3n-6	0.76 ± 0.08^{a}	0.77 ± 0.06^{a}	0.76 ± 0.04^{a}	0.73 ± 0.09^{a}
20:4n-6	2.88 ± 0.39^{a}	2.70 ± 0.12^{a}	2.63 ± 0.11^{a}	2.74 ± 0.22^{a}
20:5n-3	0.85 ± 0.08^{ab}	0.60 ± 0.01^{c}	0.72 ± 0.03^{bc}	0.96 ± 0.09^{a}
22:6n-3	0.46 ± 0.10^{a}	0.31 ± 0.05^{a}	0.29 ± 0.11^{a}	0.45 ± 0.18^{a}

Values are means ± standard deviation. $^{a, b, c, d}$ Means in the same column followed by different lowercase letters present statistically significant difference by the Tukey test ($P < 0.05$). RT: retention time. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

4. Discussion

According to Table 2, the highest values of TBARs were found in 45 days of storage in all treatments and then generally decreased, however, after 180 days of storage, a further increase of malonaldehyde occurred in all treatments. These results can be explained by the precise identification of the malonaldehyde in the initial and propagation stages (Funaro et al., 2014).

The replacement of 50% NaCl by KCl (F1: 50% NaCl + 50% KCl) resulted in the lowest malonaldehyde content ($P < 0.05$) after 90 days of storage compared to others treatments. By other hand, the salted meats containing $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$) presented the highest values of malonaldehyde ($P < 0.05$) in the first 90 days of storage if compared to FC1 (100% NaCl) and F1 (50% NaCl + 50% KCl) treatments. Dos Santos et al. (2017) obtained similar results of increase of malonaldehyde values in salami with replacement of 50% NaCl by 50% $CaCl_2$ and dos Santos et al. (2015) reported that the addition of $CaCl_2$ increase the lipid oxidation by generation of hexanal and (E)-hept-2-enal and other volatiles during processing and storage of fermented sausages.These results of TBARs demonstrate a greater lipid oxidation capacity of $CaCl_2$ when compared to NaCl and KCl during 180 days of storage, in agreement to Vitor et al. (2019) who founded similar results of malonaldehyde values during elaboration of jerked beef added of NaCl, KCl and $CaCl_2$.

To understand the mechanism of formation of primary and secondary products of lipid peroxidation from fatty acids, an example of arachidonic acid undergoing lipid peroxidation is shown in Scheme 1.

Thus, it can be assumed that arachidonic acid (AA, 20:4n-6) with m/z 303 present at the sn-2 position in phospholipid could have undergone lipolysis and released AA as a free fatty acid, which on further lipid peroxidation could have formed PGF2α product, as depicted in Figure 3. A detailed mechanism demonstrating the formation of secondary products of lipid peroxidation from AA is shown in Scheme 1. Similarly, various oxidized peaks were detected in the full scan spectra in negative ion mode from different salted meat samples (Figures 5 and 6), for instance, peak m/z 293 is observed due to oxidation of α-linolenic acid (18:3n-3), which may form 9-oxo or 13-oxo octadecadienoic acid. Likewise, peak of m/z 295 was observed due to oxidation of linoleic acid (18:2n-6) and peaks of m/z 317 and 319 were found due to oxidation of arachidonic acid (20:4n-6), respectively.

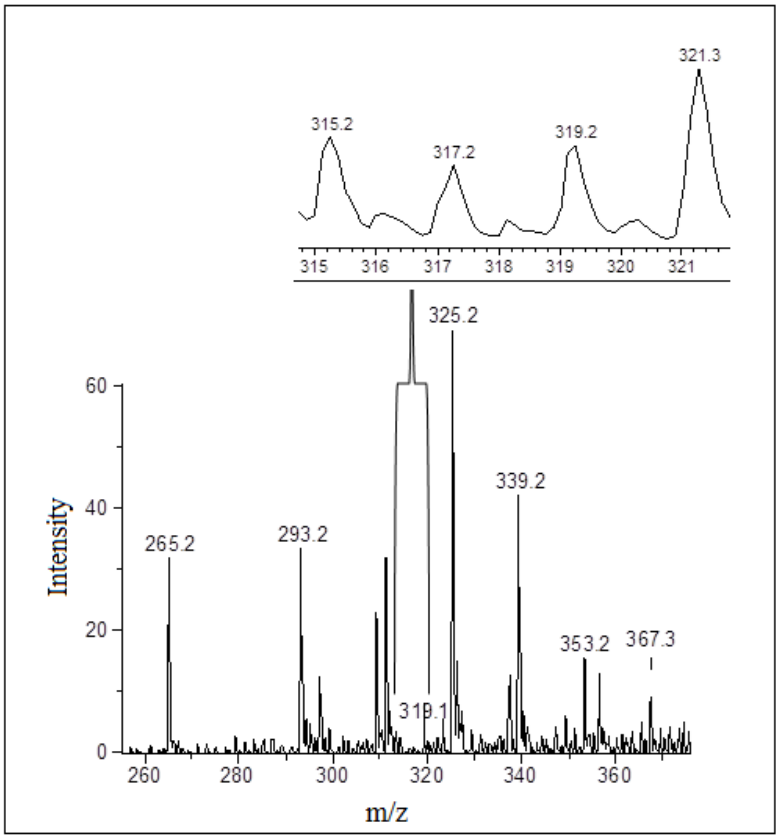


Figure 5. Detection of oxidized polyunsaturated fatty acid from ESI-MS spectra of FC1 T180 sample at *m/z* range of 250-400 in negative ion mode. Precursor ions with *m/z* 293. 295. 317. 319 are characteristic peaks for oxidation of linoleic and Arachidonic acid.

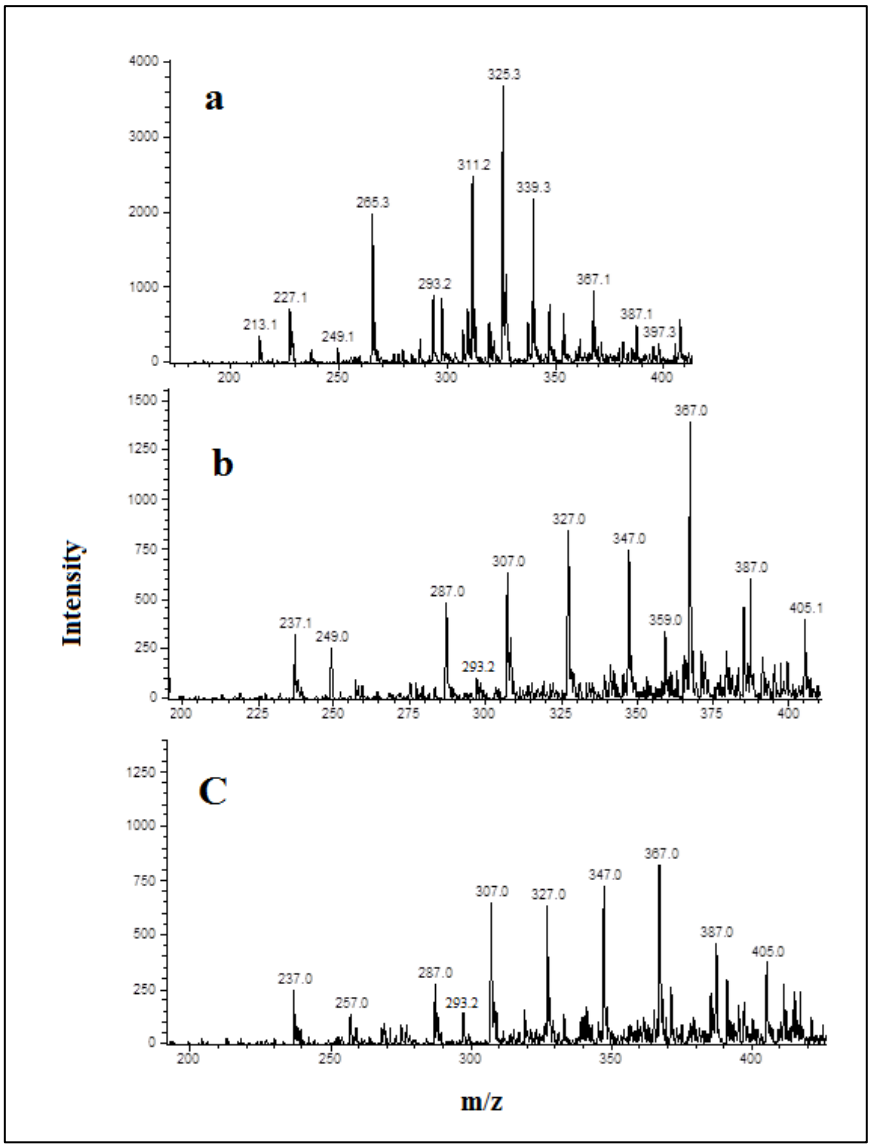


Figure 6. Detection of oxidized polyunsaturated fatty acid from ESI-MS spectra of samples a) F1 T180, b) F2 T180 and c) F3 T180 at *m/z* range of 250-400 in negative ion mode. In the spectra, it demonstrates characteristic peaks for oxidation of linoleic acid with peak ions m/z 293 and 295 respectively.

The products of lipid peroxidation were extensively studied by Esterbauer and his colleagues in the 80s. In their work, they found that MDA appears to be the most mutagenic product of lipid peroxidation, whereas 4-HNE is the most toxic compound (Esterbauer et al., 1982; Esterbauer et al., 1980; Esterbauer et al., 1984; Esterbauer et al., 1990). Further advanced studies on the identification of positional isomers of hydroxy, hydroperoxy and keto phospholipids including those derived from the oxidation of PUFAs were reviewed (Spickett et al., 2015). From the reported studies, it was evident that secondary products of lipid

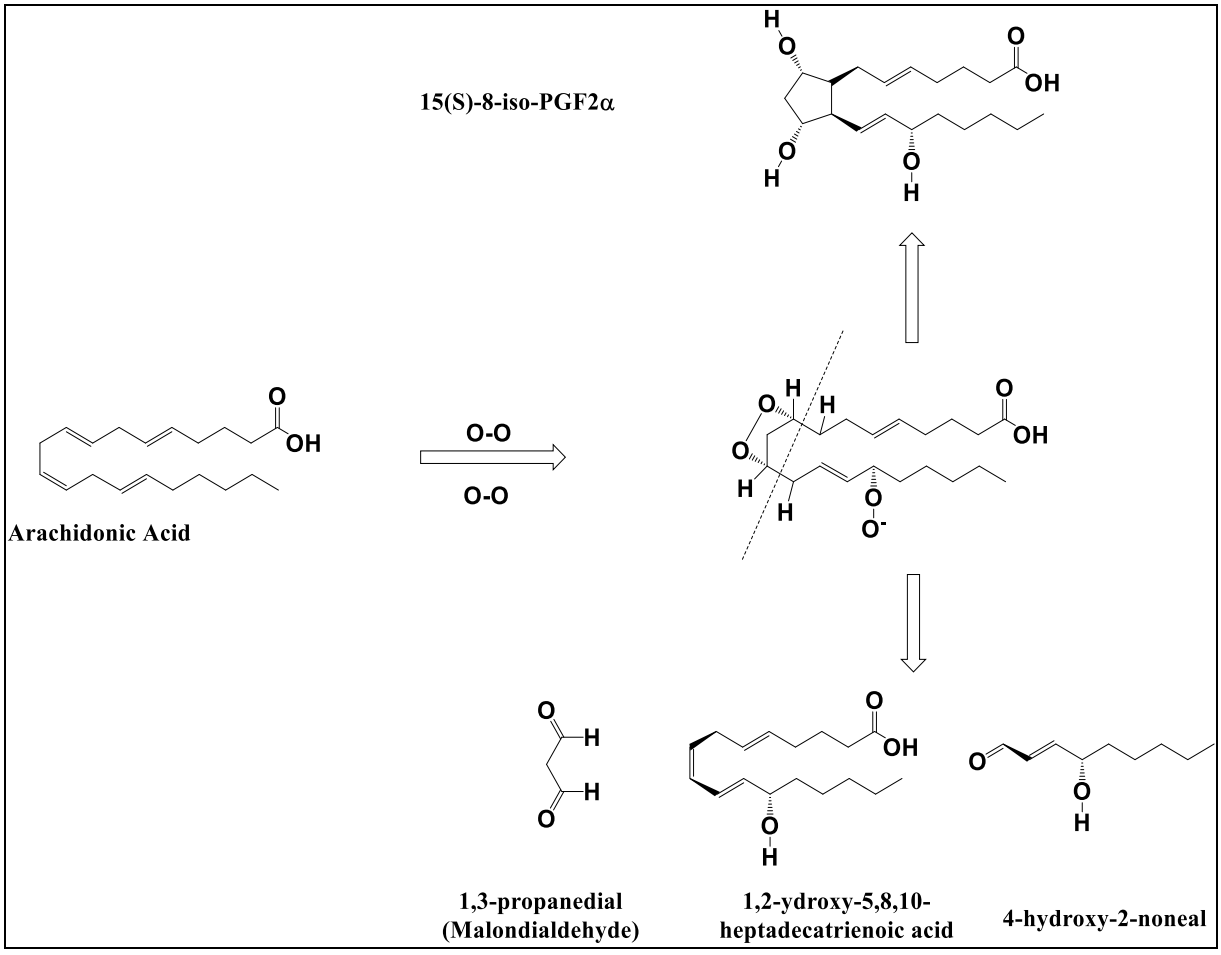
peroxidation appear to be carcinogenic due to excessive lipid oxidation. Hence, the reduction of the NaCl, main source of sodium, during meat processing is quintessential.

Some MUFAs such as oleic acid (18:1) and SFAs like myristic acid (14:0), as well as arachidic acid (20:0) were observed in samples with 0 day of storage. This could be due to the increased enzymatic activity of phospholipase enzymes, which could have released FFA from Sn-2 position of phospholipids (Motilva, Toldra, Nieto & Flores, 1993; Coutron-Gambotti & Gandemer, 1999; Motilva et al., 1993). However, with an increased period of storage i.e., at 180 days, it was observed that FC1 T180 and F2 T180 samples have demonstrated changes in the free fatty acids composition.

It was also observed that many peaks corresponding to PUFAs decreased. Perhaps, it could be due to the effect of $CaCl_2$ salt on lipolysis that produced oxidation of FFA. However, the effect of salt directly or indirectly plays an important role in the generation of volatile and non-volatile compounds that enhance the flavor of the meat (Buscailhon et al., 1993; Careri at al., 1993; Flores et al., 1997), which are in agreement with previous reported studies. It was observed that semimembranosus and biceps femoris muscle from ham with higher salt content showed a marked decrease in the total fatty acid content especially PUFAs (Andres et al., 2005).. Similar effects were observed in ham during lipolysis (Coutron-gambotti et al.. 1999; Motilva & Toldrá 1993). In order to confirm if really lipid peroxidation was occurring during a prolonged period of storage, we studied the full scan spectra of all treated meat samples obtained in negative ion mode by ESI-MS.

Regarding volatile compounds, NaCl replacement increased the total VOC. In similar way, Armenteros et al. (2012) found in ham samples that the replacement of NaCl by a blend of chloride salts (potassium, calcium and magnesium) resulted in an increase of total VOC. In contrast, Domínguez et al. (2016) described the highest values in control samples, mainly due to the highest amounts of hexanal found in this batch. Not only the total VOC, but also the volatile profile was also affected by the salt treatment. In FC1 and F3 batches, the major VOC were the ketones (Figure 4), representing 42.3% and 27.9% of total VOC, respectively, while in F1 and F2 batches, acids were the major group (about 32% in both treatments). In contrast with our values, other authors found that aldehydes were the more abundant volatiles in meat products (Armenteros et al., 2012; Domínguez et al., 2019; Marušić, Vidaček, Janči, Petrak, & Medić, 2014; Petričević, Marušić Radovčić, Lukić, Listeš, & Medić, 2018). However, not all researches show the same results. In fact, ketones were also reported as the major chemical group in sausages (Domínguez et al., 2019) and also in cured loin (Ventanas et al., 2008),

while acids were found the main group in cured loin (Domínguez et al., 2019). As could be seen in the Figure 4, although there are differences among treatments, the major volatile groups in all samples were ketones, acids and aldehydes (in different proportions). The major individual VOC, except for FC1 treatment, was butanoic acid (352–447 AU × 104/g) followed by hexanal (183-299 AU × 104/g) and acetoin (171-204 AU × 104/g). Contrary, in the FC1 samples, the major VOC was acetoin (145 AU × 104/g), followed by hexanal (135 AU × 104/g) and butanoic acid (101 AU × 104/g). High amounts of these 3 compounds were found in other dry-cured products (Domínguez et al., 2019). Therefore, it seems that these compounds had an important influence in the aromatic characteristics of the salted meat.



Scheme 1. Mechanism of formation of 1,3-propanedial (malondialdehyde). 1,2-hydroxy-5,8,10-heptadectrienoic acid, 4-hydroxy-2-noneal and 15(s)-8-iso-PGF2α from peroxidation of Arachidonic acid (AA).

However, the major differences among control batch (FC1) and the other treatments were observed in the content of acids, ketones and aldehydes, although the other chemical families also showed minor differences. With this regards, it is well known that the compounds derived from lipid oxidation reactions are aldehydes, ketones, some carboxylic

acids and also alcohols (Ramírez & Cava, 2007). Therefore, as will be discussed below, it seems that the compounds derived from lipid oxidation had high influence in total volatile compounds content in salted meat.

A total of 6 organic acids were identified in samples. In this case, all experimental treatments had higher values than samples from control batch. The major amount of total acids was found in F2 (525 AU × 104/g) followed by F1 (449 AU × 104/g), F3 (433 AU × 104/g) and finally FC1 (135 AU × 104/g). The major acid, as commented above, was butanoic acid, followed by acetic and hexanoic acids. In the present study, the content of these three acids was higher in the samples with partial replacement of NaCl than in control. These findings were also reported in dry-cured loin (Domínguez et al., 2016). Additionally, all these acids were also found in dry-cured loin (Muriel et al., 2004; Ventanas et al., 2008) and hams (Ramírez & Cava, 2007). In contrast with our results, Armenteros et al. (2012) reported that the replacement of NaCl by other chloride salts resulted in a decrease of total acids content. The main origin of the acetic acid is frequently related with carbohydrate fermentation and Maillard reaction (Domínguez et al., 2019; Pérez-Santaescolástica et al., 2018). In salted meat as performed in this study, it makes sense, considering the last processing step when the dry meat is put under 35–40 °C to improve the ripening. In this condition, the Maillard reactions can be favored. Other straight-chain carboxylic acids are derived from the hydrolysis of lipids (triglycerides and phospholipids) (Pugliese et al., 2015). This fact could explain the differences found between studies and meat products, due mainly to the different ingredients and dry-cured conditions. Additionally, the content of NaCl and the partial replacement by other salts also affect the activity of the enzymes and therefore, the release of precursor compounds for the formation of VOC.

Regarding ketones content, acetoin showed the major values, following by 2,3-butanedione. Also, important amounts of 2-butanone and 2-heptanone were found. Acetoin also was the major ketone in other meat products as loin, salchichón, shoulder and chorizo (Domínguez et al., 2019). The highest contents of acetoin were observed in samples from F1 and F3 samples, while the lowest values were found in control batch. The content of 2-butanone and 2-heptanone, both related with lipid oxidation, was higher in F2 and F3 treatments (salt blends that include calcium in their formulation) than in F1 and FC1. The origin of ketones can be diverse. Linear ketones, especially 2-ketones and methyl ketones arise from the oxidation of free fatty acids (Narváez-Rivas, Gallardo, León-Camacho, &

León-Camacho, 2012; Ramírez & Cava, 2007), while other ketones such acetoin are formed through Maillard reactions (Pérez-Santaescolástica et al., 2018).

Aldehydes represented around 20% of total VOC in all batches. However, if we observe the Figure 4, it is clear that their content (AU × 104 /g) suffered a significant increase in F2 and F3 batches, in comparison with F1 and control samples. In fact, the major increment was detected in F2 samples, which contain the highest amount of calcium in their formulation. In all samples, the major aldehyde was hexanal, followed by pentanal and heptanal. In dry-cured lacón also was reported that these 3 compounds were the major aldehydes detected in the final product (Domínguez et al., 2016)Additionally, there are other researches that found hexanal was the major aldehyde in dry-cured products (Armenteros et al., 2012; Gomez, Dominguez, Fonseca, & Lorenzo, 2015; Lorenzo, 2014). The content of other aldehydes also increased with the partial replacement of NaCl. The amount of octanal, pentadecanal and propanal, 2-methyl were higher in F2 and F3 samples than in control samples. It is well known that hexanal, and pentanal deriving from the oxidation of linoleic, linolenic and arachidonic fatty acids, while heptanal, octanal and nonanal come from oleic acid autoxidation (Lorenzo, 2014; Montanari et al., 2018). Additionally, aldehydes due to their low odor threshold values, have and important role in the aroma of dry-cured meat products (Petričević et al., 2018; Ramírez & Cava, 2007). However, the presence of some aldehydes is not always related with quality decrease. The aldehydes derived from oleic acid oxidation, such as heptanal, octanal and nonanal have been related to pleasant meaty notes (Domínguez et al., 2019).

Finally, other important VOCs that had influence in the final aroma were also found in the present study. These compounds were all related with lipid oxidation. To this regard, the content of linear hydrocarbons as pentane, heptane, octane, dodecane and tridecane was higher in samples from treatments F2 and/or F3 than in F1 and control samples. In the same way, the content of lipid oxidation-derived alcohols such as 1-propanol, 1-butanol, 1-octen-3-ol showed the highest values in F2 samples (which contains the major amount of calcium in their formulation), and the content of furan, 2-penthyl, also derived from the oxidation of linoleic acid was higher in the samples from F2 and F3 than in the other 2 batches.

As a general comment, the F2 and F3 batches showed the highest contents of lipid-derived VOC, while F1 had intermediate values, in comparison with control samples. It is well known that during processing chemical reactions and enzyme activity are involved in the generation of volatile compounds affecting the sensory properties of dry-cured meat products.

There are multiple researches that studied the influence of salt replacement on lipolytic and oxidative processes, which are directly related with volatile generation. The high content of NaCl influences the progression lipolytic/oxidation reactions during the ripening and dry-curing steps (Lorenzo, Fonseca, Gómez, & Domínguez, 2015; Mariutti & Bragagnolo, 2017). Therefore, it is expected that its partial replacement exerted a significant effect on the final content of volatile compounds.

Furthermore, the replacement of NaCl also influenced the free fatty acids contents. The use of blends of substitutes salts (KCl and $CaCl_2$) affected the proportion of: caprylic acid (C8:0), palmitic acid (C16:0), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (9t-C18:1), cis-vaccenic acid (C18:1n-7), linoleic acid (C18:2n-6), γ-linolenic (C18:3n-6), α-linolenic (C18:3n-3), 9-cis 11-trans-octadecadienoic (9c,11t-C18:2 (CLA)) and eicosapentaenoic acid (C20:5n-3).

Moreover, different authors found an increase in the fatty acid release and FFA content as results of NaCl replacement, mainly in samples salted with blends that include chloride salts with divalent ions (Lorenzo, Cittadini, Munekata, Domínguez, & Bermúdez, 2015). In similar way to lipolysis, other authors found that lipid oxidation was also higher in samples salted with blends containing divalent ions than samples salted with NaCl (Vidal et al., 2019; Wu et al., 2016), which indicate that these salts favored the progression of oxidative reactions in the lipids of dry-cured meats. Therefore, the reduction of NaCl proportion in the salting step increased lipolytic and oxidative processes. The role of divalent salts in both lipolysis and lipid oxidation remains unclear due to contrasting results observed among studies (Armenteros, Aristoy, Barat, & Toldrá, 2009; Lorenzo, Cittadini, et al., 2015). However, it seems that the use of $CaCl_2$ in the blend composition increase lipid oxidation. Furthermore, another study concluded that the amount of $CaCl_2$ used was important because larger amount favored the lipid oxidation (Flores, Nieto, Ferrer, & Flores, 2005), which is in agreement with our results. The samples containing $CaCl_2$ in the salt blend (F2 and F3) showed the highest TBARs values (1.10 and 1.74 mg MDA/kg, respectively), in comparison with the values of the other batches (about 0.9 mg MDA/kg). Therefore, it easy to conclude that the main differences found in volatile contents and profiles among the samples analyzed in the present study are due to the differences in the oxidation processes between the different batches.

5. Conclusion

Altogether, our results showed that the partial replacement of NaCl by KCl and $CaCl_2$ influenced the lipolysis reactions and lipid profile in reduced sodium salted meats. $CaCl_2$ promoted the most several changes in lipid oxidation along shelf life (180 days) being responsible for the highest values of malonaldehyde. The total volatile compounds, volatile profile and free fatty acids profile were affected by salt used in salting steps. Ketones, acids and aldehydes were reported as the major groups of volatile compounds founded in different proportions and oleic acid (C18:1n-9), palmitic acid (C16:0) and stearic acid (C18:0) were the main free fatty acids founded in all salted meat treatments. Considering the differences of TBARs values among treatments and all the results present in this study, it is evident the highest oxidative capacity and impact of $CaCl_2$ on lipid profile compared to NaCl and KCl in salted meat treatments at same ionic strength.

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6. Abbreviations

Saturated fatty acids (SFAs), free fatty acids (FFA), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), sodium chloride (NaCl), potassium chloride (KCl) and calcium chloride ($CaCl_2$), sodium hypochlorite (NaOCl), thiobarbituric acid-reactive substances (TBARs), malonaldehyde (MDA), arachidonic acid (AA), 4-hydroxynonenal (4-HNE), volatile organic compounds (VOC).

Funding

Leonardo S. Santos and Fabiane M. Nachtigall thank FONDECYT 1180084 to support research activities. Radha D. Pyarasani wishes to acknowledge Vicerectoria de Investigacion y postgrado. Universidad Catolica del Maule for the postdoctoral position. Vitor A. S. Vidal and Marise A. R. Pollonio would like to thank CNPq (process numbers: 140533/2015-0 and 406671/2016-8) for its financial support.

Compliance with Ethical Standards

Conflict of Interest

Fabiane M. Nachtigall declares that she has no conflict of interest. Vitor A. S. Vidal declares that he has no conflict of interest. Radha D. Pyarasani declares that she has no conflict of interest. Rubén Domínguez declares that he has no conflict of interest. José M. Lorenzo declares that he has no conflict of interest. Leonardo S. Santos declares that he has no conflict of interest. Marise A. R. Pollonio declares that she has no conflict of interest

Ethical Approval

This article does not contain any studies with human or animal subjects.

Informed Consent

Not applicable

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CAPÍTULO 6

HOW DOES THE PROTEOLYSIS IMPACT THE TEXTURE IN REDUCED SODIUM SALTED MEAT ALONG SHELF LIFE?

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Submitted to Meat Science

Abstract

Salted meats are widely consumed and have high nutritional relevance, especially in areas with limited cold chain. Despite this significant impact, the high levels of sodium present in salted meats, its consumption may increase the risk of developing hypertension and cardiovascular diseases, among others. Sodium reduction in salted meat is a major technological challenge due to the technological functions of NaCl, responsible for the reduction of water activity and consequent microbiological safety, stability during shelf life, development of characteristic texture and flavor, proteolysis and lipolysis. The objective of this research is to evaluate the effects of partial replacement of NaCl by KCl and $CaCl_2$ on the texture properties during 180 days of storage in salted meat. During 180 days of storage, a significant decrease ($P < 0.05$) of shear force values have been observed in all treatments. The results showed the intense effects of $CaCl_2$ on texture and proteolysis reactions and the blend NaCl + KCl had a similar impact to salted meat product compared to control treatment (100% NaCl).

Keywords: Salted meat, sodium reduction, proteolysis

1. Introduction

The salted meat is widely consumed worldwide due to its unique sensorial characteristics besides its relevance in regions where the cold chain is not fully established (Ishihara & Madruga, 2013; Garcia et al., 2003). The elaboration of these products is based on hurdle technology, using mainly dehydration by salt and vacuum packaging (Leistner, 1987; Shimokomaki et al., 1998). Thereby, the growth of microorganisms is inhibited and the product becomes stable up until 180 days at room temperature.

Sodium chloride (NaCl), the main source of sodium in processed foods, playing a critical role in the human diet, being necessary for many physiological functions. But its excessive intake is associated with several health problems such as the increase of risk development of hypertension and cardiovascular diseases besides certain types of cancer (McGough, Sato, Rankin, & Sindelar, 2012; Pires, Munekata, Baldin, Rocha, Carvalho, dos Santos, et al., 2017; Ripollés, Campagnol, Armenteros, Aristoy, & Toldrá, 2011). Although the salted meat has the technological benefits already mentioned, the salting used to elaborate the product provides a high sodium content, needing a desalting process for consumption. As this procedure not always is standardized by consumer, due to domestic procedures, the partial substitution of NaCl as the main component of the salting steps by other chloride salts is an excellent strategy to effectively guarantee sodium reduction in this category of products.

In salted meat, several biochemical reactions that occur during drying are particularly important to determinate the final quality of the processed product. Among them, the proteolysis is dependent on a large number of variables related to enzymatic activity, raw material, processing conditions, microbiota, components of the formulation, in addition, salt is considered one of the most important ingredients (Mora, Gallego, Escudero, Reig, Aristoy, & Toldrá, 2015).

Proteolysis is a result of the action of microbial enzymes and tissues, which is affected by water activity, processing conditions, and mainly salt levels. Salt regulates the activity of proteolytic enzymes, which can be inhibited by increasing salt concentration during drying, the reduction of NaCl can activate more muscular proteases, being able to increase the proteolytic activity and consequent release of free amino acids (Toldrá, Rico, & Flores, 1992). Thus, to understand the effect of NaCl reduction and/or substitution by other salts on the proteolysis is one of the main problems to be approached and explained.

In this context, the objetive of this study was investigate the effect of NaCl replacement by KCl and $CaCl_2$ blends on texture properties by comparing electrophoretic profile and shear force analysis of salted meat, aiming to understand the effect of different salts.

2. Materials and methods

2.1 Treatments, material and additives

The additives sodium nitrite and sodium erythorbate were donated by the company Kerry of Brazil. The NaCl, KCl and $CaCl_2$ salts added in the treatments are food grade (Anidrol, Brazil). The selected meat cut (bovine raw meat) was the *biceps femoris* obtained from slaughterhouses with assured sanitary quality at Campinas, São Paulo, Brazil.

Four treatments of salted meat were performed. The NaCl replacement by KCl and $CaCl_2$ was based on ionic strength, obtaining the same ionic strenght in all treatments. The additive sodium nitrite and sodium erythorbate was added at fixed content in the wet step. The variable was the salt used during the wet and dry salting steps.

Table 1. Salts used to performed the salted meat treatments

Treatments	NaCl (%)	NaCl (mg)*	KCl (%)	KCl (mg)*	$CaCl_2$ (%)	$CaCl_2$ (mg)*
FC1	100	1000	-		-	
F1	50	441	50	560	-	
F2	50	614	-	-	50	387
F3	50	513	25	326	25	162

The amount of salt added was based on the ionic strength, all treatments obtained the same ionic strength. * Salt proportion added according to ionic strength, for each 1000mg of bovine raw meat was utilized 2000mg of salt

2.2 Salted meat processing

The salted meat processing was performed according to Vidal et al. (2019). In wet salting step, the meat pieces of each treatment were submerged in saturated solution with

respective salts described in Table 1 and 0.015% of sodium nitrite and 0.05% of sodium erythorbate for 1 hour at 13 °C.

For dry salting step, was used chamber at 13 °C for a period of 144 hours (6 days), putting the treatments in contact with respective salts. The ripening step was carried out in a controlled climatic chamber (Instala Frio, Paraná, Brazil) with 55% humidity, 25 ºC and 0.5 m/s forced ventilation for 24 hours. After the process, the final products were packed under vacuum packed of polyethylene (Spel, São Paulo, Brazil) and stored at room temperature. (25 °C).

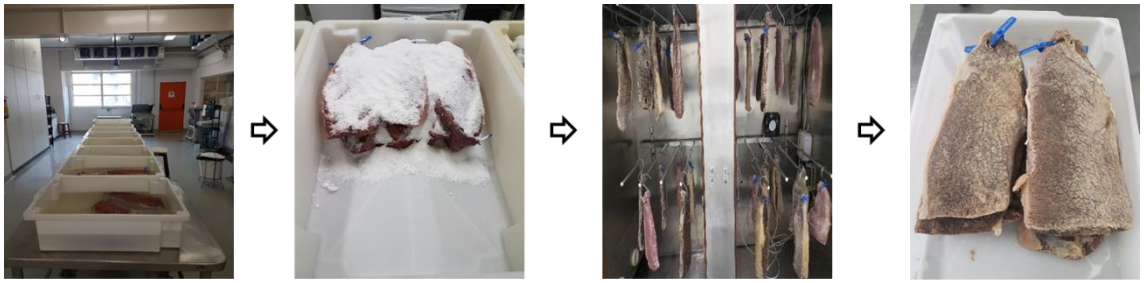

Figure 1. Salted meat processing. A: wet salting; B: dry salting; C: ripening; D: final product

The process to elaborate the treatments was performed in the Pilot Plant of the Meat Area, Faculty of Food Engineering, University State of Campinas (UNICAMP). The process was performed in triplicate.

2.3 Desalting, cooking and shear force

The desalting and cooking procedures were according to Vidal et al (2019). Cooking was done in a water bath (RSA-1708, RSA, Campinas, Brazil) at 80 ºC.

The desalted and cooked samples were stored under refrigeration for 24 hours prior to the analysis of Warner-Bratzler shear force (Stock & Board, 1995). The shear force was measured in a TA-XT 2i texture analyzer (Texture Technologies Corporation / Stable Micro Systems, Hamilton, UK). For Warner-Bratzler shear force analysis, the distance to the platform was 25 mm and the ascent/descent speed was 200 mm/min. The analysis of Warner-Bratzler shear force was performed in six replicates for each replicate process. The shear force was measured at 0, 45, 90, 135 and 180 days of storage. The analysis was performed in six replicates in each process replicate.

2.4 Myofibrillar and sarcoplasmic proteins extraction

The sarcoplasmic and myofibrillar proteins extraction were performed according to the methodology described by Hughes, Kerry, Arendt, Kenneally, McSweeney, and O'Neill (2002), with some modifications.. Accordingly, 5 g of sample were homogenized with a 35 mL phosphate buffer 0.03 mol/L and pH 7.4 for 2 min at 13500 rpm at 4 °C using an Ultra-Turrax T25 (IKA, Leicestershire, UK). Then, the sample were centrifuged at 10000 g for 20 minutes at 4 °C to extract the sarcoplasmic protein, the resulting supernatant were filtered through glass wool and kept in refrigeration (4 °C). The process described above was repeated twice to remove any remaining sarcoplasmic proteins. After the washes, the resulting pellet were homogenized with a 25 mL solution of 8 mol/L urea and 1% β- mercaptoethanol for 2 min at 13500 rpm at 4 ° C using an Ultra-Turrax T25 (IKA, Leicestershire, UK), followed by centrifugation at 10000 g for 20 minutes at 4 °C. The supernatant containing the myofibrillar proteins were filtered through glass wool, and kept in refrigeration (4 °C) until analysis. The concentration of myofibrillar and sarcoplasmic proteins were determined by the method of Bradford (1976). Samples at 0, 45, 90, 135 and 180 days of storage were evaluated

2.5 SDS-PAGE electrophoresis

To evaluate the proteolysis, was performed sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) described by Laemmli (1970) using a Mini Protean II apparatus (Bio Rad, California, USA) and 8% and 17.5% polyacrylamide gradient gels. The extracted sarcoplasmic and myofibrillar proteins with 4 mg protein/mL were dissolved in a sample buffer (2% SDS and 5% β-mercaptoethanol).

3. Results

3.1 Shear force determination

The high content of salts applied in the salting steps directly change texture characteristics due to modification of the solubility of myofibrillar proteins and swelling of muscle fibers (Offer et al., 1989), being this change related to effects on water retention and

binding capacity by different concentration of salt and pH (Offer & Trinick, 1983; Oliveira et al., 2012).

In this study, the impact of using different salts on texture can be analyzed by the values of shear force presented in Table 2. During 180 days of storage, a significant decrease ($P < 0.05$) of shear force values have been observed in all treatments. It can be explained, possibly due to the intense proteolytic reactions promoted by the different salts. On the other hand, treatments containing $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$) maintained the highest shear force values during 180 days of storage in relation to treatments containing NaCl (FC1: 100% NaCl) or NaCl + KCl (F1: 50% NaCl + 50% KCl). These results may have reported due to the higher dehydrating effect of $CaCl_2$ in relation to NaCl and KCl. Many authors have described an increase in the rate of dehydration using calcium salts in various foods (Lewicki, Vu Le, & Pomarańska-Łazuka, 2002; Mastrantonio, Pereira, & Hubinger, 2005; Pereira, Carmello-Guerreiro, Bolini, Cunha, & Hubinger, 2007) which directly affect the texture characteristics (Aliño et al., 2009; Aliño et al., 2010; Offer et al., 1989; Offer & Trinick, 1983; Oliveira et al., 2012). Along the storage, the treatments which had $CaCl_2$ in blend salt composition, shown a hard surface layer resulted by fast and higher rate of water released.

Table 2. Shear force (N) values in salted meat treatments during storage

Treatments	0 day	45 days	90 days	135 days	180 days
FC1	39.55^{bA}	38.65^{bAB}	37.94^{bAB}	31.27^{bcBC}	27.49^{bC}
F1	41.87^{bA}	37.49^{bAB}	35.56^{bABC}	30.87^{cBC}	28.19^{bC}
F2	54.23^{aA}	52.91^{aA}	51.60^{aA}	39.87^{abB}	40.49^{aB}
F3	58.77^{aA}	48.73^{aBC}	50.77^{aAB}	42.00^{aBC}	38.51^{aC}
Standard error	1.57	1.14	1.35	1.86	2.16

Values are means. $^{a, b, c, d}$ Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test. ^{A,B,C,D} Values in the same line with the same capital letters do not differ significantly ($P < 0.05$) according to Tukey's test. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

3.2 SDS-PAGE electrophoresis

Electrophoresis gels containing the profile of myofibrillar proteins can be seen in Figure 2 and sarcoplasmic proteins in Figure 3. The average intensities of the main bands in Figure 2, corresponding to myosin heavy chain (~205 kDa), C-protein (~137 kDa), actin (~43 kDa), tropomyosin (~36 kDa) and myosin light chain (~24 kDa) (dos Santos et al., 2015). Analyzing the electrophoretic profile of myofibrillar proteins of salted meat treatments with different salts, is possible to verify the disappearance of some bands of high molecular weight proteins during the storage simultaneously in all treatments. However, after 135 days, treatments containing $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ e F3: 50% NaCl + 25% KCl + 25% $CaCl_2$) decreased the intensity of bands with molecular weights greater than 100 kDa as myosin heavy chain and C-protein and increased the intensity of bands with lower molecular weights such as actin, tropomyosin and myosin ligh chain. This suggests that $CaCl_2$ can promote denaturation of high molecular weight proteins resulting in lower molecular weight proteins.

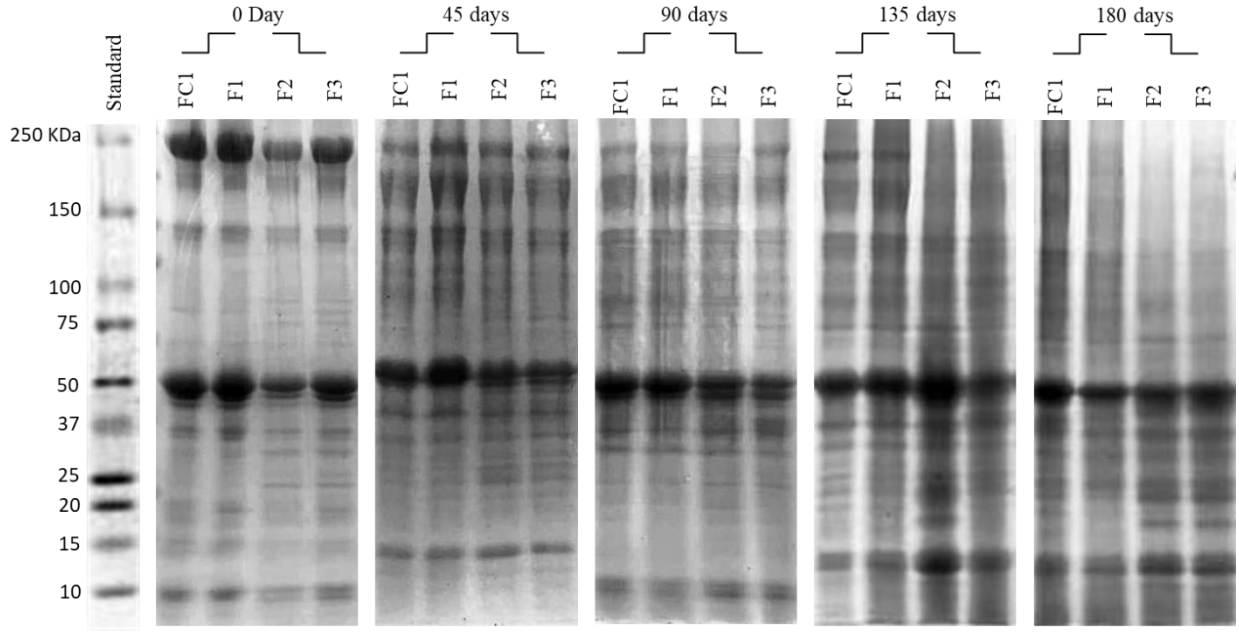

Figure 2. 8 and 17.5% SDS-PAGE gels of myofibrillar proteins in the salted meat treatments. Standards: BioRad molecular weight standards (Da: 10000 to 250000). FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

Observing Figure 3, there was a significant change in the electrophoretic profile of sarcoplasmic proteins during storage of salted meat treatments with the addition of $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$), promoting the

disappearance of several bands compared to treatments FC1 (100% NaCl) and F1 (50% NaCl + 50% KCl). It can be noted that after 45 days of storage, $CaCl_2$ caused an intense proteolysis of sarcoplasmic proteins of all weights. According to Lorenzo et al. (2015), salt type influences chemical and biochemical reactions such as proteolysis.

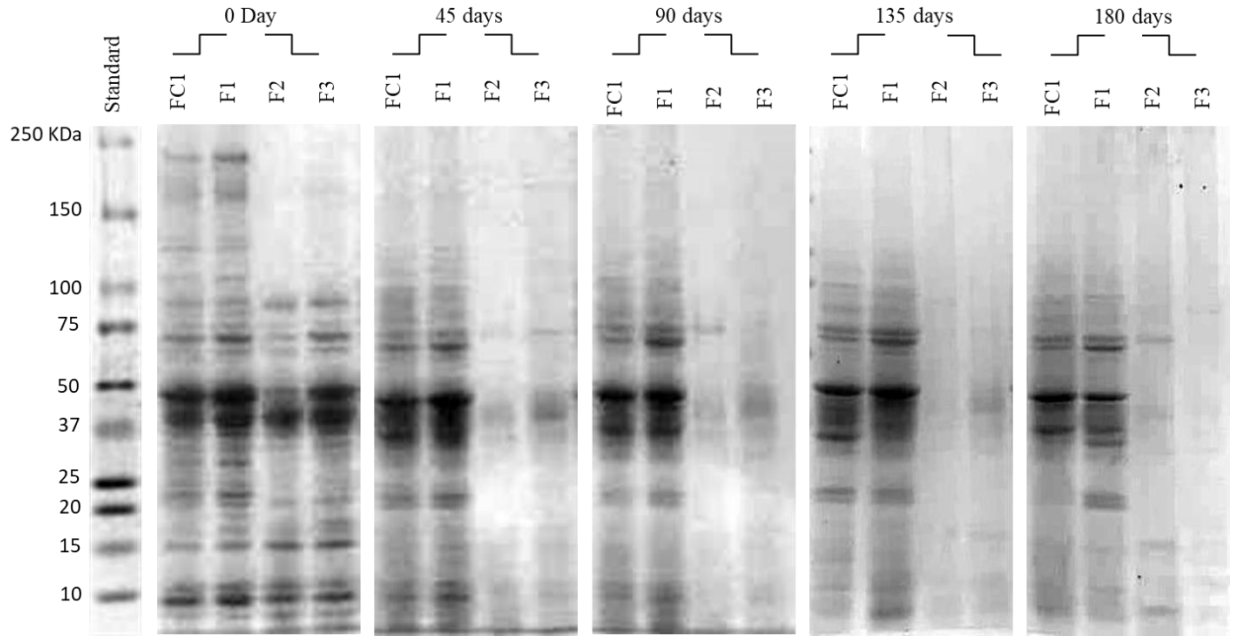

Figure 3. 8 and 17.5% SDS-PAGE gels of sarcoplasmic proteins in the salted meat treatments. Standards: BioRad molecular weight standards (Da:10000 to 250000). FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

In addition, the electrophoretic profile both myofibrillar and sarcoplasmic proteins of treatment containing 100% NaCl (FC1) were similar to the treatment containing partial replacement of NaCl by KCl (F1: 50% NaCl + 50% KCl).

4. Conclusion

The use of $CaCl_2$ to elaborated salt blends aiming to reduce sodium in salted meat intensily affected texture and proteolysis reactions during 180 days of storage. In contrast, the blend containing NaCl + KCl had a similar impact to salted meat samples compared to NaCl control treatment, being a promising alternative for reducing or replacing NaCl in salted meat products without impact in texture properties.

Acknowledgment

The authors would like to thank National Council for Scientific and Technological Development (CNPq, Brazil) (process numbers: 140533/2015-0 and 406671/2016-8) for the financial support.

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CAPÍTULO 7

INFLUENCE OF THE ADDITION OF KCL AND $CACL_2$ BLENDS ON THE PHYSICOCHEMICAL PARAMETERS OF SALTED MEAT PRODUCTS THROUGHOUT THE PROCESSING STEPS

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Food Science and Technology (Campinas)

In Press

DOI: 10.1590/fst.14919

Abstract

The objective of this study was to evaluate the effects of different chloride salts (NaCl, KCl, and $CaCl_2$) on the characteristics of salted meat products through the determination of moisture, pH, aw, chloride, ash levels, cooking loss, and instrumental color during the processing steps. Four salted meat treatments were elaborate using the following salts in the wet and dry salting steps: FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 25% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$. The addition of $CaCl_2$ led to the lowest pH and changes in aw, moisture, ash levels, and instrumental color when compared to the other treatments, which was different from the control (100% NaCl) and F1 (50% NaCl + 50% KCl), thus evidencing the great effect of $CaCl_2$ on the characteristics of salted meat products during the whole processing. The partial replacement of NaCl by KCl and/or $CaCl_2$ greatly increased the cooking loss of salted meat products. The replacement of NaCl by KCl promoted similar quality parameters.

Keywords: salted meat; sodium reduction; potassium chloride; calcium chloride

Pratical Application

Use of KCl and $CaCl_2$ as a strategy to reduce sodium content in salted meat

1. Introduction

Salted meat products are consumed and appreciated worldwide due to their unique sensory characteristics and long shelf-life (Liu et al., 2014). The manufacture of salted meat products is based on the hurdle technology (Leistner, 1987), and several steps such as salting (wet or dry), drying and ripening can be used during processing (Mora et al., 2015), besides the addition of sodium chloride (NaCl) and additives, and vacuum packaging (Shimokomaki *et al.*, 1998). The combination of these steps provides the sensory characteristics and microbiological stability for the processed product (Ishihara et al., 2013).

Changes in lifestyle associated with the modernization of society and the development of new products have led to a drastic change in eating habits, with increased consumption of processed products. Some of these products can be a major source of fat, sodium, and sugars, which can cause various health problems when consumed in excess, such as obesity, diabetes, and cardiovascular disorders (Roberfroid, 2002). Therefore, there is a growing consumer's demand for healthy eating perceptions and healthy lifestyle, with a preference for meat products rich in proteins and low in lipids, cholesterol, and sodium (Lorenzo & Carballo, 2015).

Sodium chloride is an ingredient extensively used and very important to the development of numerous desirable sensory and technological characteristics in meat products (Inguglia et al., 2017). It plays an important role in salted meat products, once when combined with other techniques, it can preserve the product for months without refrigeration for later consumption (Torres et al., 1989). However, NaCl is the main source of sodium in the human diet (Desmond, 2006), and the excessive sodium intake causes several deleterious health effects such as high blood pressure, cardiovascular and renal diseases (Cook et al., 2016, Denton et al., 1995, Frieden, 2016, Strazzullo et al., 2009). The World Health Organization (WHO) recommends a daily intake of 2 g of sodium, equivalent to 5 g NaCl. In this context, an effective reduction of NaCl during the manufacture of salted meat product, which presents a high sodium content after processing, is extremely necessary to make the product healthier.

There are many ways to reduce sodium content in meat products. One of the most used strategies for reducing or replacing NaCl is the use of other chloride salts as KCl (potassium chloride), $CaCl_2$ (calcium chloride) and $MgCl_2$ (magnesium chloride) (Aliño et al., 2010, Ripollés et al., 2011). Among the chloride salts, KCl is widely used due to the development of

similar characteristics to NaCl in meat products; however, the addition of KCl promotes bitter and metallic taste, thus impairing the use in excess (Doyle & Glass, 2010, Grummer et al., 2013). Although $CaCl_2$ is also used as a substitute for NaCl, in some cases it can negatively affect the texture and flavor characteristics (Vidal et al., 2019).

Taking into account the deleterious health effects caused by the excessive consumption of sodium in salted meat products, the objective of this study is to evaluate the effects of blends containing NaCl, KCl, and $CaCl_2$ on the characteristics of salted meat treatments.

2. Material and Methods

2.1 Treatments, raw materials, and additives

The bovine raw meat (*biceps femoris*) was purchased from slaughterhouses with assured hygienic quality. The additives sodium nitrite and sodium erythorbate were donated by the company Kerry of Brazil. The salts NaCl, KCl, and $CaCl_2$ were purchased from Anidrol, Brazil.

Table 1. Salts used to perform the salted meat treatments

Treatments	NaCl (%)	NaCl (mg)*	KCl (%)	KCl (mg)*	$CaCl_2$ (%)	$CaCl_2$ (mg)*
FC1	100	1000	-	-	-	-
F1	50	441	50	560	-	-
F2	50	614	-	-	50	387
F3	50	513	25	326	25	162

The amount of salt added was based on the ionic strength, all treatments obtained the same ionic strength. * Salt proportion added according to ionic strength, for each 1000mg of bovine raw meat was utilized 2000mg of salt

Four salted meat treatments were made, as shown in Table 1. The concentration of KCl and $CaCl_2$ substitute salts was based on the calculation of ionic strength to make up the ionic strength of 50% and 25% of NaCl, obtaining the same final ionic strength in all treatments. Then, the blends were made in sufficient quantity for the salting steps, depending on the weight of the raw meat, using 2 kg salt per kg of meat. Similar amounts of the

additives sodium nitrite (150 ppm) and sodium erythorbate (500 ppm) were added in the wet salting step, and the salt was the variable of the wet and dry salting steps.

2.2 Processing

The manufacturing process was carried out according to Vidal et al. (2019) and the salts added were described in Table 1. The bovine raw meat pieces have been cut standardized to be submitted to the salting steps (wet and dry). In the wet salting step, the treatment were submerged in a respective saturated solution with respective salts, sodium nitrite and sodium erythorbate for 1 hour. During the dry salting period, the treatments were in contact with respective salts for 144 hours (6 days) at 13 ° C. The ripening step were carried out in a controlled climatic chamber (Instala Frio, Curitiba, Brazil) with 55% humidity, 25 ° C and 0.5 m/s forced air ventilation for 24 hours. After the process, the pieces were vacuum packed of polyethylene (Spel, São Paulo, Brazil) and stored at 25 °C.

All the manufacture process was performed in three replicates on different days with the same methodology, formulation and technology. All the processing steps were carried out in the Meat Laboratory of the Department of Food Technology (DTA) at University of Campinas (UNICAMP).

2.3 Physicochemical characterization

The chloride content was determined according Doughty (1924) using silver nitrate for reaction and potassium chromate as indicator. The moisture and ash content was determined according to Horwitz and Latimer (2005). The pH was determined by homogenizing 10 g sample and distilled water (1:10), utilizing combined electrode (22 DM, Digimed, São Paulo, Brazil). The water activity (aw) was measured at 20 °C using the Aqualab apparatus (Decagon Devices Inc., Pullman, USA).

The instrumental color was measured using the Hunter Lab colorimeter (Colourquest II, Hunter Associates Laboratory Inc., Virginia, USA) with D65 illuminant, 20 mm aperture and standard 10 ° observer. CIELAB L*, a*, and b* parameters were determined as an indicator of luminosity, red intensity, and yellow intensity, respectively. The whiteness index (W) was calculated by the following equation: 100 - [(100-L *) 2 + a * 2 + b * 2] 1/2. The

samples were kept at room temperature (25 ° C) during analysis. All analyses were performed in triplicate for each replicate of the experiment.

2.4 Cooking loss

The samples of the different treatments were cut into portions of 6x6 cm and desalted using a ratio of 1:6 (sample:water), with continuous water exchange every 2 hours for 30 hours, and then vacuum packed for cooking. Cooking was carried out in a water bath (RSA-1708, RSA, Campinas, Brazil) at 80°C, and the temperature of the samples was monitored by a thermocouple. From the moment the center of the sample reached 72 ° C, remaining at this temperature for 60 minutes.

After cooking procedure, the cooked samples were weighed after 30 minutes at room temperature. The cooking loss was calculated as a percent of weight difference between raw meat and cooked sample using the following equation: cooking loss= (raw sample - cooked sample / raw sample) x 100.

2.5 Statistical analysis

For each process, at least three samples were taken for each analysis. The results were expressed as the averages from all data. Data were analyzed using a General Linear Model (GLM) considering the treatments as a fixed effect and the replicates as a random effect. Significant differences were analyzed by the Tukey's test at the 5 % level of significance utilizing the commercial software Statistica v. 8 (Statsoft Inc., Tulsa, Oklahoma, USA).

3. Results and Discussion

3.1 Chloride, ash, and moisture contents

The moisture contents are presented in Table 2, chlorides levels in Table 3 and ash in Table 4. There is a relationship among the chloride levels and the ash and moisture contents of the samples.

Table 2. Moisture (%) in salted meat treatments during process

Treatments	Raw meat	AWS	ADS	FP	Standard error

FC1	75.15	72.54^{aA}	52.75^{bB}	50.61^{bC}	1.94
F1	75.19^{a}	72.52^{aA}	52.39^{bB}	50.55^{bC}	1.95
F2	75.22^{a}	72.90^{aA}	51.28^{cB}	51.44^{aB}	1.99
F3	75.21^{a}	72.98^{aA}	55.16^{aB}	50.06^{bC}	1.92
Standard error	0.13	0.11	0.24	0.12	

Values are means. $^{a, b, c, d}$ Means in the same column followed by different lowercase letters present statistically significant difference by the Tukey test ($P < 0.05$). ^{A,B,C,D} Means in the same line followed by different capital letters present statistically significant difference by the Tukey test ($P < 0.05$). AWS: after wet salting; ADS: after dry salting; FP: final product. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

The treatment F2 (50% NaCl + 50% $CaCl_2$) had the lowest ash ($P < 0.05$) and the highest moisture contents ($P < 0.05$) in the final product when compared to the other treatments. These results may be due to the difficulty of $CaCl_2$ to penetrate into the product, once it was used in excess (equivalent to 50% of ionic strength).$CaCl_2$ is used in several products as a dehydrating agent, once calcium ions increase the mass transfer leading to a higher dehydration rate (Lewicki & Michaluk, 2004). However, the high dehydration may have formed a dry barrier on the surface of the samples, impairing the water release from meat and the penetration of salt (Vidal et al., 2019).

Table 3. Chlorides (%) values in salted meat treatments during process

Treatments	Raw meat	AWS	ADS	FP	Standard error
FC1	0.21^{a}	1.86^{cC}	16.44^{aA}	14.53^{abB}	1.29
F1	0.20^{a}	2.34^{bB}	16.76^{aA}	15.56^{aA}	1.31
F2	0.20^{a}	2.39^{abC}	16.41^{aA}	13.23^{bB}	1.19
F3	0.20^{a}	2.54^{aB}	16.37^{aA}	15.28^{aA}	1.27
Standard error	0.01	0.05	0.29	0.28	

Values are means. $^{a, b, c, d}$ Means in the same column followed by different lowercase letters present statistically significant difference by the Tukey test ($P < 0.05$). ^{A,B,C,D} Means in the same line followed by different capital letters present statistically significant difference by the Tukey test ($P < 0.05$). AWS: after wet salting; ADS: after dry salting; FP: final product. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

158 **Table 4**. Ash (%) and cooking loss values in salted meat treatments during process

Treatments	Ash				Standard error	Cooking Loss
	Raw meat	AWS	ADS	FP		
FC1	1.07^{a}	2.66^{cC}	17.93^{bA}	17.32^{cB}	1.23	16.94^{b}
F1	1.04^{a}	3.55^{aC}	19.55^{aA}	18.79^{aB}	1.39	26.67^{a}
F2	1.03^{a}	3.15^{bC}	15.85^{cA}	15.16^{dB}	1.08	25.60^{a}
F3	1.09^{a}	3.48^{aB}	19.22^{aA}	18.99^{aA}	1.34	25.55^{a}
Standard error	0.01	0.07	0.26	0.32		0.78

159 Values are means. [a, b, c, d] Means in the same column followed by different lowercase letters
160 present statistically significant difference by the Tukey test ($P < 0.05$). [A,B,C,D] Means in the
161 same line followed by different capital letters present statistically significant difference by the
162 Tukey test ($P < 0.05$). AWS: after wet salting; ADS: after dry salting; FP: final product. FC1:
163 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25%
164 KCl + 25% $CaCl_2$

165

166 **3.2 pH and aw**

167 The results of pH of salted meat treatments are shown in Table 5. In general, a
168 decrease in the pH values was observed during the process. The addition of $CaCl_2$ decreased
169 the pH values when compared to the treatments containing only NaCl and KCl, once the
170 treatments F2 (50% NaCl + 50% $CaCl_2$) and F3 (50% NaCl + 25% KCl + 25% $CaCl_2$)
171 presented lower pH values when compared to FC1 (100% NaCl) and F1 (50% NaCl + 50%
172 KCl). Other authors have reported the effect of $CaCl_2$ on the pH reduction of meat products
173 with reduced NaCl content (Gimeno et al., 2001, Lawrence et al., 2003, Gimeno et al., 1999;
174 Vidal et al., 2019).

175 **Table 5**. pH values in salted meat treatments during process

Treatments	Raw meat	AWS	1º day	2º day	3º day	4º day	5º day	ADS	FP	Standard error
FC1	5.63^{a}	5.61^{abB}	6.05^{aA}	5.46^{aD}	5.45^{aD}	5.47^{aD}	5.24^{aE}	5.52^{aC}	5.25^{bE}	0.12
F1	5.63^{a}	5.63^{abB}	5.93^{bA}	5.46^{aBC}	5.41^{bCD}	5.38^{bCD}	5.26^{aD}	5.49^{aBC}	5.39^{aCD}	0.08
F2	5.64^{a}	5.42^{bB}	5.83^{cA}	5.40^{bB}	5.28^{cC}	5.16^{cD}	5.17^{bD}	5.31^{bB}	5.13^{cD}	0.05
F3	5.65^{a}	5.73^{aB}	5.88^{bcA}	5.41^{bC}	5.26^{dD}	5.12^{dE}	4.99^{cF}	5.18^{cDE}	5.00^{dF}	0.07

Standard error	0.01	0.09	0.02	0.01	0.01	0.02	0.02	0.12	0.13

Values are means. [a, b, c, d] Means in the same column followed by different lowercase letters present statistically significant difference by the Tukey test ($P < 0.05$). [A,B,C,D] Means in the same line followed by different capital letters present statistically significant difference by the Tukey test ($P < 0.05$). AWS: after wet salting; ADS: after dry salting; FP: final product. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

Aw is a very relevant parameter to ensure food safety, and especially in salted meat products, the low aw can confer stability during several months of storage (Toldrá, 2006). As expected, the addition of salts to the treatments significantly reduced the aw values during the process, as shown in Table 6. The treatment F2 (50% NaCl + 50% $CaCl_2$) presented the highest aw values ($P < 0.05$) during the dry salting and in the final product. As previously discussed, the higher addition of $CaCl_2$ during the dry salting may have caused a rapid surface drying, impairing the water release in the treatments.

Table 6. Aw values in salted meat treatments during process

Treatment	Raw meat	AWS	1º day	2º day	3º day	4º day	5º day	ADS	FP	Standard error
FC1	0.988^{a}	0.977^{aA}	0.948^{bB}	0.893^{bC}	0.851^{bD}	0.827^{bE}	0.786^{bF}	0.778^{bG}	0.769^{bH}	0.005
F1	0.989^{a}	0.973^{aA}	0.918^{dB}	0.864^{cC}	0.845^{cD}	0.803^{cE}	0.775^{cF}	0.765^{cG}	0.752^{cH}	0.009
F2	0.988^{a}	0.974^{aA}	0.956^{aB}	0.904^{aC}	0.901^{aC}	0.846^{aD}	0.827^{aE}	0.799^{aF}	0.781^{aG}	0.009
F3	0.989^{a}	0.976^{aA}	0.939^{cB}	0.889^{bC}	0.855^{bD}	0.826^{bE}	0.783^{bF}	0.776^{bG}	0.756^{cH}	0.008
Standard error	0.001	0.001	0.002	0.003	0.004	0.003	0.003	0.002	0.002	

Values are means. [a, b, c, d] Means in the same column followed by different lowercase letters present statistically significant difference by the Tukey test ($P < 0.05$). [A,B,C,D] Means in the same line followed by different capital letters present statistically significant difference by the Tukey test ($P < 0.05$). AWS: after wet salting; ADS: after dry salting; FP: final product. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

3.3 Instrumental color

The color characteristics of meat and meat products are fundamental for the consumers' acceptance of the product, and myoglobin is the only pigment present in sufficient amount capable of providing red color (Mancini & Hunt, 2005). As can be seen in Table 7,

the color parameters L* (luminosity), a* (red-green dimension), b* (yellow-blue dimension) and W (whiteness index) of the treatments were affected by the addition of different salts. A lower intensity of red color was observed in the salted meat products ($P < 0.05$) with the addition of KCl (F1: 50% NaCl + 50% KCl), which was more pronounced ($P < 0.05$) in the treatments with the addition of $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$ and F3: 50% NaCl + 25% KCl + 25% $CaCl_2$) in relation to the control made with 100% NaCl (FC1). In addition, the parameter W (whiteness index) increased ($P < 0.05$) in all treatments during the manufacturing process of the salted meat products. Similar results were found by Vitor et al. (2019) who replaced NaCl by KCl and $CaCl_2$ in jerked beef.

Table 7. L* (luminosity), a* (red-green dimension), b* (yellow-blue dimension) e W (whiteness index) values in salted meat treatments during process

Tr.	Raw meat	AWS	1º day	2º day	3º day	4º day	5º day	ADS	FP	Standard error
					L*					
FC1	37.09^{a}	37.61^{aD}	32.07^{bcF}	32.41^{cF}	35.94^{bE}	41.91^{bC}	49.83^{aA}	41.53^{dC}	47.93^{bB}	0.74
F1	37.33^{a}	34.00^{bE}	32.61^{bF}	33.21^{cF}	35.67^{bD}	39.29^{cC}	42.88^{bB}	46.61^{bA}	47.51^{bA}	0.68
F2	37.54^{a}	36.84^{aE}	31.38^{cG}	35.45^{bF}	34.19^{cF}	39.33^{cD}	40.06^{cC}	44.00^{cB}	48.15^{bA}	0.61
F3	36.89^{a}	37.59^{aF}	34.95^{aG}	36.53^{aF}	44.43^{aD}	45.83^{aC}	43.12^{bE}	48.58^{aB}	54.26^{aA}	0.74
Standard error	0.54	0.29	0.25	0.31	0.69	0.47	0.62	0.49	0.50	
					a*					
FC1	16.94^{a}	8.80^{cD}	15.23^{dA}	14.58^{aA}	14.44^{aA}	11.89^{aB}	12.34^{aB}	10.53^{aC}	12.03^{aB}	0.25
F1	16.74^{a}	9.87^{bE}	16.21^{cA}	13.61^{bB}	12.08^{bC}	10.66^{bD}	11.76^{aC}	9.78^{bE}	10.36^{bDE}	0.25
F2	16.23^{a}	11.54^{aE}	18.31^{aA}	12.77^{cD}	14.55^{aC}	9.41^{cF}	9.45^{bB}	8.41^{cG}	7.81^{cG}	0.42
F3	16.41^{a}	9.89^{bD}	16.90^{bA}	12.75^{cB}	11.99^{bC}	9.79^{cD}	9.64^{bD}	8.80^{cE}	6.78^{dF}	0.34
Standard error	0.62	0.18	0.20	0.16	0.21	0.18	0.44	0.15	0.37	
					b*					
FC1	18.05^{a}	13.79^{aF}	15.66^{aDE}	16.03^{aCD}	15.19^{bE}	16.65^{abBC}	19.12^{aA}	16.39^{bBCD}	17.12^{aB}	0.18

F1	18.39^{a}	12.02^{bE}	14.76^{bCD}	14.51b^{D}	14.31^{cD}	15.89^{bBC}	16.46^{cAB}	17.64^{aA}	16.32^{abBB}	0.21
F2	17.84^{a}	13.24^{aE}	16.05^{aB}	14.22^{bCD}	14.95^{bC}	14.54^{cCD}	17.81^{bA}	13.91^{cDE}	15.72^{bB}	0.17
F3	18.22^{a}	13.15^{aE}	16.23^{aC}	14.93^{bD}	17.28^{aAB}	16.84^{aBC}	15.36^{dD}	17.86^{aA}	15.55^{bD}	0.17
Standard error	0.22	0.14	0.12	0.15	0.19	0.19	0.25	0.30	0.15	
					W					
FC1	32.40^{a}	35.50^{aC}	28.65^{bE}	29.02^{dE}	32.60^{bD}	38.41^{bB}	44.91^{aA}	38.36^{cB}	43.86^{cA}	0.69
F1	32.58^{a}	32.18^{bD}	29.14^{bE}	30.31^{cE}	33.00^{bD}	36.34^{cC}	39.40^{bB}	42.91^{bA}	44.05^{cA}	0.64
F2	33.05^{a}	34.43^{aD}	27.18^{cG}	32.68^{bE}	30.96^{cF}	36.91^{cC}	35.45^{cD}	41.69^{bB}	45.26^{bA}	0.65
F3	32.29^{a}	35.46^{aE}	30.85^{aG}	33.56^{aF}	40.59^{aD}	42.43^{aC}	40.29^{bD}	44.85^{aB}	51.21^{aA}	0.73
Standard error	0.37	0.27	0.25	0.32	0.64	0.42	0.58	0.44	0.53	

Values are means. $^{a, b, c, d}$ Means in the same column followed by different lowercase letters present statistically significant difference by the Tukey test ($P < 0.05$). ^{A,B,C,D} Means in the same line followed by different capital letters present statistically significant difference by the Tukey test ($P < 0.05$). AWS: after wet salting; ADS: after dry salting; FP: final product. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% $CaCl_2$; F3: 50% NaCl + 25% KCl + 25% $CaCl_2$

3.4 Cooking loss

The heat treatment induces the water loss in meat and meat products, and the determination of this parameter during cooking is very important to predict yielding, the nutritional quality, and the sensory properties of the product, mainly regarding the juiciness perception (Bertram et al., 2003). The cooking loss values are presented in Table 4.

The parcial replacement of NaCl by KCl and $CaCl_2$ increased considerably ($P < 0.05$) the cooking loss values. The control treatment (FC1: 100% NaCl) presented 16.94% of cooking loss in relation to the treatments containing NaCl + KCl (F1: 50% NaCl + 50% KCl), NaCl + $CaCl_2$ (F2: 50% NaCl + 50% $CaCl_2$) and NaCl + KCl + $CaCl_2$ (50% NaCl + 25% KCl + 25% $CaCl_2$), which exhibited values from 25.55 to 26.67%, with no significant difference ($P < 0.05$) between them. This substitution may have increased the protein denaturation during cooking, with a lower trapping of water molecules within the protein structures maintained by the capillary forces (Aaslyng et al., 2003).

As mentioned, the cooking loss is a very important parameter affecting several characteristics, and the differences in cooking loss around 9% between the control and the treatments with partial replacement of NaCl by salt substitutes can directly affect the quality of the final product.

4. Conclusion

The addition of $CaCl_2$ during the processing of salted meat products significantly affected all the parameters studied when compared to the treatments containing only NaCl (control) or NaCl + KCl, with a consequent impact on product's quality.

The replacement of NaCl by KCl and $CaCl_2$ significantly increased the cooking loss, which may affect the sensory characteristics of the salted meat product. In general, the treatment containing NaCl + KCl presented similar characteristics to the control treatment containing only NaCl; however, the use of KCl should be carried out with caution due to the risk of hyperkalemia in patients with kidney disease.

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CAPÍTULO 8
DISCUSSÃO GERAL

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Durante o processamento de produto cárneo salgado, é adicionado um grande teor de NaCl (principal fonte de sódio na dieta) com finalidade de desenvolver todas as características tecnológicas, sensoriais e segurança microbiológica desejáveis. O princípio deste processo baseia-se na extensiva salga, com redução da atividade de água do produto resultante da secagem e entrada de NaCl na matriz cárnea. Nesse contexto, alguns produtos cárneos salgados típicos como o jerked beef e charque podem chegar a ter mais de 5000 mg/100g de sódio, e se as operações de dessalga não forem realizadas adequadamente, o consumidor acabará por ingerir grande quantidade de sódio. A Organização Mundial da Saúde recomenda uma ingestão diária de 2000 mg de sódio (equivalente a 5000 mg de NaCl) para evitar o aumento de risco de desenvolver doenças crônicas como hipertensão, câncer, doenças cardiovasculares e renais. Levando isso em consideração, o objetivo deste estudo foi avaliar a substituição parcial do NaCl por KCl e/ou $CaCl_2$ em produto cárneo salgado sobre as características sensoriais, processamento, propriedades físico-quimicas, estabilidade microbiológica, reações proteolíticas e lipolíticas.

Na primeira etapa (Capítulo 2) intitulada "*Reducing 50% sodium chloride in healthier jerked beef: an efficient design to ensure suitable stability, technological and sensory properties*", foram investigados os efeitos da substituição parcial de NaCl por blends de KCl e $CaCl_2$ sobre as propriedades físico-químicas, microbiológicas e sensoriais da carne salgada. Durante o processamento, observou-se que o $CaCl_2$ promoveu maior desidratação em relação ao NaCl e ao KCl. Segundo Lewicki e Michaluk (2004), os íons de cálcio aumentam a transferência de massa. Portanto, o uso de *blends* contendo 50% de $CaCl_2$ causou uma elevada desidratação superficial, dificultando a liberação de água e diminuição da atividade de água contida no interior das amostras.

Outra característica observada ao longo do processamento foi a diferença de cor da carne salgada e do líquido liberado durante a etapa de salga seca. O $CaCl_2$ possivelmente deslocou o ferro e desnaturou a mioglobina durante a desidratação osmótica, observando-se que o líquido drenado durante a etapa de salga foi transparente, o que não foi verificado para os demais tratamentos. Além disso, o $CaCl_2$ causou um maior declínio do pH durante o processamento do produto cárneo salgado. O efeito de redução de pH do $CaCl_2$ foi relatado por outros autores em diferentes produtos cárneos. (Fieira, Marchi, Marafão e Alfaro, 2018; Gimeno, Astiasarán e Bello, 1999; Gimeno, Astiasarán e Bello, 2001; Horita, Messias,

Morgano, Hayakawa e Pollonio, 2014; Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2003; Lorenzo, Cittadini, Bermúdez, Munekata, & Domínguez, 2015). A substituição parcial de NaCl por KCl e/ou $CaCl_2$ adicionado nas etapas de salga úmida e seca diminuiu consideravelmente o teor de sódio.

Em relação às características sensoriais, a adição de $CaCl_2$ desenvolveu fibrosidade aparente, aroma de ranço, sabor amargo, dureza, fibrosidade e sabor residual, enquanto NaCl e KCl foram associados a coloração vermelha, sabor salgado e suculência. Diversos autores relataram os efeitos indesejáveis do $CaCl_2$ sobre as características sensoriais de diferentes produtos cárneos (Armenteros, Aristoy, Barat, & Toldrá, 2012; dos Santos et al., 2015; Horita, Morgano, Celeghini, & Pollonio, 2011).

A aceitação sensorial apresenta-se como um dos maiores desafios em carne salgada com redução de sódio. Apesar do tratamento com KCl ter sido avaliado como uma promissora solução, o uso indiscriminado desse sal como substituto ao NaCl vem sendo questionado por problemas de excesso de ingestão de potássio – a hiperpotassemia. Para minimizar os efeitos sensoriais negativos do $CaCl_2$, no Capítulo 3 "*Adding lysine and yeast extract improves sensory properties of low sodium salted meat*", foram testados: lisina, taurina, arginina, inosinato + guanilato e intensificadores de sabor naturais. Após os testes de aceitação e diferença do controle, a lisina e o extrato de levedura foram selecionados. A lisina e o extrato de levedura demonstraram ser uma boa opção para diminuir os efeitos sensoriais indesejáveis desenvolvidos pelo $CaCl_2$ no produto cárneo salgado.

A mobilidade da água nos diferentes tratamentos foi estudada no Capítulo 4 "*Understanding the effect of different chloride salts on the water behavior in the salted meat matrix along 180 days of shelf life*" através de análises físico-químicas, microestrutura e ressonância magnética de baixo campo. A adição de *blends* contendo $CaCl_2$ modificou a estrutura do produto cárneo salgado, aumentando os espaçamentos na matríz, atividade de água, desidratação e perda de peso durante 180 dias de armazenamento. A substituição parcial do NaCl por KCl durante a elaboração da carne salgada promoveu impactos semelhantes na microestrutura, desenvolvendo topografia densa, compacta e homogêna. Por outro lado, o uso de $CaCl_2$ resultou em uma microestrutura heterogênea com cristais de sal aparentes, podendo ser associada migração de cristal ao efeito desidratante deste sal.

No Capítulo 5 "*Substitution effects of NaCl by KCl and $CaCl_2$ on lipolysis of salted meat*", foi constatado que a substituição parcial de NaCl por KCl e CaCl2 influenciou as reações de

lipólise e perfil lipídico do produto cárneo salgado. O $CaCl_2$ aumentou a oxidação lipídica ao longo da vida de prateleira (180 dias) e os compostos voláteis e perfil de ácidos graxos foram afetados pelo sal utilizado nas etapas de salga. A maior capacidade oxidativa e o impacto do CaCl2 no perfil lipídico em comparação com o NaCl e o KCl em tratamentos de carne salgada com a mesma força iônica foram observados.

Foi investigado o impacto das reações proteolíticas através da análise de força de cisalhamento e perfil eletroforético durante 180 dias de armazenamento de carne salgada no Capítulo 6 "*How does the proteolysis reactions impact the texture in reduced sodium salted meat along shelf life*?". Os valores de força de cisalhamento (N) foram significativamente aumentados pela adição de $CaCl_2$, podendo ser devido à ação desidratante característica deste sal. Além disso, em todos os tratamentos, ao longo do armazenamento, ocorreu uma diminuição gradual dos valores de força cisalhamento, possivelmente provocada pelas reações proteolíticas influenciadas pelos sais. Em relação ao perfil eletroforético, pode-se notar que ocorreu a proteólise das proteínas miofibrilares e sarcoplasmáticas durante o armazenamento de 180 dias de carne salgada. O $CaCl_2$ intensificou as reações de proteólise, podendo ser notado pela diminuição da intensidade das bandas de proteínas miofibrilares maiores que 100 kDa (sendo a cadeia pesada de miosina a mais afetada) após 135 dias armazenamento e intensa proteólise das proteínas sarcoplasmáticas após 45 dias de armazenamento

No Capítulo 7 "*Influence of the addition of KCl and $CaCl_2$ blends on the physicochemical parameters of salted meat products throughout the processing steps*", os parâmetros físicos-químicos de tratamentos de carne salgada ao longo das etapas de salga úmida, salga seca e maturação foram analisados. A substituição do NaCl por KCl e/ou $CaCl_2$ aumentou a perda por cozimento, podendo impactar as características sensoriais. O uso de $CaCl_2$ durante o processamento afetou significativamente todos parâmetros de qualidade estudados quando comparados com os tratamentos contendo apenas NaCl ou NaCl + KCl.

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CAPÍTULO 9
CONCLUSÃO GERAL

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O presente estudo demonstrou a viabilidade tecnológica da redução de sódio em carnes salgadas através da utilização parcial de blends de sais substitutos ao cloreto de sódio com aspectos de qualidade físico-química, sensorial e microbiológica preservados.

A substituição parcial de 50% de cloreto de sódio por blends de sais de cloreto de cálcio e cloreto de potássio resultou em significativa redução de sódio nos produtos reformulados, variando entre 27,57% a 41,58%.

O uso do $CaCl_2$ promoveu características indesejáveis ao produto cárneo salgado, aumentando a dureza, taxa de oxidação lipídica, afetando as reações de proteólise, e reduzindo significativamente a aceitação sensorial devido ao desenvolvimento do aroma de ranço, sabor amargo, sabor residual e fibrosidade. Para minimizar os efeitos sensoriais adversos causados pelo $CaCl_2$, a adição de lisina e extrato de levedura durante a elaboração da carne salgada provou ser eficiente além de não provocar mudanças nos parâmetros de qualidade fisico-química e segurança dos tratamentos de carne salgada.

O *blend* contendo 50% da força iônica fornecida pelo $CaCl_2$ prejudicou a redução da atividade de água quando comparado ao NaCl e KCl. O tempo de desidratação durante o processo de salga da carne pode ser prolongado com a adição de grande teor de $CaCl_2$, o que pode acarretar problemas durante a elaboração e requerer adaptações de processo para não afetar adversamente o produto. A estabilidade microbiológica dos produtos cárneos salgados é um requisito muito importante, pois esta categoria é uma fonte relevante de proteína e outros nutrientes principalmente em regiões onde a cadeia de frio é ineficaz ou inexistente. Por outro lado, a substituição parcial do NaCl por KCl forneceu características tecnológicas e sensoriais semelhantes à carne salgada tradicionalmente feita com 100% de NaCl, porém, o consumo em excesso de potássio pode prejudicar a saúde de pessoas com problemas renais devido a hiperpotassemia.

Finalmente, o presente estudo contribuiu para o desenvolvimento de um produto cárneo salgado com expressiva redução de sódio, seguro, estável durante sua vida de prateleira, bom desempenho sensorial, com grandes benefícios nutricionais e impactos positivos sobre a saúde para públicos consumidores desse produto.

CAPÍTULO 10
REFERÊNCIAS

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ANEXOS

ANEXO 1

PARECER CONSUBSTANCIADO DO COMITÊ DE ÉTICA EM PESQUISA

UNICAMP - CAMPUS CAMPINAS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Redução de sódio em carnes salgas: efeito da adição de sais e realçadores de sabor sobre padrões de identidade, qualidade e segurança

Pesquisador: VITOR ANDRE SILVA VIDAL

Área Temática:

Versão: 2

CAAE: 65255316.9.0000.5404

Instituição Proponente: Faculdade de Engenharia de Alimentos

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.017.279

Apresentação do Projeto:

Produtos cárneos salgados são amplamente consumidos no Brasil e possuem alta relevância nutricional, especialmente em áreas onde a cadeia de frio é limitada, permitindo o acesso ao consumo de carne através da salga como método de conservação, o que os coloca como alimentos de grande relevância nutricional. Apesar desse significativo impacto, os elevados teores de sódio presente nos produtos cárneos salgados, principalmente

charque e Jerked Beef, quando consumidos em excesso podem aumentar o risco de desenvolvimento de hipertensão e doenças cardiovasculares, entre outras. A OMS recomenda o consumo de 2 g de sódio por dia, equivalente a 5 g de cloreto de sódio (NaCl). A redução sódio em carne salgada é um grande desafio tecnológico devido às funções tecnológicas do sal, cloreto de sódio, principal fonte de Na, responsável por redução da atividade de água e consequente segurança microbiológica, estabilidade durante vida de prateleira, desenvolvimento de textura e sabor característicos, além das demais propriedades sensoriais. O objetivo do presente projeto de pesquisa é avaliar os efeitos da substituição parcial do NaCl por KCl, CaCl2 e sobre as reações proteolíticas e lipolíticas de carne bovina salgada visando a redução de Na nos produtos finais. Na primeira etapa, serão avaliados os efeitos da redução de NaCl e adição de blends de KCl e CaCl2 com base na força iônica, sobre a qualidade

microbiológica, físico-química e sensorial de carnes salgadas combinada à seleção de diferentes realçadores de sabor. Na segunda etapa, serão avaliados os efeitos da redução de NaCl e adição de blends de KCl e CaCl2 sobre as reações de lipólise e oxidação lipídica em carnes salgadas. E na terceira etapa serão avaliados os efeitos da redução de NaCl e adição de blends de KCl e CaCl2 sobre as reações de proteólise em carnes salgadas. Espera-se com esse trabalho contribuir efetivamente na melhoria da qualidade nutricional de carnes salgadas com menores teores de Na, bem como com a indústria de processamento com recomendações de estratégias
tecnológicas viáveis para esse fim e junto a órgãos regulatórios na obtenção de um novo padrão de identidade de qualidade para essa categoria de produto. Serão produzidas amostras de Jerked Beef para análise sensorial de aceitação e Análise Descritiva Quantitativa (ADQ). Análises microbiológicas serão realizadas previamente para determinar a segurança do produto. Para análise dos dados será utilizado nível de significância de 5% ($p<0,05$). Os resultados serão avaliados por Análise de Variância e teste de médias de Tukey utilizando o programa Statistica software v.8 (Statsoft, Inc., Tulsa, OK, USA). Quanto às respostas sensoriais serão avaliados por análise de variância e aplicado o teste de Friedman e a Análise Múltipla de Fatores utilizando o programa XLSTAT-sensory (Microsoft, USA).

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar os efeitos da substituição parcial do NaCl por KCl e CaCl2 e adicionados de diferentes realçadores de sabor sobre as reações proteolíticas, lipolíticas e vida de prateleira de carne bovina salgada visando a redução de Na nos produtos finais.

Objetivo Secundário:

Avaliar o efeito substituição parcial do NaCl por KCl e CaCl2 sobre a segurança microbiológica e estabilidade durante vida-de-prateleira.

Investigar o efeito substituição parcial do NaCl por KCl e CaCl2 sobre as reações proteolíticas e lipolíticas de carnes salgadas com redução de Na.

Avaliar as propriedades físico-químicas e sensoriais de carnes bovinas salgadas com redução de Na, adicionadas de sais substitutos ao NaCl e adicionados de diferentes realçadores de sabor.

Elaborar um Padrão de Identidade e Qualidade para carnes bovinas salgadas com redução de Na de forma a contribuir com órgãos regulatórios na implementação dos resultados da presente pesquisa.

Avaliação dos Riscos e Benefícios:

Riscos

Em caso de dúvidas ou reações adversas o contato do responsável pela pesquisa estará no Termo de Consentimento Livre e Esclarecido, que será entregue ao provador previamente à análise sensorial. O participante deverá ler o que está contido no documento e avaliar se participará ou não do estudo. O risco associado ao teste é extremamente baixo devido ao rigor do controle higiênico-sanitário da planta piloto onde serão elaborados os produtos cárneos salgados. Além disso, serão realizadas análises microbiológicas prévias que garantam inocuidade do alimento

Benefícios:

Não haverá benefícios diretos aos provadores. As respostas dos voluntários ajudarão os pesquisadores a elaborar um produto cárneo com reduzido teor de Na com consequente apelo de saudabilidade.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um projeto de doutorado a ser desenvolvido por um aluno na Faculdade de Engenharia de Alimentos (FEA)-Unicamp, sob supervisão de uma docente do Departamento de Tecnologia de Alimentos (DTA) dessa Unidade. Os experimentos serão realizados no Laboratório de Carnes do referido departamento. O Cronograma mostra que este trabalho foi iniciado no primeiro trimestre de 2016 com revisão bibliográfica e testes também iniciados no primeiro trimestre de 2016, perdurando durante todo esse ano (2016). Foi apresentado orçamento, sendo dada a informação que o projeto será custeado com recursos próprios do proponente. Serão realizadas análises físico-químicas: umidade, pH, Aw, cinzas, proteínas, lipídios, textura objetiva, potencial de óxido-redução, cor, teor de sódio, potássio e cálcio. A descrição completa e pormenorizada do processamento do material e das análises físico-químicas, microbiológicas e sensoriais encontra-se no projeto detalhado. Para avaliar o perfil sensorial das amostras será realizada a análise de aceitação e Análise Descritiva Quantitativa (ADQ). Para a análise de aceitação será utilizada uma escala hedônica estruturada de nove pontos, com extremos variando de desgostei muitíssimo a gostei muitíssimo. Os atributos de cor, aroma, sabor, textura e impressão global serão avaliados por 130 consumidores recrutados entre estudantes, funcionários e professores da Universidade Estadual de Campinas, com idades entre 18 e 60 anos (Meilgaard et al, 1999). As amostras serão avaliadas logo após a finalização do processo de fabricação. As amostras serão apresentadas aos consumidores de forma monádica e a ordem de apresentação seguirá um delineamento balanceado conforme descrito por Macfie et al. (1989). Será utilizado a ADQ descrita por Stone e Sidel, 1985. Os provadores treinados irão realizar o levantamento dos termos descritores sensoriais através do método tradicional de análise descritiva quantitativa (Stone e

Sidel, 1993) sendo que as amostras serão apresentadas aos provadores de forma monádica e em cabines individuais de avaliação sensorial. Os provadores serão solicitados a avaliar as amostras, utilizando a Ficha de Aplicação do Método de Análise Descritiva Quantitativa. Após cada provador gerar seus próprios termos para descrever todas as características de cada amostra, ocorrerá uma discussão em grupo com objetivo de agrupar os termos descritivos semelhantes e gerar amostras de referência. Com os termos descritores gerados será elaborada a ficha de avaliação, utilizando escalas não estruturadas de 9 cm, com extremos que irão variar de desgostei muitíssimo a gostei muitíssimo. A equipe definitiva para a análise descritiva quantitativa será selecionada através de testes que irão utilizar uma ficha elaborada com as escalas de intensidade para os termos descritivos definidos. Serão selecionados os candidatos com base no poder de discriminação entre amostras, repetibilidade e concordância entre os provadores (Damásio e Costel, 1991), que será verificada através de análise de variância de dois fatores (F amostra e F repetição) para cada provador em relação a cada atributo (Stone e Sidel, 1985). A equipe selecionada de provadores deverá apresentar valores de F amostra significativa para $p<0,30$ e F repetição não significativo para $p>0,05$ e concordância com as médias da equipe. Os provadores selecionados irão avaliar as amostras através de três repetições, com auxilio da ficha sensorial de escala não estruturada de 9cm. As amostras serão apresentadas codificadas com algarismos de três dígitos, em blocos completos balanceados (Macfie e Bratchell, 1989) de forma monádica (Stone e Sidel, 1985) e em cabines individuais, no Laboratório de Análise Sensorial do Departamento e Tecnologia de Alimentos.

Critério de Inclusão:

O pesquisador responsável irá se comprometer em recrutar apenas provadores que não tenham restrições de qualquer ordem (como alergia ou intolerância alimentar, religiosa, etc.) para consumo de produtos cárneos com matéria-prima bovina e sais.

Metodologia de Análise de Dados:

Os resultados serão avaliados por Análise de Variância e teste de médias de Tukey utilizando o programa Statistica software v.8 (Statsoft, Inc., Tulsa, OK, USA). Quanto às respostas sensoriais serão avaliados por análise de variância e aplicado o teste de Friedman. e a Análise Múltipla de Fatores utilizando o programa XLSTAT-sensory (Microsoft, USA) ambos ao nível de significância de 5% para comparação entre as médias.

Considerações sobre os Termos de apresentação obrigatória:

Foram apresentados a Folha de Rosto, assinada pelo Diretor da Faculdade de Engenharia de

Alimentos- Unicamp, o documento com Informações Básicas do projeto, o projeto detalhado e o Termo de Consentimento Livre e Esclarecido.

Recomendações:

Nenhuma.

Conclusões ou Pendências e Lista de Inadequações:

Pendência 1:

Apresentar os critérios de exclusão dos participantes da pesquisa.

Resposta:

Foram adicionados os critérios de exclusão dos participantes da pesquisa no tópico 5. População.

Pendência 2:

Esclarecer se os testes iniciados no primeiro trimestre de 2016 e durante todo o ano de 2016, conforme mencionado no cronograma do projeto, envolveram participantes da pesquisa.

Resposta:

Os testes iniciados no primeiro trimestre de 2016 mencionados no cronograma se referem aos testes de procedimentos e análises, excluindo testes sensoriais.

Pendência 3:

Esclarecer de que forma (cartaz,, etc.) será feito o recrutamento dos participantes da pesquisa. Em caso deste recrutamento ser feito via cartaz ou pôster, anexar ao projeto o texto que constará do mesmo.

Resposta:

O recrutamento de participantes para o teste de aceitação e análise descritiva quantitativa será realizado via cartazes espalhados pela Faculdade de Engenharia de Alimentos da UNICAMP.

Considerações Finais a critério do CEP:

- O participante da pesquisa deve receber uma via do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (quando aplicável).

- O participante da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (quando aplicável).

- O pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado. Se o pesquisador considerar a descontinuação do estudo, esta deve ser justificada e somente ser

realizada após análise das razões da descontinuidade pelo CEP que o aprovou. O pesquisador deve aguardar o parecer do CEP quanto à descontinuação, exceto quando perceber risco ou dano não previsto ao participante ou quando constatar a superioridade de uma estratégia diagnóstica ou terapêutica oferecida a um dos grupos da pesquisa, isto é, somente em caso de necessidade de ação imediata com intuito de proteger os participantes.

- O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo. É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

- Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas e aguardando a aprovação do CEP para continuidade da pesquisa. Em caso de projetos do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma, junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial.

- Relatórios parciais e final devem ser apresentados ao CEP, inicialmente seis meses após a data deste parecer de aprovação e ao término do estudo.

-Lembramos que segundo a Resolução 466/2012 , item XI.2 letra e, "cabe ao pesquisador apresentar dados solicitados pelo CEP ou pela CONEP a qualquer momento".

-O pesquisador deve manter os dados da pesquisa em arquivo, físico ou digital, sob sua guarda e responsabilidade, por um período de 5 anos após o término da pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_837945.pdf	31/03/2017 14:10:27		Aceito

Continuação do Parecer: 2.017.279

Projeto Detalhado / Brochura Investigador	Comite_de_Etica_Vitor_Vidal.pdf	31/03/2017 14:09:13	VITOR ANDRE SILVA VIDAL	Aceito
Outros	Carta_Resposta.pdf	31/03/2017 14:03:29	VITOR ANDRE SILVA VIDAL	Aceito
Outros	Cartaz_Vitor_Vidal.pdf	31/03/2017 14:01:16	VITOR ANDRE SILVA VIDAL	Aceito
Outros	Vitor_Vidal.jpg	22/02/2017 14:13:56	VITOR ANDRE SILVA VIDAL	Aceito
Outros	Atestado_Matricula_Vitor_Vidal.pdf	22/02/2017 14:09:12	VITOR ANDRE SILVA VIDAL	Aceito
Folha de Rosto	Folha_de_rosto_Vitor_Vidal.pdf	16/12/2016 14:39:45	VITOR ANDRE SILVA VIDAL	Aceito
Cronograma	Cronograma.pdf	06/12/2016 00:06:31	VITOR ANDRE SILVA VIDAL	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Termo_de_consentimento_Vitor_Vidal.pdf	06/12/2016 00:02:56	VITOR ANDRE SILVA VIDAL	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CAMPINAS, 17 de Abril de 2017

Assinado por:
Renata Maria dos Santos Celeghini
(Coordenador)

Endereço: Rua Tessália Vieira de Camargo, 126
Bairro: Barão Geraldo **CEP:** 13.083-887
UF: SP **Município:** CAMPINAS
Telefone: (19)3521-8936 **Fax:** (19)3521-7187 **E-mail:** cep@fcm.unicamp.br

ANEXO 2

CADASTRO NO SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL. ASSOCIADO

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº A6C3B82

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A6C3B82**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie
Manihot esculenta
Capsicum annuum
Myristica fragrans
Piper nigrum
Bixa orellana
Cichorium endivia
Salvia hispânica
Bambu Bambuseae
Brassica napus
Chondrus crispus
Staphylococcus xylosus
Pediococcus pentosaceus
Saccharomyces cerevisiae

Listeria innocua

Título da Atividade: **REDUÇÃO DE SAL E GORDURA SATURADA EM PRODUTOS CÁRNEOS PROCESSADOS**

Equipe

Claudia Nakamura Horita	**UNICAMP**
Mária Herminia Ferrari Felisberto	**UNICAMP**
Bibiana Alves dos Santos	**UNICAMP**
Paulo Cezar Bastianello Campagnol	**UNICAMP**
Vanessa Cristina Messias	**UNICAMP**
Andrea Carla da Silva Barreto	**UNICAMP**
Iara Maria Cerqueira Magalhães	**UNICAMP**
Maria Raquel Manhani	**UNICAMP**
Carla Ivone Carraro	**UNICAMP**
Maristela Midori Ozaki	**UNICAMP**
Camila de Souza Paglarini 023.652.051-27	**UNICAMP**
Vitor Andre Silva Vidal	**UNICAMP**

Resultados Obtidos

Divulgação de resultados em meios científicos ou de comunicação

Identificação do meio onde foi divulgado:	**Dissertação de Mestrado. Maristela Midori Oza**
Identificação do meio onde foi divulgado:	**Dissertação de Mestrado - Vitor André Vidal. E**
Identificação do meio onde foi divulgado:	**Dissertação de Mestrado: Redução de sal e gor**
Identificação do meio onde foi divulgado:	**Dissertação de Mestrado: Aplicação de amidos**
Identificação do meio onde foi divulgado:	**Dissertação de Mestrado: Efeito da adição e fib**
Identificação do meio onde foi divulgado:	**Dissertação de Mestrado: Redução de cloreto d**
Identificação do meio onde foi divulgado:	**Dissertação de Mestrado: Redução de cloreto d**
Identificação do meio onde foi divulgado:	**Dissertação de Mestrado: Reformulação de pro**

Identificação do meio onde foi divulgado:	**Teses de doutorado: Efeito de lactato de potás**
Identificação do meio onde foi divulgado:	**Teses de doutorado: Estratégias tecnológicas**
Identificação do meio onde foi divulgado:	**Teses de doutorado: Redução de sódio em sal**
Identificação do meio onde foi divulgado:	**Teses de doutorado: Redução de sódio em sal**
Identificação do meio onde foi divulgado:	**Teses de doutorado: Efeito da adição de fibras**
Identificação do meio onde foi divulgado:	**Teses de doutorado: Influência da redução de**
Identificação do meio onde foi divulgado:	**Dissertação de Mestrado: Estruturação de óleo**
Identificação do meio onde foi divulgado:	**Teses de doutorado: Aplicação de polissacarid**
Identificação do meio onde foi divulgado:	**Teses de doutorado: Efeitos da adição de Chia**
Identificação do meio onde foi divulgado:	**Teses de doutorado: Redução de sódio em car**
Identificação do meio onde foi divulgado:	**Teses de doutorado:Desenvolvimento de géis**
Identificação do meio onde foi divulgado:	**Meat Science, v. 145, p. 55-62, 2018. https://doi.**
Identificação do meio onde foi divulgado:	**Journal of Food Quality, v. 2017, p. 1-8, 2017. ht**
Identificação do meio onde foi divulgado:	**. Meat Science, v. 131, p. 90-98, 2017.**
Identificação do meio onde foi divulgado:	**. Journal of Food Engineering, v. 222, p. 29-37,**
Identificação do meio onde foi divulgado:	**Food Research International, v. 84, p. 1-8, 2016.**
Identificação do meio onde foi divulgado:	**Journal of Food Engineering, v. 151, p. 16-24, 2**
Identificação do meio onde foi divulgado:	**Journal of Food Quality, v. 38, p. 201-212, 2015.**

Identificação do meio onde foi divulgado:	**Food Science and Technology (Campinas), v. 3**
Identificação do meio onde foi divulgado:	**Food Research International, v. 76, p. 725-734, 2**
Identificação do meio onde foi divulgado:	**Journal of Food Science, v. 80, p. S1093-S1099,**
Identificação do meio onde foi divulgado:	**Meat Science, v. 104, p. 44-51, 2015. https://doi.**
Identificação do meio onde foi divulgado:	**Meat Science, v. 104, p. 44-51, 2015. https://doi.**
Identificação do meio onde foi divulgado:	**Food Research International, v. 74, p. 306-314, 2**
Identificação do meio onde foi divulgado:	**Meat Science, v. 96, p. 509-513, 2014. https://do**
Identificação do meio onde foi divulgado:	**Ciência e Tecnologia de Alimentos (Online), v. 3**
Identificação do meio onde foi divulgado:	**. Food Research International, v. 66, p. 29-35, 2(**
Identificação do meio onde foi divulgado:	**Lebensmittel-Wissenschaft + Technologie / Foc**
Identificação do meio onde foi divulgado:	**Lebensmittel-Wissenschaft + Technologie / Foc**
Identificação do meio onde foi divulgado:	**Journal of Food Quality, v. 36, p. 41-50, 2013. ht**
Identificação do meio onde foi divulgado:	**Meat Science, v. 91, p. 334-338, 2012. https:**
Identificação do meio onde foi divulgado:	**International Journal of Food Science & Techn**
Identificação do meio onde foi divulgado:	**Meat Science, v. 90, p. 36-42, 2011. https://doi.o**
Identificação do meio onde foi divulgado:	**Meat Science, v. 87, p. 239-243, 2011. https:/**
Identificação do meio onde foi divulgado:	**Meat Science, v. 87, p. 290-298, 2011. https://do**
Identificação do meio onde foi divulgado:	**Meat Science, V. 89, P. 426-433, 2011. https**

Data do Cadastro:	**03/11/2018 23:17:45**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **23:19** de **03/11/2018**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN**

ANEXO 3

PERMISSÃO PARA O USO DO ARTIGO CORRESPONDENTE AO CAPÍTULO 2

Title: Reducing 50% sodium chloride in healthier jerked beef: An efficient design to ensure suitable stability, technological and sensory properties

Author: Vitor A.S. Vidal,João P. Biachi,Camila S. Paglarini,Mariana B. Pinton,Paulo C.B. Campagnol,Erick A. Esmerino,Adriano G. da Cruz,Marcelo A. Morgano,Marise A.R. Pollonio

Publication: Meat Science

Publisher: Elsevier

Date: June 2019

ANEXO 4

PERMISSÃO PARA O USO DO ARTIGO CORRESPONDENTE AO CAPÍTULO 3

Title: Adding lysine and yeast extract improves sensory properties of low sodium salted meat

Author: Vitor A.S. Vidal,Jaqueline B. Santana,Camila S. Paglarini,Maria A.A.P. da Silva,Mônica Q. Freitas,Erick A. Esmerino,Adriano G. Cruz,Marise A.R. Pollonio

Publication: Meat Science

Publisher: Elsevier

Date: Available online 29 August 2019

ANEXO 5

PERMISSÃO PARA O USO DO ARTIGO CORRESPONDENTE AO CAPÍTULO 4

Title: Understanding the effect of different chloride salts on the water behavior in the salted meat matrix along 180 days of shelf life

Author: Vitor A.S. Vidal,Oigres Daniel Bernardinelli,Camila S. Paglarini,Edvaldo Sabadini,Marise A.R. Pollonio

Publication: Food Research International

Publisher: Elsevier

Date: Available online 23 August 2019